[Frontiers in Bioscience, Elite, 5, 341-360, January 1, 2013]

Biomaterial scaffolds for tissue engineering

Kajal K. Mallick, Sophie C. Cox

Warwick Manufacturing Group, School of Engineering, University of Warwick, Coventry, CV4 7AL, United Kingdom

TABLE OF CONTENTS

1. ABSTRACT

Reconstruction and regeneration of new tissues are challenges facing scientists, technologists and clinicians. This review describes strategies of selection and design of biomaterials having significant impact on various possible synthesis routes for scaffold fabrication. The criteria for three-dimensional (3D) scaffold architectures are explored in tandem with biomaterial properties such as porosity, interconnectivity and mechanical integrity. The cell-surface biointerface is outlined in terms of biomaterial composition, target tissues and biological evaluation with emphasis on bone tissue engineering. Comparative merits and demerits of conventional and rapid prototyping (RP) approaches of fabrication are discussed. The conventional methods are often simple to design, inexpensive and flexible to optimise or modulate physicochemical properties. Despite being expensive and suffering from certain drawbacks of choice of materials and capital costs many generic RP techniques are extremely attractive in their ability to mimic new tissue structures and possibility of incorporating pharmaceutical agents. The future directions include scaffold development using nanobiomaterial based biosystems /biointerfaces where cell biology including genetically modified tissue engineering approaches can play a cross-disciplinary role for the success of tissue augmentation.

2. INTRODUCTION

For the past decade, the field of tissue engineering is in the process of evolution and is still growing rapidly in order to formulate specific strategies to offer alternative solutions to the replacement of diseased or damaged/malfunctioned tissues. Traditionally, allograft and autograft procedures are used for tissue grafting and organ transplantation but with known associated problems of morbidity and pathogen transmission (1). Thus, biodegradable tissue engineered scaffold constructs for clinical applications, either in-vitro or in-vivo, have become an important focus of the multidisciplinary biomedical research involving material science and engineering, scaffold fabrication techniques, cell biology and associated biointerfacial studies. These synthetic or biologic scaffolds, amenable for tissue regeneration, present an alternative approach to allograft and autograft for regeneration of new tissue growth. The goal here is to facilitate a clinically viable strategy to augment hard or soft tissues (1, 2-5). It is imperative however that such an engineered scaffold must successfully mimic the natural tissue in terms of its structure, composition and function with desired level of hierarchy, interconnectivity, porosity and mechanical strength so as to allow cell attachment and proliferation resulting in adequate level of integration with surrounding tissues (3). In this review, we intend to focus

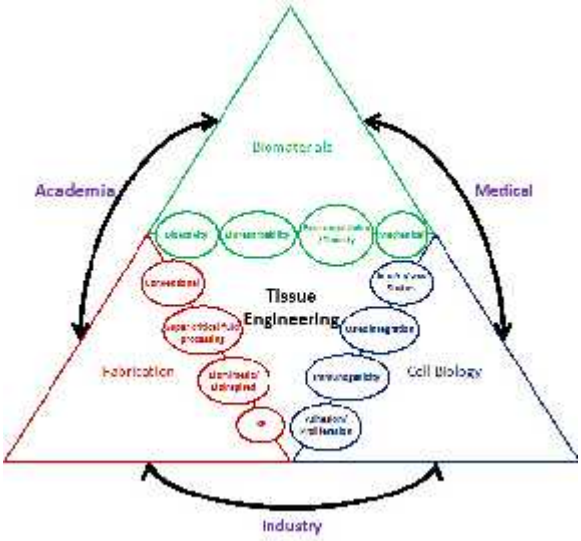

Figure 1. Cross-disciplinary tissue engineering approach

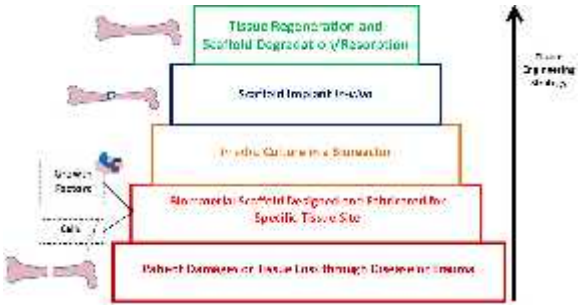

Figure 2. Tissue engineering strategy for bioscaffolds

on the current status of the development of biomaterials, synthesis, fabrication, processing methodologies and applications of porous scaffolds for tissue engineering but limited to particular emphasis on hard tissue replacement.

3. THE STRATEGY

The strategies relating to the cross-disciplinary tissue generation approaches as well as concepts of bone tissue engineering of a defect are schematically presented in Figures 1 and 2, repectively. The protocol and an approach similar to that shown in Figure 2 can also be easily adpated to find solutions for soft tissue replacement.

4. SCAFFOLD SPECIFICATIONS/CRITERA

4.1. Design and Function of 3D Architecture

Human tissues are organized into 3D structures as functional organs and organ systems. To engineer functional tissues and organs successfully, the scaffolds have to be designed to facilitate cell distribution and guide tissue regeneration in three dimensions. The primary function of a porous scaffold is to provide a suitable substrate for cell mediated adhesion and growth, either by seeding within the construct or allowing cells migrating from surrounding tissue, differentiated function and migration. Mammalian cell types are intrinsically dependent on anchorage mechanisms and generally lysing occurs if the adhesion is poor. The prerequisite physicochemical properties of scaffolds are as follows: (i) support, supply and delivery of cells (ii) induce, differentiate and conduit tissue growth (iii) promote cell-substrate adhesion and stimulate cellular response by surface conduction (v) elicit minimal immunogenicity (vi) act as wound healing barrier (vii) good cytocompatibility, hemocompatibility and bioresorbability to degrade at a similar rate to new tissue generation and remodeling (viii) easy to fabricate to desired shapes and hierarchy with dimensional stability (ix) highly porous and permeable with large surface/volume ratio to allow cell infiltration, nutrient and metabolite diffusion (x) appropriate mechanical properties to suit microstress environment for cell types (xi) sterilizability (6, 7). These properties will vary from material to material and can affect the overall cellular response. Additionally, since successful design of scaffold materials serves also as synthetic extracellular matrices (ECMs) a thorough knowledge base and understanding of molecular interaction that occur within the tissues and between the cells and the ECM are required (8-10).

4.2. Pore Morphology and Structure

3D architecture of the scaffold is very important when attempting to mimic the structure and functions of the natural ECM. One pre-requisite characteristic required for bone tissue engineering is that the scaffolds must have a porous and interconnected structure, enabling the migration and infiltration of cells (11). There is no clear consensus in the literature regarding the pore size that best promotes osteoconduction. The established view is that bone growth occurs when the pore is large enough to host cells and facilitates vascularisation. A pore size larger than 100µm and smaller than 400µm is usually considered optimal for osteoconduction (15). However, there are conflicting scientific reports demonstrating that bone growth can occur in pores smaller than 100µm and larger than 500µm (3, 13-16). The structural configuration of, for example, hydroxyapatite (HAP) within the fabricated scaffold including pore size, morphology and surface area has been shown to be critical in allowing osteoconduction, growth and migrations of tissue and transfer of nutrients through the scaffold (3, 17). Macro and microporosity within the scaffold facilitates the transfer of nutrients and encourages, via biomolecular signals, the deposition of ECM by promoting favourable cellular interactions. Apart from porosity another important factor is to create optimal strut dimension of interconnecting pores in order to maintain scaffold mechanical strength, complete penetration of cells and nutrients throughout the scaffold, thus preventing the formation of necrotic tissue in the centre of the scaffold (18).

4.3. Mechanical

Several authors have drawn attention to the need of good mechanical properties in scaffolds intended to be used in bone tissue engineering. Although not universally agreed there is a view in the literature that supporting mechanical forces is secondary as the main function of a scaffold is to support bone growth (3, 11). All factors

affecting the porosity must be considered and be balanced with the mechanical strength of the tissue engineered scaffolds that are designed for specific application since bone density at different anatomical locations vary. The compressive strength of compact bone is approximately 133MPa whereas cancellous bone ranges from 10-80MPa. Engineering of porosity balanced with strength is challenging. For example, highly porous (greater than 80% porosity) HAP scaffolds suffer from a reduction in mechanical strength and therefore can only be used in non-load bearing application but can be supported by several forms of fixation devices. The advantage here is that once the process of tissue mineralisation has started the initial weak mechanical strength of such highly porous HAP or other biomaterials would increase and provide adequate mechanical support. Microporosity can also compromise the mechanical integrity of the struts within the scaffold. If the scaffold is unable to provide a mechanical modulus in the range of hard (10-1500MPa) (18) or soft tissues (0.4-350MPa) any nascent tissue formation will probably fail due to excessive deformation.

4.4. Cell Attachment

Cell adhesion to scaffolds is a prime requirement for the success of scaffold based tissue engineering strategy. Cell/scaffold biointerfaces play a prominent role via cell adhesion molecules (CAMs). CAMs are glycoproteins which act as receptors on the cell surface for enhancing cell/cell and cell/ECM adhesions (8). Intergrins are one such major class of CAMs that is known to regulate many cellular functions such as cell adhesion, differentiation, motility and growth (19). Although there are many cell types that are routinely used *in vivo* and in-vitro for a diverse range of tissue engineering applications the regenerative medicine using stem cells is a rapidly developing field in the treatment of tissue defects (20). The platform technology is based on judicious selection of an appropriate type of cell and a cytocompatible and biodegradable scaffold matrix or carrier in order to develop a regulatory biosystem that mimics the structure, function or even composition of a specific type of tissue (21).

5. BIOMATERIALS FOR SCAFFOLD

Target tissues for regenerative therapies where appropriate scaffolds include hard tissues such as bone (various types) and teeth whereas cartilage, tendon, ligament, skin, liver, muscles, nerve, vascular, neural and others form the class of numerous soft tissues. Since the dynamics of different tissues vary significantly, appropriate materials need to be carefully selected to satisfy the necessary psychochemical and biological properties. Over the last decade, much scientific understanding in these areas has led to the design and development of several bioresorbable biomaterials to fabricate scaffolds for engineering different tissue types (31). Table 1 summarizes the different classes of biomaterials used to fabricate scaffolds for a variety of indications for both structural and non-structural augmentation. A number of materials have been used for bone tissue engineering that include metallic, polymeric either synthetic or biologic (natural), apatite based bioceramics as well as bioglass and bioglass-ceramics. Also, there is considerable research interest in the bioinspired, biomimetic and bioactive biomaterials that may be used as desirable tissue engineered scaffolds due their ability to mimic natural environments of the extracellular matrix (2).

5.1. Extracelluar Matrix (ECM)

The nature of the development of an ECM during tissue generation is very complex. However, its presence is crucial for the success of any tissue engineering approach as it plays a critical role in controlling or mimicking specific cell function. The ECM glycoproteins develop an interwoven network of fibers and fibrils. This matrix is known to function as a structural as well as a signalling scaffold for cells. The degree to which the spatial arrangement, composition and immobilisation of ECM vary is dependent on each tissue type. Bone ECM, for example, consists mostly of collagen type I structural proteins, apatite mineral and non-collagenous proteins such as osteocalcin, fibronectin and vitronectin (6). When designing exogenous synthetic ECM one approach might be is to incorporate bioactivity in the scaffold by introducing soluble bioactive agents e.g. growth factors or plasmid DNA, which can trigger or modulate new tissue formation. The second strategy is to incorporate analogues of well known CAMs into the biomaterials. This is achieved via physicochemical, photochemical or ionic cross-linking methods. Integrins, a family of cell/surface transmembrane receptors, play an important role in mediating transmission of chemical and mechanical signals from the ECM. Biomimetic scaffolding using specific peptides or bioactive molecules is also attractive. In this case, the ECM recapitulation via incorporation of adhesive peptide sequences can be made bound to molecules that are correspondingly bound to the scaffold material and growth factors.

5.2. Natural and Synthetic Polymers

Many synthetic and naturally occurring (biologic) macromolecular polymers are ECMs generally used as scaffold biomaterials for tissue engineering applications. The natural polymers are proteins, polysaccharides collagens (gelatin), alginate, cellulose, starch, chitosan (chitin), fibrins, albumin, gluten, elastin, fibroin, hyarulonic acid, sclerolucan, elsinan, pectin (pectinic acid), galactan, curdlan, gellan, levan, emulsan, dextran, pullulan, heparin, silk, chondroitin 6-sulfate, small intestine submucosa (SIS), acellular dermis, polyhydroxyalkanoates and others (23-32). These relatively abundant natural polymers exhibit notable biocompatibility and minimal adverse immunogenic response. However, although commercially available mainly due to ease of processing and notwithstanding compositional variation that can occur from batch-to-batch processing there are significant issues that need to be addressed. Typically, these are expensive and there exist shortages of some of these natural polymers. There is also the likelihood of cross-contamination and transmission of virus or disease during extraction and enzymatic processing from plant, animal or human tissue (33-35). In contrast, for the synthetically derived polymers, various physicochemical properties, nature of the by-products during polymerization, purity, batch weight,

Table 1. Summary of biomaterial classes used for scaffold fabrication

Material Class	**Scaffold Composition**	**Fabrication Technique**	**Pore Size/ Porosity (μm/%)**	**Target Tissue**	***In vitro/In-vivo* Study**	**Ref**
Ceramic	HAP	Porous block sintering	90-600/	Bone	*In-vivo:* BMP-2 delivery, ectopic bone formation in rats	168-170
		SFF and image based design	366, 444/38, 44	Bone	*In-vivo:* Mandible defects in Yucatan minipigs	171
		Porous sintering	200-800/60-80	Bone	*In-vitro* and *in-vivo:* Goat bone marrow stromal cells, femoral defects in rabbits, ectopic bone formation in goats, dogs and mice	172
		Sintering (solid, porous particles and porous coral replication)	150, 230/66, 70	Bone	*In-vivo:* Ectopic bone formation in rats	173
	TCP cement	Salt-leaching	0.2, 8.7/31, 62	Bone	-	174
	Calcium metaphosphate	Porous sintering	200/	Bone	*In-vitro* and *in-vivo:* Rat bone marrow stromal cells *ex vivo* and ectopic bone formation in mice	175
	Natural coral	Porous sintering	150-220/36	Bone	*In-vitro* and *in-vivo:* Rabbit marrow mesenchymal cells *ex vivo* and ectopic bone formation in mice	176
	HAP-TCP	Porous cylinder sintering	100-150/36	Bone	*In-vivo:* Femoral defects in dogs	177
	Calcium phosphate	Compaction	-	Drug delivery	*In-vitro*	178
Glass and Glass Ceramic	Porous-glassy-carbon	Porous pellet sintering	100-200/40	Bone	*In-vivo*: Tibia defects in rabbits	179
	Na2O-CaO-SiO2-P2O5	Reaction sintering under isostatic pressure	100-200/	-	*In-vitro:* Simulated body fluid	180
	Bioglass 45S5	Melt derived	-	Bone	*In-vitro:* Human osteoblasts *in vitro*	181, 182
		Foaming and porous sintering	100-600/	Bone	*In-vivo*: Ectopic bone formation in dogs	193
	SiO2 based Sol-gel foams	Foaming	<10-500/	Bone	*In-vitro*	182
	Silica-calcium phosphate	Phase transformation	10-300/43-51	Bone	*In-vivo*: Femoral defects in rabbits	184
	Hyaluronic acid	Phase inversion, particulate leaching	100-600/80-90	Bone	*In vitro*: BMP-2 delivery and C3H10T1/2 cells	185
	Mesoporous bioactive glass	Polyurethane sponge technique, immersion in phosphate buffered saline solution	0.002-0.05/	Drug delivery	*In-vitro*	186
Natural Polymers	Collagen	Freeze drying	11-134/	Bone	*In-vivo*: Tibia defects in rats	187
	Collagen/hyaluronate	Cross linking	45.7, 35.4/	Bone	*In-vitro* and *in-vivo:* Cranial defects in rats	188
	Silk fibroin	Freeze drying, salt-leaching, gas foaming	15-100/84-99	-	-	189
		Salt leaching	202/84-98	Bone	*In-vitro*: Human bone marrow stromal cells in-vitro	189, 190
Chemically Synthesized Polymers	PLA	Phase separation, emulsion-solvent diffusion and porogen leaching	50-800/	Bone	-	191
	PLA/PGA	Melt processing	800	Bone	-	191
	PLDLA	Solution coating, porogen decomposition	/58-80	Bone	-	192
	PLGA	Sintered microsphere method	83-300/35	Bone	*In-vitro*	193
		Consolidation by pressure drop	100/60-66	Teeth implants	-	194
		Sintering	187/31	Bone	*In-vitro*	195
		Gas foaming	200/	Bone	*In-vitro*: Human mesenchymal cells in-vitro	195
		Electrospinning	2-465/92	-	-	197
Chemically Synthesized Polymers	PLGA/PEG	Porogen dissolving	300-500/85	Articular cartilage	*In-vitro*	198
	PLGA/PVA	Melt molding particulate leaching method	200-300/90	Bone	*In-vitro* and *in-vivo:* Cranial defects on rabbits	199
	Poly(multifunctional lactic acid based oligomer)	Salt leaching	45-150, 300-600/80	Bone	*In-vitro* and *in-vivo*	200, 201
	PPF	Gas foaming with	50-	Bone	*In-vivo*: Tibia defects in rats	202

		effervescent reaction	1000/51			
		Porogen leaching and photo-cross linking	300-800/57-80	Hard and soft tissue	*In-vivo*: Cranial defects in rabbits	203, 204
		Gas foaming with effervescent reaction	100-500/	Bone	*In-vivo*: Cortical defects in rats	205
	PET	Melt blowing	/93-97	Bone	*In vitro*: Rat mesenchymal stem cells *in vitro*	206
	Polymeric foams	High internal phase emulsion	40, 100/70-97	Bone	*In vitro*: Rat osteoblasts *in vitro*	207
	Poly(desaminotyrosl-tyrosine ethyl ester carbonate)	Solid freeform	486/80-87.5	Bone	*In-vivo*: Cranial defects in rabbits	208
Composites	PLLA/collagen/chitosan	Solvent castings Salt leaching	125-500/	Bone	*In-vivo*: Rat calvaria stromal cells	209
	PLGA/bioactive glass microsphere	Microsphere Heating mold	350-500/	Bone	*In-vitro*: Marrow stromal cells (MSC)	210
	PPF/PLGA-PEG microparticles	Salt leaching	-	Bone	*In-vitro*	211
	PLA/calcium metaphosphate	Porous sintering	100-400/	Bone	*In-vitro* and *in-vivo*: Mice osteoblast cells	212
	PLLA/nHAP/collagen/chitin fibres	Ultrasonication, Lyophilised	200/	Bone	*In-vitro*	213
	PLGA/HAP	Gas forming Particulate leaching	100-250/	Bone	*In-vitro*	214
	L-PLGA/TCP D, L PLGA/L-PLA	TheriForm 3D printing	40-150/55, 90	Articular cartilage	*In-vitro*	215
	PGA fiber/fibrin	Freeze drying	300/	Skin	*In-vitro* and *in-vivo*: Fibroblasts	216
	PLGA mesh/collagen gel, sponge	Knitted mesh	-	Urinary bladder	*In-vitro*: Urothelial and smooth muscle cells	217
	PLLA braid/collagen coating	Freeze drying	50-100/	Ligament	*In-vitro* and *in-vivo:* Fibroblasts	218
	PCL fiber/pHEMA hydrogel	Polymerisation, etched acetone	100-400/34-41	Neural TE	-	219
	PLGA/chitin	Electrospinning	-	Skin	*In-vitro*	220
	PGA mesh/Bioglass 45S5	Bioglass particle in distilled water Immerses PGA mesh	-	Soft tissue	*In-vitro* and *in-vivo*	221
Composites	PLGA/Bioglass® tubular foam	Dispersion Freeze drying	10-50, 100/	Intestine, trachea, blood vessel	*In-vitro*: Mouse fibroblasts	222
	PLA/nHAP/collagen	Phase separation	-	Periodontal tissues	*In-vitro*	223
	HAP/PCL	Polymeric reticulate method	150-200/87	Drug delivery	*In-vitro*	224
	HAP/chitosan-gelatin	Freeze drying	300-500/91	Bone	*In-vitro*: Rat calvarial osteoblasts	225
	HAP/β-TCP/chitosan	Porogen leaching Freeze drying	300-600/	Bone	*In-vitro*	226
	Collagen/HAP	Freeze drying	30-100/85	Bone	*In-vitro*: Rabbit periosteal cells	227
	CO3Ap/collagen	Freeze drying	50-300/49, 73, 79	Bone	*In-vitro*: MC3T3-E1 osteoblasts	228
	Titanium alloy (Ti-6Al-4V)/CaP sol-gel	Porous sintering	50-200/35	Bone	*In-vivo:* Femoral defects in rabbits	229
		Sintering (porous)	250/86	Bone	*In-vivo*: Ectopic bone formation in rats	230
	Titanium/nHAP	Soaking	-	Bone	*In vitro*: Human osteoblasts, canine bone-ingrowth chamber	231
	Ti-TiBx, NiTi	Self-propagating high	/15-55	Bone	-	232

		temperature synthesis (porous)				
	PPF/β-TCP	Salt leaching	150-300/69, 74	Bone	*In-vitro*	233
	PLLA/Bioglass	Phase separation	<10, >100/	Bone	*In-vitro*	234
	Silica-calium phosphate	Sintering	10-300/43-51	Bone	*In-vitro* and *in-vivo:* Femoral defects in rabbits	184
	PLGA/collagen/apatite	Salt leaching	355-425/87	Bone	-	235
	β-TCP/calcium phosphate invert glass/chitosan	Thermally induced phase separation, immersion in phosphate buffed-saline solution	-	Drug delivery	*In-vitro*	236
	Bioactive glass/β-cyclodextrin	Sol-gel	-	Drug delivery	*In-vivo*	237
	Starch/PLA	Supercritical phase inversion	-	Drug delivery	*In vitro*	238
Metals	Nickel/Titanium	Self-propagating high temperature synthesis	229-641/47-66	Bone	*In-vivo*: Femoral defects in rats	239
	Titanium fiber meshes	Sintering	250/86	Bone	*In-vivo*: TGF-β1 delivery in cranial defects in rabbits	240
	Titanium	Plasma-spraying	200-400/50-60	Bone	*In-vivo*: Femoral defects in dogs	241
		Porous sintering	50-200/35	Dental	-	242
		Diffusion	350/45	Bone	*In-vivo*: Hip arthroplasty in dogs	243
		Laser-texture (Ti-6Al-4V)	100, 200, 300/	Bone	*In-vivo*: Femoral defects in rabbits	244
		Electrochemical oxidation	1-8/13-24	Bone	*In-vivo*: Tibia defects in rabbits	245
		Shot-blasting	<10/44, 48	Bone	*In-vivo*: Mandible and femoral defects in dogs	242, 246
		Acid-etching	400/	Bone	*In-vitro*: Femoral defects in rabbits	247
		Deposition through polystyrene latex beads	0.5, 16, 50/	Bone	*In-vivo*: Human bone derived cells *in vitro*	248

compositional consistency and absence of immunogenicity of synthetically derived polymers can be easily controlled. Also, various processing methods are readily available that are able to optimize the physical and mechanical properties, molecular weight and molecular structure, for example, of tissue engineered scaffolds designed for specific *in-vivo* locations and clinical indications.

The materials in the category of synthetic polymers as porous scaffold are numerous (36, 37). The most commonly used formulations belong to a family of linear aliphatic polyesters such as polyglycolic acid (PGA), polylactic acid (PLA) and their copolymers (PLGA) (38-40). The choice of these polymeric scaffolds is dictated by the fact that degradation rate can be tuned or controlled to the rate of new tissue in-growth, with times ranging from two to twelve weeks for PGA and for up to a year for PLA. This is due to PGA being more hydrophilic than PLA resulting in its relatively rapid rate of hydrolytic dissolution (41) in-vivo. These polymers are also a family of few synthetic polymers approved by the US Food and Drug Administration (FDA) (42). The other examples include polycaprolactone (PCL) used as long term implants and drug release microcapsules (43), polyhydroxybtyrate (PHB) for slow degradation rate (44), polypropylene fumerate (PPF), α-hydroxyesters, polyanhydrides (45, 46), polyphosphazenes and polyphosphoesters (47-50). The hydrogel character of the natural ECM has inspired the development of several synthetic hydrogels for tissue engineering applications such as artificial soft tissues, ophthalmological and drug delivery devices (51-56).

5.3. Bioceramics and Bioglasses

Of many hundreds of thousands of inorganic compounds, bioceramics and bioglass derived ceramics are the most widely used for hard tissue substitution due to their chemical and crystallographic similarity to natural mineral, bioactivity and good biocompatibility and ability to form stable interface with the host tissue (57). This class constitutes primary candidate biomaterial scaffolds for their use in tissue engineering (58). The third generation bioceramics is now focussed to provide suitable scaffolding system in order for the bone cells to mimic biomineralisation functionalities (59). Porous bioceramics are a family of calcium phosphates and hydroxy or fluroapatites (60-52) where Ca/P ratio can vary between 1.5 and 1.67 in HAP, α and β tricalcium phosphates (TCP) thus offering the ability to control the rate of in-vivo degradation. These are widely used in the fabrication for the tissue engineering applications as porous scaffolds and coatings. Synthetic HAP has been shown to stimulate osteoconduction and can be integrated into bone without triggering an immune reaction (63, 64). Bioglasses also fall into this class (60) and are highly versatile when fabricating 3D scaffold constructs. They can be used as nonresorbable (relatively inert), surface active (semi-inert) where composition can be varied to suit the bioactivity and biodegradability or resorbability (65). The advantages are their well known properties of osteoconduction, and osteoinduction with the ability to incorporate pharmaceutical agents such as bone morphogenetic protein (BMP) and growth factors such as TGFs to promote cell growth. Bioactive glasses and glass ceramics interact well with osteoblasts and chondrocytes; cells attach, spread, proliferate, and synthesize extracellular matrix on the

bioactive glass and glass-ceramic surface (66, 67). Bioglass®, a bioactive glass, the first of its kind belonged to the SiO_2-Na_2O-CaO-P_2O_5 system and now there are many other derivative compositions in the silicate, borate, and phosphate glass systems as well as compositions that can be coverted to glass-ceramics. Many have been used to produce porous 3D bone tissue engineered scaffolds. The bioactivity mechanism in bioglass and glass-ceramics is typified by the dissolution of surface moeties and formation of hydroxylated calcium apatite (HCA) (68) layer similar to chemical and crystallographic properties of bone (69). Recent research has demonstrated that doping of certain ions such as Zn, Mg and Sr can stimulate osteogenesis, osteoblast proliferation, differentiation, and bone mineralization (70-76).

The extensive use of Ca-apatites, bioglass and bioglass-ceramics for load bearing applications are limited by their inherent brittleness, poor elastic stiffness and compressive strength compared to human bone. These properties vary greatly from scaffold to scaffold but are more evident for highly porous scaffolds. As new bone grows into the scaffold the mechanical properties are likely to continue to improve until tissue regenerates fully over a period of time. Consequently, the apatite based bioceramics are also used as non-structural biomedical applications such as grafts, fillers and coatings (77). However, the composite scaffolds using variations of bioceramic, bioglass and polymers have successfully demonstrated to exhibit excellent mechanical properties and support new tissue growth (78-83).

5.4. Biocomposites

Much of the current research is devoted to the development of composite scaffolds using natural or synthetic polymers, bioactive ceramics, glasses and glass ceramics. Increasing research efforts in this area (84, 85-93, 94-99) have demonstrated that biocomposite systems have great advantages in combining various biomaterial properties such as biodegradation, biocompatibility and mechanical strength into one ideal structure for applications in both hard and soft tissue engineering. The aims are twofold: (1) to increase the mechanical stability and formability of the scaffold and enhance tissue interaction (85-90) (2) to incorporate biomolecules, and activate surface functionalization and (3) design, fabricate and characterize 3D scaffolds to find strategies for clinical solutions. Stem cell impregnated scaffolds also promise immense potential for next generation synthetic and biomimetic composite biomaterials that can be tuned to the biological environment.

Some of the most extensively studied polymers, both synthetic and natural, in the fabrication of interconnected 3D polymer-ceramic composites include PLA, PGA and their co-polymers, polymethyl methacrylate (PMMA), chitin, chitosan, collagen, gelatin, starch and their derivatives (88, 100-104). The ceramics are normally micro or nanocrystalline HAP and TCP and bioactive glass and glass-ceramics. The filler ceramic constituents of inorganic bioactive phases in the biopolymer matrix have the dual purpose of mimicking the nanostructure of natural bone and enhancing the mechanical properties (105). Chitosan and its derivatives and collagen are particularly attractive candidates in the scaffold composites due to their minimal inflammatory reactions or toxic degradation and good biodegradability coupled to excellent osteoconductivity in the case of bone graft (106-109). There are conflicting literature reports as to whether or not the inclusion of the ceramic phase improves strength. Whilst some studies suggest an increase (110) others report either no change or decrease in strength (111-114). Recently compiled data however concluded that apatite/polymer composites could potentially improve both biocompatibility and mechanical properties of load bearing bone grafts particularly since HAP and TCP scaffolds overlap with cortical and most are at the upper limit of the cancellous bone in either strength, porosity or both (115). In this case, the challenging task would be to control the kinetics of the simultaneous resorption of both constituent phases so tuned to the rate of new tissue growth in order to achieve the strength and porosity of native bone.

Since bioactive glasses exhibit good bonding ability with both hard and soft tissues (116-119) and ability to form apatite in the presence of bioactive glass particles it is possible to control mineralization in the composite by changing the glass content and also strengthen the porous polymer matrix. There are only a few studies on polymer-bioglass composites. Porous bioresorbable composite materials using PLGA containing bioactive glass particle additions (up to 50 wt %) have been fabricated with good interconnected porosity, *in vitro* bioactivity and the degradability of the composite foams (120-122). Asymmetric structures of porous composites comprising a non-biodegradable polysulfone (or cellulose acetate) polymer matrix and bioactive glass particulates and successful in-vitro growth of HAP crystals have demonstrated their potential bone graft integration with microstructural features (20-150μm pores) being strongly dependent on the polymer type, the interaction between the polymer and bioactive glass. Composite modulus and failure strength increased with increasing glass content due to the change in composition and pore content (123).

6. SCAFFOLD FABRICATION METHODS

Over the last decade the development of bioengineered porous scaffold structures for hard and soft tissue augmentation is motivated to replicate the inherently 3D nature of functional tissues especially natural bone (124, 125). Since there is an inherent lack of strength associated with porosity much attention has been devoted to fabrication of porous scaffolds that can meet the engineering challenges in order to overcome the problem of optimising strength and porosity. Hence, the development of 3D porous scaffolds has been hindered to non-load bearing applications. To design and engineer functional tissues and organs successfully, interconnected porous structures using bioresorbable materials, fabrication processes and technologies for tissue engineering must meet certain critical criteria, as elucidated earlier, i.e. to promote and guide tissue growth in three dimension by cell attachment, proliferation and differentiation (126). There

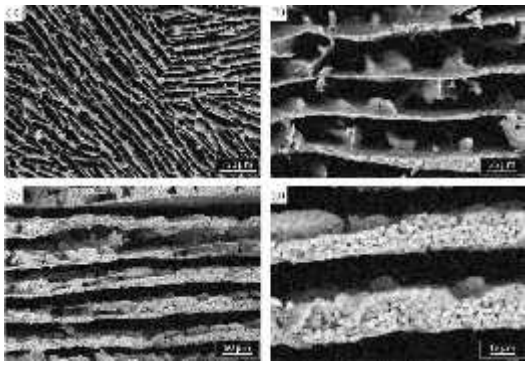

Figure 3. Microstructure of porous HAP samples parallel to the ice front (a) and detail (b), and perpendicular to the ice front (c) and detail (d). (Reproduced with permission from ref 128)

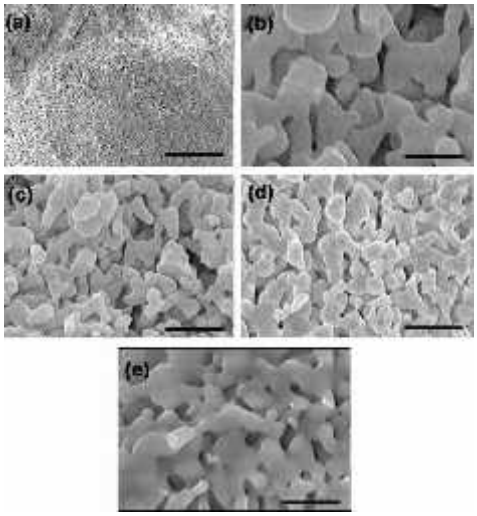

Figure 4. SEM micrographs of freeze cast Bioglass® scaffolds sintered at 730ºC at -10ºC using solid loading of (a) General microstructure, (b) 10 wt%, (c) 20 wt%, (d) 40 wt%, (d) 60 wt% and (e) 70 wt% [(a) bar = 1mm ; (b), (c), (d), (e) bar = 200µm]. (Reproduced with permission from ref 121).

are mainly two unique fabrication approaches to produce 3D porous scaffolds, namely conventional and rapid prototyping. These are discussed in this section.

6.1. Conventional Methods

The many conventional methods available for scaffold fabrication can be listed as freeze casting, solvent casting and particulate leaching, phase separation, foam reticulate method, gel casting, conventional sintering with fugitive phases, gas foaming, melt moulding, emulsion freeze drying and electrophoretic deposition. Some important methods are discussed here.

6.1.1. Freeze Casting

Freeze casting of biomaterial scaffolds is a simple method originally used to produce complex porous structures of ceramic or polymeric components (127). The technique was subsequently adopted to produce a variety of porous scaffolds for tissue engineering using HAP, TCP, bioglasses and their composites using water, camphene and glycerol as solvents. The solvent vehicle acting as a binder holding the slurry intact is then removed from the slurry by sublimation by freeze drying leaving an interconnected porous network. The green body is further sintered at appropriate temperatures to provide improved mechanical strength (121, 122, 128, 129). A wide range of compressive strength (65-145MPa) and porosity (56-85%) for HAP, bioglasses, composites of HAP-TCP and Bioglass-HAP have been reported. These may point the ways to design scaffolds for structural grafts. It is also possible to produce anisotropic microstructures with control of the pore alignment, orientation and morphology of the scaffolds by controlling the growth direction of the solvents. Although not having stiffness and strength the polymeric scaffolds produced by this method for non-load bearing applications include chitin (130-133), collagen (134), PLA, PDLA and PLGA (135, 136) and poly(HEMA) (137) agarose (138) sericin (139) and alginate (140-142). Figure 3 shows an example of the formation of lamellar microstructure of HAP with flat interconnecting macropores. Figure 4 shows the coralline/dendritic nature of highly interconnected porous Bioglass 45S5 scaffold structures.

(d), (e) bar = 200µm] (from Ref 121)

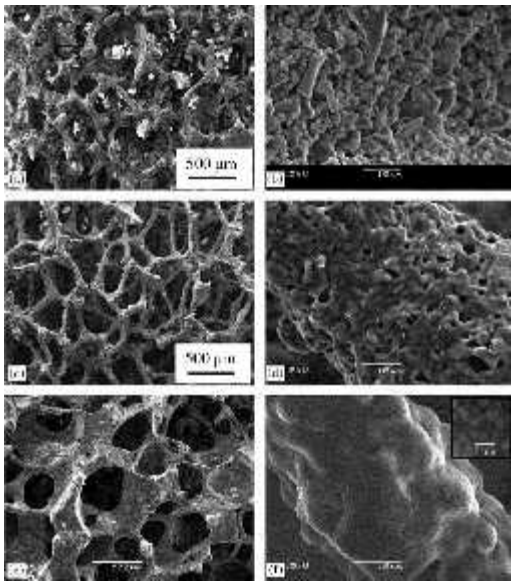

Figure 5. Pore structure and strut mmicrostructure of 45S5 Bioglass derived foams (Reproduced with permission from ref 165).

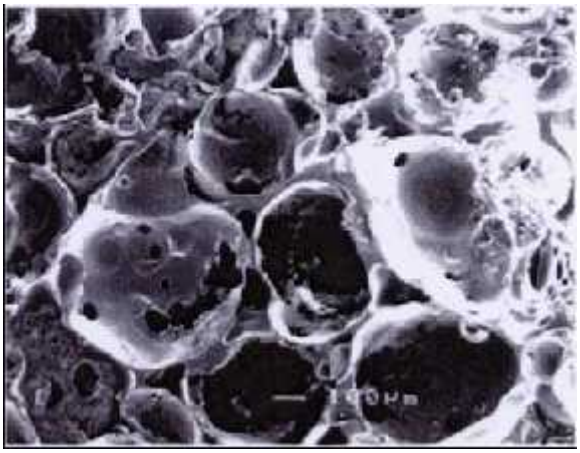

Figure 6. Scanning electron micrograph of a poly (L-lactic acid) (PLLA) foam fabricated using the salt-leaching technique. (Reproduced with permission from ref 144)**.**

6.1.2. Ceramic Powders with Fugitive Phases

Synthetic porous structures are produced by this popular method where a mixture of bioceramic and beads of polymer. Subsequent melting and vaporization of the combustible pore forming fugitive phase results in the creation of porous construct (114). Considerable difficulty is encountered in the optimization of the size of the pore forming feed that results in poor interpore connectivity and combustion kinetics due to variable morphology of the feed.

6.1.3. Foam reticulation and gel casting

Similar to slip casting, this method is extremely versatile and is able to generate net shape and complex scaffold profiles. Commercially available polymeric foams (usually polystyrene) of desired pore sizes are infiltrated with organic or aqueous slurry of ceramic, glass or composite powders. The solvent is then removed using drying as it sublimes. The process can be cost effective and the negative issues are additive toxicity, control of the drying process and the difficult polymerization reactions where organic gel is used. Figure 5 shows an example of Bioglass derived porous structure using this method. Using this method, a variety of other variant composites such as PLLA/Bioglass has been fabricated. The advantage of such these scaffolds is in their ability to make the surface more bioactive and controlling the resorption rate for accelerated tissue growth.

6.1.4. Solvent Casting and Solute Leaching

These methods have been used with limited success to fabricate scaffolds for tissue engineering applications (144-147) and can be used separately or can be combined to circumvent processing problems. The method involves intimate mixing of salt or sugar in a polymeric solution PLA, PGA, PCL, PMAA and others (Figure 6 shows an example). The mixture is then cast in a mould and following evaporation of the solvent the water soluble particulates are leached out to produce a porous structure. Polymer-bioceramic constructs can also be fabricated but the main disadvantages of this method are the likelihood of retaining toxic solvent, trapped solute and denaturation of the proteins and other molecules in the polymer by the presence of solvents. The porosity can be controlled by solute/polymer ratio and solute concentration. The porosity up to 90% and pore sizes can exceed 100 micron with poor mechanical properties.

6.1.5. Phase separation

Phase separation techniques rely on inducing thermodynamic instability during solvent removal from a multicomponent polymeric solution that results in separation of two phases and the solidification of the polymer rich phase. There are many variants of this technique but mainly two types are used, namely solid-liquid phase separation (solid phase formation in a liquid phase) and liquid-liquid phase separation. Solid-liquid phase separation has been used to fabricate various types of polymers and their composites (148-150). Although the pore size ranges can be in the order of 15-120 micron with porosity up to 95% the methods suffer from non-uniform pore size distribution. Research has shown that this method is capable of forming oriented tubular or fibrous architecture with anisotropic mechanical properties and may be suitable for soft tissue structures such as nerve, muscle, tendon, ligament, or dentin (151). Controlled thermally induced phase separation has also been developed for scaffold fabrication (152, 153). During liquid-liquid phase separation, temperature is lowered to induce the liquid-liquid phase separation of a polymeric solution with an upper critical solution temperature leading to the formation of a bicontinuous open pore scaffold structure. A mixture of dioxane and water has been used for liquid-liquid phase separation to fabricate PLA and PLGA scaffolds (154-156). Single phase or multicomponent composite scaffolds with high porosity (90%) and well-

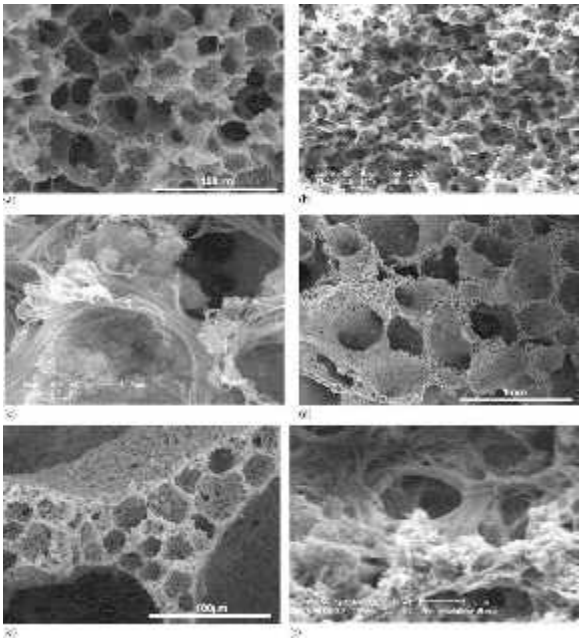

Figure 7 SEM micrographs of NHAP/PLLA (30:70) scaffolds fabricated using dioxane/water mixture solvents. (a) Dioxane:water=95:5, ×500. (b,c) Dioxane:water=90:10, ×500, ×8000. (d,e,f) Dioxane:water=87:13, ×45, ×500, ×10000. (Reproduced with permission from ref 144).

controlled pore architectures have been fabricated using thermally induced phase separation (TIPS) techniques. The morphologies, mechanical properties and protein adsorption capacities of the composite scaffolds can be suitable for hard and soft tissue replacements. Figure 7 shows examples of different morphologies of 3D porous PLLA and nanoHAP/PLLA composites.

6.2. Rapid Prototyping (RP)

In the past two decades over 20 RP systems have been presented with respect to hard tissue engineering. The notable methods are stereolithography, selective laser sintering (SLS), 3-D printing, fused deposition modeling (FDM), wax printing and bioplotter. A summary and the literature exploring the advantages and disadvantages of the most important systems have been extensively reviewed (157). RP allows precise control of otherwise complex scaffold architecture, size, shape, interconnectivity, branching, geometry and orientation. Any design of 3D pore architecture generated using hierarchical image based or computer-assisted design and manufacture (CAD/CAM) techniques cannot readily be built using conventional techniques (158, 159). Scaffold architectures must therefore be built using layer by layer manufacturing processes (160-163). The processes are computer controlled and can be integrated with medical imaging systems thus allowing accurate patient specific geometries to be produced. These methods can produce scaffolds with anisotropic properties allowing different areas of the scaffold to be optimised for certain cells. Most RP processes do not use toxic organic solvents and some allow cells and growth hormone to be incorporated into the scaffold during manufacture. However, RP can be capital intensive, low image resolution, time consuming and very much dependant on the availability of biomaterials with exact morphology and curability in case of bioresorbable feedstock.

7. CONCLUSIONS AND FUTURE DIRECTIONS

Biological systems are hierarchical and spanning about eight orders of magnitude in length scales from cells to the biomaterial scaffolds. Synthesis of well optimised properties of scaffolds that can successfully interact with the physiological environment of the human body is critical for tissue regeneration. The review presented here clearly demonstrated the need for this dynamic process and a plethora of synthesis routes and suitable biomaterial properties available to the scientists, practitioners and clinicians alike. Although not covered in this review gene mediated tissue engineering is necessary to have a more unifying tissue engineering strategy and some researchers are already engaged toward this effort.

Nanotechnological aspects of tissue engineering have focussed on both the design and fabrication of structures with control and manipulation of individual constituent molecules. The importance of nanostructured scaffolds or constructs that make use of nanobiomaterials cannot be more emphasized. The developed ECM structures would act as ideal scaffolds and be able to interact with cells and tissues at subcellular level with a high degree of functional specificity. Recent efforts in tissue engineered biomaterial development have focused on ECM derived peptides or proteins into biomaterials in order to mimic natural ECM (164). The application of nanotechnology to tissue engineering has been focusing on non-biochemical and nanotopographical aspects of ECM (165-167). Hence design of synthetic scaffold at a nanoscale is one of the exciting emerging areas in tissue engineering particularly if biomolecules can be incorporated in the scaffolds.

8. REFERENCES

1. R Langer, JP Vacanti: Tissue Engineering. *Science* 260, 920-926 (1993)

2. KG Sreejalekshmi, PD Nair: Biomimeticity in tissue engineering scaffolds through synthetic peptide modifications-altering chemistry for enhanced biological response. *J Biomed Mater Res A* 96, 477-491 (2011)

3. LA Cyster, DM Grant, DM Grant, SM Howdle, FR Rose, DJ Irvine, D Freeman, CA Scotchford, KM Shakesheff: The influence of dispersant concentration on the pore morphology of hydroxyapatite ceramics for bone tissue engineering. *Biomaterials* 26, 697-702 (2005)

4. SJ Hollister: Porous scaffold design for tissue engineering. *Nature Materials* 4, 518-524 (2005)

5. A Shekaran, AJ Garcia: Nanoscale engineering of extracellular matrix-mimetic bioadhesive surfaces and implants for tissue engineering. *Biochim Biophys Acta* 1810, 350-360 (2011)

6. G Khang, SJ Lee, MS Kim, and HB Lee: Scaffolds; tissue engineering. In: Webster's Biomedical Engineering

Handbook. Ed: S Webster, John & Wiley Press, NY, USA 366–383 (2006)

7. HB Lee, G Khang, J H Lee: Biomaterials principles and applications, Chapter 3, CRC Press, p55 (2003)

8. RO Hynes, Q Zhao: The evolution of cell adhesion. *J Cell Bio* 150, F89-96 (2000)

9. JC Adams, FM Watt: Regulation of development and differentiation by the extracellular matrix. *Development* 117, 1183-1198 (1993)

10. MM Stevens, JH George: Exploring and engineering the cell surface interface. *Science* 310, 1135-1138 (2005)

11. D Logeart-Avramoglou, F Anagnostou, R Bizios, H Petite: Engineering bone: challenges and obstacles. *J Cellular and Molecular Med* 9, 72-84 (2005)

12. BD Boyan, TW Hummert, TW Hummert, DD Dean, Z Schwartz: Role of material surfaces in regulating bone and cartilage cell response. *Biomaterials* 17, 137-146 (1996)

13. A G Mikos, G Sarakinos, MD Lyman, DE Ingber, JP Vacanti, R Langer: Prevascularization of porous biodegradable polymer. *Biotech and Bioeng* 42, 716-723 (1993)

14. O Gauthier, JM Bouler, E Aguado, P Pilet, G Daculsi: Macroporous biphasic calcium phosphate ceramics: influence of macropore diameter and macroporosity percentage on bone ingrowth. *Biomaterials* 19, 133-139 (1998)

15. KA Hing, SM Best, W Bonfield: Characterization of porous hydroxyapatite. J Mater Sci: Mater in Med 10, 135-145 (1999)

16. CM Agrawal, RB Ray: Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. *J Biomed Mater Res* 55, 141-150 (2001)

17. M Fabbri, GC Celotti, A Ravaglioli: Hydroxyapatite-based porous aggregates - physicochemical nature, structure, texture and architecture. *Biomaterials* 16, 225-228 (1995)

18. RW Goulet, SA Goldstein, MJ Ciarelli, JL Kuhn, MB Brown, LA Feldkamp: The Relationship between the Structural and Orthogonal Compressive Properties of Trabecular Bone. *J Biomech* 27, 375-389 (1994)

19. MC Siebers, PJ ter Brugge, XF Walboomersa, JA Jansen: Integrins as linker proteins between osteoblasts and bone replacing materials. A critical review. *Biomaterials* 26, 137-146 (2005)

20. P Kasten, I Beyen, P Niemeyer, R Luginbuhl, M Bohner, W Richter: Porosity and pore size of beta-tricalcium phosphate scaffold can influence protein production and osteogenic differentiation of human mesenchymal stem cells: An in vitro and in vivo study. *Acta Biomater* 4, 1904-1915 (2008)

21. H Guo, JC Su, J Wei, H Kong, Changsheng Liu: Biocompatibility and osteogenicity of degradable Ca-deficient hydroxyapatite scaffolds from calcium phosphate cement for bone tissue engineering. *Acta Biomater* 5, 268-278 (2009)

22. R Langer, DA Tirrell: Designing materials for biology and medicine. *Nature* 428, 487-492 (2004)

23. E. Bell, M. Rosenberg, P Kemp, R. Gay, GD Green, N. Muthukumaran, C Nolte: Recipes for Reconstituting Skin *J Biomech Eng* 113, 113-119 (1991)

24. AK Burkoth, JA Burdick, KS Anseth: Surface and bulk modifications to photocrosslinked polyanhydrides to control degradation behavior. *J Biomed Mater Res* 51, 352-359 (2000)

25. IV Yannas: Applications of ECM analogs in surgery. *J Cell Biochem* 56, 188-191 (1994)

26. SM Mueller, S Shortkroff, TO Schneider, HA Breinan, IV Yannas, M Spector: Meniscus cells seeded in type I and type II collagen–GAG matrices in vitro. *Biomaterials* 20, 701-709 (1999)

27. Y.S. Choi, S.R. Hong, YM Lee, KW Song, MH Park, YS Nam: Studies on gelatin-containing artificial skin: II. Preparation and characterization of cross-linked gelatin-hyaluronate sponge. *J Biomed Mater Res* 48, 631-639 (1999)

28. L Shapiro, S Cohen: Novel alginate sponges for cell culture and transplantation. *Biomaterials* 18, 583-590 (1997)

29. SV Madihally, HW Matthew: Novel alginate sponges for cell culture and transplantation. *Biomaterials* 20, 1133 (1999)

30. LA Solchaga, J Gao, JE Dennis, A Awadallah, M Lundberg, AI Caplan, VM Goldberg: Treatment of osteochondral defects with autologous bone marrow in a hyaluronan-based delivery vehicle. *Tissue Eng* 8, 333-347 (2002)

31. SF Badylak: The extracellular matrix as a scaffold for tissue reconstruction. *Seminars in Cell and Developmental Biology* 13, 377-383 (2002)

32. MK Chen, SF Badylak: Small bowel tissue engineering using small intestinal submucosa as a scaffold. *J Surg Res* 99, 352-358 (2001)

33. M Probst, HJ Piechota, R Dahiya, EA Tanagho: Homologous bladder augmentation in dog with the bladder acellular matrix graft. *BJU Int* 85, 362-371 (2000)

34. PB van Wachem, LA Brouwer, F Moatamed, A Schindler, CG Pitt: Tissue reactions to epoxy-crosslinked porcine heart valves post-treated with detergents or a dicarboxylic acid. *J Biomed Mater Res* 55, 415-423 (2001)

35. K Izumi, H Terashi, CL Marcelo, SE Feinberg: Development and characterization of a tissue-engineered human oral mucosa equivalent produced in a serum-free culture system. *J Dent Res* 79, 798-805 (2000)

36, AK Burkoth, J Burdick, KS Anseth: Surface and bulk modifications to photocrosslinked polyanhydrides to control degradation behaviour. *J Biomed Mater Res* 51, 352-359 (2000)

37. KP Andriano, Y Tabata, Y Ikada, J Heller: In vitro and In vivo comparison of bulk and surface hydrolysis in absorbable polymer scaffolds for tissue engineering. *J Biomed Mater Res* 48, 602-612 (1999)

38. PX Ma: Tissue Engineering. In: Encyclopedia of Polymer Science and Technology (3rd Edition), Eds: JI Kroschwitz, John Wiley & Sons, New Jersey, USA (2004)

39. R Zhang, PX Ma, Processing of polymer scaffolds: Phase separation. In: Methods of Tissue Engineering, Eds: A Atala, R Lanza, Academic Press, San Diego, USA (2001)

40. JM Pachence, J Kohn: Biodegradable Polymers. In: Principles of Tissue Engineering (2nd Edition), Eds: RP Lanza, J Vacanti, Academic Press, San Diego, USA (2000)

41. SM Li: Hydrolytic degradation characteristics of aliphatic polyesters derived from lactic and glycolic acids. *Biomed Mater Res* 48, 342-353 (1999)

42. RC Thomson, MC Wake, MJ Yaszemski, AG Mikos: Biodegrdable polymer scaffolds to regenerate organs. *Adv Polym Sci* 122, 245-274 (2001)

43. GG Pitt, MM Gratzl, GL Kimmel, J Surles, A Sohindler: Aliphatic polyesters II. The degradation of poly (DL-lactide), poly (ε-caprolactone), and their copolymers in vivo. *Biomaterials* 2, 215 (1981)

44. SC Woodward, PS. Brewer, F Moatamed, A Schindler, CG Pitt: The intracellular degradation of poly(ε-caprolactone). *J Biomed Mater Res* 19, 437 (1985)

45. KW Leong, J Kost, E Mathiowitz, R Langer: Polyanhydrides for controlled release of bioactive agents. *Biomaterials* 7, 364(1986)

46. NS Choi, J Heller: Drug delivery devices manufactured from poly(orthoesters) and poly(orthocarbonates). *US Patent* 4,093,709 (1978)

47. J Wang, HQ Mao, KW Leong: A novel biodegradable gene carrier based on polyphosphoester. *J Am Chem Soc* 123, 9480-9481 (2001)

48. M Spector, MJ Michno, WH Smarook, GT Kwiatkowski: High-modulus polymer for porous orthopedic implants – Biomechanical compatibility of porous implants. *J Biomed Mater Res* 12, 665-677 (1978)

49. RJ Zdrahala, IJ Zdrahala: Biomedical applications of polyurethanes: A review of past promises, present realities, and a vibrant future. *Biomater Appl* 14, 67–90 (1999)

50. K Messner: Durability of artificial implants for repair of osteochondral defects of the medial femoral condyle in rabbits. *Biomaterials* 15, 657–664 (1994)

51. CK Kuo, PX Ma: Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: Part 1. Structure, gelation rate and mechanical properties. *Biomaterials* 22, 511-521 (2001)

52. JP Fisher, TA Holland, D Dean, PS Engel, AG Mikos: Synthesis and properties of photocross-linked poly(propylene fumarate) scaffolds. *J Biomater Sci Polym Ed* 12, 673-687 (2001)

53. JA Burdick, AJ Peterson, KS Anseth: Conversion and temperature profiles during the photoinitiated polymerization of thick orthopaedic biomaterials. *Biomaterials* 22, 1779 (2001)

54. BK Mann, AS Gobin, AT Tsai, RH Schmedlen, JL West: Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: Synthetic ECM analogs for tissue engineering. *Biomaterials* 22, 3045-3051 (2001)

55. DL Elbert, JA Hubbell: Conjugate addition reactions combined with free-radical cross-linking for the design of materials for tissue engineering. *Biomacromolecules* 2, 430-441 (2001)

56. J Elisseeff, K Anseth, D Sims, W McIntosh, M Randolph, M Yaremchuk, R Langer: Transdermal photopolymerization of poly(ethylene oxide)-based injectable hydrogels for tissue-engineered cartilage. *Plast Reconstr Surg* 104, 1014-1022 (1999)

57. W Suchanek, M Yoshimura: Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants. *J Mater Res* 13, 94-117 (1998)

58. L.L Hench, JM Polak: Third-generation biomedical materials. *Science* 295, 1014 (2002)

59. J Livage, M Henry, C Sanchez: Sol-gel chemistry of transition metal oxides. *Progress Solid State Chemistry* 18 (4), 259-342 (1988)

60. RZ LeGeros: Properties of osteoconductive biomaterials: Calcium phosphates. *Clin Orthop Rel Res* 395, 81-98 (2002)

61. H Oonishi: Orthopedic applications of hydroxyapatite. *Biomaterials* 12, 171-178 (1991)

62. KJL Burg, S. Porter, JF Kellam: Biomaterial developments for bone tissue engineering. *Biomaterials* 21, 2347-2359 (2000)

63. CP Klein, AA Driessen, K de Groot, A van den Hooff: Biodegradation behavior of various calcium-phosphate materials in bone tissue. *J Biomed Mater Res* 17, 769-784 (1983)

64. P Ducheyne, Q Qiu: Bioactive ceramics: The effect of surface reactivity on bone formation and bone cell function. *Biomaterials* 20, 2287-2303 (1999)

65. LL Hench: Biomaterials: A forecast for the future. *Biomaterials* 19, 1419–1423 (1998)

66 C Loty, N Forest, H Boulekbache, T Kokubo, JM Sautier: Behavior of fetal rat chondrocytes cultured on a bioactive glass-ceramic. *J Biomed Mater Res* 37, 137–149 (1997)

67. JM Sautier, T Kokubo, T Ohtsuki, JR Nefussi, H Boulekbache, M Oboeuf, S Loty, C Loty, N Forest: Bioactive glass-ceramic containing crystalline apatite and wollastonite initiates biomineralization in bone cell-cultures. *Calcif Tissue Int* 55, 458–466 (1994)

68. LL Hench: Bioceramics. *J Am Ceram Soc* 81, 1705–1728 (1998)

69. LL Hench, J Wilson: Introduction to bioceramics. Singapore, World Scientific (1993)

70. A Oki, B. Parveen, S Hossain, S Adeniji, H Donahue: Preparation and in vitro bioactivity of zinc containing sol-gel-derived bioglass materials. *J Biomed Mater Res A* 69, 216–221 (2004)

71. A Balamurugan, G. Balossier, S Kannan, J Michel, AH Rebelo, JM Ferreira: Development and in vitro characterization of sol-gel derived CaO-P2O5-SiO2-ZnO bioglass. *Acta Biomater* 3, 255–262 (2007)

72. A Saboori, M Sheikhi, M Sheikhi, F Moztarzadeh, M Rabiee, S Hesaraki, M Tahriri, N Nezafati: Sol-gel preparation, characterisation and in vitro bioactivity of Mg containing bioactive glass. *Adv Appl Ceram* 108, 155–161 (2009)

73. E Canalis, M. Hott, P Deloffre, Y Tsouderos, PJ Marie: The divalent strontium salt S12911 enhances bone cell replication and bone formation in vitro. *Bone* 18, 517–523 (1996)

74. D Boyd, MR Towler, A Wren, O M Clarkin: Comparison of an experimental bone cement with surgical Simplex (R) P, Spineplex (R) and Cortoss (R). *J Mater Sci Mater Med* 19, 953–957 (2008)

75. S Hesaraki, M Alizadeh, H Nazarian, D Sharifi: Physico-chemical and in vitro biological evaluation of strontium/calcium silicophosphate glass. *J Mater Sci Mater Med* 21, 695–705 (2010)

76. E Gentleman, YC Fredholm, G Jell, N Lotfibakhshaiesh, MD O'Donnell, RG Hill, MM Stevens: The effects of strontium-substituted bioactive glasses on osteoblasts and osteoclasts in vitro. *Biomaterials* 31, 3949–3956 (2010)

77. SV Dorozhkin: Bioceramics of calcium orthophosphates. *Biomaterials* 31, 1465-1485 (2010)

78. R Zhang, PX Ma: Poly(α-hydroxyl acids)/hydroxyapatite porous composites for bone- tissue engineering. I. Preparation and morphology. *J Biomed Mater Res* 44, 446-455 (1999)

79. PX Ma, R Zhang, G Xiao, R Franceschi: Engineering new bone tissue in vitro on highly porous poly(α-hydroxyl acids)/hydroxyapatite composite scaffolds. *J Biomed Mater Res* 54, 284-293 (2001)

80. X. Liu, PX Ma: Polymeric scaffolds for bone tissue engineering. *Ann Biomed Eng* 32, 477-486 (2004)

81. F Zhao, Y Yin, WW Lu, JC Leong, W Zhang, J Zhang, M Zhang, K Yao: Preparation and histological evaluation of biomimetic three-dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds. *Biomaterials* 23, 3227-3234 (2002)

82. KG Marra, JW Szem, PN Kumta, PA DiMilla, L Weiss: In vitro analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering. *J Biomed Mater R*es 47, 324-335 (1999)

83. CT Laurencin, MA Attawia, LQ Lu, MD Borden, HH Lu, WJ Gorum, JR Lieberman: Poly(lactide-co-glycolide)/hydroxyapatite delivery of BMP-2-producing cells: A regional gene therapy approach to bone regeneration. *Biomaterials* 22, 1271-1277 (2001)

84. JF Mano, RA Sousa, LF Boesel, NM Neves, RL Reis: Bioinert, biodegradable and injectable polymeric matrix composites for hard tissue replacement: state of the art and recent developments. *Compos Sci Technol* 64, 789–817 (2004)

85. HW Kim, JC. Knowles, HE Kim: Hydroxyapatite/poly((epsilon)-caprolactone) composite coatings on hydroxyapatite porous bone scaffold for drug delivery. *Biomaterials* 25, 1279–1287 (2004)

86. AR Boccaccini, JJ Blaker, V Maquet, RM. Day, R Jerome: Preparation and characterisation of poly(lactide-co-glycolide) (PLGA) and PLGA/Bioglass(R) composite tubular foam scaffolds for tissue engineering applications. *Mater Sci Eng C* 25, 23–31 (2005)

87. EL Hedberg, CK Shih, JJ Lemoine, MD Timmer, MA Liebschner, JA Jansen, AG Mikos: In vitro degradation of porous poly(propylene fumarate)/poly(dl-lactic-co-glycolic acid) composite scaffolds. *Biomaterials* 26, 3215–3225 (2005)

88. K Zhang, Y Wang, MA Hillmayer, LF Francis: Processing and properties of porous poly(l-

lactide)/bioactive glass composites. *Biomaterials* 25, 2489–2500 (2004)

89. H Niiranen, T Pyhalto, P Rokkanen, M Kellomaki, P Tormala: In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites. *J Biomed Mater Res A* 69A, 699–708 (2004)

90. J Yao, S Radin, PS. Leboy, P Ducheyne: The effect of bioactive glass content on synthesis and bioactivity of composite poly (lactic-co-glycolic acid)/bioactive glass substrate for tissue engineering. *Biomaterials* 26, 1935–1943 (2005)

91. SA Gittens, H Uludag: Growth factor delivery for bone tissue engineering. *J Drug Target* 9, 407–429 (2001)

92. V Luginbuehl, L Meinel, HP Merkle, B Gander: Localized delivery of growth factors for bone repair. *Eur J Pharm Biopharm* 58, 197–208 (2004)

93. L Di Silvio, W Bonfield: Biodegradable drug delivery system for the treatment of bone infection and repair. *J Mater Sci, Mater Med* 10, 653–658 (1999)

94. V Maquet, AR Boccaccini, L Pravata, I Notingher, R Jerome: Porous poly([alpha]-hydroxyacid)/Bioglass(R) composite scaffolds for bone tissue engineering. I: Preparation and in vitro characterisation. *Biomaterials* 25, 4185–4194 (2004)

95. H Li, J Chang: Preparation and characterisation of bioactive and biodegradable wollastonite/poly(d,l-lactic acid) composite scaffolds. *J Mater Sci Mater Med* 15, 1089–1095 (2004)

96. HH Lu, A Tang, SC Oh, JP. Spalazzi, K Dionisio: Compositional effects on the formation of a calcium phosphate layer and the response of osteoblast-like cells on polymer-bioactive glass composites. *Biomaterials* 26, 6323–6334 (2005)

97. G Jiang, ME Evans, I Jones, CD. Rudd, CA. Scotchford, GS Walker: Preparation of poly(e-caprolactone)/continuous bioglass fibre composite using monomer transfer moulding for bone implant. *Biomaterials* 26, 2281–2288 (2005)

98. YM Khan, DS Dhirendra, S Katti, CT Laurencin: Novel polymer-synthesized ceramic composite-based system for bone repair: an in vitro evaluation. *J Biomed Mater Res A* 69A, 728–737 (2004)

99. HHK. Xu, JB. Quinn, S Takagi, LC Chow: Synergistic reinforcement of in situ hardening calcium phosphate composite scaffold for bone tissue engineering. *Biomaterials* 25, 1029–1037 (2004)

100. SJ Polak, SK Lan Levengood, MB Wheeler, AJ Maki, SG Clark, AJ Wagoner Johnsone: Analysis of the roles of microporosity and BMP-2 on multiple measures of bone regeneration and healing in calcium phosphate scaffolds. *Acta Biomater* 7, 1760-1771 (2011)

101 K Rezwan, QZ Chen, JJ Blaker, AR Boccaccini: Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. *Biomaterials* 27, 3413-3431 (2006)

102 M Swetha, K Sahithi, A Moorthi, N Srinivasan, K Ramasamy, N Selvamurugan: Biocomposites containing natural polymers and hydroxyapatite for bone tissue engineering. *Int J Biological Macromolecules* 47, 1-4 (2010)

103. PX Ma, R Langer: Morphology and mechanical function of long-term in vitro engineered cartilage. *J Biomed Mater Res* 44, 217-221 (1999)

104. DW Huntmacher: Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 21, 2529–2543 (2000)

105. X Cai, H Tong, X Shen, W Chen, J Yan, J Hu: Preparation and characterization of homogeneous chitosan-polylactic acid/hydroxyapatite nanocomposite for bone tissue engineering and evaluation of its mechanical properties. *Acta Biomaterialia*. 5, 2693–2703 (2009)

106. X J Tang, L Gui, XY Lu: Hard tissue compatibility of natural hydroxyapatite/chitosan composite *Biomed Mater* 3(4), (2008)

107. S Kim MS Park, O Jeon, CY Choi, B Kim: Poly(lactide–co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* 27, 1399–1409 (2006)

108. C Du, F-Zi Cui: Hydroxyapatite/collagen scaffolds. In: Scaffolding in tissue engineering, Eds: PX Ma, J Elisseeff, CRC Press, 265 (2006)

109. KT Laurencin, Y Khan: Polymer/calcium phosphate scaffolds for bone tissue engineering. In: Scaffolding in tissue engineering, Eds: PX Ma, J Elisseeff, CRC Press, 253 (2006)

110. H Wang, Y Zuo, Q Zou, L Cheng, D Huang, L Wang, Y Li: Nano-hydroxyapatite/polyamide66 composite tissue-engineering scaffolds with anisotropy in morphology and mechanical behaviors. *J Polym Sci Polym Chem* 47, 658–669 (2009)

111. Y Shikinami, M Okuno: Bioresorbable devices made of forged composites of hydroxyapatite (HA) particles and poly-l-lactide (PLLA): Part I. Basic characteristics. *Biomaterials* 20, 859–877 (1999)

112. X Cai, H Tong, X Shen, W Chen, J Yan, J Hu: Preparation and characterization of homogeneous chitosan–polylactic acid/hydroxyapatite nanocomposite for bone tissue engineering and evaluation of its mechanical properties. *Acta Biomater* 5, 2693–2703 (2009)

113. G Converse, T Conrad, R Roeder: Mechanical properties of hydroxyapatite whisker reinforced

polyetherketoneketone composite scaffolds. *J Mech Behav Biomed Mater* 2, 627–635 (2009)

114. Y Cui, Y Liu, Y Cui, X Jing, P Zhang, X Chen: The nanocomposite scaffold of poly(lactide–co-glycolide) and hydroxyapatite surface-grafted with l-lactic acid oligomer for bone repair. *Acta Biomater* 5, 2680–2692 (2009)

115. AJW Johnson, BA. Herschler: A review of the mechanical behavior of CaP and CaP/polymer composites for applications in bone replacement and repair. *Acta Biomater* 7, 16-30 (2011)

116. LL Hench, RJ Splinter, WC Allen, TK Greenlee Jr.: Bonding mechanisms at the interface of ceramic prosthetic materials. *J Biomed Mater Res* 2, 117–141 (1971)

117. T Kokubo, M Shigematsu, Y Nagashima, M Tashiro, T Yamamuro, S Higashi: Apatite-and wollastonite-containing glass-ceramics for prosthetic application. *Bull Inst Chem Res* 60, 260–268 (1982)

118. J Wilson, GH. Pigott, FJ Schoen, LL Hench: Toxicology and biocompatibility of Bioglass. *J Biomed Mater Res* 15, 805–817 (1981)

119. J Wilson, D Nolletti: Bonding of Soft Tissues to Bioglass. In: Handbook on bioactive ceramics: Bioactive glasses and glass-ceramics, Vol 1, Eds: IT Yamamuro, LL Hench, J Wilson, Boca Raton, FL, USA 283–302 (1990)

120. AR Boccaccini, V Maquet: Bioresorbable and bioactive polymer/Bioglass® composites with tailored pore structure for tissue engineering applications. *Composites Science and Technology* 63, 2417-2429 (2003)

121. KK Mallick: Freeze Casting of Porous Bioactive Glass and Bioceramics. *J Amer Ceram Soc* 92(1), 85-S94 (2009)

122. KK Mallick: Development, synthesis and characterization of porous biomaterial scaffolds for tissue engineering. Advances in biomedical and biomimetic materials, Ceramic Transactions Book Series 206, 115-128 (2009)

123. K Zhang, Y Ma, LF Francis: Porous polymer/bioactive glass composites for soft-to-hard tissue interfaces *J Biomed Mater Res A* 61, 551-563 (2002)

124. PX Ma: Tissue Engineering. In: Encyclopedia of Polymer Science and Technology (3rd Edition), Ed: JI Kroschwitz, John Wiley & Sons, NJ, USA (2004)

125. R Zhang, PX Ma: Synthetic nano-fibrillar extracellular matrices with predesigned macroporous architectures. *J Biomed Mater Res* 52, 430-438 (2000)

126. CM Agrawal, RB. Ray: Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. *J Biomed Mater Res* 55, 141-150 (2001)

127. T Fukasawa, ZY Deng, M Ando, T Ohji, Y Goto: Pore structure of porous ceramics synthesized from water-based slurry by freeze-dry process. *J Mater Sci* 36, 2523–2527 (2001)

128. S Deville, E Saiz, AP Tomsia: Freeze casting of hydroxyapatite scaffolds for bone tissue engineering. *Biomaterials* 27, 5480-5489 (2006)

129. S Deville: Freeze-casting of porous ceramics: A review of current achievements and issues. *Adv Eng Mater* 10, 155-169 (2008)

130. SV Madihally, HWT Matthew: Porous chitosan scaffolds for tissue engineering. *Biomaterials* 20, 1133–1142 (1999)

131. HW Kang, Y Tabata, Y Ikada: Fabrication of porous gelatin scaffolds for tissue engineering. *Biomaterials* 20, 1339–1344 (1999)

132. Y Huang, S Onyeri, M Siewe, A Moshfeghian, SV Madihally: In vitro characterization of chitosan-gelatin scaffolds for tissue engineering. *Biomaterials* 26, 7616–7627 (2005)

133. H Schoof, L Bruns, A Fischer, I Heschel, G Rau: Dendritic ice morphology in unidirectionally solidified collagen suspensions. *J Cryst Growth* 209, 122–129 (2000)

134. R Zhang, PX Ma: Poly(alpha-hydroxyl acids) hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. *J Biomed Mater Res* 44, 446–455 (1999)

135. V Maquet, AR Boccaccini, I Notingher, R Jérôme: Porous poly(alpha-hydroxyacid)/Bioglass (R) composite scaffolds for bone tissue engineering. I: preparation and in vitro characterisation. *Biomaterials* 25, 4185–4194 (2004)

136. HR Oxley, PH Corkhill, JH Fitton, BJ Tighe: Macroporous hydrogels for biomedical applications - methodology and morphology. *Biomaterials* 14, 1064-1072 (1993)

137 S Stokols, MH Tuszynski: The fabrication and characterization of linearly oriented nerve guidance scaffolds for spinal cord injury. *Biomaterials* 25, 5839–5846 (2004)

138. W Tao, M Li, R Xie: Preparation and structure of porous silk sericin materials. *Marcomol Mater Eng* 290, 188-194 (2005)

39. S Zmora, R Glicklis, S Cohen: Tailoring the pore architecture in 3-D alginate scaffolds by controlling the freezing regime during fabrication. *Biomaterials* 23, 4087–4094 (2002)

140. TW Chung, J Yang, T Akaike, KY Cho, JW Nah, SI Kim, CS Cho: Preparation of alginate/galactosylated

chitosan scaffold for hepatocyte attachment. *Biomaterials* 23, 2827–2834 (2002)

141. A Tampieri, M Sandri, E Landi, G Celotti, N Roveri, M Mattioli-Belmonte, L Virgili, F Gabbanelli, G Biagini: HA/alginate hybrid composites prepared through bio-inspired nucleation. *Acta Biomater* 1, 343–351 (2005)

142. A Uchida, SM Nade, ER McCartney, W Chiang: The use of ceramics for bone replacement: A comparative study of three different porous ceramics. *J Bone Joint Surg Br* 66, 269-275 (1984)

143. PX Ma, R Langer: Fabrication of biodegradable polymer foams for cell transplantation and tissue engineering. In: Tissue Engineering Methods and Protocols, Eds: J Morgan, M Yarmush, Humana Press, NJ, USA 47 (1999)

144. G Wei, PX Ma.: Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* 25(19) 4749-4757 (2004)

145. AG Mikos, AJ Thorsen, LA Czerwonka, Y Bao, R Langer: Preparation and characterization of poly(L-lactic acid) foams. *Polymer* 35, 1068–1077 (1994)

146. AG Mikos, G Sarakinos, SM Leite, JP Vacanti, R Langer: Wetting of poly(l-lactic acid) and poly(dl-lactic-co-glycolic acid) foams for tissue culture. *Biomaterials* 14, 323–330 (1993)

147. R. Zhang, PX Ma: Poly(alpha-hydroxyl acids) hydroxyapatite porous composites for bone-tissue engineering. I. Preparation and morphology. *J Biomed Mater Res* 44, 446-455 (1999)

148. PX Ma, R Zhang, G Xiao, R Franceschi: Engineering new bone tissue in vitro on highly porous poly(α-hydroxyl acids)/hydroxyapatite composite scaffolds. *J Biomed Mater Res* 54, 284-293 (2001)

149. PX Ma, R Zhang: Fibrillar Matrices. *US Patent* 6,146,892 (2000)

150. PX Ma, R Zhang: In vitro effects of low-intensity ultrasound stimulation on the bone cells. *J Biomed Mater Res* 56, 449-456 (2001)

151. R Zhang, PX Ma: Processing of polymer scaffolds: Phase separation. In: Methods of Tissue Engineering, Editors: A Atala, R Lanza, Academic Press, San Diego, USA 715 (2001)

152. PX Ma, RY Zhang: Synthetic nano-scale fibrous extracellular matrix. *J Biomed Mater Res* 46, 60–72 (1999)

153. YS Nam, TG Park: Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation. *J Biomed Mater Res* 47, 8-17 (1999)

154. W Guobao, PX Ma: Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* 25, 4749-4757 (2004)

155. Y Hu, C Zhang, S Zhang, Z Xiong, J Xu: Development of a porous poly(L-lactic acid)/hydroxyapatite/collagen scaffold as a BMP delivery system and its use in healing canine segmental bone defect. *J Biomed Mater Res* 67A(2), 591-598 (2003)

156. SJ Hollister: Porous scaffold design for tissue engineering. *Nature Materials* 4, 518-524 (2005)

157. S. Yang, KF Leong, Z Du, CK Chua: The design of scaffolds for use in tissue engineering. Part II. Rapid prototyping techniques. *Tissue Eng* 8, 1-11 (2002)

158. A Park, LG Griffith: Integration of surface modification and 3D fabrication techniques to prepare patterned poly(L-lactide) substrates allowing regionally selective cell adhesion. *J Biomater Sci Polymer Edition* 9, 89–110 (1998)

159. J Zeltinger, JK Sherwood, DA Graham, R Müeller, LG Griffith: Effect of pore size and void fraction on cellular adhesion, proliferation, and matrix deposition. *Tissue Eng* 7, 557–72 (2001)

160. DW Hutmacher, T Schantz, I Zein, KW Ng, SH Teoh, KC Tan: Mechanical properties and cell cultural response of polycaprolactone scaffolds designed and fabricated via fused deposition modeling. *J Biomed. Mater Res* 55, 203-216 (2001)

161. R Sodian, M Loebe, A Hein, DP Martin, SP Hoerstrup, EV Potapov, H Hausmann, T Lueth, R Hetzer: Application of stereolithography for scaffold fabrication for tissue engineered heart valves. *Asaio Journal* 48, 12–16 (2002)

162. TMG Chu, JW Halloran, SJ Hollister, SE Feinberg: Hydroxyapatite implants with designed internal architecture. *J Mater Sci Mater Med* 12 (6), 471–478 (2001)

163. SN Bhatia, UJ Balis, ML Yarmush, M Toner: Microfabrication of hepatocyte/fibroblast co-cultures. *Biotechnol Prog* 14, 378−387 (1998)

164. A Shekaran, AJ Garcia: Nanoscale engineering of extracellular matrix-mimetic bioadhesive surfaces and implants for tissue engineering. *Biochem Biophys Acta* 1810, 350-360 (2011)

165 QZ Chen, ID Thompson, AR Boccaccini: 45S5 Bioglass®-derived glass–ceramic scaffolds for bone tissue engineering, *Biomaterials* 27(11), 2414-2425 (2006)

166. CJ Bettinger, R Langer, JT Borenstein: Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew Chem Int Ed Engl* 48, 5406-5415 (2009)

167. EM Christenson, KS Anseth, JJ van den Beucken, CK Chan, B Ercan, JA Jansen, CT Laurencin, WJ Li, R Murugan, LS Nair, S Ramakrishna, RS Tuan, TJ Webster,

AG Mikos: Nanobiomaterial applications in orthopedics. *J Orthop Res* 25, 11-22 (2007)

168. QM Jin, H Takita, T Kohgo, K Atsumi, H Itoh, Y Kuboki: Effects of geometry of hydroxyapatite as a cell substratum in BMP-induced ectopic bone formation. *J Biomed Mater Res* 52, 491-499 (2000)

169. Y Kuboki, H Takita: Geometry of carriers controlling phenotypic expression in BMP-induced osteogenesis and chondrogenesis. *J Bone Joint Surg Am* 83, S105-115 (2001)

170. Y Kuboki, Q Jin, J Mamood, H Takita: Geometry of artificial ECM: sizes of pores controlling phenotype expression in BMP-induced osteogenesis and chondrogenesis. *Connect Tissue Res* 43, 529-534 (2002)

171. TMG Chu, DG Orton, SJ Hollister, SE Feinberg, JW Halloran: Mechanical and in vivo performance of hydroxyapatite implants with controlled architectures. *Biomaterials* 23, 1283-1293 (2002)

172. E Damien, K Hing S Saeed, PA Revell: A preliminary study on the enhancement of the osteointegration of a novel synthetic hydroxyapatite scaffold in vivo. *J Biomed Mater Res* 66, 241-246 (2003)

173. Y Kuboki, H Takita, D Kobayashi, E Tsuruga, M Inoue, M Murata, N Nagai, Y Dohi, H Ohgushi: BMP-induced osteogenesis on the surface of hydroxyapatite with geometrically feasible and nonfeasible structures: topology and osteogenesis. *J Biomed Mater Res* 39, 190-199 (1998)

174. JE Barralet, L Grover, T Gaunt, AJ Wright, IR Gibson: Preparation and analysis of macroporous calcium phosphate cement tissue engineered scaffold. *Biomaterials* 23, 3063-3072 (2002)

175. YM Lee, YJ Seol, YT Lim, S Kim, SB Han, IC Rhyu, SH Baek, SJ Heo, JY Choi, PR Klokkevold, CP Chung: Tissue-engineered growth of bone by marrow cell transplantation using porous calcium metaphosphate matrices. *J Biomed Mater Res* 54, 216-223 (2001)

176. F Chen, T Mao, K Tao, S Chen, G Ding, X Gu: Bone graft in the shape of human mandibular condyle reconstruction via seeding marrow-derived osteoblasts into porous coral in a nude mice model. *J Oral Maxillofac Surg* 60, 1155-1159 (2002)

177. C Zhang, J Wang, H Feng, B Lu, Z Song, X Zhang: Replacement of segmental bone defects using porous bioceramic cylinders: a biomechanical and X-ray diffraction study. *J Biomed Mater Res* 54, 407-411 (2001)

178. S Kimakhe, S Bohic, C Larrose, A Reynaud, P Pilet, B Giumelli, D Heymann, G Daculsi: Biological activities of sustained polymyxin B release from calcium phosphate biomaterial prepared by dynamic compaction: an in vitro study. *J Biomed Mater Res* 47, 18-27 (1999)

179. T Tarvainen, T Tunturi, J Rautavuori, P Tormala, H Patiala, P Rokkanen: Shear strength of loaded porous-glassy-carbon/bone interface – an experimental study on rabbits. *Ann Biomed Eng* 14, 417-424 (1986)

180. W Gong, A Adelouas, W Lutze: Porous bioactive glass and glass-ceramics made by reaction sintering under pressure. *J Biomed Mater Res* 54, 320-327 (2001)

181. ID Xynos, MV Hukkanen, JJ Batten, LD Buttery, LL Hench, JM Polak: Bioglass 45S5 stimulates osteoblast turnover and enhances bone formation in vitro: implications and applications for bone tissue engineering. *Calcif Tissue Int* 67, 321-329 (2000)

182. P Sepulveda, JR Jones, LL Hench: Bioactive sol-gel foams for tissue repair. *J Biomed Mater Res* 59, 340-348 (2002)

183. H Yuan, JD de Bruijn, X Zhang, CA van Blitterswijk, K de Groot: Bone induction by porous glass ceramic made from Bioglass (45S5). *J Biomed Mater Res* 58, 270-276 (2001)

184. AR El-Ghannam: Advanced bioceramic composites for bone tissue engineering: design principles and structure-bioactivity relationship. *J Biomed Mater Res A* 69, 490-501 (2004)

185. HD Kim, RF Valentini: Retention and activity of BMP-2 in hyaluronic acid-based scaffolds in vitro. *Biomaterials* 26, 6314-6322 (2002)

186. C Wu, Y Ramaswamy, Y Zhu, R Zheng, R Appleyard, A Howard, H Zreiqat: The effect of mesoporous bioactive glass on the physiochemical, biological and drug-release properties of poly(DL-lactide-co-glycolide) films. *Biomaterials* 30: 2199-2208 (2009)

187. LB Rocha, G Goissis, MA Rossi: Biocompatibility of anionic collagen matrix as scaffold for bone healing. *Biomaterials* 23, 449-456 (2002)

188. LS Liu, AY Thompson, MA Heidaran, JW Poser, RC Spiro: An osteoconductive collagen/hyaluronate matrix for bone regeneration. *Biomaterials* 20, 1097-1108 (1999)

189. R Nazarov, HJ Jin, DL Kaplan: Porous 3-D scaffolds from regenerated silk fibroin. *Biomacromolecules* 5, 718-726 (2004)

190. L Meinel, V Karageorgiou, S Hofmann, R Fajardo, B Snyder, C Li, L Zichner, R Langer, G Vunjak-Novakovic, DL Kaplan: Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. *J Biomed Mater Res A* 71, 25-34 (2004)

191. JM Taboas, RD Maddox, PH Krebsbach, SJ Hollister: Indirect solid free form fabrication of local and

global porous, biomimetic and composite 3D polymer-ceramic scaffolds. *Biomaterials* 24, 181-194 (2003)

192. AS Lin, TH Barrows, SH Cartmell, RE Guldberg: Microarchitectural and mechanical characterisation of oriented porous polymer scaffolds. *Biomaterials* 24, 481-489 (2003)

193. M Borden, SF El-Amin, M Attawia, CT Laurencin: Structural and human cellular assessment of novel microsphere-based tissue engineered scaffold for bone repair. *Biomaterials* 24, 597-609 (2003)

194. FA Maspero, K Ruffieux, B Muller, E Wintermantel: Resorbable defect analog PLGA scaffolds using CO2 as solvent: structural characterisation. *J Biomed Mater Res* 62, 89-98 (2002)

195. EA Botchwey, SR Pollack, EM Levine, CT Laurencin: Bone tissue engineering in a rotating bioreactor using a microcarrier matrix system. *J Biomed Mater Res* 55, 242-253 (2001)

196. XB Yang, HI Roach, NMP Clarke, SM Howdle, R Quirk, KM Shakesheff, ROC Oreffo: Human osteoprogenitor growth and differentiation on synthetic biodegradable structures after surface modification. *Bone* 29, 523-531 (2001)

197. WJ Li, CT Laurencin, EJ Caterson, RS Tuan, FK Ko: Electrospun nanofibrous structure: a novel scaffold for tissue engineering. *J Biomed Mater Res* 60, 613-621 (2002)

198. D Schaefer, I Martin, P Shastri, RF Padera, R Langer, LE Freed, G Vunjak-Novakovic: In vitro generation of osteochondral composites. *Biomaterials* 21, 2599-2606 (2000)

199. SH Oh, SG. Kang, ES Kim, SH Cho, JH Lee: Fabrication and characterization of hydrophilic poly(lactic-co-glycolic acid)/poly(vinyl alcohol) blend cell scaffolds by melt-molding particulate-leaching method. *Biomaterials* 24, 4011-4021 (2003)

200. JA Burdick, RF Padera, F Robert, JV Huang, KS Anseth: An investigation of the cytotoxicity and histocompatibility of in situ forming lactic acid based orthopedic biomaterials. *J Biomed Mater Res* 63, 484-491 (2002)

201. JA Burdick, D Frankel WS Dernell, KS Anseth: An initial investigation of photocurable three-dimensional lactic acid based scaffolds in a critical-sized cranial defect. *Biomaterials* 24, 1613-1620 (2003)

202. KU Lewandrowski, DD Hile, BM Thompson, DL Wise, WW Tomford, DJ Trantolo.: Quantitative measures of osteoinductivity of a porous poly(propylene fumarate) bone graft extender. *Tissue Eng* 9, 85-93 (2003)

203. JP Fisher, TA Holland, D Dean, PS Engel, AG Mikos: Synthesis and properties of photocross-linked poly(propylene fumarate) scaffolds. *J Biomater Sci Polym Ed* 12, 673-687 (2001)

204. JP Fisher, JW Vehof, D Dean, JP van der Waerden, TA Holland, AG Mikos, JA Jansen: Soft and hard tissue response to photocrosslinked poly(propylene fumarate) scaffolds in a rabbit model. *J Biomed Mater Res* 59, 547-556 (2002)

205. DJ Trantolo, ST Sonis, BM Thompson, DL Wise, KU Lewandrowski, DD Hile: Evaluation of a porous, biodegradable biopolymer scaffold for mandibular reconstruction. *Int J Oral Maxillofac Implants* 18, 182-188 (2003)

206. Y Takahashi, Y Tabata: Effect of the fiber diameter and porosity of non-woven PET fabrics on the osteogenic differentiation of mesenchymal stem cells. *J Biomater Sci Polym Ed* 15, 41-57 (2004)

207. G Akay, MA. Birch, MA Bokhari: Microcellular polyHIPE polymer supports osteoblast growth and bone formation in vitro. *Biomaterials* 25, 3991-4000 (2004)

208. JL Simon, TD Roy, JR Parsons, ED Rekow, VP Thompson, J Kemnitzer, JL Ricci: Engineered cellular response to scaffold architecture in a rabbit trephine defect. *J Biomed Mater Res A* 66, 275-282 (2003)

209. Y Chen, AFT Mak, M Wang, J Li, MS Wong: PLLA scaffolds with biomimetic apatite coating and biomimetic apatite/collagen composite coating to enhance osteoblast-like cells attachment and activity. *Surface and Coatings Technology* 201, 575-580 (2006)

210. J Yao, S Radin, P Leboy, P Ducheyne: The effect of bioactive glass content on synthesis and bioactivity of composite poly (lactic-co-glycolic acid)/bioactive glass substrate for tissue engineering. *Biomaterials* 26, 1935-1943 (2005)

211. EL Hedberg, HC Kroese-Deutman, CK Shih, RS Crowther, DH Carney, AG Mikos, JA Jansen: In vivo degradation of porous poly(propylene fumarate)/poly(DL-lactic-co-glycolic acid) composite scaffolds. *Biomaterials* 26, 4616-4623 (2005)

212. Y Jung, SS Kim, YH Kim, SH Kim, BS Kim, S Kim, CY Choi, SH Kim: A poly(lactic acid)/calcium metaphosphate composite for bone tissue engineering. *Biomaterials* 26, 6314-6322 (2005)

213. X Li, Q. Feng, F Cui: In vitro degradation of porous nano-hydroxyapatite/collagen/PLLA scaffold reinforced by chitin fibres. *Materials Science and Engineering C* 26, 716-720(2006)

214. SS Kim, MS Park, O Jeon, CC Yong, BS Kim: Poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* 27, 1399-1409 (2006)

215. JK Sherwood, SL Riley, R Palazzolo, SC Brown, DC Monkhouse, M Coates, LG Griffith, LK Landeen, A Ratcliffe: A three-dimensional osteochondral composite scaffold for articular cartilage repair. *Biomaterials* 23, 4739-4751 (2002)

216. A Hokugo, T Takamoto, Y Tabata: Preparation of hybrid scaffold from fibrin and biodegradable polymer fiber. *Biomaterials* 27, 61-67 (2006)

217. Y Nakanishi, G Chen, H Komuro, T Ushida, S Kaneko, T Tateishi, M Kaneko: Tissue-engineered urinary bladder wall using PLGA mesh-collagen hybrid scaffolds: a comparison study of collagen sponge and gel as a scaffold. *J Pediatr Surg* 38, 1781-1784 (2003)

218. A Ide, M Sakane, G Chen, H Shimojo, T Ushida, T Tateishi, Y Wadano, Y Miyanag: Collagen hybridization with poly(-lactic acid) braid promotes ligament cell migration. *Materials Science and Engineering C* 17(1-2), 95-99. (2001)

219. L Flynn, PD Dalton, MS Shoichet: Fiber templating of poly(2-hydroxyethyl methacrylate) for neural tissue engineering. *Biomaterials* 24, 4265-4272 (2003)

220. BM Min, Y You, JM Kim, SJ Lee, WH Park: Formation of nanostructured poly(lactic-co-glycolic acid)/chitin matrix and its cellular response to normal human keratinocytes and fibroblasts. *Carbohydrate Polymers* 57, 285-292 (2004)

221. RM Day, AR Boccaccini, S Shurey, JA Roether, A Forbes, LL Hench, SM Gabe: Assessment of polyglycolic acid mesh and bioactive glass for soft-tissue engineering scaffolds. *Biomaterials* 25, 5857-5866 (2004)

222. AR Boccaccini, JJ Blaker, V Maquet, RM Day, R Jerome: Preparation and characterisation of poly(lactide-co-glycolide) (PLGA) and PLGA/Bioglass composite tubular foam scaffolds for tissue engineering applications. *Materials Science and Engineering C* 25, 23-31 (2005)

223. SX Pan, Y Li, HL Feng, W Bai, YY Gu: In vitro aging of mineralized collagen-based composite as guided tissue regeneration membrane. *Materials Science and Engineering C* 26, 724-729 (2006)

224. HW Kim, JC Knowles, HE Kim: Hydroxyapatite/poly(epsilon-caprolactone) composite coatings on hydroxyapatite porous bone scaffold for drug delivery. *Biomaterials* 25, 1279-1287 (2004)

225. F Zhao, Y Yin, WW Lu, JC Leong, W Zhang, J Zhang, M Zhang, K Yao: Preparation and histological evaluation of biomimetic three-dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds. *Biomaterials* 23, 3227-3234 (2002)

226. Y Zhang, M Zhang: Three-dimensional macroporous calcium phosphate bioceramics with nested chitosan sponges for load-bearing bone implants. *J Biomed Mater Res* 61, 1-8 (2002)

227. D Lickorish, JA Ramshaw, JA Werkmeister, V Glattauer, CR Howlett: Collagen-hydroxyapatite composite prepared by biomimetic process. *J Biomed Mater Res A* 68, 19-27 (2004)

228. M Itoh, A Shimazu, I Hirata, Y Yoshida, H Shintani, M Okazaki: Characterization of CO3Ap-collagen sponges using X-ray high-resolution microtomography. *Biomaterials* 25, 2577-2583 (2004)

229. A Tache, L Gan, D Deporter, RM Pilliar: Effect of surface chemistry on the rate of osseointegration of sintered porous-surfaced Ti-6Al-4V implants. *Int J Oral Maxillofac Implants* 19, 19-29 (2004)

230. JW Vehof, PH Spauwen, JA Jansen: Bone formation in calcium-phosphate-coated titanium mesh. *Biomaterials* 21, 2003-2009 (2000)

231. P Li: Biomimetic nano-apatite coating capable of promoting bone ingrowth. *J Biomed Mater Res A* 66, 79-85 (2003)

232. X Zhang, RA Ayers, K Thorne, JJ Moore, F Schowengerdt: Combustion synthesis of porous materials for bone replacement. *Biomed Sci Instrum* 37, 463-468 (2001)

233. MS Wolfe, D Dean, JE Chen, JP Fisher, S Han, CM Rimnac, AG Mikos: In vitro degradation and fracture toughness of multilayered porous poly(propylene fumarate)/beta-tricalcium phosphate scaffolds. *J Biomed Mater Res* 61, 159-164 (2002)

234. K Zhang, Y Wang, MA Hillmyer, LF Francis: Processing and properties of porous poly(L-lactide)/bioactive glass composites. *Biomaterials* 25, 2489-2500 (2004)

235. G Chen, T Ushida, T Tateishi: Poly(DL-lactic-co-glycolic acid) sponge hybridized with collagen microsponges and deposited apatite particulates. *J Biomed Mater Res* 57, 8-14 (2001)

236. Y Zhang, M Zhang: Calcium phosphate/chitosan composite scaffolds for controlled in vitro antibiotic drug release. *J Biomed Mater Res* 62, 378-386 (2002)

237. ZR Domingues, ME Cortes, TA Gomes, HF Diniz, CS Freitas, JB Gomes, AM Faria, RD Sinisterra: Bioactive glass as a drug delivery system of tetracycline and tetracycline associated with beta-cyclodextrin. *Biomaterials* 25, 327-333 (2004)

238. AR Duarte, JF Mano, RL Reis: Dexamethasone-loaded scaffolds prepared by supercritical-assisted phase inversion. *Acta Biomater* 5, 2054-2062 (2009)

239. S Kujala, J Ryhänen, A Danilov, J Tuukkanen: Effect of porosity on the osteointegration and bone ingrowth of a weight-bearing nickel-titanium bone graft substitute. *Biomaterials* 24, 4691-4697 (2003)

240. JW Vehof, MT. Haus, AE de Ruijter, PH Spauwen, JA Jansen: Bone formation in transforming growth factor beta-

I-loaded titanium fiber mesh implants. *Clin Oral Implants Res* 13, 94-102 (2002)

241. S Nishiguchi, H Kato, M Neo, M Oka, HM Kim, T Kokubo, T Nakamura: Alkali- and heat-treated porous titanium for orthopedic implants. *J Biomed Mater Res* 54, 198-208 (2001)

242. RM Pilliar: Overview of surface variability of metallic endosseous dental implants: textured and porous surface-structured designs. *Implant Dent* 7, 305-314 (1998)

243. EJ Harvey, JD. Bobyn, M Tanzer, GJ Stackpool, JJ Krygier, SA Hacking: Effect of flexibility of the femoral stem on bone-remodeling and fixation of the stem in a canine total hip arthroplasty model without cement. *J Bone Joint Surg Am* 81, 93-107 (1999)

244. HE Gotz, M Muller, A Emmel, U Holzwarth, R.G Erben, R Stangl: Effect of surface finish on the osseointegration of laser-treated titanium alloy implants. *Biomaterials* 25, 4057-4064 (2004)

245. YT Sul, CB Johansson, Y Jeong, A Wennerberg, T Albrektsson: Resonance frequency and removal torque analysis of implants with turned and anodized surface oxides. *Clin Oral Implants Res* 13, 252-259 (2002)

246. BJ Story, WR Wagner, DM Gaisser, SD Cook, AM Rust-Dawicki: In vivo performance of a modified CSTi dental implant coating. *Int J Oral Maxillofac Implants* 13, 749-757 (1998)

247. DD D'Lima, SM Lemperle, PC Chen, RE Holmes, CW Colwell: Bone response to implant surface morphology. *J Arthroplasty* 13, 928-934 (1998)

248. FA Akin. H Zreiqat, S Jordan, MB Wijesundara, L Hanley: Preparation and analysis of macroporous TiO2 films on Ti surfaces for bone-tissue implants. *J Biomed Mater Res* 57, 588-596 (2001)

Key Words: tissue engineering, fabrication, conventional, rapid prototyping, scaffold, biomaterial, bioscaffold, porous, three dimensional architecture, bone

Send correspondence to: Kajal K. Mallick, Warwick Manufacturing Group, School of Engineering, University of Warwick, Coventry, CV4 7AL, United Kingdom, Tel: 4402476522342, Fax: 4402476524783, E-mail: k.k.mallick@warwick.ac.uk