Bioceramic-based composites reinforced with carbonaceous nanomaterials

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NANYANG
TECHNOLOGICAL
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**BIOCERAMIC-BASED COMPOSITES REINFORCED
WITH CARBONACEOUS NANOMATERIALS**

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SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

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WITH CARBONACEOUS NANOMATERIALS**

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School of Mechanical and Aerospace Engineering

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Abstract

Carbon nanotubes (CNTs) and graphene have been widely used to toughen bioceramics such as bioactive glasses and hydroxyapatite (HA). However, it remains a challenge to homogeneously disperse them in the matrix and achieve adequate interfacial strength. In this thesis, two approaches have been successfully used to address these issues. They are: (1) using graphene oxide (GO) as a precursor to graphene, and (2) coating CNTs with a SiO₂ shell. Spark plasma sintering (SPS) was used to fabricate the pure bioceramic samples as well as the composites, as it can provide high heating and densification rates and preserve the structural integrity of carbon-based reinforcements.

Dense 45S5 Bioglass® (45S5) pellets with different degrees of crystallinity were prepared by sintering melt-derived 45S5 powder in the range of 500–600 °C for 3 min. The samples sintered at 500 °C were amorphous and those sintered at 550 and 600 °C contained the crystalline phase Na₂CaSi₃O₈. The cytocompatibility of the 45S5 compacts was assessed with human osteosarcoma cell line MG63 and mouse fibroblast cell line L929. Two types of pellets were used for the biological tests: the freshly sintered and those aqueously aged for 21 days. A lower sintering temperature and aqueous aging could both lead to faster proliferation and higher viability of the MG63 and L929 cells cultured in the pellet-conditioned media. On the other hand, the samples sintered at 550 °C resulted in higher alkaline phosphatase (ALP) activity in the MG63 cells grown on their surfaces than those prepared at 550 or 600 °C. Therefore, 550 °C was selected to prepare graphene/45S5 composites.

Prior to the fabrication of graphene-reinforced 45S5, SPS was employed as a thermal reduction process for GO. The influences of temperature, holding time, and number of processing cycles on the properties of the reduced graphene oxide (rGO) were studied. It was found that GO could be successfully exfoliated, reduced and hierarchically roughened at 500 °C, which is lower than the critical temperature (550 °C) required for GO exfoliation to occur in conventional thermal reduction processes. The rGO obtained by 1 min of SPS treatment at 1050 °C possessed a remarkably high C:O ratio of 83.03 (atom %) and its surface roughness was 10 times as large as that of GO. Among the three SPS parameters investigated, the temperature was found to play a decisive role, while the

holding time and number of processing cycles showed minor influence on the properties of rGO. The reduction mechanism was proposed to be the synergistic action of local high temperature, spark pressure, and high-speed ion migration.

GO/45S5 composite powders with 0.1, 0.5, and 1 wt.% GO were sintered at 550 °C for 3 min. Biological tests of pure 45S5 pellets indicated that the optimum sintering temperature was 550 °C, the temperature at which *in situ* GO reduction was expected to occur. The optimum GO loading was found to be 0.5 wt.%, which increased the fracture toughness by 130.2 % and decreased the specific wear rate and friction coefficient by 62.0 % and 21.3 %, respectively. Moreover, the viability of MG63 cells cultured on the composite and pure pellets showed equivalent viability, while generally faster proliferation and higher viability were observed for the MG63 cells grown in the media conditioned by the composites.

Pure HA compacts were fabricated to study the influences of starting powder and sintering parameters (temperature and pressure) on their properties. Three types of raw powder, i.e. HA micro-spheres (MS), nano-rods (NR), and nano-spheres (NS), were sintered for 3 min in the range of 900–1100 °C. The sintered MS pellets had higher transmittance and cytocompatibility than the other two types, and 1000 °C was found to be the optimum temperature. The NS pellets showed higher mechanical properties than the MS and NR ones. For the sintering of the NR powder, a small initial pressure instead of an invariably high pressure should be applied to minimize grain coarsening and microstructural defects.

Silica-coated CNTs (S-CNTs) synthesized via a sol-gel process were mixed with HA NRs obtained from a wet chemical precipitation method. XPS and FTIR results confirmed the formation of covalent bonding between the CNT core and the SiO₂ shell. The composite powders were sintered in the range of 900–1200 °C for 3 min. The most desirable temperature for preparing the composite pellets was 1100 °C, as it led to the highest Young's modulus and hardness. When 0.1–2 wt.% S-CNTs were incorporated in HA, 1 wt.% was found to be the optimum and could enhance the Young's modulus, hardness and fracture toughness by 8.6 %, 7.3 % and 101.7 %, respectively. By contrast, these properties were either reduced or increased to a much smaller extent with the addition of 1 wt.% raw CNTs. Furthermore, MG63 cells grown on the S-CNT/HA pellets

could proliferate faster and produce significantly higher ALP than the pellets reinforced with raw CNTs. This was probably caused by the release of Si ions from the SiO₂ shell.

Toughening mechanisms such as crack bridging, crack deflection, and filler pull-out were observed for both the graphene/45S5 and S-CNT/HA composites. Additionally, S-CNTs were found to be able to bond more tightly to the HA matrix than raw CNTs.

The markedly increased fracture toughness achieved for the two composite systems can provide strong evidence for the effectiveness of the two strategies proposed in this research to cope with the challenges of the fabrication of graphene- and CNT-reinforced bioceramics. The improved biological properties further support the advantages and superiority of these strategies. This research has therefore opened new doors for the use of carbonaceous nanomaterials to enhance various bioceramics.

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Abbreviations

45S5	45S5 Bioglass®
AFM	atomic force microscope
ALP	alkaline phosphatase
BET	Brunauer–Emmett–Teller
BJD	Bone and Joint Decade
CAPAD	current-activated pressure-assisted densification
CHA	carbonated HA
CM	conditioned medium / media
CNT	carbon nanotube
DI water	deionized water
DMEM	Dulbecco's Modified Eagle's Medium
DMF	dimethylformamide
DSC	differential scanning calorimetry
EDS	energy dispersive X-ray spectroscopy
FBS	fetal bovine serum
FESEM	field emission scanning electron microscopy / microscope
FFT	fast Fourier transform
FM	fresh medium / media
FTIR	Fourier transform infrared spectroscopy
GNP	graphene nanoplatelet
GO	graphene oxide
HA	hydroxyapatite
HRTEM	high resolution transmission electron microscopy / microscope
ICP-OES	inductively coupled plasma optical emission spectrometer
ILT	in-line transmittance
MS	micro-spheres
MWCNT	multi-walled carbon nanotube
NM	non-conditioned medium / media

NR	nano-rods
NS	nano-spheres
PDF	Powder Diffraction File
PLA	poly(lactic acid)
PMMA	poly(methyl methacrylate)
R-CNT	raw carbon nanotube
rGO	reduced graphene oxide
S-CNT	silica-coated carbon nanotube
SAED	selected area electron diffraction
SPS	spark plasma sintering
SPSrGO	spark plasma sintering-reduced graphene oxide
STA	simultaneous thermal analysis
STEM	scanning transmission electron microscopy
TCP	tricalcium phosphate / tissue culture plastic
TEM	transmission electron microscopy / microscope
TEOS	tetraethyl orthosilicate
TFT	total forward transmittance
TGA	thermal gravimetric analysis
TTCP	tetracalcium phosphate
XPS	X-ray photoelectron spectroscopy / spectrometer
XRD	X-ray diffraction / diffractometer

Chapter 1 Introduction

The topic of this dissertation concerns the investigation into the structure, processing, performance and properties of bioceramic-based nanocomposites. The present introductory chapter begins with a problem statement on the low fracture toughness of two bioceramics: Bioglass® and hydroxyapatite. Then, a hypothesis pertaining to the use of two types of carbonaceous nanostructured reinforcements is proposed. In turn, the objectives and scope of this research are presented, and are followed by an outline of the dissertation. Finally, the novel contributions from the work of this dissertation are summarized.

1.1 Preamble and hypothesis

The world's population is aging rapidly, thus creating a growing demand for bone repair and regeneration. To overcome the many drawbacks of using bone transplants, which is the conventional approach to bone defect management, a wide range of synthetic biomaterials have been developed and utilized in bone grafts.

Biomaterials research interfaces with multiple disciplines including materials science, engineering, chemistry, biology, and medical science [1]. It lays the foundation for tissue engineering and the development of medical devices, and has become one of the focal points in materials science [2]. The last few decades have witnessed an increasing amount of attention drawn to biomaterials research all around the world.

Biomedical ceramics, or bioceramics for short, are among the most frequently used biomaterials in bone defect treatment. The bioactive ceramics research field was launched with the invention of Bioglass® [3-5]. Since then, a large number of bioceramics have been employed in bone defect treatment, with 45S5 Bioglass® (45S5) and hydroxyapatite (HA) being two of the most extensively used. 45S5 is the first material found to be able to chemically bond to human bone, and HA closely resembles bone mineral. Although both bioceramics have achieved commendable clinical outcomes in bone repair, the utilization of bulk 45S5 and HA in major load-bearing applications is very limited due to their insufficient fracture toughness.

To extend the applications of 45S5 and HA in orthopedic implants, various reinforcements have been incorporated to enhance their fracture toughness. Carbon nanotube (CNT) and graphene, two carbonaceous nanomaterials, possess eminent mechanical properties and are widely used to reinforce bioceramics such as 45S5 and HA [6-8]. However, like other nanomaterials, they tend to agglomerate and are very difficult to be homogeneously dispersed in the bioceramic matrix. Another issue that needs to be addressed is their bonding strength to the matrix, which has to be sufficiently strong to maximize the toughening effects. In addition, controllable and cost-effective processes for the production of high-quality pristine graphene on an industrial scale are still rare.

This research proposes two strategies to tackle the above challenges. First, graphene oxide (GO), which can be produced on a large scale and uniformly dispersed in

a variety of organic and inorganic solvents, is used as a precursor to graphene [9]. Second, raw CNTs are coated with a SiO₂ shell, as it possesses higher wettability and is likely to bond more firmly to the bioceramic matrix [10]. Another motivation for the use of SiO₂ is the presence of Si ions in the HA minerals in human bone. SiO₂ itself has been proven to be highly compatible with bone cells [11]; previous studies have shown that enriching HA with trace levels of Si could facilitate the bone remodeling process [12, 13]. The following hypothesis will be tested in this thesis:

Utilizing GO as a graphene precursor and coating CNTs with a SiO₂ layer are two sound and effective strategies for the fabrication of tough bioceramic-based composites with carbonaceous nanostructured reinforcements.

The selection of processing route for CNT- and graphene-reinforced bioceramics is of critical importance to minimize the structural damage and decomposition of the carbon phase. An advanced sintering technique, spark plasma sintering (SPS), is exploited to consolidate the composites [14]. SPS is carried out either in vacuum or inert atmosphere, and has been frequently used to sinter ceramic composites enhanced with carbonaceous nanomaterials [15-17].

1.2 Objectives and scope

This research addresses the aforementioned challenges of using carbonaceous nanomaterials to toughen bioceramics. Specifically, it aims at effectively enhancing the fracture toughness of 45S5 and HA through the incorporation of graphene (reduced graphene oxide, or rGO) and S-CNT without sacrificing the other key properties. First, the pure bioceramics and their composites will be compacted by SPS. Then, the mechanical properties (fracture toughness, Young's modulus, and hardness) will be compared. The *in vitro* biological properties of the samples will be evaluated by cell culture. The wear resistance of the graphene-reinforced 45S5 pellets will also be assessed.

The rationale behind this research is the materials science tetrahedron presented in Fig. 1.1. In essence, it concerns the optimization of the processing route and

microstructure to enhance the properties and performance of bioceramic-based systems. The execution of this project will mainly comprise the following research activities:

1. Preparing dense 45S5 samples by SPS to investigate the biological properties of the pellets sintered at different temperatures;
2. Verifying the feasibility and efficacy of SPS in the thermal reduction of GO and identifying the reduction mechanisms;
3. Fabricating graphene/45S5 composites by SPS through *in situ* GO reduction and assessing the properties of the composites with different graphene loadings;
4. Studying the influence of the starting powder and SPS parameters on the properties of pure HA pellets;
5. Synthesizing HA nanorods and S-CNTs and sintering S-CNT/HA composite pellets by SPS; and
6. Investigating the enhancement effects and underlying mechanisms induced by S-CNT incorporation in the HA-based composites.

Fig. 1.1 The materials science tetrahedron. Reproduced from Ref. [18].

1.3 Dissertation overview

This thesis addresses the gaps in knowledge on the toughening of bioceramics using carbonaceous nanostructured reinforcements. It is divided into 7 chapters:

Chapter 1 points out the vital importance of improving the mechanical properties, especially the fracture toughness, of two most commonly used bioceramics: Bioglass® and HA. Following a discussion of the potential and challenges of using CNTs and graphene as toughening agents, the hypothesis as well as research objectives and scope are proposed. Then, the thesis outline is presented. It ends by summarizing the novel scientific and scholarly contributions.

Chapter 2 reviews the literature concerning bone repair, biomaterials, and the SPS technique. It begins by briefly describing the unmet clinical need for bone defect treatment, and subsequently discusses the basics of bone structure and properties. Following that, an overview of biomaterials is presented; the properties of different types of bone-substitute materials are collated, with particular emphasis placed on two widely used bioactive ceramics, Bioglass® and HA, and their insufficient fracture toughness. Finally, it presents a thorough analysis of the mechanisms of SPS and its application in the consolidation of bioceramics and their composites, especially those reinforced with CNT and graphene. Through literature survey this chapter highlights the existing gaps in knowledge on the fabrication of bioceramics enhanced with carbonaceous nanomaterials and the novelty of this research.

Chapter 3 attempts to unveil the *in vitro* biological properties of dense 45S5 pellets with controlled crystallinity and satisfactory mechanical properties prepared by SPS. The influences of sintering temperature on the densification behavior as well as the structural, mechanical and biological properties of the compacted samples are systematically investigated. The work described in this chapter is a prerequisite for the fabrication of 45S5-based nanocomposites developed in Chapter 4.

Chapter 4 begins with a study on the outcomes and mechanisms of GO reduction by SPS. It then elaborates the preparation and properties of graphene-reinforced 45S5, where GO is used as a precursor to graphene. The sintered composites are characterized in terms of their mechanical, tribological and biological performances. An in-depth analysis of the

toughening effects and mechanisms is carried out.

Chapter 5 examines the influences of starting powder and SPS parameters (temperature and pressure) on the structural, mechanical, optical, and biological properties of sintered HA compacts. Special attention is paid to the transparent HA compacts obtained and their interaction with visible light, because they can facilitate direct visual observation of living cells cultured on them. This chapter lays a foundation for the HA-based composites studied in Chapter 6.

Chapter 6 employs S-CNTs to reinforce HA and considers the effects of sintering temperature and S-CNT loading on the mechanical properties of the composite pellets. A detailed description of the synthesis and characterization of the S-CNTs and HA nanorods is provided. Comparisons between S-CNT/HA composites with pure HA and raw CNT-reinforced HA pellets were made in the structural, mechanical, and biological aspects. The possible enhancement mechanisms brought about by S-CNTs are explored.

Chapter 7 presents a general discussion of three key factors influencing the toughening behavior, summarizes the major outcomes of this research, and recommends future work toward developing bioceramic-based nanocomposites.

Fig. 1.2 shows the key components of this thesis and how they interconnect with one another.

Fig. 1.2 Key components of this thesis.

1.4 Originality

The originality of this research arises from the enhancement of mechanical and other properties, especially the fracture toughness, of bioceramics through using two innovative strategies for the incorporation of carbonaceous nanostructured reinforcements. The major novel outcomes generated by this research are as follows:

1. Rapid fabrication of dense 45S5 pellets through SPS and a systematic investigation into their *in vitro* biological properties;
2. Effective thermal reduction of GO by SPS;
3. Enhancement of the fracture toughness, wear resistance and cytocompatibility of 45S5 by incorporating *in situ* reduced GO;
4. Identification of the influences of starting powder and SPS parameters on the optical and *in vitro* biological properties of compacted HA pellets;
5. Successful fabrication of transparent HA pellets using a direct and fast SPS process with the application of a moderate pressure;
6. The first study on HA-based composites reinforced with S-CNTs; and
7. Discovery of the unique toughening mechanisms induced by S-CNTs in a ceramic system.

Chapter 2 Literature Review

The world's aging population has led to the steeply increasing clinical need for bone defect treatment, which has in turn fueled enormous research into bone graft materials. The structure and properties of human bone are discussed in this chapter, as a deep understanding of them lays a solid foundation for the design and development of biomaterials intended for bone repair and regeneration. Then it presents a broad view of the biomaterials field, which is followed by the state-of-the-art of bone-substitute materials. Special attention will be attached to Bioglass® and hydroxyapatite, two of the most widely used bioceramics in bone tissue engineering. SPS, the primary materials processing technique utilized in this dissertation, has demonstrated distinctive advantages in sintering bioactive glasses and hydroxyapatite as well as their composites reinforced with carbonaceous nanomaterials. This chapter thus provides a detailed review of its mechanisms and applications, with special attention riveted to the recent progress in spark plasma sintered bioceramics enhanced with CNT and graphene.

2.1 Bone

Human bones possess exquisitely designed and assembled structures. They form the skeletal system and provide structural support to the body. Natural bones can offer a wealth of inspiration for researchers working on bone-substitute materials. There has been a relentless effort to fabricate bone graft materials possessing properties that can emulate those of natural bones. The development of bioceramics and bioceramic-based composites is primarily intended for bone tissue engineering. In this thesis the term *bioceramic* may refer to ceramics and glasses utilized in biomedical applications. Therefore, it is essential for researchers and engineers working in this field to understand clinical need as well as fundamental knowledge on the composition, structure and mechanical properties of bones.

2.1.1 Clinical need for bone repair and regeneration

The United Nations endorsed the Bone and Joint Decade (BJD) global initiative in 1999. The year 2000 witnessed the official launch of the BJD at the headquarters of the World Health Organization in Geneva, Switzerland. We are now in the middle of the BJD Joint Motion 2010–2020 [19]. This initiative aims at raising public awareness of the increasing importance of musculoskeletal conditions on the socioeconomic development – especially the healthcare of an aging population – at international, regional and national levels. In Asia-Pacific a Call for Action has been supported by 31 regional and national organizations [20].

Healing of large orthopedic defects usually requires mechanical and structural support from bone grafts implanted through surgical intervention. The bone grafts are able not only to fill bone defects, but also to enhance and facilitate the regeneration process of the host bone in the body. As a matter of fact, bone is the second most commonly transplanted human tissue (the first being blood) [21]. In the United States, more than 500,000 patients undergo bone defect treatment annually, at a total cost of over 2.5 billion USD; this number has been trending upward steeply and is expected to double by 2020 [22].

Depending on the source of the graft material, bone transplants can be classified into three major categories: autograft (from the same patient), allograft (from a human donor), and xenograft (from an animal). Each of these has its own advantages and disadvantages. Receiving an autograft has remained the gold standard treatment thus far and can lead to the best clinical outcome. Autografts are usually harvested from the patient's iliac crest, which significantly increases the patient's suffering and treatment cost. The scarcity of donor tissue also severely hinders the use of autografts. In addition, there may be serious donor site injury and morbidity associated with infection, hematoma, and chronic pain [23]. Furthermore, it is not feasible to use autografts when the required bone size exceeds what is available in the patient's body.

Allografts are the second most widely used bone grafts. Allogenic bone is often collected from a cadaver, followed by devitalization and freeze-drying. The absence of any cellular component makes it less osteoconductive than autografts. Although they are present at higher availability than autografts, the shortage of allogenic bones still causes a huge unmet supply demand in the bone grafting market.

Xenografts come from animal sources. It is thus necessary to have even more stringent requirements on the sterilization and preservation procedure. Even so, many risks occur with the use of xenografts, such as infection, disease transmission, and immunogenic response [24]. These challenges are also faced by allograft procedures.

Other techniques utilized to manage bone defects may involve bone cement [25], bone morphogenetic proteins [26], and distraction osteogenesis [27]. Although the various methods described above have been proven to be capable of enhancing bone repair, none of them possess all the characteristics of an ideal bone graft: biological safety, no size constraints, high availability, affordable cost, low patient morbidity, as well as satisfactory osteoconductivity and osteogenicity.

2.1.2 Bone structure and properties

Bone is an elegantly designed nanocomposite structure consisting of both organic and inorganic phases. The organic matrix is comprised predominantly of collagen, the most abundant structural protein in the human body, and the mineralized inorganic phase refers to carbonated HA (CHA) crystals [28]. As the two major components, HA and

collagen take up ~60 wt.% and ~20 wt.% of bone, respectively [24]; it should be noted that bone also has a liquid portion which occupies ~9 wt.% (~25 vol.%) and permeates it by filling the many interconnected pores [24, 29]. The HA minerals are enriched with a few subtle but significant ions such as K^+ , Mg^{2+} , Na^+ , F^- and SO_4^{2-} , which play a critical role in their bioactivity and functionality [30, 31]. The cellular component of bone is comprised of three major types of cells: osteoblasts (bone-forming cells), osteocytes (cells inside the bone) and osteoclasts (bone-resorbing cells) [32]. Fig. 2.1 presents a schematic of the three phases (initiation, transition, and formation) of the bone remodeling cycle and the roles played by various bone cells.

Fig. 2.1 Bone remodeling cycle. RANKL: receptor activator of nuclear factor kappa-B ligand. M-CSF: macrophage colony-stimulating factor. Reproduced from Ref. [33].

The hierarchical structure of bone over different length scales is presented in Fig. 2.2. As shown in Fig. 2.2, bone can be regarded as an assemblage of hierarchical structures from macro- to nanoscale. Mature lamellar bones are arranged in either a compact configuration or a trabecular form, resulting in two distinctive types: cortical

bone (compact bone) and cancellous bone (spongy bone). They each possess a porosity of 5–30 % and 30–90 %, respectively. The pores are at various scales ($\sim 1\text{--}5000\text{ }\mu\text{m}$) and highly interconnected to allow the transportation of body fluid and nutrients as well as the formation of a vascular network. The structural and functional unit of cortical bone is osteon (Fig. 2.2b), a microscopic structure comprising concentric tubular layers. Most osteons have a length of several millimeters and a diameter of $\sim 0.2\text{ mm}$. Approximately 20 % of the total bone is cancellous bone, with the rest being compact bone.

Fig. 2.2 Dimension-dependent hierarchical structure of bone. Reproduced from Ref. [34].

Bones perfectly exemplify hard yet tough materials, although nature uses only brittle (HA) and soft (collagen) ingredients to build them. The two components act in a synergistic manner, leading to the eminent mechanical properties of bone (Table 2.1). The HA minerals are principally responsible for the hardness and rigidity of bone, while the collagen fibrils endow bone with flexibility and intrinsic toughness. It is believed that the outstanding mechanical strength of bone results from the unique and complex arrangement of the two major phases in a self-assembling manner. It can be seen in Table 2.1 that cancellous and cortical bones vary drastically in mechanical properties. This is

closely related to their respective mechanical functions in different scenarios.

Table 2.1 Properties of cortical and cancellous bones [24]

Property	Cortical bone	Cancellous bone
Density (g/cm ³)	18–22	0.1–1.0
Apparent density (g/cm ³)	1.8–2.0	0.1–1.0
Total bone volume (mm ³)	1.4×10^6	0.35×10^6
Total internal surface (mm ²)	3.5×10^6	7.0×10^6
Surface/bone volume (mm ² /mm ³)	2.5	20
Strain to failure	1–3	5–7
Compressive strength (MPa)	170–193	7–10
Tensile strength (MPa)	50–150	10–20
Fracture toughness (MPa m ^{1/2})	2–12	0.1
Young's modulus (GPa)	14–20	0.05–0.5

Although the composition and the seven-level hierarchy of bone have been identified [28, 35], the precise biological mechanism by which human bone is generated by osteoblasts is still unclear. Additionally, further investigation is needed to understand how the bone composition and structure are related to its mechanical properties.

2.1.3 Summary

The world's aging population has resulted in a rapidly increasing need for bone repair and regeneration. Bone transplants and many other methods have been used for bone defect management, but none of them possess all the properties of an ideal bone graft. Engineered bone tissue based on a comprehensive understanding of the bone structure and properties provides a promising alternative to fulfill this unmet need.

2.2 Biomaterials

2.2.1 Overview

The definition of biomaterials has been evolving with their wider applications and our deeper understanding of their characteristics. The definition given by Williams in 2009 is currently the most acknowledged worldwide [1]:

“A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions

with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine.”

Three critical factors should be noted to correctly understand this definition: (1) the scope of biomaterials is solely in the healthcare domain, (2) the material has to interface tissue, and (3) it has to be engineered in one way or another.

Biocompatibility is a general term used to characterize the host response to biomaterials with which they come into contact, and it may be positive, neutral, or hostile. Over the years, there has also been continuous improvement on the concept of biocompatibility as we gain more accurate and comprehensive understanding of its mechanism [36]. During 1940–1980, researchers who developed and utilized the first generation of implantable medical devices emphasized that the material should “*do no harm to the host tissues*”. This definition was later modified to incorporate the term “*an appropriate host response*” as this gives a more proper description of the expected functionality of medical devices that are not intended for long-term contact with human tissue. The last few decades have witnessed rapid progress of biomaterials science and engineering, which provides us with opportunities to gain new insights into the mechanisms of biocompatibility. Williams [37] critically reviewed the evolution of this concept and reported a unified definition in 2008, which can be considered the current consensus. This latest definition underlines not only the elimination of undesirable effects in the recipient but also the attainment of beneficial host response.

Depending on the biocompatibility or the level of interaction with host tissue, biomaterials can be divided into three categories: bioinert, bioresorbable (biodegradable), and bioactive. Bioinert materials can remain chemically stable for a long term *in vivo*, and are usually encapsulated by a fibrous tissue [38]. They are frequently used in dental replacements and orthopedic implants. As is the minimum requirement for a biomaterial, it causes no harm to the surrounding tissue because of its bioinertness. Bioinert materials may need to be removed from the body through a second surgery, which causes extra suffering to the patient. Therefore, there have been continuous clinical and scientific trials attempting to replace them with bioresorbable or bioactive materials.

Bioresorbable materials are able to be degraded and resorbed gradually *in vivo*. The degraded part is replaced little by little by newly grown tissue, and the degradation

process continues until the material is fully resorbed. This type of material has won wide popularity in controlled drug release systems [39].

Bioactive materials can cause a thin layer of apatite to form on their surfaces when implanted *in vivo*, which bonds the surrounding hard tissues and the materials together directly and tightly. There have been increasingly high expectations of the bioactivity of today's biomaterials so as to initiate certain direct chemical and biological reactions with human tissue [40]. Bioactive ceramics in the form of a sintered bulk or a coating on Ti or Ti alloy implants, or as a component in organic-inorganic hybrids, are common examples [41].

Whereas first-generation biomaterials are neither bioresorbable nor bioactive, second-generation biomaterials are either bioresorbable or bioactive. In the year of 2002, the concept of third-generation biomaterials was proposed for those materials that possess both bioresorbability and bioactivity and can assist the body to heal itself once implanted [42].

2.2.2 Bone-substitute materials

As mentioned in Section 2.1.1, the use of bone transplants is severely hampered by their short supply and many drawbacks. From a materials perspective, they can be regarded as natural biomaterials. Various synthetic biomaterials have been developed as bone-substitute materials and significantly contributed to progress in bone tissue engineering. A schematic of the evolution of bone-substitute materials is presented in Fig. 2.3.

According to their chemical composition and properties, bone-substitute materials can come from four categories: metal and alloy, polymer, glass and ceramic, and composite. Table 2.2 shows a general comparison between the four types of materials used for bone repair and regeneration. Metals and alloys represent the first-generation biomaterials but still take up a substantial percentage of bone-substitute materials due to their wide availability and eminent mechanical properties. Since metallic biomaterials are inherently bioinert, their interfacial strength with surrounding tissue and *in vivo* fixation are critical issues affecting their service life. Metallic materials usually have a much higher stiffness than human bone, which in the long run can lead to the stress-shielding

phenomenon, namely decreased bone density caused by the reduced normal load on a bone [43]. In addition, *in vivo* corrosion and erosion lead to the release of ions and the generation of wear debris, respectively, both of which tend to induce toxic effects.

Fig. 2.3 Evolution of bone-substitute materials. Reproduced from Ref. [24].

Table 2.2 Comparison of four types of bone-substitute materials*

Material	Bonding(s)	Microstructural unit	Pros	Cons	Examples
Metal & alloy	Ionic	Grain	Strong, tough, stiff, ductile, machinable	Heavy, poor resistance to corrosion, wear and fatigue	Ti, Ti6Al4V, Co-Cr alloy
Polymer	Covalent	Molecular chain	Shapable, flexible, low density, various surface functional groups	Low strength and stiffness, poor wear and fatigue resistance	Collagen, PMMA, PLA
Glass and ceramic	Ionic/covalent	Grain/amorphous phase	Strong, hard, stiff, good resistance to corrosion, wear and high-temperature	Low toughness and tensile strength	Al ₂ O ₃ , TCP, HA, ZrO ₂ , Bioglass®
Composite	Depending on constituents	Depending on constituents	Good overall properties	High cost, possible delamination/disintegration	Collagen/HA, Ti/HA, ZrO ₂ /HA

* PMMA: poly(methyl methacrylate), PLA: poly(lactic acid), TCP: tricalcium phosphate. Hereinafter TCP stands for tricalcium phosphate when it refers to a bioceramic, and means tissue culture plastic when this acronym is used in cell culture tests.

Major applications of Bioglass® and bioceramics are found in hard tissue repair

because they inherently combine good mechanical strength and chemical stability. Moreover, they have demonstrated unequivocal suitability in a wide variety of applications outside the skeletal system [44, 45]. In addition, many ions present in bioactive glasses and ceramics (e.g., Ca^{2+} , Na^+ , Mg^{2+} , and K^+) are frequently found in the physiological environment as well. This is an important reason for their biocompatibility. Among the many different bioceramics, alumina and zirconia are superior to others in terms of strength and toughness, leading to their creditable clinical success when used in total hip replacement surgeries [46-48]. Nonetheless, they are bioinert and thus suffer from weak bonding strength to host tissues. Many members in the calcium phosphate family are either bioactive or bioresorbable ceramics, among which HA has attracted the greatest amount of research interest. HA and Bioglass® are both highly acclaimed for their prominent bioactivity, but their poor crack resistance restrains their applications as major load-bearing implants. Thus, there has been a continuing quest for approaches to enhance their fracture toughness [49].

It can be concluded from the above discussions that a single-phase biomaterial rarely possesses all the desired properties for a bone graft. Properly designed and manufactured composite materials can combine the advantages of different constituents and are thus highly promising solutions to the challenge of materials selection in bone grafting. Different phases in a composite material work in a synergistic manner to produce properties that cannot be achieved by each single constituent alone. The properties of composite materials are heavily dependent on three aspects: (1) the intrinsic properties of the constituents, (2) the dispersion of the reinforcing phase(s) in the matrix, and (3) the interfacial strength between the constituents. Conventionally, composites are defined as the combination of two or more heterogeneous materials with different composition or morphology on a microscale [50]. Therefore, they may be referred to with a more accurate term – microcomposites. Micrometer-sized particles and fibers that are superior to the matrix in mechanical properties are typical reinforcements utilized in microcomposites. When at least one of the constituent materials is on the nanometer scale, the composite can be called a nanocomposite [24]. Materials present unique properties when they are miniaturized to the nanometer scale [7, 51-53]. The reason is that nanomaterials have significantly more surface atoms (and hence much higher surface

energy) than materials on larger scales. These surface atoms directly affect the materials' physicochemical properties such as mechanical strength, reactivity, and stability. Therefore, when nanostructured reinforcements are used, they differ from micrometer-sized reinforcements in all the three aforementioned aspects. A larger surface area not only drastically changes the materials' intrinsic properties, but also significantly alters the way they interact with the matrix.

Over the last few years, rapidly increasing research interest has been devoted to the development of nanocomposites for the repair of bone defects [24, 49, 54-62]. This motivates us to investigate bioceramic-based nanocomposites with enhanced damage tolerance and satisfactory biological performances.

2.2.3 Bioglass®

Bioactive glasses, invented by Hench in 1969, marked the launch of the bioactive ceramics research field and triggered enormous research interest since then [3, 5, 63]. The first composition developed by Hench is 45S5, and was the first artificial material found to be able to form strong chemical bonds with human bone. It was first employed in clinical use in 1985. Table 2.3 summarizes the composition of several frequently studied types of Bioglass®.

Table 2.3 Composition of four types of typical Bioglass®

Glass	SiO ₂	Na ₂ O	CaO	P ₂ O ₅	B ₂ O ₃	K ₂ O	MgO	Notes & references
45S5	45 (46.1)	24.5 (24.4)	24.5 (26.9)	6 (2.6)	0 (0)	0 (0)	0 (0)	wt.% (mol.%); original Bioglass® formulation; ref [64]
58S	60.0	0	36.0	4.0	0	0	0	mol.%; ref [64]
S53P4	53.0	23.0	20.0	4.0	0	0	0	wt.%; inhibits bacterial growth; ref [65]
1-98	53.0	6.0	22.0	2.0	1.0	11.0	5.0	wt.%; ref [66]

The discoveries of bioactive glasses bonding to bone and soft connective tissue are important milestones in their development [67]. The 12-stage interfacial reaction kinetics has been revealed by many a previous study. The first five stages proceed in a rapid manner and are summarized as follows: rapid exchange of Na⁺ or K⁺ with H⁺ or H₃O⁺ from body fluids (stage 1), dissolution of the Si–O–Si network (stage 2), condensation and repolymerization of the silica gel layer (stage 3), migration of Ca²⁺ and PO₄³⁻ groups

(stage 4), and crystallization of the CHA layer (stage 5) [4, 68]. The chemical and structural equivalence of this CHA layer is the primary reason for the prominent bonding strengths with surrounding tissues [3].

Conventionally bioactive glasses were predominantly used for hard tissue repair and regeneration. Extensive research activities dedicated to understanding their structure and properties have expanded the use of such materials to various special applications outside the skeletal system [44]. Bioglass® products have achieved commendable success in a variety of clinical applications [5]. Monolithic medical devices made from 45S5 have been used as middle ear prostheses [69], postextraction ridge maintenance implants [70], and orbital floor implants [71]. Particulate 45S5 products for bone regeneration include PerioGlas® released in 1993 [72], NovaBone® released in 1999 [73], and Biogran®. As a representative of products developed with other Bioglass® compositions, BonAlive®, approved as a bone graft substitute in Europe in 2006, is based on S53P4 particulates [74]. NovaMin®, another product produced with fine 45S5 particles ($D_{50} = \sim 18 \mu\text{m}$), has shown praiseworthy treatment outcomes for tooth hypersensitivity. It can be used as a toothpaste additive or in air-polishing powder abrasives [75, 76]. Bioglass® was also utilized to form a bioactive coating on metallic implants. Moritz et al. [77] found that compared with the control group (NaOH-treated and grit-blasted Ti implants), those coated with bioactive glass 1-98 resulted in the formation of significantly more bone after 8 weeks of implantation in rabbit femurs.

Bioglass® can be obtained through the traditional melt-quenching process or the sol-gel method. While melt-derived Bioglass® is inherently dense, the sol-gel route offers greater versatility as it can result in monoliths or powders with nanoporosity [64]. One of the limitations of the sol-gel process, however, is that monoliths with diameters larger than 1 cm are prone to crack during drying.

The sintering window is defined as the temperature difference between the glass transition temperature (T_g) and the onset temperature for crystallization ($T_{c,onset}$) [78]. Commercially available bioactive glasses such as S53P4 and 45S5 possess a very small sintering window and thus usually experience rapid crystallization during sintering. As a consequence, the sintered products either suffer from a large number of structural defects in order to retain their amorphousness, or gain higher structural integrity at the expense of

bioactivity or stability [79]. Therefore, there have been no commercialized porous Bioglass® scaffolds to date. To overcome this challenge, either modification of the chemical composition or employment of advanced sintering techniques such as SPS would be necessary [80, 81].

Additive manufacturing techniques have emerged as relatively new but highly promising methods for fabricating porous Bioglass® scaffolds. They are able to construct complex-shaped implants and more accurately control the size, morphology and distribution of pores than direct foaming procedures [82]. Porous scaffolds with strengths comparable to those of natural bones have been manufactured using methods such as freeze extrusion fabrication [83], selective laser sintering [84] and direct-ink-write assembly [85]. Nonetheless, as pointed out by Fu et al. [82], insufficient fracture toughness and mechanical reliability are still the main limitations of Bioglass® scaffolds.

According to Hench, the inventor of Bioglass®, the future of bioactive glasses lies in further unleashing their potential in gene activation and the stimulation of specific cellular responses at the molecular level [42, 63, 86]. The ultimate objective is to design and produce a new generation of biomaterials that can be tailored for specific diseases and individual patients so as to prevent the deterioration and loss of tissue as people age [87]. A promising way to realize this objective is to control the biological responses to the ionic products released from Bioglass® [86, 88]. The schematic shown in Fig. 2.4 gives typical examples of such responses.

Fig. 2.4 Schematic of typical biological responses to ions released by Bioglass®. Reproduced from Ref. [88].

2.2.4 Hydroxyapatite

Pioneering work by Hench has stimulated and fueled enormous research activities in biomaterials, especially bioactive glasses and ceramics. Although 45S5 is the first bioactive material discovered, HA has attracted significantly more attention from biomaterials researchers around the world. Additionally, calcium phosphates such as TCP and synthetic HA have been more widely used in clinical applications than Bioglass®, mainly because of the scientific limitations of Bioglass® described in Section 2.2.3. Among the many members in the calcium phosphate family, silicon-substituted HA granules are leading the market [89]. The privileged position of HA is rooted in its close chemical resemblance to bone mineral and tooth enamel. As shown in Table 2.4, synthetic HA and the calcium apatite found in human hard tissue have very close Ca/P ratios, albeit the variations in their crystallinity, stoichiometry and constituents.

Table 2.4 Comparison between synthetic HA with apatite in human bone and tooth enamel [90, 91]

	Synthetic HA	Bone mineral	Tooth enamel
Constituents (wt.%)			
Ca/P molar ratio	1.67	1.65	1.62
Ca ²⁺	39.6	24.5	36.0
P	18.5	11.5	17.7
Na ⁺	traces	0.5	0.7
K ⁺	traces	0.03	0.08
Mg ²⁺	traces	0.55	0.44
Cl ⁻	/	0.10	0.30
F ⁻	/	0.02	0.01
CO ₃ ²⁻	/	5.8	3.2
Total inorganic	100	65.0	97.0
Total organic	/	25.0	1.0
Absorbed water	/	9.7*	1.5
Crystallographic properties			
Crystallinity (%)	100	33 – 37	70–75
Crystallite size (nm)	process-dependent	25 × 2.5–5	130 × 30
<i>a</i> -axis (nm)**	0.9422	0.9419	0.9441
<i>c</i> -axis (nm)**	0.6880	0.6880	0.6882

* Value for cortical bone.

** ±0.0003 nm.

The stoichiometric formula of crystalline HA is $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$, but is usually presented as $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, or more precisely as $\text{Ca}_4(\text{I})\text{Ca}_6(\text{II})(\text{PO}_4)_6(\text{OH})_2$. Two crystallographically independent calcium atoms can be found in the HA unit cell. The Ca(I) atoms are at the fourfold symmetry 4(f) position, with the Ca(II) atoms located at the sixfold symmetry 6(h) position [92]. Specifically, Ca(I) is surrounded by six oxygen atoms which form a distorted octahedron; Ca(II) is surrounded by six oxygen atoms belonging to PO_4^{3-} and two oxygen atoms belonging to OH^- . The spatial structure of the HA crystal is shown in Fig. 2.5.

Like Bioglass®, HA has excellent biocompatibility and bioactivity and can directly bond to hard and soft tissues. When exposed to physiological solutions, HA implants induce the formation of an amorphous calcium phosphate layer on their surfaces which will crystallize in a few days into HA apatite [93]. This apatite HA layer is structurally and chemically equivalent to the inorganic part of bone tissue and contributes to the interfacial bonding between the implant and surrounding tissues.

Fig. 2.5 Three dimensional crystal structure of HA. Reproduced from Ref. [94].

It is of fundamental importance to understand some basic properties of HA which are summarized in Table 2.5. Comparing Table 2.1 with Table 2.5, it appears that HA possesses higher Young's modulus and compressive strength than cortical bone. However, the tensile strength and fracture toughness of HA are much lower, thus limiting its application as load-bearing implants.

Table 2.5 Basic properties of HA

Property	Typical value
Crystal system	Hexagonal [24]
Space group	P6 ₃ /m [24, 95]
Unit cell volume (nm ³)	0.5276 [95]
Molecular weight	502.31 [96]
Theoretical density (g/cm ³)	3.156 [97]
Thermal diffusivity (cm ² /s)	5×10 ⁻³ [96]
Thermal conductivity (W/(cm K))	0.013 [24]
Hardness (Mohs)	5 [96]
Hardness (Vickers)	600 [24]
Young's modulus (GPa)	80–110 [24]
Compressive strength (MPa)	400–900 [24, 49]
Bending strength (MPa)	115–200 [24]
Flexural/tensile strength (MPa)	17–110 [49]
Fracture toughness (MPa m ^{1/2})	0.7–1.2 [24, 49]
Dielectric constant	7.40–10.47 [24]

As one of the most sought-after materials for orthopedic applications, HA is usually used in close contact with living tissue. Since the most abundant substance in human body is water, it is of special importance to understand the stability and solubility

of HA in aqueous environments. When exposed to an aqueous solution, a metastable compound may undergo two different processes:

(1) A continuous dissolution of the compound surface. This process takes place when the surrounding solution is undersaturated, and continues until it becomes saturated. For implants coated with metastable materials, this can lead to the exposure of the substrate and subsequent failure of the implant.

(2) Formation of a second, stable phase caused by the surface reactions. Precipitation of the second phase occurs when the surrounding solution is supersaturated.

The driving force for the precipitation / dissolution process depends on the degree of supersaturation / undersaturation. The relative rate of precipitation to dissolution determines the overall stability of the compound and hence the service life of the implant. HA is the most stable calcium apatite when $\text{pH} > 4.2$, but is less stable in more acidic solutions.

Fig. 2.6 presents the phase diagram of $\text{CaO-P}_2\text{O}_5$ at a vapor pressure of 66.7 kPa. It can be seen that the melting temperature (T_m) of HA is $\sim 1570^\circ\text{C}$. At elevated temperatures HA may decompose into different Ca-P phases including TCP, CaO and tetracalcium phosphate (TTCP). The following reactions represent the chemical decomposition of HA during high-temperature processing [98]:

HA can be synthesized via various routes such as mechanical alloying [99, 100], electrochemical deposition [101], hydrothermal process [102], and precipitation techniques [54]. It can also be derived from natural sources such as pig or bovine bone [103-105], eggshell [106, 107], and coral skeleton [108]. Precipitation methods are the most popular techniques for preparing HA powder. Two widely utilized precipitation reactions are as follows [109, 110]:

Fig. 2.6 Phase diagram of CaO–P₂O₅ (vapor pressure = 66.7 kPa). Reproduced from Ref. [111]

Despite of the faster production rate of the first reaction, its drawbacks include the use of ammonium hydroxide as a buffer solution and repetitive washing for the removal of ammonium hydroxide and nitrate by-product. By contrast, processes based on the second reaction are more convenient to control and monitor and lead to the generation of H₂O as the only by-product.

A wide variety of processes have been utilized to manufacture HA-based implants and devices. Thermally sprayed HA coatings on Ti and Ti alloy substrates have attracted large quantities of research interest and resulted in widespread clinical applications. Such HA-coated implants combine the fracture resistance and bending strength of the metallic substrates with the eminent biocompatibility and bioactivity of HA. Commonly used thermal spray processes to fabricate HA coatings include plasma spraying [111, 112], high velocity oxy-fuel spraying [113, 114], and cold spraying [115]. Thermally sprayed HA coatings are confronting a number of challenges, such as undesirable phase change, insufficient bonding strength, corrosion of the substrate and the resultant ion release, as well as unsatisfactory fretting wear behavior and fatigue life.

Additive manufacturing techniques such as 3D printing [116, 117] and selective laser sintering [118, 119] have emerged as promising methods to produce HA-based porous scaffolds. The layer-by-layer addition of materials allows versatile design of the shape and microstructure of the final products, which can be customized to be patient and disease specific. However, binders are usually employed in these processes and need to be removed by an extra sintering step [120]. The difficulty in eliminating the residual binders and loose powders, together with cracking of the parts due to non-uniform shrinkage, poses severe challenges to post-processing sintering. Furthermore, it is imperative to optimize the process-structure-property relationship to meet the stringent criteria on the quality and reproducibility of HA-based implants. The most critical issue to be addressed, as pointed out by Bose et al. [121], is the poor mechanical properties of additive manufactured porous ceramic scaffolds.

Various sintering processes have been utilized to directly manufacture bulk HA parts with a high relative density [122]. The properties of sintered HA compacts are influenced by the physicochemical properties of the raw powder and the sintering conditions utilized. Previous studies have shown that both nanosized powder and ultrahigh pressure can substantially reduce the sintering temperature and assist the densification of the particles [123-125]. A reduced sintering temperature is desirable as it minimizes the dehydroxylation and decomposition of HA [126]. Much emphasis has been placed on the sintering of nanocrystalline HA pellets that can potentially provide superior mechanical and biological properties [127, 128]. To this end, unconventional sintering techniques such as two-step sintering and SPS have been employed to obtain nanosized grains in sintered HA [129, 130]. Compared with conventional sintering processes, SPS generally requires a lower temperature and a shorter duration to reach comparable relative densities, leading to decreased grain sizes and enhanced mechanical properties. It has thus been exploited to prepare a wide variety of HA-based biomaterials.

Nevertheless, HA compacts fabricated by SPS still suffer from insufficient fracture toughness. To improve the reliability of HA-based implants in load-bearing applications, different reinforcements have been used to toughen HA. Widely utilized reinforcing phases include Ti and Ti alloy [131-133], titania [134, 135], and zirconia [54, 59, 136, 137]. Nanostructured reinforcements such as CNT and graphene particularly

have been extensively used to reinforce HA by virtue of their prominent mechanical properties [49, 138]. When such composites are created several key aspects have to be taken into consideration. First, it is challenging to obtain homogeneously dispersed nanomaterials in the HA matrix and achieve optimum interfacial strengths. Second, the addition of a nanostructured phase alters the densification behavior of HA, which warrants modification and optimization of the sintering parameters. Third, the effects of nanomaterial incorporation on the biological properties of HA have to be critically assessed before the composites can be utilized in clinical applications.

2.2.5 Summary

The field of biomaterials science and engineering has undergone rapid development and propelled praiseworthy progress in bone tissue engineering in the past few decades. Bone defect management in clinical scenarios has made use of different categories of bone-substitute materials. Metallic biomaterials possess outstanding mechanical properties and are widely utilized in load-bearing applications. However, their poor corrosion resistance is an evident disadvantage and leads to the release of metallic ions which may be toxic *in vivo*. Polymeric biomaterials can be easily fabricated into different shapes and forms. Nevertheless, their mechanical strength is not high enough for hard tissue replacement. Ceramic biomaterials are popular candidates for bone repair and regeneration by virtue of their excellent corrosion resistance, high compressive strength, long-term stability *in vivo*, and good biocompatibility. Bioglass® and HA show remarkable bioactivity and can directly bond to human hard and soft tissues. However, the toughness of both materials has to be increased to satisfy the requirements of the mechanical stability of bioceramic-based bone implants. Numerous previous studies on bone-substitute materials clearly indicate that no single-phase material has all the desired mechanical and biological properties for bone graft materials. Therefore, a wide range of composite materials have been developed and investigated to achieve satisfactory comprehensive properties, among which bioceramic-based nanocomposites have been receiving high levels of research interest.

2.3 Spark plasma sintering

2.3.1 Overview

Two kinds of porosity are generated when crystallites agglomerate into particles: inter-particle pores and intra-particle pores. Densification or consolidation is defined as a process of removing internal pores or voids in powder compacts to prepare an essentially pore-free sample whose density is usually 95–98 % of the theoretical density [14]. Densification of powder compacts can be realized by one of the following three mechanisms: sintering, plastic deformation, and particle or crystallite rearrangement.

Sintering is a method used to produce bulk objects from powders through atomic diffusion. The underlying mechanism is the formation of connections between particles due to the accelerated diffusion of atoms at elevated temperatures. In a strict sense, sintering means the densification caused by reduced surface curvature driven by a decrease in surface energy [14]. The mechanisms of mass transport involved in sintering are shown in Fig. 2.7a. It can be evidently seen that volume / grain boundary diffusion can contribute to densification, while evaporation and surface diffusion only lead to grain coarsening. Fig. 2.7b illustrates the densification caused by particle / crystallite rearrangement. It is directly related to the applied pressure, but is still temperature activated and can be expedited by surface / grain boundary diffusion.

Fig. 2.7 (a) Mass transport mechanisms associated with sintering. (b) Schematic of pressure-induced particle / crystallite rearrangement. Reproduced from Ref. [14].

Sintering temperatures are generally below the T_m of the material, and typically fall within the range of $(0.5-0.9)T_m$ in solid-state sintering [139]. Sintering can thus be an effective technique for fabricating and shaping bulk materials with a high T_m .

SPS is an unconventional and rapid sintering technique that was first developed in Japan in the early 1960s, but its widespread application was not realized until the late 1980s [140]. Although the name SPS is the most commonly used, quite a few other terms have been used to refer to this process and they emphasize its different characteristics. Some other commonly used names for this process are listed in Table 2.6.

Table 2.6 Other terms used to describe the SPS process

Name	Ref.	Name	Ref.
Plasma activated sintering	[140, 141]	Spark plasma consolidation	[140]
Spark plasma joining	[140]	Spark plasma growth	[140]
Spark plasma reaction	[140]	Pulsed electric current sintering	[14, 141, 142]
Field-activated sintering	[141, 142]	Current-activated pressure-assisted densification	[14, 142]
Field-assisted sintering	[14]	Electric current activated/assisted sintering	[141]
Pulsed discharge sintering	[141]	Resistance sintering	[141]
Pulse current sintering	[141]	Plasma pressure compaction	[141]
Plasma assisted sintering	[143]	Electrocondensation	[143, 144]
Electric pulse assisted consolidation	[143, 144]	Instrumented pulse electro-discharge consolidation	[145]
Resistance/spark sintering under pressure	[145]	Plasma pressure consolidation	[145]
Pulsed electrical discharge with pressure application	[145]	Pulse-current-assisted sintering	[146]
Pulse current hot pressing	[146, 147]		

Among the different names describing the SPS process, the most reasonable one should be current-activated pressure-assisted densification (CAPAD). First, the uniqueness of this process is the simultaneous application of an electric current and the pressure. In addition, as will be stated later, direct evidence for the role and even the existence of plasma cannot be unambiguously presented. Furthermore, densifying mechanisms other than sintering may also play a role. It should be also noted that, when referring to the term SPS, the current utilized is more or less continuous (DC or pulsed DC), while other types of current may also be employed in CAPAD. In this thesis the most common name, i.e. SPS, is used for convenience.

Conventionally, ceramic processing usually involves three steps: powder synthesis, forming / shaping and sintering. SPS can serve as a technique combining two or all of the three steps. For example, it has been reported that SPS is capable of synthesizing and densifying a material at the same time [148-150]. Specifically, Omori et al. [151] successfully obtained HA through the reaction between $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and Ca(OH)_2 during SPS. In most cases, forming / shaping is not necessary and loose powders are directly loaded and sintered in a graphite die.

As a versatile and efficient sintering technique, SPS can be utilized to sinter a wide variety of conventional materials such as ceramics and metallic materials. The strengths of this process are most noticeable for difficult-to-sinter materials. A number of novel materials such as functionally graded materials [133, 152], superplastic ceramics [153, 154] and porous materials [152, 155-157] have also been successfully fabricated by this method.

2.3.2 Mechanisms of SPS

A schematic of the SPS system is shown in Fig. 2.8. The basic configuration of an SPS system was given in a pioneering work by Tokita et al. [158]. The powders to be sintered are placed in a graphite die. Graphite is electrically and thermally conductive and can maintain its mechanical properties under high temperature conditions. For most SPS apparatuses the two electrodes and spacers are also made of graphite, hence the absence of elements with high heat capacity in the system. This serves as the main cause of its much faster heating and cooling rates as compared with other sintering methods. Heating rate as high as 2000 °C/min is achievable [142], while the typical value is 100–600 °C/min [14]. A pulsed DC current is generated by applying a voltage which is typically below 10 V [142]. The pattern comprises a sequence of pulses with DC current followed by absence of current. Pulses in the SPS apparatus manufactured by Sumitomo Coal Mining Co. Ltd., Japan, a leading SPS system manufacturer, typically have 3.3 ms in duration [141]. The incorporation of an electric current in the compaction process is the major difference between SPS and other sintering processes such as free sintering and hot isostatic pressing.

Fig. 2.8 Schematic of the SPS system.

Sintering pressure is applied through two punches. The magnitude of the sintering pressure has an upper limit due to the strength constraint of the graphite die, with the magnitude commonly utilized being 30–150 MPa [14, 142]. Typically, the load capacities of SPS units range from 50 kN to 250 kN [14]. The sintering process can be carried out in a vacuum or a controlled gaseous medium with alterable atmospheric pressure in the water-cooled vacuum chamber. In most cases the short processing time and relatively low sintering temperature make it appropriate to use vacuum sintering without extra atmospheric control. A reducing environment is created if a graphite die is used, which can contribute to good particle bonding due to the decomposition of surface oxide in a reducing vacuum environment at high temperatures.

A schematic of a typical SPS process is given in Fig. 2.9 that includes important experimental parameters such as heating rate, hold temperature, cooling rate, load application rate, hold load, and load removal rate.

Fig. 2.9 Schematic of a typical SPS process.

To make the most of the SPS technique, it is essential to understand the role of a number of important influencing factors, such as electric current, pulse pattern, spark plasma, heating rate, as well as sintering duration, temperature, and pressure.

Electric current. Typical SPS units utilize an electric current ranging from 1,000 to 10,000 A at 10–15 V [14]. A schematic showing the pulsed current passing through conductive particles is given in Fig. 2.10 [158]. Tokita et al. [158] pointed out that the pulse DC in SPS can generate (1) spark plasma, (2) spark impact pressure, (3) Joule heating, and (4) an electric field diffusion effect. Depending on the electrical conductivity of the green body, thermal effects caused by current flow may take place in only the graphite die or both the die and the sample. Conductive powders, therefore, are subjected to the Joule heat generated inside themselves as well as heat treatment by the die and the electrodes. At least one of the green body and the die has to be electrically conductive in order to form a closed circuit in the sintering process.

Munir et al. [142] summarized in a review article that the current can promote point defect generation and enhance mass transport (through electromigration) [159] and defect mobility (by decreasing defect migration energy) [160-162]. It has been reported that the presence of an electric field plays a part in retarding grain growth [163]. In addition, Chen et al. [164] proposed that the thermo-mechanical fatigue effect induced by the pulse current can break grains on the micrometer scale into nanosized grains.

Fig. 2.10 Schematic of pulsed current flowing through conductive particles. Reproduced from Ref. [158].

Using a piece of custom built apparatus, Friedman et al. [165] investigated the influence of DC current varying from 0 A/cm² to 764 A/cm² on the interfacial reactions in Ag-Zn diffusion couples. Their work indicates that a high-density current can promote the nucleation of product phases and remarkably enhance their growth kinetics in solid-solid interfacial reactions. It also implies that the electric current possibly leads to an increased atomic mobility or defect concentration.

Song et al. [166] investigated the microstructure of Cu particles sintered at different stages of the SPS process and also conducted theoretical analysis to understand the neck formation and growth mechanisms. They concluded that because of the variation in the intensity of current (and hence Joule heating) from the contact area between adjacent particles to the particle center, the distribution of temperature increase is very inhomogeneous. The local high temperature at the contact surface may reach the boiling point of Cu, causing neck formation to be realized through a local melting and fast solidification process at a relatively low sintering temperature.

It needs to be pointed out that thus far the only experimentally proven role played by the current is the Joule heating effect, which is probably the most dominant one. While other effects may occur simultaneously, they are difficult to prove with experiments since they can be easily masked by Joule heating.

Pulse pattern. Although quite a few current waveforms can be applied in SPS, the most frequently utilized is pulse DC. Munir et al. [142] reported that neither the frequency nor the pulse pattern influences the densification, microstructure, or the grain

growth of the sintered powders. Their experiments also proved that the pulse pattern has no effect on the reactivity (and hence mass transport) and growth rate of the product [162, 167]. They further found out that Joule heating is only associated with the root mean square of the instantaneous current density [167].

Song et al. [168] pointed out that the high frequency components of the pulsed current contribute more to the sintering of materials because of the current skin effect. However, after studying the Fourier transform of an 8:2 (ON:OFF) pulse pattern, Anselmi-Tamburini et al. [169] found out that most of the overall heating power was generated by components with a frequency < 100 Hz. Thus, the current in the die was not expected to present any skin effects. Additionally, it was observed that the patterns did not contain ideal square waves, and that the current usually did not reach zero during the OFF time.

Spark plasma. Plasma is a state of matter that is an ionic mixture containing charged particles such as ions and electrons. Ionization can be induced by providing energy to gases through, for example, heating or strong electromagnetic fields. It is generally believed that the SPS process is primarily characterized by the generation of high-energy plasma between two electrodes. However, convincing evidence has yet to be observed to explicitly prove it. On the other hand, it is commonly recognized that electric discharge occurs on a microscopic level at the initial stage of SPS. Numerous small capacitors are formed with the presence of gaps or insulating surface layers between neighboring particles.

Theoretically, the quantity of spark plasma is the highest at the early stage of sintering and decreases gradually as the gaps between particles diminish [140]. The impact caused by the generated spark plasma can increase the sintering pressure / internal stress [140]. Additionally, it plays a large part in surface activation by clearing or destroying the impurities on particle surfaces such as oxides and absorbed gas and generating different types of surface defects which can promote the grain boundary diffusion [144].

It has been suggested that the term SPS is misleading since the findings of some researchers indicated the absence of plasma in this process. For example, Hulbert et al. [170] used direct visual observation, *in situ* atomic emission spectroscopy and ultrafast *in*

situ voltage measurements to test a variety of raw powders under different SPS conditions and his results indicated no generation of plasma, sparking, or arching. Garray et al. [14] believe that plasma is difficult to form in SPS. Plasma generation requires high voltage and is enhanced by the presence of gases, while SPS usually takes place in a vacuum. In such situations, the plasma, which is essentially an ionized gas, can only be derived from the evaporated surface material. Furthermore, the existence of a static electric field needs to be verified since the current can flow through the highly conductive die.

This thesis does not intend to unambiguously prove the existence of spark plasma. Like many previous studies [171], it assumes that spark plasma exists in the SPS process and exerts various influences on the sintering process and sample properties.

Heating rate and sintering duration. A typical SPS run is approximately 20 min long. The short processing time primarily results from fast heating rates. Shorter sintering duration means higher efficiency and less energy consumption. A high heating rate favors the densification process driven by volume and grain boundary diffusion and shortens the period at lower temperatures when non-densifying mechanisms (grain coarsening through evaporation, condensation and surface diffusion) dominate [142]. Thus, smaller grain sizes can generally be expected when higher heating rates are employed. As shown in Fig. 2.7a, a sintering process is essentially a competition between densification and particle coarsening. A high heating rate therefore benefits the sintering process by offering favorable densification mechanisms. Furthermore, the large temperature gradient caused by a high heating rate serves as an additional driving force for sintering [172]. The capability of separating grain growth from densification is a unique notable characteristic of SPS.

To understand the mechanisms behind the fast heating rates in SPS, Song et al. [168] semi-quantitatively calculated the heat generated by the pulsed DC when conductive and insulating materials are sintered. They concluded that the DC component in pulse current enables more rapid temperature rises in conductive samples.

The effect of heating rate on the relative density of the sintered sample may vary at different temperatures, and is probably the most significant at intermediate temperatures. At low temperatures, heating rates do not have a significant effect due to insufficient energy for thermal activation to occur. On the other hand, its effect may be masked at

high temperatures because a full density can be readily obtained at a wide range of heating rates.

Sintering temperature. Full density can be obtained for most materials at 50–80 % of their homologous temperature [14]. It has been reported that under the same temperature SPS can bring about a higher density than conventional sintering processes. A low sintering temperature, together with a shorter sintering duration, not only minimizes undesirable phase transformation and vaporization-induced material loss, but also inhibits grain growth. Munir et al. [142] believe that most of the enhanced material properties reported in literature are likely to be the consequences of the reduced sintering temperature. This is because lower sintering temperatures significantly affect the materials' microstructure and composition, which in turn can lead to varying properties.

According to Nygren et al. [144], the grain growth rate is greatly increased above a critical temperature, whose value relies largely on the raw powder properties, sintering pressure, and heating rate. By comparing his experimental data with those of other researchers, Garraý et al. [14] found out that the sintering temperature has a stronger influence on metals than on ceramics. They attributed this phenomenon to the larger plastic deformation and faster electromigration in metallic materials. In addition, compared to more ionic material, materials with a larger portion of covalent bonds generally require higher temperatures to sinter.

It is of fundamental significance to note that homogeneous temperature distribution in the sample is difficult to achieve. A large temperature gradient probably exists along both the radial and the axial directions within the sample. In addition, the recorded sintering temperature, namely the surface temperature of the die, is different from the actual sample temperature. Another consideration is the dimension of the sample. When the sample size is augmented, the skin effect plays a larger role, which in turn affects the distribution of current and Joule heat in the sample. Furthermore, the generation and loss of heat are associated with the sample volume and surface area, respectively. In this regard, the dimension of the green body also alters the temperature distribution within it.

Sintering pressure. Munir et al. [143] believe that pressure can play both intrinsic and extrinsic roles in sintering. Its intrinsic effects refer to creep, plastic and viscous flow,

and an augmented chemical potential (diffusion-related mass transport) [173]. The extrinsic effects, on the other hand, include destruction of agglomerates and particle rearrangement (Fig. 2.7b). The application of pressure is of special importance when nanosized powders are sintered. The coalescence of nano particles caused by van de Waals forces can be destroyed by the external pressure, which would otherwise hinder the formation of fine grains.

Generally, the larger the external pressure, the higher the relative density of the sintered compacts. This trend is more evident at lower sintering temperatures when the atomic diffusion and material flow are slower. The effects of pressure on the relative density of compacted metals tend to be more obvious than on those of sintered ceramics, which should be attributed to the difference in their sensitivity to mechanical deformation [14]. Furthermore, it has been found that the application of a higher pressure results in a smaller temperature difference between the sample and the die [174]. The finding of Xu et al. [175] evidenced that a higher densification rate can be achieved when the sintering pressure is applied at a higher rate.

As a consequence of the built-up stresses caused by thermal expansion differences during SPS, there exists large pressure gradients in the sample. The pressure is comprised of the axial, angular, and radial components [176]. The hydrostatic pressure, i.e. the average of the three, plays a crucial role in densification. The nominal pressure σ is frequently used to study the influences of sintering pressure on the sample properties and can be calculated by the following equation:

$$\sigma = F/S \quad (2.5)$$

where F denotes the load applied and S the area of the two punches. Nevertheless, it should be noted that the nominal pressure does not appropriately indicate the actual sintering pressure / stress within the sample.

2.3.3 SPS of carbonaceous nanomaterials-reinforced bioceramics

Based on the number of dimensions outside the nanometer range, nanomaterials can be classified into three broad categories: 0D, 1D and 2D nanomaterials [177]. 0D nanomaterials include various nanoparticles and have three dimensions ≤ 100 nm. 1D nanomaterials have two dimensions at the nanoscale, and typical examples are nanowires,

nanorods and nanotubes. Nanoplatelets, nanofilms and other 2D nanostructured materials have only one dimension ≤ 100 nm.

By virtue of its high sintering efficiency and strong densification capability, SPS has become one of the most popular and successful techniques to fabricate nanocrystalline bioceramics [178]. In addition, bioceramic-based composites enhanced with various nanostructured reinforcements have been prepared by SPS. Examples of 0D nanomaterials employed to reinforce HA include ZrO₂ nanoparticles [54, 179-182], Ag nanoparticles [183], and TiO₂ nanoparticles [134, 135].

Carbon is the 6th most abundant element in the universe and a key element for all known life on earth as the backbone of nearly all biological molecules is formed by carbon atoms. Among the many 1D and 2D nanostructured materials utilized to enhance bioceramics, carbonaceous nanomaterials such as graphene and CNT have been extensively and intensively investigated. They both possess a low density and outstanding mechanical properties, making bioceramics reinforced by CNT or graphene highly promising bone-substitute materials [6, 15, 52, 184, 185]. To consolidate such composites, SPS is particularly advantageous to conventional sintering techniques. While CNTs and graphene usually experience serious structural damage or even decompose completely in conventional sintering processes, they have been proven to be capable of surviving the SPS process and retaining their structural integrity [16, 186-188].

This dissertation is focused on using SPS to fabricate tough and biocompatible bioceramic-based composites with CNT or graphene as the reinforcing material. Therefore, this section reviews the literature available on CNT- and graphene-reinforced bioceramics consolidated by SPS. Special attention will be paid to the dispersion method and the mechanical and biological properties of the composites.

Table 2.7 compares various bioceramic-based composites prepared by SPS with CNT and/or graphene as the reinforcements. It can be seen in Table 2.7 that HA- and 45S5-based systems have received the greatest amount of research interest. HA and 45S5 composites were typically sintered in the range of 1000–1100 °C and 550–600 °C, respectively. A wide range of methods have been attempted to deagglomerate and disperse CNTs and graphene, including sonication, mechanical stirring, ball milling, and spray drying. Still, inhomogeneous dispersion of the nanosized reinforcements has

remained a major hurdle to overcome to achieve the desired enhancement effects. Besides looking at different ways to properly mix the two as-synthesized materials, researchers have also tried to alter the wettability and dispersibility of CNTs and graphene through, for instance, surface functionalization [189]. Another promising approach is the *in situ* mineralization of HA on CNT or graphene [190, 191], which not only brings about homogeneous dispersion of the nanostructured reinforcement, but also possibly creates strong interfacial strengths between the two phases.

Table 2.7 Comparison of CNT- and graphene-reinforced bioceramics fabricated by SPS*

Reinforce- ment & matrix	Dispersion method & SPS parameters	Enhancement in mechanical properties by %**		Biological properties	Ref. & year
		K_{IC}	Others		
CNT-reinforced bioceramics					
2.5 vol.% MWCNT; HA	Using surfactant & ball milling; 1100 °C, 30 MPa, 5 min	29.6	HV : 469.6***	NA	[192] 2007
2 vol.% MWCNT; HA	Mechanical stirring; 1100 °C, 3 min	NA	E : 131.1 GPa***, H : 6.9 GPa***	CNT incorporation improved proliferation and induced different protein profile in human fetal osteoblastic 1.19 cell line	[193] 2008 [194] 2009
1–15 wt.% MWCNT; HA	MWCNT, CaHPO ₄ ·H ₂ O and Ca(OH) ₂ mixed with surfactant in a rotation revolution mixer; 1200 °C, 120 MPa	NA	H decreased while $\sigma_{bending}$ unchanged at higher CNT loadings	NA	[195] 2009
4 wt.% MWCNT; HA	Spray drying; 1100 °C, 60 MPa, 5 min	92	25.0 in E , 28.6 in H , 66 in wear resistance; –60 in friction coefficient	NA	[61] 2010
5 wt.% MWCNT; HA	Ultrasonication; 900 °C for 2 min + 1000 °C for 1 min, 40 MPa	NA	~50 in H	NA	[196] 2010
1.6 wt.% MWCNT & 18.4 wt.% Al ₂ O ₃ ; HA	Spray dried Al ₂ O ₃ -CNT mixed with HA by ball milling; 1100 °C, 50 MPa, 5 min	130.5	–21.7 in E , –12.5 in H (measured via nanoindentation)	Similar attachment, proliferation and viability of mouse fibroblast cells (L929)	[197] 2010
0–2 wt.% MWCNT; HA	Sonication; 900 °C for 2 min + 1000 °C for 1 min, 40 MPa	NA	Increased $\sigma_{bending}$ at higher MWCNT loadings	Improved adhesion and proliferation of osteoblast cells (MC3T3-E1)	[198] 2011

Table 2.7 Continued

5 wt.% MWCNT; 45S5	High-energy ball milling; 850 °C, 40 MPa, 10 min	105.3	158.5 in flexural strength, 40.5 in flexural modulus, -36.3 in H	NA	[199] 2011
40–80 wt.% MWCNT; HA	Mixing in DI water; 1100 °C, 120 MPa, 14 min	NA	Increased H , E and compressive strength at higher CNT loadings	Better osteointegration for MWCNT/HA than pure MWCNT in rat femur	[58] 2012
3 wt.% oxidized MWCNT; HA	Ultrasonication + mechanical stirring; 900 °C, 40 MPa, 5 min	30	-1.1 in H	NA	[200] 2013
10 vol.% MWCNT; HA	High-energy ball milling & ultrasonication; 900 °C, 45 kN, 10 min	240	290 in H	Compatible with osteoblast cells (MC3T3-E1)	[201] 2014
6.35 wt.% MWCNT; 45S5	Acid-treated CNT mixed with 45S5 by sonication and mechanical stirring; 550 °C, 70 MPa, 2 min	NA	NA	Inhibited attachment, proliferation, viability, and phenotype expression of MG63 cells	[202] 2015
1.5 vol. MWCNT & 48.5 vol.% Fe; α -TCP	Ball milling; 1000 °C, 35 MPa, 10 min	NA	Significantly increased diametral tensile strength	MG63 osteogenic cell attached onto the composite but had low viability	[203] 2016
Graphene-reinforced bioceramics					
0.5 wt.%; HA	Sonication; 900 °C for 2 min + 1000 °C for 1 min, 40 MPa	NA	11.6 in $\sigma_{bending}$	Impeded osteoblast cell (MC3T3-E1) adhesion and proliferation	[198] 2011
1 wt.%; HA	Precipitation of HA grains in graphene solution; 950 °C, 30 MPa, 5 min	203	25.8 in E , 47.6 in H	Improved proliferation and ALP activity of human osteoblast cells (1.19 SV40 transfected osteoblasts)	[57] 2013
1 wt.%; HA	Using surfactant & ultrasonication; 1150 °C, 40 MPa, 3 min	82.8	43.9 in E , 31.0 in H	Graphene provides preferable locations for osteoblast (MC3T3-E1) adhesion and apatite mineralization	[138] 2013
1–5 vol.%; 45S5	Graphene dispersed by ultrasonication and mixed with 45S5 by ball milling; 600 °C, 70 MPa, 2 min	NA	NA	More HA mineralization after 3 day immersion in SBF	[204] 2014

Table 2.7 Continued

3 vol.% / 5 vol.%; HA	Sonication; 900–1100 °C, 45 kN, 20 min	NA	E and H both decreased	NA	[205] 2015
2 wt.%; HA	Attritor milling; 700 °C, 30 MPa, 5 min	NA	H : 4.0 GPa***, $\sigma_{bending}$: 119.0 MPa***	NA	[206] 2015
1 wt.%; HA	Precipitation of HA grains in graphene solution; 950 °C, 30 MPa, 5 min	203	Improved fracture behavior under impact loading	NA	[207] 2016

* MWCNT: multi-walled carbon nanotube, DI water: deionized water, K_{IC} : fracture toughness, HV : Vickers hardness, E : Young's modulus, H : hardness, NA: not available, SBF: simulated body fluid.

** Negative values mean the composites had decreased values than the pure bioceramics.

*** Absolute value.

In all those studies where the K_{IC} values were available, CNT and graphene were reported to increase the K_{IC} to a certain extent. It should be noted that the K_{IC} values obtained by Vickers indentation tests may not be used for comparison between different studies due to a lack of testing standards. Nevertheless, they can serve as an indicator of the enhancement effect achieved when the K_{IC} values of the pure bioceramic and the composite are reported in pairs. Microscopic toughening mechanisms induced by CNT or graphene (e.g. crack deflection and bridging) were observed in many previous studies, but few have examined the toughening effects from the perspective of interfacial strength [\[208\]](#).

As shown in Table 2.7, there have been quite a few contradicting results on the change in E , H and biocompatibility caused by CNT or graphene incorporation. Such inconsistencies could be caused by the different raw materials and processing routes (such as dispersion method, heating and cooling rates, sintering atmosphere, and applied pressure) employed. Short-term *in vitro* tests were used to assess the composites' biological properties in most previous investigations. Further studies are warranted to comprehend their compatibility with various bone-specific cells. To push them from concept to clinic, a deeper understanding of the *in vivo* biocompatibility of such composites has to be gained through implantation in bone tissue for longer periods.

2.3.4 Summary

The enormous amount of research work devoted to SPS in the past few decades stems from the intrinsic advantages of this process as well as from the superior material properties obtained as compared with those resulting from conventional sintering methods. The underlying mechanism of SPS is still not completely understood, which will thus remain a major research trend. A second one, which will surely benefit from the discoveries in the first, is to explore possible material systems where SPS can be applied, and to continuously optimize the processing parameters. Additionally, as the SPS technology gets more mature and widespread, large-scale engineering applications will call for knowledge and approaches to upgrade and transform it to meet the evolving industrial requirements and expectations.

To use SPS as a technique for fabricating bioceramic composites with carbonaceous nanostructured reinforcements, it is crucial to understand comprehensively the influences of this process on the structure and properties of the different constituents. Two key issues that considerably influence the mechanical properties of such composite systems are the distribution of the carbon-based nanostructures in the matrix and the interfacial strength between the two phases. More importantly, to accomplish the optimum toughening effects, the underlying mechanisms have to be carefully investigated and validly illustrated. Prior to the clinical application of such composites as bone graft materials, it is a must to present solid data from *in vitro* and *in vivo* biocompatibility studies that can support their safety and efficacy.

Chapter 3 Spark Plasma Sintering of 45S5 Bioglass®

It is challenging to obtain dense 45S5 with controlled crystallinity and satisfactory mechanical properties by conventional sintering processes due to its fast crystallization above the first glass transition temperature. SPS has stood out in this respect by virtue of its capability to provide fast heating and densification rates. However, there have been insufficient investigations into the in vitro biological properties of 45S5 compacts obtained by SPS. In this chapter, fully densified 45S5 pellets were fabricated in the temperature range of 500–600 °C through a rapid SPS process (with a hold time of 3 min) to assess the in vitro biological properties of both fresh and aqueously aged samples with L929 and MG63 cells. The findings can provide valuable guidance on the preparation of 45S5-based composites to be investigated in Chapter 4.

3.1 Introduction

45S5 is the first Bioglass® formulation developed by Hench et al. [3, 40, 63] and has the following composition: 45.0 wt.% SiO₂, 24.5 wt.% CaO, 24.5 wt.% Na₂O, 6.0 wt.% P₂O₅. By virtue of its outstanding bioactivity as well as osteoinductivity and osteoconductivity, 45S5 has aroused an immense amount of research interest and achieved commendable success in clinical applications ever since it was developed in the late 1960s [63, 87, 209]. However, the tendency of 45S5 to quickly crystallize above its first glass transition temperature ($T_{gl} = 550$ °C) restrains its full densification to be obtained via viscous flow [210, 211]. Achieving fully dense bulk 45S5 with satisfactory mechanical properties is thus a major challenge of sintering 45S5.

SPS is an advanced sintering technique with the simultaneous application of a pulsed DC current and sintering pressure. It thus enables highly densified pellets to be achieved at a lower temperature and within a shorter duration than conventional sintering processes [143, 212]. Therefore, SPS is especially advantageous for sintering 45S5. Grasso et al. [81] demonstrated that by using SPS, even fully dense amorphous 45S5 compacts could be fabricated. In our previous study, we reported the first use of SPS to densify sol-gel derived 45S5 and found that SPS could lead to enhanced mechanical properties and better early-stage cytocompatibility than the conventional heat treatment [213]. Although SPS has been utilized to manufacture bulk 45S5 and various 45S5-based composites, there is a lack of comprehensive investigations into the *in vitro* biological properties of such materials [81, 199, 204]. For instance, although Peitl Filho et al. [79] reported 20 years ago the effect of annealing-induced crystallization on the bioactivity of 45S5, such influences have been inadequately studied for spark plasma sintered 45S5 compacts.

In vitro cell behavior in the presence of bioactive materials such as 45S5 and Ca-P ceramics can be studied by two general approaches: directly seeding them on the material surface and culturing them in the material-conditioned media [88, 214]. The release of ionic dissolution products of 45S5 powder and plasma sprayed Ca₂SiO₄ has been reported to significantly influence the proliferation, differentiation and mineralization of

mesenchymal stem cells and osteoblasts [209, 215, 216]. However, such studies are rare for 45S5 pellets prepared by SPS.

The outstanding bioactivity of 45S5 stems from a series of reactions at the interface between the material and biological fluid, which are determined by its unique chemical composition [3, 68]. The underlying mechanisms for the bioactivity and degradation of 45S5-based biomaterials have thus been frequently studied by aqueous aging [88, 217, 218]. In the aging period, the rapid ion exchange of 45S5 with the surrounding solution and the subsequent interfacial reactions alter not only the surface condition of the material, but also the chemical composition of the media [4, 88, 216]. Both changes, especially those induced by extended aging, are believed to influence the cell behaviors when the aforementioned two cell culture approaches are employed [219].

It is hypothesized in this chapter that fully dense 45S5 pellets can be obtained through a rapid SPS process, and that the *in vitro* biological properties of the 45S5 pellets, either as a substrate or as a source of ionic dissolution products, can be affected by the sintering temperature and aqueous aging. Two well established cell lines, mouse fibroblast cell L929 and human osteoblast-like cell MG63, were cultured in 45S5-conditioned media to study their growth and viability. Furthermore, MG63 cells were directly seeded on the 45S5 pellets' surface to examine the activity of an important osteogenic marker, alkaline phosphatase (ALP).

3.2 Experimental

3.2.1 Sample preparation

The melt-derived 45S5 powder used in this study was supplied by XL Sci-Tech Inc., US, and the particle size was measured by laser diffraction particle size analysis (Mastersizer 2000, Malvern Instruments, UK). DI water was used as the dispersant, and particle size was recorded when the laser obscuration values were between 13 % and 15 %. Elemental composition of the as-received 45S5 powder was checked by X-ray photoelectron spectroscopy (XPS; Kratos, Axis-ULTRA, UK). Acquisition of XPS spectrum was carried out with an Al K α (1486.6 eV) monochromatic X-ray source with emission of 10 mA and anode HT of 15 kV when the sample analysis chamber pressure

reached $\sim 10^{-9}$ Torr. Binding energy calibration was carried out by referring to the C 1s component located at 284.6 eV. Simultaneous thermal analysis (STA) comprising thermogravimetry and differential scanning calorimetry (TGA/DSC; SDT Q600, TA Instruments, US) was carried out to reveal the thermal stability and crystallization kinetics of the as-received 45S5 powder. The thermal analysis was conducted under 100 mL/min nitrogen flow from RT to 1000 °C with a heating rate of 50 °C/min.

Based on the STA results, as-received 45S5 powder was sintered for 3 min in an SPS system (Dr. Sinter 1050, Sumitomo Coal Mining, Japan) at three different temperatures: 500 °C, 550 °C and 600 °C, and the sintered compacts were denoted as BG500, BG550 and BG600, respectively. One gram of 45S5 powder was compacted in a graphite die with an inner diameter of 10 mm at a heating and cooling rate of 50 °C/min and 100 °C/min, respectively. Real-time temperature measurement was conducted by inserting a thermocouple into a small hole drilled in the wall of the graphite die. The sintering pressure was exerted on the green body through two graphite punches. A pre-SPS pressure of ~ 7.6 MPa was first applied, and the pressure was increased and kept constant at 50 MPa for 3 min when the sample was held at the sintering temperature. A layer of graphite paper was used to prevent direct contact of the powder with the die or punches and to enable easy removal of the pellet after sintering. The sintered pellets were ground under running water sequentially with P180, P400, P800, P1200 and P4000 sand papers to remove the graphite affected layer. Sintering parameters were recorded using the data acquiring software of the SPS system and data points were collected every 5 s.

3.2.2 Characterization of sintered compacts

A field emission scanning electron microscope (FESEM; JEOL JSM 7600F, Japan) was employed to observe the as-received 45S5 particles and the fracture surface morphology of 45S5 compacts. The density of the sintered 45S5 compacts was determined by Archimedes method and the relative density was calculated assuming a theoretical density of 2.72 g/cm³ [204]. X-ray diffraction (XRD; PANalytical EMPYREAN, The Netherlands) in the 2θ range of 10–80° was utilized at an operating voltage and current of 40 kV and 40 mA, respectively, to identify the phase composition of the samples. The strongest diffraction peak ($2\theta \approx 34^\circ$) was used to calculate the

crystallite size of the main phase in the crystalline samples according to the Scherrer equation:

$$\zeta = (k \cdot \lambda) / (\beta \cdot \cos\theta) \quad (3.1)$$

where ζ is the crystallite size, k the Scherrer constant equal to 0.89, λ the wavelength of the Cu K α X-ray, and β the full width at half maximum of the diffraction peak.

Young's modulus and hardness of the sintered pellets were tested by nanoindentation (Agilent G200, Keysight Technologies, US), assuming the Poisson ratio to be 0.261 [220]. The continuous stiffness measurement technique was employed, with the depth limit, strain rate target, harmonic displacement target, and frequency target set at 2000 nm, 0.05 s⁻¹, 2 nm, and 45 Hz, respectively. In total 30 indentations were conducted on each sample.

3.2.3 Cell culture

In vitro cell response to sintered 45S5-based materials can be tested by both direct and indirect methods, the former being seeding cells on sintered pellets and the latter introducing 45S5 ionic extracts to the cell culture media in advance [88].

A schematic showing the procedure of cell culture is given in Fig. 3.1. For all the *in vitro* biological tests, two types of 45S5 pellets were used: the fresh samples were sintered and newly polished and had not been soaked in any medium, while each of the aged samples had undergone prolonged aging (~21 days) in 1.5 mL fresh media (FM, Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % (v/v) HyCloneTM research grade fetal bovine serum (FBS) and 1 % (v/v) HyCloneTM Penicillin-Streptomycin solution, all from GE Healthcare) in a 12-well plate. Throughout the aging period the media in each well was changed into FM for 6 times. All samples were thoroughly cleaned with ethanol and DI water, sterilized (autoclaved at 121 °C for 20 min), and dried at 60 °C prior to the current study. Fourier transform infrared spectroscopy (FTIR; PerkinElmer Frontier, US) was used to check the chemical bonds present in both types of samples. FTIR spectra were obtained from 650 to 1800 cm⁻¹ at a step size of 1 cm⁻¹ by using an attenuated total reflection accessory. A total number of 32 scans were recorded for each sample to obtain the average spectrum.

Fig. 3.1 Schematic of the cell culture procedure.

Cell viability and proliferation were assessed after exposing L929 and MG63 to 45S5-conditioned media for 3 days. The conditioned media (CM) containing the dissolution products of 45S5 was prepared by incubating each polished pellet in 1.5 mL of FM in a 12-well plate at 37 °C in a humidified environment with 5 % CO₂ for 5 days. For comparison purposes, 1.5 mL of FM was incubated under the identical conditions and denoted as non-conditioned media (NM). A 100 µL volume of both cell suspensions were initially seeded at a density of 10,000 cells/mL in a 96-well plate containing FM. The cells were incubated for ~18 h to allow them to attach to the well bottom, after which the FM was changed to CM. The cell numbers were quantified using a CyQUANT Cell Proliferation Assay Kit (Molecular Probes, US) after 3 day culture. Cell viability, represented by the percentage of live cells, was assessed with the LIVE/DEAD[®] Viability/Cytotoxicity Assay Kit (Molecular Probes, US). The stained cells were observed by an optical microscope equipped with an Olympus U-RFLT50 power supply unit and an Olympus DP70 digital camera (Olympus, Japan). The percentage of live cells was obtained by manually counting the numbers of live and dead cells using the Cell Counter plugin available in Image J. In the cell viability experiment, cells grown in NM were regarded as the positive control while the negative control used was prepared by exposing the positive control to sodium dichloroisocyanurate for ~45 min before the assay. The concentration of the ions present in CM and NM was measured by an

inductively coupled plasma optical emission spectrometer (ICP-OES; Optima™ 8300, PerkinElmer, US)

In addition, ALP activity was evaluated using an ALP Assay Kit (Abcam, UK). The surface of each 45S5 pellet, obtained after removing the CM, was seeded with 4,000 MG63 cells in a 100 μ L drop of FM. After $\sim$ 3.5 h incubation, the level of FM in each well was increased to immerse the pellets. Cells directly seeded and grown on the tissue culture plastic (TCP) were used as the control. Quantification of ALP was carried out after culturing the MG63 cells for 4 days. All three assays were carried out following the manufacturers' protocols.

Student's *t*-test was used for data analysis. Significance level was set at $p=0.05$, with *, **, ***, and **** representing $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and ≤ 0.0001 , respectively in the relevant figures. Cell culture data was obtained from triplicate measurements and presented as the mean $\pm$ standard deviation.

3.3 Raw powder analysis

Fig. 3.2a and b present the FESEM images of pristine 45S5 powder. As can be seen in Fig. 3.2a, the 45S5 particles are irregular in shape and their sizes range from sub-micron to tens of microns. The high-magnification FESEM micrograph of a single particle given in Fig. 3.2b shows the typical surface morphology of melt-derived 45S5 [221].

The TGA/DTA curves for the pristine 45S5 powder are shown in Fig. 3.2c. The evident endothermic peak located at 593 $^{\circ}$ C corresponds to the first glass transition temperature (T_{gl}). This value is higher than those typically reported in literature ($\sim$ 550 $^{\circ}$ C) [84, 221-223], possibly due to the high heating rate used in our study. It has been found by Bretcanu et al. [211] that an increase in heating rate leads to a higher T_{gl} . Crystallization of the amorphous 45S5 is evidenced by the exothermic peak seen at 700 $^{\circ}$ C, and this T_c value is in good agreement with the temperature range (600–750 $^{\circ}$ C) reported for 45S5 to crystallize [211, 223]. The TGA curve shows a total weight loss of 4.5 %. However, the majority of weight reduction (3.9 %) was confined to RT–400 $^{\circ}$ C, before the onset of crystallization. Weight loss below 100 $^{\circ}$ C (1.7 %) occurred as a result

of the evaporation of free water, and the others should be attributed to the loss of –OH groups.

Fig. 3.2 FESEM images (a, b), TGA/DSC curves (c), XPS spectrum (d) and particle size distribution (e) of as-received 45S5 powder.

The XPS spectrum of the raw 45S5 powder is presented in Fig. 3.2d. Based on its formulation, 45S5 is supposed to contain the elements of Si, P, Ca, Na and O [63], and all the corresponding peaks could be identified in the obtained XPS spectrum. It should be noted that the C 1s peak was caused by adventitious carbon and is commonly seen for air-

exposed materials [224]. It is thus safe to conclude that the starting powder was free from elemental contamination.

It can be seen in Fig. 3.2e that most particles were $<30\text{ }\mu\text{m}$, with the surface weighted mean diameter being only $5.3\text{ }\mu\text{m}$.

3.4 Sintering behavior and pellet properties

3.4.1 Sintering behavior

Taking into account the fact that in SPS the actual sample temperature would be slightly higher than the measured value [225], the sintering temperature was initially set at $600\text{ }^{\circ}\text{C}$, a temperature very close to the T_{gl} of 45S5. In order to fabricate 45S5 pellets with varying degrees of crystallinity, which were revealed by XRD, we also experimented subsequently with two lower temperatures, i.e. 550 and $500\text{ }^{\circ}\text{C}$.

Fig. 3.3a-c show the sintering behaviors exhibited by 45S5 samples when compacted at three different temperatures. The linearity observed in the heating up stage of the temperature curves was an indication of accurate temperature control. It can be seen in the displacement and derivative displacement curves that densification of the green body started at $\sim 515\text{ s}$ and it corresponded to $\sim 423\text{ }^{\circ}\text{C}$. At $\sim 24\text{ s}$ before reaching the preset sintering temperature, the applied pressure started increasing, as can be seen in the respective pressure curves. The temperature effect and pressure increment resulted in two peaks in the derivative displacement curves for BG550 and BG600 (Fig. 3.3b, c), while only one peak could be observed for BG500 because of the overlapping influences of these two factors (Fig. 3.3a). As a result, the fastest densification occurred at $\sim 595\text{ s}$ ($\sim 489\text{ }^{\circ}\text{C}$) for BG500, but the largest derivative displacement was seen at $\sim 610\text{ s}$ ($\sim 499\text{ }^{\circ}\text{C}$) for BG550 and BG600 because it was only contributed by the temperature factor for these two samples.

It has been reported that 45S5 densifies in two major stages, i.e. $500\text{--}600\text{ }^{\circ}\text{C}$ and $850\text{--}1100\text{ }^{\circ}\text{C}$, because of its two glass transitions [210, 211]. This study took advantage of the first densification stage; the low onset temperature for densification seen in this study ($\sim 423\text{ }^{\circ}\text{C}$) probably derived benefit from the high heating rate used and the application of sintering pressure. The relative density was calculated to be very close to

100 % for all compacts (Table 3.1), which proves that they were almost fully densified. It is worth a mention that the SPS processes we exploited, which comprised 10–12 min heating and 3 min holding, were among the fastest ones that could lead to fully densified 45S5 compacts reported thus far [81, 199, 202, 204, 222].

Fig. 3.3 Recorded sintering curves (a-c) and fracture surface morphology (d-f) for 45S5 samples sintered at 500 °C (a, d), 550 °C (b, e) and 600 °C (c, f).

3.4.2 Structural characteristics

The XRD patterns of sintered 45S5 pellets are compiled in Fig. 3.4a. While BG500 was amorphous, 45S5 compacts sintered at two higher temperatures exhibited sharp diffraction peaks from the crystalline phase $\text{Na}_2\text{CaSi}_3\text{O}_8$. It is well known that 45S5 possesses a high crystallization ability, and a surface crystallization mechanism has been reported by Bretcanu et al. [211]. Lefebvre et al. [221] found that after the first glass transition and prior to the crystallization process, a glass-in-glass phase separation occurred, leading to two immiscible phases which subsequently crystallized at different temperatures. Therefore, a trace amount of secondary phase was likely to exist in the samples sintered at 550 °C and 600 °C in this study. Further investigation is needed to reveal the detailed structural transformation of 45S5 in the SPS process. Interestingly, 550 °C is the lowest temperature reported thus far that can enable 45S5 to crystallize [81, 202]. This should be primarily attributed to the high heating rate used in the current study [211].

According to Equation (3.1), the size of $\text{Na}_2\text{CaSi}_3\text{O}_8$ crystallites was estimated to be 17.39 and 18.95 nm for BG550 and BG600, respectively (Table 3.1). The values are in good agreement with crystallite sizes reported for 45S5 heat-treated at similar temperatures [221]. The larger crystallite size of BG600 implies that it experienced a higher degree of crystallization than BG550.

Fig. 3.4 (a) XRD patterns of 45S5 powder and sintered pellets. (b) Mechanical properties of 45S5 pellets sintered at different temperatures.

Table 3.1 Relative density, crystallite size and mechanical properties of sintered 45S5 compacts

Sintering temperature (°C)	Crystallite size (nm)	Relative density (%) [*]	Young's modulus (GPa) [*]	Hardness (GPa) [*]
500	N.A.	99.5 ± 0.6	82.55 ± 0.03	6.45 ± 0.04
550	17.39	100.2 ± 0.1	96.3 ± 0.7	6.6 ± 0.1
600	18.95	100.4 ± 0.2	98.1 ± 0.8	6.7 ± 0.2

^{*} Data presented as average ± standard error.

There were marked differences in the fracture surface morphology between amorphous and crystalline 45S5 pellets, as shown in Fig. 3.3d–f. Unlike BG550 and BG600, the inter-particle boundaries were still retained in the amorphous BG500 pellet, as indicated by the arrows in Fig. 3.3d. It suggests that the compaction of BG500 was principally driven by particle deformation. When BG550 and BG600 were sintered, the sintering temperature and activation energy were sufficiently high to enable the massive transport and relocation of atoms (including those at the inter-particle boundaries) in the crystallization process, which in turn led to the disappearance of those boundaries in the compacted samples. In addition, BG550 and BG600 showed a much rougher fracture surface than BG500 (Fig. 3.3d–f). This was caused by the fact that BG550 and BG600 were essentially glass-ceramics with many nano-sized crystallites embedded in the amorphous matrix. The $\text{Na}_2\text{CaSi}_3\text{O}_8$ crystallites are believed to possess superior mechanical properties and could serve as a reinforcing phase in BG550 and BG600. Therefore, crack propagation in those pellets was obstructed by the crystalline phase, leading to a rougher fracture surface. No residual pores were visible on the fracture surface of any sintered compact, further supporting the successful fabrication of dense 45S5 pellets by our rapid SPS process.

3.4.3 Mechanical properties

Besides phase composition, the mechanical properties of sintered Bioglass® pellets are also closely related to the sintering temperature [226]. Nanoindentation was thus carried out to investigate the mechanical properties of the 45S5 compacts and the results are presented in Fig. 3.4b and Table 3.1. It was found that a higher sintering temperature could generally enhance both E and H of the sintered compacts. Particularly, E of BG550 was 16.7 % larger than BG500, which implies that E of 45S5 compacts could

be markedly augmented by the formation of a crystalline phase; by contrast, E was only slightly increased (by 1.9 %) in BG600 as compared to BG550. Besides, overall the E and H values of BG500 showed the smallest variation, which should be attributed to the presence of only one glassy phase.

Desogus et al. [222] reported that the devitrification of 45S5 in the SPS process led to a reduction in pellet density, which in turn was detrimental to its mechanical properties. Such a negative effect caused by the crystallization of 45S5 was avoided in our study. This was probably because of the application of a higher sintering pressure, which caused much higher E values to be achieved for our glass-ceramic pellets [222]. Furthermore, it is worth mentioning that the E values obtained in the current study are comparable to that of compact bone [213], signifying their enormous potential as orthopedic implants.

3.4.4 Biological properties

Fig. 3.5a compares the number of L929 and MG63 cells after 3 days of culture in CM and NC. In terms of L929 cell number, no difference was seen for the aged compacts, whereas BG500 (fresh) and BG550 (fresh) both resulted in more cells than BG600 (fresh). When it comes to MG63 cell number, BG500 and BG550 from both the fresh and aged categories performed better than BG600. No statistical difference could be identified between BG500 and BG550 with respect to the number of either L929 or MG63 cells. In addition, the aged samples performed better than their fresh counterparts in supporting the growth of either one or both of the cell lines. It was further found that NM could result in the highest cell number for both cell lines.

Fig. 3.5 (a, b) Cell number and viability of L929 and MG63 cells after 3 day culture in CM and NM: (a) cell number, (b) cell viability. (c) ALP activity of MG63 cells after 4 day culture on 45S5 pellets. Asterisks in parentheses mean statistical differences between aged and fresh 45S5 pellets sintered at the same temperature. *, **, ***, and **** represent $p \leq 0.05$, ≤ 0.01 , ≤ 0.001 , and ≤ 0.0001 , respectively.

The sintering temperature effect on cell viability was more noticeable, as evidenced by Fig. 3.5b. First, the percentage of live L929 cells obviously decreased as the sintering temperature was augmented for both the aged and fresh pellets; for MG63 cells, the CM produced with pellets sintered at lower temperatures could bring about a higher percent of live cells, except that no statistical difference existed between BG500 (aged) and BG550 (aged). Second, comparing the viability of L929 and MG63 cells grown in CM prepared with the aged compacts with their fresh counterparts, generally larger differences could be found at higher sintering temperatures. Overall CM prepared with BG500 (aged) was the most desirable and BG600 (fresh) the most unfavorable to achieve satisfactory cell viability, which can be supported by the representative images of stained viable and dead cells shown in Fig. 3.6a-c and Fig. 3.7a-c as well. In addition, it was found that the positive control could outperform BG500 (aged)-conditioned media regarding L929 cell viability, and BG600 (fresh)-conditioned media resulted in even lower percentages of live L929 and MG63 cells compared with the negative control.

Fig. 3.6 Representative images showing stained live (green) and dead (red) L929 cells.

Fig. 3.7 Representative images showing stained live (green) and dead (red) MG63 cells.

The differences in cell number and viability between CM prepared with aged and fresh samples probably stemmed from the stronger alkalizing ability of the fresh pellets [213]. It has been widely accepted that a shift of pH to higher values usually occurs for Bioglass®-conditioned media due to the release of sodium ions [202]. It can be seen in Fig. 3.8 that the fresh 45S5 pellets could generally lead to higher Na concentrations in the CM than the aged ones, and that the CM all possessed significantly higher Na concentrations than the NM. It was found in our previous research that the rapid reaction between Na_2O in sintered 45S5 pellets and water could lead to the formation and growth of Na-rich surface patterns on the samples when aged in air [227]. In the current study, it is believed that sodium ions could be quickly and continuously released when aged in cell culture media. FTIR was used to identify the changes in surface composition of the 45S5 pellets after aging. Compared with those of the freshly ground pellets, after aqueous aging the FTIR spectra of the 45S5 pellets displayed two distinct changes (Fig. 3.9). First, an intensified absorption band centered at $\sim 1650 \text{ cm}^{-1}$ was observed, which can be

ascribed to absorbed water. Second, the bands corresponding to Na_2CO_3 were obviously stronger, especially for the amorphous BG500 (aged) sample. This indicates that after aging there existed a higher amount of Na_2CO_3 on the pellet surface, probably because of the reaction of Na_2O with absorbed water and CO_2 in air [227].

Fig. 3.8 Concentrations of Na, Ca, P, Si ions in CM and NM.

Fig. 3.9 FTIR spectra of sintered and newly ground 45S5 samples (a) and the aged pellets (b).

The slower proliferation and lower viability of cells grown in CM prepared with BG600 were possibly linked to the deficiency of essential ions such as Ca and Si (Fig. 3.8 and Fig. 2.4). Kubo et al. [228] reported that the combination of Ca and Si leached from

45S5 could promote the growth, ALP activity and mineralized nodule formation in periodontal-ligament fibroblasts. In this study both of these two ions were present at the lowest concentration in BG600-conditioned media. Since BG600 had the highest degree of crystallinity, the largest number of Si and Ca atoms was confined in the $\text{Na}_2\text{CaSi}_3\text{O}_8$ lattice by the strong bonds between them [229]. Therefore, the release of Si and Ca to the surrounding media was probably the slowest for BG600. It should be noted that in our previous research, apparently slower cell growth was observed for 45S5 pellets with low crystallinity [213]. Possible reasons for the different phenomena observed here include the longer media-conditioning period (5 days) employed, a different starting 45S5 powder (melt-derived) utilized for sintering, and the extended culture period (3 days) used for comparison.

A number of previous studies have reported the decreased bioactivity of Bioglass® with a higher degree of crystallization [230, 231]; on the other hand, it was found in some other investigations that crystallization of Bioglass® did not induce cytotoxicity or inhibit the formation of an apatite layer on its surface in simulated body fluid [232, 233]. We believe an unambiguous understanding of such discrepancies calls for careful examination of the degradation behavior and surface condition of the material in the immediate environment, be it *in vitro* or *in vivo*, utilized for the assessment [234, 235].

It can be found in Fig. 3.5c that the MG63 cells cultured on 45S5 compacts all showed remarkably higher ALP activity, an important feature of the osteoblast phenotype and an early marker for osteoblast differentiation, than those on the TCP control, on which the cells were routinely grown. The 45S5 pellets were thus obviously advantageous over TCP in promoting MG63 cell differentiation and bone formation, although TCP could probably lead to faster cell growth and higher cell viability than the 45S5 compacts. Similarly, in the study on another bioactive glass composition by Haimi et al. [236], it was observed that compared with scaffolds made from only polylactic acid, the bioactive glass incorporated scaffolds resulted in slower growth of adipose stem cells but significantly higher relative ALP activity of a single cell. In addition, Fig. 3.5c shows that the fresh samples all resulted in significantly higher ALP activity than their aged counterparts. This phenomenon can be attributed to two possible reasons. First, the

interfacial pH values at the surface of fresh pellets were probably higher, which may have promoted the ALP activity of the MG63 cells [237]. Second, the quantity of bioactive substances released from the fresh pellets was probably higher, which might facilitate cell differentiation and bone formation.

Particularly, the content of Si, which is considered as a vital factor in regulating the metabolism of MG63 [215], was found to vary drastically in the different CM used. As shown in Fig. 3.8, the fresh pellets led to much higher Si ion concentrations in the corresponding CM than their aged counterparts, and Si content in the NM was negligible. The enhancement effect of Si ions on the bone mineralization process of osteoblasts has been extensively studied [238]. We reported in our previous study that trace levels of silicon in biomaterials could enhance the differentiation of human fetal osteoblast partly due to the rapid release of silicate ions [239]. Porter et al. [12] also found that silicon-substituted hydroxyapatite possessed enhanced osteoconductive properties than pure HA. By examining 45S5 filled holes in the femoral condyles of mature rabbits, Oonishi et al. [240] reported that the bone ingrowth rate strongly correlated with the soluble silica provided to osteoblasts in the growing bone.

Interestingly, both aged and fresh BG550 pellets brought about a higher Si ion concentration in the CM and hence much higher ALP activity in MG63 cells than the BG500 and BG600 compacts from the same batch. This phenomenon suggests that for the sintered 45S5 samples a moderate degree of crystallization favors the Si leaching into the media. It is well known that the loss of soluble silica to the surrounding solution is a critical reaction step contributing to the outstanding bioactivity of 45S5 based biomaterials [68]. According to Grasso et al. [81], the bioactivity of mildly crystallized 45S5 based glass-ceramics obtained through SPS was superior to fully amorphous or highly crystalline samples. Therefore, as a result of the optimum amount of nano-crystalline $\text{Na}_2\text{CaSi}_3\text{O}_8$ phase, the moderately crystallized pellet fabricated at 550 °C in this study was possibly the most bioactive and could release Si ions more quickly when immersed in cell culture media [81]. This interesting finding is of special importance as it provides useful guidance on the selection of SPS temperature for the fabrication of bulk 45S5 implants. Further investigations will be carried out to gain an in-depth understanding of this phenomenon.

3.5 Summary

In this chapter 45S5 compacts with a relative density of > 99 % were fabricated by sintering in the temperature range of 500–600 °C for 3 min through a rapid SPS process. The cytocompatibility of the sintered compacts was investigated with two different cell lines, i.e. L929 and MG63, and the ALP activity of MG63 cells cultured on the densified 45S5 pellets fabricated by SPS was reported for the first time. It was found that compared with the fresh samples, the samples that had been aged for 21 days could generally increase the growth rate and viability of both cell lines because of slower alkalization of the cell culture media. However, they led to lower ALP activities in MG63 cells due to the presence of less Si ions in the media during cell culture. Although a lower SPS temperature in the 500–600 °C range tended to bring about faster cell proliferation and higher cell viability, a moderate temperature of 550 °C could result in the generation of more ALP by MG63 cells possibly caused by the highest Si content in the media and high interfacial pH. It is thus concluded that an SPS temperature of 550 °C should be used for fabricating dense 45S5 compacts to achieve the optimum cell response when used in orthopedic applications.

Chapter 4 Graphene-reinforced 45S5 Bioglass®

Notwithstanding its excellent biocompatibility and bioactivity, the fracture toughness of 45S5 has to be improved to extend its application in load-bearing orthopedic implants. Satisfactory wear resistance plays an important role in the in vivo performance of orthopedic implants. This chapter explores the potential of using graphene nanoplatelet (GNP) to enhance the fracture toughness and wear resistance of 45S5. GO was used as a precursor to GNP and in situ thermal reduction of GO was realized in the SPS process. Prior to the fabrication of GNP/45S5 composites, SPS treatment of GO was systematically investigated to understand the reduction effect and underlying mechanisms. In light of the results of GO reduction experiments as well as conclusions drawn in Chapter 3, composite powders with 0–1 wt.% GO were sintered by SPS at 550 °C. The effects of GNP incorporation on the mechanical, tribological, and biological properties of the sintered pellets were comprehensively studied.

4.1 Introduction

As the original Bioglass® formulation, 45S5 is able to directly bond to bone and soft tissues and has been thus widely used for hard and soft tissue repair [3, 40, 42, 63]. However, like many other types of bioactive glasses, 45S5 is intrinsically brittle and the insufficient fracture toughness constraints its extensive applications as major load-bearing components. Furthermore, there is a paucity of investigations into the fracture toughness of bioactive glasses in the scientific community [82].

Satisfactory wear resistance is crucial for the *in vivo* fixation, integration and lifetime of an implant because wear debris can lead to severe inflammatory responses of surrounding tissue [241, 242]. Tribological properties are especially critical for the bearing surfaces of orthopedic and dental implants, where bioactive glasses have been widely used [243-245]. In addition, when bulk bioactive glasses are employed in orthopedic applications, their wear resistance has to be improved as well [246].

Graphene is a fascinating 2D material with exceptional mechanical properties which bring about its intriguing application as a reinforcement in various composites [7]. By virtue of its outstanding intrinsic strength and high specific surface area, graphene has been extensively exploited to toughen a wide variety of structural and biomedical ceramics, such as silicon nitride [17], hydroxyapatite [57] and 58S Bioglass® [247]. Moreover, it has been reported that the presence of graphene did not impair the *in vitro* bioactivity and cytocompatibility of graphene coated 45S5-derived scaffolds [248]. However, the influence of graphene addition on the fracture toughness of 45S5-based composites has not been reported thus far, nor has any study investigated the wear behavior of such composites.

It is imperative to address these two issues in the manufacture of graphene-enhanced composites: (1) graphene tends to agglomerate, making it a challenge to homogeneously disperse graphene sheets in the matrix, and (2) it is difficult to fabricate high-quality pristine graphene on a large scale. Therefore, GO, known for its higher availability and eminent dispersibility in a variety of organic and aqueous solvents, has been utilized as one of the major precursors to graphene-reinforced composites [9, 249].

The numerous similarities between rGO and pristine graphene have made the reduction of GO one of its most important reactions [250-254].

The simultaneous application of pulsed DC and pressure endows SPS with high heating rates (up to 2000 °C/min) and short process durations (typically < 20 min) [212]. SPS is especially advantageous in sintering bioceramics such as hydroxyapatite and Bioglass® to avoid undesirable phase transformation and grain coarsening caused by prolonged exposure to high temperature [255]. It has been proven in Chapter 3 that SPS was capable of effectively compacting 45S5 powders into dense pellets.

Several unique characteristics of SPS solidify its great potential in the thermal reduction of GO: (1) it can offer a heating rate as high as 2000 °C/min [142], which is desirable in the thermal reduction processes of GO, (2) the process is conducted in vacuum (~5 Pa) or protected with inert gas, meaning that GO can be protected from reacting with air at high temperatures, (3) GO is sealed in a graphite die–punch combination, which minimizes contamination and sample loss, and (4) the generated spark plasma and the high temperature can possibly result in a synergetic effect to minimize residual oxygen-containing functional groups and achieve a high reduction degree of rGO [140]. However, there have been no systematic investigations into the reduction of GO by SPS to date. Such studies are believed to be able to provide useful guidance on the preparation of various rGO-reinforced materials sintered by SPS. Additionally, SPS is usually the first-choice method for sintering ceramics reinforced with carbonaceous materials such as CNT and graphene which cannot survive atmospheric sintering processes [57, 194]. It has been reported that GO could be reduced *in situ* when SPS was used to fabricate rGO–ceramic composites with matrixes such as silica and silicon nitride [256-258]. Porwal et al. [202, 204] reported the preparation of CNT/45S5 and graphene/45S5 composites by SPS. However, the fracture toughness and tribological properties of the composites were not studied; neither was the cytocompatibility of the graphene-reinforced 45S5 pellets reported.

This chapter uses SPS to fabricate graphene nanoplatelet (GNP) reinforced 45S5 composites with improved fracture toughness and wear properties. It starts with a study into the thermal reduction of GO by SPS, which can overcome the aforementioned drawbacks of conventional thermal reduction processes. GO was then used as the

precursor to graphene when fabricating the GNP/45S5 composites. Unlike the processing route used by Porwal et al. [204] where pristine graphene was mixed with the 45S5 powder, in this study *in situ* reduction of GO and enhancement of 45S5 matrix were simultaneously realized in the SPS process.

4.2 Experimental

4.2.1 GO reduction by SPS

GO was synthesized from natural graphite flakes using a modified Hummers method [259, 260]. A 0.24 g sample of freeze-dried GO powder was loaded into a graphite die with an inner diameter of 20 mm and clamped between two graphite punches. All the reduction processes were conducted by holding the GO sample in an SPS system (Dr. Sinter 1050, Sumitomo Coal Mining, Japan) at preset temperatures for 1 min, unless otherwise specified. A schematic of the SPS setup used is given in Fig. 4.1. Heating and cooling rates were both set to be 100 °C/min. Sample names of SPS-reduced GO (SPSrGO) and the corresponding SPS parameters are summarized in Table 4.1. Temperature measurement was realized by an infrared pyrometer focused on a 2 mm hole on the graphite die or, when the highest temperature was < 600 °C, a thermocouple inserted into the hole.

Fig. 4.1 Schematic of the setup for reducing GO by SPS.

Table 4.1 Processing parameters and properties of GO and SPSrGO samples*

Sample name	Processing parameters	C:O ratio (by at. %/wt. %)	BET surface area (m ² /g)	Interlamellar spacing (Å)	I_D/I_G ratio	Buckypaper sheet resistance (Ω/sq.)
GO	N.A.	2.26/1.70	83	8.07	0.85	$> 2 \times 10^6$
SPSrGO-500	500–1–1	9.62/7.22	734	3.56	0.80	12.4 ± 0.8
SPSrGO-900	900–1–1	82.33/61.89	806	3.55	0.91	1.77 ± 0.05
SPSrGO-1050	1050–1–1	83.03/62.69	859	3.57	1.05	1.85 ± 0.03
SPSrGO-2C500	500–1–2	15.13/11.35	818	3.42	0.74	13.61 ± 0.04
SPSrGO-3C500	500–1–3	14.90/11.18	1266	3.43	0.76	17 ± 1
SPSrGO-500E	500–3–1	11.71/8.78	800	3.40	0.79	14.0 ± 0.2

* Processing parameters: highest temperature (°C)–holding time (min)–number of processing cycles. I_D/I_G ratios were calculated from Raman spectra. C:O ratios were determined from the XPS detailed scan spectra. Interlamellar spacings were calculated from XRD patterns. Buckypaper sheet resistance data are given as the average $\pm$ standard deviation. The sheet resistance of GO could not be obtained as it exceeded the measuring range (1–2 MΩ/sq.) of the four-point probe.

To elucidate the role of the spark plasma generated during the heating stage in reducing the GO, two samples, denoted as SPSrGO-2C500 and SPSrGO-3C500, were fabricated, with the number of processing (i.e., heating–holding–cooling) cycles being 2 and 3, respectively, using 500 °C as the highest temperature. For comparison SPSrGO-500E was also fabricated by holding at 500 °C for an extended duration of 3 min in one processing cycle.

4.2.2 Characterization of SPSrGO

The chemical state of C and O in GO and SPSrGO samples was investigated by XPS (Kratos, Axis-ULTRA, UK) equipped with an Al K α (1486.6 eV) monochromatic X-ray source. The thermal stability of the GO and SPSrGO samples was investigated by TGA (TA Instruments Q500, US) using a heating rate of 10 °C/min. The specific surface area of the powders was determined by a gas sorption analyzer based on the Brunauer–Emmett–Teller (BET) method, using a QuadraSorb Station 2 surface area and pore size

analyzer (Quantachrome Instruments, US). The crystallinity of the SPSrGO samples was tested using XRD (Philips MPD 1880 diffractometer, The Netherlands) at 0.01 deg/s with a Cu K α radiation. FTIR (PerkinElmer Frontier) was used to compare the functional groups in GO and SPSrGO powders. The samples were mixed with KBr using a mortar and pestle, compressed into translucent discs and tested in the FTIR transmission mode. Raman spectra of GO and SPSrGO samples were collected using a Rapid Imaging Micro-Raman spectrophotometer system (Renishaw Invia, UK) with a 488 nm line of a HeNe laser. The sheet resistance of GO and SPSrGO buckypaper prepared with a hydraulic press was measured by a four-point probe (CMT-SR2000NW, Materials Development Corp., US) and averaged from six measurements.

The morphology of the samples was observed using both transmission electron microscope (TEM; JEOL JEM 2010, Japan) and FESEM (JEOL JSM 7600F, Japan). Atomic force microscope (AFM; Park Systems, Suwon, South Korea) imaging was conducted in an ambient environment to obtain topographic and phase images of the samples as well as to determine the thickness of the GO sheets.

4.2.3 Preparation and sintering of composite powder

A schematic of the colloidal processing route to the GO/45S5 composite powder is presented in Fig. 4.2a. Dimethylformamide (DMF) was utilized as the solvent, as it has been proven to be suitable for the preparation of GO dispersion with long-term stability and was successfully utilized to prepare GO/45S5 composite powder with outstanding degree of homogeneity [204, 249]. GO and 45S5 were sonicated separately in DMF for 30 min at 1 mg/mL and 86 mg/mL, respectively. GO solution was subsequently added drop by drop to 45S5 solution over 30 min, during which both sonication and mechanical stirring were utilized to maximize the degree of homogeneity. The mixed solution was then dried on a combined hot-plate magnetic-stirrer device at ~100 °C until it became gelatinous. Further drying was conducted at 80 °C in a vacuum oven for 24 h to fully evaporate residual DMF. Composite powders with GO loadings of 0.1, 0.5, and 1 wt.% were obtained by carefully grinding the dried cake using a mortar and pestle.

Fig. 4.2 Schematics depicting the processes of (a) the preparation of composite powder and (b) cell culture.

The GO/45S5 powder was sintered at 550 °C, as it was found in Chapter 3 that 550 °C could result in the optimum *in vitro* biological properties of the sintered pellets. The heating and cooling rates as well as sintering pressure were the same as those specified in Chapter 3. For comparison, pure 45S5 pellets were sintered under the same conditions. Sintered pellets with 0, 0.1, 0.5, and 1 wt.% GO added in the raw powder are denoted as BG0, BG0.1, BG0.5 and BG1, respectively. The sintered pellets were ground sequentially with 400#, 800#, 1200#, 2400#, and 4000# sand papers to remove the graphite-contaminated surface layer before various characterizations.

4.2.4 Characterization of composite powders and pellets

Chemical, structural, and morphological properties of the materials were extensively characterized using XPS, FTIR, Raman spectroscopy, FESEM, and TEM (experimental details given in Section 4.2.2). The phase composition of the pristine 45S5 powder and sintered pellets was identified by XRD, as described in Section 3.2.2.

4.2.5 Mechanical and tribological tests

E and H of the sintered pellets were measured by nanoindentation, using the same parameters as those specified in Section 3.2.2. In total 20 indentations were made on each pellet to obtain the average value and standard deviation. A micro Vickers hardness tester (FE-300e, FUTURE-TECH, Japan) was utilized to conduct the Vickers indentation fracture toughness test by applying an indentation load of 500 g for 10 s. The K_{IC} of the polished pellets was calculated based on the model proposed by Anstis et al. [261]:

$$K_{IC} = 0.016(E/H)^{1/2} \cdot P/c^{3/2} \quad (4.1)$$

where P is the indentation load and c the characteristic dimension of the radial crack.

Friction and wear characterization was carried out with a ball-on-disk micro-tribometer (CSM High Temperature Tribometer, Switzerland). The sintered pellets were tested against an Al_2O_3 ball (3 mm in radius) in a circular path (2 mm in diameter) for 50,000 laps at a sliding speed of 2 cm/s under a normal load of 3 N. Three measurements were carried out for each sample to get an average friction coefficient. Wear track topography was studied by surface profilometry using a Talyscan 150 3D scanner equipped with a diamond stylus of 4 μm in diameter (Taylor Hobson, US). The wear volume was calculated by measuring the width and depth of wear tracks using surface profilometry. The specific wear rate, k_i , was calculated based on the following equation:

$$k_i = V_i / (F \cdot s) \quad (4.2)$$

where V_i is the wear volume, F the normal load and s the sliding distance. Wear track morphology was examined by FESEM.

4.2.6 Cell culture

To evaluate the cytocompatibility of the sintered pellets, human osteosarcoma cell line MG63 was directly seeded on polished pellet surfaces as well as cultured in BG0 and BG1 pellet leachates at two different concentrations (Fig. 4.2b). All polished samples used for cell work were cleaned by brief sonication in ethanol and sterilized in an autoclave. The cell culture medium (denoted as fresh medium or FM hereafter) used in this study was DMEM (GlutaMAXTM-I, Life Technologies) supplemented with 10 % (v/v) FBS (HyCloneTM research grade, South American Origin, GE Healthcare) and 1 % (v/v) HyCloneTM Penicillin-Streptomycin solution (GE Healthcare). To prepare the pellet leachates, each sterilized pellet was soaked in 2.5 mL FM at 37 °C in 5 % CO₂ for 3.5 days in a 12 well plate, leading to a (pellet mass)/(medium volume) ratio of 0.4 g/mL. This high concentration leachate was then used to prepare the 0.08 g/mL leachate dilution by adding FM. For comparison purposes, non-conditioned media (NM) were prepared by incubating FM (without soaking any pellet in it) under the identical conditions.

MG63 cells were directly seeded on the soaked pellets after removing the leachates from each well. A 100 µL drop of FM containing 1000 MG63 cells was carefully pipetted onto the top surface of each pellet, leading to a seeding density of ~1274 cells/mm². They were then incubated at 37 °C in a humidified environment supplied with 5 % CO₂ for ~3.5 h to let the cells adhere to the sample surface, after which medium level was topped up by 2 mL to fully immerse the pellets. Tissue culture plastic, on which the cells were routinely grown, was used as the material control. After 72 h culture, cell viability was evaluated with a LIVE/DEAD[®] Viability/Cytotoxicity Assay Kit (Molecular Probes, US), using the protocols recommended by the manufacturer. An optical microscope equipped with an Olympus DP70 digital camera and an Olympus U-RFLT50 power supply unit (Olympus, Japan) was used to visualize the stained cells. Numbers of live and dead cells were manually counted by using the Cell Counter plugin available in ImageJ.

Cell culture in a 96 well plate was carried out to investigate the proliferation and viability of MG63 cells in BG0 and BG1 leachates. A 100 µL volume of cell suspension containing 1000 cells was pipetted into each well and incubated for ~18 h to allow the

cells attach to the well bottom. The media was then carefully removed from each well and replaced with 200 μ L pellet leachates, after which the cells were left to proliferate for another ~54 h. Cell viability was then assessed and analyzed using the method described above. For cell number quantification, a CyQUANT Cell Proliferation Assay Kit (Molecular Probes, US) was utilized according to the manufacturer's protocols.

Cell work data was gained from triplicate measurements and presented as mean $\pm$ standard deviation. Student's *t*-test was performed for data analysis, with statistical difference represented by * ($p \leq 0.05$) and ** ($p \leq 0.01$) in the relevant figures.

4.3 Chemical and structural characteristics of SPSrGO

Fig. 4.3 shows the XPS survey and C 1s detailed scan spectra for GO and SPSrGO. Only peaks corresponding to C and O were present in the spectra (Fig. 4.3a), demonstrating no contamination from other elements. It can be seen that SPSrGO samples all showed an enhanced C 1s peak and weakened O 1s peak as compared to GO, and this phenomenon was more obvious as the reduction temperature increased. To quantify the change in the relative intensity of those two peaks, we calculated each sample's C:O ratio, which has been widely used to indicate the extent of reduction for rGO, where a higher value means elimination of more oxygen-containing functional groups and a greater extent of reduction. As shown in Fig. 4.3b and Table 4.1, GO has a low C:O ratio of 2.26 (by at.%). This value was improved by over 4 fold to 9.62 for SPSrGO-500. SPSrGO-900 and SPSrGO-1050 achieved an extraordinarily high C:O ratio of 82.33 and 83.03, respectively, corresponding to more than a 30-fold increase as compared with GO. Such a high C:O ratio has rarely been reported for rGO, and to the best of our knowledge, this is the highest value achieved for thermally reduced rGO thus far (Table 4.2). It is worth mentioning that although an even higher value was reported by Gao *et al.* [262], a complex chemical reduction process was used in conjunction with thermal annealing at 1100 $^{\circ}$ C.

Fig. 4.3 (a, c–f) XPS spectra of GO and SPSrGO: survey scan spectra (a) and C 1s spectra (c–f), where the purple line stands for fitted envelopes, the open circle for measured data, and the numbers denote the content (at.%) of major deconvoluted components. (b) C:O ratios and O content (wt %) in GO and SPSrGO, where the star represents the C:O ratio from ref [263] where rGO was fabricated by conventional thermal reduction at 400 °C for 30 min followed by 1200 °C for 30 min.

Table 4.2 Comparison of properties between SPSrGO and other thermally reduced GO

References	Method	C:O ratio (at.%)	BET surface area (m ² /g)	Interlamellar spacing (Å)	I _D /I _G ratio	Electrical conductivity (S/m)
Current study	SPS at 1050 °C for 1 min	83.03	859	3.57	1.05	1564 (900 °C)*
[263]	30 min annealing at 1200 °C in Ar	50.55	Not available	3.44	1.06	Not available
[264]	10 min microwave treatment at 800 W	5.46	Not available	Not available	0.96	200
[265, 266]	30 s treatment at 1050 °C in Ar	9.75	600–1500	Not available	Not available	1000–2300
[267]	Flash reduction	4.23	Not available	Not available	Not available	~1000
[268]	1 min microwave treatment in air at 700 W	2.75	463	3.6	Not available	274

* Sheet thickness was measured to be 362 μm. Conductivity was calculated by 1/(sheet resistance × thickness).

Parts c–f of Fig. 4.3 show the as-obtained and deconvoluted C 1s detailed scan spectra for GO and SPSrGO. A C–O component stronger than the C–C component was seen for GO, together with two other components corresponding to C=O and O=C–OH. This proves the existence of a large quantity of oxygen-containing groups. The C–O component intensity was significantly decreased in all SPSrGO samples, and correspondingly, the C–C component obviously intensified. In addition, after the SPS process one new component located at ~291.2 eV appeared, which was the shakeup satellite (π - π^* bonding) stemming from the aromatic character of graphene. This distinct π - π^* bonding clearly demonstrates the restoration of the conjugated network that did not exist in GO.

The structural characteristics of SPSrGO were extensively characterized by TGA, XRD, selected area electron diffraction (SAED), FTIR, Raman spectroscopy, and BET surface area measurement. SPSrGO-500, SPSrGO-900 and SPSrGO-1050 all showed a significantly higher thermal stability and surface area than GO (Fig. 4.4 and Table 4.1). Interestingly, it has been reported that a critical temperature of 550 °C has to be exceeded for GO to be thermally exfoliated [265]. Nevertheless, it is clearly seen in the current study that a lower SPS temperature of 500 °C could effectively exfoliate GO. SPS thus appears to be a superior thermal treatment procedure for GO exfoliation and reduction.

Fig. 4.4 TGA curves of GO and SPSrGO.

XRD patterns shown in Fig. 4.5 clearly signified decreased crystallinity in SPSrGO, which is supported by the TEM SAED patterns (Fig. 4.8c and d insets). A typical sharp polycrystalline ring pattern was observed for GO, while diffuse and weak rings without any bright spots were seen for SPSrGO-1050. The calculated interlamellar spacing (Table 4.1) decreased from 8.07 Å for GO to 3.4–3.6 Å for SPSrGO, mainly due to the decomposition of oxygen-containing groups and their associated structural defects as well as the evaporation of intercalated water.

Fig. 4.5 XRD patterns of GO and SPSrGO.

The FTIR spectra of GO and SPSrGO in the 600–2000 cm^{-1} region are compiled in Fig. 4.6. Four major absorption peaks occur in these regions of the GO spectrum: 1730, 1625, 1380, and 1070 cm^{-1} , which can be attributed to C=O carboxyl/carbonyl, aromatic C=C, C–O epoxy, and C–O alkoxy, respectively [269, 270]. FTIR spectra showed significantly suppressed absorption peaks for C=O carboxyl/carbonyl and C–O epoxy in all SPSrGO samples (Fig. 4.6), indicating that most of those groups had been removed in the SPS process. Peaks corresponding to the D and G bands were explicitly seen at ~ 1357 and ~ 1595 cm^{-1} , respectively, in Raman spectra of GO and SPSrGO (Fig. 4.7). In addition, it was found that all SPSrGO samples reduced at 500 $^{\circ}\text{C}$ had decreased I_D/I_G (defect index: intensity ratio of the D band and G band) compared with GO. This could be attributed to the removal of oxygen-containing functional groups and restoration of sp^2 bonds in the graphite lattice [263]. On the contrary, SPSrGO samples reduced at above 500 $^{\circ}\text{C}$ showed I_D/I_G higher than that of GO. This was probably caused by an increased number of structural defects induced at higher temperatures due to the generation of more intense spark plasma.

Fig. 4.6 FTIR spectra of GO and SPSrGO. Note the obviously decreased intensity for the two peaks in red rectangles.

Fig. 4.7 Raman spectra of GO and SPSrGO.

It is worth mentioning that the SPSrGO-900 and SPSrGO-1050 still showed remarkably lower sheet resistance than SPSrGO treated at 500 °C (Table 4.1). As evidenced by the much higher C:O ratios, both 900 and 1050 °C could enable a significantly higher number of oxygen-containing groups to be removed than 500 °C, although the I_D/I_G values imply that more SPS-induced structural defects would probably be introduced at those temperatures. The much higher reduction degree should be the main contributor to the lower sheet resistance of SPSrGO reduced at above 500 °C. SPSrGO-900 demonstrated a notably high electrical conductivity of 1564 ± 40 S/m, which is higher than or comparable to those from other thermal processes (Table 4.2) [262, 265-267]. It has been reported previously that I_D/I_G was only observed to decrease with a thermal annealing temperature higher than 1000 °C [263]. Our results further confirm the superior effectiveness of SPS in restoring sp^2 carbon-carbon bonds as it enabled a decreased I_D/I_G to be achieved at a low temperature of 500 °C.

4.4 Morphological characteristics of SPSrGO

Parts a, b, e, f of Fig. 4.8 show the FESEM images of dried GO and SPSrGO after brief bath sonication in ethanol. While GO sheets were smooth, SPSrGO samples were characterized by microscale sheet bending as well as submicrometer or nanosized rough structures.

Fig. 4.8 FESEM (a, b) and TEM (c, d) images of GO (a, c) and SPSrGO-1050 (b, d). SAED patterns are shown in the insets of (c) and (d). (e, f) FESEM images of SPSrGO-500 (e) and SPSrGO-900 (f).

The morphological difference between GO and SPSrGO-1050 was further compared. When dispersed in DI water by sonication and dropped onto Si wafer, GO sheets were able to stretch out and overlap each other, whereas SPSrGO-1050 demonstrated an agglomerated and granular appearance (Fig. 4.9). In TEM images (Fig. 4.8c and d), the morphology of GO contrasted dramatically with that of SPSrGO-1050. While GO presented very smooth sheets, SPSrGO-1050 was wrinkled and corrugated with both micro- and nanosized structures.

Fig. 4.9 FESEM images of a) GO and b) SPSrGO-1050 cast from a dispersion in water.

The drastic difference in topography between GO and SPSrGO-1050 was also obvious under AFM (Fig. 4.10). GO sheets tend to spread and adhere to the silicon substrate, with the majority of their surface being flat and smooth. On the contrary, SPSrGO-1050 showed a much rougher surface with numerous micrometer- and submicrometer-scale protrusions. The surface roughness (R_a) was obtained from three $0.5\ \mu\text{m} \times 0.5\ \mu\text{m}$ regions randomly selected in Fig. 4.10a and b. It was found that the average R_a for SPSrGO-1050 ($19 \pm 4\ \text{nm}$) was increased by a factor of ~ 10 compared with that of GO ($1.9 \pm 0.3\ \text{nm}$). The height profile was recorded for one GO sheet (Fig. 4.10g) and gave an average sheet thickness of $\sim 2\ \text{nm}$, which is typical for few-layer GO sheets. The FESEM, TEM, and AFM results confirm that SPS induced the formation of hierarchical surface roughness in the SPSrGO samples.

Fig. 4.10 AFM images of GO cast from a dispersion in water (a, c, and e-g) and SPSrGO-1050 cast from a dispersion in DMF (b, d). (a, b) 3D topography images; (c, d) 3D phase images. (e, f) 2D topography images. (g) Height profile of a GO sheet in (f). Note that different GO concentrations were used for samples in (a, c) and (e, f).

4.5 Mechanisms for GO reduction by SPS

Wrinkled structures in rGO thermally treated by a tube furnace have also been observed [265, 266]. However, in the previous studies the corrugated appearance could no longer be retained after dispersion of the rGO sample by sonication [265], while in the current study it remains well preserved. Consideration is thus directed to the unique characteristics of the SPS process, which are the major differences between our thermal reduction route and the previously reported ones. It has long been recognized that the application of a pulsed DC grants SPS a number of advantages over conventional sintering techniques, such as generation of speak plasma, a high-density energy supply, and a local high temperature [142, 158]. In our previous studies SPS has been applied as an effective heat treatment technique [271, 272]. This idea was also adopted by Cao *et al.* [273] who processed rGO by SPS at 1300 °C for 5 min. It was found that SPS induced the formation of many ripples which changed smooth rGO surfaces into rough ones. They proposed that on rGO surfaces there exist high/low regions with a larger electronic density which are susceptible to discharge and plasma striking during the SPS process. Unlike this previous work, in the current study smooth GO sheets were directly treated by SPS with no prior heating/reduction. GO reduction and ripple formation thus occurred simultaneously. It is interesting to note that in the study by Cao *et al.* [273], GO sheets thermally reduced by the conventional method at 700 °C for 2 h were still very smooth while in our study SPSrGO-500 already showed obvious hierarchical surface roughness. This further confirms the superiority of SPS in inducing rough rGO surfaces.

Because of the low density of freeze-dried GO, the 0.24 g GO clusters used in all SPS experiments were in good contact with other GO flakes as well as with the graphite die and punches. During SPS, electric current flows through both the graphite die and GO powder, with the majority of current likely flowing through the die due to its higher conductivity. Current flow results in Joule heating from both the die and the GO powder leading to uniform heating across the whole sample. SPS has been well-known to result in surface purification and activation due to the spark plasma [158]. In our study, the spark plasma likely contributes to the cleavage of oxygen-containing functional groups and assists the reduction of GO, as depicted in the schematic reduction mechanism

proposed in Fig. 4.11. Local high-density energy enables electrical breakdown and discharge at narrow gaps between adjacent GO sheets (Fig. 4.11a, b). The generated local high temperature can be particularly beneficial to the efficient removal of the functional groups (as shown in Fig. 4.11a, c). Furthermore, the spark impact pressure, together with high-speed ion migration, may also have a deoxygenation effect and contribute to local sheet deformation through striking and sputtering (as depicted in Fig. 4.11b, d). The generated gaseous products, mainly water vapor and carbon dioxide, escape from GO laminae and lead to further GO expansion and exfoliation (Fig. 4.11a). As a result, it was observed that the SPSrGO samples had increased in volume during the process of SPS. Unlike conventional thermal reduction processes, SPS is conducted in vacuum, which probably facilitates the escape of those gaseous byproducts from GO laminae and thus expedites GO expansion and exfoliation. This is possibly an important reason for the notably high surface area values (up to $1265.5 \text{ m}^2/\text{g}$) achieved in this study (Table 4.1). Therefore, SPS can be regarded as a multifunctional process that integrates surface roughening into the exfoliation and reduction of GO. In this process, the local high temperature, spark impact pressure, and rapid ion migration act in concert and result in hierarchical roughness which can be well maintained during subsequent ultrasonication processes.

Fig. 4.11 Proposed mechanism for GO reduction by SPS. The local high temperature, spark impact pressure, and rapid ion migration effectively convert most oxygen-containing functional groups into gaseous products (a, c) and generates lots of hierarchical rough structures on the SPSrGO surface (b, d).

It is believed that the quantity of spark plasma is highest in the early stage of SPS and decreases gradually [140]. Since spark plasma was possibly a significant contributor to GO reduction, cyclic SPS was attempted in hope of achieving a higher degree of reduction by introducing more “early stages”. At the SPS temperature of 500 °C, the number of sintering cycles was increased to two (SPSrGO-2C500) and three (SPSrGO-3C500). It was found that the C:O ratio was increased from 9.62 for SPSrGO-500 to 15.13 and 14.90 for SPSrGO-2C500 and SPSrGO-3C500 (Table 4.1), respectively, corresponding to a much smaller change as compared with that induced by increasing the SPS temperature. Furthermore, increasing the number of SPS cycles from two to three had virtually no influence on the C:O ratio. Such phenomena can be attributed to several causes. First, a higher temperature may have brought about more intense heating which, as in all other thermal reduction processes, was beneficial for the removal of oxygen-containing groups. It should be noted that the thermal stability of oxygen-containing

groups varies (e.g., epoxide < carbonyl < hydroxyl) and a temperature higher than 500 °C is required to remove certain groups [274]. This was probably the reason why extending the holding time at 500 °C from 1 min to 3 min (SPSrGO-500E, Table 4.1) only achieved a minor increase in the C:O ratio (from 9.62 to 11.71, Table 4.1). In SPS, higher sintering temperatures are realized by exerting a higher voltage and current. This leads to a stronger electric field, higher spark impact pressure and faster ion migration, which possibly further contributed to the removal of the surface functional groups. Second, in the later cycle(s) it was likely more difficult to generate spark plasma due to higher vacuum in the chamber. During SPS, the chamber was actively being evacuated, resulting in less and less residual air inside the chamber as the process continued, as confirmed by the increasing vacuum level in the chamber indicated by the Pirani gauge. This weakens the generation of plasma, because the presence of gases is essential for creating plasma. It is worth mentioning that both oxygen-containing groups and intercalated species (mainly water) in GO turned into gaseous products during the reduction process, which could enhance plasma generation. However, their conversion is believed to happen mainly in the early stage of the first SPS cycle, which can be supported by the fact that major weight loss occurred at below 250 °C during the TGA test of GO (Fig. 4.4). The significantly reduced sample resistance and increased electrical conductivity in the first SPS cycle may also have made spark generation more difficult in the subsequent cycles. As shown in Table 4.1, SPSrGO-500 possessed a buckypaper sheet resistance as low as $12.4 \pm 0.8 \Omega/\text{sq}$. As a result, there already existed a highly conductive sponge-like network inside the graphite die during the later SPS cycles which likely facilitated current flow and reduced spark generation.

4.6 Properties of SPSrGO/45S5 composites

4.6.1 Structural properties

Fig. 4.12a presents the FTIR spectra of pure 45S5 powder and GO/45S5 composite powders. The peaks located at ~ 505 , ~ 930 , and $\sim 1030 \text{ cm}^{-1}$ can be attributed to Si–O–Si bending mode, Si–O and Si–O–Si stretching modes, respectively [234]. The weak absorption bands seen at ~ 600 and $\sim 1650 \text{ cm}^{-1}$ should be ascribed to amorphous

phosphate and absorbed water, respectively [275]. The absorption band centered at $\sim 1454\text{ cm}^{-1}$ probably corresponds to CO_3^{2-} groups produced by the reaction of Na_2O and CaO with CO_2 from air [227]. The band located at $\sim 880\text{ cm}^{-1}$ for composite powders, which are more obviously displayed in Fig. 4.12b, should be assigned to the bending vibration of the C–O bond [248]. This band was not seen for pure 45S5 powder due to the absence of GO.

Fig. 4.12 FTIR spectra for the starting powders used for sintering. A magnified view of the part in the dashed rectangle in (a) is given in (b).

Fig. 4.13a presents the XRD patterns of the sintered pellets. It can be seen that the pristine 45S5 powder was amorphous, as only a slight hump was visible in its XRD pattern. The sharp diffraction peaks seen for all sintered pellets indicate that crystallization occurred in the SPS process. The crystalline phase was identified to be $\text{Na}_2\text{CaSi}_3\text{O}_8$ (PDF 120671). Due to the overlap between the (002) peak of GNP and two nearby peaks of $\text{Na}_2\text{CaSi}_3\text{O}_8$ located at $\sim 26.35^\circ$ and 26.75° , respectively, no noticeable difference in XRD peaks between BG0 and the composite pellets could be seen. It is worth mentioning that 550°C is the lowest SPS temperature reported thus far which can result in crystalline phase in 45S5 matrix [81, 199, 204, 213]. In addition, unlike those previous studies, extended hold time (10 min or longer) was replaced by a short period of only 3 min in our SPS program. One possible reason is the higher heating rate ($50^\circ\text{C}/\text{min}$) used which could result in higher spark impact pressure, more plasma generation and faster ion migration [142].

Fig. 4.13 (a) XRD patterns of powder samples and sintered pellets; (b) Raman spectra recorded on powder samples (P), sintered pellets (S) and wear tracks (W), with corresponding I_D/I_G values given in the parentheses.

It has been reported that 45S5 pellets with crystalline structure embedded in the matrix have superior bioactivity as compared with the fully amorphous ones sintered by low-temperature SPS or highly crystalline samples obtained through high-temperature conventional sintering [81]. In addition, a lower sintering temperature is also beneficial for achieving finer grains and hence better mechanical properties. Daguno et al. [233] found that excessively high sintering temperatures notably increased the porosity and thus deteriorated the mechanical properties of partially crystallized bioactive glass. Therefore, our SPS program shows great potential in fabricating highly bioactive 45S5 based pellets with improved mechanical properties.

The Raman spectra of sintered pellets are compiled in Fig. 4.13b. The D band and G band, arising from structural imperfections and graphitic carbon, respectively, are commonly seen in the Raman spectra of carbonaceous materials. While no obvious peaks were observed for 45S5 powder and pellet, both D band (at $\sim 1350 \text{ cm}^{-1}$) and G band ($\sim 1600 \text{ cm}^{-1}$) could be explicitly seen for GNP/45S5 composites. It thus proves that GNP survived the SPS process and was successfully incorporated in the sintered pellets. Furthermore, compared with the corresponding value of the composite powders, the sintered pellets demonstrated significantly decreased I_D/I_G . This means that less structural

defects existed in the composites after the SPS process, which should be attributed to the removal of oxygen-containing functional groups from GO sheets and restoration of sp^2 bonds in the graphite lattice [186]. It has been reported in Section 4.3–4.5 that SPS is a superior thermal reduction technique for GO which can produce reduced GO with high C:O ratio in an efficient and effective manner [186]. Since an SPS temperature of 500 °C was found to be capable of enabling successful GO reduction and exfoliation, it is believed that GO reduction was realized when the composite powders were compacted at 550 °C. Therefore, when used to fabricate GNP/45S5 composite pellets, SPS should be considered as a multifunctional process which combined powder densification and *in situ* GO reduction.

4.6.2 Mechanical properties

E and H of the sintered pellets at different indentation depths are presented in Fig. 4.14a. As a consequence of the indentation size effect, generally decreased E and H values were obtained with increased displacements [276]. It was found that both E and H were lower for GNP/45S5 composites as compared to BG0. Such decreases have also been reported for other GNP-reinforced ceramics [17, 277]. Two possible reasons that may have contributed to this phenomenon are: (1) the interface between the matrix and reinforcements may be weak and thus easier to be deformed than either phase, and (2) due to the weak van der Waals forces between the graphene layers, it is expected that sliding and even cleavage of the graphene layers would occur under in-plane stress, while a large deformation would probably happen under out-of-plane stress [277, 278]. Nevertheless, the phenomenal in-plane elastic modulus and intrinsic strength of graphene should never be underestimated when studying the deformation behavior of the composites [7]. GNP loading is thus a key factor that has to be optimized to balance the pros and cons of GNP addition to achieve the desired E and H values. It can be seen in Fig. 4.14a that among the composite pellets BG0.5 possessed the highest E (90.6 GPa) and H (5.82 GPa).

Fig. 4.14 (a, b) Mechanical properties of sintered pellets: Young's modulus and hardness (a) and fracture toughness (b). (c–f) Graphene induced toughening mechanisms in sintered GNP/45S5 pellets: crack bridging (c), crack deflection (d, e), and sheet pull-out (e, f).

Fig. 4.14b shows the K_{IC} of the sintered pellets with 4 different GNP loadings. Compared with BG0, all the GNP-reinforced pellets showed higher K_{IC} . The highest K_{IC} of $1.22 \text{ MPa}\cdot\text{m}^{1/2}$ was achieved for BG0.5, corresponding to an exciting improvement of 130.2 % compared with that of BG0. Therefore, this study has verified the feasibility and efficacy of toughening bioactive ceramics with graphene. Although the highest K_{IC}

achieved is still lower than that of cortical bone ($2\text{--}12 \text{ MPa}\cdot\text{m}^{1/2}$), it is higher than the K_{IC} of sintered HA ($\sim 1.0 \text{ MPa}\cdot\text{m}^{1/2}$) and comparable to that of a number of commercial glass-ceramics such as Bioverit® ($0.5\text{--}2.0 \text{ MPa}\cdot\text{m}^{1/2}$) and Biosilicate® ($\sim 1.0 \text{ MPa}\cdot\text{m}^{1/2}$) [4, 233]. Different toughening mechanisms revealed by SEM are shown in Fig. 4.14c–f. Crack deflection, crack bridging and sheet pull-out could be clearly seen when examining the crack propagation paths (Fig. 4.14c–e). As a 2D nanomaterial, the unique 2D structure and remarkably high surface area of graphene should be taken into account when analyzing graphene-induced toughening mechanisms. GNP could not only divert crack propagation in one plane, but also lead to 3D crack deflection [17]. Compared with particulate or fibrous reinforcements, GNP can usually provide a larger contact area with the 45S5 matrix, which enables it to absorb more energy at crack tip and brings about enhanced bridging and deflection effects to hinder crack propagation. Pulled-out GNP sheets were also explicitly visible on the fracture surface (Fig. 4.14f). The large exposed area of GNP after being separated from the matrix is an indication of weak interfacial strength between the two phases.

4.6.3 Tribological properties

The friction coefficient and specific wear rate of sintered pellets are presented in Fig. 4.15a. The lowest friction coefficient of 0.59 and smallest specific wear rate of $1.75 \times 10^{-14} \text{ m}^3/\text{Nm}$ were achieved for BG0.5, leading to a decrement of 21.3 % in friction coefficient and 62.0 % in specific wear rate compared with BG0. A clear decreasing trend in specific wear rate could be seen from BG0 to BG0.5, probably as a result of the improved fracture toughness as well as the lubrication effect of GNP. A higher fracture toughness means slower crack propagation and hence the generation of less wear debris. The lubrication effect originates from the weak van der Waals interactions between adjacent graphene layers which results in easy sliding between them when in-plane stress is exerted. A reduced specific wear rate means smaller width and depth of a wear track, as can be seen in Fig. 4.15c. The contact area between the two rubbing surfaces was thus decreased during the ball-on-disc tests, which in turn led to decreased friction coefficient as seen in Fig. 4.15a. Both friction coefficient and specific wear rate increased from BG0.5 to BG1, probably due to excessive GNP loading in the composite pellet. Due to

the superfluity of GNPs in the matrix, the GNPs were likely to be less homogeneously dispersed in the 45S5 matrix, which was detrimental to its wear resistance. In addition, since the GNPs probably had a deficient binding strength to the matrix, an excessive GNP loading caused easier material removal of the composite pellet during the ball-on-disc test as a consequence of the incapability of the matrix to hold all the GNPs. It is consistently found in Fig. 4.15c that when GO loading was increased from 0.5 wt.% to 1 wt.%, a wider and deeper wear track remained on the BG1 surface after the test. It can thus be concluded that 0.5 wt.% is the optimum GO content for achieving the best enhancement effect in fracture toughness and wear resistance for the GNP-reinforced 45S5 pellets.

Fig. 4.15 (a) Friction coefficient and specific wear rate of sintered pellets. (b) Friction coefficient of the same pellets as a function of number of laps. (c) Wear track topography of sintered pellets. Inset in (b) shows the magnified view of the evolution of friction coefficient in the first 100 laps.

Fig. 4.15b compares the evolution of friction coefficient with the number of laps for the sintered pellets with different GNP loadings. It can be clearly seen that the GNP modified 45S5 pellets generally showed more stable friction during the whole test

without abrupt changes in their friction coefficients. The sudden drops in the friction coefficient of BG0 at ~30,000 and ~40,000 laps, indicated by the two arrows in Fig. 4.15b, were probably caused by the removal of a chunk of material which decreased the contact area between the rubbing surfaces. A similar phenomenon was also seen in the first 100 laps for BG0 and BG1 (Fig. 4.15b inset). While large wear debris probably peeled off BG0 due to its intrinsic brittleness, removal of material chunks in BG1 could be possibly caused by inhomogeneous GNP dispersion and weak GNP-45S5 interfaces. It was found that both BG0.1 and BG0.5 showed a stable friction coefficient after ~40 laps, whereas it took approximately twice as many laps for the friction coefficient of the other two pellets to be stabilized. Among the three GNP-reinforced pellets, BG1 showed the most unstable friction coefficient, especially towards the end of the 50,000 laps. This also serves as an indication of excessive GNP loading in BG1, which is consistent with the fracture toughness and specific wear rate results discussed earlier.

In Fig. 4.16 the wear morphology of BG0 and BG1 is compared. Although BG1 probably had excessive GNP loading, it was expected to possess the most noticeable GNP-induced lubrication effect. BG0 showed a very rough wave-like wear track with numerous “steps” and deep valleys in between. Besides, a number of cracks were clearly seen. During the repeated sliding, cyclic stress concentration occurring below the Al_2O_3 counter ball initiated micro-cracks perpendicular to the sliding direction, which subsequently propagated into the subsurface. The prolonged sliding caused the formation of a micro-crack network that in turn resulted in the wave-like features on the wear track of BG0 seen in Fig. 4.16. Such wear morphology can be attributed to surface fatigue that occurred on BG0. In contrast, the worn surface on BG1 was a lot smoother, probably because GNP acted as a solid lubricant. A smoother surface could contribute to a lower friction by reducing the mechanical interlocking between the asperities on the two mating surfaces [279]. Furthermore, tribo-layers could be apparently seen on the worn surface of BG1 (indicated by white arrows) [280]. The formation of such tribo-layers is believed to originate from the wear debris generated during repeated sliding which was subsequently compacted by the Al_2O_3 ball. They could serve as a spacer between the two mating surfaces to prevent a direct contact between the ball and the pellet. Such tribo-layers were not found on BG0 because of its more brittle microstructure and severe wear that did not

allow them to form [281]. Additionally, BG1's wear track presented much fewer sharp edges and no obvious cracks, which is indicative of its tougher microstructure.

Fig. 4.16 Wear track morphology of BG0 and BG1.

The lubrication and toughening effects of GNP during the wear tests can be supported by the FESEM images shown in Fig. 4.17. The layered structure of GNPs, which can lubricate the rubbing surfaces, was explicitly seen on the fracture surface of the sintered composites (indicated by arrows in Fig. 4.17a, b). In Fig. 4.17c, d the GNPs with wavy edges were clearly seen on the wear tracks. It is believed that the detachment of GNP occurred gradually and their peripheries were the first to delaminate during the wear test, hence the formation of wavy edges. The GNPs also demonstrated their bridging effect on the wear track (indicated by the arrows in Fig. 4.17c, d). When the GNPs bridged the gap between two 45S5 pieces, removal of the 45S5 material could be possible only when the GNPs were detached on either end or broken in the center. From the FESEM images the latter seems to be more likely to occur, probably due to the large area of GNPs that were bound to the matrix. Considering the outstanding in-plane elastic modulus and intrinsic strength of graphene, it is deduced that a very long sliding distance

was needed to exert the in-plane stress that was sufficient to break the GNP sheets, which could thus significantly retard material removal during the wear test.

Fig. 4.17 FESEM images showing GNPs on the (a-b) fracture surface and (c-d) wear track of sintered GNP/45S5 composites. Samples used were BG1 (a), BG0.1 (b), and BG0.5 (c, d).

New structural defects were probably introduced to the GNPs during the wear test due to the tearing off of the graphene layers by the repeated sliding and shearing force. As a consequence, Raman spectra recorded on the wear tracks had obviously increased I_D/I_G values for all the composite pellets (Fig. 4.13b). Nevertheless, all the values were still smaller than the corresponding values for the GO/45S5 powders, meaning that after undergoing repeated wear, the GNPs still possessed a better structural integrity than the GO in the starting powders.

4.6.4 Biological properties

Apart from the mechanical and tribological properties, the biocompatibility of the compacted GNP/45S5 composites has to be critically evaluated prior to their clinical application. Fig. 4.18a depicts the viability of MG63 cells on polished pellets and TCP after 72 h culture. It was found that the percentage of live cells on the pellets was either

as high as or higher than that on TCP, indicating the commendable cytocompatibility of all pellets. It can be seen in Fig. 4.19 that the metabolically active cells, which were stained in green, took up the vast majority of the cells on all samples. In addition, there existed no statistical difference between the percentage of live cells on pure 45S5 sample and those on the composite pellets. This signifies that the cytocompatibility of GNP-incorporated 45S5 pellets was comparable to that of pure 45S5 which have achieved widespread and meritorious applications in bone tissue engineering. A number of previous studies have reported the cytocompatibility of GNP with osteoblast cells, where it was tested either as a single phase [282, 283] or as a reinforcement in bioceramic-based composites [57, 138, 284, 285]. Zhang et al. [138] proposed that the nano-roughness introduced by GNP addition may play a beneficial role in cell-material interactions. It was interesting to find the notably high viability of cells on BG0.1, which implies an unobvious correlation between GNP loading and MG63 cell behavior and warrants further investigations [57, 286].

Fig. 4.18 (a) Viability of MG63 cells after 72 h culture on sintered pellets and TCP. (b, c) Viability (b) and number (c) of MG63 cells after 72 h culture in pellet leachates and NM. Statistical difference is represented by * ($p \leq 0.05$) and ** ($p \leq 0.01$).

Fig. 4.19 Representative images showing the stained live (green) and dead (red) cells after 72 h culture on the sintered pellets and TCP.

Part b and c of Fig. 4.18 present the influences of BG0 and BG1 leachates at two different concentrations on the viability and proliferation of MG63 cells after 72 h culture. It can be seen in Fig. 4.18b that the percentage of live cells in all the solutions was above 97 %, proving that the leachates and NM were highly cytologically compatible. This can also be supported by Fig. 4.20, where only a scattering of injured/dead cells (stained in red color) could be seen. It was found in Fig. 4.18b that at 0.4 g/mL both BG1 leachates and NM could bring about significantly higher cell viability than the BG0 leachate, while there was no statistical difference between any two of the 0.08 g/mL leachates. Similar results were observed for the cell proliferation results (Fig. 4.18c). In addition, compared with their 0.08 g/mL dilutions, the 0.4 g/mL leachates generally led to lower cell viability and a smaller number of cells after 72 h culture. Nonetheless, the 0.4 g/mL BG1 leachate resulted in a higher percentage of live cells than its 0.08 g/mL leachate dilution.

Fig. 4.20 Representative images showing the stained live (green) and dead (red) cells after 72 h culture in the pellet leachates and controls.

Such differences were likely to be caused by the different degrees of alkalization of the leachates. As a consequence of the release of sodium ions, it has been found that soaking 45S5 in cell culture media could lead to a shift of its pH to higher values [213]. This justifies the common practice of cultivating 45S5 compacts in culture media prior to seeding the cells to mitigate the alkalization effect [202]. In this study, the 0.4 g/mL leachates are believed to be more alkaline than their corresponding 0.08 g/mL dilutions. Therefore, the leachates at the lower concentration generally led to higher cell viability and faster cell proliferation. It is further believed that the 45S5-induced alkalization could be possibly alleviated by adding GO. In the colloidal process of composite powder fabrication, the change in the color of the GO/45S5 solution from light grey to light black suggests that GO was partially reduced. This may be partly caused by the heat generated in the sonication and mechanical stirring process. In addition, the reaction of GO, the

oxygen-containing functional groups in particular, with the alkaline solution probably contributed to its reduction as well, since alkalis are commonly utilized as chemical reducing agents for GO [250]. After SPS, there would still be a number of residual oxygen-containing functional groups on the GNPs. When exposed to the FM, such groups could form acids and thus decrease the pH value. Mehrali et al. [287] reported in their study on GNP-reinforced calcium silicate composites that the incorporation of GNP could decrease the pH of SBF in which the compacted pellets were soaked. Furthermore, the “sheet wrapping” around the matrix grains might have acted as a barrier between the 45S5 matrix and the aqueous solution during the 84 h soaking [17, 138]. Therefore, the leaching of sodium ions and the resultant alkalization effect were likely to be retarded. This might explain the less consistent concentration-induced differences in cell viability and cell number for the BG1 leachates as compared with the BG0 leachates (Fig. 4.18b, c). Further investigations will be required to obtain a comprehensive understanding of the mechanisms described above.

Considering the rapid reaction between 45S5 and aqueous media and the long soaking period utilized [68], it is worth mentioning that small quantities of GNPs would be inevitably incorporated in the BG1 leachates as a dissolution product. Hence, the current study detected no cytotoxicity of GNP-containing culture media, possibly because of the low GNP concentrations [288].

4.7 Summary

This chapter demonstrates for the first time the successful fabrication of highly reduced GO through SPS. GO could be successfully exfoliated, reduced, and hierarchically roughened by SPS at a temperature as low as 500 °C, which is lower than the critical temperature (550 °C) required for GO exfoliation in a conventional thermal process. In addition, SPS demonstrated superior efficacy in minimizing the residual oxygen content and creating surface roughness on both the micrometer scale and nanoscale. It is believed that the efficiency and effectiveness of SPS in reducing GO stemmed from the synergistic effect of local high temperature, spark impact pressure and rapid ion migration. Since SPSrGO has great promise to be utilized as a reinforcing agent

in ceramic-based composites which can be fabricated by SPS, the study on the fabrication and properties of SPSrGO thus provides valuable insights into the *in situ* reduction and enhancement effects in those composite systems.

Subsequently, the strategy of *in situ* GO reduction by SPS was employed to compact novel GNP-reinforced 45S5 composite pellets. A relatively low sintering temperature of 550 °C was utilized to avoid over-heating and grain coarsening of the crystalline phase $\text{Na}_2\text{CaSi}_3\text{O}_8$. Among the composites reinforced with different GNP loadings, the addition of 0.5 wt.% GO was proven to be the optimum which could enhance the fracture toughness by 130.2 % and decrease the friction coefficient and specific wear rate by 21.3 % and 62.0 %, respectively. The GNPs were found to be able to effectively toughen the 45S5 pellets through crack bridging, crack deflection, and sheet pull-out. The augmented fracture toughness, together with the lubrication effect caused by the GNPs, resulted in improved tribological behavior of the composite pellets. In addition, incorporation of GNPs had no influence on the pellets' cytocompatibility with MG63 cells directly grown on their surfaces, while the GNP/45S5 leachates tended to result in faster proliferation and higher viability of the MG63 cells than the pure 45S5-conditioned media. The encouraging enhancement in fracture toughness, wear resistance and *in vitro* cytocompatibility achieved in this study can contribute to the advancement in extending the clinical applications of 45S5-based materials in bone tissue engineering.

Chapter 5 Spark Plasma Sintering of Hydroxyapatite

This chapter intends to investigate the influences of starting powder as well as the sintering temperature and pressure on the structural, mechanical, optical, and biological properties of sintered HA pellets. Special attention is attached to the fabrication and characterization of transparent HA pellets as they can enable direct visual observation of cells cultured on their surfaces and thus potentially extend the application of HA. The findings of this chapter will shed light upon the manufacture of HA-based composites to be studied in Chapter 6.

5.1 Introduction

By virtue of its close resemblance to natural bone mineral and tooth enamel, HA has become a key bioceramic which has attracted significant interest in both materials research and clinical applications [42, 194]. The properties of sintered HA compacts is affected by the properties of starting powder as well as processing parameters, as both can affect its microstructure. Additionally, major differences in the structural, mechanical, and biological properties of sintered samples can be caused by markedly diverse stability and sinterability of different starting HA powders [239, 289, 290]. However, there have been insufficient studies which examined the influence of HA raw powder on the properties of sintered pellets [291, 292].

It is envisioned that the application of HA can be extended by fabricating transparent HA-based structures. This is because high degree of transparency enables direct visual observation of interactions occurring at biomaterial – cell/tissue interfaces, which will be beneficial to various *in vitro* and *in vivo* evaluations of HA such as cell culture and animal testing [293, 294].

To date, the fabrication of transparent HA usually involves the application of very high sintering pressure and/or long sintering duration, making the manufacturing process energy- and time-consuming [123, 125, 178, 295]. To avoid HA dehydration in the sintering process and fabricate transparent HA samples, Eriksson et al. [178] employed high-pressure SPS which permits the application of pressure up to 500 MPa. By sintering HA nanorods at 900 °C in a box furnace for 1 h followed by hot isostatic pressing at 875 °C for 1 h under 150 MPa, Wang et al. [125] achieved an in-line transmittance (ILT) value of 66 % at 645 nm for a 0.9 mm thick HA disc. Tan et al. [123] also reported the preparation of highly transparent HA samples, where a sintering pressure of 2.0–5.0 GPa and sintering duration of 20 min were utilized. Nonetheless, in an earlier study by our group, translucent oxyapatite compacts could be obtained through SPS with a sintering duration of only 3 min and the application of a relatively low sintering pressure (23 MPa) [296].

SPS has been one of the most widely used densification techniques for achieving transparent ceramic compacts [297-301]. The uniqueness of SPS stems from the

simultaneous application of an electric current and pressure [213]. Unlike traditional sintering methods, in SPS the ceramic green body is heated externally (by the graphite die and punches) as well as internally (by itself) [143, 186]. During the SPS process the sample acts as a resistor which electric current passes through and causes Joule heating. In addition, the generation of spark plasma in the SPS process helps activate particle surface by clearing impurities such as absorbed gases [14]. Previous studies have proved that parameters such as sintering temperature and pressure can exert a major influence on the mechanical and structural characteristics of spark plasma sintered HA [126, 212, 255, 292, 302]. Many other investigations have extensively examined the dependence of optical properties on various SPS parameters for ceramics such as zirconia [297, 298] and alumina [299, 303]. However, the influences of such SPS parameters on the optical and biological properties of HA have been much less studied [304, 305].

In this chapter, three types of HA powder were compacted to compare the structural, mechanical, optical and biological properties of the sintered samples. Particularly, it is attempted to obtain transparent HA pellets through a direct and fast SPS process with a moderate sintering pressure (50 MPa).

5.2 Experimental

5.2.1 Analysis of starting powders

Three different types of HA powder were utilized in this study, namely micro-spheres (MS, Fluidinova, Portugal), nano-rods (NR, Nanjing Emperor Nano Materials, China) and nano-spheres (NS, Sigma-Aldrich, US). The elemental composition of the powders was checked with XPS, using the measurement conditions described in Section 3.2.1. Powder morphology was observed by either FESEM or TEM. Specific surface area of the raw powders was determined by the BET method (see Section 4.2.2). The particle size of the MS powder was measured by laser diffraction particle size analysis (see Section 3.2.1).

5.2.2 Sintering

The HA powders were sintered by SPS, with the sintering program schematically shown in Fig. 5.1a [289]. Briefly, 0.6 g of raw powders was compacted at the designated temperature (900/950/1000/1050/1100 °C) for 3 min in a graphite die with an inner diameter of ~10 mm. Heating rate was set to be ~200 °C/min below 600 °C, while above that a heating/cooling rate of 100 °C/min was used. Sintering temperature was measured by an infrared pyrometer focused on a small hole in the outer wall of the die. Initially a constant sintering pressure of 50 MPa was applied before cooling (Stages ①-③) for all three types of powder. However, the sintered NR pellets frequently broke when removed from the die, probably as a result of internal stresses and layering process of hexagonal HA structures [178, 306, 307]. To overcome this problem, a small initial pressure of 7.5 MPa was used and the constant pressure of 50 MPa was only applied during the holding time at the highest temperatures (Stages ③) when NR was sintered. Hereafter NR powders/pellets sintered with the smaller initial pressure and constant pressure are denoted as NR-L and NR-H, respectively. Fig. 5.1b depicts the different sintering pressures utilized when fabricating NR-H/MS/NS and NR-L at 1000 °C.

Fig. 5.1 (a) Schematic five-stage sintering program where A and F represents room temperature, C and D denotes holding/sintering temperature, B and E stand for 600 °C (650 °C) when C/D = 900 °, 1000 ° or 1100 °C (950 ° or 1050 °C). Stage ① lasts for 3 min. The five stages and their denotations are applicable to all sintering processes in this study; (b) Sintering pressure applied when fabricating NR-H/MS/NS and NR-L at 1000 °C.

5.2.3 Evaluation of phase, microstructure, and mechanical properties

As-sintered pellets were ground sequentially by 400–4000# sand papers and polished to ~0.25 μm surface finish with diamond paste for various characterizations. Phase composition of the as-received powder and sintered pellets was examined by XRD (PANalytical EMPYREAN, The Netherlands). XRD patterns were collected in the 2θ range of 20–55 ° at a step size of 0.026 °. For the HA pellets the incident X-ray beam was shed on the circular surface perpendicular to the direction of sintering pressure (Fig. 5.7f). The density of the pellets was determined with Archimedes' method and the relative density calculated assuming the theoretical density of HA to be 3.156 g/cm^3 (Table 2.5).

Polished HA pellets were thermally etched for 0.5 h at temperatures 100 °C lower than the sintering temperatures to examine the grain morphology. Average grain size of the pellets was measured on the FESEM images of their thermally etched surfaces with a correction factor of 1.56 [308].

Assuming the Poisson ratio to be 0.3, the E and H of the sintered pellets were tested by nanoindentation. The results are presented as the mean $\pm$ standard deviation with the total number of indents $n=20$. The other measurement parameters were the same as those given in Section 3.2.2. The fracture toughness of sintered HA pellets was assessed by Vickers indentation fracture toughness test, using the method and parameters mentioned in Section 4.2.5.

5.2.4 Optical characterization

Total forward transmittance (TFT) of the polished pellets was measured in the visible range from 450 nm to 700 nm using an integrating-sphere system (RT-060-SF, Labsphere, US) equipped with a USB4000 Fiber Optic Spectrometer (Ocean Optics, US). A Cary 5000 UV-VIS-NIR Spectrometer (Agilent, US) was used to measure the in-line transmittance of the pellets in the range of 175–1300 nm. This spectrometer was also employed to obtain the reflection of the pellets in the same wavelength range with the installation of an external Diffuse Reflective Accessory (DRA-2500, Agilent, US). Raman measurements in the range of 100–4000 cm^{-1} were carried out at room

temperature using the Raman spectrophotometer system and measurement parameters described in Section 4.2.2.

5.2.5 Cell culture

The *in vitro* biological properties of the HA pellets were investigated using two established cell lines: L929 and MG63. They are extensively used in various biocompatibility tests for their reproducible biological responses and growth rates [309, 310]. L929 is a mouse fibroblastic cell line, often used to observe the general cytocompatibility and toxicity of novel materials, and MG63 is a human osteoblast-like cell line used to assess the response of bone-specific cells to materials for potential use in bone tissue engineering. Cell proliferation on sintered HA pellets, each having a culture area of $\sim 0.78 \text{ cm}^2$, was assessed with L929 and MG63 cells. The cell culture medium used was DMEM (GE Healthcare) supplemented with 10 % (v/v) HyCloneTM research grade FBS (South American Origin, GE Healthcare) and 1 % (v/v) HyCloneTM Penicillin-Streptomycin solution (GE Healthcare). A 100 μL drop of cell culture medium containing 2000 L929 cells or 1000 MG63 cells was pipetted onto the top surface of each autoclave sterilized HA pellet, after which they were incubated at 37 °C in 5 % CO_2 for ~ 3.5 h to let the cells attach to the ceramic surfaces. The medium level in each well was then topped up by 1.5 mL to immerse the pellets completely in culture media. After 24 or 72 h of cell culture, cell numbers on the HA pellets were quantified with a CyQUANT Cell Proliferation Assay Kit (Molecular Probes, US) using standard manufacturer's protocols. In each experiment, a tissue culture plastic (TCP) control was also measured, wherein an identical number of cells in an identical seeding volume (2000 L929 or 1000 MG63 cells in 100 μL) was spread over the same culture area as a HA pellet. This was also incubated for 3.5 h to allow cells to fully attach to the culture well before the volume of the well was topped up to 1.6 mL. The cells were observed to remain in the seeding area at 24 h, and spread only slightly outside of the seeding area at 72 h.

Using the same cell culture procedure, the viability of L929 cells grown on HA pellets was assessed with the LIVE/DEAD[®] Viability/Cytotoxicity Assay Kit (Molecular Probes, US) after 72 h culture. Stained cells were imaged with an optical microscope equipped with an Olympus DP70 digital camera and an Olympus U-RFLT50 power

supply unit (Olympus, Japan). Quantification was completed by manual counting of calcein-stained cells (total metabolically active cell number) and ethidium homodimer-1-stained nuclei (dead or dying cell number) using ImageJ software [311].

Furthermore, the differentiation of MG63 cells cultured on HA pellets was estimated by evaluating the amount of ALP, an important osteogenic marker, with an ALP Assay Kit (Abcam, UK), using the protocol recommended by manufacturers. The aforementioned cell culture parameters were kept constant, except that 4000 MG63 cells in 100 μ L medium were seeded on each sample and cultured for 96 h.

In the cell culture process, attempts to directly observe L929 and MG63 cells grown on the HA pellets were made by using the optical microscope mentioned above.

5.3 Properties of raw materials

The XPS survey-scan spectra for the raw HA powders are compiled in Fig. 5.2, which proves that the powders used were free from extraneous elements. It should be noted that adventitious carbon contamination is inevitable and commonly used as a charge reference [224].

Fig. 5.2 XPS survey-scan spectra for three different types of HA powder.

Morphology of the raw powders is shown in Fig. 5.3, together with the high resolution transmission electron microscopy (HRTEM) images of NR and NS particles. It was found that although MS particles were several microns in diameter (Fig. 5.3a), they consisted of numerous agglomerated nano-sized structures (Fig. 5.3b). It was revealed by particle size analysis that MS particles had a mean diameter $<15\ \mu\text{m}$, and 90 % of them were $<30\ \mu\text{m}$ (Fig. 5.4). Crystallinity of both types of nano powder was confirmed by the clearly seen lattices (Fig. 5.3d and f). The specific surface area of MS, NR, and NS was determined to be 124.5, 63.8, and $20.4\ \text{m}^2/\text{g}$, respectively. Surprisingly, MS was found to possess a specific surface area almost twice as large as NR and over 5 times larger than NS. This was probably due to the presence of the very fine nanostructures in MS particles.

Fig. 5.3 (a, b) FESEM images of MS powder; (c-f) TEM/HRTEM images of (c, d) NR and (e, f) NS powders.

Fig. 5.4 Particle size distribution of HA-MS powder.

5.4 Sintering behavior

Fig. 5.5 shows the densification behavior of different types of HA powder when sintered at 1000 °C by SPS. The relative densities of the four sintered pellets proved that all of them were almost fully densified (Fig. 5.6). This can also be confirmed by the fact that the displacement curves became flat towards the end of Stage ③. Besides, a bigger final displacement is an indication of smaller green body density (ρ_g) before sintering. The relationship between the ρ_g values of the green bodies can thus be expressed as:

$$\rho_{g\text{ NR-L}} < \rho_{g\text{ NR-H}} \approx \rho_{g\text{ MS}} < \rho_{g\text{ NS}} \quad (5.1)$$

The smallest ρ_g shown by NR-L resulted from the small initial pressure applied, which caused the NR powders to be relatively loosely packed in the die before sintering. Both types of HA spheres were found to be easier to be compacted than NR. It should be noted that the average particle size of MS ($\sim 10\text{ }\mu\text{m}$) was 1–2 orders of magnitude larger than that of NR ($\sim 20\text{ nm}$ in diameter and $\sim 150\text{ nm}$ in length). As a consequence, MS induced much larger gaps/voids between the particles as compared with NR. The MS powder thus possess superior compactability considering that $\rho_{g\text{ MS}}$ approximates $\rho_{g\text{ NR-H}}$.

Fig. 5.5 Densification behaviors of HA powders sintered by SPS at 1000 °C: (a) displacement, (b) derivative displacement.

Fig. 5.6 Relative densities of the sintered HA pellets.

In Stage ①, a direct current was started with the ON and OFF time being 12 ms and 2 ms, respectively. The pulsed current flowed through both the graphite die and sample, resulting in discharge between the HA particles. It is believed that the largest quantity of spark plasma were initially generated and gradually decreased as the gaps between particles diminished. The generated spark plasma exerted an impact on the HA powders. The green body was thus pressed by both external and internal forces: the uniaxial pressure by the graphite punches and the impact of spark plasma. Desorption of absorbed moisture and gases also occurred in this stage. This phenomenon was especially

obvious for MS as considerable increase in chamber pressure was observed on the pirani gauge. This was probably the main reason for the larger shrinkage of MS as compared with other powders in this stage. Negative displacement was seen for NS because of the rapidly increased pressure caused by spark plasma as well as the dilation of the graphite punches and NS green body.

Significant densification/shrinkage took place in Stage ②, as evidenced by the large displacements seen in Fig. 5.5a. Except NR-L, all samples stopped shrinking within this stage. Although the displacement of NS increased again towards the end of Stage ②, it should not be attributed to shrinkage but to the escape of viscous HA through the gap between the die and punch. This was supported by the smaller thickness of the sintered MS pellet compared with the other three.

Although accurate quantitative study of shrinkage behaviors usually requires the use of a dilatometer [302], a comparative analysis is supported by Fig. 5.5b. Two important parameters obtainable from Stage ② and ③ are the starting and ending temperatures of densification which are denoted as T_s and T_e , respectively. The corresponding time points are represented by t_s and t_e and the shrinkage duration d_s is defined as $(t_e - t_s)$. In addition, the temperature and time at which the peak derivative displacement occurred in Fig. 5.5b are denoted as T_p and t_p , respectively. These parameters for different HA powders are summarized in Table 5.1.

Table 5.1 T_s , T_e , t_s , t_e , d_s , T_p , and t_p of HA samples sintered at 1000 °C

	T_s (°C)	t_s (s)	T_e (°C)	t_e (s)	$d_s = t_e - t_s$ (s)	T_p (°C)	t_p (s)
MS	708	245	1000	420	175	800	300
NR-L	758	275	1000	465	190	975	405
NR-H	708	245	950	390	145	833	320
NS	658	215	817	310	95	758	275

It can be seen in Table 5.1 that NS showed the smallest values for all seven parameters; compaction of the NS sample stopped even before 900 °C was reached, and its shrinkage lasted for only 95 s. Its highest displacement rate was seen at 275 s/758 °C, well before the highest temperature was reached. Thus, NS possesses intrinsically outstanding sinterability which results from not only their nanostructure and hence high surface energy but also their superior compactability caused by their spherical shape. MS

showed the same T_s (708 °C) as NR-H, but required a longer d_s to be fully densified due to the difficulty in filling the bigger inter-particle voids.

All the seven parameters were the highest for NR-L. By comparing the parameters of NR-L and NR-H, it is found that the application of a high initial sintering pressure could lower T_s and T_e and shorten d_s ; the T_p and t_p values were also greatly decreased. While NR-H reached its full density 30 s before the sintering temperature was held at 1000 °C, NR-L did not stop shrinking until holding at 1000 °C for 45 s.

5.5 Phase composition

The identification of phase composition was carried out for the raw powders and sintered pellets by using XRD. By analyzing the XRD patterns, no decomposition was found for any sintered pellet as all peaks could be attributed to HA (Fig. 5.7a-e).

For all four types of HA pellet enhanced crystallinity was observed in comparison with raw powders, as proven by the much sharper diffraction peaks. Among the three types of HA powder, MS showed the most unresolved XRD peaks, which is an indication of the lowest crystallinity. For NR powder the strongest diffraction peak was located at $\sim 31.8^\circ$ and corresponded to the (121) plane (Fig. 5.7b, c). However, the (300) peak gradually intensified with the increase of sintering temperature, and the (300) peak became the highest for NR-L pellets sintered at 950 °C or above (Fig. 5.7b). This was even more evident for NR-H, with all pellets showing a (300) peak stronger than the (121) one (Fig. 5.7c). By contrast, reinforcement of the (300) peak at higher sintering temperatures was not obviously observed for sintered MS and NS pellets (Fig. 5.7a, d).

Fig. 5.7 (a-d) XRD patterns of as-received HA powders and sintered HA pellets: (a) MS, (b) NR-L, (c) NR-H and (d) NS; (e) reference XRD pattern for HA; (f) the circular surface, which was perpendicular to the applied sintering pressure, was subjected to XRD measurement.

Compared with the corresponding raw powders, the NR pellets showed a stronger tendency to intensify the (300) peak than the MS and NS pellets. It implies that in the NR compacts, there was a more vigorous enhancement in the alignment of the c -axis, or [001] direction of the crystals along the circular surface depicted in Fig. 5.7f. This probably resulted from the application of a pre-SPS pressure which caused the [001] direction of

many NR crystals to become perpendicular to the pressure direction (Fig. 5.8). Such an alignment could be reinforced by applying a higher pre-SPS pressure, as in the case of NR-H pellets. Therefore, it is more likely for the (100) plane of NR pellets to be parallel to the circular surface, which led to increased intensity of their (300) peak. The preferential crystallization along the [001] direction was further enhanced in the subsequent SPS process, thus higher (300) peaks were observed for NR pellets sintered at higher temperatures. Watanabe et al. [295] also observed such a crystal orientation in the HA body sintered by SPS, and their study agrees well with ours in that the c -axis in those HA compacts was aligned perpendicular to the pressure direction. For MS and NS particles, a pre-SPS pressure was able to affect only the ρ_g and should not favor any crystallite orientation (Fig. 5.8).

Fig. 5.8 A schematic depicting the effects of the pre-SPS pressure: (a, b) the pre-SPS pressure could lead to a higher ρ_g and cause the c -axis of more NR crystals to align perpendicular to the pressure direction; (c, d) applying a pre-SPS pressure could increase the ρ_g of MS/NS green body but did not influence the crystallite orientation.

5.6 Microstructure and mechanical properties

Microstructural characteristics of the HA pellets are presented in Fig. 5.9. It was found that MS and NR-L pellets sintered at 1000 °C or 1050 °C all consisted of sub-micron grains with few visible intergranular pores (Fig. 5.9a, b, d). Such fine microstructures are particularly desirable as they can potentially improve the mechanical behaviors of HA and enhance its biological properties [127]. As can be found in Fig. 5.9b and c, NR-H possessed much coarser grains than NR-L, implying that significant grain growth occurred when a high SPS pressure was exerted from the beginning of the SPS cycle. The average grain size of NR-H sample sintered at 1050 °C was calculated to be 0.77 μm , corresponding to > 100 % increase as compared to that of the NR-L pellet fabricated at the same temperature, whose average grain size was only 0.36 μm (Fig. 5.10). This was caused by two possible reasons. First, as shown in Eq. (5.1), the fact that NR-L had a lower ρ_g than NR-H could result in a larger number of nucleation sites in NR-L in the heating up stage and consequently give rise to refined grains. Furthermore, the t_e of NR-L was 75 s later than NR-H (Table 5.1), meaning that the post- t_e period of the SPS cycle, which no longer promoted densification but favored fast grain growth, was shorter for NR-L.

On the other hand, as can be seen in Fig. 5.9a and b, increasing the sintering temperature from 1000 °C to 1050 °C only led to minor increase in the grain size of NR-L, with the average grain size slightly coarsened from 0.33 to 0.36 μm . Very fine grains could also be seen in the MS pellet sintered at 1050 °C (Fig. 5.9d) and the average size was calculated to be 0.41 μm (Fig. 5.10).

While most grains in MS and NR pellets were equiaxed, NS pellets contained many elongated grains (Fig. 5.9e, f). In addition, when the sintering temperature was increased from 1000 °C to 1050 °C, a larger number of elongated grains could be seen, probably as a result of the longer sintering duration and higher energy supplied for selective grain growth and ripening. The small grains found at the HA grain boundaries were calcium oxide, whose formation was due to the decomposition of HA in the thermal etching process (phase composition shown in Fig. A.1).

Fig. 5.9 Thermally etched surface of HA pellets sintered at 1000 °C (a, e) and 1050 °C (b-d, f): (a, b) NR-L, (c) NR-H, (d) MS, (e, f) NS. Images were obtained from sample sections parallel to the pressure direction.

Fig. 5.10 Young's modulus, hardness and average grain size of the HA pellets sintered at 1050 °C.

Interestingly, as indicated by the arrows in Fig. 5.9f, the elongated grains were randomly oriented, thus forming an interlocking microstructure. This type of ceramic microstructure is highly sought-after as it enables the damage tolerance to be remarkably improved [225]. It has been reported by Shen et al. [225] that the key to achieving such microstructures is the application of a fast heating rate. Toughening mechanisms induced by the elongated HA grains can be clearly seen in Fig. 5.11a-c, where absorption of fracture energy was realized by intragranular crack propagation (indicated by arrows in Fig. 5.11a) as well as crack deflection by elongated grains (indicated by arrows in Fig. 5.11b, c). When a Vickers indentation load of 500 g was applied, the NS pellet showed a K_{IC} value of $1.06 \pm 0.17 \text{ MPa m}^{1/2}$ (number of indents $n=12$). However, severe spalling occurred for MS and NR pellets even when the indentation load was as low as 200 g, hence the impossibility of obtaining reliable K_{IC} for them. Nevertheless, it is safe to conclude that the NS pellet possesses the highest E , H and K_{IC} among the four samples (Fig. 5.10).

Fig. 5.11 Thermally etched surface of HA pellets sintered at 1050 °C: (a-c) NS, (d) NR-H. Arrows in (d) indicate two columnar HA crystallites. Images were obtained from sample sections parallel to the pressure direction.

It is worth mentioning that although a small quantity of elongated grains were also observed in NR-H, they did not bring about the desired interlocking microstructure and

their formation should be attributed to the preferred crystal orientation produced in the SPS process. The major axis of the columnar HA crystallites seen in Fig. 5.11d should correspond to their c -axis [295].

The E and H of the HA pellets sintered at 1050 °C are shown in Fig. 5.10, which clearly indicates the influences of raw powder and sintering pressure on the mechanical properties of sintered pellets. Among the four samples tested, highest E and H were again achieved by the NS pellet, being 130 ± 5 and 7.2 ± 0.6 GPa, respectively.

5.7 Optical properties

5.7.1 Transmittance

The macroscopic image of polished HA pellets with a thickness of ~2 mm is shown in Fig. 5.12, with the corresponding TFT curves in the visible spectrum plotted in Fig. A2. It can be seen that transparent pellets could be readily obtained when MS powder was sintered at above 950 °C, which can be attributed to coalescence phenomena and considerable increase in relative density of the pellets (from 93 % to above 99 %, Fig. 5.6) [289, 312].

Fig. 5.12 Macroscopic images of the polished sintered HA pellets.

Most sintered NR pellets were translucent, as shown in Fig. 5.12. The yellowish brown appearance of NR pellets probably stemmed from their oxygen vacancies. This

type of defect tends to develop in thermal reduction and electro-reduction environments, both of which exist in SPS [301]. Fig. 5.12 shows that all sintered NS pellets were opaque. Their TFT was measured to be all below 12 % (Fig. A2d).

Although the same SPS parameters were applied to fabricate MS, NR-H and NS pellets, drastically different optical properties were observed. This justifies the critical role of the physicochemical properties of the HA powder in influencing the transparency of sintered pellets. Compared with the two types of nano-sized powder, MS particles are believed to form less agglomeration, which promotes densification in the SPS process. In addition, under identical sintering conditions, MS particles were found to be able to yield a narrower grain size distribution, which is also beneficial for achieving high transparency and will be further discussed later.

It should be noted that thickness of the polished HA pellets varied and measured transmittance values were thus influenced by the sample thickness. In order to make more accurate comparison of transmittance between various HA pellets, ILT of the samples was measured. Based on the as-measured ILT value T_1 for a sample with thickness t_1 , the ILT value T_2 for a sample which is made from the same material and has a thickness t_2 can be calculated by the following equation [301]:

$$T_2 = (1 - R_S) \left(\frac{T_1}{1 - R_S} \right)^{t_1/t_2} \quad (5.2)$$

In this chapter a value of 2 mm is assigned to t_2 , and R_S is the reflection loss at the two sample surfaces at normal incidence:

$$R_S = \frac{2R'}{1+R'} \text{ with } R' = \left(\frac{n-1}{n+1} \right)^2 \quad (5.3)$$

where n is the refractive index of HA.

Fig. 5.13a shows the calculated ILT values of MS pellets in the range of 250–1300 nm. Fig. A2a seems to support that a higher sintering temperature resulted in improved TFT in MS pellets. However, after excluding the influence of sample thickness, the optimum sintering temperature given by Fig. 5.13a is 1000 °C, above which ILT decreased as sintering temperature was augmented. In this study, the highest ILT achieved by a 2 mm MS pellet was 69 % at 700 nm and 84 % at 1300 nm. According to Eq. (5.2), it corresponds to an ILT value of 78 % at 700 nm and 86 % at 1300 nm for a 1 mm thick pellet, and they are among the highest ILT values reported thus far for

transparent HA [123, 125, 178, 295, 313]. The result is even more encouraging if we consider the fast and direct SPS process, which avoided extremely high sintering pressure and long sintering duration and is thus distinctly advantageous over the approaches adopted previously by other researches [125, 178, 295]. We therefore believe that the SPS parameters and MS powder selected in this study are highly advantageous and promising in the manufacture of transparent HA.

Fig. 5.13 (a) ILT curves of MS pellets and (b) ILT values of all HA pellets at 1300 nm. All transmittance values were converted for a 2 mm thick sample based on the measured values.

ILT values at 1300 nm for all polished HA pellets are shown in Fig. 5.13b. It is found that NR-L pellets tended to show higher ILT as sintering temperature was augmented, whereas NR-H demonstrated the opposite tendency. The former phenomenon was due to the more compact microstructure for samples fabricated at higher temperatures, while the latter is believed to be partly caused by internal defects such as micro cracks developed during SPS. Compared with NR-L, NR-H could reach its full density in a faster manner (Table 5.1), and the already densified pellets thus experienced extended exposure to the harsh sintering conditions with high temperature and pressure. Both the size and density of residual pores were expected to increase in this process, which was detrimental to light transmission. Moreover, higher internal stresses could be induced in this process, which led to the frequent breakdown of the sintered NR-H pellets (Fig. 5.12).

Another possible reason for the decreased ILT of NR-H at higher sintering temperatures is the loss of more hydroxyl groups. It has been reported by Fujimori et al. [314] that dehydration of HA occurs at above 800 °C, and Raman spectroscopy served as a powerful tool to identify this change in their study. Raman spectra were therefore recorded for NR-H sintered at 900 °C and 1100 °C to compare the relative intensity of the band corresponding to hydroxyl group at $\sim 3570\text{ cm}^{-1}$ (Fig. A3a). The strongest peak located at $\sim 960\text{ cm}^{-1}$ was used to normalize the spectra and the normalized hydroxyl absorption bands were compared and plotted in Fig. A3b. The weaker band at $\sim 3570\text{ cm}^{-1}$ signifies a smaller amount of hydroxyl groups in NR-H sintered at 1100 °C.

Furthermore, it is believed that spark plasma played a more significant role in densifying NR-L particles, which might contribute to improved densification and higher transparency. As illustrated in Section 5.4, NR-L green body had a much lower density than NR-H. As a consequence, gaps and voids probably existed in larger quantity and size in NR-L green body. Additionally, major pores/gaps persisted for a longer time in NR-L pellets, as evidenced by its bigger t_e (Table 5.1). As a result, NR-L could probably provide better conditions for the generation and development of spark plasma when pulsed electric current was applied.

The ILT values of NS pellets were all smaller than 3 % at 1300 nm (Fig. 5.13b), suggesting that NS powder is not suitable for the fabrication of transparent HA pellets. One possible reason is that NS pellets had a much wider grain size distribution, with the coexistence of equiaxed and elongated grains. It has been reported by Kim et al. [300] that larger grain size variations reduced the ILT of transparent alumina fabricated by SPS. Besides, pore growth, which is usually unavoidable when grains coarsen, probably proceeded faster in NS pellets as the elongated grains ripened in random orientations. This can be supported by two facts: (1) NS pellets generally had a lower relative density than the other three types (Fig. 5.6), and (2) NS pellets sintered at higher temperatures tended to show decreased ILT.

5.7.2 Reflection, absorption and scattering

The measured reflection of MS pellets in the 250–1300 nm range is shown in Fig. 5.14a. Obvious decrease in measured reflection was observed when sintering temperature

was increased from 900 °C to 1000 °C. The measured reflection was contributed by reflection at two sample surfaces (R_S) as well as back scattering by grain boundaries and residual pores. Since R_S is the same for all HA pellets (Eq. (5.3)), the decrease in measured reflection should be due to reduced back scattering.

Fig. 5.14 (a) Dependence of the measured reflection of MS pellets on wavelength. (b) Measured reflection values at 1300 nm for all HA pellets.

The hexagonal crystal structure makes HA birefringent, meaning that its refractive indexes vary along different crystallographic directions. As a result, there would be discontinuity in refractive index at grain boundaries if the adjacent grains have different crystallographic orientations. For perpendicular incidence, grain boundary reflection ($R_{\perp}$) can be calculated by the following equation [315]:

$$R_{\perp} = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2} \quad (5.4)$$

where n_1 and n_2 are the refractive indexes of two adjacent grains. For HA the maximum difference between n_1 and n_2 is 0.007. Therefore, the maximum $R_{\perp}$ value (R_{max}) can be calculated to be 4.5×10^{-6} . Based on this value, if the light passes all grains boundaries at 90°, the maximum grain boundary reflection can be calculated using the following equation:

$$R_{max} \approx 1 - (1 - R_{\perp})^m \quad (5.5)$$

where m is the number of grain boundaries. For a 2 mm thick sample with an average grain size of 411 nm (average grain size of MS pellet sintered at 1050 °C), a light beam

passes ~4863 grains. In this case the maximum grain boundary reflection is as low as 2.2 %. Considering that actual grain boundary induced reflection would be much lower than this value, it is safe to conclude that the contribution of grain boundary reflection is minor and can thus be neglected when the boundaries are free from pores and impurity phases. This conclusion can also be supported by a previous study on transparent HA [125]. Therefore, reduced backscattering by residual pores should be the primary cause of the significant decrease in measured reflection seen in Fig. 5.14a. With high sintering temperatures major residual pores could be effectively removed in the HA pellets.

The measured reflection values at 1300 nm of all HA pellets are shown in Fig. 5.14b. The measured reflection was markedly reduced when the sintering temperature was increased from 900 °C to 950 °C for NR-L, which should be attributed to the much denser microstructure achieved at 950 °C. As discussed earlier, concurrent pore growth may take place when HA grains coarsen. Consequently, light reflection at 1300 nm increased with sintering temperature for all NS and NR-H pellets, and NR-L pellets sintered in the range of 1000–1100 °C showed higher reflection loss than that fabricated at 950 °C.

In order to better understand the light-material interactions, the absorption coefficient (C_{abs}) and scattering coefficient (C_{sca}) were calculated for all sintered HA pellets by the following equations [301]:

$$C_{abs} = -\frac{\ln[T/(1-R)]}{t} \quad (5.6)$$

$$C_{sca} = -\frac{\ln(I/T)}{t} \quad (5.7)$$

where T , I , R and t are the TFT, ILT, measured reflection and thickness of the sample, respectively.

The C_{abs} and C_{sca} values for all polished HA pellets are plotted in Fig. 5.15. It can be seen in Fig. 5.15a that C_{abs} of MS pellets continuously decreased as sintering temperature was raised. This was mainly caused by the progressive reduction in the quantity and size of residual pores in the sintered pellets. The C_{abs} of NR-L pellets evolved in a similar trend (Fig. 5.15b). For NR-H and NS pellets, generally both their C_{abs} and C_{sca} values were increased at higher temperatures (Fig. 5.15b and d), probably due to the presence of a larger number of internal defects. Interestingly, for MS and NR-L

pellets, their C_{sca} turned out to be more sensitive to high temperatures than C_{abs} . In the range of 450–700 nm, the one sintered at 1000 °C had the lowest C_{sca} among all MS pellets, while a sintering temperature of 950 °C resulted in the smallest C_{sca} at 700 nm for NR-L pellets (Fig. 5.15c and d). For both types of HA pellets, the evolution of the C_{sca} with sintering temperature agrees well with that of the measured reflection (Fig. 5.15d and Fig. 5.14b), because light scattering was the major contributor to reflection loss. The higher C_{sca} values for MS pellets sintered at >1000 °C and NR-L pellets sintered at >950 °C can be attributed to the development of more internal defects. In SPS a higher sintering temperature is realized by applying a higher pulsed DC current, which in turn leads to more intense spark plasma generation and possibly a higher defect density [300].

Fig. 5.15 Absorption (a, b) and scattering (c, d) coefficients of HA pellets. (a, c) Curves for MS pellets. (b, d) Values obtained at 700 nm for all HA pellets.

Comparing Fig. 5.13a, Fig. 5.14a and Fig. 5.15c, it is consistently found that MS sintered at 1000 °C possessed the highest ILT, lowest reflection loss and smallest C_{sca} . Based on the analysis of optical properties, it is recommended that MS powder should be sintered at 1000 °C by SPS in order to obtain HA pellets with high transparency.

5.8 *In vitro* biological properties

5.8.1 Cell proliferation, viability and differentiation

Critical assessment of biocompatibility has to be carried out before the actual clinical application of any implant material. After 24 h and 72 h of culture, the number of L929 and MG63 cells on different HA pellets was quantified and normalized to the number of cells in the TCP controls, which the cells were routinely grown on. The cell proliferation data is presented in Fig. 5.16a and b. In Section 5.8, the number following a sample name refers to its sintering temperature (e.g. MS/950 for MS pellet sintered at 950 °C). In terms of L929 cell number, although there were fewer cells on HA pellets at 24 h compared to TCP controls (normalized cell number < 100 %), the performance of sintered HA seemed to be similar to TCP at 72 h (Fig. 5.16a). It can be seen in Fig. 5.16b that except NR-H/950, the HA pellets performed almost as well as or even better than TCP in supporting the growth of MG63 cells. The number of MG63 cells on NR-H/950 was found to be significantly smaller than on MS/1100 ($p < 0.05$).

The results of L929 cell viability tests are shown in Fig. 5.16c and Fig. 5.17. While a higher percentage of live cells were observed on TCP than on NR-H/950, NR-L/950 and NS/950, no statistical difference was found between TCP and the two MS pellets. Furthermore, cells grown on MS/950 had much higher viability than those grown on NR-L/950, NR-H/950 and NS/950 (Fig. 5.16c and Fig. 5.17). It can be seen in Fig. 5.16c that the MS/1100 culture surfaces led to higher cell viability than the NR-H/950 pellet (~95 % and ~86 % live cells, respectively). In addition, live cells were present at a significantly higher percentage on sintered NR-L/950 than on NR-H/950 pellet.

Fig. 5.16 (a, b) The number of cells on HA pellets normalized to that on TCP: (a) L929 and (b) MG63. (c) Percentage of live L929 cells after 72 h culture on HA pellets and TCP controls. (d) ALP activity of MG63 cells on HA pellets normalized to that on TCP. Dashed lines correspond to values for TCP (100 %). Error bar represents standard deviation of triplicate measurements. Statistical difference is indicated by * ($p < 0.05$), ** ($p < 0.01$), and *** ($p < 0.001$).

Fig. 5.16c indicates that sintered MS pellets led to higher cytocompatibility than the pellets sintered from NR and NS and could result in equivalently high cell viability as TCP controls. Fig. 5.16b suggests that NR-H/950 was the most disadvantageous in supporting the proliferation of MG63 cells at both 24 h and 72 h, and Fig. 5.16c further proved that NR-H/950 was the most unfavorable as it led to the highest percentage of dead L929 cells. One important reason for the relatively low biocompatibility of NR-H/950 may be its coarser grains compared to other HA pellets. Fig. 5.9 and Fig. 5.10 show that NR-H possessed a larger average grain size than the other three pellets sintered at the same temperature. Larger grains in NR-H mean a lower number of grain boundaries, which does not favor the adhesion and proliferation of cells because it decreases the wettability of sample surface [127]. This may also explain the decreased cell viability of NS/950 as compared with MS/950 (Fig. 5.16c).

Fig. 5.17 Representative images showing stained live (green) and dead (red) L929 cells on (a) MS/1100, (b) MS/950, (c) NR-H/950, (d) NR-L/950, (e) TCP, and (f) NS/950.

Additionally, the high crystal orientation along the [001] direction may have negatively affected the cytocompatibility of sintered NR pellets. In this study the sample section subjected to XRD tests was the same as that used for cell culture, to which the *c*-plane of HA crystals were aligned (see Section 5.5). HA granules have long been used for the purification and separation of biological molecules, and it has been reported that the *c*-plane absorbs significantly fewer proteins than the *a*-plane because of differences in electrostatic interactions [316-318]. Generally, better cell attachment can be expected when a larger number of proteins from the serum in the media bind to the sample surface. Therefore, the preferred crystal orientation in sintered NR seen in Fig. 5.7b, c may need to be avoided for the sake of biocompatibility.

ALP activity has been recognized as an early marker for osteoblast differentiation [319]. In this study, although there existed no statistical difference in normalized ALP

activity between different HA pellets, the average values were all above 130 % of the TCP control for the five HA pellets tested (Fig. 5.16d). This implies that the sintered HA was superior to TCP in promoting the differentiation of MG63 cells, which resulted in the generation of more ALP during *in vitro* bone formation [209, 320]. Interestingly, NR-H/950 pellets (which had resulted in the lowest L929 and MG63 cell proliferation and lowest L929 viability) showed the greatest ALP result. This could indicate either that the cells had stopped proliferating in favor of osteoblastic differentiation, or ALP expression may be increasing as a stress response of the MG63 cells to an incompatible culture environment.

Based on the above analysis, it can be concluded that MS pellets resulted in the highest viability of L929 cells than the other three types and significantly faster MG63 cell proliferation than NR-H pellets. Therefore, this chapter confirms that among the three starting powders used in this study, MS is the most desirable for sintering transparent HA with satisfactory *in vitro* biological properties.

5.8.2 Observation of living cells on transparent HA

Fig. A4 shows the optical images of HA pellets seeded with L929 cells after 48 h incubation. It can be seen that the degree of transparency strongly influenced the ease of cell observation. Cells could be barely seen on the translucent NR-L and NR-H pellets, while no cells could be observed at all on the opaque NS pellet (Fig. A4a1-c1). Cell observation became easier on MS/950, where quite a few cells could be found on the pellet surface (Fig. A4d). When it came to the transparent MS/1100, many cells could be clearly seen across the whole sample surface (Fig. A4e).

After culturing the L929 cells for a longer period of 72 h, an obvious increase in cell density could be observed on the transparent MS pellet, as shown in Fig. 5.18a. Comparing Fig. 5.18a to Fig. 5.18b, it was found that L929 cells on TCP and MS/1100 demonstrated very similar density and morphology. This again proves that growth and proliferation of L929 cells could be well supported by the sintered MS. In addition, there was minor difference in the resolution of L929 cells observed on TCP and the MS/1100 pellet.

Fig. 5.18 Optical images of L929 cells on MS/1100 (a) and TCP (b) at three magnifications after 72 h culture.

Fig. A5 presents the images of MG63 cells on MS/1100 and TCP captured at 24 h and 72 h. For both MS/1100 and TCP, a significantly higher cell density was observed at 72 h because of the proliferation of MG63 cells. In addition, most of the cells on MS/1100 and TCP showed the typical fibroblast morphology of healthy MG63 cells. It indicates that the cytocompatibility of MS/1100 for MG63 cell line could well match that of the culture-optimized TCP material.

It is worth mentioning that the highest ILT was demonstrated by MS/1000 (Fig. 5.13). Therefore, cell features on sintered HA can be observed with an even higher resolution when MS/1000 is used. Additionally, light transmission through the HA sample could be further enhanced by using a thinner pellet, as in most previous studies [309, 313, 321]. Using transparent HA as the substrate, Kotobuki et al. [293] has observed the attachment, proliferation and osteogenic differentiation of rat mesenchymal stem cells by light microscopy. However, to the best of our knowledge, to date *in situ* viewing of cell behavior on transparent HA has been limited to animal cell lines [313, 321]. Since the application of HA has achieved tremendous success in bone tissue engineering, we believe it would be of great interest and special importance to study the interactions between transparent HA and human bone marrow derived cells [22, 322]. Such investigations are not the focus of this thesis, but will be carried out in the future.

5.9 Summary

In this chapter three types of HA powder – MS, NR and NS – were sintered in the range of 900–1100 °C by SPS for 3 min. The densification behavior, phase composition, microstructure, mechanical behavior, as well as optical and *in vitro* biological properties of the samples were systematically investigated. It was found that transparent MS pellets could be fabricated at a sintering temperature > 950 °C, while most sintered NR were translucent and all NS pellets turned out to be opaque. A sintering temperature of 1000 °C was proven to be the optimum sintering temperature for MS powder and resulted in an outstanding ILT of 84 % at 1300 nm for a 2 mm thick sample. Keeping a constant sintering pressure of 50 MPa for the entire sintering process was found to be inappropriate for NR as it led to significant grain growth and more microstructural defects. Although NS powder was the most unfavorable for producing transparent HA, the sintered NS pellets possessed the largest *E* and *H* values as well as we the highly promising self-toughened microstructure due to the presence of randomly oriented elongated grains.

In the cell proliferation and viability tests, MS pellets either outperformed or performed as well as the three other types. These phenomena were possibly caused by the high crystal orientation in NR pellets as well as the larger grain size and hence reduced surface hydrophilicity of NR-H and NS compacts. Living L929 and MG63 cells could be readily observed on a transparent MS pellet using a normal optical microscope, and very similar cell density and morphology were seen on the sintered MS and TCP for both cell lines. Compared with TCP, the HA pellets all resulted in the generation of higher ALP activity in MG63 cells.

Therefore, while NS powder is preferred to obtain mechanically strong compacts, MS powder is the most desirable for fabricating transparent HA pellets which possess notable biocompatibility and great promise in studies on cell-bioceramic interactions.

Chapter 6 Silica-coated CNT Reinforced Hydroxyapatite

CNTs possess eminent mechanical properties and have been widely used to toughen bioceramics such as HA. Major challenges associated with the preparation of CNT-reinforced bioceramics include the inhomogeneous dispersion of CNTs and the poor interfacial strength between the two phases. To address such issues, this chapter proposes to coat CNTs with a silica layer and use the core-shell structures to toughen HA. HA-based composites with 0.1–2 wt.% S-CNTs were sintered by SPS to investigate their mechanical and biological properties. Pure HA and raw CNT-reinforced HA compacts were prepared for comparison purposes.

6.1 Introduction

HA possesses close chemical resemblance to the inorganic part of human hard tissue and thus demonstrates outstanding biocompatibility and bioactivity. However, its insufficient mechanical properties, especially low fracture toughness, limit its major orthopedic applications to surface coatings on metallic implants [271]. It has been a constant pursuit to improve the mechanical properties of bulk HA intended for major loading bearing applications by utilizing advanced fabrication techniques and / or incorporating reinforcing phases [212].

CNTs have aroused intense research interest in their potential biomedical applications by virtue of their outstanding mechanical, electrical, thermal and optical properties [323]. As an example, enthusiasm for the exploration of CNTs' application in nanomedicine has led to exciting advances [324]. Moreover, CNTs have been effectively used to enhance the mechanical properties of various bioceramics such as Bioglass® and HA [194, 202].

A growing concern regarding using CNTs in biological systems is their potential cytotoxicity [325, 326]. Although some studies have proved the compatibility of CNTs with several cell lines (see Table 2.7), in some other investigations CNTs were found toxic to a variety of cells such as mouse macrophages [327, 328], guinea pig alveolar macrophages [329], A549 human pneumocytes [330], and human MSTO-211H cells [331]. To address this issue, various pathways have been attempted, a major one being covalent / non-covalent surface modification of CNT [332]. For instance, CNTs coated / functionalized by polyethylene glycol [333], ammonium [334], taurine [335], and carboxylic acid [332] have been reported. However, when such surface-modified CNTs are used as reinforcements in bulk bioceramics, none of them can survive high-energy processing routes such as sintering.

Surface modification can also effectively contribute to the deagglomeration of CNT bundles [336, 337]. Due to their flexibility, high aspect ratios, and van der Waals interactions, CNTs intrinsically tend to agglomerate. That not only place restricts on deploying their physical and chemical properties, but also hinders their enhancement effects on the mechanical and / or electrical properties of various composite materials.

Recently, endowing CNTs with a SiO₂ coating has attracted a lot of research interest [338, 339]. The silica coating, by virtue of its higher wettability in solvents such as water and ethanol than CNT, reduces agglomeration of the nanosized fibrous material and enables more homogeneous dispersion. It has been reported by Wick et al. [331] that a higher degree of homogeneity contributes to lower cytotoxicity of CNTs. Furthermore, in our previous study and those by Best et al. [12, 13, 193], it was found that the incorporation of silicon in HA-based bioceramics could enhance the differentiation of human osteoblasts and promote bone growth and remodeling. In addition, such an inorganic coating not only provides a new platform for side wall reactions [11], but also opens up new possibilities of fabricating novel composites by exploiting the high stiffness and rigidity of the SiO₂ shell. By adding 4 wt.% of SiO₂-coated multi-walled carbon nanotubes (MWCNTs) to PMMA, Olek et al. [340] found that the ultimate hardness and Young's modulus of the composite were 2 and 3 times higher than the matrix, respectively. According to Zeng et al. [341], bismaleimide-triazine with 0.8 wt.% SiO₂-coated MWCNTs showed significantly enhanced tensile strength and Young's modulus as well as improved thermal stability and electrical insulation. However, to the best of the author's knowledge this type of core-shell structure has rarely been used to reinforce ceramics.

In this study, it is hypothesized that coating CNTs with a layer of silica can enhance their dispersibility and bring about improved mechanical and biological properties when they are employed as a reinforcing phase in HA. S-CNTs at four different loadings (0.1–2 wt.%) were added to HA and the corresponding composite pellets were prepared by SPS. Sintered S-CNT/HA composites were extensively assessed to reveal their structural, mechanical and *in vitro* biological properties; the S-CNT/HA composites were compared with pure HA as well as HA enhanced with raw CNTs (R-CNTs).

6.2 Experimental

6.2.1 Synthesis of HA nanorods

It was found in Chapter 5 that the HA MS powder was the most desirable to fabricate HA pellets with good optical and biological properties. Nonetheless, it has been reported that the use of nanosized powders can lead to reduced sintering temperatures, nanocrystalline compacts, and enhanced mechanical properties of the sintered samples [124, 342]. Although the NS pellets possessed the highest mechanical properties, they showed inferior cytocompatibility. Additionally, most commercially available HA powders are supplied in dry form, but usually needs to be dispersed in a solution before they can be mixed with nanostructured reinforcements such as CNT. Therefore, when HA suspensions produced by wet chemical processes are directly used, the mixing process is simplified and a higher degree of homogeneity can be possibly achieved.

Because of the reasons mentioned above, a wet chemical precipitation method based on Eq. (2.4) was used to obtain HA nanorods. Briefly, 148.2 g of calcium hydroxide powder ($\geq 96.0\%$, Merck Singapore) was dissolved in 2 L DI water and the solution was maintained at $\sim 36.8\text{ }^{\circ}\text{C}$ in a water bath. 95.2 mL ortho-phosphoric acid (concentration 85 wt.%, Merck Singapore) was diluted with 2 L DI water and heated to $\sim 42.0\text{ }^{\circ}\text{C}$. Using a mechanical pump, the acid was added drop by drop at $\sim 0.9\text{ L/h}$ to the Ca(OH)_2 solution, whose pH value was continuously monitored. Acid addition was terminated when the pH value dropped below 9. To discard the soluble impurities, the supernatant was replaced with fresh DI water after 24 h of settling. The HA sediment was then agitated to mix with DI water and left to settle again. This washing process was conducted for 5 times. Afterwards the concentration of as-obtained HA solution was determined to be 51.24 mg/mL. Dry HA powder, obtained by evaporating DI water from the HA solution and grinding the dried cake with a mortar and pestle, was utilized for sintering pure HA pellets. The purified HA solution was directly used to mix with the S-CNT or R-CNT solution to prepare the composite powders.

6.2.2 Fabrication of silica-coated CNT

The MWCNTs used were supplied by Nanocyl SA, Belgium and had an average diameter and length of 10 nm and 1.5 μm , respectively. (Caution: emerging data indicates the potential risks from exposure to CNTs. It is crucial to ensure sufficient risk management and follow the guidance on the safe handling and use of them. See Ref. [343] for more information). Tetraethyl orthosilicate (TEOS) was procured from Sigma Aldrich, and other reagents were purchased from Thermo Fisher Scientific Inc. As-received chemicals were utilized directly without any further purification.

S-CNTs were fabricated at room temperature using the sol-gel method proposed by Xie et al. [344, 345]. The R-CNTs were first acidified by stirring them in the mixed solution of HNO_3 and H_2SO_4 ($V(\text{HNO}_3):V(\text{H}_2\text{SO}_4) = 1:3$) at 60 $^\circ\text{C}$ for 3 h. They were subsequently washed repeatedly by DI water via centrifugation until the solution's pH value was approximately 7. A vacuum freeze-drying process was employed to obtain dry acidified MWCNT powder. A mixed solution of DI water, ethanol and NH_4OH (25.0–28.0 wt. %) with a volumetric ratio of 45:125:4 was used to disperse the acidified CNTs. A homogeneous and stable suspension of the acidified CNTs at a concentration of 0.29 g/mL could be obtained after 30 min of ultrasonication followed by another 30 min of mechanical stirring. After that an appropriate amount of TEOS was immediately added to the solution, and the mixture was stirred for 12 h to allow the reaction to complete. The sediment was obtained by centrifuging the mixture at 4000 rpm. It was then re-dispersed in DI water by ultrasonication and collected by vacuum filtration through a nylon membrane. This procedure was repeated for at least four times to completely remove the free silica particles. The final S-CNT product was obtained after a vacuum freezing-drying process.

6.2.3 Production of S-CNT/HA composites

An appropriate volume of 1 mg/mL S-CNT ethanol solution was sonicated for 30 min before being added to 200 mL HA solution in a dropwise manner. The addition lasted 30 min, during which both sonication and mechanical stirring (~ 285 rpm) were employed. The mixed solution was heated at 60 $^\circ\text{C}$ and stirred at 250 rpm on a combined

hot-plate magnetic stirrer until it became gelatinous. To fully evaporate any residual solvent, further drying was conducted overnight at 80 °C in a vacuum oven. Composite powders with S-CNT content of 0.1 wt.%, 0.5 wt.%, 1 wt.% and 2 wt.% were typically obtained. For comparison 1 wt.% R-CNT/HA was prepared through the same processing route.

The composite powders were compacted by SPS with a heating and cooling rate of 100 °C/min. For each sample, 0.6 g powder was loaded in a cylindrical graphite die with an inner diameter of 10 mm and held at the sintering temperature for 3 min with a pressure of 50 MPa applied. Based on the conclusions of Chapter 5, a small initial pressure (7.5 MPa) was used and 50 MPa was only exerted during the 3 min hold. To investigate the influence of sintering temperature, 1 wt.% S-CNT/HA was first sintered in the range of 900–1200 °C. Afterwards, 1100 °C was selected to sinter the pure HA and other composites. The optimization of sintering temperature was deemed necessary as it was found out in Chapter 5 that the sinterability of different starting powders varied drastically. In addition, previous studies suggest that HA composites containing SiO₂ may require a higher sintering temperature than pure HA [118, 346, 347]. The sintered pellets were ground with sand paper and polished to 1 µm surface finish for various characterizations.

6.2.4 Characterization

A D8-ADVANCE Bruker-AXS diffractometer was used to identify the phase composition of the starting powders using a Cu K α radiation operated at 40 kV and 30 mA. Morphology of the as-synthesized HA, R-CNT, and S-CNT powders was observed by TEM / HRTEM (JEOL JEM 2100F, Japan) and FESEM (JEOL JSM 7600F, Japan). Thermal stability of the powders in air was checked with TGA at a heating rate of 10 °C/min (TGA 2950, TA instrument). A surface area and porosity analyzer based on the BET method was employed to measure the specific surface area of the powders at liquid nitrogen temperature, i.e. 77.3 K (Micrometrics ASAP 2020 M, U.S.). The samples had been degassed at 10⁻⁵ bar, 150 °C for 10 h prior to the BET tests. The elemental composition of the powders was determined by XPS (Kratos, Axis-ULTRA, U.K.), which was equipped with an Al K α (1486.6 eV) monochromatic X-ray source.

Phase analysis was conducted for the sintered pellets by XRD (PANalytical EMPYREAN, The Netherlands) in the 2θ range of $20\text{--}60^\circ$. A Cu K α radiation operated at 40 kV and 40 mA was used. The chemical bonds in the samples were identified by FTIR (Bruker VERTEX-70) and Raman spectroscopy (Renishaw Invia, U.K.). For FTIR measurements, the powders were ground with KBr and compressed into translucent pellets. A 488 nm line of HeNe laser was used for the Raman tests. Relative density of the sintered pellets was determined based on Archimedes' method, assuming the theoretical density of HA to be 3.156 g/cm^3 (Table 2.5). Young's modulus and hardness of the pellets were measured by Nanoindentation, using the instrument and parameters specified in Section 3.2.2. Their fracture toughness was tested by Vickers indentation fracture toughness test (FE-300e, FUTURE-TECH, Japan), where an indentation load of 200 g was applied for 10 s. Microstructural observation was carried out for the sintered pellets using FESEM. HRTEM and scanning transmission electron microscopy (STEM; JEOL JEM 2100F, Japan) were employed to observe the HA lattice structure in the sintered pellets and the interface between the two constituents, respectively. Elemental mapping was conducted in the STEM mode using Energy dispersive X-ray spectroscopy (EDS). To prepare the samples for HRTEM and STEM, HA pellets reinforced with S-CNTs and R-CNTs were carefully ground in ethanol using a mortar and pestle. The ethanol solutions were then dropped onto copper grids, which were afterwards dried overnight at 40°C in air.

6.2.5 Cell culture

The *in vitro* biological properties of the sintered pellets were investigated by examining the proliferation, viability and differentiation of the human osteoblast-like cell line MG63. The cell culture media used was Dulbecco's Modified Eagle's Medium supplemented with 10 % (v/v) HyCloneTM research grade fetal bovine serum and 1 % (v/v) HyCloneTM Penicillin-Streptomycin solution (all from GE Healthcare). Quantification of the cell number was conducted at both 24 h and 72 h using a CyQUANT Cell Proliferation Assay Kit (Molecular Probes, US), and a LIVE/DEAD[®] Viability/Cytotoxicity Assay Kit (Molecular Probes, US) was utilized to obtain the percentage of live cells at 72 h. The ALP activity, an important osteogenic marker, was

evaluated with an ALP Assay Kit (Abcam, UK) after culturing the MG63 cells for 4 days. All the assays were performed according to the standard manufacturer's protocols.

Initially, 100 μL of cell suspension, which contained 1000 cells for the first two assays or 4000 cells for the ALP assay, was seeded onto each pellet. After incubating them at 37 $^{\circ}\text{C}$ in 5 % CO_2 for ~ 3.5 h, 1.5 mL media was added into each well of the 12-well plate to immerse the pellets. This allowed the vast majority of the cells to adhere to the sample surface rather than the bottom of the well. TCP was used as a material control in all cell experiments. Triplicate measurements were carried out to obtain the cell culture data. Student's *t*-test was used for data analysis. The results are presented as mean $\pm$ standard deviation, with statistical difference indicated by * ($p \leq 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$) in the relevant figures.

6.3 Raw powder analysis

The morphology of as-synthesized HA nanorods is shown in Fig. 6.1a. They typically had a diameter of 15–25 nm, and were several hundred nanometers long. Lattices of HA could be clearly seen by HRTEM (Fig. 6.1b), which confirms the crystallinity of the HA powder. The R-CNTs shown in Fig. 6.1c had an average diameter of ~ 10 nm. The presence of kinks, which can also be seen in Fig. A6a, is an indication of their structural flexibility [323]. After the sol-gel process, the major part of each CNT was covered by a continuous SiO_2 layer with a few SiO_2 nanoparticles attached (Fig. 6.1d and Fig. A6b), although the caps of some S-CNTs were not covered by SiO_2 (Fig. 6.1d, e). The thickness of the SiO_2 coating was 20–30 nm, and the S-CNT core-shell structure thus possessed a diameter of 50–70 nm (Fig. 6.1d). To confirm the formation of a continuous shell, the S-CNTs were calcined in air at 600 $^{\circ}\text{C}$ for 4 h to completely remove the carbonaceous core. It was found that after calcination SiO_2 nanotubes having similar morphology to S-CNTs were left behind (Fig. 6.1f and Fig. A6c), indicating that continuous SiO_2 shells had been formed.

Fig. 6.1 TEM/HRTEM images of HA nanorods (a, b), raw CNTs (c), S-CNTs (d, e), and calcined S-CNTs (f). Inset in (b) shows the (102) lattice with an interplanar spacing of 3.17 Å.

The specific surface area of HA nanorods, S-CNTs, R-CNTs were measured to be 81 m²/g, 39 m²/g, and 216 m²/g, respectively. The S-CNTs had a smaller surface area than R-CNTs, mainly because silica has a higher density than R-CNT. The XRD patterns of the three types of powder are compiled in Fig. 6.2. Only peaks corresponding to HA were seen for the HA nanorods (PDF 01-084-1998), indicating their phase purity. In the XRD pattern of R-CNT, the two main peaks located at ~25.6° and ~42.8° can be ascribed to the 002 and 100 Bragg reflections of graphite, respectively [348]. The S-CNT powder showed a similar XRD pattern. However, due to the presence of amorphous silica, its first order diffraction peak became broader and its position shifted to a smaller 2θ value of 23.1° (Fig. 6.2) [349].

Fig. 6.2 XRD patterns of the HA, R-CNT and S-CNT powders.

Fig. 6.3a shows the TGA curves of the starting powders. HA experienced a very small weight loss of 3.4 % from RT to 850 °C. The 0.8 % weight loss from RT to 100 °C was caused by evaporation of absorbed water, while the rest should be attributed to the loss of –OH groups. A significant weight loss of 90.0 % was seen for R-CNT when it was heated to 680 °C, above which its weight remained constant. It signifies that most pristine carbon could be burnt into volatile gases at this temperature, and that ~10 wt.% impurity (mainly catalyst used for growing CNTs) existed in the R-CNT powder. By contrast, the weight of S-CNT stopped decreasing at a higher temperature (~760 °C), and the total weight loss was only 16.0 % up to 850 °C. Therefore, the SiO₂ shell took up 84.0 wt.% of the S-CNTs and endowed them with a higher thermal stability compared with R-CNTs.

Fig. 6.3 TGA curves (a) and FTIR spectra (b) of the HA, R-CNT and S-CNT powders.

The FTIR spectra of the powders are presented in Fig. 6.3b. In the FTIR spectrum of HA, the double peaks at ~ 568 and ~ 604 cm^{-1} were caused by the O–P–O bending mode (ν_4) of the phosphate group, and the one at ~ 964 cm^{-1} corresponded to the P–O symmetric stretching mode (ν_1) of this group [248]. The peaks at ~ 1041 and ~ 1095 cm^{-1} should be attributed to the asymmetric stretching mode (ν_3) of the P–O bond [305]. The weak band at ~ 877 cm^{-1} and those between 1410 and 1460 cm^{-1} can be ascribed to carbonate groups, which possibly substituted the PO_4^{3-} or $-\text{OH}$ groups in HA [194, 350]. The hydroxyl stretching band of HA appeared at ~ 3569 cm^{-1} , and the absorbed water led to the broad bands centered at ~ 1637 and ~ 3439 cm^{-1} .

The two peaks centered at ~ 801 and ~ 957 cm^{-1} in the FTIR spectrum of S-CNT were due to the Si–O–Si symmetric stretching and Si–OH stretching vibration, respectively [344]. The strong band at ~ 1098 cm^{-1} was likely to stem from the asymmetric stretching of Si–O–Si and Si–O–C, suggesting the possibility of covalent bond formation between SiO_2 and CNT [351]. The band centered at ~ 1400 cm^{-1} should be attributed to the $-\text{OH}$ bond introduced during the acidification process [351]. Only a weak band located at ~ 1580 cm^{-1} was observed for R-CNTs, which can be ascribed to the C=C stretching mode of the CNT backbone.

Raman spectroscopy is extensively used to assess the quality of carbonaceous materials. Fig. 6.4a shows the Raman spectra of the three powders. The D band and G band were seen at ~ 1356 and ~ 1590 cm^{-1} , respectively, for both R-CNT and S-CNT. As mentioned in Section 4.6.1, the D band serves as an indication of the structural

imperfections, while the G band arises from graphitic carbon. The defect index I_D/I_G was calculated to be 1.1 for R-CNTs and 0.98 for S-CNTs. The slightly smaller defect index of S-CNT possibly resulted from the formation of covalent bonds between the SiO_2 layer and CNTs at the defect sites, where oxygen-containing functional groups were introduced when R-CNTs were acidified. The 2D band could be clearly seen for R-CNT at $\sim 2700 \text{ cm}^{-1}$. All the three bands were less obvious for S-CNT because most CNTs were covered by a SiO_2 coating. In the Raman spectra of HA, the strongest peak at $\sim 961 \text{ cm}^{-1}$ can be attributed to the ν_1 symmetric stretching vibration mode of PO_4^{3-} ; the peaks at $\sim 430 \text{ cm}^{-1}$, $\sim 590 \text{ cm}^{-1}$, and $\sim 1047 \text{ cm}^{-1}$ corresponded, respectively, to the bending ν_2 , the anti-symmetric bending ν_4 , and the anti-symmetric stretching ν_3 modes of PO_4^{3-} . The hydroxyl absorption band was observed at $\sim 3574 \text{ cm}^{-1}$ [305].

Fig. 6.4 Raman spectra of the raw powders used (a) and pellets sintered at 1100 °C (b-c). A magnified view of the dashed rectangles in (a) and (b) is given in the inset of (a) and image (c), respectively.

XPS spectra were recorded for the R-CNT and S-CNT powders to confirm the formation of covalent bonds between the SiO_2 shell and the CNT core (Fig. 6.5). In the survey scan spectra shown in Fig. 6.5a, only peaks corresponding to C and O were detected for R-CNTs, while additional peaks pertaining to Si 2s and Si 2p electron orbitals could also be explicitly seen for S-CNTs. In addition, S-CNTs led to a significantly stronger O 1s peak and a much weaker C 1s peak than R-CNTs. The peaks seen in the survey scan spectra of HA can be attributed to its elemental composition (O, Ca, and P) and adventitious carbon. In the O 1s and Si 2p spectra of S-CNTs (Fig. 6.5b, c), the deconvoluted components located at 532.7, 533.5 and 103.7 eV signify the presence of SiO_2 [351]. The formation of covalent bonds between SiO_2 and CNT can be evidenced by the components at 531.8 and 102.7 eV [352]. Based on the FTIR, Raman and XPS results, it can be concluded that the CNT core coupled to the SiO_2 shell through strong covalent bonding.

Fig. 6.5 (a) XPS survey scan spectra of the powder samples and two pellets sintered at 1100 °C. (b, c) Detail scan spectra of S-CNTs: (b) O 1s, (c) Si 2p, where the solid black line, dashed magenta line, and dotted cyan line stand for measured data, fitted envelopes, and the background, respectively.

6.4 Properties of S-CNT/HA composites

6.4.1 Structural and mechanical properties

To find out the optimum sintering temperature for S-CNT/HA composites, the composite powder with 1 wt.% S-CNT was sintered in the range of 900–1200 °C. Fig. 6.6a depicts the effects of sintering temperature on the E , H and grain size of the compacted pellets. An increasing trend in both E and H was observed when the temperature was augmented from 900 to 1100 °C, probably as a result of the denser microstructure. At the same time, the average grain size increased from 89.2 nm at 900 °C to 893.5 nm at 1100 °C. Further grain growth occurred at 1200 °C, leading to an average size of 4.15 μm . This was possibly the main reason for the decreased E and H seen at 1200 °C. 1100 °C was thus selected as the optimum temperature to sinter pure HA and HA-based composites.

Fig. 6.6 (a) E , H and grain size of 1 wt.% S-CNT/HA samples sintered at different temperatures. (b, c) Density and mechanical properties of the HA, S-CNT/HA and R-CNT/HA pellets sintered at 1100 °C: (b) density, (c) mechanical properties. (d) Representative load-displacement curves for the sintered pellets. TD: theoretical density; RD: relative density. A magnified view of the dashed rectangle in (d) is given in its inset.

The XRD patterns of the 1 wt.% S-CNT/HA pellets sintered in the range of 900–1200 °C are presented in Fig. 6.7a. No decomposition of the pellet sintered at 900 °C could be identified as all peaks corresponded to HA. An additional weak peak at 31.3° appeared for the pellets sintered at the three higher temperatures, which could be attributed to the decomposition of HA into α -TCP (PDF 00-009-0348).

The macroscopic image and density of the pellets sintered at 1100 °C are presented in Fig. A7 and Fig. 6.6b, respectively. The translucency of the pure HA pellet is an indication of its high relative density. It can be seen in Fig. 6.6b that while the pure HA and S-CNT/HA pellets all possessed a relative density of > 99 %, R-CNT/HA had a smaller relative density of 98.0 %, signifying its inferior sinterability. Fig. 6.7b presents the XRD patterns of the sintered pellets containing 0–2 wt.% S-CNTs. The α -TCP peak at 31.3° was only seen for all S-CNT/HA pellets, and it intensified at higher S-CNT loadings (Fig. 6.7b). It signifies that the addition of S-CNTs promoted the decomposition of HA. Nikom et al. [347] also found that the addition of SiO₂ enhanced the phase transformation of HA to TCP during sintering. The average grain size of pure HA pellet sintered at 1100 °C was measured to be 1.12 μ m, which was 25.3 % larger than that of the 1 wt.% S-CNT/HA pellet sintered at the same temperature (Fig. 6.6a). This proves that the addition of S-CNT could refine the grain size of HA.

Fig. 6.7 XRD patterns of the sintered pellets: (a) 1 wt.% S-CNT/HA pellets sintered at different temperatures, (b) pure HA and Si-CNT/HA pellets sintered at 1100 °C. The arrows point at a peak corresponding to α -TCP.

HRTEM observation was carried out to gain a deeper understanding of the composition of the composites, with two representative images of 1 wt.% S-CNT/HA shown in Fig. 6.8. It was found that HA was the predominant phase in the sample, which was in agreement with the XRD results. The periodicity of the HRTEM image in Fig. 6.8a was confirmed by the fast Fourier transform (FFT) spectrum (Fig. 6.8a inset). The FFT spectrum also enabled the measurement of the d spacing. Based on the d spacing value of 3.44 Å, it was determined that the image displayed the (002) plane of HA. Fig. 6.8b shows the boundary between two adjacent HA grains, and no second phase could be found.

Fig. 6.8 HRTEM images of the HA phase in the 1 wt.% S-CNT/HA sample sintered at 1100 °C: (a) (002) lattice, (b) grain boundary. The inset in (a) is the FFT image.

The Raman spectra of the pellets sintered at 1100 °C are compiled in Fig. 6.4b. It was found that when the filler content was 1 wt.% or higher, Raman bands arising from both HA and graphitic materials were observed for the composites, indicating that both raw and modified CNTs were able to survive the SPS process. As shown in Fig. 6.4c, R-CNTs without the SiO₂ shell led to more intense D, G and 2D bands. The absence of these bands in the composites with 0.1 wt.% and 0.5 wt.% S-CNTs was due to their low loading. In Fig. 6.5a the XPS survey scan spectra of the pellets reinforced with 1 wt.% raw and modified CNTs are compared. The Si 2p peak was seen for S-CNT reinforced HA (Fig. A8), but was absent in the XPS spectrum of R-CNT/HA.

The mechanical properties of the pure HA and composite pellets sintered at 1100 °C are compared in Fig. 6.6c. The highest E and K_{IC} were achieved for 1 wt.% S-CNT/HA pellet, while 0.5 wt.% S-CNT/HA had the largest H . Compared with the pure HA pellet, the E , H and K_{IC} values of 1 wt.% S-CNT/HA were increased by 8.6 %, 7.3 % and 101.7 %, respectively. By contrast, the E of 2 wt.% R-CNT/HA pellet was 3.5 GPa lower than that of the pure HA sample, and its H and K_{IC} were augmented by only 0.7 % and 57.6 %, respectively. The deteriorated mechanical properties associated with increasing the S-CNT content from 1 wt.% to 2 wt.% was probably a consequence of the presence of superfluous S-CNTs in the HA matrix. It can be therefore concluded that the optimum S-CNT loading is 1 wt.%. On the other hand, when 1 wt.% R-CNTs were added into HA, the H was reduced by 0.02 GPa, and the E and K_{IC} were increased by only 3.2 % and 25.4 %, respectively. Thus, R-CNTs were much less effective in enhancing the mechanical properties of sintered HA than S-CNTs.

There are several possible reasons for the drastically different enhancement effects brought about by the raw and modified CNTs. First, a denser microstructure could be obtained for the S-CNT/HA composites (Fig. 6.6b). The representative load-displacement curves for sintered HA, 1 wt.% S-CNT/HA and 1 wt.% R-CNT/HA samples are compared in Fig. 6.6d. It could be apparently seen that to achieve the same indentation depth, the largest load was needed for the 1 wt.% S-CNT/HA pellet, and it experienced the smallest displacement when the same load was applied. In addition, a plateau region was observed for 1 wt.% R-CNT/HA. It probably resulted from the presence of voids and hence inhomogeneous microstructure of this pellet.

Furthermore, the inorganic SiO₂ shell possibly enhanced the interfacial strength between the matrix and the reinforcements [10]. The TEM/HRTEM and STEM images in Fig. A9 clearly show the morphology of HA debris and rod-shaped fillers. Comparing Fig. A9 with Fig. 6.1c and d, it seems that after being isolated from the HA matrix, the diameter of S-CNTs was enlarged while the R-CNTs had a very similar diameter to the as-received CNTs. EDS mapping was therefore carried out to identify the possible changes in the surface composition of both fillers, with the results presented in Fig. 6.9. A peak corresponding to Si could be seen in the S-CNT/HA spectra, which was absent in that of the R-CNT/HA (Fig. 6.9a, b). In the element maps shown in Fig. 6.9c, the

distribution of Si was found to be confined in the area of rod-like structures; it was interesting to find that Ca, P and O were also detected in those regions in addition to the locations of HA debris. It indicates that a layer of HA adhered onto the S-CNT surface, and that the fracture occurred within the HA phase rather than along the SiO₂–HA interface when the rod-shaped structures were separated. Such a phenomenon was not observed for the R-CNT/HA sample. Fig. 6.9d shows that the elements of Ca, P, and O were primarily detected where the HA debris was located and could not be explicitly identified in the area of R-CNTs. The difference in elemental distribution thus proved that the SiO₂–HA interfacial strength was stronger than the adhesion strength between R-CNT and HA. Furthermore, the rod-like structures (HA coated S-CNTs) were all intact, and no delamination of the SiO₂ shell was observed (Fig. A9a, c). This signifies strong interfacial strength between CNT and the SiO₂ layer as well.

Fig. 6.9 STEM-EDS mapping results of sintered S-CNT/HA (a, c) and R-CNT/HA (b, d) composites: (a, b) EDS spectra, (c, d) STEM bright field images and the corresponding elemental mappings. Loading of reinforcements: 1 wt.%. The outlines of R-CNTs and S-CNTs are indicated by the dashed lines in the Si and C maps.

The propagation path of indentation-induced cracks as well as the polished and fracture surfaces of the 1 wt.% S-CNT/HA pellets were examined by FESEM to identify the possible mechanisms behind their outstanding toughness. Pull-out of S-CNTs was clearly seen on both the polished and fracture surfaces (Fig. 6.10a–c); along the crack propagation paths pulled out S-CNTs which were able to bridge the crack could also be explicitly observed (Fig. 6.10d, e). Considering the stronger interfacial strength between S-CNT and HA, the pull-out of S-CNTs was expected to dissipate and absorb a larger amount of fracture energy than that of the R-CNTs. In addition, wavy textures were seen on both sides of the crack paths (Fig. 6.10d–f); Fig. 6.10f shows that by virtue of the submicrometer grain size, the prevailing fracture mode was intergranular. The addition of S-CNTs, therefore, indirectly toughened HA by reducing the grain size.

Fig. 6.10 Toughening mechanisms for the sintered 1 wt.% S-CNT/HA pellets. (a–c) S-CNT pull-out on the polished surface (a) and fracture surface (b, c). (d, e) S-CNT pull-out and crack bridging effect. (f) Crack deflection by fine HA grains.

Another factor affecting the mechanical properties of the composites is the degree of homogeneity for nanotube dispersion in the HA matrix. As mentioned in Section 6.2.3, both types of CNTs were dispersed in ethanol and added into aqueous HA suspensions to obtain the composite powders. Fig. A10 shows the state of S-CNTs and R-CNTs in the

two solvents shortly after a brief period of ultrasonication. While R-CNT sediments were observed in both solvents, the S-CNT solutions were much more homogeneous. Thus, coating CNTs with a SiO₂ shell improved their dispersibility in both DI water and ethanol, which in turn could result in more homogeneous filler distribution in the HA matrix. As can be seen in Fig. A11, severe agglomeration of CNTs was observed on the fracture surface of the 1 wt.% R-CNT/HA pellets. This was probably an important reason for the very limited enhancement effect or even detrimental impact of R-CNT addition on the mechanical properties.

The largest K_{IC} achieved for S-CNT/HA composites (1.2 MPa·m^{1/2}) is comparable to that of a number of commercial glass-ceramics such as Biosilicate® (~1.0 MPa·m^{1/2}) and Bioverit® (0.5–2.0 MPa·m^{1/2}) [4, 233]. Although Lahiri et al. [61] reported an even higher K_{IC} for CNT-reinforced HA, it is not intended to compare our K_{IC} value with theirs. As mentioned in Section 2.3.3, when Vickers indentation test is used to assess the fracture toughness, it may not be appropriate to compare the values reported by different studies because of a lack of testing standards. They can, nevertheless, reflect the toughening effect accomplished by S-CNT addition in this study.

6.4.2 *In vitro* biological properties

The cell culture results are shown in Fig. 6.11. It was found that after a short culture period of 24 h, the number of cells was higher on all the pellets than on TCP (Fig. 6.11a); in addition, there were more cells on the pure HA pellet than most composite pellets. These results indicate that all the bioceramic samples were highly biocompatible and that the addition of S-CNTs and R-CNTs into HA possibly retarded the attachment and early growth of MG63 cells. Nevertheless, after 72 h no statistic difference in cell number could be identified between pure HA and the composites, and the slowest cell proliferation was observed on the 2 wt.% S-CNT/HA pellet among all the composites. It signifies that in the long run, low loadings of S-CNTs did not exert adverse influences on the proliferation of MG63 cells, but could slow down their proliferation at a concentration of 2 wt.%. At higher S-CNT concentrations, the number of nanotubes protruding from the polished surface is expected to increase. This would probably affect the cell–material interactions and hence the proliferation and growth of the cells [202]. Moreover, the

dispersion of S-CNTs is expected to be less homogeneous at higher loadings, which may also influence the behavior of cells grown on them [326]. In addition, there were a larger number of cells on the 1 wt.% S-CNT pellets than on the 1 wt.% R-CNT/HA samples at 72 h. It implies that for the HA-based composites reinforced with CNTs, coating CNTs with a SiO₂ shell could lead to faster proliferation of MG63 cells cultured on them. This was probably due to the release of Si ions to the cell culture media, which have been proven to be able to enhance the proliferation of bone-specific cells in many a previous study [238].

The percentage of live MG63 cells on the sintered samples and TCP is compared in Fig. 6.11b, and Fig. 6.12 shows the representative images of the stained live and dead cells. All the pellets led to a significantly higher percentage of live cells (stained green in Fig. 6.12) than the TCP control, which further confirms the high biocompatibility of the HA-based pellets. There existed no statistical difference between the pure HA sample and the composites. Reinforcing HA compacts with 0.1–2 wt.% S-CNTs or 1 wt.% R-CNTs, therefore, showed no negative influence on the viability of MG63 cells cultured on them. The biocompatibility and cytotoxicity of CNT have been experiencing massive investigations [190, 353]. Akasaka et al. [190] reported that CNTs can provide effective nucleation surfaces for the initial precipitation and crystallization of an apatite layer. It is also believed that the nanosized tubular structures may also serve as favorable sites for protein adsorption [353]. Both factors may have contributed to the high cell viability observed for the composite pellets.

Fig. 6.11 (a) The number of MG63 cells on the sintered pellets normalized to that on TCP. (b) Percentage of live MG63 cells after 72 h culture on the sintered pellets and TCP. (c) ALP activity of MG63 cells after 4 day culture on the sintered pellets and TCP. Statistical difference is indicated by * ($p \leq 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.001$).

Fig. 6.12 Representative images showing stained live (green) and dead (red) MG63 cells on the sintered pellets and TCP.

The ALP activity, an early marker for osteoblast differentiation [319], was compared for the MG63 cells grown on different samples. It can be seen in Fig. 6.11c that generally the normalized ALP activity was augmented at higher S-CNT loadings; additionally, at a filler concentration of 1 wt.% in the HA matrix, S-CNTs led to significantly higher ALP activity than R-CNTs ($p < 0.05$). Such phenomena probably stemmed from the more Si ions in the vicinity of pellets with higher S-CNT loadings during cell culture. Many previous studies have proven that the incorporation of trace

amounts of Si in HA-based bioceramics plays a major part in the enhanced osteoblast differentiation and bone remodeling [12, 13, 193]. Fig. 6.11c also shows that all the bioceramic samples resulted in remarkably higher ALP activities than the TCP control, suggesting that they are advantageous to TCP in promoting osteoblast differentiation and bone formation [209, 320].

6.5 Summary

This chapter reported the first use of S-CNTs to reinforce HA. The S-CNTs were successfully prepared via a sol-gel method, and strong covalent bonding was formed between the MWCNT core and the SiO₂ shell. Ultrasonication and mechanical stirring were employed to homogeneously mix the S-CNTs with highly pure and crystalline HA nanorods synthesized through a wet chemical precipitation method. SPS was utilized to consolidate the HA composites with 0.1–2 wt.% S-CNTs. The influence of sintering temperature on the properties of 1 wt.% S-CNT/HA was systematically investigated, and it was found that 1100 °C was the most suitable temperature. Among the four different S-CNT loadings, 1 wt.% was found to be the optimum, which led to the E , H and K_{IC} to be increased by 8.6 %, 7.3 % and 101.7 %, respectively, as compared to the pure HA compacts. By contrast, incorporating 1 wt.% R-CNTs into HA caused its H to decrease and resulted in very limited increases in E (3.2 %) and K_{IC} (25.4 %). The advantages of S-CNTs over R-CNTs in this application include the higher degree of homogeneity of their dispersion as well as the enhanced sinterability and stronger interfacial strength between the constituents it could bring about. Toughening mechanisms such as crack bridging, pull-out of S-CNTs, and crack deflection by fine HA grains were identified for the S-CNT/HA compacts. After 72 h culture of the MG63 cells, the 1 wt.% S-CNT/HA pellets brought about equivalent cell viability and growth rate to the pure HA, but led to faster cell proliferation than the 1 wt.% R-CNT/HA and 2 wt. % S-CNT/HA samples as well as the TCP control. The MG63 cells cultured on the composites with increased S-CNT loadings tended to have higher ALP activities, and the 1 wt.% S-CNT/HA pellets resulted in significantly higher ALP activities in the cells than the 1 wt.% R-CNT/HA ones. The cells cultured on the S-CNT/HA pellets probably derived benefit from the released Si

ions from the SiO_2 shell. Therefore, by replacing conventionally used R-CNTs with S-CNT core-shell structures to reinforce HA, the current study provides an innovative and effective pathway to the improvement of both mechanical and biological properties. It is further believed that the S-CNTs also possess enormous potential in the enhancement of many other structural and biomedical ceramics.

Chapter 7 Discussion, Conclusions and Recommendations

7.1 General discussion

The key motivation of this thesis is to effectively enhance the fracture toughness of bioceramics through the incorporation of carbonaceous nanomaterials. On the basis of the materials science tetrahedron shown in Fig. 1.1, this section presents a general discussion of three pivotal factors influencing the toughening effect.

7.1.1 Microstructural design of matrix

This research has revealed a number of toughening mechanisms induced by the reinforcing phase, such as crack bridging and filler pull-out. It is noted that properly designed microstructure of the bioceramic matrix can also significantly contribute to the toughening behavior. Outstanding examples of tough ceramic microstructures include interlocking Si_3N_4 grains, [225], nacre-like Al_2O_3 ceramics [354], nanostructured Y_2O_3 compacts [355], and nanocrystalline HA [128].

It is believed that an approach of remarkable potential is to couple different toughening mechanisms in the same ceramic-based composite system [356]. Therefore, tailoring the matrix microstructure and incorporating a reinforcing phase can be used simultaneously to realize synergistic toughening effects. For instance, by lowering the sintering temperature for HA-based composites, the size of HA grains can be decreased and the fracture toughness may be further increased. Fig. 7.1 shows the evolution of the fracture surface morphology of HA samples sintered at different temperatures by SPS. It can be clearly seen that obvious grain growth occurred at higher temperatures. This is not desirable because when grain size coarsens, the transformation from intergranular fracture to intragranular fracture decreases the fracture toughness [128]. Nevertheless, it should be pointed out that the temperature has to be high enough to obtain satisfactory relative density.

Fig. 7.1 FESEM images showing the fracture surface of HA pellets sintered at different temperatures: (a) 900 °C, (b) 950 °C, (c) 1000 °C, (d) 1050 °C, and (e) 1100 °C. Spherical HA particles (diameter < 200 nm) were used as the raw powder. Pressure: 50 MPa; hold time: 3 min.

As another example, CNT or graphene may be used to toughen NS pellets to make use of its interlocking microstructure (see Section 5.6). In this research, the NS powder was not selected to prepare the S-CNT/HA composites due to the reasons mentioned in Section 6.1. However, it is a highly potential starting powder which may enable us to go beyond the currently obtainable K_{IC} values of HA-based ceramics.

7.1.2 Interfacial strength

Markedly different interfacial strengths were gained for the two composites studied in this research. It was found in Section 4.6.2 that weak interfacial strength

existed between GNP and the 45S5 matrix. By contrast, Section 6.4.1 proved that S-CNTs could bond to HA tightly. The fracture energy was therefore dissipated and absorbed through different mechanisms in the two composites.

For the GNP-reinforced 45S5 pellets, the majority of the fracture energy was probably absorbed by the interfacial debonding, which may subsequently lead to crack bridging by partially debonded GNP and the pull-out of graphene sheets. In this case, a weak interfacial strength is needed to ensure the occurrence of interfacial debonding [357]. On the other hand, frictional sliding caused by a certain level of interfacial strength may enhance the toughening effect. Ramirez et al. [258] found that SPSrGO could increase the fracture toughness of Si_3N_4 to a greater extent than pristine GNP. They proposed two possible reasons: (1) the presence of more defects in SPSrGO may promote the chemical interactions between the two phases, and (2) additional mechanical interlocking could be induced by SPSrGO because of its rougher surface morphology. In this research, highly corrugated SPSrGO sheets were also obtained (Fig. 4.8 and Fig. 4.14), and those two reasons probably made a contribution toward the enhanced fracture toughness of GNP/45S5 composites as well.

By contrast, the strong interfaces made it more difficult for debonding and nanotube pull-out to occur in the S-CNT/HA composites. As a result, a higher density of matrix cracking can be expected [358]. This was probably responsible for the consumption of most fracture energy in the S-CNT/HA pellets. Nonetheless, crack bridging and S-CNT pull-out were still among the toughening mechanisms identified, as shown in Fig. 6.10.

7.1.3 Properties of reinforcing phase

The characteristics of the reinforcing materials can influence both the matrix microstructure and interfacial strength. It has been shown in Section 6.4.1 that adding S-CNTs refined the HA grains; the study by Liu et al. [207] proved that the incorporation of graphene could inhibit HA grain growth. In this regard, CNTs and GNPs with higher dispersibility are expected to play a more effective role, and those comprising fewer graphene monolayers are preferable as they have a higher specific surface area. In addition, the number of graphene layers in both materials affects not only their

mechanical properties [7, 16], but also the interfacial sliding resistance and hence crack propagation [358].

When GO is used as a precursor to graphene in bioceramic-based composites, the extent of oxidation and exfoliation of GO has to be carefully taken into account. It can influence the reactivity of GO in the sintering process as well as the surface roughness of rGO, which in turn affects the interfacial strength [258].

Structural changes in the carbonaceous nanomaterials, which may augment or hamper the enhancement effects, can be induced by the processing routes employed to disperse the nanostructured reinforcements. Acid treatment [202], ultrasonication [16] and ball milling [15] are among the most frequently used methods to disperse them. The SPS process can further alter the characteristics of the carbon-based reinforcements, as reported in many previous studies [187, 273, 359-362]. A definitive understanding of such process-dependent changes of the carbonaceous nanomaterials can greatly enrich our interpretation of the toughening effects and mechanisms.

7.2 Conclusions

In this thesis, two strategies were successfully employed to overcome the challenges of using two carbonaceous nanomaterials, namely CNT and graphene, to toughen bioceramics. The representative base materials were selected to be 45S5 and HA, both of which have eminent biological properties but low fracture toughness. Prior to the fabrication of the two types of nanocomposites, pure 45S5 and HA samples were prepared by SPS to gain a deep understanding of their sintering behavior and properties; the thermal reduction of as-synthesized GO by SPS was systematically investigated to evaluate the properties of SPSrGO and unveil the underlying reduction mechanisms. These findings served as stepping stones for the subsequent fabrication and investigation of graphene/45S5 and S-CNT/HA composites. The major outcomes and conclusions are summarized as follows:

1. Fully dense 45S5 pellets with varying degrees of crystallinity could be obtained by sintering in the range of 500–600 °C for 3 min through SPS. Crystallization of the

amorphous 45S5 into $\text{Na}_2\text{CaSi}_3\text{O}_8$ took place at 550 and 600 °C, leading to obviously improved mechanical properties. Both 21-day aqueous aging and a lower sintering temperature could lead to faster proliferation and higher viability of L929 and MG63 cells cultured in the pellet-conditioned media. MG63 cells grown on the fresh sample (not aqueously aged) sintered at a moderate temperature of 550 °C had the highest ALP activity, possibly as a result of the high interfacial pH and the highest Si content in the media. Therefore, dense 45S5 compacts prepared at 550 °C by SPS had the optimum comprehensive properties for orthopedic applications.

2. The thermal reduction of GO by SPS was systematically studied for the first time. After 1 min of SPS treatment at 500 °C, which is lower than the critical temperature (550 °C) required for GO exfoliation to occur in a conventional thermal process, GO was found to be successfully exfoliated, reduced, and hierarchically roughened. Moreover, SPS was proven to be particularly effectual in minimizing the residual oxygen content and creating hierarchical surface roughness on the rGO sheets. SPSrGO-1050 achieved an extraordinarily high C:O ratio of 83.03 (atom %), and its R_a was 10 times as large as that of GO. The reduction mechanism was proposed to be the synergistic action of local high temperature, spark impact pressure and rapid ion migration. These findings can provide practical guidance on the preparation and properties of composite systems reinforced with SPSrGO.
3. To obtain homogeneous dispersion of the reinforcing phase, novel GNP/45S5 composites were prepared using the strategy of *in situ* GO reduction by SPS. Composite powders with three different GO concentrations (0.1, 0.5 and 1 wt.%) were densified by SPS at 550 °C. As compared with those of the mixed powders, the Raman spectra of the corresponding sintered composites showed decreased I_D/I_G , indicating the restoration of sp^2 bonds in the graphite lattice during SPS. The addition of 0.5 wt.% GO was found to be the optimum, which increased the fracture toughness of the sintered pellets by 130.2 % and decreased the friction coefficient and specific wear rate by 21.3 % and 62.0 %, respectively. The GNP-induced toughening mechanisms were identified to be crack bridging, crack

deflection, and sheet pull-out. Furthermore, MG63 cells grown on the pure 45S5 pellets and the composites had comparably high cell viability. The GNP/45S5 leachates tended to bring about faster proliferation and higher viability of the MG63 cells than the pure 45S5-conditioned media. The GNP-induced enhancement effects in both mechanical and biological properties have thus opened a new window for broader applications of 45S5 in bone tissue engineering.

4. Three types of raw HA powder, namely MS, NR and NS, were sintered in the range of 900–1100 °C by a 3 min SPS process with the application of a moderate pressure of 50 MPa. The MS powder was found to be able to result in the most transparent pellets, with an in-line transmittance of 84 % accomplished at 1300 nm for a 2 mm thick sample sintered at 1000 °C. To minimize grain coarsening and microstructural defects in the NR pellets, the application of a low initial pressure was preferable to a constant pressure exerted throughout the sintering process. Although the NS pellets possessed the lowest transmittance, they had the highest mechanical properties. In addition, the MS pellets demonstrated the highest cell viability in the *in vitro* biological tests with L929 cells, possibly due to the preferred crystal orientation or coarser grains in the other pellets. Living MG63 and L929 cells cultured on a transparent MS pellet could be effortlessly and clearly observed by light microscopy, and the cell density and morphology were very similar to those on the TCP control. Therefore, NS powder is preferable to achieve high mechanical properties, while MS powder is the most desirable for fabricating transparent and biocompatible HA pellets.
5. Using HA as the representative material system, S-CNTs were utilized to reinforce bioceramics for the first time. The S-CNTs with strong covalent bonds between the MWCNT core and the SiO₂ shell were successfully synthesized through a sol-gel process. HA composites with 0.1–2 wt.% S-CNTs were compacted by SPS. When sintered in the range of 900–1200 °C, the 1 wt.% S-CNT/HA composites sintered at 1100 °C had the highest *E* and *H*. Among the four different S-CNT loadings, 1 wt.% was found to be the optimum and could increase the *E*, *H* and *K_{IC}* by 8.6 %, 7.3 % and 101.7 %, respectively, as compared to the pure HA compacts. By contrast, when 1 wt.% R-CNT was added, the *H* was decreased and very

limited increases in E (by 3.2 %) and K_{IC} (by 25.4 %) were observed. It was found that the S-CNTs could lead to higher sinterability of the composite powder than the R-CNTs, and were easier to be dispersed and more tightly bonded to the HA matrix. The incorporated S-CNTs could refine the HA grains and absorb fracture energy through their pull-out and by bridging the cracks, which led to improved fracture toughness. MG63 cells cultured on the 1 wt.% S-CNT/HA pellets were found to proliferate faster and possess significantly higher ALP activities than those grown on the 1 wt.% R-CNT/HA compacts, probably by virtue of the released Si ions from the SiO_2 shell. Therefore, the S-CNT core-shell structures could improve both mechanical and biological properties of HA more effectively than the conventionally used R-CNTs. The current study also presents a novel and effective approach to the enhancement of many other biomedical and structural ceramics through S-CNT incorporation.

In conclusion, the work reported in this thesis demonstrated the effectiveness for fabricating tough bioceramic-based composites with carbonaceous nanostructured reinforcements with the following two strategies: (1) utilizing GO as a graphene precursor, and (2) coating CNTs with a SiO_2 layer.

7.3 Recommendations for future work

The following directions and opportunities for future work are suggested:

1. *Investigation of the changes in the densification behavior and possible decomposition of the base ceramics.*

Marked differences may exist between the constituents of a bioceramic-based composite in various aspects such as electrical and thermal conductivity, thermal expansion coefficient, reactivity, and stability. Because of such differences, significant changes in the sintering behavior can be induced by the reinforcing material. Additionally, the decomposition of the base bioceramic may be promoted by the incorporation of a second phase [347]. The role of the reinforcing phase in

such changes should be unambiguously understood as it closely relates to the properties and performance of the sintered samples.

2. *Protein profile analysis and quantification of adhesion for bone cells.*

Previous studies have reported contradicting results on the biocompatibility of CNT- and graphene-reinforced bioceramics (Table 2.7). Exhaustive proteomics profiling of bone cells can reveal the molecular mechanisms by which the cells interact with the composites and provide explicit and strong evidence for the evaluation of their biocompatibility [193]. The adhesive strength between bone cells and a bioceramic implant can be quantified by techniques such as AFM to reflect the implant's *in vivo* performance [363]. Future studies entailing these two aspects would greatly contribute to our understanding of the biocompatibility of newly developed bioceramic-based systems.

3. *Assessment of the composites' in vivo biological properties.*

It can be difficult and dubious to extrapolate the *in vivo* performance of a bone substitute from *in vitro* studies. Rigorous *in vivo* testing through the use of animal models is usually a must to determine if a bone graft material conforms to the stringent clinical requirements on safety and efficacy. A comparison of the animal models commonly used to assess bone substitutes can be found in a review article by Pearce et al. [364].

4. *Evaluation of the findings in other composite systems.*

Future investigations can be extended to composite systems based on other bioceramics and / or reinforced with other nanostructures to further evaluate the findings of this research. Examples of other typically used bioceramics in bone tissue engineering can be found in Table 2.2 and Table 2.3. Other 1D and 2D nanosized reinforcements, being either as-synthesized or modified, can be utilized to verify the toughening mechanisms proposed in this thesis.

5. *Exploration of other strategies for incorporating nanomaterials in bioceramics.*

To maximize the enhancement effects caused by nanomaterial addition, innovative strategies should be proposed to achieve homogeneous mixture of the two phases and sufficiently strong bonding strength between them. Growing one phase *in situ* in the vicinity of the other can be a promising approach to simultaneously realize the two aforementioned objectives [[189](#), [191](#)].

Appendix

This section presents supplementary information for two chapters of this dissertation. Fig.A1 – Fig.A5 are relevant to Chapter 5, and Fig. A6 – Fig.A11 support Chapter 6.

Fig. A.1 XRD pattern of thermally etched NS pellet sintered at 1000 °C.

Fig. A.2 TFT curves of (a) MS, (b) NR-L, (c) NR-H, and (d) NS pellets in the visible spectrum.

Fig. A3 (a) Raman spectra of NR-H sintered at 900 °C and 1100 °C. (b) Hydroxyl absorption band of the two pellets displayed in normalized intensity.

The peak at 961 cm^{-1} is attributed to the ν_1 symmetric stretching vibration mode of PO_4^{3-} . The peaks at 430 cm^{-1} , 590 cm^{-1} , and 1047 cm^{-1} correspond, respectively, to the bending ν_2 , the anti-symmetric bending ν_4 and the anti-symmetric stretching ν_3 modes of the PO_4^{3-} ions. The hydroxyl absorption band is observed at 3574 cm^{-1} [305].

Fig. A4 Optical images of HA pellets after culture L929 cells on their surfaces for 48 h: (a) NR-H/950, (b) NR-L/950, (c) NS/950, (d) MS/950, (e) MS/1100. Images in d1-3 and e1-3 were taken at different magnifications to demonstrate degree of cell detail that could be observed.

Fig. A5 Optical images of MG63 cells after being cultured on MS/1100 (a, c) and TCP (b, d) for 24 h (a, b) and 72 h (c, d).

Fig. A6 FESEM images of raw CNTs (a), S-CNTs (b), and calcined S-CNTs (c).

Fig. A7 Macroscopic image of HA and HA-based composite pellets sintered in a 10 mm die at 1100 °C.

Fig. A8 XPS Si 2p spectrum of sintered 1 wt.% S-CNT/HA pellet.

Fig. A9 TEM/HRTEM (b, c) and STEM (a, d) images of sintered 1 wt.% S-CNT/HA (a–c) and 1 wt.% R-CNT/HA (d) samples.

Fig. A10 Macroscopic image of R-CNT and S-CNT dispersions in two different solvents. Concentration: 1 mg/mL.

Fig. A11 FESEM images showing the fracture surface of a sintered 1 wt.% R-CNT/HA pellet. Panel (a) and (b) represent two different locations.

List of Publications

Journal publications:

1. **Z. Li**, N.W. Khun, X.-Z. Tang, E. Liu, K.A. Khor, Mechanical, tribological and biological properties of novel 45S5 Bioglass® composites reinforced with *in situ* reduced graphene oxide, *J. Mech. Behav. Biomed. Mater.* 65 (2017) 77-89.
2. **Z. Li**, X.-Z. Tang, W. Zhu, B.C. Thompson, M. Huang, J. Yang, X. Hu, K.A. Khor, Single-step process toward achieving superhydrophobic reduced graphene oxide, *ACS Appl. Mater. Interfaces* 8 (2016) 10985-10994.
3. **Z. Li**, B.C. Thompson, H. Hu, K.A. Khor, Rapid fabrication of dense 45S5 Bioglass® compacts through spark plasma sintering and evaluation of their *in vitro* biological properties, *Biomed. Mater.* 11 (2016) 065006.
4. **Z. Li**, H. Hu, K.A. Khor, Design of air aging induced surface patterns on 45S5 Bioglass® compacted by spark plasma sintering, *J. Non-Cryst. Solids* 445-446 (2016) 69-76.
5. **Z. Li**, B.C. Thompson, Z. Dong, K.A. Khor, Optical and biological properties of transparent nanocrystalline hydroxyapatite obtained through spark plasma sintering, *Mater. Sci. Eng. C* 69 (2016) 956-966.
6. R. Varghese Hansen, **Z. Li**, K.A. Khor, L. Zheng, J. Yang, Tuneable electrochromism in weavable carbon nanotube/polydiacetylene yarns, *Carbon* 106 (2016) 110-117.
7. R. Li, **Z. Li**, Z. Dong, K.A. Khor, A review of transmission electron microscopy of quasicrystals—how are atoms arranged?, *Crystals* 6 (2016) 105.
8. N.W. Khun, **Z. Li**, K.A. Khor, J. Cizek, Higher in-flight particle velocities enhance in vitro tribological behavior of plasma sprayed hydroxyapatite coatings, *Tribol. Int.* 103 (2016) 496-503.
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10. Z.-Z. Luo, Y. Zhang, C. Zhang, H.T. Tan, **Z. Li**, A. Abutaha, X.-L. Wu, Q. Xiong, K.A. Khor, K. Hippalgaonkar, J. Xu, H.H. Hng, Q. Yan, Multifunctional 0D-2D Ni₂P nanocrystals-black phosphorus heterostructure, *Adv. Energy Mater.* (2016) 1601285.
11. Y. Zhang, H. Wang, Z. Luo, H.T. Tan, B. Li, S. Sun, **Z. Li**, Y. Zong, Z.J. Xu, Y. Yang, K.A. Khor, Q. Yan, An air-stable densely packed phosphorene-graphene composite toward advanced lithium storage properties, *Adv. Energy Mater.* 6 (2016) 1600453.
12. C. Zhang, Z. Peng, **Z. Li**, L. Yu, K.A. Khor, Q. Xiong, Controlled growth of bismuth antimony telluride Bi_xSb_{2-x}Te₃ nanoplatelets and their bulk thermoelectric nanocomposites, *Nano Energy* 15 (2015) 688-696.
13. M. Huang, **Z. Li**, J. Wu, K.A. Khor, F. Huo, F. Duan, S.C. Lim, M.S. Yip, J. Yang, Multifunctional Alumina Composites with Toughening and Crack-Healing Features via Incorporation of NiAl Particles, *J. Am. Ceram. Soc.* 98 (2015) 1618-1625.

Conference publications:

1. **Z. Li**, B.C. Thompson, H. Hu, K.A. Khor, In vitro biological properties of 45S5 bioglass fabricated by spark plasma sintering (SPS), *Frontiers in Bioengineering and Biotechnology*. Presented in: 10th World Biomaterials Congress, May 17-22, 2016, Montreal, Canada. DOI: 10.3389/conf.FBIOE.2016.01.02398.
2. **Z. Li**, N.W. Khun, X.-Z. Tang, K.A. Khor, Novel graphene nanoplatelet reinforced 45S5 bioglass composites fabricated by Spark Plasma Sintering (SPS), *Frontiers in Bioengineering and Biotechnology*. Presented in: 10th World Biomaterials Congress, May 17-22, 2016, Montreal, Canada. DOI: 10.3389/conf.FBIOE.2016.01.01797.
3. **Z. Li**, K.A. Khor, Transparent hydroxyapatite obtained through spark plasma sintering: optical and mechanical properties, *Key Eng. Mater.* 631 (2015) 51-56. Presented in: the 26th Symposium and Annual Meeting of the International Society for Ceramics in Medicine, November 6-8, 2014, Barcelona, Spain.
4. M. Huang, **Z. Li**, J.L. Yang, F. Duan, S.C. Lim, M.S. Yip, Toughening and self-healing of alumina ceramics via spark plasma sintering, presented in: Materials Science and Engineering 2014 (MSE 2014), September 23-25, 2014, Darmstadt, Germany.

Manuscripts in preparation:

1. **Z. Li**, S.G. Bi, B.C. Thompson, R. Li, C.Y. Yue, K.A. Khor, Reinforcing hydroxyapatite with silica-coated carbon nanotubes, *in preparation*.

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