Biomaterials for Bone Tissue Engineering

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Bone tissue loss caused by various reasons including the accident trauma, tumor removal, or congenital deformity, etc., is a challenging problem in the clinic of orthopeadics, which brings the issue of bone grafting. Reconstructive surgery is based upon the principle of replacing these types of defective tissues with viable, functioning alternatives. It is reported that over 450,000 bone-grafting procedures are performed each year in the United States, and the number is expected to increase with the life expectancy increases [1]. Up to now, autologous transplantation is still considered as the golden standard procedure to orthopedic surgeons [2]. However, although autograft has good compatibility and no immunological response, the limited donor bone supply and additional trauma have limited its applications. Severe immunological problems and high risks of disease transmission have also limited the allograft applications, although a very careful screening process has eliminated most of the disease-carrying tissue [3]. Tissue engineering has emerged as a promising way to reconstruct and regenerate the lost or damaged bone tissues. Since the late 1980s, tissue engineering has been attracted much attentions in the fields of science, engineering, medicine and the society [4]. Tissue engineering has been defined by Laurencin et al. [5] as "the application of biological, chemical, and engineering principles towards the repair, restoration, or regeneration of tissues using cells, scaffolds and growth factor alone or in combination". There are two tissue-engineering approaches in regeneration of tissues or organs [6]. The initially described approach is that a small amount of cells harvested from the patients themselves are proliferated in vitro and then seeded into the appropriate three-dimensional scaffold in the presence of growth factors. The cells with growth factors under proper conditions will secret various extracellular matrix materials to create an actual living tissue in vitro, which will be implanted back to replace the damaged or defected tissues. Another approach is that the scaffold materials loaded with or without growth factors are implanted into the aim sites directly, which will guide the tissue formation in situ combining the degradation of scaffold materials. In the past several years, scaffolds, cells and growth factors have been considered as the three main factors for tissue engineering [1, 7]. Recently, with the development of materials science, it is controversial that growth factors are essential for bone tissue engineering. The new viewpoint is that the growth factor is not necessary for the bioactive material, which can induce

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the tissue formation and enhance the secretion of growth factors from the host bone cells.

It is now generally accepted that one of the important issues for tissue engineering is the development of ideal scaffold materials. Since the human body is a complex and sensitive system, the requirements of the scaffold materials for tissue engineering are strict and extremely challenging. Up to now, the optimum material for tissue engineering scaffold has not yet been developed [8]. Nontoxicity and biocompatibility are the basic requirements for scaffold materials. The material should not have the potential to elicit an immunological or clinically detectable primary or secondary foreign body reaction [9, 10]. Suitable biodegradability is another essential requirement of the scaffold materials for tissue engineering; the resorption rate should match the tissue growth. Furthermore, the material should have proper mechanical properties matching those at the implant site, and can provide sufficient support to the new tissue during degradation until the new tissue is able to support itself [9]. In addition, the ideal scaffold materials for bone tissue engineering should also promote cell growth, cell differentiation and tissue regeneration. Synthetic materials for the bone tissue engineering have been studied extensively in the recent decades with the development of material sciences. Ceramics, polymers and their composites have all been investigated as scaffold materials for bone tissue engineering [1, 4, 8, 11–15].

Biodegradable Polymers

The biodegradable polymers used in bone tissue engineering can be classified into two categories. One is the natural-based polymers, such as starch, alginate, chitin/chitosan, collegen, silk, hyaluronic acid [16–23]. Another type is synthetic biodegradable polymers, like PLA, PGA, PLGA, PCL [24–26]. Most natural polymers are biocompatible, degradable and readily solubilized in physiological solution. However, they have some drawbacks, like immunogenecity, difficulty in processing, and a potential risk of transmitting animal-originated pathogens [2]. Among all the natural polymers, collagen is the most widely studied one. It is well known that collagen is the most abundant extra cellular matrix (ECM) protein and is originally secreted by osteoblasts, so it has a good biocompatibility with bone tissues [27]. However, the poor mechanical strength and rapid degradation rate greatly limited its applications as implantable porous scaffolds for bone tissue engineering.

Compared to natural polymers, synthetic polymers indeed have better chemical and mechanical properties. Moreover, synthetic polymers can eliminate the risk of disease transmission and immunogenecity. Synthetic polymers can provide versatile properties, since they can be synthesized under controlled conditions. The chemical and mechanical properties, degradation rate of synthetic polymers can be tailored by molecular weights, functional groups, configurations, and confirmations of polymer chains [2].

The most commonly used biodegradable synthetic polymers for bone tissue engineering are saturated poly-α-hydroxy esters such as poly lactic acid (PLA), poly glycolic acid (PGA), and their co-polymers (PLGA). The degradation of these polymers is through the procedure of de-esterification. The degradation products of these polymers are lactic and glycolic acids, which could be safely absorbed or derived by body metabolism. PLA, PGA and their copolymers have been approved by the US Food and Drug Administration to use as products and devices in clinic.

However, there are some drawbacks, which have limited their further applications [4, 25, 28, 29]. The hydrophobic characteristics of these polymers resulted in a poor cell attachment. The hydrophilicity of PLA and PGA scaffolds was effectively improved by Mikos et al. [25] using a two-step immersion in ethanol and water. Another problem of PLA, PGA and their co-polymers are aseptic inflammations, which is caused by the excessively low local pH value resulted from the accumulation of acidic degradation products. It is reported that aseptic inflammation occurred in a small but significant percentage (8 %) of patients [28]. In addition, PLA and PGA have no ability to induce apatite

formation in SBF, indicating a low bioactivity. Insufficient mechanical strength also inhibits their applications in bone tissue engineering

Poly (ε-caprolactone) (PCL) is another type of aliphatic polyester polymer for bone tissue engineering. It has been used to enhance bone ingrowth and regeneration in the treatment of bone defects. The degradation rate of PCL is much lower than that of PLA and PGA, which makes it less attractive for tissue engineering [30]. It has been reported that it took 3 years for PCL with a molecular weight of 50,000 to be completely removed from the host body [26, 31].

Polyhydroxyalkanoates (PHA) are also polyesters used in the field of bone tissue engineering, which are produced by microorganisms under unbalanced growth conditions. Up to date, only several polymers in the PHA family are available in sufficient quantity for applications in the bone tissue engineering, such as poly 3-hydroxybutyrate (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly 4-hydroxybutyrate (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly3-hydroxyoctanoate [32]. This kind of polymers is also characterized with good biocompatibility and biodegradability and has been investigated as bone graft substitutes. The copolymerizing among the PHA polymers can dramatically change the properties of the material [33]. Among all the PHA polymers, PHB has been attracted the most attention as materials for bone tissue engineering, since it has been demonstrated that PHB showed a consistent favorable bone tissue adaptation response with no evidence of an undesirable chronic inflammatory response after implantation up to 12 months [34]. Doyle's work also showed that bone is rapidly formed close to the material and subsequently becomes highly organized, with up to 80 % of the implant surface lying in direct apposition to new bone [34]. However, pyrogens like endotoxin incorporated in the PHA polymers during the producing process may be a problem for its implantation uses. The investigations showed that pyrogens incorporated in the PHA polymers can be reduced by oxidizing agent, like hydrogen peroxide or benzoyl peroxide [35]. In addition, the limited availability and time-consumption extraction procedure are also the challenging issues for PHA polymers as bone tissue engineering materials [13].

In addition, copolymers of polyethylene glycol (PEG) and poly butylene terephathalate (PBT), commercially named as Polyactive™, are another type of polymers for bone tissue engineering [6, 36]. It seems the Polyactive™ is the only polymer which can form a bone bonding when implanted *in vivo* [37, 38]. It has been reported that the apatite layer formed on the surface of Polyactive™ is similar to that formed on the surface of bioactive ceramics [39]. Due to its bone bonding properties, Polyactive™ has been studied as bone tissue engineering material.

Three dimensional polymer scaffolds have been prepared by the following techniques.

Solvent Casting/Particulate Leaching

Solvent casting/particulate leaching is the most conventionally used methods to prepare porous polymer scaffold. In this technique, the polymer is first dissolved in an organic solvent, such as chloroform and methylene chloride. Salt particles with a desired particle size are then dispersed uniformly in the polymer solution. The polymer solution with salt particles is then cast in a glass container. After the evaporation of organic solvent, the polymer-salt particle composites were then immersed in water to leach out the salt particles to get a porous polymer structure [40]. The porous scaffold prepared by this technique could have a porosity ranging 87–91 %, which are predominated by the amount of salt particles. Moreover, the pore size of the scaffold could be controlled by the size of salt particles. Both porosity and pore size are undependent on the particle type. However, the solvent casting/particulate leaching technique only works for thin membranes or 3-D specimens with very thin wall sections. Otherwise, it is not possible to remove the soluble particles from within the polymer matrix [8, 41]. Mikos et al. [42] tried to fabricate 3-D structures by laminating the porous sheets using the technique. Another drawback of this technique is the extensive usage of highly-toxic solvents.

Emulsion Freeze-Drying/Thermally Induced Phase Separation

The emulsion freeze-drying technigue was first introduced to the field of tissue engineering by Whang and his colleagues [43, 44]. This technique consists of creating an emulsion by homogenization of a polymer solvent solution and water, rapidly cooling the emulsionto lock in the liquid-state structure, and removing the solvent and water by freeze-drying. Scaffolds with porosity greater than 90 %, pore size ranging from 15 to 200 μm were obtained using this method [44]. The scaffold also showed high volume of interconnected micropores and a high specific surface area (58–102 m^2/g) [45].

Gas Foaming

In the gas foaming technique, a small amount of gas (CO_2 or N_2) was dissolved into polymers under certain pressure and temperature levels. After the gas was released, a porous polymer scaffold formed. The concentration of CO_2 in the polymer, temperature, pressure, soaking time, depressurization, molecular weight and chemical composition of the polymer will all have significant effects on the pore structure [46, 47]. Barry et al. [48] reported that a rapid release of CO_2 gives smaller pores, while a slow release gives larger pores. This technique can get a pore size in a very wide range of 88–198 μm [49]. A porosity ranging 64.5–83.4 % are achieved in PLA scaffold [49]. The highlight of this technique is that it is a fully solvent-free technique.

Rapid Prototyping

Since the middle 1990s, rapid prototyping method (RP) has been introduced into the field of tissue engineering to fabricate scaffolds [50–52]. Rapid prototyping is a technique based on the advanced development of computer and manufacturing, which is also called solid free form fabrication (SFF) [53]. The potential to intimately control the microstructure of porous channels and the overall macroscopic shape of the scaffolds makes rapid prototyping an ideal process for fabricating scaffolds [54]. It can produce complex products rapidly from a designed model in the computer as well as digital data produced by an imaging source as computer tomography (CT) or magnetic resonance imaging (MRI) [55]. Another advantage of this technique is the structure of the scaffold is 100 % interconnected macropororous [8]. In addition, parameters, such as the porosity, interconnectivity, pore size and geometric stability of the scaffolds fabricated by the rapid prototyping can be controlled more precisely than conventional fabrication techniques [40, 56].

In addition, the polymer scaffolds have been also prepared by microsphere sintering, replication from natural materials, etc. Li et al. [57] prepared PDLLA scaffolds with a similar macroporous structure to natural cancellous bone using calcined bone as a negative mould. The scaffolds were fabricated by immersing the calcined bovine cancellous bone into PDLLA solution under repeated vacuum. The negative template was removed by a following treatment of the scaffolds in hydrochloric acid. The morphology and structure of the obtained scaffolds are similar to the organic matrix of natural concellous bone blocks. Moreover, the compressive strength and modulus of the obtained scaffolds could be adjusted by the concentration of polymer solution, which are significantly improved as compared to the scaffolds prepared by sovent casting/particulate leaching technique.

The most common problems for synthetic polymers are acute or chronic inflammatory response, which was due to the decreased local pH value caused by the acidic hydrolytic degradation products. No bioactivity is also a common problem for polymeric materials. Incorporation of basic ceramics into polymers could neutralize the local acidity effectively and could increase the bioactivity simultaneous.

Bioceramics

The use of ceramics in bone repair has a very long history, which can be traced back to thousands of years ago. The ceramics used at the early

stage are nearly bioinert in the biological environment, such as alumina (Al_2O_3) [58], zirconia (ZrO_2) [59], calcium sulphate ($CaSO_4$) [60] and calcium carbonate (coral) [61]. Compared with the bioinert ceramics, calcium phosphates and bioactive glasses and glass-ceramics can form a bonding interface with host tissues. Due to the good biocompatibility and bioactivity, they have been widely investigated as bone graft materials and some products have been successfully used in clinic.

Calcium Phosphates

It is well known that the inorganic components (over 60 wt%) of bone are hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HA) [13]. Therefore, some calcium phosphates, like HA, tricalcium phosphates (α-TCP and β-TCP), octacalcium phosphate (OCP), calcium pyrophospates ($Ca_2P_2O_7$) have been intensively investigated as bone grafts [62–65]. The study of calcium phosphates as biomaterials for bone repair was started from the middle of 1970s, by Jarcho from the USA [66], de Groot from Europe [67], and Aoki from Japan [68], simultaneously. Calcium phosphate ceramics have been proved having good biocompatibility with bone and they can bond to bone without any fibrous capsule [69, 70]. Synthetic hydroxyapatite with a stoichiometric composition has been extensively studied as bone replacement material [63, 69]. It has been proved that porous HA has excellent biocompatibility and osteoconductivity, and some commercial products of HA have been used in clinic [69, 71, 72]. Porous hydroxyapatite (such as ProOsteon® and Interpore®) has been prepared by the hydrothermal conversion of corals, which caused a replacement of phosphate ions for the carbonate ions and changed the crystal structure to calcium phosphate [73–75]. The porous hydroxyapatite scaffolds prepared by this method have a uniformity of pore size ranging from 60 to 500 μm and have complete pore interconnection. However, the porosity of the HA scaffolds prepared by this method have a narrow porosity distribution ranging from 46 to 48 %, which is not good to the mechanical properties and biological applications. In addition, the final composition of the scaffold is hard to control, due to the impurities in the original corals [76].

The porous HA scaffolds can also be prepared by the demineralization of natural bone (Endobon®) [77], polymer foaming [78], H_2O_2 foaming [79], freezing casting [80], replicas of porous structures [81, 82], etc. The most simple and commonly used method to prepare porous ceramics is the polymer porosifier method. The parameters such as porosity, pore size and interconnectivity can be adjusted by the amount and size of porogen particles. It was reported that bone formation occurred in porous HA scaffolds mixed with fresh bone marrow cells after 3 weeks implantation, which was enhanced by a preculture process of bone marrow cells [83, 84].

However, it has been proved that the stoichiometric HA has limited ability to form chemical bonding with the host tissues and it also has limited ability to stimulate the bone formation [85]. Moreover, the stoichiometric HA has a very low degradation rate, and it almost remains as a permanent fixture susceptible to long-term failure [86]. The above drawbacks have limited its application in bone tissue engineering. Actually, the mineral phases of the natural bone differ from stoichiometric HA in composition, stoichiometry, and some properties, which are calcium deficient hydroxyaptite with some positive (Na^+, Mg^{2+}, K^+, etc.) and negative (CO_3^{2-}, F^-, Cl^-, etc.) ion substitutions. In particularly, the carbonate ion concentration in the bone apatite is up to 8 wt% [87]. These substitutions in the bone apatite structure play important roles in its biological activity. Recent years, substituted apatites have been attracted increasing interests [88–96]. The use of these substituted apatites in bone tissue engineering is still exploring. The substitution in the structure of HA indeed increased the bioactivity and bioresorbability of the material. In addition, Si incorporation in the calcium phosphates has been shown to increase osteogenesis of osteoblast-like cells [97]. Precipitation of a biological carbonated hydroxyapatite onto the surface of a scaffold by biomimetic method has also been extensively studied to improve the bioactivity of the scaffold [98–105].

Of all the substituted HA, Si-substituted HA have been investigated widely. Si has been found

to be essential for new bone formation, and it was found that Si localized at active calcification sites in the bones of young mice and rats [106]. A recent research has been found that a dietary Si intake was positively and significantly affecting the bone mineral density of humans [107]. Trace levels of Si in the structure of hydroxyaptite have remarkably increased the biological performance in comparison to stoichiometric HA [108]. The Si-HA has always been synthesized by wet chemical methods where Si is added through a silicon source, such as tetraethylorthosilicate (TEOS) and Si IV acetate ($Si(COOCH_3)_4$) [109–111]. Some research also added nanoparticulate silica during the precipitation and sintering of an amorphous calcium phosphate to fabricate silicon doped HA [112]. Si substituted hydroxyapatites have been proved to have the ability to induce biomimetic precipitation in a physiological solution due to the release of silicon [113]. The in vivo investigates have also shown that bone ingrowth into silicon-substituted HA granules was remarkably greater than that into pure HA [114]. Currently, two different Si-substituted calcium phosphates have been developed as bone substitute applications commercially [85]. Single phase Si-HA have been manufactured commercially by Apatech Ltd. under the trade name Actifuse™. Multiphase Si-stabilized calcium phosphates have been produced by Millenium Biologix Corporation under the trade name Skelite™.

β-TCP is another calcium phosphate material widely used for bone tissue engineering. Compared to stoichiometric hydroxyapatite, β-TCP has a much higher dissolution rate. Many researches have shown that the dissolution rates of β-TCP are much higher than that of HA, which is strongly dependent on the testing media [76, 115]. β-TCP has been accepted and used as a biocompatible, and resorbable material for bone repair. However, some studies have also showed that the high dissolution rate of β-TCP adversely accelerates material resorbability and elicits immunological response [116, 117]. There are some different reports about the degradation rate of β-TCP *in vivo*, which is dependent on the characteristics of the material used and the sites where the material is used. Similar to HA, substituted β-TCP have also been intensively investigated to pursue various properties [118–120]. It has been shown that magnesium substitution in the structure of β-TCP could decrease the biodegradation rate. The Si substitution enhanced the biological properties of β-TCP. The impurities in β-TCP may affect its sintering properties.

Parameters, like pore size and distribution, porosity and connectivity of porous β-TCP scaffolds prepared by the traditional methods are difficult to be controlled precisely. Recently, a new method has been developed to prepare porous β-TCP scaffolds, which can fully control the macroporosity, in terms of shape and size of pores and their interconnectivity [121, 122]. In this technique, β-TCP scaffolds were prepared by impregnating of an organic edifice with proper β-TCP suspension followed by sintering at elevated temperatures. The organic edifice served as the template, which was prepared by preheating polymer microspheres at a temperature higher than polymer glass transition point to make them bind together. The pore structure of the scaffolds can be controlled by the treatment parameters of polymer microspheres. Pore size, shape and porosity are controlled by the size, shape and amount of polymer spheres. The interconnection between the macropores depends on the amplitude bridging between polymer balls, which are controlled by the temperature and dwell time of the treatment of polymer frame. β-TCP scaffolds prepared by this method have good pore connectivity. Xie et al. [123] investigated the proliferation of stem cells inside the β-TCP scaffold prepared by the above described method, and showed that after a flow perfusion culture, the cells survived and proliferated through the whole scaffolds indicating its good connectivity and nutrition supply. The in vivo results also showed that these scaffolds had good osteoconductivity and good vascularization [124, 125]. In addition, parts with a gradient distribution of pore size, or interconnectivity to pursue specific properties can be easily handled by this technique. The β-TCP scaffolds prepared by this method have been commercialized by Shanghai Bio-Lu Biomaterial Corporation.

The poor mechanical property is the major problem of the porous bioceramic scaffold. Zhang et al. [126] prepared porous β-TCP scaffold inspired from the structure of natural bone, which is characterized by a macrostructure feature of porous cancellous bone inside with compact (or cortical) bone outside. The bioinspired structural β-TCP scaffolds were designed with a structure of porous cancellous structure (porosity: 70–95 %) inside and dense compact shell (porosity: 5–10 %) outside. The scaffold with this kind of bioinspired structure improved the mechanical properties.

Biphasic Calcium Phosphate

Biphasic calcium phosphates (BCP) are also important members of the calcium phosphates family. BCP are ceramics which containing both hydroxyapatite and TCP. It has shown that BCP exhibited prior bone repair and regeneration abilities than pure HA or β-TCP [127, 128]. The degradation rate as well as other properties can be controlled by the HA/TCP ratios to a certain degree [127–131]. It has been reported that BCP have osteoinductivity when implanted in muscle tissue [132, 133]. Grundel Ng et al. [134] seeded osteoprogenitor cells derived from periosteum onto HA/TCP scaffolds and then intramuscularly implanted them in nude mice after 4 weeks i*n vitro* culture, which indicated that HA/TCP showed superior in early bone formation than pure HA. BCP with 60 % HA and 40%TCP has been manufactured commercially under the trade name Triosite. Another BCP with 65%HA and 35%TCP has also been commercialized by Teknimed Limited Company under the name Ceraform.

Bioactive Glass and Glass-Ceramics

In 1969, Hench et al. found that some glasses with specific compositions had excellent biocompatibility with natural bone and they can form a chemical bonding with the host bone [135]. These glasses have been called as bioactive glasses, which contain SiO_2, Na_2O, CaO and P_2O_5 in specific proportions and have been commercially available as Bioglass®. The concept of "bioactive" has been aroused since then. The bioactive material was defined as "one that elicits a specific biological response at the interface of the material which results in the formation of a bond between the tissues and the material" [136]. The bioactive glasses have the ability to induce calcium-deficient, carbonated hydroxyapatite formation when in contact with physiological solutions or implanted in vivo [137, 138]. It has been accepted that the essential requirement for an artificial biomaterial to exhibit a bone bonding to living bone is the formation of a bone-like apatite layer on its surface in body environment and it has been used as a criteria to evaluate the bioactivity of biomaterials [135–138]. The mechanism of the bioactivity of bioactive glasses has been thoroughly investigated by Hench [137], which is due to complex ion exchanges occurred on the surface of bioactive glasses. Silicon is considered to play a key role in the bioactivity of bioactive glasses, which can induce the apatite nucleation. The ionic dissolution products from bioactive glasses were shown to enhance the proliferation of osteoblasts, upregulate seven families of genes that control osteogenesis and induce the synthesis of growth factors [114, 139–141]. The bioactive glasses have also been found to enhance enzyme activity, vascularization and the differentiation of mesenchymal cells into osteoblasts [13].

The bioactivity of the bioglass is composition dependent, which has been systematically summarized previously by Hench [140]. Only a limited range of bioactive glass compositions in the system SiO_2-Na_2O-CaO-P_2O_5, with less than 55 wt% SiO_2 exhibit Class A bioactivity [142], which are osteoproductive as well as osteoconductive, and can bond to both bone and soft connective tissues. Class B bioactive materials only exhibit osteoconductivity. In recent years, sol–gel technique has been used to prepare bioactive glasses [143–148]. The bioactive glasses produced by sol–gel method, also termed bioactive gel-glasses, have a higher bioactivity and resorb faster than the conventional glasses with the same composition [148]. The compositional range of Class A bioactive behaviour is considerable

extended for the sol–gel derived bioactive glasses over the conventional ones [142]. It is reported that P_2O_5-free bioglasses in the system SiO_2-Na_2O-CaO are also bioactive, which implies that P_2O_5 is not an essential component for bioactivity of the material. The great characteristics of the gel-glasses are the high specific surface area and fine porous structure [149–151]. In addition, the structure and chemistry of bioactive glasses can be tailored at a molecular level by sol–gel method [152]. The above features can further influence the biological activities, such as cell differentiation and proliferation, enzyme activity and tissue regeneration of the bioactive glasses [149, 150]. 45S5 Bioglass® has achieved much success in clinic as a treatment for periodontal disease (Perioglas) and as bone filling material (Novabone) [137, 140].

At the very beginning, the bioglass® were used in clinical applications only in the granule or bulk forms, since the bioglasses produced by high temperature melting have a poor machinability and are hard to be processed. Bioactive glasses have gained new attention recently as promising scaffold materials. Some works have attempted to introduce porous structure into melt-derived bioactive glasses. Yuan et al. [153] reported a method to prepare porous bioglass ceramic by H_2O_2 foaming method using ball-milled Melt-derived 45S5 Bioglass® powder. However, the porosity and pore interconnectivity of the porous scaffold prepared by the above method are not satisfied. Chen et al. [154] prepared porous bioactive glass scaffolds with porosity over 90 % by the replication method using polyurethane foam as a sacrificial template. The Melt-derived 45S5 Bioglass® powders were also mixed with polymer porogen to make porous scaffold using the traditional porosifier method [155, 156]. However, the scaffolds made by these method all had high-temperature treatment histories. It has been reported that crystallization of bioactive glasses will result in a decrease in bioactivity [157] and even turns a bioactive glass into bioinert material [158].

Hench group at Imperial College has produced scaffolds with hierarchical pore structure by foaming sol–gel derived bioactive glasses [10, 159–162]. In the first step of this method, a sol is prepared from a silica based alkoxide precursor, such as tetraethyloxysilane (TEOS). After complete hydrolysis, the sol is foamed using surfactants under vigorous agitation in air. The foamed sol with a high viscosity is then cast into sealable moulds, followed by aging, drying and thermal stabilization at 600–800 °C. To get a better mechanical property, the foamed scaffold can be further sintered at an elevated temperarure. Unary, binary and tertiary systems have all been successfully foamed as scaffolds [162]. The scaffold prepared by this method is comprised of large interconnected macropores (10–500 μm) and mesoporous pores (2–50 μm). The macropores with diameters over 100 μm, enable cells growing into 3D structures. The mesoporous structure is the inherent characteristic of sol–gel derived bioactive glasses, which can dissolve at a rate that releases the proper ionic concentration for osteogenesis. The pore interconnects in the foamed scaffolds are larger than 100 μm, which benefits to a 3D cellular structure formation and vascularization [142, 161]. Various parameters, including glass composition, surfactant concentration, gelling agent concentration, treating temperature, etc. all have an effects on the 3D structure of the foamed scaffolds [161].

In addition, many researchers are trying to prepare bone scaffolds with biomorphic structure to cancellous bone by using natural materials as templates [57, 82, 163]. However, most of the previous works just mimicked the macroporous structure of the natural materials. The fine microstructures of natural cancellous bone, such as ordered assembly of the nanoparticles on the pore wall, multimodal pore distribution on the micro- and nanometer scale, are challenging to mimic. The macroporous structure enables cell ingrowth, while the micro/nanoporosity improves fluid flow through the ceramics, providing nutrition for cells inside the scaffold [164]. In our lab, Xia et al. (unpublished data) produced porous bioactive glass scaffold with both similar macrostructure and microstructure to those of natural cancellous bone using a replication method. The obtained bioactive glass scaffold possessed a porosity of 89.3 %, which is similar

to the calcined bone (86.6 %). The compressive strength of the obtained scaffold is also similar to that of the calcined bone.

The degradability of these bioactive glasses is mainly based on dissolution process, which is influenced by the particle size, glass composition, etc. [165]. However, overall the biodegradation of these materials is considerably low [164].

Poor mechanical properties are the big drawbacks for bioactive glasses as scaffolds for tissue engineering. A glass can be converted to glass-ceramic by heat treatment. The crystallized glass-ceramic exhibits superior mechanical properties to the parent glass. In the early 1980s, Kokubo and co-workers developed a glass-ceramic, which contains crystalline phases of apatite and β-wollastonite and was termed A-W glass-ceramic [166, 167]. A-W glass-ceramic is prepared from the parent glass in the pseudoternary system $3CaO \cdot P_2O_5$-$CaO \cdot SiO_2$-$MgO \cdot CaO \cdot 2SiO_2$ with a composition of 38 wt% apatite, 34 wt% wollastonite and 28 % residual glass. This glass-ceramic material possesses both excellent mechnical properties and good bioactivity, and can be easily machined into various shapes, which has been used successfully in clinic as bone replacement under the trade name of Cerabone® [167]. The bending strength of A-W glass-ceramic is about 215 MPa, which is almost twice that of dense hydroxyapatite, and also much higher than that of bioglasses [11]. The fracture toughness is also much higher than that of hydroxyapatite and bioactive glasses [13]. The higher mechanical properties of A-W glass-ceramic are attributed to the precipitation of wollastonite. A-W glass-ceramic can form a bone bonding with natural bone through a thin layer of biologically active apatite and it can also induce apatite formation in an acellular simulated body fluid having ion concentrations nearly equal to those of human blood plasma (termed SBF). The mechanism of bioactivity of the A-W glass-ceramic is similar to that of bioactive glasses, which is attributed to the release of soluble Si, Ca and P ions into the physiological fluid. Dyson et al. [168] evaluated the behavior of mesenchymal stem cells on A-W glass-ceramic scaffolds produced by the layer manufacturing technique and selective laser sintering, showing that the expression of the osteogenic markers was significantly higher than that on the commercial calcium phosphate scaffold. However, it is definite that A-W glass-ceramic exhibits Class B bioactivity, which is lower than that of Bioglass® [169].

Ceravital® [170, 171] and BIOVERIT® [172, 173] are also commercially available glass-ceramics for bone replacement. However, there are very few reports on these two materials for applications in the field of bone tissue engineering. Recently, Vitale-Brovarone et al. [174–176] developed a series of K_2O-containing bioactive glass-ceramics. The glass with a molar composition of 50%SiO_2-44%CaO-6%K_2O (termed SCK) showed a crystalline phase of β-wollastonite (β-$CaSiO_3$), which exhibited good in vitro bioactivity. It is reported that a too higher pH can inhibit osteoblast activity and cause cell necrosis or apoptosis [177, 178]. To avoid the severe pH changes in the physiological solution, a new bioactive glass-ceramic, in the SiO_2-P_2O_5-CaO-MgO-K_2O-Na_2O system, was developed with a lower monovalent oxide content and a slightly higher P_2O_5 content compared to commercial bioactive glasses [175, 176]. $Ca_3Mg(SiO_4)_2$ and $Ca_2MgSi_2O_7$ were identified as crystalline phases of the above glass-ceramic. Macroporous scaffolds with a porosity over 70 % and pores in the range of 100–500 μm prepared from the above glass-ceramic showed high bioactivity and promoted a high cell differentiation. In addition, some researchers have also shown that some borate glasses can convert to hydroxyapatite and bond to bone chemically [179–181] like the silicate-based bioactive glass.

Silica-free calcium phosphate glass-ceramics have also been developed for bone tissue engineering [182–187]. Kasuga et al. [182–186] developed series calcium phosphate ceramics in CaO-P_2O_5-TiO_2 and CaO-P_2O_5-Na_2O-TiO_2 systems, which were initially used as coatings on titanium implants. The bioacvitity of these calcium phosphate glass-ceramics are composition dependent. The glasses with orthophosphate and pyrophosphate groups have the ability to induce apatite deposition, while the glass containing

no orthophosphate group does not deposit apatite. Moreover, the replacement of 7wt%TiO_2 by 7wt%Na_2O results in a significant increase in bioactivity. In addition, the apatite-forming ability of the above glass-ceramics is also strongly influenced by a small amount (3 %) of additive such as TiO_2 and MgO [186]. However, the mechanical properties of the calcium phosphate glass-ceramic are a little lower than those of silica-based A-W glass-ceramic [184].

Silicate Bioceramics

Inspired from the success of silicate –based bioactive glasses and glass-ceramics, some silicate ceramics have also been explored for bone tissue engineering applications, including wollastonite (low temperature calcium silicate, β-$CaSiO_3$) [188–193], pseudowollastonite (high temperature calcium silicate, α-$CaSiO_3$) [194–199], dicalcium silicate (Ca_2SiO_4) [200, 201], tricalcium silicate (Ca_3SiO_5)[202, 203], akermanite ($Ca_2MgSi_2O_7$) [204–206], bredigite ($Ca_7MgSi_4O_{16}$) [207, 208], diopside ($CaMgSi_2O_6$) [209–211], combeite ($Na_2Ca_2Si_3O_9$) [212], Silicocarnotite ($Ca_5(PO_4)_2SiO_4$) [213, 214] and silicate-based composites [215, 216].

As stated above, β-wollastonite is one of the crystalline phases of A-W glass-ceramic, which is mainly responsible for the bioactivity of A-W glass-ceramic. de Aza et al. [188] fabricated polycrystalline wollastonite by solid-state reactions at elevated temperature using solid calcium carbonate and silica with a CaO/SiO_2 molar ratio equal to one. The polycrystalline wollastonite showed a high "in vitro" bioactivity with the formation of apatite in the simulated body fluid. According to de Aza, the ionic interchange of Ca^{2+} for $2H^+$ between wollastonite and SBF resulted in an amorphous silica phase on the wollastonite surface and increased the calcium concentration and pH in the surrounding SBF, giving the conditions for HA precipitation.

The ex vivo cell culture studies have shown that β-wollastonite can enhance the attachment and proliferation of mesenchymal stem cells, and induce the differentiation of MSC to osteoblasts [217, 218]. In addition, the in vitro degradation rate of β-wollastonite scaffolds was substantially faster than that of the β-TCP [218]. In vivo evaluation of the plasma sprayed wollastonite coating showed that the wollastonite coating could form a tight bone-bonding with the surrounding bone tissue through a bone-like apatite layer [192]. Moreover, the wollastonite coating could also induce the apatite formation after 1-month implantation in muscle and could induce bone formation in marrow sites, indicating good bioactivity and osteoinductivity. Recently, the in vivo bone regenerative capacity and resorption of porous β-wollastonite scaffolds were investigated in a rabbit calvarial defect model using porous β-TCP scaffolds as a parallel by Xu et al. [219], showing that the β-wollastonite has a much higher resorption rate and more bone formation than β-TCP. After 16-week implantation, only 3.81 % of β-wollastonite remained (as shown in Fig. 4.1).

The pseudowollastonite (α-$CaSiO_3$), which is a high temperature form of calcium silicate, has also been found exhibiting good biocompatibility and bioactivity. Dufrane et al. [220] showed that the pseudowollastonite extract did not show significant cytotoxic effects confirming its biocompatibility. Lin et al. [221] also demonstrated good biocompatibility of α-$CaSiO_3$. The bioactivity of pseudowollastonite has been observed in vitro (in SBF) and in vivo (implanted in animals). Apatite formation on the surface of α-$CaSiO_3$ scaffold after soaking in SBF is shown in Fig. 4.2. Similar to wollastonite, pseudowollastonite also has the ability to induce apatite formation when immersed in SBF [197]. It can even induce apatite formation in human parotid saliva [195] and serum-containing media [10]. It has been reported that the rate of hydroxyaptite precipitation on the surface of pseudowollastonite surface are higher than those on all the reported bioglasses and glass-ceramics [222]. Sarmento et al. [198] found that osteoblasts could attach and proliferate well on the surface of pseudowollastonite. In addition, the cell attachment could be enhanced by preincubation of pseudowollastonite in serum or media containing fibronectin. The in vivo bioacticity of

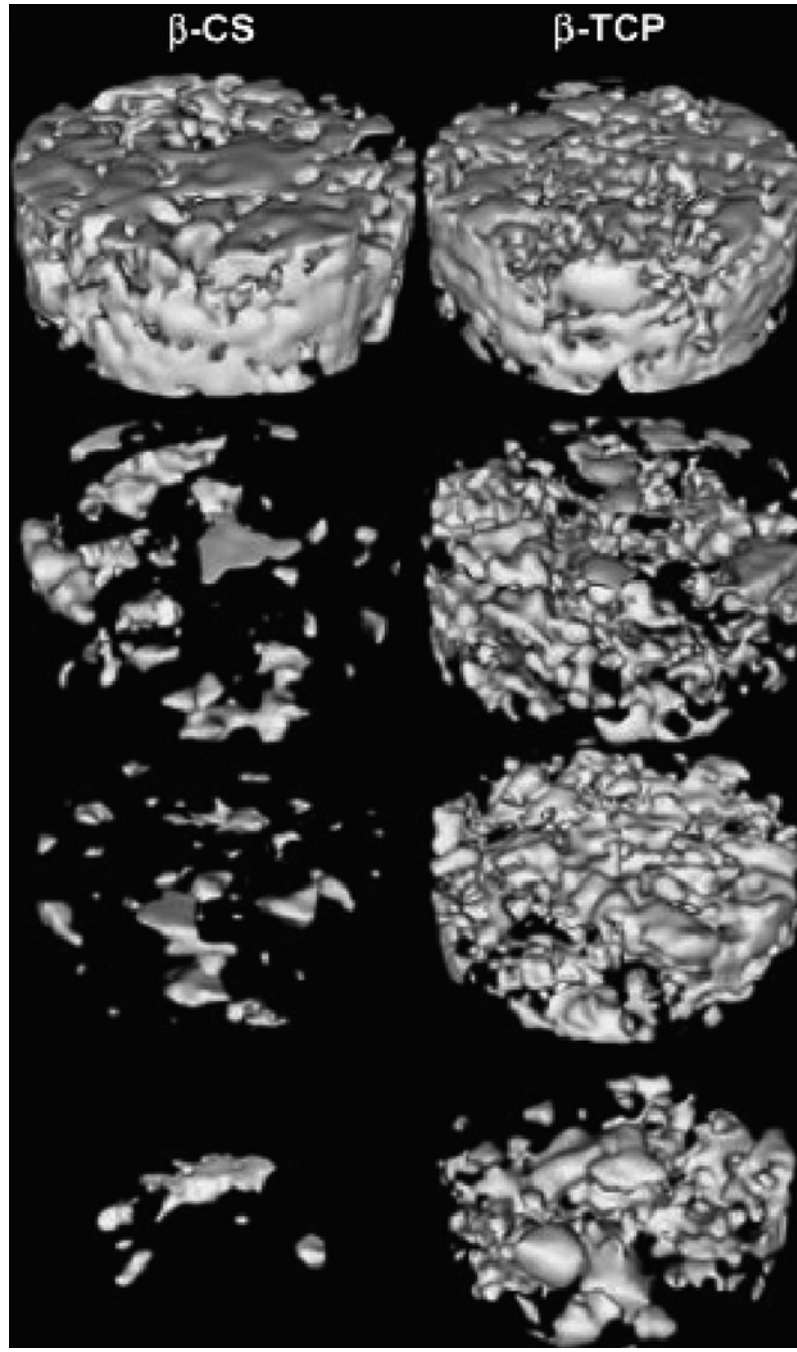

Fig. 4.1 3D reconstruction images of residual β-CS and β-TCP after implantation in the rabbit calvarial defects for different periods using Micro-CT analysis (From Xu et al. [219], with permission)

pseudowollastonite was evaluated by De Aza and co-workers through implantation into rat tibias [196, 199]. The SEM and EDS analyses showed that a calcium phosphate layer was formed at the implant interface, which had characteristics of new bone tissue. High resolution transmission electron microscopy observations confirmed the newly formed bone at the interface between the pseudowollastonite implant and the host bone as composed of hydroxyapatite-like nanocrystals growing epitaxially across the interface in the [002] direction [196]. It was shown that the rate of

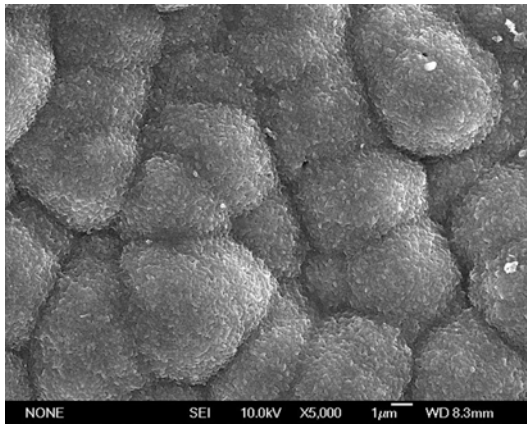

Fig. 4.2 Apatite formation on the surface of α-$CaSiO_3$ scaffold after soaking in SBF (From Lin et al. [221], with permission)

new bone formation around pseudowollastonite decreased after the first 3 weeks and reached constant value over the following 9 weeks, which coincided with the results of β-wollastonite reported by Xu and co-workers [219].

Sahai et al. [223] used crystallographic constraints with ab initio molecular orbital calculations to identify the active site and reaction mechanism for heterogeneous nucleation of calcium phosphate. It is proposed that the cyclic silicate trimer is the universal active site for heterogeneous, stereochemically promoted nucleation on silicate-based bioactive ceramics. A critical active site density and a less point of zero charge of the biomaterial than physiological pH are considered essential for bioactivity.

Chang and his colleagues find that dicalcium silicate and tricalcium silicate also show good bioactivity, and they can rapidly induce apatite formation in the SBF [201–203, 224]. Besides the binary calcium-silicates, some ternary calcium-silicate ceramics have also been attracted much attention in recent years. The investigation of diopside as implant material started by Nakajima in the late 1980s [225]. It was found that diopside can induce apatite formation in SBF and can form a bone bonding with surrounding bone tissues [209, 226, 227]. Calcium released from the material into SBF plays a key role in the apatite formation on the surface of diopside, which is initially released rapidly and eventually reaching steady-state. On the contrary, Mg and Si are released more slowly at similar rates to each other [12, 211, 228]. And Mg does not play a role for apatite nucleation on diopside [211]. It is proposed that the (100) plane of diopside epitaxially nucleates the (010) plane of octacalcium (OCP), which has a similar cell parameters to hydroxyapatite and has been considered as a precursor to hydroxyaptite in normal bone growth [12, 227]. The reported bending strength and fracture toughness of the diopside is 300 MPa and 3.5 $MPa \cdot m^{1/2}$, respectively [209]. These values are about two or three times higher than those of hydroxyapatite. However, the degradation rate of diopside is very poor, which is even lower than that of hydroxyaptite [209].

Besides diopside ceramics, akermanite and bredigite in the Ca-Si-Mg system have also been investigated for bone tissue engineering. Wu et al. [206, 229] synthesized pure akermanite and bredigite powders by sol–gel methods. Both akermanite and bredigite have the ability to induce apatite formation in SBF. The apatite formation ability decreases with the increase of Mg in the Ca-Si-Mg ceramics, i.e. bredigite has better apatite formation ability than akermanite, which is indicated by higher calcium content and lower phosphorus content in SBF after immersion. The increase in activation energy of Si release should be responsible for the reduced apatite formation ability [230]. In addition, activation energy of Si release also predominates the degradation rate of the Ca-Si-Mg ceramics. With the increase in Mg content, the degradation rate of the Ca-Si-Mg ceramics decreases. Considering the poor degradability of diopside, it may not be suitable as bone tissue engineering materials as the akermanite and bredigite. Akermanite prepared by two-step precipitation method has a higher bioactivity than that prepared by sol–gel method, due to its finer particle size. Akermanite and bredigite have all shown the ability to stimulate osteoblasts proliferation. The intensive investigation by Sun et al. showed that akermanite ceramics enhanced the expression of osteoblast-related genes, including alkaline phosphate (ALP), osteopontin (OPN), bone sialoprotein (BSP), and osteocalcin (OC) [231]. It also

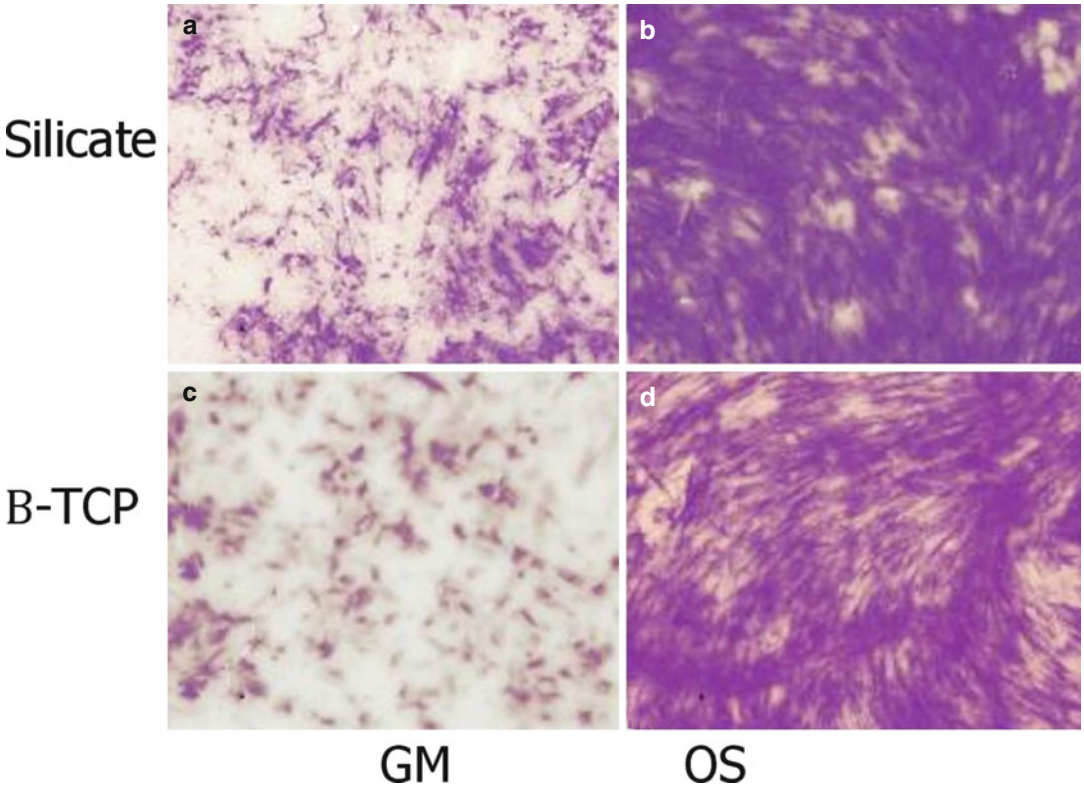

Fig. 4.3 ALP staining of differentiating hBMSC on the surface of different material. The hBMSC were cultured on akermanite disks (**a**, **b**) and β-TCP disks (**c**, **d**) for 7 days in growth medium (**a**, **c**) or osteogenic medium (**b**, **d**). ALP-positive cells are shown in purple. The bars in the pictures present 200 mm (From Sun et al. [231] with permission)

showed that akermanite could promote osteoblastic differentiation of human bone marrow stromal cells (hBMSC) in normal growth medium without osteogenic reagents, such as L-ascorbic acid, glycerophosphate and dexamethasone (as shown in Fig. 4.3). Highly connective porous akermanite and bredigite scaffolds, with porosity about 90 % and pore size ranging 300–500 μm, were prepared using polymer sponge as templates by Wu and his co-workers [208]. Both alkermanite and bredigite scaffolds could support osteoclasts-like cells growth, proliferation and differentiation. The biomimetic treatment of alkermanite and bredigite scaffolds in the SBF could enhance the cell proliferation and differentiation.

To combine the advantages of phosphates and silicates, Ning and her co-workers synthesized pure $Ca_5(PO_4)_2SiO_4$ (CPS) by a sol–gel method using triethyl phosphate (TEP), tetraethoxysilane (TEOS) and calcium nitrate tetrahydrate as original materials [213]. It is revealed that CPS has a greater in vitro apatite-forming ability than HA. In addition, the proliferation of rBMSC on CPS is significantly higher than that on HA. Moreover, the expression of alkaline phosphatase activity (ALP) and osteogenic-related genes, including Runx-2, osteopontin (OPN), bone sialoprotein (BSP) and osteocalcin (OC), demonstrated that CPS has enhanced the osteogenic differentiation of rBMSC and accelerated the differentiation process [214].

Silicate/Phosphate Based Composites

As stated above, silicate-based bioceramics exhibit excellent bioactivity, which can promote the osteoblast proliferation, induce the osteoblastic differentiation of marrow stem cells, and enhance the bone formation. On the other hand,

calcium phosphate ceramics have excellent biocompatibility due to their similar compositions to the bone minerals, while they have no obvious stimulatory effect on the proliferation and differentiation of osteoblasts. A composite strategy is applied to combine the advantages of silicates and phosphates, which is effective way to make materials with tailorable properties, such as mechanical property, bioactivity and biodegradation rate.

De Aza et al. [232, 233] developed a bioeutectic wollastonite-tricalcium phosphate ceramic, with a composition of 60 wt% wollastonite and 40 wt% TCP, by a specific high temperature treatment (termed W-TCP). The eutectic W-TCP material presented a high bioactivity in SBF [232] and human parotid saliva [233], with the formation of two well-differentiated zones of hydroxyapatite. The inner layer formed by pseudomorphic transformation of the tricalcium phosphate into hydroxyapatite after the dissolution of wollastonite into SBF, and the outer layer formed by the deposition of hydroxyapatite onto the surface of the material in the later stages of immersion.

Huang et al. [234] and Ni et al. [235] prepared β-$CaSiO_3$/β-$Ca_3(PO_4)_2$ composite materials by in-situ precipitation method. The mechanical properties of the CS-TCP composites increased with the increase in TCP content. A higher CS content also resulted in a higher dissolution rate. The CS-TCP composites exhibited good bioactivity. Compared with pure β-TCP, the CS-TCP composites, especially the composites with over 50 % wollastonite, enhanced the adhesion, growth and ALP activity of the osteoblast-like cells [235]. Zhang et al. [193] prepared nanocrystalline wollastonite/β-TCP composite powders by a two-step chemical precipitation method. Porous scaffolds were fabricated using these composite powders by porogen burnout technique. The mechanical properties of these scaffolds sintered from nano-scale composite powder were significantly improved, which were about twice as high as those of the scaffolds sintered from submicron powders. In addition, these scaffolds sintered from nano-powders showed less strength loss during the degradation process.

The silicate/phosphate composite ceramic with the composition of 32.9 mol% Na_2O, 32.9 mol% SiO_2, 22.8 mol% CaO and 11.4 mol% P_2O_5 were prepared by El-Ghannam and his co-workers [216], which showed main crystalline phases of Na_2CaSiO_4 and $NaCaPO_4$ (termed SCPC). This composite ceramic has compositional components similar to 45S5 bioglass. SCPC provided a superior release profile of biologically active rhBMP-2 compared to commercial porous hydroxyapatite. Moreover, cells attached to the SCPC produced mineralized extracellular matrix and bone-like tissue covered the entire material surface after 3 weeks culture in vitro, while the hydroxyapatite only produced limited amount of unmineralized ECM. Porous SCPC scaffold was prepared by rapid prototyping technique using a segment of a rabbit ulnar bone as prototype model [215]. After 4-weeks, CT scans showed that the defect filled by the above SCPC composite scaffold loaded with rh-BMP-2 had already been replaced by newly formed bone, indicating that SCPC are highly resorbable and have good bone formation ability.

Polymer/Inorganic Composites

The composite materials for bone tissue engineering have been pursued in the near decade, since the composites combine the advantages of the different components, which offered superiorities over single-phase materials.

Compared to the strengths of metals and ceramics, the strengths of biodegradable polymers are low. The porous structure of the scaffolds further decreases their strengths. Moreover, the synthetic polyesters are often non-osteoconductive. To enhance the strength and bioactivity of the polymer scaffolds, an inorganic component is always introduced to make polymer/inorganic composites. Studies have demonstrated that such composites could result in scaffolds with tailorable physical and biological properties for specific applications. The addition of an inorganic phase to a biodegradable polymer may also change the *in vitro* and *in vivo* polymer degradation behaviour.

Bioglass, glass-ceramics, calcium phosphates and silicates, etc. have all been used to reinforce polymers. The development of polymer/inorganic composites has been well reviewed in literatures [4, 13, 32]. In the recent years, the polymer/silicate ceramic composites have been intensively investigated. For example, wollastonite was incorporated into the PDLLA to prepare a bioactive PDLLA/wollastonite composite [236]. The composite scaffold was prepared using a solvent casting/particulate leaching method. With the same salt content, the porosity of the PDLLA deceased from 95 to 85 % as the wollastonite content increased from 0 to 40 %. The bioactivity of the PDLLA/wollastonite composite was confirmed by the formation of an apatite layer on its surface after immersing in SBF for seven days. The interesting and important advantage of the PDLLA/wollastonite composite is that the acidic degradation products of the PDLLA could be neutralized by the basic ions released from wollastonite due to its dissolution in the SBF solution. For the PHBV/wollastonite porous scaffolds, there were no significant differences in porosity between the samples with different wollastonite content [237]. However, the mechanical strength of the composite scaffolds was significantly enhanced by the incorporation of wollastonite. In addition, the incorporation of silicates into polymers will result in an improvement in hydrophilicity, expressed by a decrease in water contact angle [237, 238]. This implied that wollastonite could be used as a good candidate for preparation of bioactive polymer/ceramic composites for tissue engineering applications.

During the process of polymer/ceramic composites preparation, a common problematic issue is that it is difficult to get a uniform polymer/inorganic particle suspension, since the inorganic particles have the tendency to agglomerate. This problem makes it difficult to fabricate composites with a uniform microstructure [236, 237]. And it was found that some of the PDLLA/β-$CaSiO_3$ composites lost their strength rapidly under physiological environment, and failures mainly occurred at the interface between the β-$CaSiO_3$ agglomerates and the polymer matrix. Consequently, it is necessary to increase the compatibility between the inorganic component and the polymer matrix by improving the dispersion of inorganic particles in preparing polymer/inorganic composites.

Mechanical stirring [239] and ultrasonic energy [240] have been used to reduce agglomerate formation and provide some level of particle dispersion during the blend processing of composite. However, these effects are just temporary and particle agglomeration ensues once the mixing energy is removed.

It is supposed that chemical techniques can provide more permanent effect to solve this problem and various methods have been developed to match the surface properties between filler powders and a specific polymeric matrix [241–244]. Zhang et al. [241] used silane derivatives as modification molecules to shield hydroxyl groups (–OH) formed on the surface of HA to improve the interfacial property between the ceramic phase and the polymer phase, which resulted in a 27.8 % increase in maximum bending strength of the HA/PLA composites. Qiu et al. [242] modified the surface of HA with L-lactic acid oligomer, and the dispersion of HA particles in the polymer solution was improved significantly. The mechanical strength of the L-lactic modified HA/PLLA composite film was also increased [242, 243].β-$CaSiO_3$ particles treated with dodecyl alcohol can react with the Si-OH groups on the surface of β-$CaSiO_3$ particles in an aqueous solution by esterification reaction. This modification could make the β-$CaSiO_3$ particle hydrophobic and thus enhance its dispersion in the organic solvent (as shown in Fig. 4.4). The tensile strength of the modified β-$CaSiO_3$/PLLA composite film with 15 wt% ceramic phase increased 52.2 % compared to that of the unmodified one [244]. In addition, the modification had no effects on the bioactivity of the β-$CaSiO_3$/PLLA composite. Our experiments also showed that the dodecyl alcohol on the modified $CaSiO_3$ particles in the composite could be removed by hydrolysis in boiling water. The valuable results are that the esterification-hydrolysis process has improved the mechanical properties of β-$CaSiO_3$/PLLA composites, while without impairing their wettability and bioactivity. The same phenomenon was found for the 45S5/PLLA composites.

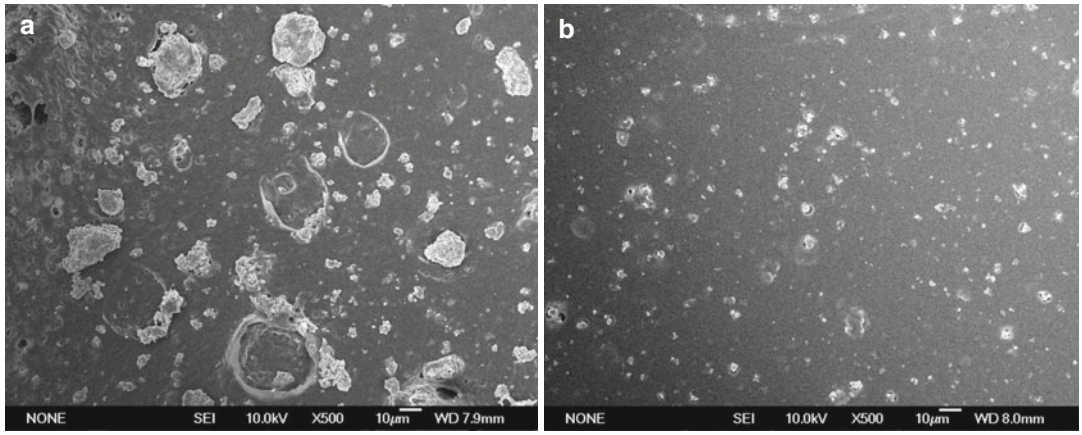

Fig. 4.4 SEM micrographs of the composite films. (**a**) composite film with 15wt%β-$CaSiO_3$ and (**b**) composite film with 15 wt% modified β-$CaSiO_3$ (From Ye et al. [244], with permission)

Concluding Remarks

The concept of replacement of tissues has been shifting to a new concept of regeneration of tissues in the new century [142]. Tissue engineering is an effective way to achieve the goal of tissue regeneration. From the perspective of materials science, the present challenge in tissue engineering is to develop bioactive and bioresorbable biomaterials, which should have the ability to activate the body's own repair mechanisms. An ideal biomaterial for bone tissue engineering should have favorite composition and structures which can facilitate cellular attachment, proliferation and stimulate osteoblastic differentiation of bone marrow stromal cells, and should initiatively participate in the activities of bone formation.

Generally speaking, silicate ceramics have superior bioactivity than phosphate ceramics. The former are considered as osteoconductive and may be considered as osteoinductive, while the latter are only considered as osteoconductive. Therefore, silicate ceramics have a more wide application perspective for bone tissue engineering than phosphate ceramics.

On the other hand, since the hard tissues in human body are natural composite materials, the composite strategy provides an effective way to fabricate scaffold biomaterial with tailorable physiochemical and/or mechanical properties. The composite scaffolds possessing both osteoconductivity and osteoinductivity appear to have great potential for bone tissue engineering applications.

In addition, the architecture of the scaffolds not only influences its mechanical properties and degradation behavior, but also strongly affects the cellular activities and nutrition supplies in the scaffold, which are also important factors for bone regeneration. Thus, the ideal scaffold material should also have highly connective porous structure.

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