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ABSTRACT

Tissue-engineering solutions often harness biomimetic materials to support cells for functional tissue regeneration. Three-dimensional scaffolds can create a multi-scale environment capable of facilitating cell adhesion, proliferation, and differentiation. One such multi-scale scaffold incorporates nanofibrous features to mimic the extracellular matrix along with a porous network for the regeneration of a variety of tissues. This review will discuss nanofibrous scaffold synthesis/fabrication, biological effects of nanofibers, their tissue-engineering applications in bone, cartilage, enamel, dentin, and periodontium, patient-specific scaffolds, and incorporated growth factor delivery systems. Nanofibrous scaffolds cannot only further the field of craniofacial regeneration but also advance technology for tissue-engineered replacements in many physiological systems.

KEY WORDS: Nanofibrous scaffold, stem cell, biomaterials, tissue engineering, craniofacial, drug delivery.

Nanofibrous Scaffolds for Dental and Craniofacial Applications

INTRODUCTION

In the dental and craniofacial region, tissue regeneration is in high demand due to trauma, post-cancer surgery, skeletal disease, congenital malformations, and periodontal disease, among others. Successful regeneration of affected tissues is necessary to reconstruct facial support, allow for mastication, and coordinate with sensory organs (eyes, nose, ears, mouth). One common approach in the field of tissue engineering aims to restore tissue function by growing cells on a designed scaffold that creates a three-dimensional microenvironment for cell support.

When designing the appropriate scaffolding to provide this support, material selection is important. Scaffolds have been fabricated for dental applications with a variety of natural and synthetic biomaterials, such as proteins (Kumada and Zhang, 2010), ceramics (Miura *et al.*, 2003), metals (Lin *et al.*, 2010), and polymers (Young *et al.*, 2002). Synthetic polymers are gaining popularity, because they can be designed to have a high processing capability, mechanical stability, biocompatibility, and biodegradability (Ma, 2008). These features allow a polymer scaffold to be integrated into biological systems and tailored to mimic the natural cell environment of the extracellular matrix (ECM). The ECM is the nanofibrous protein network that surrounds cells in all tissues to support their many functions (Ekblom *et al.*, 1986) and can be emulated with a nanofibrous polymer scaffold. The nanofibers can be designed to promote cell functions such as adhesion, proliferation, differentiation, and tissue neogenesis (Ma, 2008). In addition to nanofibers, the scaffold should have an internal interconnected porous network, a common scaffold design requirement, to allow for cellular integration into the scaffold among other functions. It can even be designed to release growth factors to tailor tissue development (Wei *et al.*, 2007).

Many cell types have been cultured on nanofibrous materials for the regeneration of craniofacial tissues. Embryonic stem cells (ESCs) (Thomson *et al.*, 1998) and adult stem cells (Bianco and Robey, 2001) are attractive cell sources due to their ability to differentiate into multiple lineages and self-renew. For example, bone marrow contains progenitor cells that have been called mesenchymal stem cells (MSCs) (Caplan, 1991), skeletal stem cells (Bianco and Robey, 2001), and so forth, because they can differentiate into multiple mesenchymal or skeletal phenotypes, including osteoblasts, chondrocytes, and adipocytes. These stem cells have been commonly identified by a few markers such as STRO1, CD146, and CD44 (Pittenger *et al.*, 1999). For simplicity, we will use the term “MSCs” in this article to indicate the pool of multipotential skeletal (mesenchymal) tissue progenitors. Dental-specific stem cells have recently been derived from several dental tissues such as dental pulp (Gronthos *et al.*, 2000), exfoliated deciduous teeth (Miura *et al.*, 2003), periodontal ligament (Seo *et al.*, 2005), apical dental papilla (Sonoyama *et al.*, 2006), and dental follicle (Zhao *et al.*, 2002). Differentiated cells have

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also been utilized to form their resident tissue: osteoblasts for bone, chondrocytes for cartilage, etc. Each cell source can be successfully implemented to promote desired tissue formation when provided with adequate support of cell function. 3D porous, nanofibrous scaffolds have supported various stem cells and differentiated cells to regenerate many hard and soft tissues. However, this review will focus on the use of the nanofibrous scaffold for dental and craniofacial tissues such as bone, cartilage, and teeth.

NANOFIBROUS SCAFFOLD SYNTHESIS AND FABRICATION

Many methods have been developed to synthesize scaffolds for tissue-engineering applications; however, only a limited number of methods can generate the nano-scale features necessary to mimic the extracellular matrix. Nanofibrous scaffolds have been fabricated by 3 techniques: electrospinning, self-assembly, and phase separation.

Electrospinning

The electrospinning process creates polymer nanofibers by applying a high voltage to a syringe filled with a polymer solution (Reneker and Chun, 1996). The applied voltage creates an electric field, which causes a jet stream of polymer solution by creating a force greater than the surface tension of the solution. The jet then bends and elongates due to electrical instability, causing a spiraling motion and smaller-diameter jet. The solvent then evaporates, leaving only a charged polymer nanofiber. The nanofiber is attracted to a grounded collector, where it solidifies into a non-woven mat. The collector can be rotated to produce a desired fiber orientation.

Electrospinning can be used for many synthetic and natural polymers and can produce scaffolds to which many cell types can adhere. In the dental field, electrospinning has been used to form a gelatin membrane for periodontal tissue regeneration. When seeded with periodontal ligament cells, the electrospun gelatin membrane showed good cell attachment and proliferation over 7 days (Zhang *et al.*, 2009). This process also supports fabrication of nanofibers from copolymers or polymer composites with biological molecules (Binulal *et al.*, 2010) or minerals (Asran *et al.*, 2010; Deng *et al.*, 2007; Jose *et al.*, 2010). Electrospinning is commonly used to produce fine synthetic and natural polymer fibers, due to its ease and compatibility with almost any dissolvable polymer; however, it often cannot produce true nanofibers that are at the order of 100 nm or less with frequently used biodegradable polymers. More importantly, electrospinning cannot produce complex 3D scaffolds or designed pore geometry.

Self-assembly

Self-assembly is the autonomous organization of components into a specific structure (Whitesides *et al.*, 1991). This important process occurs naturally with the self-assembly of nucleic acids and proteins like collagen, a main extracellular matrix protein of

many tissues (bone, cartilage, blood vessels, skin, tendons, etc.). These natural self-assembly processes can be mimicked to form nanofibrous polymer scaffolds from engineered self-assembling peptides (Hartgerink *et al.*, 2001; Zhang, 2003). These peptides can be designed to form a stable organized structure through spontaneous organization of molecules due to non-covalent interactions.

Molecular self-assembly can often result in hydrogel formation containing nanofibers of a much smaller diameter than electrospun fibers (Hartgerink *et al.*, 2001). They can also be useful in injection applications, since the self-assembly process can occur *in vivo* following injection. However, self-assembled hydrogels do not allow for control over internal pore shape and can have poor mechanical strength. Self-assembly peptides are also susceptible to enzymatic degradation, which can affect the scaffold degradation rate in an uncontrolled fashion. These issues need to be addressed, especially when self-assembling scaffolds are utilized for tissue-engineering applications.

Phase Separation

Phase separation is a process where a single-phase homogenous polymer solution is critically quenched, causing separation into a polymer-rich phase and a solvent-rich phase. This separation occurs to lower the system free energy due to the thermodynamically unstable state of the solution (Ma, 2008).

Our laboratory has developed a novel method that uses thermally induced phase separation to produce a synthetic biodegradable polymer scaffold with nanofiber features. This is done by the dissolution of a polymer such as poly(L-lactic acid) (PLLA) in a solvent such as tetrahydrofuran (THF) and induction of the solution to phase-separate at a low temperature, resulting in two distinct phases of PLLA nanofibers and THF. The THF can then be extracted with another solvent and sublimated, leaving only the PLLA nanofibers (Ma and Zhang, 1999). We have also applied this method to create a nanofibrous gelatin scaffold using different solvents (Liu *et al.*, 2009).

In biological applications, it is advantageous for nanofibrous scaffolds to have interconnected, internal pore structures to aid in cell migration, nutrient/waste exchange, and uniform cell and nutrient distribution (Ma, 2008). Porous scaffolds can be manufactured by this method because a porogen such as sugar or paraffin can be used to form pores within a bulk material and then leached out following phase separation, leaving open pores as well as nanofibers. The entire process to make a nanofibrous, macroporous scaffold is shown in Fig. 1: spherical porogen template formation, heat treatment to interconnect individual porogen spheres, polymer casting, phase separation, solvent exchange, porogen leaching, and freeze-drying (Wei and Ma, 2006). Because of this complex procedure, phase separation is more time-consuming than electrospinning. However, with this method, nanofibers with an average diameter on the order of 100 nm can be formed (Fig. 1f) (Wei and Ma, 2006), which can be difficult with other techniques. Phase separation also beneficially allows for the incorporation of internal macro-pores and complex scaffold geometries (Chen *et al.*, 2006). Therefore, phase separation is a valuable method for nanofibrous scaffold preparation.

EFFECTS OF NANOFIBERS ON CELL FUNCTION

The natural extracellular matrix (ECM) consists of nano-scale proteins such as collagen, fibronectin, and vitronectin. Cell-ECM interactions affect many signaling pathways that alter cell responses such as adhesion, proliferation, differentiation, and tissue neogenesis (Eklom *et al.*, 1986). Similarly, nanofibers affect these cell behaviors, as seen in numerous cell types cultured on various nanofibrous materials.

Adhesion and Proliferation

Without sufficient adhesion to surroundings, cell death can occur. Nanofibrous materials have been shown to promote adhesion so cells can take on morphology similar to that *in vivo* as compared with smooth materials. This has been demonstrated by human embryonic stem cell derived osteoprogenitor cells cultured on thin phase-separated nanofibrous matrix (Smith *et al.*, 2010). On nanofibrous matrix, cells beneficially maintain 3D morphology and form adhesions with phase-separated nanofibers. However, on both solid films and tissue culture plastics, cells spread into a non-physiological 2D morphology, which is not conducive to tissue development (Fig. 2). In contrast, mouse ESCs lack interaction with solid films and take on a rounded morphology, another undesirable result (Smith *et al.*, 2009b). Dental pulp stem cells (DPSCs) cultured on electrospun nanofibers showed similar behavior, since nanofibers induced a more elongated shape (Deng *et al.*, 2007).

Following adhesion, cells must proliferate for successful tissue formation. This has been reported with many cell types such as human DPSCs (Wang *et al.*, 2011) and pre-osteoblasts (Liu *et al.*, 2009) cultured on phase-separated PLLA nanofibrous scaffolds or MSCs cultured on electrospun poly(lactic-co-glycolic) acid (PLGA) scaffolds (Li *et al.*, 2002).

Differentiation and Tissue Formation

Nanofibrous scaffolds have been shown to improve differentiation of numerous stem cell populations (Hu *et al.*, 2009, 2010; Smith *et al.*, 2009a; Sun *et al.*, 2010; Wang *et al.*, 2011). For example, the phase-separated nanofibrous scaffold supported both osteogenic and chondrogenic differentiation of human MSCs when combined with appropriate chemical cues

Figure 1. SEM micrographs of nanofibrous, macroporous PLLA scaffold with sugar sphere template leaching and thermally induced phase separation method. (a) Sugar crystals; (b) sugar spheres (250- to 425- μ m diameter) made from sugar crystals; (c) interconnected sugar sphere template after heat treatment at 37°C for 15 min; (d) polymer/sugar composite following polymer casting, phase separation at -20°C, solvent exchange, prior to sugar template leaching; (e, f) final 3D nanofibrous, macroporous scaffold after sugar template leaching and freeze-drying at low (50x to show macro/micro pores) and high (10,000x to show nanofibers) magnifications. From Wei and Ma (2006). Copyright © 2006 by John Wiley & Sons.

(Hu *et al.*, 2009). Further examples of differentiation will be detailed with respect to specific tissues later in the review.

While most studies support that nanofibers promote differentiation better than other materials, it has been shown that nanofibers can aid in maintaining pluripotency of proliferating mouse ESCs (Nur-E-Kamal *et al.*, 2006). This suggests that nanofiber effects may be dependent on culture conditions and may involve several complex signaling pathways. Another study claims that electrospun PLLA microfibers better promote chondrogenic differentiation of human MSCs compared with nanofibers (Shanmugasundaram *et al.*, 2011). However, this result is likely due to the minuscule pore size (2-3 μ m) of electrospun nanofiber scaffolds, one criticism of electrospinning, not to the nanofibers themselves. While the experimental data on the effects of various nanofibers are still accumulating, further work

Figure 2. SEM micrographs of hESC-derived bone progenitor cells after 48 hrs of culture in osteogenic supplemented media on a thin phase-separated nanofibrous film (Nano), a solid film with no nanofibrous features (Solid), and gelatin-coated tissue plastic (Control). Cells maintain 3D morphology on nanofibers but spread into an undesirable 2D morphology on solid film and control tissue plastic. Scale bars = 20 μ m for Nano & Control, 50 μ m for Solid. From Smith *et al.* (2010). Copyright © 2010 by Elsevier.

is required to elucidate the role of nanofibrous features in stem cell differentiation.

Potential Mechanisms

There are a few proposed mechanisms that may explain why nanofibers induced these positive biological effects: increased protein adsorption, increased integrin expression, and altered signaling pathways. It has been suggested that initial attachment of cells may be due to increased selective adsorption of ECM proteins such as fibronectin, vitronectin, and laminin (Woo *et al.*, 2003). Increased protein adsorption may, in turn, increase expression of integrins, which are transmembrane proteins that mediate cell-ECM attachment. For example, mouse ESCs have shown increased expression of $\alpha 2$, $\alpha 5$, and $\beta 1$ integrins when cultured on nanofibrous matrix vs. smooth films. Increased integrins were furthermore linked to increased mesodermal and osteogenic differentiation in this study (Smith *et al.*, 2009b). It has been proposed that integrins may aid in differentiation by activating paxillin and focal adhesion kinase, which are involved in differentiation pathways (Woo *et al.*, 2007). Differentiation, morphology, and adhesion responses have specifically been attributed to RhoA (Hu *et al.*, 2008) and Rac (Nur-E-Kamal *et al.*, 2006) expression, which regulate cytoskeletal organization and other cell behaviors. While these are possible underlying mechanisms of nanofiber effects, more work, especially *in vivo*, is required for full investigation of the complex cell signaling pathways that may be involved. However, the effects of nanofibers on the craniofacial regeneration of bone, cartilage, enamel, dentin, and periodontium will further be discussed.

BONE TISSUE REGENERATION

In the craniofacial region, bone tissue forms the mandible; the auditory ossicles; the neurocranium, which protects the brain; and the splanchnocranium, which supports the face (Cesani *et al.*, 2006). Regenerating bone tissue in this region following injury or genetic defects can be vital to allow for normal brain function, vision, hearing, speech, and food consumption. While tissue-engineered bone grafts have been researched for years, challenges still lie in achieving *in vivo* mechanical properties and vascularization. The solution to overcoming these challenges

may lie in combining the right cell source with a nanofibrous scaffold.

Nanofibrous scaffolds may be an advantageous microenvironment for bone tissue formation by mimicking the Type I collagen fibers that are a major component of bone. The large macropores generated by porogens allow for necessary blood vessel in-growth for bone tissue regeneration and survival. Even without seeded cells, the phase-separated nanofibrous scaffold has shown promising results *in vivo* following scaffold implantation into a rat calvarial defect model, likely due to host MSC migration and subsequent osteo-

genic differentiation (Woo *et al.*, 2009). Several cell types and composite scaffold materials have been successfully utilized by our laboratory and others to produce bone tissue.

Mouse ESCs (Smith *et al.*, 2009b), human ESC-derived mesenchymal stem cells (hESC-MSCs) (Hu, 2010), human MSCs (Hu *et al.*, 2009), and human amniotic-fluid-derived stem cells (hAFSCs) (Sun *et al.*, 2010) have all been directed to an osteogenic lineage when cultured on phase-separated nanofibrous materials. In comparison with smooth materials, nanofibers promoted adhesion and differentiation, often with the addition of osteogenic factors such as bone morphogenetic protein (BMP)-7.

A mineral phase such as hydroxyapatite (HA) can also be incorporated into the nanofibrous scaffolds to form a composite bone matrix-mimicking scaffold (Zhang and Ma, 1999). HA was hypothesized to aid in bone tissue engineering because it is the main inorganic component in natural bone. The phase-separated nanofibrous PLLA scaffold was previously modified by HA crystals using simulated body fluid incubation (Zhang and Ma, 1999; Wei and Ma, 2006). Most recently, Liu *et al.* (2009) incorporated nano-HA into a 3D phase-separated nanofibrous gelatin scaffold. Nanofibers allow for adequate adhesion of HA crystals (Fig. 3a) while preventing blockage of pore interconnections (Fig. 3b). When seeded with MC3T3-E1 pre-osteoblasts, the nanofibrous gelatin/HA scaffold supported differentiation, shown by bone sialoprotein (BSP) and osteocalcin (OCN) expression (Figs. 3c, 3d), and was mechanically superior to commercially available Gelfoam® (Liu *et al.*, 2009). Other studies have shown successful bone formation with nanofibrous synthetic and natural polymer scaffolds such as electrospun polycaprolactone (Yoshimoto *et al.*, 2003), poly(lactic-co-glycolic acid) (PLGA) (Jose *et al.*, 2010), polyvinyl alcohol/type I collagen blend (Asran *et al.*, 2010), and many others (Venugopal *et al.*, 2010).

Nanofibrous scaffolds provide a cellular platform for bone formation. However, although calvarial defect models exist (Woo *et al.*, 2009), bone regeneration research is often geared toward the engineering of long bones. Because of differing developmental origins and ossification processes of the cranial skeleton and appendicular skeleton (Mao *et al.*, 2006), current approaches to the engineering of long bones may require some optimization to restore craniofacial bones. Therefore, additional

studies that focus on craniofacial bone regeneration are needed to improve restoration of this important tissue.

CARTILAGE REGENERATION

Although cartilage regeneration is often in demand for arthritic knees, hips, or shoulders, all three types of cartilage can be found in the craniofacial region. Like articulating joints, the nasal septum is also hyaline cartilage. The mandibular condyles in the temporomandibular joint (TMJ) are covered with fibrocartilage, which, when diminished, can cause TMJ disorder (Girdler, 1993). Last, the outer ear and nasal tip consist of elastic cartilage. While each type of cartilage differs in cell and matrix arrangement and proportion, the constituents of cartilage remain the same: namely, chondrocytes, collagen type II, and proteoglycans (Wachsmuth *et al.*, 2006). Therefore, successful cartilage formation can be widely applied within the craniofacial region.

While hydrogels are commonly used in cartilage tissue engineering for stem cell encapsulation (Salinas and Anseth, 2008), they cannot create complex architectural features to mimic the cell micro-environment as well as a nanofibrous scaffold. When seeded with human mesenchymal stem cells (hMSCs), the 3D phase-separated nanofibrous scaffold supported chondrogenic differentiation, shown by gene expression of chondrogenic markers. After 6 wks of culture, GAG accumulation and Type II collagen deposition occurred in the presence of TGF- β 1 (Hu *et al.*, 2009). In addition to ECM deposition, scaffold's pore architecture favorably forced hMSC aggregation to improve stem cell condensation for chondrogenic commitment. This study shows that nanofibers can support not only bone formation but also cartilage formation, with the inclusion of appropriate growth factor(s), to create a tissue-specific synthetic environment. Positive results have also been shown with thin electrospun nanofibrous meshes for cartilage regeneration through hMSC differentiation (Xin *et al.*, 2007; Alves da Silva *et al.*, 2010), though these meshes do not provide a highly 3D environment. Therefore, with the ability to form both cartilage and bone, the entire osteochondral interface found in most joints, like the temporomandibular joint, can potentially be restored following injury or disease.

OTHER DENTAL AND CRANIOFACIAL APPLICATIONS

Like many tissues throughout the body, several craniofacial tissues are hybrid tissues, such as periodontium, TMJ, cranial sutures, and mineralized layers of the tooth (enamel and dentin).

Figure 3. Effect of phase-separated nanofibrous nano-hydroxyapatite/gelatin scaffold on MC3T3-E1 pre-osteoblast differentiation. SEM micrographs of scaffolds incubated in 1.5x SBF for 7 days. (a) SEM image at a high magnification shows significant apatite deposition on nanofibers. (b) SEM image at a low magnification shows that apatite on pore walls does not block pore interconnections. Real-time PCR results of (c) bone sialoprotein (BSP) and (d) osteocalcin (OCN) after 1 and 4 wks of culture on gelatin or gelatin/apatite scaffold, normalized to beta-actin expression. Gene expression of both bone markers is significantly higher at 4 wks for gelatin/apatite composite scaffold than for gelatin scaffold alone. (*) represents statistically significant difference, $p > 0.05$. From Liu *et al.* (2009). Copyright © 2009 by Elsevier.

This aspect makes regeneration difficult. Although limited studies currently exist where nanofibrous scaffolds were used for dental applications, nanofibrous scaffolds hold potential in supporting the formation of these composite tissues. Specifically, enamel, dentin, and periodontium regeneration will be addressed, as well as patient-specific mandible regeneration.

Enamel

One recent study used a nanofibrous self-assembling peptide amphiphile to support enamel formation (Huang *et al.*, 2010). Following injection of the peptide into a mouse incisor, the incisor was cultured in a kidney capsule for 8 wks. Ordered crystalline hydroxyapatite was found in "pearls" near the incisor. Although nanofibrous organization was not verified following injection, these results may suggest that self-assembling peptides could form initial stages of tooth development.

Dentin

Dental pulp stem cells (DPSCs) are an established cell source for the formation of dentin (the mineralized layer below enamel) because they can differentiate into odontoblasts. When seeded onto a phase-separated nanofibrous PLLA scaffold, nanofibers promoted attachment and proliferation of human DPSCs *in vitro* (Wang *et al.*, 2011). Electrospun nanofibrous polymer scaffolds

Figure 4. Patient-specific nanofibrous (NF) scaffold for mandible section from CT-scan. **(a)** 3D reconstruction of entire human mandible with highlighted section molded for scaffold fabrication. **(b)** Resulting bulk phase-separated NF scaffold (scale bar = 10 mm). SEM micrographs of **(c)** nanofibers (scale bar = 5 μ m) and **(d)** interconnected pore structure of scaffold (scale bar = 500 μ m). From Chen *et al.* (2006). Copyright © 2006 by Elsevier.

have also supported adhesion of DPSCs (Deng *et al.*, 2007; Xu *et al.*, 2007). Odontogenic differentiation of DPSCs was enhanced on the phase-separated nanofibrous scaffold compared with the solid-walled scaffold *in vitro*, shown by increased alkaline phosphatase (ALP) activity, dentin marker gene expression, and calcium deposition (Wang *et al.*, 2011). Furthermore, these nanofibers promoted collagen matrix deposition, dentin sialoprotein secretion, blood vessel in-growth, and mineralization compared with solid-walled scaffolds in a subcutaneous implantation study of cultured DPSC-scaffold constructs in nude mice (Wang *et al.*, 2011). Similar to osteogenic differentiation, odontogenic differentiation was enhanced by nanohydroxyapatite on electrospun nanofibers (Yang *et al.*, 2010).

Periodontium

Periodontal tissue engineering has also been of recent interest for the repair of defects in alveolar bone, periodontal ligament, and cementum due to periodontal disease. Recent studies have shown good attachment and proliferation of periodontal ligament cells on electrospun PLGA (Inanc *et al.*, 2009) and electrospun gelatin (Zhang *et al.*, 2009) scaffolds, as well as periodontal ligament cells' osteogenic differentiation potential (Inanc *et al.*, 2009). Furthermore, human periodontal ligament cells cultured on self-assembled peptide nanofibrous scaffolds promoted deposition of the main periodontal ligament ECM components, collagen type I and type III (Kumada and Zhang,

2010). Much work is still required to determine the nanofiber effect on periodontal tissue regeneration as well as the underlying mechanisms.

Patient-specific Mandible

Because of the highly intricate and unique features of the face and widely varied craniofacial sizes and complex shapes, there is a need for patient-specific tissue regeneration. To serve this need, our laboratory has developed technology to create patient-specific, anatomically shaped scaffolds. A scaffold unique to each patient can be helpful in regenerating the mandible shape, for example, since it has complex geometry and can vary highly between individuals. This can be done by the creation of a wax mold from computed tomography (CT) scans of the patient's mandible (Fig. 4a). The mold is then used to form the phase-separated nanofibrous scaffold (Figs. 4b, 4c) with macroporous structure (Fig. 4d) and patient-specific geometry. Such nanofibrous scaffolds supported bone formation when seeded with MC3T3-E1 pre-osteoblasts (Chen *et al.*, 2006). Similar nanofibrous scaffolds have been created for an anatomical ear (Chen *et al.*,

2006). This technique holds great potential for dental/craniofacial tissue replacements that are specific to each patient for best restoration of natural appearance and function.

DRUG DELIVERY BY IMMOBILIZED NANOSPHERES

As shown for each tissue described previously, growth factors can have a large influence on stem cell differentiation for craniofacial tissue formation. Drug delivery could aid in developing craniofacial tissue as well as for the delivery of therapeutic drugs following implantation, such as for periodontal disease treatment with antibiotics (Zamani *et al.*, 2010). Controlled release of drugs or growth factors *in vivo* is highly desired to sustain their bioactivity (Krishnamurthy and Manning, 2002). Hydrogels such as polyethylene glycol (PEG) are often used as drug carriers because drugs can be easily incorporated into the hydrogel solution (Elisseeff *et al.*, 2001). However, biodegradable polyesters such as PLGA can be made into nanospheres by a double-emulsion technique to achieve significantly longer controlled release compared with that of hydrogels.

PLGA nanospheres were used to deliver BMP-7 to induce ectopic bone formation. Nanospheres were immobilized on the nanofibers (Fig. 5a) of a phase-separated nanofibrous scaffold without blocking interpore connections (Fig. 5b). Scaffolds with BMP-7 nanospheres without cells were implanted into rats and evaluated after 3 wks. Scaffolds soaked with BMP-7 (Fig. 5c) or with blank nanospheres (not shown) contained only fibrous

tissue, but scaffolds with BMP-7 nanospheres revealed initial bone formation (Fig. 5d) (Wei *et al.*, 2007). A longer implantation time resulted in more significant bone formation in the nanofibrous scaffolds incorporated with BMP-7 nanospheres. Similarly, PDGF-releasing nanospheres immobilized on a phase-separated nanofibrous scaffold have been shown to promote angiogenesis (Jin *et al.*, 2008). The temporally and spatially controlled drug-delivering PLGA nanospheres on the nanofibrous scaffolds can be beneficially applied to dental and craniofacial tissue regeneration.

CONCLUSION

The fields of biomaterials and tissue regeneration have advanced to create a suitable microenvironment for cell development for the regeneration of various tissues. Nanofibrous scaffolds are one such biomaterial approach shown to enhance bone, cartilage, enamel, dentin, and periodontium regeneration. Controlled biomolecule delivery on the scaffolds has further improved tissue formation through sustaining the bioactivity of the biological factors. However, more studies are required to understand the mechanisms of the nanofiber effects. There remain significant technical challenges for the synergistic integration of structural cues with biological cues for cell-based therapies to achieve functional dental and craniofacial tissue regeneration.

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Figure 5. BMP-7 releasing nanospheres (NS) immobilized on phase-separated nanofibrous scaffold and *in vivo* histological analysis. SEM micrographs of (a) NS immobilized on nanofibers with (b) undisturbed porous structure. H-E staining following 3 wks of rat implantation *in vivo* for (c) BMP-7 absorbed scaffold and (d) BMP-7 NS scaffold at 200x magnification. Bone formation is observed in BMP-7 NS scaffold only, shown by matrix formation within pores. From Wei *et al.* (2007). Copyright © 2007 by Elsevier.

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