Review

Regenerative Medicine

Recreating composition, structure, functionalities of tissues at nanoscale for regenerative medicine

Nanotechnology offers significant potential in regenerative medicine, specifically with the ability to mimic tissue architecture at the nanoscale. In this perspective, we highlight key achievements in the nanotechnology field for successfully mimicking the composition and structure of different tissues, and the development of bio-inspired nanotechnologies and functional nanomaterials to improve tissue regeneration. Numerous nanomaterials fabricated by electrospinning, nanolithography and self-assembly have been successfully applied to regenerate bone, cartilage, muscle, blood vessel, heart and bladder tissue. We also discuss nanotechnology-based regenerative medicine products in the clinic for tissue engineering applications, although so far most of them are focused on bone implants and fillers. We believe that recent advances in nanotechnologies will enable new applications for tissue regeneration in the near future.

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Regenerative medicine aims to restore the function of human tissues and organs by stimulating the intrinsic regenerative capacity of the body by utilizing cells, biomaterials and growth factors [1,2]. Current advances in regenerative medicine have led to the creation of bioengineered tissues and organs that can perform key biological functions. For example, biomimetic tissues including bone, blood vessels, urethra, skin, liver, lung, bladder and trachea transplants have been successfully engineered and implanted *in vivo* [3–10]. Bioengineered tissue constructs can grow and remodel *in vivo* since they are composed of living cells, or can stimulate body cells to migrate and integrate into scaffolding materials.

Currently, by virtue of recent achievements in nanotechnology, the composition and structure of bioengineered tissues are becoming more analogous to natural tissues at the nanoscale, providing a biomimetic niche for cells. The activities of cells depend on biochemical and physical signals from surrounding tissues, and since cells dynamically interact with their local microenvironment at the nanoscale, it is necessary to control properties of engineered tissues at these scale lengths. In addition, nanostructured biomaterials can decrease inflammatory response and increase wound healing in comparison to conventional biomaterials, possibly due to their high surface energy affecting protein adsorption and cell adhesion [11]. In this sense, advanced nanotechnologies for mimicking native tissues can also overcome the disadvantages of using autografts or allografts, such as the risk of immune reaction, infection and disease transmission.

In this paper, we highlight key achievements in the nanotechnology field to recreate the composition, structure and functionality of major tissues and organs, using bio-

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mimetic and bio-inspired approaches to improve tissue regeneration. In addition, we report on clinically approved nanotechnology-based regenerative medicine products for tissue engineering applications. By providing an overall view of the recent status of nanotechnology applications in the regeneration of various tissues, we expect that this article will be particularly helpful for those who are investigating the regeneration of complex tissues.

Biomimicking tissue composition at nanoscale

Every tissue in the body has its own nanoscale composition which provides a suitable microenvironment to direct cellular differentiation toward a particular lineage. Since engineered nano-architecture features a high surface area to volume ratio, it can systematically expose cells to multiple biological components with different functionalities. The ability to control the spatial distribution of materials at the nanoscale can also enhance tissue regeneration by enabling better integration with host tissue [12]. For example, bone tissue is mainly composed of inorganic calcium phosphate nanocrystals and organic components (mainly collagen type I) [13–15]. It is reported that a nanocomposite scaffold that is composed of both organic and inorganic components of bone tissues can promote bone regeneration [16,17]. In addition, the inorganic phase of human bone tissue is composed of two major bone minerals: hydroxyapatite (HAP: $Ca_{10}[PO_4]_6[OH]_2$) and whitlockite (WH: $Ca_{18}Mg_2[HPO_4]_2[PO_4]_{12}$) nanocrystallites, with different physicochemical properties [14,15]. For example, Mg^{2+} ions are too small in size to maintain a HAP crystal structure, and so are mostly incorporated in the WH crystal structure [14,18]. Furthermore, it is reported that these two bone crystals are distributed in different ratios depending on certain regions of bone tissue [14], implying that HAP and WH have distinguished biological roles. Therefore, controlling their spatial distribution at the nanoscale is important for mimicking native bone tissue.

In Table 1, we have listed representative examples of recent research achievements to recreate the nanoscale composition of each tissue type. However, despite many outstanding achievements in both the nanotechnology and tissue engineering fields, so far, most bioengineered tissues are still dependent on the usage of bulk materials with micrometer scale designs or larger, which have limited tissue functions. Therefore, there remains a strong need to further develop nanomaterials that mimic the major components of tissues at the nanoscale and apply them for tissue regeneration.

Mimicking nanoscale tissue structure

Human tissues have complex topographical features at the nanoscale that can physically influence the behavior of cells by directly modulating their migration, orientation, differentiation and proliferation. For example, skeletal and cardiac muscles are composed of perpendicularly interwoven collagen strips and elastin bundles at the nanometer scale [28]. Also, bone tissue is composed of HAP nanocrystals that form nanopatterns along collagen fibers [29]. In addition, highly connected nanopores/channels in tissues can continuously supply a sufficient level of oxygen and nutrients to cells, and allow for intercommunication between different cell types. For example, there exist three levels of hierarchical pore architectures within cortical and cancellous bone, ranging from 10 to 20 μm in radii, which support blood or interstitial fluid transportation [30].

To mimic the nanoscale structure of each tissue type to stimulate cells with the proper topographical cues, nanofibrous and nanocomposite structures, nanoscale surface topographies and nanoporous/nanochannel networks in the scaffold have been engineered by nanotechnologies such as electrospinning, nanolithography, self-assembly, phase separation and sacrificial template methods (Table 2).

Since the cellular microenvironment includes ECM components such as fibril structured proteins and polysaccharides [43], engineered nanofiber networks can support cellular growth and regulate cellular behaviors in a physiologically similar manner [44]. Aligned nanofibers are especially useful in guiding cellular orientation to mimic the anisotropy of natural tissues, including heart, nerve, tendon and blood vessels. For example, when human tendon progenitor cells were seeded on aligned poly (L-lactic acid) nanofibers that recapitulated parallel collagen fibers in tendon, these cells expressed higher level of tendon specific genes compared with cells grown on random fibers [34].

Nanocomposite structures are used widely, as they can enhance the mechanical strength of hybrid organic/inorganic composites, and thus influence cellular proliferation and differentiation. To mimic the organization of bone tissue that is composed of inorganic minerals and organic collagen matrix, silicate nanoparticles were incorporated into organic materials, enhancing mechanical properties (i.e., compressive strength, tensile strength and elastic modulus) and further promoting cellular proliferation [37,38,45]. In fact, stiffness is one of the key parameters for altering cell growth and differentiation [46,47]. Recently, Alakpa *et al.* fabricated supramolecular nanofiber hydrogels and controlled their stiffness to direct the differentiation of stem cells without any biochemical functionalization [47].

Table 1. Examples of biomimicking composition of tissues at nanoscale.

Tissue	Nanotechnologies	Functionality	Tissue regeneration capacity	Ref.
Bone	Hydroxyapatite composite sponge with concentrated collagen nanofibers	Mimicking bone chemistry based on osteoconductive scaffolds composed of inorganic material and natural polymers	Induced continuous deposition of lamellar bone tissue while maintaining osteoblast activity	[17]
	Synthesis of the two major bone crystals: hydroxyapatite and whitlockite nanoparticles	Mimicking inorganic composition of bone, providing mechanical stability and stimulating osteogenic differentiation of stem cells	Enhanced proliferation and differentiation of bone cells and induced rapid regeneration of bone tissues	[19,20]
	Self-assembled peptide amphiphile nanofibrous matrices to induce biomimetic nucleation of hydroxyapatite crystals	Mimicking bone mineralization with collagen-like fibril structure and nucleation of hydroxyapatite crystals	Promoted new bone formation in a rat femoral defect model	[21]
Cartilage	Peptide amphiphilic nanofibers functionalized with chemical groups of GAG molecules	Mimicking composition, structure and function of the ECM	Enhanced aggregation of MSCs and deposition of cartilage-specific matrix elements	[22]
	Self-assembled supramolecular GAGs like glycopeptide nanofibers	Mimicking composition and functions of HA, the major component of cartilage	Induced chondrogenic differentiation of MSCs and enhanced formation of hyaline-like cartilage	[23]
Heart	Nanofibrous collagen scaffold made by electrospinning and crosslinking for cardiac tissue regeneration	Mimicking composition of myocardial connective stroma and delivery of cardiomyocytes	Improved vascularization of scaffold with upregulation of gene expression related to ECM remodeling, after implanted *in vivo*	[24]
	MSC seeded polycaprolactone nanofiber cardiac patch by fibronectin immobilization	Mimicking ECM of heart by using fibronectin, which is a major component of normal heart for cell adhesion and activity	Enhanced cellular adhesion increased angiogenesis, and improved cardiac function	[25]
Skin	Multilayer nanofilm composed of HA and poly-L-lysine on top of a HA scaffold by using layer-by-layer assembly for skin tissue engineering	Mimicking epidermal–dermal composition and structure of skin at nanometer scale	Promoted adhesion of keratinocytes, enhancing epidermal protective barrier function of skin	[26]
Muscle	Laminin mimetic peptide nanofibrous network	Mimicking composition and structure of skeletal muscle basal lamina	Enhanced cellular gene expression related to skeletal muscle specific marker	[27]

ECM: Extracellular matrix; GAG: Glycosaminoglycan; HA: Hyaluronic acid; MSC: Mesenchymal stem cell.

Nanopatterns play an important role in directing various cellular behaviors, due to their structural consistency with many vital components of native ECM, such as basement membrane and focal adhesion complexes, ranging from a few to a hundred nanometers [48,49]. Patterning techniques at the nanoscale allow for the mimicking of native ECM, thus modulating cell-matrix interactions [50]. Interestingly, nanoscale disorders can direct osteogenic differentiation of human MSCs in the absence of osteogenic supplements [40]. On the other hand, when the pattern contains absolute square lattice symmetry, nanoscale patterning can also promote the growth of stem cells and the retention of multipotency, indicating that nanoscale surface topographies can determine cell fate and functions [41]. Likewise, since cell orientation strongly correlates with the direction of underneath patterns, nanoscale structural cues can further control the macroscopic function of tissue constructs. For example, nanotopographically controlled heart tissue constructs that mimic the ECM structure of myocardium have successfully demonstrated anisotropic action potential conduction and contractility characteristics of native cardiac tissue [39].

Nanopores/channels in natural tissues are also vital for maintaining the activity of cells, as they provide transport paths for oxygen and nutrients [51,52]. While it seems that the two concepts of permeability

Table 2. Examples of mimicking nanoscale tissue structure for tissue regeneration.

Nanostructure	Tissue	Nanotechnology	Tissue regeneration capacity	Ref.
Nanofibrous structures	Heart	Electrospun aligned poly(lactide)- and poly(glycolide)-based scaffold	Demonstrated directionally dependent mature contractile machinery of cardiomyocytes and increased their synchronized beating	[31]
		Highly aligned nanofiber engineered by rotary jet spinning	Induced alignment of rat ventricular myocytes along with the nanofiber	[32,33]
	Tendon	Electrospun aligned PLLA nanofibers	Upregulated tendon-specific genes	[34]
	Cartilage	Nanofibrous hollow microspheres with ECM mimetic architecture as an injectable cell carrier	Induced successful cartilage regeneration in a critical-size osteochondral defect in a rabbit model	[35]
	Skin	3D Multilayered nanofibrous scaffold	Produced dermal-like tissues or bilayer skin tissues with both epidermal and dermal layers	[36]
Nanocomposite structures	Bone	Nanocomposite made from poly(ethylene oxide) and silicate nanoparticles	Induced direction-dependent mechanical properties with increased mechanical strength and extensibility, enhancing cellular activities and mineralization	[37,38]
Nanotopographies	Heart	Myocardium model with controlled nanoscale surface topographies mimicking function of mayocardial tissue and ECM architecture	Displayed anisotropic action potential conduction and contraction of native cardiac tissues	[39]
	Bone	Nanostructured surfaces with symmetry or disorder to modulate stem cell differentiation	Enabled to control MSCs to maintain multipotency or to produce bone minerals depending on nanopatterns	[40,41]
Nanoporous/ nanochannel structures	Bone	Self-assembled hierarchical nanochannel network in bone ceramic	Provided both sufficient mechanical strength and efficient nutrient supply for bone cell growth and differentiation	[42]
	Vessel	Nanopores in the vessel wall mimicking a vascular bed	Enhanced permeability and intercellular crosstalk	[4]

ECM: Extracellular matrix; MSC: Mesenchymal stem cell; PLLA: Poly(L-lactic acid).

and mechanical strength are contradictory, as they are directly or inversely correlated with the porosity of the structures, nanoporous/channel structures can simultaneously satisfy these properties due to their enhanced permeability compared with microporous/channel structures. In fact, the amount of nutrients that are delivered by nanochannels is known to be sufficient to sustain cellular vital activities. Nanopores/channels have been incorporated in vascularized cardiac or hepatic tissue constructs and bone scaffolds by using self-assembled and porogen methods to enhance permeability and permit cellular crosstalk, while maintaining mechanical properties [4,42].

Developing bioinspired nanotechnologies & functional nanomaterials

The function of human tissue occurs based on the localized microenvironment where cells interact with specific types of ECM at the nanoscale. In this respect, nanoscale delivery systems and functional nanomaterials have been applied for directing cellular differentiation and tissue specific activities to restore function of damaged tissues.

In the past two decades, nanoscale delivery systems have attracted a great deal of attention by researchers in the field of regenerative medicine based on their unique features, such as high surface area and easiness of surface functionalization, which can promote the adsorption of growth factors and drugs [53,54]. For example, nanofibers are one of the most widely used nanoscale delivery platforms based on their similarity with the physical structure of ECM [55,56]. Hartgerink *et al.* developed an injectable, self-assembled peptide-based nanofibrous hydrogel that contains peptides for pro-angiogenic moieties which can rapidly form mature vascular networks and induce tissue integration after subcutaneous delivery *in vivo* via a syringe needle [56].

Functional nanomaterials can actively support damaged tissues with functional loss, and thus can enhance their regeneration. For example, electroconductive

Table 3. Developing bioinspired nanotechnologies and functional nanomaterials for tissue regeneration.

Tissue	Nanotechnologies	Functionality	Tissue regeneration capacity	Ref.
Bone	Biomimetic ECM nanostructures constructed through layer-by-layer self-assembly of biodegradable nanoparticles and polysaccharides	Preservation of the activity of osteoinductive growth factors and induced their sustained release	Promoted the attachment, proliferation and differentiation of BMSCs and enhanced new bone formation by sustained release of biomolecules	[60]
	Intermediate precursors-loaded mesoporous silica nanoparticles as delivery devices for biomineralization	Sustained release of amorphous calcium phosphate precursors	Induced biomimetic intrafibrillar mineralization of collagen	[61]
Cartilage	ECM mimetic chondroitin sulfate/polyethylene glycol/GO hybrid nanocomposite scaffold for cartilage engineering	Improvement of overall mechanical properties and electrical conductivity of scaffold by GO	Enhanced regeneration of cartilage tissue with improved subchondral bone reconstruction	[62]
	Bioprinted nanoliter droplets encapsulating stem cells and growth factors to mimic native fibrocartilage microenvironment	Mimicking the complex anisotropic fibrocartilage tissue by 3D printing nanoliter droplets encapsulating MSCs along with biochemical gradient and ECM components	Upregulated osteogenic and chondrogenic related genes in the 3D fibrocartilage model	[63]
	Self-assembled supramolecular peptide amphiphile nanofibers containing binding epitopes to TGF-β-1 for cartilage regeneration	Prolonged release of TGF-β-1 from PA gels containing high density of TGFβ-1 binding sites	Promoted articular cartilage regeneration in a rabbit chondral defect model without any exogenous growth factor	[64]
Vessels	VEGF-loaded heparin-functionalized PLGA nanoparticle–fibrin gel complex	Localized and sustained delivery of growth factor	Improved the therapeutic angiogenic effect in an ischemic hind limb model by increasing blood pressure, angiographic score and the capillary density	[65]
	Biodegradable porous silicon nanoneedles for local intracellular delivery of nucleic acids to induce tissue neovascularization	Codelivery of DNA and siRNA into cell cytosol by nano-injection	Induced localized neovascularization and increased blood perfusion *in vivo*	[66]
	Peptide amphiphile nanostructures that display VEGF mimetic peptide on the surface of nanofibers	Mimicking the activity of VEGF by generating phosphorylation of VEGF receptors	Enhanced proangiogenic activities of endothelial cells and microcirculatory angiogenesis in the ischemic tissue	[67]
Heart	Pluripotent stem cell-derived cardiomyocyte spheroids that incorporate electrically conductive silicon nanowires	Formation of electrically conductive microenvironment in cardiac spheroids which can synergize with exogenous electrical stimulation	Enhanced cell–cell junction formation, increased contractile machinery expression, while regulating the endogenous spontaneous beating of pluripotent stem-cell-derived cardiac spheroids	[57]
	Hybrid hydrogel scaffold incorporating aligned carbon nanotubes	Tunable and anisotropic mechanical and electrical characteristics	Enhanced cardiac differentiation of embryoid bodies with increased beating activity	[58]
Bladder	PLGA nanoparticle thermosensitive gel scaffold for bladder tissue regeneration	Codelivery of growth factors by a PLGA nanoparticle carrier	Promoted bladder tissue regeneration with rapid vascularization while inhibiting graft contracture in a rabbit model	[9]
Nerves	PLGA nanoparticles including LIF as a cargo with surface modification to target OPCs for myelin repair	Sustained and controlled release of LIF by PLGA nanoparticles after selectively attached to OPCs	Induced remyelination with increased myelinated axon numbers and myelin thickness per axon	[68]

BMSC: Bone marrow stem cell; ECM: Extracellular matrix; GO: Graphene oxide; MSC: Mesenchymal stem cell; OPC: Oligodendrocyte precursor cell; PA: Peptide amphiphile; PLGA: Poly(D,L-lactic-co-glycolic acid).

Table 4. Selective list of FDA approved nanotechnology products for tissue regeneration.

Name/company	Approved applications	Product description	Function and clinical outcomes	US FDA approval year	Ref.
Vitoss® scaffold synthetic cancellous bone void filler/Stryker Corporation	Filler, osseous defects	Highly porous 3D β-tricalcium phosphate scaffold based on calcium phosphate nanoparticles	This filler has similar composition to natural bone minerals, enhancing bone regeneration, along with increased spinal fusion rates	2003	[70–72]
Ostim® bone grafting material/Heraeus Kulzer, Inc.	Filler, osseous defects	Nanocrystalline hydroxyapatite paste that is injected into a bone void or defect	This filler facilitates bone regeneration, based on its bone mimetic chemical composition and crystalline structures	2004	[70,73]
NanOss™ bone void filler/Angstrom Medica, Inc.	Filler, osseous defects	Osteoconductive, resorbable bone graft that uses calcium phosphate nanocrystals	This dense, nanocrystalline material mimics the microstructure and composition of bone and has strong mechanical properties and osteoconductive effects	2005	[74,75]
BoneGen-TR/BioLok International, Inc.	Filler, oral surgery, periodontics, endodontics, implantology	Calcium sulfate-based nanocomposite	The filler can control timed release of calcium sulfate that supports bone augmentation	2006	[76]
EquivaBone osteoinductive bone graft substitute/ETEX Corporation	Filler, osseous defects	Resorbable, osteoinductive bone graft substitute that is composed of demineralized bone matrix and nanocrystalline hydroxyapatite	This scaffold has osteoconductive effect by providing hydroxyapatite nanocrystalline and osteoinductive growth factors	2009	[77,78]
Beta-BSM injectable bone substitute material/ETEX Corporation	Filler, osseous defects	Synthetic calcium phosphate bone graft material in a nanocrystalline matrix	This filler has osteoconductive properties based on bone mimetic chemical structure	2010	[78]
NanoGen/Orthogen, LLC	Filler, osseous defects	Medical grade calcium sulfate hemihydrate based nanocomposite	This filler is controlled to be degraded over a period of 12 weeks, stimulating bone regeneration	2011	[79]
FortiCore™/Nanovis, Inc.	Implant, spinal fusion procedures	Implant composed of a highly porous titanium scaffold that is integrated with a PEEK-OPTIMA (high-performance, implant-grade polymer) core	This implant has nanotube-enhanced surface which can promote bone regeneration around the implant	2014	[80]
NB3D bone void filler/ Pioneer Surgical Technology, Inc.	Filler, osseous defects	3D construct that is composed of porous hydroxyapatite nanogranules suspended in a porous gelatin-based foam matrix	This filler has interconnected porosity similar to human cancellous bone and also has equivalent crystal size and structure as natural bone, promoting tissue interaction and regeneration	2014	[81]

nanomaterials have been applied for the treatment of cardiac tissues to generate electrical function of these tissues. The incorporation of electrically conductive silicon nanowires in cardiac spheroids can provide an endogenous electrical microenvironment for cardiomyocytes, and synergize with exogenous electrical

stimulation, enhancing cardiac microtissue development [57]. In addition, when carbon nanotubes are integrated into hydrogels and oriented in an aligned manner, the cardiac differentiation of embryoid bodies and their beating activities are enhanced. The incorporation of carbon nanotubes in a hydrogel scaffold has been reported to further enhance the mechanical properties of tissue constructs [58]. The functionalization of biomaterials by the internalization of biological motifs can also control cellular behavior; for instance, Gouveia *et al.* incorporated peptide amphiphile composed of the N-(fluorenyl-9-methoxycarbonyl) (Fmoc) molecule linked to the cell-adhesion Arg–Gly–Asp–Ser (RGDS) motif into biomimetic collagen gels. These functionalized hydrogels promoted attachment and proliferation of human corneal stromal fibroblasts [59].

In Table 3, we have listed representative examples of the current use of nanotechnologies and nanomaterials to enhance tissue regeneration.

FDA approved regenerative medicine products for tissue regeneration based on nanotechnologies

In the 2014 Guidance for Industry entitled "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology," the US FDA defined nanotechnology products as those which have at least one dimension between 1 and 100 nm in size [69]. The FDA also recognized materials that are as large as 1000 nm as nanomaterials if they can demonstrate similar 'properties or phenomena' as other nanotechnology-based products [69]. During the process of commercialization, a nanotechnology product moves through various developmental phases, starting with the basic concept product and culminating with clinical investigations and commercialization. The resulting nanotechnology products can belong to various FDA classifications, such as biologicals, devices, genetics, drugs and others [70].

Based on recent achievements in nanotechnologies for recreating the composition, structure and functions of tissues in a more precise way than ever before, the related nanotechnologies are starting to be applied in clinics to repair diseased/damaged tissues [2,70]. In Table 4, we have selectively listed nanotechnology based products for tissue regeneration that have obtained approval from FDA and are currently on the market.

Conclusion & future perspective

In this special issue, we selectively highlighted state-of-the-art nanotechnologies that successfully mimic the composition and structure of different tissue types, as well as bio-inspired nanotechnologies and functional nanomaterials for tissue regeneration. Based on recent advances in nanotechnologies and tissue engineering, bioengineered tissues are becoming more similar to natural tissues, thus enabling the partial recovery of damaged/diseased tissues. However, there are still many biological components that are not fully understood or ignored in regenerative medicine due to the

Executive summary

- This paper highlights the key achievements in the nanotechnology field for regenerative medicine to recreate functional biomimetic tissues and organs.

Biomimicking tissue composition at nanoscale

- Every tissue in the body has its own nanoscale composition.
- Controlling nanoscale composition is important as each tissue type has a unique spatial distribution of materials at the nanoscale which then provides different types of niches for cells.

Mimicking nanoscale tissue structure

- Human tissues have complex topographical features at the nanoscale.
- Nanofibrous and nanocomposite structures, nanotopographies and nanoporous/nanochannel structures have been designed and built by utilizing nanotechnologies such as electrospinning, nanolithography, self-assembly, phase separation and sacrificial template method.

Developing bioinspired nanotechnologies & functional nanomaterials

- Nanoscale delivery systems have provided the sustained and controlled release of growth factors for tissue regeneration.
- Functional nanomaterials have successfully generated similar or even better tissue functions to stimulate cells to repair tissues.

US FDA approved clinical products for regenerative medicine based on nanotechnologies

- Recently, FDA approved nanotechnology based regenerative medicine products have started to be actively used in the clinic for tissue regeneration.
- Most of the current nanotechnology based regenerative medicine products are made for bone tissue regeneration.
- We anticipate that the recent achievements in the nanotechnology field will further lead to the development of regenerative medicine products for various tissue types in the near future.

difficulty in their fabrication. Moreover, although many nanomaterials can successfully promote cellular activities *in vitro*, there still exist safety concerns about the use of these nanomaterials, as they can cause systemic side effects by crossing cell barriers in non-targeted organs. In fact, most of the newly developed nanomaterials have not been assessed in large animal models. As a result, except for bone related materials, the majority of the newly developed nanomaterials have not been applied for tissue regeneration in the clinic. These issues can be addressed by thorough physicochemical characterization of nanomaterials and restriction of undesired uptake via functionalization with targeting moieties [82,83]. Based on the understanding of the effectiveness and safety of nanomaterials, proper *in vivo* studies should be continued with selective nanomaterials for the purpose of clinical translation. We envision that the development of nanotechnologies, which is becoming faster than ever before, will overcome current challenges in regenerative medicine to heal diseased/damaged tissues in the near future.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Mason C, Dunnill P. A brief definition of regenerative medicine. *Regen. Med.* 3(1), 1–5 (2008).

2 Engel E, Michiardi A, Navarro M, Lacroix D, Planell JA. Nanotechnology in regenerative medicine: the materials side. *Trends Biotechnol.* 26(1), 39–47 (2008).

3 Sun D, Chen Y, Tran RT *et al.* Citric acid-based hydroxyapatite composite scaffolds enhance calvarial regeneration. *Sci. Rep.* 4 6912 (2014).

4 Zhang B, Montgomery M, Chamberlain MD *et al.* Biodegradable scaffold with built-in vasculature for organ-on-a-chip engineering and direct surgical anastomosis. *Nat. Mater.* 15(6), 669–678 (2016).

• **Nanopores' structure is incorporated in vascularized cardiac or hepatic tissue and bone scaffolds.**

5 Jia W, Tang H, Wu J *et al.* Urethral tissue regeneration using collagen scaffold modified with collagen binding VEGF in a beagle model. *Biomaterials* 69, 45–55 (2015).

6 Yu B, Kang S-Y, Akthakul A *et al.* An elastic second skin. *Nat. Mater.* 15(8), 911–918 (2016).

7 Mazza G, Rombouts K, Hall AR *et al.* Decellularized human liver as a natural 3D-scaffold for liver bioengineering and transplantation. *Sci. Rep.* 5, 1–15 (2015).

8 Ren X, Moser PT, Gilpin SE *et al.* Engineering pulmonary vasculature in decellularized rat and human lungs. *Nat. Biotechnol.* 33(10), 1097–1102 (2015).

9 Jiang X, Lin H, Jiang D *et al.* Co-delivery of VEGF and bFGF via a PLGA nanoparticle-modified BAM for effective contracture inhibition of regenerated bladder tissue in rabbits. *Sci. Rep.* 6, 1–12 (2016).

10 Jungebluth P, Haag JC, Sjöqvist S *et al.* Tracheal tissue engineering in rats. *Nat. Protoc.* 9(9), 2164–2179 (2014).

11 Ainslie KM, Thakar RG, Bernards DA, Desai TA. Inflammatory response to implanted nanostructured materials. In: *Biological Interactions on Materials Surfaces*. Puleo DA, Bizios R (Eds). Springer, New York, USA, 355–371 (2009).

• **This book chapter discusses advantages of nanostructured biomaterials in inflammatory response.**

12 Perez RA, Won J-E, Knowles JC, Kim H-W. Naturally and synthetic smart composite biomaterials for tissue regeneration. *Adv. Drug Del. Rev.* 65(4), 471–496 (2013).

13 Wang Y, Azaïs T, Robin M *et al.* The predominant role of collagen in the nucleation, growth, structure and orientation of bone apatite. *Nat. Mater.* 11(8), 724–733 (2012).

14 Driessens FC, Verbeeck R. *Biominerals.* CRC Press, USA (1990).

15 Elliott JC. Structure and chemistry of the apatites and other calcium orthophosphates. In: *Studies In Organic Chemistry.* Elsevier, Amsterdam, The Netherlands (1994).

16 Kikuchi M. Hydroxyapatite/collagen bone-like nanocomposite. *Biol. Pharm. Bull.* 36(11), 1666–1669 (2013).

17 Scaglione S, Giannoni P, Bianchini P *et al.* Order versus disorder: *in vivo* bone formation within osteoconductive scaffolds. *Sci. Rep.* 2, 1–6 (2012).

18 Terpstra R, Driessens F. Magnesium in tooth enamel and synthetic apatites. *Calcif. Tissue Int.* 39(5), 348–354 (1986).

19 Jang HL, Jin K, Lee J *et al.* Revisiting whitlockite, the second most abundant biomineral in bone: nanocrystal synthesis in physiologically relevant conditions and biocompatibility evaluation. *ACS Nano* 8(1), 634–641 (2013).

•• **Reports a facile synthetic methodology for the second major bone mineral in the human body.**

20 Jang HL, Zheng GB, Park J *et al. In vitro* and *in vivo* evaluation of whitlockite biocompatibility: comparative study with hydroxyapatite and β-tricalcium phosphate. *Adv. Healthc. Mater.* 5(1), 128–136 (2015).

21 Mata A, Geng Y, Henrikson KJ *et al.* Bone regeneration mediated by biomimetic mineralization of a nanofiber matrix. *Biomaterials* 31(23), 6004–6012 (2010).

22 Yaylaci SU, Sen M, Bulut O, Arslan E, Guler MO, Tekinay AB. Chondrogenic differentiation of mesenchymal stem cells on glycosaminoglycan-mimetic peptide nanofibers. *ACS Biomater. Sci. Eng.* 2(5), 871–878 (2016).

23 Ustun Yaylaci S, Sardan Ekiz M, Arslan E *et al.* Supramolecular GAG-like Self-assembled glycopeptide nanofibers induce chondrogenesis and cartilage regeneration. *Biomacromolecules* 17(2), 679–689 (2016).

24 Joanne P, Kitsara M, Boitard S-E *et al.* Nanofibrous clinical-grade collagen scaffolds seeded with human cardiomyocytes induces cardiac remodeling in dilated cardiomyopathy. *Biomaterials* 80 157–168 (2016).

25 Kang B-J, Kim H, Lee SK *et al.* Umbilical-cord-blood-derived mesenchymal stem cells seeded onto fibronectin-immobilized polycaprolactone nanofiber improve cardiac function. *Acta Biomater.* 10(7), 3007–3017 (2014).

26 Monteiro IP, Shukla A, Marques AP, Reis RL, Hammond PT. Spray-assisted layer-by-layer assembly on hyaluronic acid scaffolds for skin tissue engineering. *J. Biomed. Mater. Res. A* 103(1), 330–340 (2015).

27 Yasa IC, Gunduz N, Kilinc M, Guler MO, Tekinay AB. Basal lamina mimetic nanofibrous peptide networks for skeletal myogenesis. *Sci. Rep.* 5, 16460 (2015).

28 Parker KK, Ingber DE. Extracellular matrix, mechanotransduction and structural hierarchies in heart tissue engineering. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 362(1484), 1267–1279 (2007).

29 Dvir T, Timko BP, Kohane DS, Langer R. Nanotechnological strategies for engineering complex tissues. *Nat. Nanotechnol.* 6(1), 13–22 (2011).

30 Cowin SC, Cardoso L. Blood and interstitial flow in the hierarchical pore space architecture of bone tissue. *J. Biomech.* 48(5), 842–854 (2015).

31 Zong X, Bien H, Chung C-Y *et al.* Electrospun fine-textured scaffolds for heart tissue constructs. *Biomaterials* 26(26), 5330–5338 (2005).

32 Badrossamay MR, Balachandran K, Capulli AK *et al.* Engineering hybrid polymer-protein super-aligned nanofibers via rotary jet spinning. *Biomaterials* 35(10), 3188–3197 (2014).

33 Badrossamay MR, Mcilwee HA, Goss JA, Parker KK. Nanofiber assembly by rotary jet-spinning. *Nano Lett.* 10(6), 2257–2261 (2010).

34 Yin Z, Chen X, Chen JL *et al.* The regulation of tendon stem cell differentiation by the alignment of nanofibers. *Biomaterials* 31(8), 2163–2175 (2010).

35 Liu X, Jin X, Ma PX. Nanofibrous hollow microspheres self-assembled from star-shaped polymers as injectable cell carriers for knee repair. *Nat. Mater.* 10(5), 398–406 (2011).

36 Yang X, Shah JD, Wang H. Nanofiber enabled layer-by-layer approach toward three-dimensional tissue formation. *Tissue Eng. Pt. A* 15(4), 945–956 (2008).

37 Gaharwar AK, Schexnailder PJ, Kline BP, Schmidt G. Assessment of using Laponite® cross-linked poly (ethylene oxide) for controlled cell adhesion and mineralization. *Acta Biomater.* 7(2), 568–577 (2011).

38 Gaharwar AK, Schexnailder P, Kaul V *et al.* Highly extensible bio-nanocomposite films with direction-dependent properties. *Adv. Funct. Mater.* 20(3), 429–436 (2010).

39 Kim D-H, Lipke EA, Kim P *et al.* Nanoscale cues regulate the structure and function of macroscopic cardiac tissue constructs. *Proc. Natl Acad. Sci. USA* 107(2), 565–570 (2010).

40 Dalby MJ, Gadegaard N, Tare R *et al.* The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat. Mater.* 6(12), 997–1003 (2007).

41 Mcmurray RJ, Gadegaard N, Tsimbouri PM *et al.* Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. *Nat. Mater.* 10(8), 637–644 (2011).

42 Jang HL, Lee K, Kang CS *et al.* Biofunctionalized ceramic with self-assembled networks of nanochannels. *ACS Nano* 9(4), 4447–4457 (2015).

43 Pellowe AS, Gonzalez AL. Extracellular matrix biomimicry for the creation of investigational and therapeutic devices. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* 8(1), 5–22 (2016).

44 Liu W, Thomopoulos S, Xia Y. Electrospun nanofibers for regenerative medicine. *Adv. Healthc. Mater.* 1(1), 10–25 (2012).

45 Asran AS, Henning S, Michler GH. Polyvinyl alcohol–collagen–hydroxyapatite biocomposite nanofibrous scaffold: mimicking the key features of natural bone at the nanoscale level. *Polymer* 51(4), 868–876 (2010).

46 Caiazzo M, Okawa Y, Ranga A, Piersigilli A, Tabata Y, Lutolf MP. Defined three-dimensional microenvironments boost induction of pluripotency. *Nat. Mater.* 15(3), 344–352 (2016).

47 Alakpa EV, Jayawarna V, Lampel A *et al.* Tunable supramolecular hydrogels for selection of lineage-guiding metabolites in stem cell cultures. *Chem* 1(2), 298–319 (2016).

48 Stevens MM, George JH. Exploring and engineering the cell surface interface. *Science* 310(5751), 1135–1138 (2005).

49 Geiger B, Bershadsky A, Pankov R, Yamada KM. Transmembrane crosstalk between the extracellular matrix and the cytoskeleton. *Nat. Rev. Mol. Cell Biol.* 2(11), 793–805 (2001).

50 Rahmany MB, Van Dyke M. Biomimetic approaches to modulate cellular adhesion in biomaterials: a review. *Acta Biomater.* 9(3), 5431–5437 (2013).

51 West GB, Brown JH. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. *J. Exp. Biol.* 208(9), 1575–1592 (2005).

52 Banavar JR, Maritan A, Rinaldo A. Size and form in efficient transportation networks. *Nature* 399(6732), 130–132 (1999).

53 Perán M, García MA, López-Ruiz E *et al.* Functionalized nanostructures with application in regenerative medicine. *Int. J. Mol. Sci.* 13(3), 3847–3886 (2012).

54 Zhang L, Webster TJ. Nanotechnology and nanomaterials: promises for improved tissue regeneration. *Nano Today* 4(1), 66–80 (2009).

55 James R, Laurencin CT. Nanofiber technology: its transformative role in nanomedicine. *Nanomedicine (Lond.)* 11(12), 1499–1501 (2016).

56 Kumar VA, Taylor NL, Shi S *et al.* Highly angiogenic peptide nanofibers. *ACS Nano* 9(1), 860–868 (2015).

• **This minimally invasive injectable hydrogel significantly enhances the formation of robust mature vascular networks in a rat model.**

57 Richards DJ, Tan Y, Coyle R *et al.* Nanowires and electrical stimulation synergistically improve functions of hiPSC cardiac spheroids. *Nano Lett.* 16(7), 4670–4678 (2016).

58 Ahadian S, Yamada S, Ramón-Azcón J *et al.* Hybrid hydrogel-aligned carbon nanotube scaffolds to enhance cardiac differentiation of embryoid bodies. *Acta Biomater.* 31, 134–143 (2016).

59 Gouveia RM, Jones RR, Hamley IW, Connon CJ. The bioactivity of composite Fmoc-RGDS-collagen gels. *Biomater. Sci.* 2(9), 1222–1229 (2014).

60 Wang Z, Dong L, Han L *et al.* Self-assembled Biodegradable Nanoparticles and Polysaccharides as Biomimetic ECM Nanostructures for the Synergistic effect of RGD and BMP-2 on Bone Formation. *Sci. Rep.* 6, 25090 (2016).

61 Zhang W, Luo X-J, Niu L-N *et al.* Biomimetic intrafibrillar mineralization of type I collagen with intermediate precursors-loaded mesoporous carriers. *Sci. Rep.* 5, 11199 (2015).

62 Liao J, Qu Y, Chu B, Zhang X, Qian Z. Biodegradable CSMA/PECA/graphene porous hybrid scaffold for cartilage tissue engineering. *Sci. Rep.* 5, 9879 (2015).

• **This delivery strategy significantly enhances continuous subchondral bone formation and thicker newly formed cartilage in a rabbit model.**

63 Gurkan UA, El Assal R, Yildiz SE *et al.* Engineering anisotropic biomimetic fibrocartilage microenvironment by bioprinting mesenchymal stem cells in nanoliter gel droplets. *Mol. Pharm.* 11(7), 2151–2159 (2014).

64 Shah RN, Shah NA, Lim MMDR, Hsieh C, Nuber G, Stupp SI. Supramolecular design of self-assembling nanofibers for cartilage regeneration. *Proc. Natl Acad. Sci. USA* 107(8), 3293–3298 (2010).

65 Chung Y-I, Kim S-K, Lee Y-K *et al.* Efficient revascularization by VEGF administration via heparin-functionalized nanoparticle–fibrin complex. *J. Control. Release* 143(3), 282–289 (2010).

66 Chiappini C, De Rosa E, Martinez JO *et al.* Biodegradable silicon nanoneedles delivering nucleic acids intracellularly induce localized *in vivo* neovascularization. *Nat. Mater.* 14(5), 532–539 (2015).

•• **Codelivery of DNA and siRNA in silicon nanoneedles with high loading efficiency results in neovascularization and improved blood perfusion in a mouse model.**

67 Webber MJ, Tongers J, Newcomb CJ *et al.* Supramolecular nanostructures that mimic VEGF as a strategy for ischemic tissue repair. *Proc. Natl Acad. Sci. USA* 108(33), 13438–13443 (2011).

68 Rittchen S, Boyd A, Burns A *et al.* Myelin repair *in vivo* is increased by targeting oligodendrocyte precursor cells with nanoparticles encapsulating leukaemia inhibitory factor (LIF). *Biomaterials* 56, 78–85 (2015).

69 Savers S. FDA Guidance on Nanotechnology DOCUMENT: guidance for industry considering whether an FDA-regulated product involves the application of nanotechnology. *Biotechnol. Law Rep.* 30(5), 571–572 (2011).

•• **Describes the US FDA's position on the applications of nanotechnology, and suggests attention to safety, effectiveness, public health impact and regulatory status of nanotechnology-based products.**

70 Etheridge ML, Campbell SA, Erdman AG, Haynes CL, Wolf SM, Mccullough J. The big picture on nanomedicine: the state of investigational and approved nanomedicine products. *Nanomedicine* 9(1), 1–14 (2013).

71 Sinha R, Menon P, Chakranarayan S. Vitoss synthetic cancellous bone. *Med. J. Armed Forces India* 65(2), 173 (2009).

72 Witten CM. Summary Vitoss® Scaffold Synthetic Cancellous Bone Void Filler. (FDA Document, FDA) (2003). www.accessdata.fda.gov/cdrh_docs/pdf3/k032409.pdf

73 Lin C. Summary Ostim® Bone Grafting Material. (FDA Document, FDA, Rockville) (2004). www.accessdata.fda.gov/cdrh_docs/pdf3/K030052.pdf

74 Witten CM. Summary NanOssTM Bone Void Filler. (FDA Document, FDA) (2005). www.accessdata.fda.gov/cdrh_docs/pdf5/K050025.pdf

75 Henschke A. *Nanoscale: Issues and Perspectives for the Nano Century*. Cameron N, Mitchell ME (Eds). John Wiley & Sons, NJ, USA (2009).

76 Lin CS. Summary BoneGen-TR (FDA Document, FDA). (2006). www.accessdata.fda.gov/cdrh_docs/pdf6/K060285.pdf

77 Melkerso MN. Summary EquivaBone Osteoinductive Bone Graft Substitute. (FDA Document, FDA) (2009). www.accessdata.fda.gov/cdrh_docs/pdf9/K090855.pdf

78 Watson AD. Summary Beta-bsm Injectable Bone Substitute Material. (FDA Document, FDA) (2010). www.accessdata.fda.gov/cdrh_docs/pdf10/K102812.pdf

79 Watson AD. Summary NanoGen. (FDA Document, FDA). (2011). www.accessdata.fda.gov/cdrh_docs/pdf10/K102208.pdf

80 Melkerson Mark N. Summary Intervertebral body fusion device. (FDA Document, FDA). *Silver Spring* (2014). www.nanovisinc.com

81 Witten CM. Summary NB3D (nanOss Bioactive 3D) Bone Void Filler. (FDA Document, FDA) (2014). www.accessdata.fda.gov/cdrh_docs/pdf13/K132050.pdf

82 Verma S, Domb AJ, Kumar N. Nanomaterials for regenerative medicine. *Nanomedicine* 6(1), 157–181 (2011).

83 Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett.* 10(9), 3223–3230 (2010).