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Review Article

A review on bioscaffolds for tissue engineering application

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Abstract: This paper reviews the current practices in the field of Tissue Engineering for Bone scaffold Applications. The mechanism of bone replacement or bone graft has been covered. The synthetic biomaterials and composites that are used for fabrication of scaffolds are reviewed. Scaffold requirements in terms of their mechanical properties, pore structure along with other biological properties are discussed. Finally, this paper also highlights the challenges faced in this industry and suggestions for further research and development of this field.
Keywords: Tissue engineering (TE), bone scaffold, biomaterial, mechanical property

INTRODUCTION

Tissue Engineering is an inter-related and a multi-disciplinary field that integrates the cell behaviour and technique of growing it on an artificial substrate known as scaffold along with suitable biochemical factors that are required to create artificial tissue and organs or simply to regenerate damaged tissues [1-2]. It involves the seeding of cells on to a scaffold, which are then cultured *invitro* to form the matured tissues. Then it is fixed into the body damaged parts such as fractured bone, cartilage or skin as an implant. The natural tissue regeneration process takes place within the scaffold during which the blood vessels infiltrate the structure and the scaffold is degraded slowly while a newly formed tissue is in place as explained schematically in Figure 1 [2-3].In general, tissue engineering scaffolds must serve three primary purposes: (i) They must define a space that will shape the regenerating tissue; (ii) they must provide temporary function in a defect while tissue regeneration and (iii) they must facilitate ingrowth of tissue and possibly allow for inclusion of seeded cells, proteins and/or genes to accelerate tissue regeneration. The recent developments in tissue engineering (TE) in understanding the cell-scaffold interaction as well as the development of technologies for the production and characterization of porous scaffolds allowed the birth of "third-generation" biomaterial scaffolds; bioactive and biodegradable scaffolds designed to provide a temporary 3D microenvironment for cell and tissues and simultaneously to guide cellular processes involved in denovo tissue genesis[4].

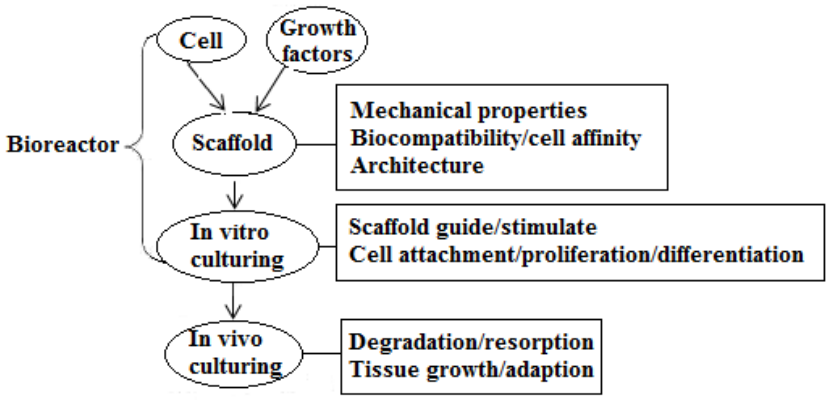

Figure 1:General concept of tissue engineering process [2,3].

The scaffold provides a framework and initial support for the cells to attach, proliferate and differentiate to form the extracellular matrix (ECM) [5].In addition to being biocompatible both in as implanted and degraded form, these scaffolds have to exhibit appropriate mechanical properties to provide the correct stress environment for the neo-tissues. The material must be designed with a degradation rate that assures that the strength of the scaffold is retained until the newly grown tissue takes over the synthetic support [6]. Non-healing bone fractures are major health problem world-wide because of a large aging population and increased occurrence of sports related injuries. The rate of bone grafting is increasing dramatically. Bone substitutes are playing a major role in repairing or replacing damaged or diseased tissue resulting from trauma pathological degradation, congenial deformation, cancer and cosmetic. It was reported that over one million bone grafts were implanted annually in USA and EUROPE and over 500 thousand bone grafting procedures performed annually in the USA alone [6-11]. Bone and cartilage injuries occur due to various reasons including degenerative, surgical and traumatic process, which significantly compromise quality of life. Currently, millions of patients are suffering from bone and cartilage defects, reportedly with over 450,000 bone grafts and approximately 250,000 knee arthoplasty procedures performed per year in the US alone [7-8]. Furthermore, the clinical needs to effectively treat such conditions are expected to increase as aged population continues to grow [9].

BONE TISSUE ENGINEERING

For bone tissues engineering, a scaffolds is used to either induce formation of bone from the surrounding tissue or act as a carrier or template for implanted bone cells or other agents. Bone regeneration generally involves few critical components; a morphogenetic signal, host cells that will respond to the signal, a template of this signal that can deliver to the damaged tissues than serve as a scaffold for the growth of the host cells and a well vascularized host bed. Bone morphogenetic protein (BMP) [12], a group of proteins responsible for a variety of events in embryogenesis and in postnatalskeleton, act as the morphogenetic signal. BMP causes puluripotential cells to differentiate into osteoblast, bone regenerating cells. One of the key biological properties of BMPs is the ability to induce new bone and cartilage [13]. The scaffold serves as a carries of BMP or functions as a template for implanted bone cells or other agents, and it also supports ingrowth of capillaries and cells from the host into 3-D substrate to form bone [14]. Some scaffolds degrade at a controlled rate that is compatible with tissues ingrowth rate; the degradation products can be easily metabolized or excreted. At the end a new, completely natural bone tissues is formed in the place of scaffold [14].

Bone tissue engineering has the potential to reach millions annually through the repair of bone defects. Therefore, researchers in bone tissue engineering are working to develop alternatives to allogenic and autologous bone grafts in order to address the growing needs of the population, and the much of the research is scaffold. A scaffold can be used to guide bone regeneration and repair defects or be combined with cell and/or biologics, which are added to further enhance bone regeneration [15].

Yoneda *et al.* researched on recombinant human bone morphogenetic protein (rhBMP)-2 in a block copolymer composed of poly-D,L-lactic acid with randomly inserted p-dioxanone and polyethylene glycol (PLA-DX-PEG) as a carrier and porous beta-tricalcium phosphate (beta-TCP) blocks were used to generate a new fully absorbable osteogenic biomaterial. The bone regenerability of the rhBMP-2/PLA-DX-PEG/beta-TCP composite was studied in a critical-sized rabbit bone defect model. In an initial study, a composite of PLA-DX-PEG (250 mg) and beta-TCP (300 mg) loaded with or without rhBMP2 (50 µg) was implanted into a 1.5 cm intercalated bone defect created in a rabbit femur. Defects were assessed by biweekly radiography until 8 weeks postoperatively. The bony union of the defect was recognized only in the BMP-loaded group. To obtain further data on biomechanical and remodeling properties, another BMP-loaded composites group was made and observed up to 24 weeks. All defects were completely repaired without residual traces of implants. Experimental results indicates that fully absorbable rhBMP-2/PLA-DX-PEG/beta-TCP is a promising composite having osteogenicity efficient enough for repairing large bone defects [16].Figure 2 shows an image of Calcium Phosphate scaffold implanted on a rat cranial bone after one month of implantation.

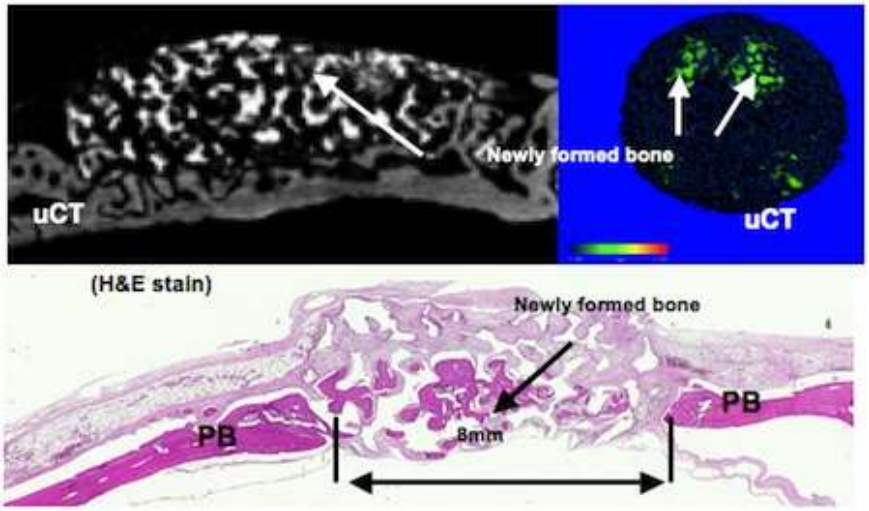

Figure 2: Image of CaP scaffolds aided bone healing in the absence of BMP-2 and cells using a rat cranial criticalsize bone defect (8 mm in diameter) after one-month implantation [15].

Tadic *et al*. studied on calcium phosphate phase that is equivalent in composition and crystallinity to the mineral phase of bone which was prepared by a continuous precipitation method. The powder was compacted by cold isostatic pressing into desired shapes with high compressive strength in the range of 20–50MPa. It is concluded that such implant materials can be prepared with a fine-tuned biodegradability in combination with a high mechanical strength. The high mechanical strength of the objects also permits further mechanical shaping procedures like drilling or cutting [17].

A successful tissue engineering method for bone replacement would imitate natural bone graft by providing the essential elements for new bone formation using synthetic scaffolds, osteogenic cell populations, and bone induction factors. Thomson *et al.* evaluated the suitability of various formulations of poly (DL-lactic-co-glycolic acid) (PLGA) foams to provide a tissue conducting scaffold in an bovine model for bone flap fabrication [18]. Three formulations were used of different copolymer ratio and molecular weight. Porous wafers of PLGA were stacked into a closed rectangular chambers with one side open. Some chambers also contained autologous morcellized bone graft (MBG)[18]. The chambers were inserted with the open face adjacent to the cambium layer of the periosteum in rib beds of seven sheep and harvested after 8 weeks in vivo. Gross and histologic examination of the resulting tissue specimens demonstrated molded units of vascularized tissue generally conforming to the shape of the chambers and firmly attached to the periosteum. Polymer degradation appeared to occur by varying degrees based on polymer formulation. New bone formation was observed only in areas containing MBG [18]. A PLGA foam scaffold is an efficient conductor of new tissue growth but not osteoinductive [19], it contributes to the shape of molded tissue, and biocompatible when used in this model. Further studies are warranted to develop practical methods to deliver bone induction factors to the system to promote osseous tissue generation throughout the synthetic scaffold.

BIOMATERIALS FOR BONE SCAFFOLD

The field of biomaterials has been rapidly growing during the last few years and we can now find a replacement of biological material with that of an artificial matter, where new biomimetic structures with a wide range of chemical and physical properties will promote the development of a novel generation of medical devices. Biomaterials are those materials which are naturally existing or man-made materials that can replace the living tissues [21].

Natural polymers, synthetic biodegradable polymer and synthetic non-biodegradable polymer are the fundamental sorts of polymers utilized as biomaterials.Natural polymers might be acknowledged as the first biodegradable biomaterials utilized clinically [22]. A natural material with bioactive properties interacts with the cells to allow them in improving the cells' performance in the biological system. Natural polymers are considered as proteins such as silk, collagen, gelatin, fibrinogen, elastin, keratin, actin, and myosin, and polysaccharides or polynucleotides.

Synthetic biomaterial facilitates the restoration of structure and functions of the harmed and sick tissues. Synthetic polymers are remarkable functions in biomedical field. Their properties for example porosity, mechanical attribution and extra could be designed for particular applications. A synthetic polymer represents as the largest group of biodegradable polymers and can be generated under controlled conditions. They display predictable mechanical and physical properties for example the tensile strength, elastic modulus, and degradation rates. Examples of commonly used synthetic polymers,copolymers are poly (lactic acid) PLA, poly (glycolic acid) [23] PGA, and poly (DL-

lactic acid-co-glycolic acid) PLGA . Poly (e - caprolactone) (PCL) is a semi crystalline and bio-resorbable polymer that belongs to an aliphatic polyester family. It is viewed as a good bio-resorbable material for soft and hard tissues to be utilized as scaffold tissue engineering.It has comparable biocompatibility to PLA and PGA with low degradation rate. Slow degradation process makes it not suitable for tissue engineering, but it is a proper applicant for a long-term drug delivery carrier.It is regularly combined together with other materials such as bio-ceramics to increase its Young Modulus and enables modification of its biodegradation rate [23].

Biomaterial plays an important role in the tissue engineering by performing as synthetic frameworks in referring as scaffolds, matrices and constructs. Biomaterials designing have constantly developed in the past few decades. Lately,priorities have been given on these materials that could be utilized as a part of biomedical fields.Biomaterials proposed for biomedical applications are focused in improving artificial materials that might be used in repair or restore function of diseased tissues in the human body and enhancing the quality of life. After an early experimental stage of biomaterials choice dependent upon its availability, the design efforts were mainly concentrated on either attaining structural or mechanical performance. Biomaterials utilized as attachments in the form of bone plates,ligaments, joint replacements, vascular grafts, intraocular lenses, heart valves dental implants, and other medical devices such as pacemakers, biosensors, etc. [24].

Organic and Inorganic biopolymers scaffold

Natural polymers used in bone tissue engineering include chitosan, collagen, fibrin, alginate, silk, hyaluronic acid, and gelatin [25]. Most natural polymers are biocompatible, degradable, and readily solubilized in physiological fluid which can be used alone as a growth factor delivery carrier or combined with other delivery materials such as synthetic polymers and inorganic materials. As a drug delivery carrier, collagen has been fabricated as gels, nanofibers, porous scaffolds, and films. Despite the biocompatibility, collagen, like other natural polymers, is mechanically weak and undergoes rapid degradation upon implantation. Therefore, optimization of degradation rate and molecular properties may be required by crosslinking of collagen with appropriate chemical reagents [25].

Perhaps synthetic polymers are the most widely used materials as growth factor delivery carriers in tissue engineering. Synthetic materials indeed provide excellent chemical and mechanical properties than that of natural polymers. The great advantages of synthetic polymers are associated with their processibility and flexibility to tailor to have appropriate chemical and mechanical properties [26]. While natural polymers are high molecular weight macromolecules, which make it difficult to process, various synthetic routes for man-made polymers provide better opportunities to control molecular weights, functional groups, configurations, and conformations of polymer chains. Tailoring polymer structure can determine the length and degradation characteristics, which may be the most influential parameter dictating release behaviour of growth factors [27].

Hydroxyapatite (HA) reinforced polyethylene was developed by Wang *et al.* (1999) as a bone replacement material. In order to improve bonding between HA and polyethylene, and hence to increase mechanical properties of the composite, chemical treatments of HA and polyethylene were investigated and new composites manufactured. Two approaches were employed in this investigation: the use of silane-treated HA as the filler, and the application of polymer grafting for polyethylene. The silane coupling agent used was 3-tri-methoxy-siyl-propyl-methacrylate and the grafting monomer for polyethylene was acrylic acid. A processing route was established with and without the application of polymer grafting. New composites with different HA contents were produced and evaluated [28-30].

Synthetic bone scaffold

In most cases, biocompatible, degradable polymers are utilized to induce surrounding tissues ingrowth or to serve as temporary scaffolds for transplanted cells to attach, grow and maintain differentiated functions. In addition to bring biocompatible both in as implanted and degraded form, these scaffolds have to exhibit an appropriate mechanical properties to provide the correct stress environment for the neo-tissues. The scaffold material must be designed with a degradation rate that assures the strengths of the scaffold is retained until the newly growth tissues takes over the synthetic support [31]. The scaffold is a 3-dimensional substrate and it serves as a template for tissues regeneration. The ideal scaffolds should be porous and permeable to permit the ingress of cells and nutrients. It also should have an appropriate surface chemistry and micro structure to facilitate cellular attachment, proliferation and differentiation. In addition, the scaffolds should possess adequate mechanical strength and biodegradation rate without any undesirable bi-products [32]. Hong *et al.* performed a study to improve the bonding between hydroxyapatite (HAP) particles and poly (L-lactide) (PLLA), and hence to increase mechanical properties of the PLLA/HAP composite as potential bone substitute material, the HAP nano-particles were surface-grafted with PLLA and further blended with PLLA. The PLLA molecules grafted on the HAP surfaces, as inter-tying molecules, played an important role in improving the

adhesive strength between the particles and the polymer matrix [33]. At a low content (approximately 4 wt%) of surface grafted-HAP (g-HAP), the PLLA/g-HAP nano-composites exhibited higher bending strength and impact energy than the pristine PLLA, and at a higher g-HAP content (e.g., 20 wt%), the modulus was remarkably increased. It implied that PLLA could be strengthened as well as toughened by g-HAP nano-particles. The results of biocompatibility test showed that the g-HAP existing in the PLLA composite facilitated both adhesion and proliferation of chondrocytes on the PLLA/g-HAP composite film [33].

Parsons *et al.* reviews the progress that has been made in fabricating biomimetic bone structures using synthetic composite materials. The specification for long bone applications are developed and identify the candidate materials for delivering cortical and cancellous bone properties and function. The role of composite materials are discussed together with the factors influencing fibre and matrix type. Challenges associated with moderating their performance in-vivo are discussed, relating to the properties of the starting materials and the dependence, for fibre reinforced systems, on interface quality. Fabrication routes for producing complex biomimetic structures are also reviewed and the state of current clinical developments is described along with the associated technical and regulatory issues [34].

Ni *et al.* reported that clinical outcome of cemented implants to revision total hip replacement (THR) is not as satisfactory as primary THR, due to the loss of bone stock and normal trabecular pattern. Various materials such as bioactive bone cement, strontium-containing hydroxyapatite (Sr-HA) bone cement, in a goat revision hip hemi-arthroplasty model, and compared outcomes with polymethylmethacrylate (PMMA) bone cement were used in their study. Nine months after operation, significantly higher bonding strength was found in the Sr-HA group (3.36+/-1.84 MPa) than in the PMMA bone cement group (1.23+/-0.73 MPa). After detached from the femoral component, the surface of PMMA bone cement was shown relatively smooth, whereas the surface of the Sr-HA bioactive bone cement mantle was uneven, by SEM observation. EDX analysis detected little calcium and no phosphorus on the surface of PMMA bone cement mantle, while high content of calcium (14.03%) and phosphorus (10.37%) was found on the surface of the Sr-HA bone cement. They found that a good bioactivity of Sr-HA bioactive bone cement would be more suitable in hip replacement model using goats. This in vivo study also suggested that Sr-HA bioactive bone cement was superior to PMMA bone cement in terms of bone-bonding strength. Use of bioactive bone cement may be a possible solution overcoming problems associated with the use of PMMA bone cement in revision hip replacement [35].

Cellular composite scaffold

Biological restoration of osteochondral defects requires suitable subchondral support material that also allows the induction of hyaline cartilage tissue. Biphasic implants consisting of pre-fabricated neocartilage and an underlying biodegradable osteoconductive base may meet these requirements. Porcine chondrocytes seeded scaffold in a closed and static bioreactor with a base of biomaterial consisting of either poly-L-lactide [P(L)LA], poly-d,l-lactide [P(D,L)LA] were studied.Figure 3 shows the SEM micrograph of interconnected porousstructure of PLLA and PLLA-Collagen scaffold. Viable neo-cartilage was produced on each biomaterial with differing amounts of cellular colonisation. P(D,L)LA breakdown was more rapid and uneven among the three biomaterials, leading to constructs of irregular shape. Little or no breakdown or chondrocyte colonisation was evident in PLA. Col-HA constructs were superior in terms of viability, implant morphology and integration between neo-cartilage and biomaterial. These materials have potential for producing biphasic implants that may be adequate for the repair of osteochondral defects [36].

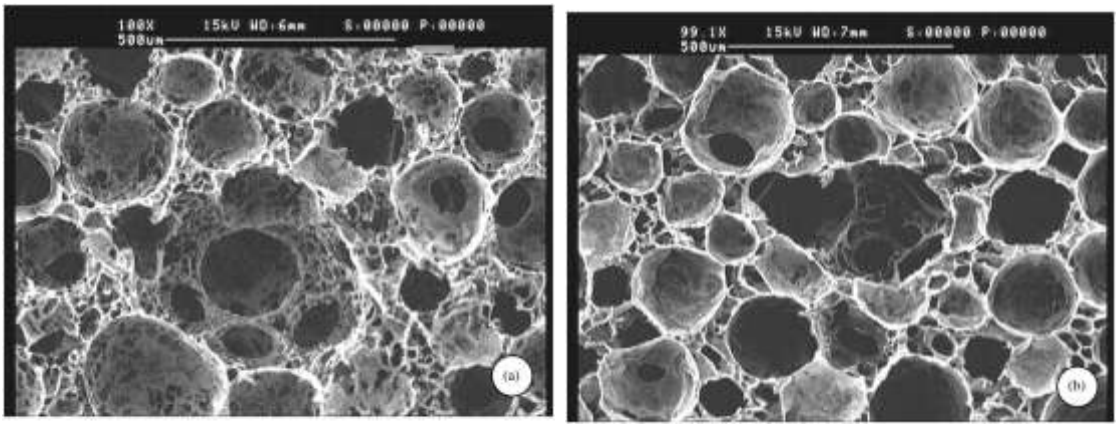

Figure 3: SEM micrograph of PLLA (a) and PLLA-Coll (b) scaffold with interconnected spherical pore structures [36].

Mendes *et al*. described an extensive biocompatibility evaluation of biodegradable starch-based materials aimed at orthopaedic applications as temporary bone replacement/fixation implants. For that purpose, a polymer (starch/ethylene vinyl alcohol blend, SEVA-C) and a composite of SEVA-C reinforced with hydroxyapatite (HA) particles, were evaluated in both *in vitro* and *in vivo* assays. For the *in vitro* analysis cell culture methods were used. The *in vivo*tissue reactions were evaluated in an intramuscular and intracortical bone implantation model on goats, using light and scanning electron microscopy. A computerized image analysis system was used to obtain histomorphometric data regarding bone contact and remodelling after 6 and 12 weeks of implantation. In both *in vitro* and *in vivo* models, the SEVA-C-based materials did not induce adverse reactions, which in addition to their bone-matching mechanical properties make them promising materials for bone replacement fixation [38].

Bioabsorbable composites

Bioactive and bioresorbable composite materials were fabricated using macroporous poly(DL-lactide) (PDLLA) foams coated with and impregnated by bioactive glass (Bioglass) particles. Stable and homogeneous Bioglass coatings on the surface of PDLLA foams as well as infiltration of Bioglass particles throughout the porous network were achieved using a slurry-dipping technique in conjunction with pre-treatment of the foams in ethanol. The Figure 4 shows the SEM micrograph of the PDLLAfoam/Bioglasss composite sample produced by slurry dipping.

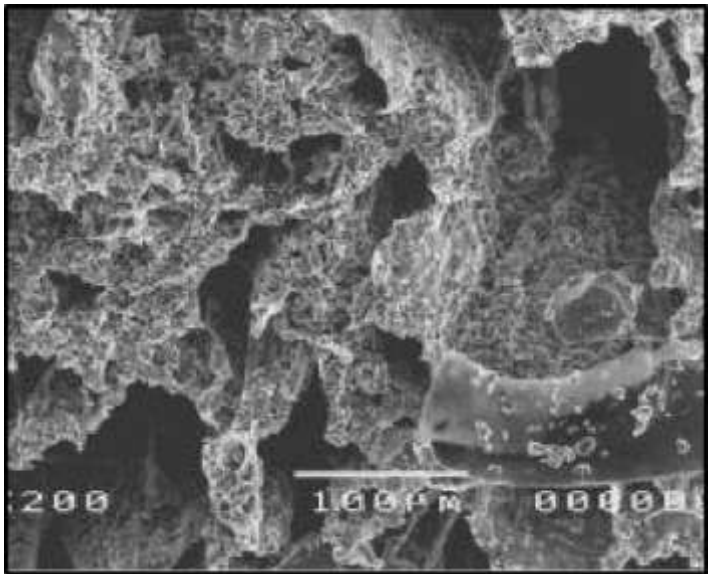

Figure 4: SEM micrograph showing the microstructure of a PDLLAfoam/Bioglasss composite sample produced by slurry dipping. The efficient infiltration of Bioglasss particles into the pores can be observed [38].

The quality of the bioactive glass coatings was reproducible in terms of thickness and microstructure. Additionally, electrophoretic deposition was investigated as an alternative method for the fabrication of PDLLA foam/Bioglass composite materials. *In vitro* studies , simulated body fluid (SBF) were performed to study the formation of hydroxyapatite (HA) on the surface of PDLLA/Bioglass composites. SEM analysis showed that the HA layer thickness rapidly increased with increasing time in SBF. The high bioactivity of the PDLLA foam/Bioglass composites indicates the potential of the materials for use as bioactive, resorbable scaffolds in bone tissue engineering [39].

GENERAL CHARACTERISTICS OF SCAFFOLD

Biological properties

The scaffold material must be biocompatible and promote cell adhesion, migration and ingrowth. As the cells produce their own extra cellular matrix (ECM), the synthetic matrix should degrade into non-toxic components that can be eliminated from the body [40].Biocompatibility may be the most important scaffold property. It is defined broadly as the ability of a material or device "to perform its intended function, including an appropriate degradation profile, without eliciting any undesirable local or systematic effects in that host" [41]. Host response both positive and negative, may include osteoblast/osteoclast response, prolonged inflammation, micro vascular changes, fibrous, encapsulation, protein adsorption and endothelial proliferation. Besides that, the scaffolds must also be osteoconductive [18]. Osteocondutivity is the ability of the scaffold to serve as a template for bone formation by encouraging cells to adhere to the surface and to proliferate and produce bone. It refers to the ability of the scaffolds properties to induce bone formation without osteoinductive agents, such as bone morphogenic proteins (BMPs) [12]. In tissue

engineering, the scaffolds must be bioactive. Bioactivity: is the tendency of the material to form a chemical bond with the host bone. For CaPs this postulated to occur through material dissolution and precipitation of a carbonated apatite that is more similar to the mineral phase of bone, crystallinity, grain size and imparity.

Internal pore structure

Both cell seeding and bone ingrowth normally are well developed with high porosity, typically among 50-90%. In general the pore size falls within a certain critical range to promote cell seeding and ingrowth [40] both upper and lower bounds are computed by different factors. Cell size controls the lower bound; the specific surface area via the availability of binding site decides the upper bound.Karageorgiou and Kaplan reviewed that the optimal pore size for bone ingrowth is in the range of 100-250μm [42] cell ingrowth and nutrients transportation are interconnected with porosities.Interconnected porosity is required for nutrients and waste transport throughout and for bone growth. The minimum pore size for bone formation has been quoted by many as 100μm[43]. However, more recently researchers have shown bone formation in interconnected micropores less than 10μm in size in scaffolds that contained both macro porosities (>100μm) [44].

Mechanical properties

Mechanical properties are the main important properties to be considered in developing scaffolds for tissue engineering.Mechanical integrity is a broad term that encompasses all mechanical properties from post-manufacture through to complete healing. The primary bone tissue has relative high compressive strength that supports the body weight. So the scaffold must provide mechanical support during the reconstruction process. Mechanical integrity for the scaffold design has to be sufficient enough to resist handling during implantation and '*invivo*' loading. An ideal scaffold would be biomechanically similar to the type of bone being replaced in order to function quickly as a synthetic bone replacement. The compressive module is in the range of 0.01 to 2.0 GPa for trabecular bone, and 14 to 18 GPa for cortical bone. The scaffold should be able to maintain sufficient mechanical properties until newly formed bone can assume a structured role and then the scaffold can be degraded and resorbed in the process of bone regeneration [45]. Numerous studies have demonstrated profound effects of mechanical forces (strain) on cells using '*invivo*' and '*invitro*' models [46]. Most of the researchers found that the mechanical properties of the substrate are significant factors affecting biological response, as the mechanical environment of the contained cell is determined by these properties [47-50].

SUMMARY AND FUTURE PERSPECTIVE

In this paper, we have reviewed the concept of tissue engineering and the importance of tissue engineering in biomedical research. The use of scaffolds for tissue regeneration has been discussed. Besides that, the materials used to develop scaffolds in tissue engineering are reviewed and presented. The composites scaffolds reviewed in this paper combine the features of bioactivity and biodegradability. Most of the composites discussed have addressed the biological aspects and mechanical factors that are necessary for *invivo* and *invitro* studies. The general characteristics and properties of tissue engineering scaffolds are also discussed. So far, an ideal scaffold material and biocomposites for bone and cartilage has not yet developed. Another major challenge in this industry is that scaffold fixation features and techniques are inadequate. Also, tissue engineering being a new emerging science for biomedical industry has to focus on gene therapy and nerve tissue regeneration in the future research.

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