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Controlled release of drugs in electrosprayed nanoparticles for bone tissue engineering

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Abstract

Generating porous topographic substrates, by mimicking the native extracellular matrix (ECM) to promote the regeneration of damaged bone tissues, is a challenging process. Generally, scaffolds developed for bone tissue regeneration support bone cell growth and induce bone forming cells by using natural proteins and growth factors. Limitations are often associated with these approaches such as improper scaffolds stability, insufficient cell adhesion, proliferation, differentiation and mineralization with less growth factor expression. Therefore, the use of engineered nanoparticles has been rapidly increasing in bone tissue engineering applications. Nanomaterials developed using electrospray technique has an advantage over other conventional methods as it generates particle sizes in micro/nanoscale range. The size and charge of the particles are controlled by regulating the polymer solution flow rate and electric voltage. The unique properties of nanoparticles like large surface area to volume ratio, small size and higher reactivity tends to attract the field of biomedical engineering. These nanomaterials are extensively used for drug delivery, as therapeutic agents, mimicking ECM, restoring and improving the functions of damaged organs. The controlled and sustained release of encapsulated drugs, proteins, vaccines, growth factors, cells and nucleotides from nanoparticles have been well developed in nanomedicine. This review provides an insight into the preparation of nanoparticles by electrospraying and illustrates how these nanoparticles are used in drug delivery for promoting bone tissue regeneration.

Keywords: Electrospray; nanoparticles; drug delivery systems; bone tissue; regenerative medicine.

1. Introduction

Nanotechnology is a multidisciplinary branch of science involving biomedical, pharmaceutical, chemical, materials, polymers, optical and electrical engineering. In the biomedical field, it has a vital role in the drug delivery, diagnostic imaging and regenerative medicine [1]. In particular, nanotechnology has been developed in regenerative medicine to improve bone tissue engineering (BTE). It aims to overcome some of the limitations related to bone tissue regeneration techniques such as inadequate mechanical strength of temporary frameworks (scaffolds), unstable production of growth factors, lack of stimulation of cell adhesion and osteogenic differentiation at the damaged site. The gap between conventional medicine and engineering aspects has been filled by biomedical engineering. The size, structure and surface area to volume ratio of nanoparticles are inter-related to the biological activity and functionalization. Nanoparticle research is therefore an area of great scientific attention due to its potential applications in tissue engineering and many research articles (>1000) related to regenerative medicine which have been published over the past few decades [2].

Bone regeneration is a well-structured, complex physiological process in normal fracture healing and includes a series of events involving various cell types, intra and extracellular signaling pathways that follow a defined temporal and spatial sequence to restore and remodel the structure of skeleton [3]. There are many potential problems that can occur during bone tissue regeneration including osteoporosis, avascular necrosis and atrophic nonunion. The treatment methods currently applied to overcome these issues include traditional autologous bone graft, allograft implants, free fibula vascularized grafts, application of growth factors to stimulate differentiation of cells, distraction osteogenesis and application of osteoconductive scaffolds [4]. When there are large defects, a two-step process called the Masquelet technique is used as

alternative method to promote bone tissue regeneration. This technique involves radical debridement of the soft tissue and insertion of a cement spacer into the defect in the bone region. After about 6-8 weeks, the inserted spacer is removed and the membrane, which has formed around it, acts as a chamber for the insertion of non-vascularized autografts [5]. During distraction osteogenesis, regeneration of bone is induced between slowly distracted osseous surfaces. This technique has been applied to treat limb length deformities, bone loss or any other bone discrepancies involving external fixators. However, these approaches have certain problems associated with psychology of patients undergoing lengthy and painful treatments of bone distraction osteogenesis [6]. In the autologous bone grafting method, the patient's own tissues, which are histocompatible and non-immunogenic, are used to reduce the possibility of immunoreactions of the grafted tissues. However, harvesting of a patient's own tissues requires further surgical procedures with potential complications and discomfort faced in addition to quantity restrictions and associated costs. Allogenic bone grafts are acquired from living donors or human cadavers and are used to overcome the limitations related to harvesting process and graft material quality, but are susceptible to rejection. Bone graft substitutes are the alternatives for autologous or allogeneic grafts. The bone grafts are generally made using natural or synthetic biomaterials, which enhance the migration, adhesion, growth and differentiation of cultured bone cells for bone tissue regeneration. The commonly used bone substitutes are made of hydroxyapatite (HA), β -tricalcium phosphate (β -TCP), collagen, glass ceramics and calcium-phosphate cements [4]. For reconstructing large bone defects or failures there is a requirement for considerable structural scaffold which is an alternative for cortical auto-allografts made of cylindrical titanium or metallic mesh cages combined with autologous bone grafts or demineralized bone matrix and cancellous allografts [7].

Drug delivery systems include the release of encapsulated therapeutic agents into the body by improving its efficiency and controlling the site, rate and time of drug release. The controlled delivery of drugs in an adequate time period without degradation of non-released drugs is essential for tissue regeneration. Drugs within their optimal concentration range will have maximum benefit and if the concentration is outside that range they might be toxic or of limited therapeutic benefit. The conventional methods for administering drugs include injection, oral ingestion, implantation and transdermal delivery. Pharmaceutical industries spend increasing amounts of money in developing new and more efficient methods for delivering successful drugs. The average cost and duration for developing a novel drug delivery system requires \$20–30 million and about 3–4 years. However, for new drug it requires ~\$500 million and about 10–12 years. Worldwide the cost of drug delivery products was estimated to be more than \$130 billion in 2012 and \$220 billion in 2015 with an annual increase rate of 37% [2, 8]. The need for efficient delivery of drugs with fewer side effects has driven researchers to innovate new drug delivery systems with an emphasis on emerging new technologies. They focus mainly on minimizing the drug degradation or loss, increase the bio-availability of drug, preventing side effects and improving drug accumulation in the targeted zone. Drug carriers are the substances used to improve the effectiveness of drug delivery and target the damaged sites. They can be made stimuli responsive, slowly degradable and also capable of directing the drug loaded system to the targeted site either by active or passive targeting mechanisms. These drug carriers can comprise of soluble synthetic polymers, biodegradable liposomes, micro/nanoparticle, nanofibers, dendrimers and micelles. Nanoparticles derived from poly (lactic-co-glycolic) acid (PLGA), polyglycolic acid (PGA), polylactic acid (PLA) are commonly applied biomaterials for delivering osteoinductive factors, plasmid DNA (for gene therapy) or anti-inflammatory drugs to

enhance bone tissue regeneration [9-11]. The size of a molecule has a significant role in delivering the drugs effectively to the particular site of the organs.

Pharmaceutical industries face major challenges when large molecules degrade rapidly in the bloodstream or gut when delivered orally or when transdermal drug delivery is not possible due to molecule size above 500 Da. These drawbacks can be improved by the active or passive targeted delivery of drug carrier systems. These targeting mechanisms are designed to regulate the drug release kinetics, to avoid nonspecific delivery of drugs and consequent side effects thereby increasing therapeutic efficiency. In some cases, like pain, the sustained release of drugs is not effective and instead pulse release is more desirable. This review summarizes the recent progress of electrohydrodynamics atomization (EHDA) method applied for the nanoformulation of micro/nanoparticles using electrospraying. This technique has been applied for the encapsulation of several therapeutic agents onto biodegradable polymeric nanoparticles to provide sustained and control release profiles with improved encapsulation efficiency. The fabrication of particulate materials using electrospray methods including the size, shape, composition, structure and morphology has also been discussed for BTE.

2. Fabrication of nanoparticles

Synthesis of nanoparticles involves various methodologies like solvent evaporation, precipitation, single and double emulsion, electrospraying, porous glass membrane emulsification, sol-gel and coacervation are used for fabricating micro/nanostructures. Among these techniques the most popular are emulsion methods, which involve the dispersion of molecules in a polymer solution and then undergo emulsification to attain micro/nanosized droplets, which are later dried after removing solvents. The major drawback of this technique is denaturation of protein based drugs due to the use of organic solvents. This causes variability in

drug loading and encapsulation efficiency, size and surface morphology of particles, which are not homogeneous. Whereas, nanoparticles synthesized by electrospray technique or EHDA have the potential method to overcome the drawbacks of conventional methods such as lower efficiency, difficulty in fabricating particle size below 100 nm, rate and time of drug delivery, difficulty in encapsulating hydrophilic drugs and degradation of drugs due to its exposure with organic solvent, high temperature and biomolecules. Owing to the simple and flexible experimental setup, it has been effectively employed to prepare nanoparticles with controllable size, structure, morphology and compositions. All these characteristics make electrospray technique a fascinating tool for generating nanoparticles in drug delivery systems [12, 13]. Patients with organ failure currently have high morbidity and limited options for treatment but bioengineering of scaffolds (by EHDA) to serve as replacement tissue may be a viable option for them [14].

Electrospraying works on the principle of applied electric fields. The applied high voltage generates the electrostatic force inside the liquid droplet, which counteracts with the surface tension of the liquid droplet. The liquid getting to the needle tip develops into a Taylor Cone and discharges a liquid jet by means of its apex. According to Coulomb's Repulsion of Charges, the highly charged liquid droplets disperse radially on the collector plate. These self-dispersing charged droplets have the higher deposition efficiency when compared with the uncharged spray. The size of the droplets ranges from hundred micrometers to around tens of nanometers. The droplet size can be precisely controlled by regulating both the flow rate and applied voltage. In practice, despite the electrospray technique enabling better control over structure, size and composition of particles as compared to other traditional fabrication methods, it is also associated with some disadvantages including low throughput of the methods and yields in the

range of milligrams/hour. Multi-jet modes have been used to obtain similar size and morphology of nanoparticles as in a single jet mode, with an increased yield ranging from milligrams to grams per hour [15].

Nanoparticles prepared by using the electrospray method are suitable for both industrial and scientific research. The technique is used widely in micro/nano film deposition, encapsulation of drugs or nanocapsule formation. The research on micro/nano encapsulation of drugs has been extensively developed due to its potential in the field of biomedical engineering such as controlled delivery of drugs, diagnostics and imaging using nanoparticles [16]. Drug-encapsulating nanoparticles are fabricated using natural/synthetic polymers and bioactive molecules. The physical morphology, shape, size, construction and composition of these micro/nanostructures can be varied by different methods and choice of materials. These nanostructures are administered in multiple ways, including: oral, inhalation and local injection. **Figure 1** shows a) the scanning electron microscope (SEM) and b) transmission electron microscope (TEM) images of polymer particles developed by electrospraying technology [17].

3. Type of materials used for fabricating nanoparticles

A material that improves bone tissue regeneration has a potential in clinical applications for treating several bone defects. Porous material scaffolds made of polymer and bioceramic components are engineered to support bone tissue for cell proliferation and mineralization. These engineered scaffold materials that can mimic both biological and mechanical context of native bone tissue matrix and provides vascularization of large tissues are of major concern. Nanoscale materials used to recreate scaffolds with novel functionality are emerging as interesting

candidates in bone tissue regeneration. Table 1 illustrates variety of materials used for fabricating nanoparticles in BTE.

3.1 Natural polymers

Electrohydrodynamics atomization techniques have been used to fabricate nanoparticles comprising a variety of biomaterials in drug delivery systems and tissue engineering applications (**Table 1**). Naturally occurring polymers that are used are generally non-toxic, highly responsive and biodegradable. The polymers are also capable of remodeling themselves based on their intrinsic properties by inducing the secretion of enzymes by cells [12]. Chitosan is a linear polysaccharide derived from chitin, which is found in crustacean shells (e.g. Shrimps, crabs). The primary amino groups of chitosan can protonate at low pH exhibiting biocompatible, biodegradable, antibacterial, analgesic, mucoadhesive and hemostatic properties [18]. Chitosan enhances bone mineral deposition by osteoblasts and exhibits low immunogenicity with better mechanical property. Hence, it has been widely explored for bone regeneration. Arya et al. obtained ampicillin loaded chitosan particles with a particle size of 520 nm using electrospraying technique. They optimized fabrication parameters such as applied voltage, needle gauge, distance from tip of needle to the collector plate and concentrations of acetic acid and chitosan solutions for generating reproducible chitosan micro/nanoparticles and enhancing their potential in drug delivery systems [19]. In a current study, chitosan nanoparticles loaded with dexamethasone (Dex), which is one of the glucocorticosteroid improved bone formation by promoting osteoblast differentiation and mineralization. The sustained delivery of Dex from chitosan nanoparticles significantly enhanced osteogenic differentiation of human mesenchymal stem cells (hMSCs) for bone tissue regeneration [20]. Zhang et al. were able to prepare solid nanoparticles by a one-step electrospraying method using chitosan/acetic acid solution at various concentrations, which in

turn produced different particle sizes [21]. Chitosan nanoparticles doped with boric acid of diameter 175 nm were synthesized using tripolyphosphate for its application in BTE. The release profiles of boron from scaffolds were found to be an early burst release for first 5 hrs followed by slow release over 120 hrs. Boron based nanoparticles loaded into chitosan scaffolds act as an osteoinductive agent by supporting proliferation and differentiation of preosteoblastic cells [22]. Dexamethasone disodium phosphate (DXP), an osteoinductive drug encapsulated within chitosan (CN) nanoparticles, was coated onto porous baghdadite (BD; $\text{Ca}_3\text{ZrSi}_2\text{O}_9$) scaffolds using nanostructured hydrogels gellan and xanthan (GX) enhanced bone regeneration. The average size of DXP-CN nanoparticle obtained was 521 ± 21 nm. The drug release profiles of DXP-CN-GX hydrogels coated with BD scaffolds showed a sustained release of DXP with an initial burst delivery. DXP released from nanostructured hydrogels integrated with BD scaffolds improved the osteogenic differentiation of cells when compared to uncoated BD scaffolds [23].

Collagen is a favourable biomaterial, which is extensively used in bone restoration due to its biocompatibility and biodegradability. Though the conversion of collagen into nanoparticles is challenging, current studies have shown that under ambient temperature and controlled pressure, it is possible to prepare collagen nanoparticles using one step electrospray deposition. Ethyl cellulose (EC) is a water insoluble polysaccharide extensively used as an encapsulated drug delivery system based on its properties: biocompatibility, non-toxic, non-irritant and its thin film shows good mechanical properties and flexibility. EC can be used as a stabilizer, binder, water conserving agent and dispensing agent. In addition, it is used for sustained release of drugs [24]. Park et al. fabricated HA nanoparticles using 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO)-oxidized bacterial cellulose scaffold as a dispersant in aqueous medium for bone tissue regeneration. Hybrid biocomposite scaffold significantly enhanced cell adhesion, migration,

growth, mineralization and differentiation by confirming it to be a possible candidate in bone tissue regeneration [25].

3.2 Synthetic polymers

The key advantages of synthetic polymers over natural polymers include tailoring of mechanical properties and the degradation process. Moreover synthetic polymers are highly attractive as they can be synthesized with desired shapes and provide a possible environment for cell growth in tissue engineering. Synthetic polymers are generally low in cost and have a highly reliable source of materials. The micro/nano particles developed, act as drug carriers with a potential to deliver drugs in a controlled and sustained manner. Polyesters represent the majority of biodegradable synthetic polymers extensively studied for its application in tissue engineering. Among the polyester family, electrosprayed nanoparticles of PGA, PLA, and poly- ϵ -caprolactone (PCL) and their copolymers are widely applied in drug delivery systems owing to their biocompatibility and biodegradability. PGA is a biodegradable, aliphatic polyester and thermoplastic polymer with high crystallinity. PGA displays a highly crystalline nature, which is responsible for slow degradation process and insolubility in water. The solubility of the polymer depends on its molecular weight and high molecular weight PGA is insoluble in most organic solvents except the fluorinated solvents (eg. hexafluoroisopropanol). PLGA is a biodegradable and biocompatible synthetic copolymer fabricated by electrospraying method acts as an excellent drug delivery carrier for tissue regeneration. PLGA polymers are widely used in drug delivery systems, tissue engineering and biomedical devices as approved by Food and Drug Administration (FDA) [26]. Berkland et al. fabricated PLGA nanoparticles for tissue regeneration using flow limited field injection electrospray method in acetonitrile with a flow rate of 1 ml/h, showed that the injection charge method inducing the ionic state of PLGA

solution is highly efficient when compared to the conventional electrospray methods [27]. Lee et al. developed anodized titanium implants layered with PLGA nanoparticles and fibroblast growth factor using electrospray to study its osseointegration in rabbit tibiae. The particle size of PLGA nanoparticles coated on the surface of implants was observed to be 268.6 ± 93.2 nm. The combination of biodegradable PLGA nanoparticles with fibroblast growth factor coated onto the surface of titanium implants resulted in the formation of new tissue next to the surface of the bone implant [28]. Almeria et al, developed monodisperse PLGA particles of size 60nm-2 μ m using electrospray drying route for tissue engineering application. By selecting appropriate electrospray parameters and polymer solution properties, it is possible to control the particle size and monodispersity. PLGA particles obtained were spherical in shape encapsulated with or without active agents, tailoring it to a particular biomedical application [29].

PCL is a semi crystalline, biodegradable, biocompatible polymer with -60 °C as a glass transition temperature. It is also chemically stable, mechanically strong and permeable. Due to these properties, PCL is accepted by the FDA for its application in drug delivery systems and tissue engineering. The polymer is highly hydrophobic in nature which makes it more difficult for the encapsulation of macromolecules like DNA, peptides, proteins and drugs [30]. Gandhimathi et al. developed biocomposite scaffold PCL/silk fibroin/ascorbic acid nanoparticles loaded with dexamethasone (DM) to improve the environment for human adipose derived stem cells (hADSCs) differentiation into osteogenesis and increased secretion of minerals for bone tissue regeneration [31]. **Figure 2** illustrates the cumulative release profiles of DM from electrosprayed nanoparticle scaffolds [31]. Polylactides (PLAs) have properties similar to PLGAs, however, the highly crystalline nature of poly (l-lactide) is responsible for its slow degradation process, whereas poly (d,l-lactide) exhibits a lower degree of crystallinity. Hence

poly (l-lactide) is used extensively as a drug delivery system due to its release kinetics and long term degradation process of the polymer [32]. Ikeuchi et al. studied phase separation process by electrospraying 1% PLA and chloroform solution in 5% ethanol at high humidity, which resulted in PLA particles of high surface porosity. The variation in polymer solution flow rate and humidity of electrospray environment influenced the diameter and surface porosity of the nanoparticles. The direct 3D patterning method combined with nanofibrous hollow structure of PLA particles showed improved cell interaction and biodegradability for its application in tissue regeneration [33].

3.3 Polymer composites

Nanoparticles incorporated into the polymer scaffolds enhance the functional characteristics of the biocomposites because of the interaction between the scaffolds and particles. These composites provide combined functional chemical and biological properties. The combination of nanohydroxyapatite (n-HA) particles with chitin, chitosan, collagen, fibrin, gelatin, PCL, PLA, PLGA, polyamide, and polyvinyl alcohol based composite scaffolds have been widely explored for bone graft substitutes. Hydroxyapatite based polymer composite scaffolds proved to be the promising candidate for bone repair and regeneration due to their chemical interaction, alkaline phosphatase (ALP) activity, biocompatibility, mineralization effect, biodegradability and mechanical property [34, 35]. Venugopal et al. developed polymeric nanofibrous scaffolds, which are loaded with 4% HA nanoparticles on the surface using electrospray method, in order to enhance cell adhesion, interaction, proliferation, migration and differentiation for bone tissue regeneration [37]. PCL nanofibers are electrospun on the rotating drum together with n-HA particles (52 nm) electrosprayed on them to get diameters of 420 ± 15 nm. Figure 3 shows the schematic representation of both electrospinning and electrospraying techniques for fabricating

PCL/HA nanofibrous scaffolds [36]. These PCL/HA nanofibrous composites enhanced the differentiation of hMSCs into osteogenic lineages for BTE. Poly (3-hydroxybutyrate) (PHB)/hydroxyapatite (PHB/HA) blend composite prepared by entrapping HA particles within the fibers were mechanically strong while a PHB/HA (spray) composite prepared by spray deposition of HA particles on the surface of fibers were highly porous and showed better biological properties by creating a rough surface for the efficient cellular interactions [37]. Gupta et al. fabricated Poly (L-lactic acid)-co-poly (3-caprolactone) (PLACL) and gelatin based biocomposite substrates incorporated with HA nanoparticles using electrospinning and electrospraying methods to achieve improved osteophilic environment for the proliferation and mineralization of osteoblasts. The biocomposite nanofibrous substrate coated with HA nanoparticles aided to achieve rough surface morphology suitable for cell adhesion, growth and also improved the mechanical stability of scaffold. It was reported that after the 30th day of cell culture the mineral formation on the surface of the scaffolds created a thick layer with the ECM production by cells. The process of mineralization refers to the deposition of calcium and phosphorous salts on the ECM, where matrix containing anionic molecules takes up Ca^{2+} and phosphate ions aids for nucleation and growth leading to calcification for bone tissue regeneration [38].

Korina et al. fabricated composites consisting of PHB nanofibers combined with Titanium dioxide (TiO_2) particles and chitosan oligomers using electrospinning, electrospraying and impregnation methods. The polymer composites obtained were compatible and provided a favorable environment for the development of hMSCs [39]. Francis et al. studied the combined effect of electrospinning and electrospraying method to fabricate polymeric composite material for bone tissue regeneration. Electrospun gelatin (Gel) nanofibers with electrosprayed HA

nanoparticle scaffolds showed better cell proliferation, interaction, enhanced osteoconductive and osteoinductive effect for bone tissue regeneration [40]. The study by Zhao et al. showed that uniform coating of calcium phosphate (CaP) on the surface of electrospun PCL-Keratin composites facilitated improved cell-matrix interactions. Further, incorporating HA nanoparticles on or within the surface of CaP coated electrospun scaffold enhanced mechanical property, biocompatibility and proliferation rate of hMSCs. The presence of CaP coating on the surface of electrospun scaffold proved to be a potential bioengineered polymeric composite for differentiation of cells into osteogenic lineage [41]. Ramier et al. synthesized electrospun PHB/Gel biocomposite scaffold and electrosprayed HA nanoparticles on the surface of the scaffold material for its application in orthopaedic defects. The hMSCs seeded on PHB/Gel/HA scaffold exhibited faster cell adhesion, proliferation, migration and osteogenic differentiation. The existence of electrosprayed HA nanoparticle induced the rate of mineralization activity by depositing high level of calcium on the surface of polymeric scaffold and makes the biocomposite scaffold a suitable candidate for bone tissue regeneration [42].

3.4 Inorganic and metallic materials

Inorganic nanoparticles have been developed for various applications due to their intriguing properties such as nanosize, optical properties, superparamagnetic and biological properties [43]. Encapsulation of inorganic nanoparticles such as silica, alumina, carbon black, titanium, calcium carbonate, magnetic iron oxides onto the polymeric matrix has been reported by several studies [44-46]. In a recent study, mesoporous silica based nanoparticles combined with bioactive factors were synthesized by incorporating bone forming peptide derived from bone morphogenetic proteins (BMPs)-7 for improving bioactivity and osteogenic factor delivery. Peptide modified silica based nanoparticles resulted in better cell proliferation, adhesion and

ALP activity, thus proved to be a likely candidate for bone repair, regeneration and bio implant coating [47]. Amorphous polyphosphate nanoparticles combined with calcium ions were synthesized in a globular size of approximately 100 nm. Polyphosphate nanoparticles regulates adenosine triphosphate (ATP) levels when exposed to sarcoma osteogenic osteoblast like cells and also acts as a metabolic fuel for the hydrolytic cleavage of phosphor-anhydride linkages, which further results in the formation of HA on the osteoblast plasma membranes [48]. The *in vivo* study of selenite incorporated bone mineral nanoparticles showed the inhibition of osteosarcoma growth and improved the healthy tissue function in nude mice model, by sustained release of selenite from the nanoparticles [49]. Mellor et al. prepared 0% or 20% TCP nanoparticles loaded PLA nanofibrous scaffolds for its application in osteochondral defects using hADSCs. Histological analysis showed that the local differentiation of hADSCs in a chondrogenic culture medium produces cartilage in the layers containing 0% TCP and calcified tissue in the layers comprising 20% TCP [50].

Bagchi et al. developed perovskite ceramic nanoparticles as fillers in biocomposite polymeric scaffolds for its use in orthopaedic treatments due to its enhanced chemical, mechanical and electrical properties. Three different perovskite particles such as barium titanate (BT), calcium titanate (CT) and strontium titanate (ST) were incorporated into PCL scaffolds to improve osteogenic proliferation. The study demonstrated that, the use of these perovskite ceramic nanoparticles might be a capable technique for developing suitable bone substrates in bone tissue regeneration [51]. Gelatin modified with calcium phosphate nanoparticles and PCL nanofibers were incorporated into fibrous gelatin matrix for facilitating cell spreading, differentiation and mineralization on the surface of scaffold for its use in BTE. PCL scaffold serves as the skeleton for enhancing the bioactivity of biocomposite constructs in bone tissue regeneration due to its

mechanical property [52]. Hickey et al. studied the effects of magnesium oxide (MgO) nanoparticles added with HA nanoparticle-poly (L-lactic acid) (PLLA) composites for orthopedic tissue engineering [53]. The HA-PLLA composites added with MgO nanoparticles exhibited better mechanical properties, proliferation and adhesion of osteoblast, fast degradation of MgO and non-toxicity. These potential characteristics of MgO nanoparticles proved to be a suitable candidate for cancellous bone applications [53]. Nguyen et al. synthesized porous gelatin-pectin (Gel-P) scaffold loaded with biphasic calcium phosphate (BCP) nanoparticles for improving the osteogenic differentiation of cell using rabbit model. The biodegradable Gel-P-BCP composite material had well defined interconnected pores which facilitates osteoconductivity and promotes cell adhesion, proliferation and viability for bone tissue formation [54].

Bioactive glass (BG) scaffolds are interesting candidates currently employed in bone tissue restoration owing to their osteoconductivity, osteoinductivity and bone bonding ability. BGs are generally used as bioactive coating material on orthopedic implants, dental applications, bone filling materials and small bone implants [55-57]. In a recent study, selenium (Se) nanoparticles showed a significant role in the modulation of cell proliferation, antioxidant protection and enhanced immune surveillance. Stevanovic et al. prepared uniform, amorphous, stable selenium nanoparticles immobilized inside spherical PLGA particles (PLGA/Se). Bioactive glass based constructs coated with PLGA/Se nanoparticles using foam replica method proved to be a promising candidate in bone tissue repair and rejuvenation by promoting bone regeneration and eliminating possible infections and inflammations [58]. In another study, nanosized mesoporous bioactive glass (NMBG) nanoparticles were prepared and coated onto PLGA-calcium silicate scaffolds for better mechanical strength, drug delivery and bioactivity in BTE. The NMBG

particles of diameter 50-100 nm showed initial burst effect of ibuprofen for 8 hrs followed by a sustained release profile after 24 hrs. Further, presence of NMBG particles in the scaffold revealed better mechanical strength, cell attachment, proliferation and mineralization activity, proving it to be a multifunctional scaffold for orthopedic treatment [59]. Gold nanoparticles synthesized with different charges using amine, carboxyl and hydroxyl groups were studied for its differential cell response on hMSCs osteogenesis. Surface functionalized gold nanoparticles treated with hMSCs showed better osteogenic differentiation, cellular uptake and cell viability with no acute toxicity [60]. Kumar et al. developed hybrid nanoparticle consisting of strontium decorated with reduced graphene oxide to demonstrate their use in bone tissue formation. The hybrid nanoparticles of size 200-300 nm were then incorporated in PCL scaffolds to improve the osteogenic differentiation process in bone tissue regeneration. This composite prepared by using hybrid nanoparticles resulted in good mechanical, osteoconductive and osteoinductive properties proved to be a suitable candidate in bone tissue regeneration [61].

4. Chemical and physical properties of nanoparticles

The physiochemical properties of nanoparticles depend on various factors like size, shape, structure, composition and morphology of particles. The ratio between surface atoms to the bulk for a larger material (around 1 μm) is low compared to the nanoparticles of size in nanometer scale. Nanoparticles are generally functionalized or surface modified by capping certain functional groups on their surfaces, which has to be flexible and biocompatible to perform multiple functionalities like targeted delivery with specific rate and particle localization. Nanoparticles commonly used in medical applications, have a comparatively large functional surface that can be used to bind, carry and absorb biomolecules like proteins, drugs, cells, genes and probes. The rapid development of these nanoparticles in nanomedicine relies completely on

this flexibility. Many biomolecules are conjugated with nanoparticles *via* surface functional groups for particular functions such as drugs for chemotherapy, aiming ligand for binding cells and genes for transfection of cells. The physiochemical property of nanoparticles reveals the biological effects, toxicity and bio-distribution. These characteristics strongly determine the reactivity and surface properties of the nanoparticles. For instance the melting property of nanoscale particles differs when compared to larger molecules. The small size nanoparticles have larger surface area to volume ratio and it is known that smaller the particle size there is a decrease in melting temperature and also melting points for smaller particles are less compared to the bulk materials [62]. The chemical properties of these particles in therapeutics influence the stability, drug release and cellular uptake in tissue engineering applications. As mentioned earlier, the size of the particle increases its reactivity and larger the surface area of nanoparticle promotes the aggregation and interaction towards the biomolecules like proteins, lipids, DNA etc. Nanoparticle aggregation is due to several interactions such as van der Waals, Born repulsion, structural solvation, electrostatic interactions, hydrophobic and hydrodynamic interactions in addition to the effect of polymer properties [63]. Different sized nanoparticles aggregate depending on the aqueous medium. For example, cobalt ferrite nanoparticles of different diameters aggregates in various medium such as cell culture medium, diethylene glycol, and phosphate buffer saline [64]. They interact and adsorb the media components like proteins and ions present in the serum due to particle size, distribution and interaction with cell changes. Smaller the size of the nanoparticle, the penetration into cellular barrier is more when compared to larger size particles [65]. For instance, the size and shape of CaP nanoparticles has a major effect on osteoblast adhesion, proliferation, apoptosis, osteogenic gene expression and viability [66-68]. Xu et al. showed that HA nanoparticles of size 20 nm significantly improved cell

adhesion, proliferation, bioactivity and inhibited cell apoptosis. Further, when cells were seeded onto sphere like HA nanoparticles results showed an improved cell migration and proliferation when compared to rod like nanoparticles [68]. The mechanical property of polymeric or ceramic nanoparticle composites depends on the morphology, composition of material, orientation and structural configuration of the matrix. For bone tissue restoration, the scaffold materials should possess a suitable mechanical strength similar to that of the native bone matrix and considerably support adhesion, growth, differentiation and mineralization of osteogenic precursor cells [69, 70]. The porous nanostructured particles have greater potential for delivery of drugs, protein and genes due to their higher drug loading capacity, targeted delivery and controlled release profiles [71, 72]. Along with size, shape and surface modification, the role of nanoparticle distribution and its accumulation on specific targeted site are also under consideration in biomedical applications [73].

5. Nanoparticles in bone tissue engineering

Nanoparticles are extensively applied in biological applications based on the surface morphology, which helps to increase its affinity towards the drugs, receptors and ligands. The approach for fabricating regenerative bone scaffolds focuses on the combination of nanoparticles due to its impact on mechanical strength; biocompatibility, bioactivity of impregnated biological factors, and osteogenic properties of the scaffolds [74, 75]. Owing to these characteristics properties of nanoparticles, there is a rapid emergence of theragnostics in biomedicine. Nanoparticles are conjugated with various biomolecules for specific functions such as drug delivery for chemotherapy and ligand targeted for cell binding in imaging technology. The nano sized particles can easily penetrate through the intracellular region or the tissue across various barriers and reach the targeted site of the damaged bone tissues. They act as a payload carrier for

delivering drugs or nucleic acids in the form of DNA and RNA. The payload carrier consists of a polymeric matrix material with several functional groups, over which the therapeutic drugs or the signal emitters are conjugated for drug delivery. Nanocarriers loaded with ligands selectively bind to the receptors and form a particular disease marker over the targeted cell and then carry the drug loaded nanoparticle to the damaged site by providing interaction with the targeted cells. The drugs can be either conjugated or encapsulated on the surface of the nanoparticles whereas the ligands are always attached to the carrier surface for better interaction with the targeted cells or tissues.

The incorporation of nanoparticles onto the electrospun fibers enhances the sustained release profiles of the targeted drugs. The most widely used HA nanoparticle helps to improve cell adhesion, interaction, growth and osteoblast differentiation due to its specific properties such as surface roughness, affinity towards biological factors and increased surface area [76, 77]. Recently many research works have been focused on improving the scaffold properties using carbon nanotubes, carbon allotrope graphene and polysaccharides such as cellulose [78, 79]. TiO₂ nanotube implants for bone interface were surface treated with silver to act as an antibiotic material and prevent any bacterial infection during postsurgical periods [80]. Synthetic polymers such as polyacrylates, polyglycolides and polycaprolactone copolymers dominate over natural polymers like collagen and gelatin; based on their capability to attain a sustained release of therapeutic agents. The most extensively studied synthetic PLGA nanoparticles are applied for bone tissue regeneration in specific due to its hydrophilic surface morphology with carboxylate at its end groups. The surface properties of these nanoparticles can be modified to reach sustained and controlled drug release rates. Choi et al. has developed a surface functionalized tetracycline loaded PLGA nanoparticle for bone specific drug delivery. These nanoparticles were

<200 nm in size and had a hydrophilic surface layer. It has been shown that these surface functionalized nanoparticles can be used to obtain controlled release profiles and site specific targeted delivery [73]. In addition, these PLGA based nanoparticles can be combined with molecules, which have a higher affinity towards CaP in bone by promoting its ability to reach the specific targeted bone sites [81]. Among natural polymers, collagen can be easily modified by reacting with its functional group, including cross-links or biological grafting molecules to promote the mechanical and biological properties of the matrix. Collagen type 1 is the most extensively available component for preparing scaffold materials, which promotes migration, interaction, proliferation, adhesion, differentiation and mineralization of bone cells [82]. A chitosan nanoparticle is non-toxic, biodegradable and promotes controlled drug delivery. They act as an antibacterial agent, drug and protein carriers and gene delivery vectors. Studies show that chitosan nanoparticles prevent infection in the damaged site and improve the wound healing process. It can also be physiochemically modified to enhance the biodistribution, circulation time, targeted delivery and DNA condensation in BTE [83].

6. Drug Delivery

The drug delivery system consisting of a carrier device targets the therapeutic agent towards the damaged site and improves its efficiency by controlling the delivery time, site and rate of release [84]. Nanoparticle based on biodegradable polymers satisfy the requirements of these drug delivery systems by its ability to transfer through aerosol, biocompatibility, targeting the specific site with the controlled release rate of drug in a sustained manner along with the degradation of drugs in a required period of time. The need for controlled and targeted delivery of drugs has stimulated the exploration of several polymeric nanoparticles as biodegradable scaffolds which are planned to degrade at an estimated rate; by releasing their encapsulated drugs in the targeted

site. In simple terms, the procedure of releasing the encapsulated bioactive agent in a specific place, rate and time is known as a drug delivery system. Although there are more advancement in discovering and designing new drugs, it is hard to turn these new methods into effective clinical use. Many therapeutic agents are in limited use owing to their poor solubility, nonspecific delivery, higher toxicity and degradation. Currently, researchers work on electrosprayed nanoparticles as targeted drug delivery systems due to their effectiveness in conveying drugs, lower degradation rate and differentiation of products with reduced cost [85]. It is widely applicable in drug delivery, cancer therapies, tissue engineering, asthma and hormonal treatments, for which tailored delivery of multiple-molecule are required for therapeutics. The main advantage of using nanoparticles as drug delivery systems is that it offers targeted drug delivery into the affected site of the body. It can either be active or passive drug delivery to the site of affected tissues. The administration of these drugs *via* oral, ocular, injection and nasal routes was reviewed by Gandhimathi et al. [86]. PLGA is generally used as a carrier in drug delivery system owing to its degradability and biocompatibility into lactic acid and glycolic acid. The surface modified carrier material improves the stability, circulation of drugs throughout the body, integration of diagnostic agents and bio-specific targeting over the cellular ligands and ECM components. Though electrospraying is considered as advantageous over other conventional methods for generating nanoparticles, it requires further more development for its application in therapeutics.

Polymeric nanoparticles fabricated using various techniques depending on the application and type of drugs used for encapsulation. Nano-encapsulation of various bioactive molecules and drugs on the polymeric nanoparticles are widely used in nanomedicine. These nanoparticles have the following properties such as controlled or sustained release, biocompatibility with cells and

tissues along with subcellular size [87]. The effective response and drug delivery of nanoparticles are influenced by the following characteristics such as surface modification, particle size and molecular weight of the drugs [88]. Current technologies applied for encapsulating the drugs includes phase separation (coacervation), solvent extraction from a double emulsion through evaporation or spray drying [89]. Although these methods are comparatively more effective, the process is very slow. Whereas electrosprayed encapsulation is a suitable technique due to its rapid mass production and it can also be reduced to portable devices that are capable of encapsulating and delivering the drug based on the demands of physicians and patients.

6.1 Encapsulation of drugs into polymeric nanoparticles

Drugs are generally related to low solubility, larger particle size and high crystallinity. Fabrication of various drug particles using electrospraying technique reduces the particle size, produces amorphous structure and increases solubility by enhancing bioavailability and its administration of the particles [90]. These particles under variable temperature X-ray diffraction displayed complete recrystallization of structure into polymorphic form of drug during heating [91]. Natha et al. investigated on PLGA loaded simvastatin drug which has the potential for bone tissue restoration. The study revealed that the encapsulation efficiency was >90% with the continuous release of drug over 3 weeks [92]. Pinto et al. reviewed various methods for encapsulating bioactive molecules on electrosprayed nanoparticles [93]. Depending on the nanosystem delivery, different drugs vary in administration, biological activity and therapeutics. The bioactive molecules such as drugs, proteins, nucleic acids or peptides tend to be bound on the surface of the nanoparticles as nanospheres or encapsulated as nanocapsule. These encapsulated nanomedicines do not change its composition in blood, non-inflammatory, non-

toxic, non-immunogenic and non-thrombogenic [94]. Midhun et al. prepared particulate formulations of drug and polymeric matrices using electrospraying method. Monodispersed budesonide encapsulated onto biodegradable polymer PCL nanobeads exhibited an encapsulation efficiency of approximately 75% with a sustained and controlled release of drugs [95]. The study on electrospun PCL-Gel nanofibrous scaffolds incorporating mesoporous bioactive glass (BG) nanoparticles loaded osteogenic drug DEX showed an improved therapeutic efficacy in bone tissue regeneration. DEX loaded BG nanoparticles displayed a loading efficiency of 63% and a sustained linear release profile upto 28 days after an early burst release kinetics of ~30% in 24 hr. The osteoinductive PCL-Gel-DEX loaded BG biopolymer scaffolds exhibited a long term osteogenic drug delivery system for bone tissue formation in a calvarium model [96].

Irmak et al. developed 17β -estradiol (E2) loaded PLGA nanoparticles incorporated in chitosan-HA scaffolds to induce osteogenic differentiation of ADSCs obtained from a rat model. The E2 loaded PLGA nanoparticles showed a diameter of ~240 nm and encapsulation efficiency of 54%. A sustained release of E2 from PLGA nanoparticles on chitosan-HA scaffolds showed a substantial effect on differentiation of cells into osteogenesis [97]. A study showed that PLGA nanoparticles used to generate cohesive colloidal gel loaded with DEX, acts as an injectable bone filler to repair skeletal defects. The drug released from colloidal gel showed a highest drug loading efficiency of 20%. Injectable PLGA colloidal gels were reported as the potential osteoconductive bone fillers capable of producing sustained drug release kinetics and repairing cranial bone defects in a rat model [98]. Schematics of developing drug loaded nanoparticles such as nanospheres or encapsulated as nanocapsule is shown in **figure 4**.

6.2 Growth factors and proteins

BTE involves delivery of biological molecules like growth factors to induce the regeneration of bone in defected sites. The bone substitute when combined with highly purified proteins such as growth factors has a tremendous improvement in the bony defect without any autogenous graft harvest. They even stimulate proliferation of mesenchymal stem cells, osteoblast or osteoprogenitor cells with the active protein molecules. Growth factors can control cell growth, adhesion, migration and differentiation into osteogenic lineages. Several growth factors that include vascular endothelial growth factors (VEGF), BMPs, platelet derived growth factor, insulin like growth factor, fibroblast growth factor and transforming growth factor- beta (TGF- β) are used as drug cargo for the promotion of osteogenic cell growth and mineralization in bone tissue engineering. Osteoblast cell growth with the successive bone formation entirely depends on the signals produced by growth factors and consecutively directs the osteogenic differentiation. Directly injecting the growth factors into solution might become ineffective due to excretion from defect site and rapid diffusion. Moreover short half-life of growth factors, unstable biological activity and negligible amount of tissue penetration might result in ineffective delivery [99]. In order to overcome these limitations, delivery carriers themselves were injected or implanted as they are biocompatible, biodegradable and more suitable for protein encapsulation. Amongst various other growth factors, the most commonly used BMPs have been extensively studied for bone regeneration process. Although BMPs are the potential osteoinductive growth factors, *in vivo* administration of BMPs are more complicated due to their localised actions, rapid local clearance and short half-life period [100]. The *in vivo* studies make use of BMP-2 or combined with several other growth factors including VEGF, TGF- β which shows the maximum inductive potential for osteoblasts differentiation of hMSCs compared to other BMPs [101]. Several bone substitutes including allogenic demineralized bone matrix,

alloplastic TCP and xenogenic bovine bone minerals are used as carriers for delivering BMP-2 in animal models resulting in excellent bone repair and regeneration. Chung et al. analysed the sustained release profile of BMP-2 growth factor loaded with functionalised nanoparticle hydrogel complex comprising of fibrin gel and heparin functionalized nanoparticle for effective bone regeneration. The release profile analysis of BMP-2 loaded onto four different carrier systems were studied in which the *in vitro* profiles resulted in a controlled and sustained release of growth factor without an early burst release that would occur *in vivo* when compared to functionalized NP fibrin gel complex and fibrin gel on its own [102]. Currently, the importance of VEGF towards bone repair and restoration has been investigated in regenerative medicine. The secreted cytokine known as VEGF, functions in the development of blood vessels, migration of cells, endothelial cell growth and prevention of apoptosis. An *in vivo* study demonstrated that delivery of VEGF is an essential substance for fracture healing and cortical defect restoration process. VEGFs consisting of (A,-B and -E) plays an important part in vascular angiogenesis, whereas VEGF (-C and -D) are used to control lymphatic vessel growth. Angiogenesis occurs through sprouting angiogenesis or by initiating extravasation and vasodilation of plasma proteins; this delivers a place for the migrating endothelial cells [103]. Few studies have been done to identify the effect of VEGFs on osteogenesis with either of the polymeric materials such as PLGA, collagen or ceramic substrates for delivering VEGF. The results showed that remarkable bone tissue regeneration was obtained using PLGA along with VEGF [104]. Wang et al. synthesised core shell sphere of diameter 1 μm using PLGA in core and poly-(D, L-lactide) (PDLLA) in shell, loaded with both BMP-2 and VEGF growth factors to stimulate osteogenesis and angiogenesis respectively. PLGA/PDLLA particles showed an early burst release profiles of VEGF, followed by continuous release of BMP-2. Both the growth factors released from the

particles maintained their bioactivity with enhanced osteogenic differentiation of mesenchymal stem cells and improved growth of endothelial cells [105]. PDGF plays a significant part in differentiation of preosteoblasts into osteoblasts and also helps in proliferation of mesenchymal stem cells. PDGF-AB and -BB are the alternatives of alpha granule platelets and are released once the platelets get attached to the defected sites. Studies show that the combination of VEGF and PDGF has a positive effect on restoration of bone, which is proved based on histologic analysis [106]. Similarly BMP-2 combined with IGF-1 resulted in no osteogenic differentiation of mouse pluripotent stem cells; however, initial delivery of BMP-2 alone led to increased release of both the growth factors followed by mineralization on the matrix surface [107]. Gelatinous matrix based carriers are used in delivering TGF- β growth factor for restoration of bone models. TGF- β growth factor resulted in potential healing of bone defects both in animal and human bones [108].

Protein based therapeutic agents are effectively used for treating several bone related diseases. Owing to the short half-life period, low tissue permeability and susceptibility, elimination from blood circulation, the need for frequent injection of protein drugs occurs in order to obtain sufficient amount of therapeutic agent at the targeted site. Multiple injections of drugs results in various problems such as side effects due to high dosage and inconvenience for patients [109]. To overcome these limitations, proteins are encapsulated within different carrier systems to succeed controlled and gradual release of drugs over a sufficient period of time. Drug loaded nanoparticles fabricated by electrospraying technique delivers therapeutics *via* different routes such as injection, oral or inhalation [110]. Jiang et al. formulated PLGA based nanoparticle with poly-aspartic acid (poly-Asp) peptide sequence bound with HA, acts as a molecular tool in bone targeted applications. Also methoxy-poly (ethylene glycol) (PEG)-PLGA and maleimide-PEG-

PLGA based nanoparticles conjugated with fluorescein isothiocyanate (FITC)-tagged with poly-ASP peptide delivery system did not induce cytotoxicity and was capable of binding with bone tissues and applicable for bone disease therapy [111]. Christopher et al. prepared ferritin based nanoparticles by electrospraying 10 mM ammonium acetate with CO₂ of flow rate 0.51/min under 2-3 kV of applied voltage. The intracellular protein molecule ferritin, stores iron in a nontoxic soluble form and then releases in a controlled manner. The size of ferritin nanoparticles obtained were 13.1 nm and that of apoferritin particles were 11.8 nm [112]. The double stranded oligonucleotides encapsulated on gelatin nanoparticles showed an ionic interaction on the surface of the particles [113]. One of the novel drug carriers are the lipids and much research has been carried out on lipid based drug delivery. Osteogenic peptides derived from soluble and insoluble constituents of bone matrix initiates osteogenesis, mineralization, vasculogenesis and bone formation. These peptides showed significantly high biological activity both *in vitro* and *in vivo* conducted in diseased animal models, once immobilized in a bone matrix by either conjugating or grafting. Osteogenic peptides proved to be an alternative growth factor in functionalizing orthopedic implants for cell adhesion, proliferation, integration and mineralization of implants with nearby tissues [114].

6.3 Antibiotics

Antibiotic loaded nanoparticles are widely used in the application of treating persistent infectious diseases. The encapsulation of nanoparticles can be achieved even at nanoscale levels which is more favourable for the particles to pass through the sputum in order to reach the colonies of microorganisms present in the targeted sites. The major advantage of using nanoparticles as a carrier is to release antibiotics in a continuous manner at the targeted site and to improve the antimicrobial efficacies towards the microbes. Some commonly used bioactive molecules like

chitosan and hyaluronic acid have the capability of inhibiting the growth and adhesion of bacteria [115, 116]. The targeted delivery of vancomycin for the cell affected by bacterial infection through surface charge switching based (PLGA-PLA-PEG) nanoparticles is an example of pH responsiveness. At acidic conditions the PLA molecules tends to form a positive charge and get attracted towards the negatively charged bacterial cell wall. Furthermore, use of PEG coating helps to maintain a sustained blood circulation of nanoparticle [117]. Sun et al. used the same antibiotic vancomycin, which is resistant to *Staphylococcus aureus* and studied its antibacterial activity in collagen scaffolds encapsulated with silver nanoparticle and BMP-2 for infected bones *in vitro* [118]. Arya et al. studied the synthesis of ampicillin loaded chitosan particles and found that it had targeted encapsulation efficiency of 80.4% and drug loading of 50.25% with a particle size of 520 nm. Initially the release of drug was increased followed by a continuous release up to 120 hours. The drug release undergoes few steps initially diffusion of drug molecules followed by degradation of polymer matrix [19].

Osteomyelitis is an infectious bone disease which causes inflammation, destruction of bones and necrosis. Acute bone infections are treated with intravenous antibiotics whereas chronic infections require operative treatments with intravenous antibiotics, debridement, local antibiotics and removal of metals. Lalidou et al. revealed that the bone grafts can be used as carriers for delivering local antibiotics for the inhibition of bone infectious diseases [119]. Apart from the treatments applied for chronic infections, several antibiotic delivery vehicles are recommended by most of the surgeons. Polymethylmethacrylate is a commonly used polymer material, which is loaded with antibiotics to act as an antibiotic delivery vehicle for orthopaedic defects. Few clinical studies have been reported that antibiotic loaded with HA and calcium sulfate can be used for local delivery of antibiotics and also it is known to be a safe method for

bone tissue regeneration. A greater amount of antibiotics have been delivered by them locally along with the serum concentrations in safe levels, aids bone repair, destroys the dead space and it doesn't require any surgery for their removal of infection [120]. Although, targeting intracellular colonies by antibiotic therapy is more difficult to prevent any infections rather than destroying only the pathogens that are present in the bone matrix. Uskokovic et al. studied intracellular delivery of plasmids using calcium phosphate nanoparticles known as non-viral agents loaded with clindamycin phosphate destroying the *Staphylococcus aureus* colonies within osteoblast cells to a greater degree when compared to the administration of pure antibiotics [121]. Prevention of invasive fungal infections caused during bone marrow transplantation is a major concern in clinical treatments. However, antifungal therapies are often toxic and the infection caused by fungi is detected in advanced stages creating serious challenge for an effective treatment in BTE. Amphotericin B (AmB) is a polyene antifungal intravenous drug mainly used for fungal infections, which are associated with ergosterol, to form pore that tends to leak potassium and thus causes fungal cell death. The interaction of AmB with the membrane sterols tends to aggregate and forms transmembrane channels. Intermolecular hydrogen bond interaction with the carboxyl, amine and the hydroxyl group helps to stabilize the channel, destroy the fungal activity and allows cytoplasm to leach out. AmB encapsulated in PCL nanoparticles showed more effectiveness when compared with free AmB to reduce the antifungal activity of *Leishmania* infected mice [122]. In a current study, bioactive glass scaffolds coated with (PLGA/Se) nanoparticles showed a substantial role in antibacterial and antiviral activity. The functionalized (PLGA/Se) nanoparticles coated bioactive glass composites prevents the antibacterial activity against the main causative agents such as *Staphylococcus epidermidis* and *Staphylococcus aureus* which are responsible for orthopaedic infections [58].

7. Bone tissue regeneration

Bone tissues are capable of repairing, remodeling and healing the diseased site, but it needs to overcome several challenges in deadly conditions due to diseases or trauma that requires replacement of damaged or affected bones. For the increasing older population, efficient treatment of bone defects and acceleration of healing process in large bone fractures is required to solve the problems. Thus tissue engineering offers the application of biocompatible and biodegradable substitutes providing enough space for cell attachment, growth, interaction and differentiation for developing new tissues. Developing engineered biomaterial substitutes is a potential approach to help the restoration of damaged bone tissues affected by trauma or inherent diseases. BTE includes the development of 3D systems combined with the bone forming cells, growth factors and porous biodegradable scaffolds. An ideal graft used for bone tissue regeneration should require extraordinary pro-osteogenesis and pro-angiogenesis qualities in order to rapidly restore the tissue function. The complex cascade of bioactivity is controlled by various biological molecules including growth factors that are liable to promote the regenerative signals at the site of injury [123]. These events further enhance bone healing by activating the inflammatory and progenitor cells that are capable of mediating the healing process [124]. To improve the regeneration process, a few strategies have been followed such as biocomposite substitutes, nanoparticles used for controlled release and delivery of drugs [125]. Researchers currently working on functionalizing the nanofibrous scaffolds with osteogenic factors or synthesizing the nanoparticles, enhance the materials properties for osteogenic differentiation in bone tissue engineering. A group of growth factors such as BMPs promote the formation of bones by differentiating the hMSCs to osteoblastic or chondroblastic cells. Among the growth factors, BMP-2 and BMP-7 are most effective in inducing the formation of complete bone

morphogenesis [126]. To study the sequential time pattern for the administration and to improve the growth factor stability, BMP-2 was loaded on PLGA nanoparticles for initial release on the other hand BMP-7 was loaded on poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanoparticles for final release of growth factors. It was reported that the *in vitro* assay in rat bone marrow hMSCs showed an enhanced osteogenic differentiation into osteoblast with sustained delivery of BMP [127]. Yilgor et al. synthesized PLGA and PHBV nanoparticles consisting of BMP-2 and BMP-7 proteins and incorporated them in PCL scaffolds for developing 3D tissue engineered constructs. These nanoparticles incorporated on PCL scaffolds delivered both BMP-2 and BMP-7 in a successive manner by improving bone marrow mesenchymal stem cells differentiation into osteogenic lineage [128]. A phosphate based ceramic called HA has been extensively used to create scaffolds of higher mechanical and osteoconductive properties [129, 130]. Moreover, HA has the potential to induce hMSCs differentiation into osteogenesis without any addition of growth factors and increase the ALP activity along with osteocalcin expression and mineralization [131]. It also enhances the surface topography of the scaffolds, promotes the growth of cells, adhesion, proliferation and differentiation [132-134]. The fabrication of Chitosan/HA biocomposite substitutes for bone tissue formation revealed that spherulites containing calcium and phosphorus is the major component in calcium phosphate apatite known as mineral phase of bone. There was a significant increase in the mineralization upto 108% in Chitosan/HA nanoparticle compared with chitosan alone [135]. HA particles induced chitosan substrates enhanced the secretion of mineral deposition on human osteoblasts for regeneration of bone tissues. **Figure 5** displays the FESEM images of biocomposite nanofibrous Chitosan/HA/osteoblast substrate consisting of needle like morphology or spherulites comprising tiny minerals of calcium phosphate in varying sizes after day 10 of cell culture. The morphology

of spherulites was changed after day 15 from needle to granules of tiny minerals. The presence of calcium and phosphate on the surface of the cells were confirmed using the elemental analyses of the mineralizing agents. HA nanoparticles were trapped by chitosan nanofibers to offer stability to the biocomposite scaffold material for mineralization and differentiation of osteoblast cells [135].

The *in vitro* study conducted by Nath et al, using electrosprayed PLGA nanoparticles loaded with simvastatin drug showed enhanced bone tissue regeneration. The cell attachment, proliferation followed by reverse transcription polymerase response revealed good biocompatibility of electrosprayed PLGA particles [92]. De Jonge et al. experimented with the electrosprayed calcium phosphate and collagen/calcium phosphate composite coatings and showed improved osteoblast differentiation, which lead to the enhanced mineral deposition [136]. The *in vitro* study of 2-*N*, 6-*O*-sulfated chitosan (26SCS) based nanoparticles (S-NP) showed dose dependent enhancement on angiogenesis. BMP-2 loaded S-NP (BMP-2/S-NP) with $1.4 \pm 0.2\%$ as the loading efficiency of protein. Gel based implant encapsulated with BMP-2/S-NP (BMP-2/S-NP/Gel) showed the formation of both peripheral vessel and new vessel along with controlled release profiles of BMP-2, which displayed encouraging vascularization at the diseased site of the bone. BMP-2/S-NP/Gel had the potential to promote and accelerate the bone augmentation by restoring the bone marrow cavity within 12 weeks. **Figure 6** shows the complete repair of bone with rich vessel network using the implant BMP-2/S-NP/Gel. The need to develop cell binding sites and biomechanical properties of electrospun nanofibers with functionalized nanoparticles are paramount but it is also the major obstacle currently faced by the tissue engineers. The promising and emerging new engineered tissues are relying on generating biocomposite scaffolds with informational function, like scaffold material comprising of growth

factor sequences that enable cell attachment, proliferation, migration and differentiation for bone tissue regeneration. These informational biocomposite scaffolds showed improved osteogenesis when compared to non-informational polymeric scaffolds [137].

7.1 Stem cells

Mesenchymal stem cells are the multipotent cells with the ability to differentiate into variable cell types as osteoblasts for bone tissue regeneration. Cells with related phenotypic characteristic properties are isolated from several adult tissues such as adipose tissue, neonatal tissues like placenta, umbilical cord blood, skeletal muscle and fetal tissues. Among these sources, bone marrow is considered to be the main source for hMSCs. Recent approach focuses on the delivery of osteogenic cells directly to the targeted site along with growth factors for effective bone tissue regeneration. Cao et al. developed a biocomposite photopolymerisable hydrogel (PH) incorporated with growth factor rhBMP-2 loaded on 2-N, 6-O-sulfated chitosan nanoparticles (PH/rhBMP-2/NPs) for bone tissue regeneration. *In vitro* hMSCs culture using the composites resulted in good cell viability, adhesion, proliferation followed by differentiation. The *in vivo* study of rhBMP-2 loaded substrate in rabbit radius serious defect undergoes gradual resorption with the replacement of new bone and reunion of the defective bones. The rhBMP-2/PH group revealed less bone binding with relatively slow rate of healing compared to PH/rhBMP-2/NPs-10 group. PH/rhBMP-2/NPs-10 group was more suitable for rapid bridging of bones with a large quantity of regenerated bone tissues [138]. Huang et al. studied the osteogenic differentiation of defected rat bone mesenchymal stem cells (rBMSCs) using the scaffold made of silk/HA nanoparticles. HA/silk nanoparticles into silk scaffolds showed an improved growth, stiffness and osteogenic differentiation of rBMSCs without any growth factors and furthermore increased the deposition of calcium and collagen type I [139]. In another study, Barium titanate (BT)

nanoparticles were combined with the hyper gravity stimulation for the betterment of hMSCs differentiation into osteoblasts. The results showed a significant development in the osteoblast expression of markers both at the protein and gene levels, the uptake of nanoparticles were substantially increased, the cellular growth and morphology confirms the differentiation of hMSCs into osteoblasts [140]. The hMSCs seeded to a biphasic calcium phosphate (BCP) nanoparticles loaded onto porous gelatin-pectin (Gel-P) composites with interconnected pores facilitated osteoconductivity and improved bone formation. Using this composite for *in vitro* studies in a rabbit model, the results were more favorable for cell attachment, interaction, proliferation and viability. The ALP activity and osteopontin marker expression confirms the formation of osteogenic lineage with mineral deposition on the surface [141]. Healing of infected or contaminated bone defects is a major concern in clinical treatments. Collagen scaffolds encapsulated with silver nanoparticles and growth factor BMP-2 was used to study the enhancement of healing process in infected bone defects. The differentiation of BMSCs into osteoblast was confirmed by Runx2 osteonectin and osteopontin protein marker expression [118]. Li et al, prepared BMP-2 loaded onto chitosan-stabilized bovine serum albumin nanoparticles (BNP) embedded to electrospun poly(ϵ -caprolactone)-co-poly(ethylene glycol) (PCE) copolymer scaffold loaded with DEX. The *in vitro* study showed the bioactivity and controlled dual release of BMP-2 and DEX from loaded nanofibrous scaffold. The *in vivo* osteogenesis studies of BNP/DEX/PCE scaffolds revealed that improved bioactivity and controlled dual delivery of BMP-2 and DEX stimulates the calvarial defect repair in rat [142]. BMP-2 loaded in fibrin-based nanocarriers resulted in ectopic bone restoration. It was stated that BMP-2 loaded fibrin nanoparticles showed an enhanced osteogenic lineage differentiation of human mesenchymal stem cells [143]. The hMSCs cultured on hybrid biocomposite nanofibrous

substrates made of poly (L-lactic acid)-co-poly-(ϵ -caprolactone)/silk fibroin/ascorbic acid/tetracycline hydrochloride (PLACL/SF/AA/TC) loaded with HA nanoparticles stimulated osteogenesis due to the presence of AA/TC/HA for bone tissue regeneration. The porous scaffold PLACL/SF/AA/TC/HA provided structural space for proliferation, differentiation and mineralization of cells allowing exchange of nutrients and biomolecules. These functionalized scaffolds with HA particles were proved to be the potential candidate for bone defect treatments [144]. PLGA based nanoparticles have been extensively formulated for the therapeutic agent delivery in bone tissue restoration. Jiang et al. prepared poly-Asp linked PLGA nanoparticles tagged with FITC for bone targeted drug delivery and tissue regeneration applications. The study showed that use of FITC-poly-Asp nanoparticles increased local drug delivery concentration, sustained drug delivery and reduced off target side effects. Further when FITC-poly-Asp nanoparticles were cultured with hMSCs and mouse bone marrow stem cells, it resulted in a better mineralized matrix, increased tibia bone section binding and reduced cytotoxicity of cells [111]. Kumar et al. developed biocomposite nanostructured substrates of PLACL, silk fibroin and HA using simultaneous electrospinning and electrospraying techniques for bone tissue regeneration. The electrospun nanofibrous substrates were electrosprayed with HA nanoparticles to attain rough surface morphology of substrates suitable for cell adhesion, proliferation and osteogenic differentiation of hMSCs. It was reported that these substrates enhanced the biological functions for cell growth, osteogenesis, ALP activity and mineralization for new bone tissue formation [145].

A nanocomposite layer made of biodegradable PCL nanofibers and HA nanoparticles coated with biphasic calcium phosphate (BCP) (BCP/PCL/nHA) scaffold cultured with ADSCs resulted in improved osteogenic differentiation with gene expression. BCP/PCL/nHA composite with

ADSCs was considered to be more suitable for bone repair and regeneration [146]. Nanostructured biocomposite scaffolds of Poly (L-lactic acid)/Poly-benzyl-L-glutamate/Collagen (PLLA/PBLG/Col) coated with nanohydroxyapatite (n-HA) were developed for bone restoration and repair. The presence of bioactive PBLG and n-HA on the polymeric biocomposite scaffolds showed improved and regulated biological functions including attachment, growth, interaction, osteogenic differentiation and mineralization of ADSCs. **Figure 7** demonstrates the SEM images of A) PLLA/PBLG/COL and B) PLLA/PBLG/COL/n-HA nanofibrous substrates and the confocal microscopic images of C) PLLA and D) PLLA/PBLG/COL/n-HA biocomposite scaffolds. The SEM images of A) and B) displayed uniform beadless structures of nanofibers trapped with n-HA, which enhanced the matrix environment suitable for cell binding, proliferation, integration and differentiation of progenitor cells into desired osteogenic lineages. The confocal images exhibited D) cuboidal morphology of ADSCs on the surface of PLLA/PBLG/COL/n-HA biocomposite scaffolds in which the arrows represents the complete differentiation of ADSCs into osteogenesis. C) In PLLA nanofibrous scaffolds the cuboidal morphology of cells are not present this is due to the absence of n-HA incorporation that might stimulate cell adhesion and also completes differentiation of into osteogenic cells. The results showed that PLLA/PBLG/COL/n-HA biocomposite substrates were suitable for bone tissue regeneration by promoting mineralization and differentiation of ADSCs into osteogenic lineage [147].

7.2 Osteoblasts

Osteoblasts play a vital role in formation, growth, repair of bone and finally responsible for the development of new bone matrix. They create basic multicellular units in the locality of vascular spaces and surface of bones that are responsible for bone remodeling through RANKL-RANK-

osteoprotegerin axis [148]. Any instance of trauma, disruption of vasculature causes hematoma formation and recruits osteoprogenitor cells from periosteum and endosteum towards the fracture sites. Later intramembranous bone formation occurs in the periosteum, leads to callus formation along with angiogenesis. The process is finally completed by remodeling of fracture site via organized osteoblast/ osteoclast activity [149]. The influence of several growth factors including BMPs, platelet-derived growth factor, fibroblastic growth factors, VEGF, TGF- β , insulin growth factor-1 on osteoblast activity is significant for the nourishment and formation of bones [149,150]. PCL nanocomposites incorporated with several perovskite ceramic nanoparticles like strontium titanate (ST), calcium titanate (CT) and barium titanate (BT) using MC3T3-E1 subclone 4 mouse pre-osteoblasts were analyzed for the enhanced bone regeneration. The cell viability and proliferation was excellent for the PCL/CT composite compared to others, which was more suitable for bone tissue regeneration [51]. Kumar et al. developed a composite made of graphene sheets and strontium metallic nanoparticles for demonstrating its application in BTE. PCL incorporated with hybrid nanoparticles (Strontium decorated reduced graphene oxide (RGO-Sr) scaffolds resulted in a significant osteoblast adhesion, proliferation and differentiation. The nanomaterial composite increased the biological activity with good osteoinductive and mechanical properties [61]. BG nanoparticles produced by multistep sol-gel method was studied for the in vitro reactivity of human fetal osteoblast (hFOB) cell model with and without HA and silicon substituted HA (SiHA) nanoparticles for the BTE. Nanocomposite scaffold in the presence of BG nanoparticles resulted in a potential HOB cell proliferation, adhesion, migration and differentiation compared to HA and SiHA nanoparticles [151].

PLACL and silk fibroin nanofibrous scaffold incorporated with HA improved an osteophilic environment for the proliferation, mineralization and differentiation of osteoblasts. Gupta et al.

studied the hFOB cell morphology on the biocomposite scaffolds by incorporation of HA with higher activity [152]. After 30th day of cell culture the mineral development on the surface of polymeric scaffolds created a thick layer with the ECM production by cells. The process of mineralization occurs through the deposition of calcium and phosphorous salts on the ECM where matrix containing anionic molecules takes up Ca^{2+} and phosphate ions aids for nucleation and growth causing calcification for bone tissue regeneration [152]. Zhang et al. developed nanocomposite scaffolds of HA/Chitosan for bone tissue restoration using human fetal osteoblast cells (hFOB). The HA nanoparticles of spindle shape were incorporated within the nanocomposite chitosan matrix. After 15 days of *in vitro* hFOB culture, HA/Chitosan scaffold resulted in a substantial bone formation compared to the pure electrospun nanostructured chitosan scaffolds. The presence of HA within the chitosan nanofibrous matrix significantly improved the structural and compositional features, which were very close to native mineralization. Differentiation of hFOB into osteogenic lineage and it proved the potential for the scaffold to promote bone tissue formation. **Figure 8** displays the FESEM images of nanostructured HA/Chitosan scaffolds (A) incorporated with 20% PEO (B) incorporated with 10% PEO (C) at high magnification (D) after alkali treatment. The presence of 10% PEO in the scaffold has considerably improved the formation of chitosan fibers when compared to 20 % PEO. The nanostructured HA/ Chitosan nanofibrous scaffolds showed a continuous, uniform geometry with granulate like morphology under higher magnification [153]. The biological analyses indicated that HA incorporated within the nanostructured biocomposite scaffolds were appeared to have considerably improved bone formation which was evident from the cell attachment, proliferation, interaction, mineral deposition, differentiation and morphology observed, owing to the tremendous osteoconductivity of HA for BTE [153,154]. In a recent

study, addition of MgO nanoparticles to HA-PLLA degradable nanocomposites resulted in an improved bone tissue regeneration. The osteoblasts cultured in degradable nanocomposite exhibited better adhesion and proliferation of cells in the presence of MgO nanoparticles. The mechanical properties of degradable nanoparticles were maintained to make it suitable for orthopedic tissue application. Furthermore presence of MgO nanoparticles in HA-PLLA nanocomposites resulted in non-toxicity towards the cells [53]. A study by Kumar et al. described that addition of diopside ($\text{CaMgSi}_2\text{O}_6$) (Dp) particles with chitosan scaffold have a potential application for BTE. Chitosan/Dp scaffolds resulted in a decreased water retention capacity however their degradation property doesn't show any changes. These biodegradable scaffolds displayed better affinity for protein adsorption and compatibility towards human osteoblastic cells by providing a suitable environment for the cell growth in bone tissue regeneration [155].

Silk fibroin scaffolds deliver high porosity, large surface area, better interaction between cell and scaffold for adhesion, interaction and proliferation of cells but lacks in mechanical stability. HA nanoparticles incorporated into SF scaffold resulted in a good mechanical strength, mineralization, cell viability and improved compatibility to osteoblasts. These mechanically reinforced biocomposite scaffolds containing HA nanoparticles proved to be a possible scaffold for bone tissue restoration [156]. The *in vitro* study of human osteoblast cultured on hybrid nanocomposite consisting of high density polyethylene, HA nanoparticles, TCP nanoparticles and MgO nanoparticles resulted in an improved ALP activity, cell adhesion, proliferation and viability. This hybrid nanocomposite supported with good mechanical strength and antibacterial property for osteogenesis prevents tissue inflammation [157].

Conclusion and future perspectives

EHDA technique used for fabricating nanoparticles demonstrates a huge potential for producing nanoparticles of controlled size, shape and morphology for application in drug delivery carriers and tissue regeneration. The unique physicochemical properties of nanoparticles have allowed the integration of various functions in a single design. It is mainly due to their nanometer size with higher surface area to volume ratio, capacity to serve as the carrier for therapeutic drugs, growth factors and genes, showing targeted delivery with controlled release in the affected sites of the tissues. Furthermore nanoparticles provide an *in situ* imaging of drug payload release and monitor the treatment response. Although there is a significant progress in developing the theragnostic nanoparticles, there are a few limitations such as tissue penetration capacity of lower and higher molecular weights when compared to small molecule drugs. In order to develop a multiple functionalizing theragnostic nanoparticle there are many challenges during manufacturing such as cost, reproducibility, colloidal stability etc. In order to fulfil these requirements, it is essential to develop nanoparticles with high stability which are capable of withstanding high salt concentration, temperature, pH and also have a minimal contact with the serum protein, that would tend to conjugate biomolecules without any changes in the colloidal stability. Many new effective drug molecules have to be identified for specific targeted delivery. Researchers should even focus on innovating new techniques to improve the efficacy in penetrating the tissue or subcellular region and offer controlled delivery of drugs in the specified site of interest. The effort on developing nanoparticles in therapeutics for higher loading capacity, payload release and antimicrobial coating is equally important. This is accomplished by exploring various functional groups for effective therapeutic loading, polymer coating and

improving the conjugation methods. The requirement of theragnostic nanoparticles varies depending on the disease for different stages of treatments.

Choice of bioactive molecules also plays a key role in delivery therapeutics. Though there are some drawbacks in maintaining stability and shear or thermal stress of biomolecules, this can be managed by choosing appropriate carrier molecules. Future work should involve more collaborative efforts for developing multifunctional particles capable of targeting drugs to the required site, diagnosing and monitoring the response of therapeutics. Nanoparticles encapsulated with multiple growth factors and signalling agents help to release these molecules in a precise temporal and spatial pattern by mimicking natural extracellular matrix for better development of damaged bone tissues. These functionalized tissue engineered scaffolds also acts as cell carriers by providing noninvasive means of cell delivery *via* direct injection of cells incorporated nanoparticles to the damaged site unlike surgical implantation. In addition electrosprayed cells also maintain their proliferation and biological functions on various substrates including electrospun scaffolds. However, further studies have to be performed in optimizing the nanoparticle material and combination of therapeutics, controlled delivery of bioactive molecules shows great potential for enhancing bone tissue repair. The advantages of using these nanomaterials are available in natural origin and biodegradable but their scalability and commercialisation are in abundance. With great scientific, commercial and public interests it is possible to lead a significant development in this field in upcoming years.

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References

- [1] W.E. Bawarski, E. Chidlow, D.J. Bharali, S.A. Mousa, Emerging nanopharmaceuticals, *Nanomedicine* 4 (2008) 273–282.
- [2] R. Sridhar, S. Ramakrishna, Electrosprayed nanoparticles for drug delivery and pharmaceutical applications, *Biomater.* 3 (2013) DOI: 10.4161/biom.24281.
- [3] T.J. Cho, L.C. Gerstenfeld, T.A. Einhorn, Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing, *J. Bone Miner. Res.* 17 (2002) 513-520.
- [4] P.V. Giannoudis, H. Dinopoulos, E. Tsiridis, Bone substitutes: an update, *Injury* 36 (2005) 20-27.
- [5] A.C. Masquelet, T. Begue, The concept of induced membrane for reconstruction of long bone defects, *Orthop. Clin. North Am.* 41 (2010) 27-37.
- [6] J. Aronson, Limb-lengthening, skeletal reconstruction, and bone transport with the Ilizarov method, *J. Bone Joint Surg. Am.* 8 (1997) 1243-1258.
- [7] P.H. Bullens, H.W.B. Schreuder, M.C. de Waal Malefijt, N. Verdonschot, P. Buma, Is an impacted morselized graft in a cage an alternative for reconstructing segmental diaphyseal defects?, *Clin. Orthop. Relat. Res.* 3 (2009) 783-791.
- [8] K. Narenda, K. Rajiv, Nanotechnology and Nanomaterials in the Treatment of Life-Threatening Diseases, Chapter 2 – Nano-based Drug Delivery and Diagnostic Systems 408 (2014) 53–107.
- [9] J.S. Son, S.G. Kim, S.C. Jin, Z.G. Piao, S.Y. Lee, J.S. Oh, C.S. Kim, B.H. Kim, M.A. Jeong, Development and structure of a novel barrier membrane composed of drug-loaded poly (lactic-co-glycolic acid) particles for guided bone regeneration, *Biotechnol. Lett.* 34 (2012) 779–787.

- [10] J. Shi, X. Zhang, J.X. Zhu, Y.B. Pi, X.Q. Hu, C.Y. Zhou, Y.F. Ao, Nanoparticle delivery of the bone morphogenetic protein 4 gene to adipose-derived stem cells promotes articular cartilage repair in vitro and in vivo, *Arthroscopy* 29 (2013) 2001–2011.
- [11] B.C.M. te Boekhorst, L.B. Jensen, S. Colombo, A.K. Varkouhi, R.M. Schiffelers, T. Lammers, G. Storm, H.M. Nielsen, G.J. Strijkers, C. Foged, K. Nicolay, MRI-assessed therapeutic effects of locally administered PLGA nanoparticles loaded with anti-inflammatory siRNA in a murine arthritis model, *J. Control Release* 161 (2012) 772–780.
- [12] J. Xie, J. Jiang, P. Davoodi, M.P.Srinivasan, C.H.Wang, Electrohydrodynamic atomization: A two-decade effort to produce and process micro-/nanoparticulate materials, *Chemical Eng. Sci.* 125 (2015) 32–57.
- [13] H. Valo, L. Peltonen, S. Vehvilainen, M. Karjalainen, R. Kostianen, T. Laaksonen, J. Hirvonen, Electrospray encapsulation of hydrophilic and hydrophobic drugs in poly(L-lactic acid) nanoparticles, *Small* 5 (2009) 1791–1798.
- [14] N. Zhang, D.H. Kohn, Using polymeric materials to control stem cell behaviour for tissue regeneration, *Birth Defects Res. C Embryo Today* 96 (2012) 63–81.
- [15] A. Jaworek, Electrostatic micro- and nanoencapsulation and electroemulsification: a brief review, *J. Microencapsul.* 25 (2008) 443–468.
- [16] O.V. Salata, Tools of nanotechnology: electrospray, *Curr. Nanosci.* 1 (2005) 25–33.
- [17] Z. Maedeh, P. Molamma, E. Thian, S. Ramakrishna, Controlled delivery of stromal derived factor-1 α from poly lactic-co-glycolic acid core-shell particles to recruit mesenchymal stem cells for cardiac regeneration. *J. Colloid Interface Sci.* 451 (2015) 144–152.
- [18] F. Croisier, C. Jerome, Chitosan-based biomaterials for tissue engineering, *Eur. Polym. J.* 49 (2013) 780–792.
- [19] N. Arya, S. Chakraborty, N. Dube, D. S. Katti, Electrospraying: A Facile Technique for Synthesis of Chitosan-Based Micro/Nanospheres for Drug Delivery Applications, *J Biomed Mater. Res. B Appl. Biomater.* 88 (2009) 17-31.
- [20] M. M. Hasani-Sadrabadi, S. P. Hajrezaei, S. H. Emami, G. Bahlakeh, L. Daneshmandi, E. Dashtimoghadam, E. Seyedjafari, K. I. Jacob, L. Tayebi, Enhanced osteogenic differentiation of stem cells via microfluidics synthesized nanoparticles, *Nanomedicine: Nanotechnology, Biology and Medicine* (2015) DOI: 10.1016/j.nano.2015.04.005.

- [21] S. Zhang, K. Kawakami, One-step preparation of chitosan solid nanoparticles by electrospray deposition, *Internat. J. Pharmaceutics* 397 (2010) 211–217.
- [22] M. Gümüşderelioglu, E. O. Tunçay, G. Kaynak, T. T. Demirtaş, S. T. Aydın, S. S. Hakkı, Encapsulated boron as an osteoinductive agent for bone scaffolds, *Biology* 31 (2015) 120–128.
- [23] R. R. Sehgal, S. I. Roohani-Esfahani, H. Zreiqat, R. Banerjee, Nanostructured gellan and xanthan hydrogel depot integrated within a baghdadite scaffold augments bone regeneration, *J. Tissue. Eng. Regen. Med.* (2015) DOI: 10.1002/term.2023.
- [24] J. Li, X. Mei, Applications of Cellulose and Cellulose Derivatives in Immediate Release Solid Dosage, Chapter 2, *ACS Symposium Series* 934 (2009) 19-55.
- [25] M. Park, D. Lee, S. Shin, J. Hyun, Effect of negatively charged cellulose nanofibers on the dispersion of hydroxyapatite nanoparticles for scaffolds in bone tissue engineering, *Colloids and Surfaces B: Biointerfaces* 130 (2015) 222–228.
- [26] R.R. Chen, D.J. Mooney, Polymeric growth factor delivery strategies for tissue engineering, *Pharm. Res.* 20 (2003) 1103–1112.
- [27] C. Berkland, D.W. Pack, K.K. Kim, Controlling surface nano-structure using flow-limited field-injection electrostatic spraying (FFESS) of poly(d,L-lactide-co-glycolide), *Biomaterials* 25 (2004) 5649–5658.
- [28] S.Y. Lee, J.Y. Koak, S.J. Heo, S.K. Kim, S.J. Lee, S.Y. Nam, Osseointegration of anodized titanium implants coated with poly(lactide-co-glycolide)/basic fibroblast growth factor by electrospray, *Int J Oral Maxillofac Implants* 25 (2010) 315-320.
- [29] B. Almeria, A. Gomez, Electrospray synthesis of monodisperse polymer particles in a broad (60 nm-2 μ m) diameter range: guiding principles and formulation recipes, *J Colloid Interface Sci.* 121 (2014) 121-130.
- [30] M.A. Woodruff, D.W. Hutmacher, The return of a forgotten polymer- polycaprolactone in 21st century, *Prog. Polym. Sci.* 35 (2010) 1217–1256.
- [31] C. Gandhimathi, N.X.H. Edwin, P. Jayaraman, J.R. Venugopal, S. Ramakrishna. S.D. Kumar. Controlled release of dexamethasone in PCL/silk fibroin/ascorbic acid nanoparticles for the initiation of adipose derived stem cells into osteogenesis, *J. Drug Metab. Toxicol.* 5 (2015) 177.

- [32] H. Cai, V. Dave1, R.A. Gross, S.P. McCarthy, Effects of physical aging, crystallinity, and orientation on the enzymatic degradation of poly(lactic acid), *Journal of Polymer Science Part B: Polymer Physics* 34 (1996) 2701-2708.
- [33] M. Ikeuchi, R. Tane, K. Ikuta, Electrospayed position and direct patterning of polylactic acid nanofibrous microcapsules for tissue engineering, *Biomed. Microdevices* 14 (2012) 35–43.
- [34] N. Ribeiro, S.R. Sousa, C.A. van Blitterswijk, L. Moroni, F.J. Monteiro, A biocomposite of collagen nanofibers and nanohydroxyapatite for bone regeneration, *Biofabrication* 6 (2014) 035015.
- [35] J. Venkatesan, S. K. Kim, Nano-hydroxyapatite composite biomaterials for bone tissue engineering-a review, *J. Biomed. Nanotechnol.* 10 (2014) 3124-3140.
- [36] J. Venugopal, R. Rajeswari, M. Shayanti, S. Low, A. Bongso, V.R. Dev, G. Deepika, A.T. Choon, S. Ramakrishna, Electrospayed hydroxyapatite on polymer nanofibers to differentiate mesenchymal stem cells to osteogenesis, *J. Biomater. Sci. Polym. Ed.* 24 (2013) 170-184.
- [37] J. Ramier, T. Boudierlique, O. Stoilova, N. Manolova, I. Rashkov, V. Langlois, E. Renard, P. Albanese, D. Grande, Biocomposite scaffolds based on electrospun poly(3-hydroxybutyrate) nanofibers and electrospayed hydroxyapatite nanoparticles for bone tissue engineering applications, *Materials Sci. Eng. C* 38 (2014) 161–169.
- [38] D. Gupta, J. Venugopal, S. Mitra, V.R. Giri Dev, S. Ramakrishna, Nanostructured biocomposite substrates by electrospinning and electrospaying for the mineralization of osteoblasts, *Biomaterials*, 30 (2009) 2085–2094.
- [39] E. Korina, O. Stoilova, N. Manolova, I. Rashkov, Multifunctional hybrid materials from poly(3-hydroxybutyrate), TiO₂ nanoparticles, and chitosan oligomers by combining electrospinning/electrospaying and impregnation, *Macromol. Biosci.* 13(2013) 707-716.
- [40] L. Francis, J. Venugopal, M. P. Prabhakaran, V. Thavasi, E. Marsano, S. Ramakrishna, Simultaneous electrospin–electrospayed biocomposite nanofibrous scaffolds for bone tissue regeneration, *Acta Biomaterialia* 6 (2010) 4100–4109.
- [41] X. Zhao, Y. S. Lui, C. K. C. Choo, W. T. Sow, C. L. Huang, K. W. Ng, J. S. C. Loo, Calcium phosphate coated Keratin–PCL scaffolds for potential bone tissue regeneration, *Materials Sci. Eng. C* 49 (2015) 746–753.

- [42] J. Ramier, D. Grande, T. Boudierlique, O. Stoilova, N. Manolova, I. Rashkov, V. Langlois, P. Albanese, E. Renard, From design of bio-based biocomposite electrospun scaffolds to osteogenic differentiation of human mesenchymal stromal cells, *J. Mater. Sci. Mater. Med.* 25 (2014) 1563-1575.
- [43] C.C. Berry, Applications of inorganic nanoparticles for biotechnology, *Nanobiotechnol. Inorg. Nanopart. vs Org. Nanopart.* 4 (2012) 159.
- [44] J. Chatterjee, M. Bettge, Y. Haik, C. Jen Chen, Synthesis and characterization of polymer encapsulated Cu–Ni magnetic nanoparticles for hyperthermia applications, *J. Magn. Magn. Mater.* 293 (2005) 303–309.
- [45] H.Y. Li, H.Z. Chen, W.J. Xu, F. Yuan, J.R. Wang, M. Wang, Polymer-encapsulated hydrophilic carbon black nanoparticles free from aggregation, *Colloids Surf. Physicochem. Eng. Asp.* 254 (2005) 173–178.
- [46] Z. Zeng, J. Yu, Z.X. Guo, Preparation of functionalized core–shell alumina/polystyrene composite nanoparticles, *Macromol. Chem. Phys.* 206 (2005) 1558–1567.
- [47] Z. Luo, Y. Deng, R. Zhang, M. Wang, Y. Bai, Q. Zhao, Y. Lyu, J. Wei, S. Wei, Peptide-laden mesoporous silica nanoparticles with promoted bioactivity and osteo-differentiation ability for bone tissue engineering, *Colloids and Surfaces B: Biointerfaces* 131(2015) 73–82.
- [48] W.E.Müller, E. Tolba, Q. Feng, H. C. Schröder, J.S. Markl, M. Kokkinopoulou, X. Wang, Amorphous Ca²⁺ polyphosphate nanoparticles regulate the ATP level in bone-like SaOS-2 cells, *J. Cell Sci.* 128 (2015) 2202-2207.
- [49] Y. Wang, H. Hao, H. Liu, Y. Wang, Y. Li, G. Yang, J. Ma, C. Mao, S. Zhang, Selenite-Releasing Bone Mineral Nanoparticles Retard Bone Tumor Growth and Improve Healthy Tissue Functions In Vivo, *Adv. Healthcare Mater.* (2015) DOI: 10.1002/adhm.201500307.
- [50] L. Mellor, M. Mohiti-Asli, J. Williams, A. Kannan, M.R. Dent, F. Guilak, E. Lobo, Extracellular Calcium Modulates Chondrogenic And Osteogenic Differentiation Of Human Derived Adipose Stem Cells: A Novel Approach For Osteochondral Tissue Engineering Using A Single Stem Cell Source, *Tissue Eng. Part A.* (2015).
- [51] A. Bagchi, S. R. K. Meka, B. N. Rao, K. Chatterjee, Perovskite ceramic nanoparticles in polymer composites for augmenting bone tissue regeneration, *Nanotechnology* 25 (2014) 13.

- [52] I. Rajzer, E. Menaszek, R. Kwiatkowski, J. A. Planell, O. Castano, Electrospun gelatin/poly(ϵ -caprolactone) fibrous scaffold modified with calcium phosphate for bone tissue engineering, *Mater. Sci. Eng. C* 44 (2014) 183–190.
- [53] D. J. Hickey, B. Ercan, L. Sun, T. J. Webster, Adding MgO nanoparticles to hydroxyapatite–PLLA nanocomposites for improved bone tissue engineering applications, *Acta Biomaterialia* 14 (2015) 175–184.
- [54] T.B. Nguyen, Y.K. Min, B.T. Lee, Nanoparticle biphasic calcium phosphate loading on gelatin-pectin scaffold for improved bone regeneration, *Tissue Eng. Part A* 21 (2015) 1376–1387.
- [55] S. Fujino, H. Tokunaga, E. Saiz, A.P. Tomsia, Fabrication and characterization of bioactive glass coatings on Co–Cr implant alloys, *Mater. Trans.* 45 (2004) 1147–1151.
- [56] J.R. Jones, Review of bioactive glass: from Hench to hybrids, *Acta Biomater.* 9 (2013) 4457–4486.
- [57] N. S. Kumar, K. Biswanath, D. Someswar, Biomaterials applications for nanomedicine, orthopedic surgery and drug delivery system (2011) 69–116.
- [58] M. Stevanovic, N. Filipovic, J. Djurdjevic, M. Lukic, M. Milenkovic, A. Boccaccini, 45S5Bioglass[®]-based scaffolds coated with selenium nanoparticles or with poly(lactide-co-glycolide)/selenium particles: Processing, evaluation and antibacterial activity, *Colloids and Surfaces B: Biointerfaces* 132 (2015) 208–215.
- [59] M. Shi, D. Zhai, L. Zhao, C. Wu, J. Chang, Nanosized mesoporous bioactive glass/poly(lactic-co-glycolic acid) composite-coated CaSiO₃ scaffolds with multifunctional properties for bone tissue engineering, *Biomed Res. Int.* 2014 (2014) 1–12.
- [60] J. J. Li, N. Kawazoe, G. Chen, Gold nanoparticles with different charge and moiety induce differential cell response on mesenchymal stem cell osteogenesis, *Biomaterials*. 54 (2015) 226–236.
- [61] S. Kumar, K. Chatterjee, Strontium eluting graphene hybrid nanoparticles augment osteogenesis in a 3D tissue scaffold, *Nanoscale* 7 (2015) 2023–2033.
- [62] J. Sun, S.L. Simon, The melting behaviour of aluminium nanoparticles, *Thermochim. Acta* 463 (2007) 32–40.
- [63] N. I. Lebovka, Aggregation of Charged Colloidal Particles, *Adv. Polym. Sci.* 255 (2014) 57–96.

- [64] P. Marmorato, G. Ceccone, A. Gianoncelli, L. Pascolo, J. Ponti, F. Rossi, M. Salomé, B. Kaulich, M. Kiskinova, Cellular distribution and degradation of cobalt ferrite nanoparticles in Balb/3T3 mouse fibroblasts, *Toxicol. Lett.* 207 (2011) 128–136.
- [65] Y.S. Chen, Y.C. Hung, L.W. Lin, I. Liao, M.Y. Hong, G.S. Huang, Size-dependent impairment of cognition in mice caused by the injection of gold nanoparticles, *Nanotechnology* 21 (2010) 485102.
- [66] M. Sadat-Shojai, M.T. Khorasani, E. Dinpanah-Khoshdargi, A. Jamshidi, Synthesis methods for nanosized hydroxyapatite with diverse structures, *Acta. Biomater.* 9 (2013) 7591–7621.
- [67] Z. Xu, C. Liu, J. Wei, J. Sun, Effects of four types of hydroxyapatite nanoparticles with different nanocrystal morphologies and sizes on apoptosis in rat osteoblasts, *J. Appl. Toxicol.* 32 (2012) 429–435.
- [68] J.L. Xu, K.A. Khor, J.J. Sui, J.H. Zhang, W.N. Chen, Protein expression profiles in osteoblasts in response to differentially shaped hydroxyapatite nanoparticles, *Biomaterials* 30 (2009) 5385–5391.
- [69] M. Lezaja, D.N. Veljovic, B.M. Jokic, I. Cvijovic-Alagic, M.M. Zrilc, V. Miletic, Effect of hydroxyapatite spheres, whiskers, and nanoparticles on mechanical properties of a model BisGMA/TEGDMA composite initially and after storage, *J. Biomed. Mater. Res.* 101B (2013) 1469–1476.
- [70] Y. Dou, K. Lin, J. Chang, Polymer nanocomposites with controllable distribution and arrangement of inorganic nanocomponents, *Nanoscale* 3 (2011) 1508–1511.
- [71] K. Lin, L. Chen, P. Liu, Z. Zou, M. Zhang, Y. Shen, *et al.*, Hollow magnetic hydroxyapatite microspheres with hierarchically mesoporous microstructure for pH-responsive drug delivery, *Cryst. Eng. Comm.* 15 (2013) 2999–3008.
- [72] K. Lin, P. Liu, L. Wei, Z. Zou, W. Zhang, Y. Qian, *et al.*, Strontium substituted hydroxyapatite porous microspheres: surfactant-free hydrothermal synthesis, enhanced biological response and sustained drug release, *Chem. Eng. J.* 222 (2013) 49–59.
- [73] S.W. Choi, W.S. Kim, J.H. Kim, Surface-functionalized nanoparticles for controlled drug delivery, *Methods Mol. Biol.* 303 (2005) 121–131.

- [74] M.A.F. Yague, S.A. Abbah, L. McNamara, D.I. Zeugolis, A. Pandit, M.J. Biggs, Biomimetic approaches in bone tissue engineering: Integrating biological and physicommechanical strategies, *Adv. Drug Deliv. Reviews* 84 (2014) 1–29.
- [75] K. L. Kelly, E. Coronado, L. Zhao, and G. C. Schatz, The optical properties of metal nanoparticles: the influence of size, shape, and dielectric environment, *J. Phys. Chem. B* 107 (2003) 668–677.
- [76] M. Jayabalan, K.T. Shalumon, M.K. Mitha, K. Ganesan, M. Epple, Effect of hydroxyapatite on the biodegradation and biomechanical stability of polyester nanocomposites for orthopaedic applications, *Acta. Biomater.* 6 (2010) 763–775.
- [77] K.R. Remya, J. Joseph, S. Mani, A. John, H.K. Varma, P. Ramesh, Nanohydroxyapatite incorporated electrospun polycaprolactone/polycaprolactone-polyethyleneglycol-polycaprolactone blend scaffold for bone tissue engineering applications, *J. Biomed. Nanotechnol.* 9 (2013) 1483–1494.
- [78] Q. Cheng, K. Rutledge, E. Jabbarzadeh, Carbon nanotube-poly(lactide-co-glycolide) composite scaffolds for bone tissue engineering applications, *Ann. Biomed. Eng.* 41 (2013) 904–916.
- [79] C.D. Gao, T.T. Liu, C.J. Shuai, S.P. Peng, Enhancement mechanisms of graphene in nano-58S bioactive glass scaffold: mechanical and biological performance, *Sci. Rep. UK* 4 (2014) DOI:10.1038/srep04712.
- [80] S.H. Uhm, D.H. Song, J.S. Kwon, S.B. Lee, J.G. Han, K.N. Kim, Tailoring of antibacterial Ag nanostructures on TiO₂ nanotube layers by magnetron sputtering, *J. Biomed. Mater. Res. B Appl. Biomater.* 102 (2014) 592–603.
- [81] P.A. McCarron, W.M. Marouf, R.F. Donnelly, C. Scott, Enhanced surface attachment of protein-type targeting ligands to poly(lactide-co-glycolide) nanoparticles using variable expression of polymeric acid functionality, *J Biomed. Mater. Res. A* 87 (2008) 873–884.
- [82] M.M. Stevens, Biomaterials for bone tissue engineering, *Mater. Today* 11 (2008) 18–25.
- [83] N. Duceppe, M. Tabrizian, Advances in using chitosan-based nanoparticles for in vitro and in vivo drug and gene delivery, *Expert Opin. Drug Deliv.* 7 (2010) 1191–1207.
- [84] K.K. Jain, *Drug Delivery Systems- An Overview*, Humana Press 437 (2008) 1-50.
- [85] T. Gaura, T. Ruchi, S. Birendra, L. Bhati, S. Pandey, P. Pandey, K.B. Saurabh, Drug delivery systems: An updated review, *Int. J. Pharm. Invest.* 2 (2012) 2–11.

- [86] G. Gandhimathi, J.R. Venugopal, S. Sundarrajan, R. Sridhar, S.S.W. Tay, S. Ramakrishna, S.D. Kumar, Breathable Medicine: Pulmonary Mode of Drug Delivery, *J. Nanosci. Nanotechnol.* 15 (2014) 2591-2604.
- [87] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, *Adv. Drug Deliv. Rev.* 55 (2003) 329-347.
- [88] A. Kumari, S. K. Yadav, S. C. Yadav, Biodegradable polymeric nanoparticles based drug delivery systems, *Colloids and Surfaces B: Biointerfaces* 75 (2010) 1–18.
- [89] R.A. Jain, The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices, *Biomaterials* 21 (2000) 2475–2490.
- [90] M.Wang, G.C. Rutledge, A.S. Myerson, B.L. Trout, Production and characterization of carbamazepine nanocrystals by electrospraying for continuous pharmaceutical manufacturing, *J. Pharm. Sci.* 101 (2012) 1178-88.
- [91] M. Nyström, J. Roine, M. Murtomaa, R. Mohan Sankaran, H.A. Santos, J. Salonen, Solid state transformations in consequence of electrospraying - A novel polymorphic form of piroxicam, *Eur. J. Pharm. Biopharm.* 89 (2015) 182-189.
- [92] S.D. Natha, S. Sona, A. Sadiasaa, Y. K. Minb, B. T. Leea, Preparation and characterization of PLGA microspheres by the electrospraying method for delivering simvastatin for bone regeneration, *Internat. J. Pharmaceutics* 443 (2013) 87– 94.
- [93] C.R. Pinto, R.J. Neufeld, A.J. Ribeiro, F. Veiga, Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles, *Nanomedicine* 2 (2006) 8-21.
- [94] A.D. Rieux, V. Fievez, M. Garinot, Y.J. Schneider, V. Preat, Nanoparticles as potential oral delivery systems of proteins and vaccines: a mechanistic approach, *J. Controlled Release* 10 (2006) Pages 1–27.
- [95] B.T. Midhun, K.T. Shalumon, K. Manzoor, R. Jayakumar, S.V. Nair, M. Deepthy, Preparation of Budesonide-Loaded Polycaprolactone Nanobeads by Electrospraying for Controlled Drug Release, *J. Biomater. Sci. Polym. Ed.* 22 (2011) 2431-2444.
- [96] A. El-Fiqi, J.H. Kim, H.W. Kim, Osteoinductive fibrous scaffolds of biopolymer/mesoporous bioactive glass nanocarriers with excellent bioactivity and long-term delivery of osteogenic drug, *ACS Appl. Mater. Interfaces* 7 (2015) 1140-52.
- [97] G. Irmak, T.T. Demirtaş, D. Çetin Altındal, M. Çalış, M. Gümüşderelioğlu, Sustained release of 17 β -estradiol stimulates osteogenic differentiation of adipose tissue-derived

- mesenchymal stem cells on chitosan-hydroxyapatite scaffolds, *Cells Tissues Organs* 199 (2014) 37-50.
- [98] Q. Wang, J. Wang, Q. Lu, M.S. Detamore, C. Berkland, Injectable PLGA based colloidal gels for zero-order dexamethasone release in cranial defects, *Biomaterials* 31 (2010) 4980-4996.
- [99] J.E. Babensee, L.V. McIntire, A.G. Mikos, Growth factor delivery for tissue engineering, *Pharm. Res.* 5 (2000) 497-504
- [100] P.Q. Ruhé, O.C. Boerman, F.G. Russel, A.G. Mikos, P.H. Spauwen, J.A. Jansen, In vivo release of rhBMP-2 loaded porous calcium phosphate cement pretreated with albumin, *J. Mater. Sci. Mater. Med.* 17 (2006) 919-927
- [101] Z.S. Haidar, R.C. Hamdy, M. Tabrizian, Delivery of recombinant bone morphogenetic proteins for bone regeneration and repair, *A Curr. Chall. BMP Deliv. Biotechnol. Lett.* 31 (2009) 1817-1824
- [102] Y. Chung, K.M. Ahn, S.H. Jeon, S.Y. Lee, J.H. Lee, G. Tae, Enhanced bone regeneration with BMP-2 loaded functional nanoparticle-hydrogel complex, *J. Controlled Release* 121 (2007) 91-99.
- [103] J. Street, M. Bao, L. deGuzman, S. Bunting, F.V. Peale Jr., N. Ferrara, H. Steinmetz, H. Hoeffel, J.L. Cleland, A. Daugherty, N. van Bruggen, H.P. Redmond, R.A.D. Carano, E.H. Filvaroff, Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover, *Proc. Natl. Acad. Sci.* 99 (2002) 9656 - 9661.
- [104] Y. Yonamine, T. Matsuyama, T. Sonomura, H. Takeuchi, Y. Furuichi, M. Uemura, Effectable application of vascular endothelial growth factor to critical sized rat calvaria defects, *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 109 (2010) 225-231.
- [105] Y. Wang, Y. Wei, X. Zhang, M. Xu, F. Liu, Q. Maa, Q. Cai, X. Deng, PLGA/PDLLA core-shell submicron spheres sequential release system: Preparation, characterization and promotion of bone regeneration in vitro and in vivo, *Chemical Eng. J.* 273 (2015) 490-501.
- [106] B.D. Riva, E. Sánchez, A. Hernández, R. Reyes, F. Tamimi, E. López-Cabarcos, Local controlled release of VEGF and PDGF from a combined brushite-chitosan system enhances bone regeneration, *J. Controlled Release* 143 (2010) 45-52.
- [107] A. Raiche, D. Puleo, In vitro effects of combined and sequential delivery of two bone growth factors, *Biomaterials* 25 (2004) 677-685.

- [108] R.M. Gruber, A. Ludwig, H.A. Merten, M. Achilles, S. Poehling, H. Schliephake, Sinus floor augmentation with recombinant human growth and differentiation factor-5 (rhGDF-5): a histological and histomorphometric study in the Göttingen miniature pig, *Clin. Oral Implants Res.* 19 (2008) 522–529.
- [109] F. Wu, T. Jin, Polymer-based sustained-release dosage forms for protein drugs, challenges, and recent advances, *AAPS Pharm. Sci. Tech.* 9 (2008) 1218–1229.
- [110] M. Zamani, M.P. Prabhakaran, S. Ramakrishna, Advances in drug delivery via electrospun and electrosprayed nanomaterials, *Int. J. Nanomed.* 8 (2013) 2997–3017.
- [111] T.Jiang, X.Yu, E.J. Carbone, C.Nelson, H.M. Kan, K.W.H. LO, Poly aspartic acid peptide-linked PLGA based nanoscale particles: Potential for bone-targeting drug delivery applications, *Internat. J. Pharmaceutics* 475 (2014) 547–557.
- [112] J.H. Christopher, B. Pratim, Narrow size distribution nanoparticles production by electro spray processing of ferritin, *Aerosol science* 39 (2008) 432–440.
- [113] J. Zillies, C. Coester, Evaluating gelatin based nanoparticles as a carrier system for double stranded oligonucleotide, *J. Pharm. Pharmaceut. Sci.* 7 (2004) 17-21.
- [114] E.Jabbari, Osteogenic peptides in bone regeneration, *Curr. Pharm. Des.* 19 (2013) 3391-3402.
- [115] A.K. Singla, M.M.Chawla, Chitosan: Some pharmaceutical and biological aspects—AN update, *J. Pharm. Pharmacol.* 53 (2001) 1047–1067.
- [116] P.H. Chua, K.G. Neoh, E.T. Kang, W.Wang, Surface functionalization of titanium with hyaluronic acid/chitosan polyelectrolyte multilayers and RGD for promoting osteoblast functions and inhibiting bacterial adhesion, *Biomaterials* 29 (2008) 1412-1421.
- [117] A.F.R. Moreno, T.K. Lu, V.A. Puscasu, C.J. Yoon, R. Langer, O.C. Farokhzad, Surface charge-switching polymeric nanoparticles for bacterial cell wall-targeted delivery of antibiotics, *ACS Nano.* 6 (2012) 4279–4287.
- [118] C.Y. Sun, Y.J. Che, S.J. Lu, Preparation and application of collagen scaffold-encapsulated silver nanoparticles and bone morphogenetic protein 2 for enhancing the repair of infected bone, *Biotechnol Lett.* 2 (2015) 467-73.
- [119] F. Lalidou , G. Kolios , A. Tavridou , G.I. Drosos , Bone grafts as carriers for local antibiotic delivery for the treatment and prevention of bone infections, *Surg. Technol. Int.* 25 (2014) 239-245.

- [120] F. Lalidou , G. Kolios , G.I. Drosos , Bone infections and bone graft substitutes for local antibiotic therapy, *Surg. Technol. Int.* 24 (2014) 353-362.
- [121] V. Uskokovic, T.A. Desai, Simultaneous bactericidal and osteogenic effect of nanoparticulate calcium phosphate powders loaded with clindamycin on osteoblasts infected with *Staphylococcus aureus*, *Mater. Sci. Eng. C Mater. Biol. Appl.* 37 (2014) 210-222.
- [122] M.S. Espuelas, P. Legrand, P.M. Loiseau, C. Bories, G. Barratt, J.M. Irache, In vitro antileishmanial activity of amphotericin B loaded in poly(epsilon-caprolactone) nanospheres, *J. Drug Target* 10 (2002) 593–599.
- [123] A. Berner, J. Boerckel, S. Saifzadeh, R. Steck, J. Ren, C. Vaquette, Biomimetic tubular nanofiber mesh and platelet rich plasma-mediated delivery of BMP-7 for large bone defect regeneration, *Cell and Tissue Research* 347 (2012) 603-612.
- [124] J. Liu, L. Xu, Y. Li, J. Ma, Temporally controlled multiple-gene delivery in scaffolds: A promising strategy to enhance bone regeneration, *Medical Hypotheses* 76 (2011) 173-185.
- [125] S. Kim, Y. Kang, C.A. Krueger, M. Sen, J.B. Holcomb, D. Chen, Sequential delivery of BMP-2 and IGF-1 using a chitosan gel with gelatin microspheres enhances early osteoblastic differentiation, *Acta. Biomater.* 8 (2012) 1768-1777.
- [126] C. Gandhimathi, J. Venugopal, R. Ravichandran, S. Sundarrajan, S. Suganya, S. Ramakrishna, Mimicking nanofibrous hybrid bone substitute for mesenchymal stem cells differentiation into osteogenesis, *Macromol. Biosci.* 13 (2013) 696-706.
- [127] P. Yilgor, N. Hasirci, V. Hasirci, Sequential BMP-2/BMP-7 delivery from polyester nanocapsules, *J. Biomed. Mater. Res.* 93 (2010) 528-536.
- [128] P. Yilgor, R.A. Sousa, R.L. Reis, N. Hasirci, V. Hasirci, Effect of scaffold architecture and BMP-2/BMP-7 delivery on in vitro bone regeneration, *J. Mater. Sci. Mater. Med.* 21 (2010) 2999–3008.
- [129] J.H. Lee, N.G. Rim, H.S. Jung, H. Shin, Control of osteogenic differentiation and mineralization of human mesenchymal stem cells on composite nanofibers containing poly[lactic-co-(glycolic acid)] and hydroxyapatite, *Macromol. Biosci.* 10 (2010) 173–182.
- [130] M.V. Jose, V. Thomas, Y. Xu, S. Bellis, E. Nyairo, D. Dean, Aligned bioactive multi-component nanofibrous nanocomposite scaffolds for bone tissue engineering, *Macromol. Biosci.* 10 (2010) 433–444.

- [131] L.X. Lu, X.F. Zhang, Y.Y. Wang, L. Ortiz, X. Mao, Z.L. Jiang, Z. D. Xiao, N. P. Huang, Effects of hydroxyapatite-containing composite nanofibers on osteogenesis of mesenchymal stem cells *in vitro* and bone regeneration *in vivo*, *ACS Appl. Mater. Interfaces* 5 (2013) 319–330.
- [132] T. Chae, H. Yang, V. Leung, F. Ko, T. Troczynski, Novel biomimetic hydroxyapatite/alginate nanocomposite fibrous scaffolds for bone tissue regeneration, *J. Mater. Sci. Mater. Med.* 24 (2013) 1885–1894.
- [133] H. Liu, H. Peng, Y. Wu, C. Zhang, Y. Cai, G. Xu, Q. Li, X. Chen, J. Ji, Y. Zhang, H.W. OuYang, The promotion of bone regeneration by nanofibrous hydroxyapatite/chitosan scaffolds by effects on integrin-BMP/Smad signaling pathway in BMSCs, *Biomaterials* 34 (2013) 4404–4417.
- [134] R. Fang, E. Zhang, L. Xu, S. Wei, Electrospun PCL/PLA/HA based nanofibers as scaffold for osteoblast-like cells, *J. Nanosci. Nanotechnol.* 10 (2010) 7747–7751.
- [135] J. Venugopal, V.R. Giri Dev, T. Senthilram, D. Sathishkumar, G. Deepika, S. Ramakrishna, Osteoblasts mineralization with composite nanofibrous substrate for bone tissue regeneration, *Cell Biol. Int.* 35 (2011) 73–80.
- [136] L. T. de Jonge, C.G. Leeuwenburgh, J.J. van den Beucken, J. te Riet, W. F. Daamen, J. G. Wolke, D. Scharnweber, J. A. Jansen, The osteogenic effect of electrosprayed nanoscale collagen/calcium phosphate coatings on titanium, *Biomaterials* 31 (2010) 2461–2469.
- [137] L. Cao, J. Wang, J. Hou, W. Xing, C. Liu, Vascularization and bone regeneration in a critical sized defect using 2-*N*,6-*O*-sulfated chitosan nanoparticles incorporating BMP-2, *Biomaterials* 35 (2014) 684–698.
- [138] L. Cao, J. A. Werkmeister, J. Wang, V. Glattauer, K. M. McLean, C. Liu, Bone regeneration using photocrosslinked hydrogel incorporating rhBMP-2 loaded 2-*N*, 6-*O*-sulfated chitosan nanoparticles, *Biomaterials* 35 (2014) 2730–2742.
- [139] X. Huang, S. Bai, Q. Lu, X. Liu, S. Liu, H. Zhu, Osteoinductive-nanoscaled silk/HA composite scaffolds for bone tissue engineering application, *J. Biomed. Materials Res. Part B: Applied Biomaterials* (2014) DOI: 10.1002/jbm.b.33323.
- [140] A. Rocca, A. Marino, V. Rocca, S. Moscato, G. de Vito, V. Piazza, B. Mazzolai, V. Mattoli, T. J. Ngo-Anh, G. Ciofani, Barium titanate nanoparticles and hypergravity stimulation improve differentiation of mesenchymal stem cells into osteoblasts, *Int. J. Nanomedicine* 10 (2015) 433–445.

- [141] L.T. Nguyen , Y.K. Min, B.T. Lee, Nanoparticle Biphasic Calcium Phosphate loading on Gelatin-Pectin scaffold for improved bone regeneration, *Tissue Eng. Part A*. 8 (2015) 1376-1387.
- [142] L. Li, G. Zhou, Y. Wang, G. Yang, S. Ding, S. Zhou, Controlled dual delivery of BMP-2 and dexamethasone by nanoparticle-embedded electrospun nanofibers for the efficient repair of critical-sized rat calvarial defect, *Biomaterials* 37 (2015) 218–229.
- [143] K. H. Park, H. Kim, S. Moon, K. Na, Bone morphogenic protein-2 (BMP-2) loaded nanoparticles mixed with human mesenchymal stem cell in fibrin hydrogel for bone tissue engineering, *J. Biosci. Bioeng.* 108 (2009) 530–537.
- [144] C. Gandhimathi, J.R.Venugopal, T.Y.Allister, S. Ramakrishna, S. Dinesh Kumar, Biomimetic hybrid nanofibrous substrate for mesenchymal stem cell differentiation into osteogenic cell, *Materi. Sci. Eng. C* 49 (2015) 776-785.
- [145] S. Kumar, C. Gandhimathi, J. Venugopal, S. Tay, S. Ramakrishna, Simultaneous electrospun-electrosprayed biocomposite nanostructured substrates for bone tissue engineering, *The FASEB Journal* 29 (2015) 876.14.
- [146] Z. F. Lu, S.I.R Esfahani, G. Wang, H. Zreiqat, Bone biomimetic microenvironment induces osteogenic differentiation of adipose tissue-derived mesenchymal stem cells, *Nanomedicine: Nanotechnology, Biology and Medicine* 8 (2012) 507–515.
- [147] R. Ravichandran, J. Venugopal, S. Mukherjee, S. Ramakrishna, Precipitation of nanohydroxyapatite on PLLA/PBLG/Collagen nanofibrous structures for the differentiation of adipose derived stem cells to osteogenic lineage. *Biomaterials* 33 (2012) 33(3), 846-855.
- [148] Y. Kobayashi, N. Udagawa, N. Takahashi, Action of RANKL and OPG for osteoclastogenesis, *Crit. Rev. Eukaryot. Gene Exp.* 19 (2009) 61–72.
- [149] J.P. Bilezikian, L.G. Raisz, T.J. Martin TJ, *Principles of Bone Biology*, New York: Academic Press (2008).
- [150] P. Jayakumar, L. Di Silvio, Osteoblasts in bone tissue engineering, *Proc. Inst. Mech. Eng. H* 224 (2010) 1415–1440.
- [151] J. P. Fan , P. Kalia, L. D. Silvio, J. Huang, In vitro response of human osteoblasts to multi-step sol–gel derived bioactive glass nanoparticles for bone tissue engineering, *Materi. Sci. Eng. C* 36 (2014) 206–214.

- [152] D. Gupta, J. Venugopal, S. Mitra, V.R. Giri Dev, S. Ramakrishna, Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts, *Biomaterials* 30 (2009) 2085–2094.
- [153] Y.Z. Zhang, J.R. Venugopal, A. El-Turki, S. Ramakrishna, B. Su, C.T. Lim, Electrospun Biomimetic Nanocomposite Nanofibers of HAp/CTS for Bone Tissue Engineering. *Biomaterials* 29 (2008) 4314-4322.
- [154] Y.Z. Zhang, J.R. Venugopal, S. Ramakrishna, C.T. Lim, Enhanced biomineralization of osteoblasts on a novel electrospun biocomposite nanofibrous scaffold of hydroxyapatite/collagen/chitosan, *Tissue Eng. A* 16 (2010) 1949-1960.
- [155] J.P. Kumar, L. Lakshmi, V. Jyothsna, D.R. Balaji, S. Saravanan, A. Moorthi, N. Selvamurugan, Synthesis and characterization of diopside particles and their suitability along with chitosan matrix for bone tissue engineering in vitro and in vivo., *J Biomed Nanotechnol.* 10 (2014) 970-981.
- [156] H. Kim, L. Che, Y. Ha, W. Ryu, Mechanically-reinforced electrospun composite silk fibroin nanofibers containing hydroxyapatite nanoparticles, *Mater. Sci. Eng. C* 40 (2014) 324–335.
- [157] F. Pourdanesh, A. Jebali, S. Hekmatimoghaddam, A. Allaveisie, In vitro and in vivo evaluation of a new nanocomposite, containing high density polyethylene, tricalcium phosphate, hydroxyapatite, and magnesium oxide nanoparticles, *Mater. Sci. Eng. C* 40 (2014) 382–388.

Figure captions

Figure 1. a) SEM micrographs and size distribution of electrosprayed core-shell particles, b) TEM micrographs of electrosprayed core-shell particles [17].

Figure 2. FESEM images of the electrosprayed particles a) PCL b) PCL/SF, c) PCL /SF/AA and d) PCL/SF/AA/DM scaffolds, e) Cumulative release profile of dexamethasone from dexamethasone-loaded scaffolds [31].

Figure 3. Diagrammatic representation of electrospraying and electrospinning system for fabricating PCL/HA nanofibers [36].

Figure 4. Schematics of developing drug loaded nanoparticles such as nanospheres or encapsulated as nanocapsules.

Figure 5. FESEM images of hFOB and its interaction with biocomposite nanofibrous substitutes a) On day 10 of cell culture the Chitosan/osteoblasts scaffolds, b) On day 15 of cell culture the Chitosan/osteoblasts scaffolds, c) On day 10 of cell culture with needle like morphology on the surface of Chitosan/HA/osteoblasts scaffolds, d) On day 15 of cell culture with granular morphology on Chitosan/HA/osteoblasts scaffolds [135].

Figure 6. The complete repair of bone with rich vessel network using the implant BMP-2/S-NP/Gel [137].

Figure 7. SEM images of a) PLLA/PBLG/COL and b) PLLA/PBLG/COL/n-HA nanofibrous substrates and the confocal microscopic images of c) PLLA and d) PLLA/PBLG/COL/n-HA biocomposite scaffolds [147].

Figure 8. FESEM images of nanostructured HA/Chitosan scaffolds a) incorporated with 20% PEO, b) incorporated with 10% PEO, c) at high magnification, d) after alkali treatment. The presence of 10% PEO in the scaffold has considerably improved the formation of chitosan fibers when compared to 20 % PEO. The nanostructured HA/Chitosan nanofibrous scaffolds showed a continuous, uniform geometry with granulate like morphology under higher magnification [153].

Abbreviations

AA- Ascorbic acid
 ALP- Alkaline phosphatase
 AmB- Amphotericin B
 Asp- Aspartic acid
 ADSCs- Adipose derived stem cells
 ATP- Adenosine tri phosphate
 BCP- Biphasic calcium phosphate
 BG- Bioactive glass
 BMP- Bone morphogenetic proteins
 BNP- Chitosan coated bovine serum albumin nanoparticles
 BT- Barium titanate
 BTE- Bone tissue engineering
 BTNPs- Barium titanate nanoparticles
 CaP- Calcium Phosphate
 CT- Calcium titanate
 CN- Chitosan
 DBM- Demineralized bone matrix
 DEX/DM- Dexamethasone
 DP- Diapside
 DXP- Dexamethasone disodium phosphate
 E2- 17 β -estradiol
 EC- Ethyl cellulose
 ECM- Extracellular matrix
 EHDA- Electrohydrodynamics atomization
 ES-DMA- Electrospray differential mobility analyser
 FESEM- Field emission electron microscope
 FITC- Fluorescein isothiocyanate
 Gel- Gelatin
 Gel-P- Gelatin-pectin
 HA- Hydroxyapatite

hFOB- human fetal osteoblast
hMSCs- human Mesenchymal stem cells
MgO- Magnesium oxide
n-HA- Nanohydroxyapatite
NMBG- Nanosized mesoporous bioactive glass
NPs- Nanoparticles
PBLG- Poly-benzyl-L-glutamate
PCL- Poly (ϵ -caprolactone)
PDGF- Platelet derived growth factor
PDLLA- Poly-(D, L-lactide)
PLLA- Poly (L-lactic acid)
PEG- Polyethylene glycol
PEO- Polyethylene oxide
PGA- Polyglycolic acid
PH- Photo polymerisable hydrogel
PEC- Poly (ϵ caprolactone) co-poly (ethylene glycol)
PHB- Poly (3-hydroxybutyrate)
PHBV- Poly (3-hydroxybutyrate-co-3-hydroxyvalerate)
PLA- Polylactic acid or polylactide
PLACL- Poly (L-lactic acid)-co-poly-(ϵ -caprolactone)
PLGA- Poly (lactic-co-glycolic) acid
PLLA- Poly (_L- lactic acid)
rBMSCs- rat bone mesenchymal stem cells
rhBMP-2- recombinant human bone morphogenetic protein-2
Runx2- Runt-related transcription factor 2
SEM- Scanning electron microscope
Se- Selenium
Si- Silicone
SF- Silk fibroin
ST- Strontium titanate
TCP- Tricalcium phosphate

TEM- Transmission electron microscope

TC- Tetracycline hydrochloride

TGF- β - transforming growth factor-beta

TiO₂- Titanium oxide

VEGF- Vascular endothelial growth factors

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Nanoparticle's in Bone Tissue Engineering

Graphical abstract

Table 1: Various materials used for fabricating nanoparticles in bone tissue engineering

	Material	Solvent	Particle size	Reference
Natural Polymers	Chitosan	Acetic acid Triphosphosphate -	520nm 175nm 521 ± 21 nm	[19] [22] [23]
	Ethyl cellulose	Dichloromethane	1 µm	[24]
Synthetic polymers	PLGA	Acetonitrile - DMSO	300 nm 268.6±93.2 nm 60nm-2µm	[27] [28] [29]
	PCL	Chloroform	10-20 µm	[31]
	PLA	Chloroform	14-19 µm	[33]
Polymer composites	PCL/HA	Methanol/chloroform	52 nm	[36]
	PHB/TiO ₂ /Chitosan Oligomers	Ethanol	<25 nm	[39]
	Gel/HA	TFE	421±75 to 679±147 nm	[40]
Inorganics and metals	Polyphosphate (polyP) complexed with Ca ²⁺	-	100 nm	[48]
	Calcium Titanate Stronium Titanate Barium Titanate	Chloroform and methanol	105 ± 20 nm 125 ± 20 nm 85 ± 10 nm	[51]
	MgO	Chloroform	20 nm	[53]
	Bioactive glass	Ethanol	<20 µm	[55]
	Bioglass 45S5	-	300–360 µm	[56]
	Calcium Silicate	Polyvinyl alcohol	<40 µm	[59]
	AuNP	Ultra-pure water and aminethanethiol	<26 nm	[60]
	Stronium based NPs	-	200-300 nm	[61]