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Effect of Biomaterials in Orthopaedic Mesenchymal Stem Cell Therapy

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Biomaterials are one of the active research areas in tissue engineering due to its high demand in regenerative medicine. And it plays a crucial role in mesenchymal stem cells therapy. A number of biomaterials and their combinations are available for the production of artificial extracellular matrix for skeletal repair like demineralised bone matrixes, polymers, bioactive glasses, coral, bioactive ceramics but unfortunately the design of scaffolds for bone tissue engineering presents several difficulties. The focus of this article is a brief narration of commonly used biomaterials for scaffold making for bone tissue engineering.

Key words: Bone, scaffold, demineralised bone matrixes, polymers, bioactive glasses, coral, bioactive ceramics

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INTRODUCTION

Bone has the unique capacity to regenerate without the development of a scar. But if patient pose significant risks like delayed healing, improper healing, congenital disorders, traumatic injury or surgery for bone cancer, artificial intervention is needed. One of the most promising approaches involves seeding Mesenchymal Stem Cells (MSCs) in highly porous biodegradable matrices or scaffolds, in the shape of the desired bone and after culturing and implanting them into the defected bones to induce and direct the growth of new bone tissues. It is thought that they respond to local injury by dividing to produce daughter cells that differentiate into multiple mesodermal tissue types, including bone, cartilage, muscle, marrow stroma, tendon, ligament, fat and a variety of other connective tissues (Mirzapour *et al*., 2011). Some medicinal plants like Cissus quadrangularis, Ficus racemosa have property to enhance the fracture healing process (Joseph and Raj, 2010a, b, 2011). *Aloe vera* has the property to stimulate the fibroblast cells (Joseph and Raj, 2010c). So if we can incorporate the bioactive compound from these plants in the scaffold, we can enhance the healing process. MSCs can be isolated from different sources like bone marrow, muscle, umbilical cord, trabecular bone, dermis, adipose tissue, periosteum, pericyte. Though MSCs can be isolated from different sources, the differentiation potential is very high in MSCs from bone marrow. As the number of MSCs obtained from a harvest is very small, the culturing of MSCs is needed to increase the cell number and to differentiate the MSCs to a particular lineage. The cells attach to the scaffold, multiply, differentiate and organize into healthy bone as the scaffold degrades. As these MSCs are autologous in origin, the immune rejection and disease transmittance can be avoided to the patients. MSC support haemopoiesis *in vitro* and express cytokines that encourage bone marrow homing and long-term engraftment, suggesting that they may have a role in improving success of HSC transplantation (Saeed and Mesaik, 2005). A wide range of biomaterials is used in single or in combination for the fabrication of scaffold. They are synthetic bioactive materials made of polymers, metals, ceramics and natural macromolecules. The adequate properties of scaffold used for bone tissue restoration include biocompatibility, bioactive, biodegradability, osteoconductivity, vascular supportive have porous and 3 D structure. But currently, there is no single scaffold, which can meet all the diverse requirements of bone tissue engineering, given the current limitation of our background knowledge on bone regeneration and material science. So in this review we are discussing about some of the widely used biomaterials for mesenchymal stem cell therapy in skeletal system repair.

DEMINERALISED BONE MATRIX

Demineralised Bone Matrixes (DBM) are decalcified cortical bones. They are produced by acid extraction of bone and contain noncollagenous proteins, growth factors, Bone Morphogenic Proteins (BMP) and type I collagen. Acid extraction reduces the potency for infection and immunogenic host response. DBM retains the trabecular collagenous structure of the original tissue and can serve as a biological osteoconductive scaffold (Giannoudis *et al*., 2005). Though DBM is an osteoconductive agent, it will not provide any structural support but it is used for filling defects and cavities (Bae *et al*., 2006). Active proteins in the DBM such as bone morphogenetic protein, transforming growth factor-beta, osteogenin, insulin-like growth factor and fibroblast growth factor are indirectly involved in bone healing cascade (Han *et al*., 2003). Active BMPs in DBM generate potent osteoinductive signals and induce bone formation *in vivo* and osteoblast differentiation from non-osseous cells *in vitro* by inducing Alkaline Phosphatase (ALP) activity (Han *et al*., 2003). Osteoblasts are sophisticated fibroblast, mononucleate cells that are responsible for bone formation (Joseph *et al*., 2010). Thus, DBM can be more osteoinductive than standard mineralised allograft (Fleming *et al*., 2000). DBM has been shown to induce bone formation in animals as well as in human studies and utilized to promote bone regeneration (Martin *et al*., 1999). Demineralized bone matrix is available in various formulations: freeze-dried powder, granules, strips, gel, chips or calcium sulphate granules (Bae *et al*., 2006). DBM is effective in decreasing the resorption of autogenous cortical bone graft (Altundal *et al*., 2005). The current recommendation for the use of demineralized bone matrix is as graft extenders (Ferreira *et al*., 2001).The use of DBM should be offered to suitable patients in the preoperative consultation as a valuable alternative for autologous grafting (Pieske *et al*., 2009).

POLYMERS

Different polymers are found to be used in bone tissue engineering. Now a days polymers are used along with polymer composites or with ceramic composites or with bioglass. Gelatin is a biological polymer derived from collagen which is an interesting candidate for tissue engineering. This material have suitable chemico-physical properties for scaffold production. It is biodegradable,

in vivo resorbable, it does not exhibit antigenicity *in vivo*, its physical and chemical properties can be easily modulated and it is cheaper than collagen (Kuijpers *et al*., 1999). One of the drawbacks of gelatin for tissue engineering applications is its solubility in aqueous media; therefore, gelatin-containing structures for long-term biomedical applications need to be crosslinked (Pulieri *et al*., 2008). Some researchers used demineralized bone matrix gelatin as scaffold (Pan *et al*., 2010). Composite scaffold made of gelatin, hydroxyapatite, bioactive glass has been evaluated for cell viability with human primary osteoblast cells (Gentile *et al*., 2010).

The most commonly used degradable synthetic Polymers are poly (Lactide-co-Glycolide) (PLGA) and their respective homopolymers, Poly(glycolide) and poly (lactide). These have been used clinically for several decades as suture materials, providing a depth of regulatory experience (Ueda and Tabata, 2003). The scaffolds based on polylactic acid (PLA), polyglycolic acid (PGA) and their copolymers (PLGA) are degradable and it can gradually replaced by a bone matrix (Borden *et al*., 2003). Poly-L-Lactic Acid (PLLA) has been found to offer the best compromise of mechanical stability and degradation rate and chondrocyte redifferentiation was significantly improved in the presence of PLLA with an increase in proteoglycans (Rahman and Tsuchiya, 2001). PLLA/HAP composite scaffolds show improved cell survival over plain PLLA scaffolds (Woo *et al*., 2007). Furthermore, long-term *in vivo* studies have shown that, unlike the rapidly degraded PGA and PLGA polymers, PLLA does not produce a significant inflammatory response and that *in vivo* degradation rates are similar to those shown *in vitro* (Weir *et al*., 2004). The porosity and tensile properties of electrospun PLGA nanofibers have been characterized (Li *et al*., 2002) and osteoblast adhesion has been observed when seeded in PLGA nanofibers (Price *et al*., 2004). Electrospun PLGA nanofiber scaffold can hold continuous differentiation of hMSCs into chondrogenic and osteogenic cells (Xin *et al*., 2007). Significant increase of chondrocyte functions (adhesion, proliferation and matrix synthesis) on 3-D nanostructured PLGA created via chemical etching was recorded (Shoichet, 2010).

Li *et al.* (2005) reported the nanofibers produced from Poly (ϵ-caprolactone) (PLC) can accommodate the differentiation of human mesenchymal stem cells (hMSCs) into adipogenic, osteogenic and chondrogenic cells. Biodegradable porous polyesters and polyurethane scaffolds have good strength and an adjustable degradation speed (Chouzouri and Xanthos, 2007) and exhibit *invivo* biocompatibility and vascularization and it can be used to generate tissue constructs, which do not induce a strong inflammatory reaction after implantation into patients (Laschke *et al*., 2009) and are widely employed in tissue engineering. Venugopal and colleagues electrospun a fibrous nanocomposite of PCL/HA/gelatin at a ratio of 1:1: 2. The results demonstrated that osteoblast proliferation, alkaline phosphatase activity and mineralization were highest on the highly flexible PCL/HA/gelatin nanocomposite when compared to other PCL nanofibrous scaffolds (Venugopal *et al*., 2008). Also the nanofibrous fibrin-based composites (polymer/calcium phosphate) promote osteoblast alkaline phosphatase activity as well as osteoblast marker gene (mRNA) expression to support bone maturation both *in vitro* and *in vivo* in a mouse calvarial defect model (Osathanon *et al*., 2008).

Silks are attractive biomaterials for bone tissue engineering because of their biocompatibility, slow degradability and excellent mechanical properties. Silk proteins, contain a highly repetitive primary sequence that leads to a high content of α-sheets, responsible for mechanical robustness and long-term degradability (Altman *et al*., 2003). Thread core protein called silk fibroins, have also been used for culturing osteoblast, hepatocyte, fibroblast cell support matrixes and for ligament tissue engineering (Li *et al*., 2006). Physical modifications, like fibers in the nanometric or micrometric scales (Bondar *et al*., 2008) or chemical modification, including growth factors incorporation can induce cell differentiation (Uebersax *et al*., 2008). It is also employed for several tissue engineering applications in combination with bone (Kim *et al*., 2005) or mesenchymal stem cells (Hagenmuller *et al*., 2007), endothelial cells (Fuchs *et al*., 2006) and co-cultures of mature endothelial cells and primary osteoblasts (Unger *et al*., 2007). For example, highly porous silk scaffolds were combined with adult MSCs for *in vitro* cartilage tissue engineering (Wang *et al*., 2005). Also from silk nano fibrous scaffold have been made in combination with isolated silk fibroins, BMP2 and nanoHAP particles by electrospinning (Li *et al*., 2006). The L-929 mouse fibroblast cell line attached and grew readily on films made of fibroin (Inouye *et al*., 1998). When applying tensile force MSCs cultured on collagen or silk fibres differentiated toward a ligament phenotype and formed tissue that morphologically and functionally resembled native ligament (Altman *et al*., 2002). Different molecular weight silk fibroins placed on a poly (D, L-lactic acid) surface supported the attachment and growth of osteoblasts *in vitro* (Cai *et al*., 2002). Osteoblast-like cells readily grew on silk films with integrin recognition sequences and the induction of markers of bone formation was observed (Sofia *et al*., 2001). Vunjak-Novakovic *et al*. (2005)

proposed a single-phase silk-based scaffold which is functionalized by covalently binding growth factors with spatial concentration gradients, with opposing gradients of a chondrogenic factor (IGF-I) and an osteogenic factor (BMP-2) for tissue engineering of osteochondral grafts.

BIOACTIVE GLASSES

Bioactive Glasses are interesting versatile class of materials and structurally all silica-based glasses have the same basic building block-SiO_4^{4-} (Thamaraiselvi and Rajeswari, 2004). Studies proved that it can form effective interaction of the scaffold with the surrounding bone tissue by generating a tenacious bond via the growth of a carbonate HA layer (Rezwan *et al*., 2006). It have a distinctive properties of bioactive glasses have the ability to convert to hydroxyapatite in body fluids and in aqueous solutions containing calcium and phosphate ions and directly bind to bone. Bioactive Glasses(BG) in the CaO-P_2O_5-SiO_2 system are similar to the natural inorganic component of bone and are regarded as class A biomaterials in medical applications, thus considered to be both osteoproductive and osteoconductive (Yang *et al*., 2006). However, bioactive glass is relatively stiff, brittle and difficult to form into complex shapes. Porous scaffolds of degradable polymers have also been coated with bioactive glass particles to produce a combination of degradable and bioactive properties (Roether *et al*., 2002). Researchers at the University of Missouri-Rolla have shown that some borate glasses can convert to hydroxyapatite and bond directly to bone in a manner comparable to the silicate-based 45S5 glass (Day *et al*., 2003). An amorphous two-dimensional glass network results in the low fracture toughness and mechanical weakness are the drawbacks of Bioglass® which is used for tissue replacement applications. Also the flexural strength (ranges from 40-60 MPa) exhibited by most Bioglass® compositions is not suitable for major load-bearing applications (Wang, 2003).

BIOACTIVE CERAMICS

Interestingly, bioactive ceramics like hydroxyapatite (HAP) have shown to be the most promising of all materials for hard tissue application due to their osteoconductive properties, ability to integrate with the bone tissue and absence of immune response (Wang, 2003) and which mimics the mineral composition of bone (Jarcho *et al*., 1977). Hydroxyapatite, with the structural formula of $Ca_{10}(PO_4)_6(OH)_2$, is the principal inorganic constituent of human bones and teeth (Hench, 1991). HAP has been well characterized chemically and mechanically as its benefits are demonstrated in both short- and long-term clinical results (Grandjean-Laquerriere *et al*., 2007). Synthetic HAP crystals are now widely used in medical applications, as implants or coatings on prostheses (Wojciech and Masahiro, 1998). Compressive strength is strongly dependent on porosity (Sopyan and Kaur, 2009). There are a large number of methods to produce porous hydroxyapatite, including incorporation of volatile organic particles in HA powder (Hench and Wilson, 1993), polymeric sponge method (Palazzo *et al*., 2005), gel casting of foams (Sepulveda *et al*., 2000), starch consolidation (Rodriguez-Lorenzo *et al*., 2002), microwave processing (Fang *et al*., 1992), slip casting (Rodriguez-Lorenzo and Ferreira, 2004), freeze casting (Fu *et al*., 2008) and electrophoretic deposition technique (Ma and Cheng, 2002). Both cell proliferation and cell apoptosis are related to the size of the HAP particles (Shi *et al*., 2009). Calcium phosphate ceramics in the nanoscale range determines the cellular performance of mesenchymal stem cells and osteoblast cells. Osteoblast shows high proliferation rate on nanophase HAP than with borosilicate glass, nanophase alumina and nanophase titania (Webster *et al*., 2000).

The precipitated apatite layer is desirable to result in the formation of bond between bone and materials. However, it also should consider carefully about the loosening HAP particles production and accumulation from the prosthetic implants which limits the longevity of prosthesis (Shu *et al*., 2003). Aseptic loosening after total joint arthroplasty is a major problem in orthopedic surgery. It is due to an osteolysis resulting from osteoclast activation by the cytokines and growth factors synthesized by the macrophages which phagocytosed the wear debris (Grandjean-Laquerriere *et al*., 2007). Interactions of bone cells with HAP surfaces are mediated by adhesion receptors belonging to the integrin family. Integrins expressed by bone cells interact with HAP surfaces via the biomolecules (eg., Fibronectin) that are absorbed to those surfaces. Calcium ions and phosphate ions are essential materials for cell mineralization and suitable concentrations of calcium and inorganic phosphates in culture media would maintain and/or enhance cell growth and proliferation (Xu *et al*., 2009). They also demonstrate superior osteoconductive properties and proven biocompatibility. While these ceramics have been employed in long bone reconstruction preclinically (Marcacci *et al*., 1999) most preclinical and clinical applications are in the area of craniofacial, dental and other non to low weight bearing skeletal site reconstructions (Matsusue *et al*., 2001). Porous hydroxyapatite bodies are mechanically weak and brittle,

which makes shaping and implantation difficult (Narulkar *et al*., 2007). However, HAP is resistant to degradation *in vivo* which occurs at a rate of 1-2% per year (Constantino and Friedman, 1994). HAP also has the ability to both adsorb and release protein; the absorption of protein increases with the increase in the surface area of HAP (Nakamura *et al*., 2006). Also it is very efficient in adsorbing adhesive proteins present within body fluids at the surgical site and therefore it isn't clear that functionalizing hydroxyapatite with RGD (Arg-Gly-Asp) would be beneficial *in vivo* (Hennessy *et al*., 2009). Reports show that fibronectin (FN), vitronectin and fibrinogen (Fbg) from the surgical environment become adsorbed to the HAP surface within minutes following implantation (Hennessy *et al*., 2008). Moreover, the combined RGD/FBS-coated HA surfaces elicited greater activation of the cell apoptotic marker, caspase 3, than FBS-coated HAP (Hennessy *et al*., 2008) suggesting that RGD had a negative effect on cell survival. RGD inhibits the osseointegration of HAP biomaterials, most probably through diminished attachment and survival of MSCs (Hennessy *et al*., 2009). HAP immersed in Simulated Body Fluid (SBF) exhibits a good proliferation and differentiation *in vitro*. This immersed condition is like that of HAP in the human bone lying alongside the collagen fibrils. So this provides the appropriate surface chemistry and thereby acts as a bone bonding interface where the cells can preferentially proliferate and differentiate into bone (Paital and Dahotre, 2009).

Rat BMSC cultured in the presence of ascorbate, b-glycerophosphate and dexamethasone form calcified nodules with biochemical, ultrastructural and morphological properties similar to woven bone (Satomura and Nagayama, 1991) and it has been shown that bFGF enhances the ability of BMSC to form these calcified nodules (Pitaru *et al*., 1993). Several calcium phosphate cements can self-harden to form hydroxyapatite and possess excellent osteoconductivity and biocompatibility (Xu *et al*., 2004). Calcium Phosphate Cement (CPC) contain an equimolar mixture of tetracalcium phosphate (TECP) and dicalcium phosphate anhydrous (DCPA). When it get mixed with water it will harden with in 30 min. As it possesses slow resorption and replacement by new bone formation with no loss in volume it is used for invivo applications (Liu *et al*., 2003). However, for certain clinical applications a more rapid resorption and replacement by new bone is desirable (Monteiro *et al*., 2003). Studies have shown that calcium phosphate ceramic implants with macropores (>100 μm) allowed ingrowth of bone tissue with functional Haversian systems and facilitated osteointegration (Almirall *et al*., 2004). Although the presence of macropores in CPC is not critical to implant resorption and replacement by bone, incorporation of macropores in CPC is likely to promote the process. As a result of the setting and hardening mechanisms operating in CPC, through dissolution and precipitation processes, CPC has an intrinsic microporosity (Takagi and Chow, 2001). Several attempts have already been made to improve the resorption behavior of CPCs e.g., by increasing the porosity of the material (Xu *et al*., 2006). Porous scaffolds in bone tissue engineering require three-dimensional interconnected porous structure, which could provide sufficient space for cell migration, adhesion and the ingrowth of new bone tissue (Wu *et al*., 2006). CPC has a composition and structure, very close to natural bone mineral and therefore has been considered to be the ideal material to build bone tissue engineering scaffold (Xu *et al*., 2007).

CORALS

Inorganic calcium carbonate phase of natural coral skeletons have a property to grow onto a charged, organized organic template (Martina *et al*., 2005). So this can be a suitable xenogenous grafting material with a reduced risk of disease transmission and viral contamination compared to allogenic or bovine bone matrix grafts. Coral is easily available and it shared close resemblance of bony mineral and made it a good alternative as bone graft (McAuliffe, 2003). At present, calcium phosphate ceramics such as hydroxyapatite produced from corals has been reportedly used for orthopedic bone defect reconstruction. These porous coral HAP scaffolds are reported to exhibit a hydrothermal exchange reaction thereby converting porous coralline skeletal materials into HAP that have similar microstructure as the starting carbonate skeletal material (White and Shors, 1990). This natural ceramic has the best mechanical properties of the porous calcium-based ceramics, given that its interconnected porous architecture is similar to that of spongy bone (Martina *et al*., 2005). The interconnected small pores pave way for efficient media perfusion and growth factor entrapment (Green *et al*., 2002) and results in the vascularization of implants and new bone formation also with subsequent coral resorption at a rate commensurate with bone formation. Bone tissue has a strong affinity to calcium carbonate-based materials (Ohgushi and Caplan, 1999). These properties make the coral skeleton a suitable material for delivering growth factors or bone marrow cells and have been used in numerous clinical applications for more than a decade (Gisep, 2002).

Bensaid *et al*. (2003) in a comparative transplantation study of coral and fibrin scaffold in nude mice showed

that HMSCs were able to migrate out of the scaffolds and penetrate deeply into the coral implants and cannot even entrapped in fibrin scaffold. However, the major drawback for the use of coralline HAP is the inability to control the pore size and chemical composition, thereby resulting in unpredictable outcomes (Oh *et al*., 2006).

CONCLUSION

Biomaterials play a pivotal role in tissue engineering. The 3-dimensional scaffold used can hold the cell growth, proliferation and finally leads to the implantation to the contextual site. The design of a scaffold is based on the sound biological principles that have evolved from understanding how autografts function and how they are remodeled after transplantation (Ostrum *et al*., 1994). The porosity, pore size distribution and continuity of the scaffold are also critical for vascular invasion and these are due to the properties of materials used, method adopted for its fabrication etc. These parameters dictate the interaction of scaffolds and transplanted cells with in the host environment. Biodegradable materials obviate the need for a second surgery, yet are complicated by the degradation products produced, which must neither be cytotoxic nor cause an inflammatory reaction and the rate of degradation which must follow that of regeneration. Understanding the cellular microenvironment and then incorporating this into biomaterials design strategies is an important focus. The implant engineering methods can be used as a way to understand the cellular microenvironment better. Combination of biomaterials for scaffold fabrication will support the growth of cells. But the level of biological complexity that needs to be recapitulated within a synthetic three-dimensional environment is still uncertain.

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