REVIEW

Biomimetic hydroxyapatite-containing composite nanofibrous substrates for bone tissue engineering

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The fracture of bones and large bone defects owing to various traumas or natural ageing is a typical type of tissue malfunction. Surgical treatment frequently requires implantation of a temporary or permanent prosthesis, which is still a challenge for orthopaedic surgeons, especially in the case of large bone defects. Mimicking nanotopography of natural extracellular matrix (ECM) is advantageous for the successful regeneration of damaged tissues or organs. Electrospun nanofibre-based synthetic and natural polymer scaffolds are being explored as a scaffold similar to natural ECM for tissue engineering applications. Nanostructured materials are smaller in size falling, in the 1–100 nm range, and have specific properties and functions related to the size of the natural materials (e.g. hydroxyapatite (HA)). The development of nanofibres with nano-HA has enhanced the scope of fabricating scaffolds to mimic the architecture of natural bone tissue. Nanofibrous substrates supporting adhesion, proliferation, differentiation of cells and HA induce the cells to secrete ECM for mineralization to form bone in bone tissue engineering. Our laboratory (NUSNNI, NUS) has been fabricating a variety of synthetic and natural polymer-based nanofibrous substrates and synthesizing HA for blending and spraying on nanofibres for generating artificial ECM for bone tissue regeneration. The present review is intended to direct the reader’s attention to the important subjects of synthetic and natural polymers with HA for bone tissue engineering.

Keywords: electrospinning; synthetic and natural polymers; nanofibres; hydroxyapatite; bone tissue engineering

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Electrospinning processes have attracted a great deal of attention as a way to mimic the structure of natural extracellular matrix (ECM) by means of producing fibres down to 5 nm. This technique is used to fabricate nanofibrous structures from natural and synthetic polymers such as collagen (Col), gelatin, chitosan (CTS), silk fibroin, poly(DL-lactide-co-glycolide), poly(lactide), polyurethane, polycaprolactone, etc. Rapid progress in nanotechnology and its far-reaching developments have triggered the use of nanostructures as scaffolds for bone tissue engineering. Fabrication of nanofibres is one of the most promising techniques for designing polymer nanofibres in tissue engineering. There are several scaffold fabrication techniques, namely electrospinning (random, aligned, core shell and vertical nanofibres), self-assembly, phase separation, melt-blown and template synthesis (Venugopal et al. 2008a). Of these techniques, electrospinning has been the most widely used technique recently, and it also seems to be demonstrating promising results for tissue engineering applications. Electrospinning has emerged as an elegant and leading technique to create fibres with diameters in the range of micrometres down to a few nanometres cost-effectively for tissue engineering.

Tissue engineering is the application of knowledge and expertise from a multidisciplinary field, to develop and manufacture therapeutic products that use the combination of matrix scaffolds with viable human cell systems or cell-responsive biomolecules derived from such cells, for repair, restoration or regeneration of cells or tissues damaged by injury or disease (Venugopal et al. 2008a,b). The concept of tissue engineering using three-dimensional scaffolds has certain advantages over direct cell injection to the tissues: (i) three-dimensional scaffolds may replace the missing or damaged infrastructure (ECM) in the infarct area and provide temporary support for self or implanted cells, (ii) by tissue engineering, one can control the size, shape, strength and composition of the graft in vitro, and (iii) tissue engineering provides a solution to the problem of bone defects and can be used to replace the injured bone. The objective was to develop a scaffold for tissue engineering that is (i) highly porous with large interconnected pores (to facilitate mass transport), (ii) hydrophilic (to enhance cell attachment), (iii) structurally stable (to withstand the shearing forces during bioreactor cultivation), (iv) degradable (to provide ultimate biocompatibility of the tissue graft), and (v) elastic (to enable transmission of contractile forces). The scaffold structure determines the transport of nutrients, metabolites and regulatory molecules to and from the cells, whereas the scaffold chemistry has an important role in cell attachment and differentiation. Mechanical properties of the scaffold should ideally match those of the native tissue, to provide mechanical integrity of the forming tissue and to support an in vivo-like mechanotransduction between cells and their environment (Radisic et al. 2007). The role of biomaterials in tissue engineering is to act as a scaffold for cells to attach and organize into tissues. The ECM is a complex arrangement of proteins and polysaccharides such as Col, hyaluronic acid, proteoglycans, glycosaminoglycans and elastin. The structure and morphology of the non-woven nanofibre matrix were found to closely match the structure of ECM of natural tissue (Li et al. 2002; Venugopal et al. 2008a).

Every year, millions of people suffer from bone defects arising from trauma, tumour or bone diseases, and many people are dying because of insufficient bone substitute. One main driving force to explore nanomaterials in bone tissue
engineering applications is that tissues in the human body are nanostructures, clearly opening the gates for numerous opportunities to improve medicine. The functional treatment of fracture non-union and bone loss associated with trauma, cancer and revision joint arthroplasty has become increasingly common for orthopaedic surgeons and remains a significant challenge in the field of musculoskeletal injury annually, and there are over several million orthopaedic procedures performed each year (Market Dynamics 2008). The global orthopaedic market is estimated by Espicom to have been worth approximately US$37.1 billion in 2008, following a growth of 9.7 per cent over the previous year. Excluding arthroscopy and ‘other’ segments (operating theatre equipment and supplies), the market totalled US$29 billion, having grown by 10.7 per cent over the previous year. To solve these problems, synthetic and natural polymeric nanofibrous scaffolds are the alternative for bone tissue engineering applications; they should be biocompatible with the surrounding biological fluids and tissues, biodegradable and highly porous with interconnected spaces, and favourable for the diffusion of nutrients as well as migration of a large number of cells. Bone tissue engineering is a rapidly expanding research area providing a new and promising approach for bone repair and regeneration. Good sterilizability, storability and processability, as well as relatively low cost, are also of great importance to permit clinical applications. Unfortunately, no artificial biomaterial is available yet which embodies all these requirements, and it is unlikely that it will appear in the near future. Until now, most of the available biomaterials appear to be either predominantly osteogenic or osteoinductive or else purely osteoconductive (Meyer et al. 2004). The current challenge in bone tissue engineering is to fabricate bioartificial bone graft mimicking the ECM with effective bone mineralization, resulting in the regeneration of fractured or diseased bones. Today, nanotechnology and nanoscience approaches to scaffold design and functionalization are beginning to expand the market for tissue engineering, which forms the basis for a highly profitable niche within the industry. This review describes recent advances for the fabrication of biomimetic nanofibrous scaffolds with hydroxyapatite (HA) for bone tissue engineering.

2. Hydroxyapatite

Hydroxyapatite is a major mineral component of calcified tissues (bone and teeth). Synthetic HA (Ca_{10}(PO_4)_6(OH)_2) has been used extensively as an implant material for bone substitute owing to its excellent osteoinductive properties (Rameshbabu et al. 2005). HA-enhanced surface properties (such as increased surface area and charge and the ability to alter adsorption of chemical species) could be used to promote cell response and proliferation to induce mineralization in bone tissue engineering. Hydroxyapatite has been used in a variety of biomedical applications such as matrices for controlled drug release, bone cements, tooth paste additive, dental implants, etc. Calcium phosphate biomaterials (HA/tricalcium phosphate) with appropriate three-dimensional geometry are able to bind and concentrate endogenous bone morphogenetic proteins (BMPs) in circulation and may become osteoinductive (Yuan et al. 1998) and can be an effective carrier of bone cells. Nanohydroxyapatite (nano-HA) can be prepared by co-precipitation and precipitation using emulsion, template and
solgel techniques, but these methods need highly controlled parameters such as reactant concentration, pH and temperature of the aqueous solution; microwave processing is a simple method for HA preparation (Rameshbabu et al. 2005). Synthetic-substituted HA is only starting to be developed in elaborate tailored biomaterials, and some of them have been shown to exhibit improved biological properties compared with stoichiometric HA (Porter et al. 2004). Currently, biocomposites with calcium orthophosphates incorporated as either a filler or a coating (or both) or into a biodegradable polymer matrix in the form of particles or fibres are increasingly considered for using as bone tissue engineering scaffolds owing to their improved physical, biological and mechanical properties (Hutmacher et al. 2007; Dorozhkin 2009). In addition, such biocomposites could fulfil general requirements for the next generation of biomaterials; these should combine the bioactive and bioresorbable properties to activate in vivo mechanisms of tissue regeneration, thus stimulating the body to heal itself and leading to the replacement of implants by regenerating tissues (Hench & Polak 2002).

3. Natural polymers/hydroxyapatite

One of the major challenges in developing porous scaffolds for bone tissue engineering is the conflicting interest between porosity, mechanical strength and biodegradability. Stable osteoblast cell adhesion is largely mediated by integrins and heterodimeric receptors that interact with ECM proteins such as fibronectin, vitronectin, fibrinogen and Col, allowing the cell to respond to its extracellular environment and modulating the cellular events that regulate remodelling of bone (Gronowicz & McCarthy 1996; Chang et al. 1998). Nanoscaled features such as surface roughness and topography of nanocrystalline bioceramics and nanofibrous scaffolds promote cell behaviour such as adhesion, proliferation, migration and differentiated functions. Natural bone being an innate example of inorganic–organic biocomposites consists of approximately 70 wt% inorganic crystals, mainly HA, and 30 wt% organic matrix, mainly Col type I. Structurally, it is hierarchically organized from macro-, micro-, to nano-scale, where the basic building blocks were pinpointed to be a plate-like HA nanocrystal incorporated into Col nanofibres (Glimcher 1959; Weiner & Traub 1986). Hydroxyapatite is considered to be a structural template for the bone mineral phase and also a major inorganic mineral component of bone, and is commonly used as a bioceramic filler in polymer-based bone substitute because of its higher level of bioactivity and biocompatibility (Hong et al. 2005).

(a) Collagen/hydroxyapatite

Col is a major ECM component that possesses fibrous structure with fibre bundles of varying diameters (50–500 nm). The nanometre size feature influences cell behaviours by allowing cells to attach to diameters smaller than the cell size of the fibres. Cells seeded on this structure tend to maintain normal phenotypic shape and guided growth according to nanofibre orientation. The main idea in biomimetic approaches is to control and fabricate the morphology and composition of developed biomaterials, in which the nanocrystallites of inorganic compounds are being dispersed with preferential orientation in the organic matrices. Owing to its large potential in biomedical applications, many studies...
reported the preparation of bone-like biocomposites of HA and bioactive organic components such as Col, gelatin, chondroitin sulphate, CTS and amphiphilic peptide by direct precipitation methods (Kikuchi et al. 2001; Chen et al. 2002), poly(lactic acid) through a solvent-cast technique (Liao et al. 2004) and polyamide by a solution method (Wei et al. 2003). Col and HA have potential in mimicking natural ECM and in replacing diseased skeletal bones. More attention has been focused on HA because its crystallographic structure is similar to that of inorganic compounds found in natural bone; it has been investigated extensively owing to its excellent biocompatibility, bioactivity and osteoconductivity.

Bone tissue contains high levels of type I Col and several non-collagenous proteins (such as osteopontin, bone sialoprotein and osteocalcin) that distinguish it from other types of tissues. The size of the bone mineral is around 50 nm in length, 25 nm in width and 2–5 nm in thickness (Sachlos et al. 2006). These crystals are oriented with their long crystallographic c-axis parallel to each other and aligned with Col tropocollagen molecules (Weiner & Traub 1989). Col is easily degraded and resorbed by the body and allows good attachment to cells. However, its mechanical properties are relatively low (E ~ 10 MPa) in comparison with bone (E ~ 2–5 GPa; Clarke et al. 1993), and it is, therefore, highly cross-linked or found in composites such as Col–glycosaminoglycans for skin regeneration (O’Brien et al. 2004) or Col/HA for bone remodelling (Venugopal et al. 2008a–e). Col and HA devices significantly inhibited the growth of bacterial pathogens, the frequent cause of prosthesis-related infection, compared with poly(lactide-co-glycolide) devices (Carlson et al. 2004). Electrostatic co-spinning of nanocomposite fibres of polymers with a nano-HA composite system in native bone is the special orientation between HA and Col molecules. However, more efforts are required in the area of nanofibrous Col/HA composite, for exactly mimicking the complex nanostructured architecture of Col matrix with c-axis orientation of nano-HA particles (Thomas et al. 2006). Col supports the cells for adhesion and proliferation, and HA acts as a chelating agent for mineralization of osteoblasts in bone tissue regeneration (Landis et al. 1993). Biologically inspired biocomposites of Col and nano-HA for bone substitute have a long history in the biomedical field (Wahl et al. 2007). A combination of Col and nano-HA materials is bioactive, osteoconductive and osteoinductive, seems to be a natural choice for bone grafting and mimics the bone components. Nano-to-microscale nano-HA/Col alignment of composite was similar to that of natural bone and thus might have been identified as ‘bone’ by the attached cells (Du et al. 1999). Itoh et al. (2001) prepared a novel HA/Col composite biomaterial using cold isostatic pressure by the co-precipitation method that has a chemical composition and crystallinity similar to that of bone and induces the development of osteogenic cells for remodelling of bone.

Skeletal bones comprise mainly Col (predominantly type I) and carbonate-substituted HA; both are osteoconductive components. Thus, an implant manufactured from such components is likely to behave similarly and to be of more use than a monolithic device. Indeed, both type I Col and HA were found to enhance osteoblast differentiation, but, combined together, they were shown to accelerate osteogenesis. A composite matrix when embedded with human osteoblastic cells showed better osteoconductive properties than monolithic HA and produced calcification of identical bone matrix (Wang et al. 1995).
Figure 1. Electrospun nanofibrous substrate interaction with human foetal osteoblast (hFOB) cells. (a) Col nanofibres (fibre diameter: 272 ± 0.63 nm) and (b) Col/HA nanofibres with hFOB mineralization.

More interest has been focused on HA because its crystallographic properties are similar to those of inorganic components found in natural bone. It has been investigated extensively owing to its excellent biocompatibility, bioactivity and osteoconductivity (Li et al. 2007). In addition, Col/HA composites proved to be biocompatible both in humans and in animals (Wahl & Czernuszka 2006). Kikuchi et al. (2001) fabricated an artificial bone material with bone-like nanostructure and chemical composition, a composite comprising HA and Col was synthesized under biomimetic conditions through a self-organization mechanism between HA and Col. The HA/Col composite fabricated and demonstrated bone-like orientation, so that c-axes of HA nanocrystals were regularly aligned along Col fibrils (Porter et al. 2005). The literature review suggests that the ‘bone bonding’ ability of calcium phosphate ceramics occurs by partial dissolution of ceramic, resulting in the elevated concentration of calcium (Ca\(^{2+}\)) and phosphate (PO\(_{4}^{3-}\)) ions within the local environment. Investigations have suggested that there is a threshold concentration of calcium ions required to stimulate the subsequent activity of osteoblasts (Nishikawa et al. 2005). The HA/Col composite, designed to simulate bone tissue, is produced using atelocollagen to reduce antigenicity by condensing Ca(OH)\(_2\)/H\(_3\)PO\(_4\) suspension (Venugopal et al. 2008c).

Venugopal et al. (2008c) found that the mineral deposition on Col/HA composite nanofibrous scaffolds cultured with osteoblasts was significantly higher than that on Col nanofibrous scaffolds (figure 1a) during 10 days of culture period. Compared with Col/HA nanofibrous scaffolds, the mineral deposition was significantly lower (by up to 56 per cent) in Col nanofibrous scaffolds. The Col/HA composite nanofibrous scaffolds have huge potential for cell adhesion and growth, as well as stimulating the cells for mineralization, exhibiting functional activity of osteoblasts for bone tissue engineering (figure 1b). These studies showed that the Col/HA composite nanofibrous scaffold has great potential for bone tissue regeneration.
The main practical problems with Col are its cost, and poor definition of commercial sources of this material makes it difficult to follow up on well-controlled processing. Col was replaced by gelatin (Gel), a protein produced by partial hydrolysis of Col extracted from skin, bone, cartilage, ligaments, etc. Mixing Gel with other synthetic polymers, sometimes called bioartificial polymeric materials, has frequently been adopted by other researchers. This approach is feasible to reduce the potential problem of cytotoxin, as a result of using a chemical cross-linking reagent, but also provides a compromise solution for overcoming the shortcomings of synthetic and natural polymers, that is, producing a new biomaterial with excellent biocompatibility and improved mechanical and physical/chemical properties (Venugopal et al. 2008e). Liu et al. (2009) reported the fabrication of three-dimensional nanofibre-Gel/apatite composite scaffolds, which mimic both nanoscale architecture and chemical composition of natural bone ECM. Three-dimensional nanofibrous Gel scaffolds with well-defined macropores were prepared by using a new thermally induced phase separation and porogen leaching technique (Liu et al. 2009). The deposition of a biomimetic apatite layer throughout the porous structure of three-dimensional scaffolds is an effective method for controlling the surface topography and chemistry within large, complex structures. These scaffolds have excellent biocompatibility and mechanical properties and showed enhanced osteoblast adhesion, proliferation and differentiation suitable for bone tissue engineering.

Although Col is used as a common matrix for scaffold design, HA seems to be a rational strategy for preparing a nanofibrous biocomposite that compositionally and structurally resembles bone for engineering of bone tissue; however, Col alone is less ideal in terms of mechanical properties and biostability owing to its rapid degradation in the biological environment and problems related to its antigenicity. Recently, a few studies have explored the feasibility of replacing Col with other natural biopolymers (e.g. silk and CTS) to prepare the HA containing electrospun biomimetic composite fibres for potential osteoregenerative applications, while the electrospinnability of the natural biopolymers of interest is no longer an issue under consideration in tissue engineering.

(b) Hydroxyapatite/silk protein

Silks are considered to be the most promising natural protein-type replacement for Cols in bone tissue engineering because of their biocompatibility, slow degradation and excellent mechanical performance. In the past few years, different natural silks (e.g. silkworm silk Bombyx mori and spider dragline silk Nephila clavipes) have been processed for making nanoscale fibres via electrospinning (Jin et al. 2002; Kim et al. 2003; Ohgo et al. 2003; Zarkoob et al. 2004). For example, to improve the electrospinnability of silk solutions and to avoid potential influences of using strong polarity organic solvents such as hexafluoroisopropanol (Zarkoob et al. 2004), hexafluoroacetone (Kim et al. 2003) and formic acid (Ohgo et al. 2003) on biocompatibility, Kaplan’s group (Jin et al. 2002) used an all-aqueous process for silk electrospinning by blending regenerated silk fibroin with a fibre forming agent, poly(ethylene oxide) (PEO), at a ratio from $\frac{1}{4}$ to $\frac{2}{3}$. A post-electrospinning treatment on the silk fibroin fibres with methanol was then performed to induce a structurally conformational transition to the original $\beta$-sheet to render water insolubility in uses. Following this success, Li et al. (2006)
recently developed silk-based composite nanofibres with the incorporation of HA nanoparticles (approx. 5 wt%) and BMP-2 (3 μg mg\(^{-1}\) of silk fibroin) growth factor to realize enhanced bone formation outcomes from culturing human bone marrow-derived mesenchymal stem cells (MSCs). They found that the inclusion of BMP-2 and HA nanoparticles within the electrospun silk fibroin fibres resulted in the highest calcium deposition and upregulation of BMP-2 transcript levels, compared with other electrospun silk-based scaffolds including silk/PEO, silk/PEO extracted, silk/PEO/BMP-2 and silk/PEO/HA.

Li et al. (2005) employed different approaches to prepare silk-based composite fibres, in which the inorganic apatite component was selectively grown on and associated with the electrospun fibrous silk fibroin matrix by a subsequent mineralization process using an alternative soaking method. Acidic protein poly(l-aspartate) (poly-Asp) as a molecular recognition motif was first introduced into the electrospun silk fibroin fibres to enable subsequent nucleation and crystal growth of apatite on the fibre surface during the mineralization process. This approach is an alternative route for preparing composite nanofibres to avoid the electrospinnability problem encountered in the electrospinning of those HA-containing solutions, which eventually leads to the formation of apatite-coated fibroin composite fibres, which is somewhat different from that obtained in the components hybridizing method (Li et al. 2006; Zhang & Lim 2008) and the nanostructure of hierarchical bone (Landis et al. 1993; Rho et al. 1998) in terms of distribution and morphology of the HA nanocrystals in the composite fibres.

Hydroxyapatite/chitosan

Amino polysaccharide CTS is derived from the structural biopolymer chitin that exists abundantly in crustacean shells (e.g. crabs) and plays a key role similar to that of collagen in higher animals. CTS possesses plenty of notable attributes such as structural similarity to glycosaminoglycan found in bone, osteoconductivity, excellent biocompatibility, tailorable biodegradability, low immunogenicity and better mechanical properties. Thus, it has become of great interest as one of the most attractive natural biopolymer matrices and alternatives to collagens for bone tissue engineering (Yamaguchi et al. 2001; Muzzarelli & Muzzarelli 2002; Hu et al. 2004). However, there are immense challenges in converting a bulk HA/CTS nanocomposite or hybrid into a fibrous form by electrospinning owing to the poor electrospinnability of the CTS itself as well as the adverse effect of the non-electrospinnable HA nanoparticles (and their aggregates) contained in the spinning dope. Formulating a robust CTS solution system consequently appears to be a prerequisite to generate nanofibrous HA/CTS scaffolds. As a result of the noted obstacles in electrospinning, so far there are very limited attempts of using nanofibrous HA/CTS for bone tissue engineering (Rusu et al. 2005; Yang et al. 2008).

By using an ultra-high-molecular-weight PEO as the fibre-forming aiding agent, Zhang et al. (2008a) demonstrated that CTS nanofibres could be prepared easily with a minimum PEO loading ratio of up to 5 wt%. This enabled an attempt to develop HA/CTS composite nanofibres for potential application in bone repair and regeneration (Rusu et al. 2005). A modified two-step approach (Zhang et al. 2008b) that combines the in situ co-precipitation synthesis route (Yamaguchi et al. 2001), which is likely to overcome the usual problem of nanoparticles...
Figure 2. Electrospun nanofibrous HA/CTS composite scaffolds for bone tissue engineering. (a) The co-precipitation method was employed for the synthesis of HA/CTS nanocomposite; (b) electrospinning of thus obtained HA/CTS nanocomposite by dissolving dilute acetic acid-dominant solvent system; (c) SEM image of the co-precipitated HA/CTS nanocomposite with needle-shaped HA nanoparticles being incorporated evenly within the CTS matrix; (d) electrospun HA/CTS nanofibres with an average diameter of ca 200 nm; (e) biomineralization of hFOBs on the nanofibrous composite substrate of HA/CTS and (f) apatite-like morphology of the deposited minerals in (e), visualized at a high magnification.

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agglomeration, with the electrospinning process was adopted for the preparation of HA/CTS nanofibres containing a higher (30 wt%) loading of HA nanoparticles (figure 2). Despite the fact that a dilute acetic acid-dominated solvent system was used for dissolving CTS, results from the selected area of electron diffraction and X-ray diffraction analysis indicated that the crystalline nature of HA remains, thus implying a minor influence of the used acid solvent system on the crystalline structure of HA incorporated within the co-precipitated HA/CTS nanocomposite. Bone formation ability assessed by conducting [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt] (MTS) and alizarin red staining (ARS) assays of human foetal osteoblasts (hFOBs) cultured for up to 15 days showed that the HA/CTS nanofibrous scaffolds had significantly encouraged bone formation-oriented outcomes compared with the electrospun CTS alone scaffolds. Biomineralization in the form of synthesizing apatite-like granular minerals, as reported elsewhere (Yoshimoto et al. 2003; Li et al. 2006), was similarly observed with the post-cultured cell-scaffold constructs (figure 2 e,f). The two-step method would pave the way for developing other sophisticated biomimetic CTS-based nanofibrous composite scaffolds for functional bone tissue engineering.

4. Synthetic polymers/hydroxyapatite and synthetic/natural polymer/hydroxyapatite composites

Biocomposite nanofibres fabricated by electrospinning mimic the nanoscale structure of ECM, and organize and provide signals for the cellular response (Gupta et al. 2009). The material for bone graft fabrication needs to provide structural integrity within the body and finally breakdown leaving new tissue formation. The material should modulate the cellular function to promote tissue regeneration in addition to mimicking the mechanical properties of the bone. Non-biodegradable electrospun polymers such as polyurethane and polyesterurethane possess good mechanical properties, but they might interfere with tissue turnover owing to their slow degradation properties (Lee et al. 2005; Riboldi et al. 2005). Non-woven poly-ε-caprolactone (PCL) nanofibrous scaffolds were developed by Yoshimoto et al. (2003) and seeded with MSCs from the bone marrow of neonatal rats with osteogenic supplements. The penetration of cells along with abundant ECM deposits was observed in the cell–polymer constructs by these researchers, favouring potential scaffolds for bone tissue engineering. In contrast, the shrinkage behaviour of natural polymeric (Col and gelatin) scaffolds is a major phenomenon problematic in bone tissue regeneration (Liao et al. 2004). Preliminary in vitro and in vivo studies using nano-HA/Col composite also showed the material as bioactive and biodegradable, but its weak mechanical properties remain a major hindrance for practical bone tissue engineering.

PCL/HA, PCL/Col/HA, PCL/Gel/HA, poly-ε-lactic acid (PLLA)/Col/HA and poly(3-hydroxy-butyrate-co-3-hydroxyvalerate (PHBV)/HA were fabricated by various research groups as a substitute for bone tissue engineering (Ito et al. 2005; Venugopal et al. 2007, 2008d,e; Prabhakaran et al. 2009). The PCL/HA nanofibrous scaffolds treated with plasma enhance the wettability and thus accelerate the biodegradation rate of nanofibrous scaffolds. PCL/HA plasma-treated nanofibre shows mineral formation on the surface of osteoblast cell
Figure 3. Mineralization of hFOBs on PCL/HA plasma-treated nanofibrous substrates. (a) PCL nanofibres (fibre diameter: $276 \pm 56 \text{nm}$) and (b) hFOB-secreted mineral deposition on the surface of the PCL/HA.

layers within 6 days of culture and morphology similar to that of HA of the natural bone (figure 3). On fabricating the composites of HA with biodegradable polyesters such as PCL, PLLA produced a scaffold with better mechanical properties, whereas HA provided excellent bioactivity and osteoconductive properties (Hamadouche & Sedel 2000; LeGeros 2002). Moreover, the optimal performance of a composite material is achieved when the small particles (HA) are uniformly dispersed throughout the scaffolds and interact strongly within the organic matrix. Electrosprun Gel/PCL nanofibres were fabricated by mixing a 1:1 ratio of Gel and PCL with Gel concentrations ranging from 2.5 to 12.5%w/v and the membrane showed improved mechanical properties and favourable wettability compared with Gel or PCL membrane (Zhang et al. 2005). Bone marrow stem cells (BMSCs) grown on the surface of these scaffolds were found to migrate inside the scaffold up to 114 μm within a week of culture, showing their biocompatibility better than PCL nanofibres. Figure 4 shows the cellular ingrowth after 4 days of osteoblasts cultured on biocomposite nanofibrous scaffolds. While loosely interlaced fibrous structure and the weak nanoscale fibres can provide the least obstruction and matched mechanical properties for cell movements, it seems that the presence of appropriate molecular signals on the nanofibre surface can also guide or attract the living cells to enter into the matrix through their amoeboid movement (Venugopal et al. 2008e).

HA/PLLA composites have been described to fulfil certain requirements for its application as a suitable substrate for bone tissue engineering (Liao et al. 2004). The compressive strength of the HA/PLLA composite has also been described as reaching the lower limit of natural cancellous bone (approx. 1 MPa). Implant experiments showed quick healing of large segmental defects after 12 weeks of scaffold implantation and also showed appropriate bone substitute material for ingrowth of the new bone. However, Prabhakaran et al. (2009) fabricated PLLA/HA and PLLA/Col/HA nanofibres by electrospinning and showed that the biocomposite PLLA/Col/HA nanofibres are superior to PLLA/HA nanofibres for effective bone regeneration and mineralization. Moreover, the tensile strength
of these electrospun PLLA/Col/HA scaffolds was higher than that of the collagen fibrous matrix (1.68 MPa) prepared by Thomas et al. (2007) and even PCL/HA scaffolds fabricated by Venugopal et al. (2008d). These authors seeded hFOBs on the nanofibres and studied the cell proliferations (MTS assay), alkaline phosphatase (ALP) activity and mineralizations (ARS staining and SEM evaluation) on these nanofibrous scaffolds. The ALP activity was 25 per cent higher on PLLA/Col/HA scaffolds than on PCL/HA scaffolds after 20 days of cell culture. The bone nodule formation of hFOBs cultured on different electrospun nanofibres was characterized by ARS staining, and the mineralization was found to be 57 per cent higher on PLLA/Col/HA scaffolds than on PLLA/HA scaffolds. Elemental analysis by EDX measurement primarily consisted of calcium and phosphorus deposition, showing that osteoblasts seeded on PLLA/Col/HA nanofibres formed mineralized tissue that primarily consists of Ca and P deposits. Scaffolds with HA-containing polymeric composites enhanced the formation of new bone tissue with increased osteoblast adhesion, osteointegration and calcium mineral deposition on its surface (Prabhakaran et al. 2009).

PCL/HA/Col nanofibres with fibre diameters of 373 ± 191 nm were electrospun by Venugopal et al. (2007), with pore sizes of 2–35 μm, providing a large surface area-to-volume ratio for cell attachment and sufficient space for bone ingrowth and nutrient transportation. The interconnected porous structure of PCL/HA/Col nanofibres provided the mechanical support and facilitated ECM production for bone tissue formation. Nanofibrous scaffolds with controlled morphology suitable for bone tissue engineering were also proved by Gupta et al. (2009) by electrospraying of HA nanoparticles on poly-ε-caprolactone (PLACL)/Gel nanofibres. These authors carried out electrospraying and electrospinning simultaneously to produce PLACL/Gel/HA nanofibres and, at the same time, compared the mechanical and cellular properties with those of electrospun PLACL/Gel/HA-blended nanofibres. Electrospun PLACL/Gel/HA (blend) nanofibres showed HA nanoparticles embedded inside the polymer

Figure 4. Field emission SEM micrographs of hFOB interaction with a nanofibrous scaffold after 6 days of culture. (a) PCL/HA/Gel nanofibres (fibre diameter 358 ± 58 nm) and (b) PCL/HA/Gel/osteoblasts with mineral deposition on the surface.
fibres (diameter 198 ± 107 nm), while the HA nanoparticles were found to be uniformly sprayed forming a layer of HA on the surface of electrospin–electrospray PLACL/Gel/Ha nanofibres (diameter 406 ± 155 nm). The tensile stress for electrospun–electrospray PLACL/Gel/HA scaffolds was higher than that of the PLACL/Gel/HA (blend) scaffolds, and this was attributed to the fact that the electrospraying resulted in superficial dispersion of HA nanoparticles compared with a mechanically mixed blend. A significant increase in hFOB proliferation was observed on PLACL/Gel/HA (spray) nanofibres compared with PLACL/Gel/HA (blend) nanofibres after 15 days of cell seeding. These scaffolds also showed 50 per cent higher mineralization than the PLACL/Gel/HA (blend) scaffolds, proving once again that the electrospraying method is superior to the blending technique for the fabrication of biomimetic nanofibrous scaffolds for bone tissue engineering.

The biocomposite nanofibres contain amino, carboxyl and apatite molecules mostly to mimic the natural ECM for hFOBs to attach, proliferate and even migrate inside the scaffolds. The biocomposites are generally composed of a mixture of biodegradable polymers such as PLLA, PCL, PLACL, PEO, etc. with natural polymers such as Col, Gel, CTS along with nano-HA molecules. For example, Col (or Gel) supports the cells for proliferation, and HA acts as a chelating agent for the mineralization of osteoblasts for bone regeneration. PHBV nanofibrous film was fabricated by electrospinning and composited with HA by soaking in simulated body fluid by Ito et al. (2005). Studying the degradation behaviour of HA/PHBV nanofibrous films using polyhydroxybutyrate depolymerase enzyme was faster, and cell adhesion on a nanofibrous film was also higher than that on a flat film.

Nanofibrous composites mimicking the bone components and characteristics were fabricated by Ngiam et al. (2009) by combining the method of electrospinning and mineralization processes. Preferential HA deposition on PLLA/Col nanofibres with better early osteoblast attachment on mineralized nanofibres was observed by these researchers. The collagen present in PLLA/Col nanofibres had carboxyl groups, which favoured Ca^{2+} ion chelation. The alternative soaking method for mineralization was effective enough, and rapid HA formation on nanofibres was achieved by increasing the concentrations of Ca and P ions up to a ratio of 1.66 (Ca/P) similar to that found in natural bone. The presence of nano-HA on the scaffolds had a greater influence on the functionality of cells at early time points of culture, as observed by a higher level of intracellular protein production by cells. These authors concluded that the mineralization on nanofibrous scaffolds could be a biomimetic method for exploitation of early osteoblast attachment and proliferation for bone tissue engineering.

5. Conclusions

This article described a number of materials/engineering approaches that are currently being explored in order to create systems that can impart clinical benefits. They reflect many of the significant challenges for producing medical implants and therapies that are capable of achieving the regeneration of viable tissues and organs for implantation. Electrospinning offers a rapid, cost-effective and convenient way for mass production of nanofibres for fabricating...
scaffolds with biomolecules and has been used across a broad range of biocomposite polymer systems and bone tissue engineering endeavours. Tissue engineers need to improve the biomechanical properties, and the cell binding sites of electrospun nanofibres are paramount but they are also the major obstacle currently facing tissue engineers. The emerging and promising next generation of engineered tissues is relying on producing scaffolds with an informational function, e.g. material containing growth factor sequences that facilitate cell adhesion, proliferation and differentiation that are far better than in non-informational polymers. More studies remain to be done on the long journey between the laboratory and the clinics, and success in this field depends on the effective cooperation of clinicians, chemists, biologists, bioengineers and materials scientists.

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References


