

Nanotechnological strategies for engineering complex tissues

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Tissue engineering aims at developing functional substitutes for damaged tissues and organs. Before transplantation, cells are generally seeded on biomaterial scaffolds that recapitulate the extracellular matrix and provide cells with information that is important for tissue development. Here we review the nanocomposite nature of the extracellular matrix, describe the design considerations for different tissues and discuss the impact of nanostructures on the properties of scaffolds and their uses in monitoring the behaviour of engineered tissues. We also examine the different nanodevices used to trigger certain processes for tissue development, and offer our view on the principal challenges and prospects of applying nanotechnology in tissue engineering.

Organ transplantation is limited by the number of available donors and high process cost, leaving thousands of people each year on the transplant waiting lists in the United States alone. Many die before an organ donor becomes available. Tissue engineering has evolved as an interdisciplinary technology combining principles from the life, material and engineering sciences with the goal of developing functional substitutes for these damaged tissues and organs¹. Rather than simply introducing cells into a diseased area to repopulate a defect and/or restore function, in tissue engineering the cells are often seeded in or onto biomaterials before transplantation. These materials serve as temporary scaffolds and promote the reorganization of the cells to form a functional tissue¹ (Fig. 1).

Until recently, it was believed that the macroporous features of scaffolds used in tissue engineering mimicked the dimension scale of the extracellular matrix (ECM), and that the matrix itself (natural or artificial) only served as a support for the cells; morphogenesis was controlled passively by defining tissue boundaries. Emphasis was placed on critical engineering and material issues, such as improving mass transfer into the core of the cell constructs and designing biocompatible and biodegradable scaffolds with mechanical properties suitable for engineering various tissues².

As the field evolved, attention focused on the biology of the scaffolds (reviewed by Place and colleagues³, and by Lutolf and Hubbell⁴) and how they affect various cell types. Tissue engineers had recognized that some of the widely used scaffolds do not fairly recapitulate the cell microenvironment and that the ECM is a dynamic and hierarchically organized nanocomposite that regulates essential cellular functions such as morphogenesis, differentiation, proliferation, adhesion and migration⁵. As a consequence, researchers developed and used existing nanotechnological tools for tissue engineering to design advanced nanocomposite scaffolds that can better mimic the ECM and eventually assemble more complex and larger functional tissues.

To explain the synthesis of nanoscience with tissue engineering, we will begin by describing the nanocomposite nature of the ECM and discuss how recreating its nanostructure could enhance functional tissue organization, noting the differences in properties and design criteria between various engineered tissues. We will review

the impact of nanostructures on matrix properties and their use in monitoring the behaviour of engineered tissues. Finally, we will discuss the principal challenges and prospects in the application of nanotechnology to the field.

Recreating the extracellular microenvironment

Engineering functional tissue requires effective organization of cells into tissue with morphological and physiological features resembling those *in vivo*. This task is difficult because the signalling factors that drive tissue assembly have not yet been fully identified. Morphogenesis in the three-dimensional (3D) scaffold should occur in a similar way to natural organ development. The cells reorganize owing to interaction with the ECM on the basis of topography⁶, mechanical properties (such as matrix stiffness, elasticity and viscosity^{7–9}), or concentration gradients of immobilized growth factors¹⁰ or ECM molecules¹¹. Recently, Ott and co-workers¹² reported a study emphasizing the importance of the ECM structure in guiding the seeded cells and promoting morphogenesis. Rat hearts were decellularized by perfusion of detergents to preserve the underlying ECM and then reseeded with cardiac and endothelial cells¹². The cells migrated and self-organized in their natural location in the matrix and by day 8, under physiological load and electrical stimulation, the constructs were able to generate pump function¹². Similar studies have shown successful engineering of liver¹³, bone¹⁴, arteries¹⁵ and lung¹⁶. These reports stress the significance of the underlying ECM in promoting a unique microenvironment that fosters tissue organization.

The ECM, which provides cells with a wealth of information, is composed of an intricate interweaving of protein fibres such as fibrillar collagens and elastins, ranging from 10 to several hundreds of nanometres. The mesh is covered with nanoscale adhesive proteins such as laminin and fibronectin that provide specific binding sites for cell adhesion (interacting with integrins, cadherins and so forth) and have been shown to regulate important cell behaviours such as growth, shape, migration and differentiation¹⁷. Polysaccharides such as hyaluronic acid and heparan sulphate fill the interstitial space between the fibres and act as a compression buffer against the stress placed on the ECM or serve as a growth factor depot¹⁸ (Fig. 2).

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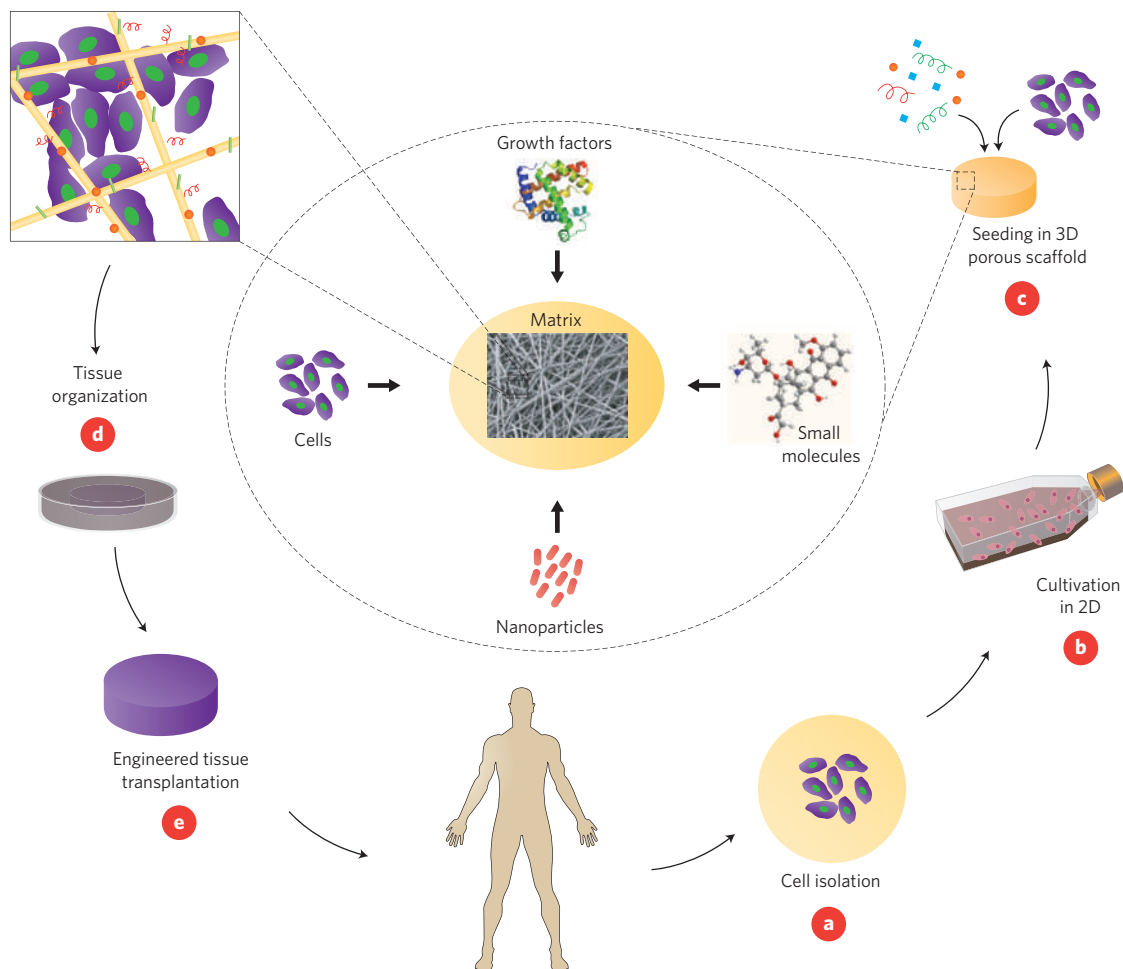


Figure 1 | An example of a tissue engineering concept that involves seeding cells within porous biomaterial scaffolds. **a**, Cells are isolated from the patient and may be cultivated (**b**) *in vitro* on two-dimensional surfaces for efficient expansion. **c**, Next, the cells are seeded in porous scaffolds together with growth factors, small molecules, and micro- and/or nanoparticles. The scaffolds serve as a mechanical support and a shape-determining material, and their porous nature provides high mass transfer and waste removal. **d**, The cell constructs are further cultivated in bioreactors to provide optimal conditions for organization into a functioning tissue. **e**, Once a functioning tissue has been successfully engineered, the construct is transplanted on the defect to restore function.

The significance of the overall fibrillar and porous nanoscale topography of the ECM in promoting essential cellular processes has led tissue engineers to replace macroporous scaffolds with biomimetic materials with nanoscale features. For example, to engineer the ECM fibrous mesh, researchers fabricated scaffolds composed of fibres with appropriate diameters that formed a highly interconnected, porous architecture allowing high mass transfer to and waste removal from the developing tissue¹⁹. In the past decade, there has been tremendous interest in exploring the potency of biomimetic fibres in tissue engineering, and several techniques originally developed for different purposes have been used for this application.

Electrospinning^{20,21} and molecular self assembly²² are common nanofabrication techniques to create 3D scaffolds composed of interwoven fibres. Electrospinning is a simple method that uses an electric field to control the deposition of polymer fibres on a target substrate. The fibres are continuous, with high porosity and high spatial interconnectivity. Moreover, the direction of the fibres can be selectively controlled by spinning speed and polymer concentration to fit the needs of various engineered tissues. The fibre bioactivity can also be designed to include delivery systems that control the release of cues for tissue assembly such as growth factors, peptides, enzymes, drugs, DNA and RNA^{23–27}. A recent example is the method developed for fabricating biocompatible polymeric electrospun

fibres that contained nanoparticles able to control the release of two proteins in parallel (Fig. 3a)²⁸. These scaffolds, which enable sequential release of multiple growth factors or cytokines, may have important applications in tissue engineering, such as promoting vasculogenesis or influencing the fate of stem cells^{29,30}.

Although considered as a 3D substrate for cell cultivation, electrospun fibres usually promote the assembly of thin tissues on their surface and do not allow proper infiltration of the cells to the core of the matrix. To address this challenge, researchers have combined micro- and nanotechnologies. Hybrid scaffolds that incorporated both electrospun fibres and 3D microprinting were fabricated to present 3D macroporous structures with interconnected pores for cell culture that also mimicked the ECM³¹. This new scaffold was able to improve cell entrapment and proliferation significantly. In addition, it increased the amount of ECM proteins produced, promoted better cell differentiation and, overall, enhanced tissue regeneration³¹.

Despite the simplicity of electrospinning in creating fibrous scaffolds, one substantial disadvantage is that the diameters of the fibres are usually at the upper limits of the 50–500-nm range seen in natural ECM. To emulate natural ECM better, both structurally and functionally, and to promote cell–matrix interaction at the molecular level, 3D scaffolds were created by molecular self-assembly of

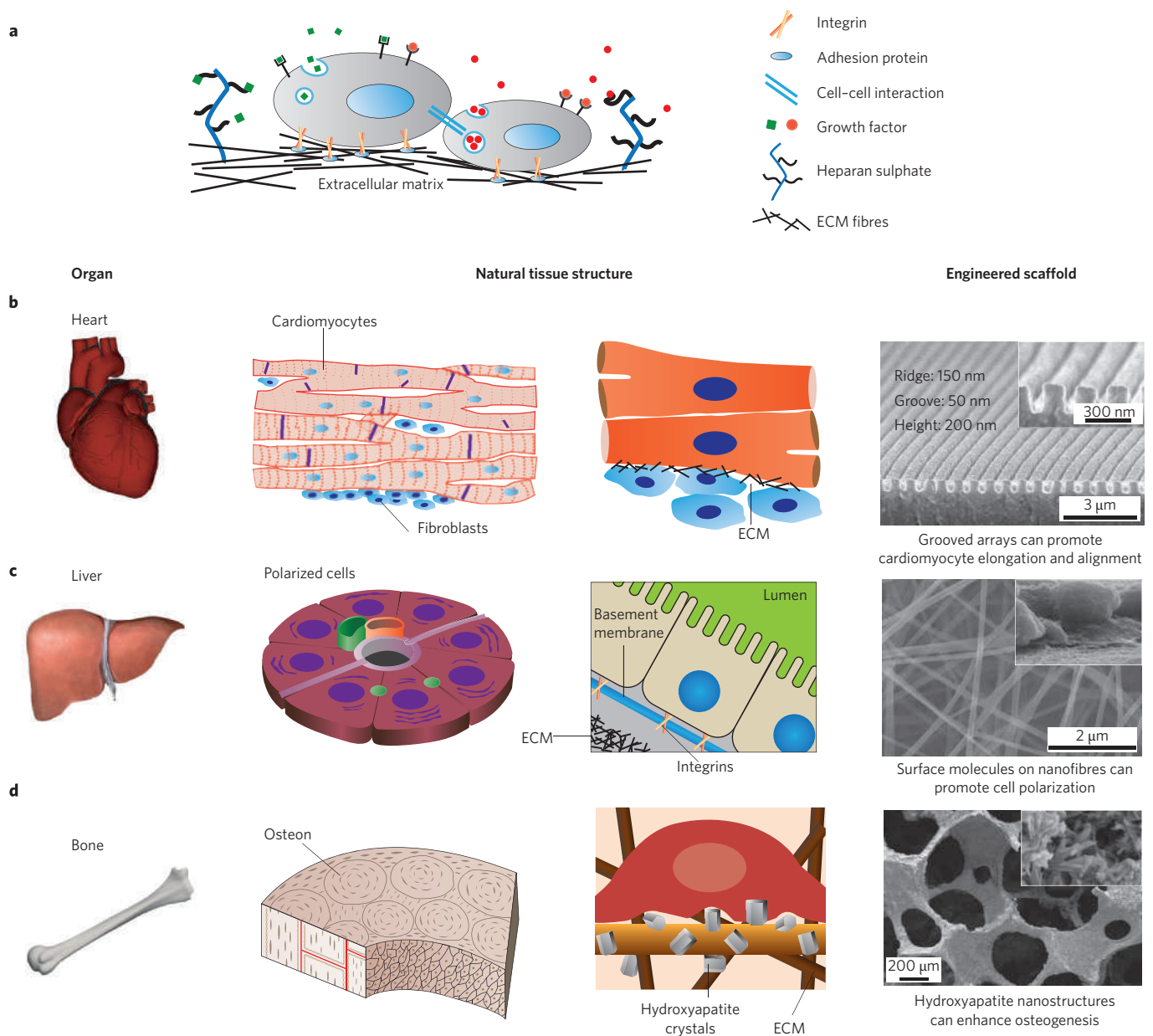


Figure 2 | The information provided to cells by the extracellular matrix (ECM). **a**, ECM fibres provide cells with topographical features that trigger morphogenesis. Adhesion proteins such as fibronectin and laminin located on the fibres interact with the cells through their transmembrane integrin receptors to initiate intracellular signalling cascades, which affect most aspects of cell behaviour. Polysaccharides such as hyaluronic acid and heparan sulphate act as a compression buffer against the stress, or serve as a growth factor depot. **b–d**, Illustrations of the heart, liver and bone at the level of organ (left) and tissue and cell/matrix interaction (centre), followed by scanning electron micrographs of engineered scaffolds (right). The ECMs of various tissues have different composition and spatial organization of molecules to maintain specific tissue morphologies. For example (**b**), the ECM of muscle tissues, such as the heart, forces the heart cells (cardiomyocytes) to couple mechanically to each other and to form elongated and aligned cell bundles that create an anisotropic syncytium. Nanogrooved surfaces (SEM image) are suitable matrices for cardiac tissue engineering because they force cardiomyocytes to align. **c**, Cells composing epithelial tissues are polarized and contact three types of surfaces for efficient mass transfer: the ECM, other cells and a lumen. Nanofibres modified with surface molecules can promote cell adhesion and tissue polarity (SEM images). **d**, Bone is a nanocomposite material consisting primarily of a collagen-rich organic matrix and inorganic hydroxyapatite nanocrystallites, which serve as a chelating agent for mineralization of osteoblasts. The scaffold structure (SEM image), stiffness and hydroxyapatite nanopatterning on the surface (inset) can enhance osteoblast spreading and bone tissue formation. SEM images reproduced with permission from: **b**, ref. 56, © 2010 NAS; **c**, ref. 59, © 2009 Elsevier; **d**, ref. 65, © 2010 Elsevier.

fibres. This technique involves the spontaneous organization of individual components into an ordered and stable structure with pre-programmed non-covalent bonds (Fig. 3b)³². The most commonly investigated self-assembled nanofibre matrix for tissue engineering applications is the peptide amphiphile, a chemical compound

possessing both hydrophobic and hydrophilic properties³³. The peptides that assemble to form the 3D scaffolds can have a fibre diameter as small as 10 nm and the scaffold pore size can range between 5 and 200 nm, significantly smaller than those produced by electrospinning²². An important advantage of this approach is the ability

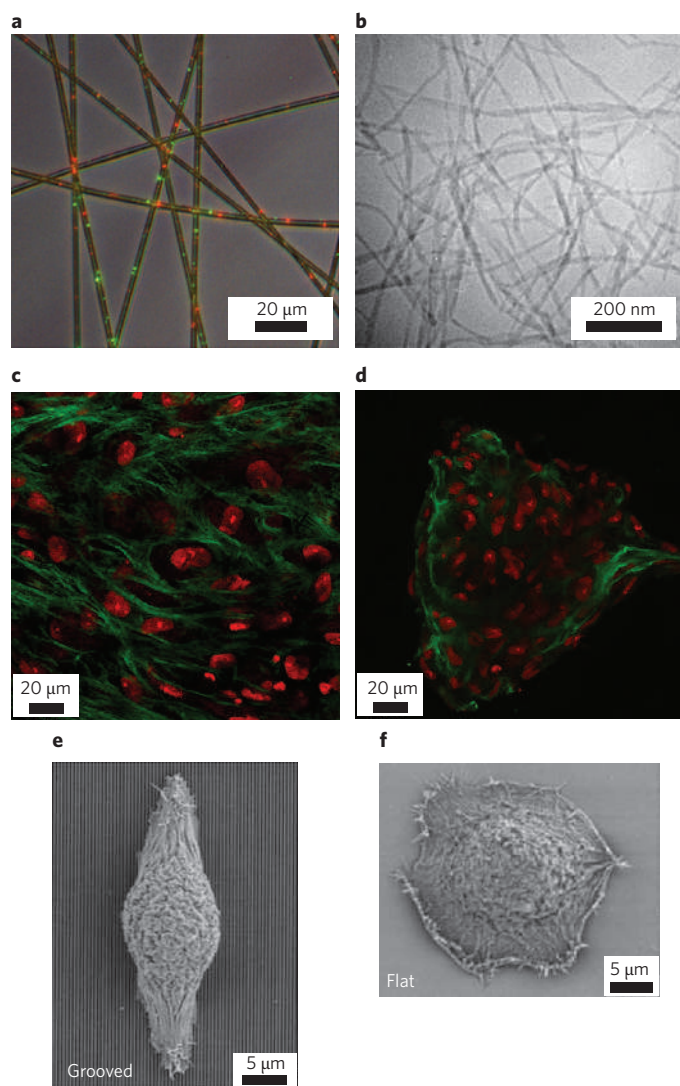


Figure 3 | Recreating ECM components using nanoscale tools.

a, ECM nanofibres produced by electrospinning polymeric fibres contain nanoparticles that release epidermal growth factor (green) and bovine serum albumin (red) in parallel. **b**, Self-assembled peptide amphiphile nanofibres. **c,d**, Alginate scaffolds containing short motifs of ECM adhesion proteins such as RGD encouraged mesenchymal stem cells to spread and attach to the matrix (**c**), whereas on unmodified scaffolds (**d**) only cell-cell interactions were seen (collagen fibres, green; nuclei, red). **e**, Epithelial cells respond to nanopatterning by alignment and elongation along the grating axis. **f**, On smooth substrates, cells are mostly rounded. Figures reproduced with permission from: **a**, ref. 28, © 2009 Wiley; **b**, ref. 32, © 2009 AAAS; **c** and **d**, ref. 42, © 2009 Elsevier; **e** and **f**, ref. 48, © 2003 Company of Biologists.

to include functional motif sequences (such as short peptides) that promote adherence, differentiation and maturation^{34–36}. This class of scaffolds can provide both mechanical support and instructive cues to the developing tissue. For example, neural progenitor cells were encapsulated within a 3D network of nanofibres³⁷, designed to present to cells the neurite-promoting laminin epitope IKVAV. Cultivation within this scaffold induced very rapid differentiation of cells into neurons, while discouraging the differentiation to other cell lineages³⁷, emphasizing the importance and simplicity of controlling cell fate by pre-designing the fibre composition. A challenge in this field is to engineer fibres effectively to provide adhesion and organization motifs that the cells can interact with, and provide

multiple cues for intracellular signalling that lead to differentiation and control of gene expression.

Other non-fibrous parts of the ECM also have an impact on cell behaviour, and their incorporation into scaffolds is essential. For example, cells interact with the ECM via nanoscale transmembrane integrin receptors that tether the cell cytoskeleton to adhesion molecules, such as fibronectin and laminin, located on the fibres³⁸. These focal adhesions initiate the intracellular signalling cascade, which affects most aspects of cell behaviour^{7,39}. Therefore, immobilizing short motifs of the ECM adhesion proteins such as RGD and YIGSR to 3D matrix surfaces enhanced functionality in terms of cell spreading and differentiation (Fig. 3c,d)^{40–42}.

In an attempt to increase the local concentration of growth factors and cytokines in the engineered tissue microenvironment, researchers recapitulated their electrostatic interactions with heparan sulphate proteoglycans^{43–45}, which act as a reservoir for growth factors¹⁷. For example, an alginate hydrogel was modified to mimic the heparin/heparan sulphate binding groups by sulphating the uronic acids in the saccharide backbone⁴⁵. The researchers demonstrated strong binding of 10 known heparin binding proteins to the matrix and studied their controlled release²⁹. The scaffolds were then used to promote pre-vascularization of engineered cardiac patches after releasing angiogenic and pro-survival factors, and after transplantation on the infarcted heart, the cardiac patches promoted its regeneration⁴⁶.

In vivo, in addition to the biochemical properties of the ECM, cells encounter and interact with many topographical features ranging from folded protein to banded collagen through a phenomenon known as contact guidance. A mimetic approach to study this effect and to create the structure and length scale (5 nm to micrometre scale) of the native ECM is by nanopatterning of different geometries such as nanogroove, nanopost and nanopit arrays⁴⁷. Although the patterning is only applied on 2D surfaces, this technique reveals aspects of cell behaviour and reactions to various substrate topographies that might be relevant to future 3D scaffolds. The topography of substrates influences a variety of cellular processes, including changes in shape, differentiation and adhesion⁴⁷. For example, epithelial cells elongate and align along patterns of grooves and ridges with feature dimensions as small as 70 nm, whereas on smooth substrates, cells are mostly rounded (Fig. 3e,f)⁴⁸. A few years ago, the control of human mesenchymal stem cell (MSC) differentiation using nanoscale symmetry and disorder was demonstrated. Highly ordered topographies produced low to negligible cellular adhesion and osteoblastic differentiation, whereas nanoscale topographic disorder stimulated MSCs to produce bone mineral⁴⁹. The precise mechanism responsible for the morphological response to different nanotopographies is still debated, although a prevalent view is that it arises from the generation of anisotropic stresses. Recent comprehensive reviews of nanoscale topographies and their effect on cells are provided by Bettinger *et al.*⁴⁷ and Kotov *et al.*⁵⁰.

Scaffold design considerations

The ECMs of various tissues in the body differ in the composition and spatial organization of the collagens, elastins, proteoglycans and adhesion molecules, to maintain specific tissue morphologies and organ specific shape and function, and to supply specific instructive cues. Therefore, the design considerations for scaffolds should vary according to the desired engineered tissue. For example, the biochemical, electrical and mechanical functions of the heart are uniquely dependent on their biological nanostructures⁵¹. The heart's 3D ECM network is composed of an intricate, micro- and nanoscale interweaving pattern of fibrillar collagen and elastin bundles that form a dense, elastic network with proteoglycans and with adhesive and non-adhesive molecules. In this defined mesh, the cardiomyocytes are forced to couple mechanically to each other, to form elongated and aligned cell bundles that interact with each other or with neighbouring capillaries

Table 1 | Mimicking ECM components to create cell microenvironment

Natural component	Method	Material used	Role	Refs
Fibres: collagens, elastins	Electrospinning	Carbon, PLA, PGA, PLGA, polycaprolactone, polydioxanone, chitosan	3D cell scaffolding, promote adhesion	19–21, 30, 31
	Self-assembly	Peptide amphiphile	3D cell scaffolding, promote adhesion, differentiation	32–34, 36, 37
	Nanopatterning	PEG, poly(ester amide)	Induce morphogenesis	56, 58
Adhesion proteins: laminin, fibronectin	Conjugation of adhesion motifs to the matrix backbone	RGD and IKVAV amino acid sequences	Promote cell adhesion to matrices	41, 42, 59
Proteoglycans: heparan sulphate	Sulphating matrix backbone	Alginate-sulphate, perlecan, sulphated chitosan	Control the release and presentation of growth factors and cytokines	29, 43–45
Minerals: hydroxyapatite		Hydroxyapatite	Induce osteogenesis and mineralization	62–65

and nerves (Fig. 2b). This multi-bundled elongated structure is essential for creating an anisotropic syncytium that provides the muscle with unique electrical and mechanical properties, allowing blood pumping to provide the body with oxygen.

Post-isolation cells lose their ultrastructural elongated morphology and their interaction with their surroundings, and they adopt a random distribution on the flat surface of the scaffold, which compromises many of their physiological properties. In recent years, various approaches to promoting elongation and alignment of engineered cardiac tissue have been used, including applying mechanical stretching⁵², interstitial fluid flow⁵³, electrical stimulation⁵⁴ or microcontact printing⁵⁵, but these have had limited success in engineering a completely aligned, thick tissue. In attempt to promote aligned structures, a nanotopographically controlled model of cardiac muscle was developed that mimics the structural and functional properties of native myocardial tissue and specifically the ECM architecture. Cardiac cells were cultured on polyethylene glycol hydrogel grooved arrays with ridge (groove) widths ranging from 150 (50) to 800 (800) nm. These cells self-assembled in the direction of the grated surfaces, became elongated and formed a highly anisotropic cell array that could produce the aligned contraction essential for heart function⁵⁶. Although this technique promotes a proper cardiac-cell organization, the challenge of patterning such arrays within 3D scaffolds and engineering thick, aligned tissues still remains.

Most of the tissues in the body do not require an aligned mesh of ECM. In epithelial tissues such as the liver, pancreas or kidney, each cell is polarized and contacts three types of surfaces: a basal surface, which contacts the ECM, a lateral surface, which contacts other cells, and an apical surface, which faces the lumen (Fig. 2c). Cells that do not contact the ECM or ones lacking apical surfaces will undergo apoptosis or generate a lumen at a region of contact with other cells⁵⁷. Engineering this precise structure is essential for the function of the tissue. In the body, epithelial tissues are involved in secretion, absorption and transcellular transport, and therefore reorganizing the cells in this complex structure, which includes layers of epithelial cells facing blood vessels, will result in functioning tissues allowing efficient diffusion to and from the adjacent lumens. To induce the interaction of the basal surface of the liver with ECM fibres, Bettinger and co-workers sought to mimic the nanotopography of native collagen films⁵⁸. Because collagen fibrils can extend to tens of micrometres in length and have a diameter between 260 and 410 nm, poly(ester amide) substrates were nanofabricated to produce pillar geometries in this size scale. Cultivation of primary hepatocytes on these substrates enhanced cell attachment and spreading, and most importantly, maintained metabolic function⁵⁸. To prepare this engineered tissue for *in vivo* applications, it is also important to pattern special geometries between the polarized tissues that

induce endothelial cell assembly to lumens. A different approach to providing hepatocytes with a microenvironment that more closely resembles the native one is by culturing them on nanofibrous galactosylated chitosan scaffolds. Nanoscale surface receptors on hepatocytes can interact with the embedded galactose ligands and lead to the formation of flat aggregates, which show a higher level of liver-specific functions⁵⁹ (Fig. 2c, SEM inset).

Among the most investigated tissues in the field of tissue engineering is bone. It contains a unique nanocomposite material mainly made of inorganic hydroxyapatite nanocrystallites and a collagen-rich organic matrix⁶⁰. Hydroxyapatite serves as a chelating agent for mineralization of osteoblasts in bone regeneration while the collagen provides mechanical support, promoting adhesion and proliferation. The crystals, 50 nm long, 25 nm wide and 2–5 nm thick, reside in the grooved regions formed by the 3D organization of the fibrils (Fig. 2d)⁶¹. In attempts to orchestrate a microenvironment that will promote the assembly of bone tissue, several groups have investigated the effect of scaffolds based on a combination of natural bone ECM and hydroxyapatite, reporting superior osteoblast adhesion, growth and stimulation for mineralization, compared with matrices without hydroxyapatite^{62–64}. In the past year, scaffolds containing various shapes and sizes of hydroxyapatite particles have been fabricated and their bioactivity investigated. The researchers found that a nanocomposite scaffold coated with needle-shaped hydroxyapatite particles showed the strongest osteoblast differentiation profile compared with rod and spherical shaped particles⁶⁵. Such observations emphasize that in addition to the dimension scale of the particles, their specific topography is of great importance for the interaction with cells.

Nanocomposites to compensate for scaffold limitations

In addition to creating ECM-like structures (Table 1), nanomaterials can be useful for other purposes. The rationale behind incorporating nanostructures is to compensate for other scaffold limitations such as weak mechanical properties, lack of electrical conductivity, the absence of adhesive and microenvironment-defining moieties, and the inability of cells to self-assemble to 3D tissues (Table 2). For example, carbon nanotubes show viscoelastic behaviour similar to that observed in soft-tissue membranes⁶⁶, so they have been used to increase the Young's modulus and tensile strength of the hybrid biomaterial⁶⁷. Carbon nanotubes were also shown to support the cultivation of neurons, and in recent years, several studies have shown that conjugation of these nanotubes to different substrates can affect cell behaviour and promote attachment, growth, differentiation and long-term survival of neurons^{68–70}.

One of the main obstacles in neural tissue engineering for the regeneration of a nerve tissue such as the spinal cord may be the

Table 2 | Nanomaterials to compensate for matrix limitations

Matrix limitation	Nanomaterial used	Effect	Ref.
Weak mechanical properties	Carbon nanotubes	Increased Young's modulus and tensile strength	67
Electrical resistance	Carbon nanotubes	Increased conductivity	74
Weak cell-matrix interaction	Nanotitanate wires	Cell adhesion to the matrix	78
Lack of biochemical cues	Synthetic and natural nanospheres	Controlled release of biomolecules	79
	Gold nanowires	Control over localization of biomolecules	80
Inability of cells to assemble to 3D structures	Magnetic nanoparticles	Engineering of tubular structures	84
	Phage, magnetic iron oxide and gold nanoparticles	Control over geometry of cell mass	85

loss of anisotropic conduction within the cell-seeded construct owing to lack of tissue consistency or to the non-conductive nature of the biomaterial. One approach to addressing this problem is to incorporate conducting nanostructures into the cell culture. Neurons that grow on a conductive nanotube meshwork display more efficient signal transmission^{70–72}. In a recent study, Cellot and co-workers provided new mechanistic insight into how nanotubes target the integrative properties of neurons, showing that nanotubes can improve the responsiveness of neurons by forming tight contacts with the cell membranes that might favour electrical shortcuts between different compartments of the neuron⁷³. Such neuronal/nanotube network hybrids may allow one to predict or engineer the interactions between nanomaterials and neurons, and guide the design of smart biomaterials for the engineering of electrically propagating tissues.

Carbon nanotubes have also been used to create 3D conductive structures. Gui and colleagues assembled nanotubes with a diameter of 44 nm into an interconnected 3D framework with ~99% porosity⁷⁴. The porous structure allowed direct polymer infiltration without disturbing the nanotube interconnection, resulting in a sponge composite with high electrical conductivity⁷⁴. The potential of these hybrid scaffolds to support the cultivation of neurons or cardiomyocytes should be further explored.

Nanostructures can also be used to increase the viability and adhesiveness of cells to pre-formed microporous scaffolds^{75–77}. Bioactive nanotitanate wires and belts were naturally grown directly inside 3D macrostructures to obtain a hierarchical architecture similar to that of human bone⁷⁸. Large amounts of nanowires and nanobelts, with an average width of 20 to 1,300 nm, covered the pores of the scaffold, growing almost perpendicular to the pore walls. The resulting nanostructures increased the overall hydrophilicity of the porous scaffold, promoting more efficient cell seeding and adhesion⁷⁸.

The process of tissue growth and development requires a constant supply of instructive cues. Another promising application for nanoscale structures in tissue engineering is the incorporation of controlled release systems into scaffolds. Controlled delivery of biomolecules, such as growth factors and cytokines *in vitro* or *in vivo*, is crucial in the support and enhancement of tissue morphogenesis, viability and functionality. Advances in nanotechnology provided the basis for fabrication of nanoparticulate delivery systems with large ratios of surface area to volume, rendering them very effective within the scaffold microenvironment. Examples of nanoparticles for controlled release of biomolecules include synthetic polymeric nanospheres, nanotubes, nanowires, liposomes and dendrimers (for a comprehensive review see ref. 79).

Recently, Fan and co-workers reported that gold nanowires can be functionalized with a cytokine and manipulated to specific locations using electric fields⁸⁰. The nanowires were able to deliver and release their payload to a pre-specific cell with subcellular resolution and activate desirable signalling pathways. Such control over

localization of biomolecules in specific zones, if applied in 3D constructs, can assist in precise engineering of the cellular microenvironment by delivering factors to specific cell types in co-cultures. For example, this technique can be used to engineer the complex microenvironment of the stem cell niche, which is composed of a variety of materials and cell types, originating from stem cells. Factors inducing self-renewal or differentiation of the cells can be manipulated to affect specific cells in different regions of the construct.

In recent years, nanoparticles have also been used to manipulate biomaterials and cells in order to create desired tissue structures. For instance, Alsberg and co-workers used magnetic forces to fabricate fibrin gels with defined nanoarchitecture. Thrombin-coated magnetic beads were manipulated to create an initial 2D hexagonal array that served as nucleation sites for the growth of fibril fibres that assembled into an ordered scaffold. Endothelial cells seeded on these scaffolds adhered to the fibres and their actin filaments aligned according to the scaffold fibres⁸¹.

Nanomagnetic actuation has also been used to culture and detach monolayers of cells. Cells were conjugated to magnetic nanoparticles and seeded on culture plates with a magnet placed underneath. The cells formed a multilayered tissue that could be detached by removing the magnet, or formed into a tubular structure by rolling a cylindrical magnet^{82–84}. Souza and co-workers reported a new approach to control the assembly of 3D tissues that could provide an alternative to biodegradable scaffolds and protein matrices⁸⁵. The technology is based on the cellular uptake and subsequent magnetic levitation of a hydrogel composed of phage, magnetic iron oxide and gold nanoparticles. By spatially controlling the magnetic field while cells divide and grow, the researchers were able to manipulate the geometry of the cell mass and engineer 3D structures. This approach can be used to engineer complex tissues composed of several cell types, organized in specialized niches by transferring cells to their specific locations.

Nanodevices for tissue engineering

After successfully engineering a tissue, nanodevices can be useful for triggering desired processes and for following tissue development, where functionality is dependent on the specific material chosen (Table 3). Moreover, the interaction of nanomaterials with existing microtechnologies may improve their function. For example, electrodes designed to stimulate neural tissue can be improved by coating them with nanostructures such as carbon nanotubes or other inorganic crystals⁸⁶. Individual carbon nanotubes have been shown to form spontaneous junctions with lipid bilayers with cleft spacings that are tight compared with the size of a single protein⁸⁷. Such tight interfaces can improve the quality of neural stimulation and recording by reducing the impedance between the device and cell membrane⁵⁰. Tungsten or stainless steel electrodes coated with carbon nanotubes were shown to be more effective at stimulating and recording from

brain tissue than their uncoated counterparts. This is mostly attributed to impedance considerations as well as the higher surface area for charge transfer and favourable stability *in vivo*⁸⁶. Other types of nanoparticle coatings enable more exquisite functionality such as light sensitivity. For instance, photovoltaic mercury telluride particles assembled into layer-by-layer electrodes were able to stimulate cultured neurons when exposed to visible light⁸⁸.

In addition to stimulation, nanostructures can be used to record the electronic signals that are transmitted through cells such as neurons and cardiomyocytes. One way to record these signals is by lithographically defining nanostructures as field-effect transistors, which are sensitive to local electric field changes. In particular, silicon nanowire transistors are useful for measuring extracellular signals because they exhibit particularly exquisite field-effect sensitivity compared with conventional, planar devices; they are just tens of nanometres in diameter and can therefore interface with cells and tissue at a subcellular level; and they show nanotopographic features that encourage tight interfaces with biological systems, a prerequisite for recording signals with high signal-to-noise ratio⁸⁹. In a recent study, arrays of nanowire devices were interfaced with patterned, cultured neurons and used to measure extracellular signal fluctuations locally at individual axons, dendrites and soma⁹⁰. Nanowire transistors were also interfaced with cardiomyocytes that were either cultured directly on top of nanowire arrays⁹¹ or cultured separately on polymer films that were then brought into direct contact with nanowire arrays^{92,93}. In each case, nanowire devices recorded signals that were substantially larger than those attainable with planar, microscale transistors, and demonstrate the added benefit of recording signals at subcellular resolution. Nanowire transistors are also useful for recording signal transmission across the surface of cardiac tissue⁹⁴ or throughout brain slices⁹⁵.

The synthesis and fabrication techniques associated with nanowire electronics are versatile and could be tailored to specific applications. Nanowire devices have been fabricated on a wide range of substrates, including silicon oxide and flexible plastic⁸⁹. Inorganic or organic transistors have also been demonstrated on resorbable substrates such as silk fibroin⁹⁶ or poly(lactic-co-glycolic) acid⁹⁷. The geometry and composition of nanowires themselves has also been elucidated, encompassing complex core-shell wires and even kinked structures with complex chemical profiles⁹⁸. These kinked nanowires together with flexible substrate materials have been used to fabricate 3D, free-standing nanowire transistor probes that were used to penetrate the membranes of living cells and measure intracellular signals (Fig. 4a)⁹⁹.

These nanowire structures may be useful in future for creating 3D array of sensors inside macroporous scaffolds.

Aside from forming electronic interfaces with cells, nanostructured devices can be used to monitor, in real time, the concentration of biomolecules or other chemical species of interest present within the scaffold. Silicon nanowire transistors, for example, have been used to selectively detect proteins at femtomolar-level concentrations or viruses at the single-particle level. In these systems, selectivity was achieved by antibodies that were covalently coupled to the nanowire surface, enabling analyte-specific detection even in complex media, such as blood serum⁸⁹. By detecting small amounts of biomolecules secreted by the engineered tissue, this technique can be used to report on the condition of the tissue before transplantation. Other types of nanoparticles can be probed with light to yield optical information that is correlated to the chemical environment. Metallic nanostructures, for example, plasmonically couple to incident light and can be engineered to produce strong surface-enhanced Raman scattering (SERS)-based signals. In a recent example, pairs of gold-silver core-shell nanoparticles were tethered with DNA to form 'nano-dumbbells' with SERS signals emerging only from coupled particles. Hence, nano-dumbbells behave as single-molecule DNA sensors and could also be engineered for other bioassays¹⁰⁰.

Carbon nanotubes have been used as multimodal sensors for chemotherapeutic drugs or reactive oxygen species, where adsorption of these species on the surface of the nanotubes uniquely altered their photoluminescence spectra (Fig. 4b)¹⁰¹. They have also been used as sensors inside hydrogels, where their emission spectra shifted with the hydration state or cross-linker density in the hydrogel. In this study, gels that were cross-linked with apo-glucose oxidase, a glucose-sensitive cross-linker, were used to reversibly detect glucose, both *in vitro* and when subdermally implanted in a rat model¹⁰². In the future such sensors could be used as part of a control system incorporated within insulin-secreting patches for the treatment of diabetes.

Challenges and prospects

Nanotechnologies clearly have had an impact on tissue engineering and still have great potential to advance therapeutic methods based on tissue engineering. The synthesis of new nanostructures and their incorporation into existing macro- and microtechnologies have led to improvements in the ability to provide a true biomimetic microenvironment to the developing tissue. But challenges still need to be addressed. It is believed that engineered nanomaterials

Table 3 | Inorganic nanoparticles in cellular interfaces and detection

Material	Forms	Applications	Physical attributes
Silicon nanowires	Intrinsic or doped composition, straight or kinked morphologies	Electronic detection	High sensitivity to local charges when operated as transistors
Carbon nanotubes	Semiconducting or metallic	Surface coatings for cell interfaces, electronic detection, optical detection	Enable surfaces with high charge-transfer area Exhibit sensitivity to local charges when used as transistors Are fluorescent in near-infrared range
Metallic nanostructures	Spheres, shells or rods. Can be composed of metals such as Au or Ag, or can be synthesized as heterostructures	Optical detection	Exhibit surface plasmon resonance and surface enhanced Raman scattering (SERS) that is dependent on ligand coupling
Optically active semiconductors	Direct bandgap materials such as HgTe	Surface coatings for optical cellular stimulation	Enable conversion of light into electric field

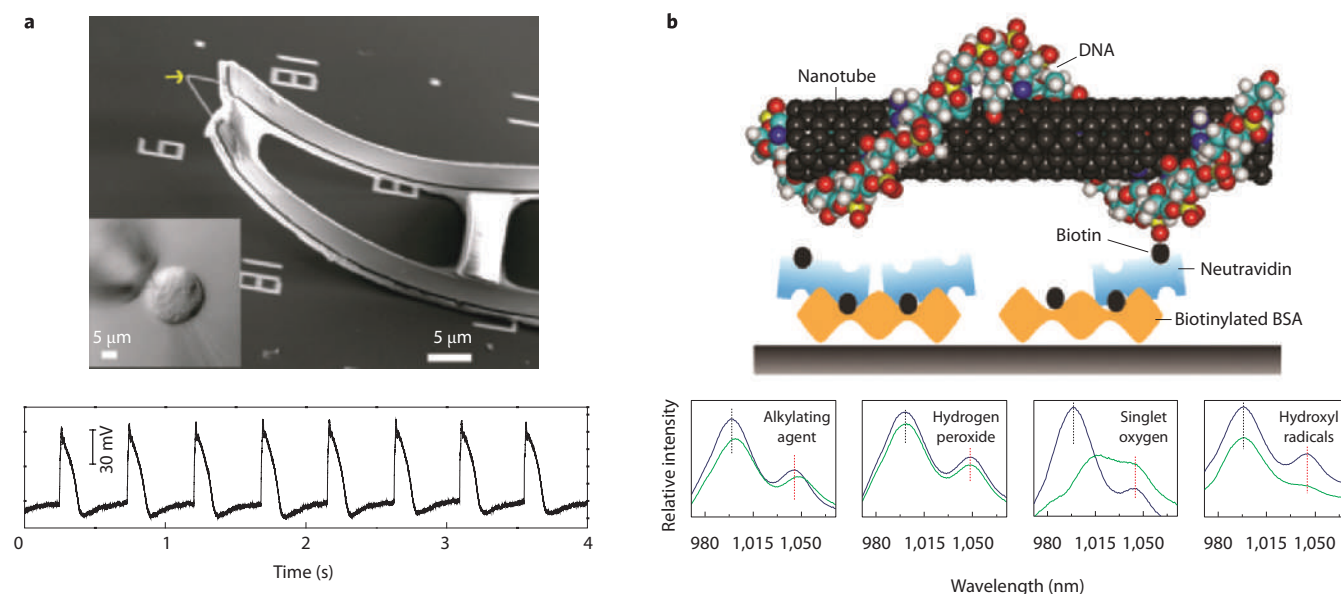


Figure 4 | Nanodevices in tissue engineering. **a**, Three-dimensional, free-standing nanowire transistor probe for electrical recording. The probe is composed of a kinked nanowire (yellow arrow) and a flexible substrate material. The device is used to penetrate the membrane of living cells (inset) and measure intracellular signals (lower panel). **b**, Biosensors based on carbon nanotubes are used for the detection of genotoxic analytes, including chemotherapeutic drugs and reactive oxygen species. Upper figure shows a schematic of a sensor made from a DNA and a single-walled carbon nanotube complex bound to a glass surface through a biotin-BSA (orange) and neutravidin (blue) linkage. Lower figures reveal the spectral changes arising from the interaction of the nanotube sensor with (from left to right): a chemotherapeutic agent, hydrogen peroxide, singlet oxygen and hydroxyl radicals (blue curve, before addition of analytes; green curve, after addition of analytes). Figures reproduced with permission from: **a**, ref. 99, © 2010 AAAS; **b**, ref. 101, © 2010 NPG.

such as carbon nanotubes, nanowires and other inorganic materials will be increasingly used in tissue engineering^{103,104}. In recent years, several reports have indicated possible negative effects of carbon nanotubes on cells and their potential to provoke oxidative stress, inflammation, genetic damage and long-term pathological effects. Therefore the biocompatibility and biodegradation of inorganic nanomaterials need to be thoroughly investigated before they can be safely applied in clinical trials. (For a comprehensive review on the toxicology of nanomaterials, see Kunzmann *et al.*¹⁰⁵.) Another challenge that must be addressed is the complexity of creating 3D porous scaffolds with nanotopographies. The interaction of cells with various nanoscale topographies has proved to be important for creating monolayers of functioning tissues^{47,56,58}, but 'scaled-up' versions of these nanopatterning technologies remain to be achieved. Moreover, it is crucial to discover the key factors promoting the assemblies of different tissues and to create specific microenvironments. Developing nanotechnological tools for controlling and guiding cells to desired locations in 3D matrices will be useful for engineering complex multicellular constructs such as epithelial and vascularized tissues.

We believe that future strategies could involve incorporating intelligent nanoscale biosensors inside scaffolds to follow the development of engineered tissues after transplantation, and according to biochemical composition or tissue behaviour, trigger the release of compensating cues. For example, when oxygen levels are low, the system would release angiogenic factors for fast new blood vessel formation. We also believe that incorporating nanomaterials, such as nanotubes or wires on the outer surfaces of 3D scaffolds, could serve to reduce inflammatory responses to transplanted engineered tissues by modulating macrophage adhesion and viability. For example, zinc oxide nanorods (50 nm in diameter and around 500 nm long) sputtered on polyethylene terephthalate discs inhibited the formation of the typical foreign body capsule occurring after subcutaneous transplantation in mice¹⁰⁶.

Finally, we envisage the use of smart nanoparticulate systems that will recruit stem cells to desired sites in the body and instruct the formation of tissues *in vivo*, on demand, by triggering the release of chemical attractants. Smart controllable nanorobots could potentially circulate inside the body, find diseased tissues and repair them by destroying defective cells and molecules, or encourage cells to regain function¹⁰⁷. These robots could be powered by biologically inspired nanomotors based on conversion of chemical energy (usually stored as adenosine triphosphate) into mechanical energy. We foresee nanowire-based brain-machine interfaces that could assist paralysed patients by re-routing movement-related signals around injured parts of the nervous system.

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Additional Information

R.L. declares competing financial interests: details accompany the paper at www.nature.com/naturenanotechnology.