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Nanotechnology in regenerative medicine: the materials side

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Regenerative medicine is an emerging multidisciplinary field that aims to restore, maintain or enhance tissues and hence organ functions. Regeneration of tissues can be achieved by the combination of living cells, which will provide biological functionality, and materials, which act as scaffolds to support cell proliferation. Mammalian cells behave *in vivo* in response to the biological signals they receive from the surrounding environment, which is structured by nanometre-scaled components. Therefore, materials used in repairing the human body have to reproduce the correct signals that guide the cells towards a desirable behaviour. Nanotechnology is not only an excellent tool to produce material structures that mimic the biological ones but also holds the promise of providing efficient delivery systems. The application of nanotechnology to regenerative medicine is a wide issue and this short review will only focus on aspects of nanotechnology relevant to biomaterials science. Specifically, the fabrication of materials, such as nanoparticles and scaffolds for tissue engineering, and the nanopatterning of surfaces aimed at eliciting specific biological responses from the host tissue will be addressed.

Introduction

Regenerative medicine has brought high expectations for a great number of current worldwide human illnesses. Diseases, such as Parkinson's disease, Alzheimer's disease, osteoporosis, spine injuries or cancer, might in the near future be treated with methods that aim at regenerating diseased or damaged tissues. The perspective of regenerating damaged or nonfunctional tissues by using an off-the-shelf synthetic product is a drive for medical science. Today's interest in nanomedicine keeps growing because the application of nanotechnology tools to the development of structures at the molecular level enables the improvement of the interactions between material surfaces and biological entities. Although cells have micrometre dimensions, they evolve *in vivo* in close contact with the extracellular matrix (ECM), a substratum with topographical and structural features of nanometre size. The interactions between cells and the ECM influence cell growth, guide cell mobility and affect the general behaviour of cells. Nanotechnologies provide the possibility to produce surfaces,

structures and materials with nanoscale features that can mimic the natural environment of cells, to promote certain functions, such as cell adhesion, cell mobility and cell differentiation.

Nanomaterials used in biomedical applications include nanoparticles for molecules delivery (drugs, growth factors, DNA), nanofibres for tissue scaffolds, surface modifications of implantable materials or nanodevices, such as biosensors. The combination of these elements within tissue engineering (TE) is an excellent example of the great potential of nanotechnology applied to regenerative medicine. The ideal goal of regenerative medicine is the *in vivo* regeneration or, alternatively, the *in vitro* generation of a complex functional organ consisting of a scaffold made out of synthetic or natural materials that has been loaded with living cells (Figure 1). Ideally, stem cells are to be used owing to their ability to generate all types of tissues and their unlimited self-renewal capacity. The functionalisation of such a porous scaffold with different biomolecules (depending on the targeted cells) or the entrapment of nanoparticles, such as growth factors, drugs or genes, could enhance the success of the TE strategy greatly. However, crucial issues, such as stem cell isolation from the patient and their proliferation, the culturing process in a bioreactor and the time delay before the engineered hybrid construct is implanted back into the patient, present major difficulties to this approach becoming a well established standardised procedure in the near future.

The aim of this article is to review the aspects related to the materials used currently for tissue regeneration and it will furthermore point out the most promising strategies in the field. Other equally important issues, such as cell therapies or diagnostic applications, fall out of the scope of this review and will not be addressed.

Nanofeatures on surface biomaterials

As discussed earlier, the behaviour of cells depends on their interactions with the environment. Consequently, the interactions between cells and implantable materials will determine the success or failure of a medical device. It is well known that cell response is affected by the physicochemical parameters of the biomaterial surface, such as surface energy, surface charges or chemical composition. Topography is one of the most crucial physical cues for cells. Microtopography influences cell adhesion, proliferation and differentiation [1,2] and, more recently, it has become clear that nanotopography also guides cell

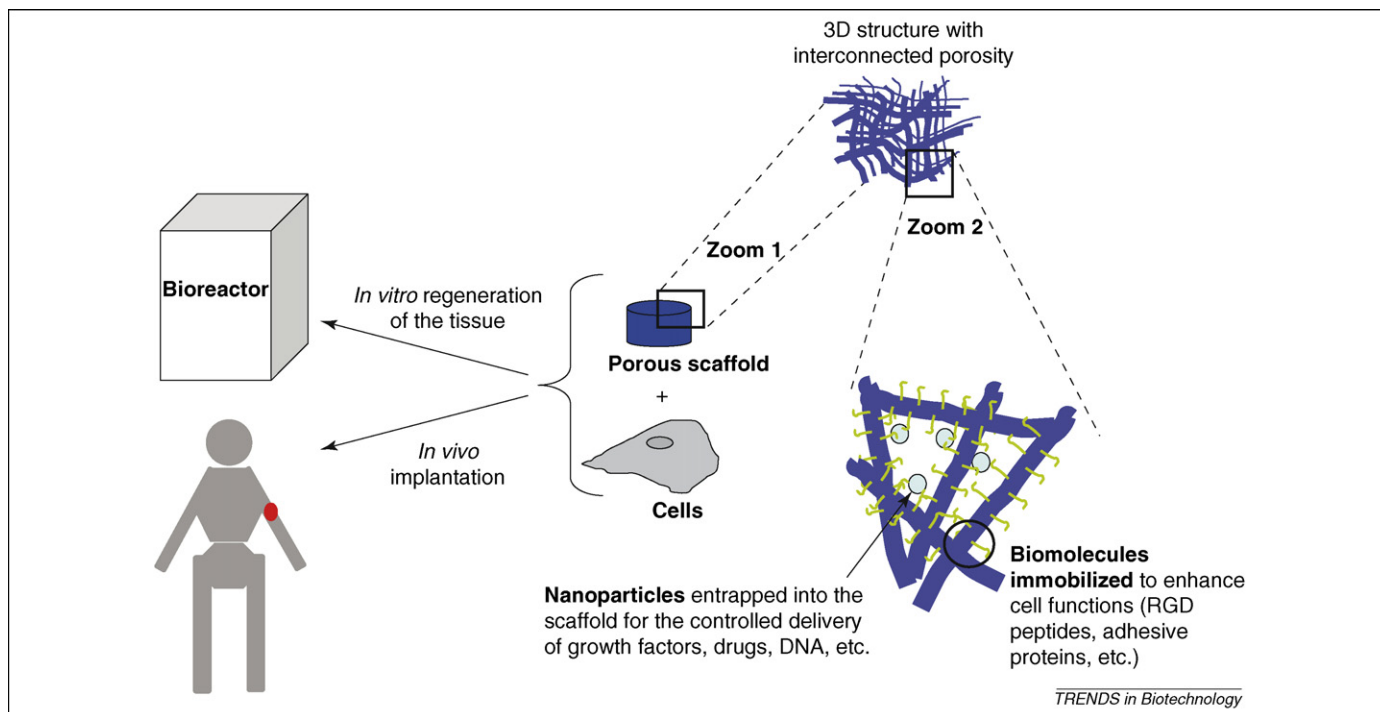*Corresponding author:* Planell, J.A. (Josep.A.Planell@upc.edu).

Figure 1. Tissue engineering approach for tissue regeneration. Two different strategies exist for regenerative medicine: *in vivo* or *in vitro* regeneration of the living tissue. Both methods use a porous scaffold that is loaded with cells. Topographical structure at the nanometre scale can be generated onto the scaffold surface. The scaffold can also be functionalised with different molecules to enhance cell functions. Components, such as drugs, genes, peptide sequences, such as arginine–glycine–aspartic acid (RGD), or proteins, can be entrapped into nanoparticles and released subsequently in a controlled manner to reach a specific target, such as cells, bacteria and viruses.

behaviour greatly [3,4]. In some applications, in which guiding the cell orientation is essential to achieve a functional tissue, such as for tendons, nerves, corneal stroma and intervertebral-disc regeneration, contact guidance of cells by micro- and nano-topographical patterns is a promising perspective [5,6]. Chemical cues in the form of various different biomolecules (nanometre scale), such as adhesive protein or growth factors, also influence cell behaviour crucially. For example, fibronectin and laminin are able to enhance the adhesion of different cell types. Therefore, considerable efforts have been made to modify biomaterial surfaces chemically by immobilisation of these biomolecules [7,8].

Nanopattern fabrication

Because nanotopography seems to have an essential role in guiding cell behaviour *in vivo*, it is now being used in biomaterials science as a tool for controlling tissue regeneration. A wide variety of techniques have been used to produce nanotopography on biomaterial surfaces. Methods leading to an ordered topography with a regular controlled pattern and methods leading to unordered topography with random orientation and organisation have been developed and are described in Box 1. Polymer demixing, chemical etching and colloidal lithography are some of the most relevant techniques to obtain randomly organised patterns. Conversely, soft-lithography techniques and other methods using different sources of radiation (electrons, ions or photons) to etch the substrate are among the techniques used most commonly to produce regular geometries. Figure 2 shows how specific nanostructured surfaces can be used to control the growth of mesenchymal stem cells (MSCs).

All the techniques of nanostructuration cited here can be combined with self-assembly for functionalisation of the surface (refer to later in the text for the definition and relevance of self-assembly). For example, the creation of a nonfouling surface, which can be useful in ophthalmological and cardiovascular application, can be achieved by the functionalisation of metal surfaces (e.g. Au) with alkanethiol self-assembled molecules, which can bind poly(ethylene-glycol) molecules. Finally, dip-pen lithography is a simple method to create chemical patterns at the nanoscale (Box 1).

Nanocoatings and nanophase materials

Nanophase materials (i.e. materials with nanometre-scaled grains) can be used to produce nanometre features on biomaterial surfaces to guide cell behaviour along a desired biological response [9,10]. In bone-regeneration applications, promising results have been obtained with the nanophase materials ceramics and metals, with which increased osteoblast adhesion, proliferation and calcium deposition have been observed compared with conventional materials (i.e. with micrometre-scaled grains) [11].

Nanophase materials can also be deposited on a biomaterial surface to improve its bioactivity and/or biocompatibility. For example, nanocrystalline apatite coatings, produced by sol-gel methods or pulse electrodeposition [12,13], are used commonly to enhance the osteointegration of an orthopaedic device. Other techniques, such as layer-by-layer assembly (LBL) deposition [14,15], lead to nanocoatings (a few nanometres thick) with a controlled structure by sequential adsorption of oppositely charged polyelectrolytes. LBL can be useful for drug-delivery systems [16] and surface functionalisation of biomaterial surfaces [14].

Box 1. Methods for topographical and chemical surface modification

Soft lithography

Soft lithography is the general term for different techniques based on the use of an elastomeric stamp [which is made of poly(dimethylsiloxane) (PDMS) most commonly] to structure a material surface. This stamp is produced by using a master with a specific pattern (for more details, refer to [48]) and is then used to reproduce the pattern on a substrate.

- **Microtransfer moulding**: See Figure Ia for details.
- **Replica moulding**: The PDMS pattern is used as a mould for casting another polymer, which, after curing, will give a negative replica of the PDMS.
- **Micromoulding in capillaries**: A PDMS stamp is placed on the substrate to be patterned to form a network of empty channels between the substrate and the stamp. A drop of polymer is placed at one end of the channels and then the channels are filled by capillarity. The PDMS stamp is removed after curing.
- **Solvent-assisted micromoulding**: A solvent is used to wet the PDMS stamp, which is then placed against the substrate. The substrate is dissolved by the solvent and the resulting fluid is moulded in the PDMS pattern. After evaporation of the solvent and solidification of the substrate, a complementary structure to that of PDMS is obtained.
- **Microcontact printing**: See Figure Ib for details. This is used to transfer molecules from the stamp to a substrate in a controlled geometry.

Colloidal lithography

This uses a nanocolloidal solution to act as an etch mask. The solution is dispersed as a monolayer on a substrate and self-assembles electrostatically. The surrounding area of the nanocolloid particles (and also of the particles themselves) is etched by reactive ion-beam bombardment, thus creating a pattern on the substrate.

Hot embossing

A rigid stamp (usually made of silicon) is pressed against a softened polymer film to produce a pattern.

E-beam or ion-beam (EUV) lithography

This uses particle exposure of a sensitive resist to produce a pattern that can be revealed chemically in a second step or used directly to initiate polymer grafting.

Dip-pen lithography

This is a direct-write method in which an AFM tip is used in tapping mode to transfer molecules (e.g. proteins, polymers) to a target region of the substrate directly.

Polymer demixing

This is based on spontaneous phase separation of a polymer mixture under the spin-casting process onto a substrate (silicon, glass). The shape (pit, island, ribbon) and size of the features can be modulated by either changing the polymer ratio or the polymer concentration. A precise control of the height of the features (vertical direction) can be achieved (feature size >13 nm).
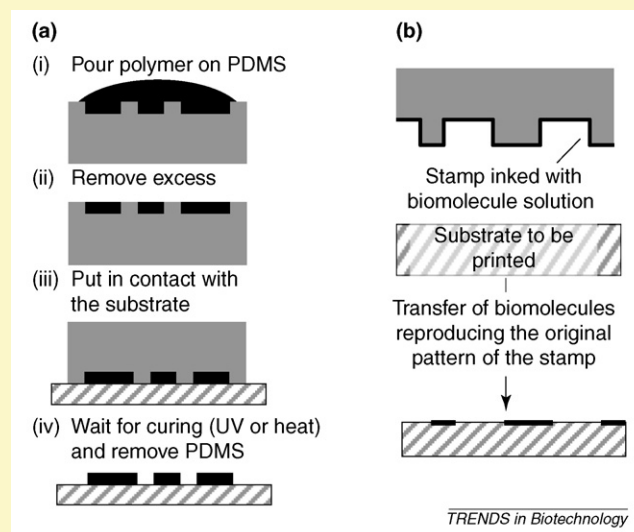

Figure I. (a) Microtransfer moulding. (b) Microcontact printing.

Applications of nanostructured materials

Numerous *in vitro* and *in vivo* studies, using nanostructured materials, have demonstrated the validity of this approach for a variety of cell types. The results of the relevant studies confirmed the potential of nanostructures in regenerating different tissues (such as bone, cartilage, bladder, nerves and vessels) by enhancing the biological performance of the cells (e.g. increased adhesion, proliferation, migration). A summary of some of these applications is given in Table 1.

Materials for nanoparticles

Nanoparticle research within regenerative medicine has been addressed mainly towards the development of entrapment and delivery systems for genetic material, biomolecules, such as growth and differentiation factors, and bone morphogenetic proteins and also as reinforcing- or bioactivity-enhancement phase for polymeric matrices in 3D scaffolds for TE.

Controlled delivery of biomolecules is crucial in the support and enhancement of tissue growth in TE applications. Nanotechnology approaches in delivery systems can enhance the success of specific therapeutic agents, such as growth factors and DNA among others, which

are of paramount importance for tissue regeneration [17]. Carriers in the nanoscale enable the intracellular delivery of molecules and the possibility of reaching targets that are inaccessible normally, such as the blood–brain barrier, tight junctions and capillaries, whereas the control over biomolecule dosage and delivery period are increased. The ultimate challenge is to develop artificial nanocarriers that can target cells with efficiency and specificity similar to that of viruses [18].

Examples of nanoparticles for delivery systems include currently microspheres, microcapsules, liposomes, micelles and also dendrimers. The different types of nanoparticles have been developed as solid, hollow or porous. The most common development methods are molecular self-assembly, nanomanipulation, bioaggregation and photochemical patterning [19,20].

Biodegradable polymers are the most commonly used materials in drug delivery. Polylactic acid (PLA), polyglycolic acid (PGA), polyethylene glycol (PEG) and its copolymers have been used widely in combination with hydrogels to attain nanocarriers that exhibit different release properties. Particularly important for the development of nanoparticles for delivery purposes are 'smart' or 'stimuli-responsive' polymers that can undergo
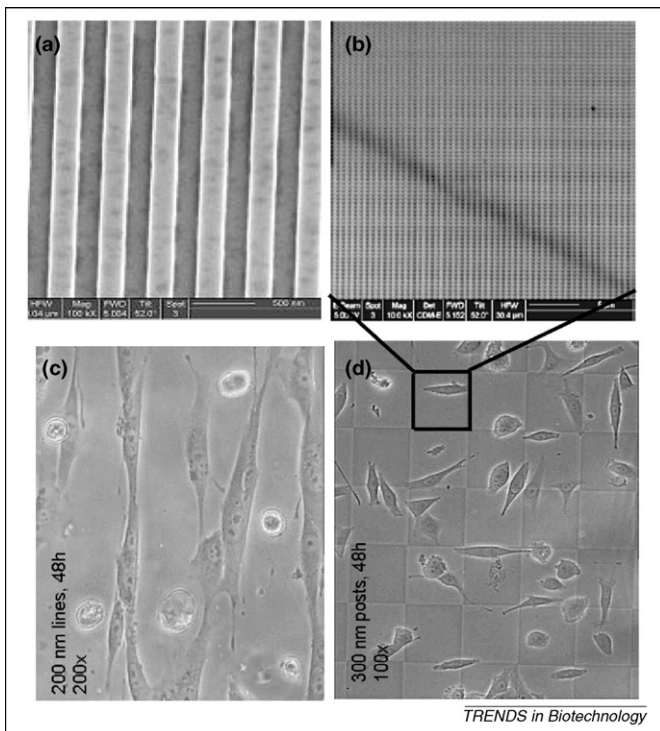
Figure 2. Nanopatterns on polymethylmethacrylate (PMMA) polymer and their influence on mesenchymal stem-cell (MSC) guiding. MSCs from bone marrow were grown on two different nanopatterned surfaces of PMMA for 48 h. **(a)** A magnified detail of the nanopattern structure, which consisted of 200 nm-wide lines. MSCs grown on this pattern show a directional alignment along the nanostructures **(c)**. **(b)** Magnification of the square shown in **(d)** to visualise the pattern of 300 nm-wide posts. Cells growing on this pattern present random distribution and morphology **(d)**.

conformational changes, such as swelling or shrinkage, on variations in temperature, pH and magnetic field. Table 2 presents several examples of nanoparticles produced with different materials. Their applications in the TE field depend mainly on the material properties.

The most widely studied temperature-sensitive polymer is poly (N-isopropylacrylamide) (PNIPAAm) [21]. Polyelectrolytes, such as polymethacrylic acid (PMAA) and polyacrylic acid (PAA), that are able to ionise under acidic or alkaline pH present interesting characteristics for drug carriers in applications in which pH changes occur, such as in the delivery of genetic material [22]. In the case of genetic-material delivery, nanoscale DNA carriers must overcome enzymatic degradation of endosomes to reach the cell nucleus and trigger specific cascades involved in tissue regeneration. Nanocarriers made of pH-sensitive polymers facilitate endosomal escape and enable the development of new nonviral genetic-material delivery systems with high transfection efficiencies [23].

In addition to biomolecule encapsulation and delivery, solid surface-modified nanoparticles might also be used for regenerative purposes. Although polymers are the most used nanoparticles in the delivery area, the use of ceramics has also been investigated. Hydroxyapatite nanoparticles functionalised with biomolecules could enhance osteoblast adhesion and bone regeneration [9].

Ceramic nanoparticles and nanofibres are also suitable in the elaboration of bio-inspired nanocomposites for bone TE applications, acting as the reinforcing phase of a polymer matrix and improving scaffold bioactivity [24].

Although the development of nanoparticles seems to have great potential for several biomedical fields, there has been only little progress in the attainment of effective results in current human therapy. However, it must be said that the use of nanoparticles also raises the need of a comprehensive understanding of their secondary effects and cytotoxicity once they enter the body. The large surface area of nanoparticles makes them very reactive in the cellular environment. Their dimensions enable them to penetrate through the lungs, skin or intestinal tract and they might deposit in several organs causing adverse biological reactions [25]. Moreover, the fact that these particles are able to penetrate the cell membrane and reach the cell nucleus might raise some concern about possible unknown risks attributed to the particle nature.

Nanofibre scaffolds
The first approach of TE was to design and fabricate 3D constructions that could mimic the structure of the tissue that required repair [26]. These scaffolds were made using

Table 1. A summary of nanostructured biomaterials with potential applications in regenerating different tissues

Application	Material	Results	Refs
Bone	Nanophased TiO_2, Al_2O_3	Osteoblast adhesion promoted; long-term osteoblast functions enhanced; osteoclast activity greater (comparison with conventional material)	[10]
	Titanium coated with nanophase apatite	Promotion of bone ingrowth and apposition (*in vivo*) and influence on human osteoblast behaviour	[49]
	Tantalum coated with nanophase apatite	Increased bone formation in comparison with conventional apatite and uncoated materials (*in vivo*)	[9]
	Nanophase Ti, Ti_6Al_4V and CoCrMo alloy	Increased osteoblast adhesion	[10,48,50]
	Nanostructured poly(lactide-co-glicolide) (PLGA) by mould-casting	Increased osteoblast adhesion; decreased fibroblast adhesion	[51]
	Ti mesh coated with nanocrystalline organoapatite	Accelerated migration and better proliferation of osteogenic cells	[52]
Cartilage	Nanostructured PLGA by chemical etching	Better chondrocyte functions than conventional materials	[53,54]
	Nanoporous Ti produced by anodisation	Increased chondrocyte adhesion and migration	[9]
Vessels	Nanostructured PLGA by chemical etching	Increased endothelial and smooth muscle cell densities compared with untreated PLGA	[54]
Bladder	Nanostructured PLGA, poly-ether-urethane and poly-caprolactone by chemical etching	Greater adhesion and proliferation of bladder smooth muscle cells compared with conventional materials	[55,56]
Nerves	Nanopatterned poly(dimethylsiloxane) by replica moulding	Induce differentiation of adult mesenchymal stem cells into neuronal lineage	[57]

Table 2. Nanoparticles and biomedical applications

Nanoparticles	Applications
Titania nanoparticles	Reinforcing phase in composite materials for TE scaffolds [58]
Quantum dots	Amplifiers, markers, biological sensors for bioreactors and TE
Hydroxyapatite nanoparticles	Reinforcing phase in composite materials for TE, biomolecule carriers [24]
Biodegradable polymer nanoparticles	Targeted-delivery systems for TE, immunotherapy [19]
Stimuli-sensitive polymeric nanoparticles	Biomolecule carriers, TE [22,59]

biodegradable materials and had a porous structure that would ensure host-cell colonisation. Among the possible structures that could replace the natural ECM, the use of nanofibres as scaffolds has several advantages compared with other techniques. Nanofibres show a high surface area and a highly interconnected porous architecture, which facilitate the colonisation of cells in the scaffold and the efficient exchange of nutrients and metabolic waste between the scaffold and its environment. These nanofibres can be made of synthetic, natural or the combination of both types of material. Several relevant methods for the production of nanofibres that are used in TE applications are summarised here now.

Electrospinning
Electrospinning is a simple and cost-effective fabrication process that uses an electric field to control the deposition of polymer fibres onto a target substrate. This system can produce fibres with diameters ranging from several microns down to 100 nm or less. The generated fibres can mimic the structural profile of the proteins found in the native ECM. Different synthetic and natural polymers have been used as materials to create 3D matrices for tissue repair. Some of these polymers have been studied in terms of fibre fabrication, diameter of the fibres and potential applications. The possibility of aligning these fibres is of interest in terms of mimicking the ECM. Different materials have been used to generate such fibres: synthetic biodegradable polymers, such as poly-L-lactic acid (PLLA) [27,28], ε-caprolactone (PCL) [29], PGA [30], and also natural polymers, such as collagen, silk and DNA. The combination of natural and synthetic fibres has been achieved as well. Table 3 summarises some of the materials used and the fibres obtained.

In addition, electrospinning is able to produce both random and aligned networks. This prospect of controlling the orientation of fibres is a prerequisite for biomimicking of natural tissues.

Phase separation
This process is another method for producing three-dimensional scaffolds of fibres in the submicron range. Current cutting-edge techniques use a thermally induced phase-separation process to produce a nanofibrous foamed material. However, this method involves several steps, such as raw-material dissolution, gelation, solvent extraction, freezing and drying, which make this process time consuming. The advantages of this method are that it does not require any sophisticated equipment and that the resulting pore sizes and morphology can be controlled by varying the processing parameters [31,32]. The most widely used material for phase-separation foams is biodegradable PLLA, which is sometimes combined with collagen. Yang *et al.* investigated the potential of PLLA nanofibrous scaffolds prepared by phase separation for nerve TE applications [31]. The resulting scaffold supported cell differentiation and neurite outgrowth. Although this technique is a good approach to mimic the ECM, it has so far failed to control fibre orientation. The distribution and arrangement of the ECM has a crucial role in controlling cell shape, regulating physiological function and defining organ architecture and it is believed that controlling the fibre orientation will increase biomimetics [33].

Self-assembly
Self-assembly is a process in which molecules and supramolecular aggregates organise and arrange themselves into an ordered structure through weak and noncovalent bonds [34]. The use of self-assembly to generate hierarchical supramolecular structures is a biomimetic strategy to produce synthetic materials that resemble biological ECMs, which are able to interact with cells at the molecular level to control the processes of tissue regeneration effectively. With this technique, nanofibres can be produced using natural or synthetic macromolecules. Several studies report promising results of this strategy. For

Table 3. Nanofibres as scaffolds for TE applications

Materials	Random/aligned	Average diameter	Potential applications	Refs
Synthetic polymers				
PLLA	Yes/Yes	50–350 nm	Neural TE	[28,31]
Poly(latide-co-glicolide) (PLGA)	Yes/No	500–800 nm	Soft tissue	[30]
PCL	Yes/?	500–900 nm	Cartilage TE	[29]
PLLA-CL	Yes/Yes	0.4–1.2 mm	Smooth muscle and endothelial cells; blood vessel engineering	[60,61]
Natural polymers				
Silk (with HAP/BMP-2)	Yes/No	?	Bone tissue engineering	[62]
Collagen	Yes/Yes	250 nm	Fibroblasts	[63]
Chitosan + polyvinyl alcohol (PVA)	Yes/No	170–700 nm	?	[64]
Chitosan + polyethylene oxide (PEO)	Yes/No	38–62 nm	Chondrocites and osteoblasts	[65]
Fibrinogen	Yes/No	320–600 nm	Cardiac fibroblasts	[66]

Abbreviation: HAP/BMP-2, hidroxyapatite/bone morphogenic protein 2.

example, a peptide amphiphile (chemical compound possessing both hydrophilic and hydrophobic properties) nanofibre network could be mineralised with hydroxyapatite to recreate the nanoscale structure of bone [35]. These amphiphile nanofibres have been designed to mimic the collagen structure-building protein-like structural motifs that incorporate sequences of biological interest [36]. These nanofibres have been also applied to promote rapid and selective differentiation of neural progenitor cells into neurons [37]. Self-assembly was also used successfully to encapsulate chondrocytes within a self-assembled peptide-hydrogel scaffold for cartilage repair [38].

Carbon nanotubes

Although carbon nanotubes (CNs) are not strictly nanofibres, they are another type of nanomaterial that can be used as scaffolds for TE applications. The discovery that carbon could form stable, ordered structures other than graphite and diamond guided Iijima to discover fullerene-related CNs by using an arc-evaporation apparatus [39]. The tubes contained at least two layers (often many more) and ranged in outer diameter from approximately 3 to 30 nm. Since their discovery in 1991, they have been proposed for many TE applications owing to their exceptional physical properties. These nanomaterials consist of graphite, either in a single layer (single-walled nanotubes) or in concentric layers (multiwalled carbon nanotubes). The unique properties of CNs, such as the fine electronic, mechanical properties and the high specific surface, might provide a three-dimensional microenvironment to facilitate the use of stem cells in tissue regeneration. [40]. CNs can be prepared by different methods but chemical-vapour deposition (CVD) is used most commonly. This process involves the reaction of a metal catalyst with a hydrocarbon feedstock at high temperatures ($>700°C$) [41]. The common use of Ni as catalyst has raised serious concern about their subsequent use in biomedical applications. In addition, the lack of a scalable production technique for high-purity CNs has impeded their use in practical applications. However, it has been possible to remove the metal catalyst and to avoid microstructural defects by using CVD at higher temperatures (1800–3000°C) [40]. Another important issue is that CNs are not biodegradable and they have to be removed once the tissue has been replaced. CNs have the potential to be used in clinical applications, such as scaffolds for neural implants, because they are highly conductive and are hence an ideal template for transmitting electrical signals to neurons [39]. Moreover, studies on bone engineering have shown that CN scaffolds are good candidates to support the differentiation of stem cells into mature osteoblasts and the production of bone tissue [40].

Related to health safety, some concerns about using CNs as scaffolds for TE have been raised. There are reports that demonstrate that nanotubes could be used to separate DNA because DNA strands wrap around CNs. A potential application seems to be separation purposes, although the potential consequences of CNs entering the human body raise a reasonable worry among the scientific community about their safe use in the humans.

Nanodevices

Recent progress in nanotechnology has led to the development of nanodevices that can act or detect at the nanoscale. This technological advance has been made possible through advances in microfabrication technology, which was developed initially for microelectronic applications. More recently, the fabrication of miomicro-electromechanical systems (BioMEMS) (see [42] for a comprehensive review of BioMEMS) has enabled the application of nanodevices in biomedicine [43]. For the purpose of this review, only BioMEMS with potential uses in regenerative medicine, such as biocapsules, bioreactors, biosensors and laboratory-on-a-chip, will be discussed and evaluated.

Biocapsules are nanodevices in the form of a shell structure that enable the storage and transport of drugs or molecules to be delivered or collected in a controlled manner. Biocapsules can be fabricated to isolate specific molecules. For example, Desai [44] has engineered micro-fabricated biocapsules with a uniform pore-size distribution that enabled the passage of molecules smaller than 6 nm but excluded molecules larger than 15 nm. Such a device has a wide range of applications owing to its versatility and simplicity in concept. Thus, the further development of biocapsules can have a tremendous impact in the future of regenerative medicine. They could be used as an accurate and locally sensitive diagnostic tool, for example, when another nanodevice is placed inside the biocapsule, which would be able to analyse entrapped molecules. Such a 'smart' biocapsule could therefore be used as a real-time diagnostic tool and could also be used for the prevention or treatment of a disease when combined with a drug-release system.

Bioreactors are devices that are used *in vitro* in which regenerative cells and tissues are grown under controlled monitoring and operating conditions (e.g. pH, temperature, pressure, fluidic and mechanical environment, nutrient supply and waste control). These devices integrate several types of BioMEMS into a single device to optimise tissue regeneration and to provide specific conditions for large-scale industrial applications [45]. Biosensors and laboratory-on-a-chip are integrated inside bioreactors to monitor and detect specific cellular processes (Figure 3). Most biosensors available currently are based on microtechnology but advances in nanotechnology foresee the potential for many applications for nanobiosensors. A nanobiosensor is a nanostructure that reacts to the local environment by providing an optical or electrical response. This response is a result of biological, chemical, mechanical or electrical reactions occurring on the sensor. Examples of nanobiosensors are quantum dots, fluorescent nanoparticles, metallic nanoparticles, CNs [41], pH sensors or oxygen sensors that use nanotechnology for locally discrete measurements or a molecule-release sensor (such as release of calcium or potassium) [46]. Energy supply of nanosensors can be provided through the integration of motor proteins that function through ATP [47]. Once nanobiosensors are integrated into bioreactors, they would in theory be able to regulate culture conditions automatically by feedback loops and this would improve tissue regeneration considerably.
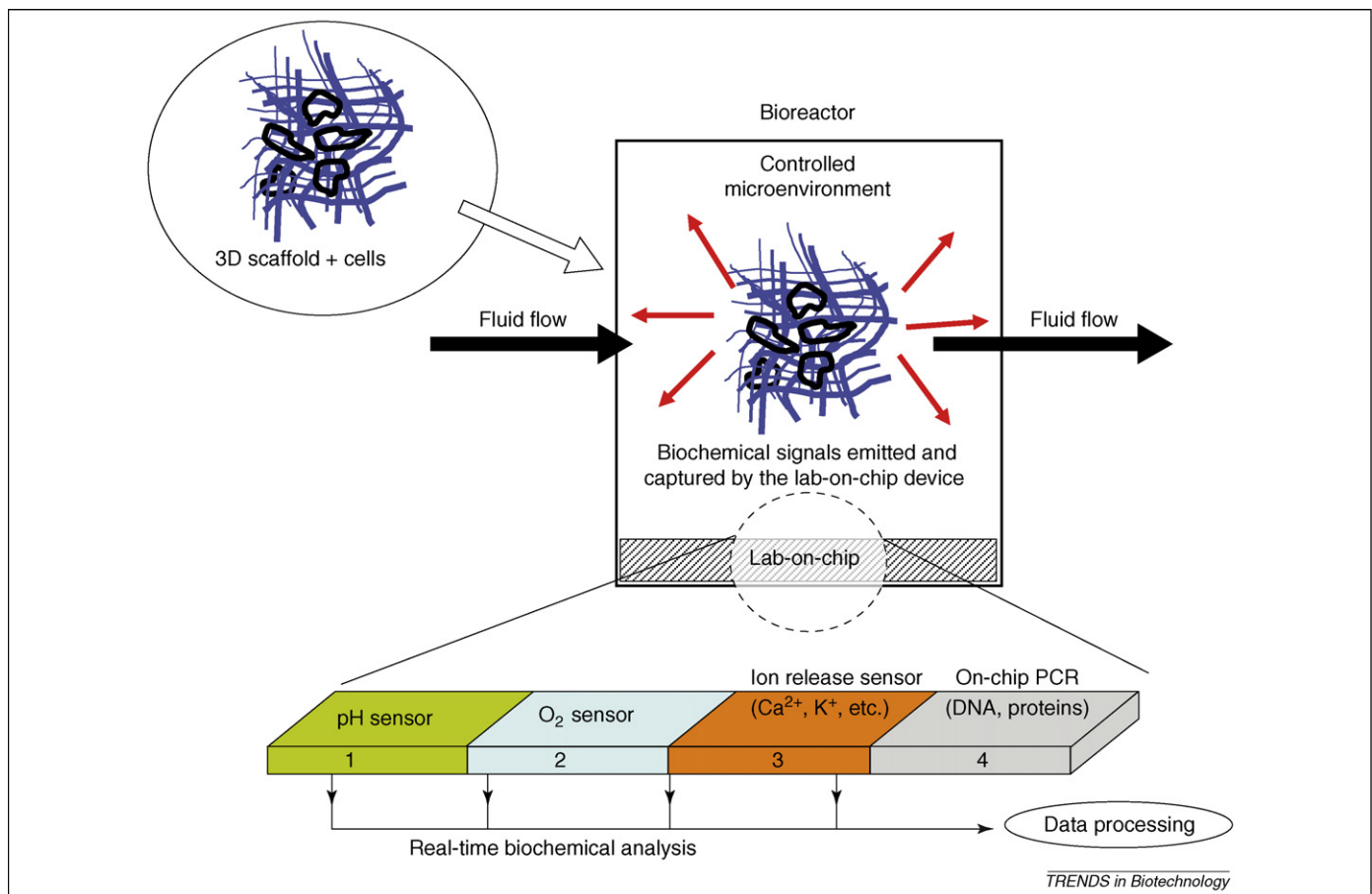
Figure 3. Bioreactors for regenerative medicine. A 3D scaffold with cells is cultured within a bioreactor in which the microenvironment is controlled. The physical and chemical stimuli, which are produced with the application of a controlled fluid flow within the scaffold, induce biochemical signals that are captured by a laboratory-on-a-chip, in which several nanosensors can be integrated to detect pH, O_2, ions, DNA or proteins. These data can be analysed in real-time to regulate the microenvironments automatically through a feedback-loop mechanism.

Conclusions and future perspectives

The prospect of replacing damaged tissue with regenerated tissue would impact and change medical science definitely and radically. Today's interest in applying nanotechnology to regenerative medicine is growing owing to its capacity for producing nanostructures that are able to mimic natural tissues as well as nanoparticles for use in delivery systems.

Regenerative medicine aspects that focus on TE have evolved into two main strategies. The first strategy consists of an elegant approach in which stem cells harvested from the patient are expanded and seeded on 3D scaffolds within a bioreactor. The resulting hybrid construct is then implanted into the patient (together with growth factors) as a tissue matrix. However, the need to harvest and expand stem cells poses great efficacy and efficiency problems that define the success of the entire process.

The second strategy relies on the development of intelligent materials that would be able to send signals to the stem cells already present in the diseased or damaged tissue niches that would then trigger the regeneration process. Nanotechnology is a powerful tool for creating these 'smart' materials. This approach is challenging and is still far from being achieved. Among other advantages, it would raise the possibility to have such cell-free materials ready 'off the shelf' and to be able to use them as and when required.

Finally, because nanotechnology is a novel tool, the concern about the safety of nanomaterials used in regenerative medicine for human health has not been addressed in a sufficient and satisfactory manner. Before nanomaterials will be used in human applications, it will be necessary to carry out consistent and comprehensive studies to ensure their innocuousness.

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