Encyclopedia of Smart Materials

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Abstract

Regenerative medicine is a promising new area of research that offers potential to treat diseased and injured tissue. In particular, the development of new biomaterials-based approaches show promise for the treatment of challenging bone and cartilage injuries. Recent trends have focused on the development of biofunctional materials that have bioactive properties and are capable of supporting and promoting new tissue formation during the tissue repair process. This article discusses current biomaterial-based approaches for bone and

cartilage repair and highlights emerging material functionalization methodologies including surface modification, growth factor delivery, cell-seeding strategies and gene therapy.

Introduction

Damage to bone and cartilage tissue frequently occurs as a result of disease and traumatic injury. The number of bone and cartilage related disorders increases year on year due to factors such as the ageing population, the growing obesity problem and a rise in sporting relating injuries (Cartilage Repair Market Global Opportunity Analysis and Industry Forecast, 2018). These conditions can significantly impact on patient quality of life, resulting in pain and limited mobility, and can lead to individuals losing their independence. As a result of the limitations of existing surgical treatment methodologies, research over the past number of years has focused on new regenerative medicine approaches involving the development of biomaterial-based and tissue engineering solutions for these clinical challenges in orthopaedic medicine. More recently, approaches to enhance the biofunctionality of materials has been a major focus within the field of bone and cartilage tissue engineering. The overall goal in the development of biofunctional materials is to achieve materials that have bioactive properties and are capable of supporting and promoting new tissue formation during the tissue repair process in order to restore tissue function. The successful design of biofunctional materials for these applications is dependent on a fundamental understanding of the structure and function of bone and cartilage tissues, and the processes involved in bone and cartilage development and healing. This article summarizes bone and cartilage repair processes, current bone and cartilage tissue engineering approaches, and scaffold functionalization methodologies including surface modification, growth factor delivery, cell-seeding strategies and gene therapy (Figure 1).

Bone structure, function and repair process

Bone is a highly dynamic and diverse tissue, both structurally and functionally (Amini, Laurencin and Nukavarapu, 2012). The major functions of bone are to provide structural support to the body, to protect our vital organs and to enable movement (Jayakumar and Di Silvio, 2010). Bone consists of an organic matrix (20%), composed mainly of type I collagen, a mineral phase (65%), composed primarily of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], water (10%), bioactive factors and cells, mainly osteoblasts and osteoclasts (Ferreira *et al.*, 2012). During embryonic development, bone is formed through one of two pathways, intramembraneous ossification or endochondral ossification (Jayakumar and Di Silvio, 2010; Amini, Laurencin and Nukavarapu, 2012). For the intramembraneous ossification pathway, bone is formed by mesenchymal progenitor cells that differentiate directly into osteoblasts. The mandible, clavicle, and many cranial bones are formed in this way. Most bones in the body (i.e., all long bones and vertebrae), however, are formed through endochondral bone

formation. This process involves mesenchymal progenitor cells first differentiating into chondrocytes, which are responsible for depositing a cartilaginous template that is later mineralized and replaced by bone. Regardless of the pathway, the bone produced is the same.

Bone has the ability to regenerate itself through a repair process that is highly regulated by biomechanical, cellular and molecular factors (Amini, Laurencin and Nukavarapu, 2012). The bone healing process involves three phases: the inflammation phase, the reparative phase and the remodeling phase (Ferreira *et al.*, 2012). When a fracture occurs, the resultant blood vessel disruption triggers the inflammation phase and results in formation of a blood clot, referred to as a hematoma. This is followed by the migration of platelets, neutrophils and macrophages which release signaling molecules including fibroblast growth factor (FGF), tumor necrosis factor-α (TNF-α), platelet-derived growth factors (PDGF), vascular endothelial growth factors (VEGF), transforming growth factor (TGF) and various cytokines (e.g. IL-1 and IL-6). IL-1 and IL-6 attract mesenchymal stem cells (MSCs) to the fracture site and these MSCs then proliferate and differentiate to form cells of the osteoblastic and chondrogenic lineage (Amini, Laurencin and Nukavarapu, 2012). During the reparative phase of bone healing, osteoblasts begin to form woven bone which aims to rebuild the damaged structure and stabilizes the wound, and chondroblasts begin to deposit hyaline cartilage. The woven bone and hyaline cartilage are replaced with lamellar tissue as a result of tissue mineralization. Blood vessels then begin to infiltrate the mineralized tissue. Lastly, during the remodeling phase, the fracture callus is transformed into new tissue. During this phase, osteoclasts resorb cancellous bone. This process involves the creation of a small pit, referred to as Howship's lacunae, where osteoclast cells release enzymes that resorb the underlying bone surface (Jayakumar and Di Silvio, 2010). These osteoclast cells also release a number of growth factors and cytokines that cause local osteoprogenitor cells to be converted to osteoblast cells. These osteoblasts are responsible for collagen type I deposition that provides a template upon which mineral is deposited. Once osteoblasts cease new bone deposition, they become trapped within the extracellular matrix (ECM) and then differentiate to form osteocytes. Osteoclasts are responsible for the balance between bone formation, maintenance and the destruction of bone tissue. This delicate balance is controlled by mechanical stimuli as well as the action of cytokines and hormones.

Cartilage structure, function and repair processes

Articular cartilage forms a thin layer covering the end of bones that allows them to glide over each other smoothly to carry out their function effortlessly. Articular cartilage has some unique properties. It is a tough but flexible tissue and, unlike other connective tissues in the body, it is avascular. Articular cartilage is composed of collagen, proteoglycan, non-collagenous

proteins, and water. Type II collagen makes up around 90-95% of the collagen present, with the remaining 5-10% composed of types V, VI, IX, X and XI (Glowacki and Mizuno, 2008). Proteoglycans are composed of 95% polysaccharide and 5% protein bound to an unbranched polysaccharide molecule called glycosaminoglycan (GAG). The water content varies from 65-80%, depending on the load status of the joint. Articular cartilage is structurally organized into four zones i) superficial, ii) middle, iii) deep and iv) calcified zone. The superficial layer is the thinnest layer and contains flattened chondrocytes and densely packed collagen fibers arranged parallel to the articular surface. Within the middle (intermediate) zone, the fibers of collagen are arranged haphazardly surrounded by ECM, where they act as a spring mechanism dissipating any forces down into the subchondral bone. Next lies the deep zone, with collagen fibers arranged like columns perpendicularly to the joint surface and anchored to the underlying calcified cartilage. The tidemark is the area between the deep zone and calcified cartilage and represents the transition from uncalcified to calcified cartilage. The subchondral bone lies below the calcified cartilage layer and is continuous with it. It acts as a crucial load-bearing structure and is essential for the resilience and durability of joints.

Chondrocytes in the articular cartilage proliferate and secrete extracellular matrix to maintain and sustain the structure and function of the cartilage tissue. These chondrocytes are derived from mesenchymal progenitor cells and occupy only 1%–5% of the total cartilage tissue (Akkiraju and Nohe, 2015). Chondrocytes by nature have low mitotic activity and due to the rigidity of the cartilage matrix they are unable to migrate (Hunziker, 1999). This means that an isolated chondral defect has minimal access to cells for regeneration and repair. Synovial fluid fills the joint space and plays an important role in lubricating the joint and providing essential nutrients for the cartilage tissue. It consists of water, electrolytes, small molecules, and glucose as well as metabolic waste from the ECM, such as oxygen and carbon dioxide (Schmidt *et al.*, 2007).

During skeletal development cartilage formation begins with the differentiation of mesenchymal cells into rapidly dividing prechondrocytes that then further differentiate into chondrocytes that secrete cartilage-specific matrix (Akkiraju and Nohe, 2015). There are two major types of chondrocytes generated at this stage: (1) periarticular chondrocytes that form the articular cartilage and (2) growth plate chondrocytes that will eventually undergo hypertrophic transformation, and be replaced by bone (Wu *et al.*, 2013). The repair responses that occur in cartilage injuries depend on the extent of the cartilage injury. Full thickness cartilage injuries that involve the subchondral bone tend to follow the same process as occurs for bone healing. As a result of bleeding from the subchondral bone, a fibrin clot fills the defect and mesenchymal cells penetrate this clot and differentiate into chondrocytes, which lay down a proteoglycan-rich ECM (Hunziker, 1999). Repair tissue usually resembles cartilage,

however, it tends to be fibrous cartilage rather than hyaline cartilage. This fibrous cartilage is less stable than hyaline cartilage and gradually breaks down over time (Hunziker, 1999). For partial-thickness defects, spontaneous defect healing does not occur. Due to the avascular nature of cartilage, no fibrin clot forms within the defect. Furthermore, there is a lack of progenitor cells in the surrounding the tissue area and, due to the dense pericellular matrix surrounding the chondrocytes, there is little or no evidence of chondrocyte migration or proliferation. These lesions do no heal and their appearance remains the same several months after the injury. Furthermore, post-traumatic osteoarthritis may occur as a result of such lesions (Anderson *et al.*, 2011).

Tissue Engineering Approaches for Bone and Cartilage Repair

Regenerative medicine can be defined as 'an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function' (Howard *et al.*, 2008). Tissue engineering, a branch within this field, aims to combine a matrix component with cells and signaling molecules to achieve tissue repair. The matrix component provides a three dimensional structure to guide new tissue formation and can be either a scaffold or hydrogel.

Scaffolds

Scaffolds designed for bone and cartilage tissue engineering applications have various biological and structural requirements. In terms of biological requirements, scaffolds should be biocompatible, non-toxic, biodegradable and capable of integrating and interacting with the surrounding environment. Structurally scaffolds are required to be porous in order to allow cellular infiltration and the transport of gases, nutrients, and regulatory factors that allow cell survival. Scaffold pore size and interconnectivity are also important. If the pore size is too big, the surface area for cell attachment is reduced. If the pore size is too small, the migration and infiltration of cells cannot occur and the diffusion of nutrients and waste is limited. Pore sizes of between 100 µm and 300 µm have been shown to be optimal for bone and cartilage tissue engineering applications (Murphy, Haugh and O'Brien, 2010; Matsiko, Gleeson and O'Brien, 2015). Scaffold permeability is also necessary for the diffusion of nutrients and waste and is influenced by the porosity, pore size, pore interconnectivity and orientation. Scaffolds also require sufficient mechanical properties to withstand the *in vivo* environment post implantation. Moreover, the ideal scaffold should degrade at the same rate as new tissue is formed. Scaffolds can be manufactured using a range of fabrication methods including solvent casting,

foaming techniques, fiber bonding. freeze-drying, electrospinning and 3D printing (Włodarczyk-Biegun and del Campo, 2017).

Hydrogels

Hydrogels are cross-linked macromolecular structures that have a high affinity for water. They are composed of three-dimensional (3D) hydrophilic chains and have the advantage of being injectable and thus can be implanted using minimally invasive surgical techniques (Vega, Kwon and Burdick, 2017). As for scaffolds, hydrogels are required to be biocompatible, non-toxic, biodegradable and capable of integrating and interacting with the surrounding environment. The setting time, injectibility and rheological properties of hydrogels are important parameters to consider when optimizing hydrogels for use in the clinical treatment of bone and cartilage defects. The properties of hydrogels can be altered using various chemical modifications and crosslinking techniques. An important parameter influencing the biological and structural properties of scaffolds and hydrogels and their ability to influence bone and cartilage repair processes is their biomaterial composition.

Biomaterials

Polymers

A range of synthetic and natural polymers have been used in bone and cartilage repair applications. Synthetic polymers offer versatile physical and chemical properties and allow precise control over attributes such as molecular weight, degradation time, and hydrophobicity. They are also pathogen-free and there is low potential for immunological rejection. They are generally cheaper than natural polymers and can be produced in large quantities with uniform properties and a long shelf life. Biodegradable synthetic polymers including poly L-lactic acid (PLLA), polyglycolic acid (PGA), poly (lactic-co-glycolide) (PLGA), polycaprolactone (PCL), and polyethylene glycol (PEG) have been successfully used to produce porous scaffolds and hydrogels. The most widely used synthetic materials in bone and cartilage repair applications include PCL, PLLA and PGA (Puppi *et al.*, 2010). These materials can be used in the form of meshes, particles or fibers to produce scaffolds in a variety of shapes, sizes. Despite the many advantages of synthetic polymers for bone and cartilage tissue engineering applications, they also have significant limitations as their biocompatibility is inferior to that of natural polymers and their products of degradation can be toxic, resulting in localized inflammation and cell death (Matsiko, Levingstone and O'Brien, 2013).

Natural polymers have numerous advantages over synthetic polymers. They are biocompatible, biodegradable with non-toxic degradation products and have bioactive properties that allows better interactions with the cells. Natural polymers used in bone and

cartilage tissue engineering include collagen, silk, gelatin, fibrinogen, elastin, keratin, actin, and myosin. Collagen, the most abundant protein in the human body, has been widely investigated for bone and cartilage repair applications. While collagen is present in many different forms within the body, from type I to type X, all share the same triple helical structure. Type I collagen is the main collagen present in bones and type II collagen is found in cartilage tissue. Collagen possesses functional groups that allow interaction with other molecules, such as polysaccharides and protein-based growth factors (Glowacki and Mizuno, 2008; Ferreira *et al.*, 2012). Additionally, the mechanical and degradation properties of collagen scaffolds can easily be tailored using cross-linking treatments (Tierney *et al.*, 2009). Silk fibroin is produced from the cocoons of the silkworm *Bombyx mori*. It is naturally biodegradable and biocompatible and has been used in a wide variety of tissue engineering applications including bone and cartilage. Fibrin is a polymerised form of fibrinogen and represents another promising biomaterial. It is found naturally in the body where its chief role is in clot formation and prevention of bleeding. Fibrin is easily resorbed by the body as regenerative cells infiltrate into the defect site (Hunziker, 1999). Fibrin-based scaffolds and hydrogels has been studied extensively in animals and also has been clinically tested in humans (Ahearne and Kelly, 2013).

Polysaccharides

Polysaccharides, for example, cellulose, amylose, dextran, chitin, and glycosaminoglycans (GAGs), have also shown potential for use in bone and cartilage repair applications (Dhandayuthapani *et al.*, 2011). In particular, the addition of GAGs such as chondroitin sulfate and hyaluronic acid has been shown to improve the biofunctionality of collagen scaffolds (Tierney *et al.*, 2009; Matsiko *et al.*, 2012). Hyaluronic acid is a glycosaminoglycan (GAG) that forms a structural component of native cartilage tissue. It also plays a role in maintaining the supplies of synovial fluid within the joint. The addition of this natural material to scaffolds has been shown to up-regulate expression of both type II collagen and aggrecan, both which are important components of hyaline cartilage (Grigolo *et al.*, 2002). Hyaluronic acid-based scaffolds have shown potential in cartilage regeneration as they are fully absorbed after being broken down to hyaluronan (a sugar molecule) (Grigolo et al., 2002).

Chitosan is a natural biopolymer derived from chitin from the shells of shrimp and other crustaceans. Chitosan contains glucosamine and hyaluronic acid, which are basic components of the native cartilage. It has a molecular weight in the range of 300 to 1000 kDa depending on its source and processing methods (Sheikh *et al.*, 2015). Chitosan is generally insoluble in aqueous solutions with a pH above 7, however, when placed in diluted acids with pH 6 or lower, the protonated free amino group of glucosamine facilitates the solubility of the

material. The *in vivo* degradation takes place primarily due to lyzozyme and is regulated via hydrolysis of the acetylated residues. The chitosan degradation rate depends on the levels of crystallinity and acetylation (Sheikh et al., 2015). Chitosan has a number of properties that make it suitable for bone and cartilage tissue engineering applications. It has an intrinsic antibacterial nature, causes minimal foreign body reaction *in vivo*, and has the ability to be molded into various geometries and forms such as porous structures that facilitate cell ingrowth (Di Martino, Sittinger and Risbud, 2005).

Alginate is a gelatinous carbohydrate polymer isolated from seaweed and is approved as a biomaterial by the FDA for clinical applications. It has been shown to support chondrogenesis by increasing cartilage-specific genes and matrix production both *in vitro* and *in vivo* (Sun and Tan, 2013). One drawback of alginate-based biomaterials is that they can potentially cause local foreign body and immunological reactions (Fragonas *et al.*, 2000).

Calcium Phosphates

Calcium phosphate ceramics have been widely used for bone repair applications. Hydroxyapatite (HA) is of particular interest as it is the most similar to the calcium phosphate phase present in bone (Amini, Laurencin and Nukavarapu, 2012). Other calcium phosphate ceramic phases, particularly α- tricalcium phosphate (α-TCP) and β-tricalcium phosphate (β-TCP), have also been widely investigated (LeGeros, 2002). Calcium phosphate ceramics have excellent biological properties and demonstrated osteoconductivity and osteoinductivity. The rate of resorption depends on the calcium phosphate phase, with α-TCP having a higher rate of resorption than β-TCP or HA. Consequently, α-TCP tends to be removed from the body as new bone is deposited, whereas hydroxyapatite tends to remain in situ for longer (LeGeros, 2002). Calcium phosphate ceramics are brittle in nature and have a low fracture toughness and are therefore often incorporated within a polymer matrix for use in bone repair applications. In such systems, the ratio of organic and inorganic phases can be altered in order to achieve the desired mechanical properties. Various ions, e.g. magnesium (Mg^{2+}), zinc (Zn^{2+}), copper (Cu^{2+}), strontium (Sr^{2+}), cobalt (Co^{2+}), lithium (Li^{+}), and fluoride (F^{-}), can also be substituted into the calcium phosphate crystal structure in order to enhance the osteogenic properties of the material or provide an anti-microbial effect.

Bioactive Glass

Bioactive glasses are inorganic glass-ceramic biomaterials composed of calcium and phosphate in a ratio that imitates the natural mineral component of bone. They are biocompatible and biodegradable and have the ability to bond to living bone and stimulate osteogenesis through the release of biologically-active ions. Bioactive glass has demonstrated

the capability to accelerate bone tissue healing. Bioactive glass products have been widely used clinically for the repair of bone and dental defects (Hench and Jones, 2015).

Biofunctional Materials for Bone and Cartilage Repair

While designing biomaterials with structural and chemical properties that match those of native tissues is important for the development of scaffolds and matrices for bone and cartilage repair, biomaterials alone do not fully replicate the chemical and biological features of the native ECM. Therefore, a range of approaches have been taken to biofunctionalize scaffolds and hydrogels to enhance their ability to successfully drive chondrogenesis and osteogenesis *in vivo*. Approaches that have been investigated include surface modification techniques, and the incorporation of cells, growth factors, drugs and genes within the scaffold.

Surface Modification

Surface modification techniques can be used to improve the functionality of scaffolds and matrices designed for the repair of bone and cartilage by improving cell affinity, biocompatibility, nontoxicity and biodegradability. Surface modification is most commonly used for synthetic materials that may lack the functional surface properties required for cellular interaction. These surface modification techniques include plasma and chemical treatments that can introduce reactive chemical groups, such as amine, hydroxyl or carboxyl groups, on the material's surface. These chemical groups are recognized by cells and form a platform for subsequent crosslinking with natural ECM components and bioactive compounds (Lim, Sardinha and Myers, 2014). For example, Gentile *et al.* developed a plasma surface polymerization process in order to graft biomimetic peptides on polymeric biomaterials for bone tissue engineering applications (Gentile *et al.*, 2015). Specifically, heparin binding peptides (KRSR and FHRRIKA) were used as they have been shown to induce cell adhesion and bone mineralization. *In vitro* analysis showed increased cell adhesion and osteoblastic differentiation on heparin binding peptide modified samples.

Coatings processes can also be effective in enhancing the surface properties of materials. Polydopamine (PDA) coatings have been shown to promote mineralization by immobilizing biomolecules on the surface of biomaterials and thus enhancing proliferation and calcium deposition of osteoblast cells (Huang *et al.*, 2016). The use of cationic zinc coatings on biomaterials has been shown to prevent inflammation by triggering immune cells in acute responses (Velard *et al.*, 2010). Furthermore, scaffolds can be coated with extracellular matrix proteins such as collagen, gelatin, fibronectin and laminin. For example, the use of collagen

and gelatin coatings has been shown to enhance the hydrophilic properties of polycaprolactone scaffolds for cartilage tissue engineering applications (Chen *et al.*, 2014).

Cells

Combining cells with scaffold and hydrogel materials can potentially enhance their functional properties and lead to an increase in bone and cartilage tissue repair. A range of cell sources have been investigated use in bone and cartilage repair applications. For bone repair, osteoblasts, as the main cell type responsible for bone formation and bone remodeling, represent the ideal choice. Similarly for cartilage repair, chondrocytes, the main cells responsible or cartilage matrix production, represent the optimal cell type. However, due to the limited availability of these osteoblasts and chondrocytes, MSCs from various sources have been widely explored for bone and cartilage tissue engineering applications.

Osteoblasts

Autologous primary pre-differentiated osteoblasts, the main bone forming cell in bone tissue, present the ideal cell source for bone tissue engineering applications as they are pre-programmed for the production of bone matrix. Osteoblasts can be isolated from bone chips using enzymatic extraction and maintained in cell culture conditions *in vitro* where they have been shown to synthesize bone-specific proteins and enzymes, including alkaline phosphatase (ALP), osteocalcin (OC), and type I collagen, and to deposit mineral in the form of calcium phosphate crystals (Jayakumar and Di Silvio, 2010). However, the use of osteoblasts in bone tissue engineering applications is limited due to their lack of availability and relatively short lifespans.

Chondrocytes

Chondrocytes are the primary cell type in hyaline cartilage and are also present in cartilaginous tissue elsewhere in the body. Chondrocytes offer the advantage of being already committed to the appropriate phenotype, and are naturally resistant to vascular invasion, mineralization and ossification (Brittberg *et al.*, 1994). However, one drawback associated with the use of articular chondrocytes is that harvesting these chondrocytes causes further trauma to the joint. The harvesting of chondrocytes from other hyaline cartilage sources, including the nasal septum, pinna of the ear and costal cartilage, have thus been explored. The harvesting of chondrocytes from the hyaline cartilage at the nasal septum is a straightforward procedure that could be easily performed by otolaryngologists or plastic surgeons (Hellingman *et al.*, 2011; Lohan *et al.*, 2013). Nasal septum cartilage has been shown to have a higher cell content than articular cartilage (Kafienah *et al.*, 2002; Sayed *et al.*, 2010). Chondrocytes have been successfully harvested from auricular cartilage in the pinna of the ear. This cartilage bears more similarity to the nasoseptal cartilage than the articular cartilage due to the elastin

expressed, and the reliance on the perichondrium for nutrition. While a high yield of chondrocytes have been harvested from auricular cartilage, these chondrocytes have been shown to proliferate at a slower rate than nasoseptal chondrocytes (Hellingman *et al.*, 2011). Costal cartilage, a hyaline cartilage that connects the rib ends to the sternum, represents another potential source of chondrocytes. It is particularly rigid and has a tendency for ossification in older individuals. Although cell yield is less than for auricular and nasoseptal cartilage it remains higher than articular cartilage with a threefold greater proliferation than articular chondrocytes and can be harvested with less morbidity (Sato *et al.*, 2008; Gelse *et al.*, 2009; Lohan *et al.*, 2013). While chondrocytes are an obvious first choice as a cell source in any tissue engineered approach to repair damaged cartilage, the low numbers of cells available and their tendency to dedifferentiate when expanded *in vitro* in two dimensional culture, represent significant limitations (Benya and Shaffer, 1982; Kato and Gospodarowicz, 1985; Sayed *et al.*, 2010). This loss of phenotypic traits could reflect a progressive loss of potential to form stable cartilage *in vivo*, thereby putting the long-term outcome at risk (Peterson *et al.*, 2010). As a result of the inherent limitations associated with chondrocytes, the use of stem cells has been investigated in order to achieve repair of chondral and osteochondral defects (Nakahara *et al.*, 1990; Wakitani *et al.*, 2002).

Mesenchymal Stem Cells (MSCs)

Mesenchymal Stem Cells (MSCs) are multipotent adult stem cells that can be derived from many different tissues in the body. These cells are defined by their ability to differentiate into adipocytes, chondrocytes or osteocytes and their expression of certain surface molecules (Dominici *et al.*, 2006). MSCs from various tissues are being explored in order to identify the optimal source for use in bone and cartilage defect repair applications.

MSCs can be easily harvested from the bone marrow (BMSCs) from the iliac crest. BMSCs have several advantages for the treatment of bone and cartilage defects, including their ability to be easily expanded *in vitro* and their ability to be differentiated down osteogenic or chondrogenic lineages as required. They have also been shown to have an immunomodulatory effect which has further therapeutic benefits (Glennie *et al.*, 2005; Eslaminejad, 2014). This immunosuppressive activity can help to modulate the inflammatory processes occurring with the site of injury, and can also enable the body to accept the foreign graft being inserted (Leijten *et al.*, 2013). While BMSCs are a valuable cell source for tissue engineering applications, they have limitations relating to the painful procedure required for bone marrow harvesting, their limited proliferation capacity and their inferior differentiation potential in aged individuals. A further disadvantage of using BMSCs for cartilage repair

applications is the difficulty in preventing their hypertrophic differentiation during *in vitro* culture (Sakaguchi *et al.*, 2005).

Adipose tissue has also emerged recently as a valuable source of adipose derived stem cells (ADSCs). ADSCs offer distinct advantages over MSCs from other sources. They can be easily harvested by means such as subcutaneous lipoaspiration, a much less painful procedure than harvesting bone marrow stem cells, and their use is less associated with ethical controversies because they are harvested from autologous fat (Miana and Prieto González, 2018). The high availability of ADSCs mean that it is possible to harvest sufficient numbers to implant without expanding the cells *ex vivo*. They thus present an excellent option for defect repair without any donor site implications (Guilak *et al.*, 2010; Jurgens *et al.*, 2013). Autologous ADSCs have shown success in the repair bony defects (Lendeckel et al., 2004; Jurgens et al., 2013). ADSCs have also been shown to produce characteristic cartilage matrix molecules *in vivo* showing their potential for cartilage tissue engineering applications (Erickson *et al.*, 2002).

ADSCs are similar to BMSCs regarding gene expression and osteogenic capacity (Niemeyer *et al.*, 2007). ADSCs can also be harvested in high yields from the fat pad within the joint. The chondrogenic potential of infrapatellar fat pad cells (FPMSCs) and their functional properties have been demonstrated, with reported advantages including a short population doubling time and ease in manipulating cells to differentiate (Ahearne, Buckley and Kelly, 2011; Ahearne, Liu and Kelly, 2014; Liu *et al.*, 2014; Mesallati, Buckley and Kelly, 2014).

Dental pulp derived MSCs have been harvested from extracted teeth and non-extracted crown fractured teeth (Ma *et al.*, 2014). These cells have a similar gene expression, a faster proliferation rate and higher percentage of stems cells in the harvested population than BMSCs (Gronthos *et al.*, 2000). Additionally some researcher report that dental pulp derived MSCs have equal bone forming capacity to BMSCs (Yamada *et al.*, 2011).

The synovium, the membrane that lines the inner surfaces of the joint, has been shown to have isolatable MSCs and has advantages as a cell source as it is also easy to harvest during arthroscopy and demonstrates full healing leaving no donor site morbidity (Sakaguchi *et al.*, 2005; Ando *et al.*, 2008). Synovial fluid also contains an inherent MSC and mesenchymal progenitor cell (MPC) population with an increased propensity toward chondrogenesis rather than osteogenesis (Sakaguchi *et al.*, 2005; Khan *et al.*, 2007). The use of synovial fluid MSCs is limited due to the very small volumes that can be aspirated from the joint (Ando *et al.*, 2008). Additionally, the synovial fluid becomes contaminated with inflammatory markers and inhibitory factors to chondrogenesis as a cartilage defect becomes chronic which may impact on tissue regeneration (Rodrigo *et al.*, 1995; Saris, Dhert and Verbout, 2003).

Peripheral blood (PB) MSCs can be harvested from a routine blood test and thus offer significant advantages as an easily accessible alternative source of MSCs in comparison to other stem cell sources where an invasive harvesting procedure. The major limitation for this cell type is the low numbers of MSCs present (Lazarus *et al.*, 1997). However, recently several authors have shown success in mobilizing MSCs from the bone marrow into the blood and thus increasing the concentration of MSCs available (Wise, Sumner and Virdi, 2012; Fu, Zhou and Yu, 2014).

Recent advances within the field of tissue engineering have led to the development of induced pluripotent stem cells (iPSCs), where adult cells are reprogrammed into pluripotent stem cells, examples include adult dermal fibroblasts from a skin biopsy and circulating lymphocytes taken from a blood sample (Takahashi *et al.*, 2007; Seki, Yuasa and Fukuda, 2011). By the transfection of certain genes through retroviruses these cells take on an appearance similar to human embryonic cells and can, from there, be guided to differentiate into multiple tissue types. iPSCs have been successfully reprogrammed to produce both bone and cartilage *in vitro* and thus they may have potential for use in bone and cartilage tissue engineering applications (Lach *et al.*, 2014; Yamashita *et al.*, 2015; Rana *et al.*, 2019). One major disadvantage of iPSCs is reported teratoma formation due to the increased proliferation rate and the reprogramming process (Uto *et al.*, 2013). While some studies have demonstrated the safe use of these cells the risk of teratoma formation remains a concern (Yamashita *et al.*, 2015).

Growth Factors

Growth factors play a vital role in the induction, promotion and maintenance of differentiation and the function of cells (Lim, Sardinha and Myers, 2014). Numerous growth factors are involved in stimulating cellular division, growth, and differentiation within bone and cartilage tissue during normal tissue repair processes. The potential for these growth factors to be used as therapeutic agents has been widely explored. In particular, transforming growth factor-β (TGF-β) 1, 2 and 3, bone morphogenic protein (BMP) 2,4,6,7 and 9, growth differentiation factors (GDFs), particularly GDF-5, insulin-like growth factor (IGF), the fibroblast growth factor (FGF) family and platelet-derived growth factor (PDGF) have all been shown to stimulate chondrogenesis (Im and Lee, 2010; Matsiko *et al.*, 2015; Deng *et al.*, 2019). Similarly, BMP 2 and 7, IGF, FGF 2, vascular endothelial growth factor (VEGF), PDGF, and parathyroid hormone (PTH) have shown the ability to stimulate osteogenesis (Liebesny *et al.*, 2016; Wang, Newman and Benoit, 2018). The successful use of growth factors as therapeutic agents is limited by their short half-life, for example insulin-like growth factor (IGF) is reported to have a half-life of only 10−12 min (Guler *et al.*, 1989; Wang *et al.*, 2017). Dose-related adverse effects are also a concern. The controlled sustained release of growth factors over a prolonged period

is recognized as being essential in order to achieve therapeutic benefits. In order to achieve this controlled sustained release, growth factors are frequently incorporated into scaffolds and hydrogels, where they enhance scaffold and hydrogel biofunctional properties. The incorporation methods used include 1) the direct blending or emulsion of the growth factors with the biomaterial during the scaffold/hydrogel synthesis and fabrication stage, 2) the physical adsorption through the immersion of the scaffold in a growth factor solution, or 3) the pre-chemical linkage to, or encapsulation within, a carrier prior to adding to the scaffold/hydrogel (Lim, Sardinha and Myers, 2014; Matsiko *et al.*, 2015).

Dual/multiple growth factor delivery approaches for bone and cartilage repair have also been investigated. For example, an approach involving the release of TGF-β1 or TGF-β3 to first chondrogenically stimulate a synthetic response by host/delivered progenitor cells, followed by the release of an anabolic or maturation factor like IGF-1 to encourage cartilage matrix production, has shown potential (Holland *et al.*, 2007). The dual delivery of TGF-β2 and BMP-7 has also been investigated (Im and Lee, 2010). A further approach is to deliver a short burst of chemotactic factors can promote early cell migration, followed by a longer-term release of chondrogenic factors to induce the recruited cells to produce cartilage matrix. While growth factor delivery strategies have been extensively investigated within the literature, the ideal therapeutic approach has yet to be identified and to date these approaches have not lived up to expectations *in vivo*. For instance, the dual delivery of TGF-β2 and BMP-7 to trochlear groove defects in rabbits failed to elicit any histological improvement in osteochondral tissue repair over controls [97]. The co-delivery of TGF-β1 and IGF-1 did not offer any additional benefits over the delivery of IGF-1 alone for osteochondral tissue regeneration *in vivo*.

Gene Therapy

While growth factor delivery has shown promise in bone and cartilage tissue engineering applications, challenges relating to the short half-life of these proteins and their poor retention within the defect site limit their ability to successful promote tissue regeneration (Kim et al., 2016). Gene therapy presents an alternative approach to enhance the functional properties of scaffolds and hydrogels, offering the potential to achieve sustained and regulated delivery of therapeutic genes. Gene therapy involves the transfer of target genes into the nucleus of cells where they achieve controlled and sustained protein expression at the site of tissue damage to enhance the formation of repair tissue. Broadly gene delivery can be classified into two categories based upon the type of carrier or ‘vectors’ used to deliver the gene into the cell nucleus: viral and non-viral. Viral vectors have high transfection efficiency but show immunogenicity and toxicity and thus non-viral vectors are recognized as a safer option. Many genes with powerful regenerative potential for bone and cartilage repair applications have

been identified (Betz *et al.*, 2018). For example, osteogenic genes such as BMPs (BMP-2, −4, −6, −7 and − 9) have been shown to enhance bone repair in pre-clinical studies. Gene therapy for cartilage repair applications has focused on transforming growth factor-β (TGF-β) and insulin-like growth factor-1 (IGF-1), due to their critical role in cartilage development and maturation (Grol and Lee, 2018).

Conclusion remarks and future prospective

Within the area of orthopaedic medicine, the need for new approaches capable of achieving the robust and long-lasting regeneration of bone and cartilage tissue remains an important requirement. In particular, there is a clear need for biofunctional materials that accurately recapitulate both the structural and functional properties of the tissue being reconstructed, while providing the bioactive properties necessary to support and promote new tissue formation during the tissue repair process. Here the recent advances relating to scaffold functionalization methodologies including surface modification, growth factor delivery, cell-seeding strategies and gene therapy are summarized. Despite numerous recent advances, the successful translation of bone and cartilage tissue engineering and regenerative medicine approaches to the clinic remains elusive. Important challenges remain relating to complex host-biomaterial interactions, lack of appropriate pre-clinic models, the high costs of clinical trials and the complex regulatory pathways involved in getting new products to the market. In order to overcome these challenges the intimate collaboration of experts from developmental biology, cell biology, immunology, material science, bioengineering, surgeons and regulatory bodies is a critical requirement. These interdisciplinary collaborations will enable the delivery of biofunctional materials to the clinic where they can positively impact on patients' lives.

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Figures