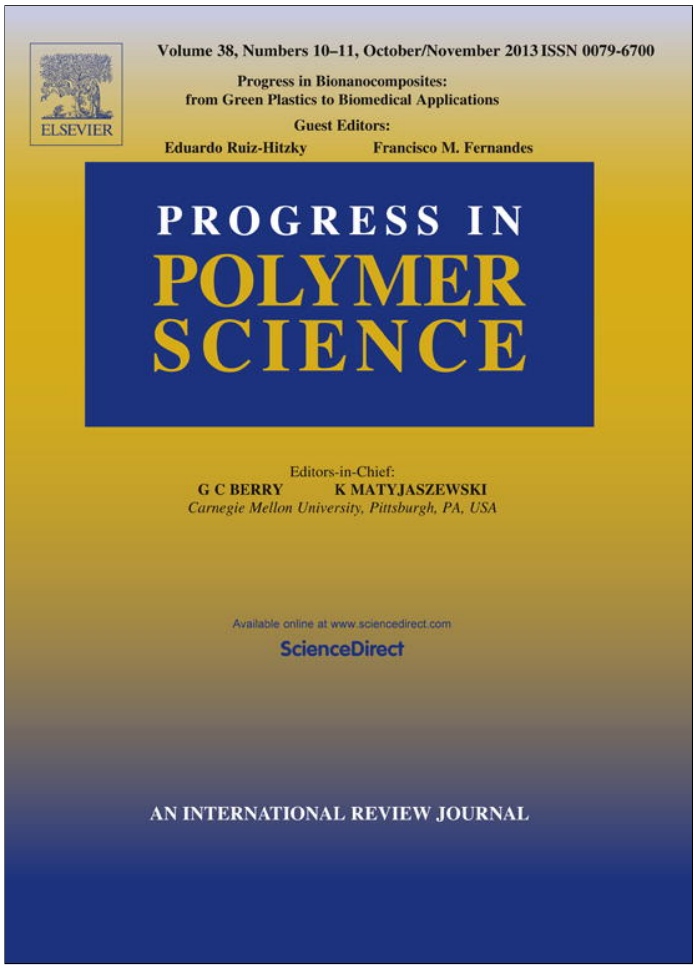

Progress in Polymer Science 38 (2013) 1487–1503

Contents lists available at ScienceDirect

Progress in Polymer Science

journal homepage: www.elsevier.com/locate/ppolysci

Synthetic biopolymer nanocomposites for tissue engineering scaffolds

Masami Okamoto*, Baiju John

Advanced Polymeric Nanostructured Materials Engineering, Graduate School of Engineering, Toyota Technological Institute, 2-12-1 Hisakata, Tempaku, Nagoya 468-8511, Japan

ARTICLE INFO

Article history:
Received 1 May 2012
Received in revised form 6 May 2013
Accepted 13 May 2013
Available online 6 June 2013

Keywords:
Biopolymer
Nanocomposites
Tissue engineering
Scaffolds
Mesenchymal stem cell
Nanoparticles

ABSTRACT

With tissue engineering we can create biological substitutes to repair or replace failing organs or tissues. Synthetic biopolymer-based nanocomposites are of interest for use in tissue engineering scaffolds due to their biocompatibility and adjustable biodegradation kinetics. The most often utilized synthetic biopolymers for three dimensional scaffolds in tissue engineering are saturated poly(α-hydroxy esters), including poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(lactic acid-*co*-glycolic acid) (PLGA), and poly(ε-caprolactone) (PCL). To enhance the mechanical properties and cellular adhesion and proliferation, the incorporation of nanoparticles (e.g., apatite component, carbon nanostructures and metal nanoparticles) has been extensively investigated. At the same time, current research is focused on the interaction between stromal cells and biopolymer interfaces. In this review, current research trends in nanocomposite materials for tissue engineering, including strategies for fabrication of nanocomposite scaffolds with highly porous and interconnected pores are presented. The results of the in vitro cell culture analysis of the cell–scaffold interaction using the colonization of mesenchymal stem cells (MSCs) and degradation of the scaffolds in vitro are also discussed.

Contents

Abbreviations: Ag, silver; BMNC, bone marrow mononuclear cell; BMSC, bone marrow stromal cell; CNF, carbon nanofiber; CNT, carbon nanotube; CO_2, carbon dioxide; ECM, extracellular matrix; EGFP, enhanced green fluorescent protein; ES, embryonic stem; FDA, US Food and Drug Administration; GF/PL, carbon dioxide foaming and solid porogen leaching; g-HA, surface-grafted-hydroxyapatite; HA, hydroxyapatite; HEK, human epidermal keratinocyte; hMSC, human mesenchymal stem cell; HRP, horseradish peroxidase; LA, bare poly(L-lactic acid) microsphere; mHA, micro-hydroxyapatite; MSC, mesenchymal stem cell; MWNT, multi-walled nanotube; ND, nanodiamond; ND-ODA, octadecylamine-functionalized nanodiamond; nHA, nano-sized hydroxyapatite; NS, nano-scaffold; $nTiO_2$, TiO_2 nanoparticles; PBS, poly(butylene succinate); PBSPTDGS, poly(butylene/thiodiethylene succinate) block copolymer; PCL, poly(ε-caprolactone); PDLLA, racemic mixture of D,L-poly(lactic acid); PGA, poly(glycolic acid); PHB, poly(hydroxy butyrate); PLA, poly(lactic acid); PLGA, poly(lactic acid-*co*-glycolic acid); PLLA, poly(L-lactic acid); PPF, poly(propylene fumarate); PVA, polyvinyl alcohol; RGD, Arg-Gly-Asp; SBF, simulated body fluid; SEM, scanning electron microscope; SWNT, single-walled carbon nanotube; 3D, three dimensional; TIPS, thermally induced phase separation; WHO, World Health Organization.

* Corresponding author. Tel.: +81 52 809 1861; fax: +81 52 809 1864.
E-mail address: okamoto@toyota-ti.ac.jp (M. Okamoto).

0079-6700/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.progpolymsci.2013.06.001

1. Introduction

Since the industrial revolution and particularly after World War II, the breakthrough in materials research has become increasingly more rapid, and has resulted in the wide use of materials such as polymers, metals, semiconductors, and agricultural chemicals (e.g., pesticides and fertilizers). The production of these materials in increasing quantities to meet the demands of a growing population has led to the increased consumption of fossil fuel and the release of wastes, resulting in regional and global environmental problems ranging from air, water and soil pollution, to climate changes.

These problems in turn limit the amount and extent to which such materials can be used, and call for a re-thinking of the design, synthesis, and production of materials in mass use. In this regard, natural materials provide not only a baseline for a comparison of the performance of man-made materials, but also a clue to the development of new materials that are more environmentally sustainable, in addition to having a desirable functionality.

Hence, with this background, biopolymers are well-known examples for renewable source based, environmental benign polymeric materials [1]. These include polysaccharides, such as cellulose, starch, alginate and chitin/chitosan, carbohydrate polymers produced by bacteria and fungi [2], and animal protein-based biopolymers such as wool, silk, gelatin and collagen. Naturally derived polymers offer interesting properties of biocompatibility and biodegradability. One of the advantages of naturally derived polymers is the biological recognition that may positively support cell adhesion and function, however, many have poor mechanical properties, and are also limited in supply and can therefore be costly [3].

On the other hand, polyvinyl alcohol (PVA), poly(ε-caprolactone) (PCL), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(hydroxy butyrate) (PHB) and poly(butylene succinate) (PBS) are examples of synthetic biodegradable polymers; it [4]. In today's commercial environment, synthetic biopolymers have proven to be relatively expensive and available only in small quantities, with applications in the textile and medical industries, as well as the packaging industry. Although this has led to limited applications, they can be produced in large-scale under controlled conditions and with predictable and reproducible mechanical properties, degradation rate and microstructure [4,5].

In this context, one of the most promising synthetic biopolymers is PLA because it is made from agriculture products. Although PLA is not a new polymer, developments in the capability to manufacture the monomer economically from agriculture products have placed this material at the forefront of the emerging biodegradable plastics industries.

PLA, PGA and their copolymers, poly(lactic acid-*co*-glycolic acid) (PLGA) are extensively used in tissue engineering for treating patients suffering from damaged or lost organs or tissues [6,7]. They have been demonstrated to be biocompatible, degrading into non-toxic components, and have a long history as degradable surgical sutures with FDA (US Food and Drug Administration) approval for clinical use. PCL and PHB are also used in tissue engineering research.

The task of tissue engineering demands a combination of molecular biology and materials engineering, since in many applications a scaffold is needed to provide a temporary artificial matrix for cell seeding. In general, scaffolds must exhibit high porosity, proper pore size, biocompatibility, biodegradability and proper degradation rate [8]. The scaffold must provide sufficient mechanical support to maintain stresses and loadings generated during in vitro or in vivo regeneration.

For some of aforementioned applications, enhancement of the mechanical properties is often needed [9]. This can be achieved by the incorporation of nanoparticles, such as hydroxyapatite (HA) carbon nanotubes and layered silicates. Polymer/layered silicate nanocomposites have become the focus of academic and industrial attention [10]. Introduction of small quantities of high aspect ratio nano-sized silicate particles can significantly improve mechanical and physical properties of the polymer matrix [11]. However, the pore structure, the porosity, the crystallinity and the degradation rate may alter the mechanical properties and, thus, the efficiency of a scaffold. As a consequence, the scaffold fabrication method should allow for the control of its pore size and shape and should enhance the maintenance of its mechanical properties and biocompatibility [6,8].

During the past year, many techniques have been applied for making porous scaffolds. Among the most popular are particulate leaching [12], temperature-induced phase separation [13], phase inversion in the presence of a liquid non-solvent [14], emulsion freeze-drying [15], electrospinning [16], and rapid prototyping [6]. On the other hand, foaming of polymers using supercritical fluids is a versatile method in obtaining porous structure [8,17].

This review highlights the synthetic biopolymer-based nanocomposites with the aim of producing porous

scaffolds in tissue engineering applications during the last decade. In addition, current research trends on nanocomposite materials for tissue engineering are also reviewed, including strategies for fabrication of nanocomposite scaffolds with highly porous and interconnected pores. The results of the in vitro cell culture analysis of the cell–scaffold interaction using the colonization of mesenchymal stem cells (MSCs), and in vitro degradation of the scaffolds are also discussed.

2. Tissue engineering applications

Tissue engineering applies methods from materials engineering and life science to create artificial constructs for regeneration of new tissue [6,7]. Tissue engineering facilitates the creation of biological substitutes to repair or replace the failing organs or tissues. One of the most promising approaches toward this direction is to grow cells on scaffolds that act as temporary support for cells during the regeneration of the target tissues, without losing the three dimensional (3D) stable structure.

Polymeric scaffolds play a pivotal role in tissue engineering through cell seeding, proliferation, and new tissue formation in three dimensions, showing great promise in the research of engineering a variety of tissues. Pore size, porosity, and surface area are widely recognized as important parameters for a tissue engineering scaffold. Other architectural features such as pore shape, pore wall morphology, and interconnectivity between pores of the scaffolding materials are also suggested to be important for cell seeding, migration, growth, mass transport and tissue formation [6].

Scaffolds made from collagen are being rapidly replaced with ultraporous scaffolds from biodegradable polymers. Biodegradable polymers are attractive candidates for scaffolding materials because they degrade as the new tissues are formed, eventually leaving nothing foreign to the body. The major challenges in scaffold manufacture lies in the design and fabrication of customizable biodegradable constructs with properties that promote cell adhesion and cell porosity, along with sufficient mechanical properties that match the host tissue, with predictable degradation rate and biocompatibility [6,7,18].

The biocompatibility of the materials is imperative. That is, the substrate materials must not elicit an inflammatory response nor demonstrate immunogenicity of cytotoxicity. The scaffolds must be easily sterilizable in both the surface and the bulk to prevent infection [19]. For scaffolds particularly in bone tissue engineering, a typical porosity of 90% with a pore diameter of about 100 μm is required for cell penetration and a proper vascularization of the ingrown tissue [20].

Bioactive ceramics such as HA and calcium phosphates are another major class of biomaterials for bone repair is [21,22]. They showed appropriate osteoconductivity and biocompatibility because of chemical and structural similarity with the mineral phase to native bone. However, their inherent brittleness and the difficulty to shape them are disadvantages. For this reason, polymer/bioactive ceramic composite scaffolds have been developed in applications for the bone tissue engineering. They exhibit good bioactivity, manipulation and control microstructure in shaping to fit bone defects [23].

3. Synthetic biopolymer and its nanocomposites for tissue engineering

The extensive literature on nanocomposite research (e.g., polymer/layered silicate) is covered in reviews [10,24–26]. The study on nanocomposites has gained greater momentum. This new class of materials is now being introduced in structural applications, such as gas barrier film, flame retardant product, and other load-bearing applications [27]. Among these nanocomposites, biopolymer-based nanocomposites are considered to be a stepping stone toward a greener and sustainable environment. Biopolymer-based nanocomposites are described in detailed studies and reviews elsewhere [28–30].

The most often utilized synthetic biopolymers for 3D scaffolds in tissue engineering are saturated poly(α-hydroxy esters) including PLA, racemic mixture of D,L-PLA (PDLLA) and PGA, as well as PLGA [31–33]. These polymers degrade through hydrolysis of the ester bonds. Once degraded, the monomeric components are removed by natural pathways. The body contains highly regulated mechanisms for completely removing monomeric components of lactic and glycolic acids. PGA is converted to metabolites or eliminated by other mechanisms. On the other hand, PLA is cleared via the tricarboxylic acid cycle. Due to these properties PLA and PGA have been used in products and devices, including degradable sutures, approved by the US FDA. The cost of PLA for medical use is about 3$/g, which is three order of magnitude higher than conventional injection moldable PLA (3$/kg) [34]. PLA and PGA may be processed easily, and their degradation rates, physical and mechanical properties are adjustable over a wide range by using various molecular weights and copolymer composition. These polymers undergo a bulk erosion process such that can cause scaffolds to fail prematurely. The abrupt release of their acidic degradation products can cause a strong inflammatory response [35]. Table 1 shows an overview of the discussed biopolymers and their physical properties. Their degradation rates decreases in the following order:

PGA > PDLLA > PLLA > PCL∼PPF

PGA degrades rapidly in aqueous solution or in vivo, and loses mechanical integrity between two and four weeks. The extra methyl group in the PLA repeating unit makes it more hydrophobic, reduces the molecular affinity to water, and leads to a slower degradation. Therefore, the scaffolds made from PLA degrade slowly both in vitro and in vivo, maintaining mechanical properties over several months [45].

PCL degrades at a significantly slower rate than PLA, PGA, and PLGA. The slow degradation makes PCL less attractive for biomedical applications, but more attractive for long-term implants and controlled release application [6,36]. PCL has been used as a candidate polymer for bone tissue engineering, where scaffolds should maintain physical and mechanical properties for at least 6 months [37].

Table 1
Physical properties of synthetic biopolymers used as scaffold materials.

Biopolymers	Thermal properties		Tensile modulus (GPa)	Biodegradation time (months)
	T_m (°C)[a]	T_g (°C)[b]		
PLLA	173–178	60–65	1.2–3.0	>24
PDLLA	–	55–60	1.9–2.4	12–16
PGA	225–230	35–40	5–7	3–4
PLGA (50/50)	–	50–55	1.4–2.8	Adjustable: 3–6
PCL	58–63	−60	0.4–0.6	>24
PPF	30–50	−60	2–3	>24

Reproduced from [48].
[a] Melting temperature.
[b] Glass transition temperature.

Poly(propylene fumarate) (PPF), an unsaturated linear polyester, has been developed for orthopedic and dental applications [38]. Its degradation products are biocompatible and readily removed from the body. The double bond of PPF main chains leads to in situ cross-linking, which causes results in a moldable composite. The preservation of the double bond and molecular weight are key characteristics to control the final mechanical properties and degradation time.

However, PPF and other biodegradable polymers lack the mechanical strength required for tissue engineering of load-bearing bones [39]. The development of composite and nanocomposite materials utilizing inorganic particles, e.g., apatite components (i.e., the main constituent of the inorganic phase of bone [6]), bioactive glasses, carbon nanostructures (e.g., nanotubes, nanofibers, and graphene), and metal nanoparticles has been investigated.

3.1. Hydroxyapatite (HA)-base nanocomposites

HA promotes bone ingrowth and biocompatible because around 65 wt% of bone is made of HA, $Ca_{10}(PO_4)_6(OH)_2$. Natural or synthetic HA has been intensively investigated as the major component of scaffold materials for bone tissue engineering [40]. The Ca/P ratio of 1.50–1.67 is the key issue to promote bone regeneration. Much better osteoconductive properties have been reported in HA by changing composition, size and morphology [41]. The nano-sized HA (nHA) may have other special properties due to its small size and huge specific surface area. A significant increase in protein adsorption and osteoblast adhesion on the nano-sized ceramic materials was reported by Webster et al. [42].

Fig. 1 shows the rod shaped morphology of the nHA with particle width ranging from 37–65 nm and length from 100 to 400 nm [43]. The compressive strength of bioceramics increases when their grain size reduced to nanolevels [44].

Nanocomposites based on HA particles and biopolymers have attracted attention for their good osteoconductivity, osteoinductivity, biodegradability and high mechanical strengths. The Ma group mimicked the size scale of HA in natural bone and showed the incorporation of nHA improved the mechanical properties and protein adsorption of the composite scaffolds, while maintaining high porosity and suitable microarchitecture [44].

Nejati et al. [43] reported that the effect of the synthesis nHA on the scaffolds morphology and mechanical properties in poly(L-lactic acid) (PLLA)-based nanocomposites.

The morphology and microstructure of the scaffolds were examined using scanning electron microscope (SEM) (Fig. 2). The nanocomposite scaffold (Fig. 2c and d) maintain a regular internal ladder-like pore structure, similar to neat PLLA scaffold (Fig. 2a and b) with a typical morphology processed by thermally induced phase separation [13]. Rod-like nHA particles are distributed within the pore walls, n with no aggregation in the pores (Fig. 2e and f). However, the nanocomposite exhibits little effect of nHA on the development of the pore morphology as compared with that of neat PLLA. Pore size of neat PLLA and nanocomposite scaffolds are in the range of 175–97 μm, respectively. In the case of microcomposite scaffold (Fig. 2g and h), micro-HA (mHA) particles are randomly distributed in PLLA matrix. Some are embedded in the pore wall and some are piled together between or within the pores. Among composite and neat PLLA scaffolds, nanocomposites scaffolds showed

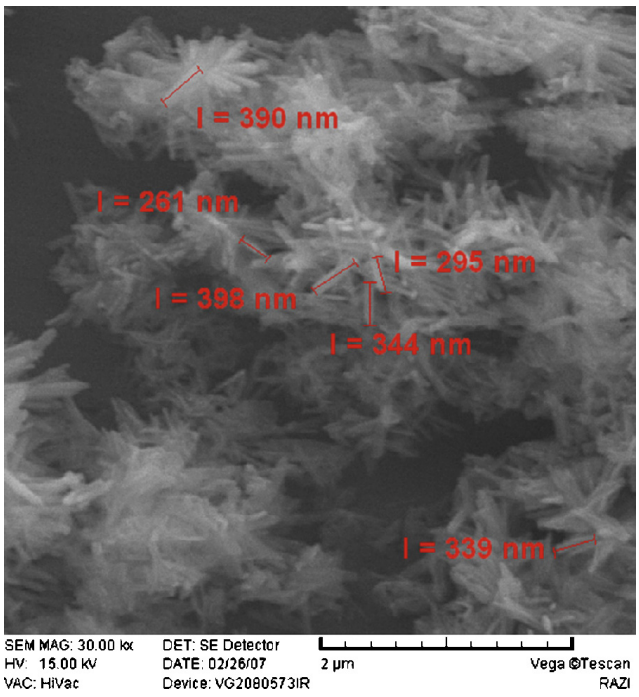

Fig. 1. SEM micrographs of the synthetic nHA rods. [43], Copyright 2008. Reproduced with permission from Elsevier Ltd.

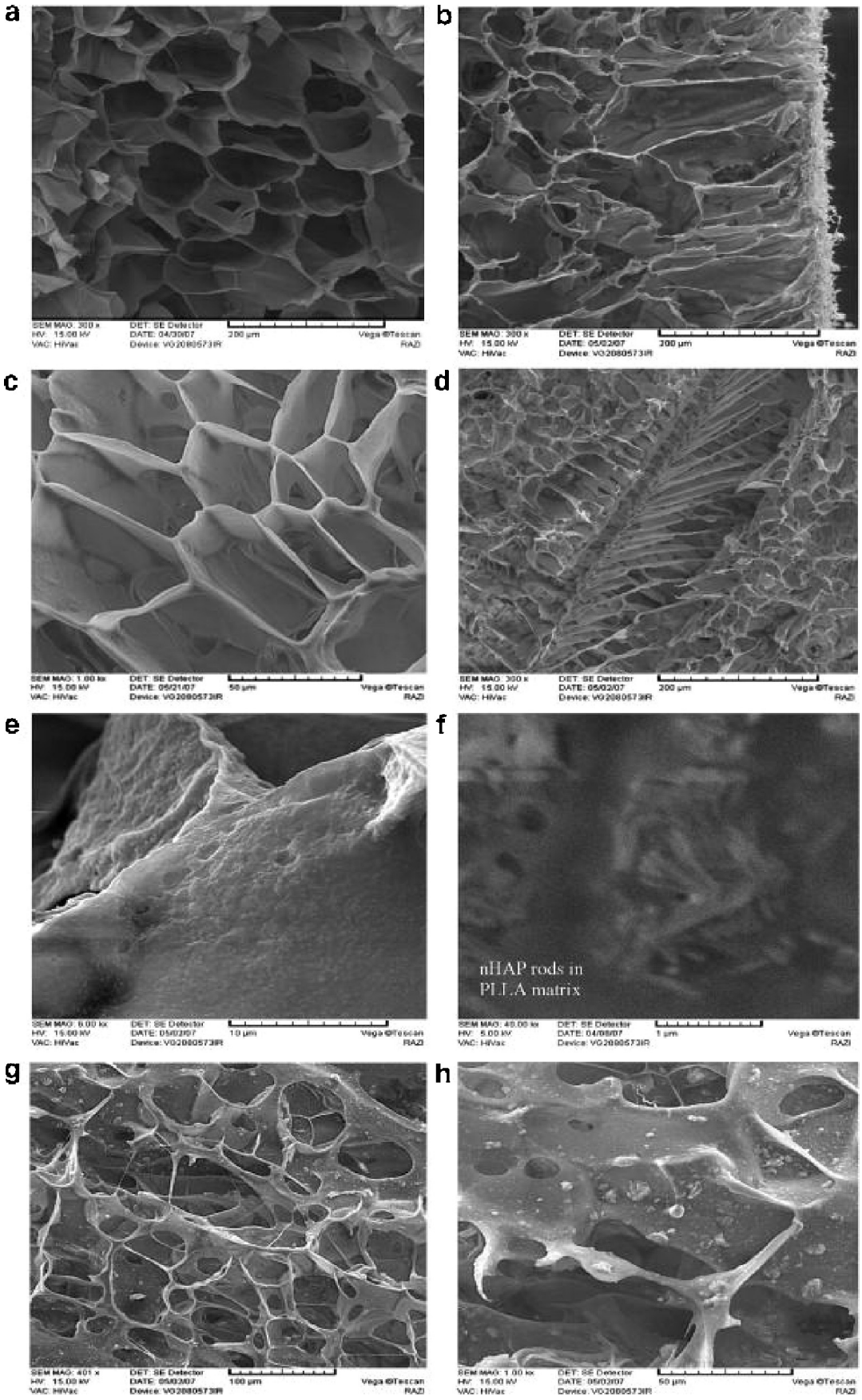

Fig. 2. SEM micrographs of pure PLLA, PLLA/nHA and PLLA/mHAP scaffolds. (a, b) Neat PLLA and cross-section; (c, d) PLLA/nHA: 50/50 and cross-section; (e, f) PLLA/nHAP: 50/50; (g, h) PLLA/mHAP: 50/50 scaffold.
[43], Copyright 2008. Reproduced with permission from Elsevier Ltd.

the highest compressive strength (8.67 MPa) with 85.1% porosity, comparable to the high end of compressive strength of cancellous bone (2–10 MPa) [46].

PCL/nHA nanocomposites were prepared and they combine the osteoconductivity and biocompatibility shown by HA ceramic with PCL properties [45,47–49]. The structural characterization of a novel electrospun nanocomposite and the analysis of cell response by a highly sensitive cell, embryonic stem cell for PCL/Ca-deficient nHA system have been investigated [47]. For higher Ca-deficient nHA contents (~55 wt%), the mechanical properties significantly decreased, and the onset decomposition temperature and crystallinity considerably decreased.

Due to the brittleness of HA and lack of interaction with polymer matrix, the ceramic nanoparticles may present deleterious effects on the mechanical properties, when loaded at high levels. Coupling agents are generally used to overcome both the lack of interaction with polymer and nHA aggregation [49–51]. In order to increase the interfacial strength between PLLA and HA, and hence increase the mechanical properties, the nHA particles were surface-grafted (g-HA) with the polymer and further blended with PLLA [50]. The PLLA/g-HA nanocomposites also demonstrated improved cell compatibility due to the favorable biocompatibility of the nHA particles and more uniform distribution of the g-HA nanoparticles on the film surface [50,51]. These nanocomposites are of interest to the biomedical community because the materials have a structure that induces and promotes new bone formation at the required site.

Calcium phosphate biomaterials certainly posses osteoconductive properties and may bind directly to bone under certain conditions [43]. Calcium phosphate materials are suitable for the calcified tissue generation.

3.2. Metal nanoparticles-base nanocomposites

Currently, metal nanoparticle-based nanocomposites are used in various biomedical applications, such as probes for electron microscopy to visualize cellular structure, drug delivery, diagnosis and therapy. The unique physical characteristics of gold nanoparticle-based nanocomposites are used in the optical and photonic fields [52], not medical applications.

Silver (Ag) is known to have a disinfecting effect, and has found applications in traditional medicines. Ag nanoparticles have aptly been investigated for their antibacterial property [53]. Biopolymer-embedded Ag nanoparticles have been investigated [54]. The nano-sized Ag permits a controlled antibacterial effect due to the high surface area. For PLLA-based nanocomposite fibers including Ag nanoparticles, a antibacterial effect longer than 20 days was shown [55]. PLGA-based nanocomposites have also been [56,57]. The metal nanoparticles induce a thermal conductivity in the nanocomposites that enhances the degradation rate [55]. Furthermore, the Ag nanoparticles change the surface wettability and roughness of the nanocomposites. For these reasons it is very difficult to control the bacterial adhesion process.

Of particular interest, it is important to note that Ag nanoparticles are recognized and listed under carcinogenic materials by the World Health Organization (WHO). These hazardous signs require immediate and thorough action not only from the environment and human health viewpoints, but also from the perspective of socio-economic benefits [58].

3.3. Carbon-base nanocomposites

Carbon nanostructures in polymer matrix have been extensively investigated for biomedical applications [59]. Carbon nanotubes (CNTs) have potential for use in biomedical scaffolds. Honeycomb-like matrices of multi-walled nanotube (MWNT) have been fabricated as potential scaffolds for tissue engineering [60]. Mouse fibroblast cells were cultured on the nanotube networks, prepared by treatment with an acid solution that generates carboxylic acid groups at the defect of the nanotubes. The carbon networks can be used as a biocompatible mesh for restoring or reinforcing damaged tissues because of no cytotoxicity of the networks. The electrical conductivity of the nanocomposites including carbon nanostructures is a useful tool to direct cell growth because they can conduct an electrical stimulus in the tissue healing process. An osteoblast proliferation on PLLA/MWNT nanocomposites under an alternating current stimulation was investigated [61]. The results showed an increase in osteoblast proliferation and extra-cellular calcium deposition on the nanocomposites as compared with the control samples. Unfortunately, no comparison was made with a currently used orthopedic reference material under electrical stimulation.

Mikos et al. [62] investigated in vitro cytotoxicity of single-walled CNT (SWNT)/PPF nanocomposites. The results did not reveal any in vitro cytotoxicity for PPF/SWNT functionalized with 4-tert-butylphenylene nanocomposites. Moreover, nearly 100% cell viability was observed on the nanocomposites and cell attachment on their surfaces was comparable with that on tissue culture polystyrene. The nature of the functional group at the CNT surface seems to play an important role to improve the dispersion of CNTs in polymer matrix and in the mechanism of interaction with cells. The sidewall carboxylic functionalized SWNTs exhibited an nucleation surface to induce the formation of a biomimetic apatite coating [63].

Nanodiamonds (NDs) synthesized by detonation are one of the most promising materials for use in multifunctional nanocomposites for various applications, including biomedical [64–66]. To fully benefit from the advantages of NDs as nanofillers for biopolymeric bone scaffolds, they need to be dispersed into single particles. The quality of the filler dispersion in the matrix is important, because it determines the surface area of the nanoparticles available for interaction with the matrix. When adequately dispersed, NDs increase the strength, toughness, and thermal stability of the nanocomposites [67]. The purified NDs are composed of particles with 5 nm average diameter. They contain an inert diamond core and are decorated by functional groups such as COOH, OH, NH_2, etc. [64].

Zhou et al. [68] prepared multifunctional bone scaffold materials composed of PLLA and octadecylamine-functionalized ND (ND-ODA) via solution casting, followed by compression molding. Addition of 10 wt% of ND-ODA

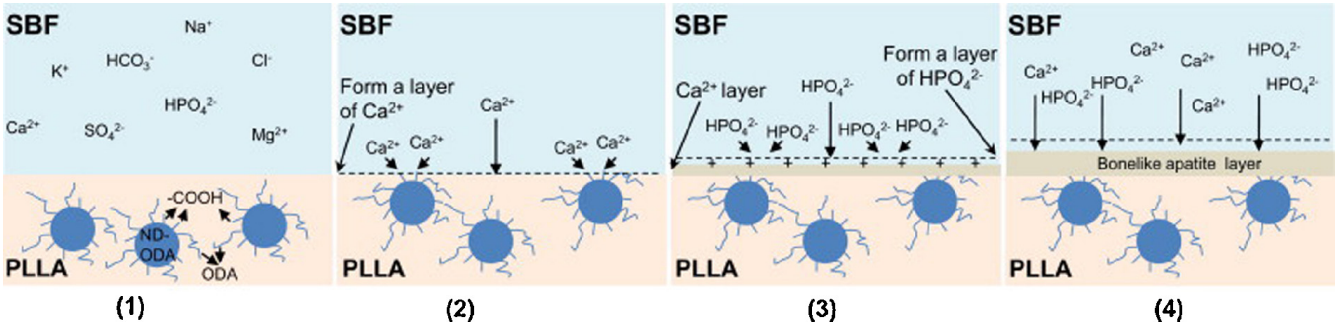

Fig. 3. A schematic representation of a biomineralization process on PLLA/ND-ODA scaffolds in SBF. (1) The initial stage. (2) While in contact with SBF, PLLA is hydrolyzed, resulting in the formation of —COOH groups on the surface of the scaffold. Due to the degradation of PLLA, ND-ODA is exposed to SBF. The exposed —COOH groups of ND-ODA dissociate and form negatively charged —COO— on the surface. In addition, the ND-ODA may speed up the degradation of PLLA to produce more —COOH groups on the PLLA surface. The negatively charged surface attracts Ca^{2+}. (3) The deposited calcium ions, in turn, interact with phosphate ions in the SBF and form bonelike apatite. (4) The bonelike apatite then grows spontaneously, consuming the calcium and phosphate ions to form apatite clusters.
[68], Copyright 2012. Reproduced with permission from Elsevier Ltd.

resulted in a 280% increase in the strain to failure and a 310% increase in fracture energy as compared to neat PLLA. Both of these parameters are crucial for bone tissue engineering and for manufacturing of orthopedic surgical fixation devices. The biomineralization of the nanocomposite scaffolds was tested in simulated body fluid (SBF) [69]. The apatite nucleation and growth occurred faster on the nanocomposites than on neat PLLA (Fig. 3) [68]. The increased mechanical properties and enhanced biomineralization make PLLA/ND-ODA nanocomposites a promising materials for bone surgical fixation devices and regenerative medicine.

Despite the evidence of research into potential biomedical applications of carbon-based nanocomposites, there have been many published studies on the cytotoxicity of the carbon nanostructures [70]. Some research groups detected high toxicity in both cells [71–78] and animals [79–81], and explained mechanisms to cell damage at molecular and gene expression levels [82].

Exposure to SWNT resulted in accelerated oxidative stress (increased free radical and peroxide generation and depletion of total antioxidant reserves), loss in cell viability and morphological alterations to cellular structure. It was concluded that these effects were the results of high levels of iron catalyst present in the unrefined SWNT. As a result, possible dermal toxicity in handling unrefined CNT was warned. Similar dermal toxicity warnings were echoed in 2005, in a study which found that MWNT initiated an irritation response in human epidermal keratinocyte (HEK) cells [74]. Purified MWNT incubated (at doses of 0.1–0.4 mg/mL) with HEK cells for up to 48 h were observed to localize within cells (Fig. 4), eliciting the production of the pro-inflammatory cytokine release and decreasing cell viability in a time- and dose-dependent manner.

These controversial results reported by different researchers reflect the complex material properties of CNTs such as SWNTs or MWNTs. In addition, different synthesis methods may produce CNTs with different diameters, lengths and impurities. The results urge caution when handling CNTs and the introduction of safety measures in laboratories should be seriously considered. Most importantly, the success of CNT technology is dependent upon

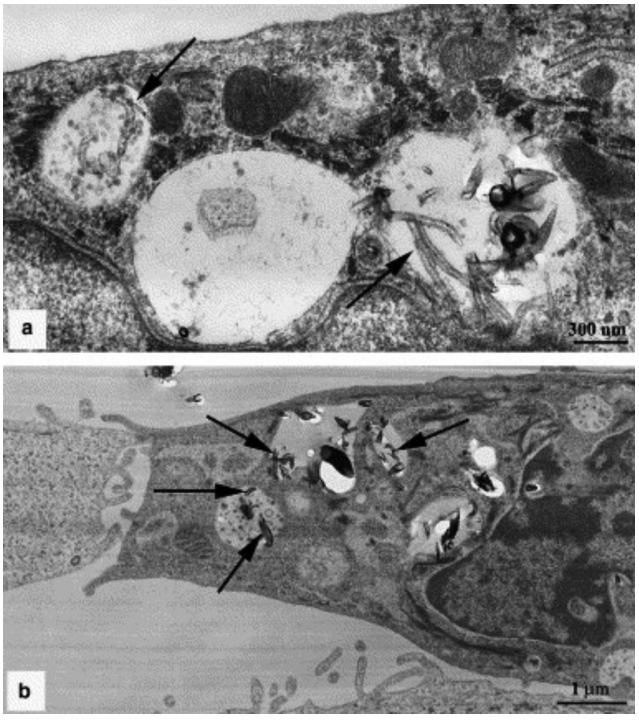

Fig. 4. Transmission electron micrograph of human epidermal keratinocytes (HEKs): (a) intracellular localization of the MWNT-arrows depict the MWNT present within the cytoplasmic vacuoles of a HEK; (b) keratinocyte monolayer grown on a Permanox surface-arrow depicts the intracytoplasmic localization of the MWNT.
[70], Copyright 2006. Reproduced with permission from Elsevier Ltd.

the continuation of research into the toxicology of CNT and CNT-based nanocomposites. At the same time, the pharmacological development must continue in parallel before providing the guidelines for the safe use in biomedical applications.

4. Three dimensional (3D) porous scaffolds

Development of composite scaffolds is attractive as advantageous properties of two or more types of materials can be combined to better suit the mechanical and

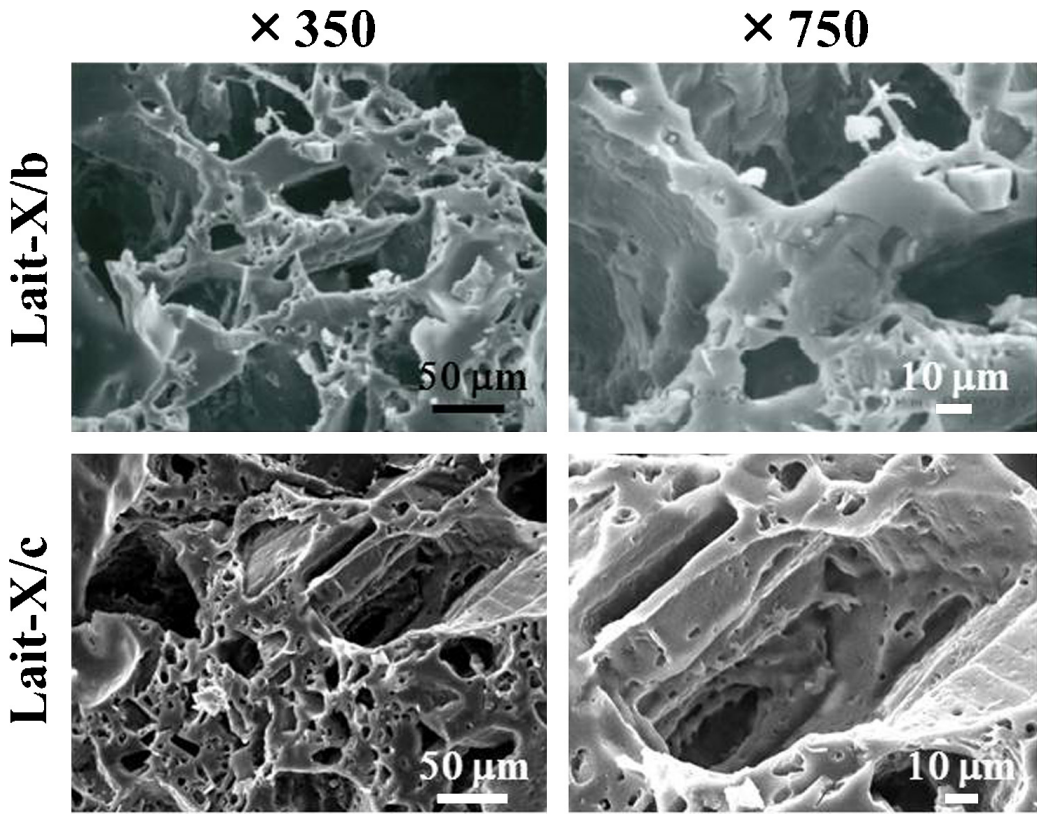

Fig. 5. SEM images of crosslinked PLA porous scaffolds: Lait-X/b and Lait-X/c, for 350× and 750× magnifications. [87], Copyright 2012. Reproduced with permission from John Wiley & Sons.

physiological demands of the host tissue. The tissues in the body are organized into 3D structure as a function of organs. The scaffolds with designed microstructures provide structural support and adequate mass transport to guide the tissue regeneration. To achieve the goal of tissue regeneration, scaffolds must meet some specific needs. A high porosity and an adequate pore size are necessary to facilitate cell seeding and diffusion throughout the whole structure of both cells and nutrients [48].

Nanocomposites 3D scaffolds based on biopolymers have been developed by using different processing methods. Most popular techniques include solvent casting and porogen (particulate) leaching, gas foaming, emulsion freeze-drying, electrospinning, rapid prototyping, and thermally induced phase separation [6,12–17,43,48,83].

4.1. Solvent casting and particulate leaching

Organic solvent casting particulate leaching is a very easy process that has been widely used to fabricate biocomposite scaffolds [84]. This process involves the dissolution of the polymer in an organic solvent, mixing with nanofillers and porogen particles, and casting the mixture into a predefined 3D mold. The solvent is subsequently allowed to evaporate, and the porogen particles are removed by leaching [85]. However, residual solvents in the scaffolds may be harmful to transplanted cells or host tissues. To avoid toxicity effects of the organic solvent, gas foaming can be used to prepare a highly porous biopolymer foam [28]. Kim et al. [86] fabricated PLGA/nHA scaffolds by carbon dioxide (CO_2) foaming and solid porogen (i.e., sodium chloride crystals) leaching (GF/PL) without the use of organic solvents. Selective staining of nHA particles indicated that nHA exposed to the scaffold surface were observed more abundantly in the GF/PL scaffold than in the conventional solvent casting and particulate leaching scaffold. The GF/PL scaffolds exhibited significant enhanced bone regeneration when compared with conventional scaffolds.

In our previous paper [87], highly porous crosslinked PLA scaffolds were successfully prepared through particulate leaching and foaming followed by leaching methods. The scaffolds were porous with good inter-connectivity and thermal stability. The SEM images confirmed the pore connectivity and structural stability of the crosslinked PLA scaffold (Fig. 5).

The Lait-X/b and Lait-X/c scaffolds have the same percentage of salt particulate with similar particle size; the former was turned into a scaffold through simple leaching, while the latter was through batch foaming followed by leaching. The qualitative evaluation of the SEM images of Lait-X/b and Lait-X/c showed a well-developed porosity and interconnectivity with pore sizes spanning over a very wide range, from a few microns to hundreds of microns.

Fig. 6 shows the relation between the pore size diameter and the cumulative and differential intrusions of mercury in Lait-X/b and Lait-X/c scaffolds. The maximum intrusion and interconnectivities of Lait-X/b was from 0.1 to 1 μm pore diameter regions and Lait-X/c it was from 0.1 to 10 μm. The porosity, total intrusion volume, total pore area

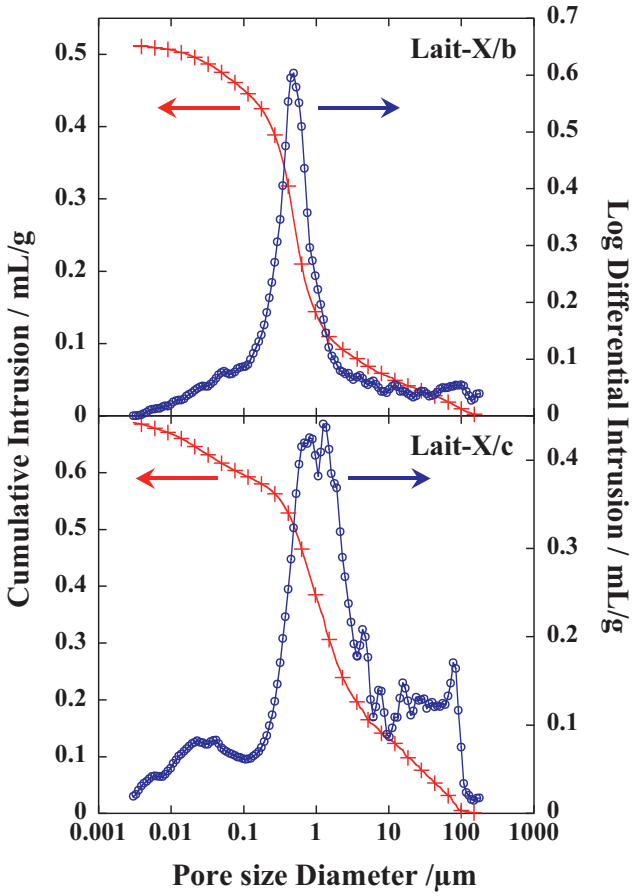

Fig. 6. Pore size distribution in crosslinked PLA porous scaffolds: Lait-X/b and Lait-X/c.
Reproduced with permission from [87], Copyright 2012. Reproduced with permission from John Wiley & Sons.

and median pore diameter (volume) of Lait-X/b calculated by mercury porosimetry were 43%, 0.511 mL/g, 13.4 m^2/g and 0.520 μm whereas for Lait-X/c it was 49%, 0.688 mL/g, 27.6 m^2/g and 1.26 μm respectively. The shift in the values of Lait-X/b and Lait-X/c was due to the effect of batch foaming which lead to the movement of salt particulate during foaming, resulting in increased porosity and total intrusion volume. The in vitro cell culture demonstrated the ability of the scaffold to support human mesenchymal stem cells (hMSCs) adhesion confirming the biocompatibility through the cell–scaffold interaction. The in vitro degradation of the PLA thermoset scaffolds in phosphate buffered solution was faster with those prepared by foaming and subsequent leaching [87]. The agglomeration of the smaller crystal (solid porogen) within 3D polymer matrix enables creation of an interconnected pore network with well-defined pore sizes and shapes.

4.2. Thermally induced phase separation (TIPS)

3D resorbable polymer scaffolds with very high porosities (~97%) can be produced using the TIPS technique to give controlled microstructures as scaffolds for tissues, such as nerve, muscle, tendon, intestine, bone, and teeth [88]. The scaffolds are highly porous with anisotropic tubular morphology and extensive pore interconnectivity. Microporosity of TIPS produced foams, their pore morphology, mechanical properties, bioactivity and degradation rates can be controlled by varying the polymer concentration in solution, volume fraction of secondary phase, quenching temperature and the polymer and solvent used [88].

When dioxane was used alone, the porous structure resulted from a solid–liquid phase separation of the polymer solution. During quenching, the solvent crystallized and the polymer was expelled from the solvent crystallization front. Solvent crystals became pores after subsequent sublimation. To better mimic the mineral component and the microstructure of natural bone, novel nHA nanocomposite scaffolds with high porosity and well-controlled pore architectures were prepared using the TIPS techniques (Fig. 2 [43]). The incorporation of nHA particles into PLLA solution perturbed the solvent crystallization to some extent and thereby made the pore structure more irregular and isotropic. The perturbation by nHA particles, however, was small even in high proportion up to 50% due to their nanometer size scale and uniform distribution. The SEM images showed that the nHA particles were dispersed in the pore walls of the scaffolds and bound to the polymer very well. PLLA/nHA scaffolds prepared using pure solvent system had a regular anisotropic, but open 3D pore structure similar to neat polymer scaffolds, whereas PLLA/mHA scaffolds had an isotropic and a random irregular pore structure.

TiO_2 nanoparticles ($nTiO_2$) have been proposed as attractive fillers for biodegradable PDLLA matrix [89]. 3D PDLLA foams containing both $nTiO_2$ and Bioglass® additions have been synthesized by TIPS. The foams demonstrated the enhancement of the bioactivity and the surface nanotopography.

4.3. Electrospinning

Electrospinning technique has attracted great interest since it facilitates the production of fibrous non-woven micro/nano fabrics for tissue engineering, mainly due to the structural similarity to the tissue extracellular matrix (ECM) [47,90]. The composition and topology of the ECM has been found to affect cell morphology, function, and physiological response [91]. The electrospun nanofibrous scaffolds aimed to mimic the architecture and biological functions of ECM, are considered as very promising substrates for tissue engineering. PCL scaffolds, a bioresorbable aliphatic polyester, have been used to provide a 3D environment for in vitro embryonic stem cell culture and differentiation. Electrospun nanocomposite scaffolds based on bioresorbable polymers and conventional HA allow osteoblast proliferation and differentiation, and are thus considered very promising as bone scaffolding materials [47].

Fibrous PCL/Ca-deficient nHA nanocomposites have been obtained by electrospinning. The electrospun mats showed a non-woven architecture, average fiber size was 1.5 μm, porosity 80–90%, and specific surface area 16 m^2/g (Fig. 7). Murine embryonic stem (ES) cell response to neat PCL and to PCL/Ca-deficient nHA (6.4 wt.%) mats was evaluated by analyzing morphological, metabolic and functional

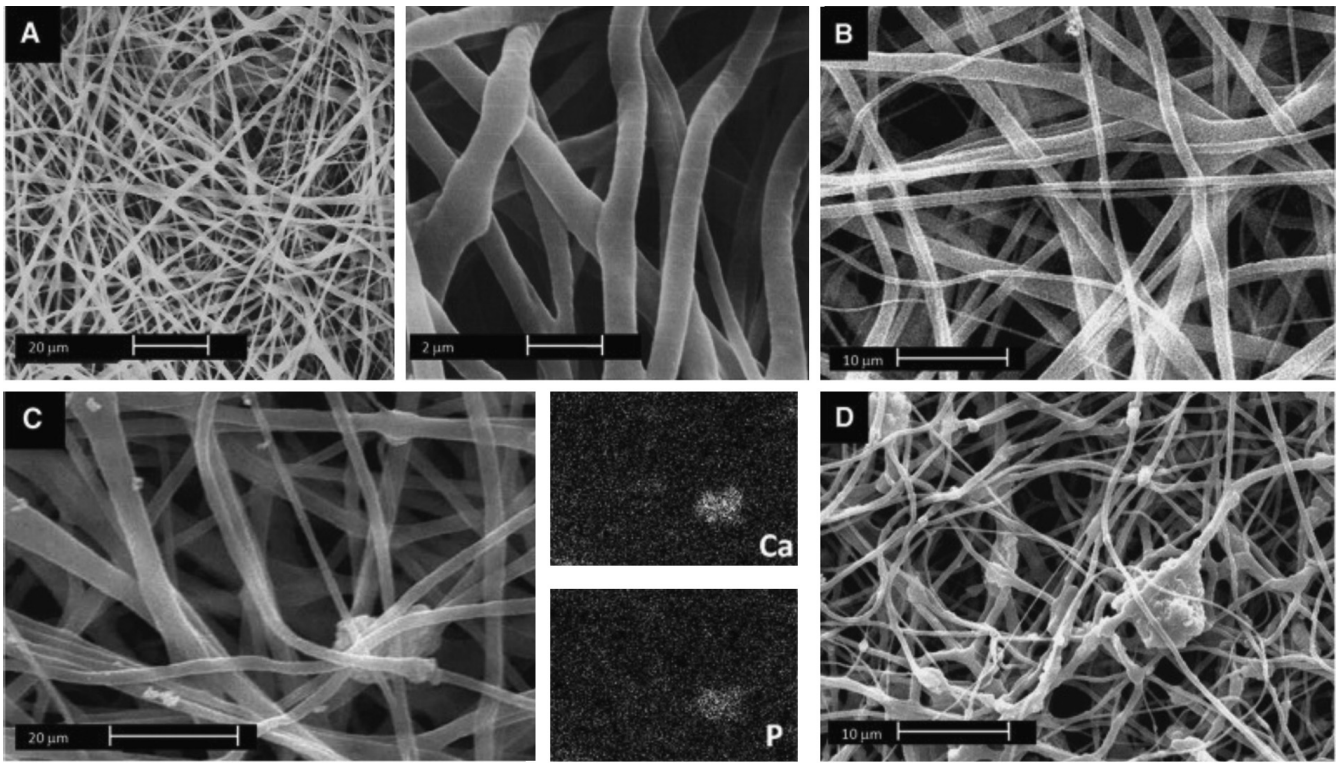

Fig. 7. SEM micrographs of neat PCL mat (A), SEM micrograph of PCL/Ca-deficient nHA 2.0 wt.% (B), SEM micrograph and EDS mappings of PCL/Ca-deficient nHA 6.4 wt.% (C), and SEM micrograph of PCL/Ca-deficient nHA 24.9 wt.% (D).
[47], Copyright 2009. Reproduced with permission from Elsevier Ltd.

markers. Cells growing on either scaffold proliferated and maintained pluripotency markers at essentially the same rate as cells growing on standard tissue culture plates, with no detectable signs of cytotoxicity, despite a lower cell adhesion at the beginning of culture. These results indicate that electrospun PCL scaffolds may provide adequate supports for murine ES cell proliferation in a pluripotent state, and that the presence of Ca-deficient nHA within the mat does not interfere with their growth.

Aligned nanocomposite fibers of PLGA/nHA were fabricated by using a rotating collector by electrospinning. At low concentrations the fibers had no agglomerates and good dispersion was achieved [92]. The presence of well-dispersed nHA particles reduced the chain mobility and hence helped to prevent shrinkage to some degree. The glass transition was affected by the incorporation of nHA into the polymer matrix which hinders chain mobility.

Interestingly, the electrospinning technique provides the opportunity to align conductive nanoparticles with high aspect ratio within the polymeric fibers. Carbo nanofibers (CNFs) can orientate along the axis of electrospun fibers due to the sink flow and the high extension of the electrospun jet [93]. The CNF alignment depends upon the CNF dispersion in the polymer solution. The idea of dispersing and aligning carbon nanostructures in polymer matrix to form highly ordered structures. The mechanical properties of PCL/CNF mats however, were only slightly affected by CNF introduction [94].

Ternary nanocomposite scaffolds involving three different materials have been developed [95,96]. The addition of MWNTs to the biopolymer creates new highly conductive material due to the 3D electrical conducting network. The results exhibited that combining two different nanostructures (e.g., MWNT/nHA or MWNT/Bioglass®) led to multifunctional biomaterials with tailored bioactivity, structural and mechanical integrity as well as electrical conductivity of the porous scaffolds.

5. In vitro degradation

Since tissue engineering aims at the regeneration of new tissues, biomaterials are expected to be degradable and absorbable with a proper rate to match the speed of new tissue formation. The degradation behavior has a crucial impact on the long-term performance of a tissue-engineered cell/polymer construct. The degradation kinetics may affect a range of processes, such as cell growth, tissue regeneration, and host response. The mechanism of aliphatic polyester biodegradation is the bio-erosion of the material mainly determined by the surface hydrolysis of the polymer. The scaffolds can lead to heterogeneous degradation, with the neutralization of carboxylic end groups located at the surface by the external buffer solution (in vitro or in vivo). These phenomena contribute to reduce the acidity at the surface whereas, in the bulk, degradation rate is enhanced by autocatalysis due to carboxylic end groups of aliphatic polyesters. In general, the amount of absorbed water depends on diffusion coefficients of chain fragment within the polymer matrix, temperature, buffering capacity, pH, ionic strength, additions in the matrix, in the medium and processing history. Different polyesters can exhibit quite distinct

degradation kinetics in aqueous solutions. For example, PGA is a stronger acid and is more hydrophilic than PLA, which is hydrophobic due to its methyl groups.

Of particular significance for application in tissue engineering are debris and crystalline by-products, as well as particularly acidic degradation products of PLA, PGA, PCL and their copolymers [97]. Several groups have incorporated basic compounds to stabilize the pH of the environment surrounding the polymer and to control its degradation. Bioglass® and calcium phosphates have been introduced [98]. Nanocomposites showed a strongly enhanced polymer degradation rate when compared to the neat polymer [99]. As mentioned in PLLA/ND nanocomposites (Fig. 3), improvement of osteoconductivity of PLLA nanocomposites, i.e., the deposition of the HA crystal on the surface was observed. Fast degradation and the superior bioactivity make these nanocomposites a promising material for orthopedic medicine application [99].

In contrast, the degradation rates of the biopolyester elastomer/MWNT nanocomposites tended to decrease with the increase of MWNT loadings (above 1 wt%) in SBF solution [100]. Continued investigation of the degradation behavior of biopolymer-based nanocomposites is required for the nanostructures incorporated into matrices.

Allen et al. [101] reported the biodegradation of SWNTs through natural, enzymatic catalysis. By incubating SWNTs with a natural horseradish peroxidase (HRP) and low concentration of H_2O_2 (~40 μM), the degradation of SWNTs proceeded at 4 °C over 12 weeks under static conditions. It is tempting to speculate that other peroxidases in plants and animals may be effective in oxidative degradation of CNTs. More studies are necessary to ascertain the byproducts of the biodegradation, as well as cellular studies for practical applications.

6. Stem cell–scaffolds interactions

Synthetic biopolymers are widely used for the preparation of porous scaffolds by different techniques set up for the purpose. The main limitation to the use of PLA-based systems is their low hydrophilicity that causes a low affinity for the cells as compared with the biological polymers. Therefore the addition of biological components to a synthetic biopolymer represents an interesting way to produce a bioactive scaffold that can be considered as a system showing at the same time adequate mechanical stability and high cell affinity [102–104]. A variety of ECM protein components such as gelatin, collagen, laminin, and fibronectin could be immobilized onto the plasma treated surface of the synthetic biopolymer to enhance cellular adhesion and proliferation. Arg-Gly-Asp (RGD) is the most effective and often employed peptide sequence for stimulating cell adhesion on synthetic polymer surfaces. This peptide sequence is present in many ECM proteins and can interact with the integrin receptors at the focal adhesion points. Once the RGD sequence is recognized by and binds to integrins, it will initiate an integrin-mediated cell adhesion process and active signal transduction between the cell and ECM [105,106]. Hollister et al. [106] reported a simple method to immobilize RGD peptide on PCL 3D scaffold surfaces. They demonstrated that rat bone marrow stromal cell (BMSC) adhesion was significantly improved on the RGD-modified PCL scaffolds in a serum-free culture condition.

Surface treatment techniques, such as plasma treatment, ion sputtering, oxidation and corona discharge, affect the chemical and physical properties of the surface layer without changing the bulk material properties. The effect of the oxygen plasma treatments on the surface of the materials have been shown to charge wettability, roughness and to enable the selective interaction between PLLA surface and the protein, further improved stem cell attachment [107].

Bone marrow derived hMSCs are an important cell source for cell therapy and tissue engineering applications.

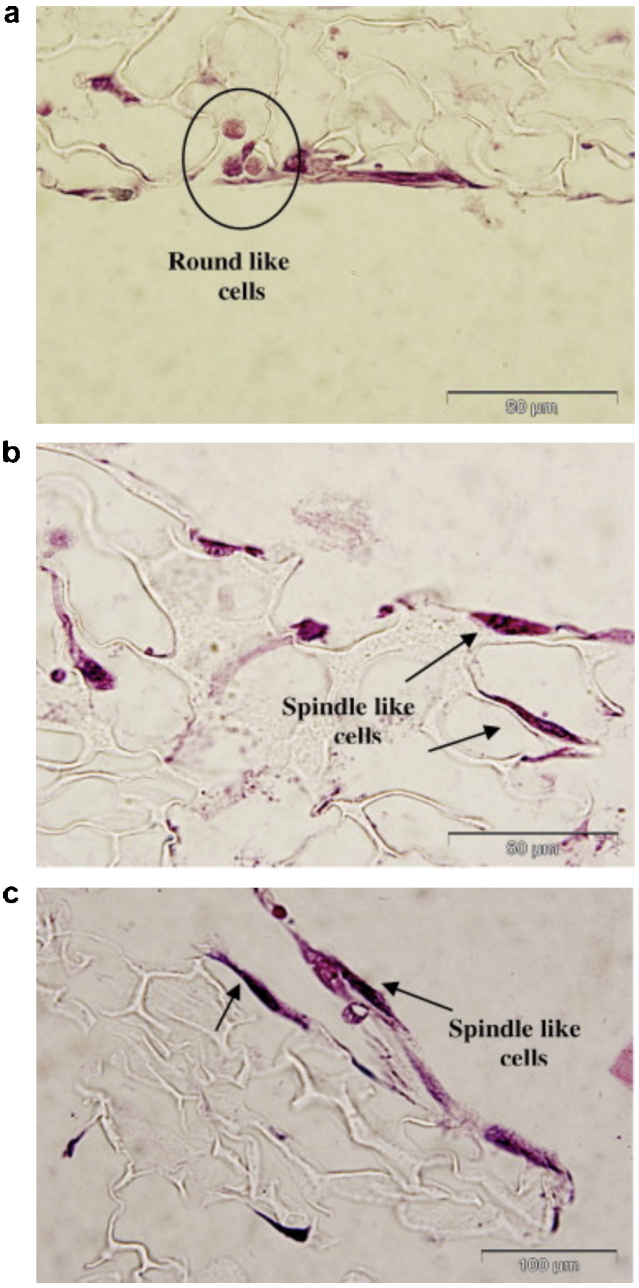

Fig. 8. Optical microscopy photographs of the colored MSCs (H&E staining) attached to the (a) neat PLLA, (b) PLLA/mHAP and (c) PLLA/nHAP. [43], Copyright 2008. Reproduced with permission from Elsevier Ltd.

The interactions between stem cell and their environment are very complex and not fully clarified. Previous work showed that cells respond to the mechanical properties of the scaffolds on which they are growing [108]. Rohman et al. [109] reported that PLGA and PCL are biocompatible for the growth of normal human urothelial and human bladder smooth muscle cells. Their analysis of the potential mechanism has indicated that differences in degradation behaviors between polymers are not significant, but that the elastic modulus is a critical parameter, relevant to biology at the microscopic (cellular) level and may also have impact at macroscopic (tissue/organ) scales. They concluded that the elastic modulus is a property that should be considered in the development and optimization of synthetic biopolymers for tissue engineering.

MSCs provided striking evidence that ECM elasticity influences differentiation. Indeed, multipotent cells are able to start a transdifferentiation process toward very soft tissues, such as nerve tissues, when the elastic modulus (E) of the substrate is about 0.5 kPa. Intermediate stiffness (~10 kPa) addresses cells toward a muscle phenotype and higher E (≥30 kPa) to cartilage/bone [110]. This should be relevant to the intelligent design of new biopolymer intended for specific applications [111]. Biopolymers presently used in tissue engineering are extremely stiff. PLA has a bulk elasticity of $E \sim 1$ GPa, that is ten-thousand times stiffer than most soft tissues. Thus engineering of soft tissue replacements needs to explore biopolymers softer than those presently available.

Poly(butylene/thiodiethylene succinate) block copolymers (PBSPTDGS) were prepared by reactive blending of the parent homopolymers (PBS and PTDGS) in the presence of $Ti(OBu)_4$ [112]. The random copolymer, characterized by the lowest crystallinity degree, exhibits the lowest elastic modulus and the highest deformation at break. When evaluated for indirect cytotoxicity, films of block PBSPTDGS30 and random PBSPTDGS240 copolymers appeared entirely biocompatible. In addition, cellular adhesion and proliferation of H9c2 cells [113] (derived from embryonic rat heat) seeded and grown up to 14 days in culture over the same films demonstrate that these new materials might be of interest for tissue engineering applications.

The biocompatibility of neat PLLA, PLLA/nHA and PLLA/mHA composite scaffolds were evaluated in vitro by observing the behavior of the stained MSCs cultured in close contact with the scaffolds [43]. Cell growth in material-free organ culture can be distinguished into four stages: Cells adhered on the surface of the composite in a round shape during the initial two days. Then the round cell attached, spread, and proliferated into the inner pores of the scaffold, exhibiting morphologies ranging from spindle shaped to polygonal. After one week, the cells reached confluence on the material while the material-free group did not reach this status (Fig. 8). The representative cell culture micrographs of cell attachment into the scaffolds after seven days are observed. It is seen that round shaped cells attached and proliferated to the scaffolds surface, became spindle like and migrated through the pores (Fig. 8a–c). The number of round shaped cells is noticeable on the surface of pure PLLA scaffold (Fig. 8a) while proliferated cells on the micro and nanocomposite scaffolds exhibit spindle shaped morphology (Fig. 8b, c). The PLLA/HA scaffolds appeared to be in vitro biocompatible and noncytotoxic to cells.

Clinical trials demonstrate the effectiveness of cell-based therapeutic angiogenesis in patients with severe ischemic diseases, however, their success remains limited.

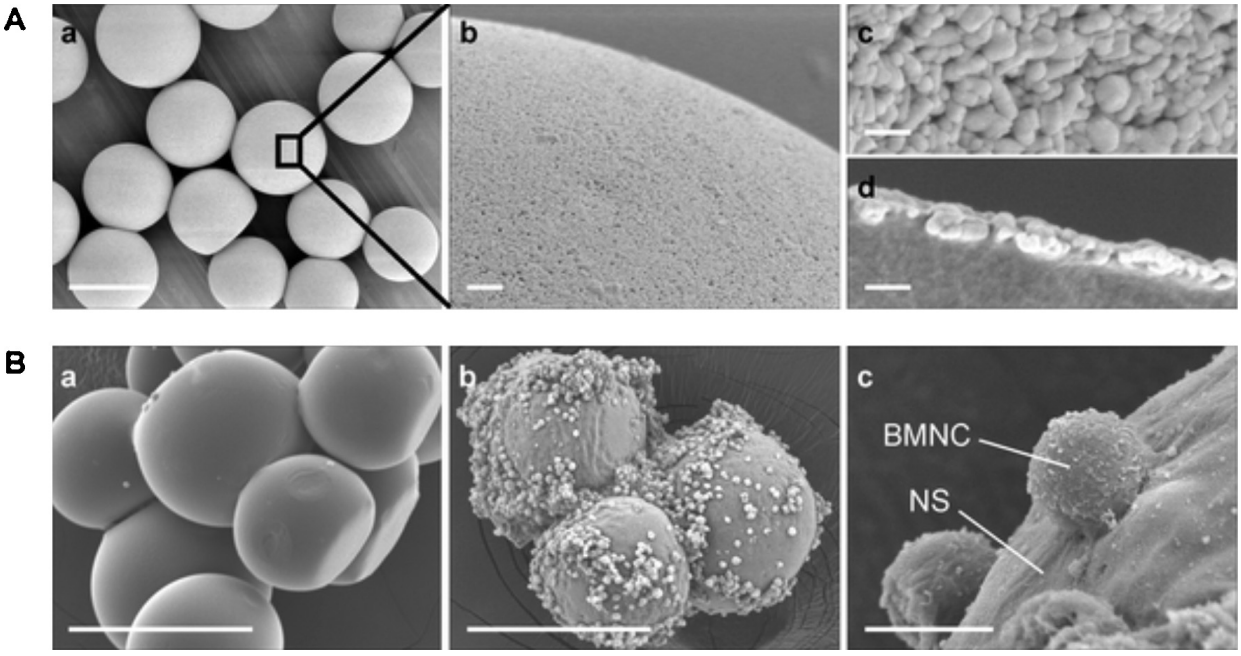

Fig. 9. SEM image of NS (A) and marked cell adhesiveness to NS in vitro (B). (A) NS are microspheres approximately 100 μm in diameter (a). The NS surface uniformly coated with nano-scale hydroxyapatite (nHA) crystals was observed at different magnifications (low and high magnification in b and c, respectively). SEM image of an NS cross-section indicating a single layer of nHA particles on the NS surface (d). (B) Murine BMNCs were incubated with LA (a) or NS (b, c) at 37 °C for 8 h. Large numbers of BMNCs adhered to NS (b, c) but not to LA (a). Scale bars: 100 μm (A-a, B-a, B-b), 5 μm (B-c), 1 μm (A-b), 100 nm (A-c, A-d).
[114], Copyright 2012. Reproduced with permission from PLoS ONE.

Maintaining transplanted cells in place are expected to augment the cell-based therapeutic angiogenesis.

In 2012, nHA-coated PLLA microspheres, named nano-scaffold (NS), were, for the first time, generated as a non-biological, biodegradable and injectable cell scaffold [114]. Fukumoto et al. investigated the effectiveness of NS on cell-based therapeutic angiogenesis. NS are microspheres approximately 100 nm in diameter (Fig. 9A-a), the surfaces of which are coated with a monolayer of nHA particles with 50 nm in diameter (Fig. 9A-b, -c, -d). To assess the cell adhesiveness of NS, SEM was performed after incubation of NS and bare PLLA microspheres (LA) as controls with murine bone marrow mononuclear cells (BMNCs) at 37 °C for 8 h in vitro. The number of cells adhering to NS was much greater than that to LA (Fig. 9B-a and b). High-magnification SEM images showed active cell adhesion to NS (Fig. 9B-c).

BMNCs from enhanced green fluorescent protein (EGFP)-transgenic mice and rhodamine B-containing PLLA microspheres (orange) as a scaffold core or control microspheres were implanted into the ischemic hind limbs of eight-week-old male (C57BL/6NCrSlc) mice to determine the co = localization of implanted cells with injected microspheres (Fig. 10A). Few implanted BMNCs were observed around LA (Fig. 10A-a), while markedly larger numbers of cells were seen with NS (Fig. 10A-b) in ischemic thigh tissue 7 days after transplantation. Intramuscular levels of GFP derived from transplanted BMNCs were consistently and significantly higher in the group injected with NS than that injected with LA or BMNCs alone at 3, 7 and 14 days after implantation, while GFP levels were not significantly different between BMNCs alone and LA+BMNCs groups (Fig. 10B).

Kaplan–Meier analysis demonstrated that NS+BMNC markedly prevented hindlimb necrosis. NS+BMNC revealed much higher induction of angiogenesis in ischemic tissues and collateral blood flow confirmed by 3D computed tomography angiography than those of BMNC or LA+BMNC groups [114]. NS-enhanced therapeutic angiogenesis and arteriogenesis showed good correlations with increased intramuscular levels of vascular endothelial growth factor and fibroblast growth factor-2. NS co-implantation also prevented apoptotic cell death of transplanted cells, resulting in prolonged cell retention.

This nano-scaffold provides promising local environment for implanted cells for the effects on angiogenesis and arteriogenesis through cell clustering, augmented expression of proangiogenic factors, and supporting cell survival without gene manipulation or artificial ECM.

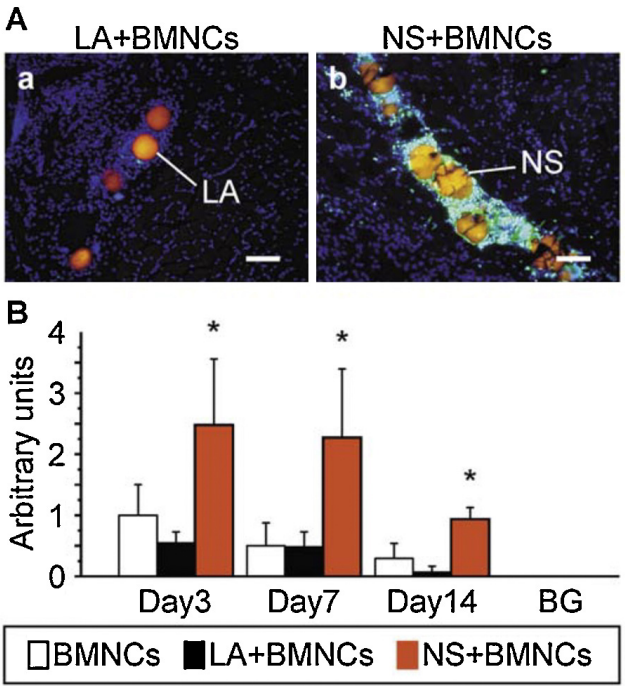

Fig. 10. Prolonged localization of implanted BMNCs in ischemic tissues by NS. (A) Colocalization of BMNCs with NS and LA in vivo. Murine BMNCs derived from EGFP-transgenic mice were transplanted together with LA or NS into the thighs in the hind limb ischemic model. Cores of NS and LA containing rhodamine B (orange) were used to indicate localization of the injected microspheres in ischemic tissues. Tissue sections 7 days after transplantation of LA+BMNCs (a) or NS+BMNCs (b) were counterstained with DAPI (blue), and merged images of DAPI, GFP and rhodamine B are shown. BMNCs (green) were observed as densely clustered around NS (b) but not LA (a). Scale bars: 100 mm. (B) Quantitative evaluation of implanted cells existing in ischemic tissues. Quantitative analysis of intramuscular GFP was performed 3, 7 and 14 days after transplantation. BMNCs were derived from EGFP-transgenic mice. BMNCs were transplanted alone or together with LA or NS into ischemic thigh muscles. Intramuscular GFP values of whole thigh muscles were corrected for total protein and expressed in arbitrary units ($n = 6$ in each group). $^{*}P < 0.05$ for the NS+BMNCs group compared to the BMNCs alone and LA+BMNCs groups. GFP concentration in normal murine muscle was measured as background (BG). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
[114], Copyright 2012. Reproduced with permission from PLoS ONE.

7. Health implication of nanoparticles

The degree of exposure to nanoparticles (TiO_2, ZnO, NiO, CNTs, Al, Cu, and Ag) and the percentage of exposed people will grow in the next few years [115]. Some properties of nanoparticles in industrial applications might be detrimental both for biological systems and environment. This led to the development of nanotoxicology, i.e., the systematic evaluation of possible toxic effects elicited by nanotechnology products on cells or biological fluids and animals. Data regarding humans are still lacking, except for isolated case-reports on accidental high dose exposure in the workplace [116]. The issue of nanoparticles toxicity for humans remains controversial, as described in Section 3.3. The main factors limiting extrapolation to humans of available finding are:

1. To date, there is no conclusive evidence of a known human toxic response that is specifically caused by nanoparticles. Effects seen in animals cannot be automatically translated to humans.
2. Data are not univocal. Some well-performed studies show no toxicity from the same nanoparticles showing adverse effects in other experiments [117]. This is due to the lack of standardization both in characterizing the nanoparticle and in the methods used to challenge the nanoparticles with biologic systems.

On the other hand, in several studies in which a direct comparison between CNTs and asbestos has been made, similar toxicity has been reported [118].

We must be clear that the nanoparticle-linked diseases related to the realm of possible risk.

8. Conclusions

The synthetic biopolymer-based nanocomposites reviewed in this article are particularly attractive as tissue engineering scaffolds due to their biocompatibility and adjustable biodegradation kinetics. Conventional materials processing methods have been adapted and incorporation of inorganic nanoparticles into porous and interconnected 3D porous scaffolds. The incorporation of nanoparticles and immobilization of biological components on the surface to enhance cellular adhesion and proliferation are promising and currently under extensive research. Current research is focused on the interaction between stromal cells and biopolymer interfaces. The synthetic biopolymer-based nanocomposite scaffolds with bioactive inorganic phases will be in the center of attention in combination with stem cell seeding.

The fundamentals for biomaterials seem to originate from introducing stem cells. In this direction, the new approach of biopolymer-based nanocomposite enables the scaffold surface to mimic complex local biological functions and may lead in the near future to in vitro and in vivo growth of tissues and organs.

In the present scenario, bioceramics entities have been used for bone tissue engineering scaffolds and drug delivery [119–121]. Osteomyelitis is a most common medical problem related to bones caused by an inflammatory process leading to bone destruction caused by infective microorganisms found worldwide in children, where the bone tissue regeneration is required [122]. Although, bioceramics scaffolds serve the purpose of tissue regeneration and drug release, but they present formidable limitations such as the lack of information relating to the long-term effects in the body. The bioceramics, especially HA, when resorbed into the biological system for a long term shall give secondary fixation (70% remains in dogs after 4 months and for humans 90% remains even after 4 years) [123]. HA crystals released from the bone scaffolds will accumulate in the joints and can stimulate an inflammatory response in the prosthesis area [124]. In order to target clinical and medical applications, in vitro and in vivo studies are inevitable and the need for additional investigations in biological system is imperative.

Acknowledgment

This work was supported by KAKENHI (22656148) and the Strategic Research Infrastructure Project of the Ministry of Education, Sports, Science and Technology, Japan (2010–2014).

References

[1] Smith R, editor. Biodegradable polymer for industrial applications. New York: CRC Press; 2005, 552 pp.
[2] Chandra R, Rustgi R. Biodegradable polymers. Progress in Polymer Science 1998;23:1273–335.
[3] Johnson RM, Mwaikambo LY, Tucker N. Biopolymer. Rapra Review Report No 159. London: Rapra Technology Ltd; 2003, 148 pp.
[4] Platt DK. Biodegradable polymers, market report. London: Rapra Technology Ltd; 2006, 158 pp.
[5] Sinha Ray S, Okamoto M. Biodegradable polylactide and its nanocomposites: opening a new dimension for plastics and composites. Macromolecular Rapid Communications 2003;24:815–40.
[6] Ma PX. Scaffolds for tissue fabrication. Materials Today 2004;7:30–40.
[7] Langer R, Vacanti JP. Tissue engineering. Science 1993;260:920–6.
[8] Quirk RA, France RM, Shakesheff KM, Howdle SM. Supercritical fluid technologies and tissue engineering scaffolds. Current Opinion in Solid State and Materials Science 2004;8:313–821.
[9] Tsivintzelis I, Marras SI, Zuburtikudis I, Panayiotou C. Porous poly(L-lactic acid) nanocomposite scaffolds prepared by phase inversion using supercritical CO_2 as antisolvent. Polymer 2007;48:6311–8.
[10] Sinha Ray S, Okamoto M. Polymer/layered silicate nanocomposites: a review from preparation to processing. Progress in Polymer Science 2003;28:1539–641.
[11] Lee JH, Park TG, Park HS, Lee DS, Lee YK, Yoon SC, Nam JD. Thermal and mechanical characteristics of poly(L-lactic acid) nanocomposite scaffold. Biomaterials 2003;24:2773–8.
[12] Mikos AG, Sarakinos G, Leite SM, Vacanti JP, Langer R. Laminated three-dimensional biodegradable foams for use in tissue engineering. Biomaterials 1993;14:323–30.
[13] Nam YS, Park TG. Biodegradable polymeric microcellular foams by modified thermally induced phase separation method. Biomaterials 1999;20:1783–90.
[14] Van de Witte P, Esselbrugge H, Dijkstra PJ, Van den Berg JWA, Feijen J. Phase transitions during membrane formation of polylactides. I. A morphological study of membranes obtained from the system polylactide–chloroform–methanol. Journal of Membrane Science 1996;113:223–36.
[15] Whang K, Thomas CH, Healy KE, Nuber GA. A novel method to fabricate bioabsorbable scaffolds. Polymer 1995;36:837–42.
[16] Bognitzki M, Czad W, Frese T, Schaper A, Hellwig M, Steinhart M, Greiner A, Wendorff JH. Nano-structured fibers via electrospinning. Advanced Materials 2001;13:70–2.
[17] Goel SK, Beckman EJ. Generation of microcellular polymeric foams using supercritical carbon dioxide. I: Effect of pressure and temperature on nucleation. Polymer Engineering and Science 1994;34:1137–47.
[18] Mohanty AK, Misra M, Hinrichsen G. Biofibers. Biodegradable polymers and biocomposites: an overview. Macromolecular Materials and Engineering 2000;276:1–24.
[19] Gilding D, Reed AM. Biodegradable polymers for use in surgery polyglycolic/poly(lactic acid) homo- and copolymers. Polymer 1979;20:1459–64.
[20] Karageorgiou V, Kaplan D. Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials 2005;26:5474–91.
[21] Hench LL. Bioceramics. Journal of the American Ceramic Society 1998;81:1705–28.
[22] Kim HW, Knowles JC, Kim HE. Hydroxyapatite/poly(epsilon)-caprolactone composite coating on hydroxyapatite porous bone scaffold for drug delivery. Biomaterials 2004;25:1279–87.
[23] Zhang R, Ma PX. Porous poly(L-lactic acid)/apatite composites created by biomimetic process. Journal of Biomedical Materials Research 1999;45:285–93.
[24] Alexandre M, Dubois P. Polymer-layered silicate nanocomposites: preparation, properties and uses of a new class of materials. Materials Science and Engineering Reports 2000;28:1–63.
[25] Gao F. Clay/polymer composites: the story. Materials Today 2004;7:50–5.
[26] Okada A, Usuki A. Twenty years of polymer–clay nanocomposites. Macromolecular Materials and Engineering 2006;291:1449–76.
[27] Okamoto M. Recent advances in polymer/layered silicate nanocomposites: an overview from science to technology. Materials Science and Technology 2006;22:756–79.
[28] Okamoto M. Biodegradable polymer/layered silicate nanocomposites: a review. In: Mallapragada S, Narasimhan B, editors. Handbook of biodegradable polymeric materials and their applications. Los Angeles: American Scientific Publishers; 2006. p. 153–97.
[29] Sinha Ray S, Bousmina M. Biodegradable polymer and their layered silicate nanocomposites: in greeting the 21st century materials world. Progress in Materials Science 2005;50:962–1079.

[30] Bordes P, Pollet E, Averous L. Nano-biocomposites: biodegradable polyester/nanoclay systems. Progress in Polymer Science 2009;34:125–55.

[31] Seal BL, Otero TC, Panitch A. Polymeric biomaterials for tissue and organ regeneration. Materials Science and Engineering Reports 2001;34:147–230.

[32] Kohn J, Langer R. Bioresorbable and bioerodible materials. In: Ratner BD, Hoffman AS, Schoen FJ, Lemmons JE, editors. Biomaterials science: an introduction to materials in medicine. New York: Academic Press; 1996. p. 64–72.

[33] Jagur-Grodzinski J. Biomedical application of functional polymers. Reactive and Functional Polymers 1999;39:99–138.

[34] Anonymous. PLA degradable. Kyoto: BMG Inc.; 2012. p. 1 http://www.bmg-inc.com/en/prod_and_res/index.html (accessed March 2013).

[35] Martin C, Winet H, Bao JY. Acidity near eroding polylactide-polyglycolide in vitro and in vivo in rabbit tibial bone chambers. Biomaterials 1996;17:2373–80.

[36] Pitt CG, Gratzel MM, Kimmel GL. Aliphatic polyesters. 2. The degradation of poly(DL-lactide), poly(ε-caprolactone) and their copolymers in vivo. Biomaterials 1981;2:215–20.

[37] Pektok E, Nottelet B, Tille JC, Gurny R, Kalangos A, Moeller M, Walpoth BH. Degradation and healing characteristics of small-diameter poly(ε-caprolactone) vascular grafts in the rat systemic arterial. Circulation 2008;118:2563–70.

[38] Shi X, Mikos AG. Poly(propylene fumarate). In: Guelcher SA, Hollinger JO, editors. An introduction to biomaterials. Boca Raton, FL: CRC Press; 2006. p. 205–18.

[39] Mistry AS, Mikos AG. Tissue engineering strategies for bone regeneration. Advances in Biochemical Engineering/Biotechnology 2005;94:1–22.

[40] Knowles JC. A review article: phosphate glasses for biomedical applications. Journal of Materials Chemistry 2003;13:2395–401.

[41] Gay S, Arostegui S, Lemaitre J. Preparation and characterization of dense nanohydroxyapatite/PLLA composites. Materials Science and Engineering C 2009;29:172–7.

[42] Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Enhanced functions of osteoblasts on nanophase ceramics. Biomaterials 2000;21:1803–10.

[43] Nejati E, Mirzadeh H, Zandi M. Synthesis and characterization of nanohydroxyapatite rods/poly(L-lactic acid) composite scaffolds for bone tissue engineering. Composites Part A Applied Science and Manufacturing 2008;39:1589–96.

[44] El-Ghannam A, Ning CQ, Mehta J. Cyclosilicate nanocomposite: a novel resorbable bioactive tissue engineering scaffold for BMP and bone-marrow cell delivery. Journal of Biomedical Materials Research Part A 2004;71:377–90.

[45] Wei G, Ma PX. Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. Biomaterials 2004;25:4749–57.

[46] Ramay Hassna RR, Zhang M. Biphasic calcium phosphate nanocomposite porous scaffolds for load-bearing bone tissue engineering. Biomaterials 2004;25:5171–80.

[47] Bianco A, Di Federico E, Moscatelli I, Camaioni A, Armentano I, Campagnolo L, Dottori M, Kenny JM, Siracusa G, Gusmano G. Electrospun poly(ε-caprolactone)/Ca-deficient hydroxyapatite nanohybrids: microstructure, mechanical properties and cell response by murine embryonic stem cells. Materials Science and Engineering C 2009;29:2063–71.

[48] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006;27:3413–31.

[49] Hong Z, Zhang P, He C, Qiu X, Liu A, Chen L, Chen X, Jing X. Nano-composite of poly(L-lactide) and surface grafted hydroxyapatite: mechanical properties and biocompatibility. Biomaterials 2005;26:6296–304.

[50] Li J, Lu XL, Zheng YF. Effect of surface modified hydroxyapatite on the tensile property improvement of HA/PLA composite. Applied Surface Science 2008;255:494–7.

[51] Hong ZK, Qiu XY, Sun JR, Deng MX, Chen XS, Jing XB. Grafting polymerization of L-lactide on the surface of hydroxyapatite nanocrystals. Polymer 2004;45:6705–13.

[52] Gansel JK, Thiel M, Rill MS, Decker M, Bade K, Saile V, von Freymann G, Linden S, Wegener M. Gold helix photonic metamaterial as broadband circular polarizer. Science 2009;325:1513–5.

[53] Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnology Advances 2009;27:76–83.

[54] Lee JY, Nagahata JLR, Horiuchi S. Effect of metal nanoparticles on thermal stabilization of polymer/metal nanocomposites prepared by a one-step dry process. Polymer 2006;47:7970–9.

[55] Xu X, Yang Q, Wang Y, Yu H, Chen X, Jing X. Biodegradable electrospun poly(L-lactide) fibers containing antibacterial silver nanoparticles. European Polymer Journal 2006;42:2081–7.

[56] Schneider OD, Loher S, Brunner TJ, Schmidlin P, Stark WJ. Flexible, silver containing nanocomposites for the repair of bone defects: antimicrobial effect against *E. coli* infection and comparison to tetracycline containing scaffolds. Journal of Materials Chemistry 2008;18:2679–84.

[57] Xu X, Yang Q, Bai J, Lu T, Li Y, Jing X. Fabrication of biodegradable electrospun poly(L-lactide-co-glycolide) fibers with antimicrobial nanosilver particles. Journal of Nanoscience and Nanotechnology 2008;8:5066–70.

[58] Anonymous. The international decade for action: water for life-2005–2015. Geneva, Switzerland: World Health Organization; 2007. p. 2 http://www.who.int/water_sanitation_health/wwd7_water_scarcity_final_rev_1.pdf (accessed March 2013).

[59] Harrison BS, Atala A. Review, carbon nanotube applications for tissue engineering. Biomaterials 2007;28:344–53.

[60] Mwenifumbo S, Shaffer MS, Stevens MM. Exploring cellular behavior with multi-walled carbon nanotube constructs. Journal of Materials Chemistry 2007;17:1894–902.

[61] Supronowicz PR, Ajayan PM, Ullmann KR, Arulanandam BP, Metzger DW, Bizios R. Novel current-conducting composite substrates for exposing osteoblasts alternating current stimulation. Journal of Biomedical Materials Research 2002;59:49–506.

[62] Shi X, Sitharaman B, Pham QP, Spicer PP, Hudson JL, Wilson LJ, Tour JM, Raphael RM, Mikos AG. In vitro cytotoxicity of single-walled carbon nanotube/biodegradable polymer nanocomposites. Journal of Biomedical Materials Research Part A 2008;86:813–23.

[63] Armentano I, Alvarez-Pérez MA, Carmona-Rodríguez B, Gutiérrez-Ospina I, Kenny JM, Arzate H. Analysis of the biomineralization process on SWNT-COOH and F-SWNT films. Materials Science and Engineering C 2008;28:1522–9.

[64] Mochalin VN, Shenderova O, Ho D, Gogotsi Y. The properties and applications of nanodiamonds. Nature Nanotechnology 2012;7:11–23.

[65] Huang HJ, Pierstorff E, Osawa E, Ho D. Protein-mediated assembly of nanodiamond hydrogels into a biocompatible and biofunctional multilayer nanofilm. ACS Nano 2008;2:203–12.

[66] Zhang QW, Mochalin VN, Neitzel I, Knoke IY, Han JJ, Klug CA, Zhou JG, Lelkes PI, Gogotsi Y. Fluorescent PLLA-nanodiamond composites for bone tissue engineering. Biomaterials 2011;32:87–94.

[67] Karbushev VV, Konstantinov II, Parsamyan IL, Kulichikhin VG, Popov VA, George TF. Preparation of polymer-nanodiamond composites with improved properties. Advances in Materials Research 2009;59:275–8.

[68] Zhang Q, Mochalin VN, Neitzel I, Hazeli K, Niu J, Kontsos A, Zhou JG, Lelkes PI, Gogotsi Y. Mechanical properties and biomineralization of multifunctional nanodiamond-PLLA composites for bone tissue engineering. Biomaterials 2012;33:5067–75.

[69] Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 2006;27:2907–15.

[70] Smart SK, Cassady AI, Lu GQ, Martin DJ. The biocompatibility of carbon nanotubes. Carbon 2006;44:1034–47.

[71] Shvedova AA, Castranova V, Kisin ER, Schwegler-Berry D, Murray AR, Gandelsman VZ, Maynard A, Baronn P. Exposure to carbon nanotube material: assessment of nanotube cytotoxicity using human keratinocyte cells. Journal of Toxicology and Environmental Health, Part A 2003;66:1909–26.

[72] Cui DX, Tian FR, Ozkan CS, Wang M, Gao HJ. Effect of single wall carbon nanotubes on human HEK293 cells. Toxicology Letters 2005;155:73–85.

[73] Jia G, Wang HF, Yan L, Wang X, Pei RJ, Yan T, Zhao YL, Guo XB. Cytotoxicity of carbon nanomaterials: single-wall nanotube, multi-wall nanotube, and fullerene. Environmental Science and Technology 2005;39:1378–83.

[74] Monterio-Riviere NA, Nemanich RJ, Inman AO, Wang YYY, Riviere JE. Multi-walled carbon nanotube interactions with human epidermal keratinocytes. Toxicology Letters 2005;155:377–84.

[75] Chen X, Tam UC, Czlapinski JL, Lee GS, Rabuka D, Zettle A, Bertozzi CR. Interfacing carbon nanotubes with living cells. Journal of the American Chemical Society 2006;128:6292–3.

[76] Margrez A, Kasas S, Salicio V, Pasquier N, Seo JW, Celio M, Catsicas S, Schwaller B, Forro L. Cellular toxicity of carbon-based nanomaterials. Nano Letters 2006;6:1121–5.

[77] Nimmagadda A, Thurston K, Nollert MU, McFetridge PSF. Chemical modification of SWNT alters in vitro cell–SWNT interactions. Journal of Biomedical Materials Research Part A 2006;76:614–25.

[78] Sayes CM, Liang F, Hudson JL, Mendez J, Guo WH, Beach JM, Moore VC, Doyle CD, West JL, Billups WE, Ausman KD, Colvin VL. Functionalization density dependence of single-walled carbon nanotubes cytotoxicity in vitro. Toxicology Letters 2006;161:135–42.

[79] Warheit DB, Laurence BR, Reed KL, Roach DH, Reynolds GAM, Webb TR. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. Toxicological Sciences 2004;77:117–25.

[80] Huczko A, Lange H, Bystrzejewski M, Baranowski P, Grubek-Jaworska H, Nejman P, Przybylowski T, Czuminska K, Glapinski J, Walton DRM, Kroto HW. Pulmonary toxicity of 1-D nanocarbon materials. Fullerenes, Nanotubes and Carbon Nanostructures 2005;13:141–5.

[81] Muller J, Huaux F, Moreau N, Misson P, Heilier JF, Delos M, Arras M, Fonseca A, Nagy JB, Lison D. Respiratory toxicity of multi-wall carbon nanotubes. Toxicology and Applied Pharmacology 2005;207:221–31.

[82] Ding LH, Stilwell J, Zhang TT, Elboudwarej O, Jiang HJ, Selegue JP, Cooke PA, Gray JW, Chen FQF. Molecular characterization of the cytotoxic mechanism of multiwall carbon nanotubes and nano-onions on human skin fibroblast. Nano Letters 2005;5:2448–64.

[83] Kretlow JD, Mikos AG. Review: mineralization of synthetic polymer scaffolds for bone tissue engineering. Tissue Engineering 2007;13:927–38.

[84] Mikos AG, Thorsen AJ, Czerwonka LA, Bao Y, Winslow DN, Vacanti JP. Preparation and characterization of poly(L-lactic acid) foams. Polymer 1994;35:1068–77.

[85] Lu L, Peter SJ, Lyman MD, Lai HL, Leite SM, Tamada JA, Vacanti JP, Langer R, Mikos AG. In vitro degradation of porous poly(L-lactic acid) foams. Biomaterials 2000;21:1595–605.

[86] Kim SS, Ahn KM, Park MS, Lee JH, Choi CY, Kim BS. A poly(lactide-co-glycolide)/hydroxyapatite composite scaffold with enhanced osteoconductivity. Journal of Biomedical Materials Research Part A 2007;80:206–15.

[87] Sakai R, John B, Okamoto M, Seppälä JV, Vaithilingam J, Hussein H, Goodridge R. Fabrication of polylactide based biodegradable thermoset scaffolds for tissue engineering applications. Macromolecular Materials and Engineering 2013;298:45–52.

[88] Boccaccini AR, Maquet V. Bioresorbable and bioactive polymer/Bioglass(R) composites with tailored pore structure for tissue engineering applications. Composites Science and Technology 2003;63:2417–29.

[89] Boccaccini AR, Blaker JJ, Maquet M, Chung W, Jerome R, Nazhat SN. Poly(DL-lactide) (PDLLA) foams with TiO_2 nanoparticles and PDLLA/TiO_2-bioglass foam composites for tissue engineering scaffolds. Journal of Materials Science 2006;41:3999–4008.

[90] Greiner A, Wendorff JH. Electrospinning: a fascinating method for the preparation of ultrathin fibers. Angewandte Chemie International Edition 2007;46:5670–703.

[91] Causa F, Netti PA, Ambrosio L. A multi-functional scaffold for tissue regeneration: the need to engineer a tissue analogue. Biomaterials 2007;28:5093–9.

[92] Jose MV, Thomas V, Johnson KT, Dean DR, Nyairo E. Aligned PLGA/HA nanofibrous nanocomposite scaffolds for bone tissue engineering. Acta Biomaterialia 2009;5:305–15.

[93] Ago H, Tobita M. Polymer composites of carbon nanotubes aligned by a magnetic field. Advanced Materials 2002;14:1380–3.

[94] Armentano I, Del Gaudio C, Bianco A, Dottori M, Nanni F, Fortunati E, Kenny JM. Processing and properties of poly(ε-caprolactone)/carbon nanofiber composite mats and films obtained by electrospinning and solvent casting. Journal of Materials Science 2009;44:4789–95.

[95] Misra SK, Watts PCP, Valappil SP, Silva SRP, Roy I, Boccaccini AR. Poly(3-hydroxybutyrate)/bioglass composite films containing carbon nanotubes. Nanotechnology 2007;18, 075701/1–8.

[96] Mei F, Zhong J, Yang X, Ouyang X, Zhang S, Hu X, Ma Q, Lu J, Ryu S, Deng X. Improved biological characteristics of poly(L-lactic acid) electrospun membrane by incorporation of multiwalled carbon nanotubes/hydroxyapatite nanoparticles. Biomacromolecules 2007;8:3729–35.

[97] Niiranen H, Pyhältö T, Rokkanen P, Kellomäki M, Törmälä P. In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites. Journal of Biomedical Materials Research Part A 2004;69:699–708.

[98] Rich J, Jaakkola T, Tirri T, Narhi T, Yli-Urpo A, Seppala J. In vitro evaluation of poly([var epsilon]-caprolactone-co-DL-lactide)/bioactive glass composites. Biomaterials 2002;23:2143–50.

[99] Dunn AS, Campbell PG, Marra KG. The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials. Journal of Materials Science Materials in Medicine 2001;12:673–7.

[100] Liu Q, Wu J, Tan T, Zhang L, Chen D, Tian W. Preparation, properties and cytotoxicity evaluation of a biodegradable polyester elastomer composite. Polymer Degradation and Stability 2009;94:1427–35.

[101] Allen BL, Kichambare PD, Gou P, Vlasova II, Kapralov AA, Konduru N, Kagan VE, Star A. Biodegradation of single-walled carbon nanotubes through enzymatic catalysis. Nano Letters 2008;8:3899–903.

[102] Lazzeri L, Cascone MG, Danti S, Serino LP, Moscato S, Bernardini N. Geratine/PLLA sponge-like scaffolds: morphological and biological characterization. Journal of Materials Science Materials in Medicine 2007;18:1399–405.

[103] Baek HS, Park YH, Ki CS, Park JC, Rah DK. Enhanced chondrogenic responses of articular chondrocytes onto porous silk fibroin scaffolds treated with microwave-induced argon plasma. Surface and Coatings Technology 2008;202:5794–7.

[104] He W, Yong T, Ma ZW, Inai R, Teo WE, Ramakrishna S. Biodegradable polymer nanofiber mesh to maintain functions of endothelial cells. Tissue Engineering 2006;12:2457–66.

[105] Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials 2003;24:4385–415.

[106] Zhang H, Lin CY, Hollister SJ. The interaction between bone marrow stromal cells and RGD-modified three-dimensional porous polycaprolactone scaffolds. Biomaterials 2009;30:4063–9.

[107] Armentano I, Ciapetti G, Pennacchi M, Dottori M, Devescovi V, Granchi D, Baldini N, Olalde B, Jurado MJ, Alava JIM, Kenny JM. Role of PLLA plasma surface modification in the interaction with human marrow stromal cells. Journal of Applied Polymer Science 2009;114:3602–11.

[108] Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. Science 2005;310:1139–43.

[109] Rohman G, Pettit JJ, Isaure F, Cameron NR, Southgate J. Influence of the physical properties of two-dimensional polyester substrates on the growth of normal human urothelial and urinary smooth muscle cells in vitro. Biomaterials 2007;28:2264–74.

[110] Engler AJ, Sen S, Sweeney HL, Disher DE. Matrix elasticity directs stem cell lineage specification. Cell 2006;126:677–89.

[111] Mitragotri S, Lahann J. Cell and biomolecular mechanics in silico. Nature Materials 2009;8:15–23.

[112] Soccioa M, Lottia N, Finellia L, Gazzanob M, Munari A. Influence of transesterification reactions on the miscibility and thermal properties of poly(butylene/diethylene succinate) copolymers. European Polymer Journal 2008;44:1722–32.

[113] Tantini B, Fiumana E, Cetrullo S, Pignatti C, Bonavita F, Shantz LM, Giordano E, Muscari C, Flamigni F, Guarnieri C, Stefanelli C, Caldarera CM. Involvement of polyamines in apoptosis of cardiac myoblasts in a model of simulated ischemia. Journal of Molecular and Cellular Cardiology 2006;40:775–82.

[114] Mima Y, Fukumoto S, Koyama H, Okada M, Tanaka S, Shoji T, Emoto M, Furuzono T, Nishizawa Y, Inaba M. Enhancement of cell-based therapeutic angiogenesis using a novel type of injectable scaffolds of hydroxyapatite-polymer nanocomposite microspheres. PLoS ONE 2012;7, e35199/1–12.

[115] Pietroiusti A. Health implications of engineered nanomaterials. Nanoscale 2012;4:1231–47.

[116] Phillips JI, Green FY, Davies JC, Murray J. Pulmonary and systemic toxicity following exposure to nickel nanoparticles. American Journal of Industrial Medicine 2010;53:763–7.

[117] Hauck TS, Anderson RE, Fischer HC, Newbigging S, Chan WCW. In vivo quantum-dot toxicity assessment. Small 2010;6:138–44.

[118] Poland CA, Duffin R, Kinlock I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nature Nanotechnology 2008;3:423–38.

[119] Dvir T, Timko BP, Brigham MD, Naik SR, Karajanagi SS, Levy O, Jin H, Parker KK, Langer R, Kohane DS. Nanowired three-dimensional cardiac patches. Nature Nanotechnology 2011;6:720–5.

[120] Wu S, Liu X, Hu T, Chu PK, Ho JPY, Chan YL, Yeung KWK, Chu CL, Hung TF, Huo KF, Chung CY, Lu WW, Cheung KMC, Luk KDK. A biomimetic hierarchical scaffold: natural growth of nanotitanates on three-dimensional microporous Ti-based metals. Nano Letters 2008;8:3803–8.
[121] Rahaman MN, Day DE, Bal BS, Fu Q, Jung SB, Bonewald LF, Tomsia AP. Bioactive glass in tissue engineering. Acta Biomaterialia 2011;7:2355–73.
[122] Gristina AG, Oga M, Webb LX, Hobgood CD. Adherent bacterial colonization in the pathogenesis of osteomyelitis. Science 1985;228:990–3.
[123] Heisel J. Animal experiment studies of callus formation by hydoxyapatite injection. Unfallchirurgie 1987;13:179–86.
[124] White RA, Weber JN, White EW. Replamineform: a new process for preparing porous ceramic, metal, and polymer prosthetic materials. Science 1972;176:922–4.