Drug-eluting scaffolds for bone and cartilage regeneration

Lee, Wei Li; Loo, Say Chye Joachim; Huang, Charlotte L.

2014

Huang, C. L., Lee, W. L., & Loo, J. S. (2014). Drug-eluting scaffolds for bone and cartilage regeneration. Drug Discovery Today, 19(6), 714-724.

https://hdl.handle.net/10356/104609

https://doi.org/10.1016/j.drudis.2013.11.007

Drug-eluting Scaffolds for Bone and Cartilage Regeneration

Multidisciplinary strategies combining cutting-edge engineering techniques, biochemistry and medicine towards the development of the ideal functional drug-eluting scaffold for tissue regeneration.

Charlotte L. HUANG, Wei Li LEE, ✉Joachim S.C. LOO.

Nanyang Technological University
School of Materials Science & Engineering
Division of Materials Technology
N4.1-01-04a, 50 Nanyang Avenue
Singapore 639798

✉Corresponding Author:
Joachim S.C. Loo: joachimloo@ntu.edu.sg, (Ph) +65-6790-4603 (Fax) +65-6790-9081

Abstract

The advancement in strategies for bone and cartilage regeneration have been centralized on a concept that describes the close relationship between osteogenic cells, osteoconductive scaffolds, delivery growth factors and its mechanical environment. The dynamic nature of tissue repair process involves intricate mimicry of signals expressed in the biological system in response to an injury. Recently, synergistic strategies involving hybrid delivery systems that provide sequential dual delivery of biomolecules and relevant topological cues received great attention. Future advances in tissue regeneration will therefore depend on multidisciplinary strategies that encompass the crux of tissue repair aimed towards constructing the ideal functional regenerative scaffold. Here, functional scaffolds delivering therapeutics are reviewed in terms of their controlled release and healing capabilities.

Comment [CHL1]: Abstract has been shortened to 117 words.

Keywords: delivery systems, biodegradable, polymers, growth factors, tissue engineering

Dr. Joachim Loo is currently Associate Professor at the School of Materials Science and Engineering (MSE), Nanyang Technological University (NTU), Singapore. His research interests include drug delivery systems, tissue engineering, nanotoxicology, and microbial community. He has published over 100 peer-reviewed journal papers, two book chapters and filed several patents in these research areas. He was awarded the Outstanding Mentor Award and the Nanyang Award for Excellence in Teaching 2012, and the Service Recognition Award 2010. He is also managing several other portfolios such as Assistant Chair (Alumni) of MSE, and Director of the NTU Solar Fuels Laboratory. Dr. Joachim Loo is currently committee member of both the ISO/TC 229 Nanotechnology National Working Group, and the Singapore National Environmental Agency (NEA) Expert Resource Panel.

Dr. Charlotte Huang is a Postdoctoral Research Fellow at the School of Materials Science & Engineering (MSE), Nanyang Technological University (NTU), Singapore. She received her PhD in Materials Science & Engineering under the NTU Postgraduate Research Scholarship Program. Her expertise is in manipulating and controlling the delivery of therapeutics from bioresorbable polymers for biomedical applications. She has published over 10 peer-reviewed journal papers and one book chapter. She was also nominated for MSE Postgraduate Research Excellence Award 2013. Her current research focuses on modulating the sequential delivery of bone morphogenetic proteins from three-dimensional scaffolds for bone tissue regeneration.

Dr. Wei Li Lee is a postdoctoral research fellow in the School of Materials Science and Engineering (MSE), Nanyang Technological University (NTU), Singapore. His research interests encompass the development of "designer" multiphase polymeric particles and microcapsules for controlled drug delivery and their biomedical applications (e.g. chemotherapy, tissue engineering and treatment of chronic diseases). He has patented 4 technologies in the field of particle fabrication and has been the first author of more than 10 peer-reviewed journal papers. Dr. Lee obtained his B. Eng (Materials Engineering) under Accelerated Bachelor's Programme from the NTU in 2008, and received the Ph.D. degree from the NTU in 2012.

I. Introduction

Tissue regeneration is a complex yet coordinated physiological process that requires spontaneous remodeling and healing cascade. It involves timely assembly of molecules, cells, proteins, gene expression and signaling pathways within the intricate physiological environment of the human body [1,2]. Bone is one of the few organs that possesses a high level of innate repair capability, even in adult life, in response to tissue destruction or loss as a result of trauma, injury, disease or aging [3]. However, this regenerative potential is not present in cartilage, which decreases with age, causing the functional decline of many tissues. According to the latest market report from Medtech Insight, approximately 20% of the more than 5 million fractures that occur are associated with healing difficulties in the United States (U.S.) alone. The U.S. market for orthopedic biomaterials for bone repair and regeneration is projected to reach an estimate value of $3.5 billion in the year 2017 (see: http://www.medtechinsight.com/ReportA321.html). Therefore, advances in science and technology constantly seek to develop functional modalities to promote healing by restoring, repairing or replacing damaged bone and cartilage tissues [3].

In recent years, developments in tissue regeneration have been centralized on the Diamond Concept that describes the inter-relationship of four elements in bone fracture healing; i.e. osteogenic cells, osteoconductive scaffolds, growth factors, and mechanical environment [4]. A plethora of different platforms and strategies aimed at combining these four elements to enhance the overall tissue regeneration process are studied and reported extensively in the literature. The future of regenerative platforms will not only provide the mechanical and structural support, but also dynamically guide and control cell attachment, migration, proliferation and differentiation, whereby the regenerated tissue could progressively replace the scaffold platform.

In a complex permutation of materials, biological, chemical and processing technologies, balancing these factors to enable the scaffold to continuously expose an array of biological signals, and at adequate doses over a desired time frame, presents a major and technological challenge, even to date. There is a need for local controlled

and sustained delivery capabilities to minimize systemic-related issues and release of these therapeutic agents to non-target sites. Therefore, besides the four elements, controlled and sustained release strategies should also be considered as another key factor in the development of novel multifunctional tissue regenerative platforms[5]. Concurrently, the delivery platform should also exhibit biodegradability over time for the regenerated tissue to progressively replace the scaffold [2]. In this review, functional scaffolds that were developed in the recent years will be reviewed in terms of their controlled and sustained release capabilities. The controlled and sustained release of biomolecular agents from ceramic-based scaffolds, biodegradable polymeric scaffolds and their combination composites will be the scope of this review.

II. Ceramic-based Scaffolds

Ceramics play an important role in repairing and regenerating defective or damaged bone by providing a robust temporal support structure for tissues to be regenerated into the desired shape and dimension. The architecture and surface properties of these ceramic-based scaffolds are often modified to induce specific biological activity that can provide cells with optimal mechanical strength, and porosity for nutrient exchange between cells and the surrounding environment. The prerequisite for a scaffold to be considered bioactive is its ability to induce the formation of apatite that serves to bond with bone tissues [6]. One class of such ceramics is the bioactive glasses. Bioactive glasses are obtained through melt-derivation, sol-gel synthesis or surfactant chemistry using sol-gel technique [7]. Bioactive glasses have played an increasingly important role in bone tissue engineering applications by virtue of their excellent osteoconductivity, and mechanical strength[8]. In order to develop a highly ordered porous architecture with improved *in-vitro* apatite mineralization, the third generation of bioactive glasses also known as mesoporous bioactive glass (MBG) were developed in 2004 through the combination of sol-gel method and supramolecular chemistry of surfactants [9]. The use of MBG in the form of particles, spheres, and fibers for drug delivery in bone tissue regeneration has also been widely studied due to their characteristics such a tunable and uniform pore size, large surface

area and high pore volume for adsorption and release of drug molecules with a sustained profile [10-12]. The apatite formed on the surface of MBG materials has also been reported to improve drug loading efficiency, reducing burst release and retarding the release rate of drugs[13-15]. Besides MBG, other popular ceramic-based systems such as hydroxyapatite (HA) and tricalcium phosphate (TCP) are widely studied for tissue regenerative applications because they also resemble the natural inorganic component of bone and possess osteoconductive properties. These bioactive calcium phosphate-based ceramics that are applied in tissue engineering can be readily synthesized through numerous routes and techniques [16]. For example, HA and HA/β-TCP have already been used via localized administration in various clinical bone-grafting procedures and orthopedic surgeries [17,18].

However, unlike natural bone tissues, synthetic ceramic materials lack biofunctionality. To improve on osteoconductivity, osteoinductivity and osteogenesis, the surfaces of synthetic ceramic-based scaffolds are typically immobilized with various peptides, proteins and biomolecules [19]. Acharya *et al*. showed enhanced bone regeneration in the calvaria defect mice model with physiological bone remodeling through the immobilization of extracellular matrix phosphogycoprotein peptide onto HA/β-TCP particles [20]. Zhao *et al.* also reported enhanced healing and extensive bone formation in rate calvarial defects through the enhanced release of growth factors with sulfated chitosan-coated calcium-deficient HA/bone morphogenetic protein-2 (BMP-2) composite [21]. Besides the common peptide-based therapeutics, the incorporation of stem cells, e.g. mesenchymal stem cells (MSCs), into ceramic-based scaffolds has also prevailed in promoting the overall biofunctionality of ceramic scaffolds. MSCs as adult stem cells are residents of multiple tissues, and can efficiently differentiate along an osteogenic lineage, have injury-seeking abilities, immunogenic properties, and are free from ethical concerns. Bone cements containing MSCs, HA, and TCP are commonly implanted directly into bone defects as fillers such as in spinal and cosmetic surgeries. Behnia*et al*. showed the incorporation of both platelet derived growth factor (PDGF) and human mesenchymal stem cells (hMSCs) in HA/TCP scaffold to enhance the regenerative capacity clinically. An overall increase in cell regeneration through this combinative

approach of peptide and stem cells in HA/TCP scaffold was assessed and validated in cleft palate and periodontal surgeries [18,22].

Ceramic-based scaffolds have been extensively studied and developed due to their robust nature in promoting mineralization of osteoblasts and the regeneration of bone tissue [8]. 3-Dimensional (3D) ceramic scaffolds have also been successfully fabricated through various engineering technologies such as fuse deposition modeling, 3D printing, selective laser sintering, and stereolithography. However, standalone ceramic scaffolds are generally brittle, have low fracture strength and poor processability for use in applications that require structural support [23]. The setbacks of pure ceramics have limited its application to just as a coating material or as an additive to complement other delivery platforms, such as biodegradable polymers, to achieve the required osteoconductive capabilities.

III. Polymeric-based Drug-Eluting Scaffolds

Compared to ceramics, polymers offer greater versatility as sustained releasing scaffolds. Polymeric materials have garnered much interest in its use for controlled and sustained release of drugs, i.e. growth factors, ions, minerals, or cells, which would enhance and accelerate functional bone/cartilage formation [5,24,25]. These systems can be prepared using a variety of polymers of synthetic or natural origin. Natural polymers such as collagen, silk fibroin, chitosan and alginate are of great importance for bone tissue regeneration due to their biocompatibility, biodegradability, biomimetic biological properties and abundant side groups that allow for further functionalization [24,26]. However, batch-to-batch variability, their poor controllable degradation rates and potential immunogenicity pose inherent concerns for these natural polymers [26]. In an attempt to address these issues, purification and recombinant technology has streamlined mass production of natural polymers with well-defined material properties [27,28]. Gamma irradiation and partial oxidation have been employed to achieve the desired biodegradability of alginate [29]. Using crosslinking methods [30,31] or composites comprising inorganic or

synthetic materials such as nano-HA/collagen/PLLA scaffold, mineralized and apatite-coated collagen [32,33] have been demonstrated to enhance physical properties. On the other hand, synthetic polymers generally offer greater versatility in overcoming the above issues, and in their ease of fabrication, adjustable physicochemical and mechanical properties [26]. The main problems associated with synthetic polymers, however, are poor cell adhesion and possible inflammatory response induced by acidic degradation by-products [24,34]. Nevertheless, polymer-based scaffolds for the delivery of therapeutic are versatile, and can be fashioned into microparticles, nanoparticles, hydrogel, porous matrix, fiber and their hybrids.

a) Polymeric-based Particulates for Tissue Regeneration

Natural polymers such as collagen, which contains RGD (Arginine-Glycine-Aspartic acid) sequences, provide a signaling extra-cellular microenvironment that promotes the attachment, migration, proliferation and differentiation of progenitor cells for the regenerative process. Gelatin, as a derivative from collagen, has been widely used to encapsulate bioactive molecules through polyion complexes, by which diffusional loading of molecules can be achieved after the formation of particles. This would minimize the exposure of bioactive factors to harsh conditions such as organic solvents during particle preparation or cross-linking process. Oppositely charged gelatin nanospheres have been developed as injectable colloidal gels as a result of electrostatic self-assembly, which showed a great potential to be used as bone fillers and programmed drug delivery systems (**Figure 1**), as demonstrated in the *in-vivo* study using a rat femoral condyle defect model [35,36]. Growth factor release from gelatin carriers was mainly controlled by the enzymatic degradability. By loading bFGF into rapidly degrading nanospheres of low crosslinking density and BMP-2 into nanospheres of lower degradability (high crosslinking density), a combination of fast release of bFGF and slow release of BMP-2 was observed. It was reported that the development of gelatin colloidal gel can overcome some of the limitations of poly(lactide-*co*-glycolide) (PLGA)-based colloidal gels, including the functionalization of polymer chain with charged groups, the lack of cell attachment sites and the release of acidic degradation products [37]. Nanostructured colloidal systems generally possess superior viscoelastic properties over microsphere-based systems in terms of injectability, structural integrity, and recovery behavior upon

network destruction, due to sufficiently strong interparticle forces [38]. Still, research into nanoparticulate delivery systems for bone tissue engineering has been comparatively limited compared to microspheres, possibly because of the requirement in manipulating microscale structures to achieve macroporosity for bone regeneration[39,40]. Furthermore, nanoparticles often lead to undesirable burst release, enhanced particle agglomeration and reduced stability, arising from its high surface-to-volume ratio[41,42]. As a result, natural polymer-based microspheres have found its usefulness in cell delivery and tissue engineering. Alginate microspheres can be prepared using ionic crosslinking in the presence of divalent cations such as Ca^{2+}[43]. This gentle gelling process would facilitate the mild encapsulation of bioactive factors. It has been recently shown that heparin-functionalized alginate microbeads displayed *in-vivo* localized delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) that induced controlled bone formation [44]. The capacity of sustained release of rhBMP-2 for approximately 3 weeks was achieved due to excellent immobilization of the growth factor within the polyelectrolyte membrane formed with heparin andpoly-l-lysine on the alginate microbead surfaces. Another unique characteristic of alginate microspheres is its ability to induce calcification *in-vivo* without using additives. Lee *et al.* demonstrated an injectable calcium cross-linked alginate microbead system that mineralized *in-vivo* to from calcium phosphate (CaP) through the interaction of calcium ions with physiological phosphate ions [45].

Synthetic polymers (e.g. polyesters- polylactides (PLA) and PLGA) have the advantage of achieving sustained release of encapsulated molecules over a prolonged period [46], as compared to natural polymers, which typically have a relatively short release period. However, the production of acidic degradation products would cause inflammatory tissue response and denaturation of bioactive factors. Jiang *et al.* developed BMP-2 surface-adsorbed chitosan/PLGA microspheres to achieve the neutralization between chitosan and PLGA degradation products, thus improving biocompatibility and guiding bone formation, as evidenced from a rabbit model [47]. A common problem with the emulsion method of producing lipophilic particles is the use of organic solvents, which may cause the denaturation of encapsulated substances. Electrospraying has emerged as an effective technique for the formation of particles

since the presence of interface between the organic and aqueous phases can be avoided [48], and unlike spray drying, high temperatures can be avoided. The controlled release of biomolecules or growth factors in the highly bioactive form from electrosprayed particles had been successfully demonstrated in the preparation of PLGA microspheres loaded with simvastatin and block copolymer loaded with BMP-6 and transforming growth factor β (TGF-β), respectively [49-51]. It was found that protein release kinetics from the amphiphilic poly(1,3-trimethylene carbonate-*co*-ε-caprolactone)-*b*-poly(ethylene glycol)-*b*-poly(1,3-trimethylene carbonate-*co*-ε-caprolactone) (P(TMC-CL)$_2$-PEG) microspheres was influenced by the protein isoelectric point, with positively charged proteins (BMP-6) releasing at a slower rate than negatively charged proteins (TGF-β). This release kinetic could be attributed to positively charged proteins adsorbing to a greater extent on the surface of the P(TMC-CL)$_2$-PEG or its negatively charged degradation products. Liposome-based particles represent another carrier for growth factors because the use of harsh organic solvents during preparation is avoided. One limitation of liposome systems though is the short-term release. A study by Haidar *et al.* reported a core-shell nanoparticulate system comprising cationic liposome core coated with layer-by-layer self-assembly of alginate and chitosan that exhibited slow release of rhBMP-7, which promoted bone formation in a rabbit model [52]. This core-shell system allows for protein entrapment within the aqueous core and the customizable polyelectrolyte layers in the shell which can modulate the release kinetics, whereby an increase in shell thickness would slow the rate of protein release. However, it is noteworthy that further studies are needed to investigate if the sustained release of growth factors can be achieved for complete bone regeneration within a physiologically relevant period.

Bone/cartilage repair occurs as a part of cascade involving several growth factors and chemokines dominantly up- or down-regulated in a specific spatiotemporal pattern. Therefore, the development of the particulate delivery system incorporating multiple signaling molecules has emerged to improve the functionality of the constructs. PLGA core- alginate shell microcapsules were developed to achieve dual release of BMP-2 and dexamethasone (DEX), which enhanced expression of osteogenic markers when co-cultured with rat bone marrow stromal cells [53]. The release profile of each biomolecule can be manipulated based on its specific localization in each

layer. Early release of the biomolecule from the shell was observed, followed by another biomolecule from the core. For cartilage repair, DEX-loaded PLGA microspheres consisting of polyplexed SOX9 genes plus heparinized TGF-β 3 were used to improve the chondrogenic differentiation of mesenchymal stem cells (MSCs) *in-vivo* **(Figure 2)** [54]. Mathiowitz *et al.* demonstrated a sequential release of IGF-I and TGF-β_1 from modular designed PLGA microsphere-based scaffolds where a combination of microspheres of different release kinetics (burst or delayed release) were fused through a dichloromethane vapor method [55]. The growth factor release kinetics could be modulated by adding bovine serum albumin (BSA) and tuning the terminal end-group of PLGA. Incorporating BSA in the IGF-I formulations decreased the initial burst from 80% to 20%, while using uncapped PLGA containing a carboxylic acid terminal group decreased the initial burst of TGF-β_1 from 60% to 0%, compared to capped PLGA. This result could be due to the BSA, growth factors and uncapped PLGA secondary interactions, which lead to a better distribution of growth factors within the microspheres. Multiple delivery systems have also been investigated for the regeneration of orthopedic interfaces. Mohan *et al.* established an oppositely oriented gradient of fused BMP-2- and TGF-β1-loaded PLGA microspheres for osteochondral regeneration in rabbit condyle defects [56]. The spatially controlled growth factors delivery induces the stem cells to differentiate along different lineages within the same construct. However, current design challenges for engineering biomimetic gradients are related to the difficulties in mimicking the natural concentration gradient (micro- or nanoscale) in living systems, and in retaining the gradient patterns for the long term due to rapid diffusion of molecules. On another system, the use of multiple growth factors and biomolecules incorporated into nanosphere-coated microspheres was demonstrated to induce chondrogenesis, osteogenesis, and adipogenesis of hMSCs, in which the delivery system can be introduced as a 3D injectable scaffold *in-vivo* via a minimally invasive procedure [57]. An *in vivo* release test of growth factors conjugated with Cy5.5 labeling, such as BMP, TGF- 3 and IGF/bFGF, were evaluated via bioimaging, and a sustained release from the nanosphere-coated microspheres was shown for 3 weeks. Heparin was selected to form oppositely charged complexes with these growth factors to increase the stabilization and bioactivities of growth factors. One should note that further investigations are still required to determine optimal doses, growth factor ratios, dosing sequence and release kinetics for long-term *in-vivo* tissue regeneration.

Compared to conventional monolithic matrix-based carriers which exhibit a rather poor ability for programmed delivery of multiple biomolecules, the independent incorporation of particulate-based systems holds great promise. This is because separate particles can be made from different materials and can contain different biomolecules, by which the release kinetics can be tailored independently. In addition, injectability/moldability of particles would allow for optimal filling of irregularly shaped defects through minimally invasive procedures. However, a concern of using particle-based scaffolds for bone tissue engineering is their poor mechanical integrity arising from weak interparticle interactions, which would lead to the migration of individual particles from defect sites. Therefore, different approaches involving the use of glues, crosslinkers, electrostatic assembly, thermal or vapor fusion have been explored to enhance the structural integrity of particle-based scaffolds.

b) 3D Matrix-type Scaffolds

Biodegradable polymers are often used to provide structural support during bone and cartilage repair due to their ease of synthesis, processability, versatility and biodegradability. Their lack of biological recognition can be readily addressed by incorporating tissue inductive and conductive components, such as cells and growth factors. With the extensive developments in 3D scaffolds that incorporate growth factors, there has been particular interest in using recombinant forms of bone morphogenetic proteins (BMPs) for bone tissue regeneration. BMPs are closely associated with growth, maturation and regulation of bone, and are considered the most potent stimulator of functional new bone formation. However, due to the short *in-vivo* half-life of BMPs, maintaining therapeutic concentrations over time has been a complex issue. The use of high dosage of growth factors not only poses long-term complications but also increases the cost of therapy [58]. Although many delivery systems for growth factors have been developed over the last decade, a robust system that sustains and controls the release of therapeutic dose from scaffolds for the promotion of bone regeneration still remains a challenge. Besides maintaining a rapid infiltration of host cells into the scaffold with low immunogenic and antigenic response, a high encapsulation efficiency that is able to sustain the delivery of the therapeutic dose at a desirable rate is also vital.

Natural polymers such as collagen sponges loaded with BMP-2 and BMP-7 growth factors generally displayed poor release kinetics and inability to provide sustained release [59,60]. The use of biodegradable synthetic polymers can better provide controlled and sustained release of biomolecules for enhanced proliferation, differentiation and bone formation capabilities. Murphy *et al.* reported 70% growth factor activity for up to 12 days by incorporating VEGF into a 3D porous PLGA scaffold that provided sustained release over a 15 day period [61]. Santana *et al.* also showed that the controlled local release of FGF-2 that was absorbed on a polyglycoate:polylactide membrane over a 2-week period stimulated bone defect healing in diabetic animal models [62]. Yilgor*et al.* on the other hand showed the ability to provide sustained and sequential release of BMP-2 and BMP-7 from biodegradable poly(caprolactone) (PCL) polymeric scaffolds with enhanced bone formation. Compared to the effect of the release of a single growth factor, sequential delivery of dual growth factors such BMP-2 and TGF-β_3, BMP-2 and VEGF, or BMP-2 and IGF-1 have showed improvement in bone formation [63-66]. The modification of scaffold geometry or architecture has also been reported to be able to control and modulate the release kinetics of these biomolecules [67-69]. Polymers such as PLLA and PCL have been widely used to entrap BMP-2 in fibers through electrospinning techniques. For example, new bone formation that filled 45% of critical-sized cranial defect in a mouse model was reported at 12 weeks with BMP-2 loaded PLLA electrospun matrix-based scaffolds [70]. Saito *et al.* showed the formation of new bone in a rat subcutaneous pocket model by seeding human gingival fibroblast cells that have been modified to deliver BMP-7 from phase-deposition modeled biodegradable synthetic poly($_L$-lactic acid) (PLLA) and PLGA [71]. Besides achieving sustained and controlled delivery, it is also important to consider the dose of biomolecules that has been loaded into these biodegradable polymers. Young *et al.* have shown that decreasing amounts of BMP-2 would result in a dose-dependent reduction in bone formation in a rat cranial critical-size defect model[65]. Therefore, in order to provide sustained release over a period of time and yet still be able to deliver efficacious doses, many strategies have been developed to improve the total loading of biomolecules or drugs in synthetic biodegradable polymers. For example, the use of supercritical fluid (SCF) improved the encapsulation efficiencies of VEGF

and BMP-2 respectively, with negligible loss in bioactivity, when incorporated into PLGA polymer [72,73].

Cross-linked biodegradable polymers such as hydrogels can also be constructed into matrix-based scaffolds for the controlled and sustained delivery of therapeutics. Hydrogels are highly hydrated polymer networks that can be constructed into 3D scaffolds upon cross-linking through methods such as chemical and photo-initiation. They can be easily constructed, can entrap molecules within their hydrated matrix and have been widely investigated for cartilage tissue regeneration. These biodegradable polymers offer tunable physicochemical properties to control the release of bioactive functional factors for bone tissue restoration. Depending on the choice of material, the delivery and physicochemical characteristics of hydrogels can be tailored to respond to changes in pH, or temperature in its surrounding environment [74]. Hydrogels tend to exhibit characteristics similar to soft tissues and therefore provide a supportive matrix for chrondrocyte activity and cartilage extracellular matrix (ECM) secretion when impregnated with chrondrocytes [75]. Peptides have also been immobilized onto a macroporous alginate scaffold and has been shown to promote transforming growth factor beta- (TGF-β) induced human MSC differentiation, which is an essential feature of the microenvironment of cells [76]. Bone tissue formation was also observed in another study using alginate scaffolds embedded with MSCs [77]. Freeman *et al.* showed 3-fold greater in blood vessel density and mature vessels in alginate scaffolds that provided the sequential release of VEGF, PDGF and TGF-TGF-$\beta_1$growth factors. The factors of release rates were correlated with the equilibrium binding constants of the factors to the alginate matrix that enabled the controlled sequential delivery of these biomolecules [78]. Kimura *et al.* showed that proper control over the release of BMP-2 enhanced the formation of bone tissue through the manipulation of water content in gelatin hydrogels [79]. Statins such as simvastatin have also been incorporated into methylcellulose gels to provide localized drug release over a period of 44 days in a murine calvarial model [80]. Continuous release of fluvastatin was also achieved through the controlled hydrolysis of lactic acid ester bonds by the co-polymerization of the statin drug with the dimethacrylated PEG hydrogel network [81]. Nelson *et al.* demonstrated extended sequential release of BSA from thermoresponsive hydrogels made of N-isopropylacrylamide(NIPAAm),

2-hydroxylethyl methacrylate (HEMA) and methacrylate polylactide modified with methacryloxy N-hydroxysuccinimide (MANHS) [82]. The constant release of drugs was therefore able to provide continuous chemical signals or triggers for bone tissue regeneration. Despite the ability to provide continuous drug releases and chemical cues, hydrogels alone are more suited to provide injectable and localized bone healing therapies. The intrinsically weak mechanical properties of hydrogel-based scaffolds would require the use of a secondary system to essentially provide structural and physical cues to further enhance bone and cartilage regeneration [83].

IV. Composite/Hybrid-based Scaffolds

To address the current limitations in bone and cartilage regeneration, studies and trials have been constantly evolving towards the ideal tissue regenerating scaffold by developing composite or hybrid systems. These include ceramic-polymer composite systems or hydrogel-polymer hybrid systems. To achieve the ideal scaffold requires the astute selection and combination of materials, biological, chemical and processing technologies. As such, next-generation systems should exploit the combination of signal and cues in promoting the synergistic repair of bone and cartilage [84].

a) Ceramic-Polymer Scaffolds for Bone Repair

While polymers provide controlled and sustained release of therapeutics, the poor cell affinity, low mechanical strength and lack of osteoconductivity necessitate the addition of a biologically active component, such as bioceramics. This allows the system to favorably promote bone regeneration because of the bioactivity of these ceramics. Therefore, polymers and ceramics have been combined to form hybrid or composite scaffolds that enhance the biological properties of polymers, while at the same time facilitating the ease of processing ceramics. Kim *et al.* developed a hydroxyapatite (HA) scaffold using DEX-loaded PLGA microspheres for bone regeneration [85]. *In-vivo* evaluation of the bone defects filled with DEX-loaded HA scaffolds indicated that new bone formation was enhanced as compared with defects that were filled only with HA scaffold. In an injectable system, PLGA/HA microspheres with adsorbed BMPs were shown to better enable osteoblast

differentiation [86]. However, polymers generally degrade faster than most ceramics, thus causing an uneven scaffold degradation which may create issues such as osteolysis [87]. To achieve a uniform erosion of the scaffolds, a more rapidly degrading amorphous CaP or slower degrading polymer could be considered for future development.

More recently, other strategies to incorporate biologically active components within biodegradable polymers that can sustain therapeutic delivery have also emerged in bone regeneration. Nandakumar *et al.* showed the expression of a number of osteogenic markers as potential indication for use in bone tissue engineering. The 3D scaffold was fabricated through the combination of rapid prototyping, electrospinning and biomimetic CaP coating in a simulated physiological environment as illustrated in **Figure 3** [88]. A nucleating agent such as sodium silicate can also be used to deposit biomimetic apatite on starch-PCL 3D scaffolds under static and dynamic conditions [89]. Carbonated apatite was also reported to be combined with gelatin and deposited onto PCL-TCP 3D scaffold through a screw extrusion system [90]. Macdonald *et al.* reported the sustained release of BMP-2 over a period of two weeks from 3D PCL scaffold coated with a tetra-layered structure of oppositely-charged chrondroitin sulfate units through layer-by-layer deposition technique as shown in **Figure 4** [91]. BMP-2 loaded PLGA composite scaffolds modified with varying concentrations of HA nanoparticles showed sustained release and complete bone healing in nude mice tibial defects over six weeks [92]. The use of ceramics and its derivatives can enhance mechanical properties, retard release of therapeutics and confer angiogenic characteristics to these biodegradable polymers. The incorporation of bioceramics into polymers through combinatorial processing strategies and techniques is undoubtedly the key towards fabricating hybrid scaffolds with synergistic properties for bone tissue engineering applications [84].

b) Hydrogel-Polymer Scaffolds in Soft Tissue Regeneration

The incorporation of hydrogels such as gelatin, alginate and poly(ethylene glycol) (PEG) to a polymer or ceramic-polymer system is more prevalent in the treatment of soft tissue injuries and dysfunction. The development of hybrid systems can provide

biomimetic multi-tissue regions that facilitate stem-cell orientation, and tissue regeneration in both the cartilaginous and bony environment [93]. Jiang *et al.* developed a multi-phased 3D scaffold composed of agarose gel and PLGA microparticles, sintered with bioactive glass, to promote chrondrocyte mineralization, calcification and bone formation [94]. Another composite composed of BMP-2 loaded PLGA microspheres embedded in a poly(propylene) scaffold was surrounded by a VEGF-loaded gelatin hydrogel [95]. This hybrid system was used for the sequential release of growth factors which demonstrated enhanced ectopic bone formation in the femoral rat model. The sequential release of VEGF and BMP-2 also demonstrated greater volume of bone formation in a cranial rat model with gelatin microparticles incorporated within porous scaffold of poly(propylene fumarate) (PPF) [64,65]. To be able to achieve sequential release of therapeutics, precise control over its release kinetics is crucial. Huang *et al*. have demonstrated that PEG-incorporated PLGA films can accurately modulate both the delivery of therapeutics as well as the biodegradation of the scaffold over a period of 2 weeks [96]. Such precise hydrogel-polymer delivery system can be further embedded with bioactive components to promote a more physiologically relevant environment for bone and cartilage repair.

Given the capabilities to control and modulate the release kinetics, dual delivery of therapeutics have become more prevalent in recent years, with reported synergistic effects on bone and cartilage regeneration both *in-vitro* and *in-vivo* [97,98]. The physiologically relevant controlled release of biomolecules tends to mimic the natural healing response that led to improved functional tissue regeneration. Moreover, the carrier properties, growth factor combination and its sequential release greatly influence the osteogenic differentiation and subsequent tissue regenerative capabilities. Tadokoro *et al*. explored an interesting route by developing a carrier for both rat MSCs and BMP-2 based on gelatin sponges that incorporates CaP [99]. The findings indicated that the combination of MSCs, gelatin-CaP composite and BMP can synergistically enhance osteogenic capabilities in bone tissue regeneration. Nandagiri*et al*. showed the increase in osteogenic activity with sequential release of BMP-2 followed by BMP-7 from chitosan-gelatin cross-linked scaffolds embedded with PLGA nanoparticles [100]. Basmanav*et al*. reported similar improvement in osteogenic differentiation with the same growth factors with PLGA scaffolds loaded

with microspheres made of poly(4-vinyl pyridine) and alginic acid polyelectrolyte complexes [101]. To further improve the environment for cell differentiation and proliferation, Lee *et al*. incorporated CPC into chemically cross-linked alginate scaffolds impregnated with MSCs. Their results showed almost complete closure of defect in the rat calvarium at 6 weeks [102]. In general, these hybrid scaffolds functioned to provide relevant and continuous topological, chemical and biological cues, mimicking the natural tissue healing response as closely as possible.

V. Conclusion & Prospective

Advancing technology through hybrid scaffolds provides the potential to sequentially deliver multiple bioactive factors with different spatial and temporal profiles. This is important in the development of novel multifunctional scaffolds aimed at mimicking the complex processes involved in natural tissue healing. In particular, the dual delivery of angiogenic factors and osteoinductive growth factors seemed to be a promising approach, as angiogenesis is essential for the recruitment of progenitor cells for subsequent osteogenic differentiation. The incorporation of biomolecules such as growth factors and bioactive ceramics into suitable biodegradable polymeric scaffolds can certainly function to promote cell attachment, differentiation and proliferation. Consequently, these strategies are focused to closely mimic the natural process of bone and cartilage regeneration.

While delivery scaffolds, by itself or hybrid, are continually being optimized to provide topological, chemical, biological cues, and relevant environment for bone and cartilage regeneration, there remain a number of challenges to be addressed. The bioactivity of growth factors are known to be affected by the various methods used to incorporate them into scaffolds. The exposure to organic solvents during processing stages would impose harsh or denaturing conditions to growth factors that are typically protein-based. The loss in bioactivity of these growth factors would therefore require some form of compensation, for example through higher protein loading, in order to achieve the desired release kinetics and therapeutic efficacy. The appropriate selection of biomolecules, carrier system and processing technique to construct an optimal functional hybrid combination, perhaps toward the ideal tissue regenerating scaffold, has to be intricately designed and engineered. Moreover, the effect of carrier sterilization has also not been comprehensively assessed and evaluated in current optimized delivery systems. The choice of sterilization method may possibly impact the stability of both the biomolecule and carrier. Sterilization techniques involving the use of chemicals or radiation may alter the biofunctionality that has been engineered on the scaffold system, possibly resulting in the loss of biochemical and signaling activities. These factors should be taken further into consideration in designing and engineering scaffolds of the next generation.

The translation from *in-vitro* to *in-vivo* evaluation is often burdened by difficulties and complex issues. As such, strong correlations are rarely established between these various avenues of assessment of any delivery system. A successful delivery system assessed *in-vitro* typically requires further optimization to translate to *in-vivo* or clinical studies. Moreover, the dosage required for tissue repair varies significantly from patient to patient. It is also difficult to determine specific dosages since they are dependent on the size and dynamic nature of the compromised zone. The degree of injury, natural cellular differentiation process, and the presence of other signaling molecules may play a role in determining the optimal dosage as well. Moreover, defects created in animal models are generally mediocre in term of signaling pathways and biomolecular expressions when compared to defects in humans such that they could have insignificant clinical relevance. Improvement in non-invasive diagnostic assessment tools should also be concurrently developed to monitor the location of biomolecules post-implantation. Future advances in bone and cartilage regeneration will therefore depend on multidisciplinary strategies that combine processing techniques, engineering principles, biochemistry and medicine aimed towards constructing the ideal functional regenerative scaffold for tissue repair, i.e. bone or cartilage.

Acknowledgements

The authors would like to acknowledge the financial support from the Agency for Science, Technology and Research (A*STAR) (Project No: 102 129 0098 – Drug-releasing bioactive materials and adipose stem cells as autologous scaffolds for bone tissue engineering).

References

1 Berthiaume, F. et al. (2011) Tissue engineering and regenerative medicine: history, progress, and challenges. *Annu Rev Chem Biomol Eng* 2, 403-430

2 Naderi, H. et al. (2011) Review paper: Critical Issues in Tissue Engineering: Biomaterials, Cell Sources, Angiogenesis, and Drug Delivery Systems. *J Biomat Appl* 26 (4), 383-417

3 Huey, D.J. et al. (2012) Unlike bone, cartilage regeneration remains elusive. *Science* 338 (6109), 917-921

4 Giannoudis, P.V. et al. (2007) Fracture healing: The diamond concept. *Injury* 38, Supplement 4 (0), S3-S6

5 Zilberman, M. et al. (2010) Drug-eluting medical implants. *Handb Exp Pharmacol* 197, 299-341

6 Giavaresi, G. et al. (2004) Poly(2-hydroxyethyl methacrylate) biomimetic coating to improve osseointegration of a PMMA/HA/glass composite implant: in vivo mechanical and histomorphometric assessments. *Int J Artif Organs* 27 (8), 674-680

7 Will, J. et al. (2012) Bioactive Glass-Based Scaffolds for Bone Tissue Engineering. In *Tissue Engineering III: Cell - Surface Interactions for Tissue Culture* (Vol. 126) (Kasper, C. et al., eds.), pp. 195-226, Springer Berlin Heidelberg

8 Jones, J.R. (2013) Review of bioactive glass: From Hench to hybrids. *Acta biomaterialia* 9 (1), 4457-4486

9 Biondi, M. et al. (2008) Controlled drug delivery in tissue engineering. *Advanced Drug Delivery Reviews* 60 (2), 229-242

10 Wu, C. and Chang, J. (2012) Mesoporous bioactive glasses: structure characteristics, drug/growth factor delivery and bone regeneration application. *Interface Focus* 2 (3), 292-306

11 Zhao, Y.F. et al. (2008) In situ SAXRD study of sol-gel induced well-ordered mesoporous bioglasses for drug delivery. *J Biomed Mater Res A* 85 (4), 1032-1042

12 Arcos, D. and Vallet-Regi, M. (2010) Sol-gel silica-based biomaterials and bone tissue regeneration. *Acta Biomater* 6 (8), 2874-2888

13 Wu, C. et al. (2010) Structure–property relationships of silk-modified mesoporous bioglass scaffolds. *Biomaterials* 31 (13), 3429-3438

14 Wu, C. et al. (2010) Bioactive mesopore-glass microspheres with controllable protein-delivery properties by biomimetic surface modification. *J Biomed Mater Res A* 95 (2), 476-485

15 Wu, C. et al. (2011) In situ preparation and protein delivery of silicate-alginate composite microspheres with core-shell structure. *J R Soc Interface* 8 (65), 1804-1814

16 Khung, Y.-L. et al. (2012) Designing calcium phosphate-based bifunctional nanocapsules with bone-targeting properties. *Journal of Nanoparticle Research* 14 (6), 1-13

17 Acharya, B. et al. (2012) Surface immobilization of MEPE peptide onto HA/β-TCP ceramic particles enhances bone regeneration and remodeling. *J Biomed Mat Res Part B: Appl Biomat* 100B (3), 841-849

18 Sarment, D.P. et al. (2006) Effect of rhPDGF-BB on bone turnover during periodontal repair. *J Clin Periodontol* 33 (2), 135-140

19 Poh, C.K. et al. (2012) In vitro characterizations of mesoporous hydroxyapatite as a controlled release delivery device for VEGF in orthopedic applications. *J Biomed Mater Res A* 100 (11), 3143-3150

20 Acharya, B. et al. (2012) Surface immobilization of MEPE peptide onto HA/beta-TCP ceramic particles enhances bone regeneration and remodeling. *J Biomed Mater Res B Appl Biomater* 100 (3), 841-849

21 Zhao, J. et al. (2012) Enhanced healing of rat calvarial defects with sulfated chitosan-coated calcium-deficient hydroxyapatite/bone morphogenetic protein 2 scaffolds. *Tissue Eng Part A* 18 (1-2), 185-197

22 Behnia, H. et al. (2012) Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: A preliminary report. *Journal of Cranio-Maxillofacial Surgery* 40 (1), 2-7
23 Best, S.M. et al. (2008) Bioceramics: Past, present and for the future. *Journal of the European Ceramic Society* 28 (7), 1319-1327
24 Wang, H. et al. (2011) The use of micro-and nanospheres as functional components for bone tissue regeneration. *Tissue Engineering Part B: Reviews* 18 (1), 24-39
25 Cartmell, S. (2009) Controlled release scaffolds for bone tissue engineering. *J Pharm Sci* 98 (2), 430-441
26 Vo, T.N. et al. (2012) Strategies for controlled delivery of growth factors and cells for bone regeneration. *Advanced drug delivery reviews* 64 (12), 1292-1309
27 Gomes, S. et al. (2012) Natural and genetically engineered proteins for tissue engineering. *Progress in Polymer Science* 37 (1), 1-17
28 Malafaya, P.B. et al. (2007) Natural-origin polymers as carriers and scaffolds for biomolecules and cell delivery in tissue engineering applications. *Adv Drug Deliv Rev* 59 (4-5), 207-233
29 Grellier, M. et al. (2009) The effect of the co-immobilization of human osteoprogenitors and endothelial cells within alginate microspheres on mineralization in a bone defect. *Biomaterials* 30 (19), 3271-3278
30 Jeon, O. et al. (2011) Affinity-based growth factor delivery using biodegradable, photocrosslinked heparin-alginate hydrogels. *Journal of controlled release : official journal of the Controlled Release Society* 154 (3), 258-266
31 He, Q. et al. (2011) Improved cellularization and angiogenesis using collagen scaffolds chemically conjugated with vascular endothelial growth factor. *Acta Biomater* 7 (3), 1084-1093
32 Yang, H.S. et al. (2011) Apatite-coated collagen scaffold for bone morphogenetic protein-2 delivery. *Tissue Eng Part A* 17 (17-18), 2153-2164
33 Niu, X. et al. (2009) Porous nano-HA/collagen/PLLA scaffold containing chitosan microspheres for controlled delivery of synthetic peptide derived from BMP-2. *Journal of controlled release : official journal of the Controlled Release Society* 134 (2), 111-117
34 Blackwood, K.A. et al. (2012) Scaffolds for growth factor delivery as applied to bone tissue engineering. *International Journal of Polymer Science* 2012
35 Wang, H. et al. (2011) Oppositely charged gelatin nanospheres as building blocks for injectable and biodegradable gels. *Advanced Materials* 23 (12), H119-H124
36 Wang, H. et al. (2013) Combined delivery of BMP-2 and bFGF from nanostructured colloidal gelatin gels and its effect on bone regeneration *in vivo*. *Journal of Controlled Release* 166 (2), 172-181
37 Wang, Q. et al. (2010) Injectable PLGA based colloidal gels for zero-order dexamethasone release in cranial defects. *Biomaterials* 31 (18), 4980-4986
38 Wang, H. et al. (2012) Comparison of micro-vs. nanostructured colloidal gelatin gels for sustained delivery of osteogenic proteins: Bone morphogenetic protein-2 and alkaline phosphatase. *Biomaterials* 33 (33), 8695-8703
39 Fu, H. et al. (2013) Evaluation of bone regeneration in implants composed of hollow HA microspheres loaded with transforming growth factor beta1 in a rat calvarial defect model. *Acta Biomater* 9 (3), 5718-5727
40 Hoekstra, J.W. et al. (2013) The in vivo performance of CaP/PLGA composites with varied PLGA microsphere sizes and inorganic compositions. *Acta Biomater* 9 (7), 7518-7526
41 Hasan, A.S. et al. (2007) Effect of the microencapsulation of nanoparticles on the reduction of burst release. *Int J Pharm* 344 (1-2), 53-61
42 Cho, W.S. et al. (2013) Surface functionalization affects the zeta potential, coronal stability and membranolytic activity of polymeric nanoparticles. *Nanotoxicology* 28, 28

43 Lim, M.P.A. et al. (2013) One-step fabrication of core-shell structured alginate-PLGA/PLLA microparticles as a novel drug delivery system for water soluble drugs. *Biomaterials Science* 1 (5), 486-493

44 Abbah, S.A. et al. (2012) Enhanced control of *in vivo* bone formation with surface functionalized alginate microbeads incorporating heparin and human bone morphogenetic protein-2. *Tissue Engineering Part A* 19 (3-4), 350-359

45 Lee, C.S. et al. (2010) Regulating *in vivo* calcification of alginate microbeads. *Biomaterials* 31 (18), 4926-4934

46 Lee, W.L. et al. (2011) Altering the drug release profiles of double-layered ternary-phase microparticles. *Journal of Controlled Release* 151 (3), 229-238

47 Jiang, T. et al. (2010) Chitosan–poly (lactide-co-glycolide) microsphere-based scaffolds for bone tissue engineering: *In vitro* degradation and *in vivo* bone regeneration studies. *Acta Biomaterialia* 6 (9), 3457-3470

48 Bock, N. et al. (2012) Electrospraying of polymers with therapeutic molecules: State of the art. *Progress in polymer science* 37 (11), 1510-1551

49 Nath, S.D. et al. (2013) Preparation and characterization of PLGA microspheres by the electrospraying method for delivering simvastatin for bone regeneration. *International journal of pharmaceutics* 443, 87-94

50 Sukarto, A. and Amsden, B.G. (2012) Low melting point amphiphilic microspheres for delivery of bone morphogenetic protein-6 and transforming growth factor-β3 in a hydrogel matrix. *Journal of Controlled Release* 158 (1), 53-62

51 Morris, M.S. et al. (2008) Injectable simvastatin in periodontal defects and alveolar ridges: pilot studies. *J Periodontol* 79 (8), 1465-1473

52 Haidar, Z.S. et al. (2010) A hybrid rhOP-1 delivery system enhances new bone regeneration and consolidation in a rabbit model of distraction osteogenesis. *Growth Factors* 28 (01), 44-55

53 Choi, D.H. et al. (2010) Fabrication of core–shell microcapsules using PLGA and alginate for dual growth factor delivery system. *Journal of Controlled Release* 147 (2), 193-201

54 Park, J.S. et al. (2012) SOX9 gene plus heparinized TGF-β 3 coated dexamethasone loaded PLGA microspheres for inducement of chondrogenesis of hMSCs. *Biomaterials* 33 (29), 7151-7163

55 Jaklenec, A. et al. (2008) Sequential release of bioactive IGF-I and TGF-beta 1 from PLGA microsphere-based scaffolds. *Biomaterials* 29 (10), 1518-1525

56 Mohan, N. et al. (2011) Continuous gradients of material composition and growth factors for effective regeneration of the osteochondral interface. *Tissue Engineering Part A* 17 (21-22), 2845-2855

57 Park, J.S. et al. (2011) The promotion of chondrogenesis, osteogenesis, and adipogenesis of human mesenchymal stem cells by multiple growth factors incorporated into nanosphere-coated microspheres. *Biomaterials* 32 (1), 28-38

58 Carragee, E.J. et al. (2011) A critical review of recombinant human bone morphogenetic protein-2 trials in spinal surgery: emerging safety concerns and lessons learned. *Spine J* 11 (6), 471-491

59 Geiger, M. et al. (2003) Collagen sponges for bone regeneration with rhBMP-2. *Adv Drug Deliv Rev* 55 (12), 1613-1629

60 Gu, Y. et al. (2011) Evaluation of an injectable silk fibroin enhanced calcium phosphate cement loaded with human recombinant bone morphogenetic protein-2 in ovine lumbar interbody fusion. *J Biomed Mater Res A* 97 (2), 177-185

61 Murphy, W.L. et al. (2000) Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering. *Biomaterials* 21 (24), 2521-2527

62 Santana, R.B. and Trackman, P.C. (2006) Controlled release of fibroblast growth factor 2 stimulates bone healing in an animal model of diabetes mellitus. *Int J Oral Maxillofac Implants* 21 (5), 711-718

63 Simmons, C.A. et al. (2004) Dual growth factor delivery and controlled scaffold degradation enhance in vivo bone formation by transplanted bone marrow stromal cells. *Bone* 35 (2), 562-569

64 Patel, Z.S. et al. (2008) Dual delivery of an angiogenic and an osteogenic growth factor for bone regeneration in a critical size defect model. *Bone* 43 (5), 931-940

65 Young, S. et al. (2009) Dose effect of dual delivery of vascular endothelial growth factor and bone morphogenetic protein-2 on bone regeneration in a rat critical-size defect model. *Tissue Eng Part A* 15 (9), 2347-2362

66 Yilgor, P. et al. (2009) Incorporation of a sequential BMP-2/BMP-7 delivery system into chitosan-based scaffolds for bone tissue engineering. *Biomaterials* 30 (21), 3551-3559

67 Bonani, W. et al. (2012) Biomolecule Gradient in Micropatterned Nanofibrous Scaffold for Spatiotemporal Release. *Langmuir* 28 (38), 13675-13687

68 Yilgor, P. et al. (2010) Effect of scaffold architecture and BMP-2/BMP-7 delivery on in vitro bone regeneration. *J Mater Sci Mater Med* 21 (11), 2999-3008

69 Ennett, A.B. et al. (2006) Temporally regulated delivery of VEGF in vitro and in vivo. *J Biomed Mater Res A* 79 (1), 176-184

70 Schofer, M.D. et al. (2011) Electrospun PLLA nanofiber scaffolds and their use in combination with BMP-2 for reconstruction of bone defects. *PLoS ONE* 6 (9), 28

71 Saito, E. et al. (2013) Effects of designed PLLA and 50:50 PLGA scaffold architectures on bone formation in vivo. *J Tissue Eng Regen Med* 7 (2), 99-111

72 Blackwood, K.A. et al. (2012) Scaffolds for Growth Factor Delivery as Applied to Bone Tissue Engineering. *International Journal of Polymer Science* 2012, 25

73 Kanczler, J.M. et al. (2007) Supercritical carbon dioxide generated vascular endothelial growth factor encapsulated poly(DL-lactic acid) scaffolds induce angiogenesis in vitro. *Biochem Biophys Res Commun* 352 (1), 135-141

74 Nguyen, M.K. and Lee, D.S. (2010) Injectable biodegradable hydrogels. *Macromol Biosci* 10 (6), 563-579

75 Kon, E. et al. (2013) Matrix assisted autologous chondrocyte transplantation for cartilage treatment: A systematic review. *Bone Joint Res* 2 (2), 18-25

76 Re'em, T. et al. (2010) The effect of immobilized RGD peptide in macroporous alginate scaffolds on TGFβ1-induced chondrogenesis of human mesenchymal stem cells. *Biomaterials* 31 (26), 6746-6755

77 Xia, Y. et al. (2012) Bone tissue engineering using bone marrow stromal cells and an injectable sodium alginate/gelatin scaffold. *J Biomed Mater Res A* 100 (4), 1044-1050

78 Freeman, I. and Cohen, S. (2009) The influence of the sequential delivery of angiogenic factors from affinity-binding alginate scaffolds on vascularization. *Biomaterials* 30 (11), 2122-2131

79 Kimura, Y. et al. (2010) Controlled release of bone morphogenetic protein-2 enhances recruitment of osteogenic progenitor cells for de novo generation of bone tissue. *Tissue Eng Part A* 16 (4), 1263-1270

80 Thylin, M.R. et al. (2002) Effects of simvastatin gels on murine calvarial bone. *J Periodontol* 73 (10), 1141-1148

81 Benoit, D.S. et al. (2006) Synthesis and characterization of a fluvastatin-releasing hydrogel delivery system to modulate hMSC differentiation and function for bone regeneration. *Biomaterials* 27 (36), 6102-6110

82 Nelson, D.M. et al. (2012) Extended and sequential delivery of protein from injectable thermoresponsive hydrogels. *J Biomed Mater Res A* 100 (3), 776-785

83 Bryant, S.J. et al. (2004) Synthesis and Characterization of Photopolymerized Multifunctional Hydrogels: Water-Soluble Poly(Vinyl Alcohol) and Chondroitin Sulfate Macromers for Chondrocyte Encapsulation. *Macromolecules* 37 (18), 6726-6733

84 Mourino, V. et al. (2013) Composite polymer-bioceramic scaffolds with drug delivery capability for bone tissue engineering. *Expert Opin Drug Deliv* 10 (10), 1353-1365
85 Kim, J.M. et al. (2012) Osteogenic evaluation of calcium phosphate scaffold with drug-loaded poly (lactic-co-glycolic acid) microspheres in beagle dogs. *Tissue Engineering and Regenerative Medicine* 9 (3), 175-183
86 Shen, H. et al. (2010) An injectable scaffold: rhBMP-2-loaded poly (lactide-co-glycolide)/hydroxyapatite composite microspheres. *Acta Biomaterialia* 6 (2), 455-465
87 Bose, S. et al. (2012) Recent advances in bone tissue engineering scaffolds. *Trends in Biotechnology* 30 (10), 546-554
88 Nandakumar, A. et al. (2013) Combining technologies to create bioactive hybrid scaffolds for bone tissue engineering. *Biomatter* 3 (2), e23705
89 Oliveira, A.L. et al. (2009) Nucleation and growth of biomimetic apatite layers on 3D plotted biodegradable polymeric scaffolds: Effect of static and dynamic coating conditions. *Acta Biomaterialia* 5 (5), 1626-1638
90 Arafat, M.T. et al. (2011) Biomimetic composite coating on rapid prototyped scaffolds for bone tissue engineering. *Acta Biomater* 7 (2), 809-820
91 Macdonald, M.L. et al. (2011) Tissue integration of growth factor-eluting layer-by-layer polyelectrolyte multilayer coated implants. *Biomaterials* 32 (5), 1446-1453
92 Fu, Y.C. et al. (2008) Optimized bone regeneration based on sustained release from three-dimensional fibrous PLGA/HAp composite scaffolds loaded with BMP-2. *Biotechnol Bioeng* 99 (4), 996-1006
93 Steele, T.W. et al. (2013) Collagen-cellulose composite thin films that mimic soft-tissue and allow stem-cell orientation. *J Mater Sci Mater Med* 14, 14
94 Jiang, J. et al. (2010) Bioactive stratified polymer ceramic-hydrogel scaffold for integrative osteochondral repair. *Ann Biomed Eng* 38 (6), 2183-2196
95 Kempen, D.H.R. et al. (2009) Effect of local sequential VEGF and BMP-2 delivery on ectopic and orthotopic bone regeneration. *Biomaterials* 30 (14), 2816-2825
96 Huang, C.L. et al. (2013) The Influence of Additives in Modulating Drug Delivery and Degradation of PLGA Thin Films. *NPG Asia Materials*
97 Kanczler, J.M. et al. (2010) The effect of the delivery of vascular endothelial growth factor and bone morphogenic protein-2 to osteoprogenitor cell populations on bone formation. *Biomaterials* 31 (6), 1242-1250
98 Park, H. et al. (2009) Effect of dual growth factor delivery on chondrogenic differentiation of rabbit marrow mesenchymal stem cells encapsulated in injectable hydrogel composites. *J Biomed Mater Res A* 88 (4), 889-897
99 Tadokoro, M. et al. (2012) Bone morphogenetic protein‐2 in biodegradable gelatin and β‐tricalcium phosphate sponges enhances the *in vivo* bone‐forming capability of bone marrow mesenchymal stem cells. *Journal of tissue engineering and regenerative medicine* 6 (4), 253-260
100 Nandagiri, V.K. et al. (2011) Incorporation of PLGA nanoparticles into porous chitosan-gelatin scaffolds: influence on the physical properties and cell behavior. *J Mech Behav Biomed Mater* 4 (7), 1318-1327
101 Basmanav, F.B. et al. (2008) Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials* 29 (31), 4195-4204
102 Lee, G.S. et al. (2011) Direct deposited porous scaffolds of calcium phosphate cement with alginate for drug delivery and bone tissue engineering. *Acta Biomater* 7 (8), 3178-3186

Figure Caption

Table 1. Summary of different biomolecular agents, carrier systems and its various carrier forms.

Figure 1.(a)Schematic and (b) experimental representation of the preparation of dual growth factor-loaded colloidal gels made of oppositely charged gelatin nanospheres (NSs) using a twin-syringe. (c) Sequential release characterized by rapid release of angiogenic basic fibroblast growth factor (bFGF) and more sustained release of osteogenic bone morphogenetic protein-2 (BMP-2) was obtained by loading bFGF onto cationic nanospheres of low crosslinking density and BMP-2 onto anionic nanospheres of high crosslinking density.
Figure adapted from Wang et al. [36]. Reprinted with permission from Elsevier.

Figure 2. The SOX9 gene plus heparinized TGF-β3-coated, DEX-loaded PLGA microspheres allowed for the simultaneous delivery of genes and transfected stem cells to a wound site for chondrogenesis.
Figure adapted from Park et al. [54]. Reprinted with permission from Elsevier.

Figure 3.A schematic illustration of the different technologies involved in fabricating the hybrid scaffolds used in this study. (a) 3-D fiber (3DF) deposition enables a controlled layer by layer deposition of extruded polymer, (b) Electrospinning (ES) to produce extra-cellular matrix like fibers and (c) Biomimetic calcium phosphate coating (ESP) to enhance osteoconductivity of the scaffolds.Scaffold morphology using SEM (A) Electrospun fibers from a 3DF + ESP scaffold that have been coated with calcium-phosphate. (B) 3DF + ESP scaffold prepared by combining rapid prototyping and electrospinning.
Figure adapted from Nandakumar et al. [88]. *Reprinted with permission from Landes Bioscience, open access journal.*

Figure 4.(a) Schematic of layer-by-layer (LbL) architecture shows that a 3-dimensional polymer (3DP) scaffold is repeatedly dipped with tetralayer units consisting of (1) Poly2 (positively charged), (2) chondroitin sulfate (negatively charged), (3) bone morphogenetic protein-2 (BMP-2) (positively charged) and (4) chondroitin sulfate. This tetralayer structure is repeated 100 times for all LbL films. (b) Release of BMP-2 from LbL films built on 3DP scaffold. 80% of the material is released over a 2-day linear release period. The remaining twenty percent is released over a period of approximately 2 weeks. Inset shows a blow up of the release profile after the initial two day period.
Figure adapted from Macdonald et al. [91].*Reprinted with permission from Elsevier.*