Review

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Nanotechnologies in tissue engineering

Abstract: As an interdisciplinary field, tissue engineering (TE) aims to regenerate tissues by combining the principles of cell biology, material science, and biomedical engineering. Nanotechnology creates new materials that might enable further tissue-engineering applications. In this context, the introduction of nanotechnology and nanomaterials promises a biomimetic approach by mimicking nature. This review summarizes the current scope of nanotechnology implementation possibilities in the field of tissue engineering of bone, muscle, and vascular grafts with forms on nanofibrous structures.

Keywords: electrospinning; nanofibers; nanomaterials; nanotechnology; tissue engineering.

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1 Introduction

The replacement of damaged or injured parts of the body with new functional tissues is an age-old dream of mankind. Recently, the previously unreachable goal has moved within easy reach as the discipline of tissue engineering (TE) has opened a new area of science and medicine. The term "tissue engineering" was defined in 1987 as the "Application of the principles and methods of engineering and life sciences toward the fundamental understanding of structure-function relationships in normal and pathologic mammalian tissue and the development of biological substitutes to restore, maintain, or improve function" [1]. Today, TE pursues three main strategies: (1) transplantation of cells through injection or transplantation into the body; (2) implantation of different types of matrices alone or in combination with growth factors and/or cells into the defect site in order to repair or regenerate tissue defects; and (3) seeding and culturing of three-dimensional biodegradable and/or biocompatible scaffolds with specific cell types in order to mimic the natural extracellular matrix (ECM). Nanotechnology is defined as the manipulation of matter over the scale of a nanometer, whereby, nanofibrous scaffolds can be produced containing nanofibers as scaffolds with two similar external dimensions in the nanoscale and the third dimension which is significantly larger. It is here that nanotechnology and nanomaterials come into play in order to fabricate optimal three-dimensional scaffolds for the attachment, proliferation, and ingrowth of cells aiming at new tissue formation. Here, the combination of the microporous structure and the high surface area to volume ratio of nanofibrous scaffolds favor cell adhesion, proliferation, migration and differentiation, thus, presenting all the desired properties for tissue engineering concepts [2]. This review summarizes the current scope of nanofibrous implementation possibilities in the field of tissue engineering of muscle, bone, and vascular tissue.

2 Methods for nanofiber synthesis

Currently, nanofibers can be fabricated using a variety of techniques such as electrospinning, phase separation, or self-assembly. Molecular self-assembly peptide systems have been developed for the fabrication of nanofibrous scaffolds in order to mimic the human ECM [3, 4]. Peptide amphiphiles (PAs) consist of a hydrophobic tail group and a short peptide sequence. The drawing method

continuously forms nanofibers by drawing and solidification of a viscous liquid polymer solution, which is pumped through a glass micropipette [5]. The phase separation technique is a relatively simple procedure with minimal equipment requirements [6]. It was first described by Ma and Zhang in 1999 and consists of five steps: polymer dissolution, phase separation process, gelation, extraction of solvent from the gel with water, freezing, and freeze-drying under vacuum [7]. Here, scaffolds with arbitrary anatomical shapes can be fabricated. Macroporosity can be achieved by incorporation of porogens. Recently, Lee et al. used room temperature ionic liquids (RTILs) as a novel porogen, which were incorporated into a poly(lactic acid) scaffold with further selective dissolution [8]. Using this technique, they were able to create an interconnected network with pore sizes of over 100 μm and a porosity of 86%–94%. The authors also studied these scaffolds for their interaction with mesenchymal stem cells (MSCs) derived from rat bone marrow. They were able to show good biocompatibility of the scaffolds *in vitro* as well as *in vivo* in a subcutaneous rat model as well as an increased osteogenic differentiation of MSCs seeded on the PLA scaffolds. Zhao et al. were able to create a porous biomimetic poly(propylene carbonate) (PPC) scaffold with a nanofibrous chitosan network using a dual solid-liquid phase separation technique [9]. In the first phase separation, the PPC scaffold was built, and in the second phase separation, the nanofibrous chitosan structure was prepared in the macropores. The advantage of the phase separation technique is the relatively simple experimental setup. Furthermore, different architectures in nano- and macrostructure can be incorporated in the matrix design.

Out of these methods, electrospinning has emerged as a very efficient technique due to its relatively simple and inexpensive design [10]. Electrospinning was first described by Formhals in 1934 and became popular in the 1990s. Electrospinning produces scaffolds with specific fiber orientation and high surface area. The electrospinning system basically consists of four major components:

1. a voltage power supply
2. a syringe pump, which determines the flow rate of the polymer solution
3. a needle shifts the polymer solution into the high electric field
4. a collector on which the nanofibers are collected [11].

Further adjustments can be made by varying the polymer solution properties, applied voltage, flow rate, or the distance between the needle and the collector. Depending on the desired tissue, different polymers can be used for electrospinning purposes. Typical polymers with relevance for biomedical applications can be divided into synthetic polymers such as polycaprolactone (PCL), poly(lactic acid) (PLA), poly(lactic acid-co-caprolactone) (PLCL), or poly(glycolic acid) (PGA) and natural polymers such as collagen, gelatin, or elastin. Synthetic polymers offer good mechanical stability but often lack biocompatibility, whereas natural polymers behave the other way. To overcome these shortcomings of natural and synthetic polymers, composites of different polymers have been created. Recently, Rim et al. reviewed the current approaches for the fabrication of electrospun nanofibers for tissue engineering [12]. The author emphasizes, that the successful fabrication of the electrospun nanofibrous scaffolds starts with the selection of proper polymers, whereas the combination of multiple polymers as well as various postmodification techniques may provide desirable properties [12]. Beachley and coworkers addressed the limited control over fiber organization within nanofiber structures when they are collected directly from an electrospinning jet [13]. Furthermore, a novel assembly technique that utilizes parallel automated tracks to orient and collect nanofibers from the electrospinning jet has been established in order to allow a better control of the fiber organization, resulting in a continuous steady-state delivery of static stabilized nanofibers [13]. Another study by Leverson and colleagues demonstrated the feasibility of fabrication of electrospun scaffolds containing two differently scaled fibers as well as scaffolds containing fibers of the two different materials fibrin and PCL using a dual extrusion electrospinning setup.

3 Muscle tissue engineering

To date, there are only a few alternatives for functional restoration of damaged muscle tissue. The surgical techniques of muscle transplantation or transposition present a reasonable possibility with good results for restoration of function, especially for the paralyzed face or the upper extremity [14]. Although the present clinically used flaps enable, in most cases, a functional satisfiable reconstruction, there are some procedural limitations: extended flaps cause significant morbidity at the donor site that can implicate long-term pain and functional limitations. As a new alternative, skeletal muscle tissue engineering may overcome problems associated with the autologous transfer of muscle tissue.

For muscle tissue engineering, fibrous matrices with a high degree of parallel orientation (Figure 1) are necessary to guarantee a high cell density and the application of

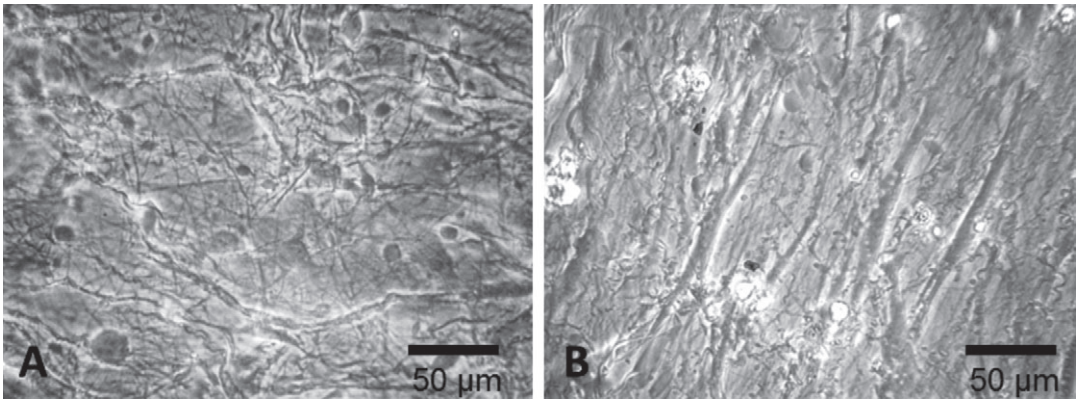

Figure 1 Cross sections of PCL nanofiber scaffolds with seeded primary myoblasts.
(A) Unstructured fiber orientation; (B) aligned fiber orientation.

longitudinal force after contraction [15, 16]. Several techniques have been described to obtain the aligned architecture, such as selective laser sintering, three-dimensional printing, or unidirectional freeze-drying [17–20]. Electrospinning as an alternative method offers the opportunity to produce nanofibers as mentioned below (Table 1). The technique allows the adjustment of matrix properties by varying multiple parameters such as the concentration and viscosity of the spinning solution, the voltage applied, or the flow rate of the spinning solution. A variety of different synthetic polymers and biopolymers can be used for electrospinning of matrices for muscle tissue engineering. Natural polymers, such as collagen [21–23], hyaluronic acid [23], or elastin [24], mimic the natural extracellular matrix perfectly and, therefore, offer the advantage of cell attachment and differentiation of cells. The main disadvantage of natural polymers is their low stability *in vivo*; therefore, these matrices are often combined with synthetic polymers. The most frequently used synthetic polymer in muscle tissue engineering is poly(ε-caprolactone) (PCL) [25, 26]. PCL is a synthetic polymer with a slow degradation rate, biocompatible but also hydrophobic and, therefore, shows poor cell attachment [27]. To combine natural and synthetic polymers, different techniques can be used. The most frequently used methods are spinning polymer-blend solutions, core-shell spinning, or co-spinning of different polymer solutions. Using the core-shell spinning technique, matrices can also be used as drug delivery systems (DDS) in order to release incorporated drugs in a controlled manner. Yoon et al. used a core-shell laminated, structured, electrospun mat of hydrophobic PCL and hydrophilic poly(ethylene oxide) (PEO)/rhodamine-B fibers in a normal PCL matrix [28]. Rhodamine was released from the scaffold by the drug delivery system, which was controlled by the thickness of the PCL layer. Matrices can also be loaded with growth factors such as angiogenic growth factor VEGF to reduce vascularization time [29] or growth factors to induce myogenic differentiation, e.g., insulin-like growth factor [30]. Rende et al. were able to show an effect of the nerve growth factor (NGF) on the differentiation and proliferation of myogenic cells *in vitro* via the tyrosine kinase receptor TrKA [31]. Furthermore, Lavasani et al. showed the effect of retroviral transduction of MDSCs with a CL-NGF vector and direct stimulation of MDSCs with an NGF protein on the muscle regeneration in dystrophic muscles [32]. They were able to show that NGF acts as a modulatory device and improves muscle regeneration. Despite other tissue engineering applications, matrices with incorporated DDS have still not been applied for muscle tissue engineering.

It has been noted that cell infiltration into dense electrospun scaffolds with reduced pore size and aligned orientation is limited [33]. The co-spinning procedure holds the opportunity to control the pore size by using the so-called sacrificial fibers such as poly-(ethylene-oxide) (PEO), which dissolves in water [34]. Baker et al. developed a dual-electrospinning method to produce scaffolds containing a PCL and a PEO component [35]. They seeded the novel matrix and a pure PCL matrix with mesenchymal stem cells (MSCs) and were able to show an increased cell infiltration and distribution after 3 weeks in culture in the PCL/PEO group. In a recent study, Baker et al. removed 50% of the initial fibers resulting in a scaffold, which provides sufficient instruction to align cells and direct the formation of a highly organized ECM [34]. After the electrospinning procedure, matrices have also been secondarily modified by coating or plasma treatment [36].

Using the electrospinning technique, electrically conductive matrices can be created by spinning conductive polymers such as polyaniline (PANi) [37]. This can lead to an increased myogenic differentiation of seeded myoblasts [38] and neurite outgrowth of cultured neural stem cells

Table 1 Properties of different nanomaterials of muscle tissue engineering.

Author, year	Material	Animal model	Cells	Results
Yoon, 2011 [28]	PCL/PEO/ rhodamine-B	*In vitro*	–	Initial burst in drug release was eliminated by thickness of PCL layer
Rende, 2000 [31]	–	*In vitro*	Myogenic cell line L6, primary cultures of adult human myoblasts, human rhabdomyosarcoma cell line TE-671	Effects of NGF are mediated by TrKA receptors, NGF is needed for increase fusion into myotubes
Baker, 2008 [35]	PCL/PEO	*In vitro*	MSC	>50% PEO fibers improved cell infiltration of scaffolds
Prabhakaran, 2011 [40]	PLLA/PANi	*In vitro*	Nerve stem cells	Electric stimulation of scaffolds resulted in extended neurite outgrowth
Guarino, 2013 [41]	PANi/PEGDA	*In vitro*	PC12/hMSC	PANi increased electric conductivity of matrices

[39]. Prabhakaran et al. fabricated an electrospun scaffold containing poly-L-lactide (PLLA) that was blended with PANi to obtain PLLA/PANi nanofibers. *In vitro* electrical stimulation of nerve stem cells seeded on the PLLA/PANi matrices resulted in extended neurite outgrowth compared to the cells grown on nonstimulated scaffolds [40]. In a recent study, Guarino et al. built a hybrid material that was fabricated by *in situ* precipitation of PANi in polyethyleneglycol diacrylate (PEGDA) solution, followed by crosslinking via UV irradiation resulting in a porous architecture by sodium chloride particle leaching [41]. The hybrid matrices improved proton conductivity and the biological response of PC12 and human mesenchymal stem cells (hMSCs).

4 Vascular tissue engineering

Tissue engineering of small-diameter vascular grafts is one of the most challenging and necessary fields of research as cardiovascular disease causes almost 1 million deaths in the US alone every year [42]. For myocardial revascularization, autologous arterial or venous grafts still represent the gold standard. The availability of vessel grafts is limited because of poor quality, limited length, previous surgical treatment, or peripheral arterial occlusive disease. Therefore, many studies have focused on the generation of vascular grafts (Table 2). Although vascular grafts composed of expanded polytetrafluoroethylene (ePTFE) or polyethyleneterephthalate (Dacron) are already used in the replacement of large- or medium-diameter vessels, these materials are not suitable for small-diameter vessels because of thrombosis and intimal hyperplasia [43, 44]. According to Ercolani et al., the optimal small-diameter vascular graft should have mechanical strength, biocompatibility, an acceptable healing response, and ease of handling [45]. Electrospinning has become an effective technique for the production of small-diameter vascular grafts because these grafts mimic the natural extracellular matrix with a high surface area, high porosity, and small pores. Different natural and synthetic materials have been used in the past for vascular tissue engineering. Poly(lactic-co-glycolic) acid (PLGA) is a synthetic polymer and has been used for small-diameter vascular grafts. Kim et al., for example, preseeded the grafts with endothelial cells and smooth muscle cells, which showed an increased patency rate compared to nonseeded grafts. One disadvantage of PLGA is its fast degradation rate, which makes this material unsuitable for vascular graft engineering [46, 47]. Diban et al. produced hollow fiber (HF) membranes via phase inversion using blends of PLGA with PCL [48]. They investigated the influence of different ratios of PLGA and PCL regarding water transport and mechanical properties as well as cell attachment and proliferation of human adipose stem cell (hASC). They showed that PCL/PLGA85/15 HFs meet the mechanical requirements of small-caliber vessels. Furthermore, the blended PCL/PLGA85/15 HFs supported more hASC attachment and proliferation than PCL and PLGA HFs. Wang et al. fabricated pillared PLGA nanoscaffolds using the femtosecond laser ablation technique [49]. They seeded bovine endothelial cells onto the scaffolds and were able to demonstrate cell adherence and growth around each branch of the pillared microvessel networks.

PCL has been used in several studies for small-diameter vascular grafts. The advantages of PCL are its slow degradation rate, good mechanical properties, and fast endothelialization [50–52]. de Valence et al. recently used PCL vascular grafts in the rat abdominal aorta replacement model [53]. They built vessels with an inner diameter of 2 mm by electrospinning of PCL. The grafts

Table 2 Properties of different nanomaterials of vascular grafts.

Author, year	Material	Animal model	Explantation time point (max.)	Cells	Stenotic changes	Degradation	Results
Diban, 2013 [48]	PLGA/PCL	*In vitro*	–	hASC	–	–	PCL/PLGA85/15 blend best results
Wang, 2012 [49]	PLGA	*In vitro*	–	Bovine endothelial cells	–	–	Cells adhere and grow around the pillared microvessels
De Valence, 2012 [53]	PCL	Rat aorta	18 months	–	No thrombosis, limited intimal hyperplasia	Fiber fragmentation	Insufficient regeneration of vascular wall
Innocente, 2009 [54]	PCL/PTX	*In vitro*, rat aorta	6 months	–	No thrombosis	–	PTX loading delays endothelialization and cellular ingrowth
Kuwabara, 2012 [55]	PCL	Rat carotid	72 weeks	–	72.5% Patency	PCL degraded	Regeneration of media layer progressed over time
He, 2005 [58]	P(LLA-CL)	*In vitro*	–	HCAEC	–	–	Scaffolds enhance viability, spreading, and attachment of HCAEC
Dong, 2008 [57]	P(LLA-CL)	*In vitro*	–	PCASMC	–	Eight month degradation time	Generation of multilayered aligned oriented PCASMCs on the scaffolds
Bergmeister, 2012 [60]	PU	Rat aorta	6 months	–	95% Patency rate	–	Cell immigration and differentiation seemed to be promoted by the scaffold
Soldani, 2009 [61]	PEtU–PDMS	Sheep carotid	24 months	–	No thrombosis at 24 month	Complete degradation	Superior handling and patency rates of PEtU-PDMS grafts compared to ePTFEE
He, 2010 [62]	PU	Rat aorta	8 weeks	Pericytes	100% Patency rate in seeded grafts	Remodeling with collagen and elastin	Increased patency by seeded pericytes
Del Gaudio, 2012 [63]	PCL/PHBV	*In vitro*	–	Rat cerebral EC	–	–	PCL-based scaffolds promote cell attachment and migration
McClure, 2010 [70]	Silk fibroin, collagen, elastin, PCL	*In vitro*	4 weeks	–	–	Signs of degradation	COL-blended samples displayed better mechanical properties than SF-blended samples
Stroncek, 2012 [71]	ePTFE	*In vitro*, rat femoral artery	28 days	EPC/+AdTM	75%–88% Patency rate in cell seeded grafts	–	EPCs increase patency rate of ePTFE grafts

were implanted in the abdominal aorta replacement model in Sprague-Dawley rats for 1.5, 3, 6, 12, and 18 months and were tested regarding patency, compliance, and scaffold degradation. They reported graft thrombosis and no significant change in graft compliance over time. Also, a decrease in molecular weight and fiber fragmentation were observed at 18 months although this lead to no measurable limitations of its mechanical properties. Cellular infiltration and vascularization were detected in the early stages followed by a progressive regression over time.

Pektok et al. compared small-diameter vascular grafts made of PCL nanofibers with ePTFE grafts regarding *in vivo* degradation and patency [50]. They implanted vascular grafts into the abdominal aorta of 15 Sprague-Dawley rats per group. After 3, 6, 12, 18, and 24 weeks, the infrarenal segment of the abdominal aorta, including the implanted

graft, was explanted and processed to histological evaluation. At the explantation time point, all grafts were patent without significant stenosis detectable in the digital subtraction angiography. Histological analysis of the PCL grafts showed a rapid endothelialization of the luminal part and a neovascularization throughout the graft starting 3 weeks after implantation with further increase over time. In the ePTFE group, the grafts revealed a delayed endothelialization and a slow cellular infiltration. Also, no neovascularization was detected in the ePTFE grafts, and the increased neointima formation led to two stenotic lesions after 18 and 24 weeks.

To reduce intimal proliferation, Innocente et al. combined small-caliber vascular prostheses using electrospun PCL with slow-releasing paclitaxel (PTX), which should show an antiproliferating effect [54]. PLX has been used to reduce in-stent restenosis, therefore, PLX-loaded PCL grafts were used in this study. The grafts were fabricated by electrospinning of PCL solutions (12% wt/vol) containing PTX (0.75 wt/wt). They were compared to plain PCL grafts in the abdominal aorta *in vivo* model in Sprague-Dawley rats. Grafts were explanted after 3, 12, and 24 weeks followed by histological evaluation. DSA showed no stenosis or aneurysm in both groups at any explantation time point. Histological evaluation revealed reduced intima formation in the PCL-PTX grafts compared to the plain PCL grafts. No differences were observed regarding cellular infiltration and neovascularization of vascular grafts.

Kuwabara et al. reported electrospun nano-scale PCL grafts with a diameter of 0.7 mm and a porosity of approximately 78% [55]. They implanted the grafts in a rat carotid artery replacement model followed by explantation after 1, 2, 6, 12, 24, 48, and 72 weeks. Histological evaluation revealed a patency rate of 72.5% without any aneurysmal formation or calcifications. The endothelium was completed in the early phase without any further modifications, whereas the tunica media only partially regenerated, and degradation of the scaffolds was visible over time without completion.

"Poly(L-lactide-co-e-caprolactone) P(LLA-CL)" nanofibers show a slow degradation rate and were used in different studies for vascular tissue engineering over past years, also combined with smooth muscle cells [56, 57]. He et al. used electrospun collagen blended P(LLA-CL) nanofibers to fabricate vascular grafts [58]. They were able to show improved cell attachment and viability compared to plain polymer nanofibers. Dong et al. used porcine coronary artery smooth muscle cells (PCASMCs) seeded and cultured on P(LLA-CL) scaffolds for up to 105 days [57]. They were able to demonstrate that PCASMCs did not influence the degradation rate of the P(LLA-CL) scaffolds, although they did attach and proliferate on the scaffolds. They also created multilayers of aligned oriented PCASMCs and a significant ECM protein secretion.

Polyurethane (PU) has become a promising material for vascular tissue engineering because of its high tensile strength, good elasticity, and biocompatibility [59]. Bergmeister et al. evaluated vascular grafts composed of PU with an inner diameter of 1.5 mm and a mean wall thickness of 70 mm [60]. They used the rat abdominal aorta model to investigate their *in vivo* biocompatibility after 1 week, 1, 3, and 6 months. They were able to show a patency rate of 95% without any visible graft degradation. Within the first month, the luminal surface was covered with endothelial cells with no further hyperplasia except for one specimen. The grafts showed ongoing cell proliferation up to 6 months after implantation. Soldani et al. fabricated small-diameter vascular grafts with a poly(ether) urethane-polydimethylsiloxane (PEtU-PDMS) semi-interpenetrating polymeric network with a diameter of 5.0 mm and a wall thickness of approximately 500 mm [61]. They implanted the grafts in the carotid artery of adult sheeps with ePTFE grafts as a control group. All PEtU-PDMS grafts were patent, while 50% of the control grafts were found to be occluded. Also PEtU-PDMS grafts showed signs of remodeling with partial degradation and no visible signs of calcification. He et al. produced bilayered elastomeric poly(ester-urethane)urea scaffolds, which were preseeded with pericytes [62]. The inner scaffold was fabricated by the thermally induced phase separation (TIPS) method, whereas the outer surface was produced by electrospinning to achieve an inner porous layer and an external fibrous layer. Seeded and unseeded scaffolds were implanted in the rat abdominal aorta model, and patency was checked using angiography. After 8 weeks, the specimens were explanted and processed to histological evaluation. Angiograms showed a significantly increased patency in the pericyte seeded group compared to the unseeded specimens. In the TIPS layer, signs of remodeling were visible, whereas in the electrospun layer, no cell infiltration was detected.

Because synthetic materials often lack cell attachment properties, they are combined with natural polymers such as collagen or elastin. This can be obtained by either blending or co-electrospinning of the synthetic and natural polymer. To improve attachment of cells, mainly PCL was blended with multiple synthetic or natural polymers such as collagen, elastin, or gelatin with different blend ratios [63–66]. Recently, del Gaudio produced PCL scaffolds blended with hard

poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a polymer of microbial origin, and evaluated these scaffolds regarding their mechanical properties and attachment and growth of rat cerebral endothelial cells [63]. They were able to demonstrate that PCL and PCL/PHBV scaffolds promoted attachment and migration of rat cerebral endothelial cells.

The normal vessel architecture consists of three layers: the intima, media, and adventitia. To mimic this structure, layered vascular grafts have gained attention over the last years. Multilayered vascular grafts consist of usually two or three different natural or synthetic polymers such as PCL, collagen, elastin, or gelatin [67–69]. McClure et al. composed a tri-layered vascular graft of polycaprolactone, elastin, collagen, and silk to mimic the native vascular wall and its mechanical properties [70].

Also, cells such as endothelial cells or endothelial progenitor cells have been investigated to improve endothelialization [71]. Stroncek et al. used small-diameter ePTFE vascular grafts seeded with endothelial progenitor cells (EPCs) or with EPCs transfected with an adenoviral vector containing the gene for human thrombomodulin (EPC+AdTM) [71]. *In vitro* results showed an increase in activated protein C (APC) in the EPC+AdTM group compared to the EPC group. They also performed *in vivo* studies in athymic rats. They implanted EPC-seeded vascular grafts, EPC+AdTM grafts, and plain ePTFE grafts bilaterally into the femoral arteries for 7 and 28 days. Control grafts showed a patency rate after 7 days of only 25%, whereas EPC seeded and EPC+AdTM seeded specimens were found to have patency rates of around 90% after 7 days and 75%–88% after 28 days.

5 Bone tissue engineering

Scaffolds with a pore size between 100 and 350 μm and a porosity >90% have been identified as optimal for cell/tissue ingrowth and, thus, enhanced bone regeneration [72]. Furthermore, the optimal nanofibrous scaffold for bone tissue engineering has to meet the physical requirements of natural bone, such as mechanical strength, porosity, pore size, rigidity, and above all, three-dimensional architecture [72–74]. A nanoscaled structure of the material surface has been found to have enhanced positive effects on osteoblastic cell response, increasing initial cell adhesion and subsequent proliferation, and finally, the osteogenic differentiation. This seems not to be surprising when we keep in mind that the natural environment of cells, the ECM, composed of organic and inorganic phase, is also organized in nanodimensions. Therefore, many of the novel bio-inspired scaffolds try to mimic this effect of ECM on cells by creating nanostructured surfaces (Table 3).

For bone tissue engineering, composite scaffolds with a mineral component such as hydroxyapatite (HA), calcium phosphate (CaP), or bioglass are commonly used. Wei et al. produced nano-hydroxyapatite (NHAP)/PLLA composite scaffolds with high porosity, well-controlled pore architectures, and improved mechanical properties using TIPS techniques and dioxane/water as a solvent [75]. Peng et al. incorporated needle-shaped nano- or micro-sized HA particles into PLLA scaffolds using the electrospinning technique [76]. The composite scaffolds revealed a higher viability of attached rat osteosarcoma ROS17/2.8 cells and higher ALP activity compared to plain PLLA specimens. Jegal et al. fabricated composite scaffolds using electrospun gelatin-apatite-poly(lactide-co-caprolactone) (PLCL) [77]. By adding only a small concentration of gelatin-apatite into PLCL, the tensile strength was significantly increased compared to plain PLCL matrices. Also osteogenic differentiation of pre-osteoblast cells was increased in the composite specimens compared to the pure PLCL nanofibers. *In vivo* studies were carried out using a rat calvarial critical size model. Composite scaffolds showed an increased defect closure compared to pure PLCL nanofibers and the blank control group.

Liu and coworkers developed nanofibrous gelatin/apatite (NF-gelatin/apatite) composite scaffolds using the TIPS technique [78]. They were able to show an increased surface area and mechanical strength of NF-gelatin scaffolds compared to commercially available gelatin foam. By incorporating apatite particles on the surface of NF-gelatin scaffolds, osteogenic differentiation of seeded cells was also improved.

Woo et al. tested the biological properties of nanofibrous PLLA scaffolds compared with solid PLLA scaffolds in the critical-size rat calvarial bone defect [79]. After 4 and 8 weeks, specimens were explanted and analyzed using micro-CT and histological evaluation. They found out that 8 weeks after implantation, nanofibrous scaffolds lead to a significantly increased bone formation compared to solid-walled specimens. They also suggested, based on the results of the immunostainings, that nanofibrous matrices lead to an osteogenic differentiation of recruited cells. Jose and coworkers tested a combination of PLGA with collagen and nano-HA to produce bioactive multicomponent nanofibrous scaffold by electrospinning [80]. They showed that nano-HA was beneficial in small amount regarding mechanical

Table 3 Properties of different nanomaterials of bone tissue engineering.

Author, year	Material	Animal model	Explantation time point (max.)	Cells	Results
Peng, 2011 [76]	HA/PLLA	*In vitro*	–	Rat osteosarcoma ROS17/2.8 cells	HA/PLLA scaffolds showed higher cell viability and ALP activity than a pure PLLA scaffolds
Jegal, 2011 [77]	Gelatin-apatite-PLCL	*In vitro*, rat calvarium	6 weeks	–	More enhanced bone formation in composite scaffolds than in pure PLCL nanofiber
Liu, 2009 [78]	NF-gelatin/apatite	*In vitro*	–	Osteoblasts	Apatite incorporation showed increased osteogenic differentiation and mechanical strength of scaffolds
Woo, 2009 [79]	PLLA	Rat calvarium	8 weeks	–	Nanofibrous scaffolds showed more new bone tissue formation than solid-walled scaffolds
He, 2010 [81]	PLLA	*In vitro*	–	–	Possible modifications of matrix characteristics by varying processing parameters
Cheng, 2013 [82]	CNT/PLGA	*In vitro*	–	MC3T3-E1 osteoblasts	CNTs increased cell attachment and proliferation and osteogenic differentiation of scaffolds
Zhang, 2011 [83]	CNT/Ti6Al4V/nano HA	*In vitro*	–	–	CNT-reinforced HA coating increased coating hardness
Schofer, 2011 [84]	PLLA+BMP-2	Rat calvarium	12 weeks	–	PLLA/BMP-2 increased bone formation in defect model compared to PLLA or bovine spongiosa alone
Srouji, 2011 [85]	PEO/PCL/PEG+BMP-2	Rat calvarium/*in vitro*	8 weeks	MSC	Significantly increased bone generation in BMP-2 group
Seyedjafari, 2010 [86]	PLLA-COL	*In vitro*	–	USSC	Enhanced proliferation and osteogenic differentiation of USSC on PLLA-COL nanofibers
Lu, 2013 [87]	PHBV/HA	Rabbit radius/*in vitro*	12 weeks	MSC	Osteogenic differentiation and bone generation by introduction of HA
Binulal, 2012 [90]	nG/PCL	*In vitro*	–	hMSC	Faster degradation and increased cell attachment by gelatin nanoparticles
Wepener, 2012 [91]	HA/βTCP	*In vitro*	–	Osteoblasts/monocytes	Scaffolds are biocompatible
Li, 2012 [92]	CelluNF/HAp	*In vitro*	–	–	CelluNF/HAp scaffolds consist of micro-, meso-, and macropores
Guo, 2012 [93]	Wnf-CPC	Rabbit femur, *in vitro*	12 weeks	MG-63	Wnf-CPC showed increased bone formation and cell attachment compared to CPC alone
Wang 2012 [95]	Silicia-titania	*In vitro*	–	MSC	Osteogenic cell differentiation was enhanced on the Ti:Si=7:3 group
Ganesh, 2012 [99]	PCL/nSiO(2)	*In vitro*	–	hMSC	Increased osteogenic differentiation and scaffold strength by incorporation of nSiO(2)

properties, whereas high concentrations were detected to be disadvantageous. The multicomponent scaffold revealed to be supportive for hMSC adhesion and proliferation.

He et al. developed a novel technique to accelerate calcium phosphate deposition on nanofibers [81]. Using an electrodisposition technique, they were able to reduce mineralization time to under an hour.

Cheng et al. produced carbon nanotube (CNT)-PLGA composite scaffolds in order to enhance mechanical strength and surface roughness [82]. CNT/PLGA scaffolds were fabricated by a dispersion of CNT in PLGA/dichloromethane solution followed by a solvent casting and particulate leaching step. The obtained results demonstrated an increased mechanical strength by incorporation of CNTs and an increased adhesion, growth, and osteogenic differentiation of MC3T3-E1 osteoblasts. Zhang and coworkers produced nanostructured HA coatings with nanosized HA powder on titanium alloy Ti6Al4V by cathodic electrophoretic deposition (EPD) [83]. Also, multiwalled CNTs were integrated into the HA coating, which lead to a markedly increased coating hardness compared to monolithic HA coating.

Although composite nanomaterials have been widely used for bone tissue engineering, pure synthetic polymers combined with growth factors have been tested to achieve osteoinductivity.

Schofer et al. incorporated bone morphogenetic protein 2 (BMP-2) into PLLA nanofibers [84]. They implanted PLLA/BMP-2 scaffolds into a critical size rat calvarial defect model and compared these samples with pure PLLA specimens, bovine spongiosa, and blank defects. Explantation time points were 4, 8, and 12 weeks after scaffold implantation. Micro-CT and Histology revealed only small amounts of bone formation in the plain PLLA scaffolds, whereas PLLA/BMP-2 specimens showed an early onset of bone regeneration after 4 weeks and the fastest bone regeneration than any other tested group. Srouji et al. developed a core-shell fiber scaffold releasing BMP-2 for bone tissue engineering [85]. The core of the matrix was made of an aqueous core solution of poly(ethylene oxide) with incorporated BMP-2, and the shell was made of polycaprolactone blended with poly(ethylene glycol). First, they demonstrated *in vitro* bioactivity of these scaffolds by measuring ALP levels using human mesenchymal stem cells. For *in vivo* testing, they implanted BMP-2-containing and pure core-shell scaffolds in a rat cranial defect model followed by explantation after 8 weeks. *In vitro* results demonstrated a significantly ninefold increased ALP activity in the BMP-2 group compared to the plain scaffolds and a slow-release kinetic of the BMP-2. Furthermore, the *in vivo* study showed an increased bone formation in rat calvarial defects of approximately 80% of the total area compared to 40% coverage in the plain group.

Seyedjafari et al. tested cord blood-derived unrestricted somatic stem cells (USSC) on collagen-grafted PLLA nanofibers [86]. They found out that the nanofibrous structure of pure PLLA promoted USSCs to osteogenic differentiate and to proliferate, whereas PLLA-COL led to an increased expression of osteogenic markers.

Recently, electrospun poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV)/HA nanofibrous scaffolds were evaluated in a critical-sized rabbit radius defect model. The investigators demonstrated good capacity of the scaffolding system for repairing rabbit bone defects [87]. PCL, as mentioned above, is a semisynthetic polymer with excellent biocompatibility and biodegradability, which make it a good candidate for bone tissue engineering applications [88, 89]. Herein, electrospun nanofibrous semisynthetic polymeric PCL scaffolds were modified with a maximum of 15 wt% of biopolymeric gelatin nanoparticles (nGs), resulting in a significant increase in the degradation rate of PCL. In addition, hMSCs attached and spread faster and better on PCL-nG scaffolds when compared with PCL-scaffolds alone [90]. In another experimental study, either osteoblasts or osteoclast-like cells were cultured separately on electrospun nanobioceramic biphasic hydroxyapatite/β-tricalcium phosphate scaffolds, showing good biocompatibility of the scaffolds without significant negative effects on either osteoblasts or osteoclast-like [91]. As another modification, electrospun cellulose nanofiber (CelluNF)/HA scaffolds were synthesized in simulated body fluid (SBF) in order to mimic the formation of collagen fiber/HA in bone tissue. As a modification, the phosphorylation of CelluNFs resulted in a highly effective guide of HA growth along the nanofibers [92]. Guo and coworkers investigated a self-setting bioactive cement by incorporation of wollastonite nanofibers (WNFs) into calcium phosphate cement (CPC). There was no effect on the setting time or the compressive strength of wnf-CPC but the hydrophilicity and degradability of wnf-CPC were significantly improved by the addition of WNFs, which was attributed to the change of microstructure. Wnf-CPCs were then evaluated in a rabbit bone defect model and showed enhanced efficiency of new bone formation when compared with CPC alone [93]. The excellent biocompatibility and bioactive properties predestine ceramic scaffolds for use as bone graft substitutes for bone regeneration applications. Furthermore, oxides such as silica as the major component of bioactive glasses and titania as the oxide complex of titanium have been found to enhance osteoblast differentiation [94]. In this context, the osteogenic potential of electrospun silica-titania nanofibrous meshes was evaluated by seeding MSCs and resulted in enhanced cell differentiation toward osteoblasts, especially with a Ti:Si ratio of 7:3 [95]. Despite its excellent biocompatibility, the relatively low bioactivity and inadequate mechanical properties are disadvantages of the native PCL for bone tissue

engineering applications [96]. As already mentioned, there is evidence in the literature that incorporating silica leads to an increase in scaffold osteogenicity, bioactivity, and mechanical properties [95, 97, 98]. Recently, Ganesh and coworkers investigated a modification of PCL nanofibrous scaffolds through adding nanoparticles of silica ($nSiO_2$) in order to take advantage of the osteogenic potential of silica while at the same time reinforcing the PCL nanofibers and, thus, improving their mechanical properties for bone tissue engineering applications. A substantial increase in scaffold mechanical strength, protein adsorption, and osteogenic differentiation of MSCs could be demonstrated by embedding silica nanoparticles [99]. The main advantage of natural polymers, such as collagen, elastin, gelatin, hyaluronic acid, and silk is the very similar structure to human macromolecular substances [100]. Based on the idea that natural polymers can be recognized and interact with the biological environment, the main components of the extracellular matrix collagen and hyaluronic acid (HyA) have been used to fabricate electrospun nanosized scaffolds. Collagen/HyA scaffolds that are insoluble in aqueous solutions have been developed and promoted cellular attachment [101].

To achieve the clinical implantation of *in vivo* and *in vitro* tested scaffolds, Wang et al. developed anatomically shaped scaffolds using a 3D printing technique [102]. Scaffolds were produced by phase separation to generate interconnected PLLA nanofibrous scaffolds showing proliferation and adhesion of osteoblasts *in vitro* as well as differentiation and bone formation.

6 Conclusions

The ECMs of various tissues in our body, like bone muscle and blood vessels, differ in the arrangement and composition of several proteins to maintain specific tissue formation, organ specific shape, and function. Therefore, the development of innovative scaffolds in regenerative medicine should vary according to the desired engineered tissue. The discussed nanotechnology approaches open the perspective to develop advanced nanomaterials that mimic the natural extracellular matrix of many tissues, and therefore, they hold great potential in tissue engineering applications. The availability of multiple natural and synthetic polymers with defined properties and the possibility of combining them during the electrospinning process represent an attractive opportunity to modulate the scaffold regarding the desired tissue. Nanofiber-based matrices show promising results to provide scaffolds with tissue-specific properties and versatility, which has been reviewed in this article for muscle, vascular, and bone tissue engineering.

Acknowledgements: We would like to thank the Xue-Hong and Hans Georg Geis, Deutsche Forschungsgemeinschaft DFG (KN 578/2-1) as well as the "Emerging Fields Initiative" of the University of Erlangen-Nuremberg and the AO Research Fund (AO Research Fund Grant S-10-36A).

Received March 7, 2013; accepted April 19, 2013; previously published online June 19, 2013

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