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TOPICAL REVIEW

Manufacture of degradable polymeric scaffolds for bone regeneration

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Abstract
Many innovative technology platforms for promoting bone regeneration have been developed. A common theme among these is the use of scaffolds to provide mechanical support and osteoconduction. Scaffolds can be either ceramic or polymer-based, or composites of both classes of material. Both ceramics and polymers have their own merits and drawbacks, and a better solution may be to synergize the advantageous properties of both materials within composite scaffolds. In this current review, after a brief introduction of the anatomy and physiology of bone, different strategies of fabricating polymeric scaffolds for bone regeneration, including traditional and solid free-form fabrication, are critically discussed and compared, while focusing on the advantages and disadvantages of individual techniques.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Bone repair and regeneration can be enhanced through implantation of biocompatible and biodegradable scaffolds with similar mechanical properties to bone and controlled degradation properties commensurate with endogenous regeneration and remodeling *in situ*. Scaffolds serve primarily as osteoconductive moieties on which newly formed bone is deposited through creeping substitution from adjacent living bone [1]. Additionally, scaffolds can also serve as delivery vehicles for transplanted cells, growth factors or even gene therapy, to further enhance the regeneration process. A popular modality is to expand progenitor cells *in vitro* and seed them onto biodegradable scaffolds in combination with growth factors that stimulate osteogenic differentiation, followed by implantation into the site of the bone defect. However, the major challenges faced with this approach are the risks of pathogenic transmission, possible immunological rejection depending on the cell source and overriding costs [2]. A better strategy may be for scaffolds to induce endogenous osteoblasts and their progenitors *in situ*, as well as work synergistically with growth factors for subsequent bone regeneration.

2. Anatomy, physiology and biomechanics of bone

Bone is a highly specialized connective tissue that provides internal support and confers marked rigidity, strength and elasticity through the secretion of a well-organized mineralized extracellular matrix (ECM). The development and remodeling of bone are tightly regulated by autocrine and paracrine mechanisms under a complex centralized control, which to date is not fully understood [3]. Collagen, inorganic calcified minerals and water are the three major constituents of bone. During bone formation, the collagen content remains essentially the same, while the increase in mineral content occurs at the expense of the water content. Type I collagen constitutes approximately 95% of the organic matrix (dry

weight), while proteoglycan and noncollagenous proteins account for the remaining 5%. The primary crystalline mineral is hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) crystals, as well as calcium carbonate. Additionally, inorganic salts of magnesium, potassium, fluoride, phosphate and citrate are also present in significant quantities [4].

There are two morphological isoforms of bone: cortical and cancellous. The matrix of cortical bone constitutes of paralleled concentric lamellae of densely packed interconnected collagen fibrils. On the other hand, cancellous bone has a loosely organized porous matrix. Four cell types are present in bone tissue: osteoblasts, osteoclasts, osteocytes and bone lining cells. Osteoblasts are fully differentiated cells responsible for the production of bone matrix and regulation of mineralization, while osteocytes are mature osteoblasts within the bone matrix that are responsible for its maintenance. Osteoclasts are large multi-nucleated cells that resorb bone under tight regulation. Bone lining cells are non-active cells with an unknown function [5]. In addition to its mechanical function, bone is also known to be a major calcium and phosphate reservoir, which is necessary for a wide variety of metabolic functions.

As mesenchymal progenitor cells mature into osteoblasts, the subsequent development of osteoblasts involves three stages: proliferation, matrix secretion and mineralization. During bone formation, multiple growth factors are expressed, such as bone morphogenetic proteins (BMPs), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1), platelet-derived growth factor-BB (PDGF-BB), tissue growth factor β-1 (TGFβ-1) and vascular epithelium growth factors (VEGF), each of which plays different roles that may overlap [6]. The bone BMP family includes a large number of factors implicated in osteoinduction, and consists of three subclasses: (1) BMP-2, BMP-4, etc; (2) BMP-5, BMP-6, BMP-7(OP-1), BMP-8 (OP-2) and (3) BMP-3, which is the least related subclass [7]. In the process of bone repair and regeneration, cellular proliferation and differentiation are tightly regulated by an ever-changing ECM and growth factor synthesis. Fracture healing is viewed by Gerstenfeld *et al* [8] as an example of specialized post-natal bone regeneration. Although the regeneration process is not exactly homologous with that which takes place during embryogenesis, fracture healing recapitulates a number of crucial processes that control bone generation during embryonic skeletal development. During fracture repair, there are three key groups of soluble factors: pro-inflammatory cytokines, the TGF-β superfamily and angiogenic factors. Pro-inflammatory cytokines which initiate the repair process are considered to arise from marrow or bone matrix within the initial injury site, and includes the macrophage colony-stimulating factor (M-CSF), receptor activator of nuclear factor kappa B ligand (RANKL), osteoprotegerin (OPG), tumor necrosis factor-alpha/beta (TNF-α/ß), etc. The TGF-β superfamily involves BMPs (1–8), GDFs (1, 5, 8 and 10), TGFb1–3, etc, which facilitate intramembranous and endochondral bone formation. The angiogenic group consists of VEGFa-d, angiopoietin-1 (Ang1), pleiotrophin (PTN), which induce formation of new blood vessels that satisfy the demand for increased blood flow during fracture repair [8].

Table 1. Mechanical properties of human bone.

Longitudinal elastic moduli	22.0×10^9 N m^{-2}
Transverse elastic moduli	11.3×10^9 N m^{-2}
Longitudinal tension	133×10^6 N m^{-2}
Longitudinal compression	193×10^6 N m^{-2}
Transverse tension	51×10^6 N m^{-2}
Transverse compression	133×10^6 N m^{-2}
Transverse torsion	68×10^6 N m^{-2}

The biomechanical properties of bone tissue vary greatly, and have been shown to exhibit structural diversity and variation in mineralization and porosity, according to the precise location and orientation of the specimens [9]. For example, the compressive strength and Young's modulus of cancellous bone is about 20-fold less than that of cortical bone [10] (table 1). A major challenge in bone regeneration is to restore cortical bone with acceptable mechanical properties [11].

The structural–mechanical function of bone tissue at the microscopic level is well reviewed by Weiner and Wagner [9], while its properties at the macroscopic level have been extensively reviewed elsewhere [12]. Biomaterials and scaffolds utilized in bone tissue engineering exhibit different structural–mechanical properties compared to normal bone, and will not be discussed here.

3. Challenges in fabricating scaffolds for bone tissue engineering

Ideally, materials and scaffolds utilized in bone tissue engineering should meet several prerequisites, in particular good biocompatibility, an essential prerequisite for all materials (including potential degradation products) utilized in tissue engineering.

3.1. Porosity, pore size, interconnectivity and microstructure

Though autologous bone grafts have long been regarded as the gold standard to which all alternatives are compared, it is not absolutely necessary for scaffolds utilized in bone tissue engineering to exactly mimic their microstructure. Other materials with different surface tensions and degradation rates will elicit varying *in vivo* responses and hence face different microenvironments. It is well accepted that higher porosity, pore size and interconnectivity result in greater bone ingrowth *in vivo*. A pore size larger than 300 μm is considered to support good tissue migration, nutrient transport and vascular formation of capillaries [13], while interconnections must be larger than 100 μm. The microstructure plays an important role in osteogenesis [14].

3.2. Mechanical properties and integration with host bone tissue

Mechanical properties of scaffolds as well as the subsequent regenerated osseous tissue must be able to bear the stress from daily activities. *In vivo* investigation on the effect of daily activities on the bone structure provides mechanical thresholds

that bone replacements will likely encounter after surgery, which are much lower than the mechanical properties of bones under subfailure and failure conditions [15]. Mechanical properties at both the microscopic and the macroscopic levels are important [16]. Except for mechanical properties, integration of scaffolds and regenerated bone within host tissue are essential for functionality, which can be further strengthened through the addition of ceramics [17].

3.3. Controlled degradation

As bone regeneration and subsequent remodeling take place over a duration of several months, it is important for scaffolds to degrade in a controlled fashion, so as to gradually allow the mechanical properties of newly regenerated bone tissue to make up for the loss of mechanical support from the original scaffold [18]. Nevertheless, most of the current research place more emphasis on the microstructure and original mechanical properties of scaffolds, with little attention on the degradation rate [19].

3.4. Retaining/enhancing osteoinductive properties of inductive/growth factors

Scaffolds ideally should be able to contribute to bone regeneration through a process commonly referred to as osteoinduction. This however is dependent on many factors appearing at different time points as well as temporal expression of genes characteristic of osteoblastic differentiation [20]. There has been extensive research on studying how scaffolds with different components and microstructure affect the differentiation and proliferation of osteogenic cells. Scaffolds are composed of various calcium phosphates (octacalcium phosphate (OCP), beta tricalcium phosphate (beta-TCP), hydroxyapatite, etc) which upon combination with different dosages of human bone morphogenetic protein exhibit different osteoinductive potencies. The scaffold microstructure (i.e. porosity) also affect the scaffold osteoinductivity [6]. Different delivery systems for growth factors have been designed in order that growth factors can target the desired cells and sustain release over appropriate durations *in vivo* [21].

Some progress has been reported on the controlled release of growth factors [22], but this is still at the preliminary stage of investigation. Osteoinductive factors can be extracted and purified from animal and human cortical bone, or recombinantly synthesized before being incorporated into the polymeric scaffold. Delivering plasmid DNA encoding the factors is another option, but there are various safety concerns with genetic modification. Controllable release of dual growth factors with distinct kinetics and effects, such as VEGF-165 and PDGF-BB, has also been reported [23]. In another study, two growth factors, bone morphogenetic protein-2 (BMP2) and transforming growth factor $\beta 3$ (TGF-$\beta 3$), have been delivered to achieve a synergistic effect, because these are not effective individually [24].

3.5. Compatibility with other technology platforms and biomaterials

Because no individual technology can induce bone regeneration optimally, a combination of different technology platforms would probably be required for bone tissue engineering. Hence, it is essential that scaffolds be compatible with other technology platforms and biomaterials. In particular, scaffolds should possess enough flexibility to also serve as vehicles or carriers for cell seeding, protein delivery and gene therapy.

3.6. Customized design

Composition and mechanical properties of bone tissue show obvious differences with regards to gender, ethnicity, age and specific location of the specimen. Furthermore, repair and regeneration processes are different with different modes of therapy, and even in the same individual, regeneration is far from homogeneous. It may be beneficial to customize scaffolds to an individual patient's biodata. The diverse arrays of commercial products for bone regeneration from each main category are listed in table 2.

4. Polymers used for bone regeneration

Polymers are large organic macromolecules formed by combining many smaller molecules (monomers) in a regular pattern, often with molecular weight between 10 000 and 1000 000. There exists a diverse array of biological and synthetic polymers. Polymers can also be classed into degradable and non-degradable, while degradable ones will be the focus of current review as they could be totally removed from human bodies, eventually as foreign bodies. The term 'biodegradable polymer' refers to the susceptibility of a polymer to be decomposed by living organisms or by environmental factors. According to the ASTM (American Society for Testing and Materials) standard definition, biodegradable means capable of undergoing decomposition into CO_2, CH_4, H_2O, inorganic compounds or biomass [25]. However, as for the materials used in tissue engineering, the biodegradable polymers could be decomposed into biologically acceptable molecules (without the production of harmful intermediates) which can be metabolized and removed from the body via naturally pathway (metabolism or excretion) [26]. The most commonly used synthetic biodegradable polymer is aliphatic polyesters because of their ease of degradation by hydrolysis of ester linkage in the body, with or without enzyme, and their degradation products being adsorbed through the metabolic pathway. This is different with the degradation process by environmental factors, in which UV light plays a key role in breaking the polymer chains [25]. For example, the degradation of PGA can be separated into two stages: at the beginning, the water diffuses into the amorphous regions of the polymer and hydrolytic chain scission starts; then the degradation involves the other crystalline areas of the polymer. The degradation product of PGA is glycolic acid which can be excreted by urine. Similarly, the degradation product of PLA is lactic acid which is normally present in

Table 2. Commercial degradable scaffolds for bone regeneration from each main category.

	Typical	Physical form	Merits	Drawbacks
HA/TCP	Ossatura (IsoTis Orthobiologics)	BCP: composite of HA and beta-TCP, 80/20 TCP: pure TCP	Osteoconductive and osteoinductive	Only as a bone void filler, but not block
Tricalcium phosphate (TCP)	Norion skeletal repair system (Norian Corp. of Cupertino)	Liquid paste	Injectable and offer mechanical integrity	Difficult to control and leak into joints [81]
Calcium carbonate	Biocoral (Biocoral Inc.)	Natural coral	Morphology and mechanics matched with bone and integrated with host bone	Quick degradation
Calcium sulphate	OsteoSet	Pellet	Antibiotic carrier	Inflammatory/allergic reaction
Collagen	Collagraft	Collagen fibrillar/calcium phosphate ceramic	Carrier for proteins, osteoprogenitor precursors, hydroxyapatite and autografts	Low mechanical strength and batch-to-batch variation
	Healos (Orquest)	Mineralized collagen sponge		potential of disease transmission
PLGA	TruGraft™ (Osteobiologics)	Granulate	Easy fabrication, excellent biocompatibility, controlled morphology and degradation	Low mechanical strength and quick degradation
Resin	Cortoss (Orthovita)	Injectable	1. Integration with bone 2. Mechanical properties match with bone	Non-degradable Bone void filler
Bioglass	Bioglass® (Porex Surgical)	Particulate	Integration with bone	Brittle in a larger size

the body. The lactic acid can enter the tricarbonxylic acid cycle and is excreted as H_2O and CO_2 [27]. In addition, some enzymes, especially those with an esterase activity, can also break down the ester chain in PGA, thus resulting in a degradation of this polymer [28].

Biological polymers, such as collagen, polysaccharides, alginate, agarose, chitin, chitosan, hyaluronan, can easily be utilized in tissue engineering according to their respective properties due to their good biocompatibility. Synthetic polymers and their composites have the advantage of ease of processing and controlled degradation [18]. However, synthetic polymeric materials are chemically and biologically inert and hence unlikely to induce cell adhesion and tissue formation. To overcome the drawback of synthetic materials, natural polymers extracted from the biological ECM have been used to modify synthetic materials to improve their cell adhesion properties [29], while ceramics are added to improve osteoconductivity and integration [30].

Unlike natural polymers which are synthesized by a diverse array of metabolic processes, most synthetic polymers are manufactured by condensation and addition processes. The two main forms (branched and network) of polymers can be chemically modified through the addition of functional chemical groups. Biodegradable polymers include polyesters, polyamides, polyphosphate esters, polyphosphazenes, polyorthoesters and polyanhydrides. The most often used in tissue engineering are polyesters, including poly-lactic acid (PLA) [31], poly-glycolic acid (PGA) [32], polycaprolactones (PCL) [33], poly-(beta-hydroxybutyrate) [30] and polycarbonate [34]. Osteoform (BoneTec) is the only commercial porous structure of the synthetic polymer, fabricated through the use of the leaching process to induce porosity [35]. However, there is no report of their mechanical properties before and after implantation. A PLA granulate (TruGraft™, Osteobiologics), serving as a bone void filler, is also commercially available.

With recent advances in manufacturing technology, degradable synthetic polymer scaffolds have attracted much more attention, mainly due to their compatibility with other technologies, as well as cost effectiveness, easy handling and biocompatibility [36–38].

To enhance osteoinductiveness, bioactive calcium phosphates hydroxyapatite (HA) and tricalcium phosphate (TCP) were incorporated into polyhydroxybutyrate-co-hydroxyvalerate (PHB-PHV) through conventional techniques such as compounding, milling, drying and compression molding.

5. Fabrication of porous polymeric scaffolds

Several techniques have been developed during the last decade, including solvent casting, membrane lamination, phase separation, freeze drying, polymerization, gas foaming, etc, which will be introduced in detail in the following section. The advantages of these traditional fabrication methods are usually the ease of the fabrication process without the need for specialized equipment, as well as easy combination with other techniques, while the disadvantages include (1) the possible retention of a toxic solvent within the polymer, (2) denaturing bioactive molecules incorporated into the polymer, (3) limitation in the shapes that can be obtained, (4) uncontrolled distribution of ceramics added (can only achieve

homogenous distribution in special areas) and (5) difficulty in aligning pores and controlling pore sizes in pre-defined modes [39].

5.1. Solvent casting and membrane lamination

Solvent casting is usually used to fabricate flat sheets and tubes by dissolving the polymer in a suitable solvent within a mold, followed by removal of the solvent. Porosity can be achieved by leaching of particles [40] or through freeze drying [41]. Tubular PLGA/PLLA conduits were made through membrane lamination for regeneration of long bone and blood vessel [42]. The acquired flat sheets from solvent casting and membrane lamination are also useful for controlled release of drugs and proteins [39].

5.2. Phase separation

Phase separation involves separation of fluid phases that contain different components, i.e. solid–liquid or liquid–liquid phase, which can be triggered by thermal changes or chemicals. In solid–liquid phase separation, the polymer is expelled from the solvent when the solvent in the polymer solution crystallizes [32]. Polyester scaffolds with isotropic pore architecture were made with a parallel array of microtubes. In liquid–liquid phase separation, the polymer solution will separate into polymer-rich and polymer-deficient phases. The porous structures form after the solvent is subsequently removed. The porosity and pore size can be controlled by the solvent used, polymer concentration, crystallization rate and temperature gradient applied.

5.3. Freeze drying

An emulsion is created by homogenizing a polymer solvent solution and water, followed by rapidly cooling the emulsion to lock in the original liquid state structure. Freeze drying is used to remove the solvent and water to create a porous structure. Porous structures for bone regeneration fabricated by freeze drying have been reported [43]. Deschamps *et al* have fabricated poly(ethylene oxide terephthalate) (PEOT)/poly(butylene terephthalate) (PBT) scaffolds by freeze drying. The copolymer was dissolved in 1,4-dioxane at 60 °C to gain 10/20% (w/w) polymer solutions, and the sample was frozen at a sequence temperature. Then at room temperature, samples were freeze dried at 0.04 mbar for 48 h, washed with ethanol (24 h) and dried for at least 3 days under reduced pressure. To combine freeze drying with particulate leaching, either sucrose (400–700 mm) or sodium chloride particles (500–700 mm) was added to the solutions. After being freeze dried at 0.04 mbar for 48 h at room temperature, the samples were washed with water to dissolve the particles for 48 h, and subsequently treated with the same methods as the last two steps mentioned earlier. It has also been used in combination with other methods to remove the solvent and create porosity [41].

5.4. Polymerization

Porous scaffolds can also be fabricated by *in situ* polymerization. In theory, it is a simple process and need not include other more complex procedures like solvent removal and salt leaching. However, the major limitations are that only a small quantity of polymer can be synthesized and that the high temperature and metal additives used may adversely affect biocompatibility. A porous polymer block of epsilon-caprolactone has been reported for bone regeneration [44]. Huang *et al* synthesized the PCL homopolymer and PCL/PEG block copolymers by bulk ring-opening polymerization of epsilon-caprolactone. In the PCL homopolymer, polymerization was realized under vacuum at 140 °C for 20 days, introducing ethylene glycol as an initiator and using zinc metal as a catalyst.

In PCL/PEG and OCL/PEG block copolymers, monohydroxyl PEG or dihydroxyl PEG was used, and the reaction time was 7 days. The polymers were dealt with by the dissolution/precipitation method, utilizing dichloromethane as a solvent and ethanol as a nonsolvent, then filtrated and vacuum dried up to constant weight.

A poly (dioxanone-co-glycolide) scaffold with a biocompatible lysine-based diisocyanate cross-linker at each termini was developed, which was mixed with hydroxyapatite or tricalcium phosphate. A nontoxic, normo-thermic crosslinking reaction hardens the scaffolds *in situ* when the catalyst (i.e. diethylaminoethanol and water) was added to the polymer. During the polymerization reaction, they used a non-toxic moiety that causes the release of carbon dioxide to produce a porous network optimal for bone ingrowth within the implant [45].

5.5. Gas foaming

When polymer particulates and porogen (i.e. sodium chloride) are equilibrated with high-pressure CO_2 and subsequently subjected to a quick drop in CO_2 pressure, nucleation and expansion of bubbles from thermodynamically unstable CO_2 together with expansion of polymer particulates will lead to fusion of the particulates. Porous polymeric scaffolds are thus formed after the porogens have been removed. The advantages of this method include an absence of organic solvents and high temperatures, as well as stable processing, which could enhance biocompatibility and application with bioactive molecules. In fact, gas-foamed poly(lactic-co-glycolic acid) (PLGA) scaffolds are most often used as vehicles for the delivery of growth factors [46]. Porous matrices from bioabsorbable materials using gas foaming combined with compression molding and particulate leaching were reported. Solid polymer (i.e. PLGA)/NaCl was compressed to disks, and the disks were then equilibrated with gases (CO_2, N_2, He) under high pressure. Subsequently, the pressure was reduced to ambience, and gas pores nucleated and grew inside the matrix. Conjoint pore structures were attained by leaching the NaCl particle. The porosity of PLGA matrices could be regulated by choosing copolymers with different ratios of lactide:glycolide, PLGA composition and molecular weights during the fabrication process, and angiogenic factors

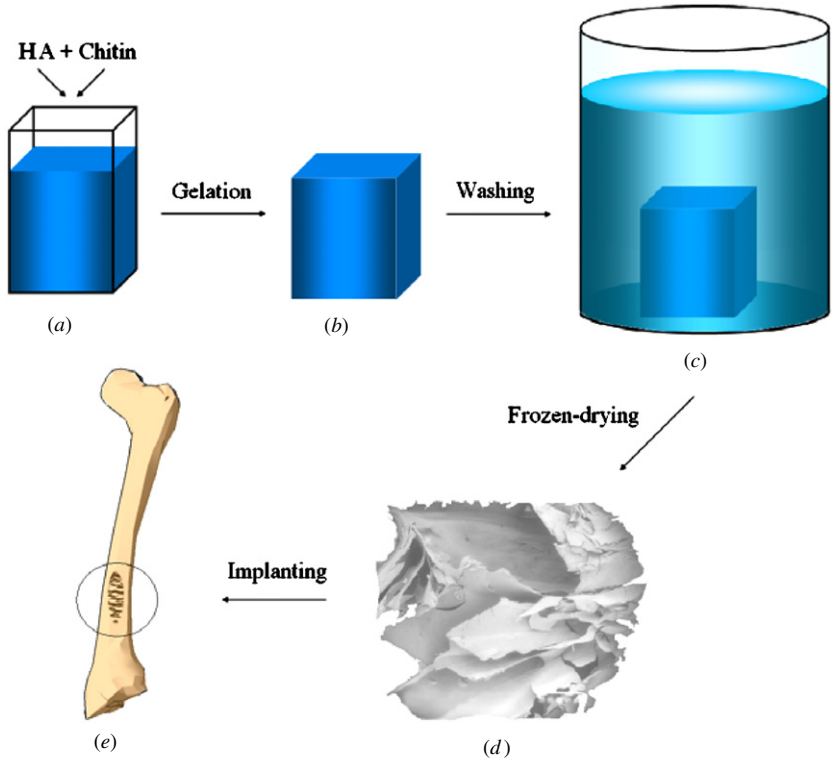

Figure 1. Fabrication of porous hydroxyapatite/chitin scaffolds for bone regeneration with solvent casting and lyophilization. (*a*) Matrix (Chitin flakes) were dissolved in a proper organic solvent (N, N-dimethylacetamide) while hydroxyapatite (HA) powders were added. (*b*) The system gelled with time. (*c*) The acquired gel was immersed in water to remove all potentially harmful solvents. (*d*) The acquired scaffold was lyophilized to make a porous structure. (*e*) The porous scaffolds could be used to fill bone defects.

(e.g., vascular endothelial growth factor) could be incorporated into matrices.

Besides the above physical method, porous scaffolds can also be fabricated by the chemical gas foaming method. Nam *et al* used ammonium bicarbonate as the gas foaming agent. PLLA-ammonium bicarbonate particle mixture was cast, and then immersed in a sufficiently hot water solution. Generation and expansion of ammonia and carbon dioxide bubbles made an interconnected macropore within the scaffold [47].

5.6. Compression molding

A force is applied to a preheated molding material within an open, heated mold cavity, thereby forcing the material into contact with all mold areas while heat and pressure are maintained till the molding material has been cured. The advantage of compression molding is fabrication of complicated forms, easy process and combination with other protocols, as well as amenability to large-scale production. Disadvantages include relatively large initial costs of the mold and tedious optimization of the protocol. Porous scaffolds for bone regeneration fabricated by compression molding have been reported [48].

5.7. Sintering

Sintering is a method of fabricating objects from powder, by heating the material until the particles adhere to each other. Sintering is traditionally used for manufacturing ceramic objects, and has also been used in fabricating polymeric scaffolds. 3D porous PLGA scaffolds were fabricated using the sintering technique. The PLGA microspheres were heated at 75 °C for 24 h after pouring into a mold; then the matrices were cooled to room temperature and de-molded [49].

5.8. Salt leaching

Leaching is a process of removing soluble constituents through the action of a percolating liquid. It is an easy, cost effective and reliable process. However, interconnectivity between individual pores is a limitation. The most often used leaching solute is sodium chloride and sucrose. It has been widely used in porous scaffold fabrication and is easily combined with other techniques, such as solvent casting [40], freeze drying [43], supercritical-fluid technology and gas foaming [50], compression molding and extrusion [42].

The main drawbacks of conventional fabrication are their lack of morphologic controls on internal structures of scaffolds and well-controlled interconnections between individual pores (figure 1). The major advantages and disadvantages of each fabrication technique are listed in table 3.

5.8.1. New generation of fabrication. It must however be noted that customized scaffold design cannot be achieved through these conventional methods. While conventional techniques are inadequate for fabricating customized scaffolds

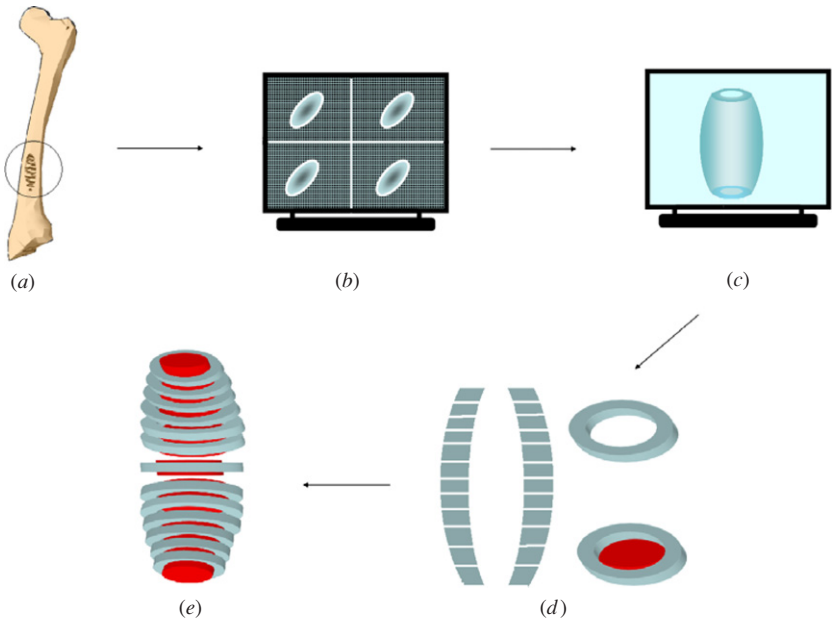

Figure 2. Fabrication of porous scaffolds with rapid prototyping technology. (*a*) The bone defect is located first. (*b*) Information of bone defects was acquired with computed tomography (CT). (*c*) The information was integrated with fabrication software (*d*) making scaffolds layer by layer with a pre-programmed mode. (*e*) The scaffold acquired with designed internal and external structures.

Table 3. Comparison of traditional polymeric scaffold fabrication.

	Ease of handling/cost	Usage of organic solvent/heat	Pros/drawbacks	Compatible with other fabrication processes	Compatible with proteins and genes
Solvent casting	Ease and cost effective	Y	Easy technique/uncontrolled pore size and distribution	N	N
Phase separation	Ease and cost effective	Y	Limited to some polymers/ uncontrolled pore size and distribution	N	Possible
Freeze drying	Ease and cost effective	N	Easy technique and compatible with most of techniques	Y	Possible
Salt leaching	Easy and cost effective	N	Easy technique and compatible with most of techniques	Y	N
Gas foaming	Mildly expensive	N	Equipment needed/ limited to some polymer	N	Y
Compression molding	Mildly expensive	Y	Equipment needed	Possible	N
Polymerization	Mildly difficult	Y	Small amount production/ limited to some polymers	N	N
Sintering	Mildly expensive	Y	Equipment needed/not suitable for polymers	N	N

used in bone tissue engineering, rapid prototyping (RP) or solid free-form fabrication (SFF) could offer a solution. These represent new fabrication technologies that can make polymeric scaffolds with sophisticated structures which are customized to biodata provided by CT and MRI scan of the patients. Rapid prototyping fabricates structures through addition of material layers and particulates in a controlled mode, as specified by a computer program (figure 2). Hutmacher and colleagues [51] provide an excellent review of the application of this new technology platform for scaffold fabrication.

5.9. Extrusion technology-based systems

Scaffold fabrication through fused deposition modeling (FDM) is achieved by laying down the melted polymer layer by layer in a predefined mode. Filaments of a heated thermoplastic polymer are squeezed out like toothpaste from a tube. The thermoplastic is deposited onto the base in an ultra-thin horizontal layer every time and then cooled rapidly since the platform is maintained at a lower temperature. The scaffold fabricated by FDM possesses excellent structural integrity. Scaffolds with a different layer structure and pore morphology which are used for regenerating various

tissues or tissue interfaces can be fabricated by regulating the deposition angle and extent, or distance between polymer elements [52]. Polycaprolactone (PCL) scaffolds made by FDM have been widely used in bone tissue engineering research; composites such as PCL-hydroxyapatite, PCL-calcium phosphate (CaP) have also been used [36, 53–55]. Layer deposition is sometimes non-uniform so that the plane can become skewed. It is not easy to distribute ceramics in a predefined heterogeneous mode.

5.10. Laser-based systems

Both selective laser sintering (SLS) and stereolithography are good examples of laser-based systems. SLS is fast and cost effective, and does not involve the use of organic solvents. The powdered biomaterial will only fuse but not decompose under a laser beam which is selectively scanned over the definite area of powder surface following the designed slice data. The temperature of the scanned surface raises to glass transition temperature; the powders start to fuse and sinter with the particles of the subsequent layer. With depositing, surface fusing and sintering layer by layer, a 3D scaffold is fabricated from a heap of powder. Some scaffolds for bone regeneration have been fabricated using this technique, such as polycaprolactone scaffolds [56] and polyetheretherketone-hydroxyapatite scaffolds [57]. Drawbacks include material shrinkage, uncontrolled porosity, degradation, cross-linkage and oxidation of polymers [51]. Compared to scaffolds obtained though conventional methods, the mechanical properties of the SLS scaffold are weak, and the micro-resolution is not ideal, because of the limitation of powder size and sintered pressure during the process. SLS is not suitable for manufacturing scaffolds which incorporate a bioactive agent such as cells and growth factors, because local temperature is too high during sintering.

In stereolithography, a UV laser is used to scan above a bath of photopolymerizable liquid polymer material. The already utilized photopolymerizable liquid polymers include derivatives of polyethylene glycol (PEG) acrylate, PEG methacrylate, polyvinyl alcohol (PVA) and modified polysaccharides such as hyaluronic acid and dextran methacrylate [51]. As polymerization is initiated, the laser beam creates a first solid plastic layer just below the bath surface. This laser polymerization process is repeated to generate subsequent layers by tracing the laser beam along the design boundaries and filling in the 2D cross-section of the model. Scaffolds made with this method have been reported [58, 59]. Nevertheless, its application in scaffold fabrication is limited, for except those described previously, and it is relatively difficult to choose photopolymerizable biomaterials that have the required biodegradability, biocompatibility and mechanical properties. When fabricating refined and complicated structures, deformation may occur, and micro-scale resolution is compromised [51].

5.11. Printing-based system

3D printing has increasingly been used in the tissue engineering field, ever since it was first developed at MIT [58]. After spreading a layer of fresh powder over a platform, a print head deposits the binder solution onto the powder bed. After the 2D layer profile is printed, a fresh layer of powder is laid down. The process will be repeated until the entire scaffold is fabricated. During the processing, the flow rate and drop position can be controlled from a computer-aided design (CAD) model [60], so it can be used to fabricate a complicated internal structure as well as macroscopic 3D shapes simultaneously, and as the most widely applied rapid prototyping technology in scaffold fabrication [61], both ceramic [62] and polymeric [63] scaffolds have been made with 3D printing. The resolution limitation, the removal of unbound powder within the porous structure and the use of organic solvent binders are the major drawbacks. 3D printing has much potential in fabricating biological and drug macromolecule controllable released scaffolds if non-toxic binder solutions are utilized, while bioactive agents (i.e. growth factor, gene, etc) can be incorporated. However, the mechanical property of scaffolds with a particle size of 100 μm drops abruptly during degradation [64]

5.12. Assembly technology-based system

A structure is assembled using small building-block units, after the building blocks of various designs are pre-fabricated using lithography, or other microfabrication technologies. The blocks are then assembled by a computer-controlled system. A robotic microassembly technique was developed to fabricate microscopic pre-fabricated blocks using a precisely controlled robot. This technique, also known as microparts microassembly, can control the dimensional distribution of blocks pre-seeded with different bioactive agents, cells, etc, which may be valuable for multiple and structural tissue interface engineering [65]. Presently, this has not been widely used for scaffold fabrication.

5.13. Indirect solid form fabrication

A negative mold is fabricated based on the scaffold design and the scaffold is cast using the desired polymeric and/or ceramic biomaterials in indirect solid form fabrication. Though SFF techniques described above could fabricate scaffolds with desired global pores and fine internal pore interconnectivity, micro-scale resolution is not ideal. On the other hand, conventional manufacturing methods could fabricate scaffolds with locally porous internal architectures, but interconnectivity among individual pores is a concern. The indirect solid form fabrication is able to combine the benefits of conventional manufacturing and direct SFF fabrication, by making local pores through conventional manufacturing methods, such as particle leaching, while controlling the 3D shape, internal interconnective network and micro-scale resolution with a pre-designed program [66]. This technology can benefit much from research on the microenvironmental effects of scaffolds on bone regeneration by providing controlled microenvironments.

6. Combination with other techniques and other materials

6.1. Enhancing the cellular microenvironment

Cellular reaction to the surface landscape of micrometer-range features such as grooves, ridges and wells has been well established for decades [67]. *In vivo*, the surface properties of the extracellular matrix probably have a profound effect on cellular physiology which in turn has important implications for development, differentiation and regeneration [68]. Optimizing the stiffness of scaffolds may be beneficial for osteoblast functionality and bone regeneration. Except for porosity and pore size, the 3D microenvironment of scaffolds is critical for osteogenesis [13, 14]. Many techniques have been used to optimize 3D microenvironments of scaffolds; the ion implantation could effectively improve the surface stiffness of polymer materials, boost up the biocompatibility as well as the capability of resisting abrasion and cauterization [69]. Utilizing aligned electrospun nanofibers [70] and mechanical topography [71] can result in improved cell orientation and differentiation. It would be advantageous to control the porosity, pore size and microenvironment with a single fabrication technique, preferably with a controlled gradient pore size, adjustable porosity and varying microenvironments at different parts of the scaffold. For this purpose, there are several techniques which have been used for fabricating internal micro-controlled scaffolds, such as the above-referenced stereolithography, 3D printing, selective laser sintering, laser-scanning lithography, microrobotics, MEMS-based fabrication, etc [72].

6.2. Cell/gene/protein delivery

Cells, genes and proteins play key roles in osteogenesis, both *in vitro* and *in vivo*. Currently, there are two strategies of incorporating cells into scaffolds: (i) seeding of cells onto the scaffold after fabrication, (ii) incorporating cells into the scaffold during the fabrication process. Obviously, the second process is considerably more attractive. Progress in cell printing techniques appears promising [73], and can potentially overcome the limitations associated with conventional cell seeding and *in vivo* cellular recruitment. However, the technique is currently immature and has not been well integrated with other fabrication technology platforms. With regards to genes and proteins, there have been some progress on their controlled delivery and integration with scaffolds [74]. Nevertheless, more research on controlled release and scaffold fabrication is necessary. As described earlier in microparts microassembly, prefabricated blocks are first seeded with different biological agents such as cells and growth factors before final assembly. Building-block units of different designs are generated using conventional methods or microfabrication technologies such as lithography. The scaffolds thus fabricated have not only the required biological and physical properties, but also the designed spatial distribution of various cells, proteins, genes, etc [65].

6.3. Addition of ceramics

Currently, there is no single scaffold which can meet all the diverse requirements of bone tissue engineering, given the current limitation of our background knowledge on bone regeneration and material science. The most commonly used biomaterials for bone regeneration fall into two categories, ceramics and polymers. Inorganic minerals including calcium phosphate, hydroxyapatite, calcium carbonate and calcium sulphate have been employed as bone substitute materials They can facilitate the migration and differentiation of osteoprogenitors and integrate with host bone tissue [75]. However, their low tensile strength and brittleness limit their usage in locations that are exposed to significant torsion, bending or shear stress [76]. Their uncontrolled degradation *in vivo* may lead to dramatically increased extracellular concentrations of calcium and phosphate ions, which in turn can trigger cell death and have other adverse side effects [77]. Careful design and polymer matrix embedment are often used to provide mechanical support and to optimize the favorable properties of ceramic-based scaffolds. The carefully designed composites of biodegradable polymers and bioactive ceramics possess a stable mechanical property, finer biocompatibility, improved tissue interaction and osteoconductivity. The most commonly used biodegradable polymers in bone tissue engineering involve saturated aliphatic polyesters (PLA, PGA and PCL), polypropylene fumarate (PPF) and polyhydroxyalkanoates (PHB, PHBV, P4HB, PHBHHx, PHO). Ideal composite scaffolds could be made through thermally induced phase separation (TIPS), solvent casting and particle leaching, solid freeform fabrication techniques (SFFT), microsphere sintering and coated scaffolds [78].

Porous PLGA/HA composite scaffolds were manufactured by the modified gas foaming and particulate leaching method. With added ceramic on the scaffold surface, osteoconductivity and wettability of the scaffolds were obviously improved [79].

7. Future directions

Current research efforts are focused on recruiting osteogenic cells to migrate into scaffolds and subsequently inducing them to undergo proliferation and differentiation. Another critical factor in scaffold design is to prevent the migration and growth of fibroblasts, which is the major cause of non-union of fractures. Besides bone tissue engineering, scaffolds can potentially also have useful applications in hyaline cartilage, vascular and neuron regeneration. Further progress will depend on deepening our understanding of cell chemotaxis and material science. Four potential strategies include

(a) use of the concentration gradient of chemicals or growth factors to repel fibroblasts,
(b) addition of chemical groups to inhibit the attachment of fibroblasts on scaffolds,
(c) selective inhibition of fibroblast growth so that they will be overwhelmed by other cell types,

(d) minimizing non-specific cell adhesion to scaffolds while maximizing the specificity of cell adhesion via incorporated peptides; alginate and polyethylene glycol (PEG) exhibit little native cell adhesion and are therefore widely used as a synthetic ECM for this reason [80].

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