

Nanotechnology in Regenerative Medicine

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3.1 Definition

For the purpose of this report, regenerative medicine describes those nanomaterials and techniques employed to repair or replace damaged soft and hard tissue.

3.2 Short Description

Tissue engineering is the use of cells and their molecules in artificial constructs that compensate for lost or impaired body functions¹. Scaffolds made of porous biomaterials which mimic the cellular environment are seeded with cells and allowed to grow there. The grown tissue construct is then implanted into the body of the patient where it replaces the diseased tissues and the scaffold degrades. Since its inception in the 1980s, the technology has grown to a stage where it has been used to replace pulmonary arteries² and to regenerate human thumb tissue³.

With the emergence of nanotechnology and new characterisation tools, it has become easier to synthesise and characterise materials at the nanoscale to enhance the activities of biological molecules and to mimic the biological functions. The advantage of nanotechnology based methods and materials in regenerative medicine is that tissues and associated extracellular matrix (ECM) which help in the regeneration of tissues in biological systems are also nanostructured materials. The interaction between ECM and the cells determines the cell growth, mobility and behaviour. The use of nanomaterials in tissue regeneration can help to create an environment which mimics the natural conditions promoting cell adhesion, cell differentiation and cell growth.

The delivery of proteins, peptides, genes and other growth factors in a sequential manner is also important in assisting cell growth. These bioactive signalling molecules trigger the regenerative activities by their entrance into the cellular matrix at the appropriate time. Methodologies used to incorporate these molecules into the artificial cellular matrix using nanotechnology have been proposed. These include the use of polymers in ECM to immobilise these triggering agents. Functionalisation of scaffolds with different biomolecules to target different types of cell has been proposed as a strategy to improve tissue adhesion and growth. This report examines some of the recent advances in regenerative medicine enabled by the use of novel materials, developments in bioreactor technologies and nanotechnology.

3.3 State of R&D

The greatest advantage of using nanotechnology in tissue engineering is that the novel properties of nanomaterials make the cell interaction and other cellular functions much more efficient than traditional materials. The building blocks of tissues have a nanoscale structure which makes nanomaterials best placed to assist in their regeneration. For example, non collagenous proteins, fibrillar collagen and embedded mineral crystals in human femur have a nanometre level structure⁴. Nanomaterials have a larger surface area than other materials which allows high protein absorption. In tissue generation processes, proteins normally get absorbed onto the surface of materials more quickly than the cells and they have the capability of enhancing or inhibiting the cellular growth processes. Vitronectin, a protein which enhances osteoblasts adhesion, can adsorb better on nanophase alumina than conventional, therefore enhancing cell adhesion⁵. It has also been recently reported that nanotopography has greater influence on cellular functions and impacts activities like cell adhesion, proliferation and differentiation⁶.

3.3.1 Nanophase Materials

The main requirements for a scaffold for regenerative applications are that it should have a highly porous structure, and be non-toxic and biodegradable. It should also have good mechanical integrity to maintain the pre-designed tissue structure, good pore size and large surface area. Finally, the scaffold should interact positively with cells enhancing cell growth, adhesion, migration, and differentiation⁷. Nanophase materials (which have grain sizes less than 100 nm) can be used to enhance tissue regeneration and to improve cell adhesion, cell spread and migration. The ability of these novel materials to replicate the characteristics and simulate the functions of several body tissues has been studied.

Nanophase titania and alumina ceramics show increased cell adhesion and could have applications in orthopaedics and dentistry.⁸ These materials were also found to enhance osteoclast and osteoblast functions⁹.

Nanophase Ti, Ti₆Al₄V and CoCrMo alloys were synthesized to examine whether nanostructured surfaces enhanced osteoblast metabolic functions¹⁰. Initial studies showed deposition of calcium and phosphorous on metal surfaces, which is an indicator of high osteoblast activity. However, it is yet to be confirmed whether this increased deposition on metal surfaces is due to the increased osteoblast metabolic activity or simply precipitation. Nevertheless, it is an indicator that nanophase metals may promote bone regeneration.

It has been reported that high pressure torsion (HPT) processed titanium nanograins improve osteoblast adhesion¹¹. The HPT technique that exerts the highest hydrostatic pressure is of particular interest, since it can most effectively refine metal grains and produce the finest nanostructures of all SPD (severe plastic deformation) techniques. It also reduces porosity, cracks, and other macroscopic defects¹². Using HPT, titanium grains less than 50 nm were found to increase adhesion and cellular growth in pre-osteoblasts attached to the nanophase. It could also effectively absorb fibronectin for assisting in cellular adhesion, growth, and spreading. Preferential growth of osteoblasts over fibroblasts was observed, which is desirable for bone regeneration as fibroblasts may cause detrimental implant loosening¹³. However, further investigation is required to understand this preference.

Nanophase coatings on the surface of biomaterials have been used to improve their biocompatibility and bioactivity. Nanophase hydroxyapatite (HA) has been coated onto titanium¹⁴ and tantalum¹⁵ to improve osteointegration and promote bone growth. Soboyejo *et al.*¹⁶ report the deposition of titanium on silica which was then functionalised with arginine-glycine-aspartic acid for improved biocompatibility. Human osteosarcoma (HOS) cells cultured on the coated surfaces showed increased cell spreading and improved cell adhesion. The method has potential applications in implantable BioMEMS devices (mainly used in *in vivo* applications). Nanocrystalline organoapatite (a biomimetic hydroxyapatite-based material) was coated on titanium mesh to increase proliferation and improve migration of osteogenic cells¹⁷.

3.3.2 Nanocomposite Scaffolds

Polymers are used widely in drug delivery applications to enhance the hydrophilicity of the non-soluble drug carriers and are functionalised with biomolecules for targeting specific cells. One of the most widely used polymers in drug delivery is polyethylene glycol (PEG). Attaching PEG to other molecules helps to increase the solubility and stability and prevents the rapid clearance of drug conjugates by the reticuloendothelial (RES) system. In tissue engineering, similar techniques have been used to deliver genes, peptides and other growth factors for biosignalling purposes. Conjugating polymers with other nanomaterials help in controlling and monitoring the cell growth while providing timely triggers to accelerate the process. The most useful of these are stimuli response polymers which respond to changes in pH, temperature, magnetic field and other external factors to enable external cell growth manipulation. The most commonly used polymers are PEG, polylactic acid (PLA), polyglycolic acid (PGA), poly (N-isopropylacrylamide) (PNIPAAm), polyelectrolytes, such as polymethacrylic acid (PMAA), polyacrylic acid (PAA) and poly(lactide-co-glicolide) (PLGA). PNIPAAm has been identified as the most temperature sensitive polymer used¹⁸.

Several studies have shown that the use of polymers in tissue engineering is desirable due to the increased biodegradability. During the natural tissue regeneration process, they degrade *in vivo* by hydrolysis into non-toxic products which enter into normal metabolic pathways and are excreted from the body as carbon dioxide and water¹⁹. The use of PLGA in bone regeneration increased osteoblastic functions and reduce fibroblast activities²⁰.

Combining nanophase materials with polymers has been shown to improve the mechanical properties in biocomposites, and improve adhesion and calcium deposition in bone regeneration processes. PLGA with nanophase titania has been used to improve osteoblast and chondrocyte adhesion *in vitro*²¹. The use of PLA with high porous nanophase HA (hydroxyapatite) scaffolds can increase the mechanical properties and osteoblast functions²².

Large improvements in mechanical properties such as hardness and tensile strength, as well as bending capabilities, have been reported due to the decrease in material size to nanosize. Composite scaffolds of needle-like hydroxyapatite (HA) nanoparticles and PLA have been reported, that mimic the mechanical strength and microstructure of bone²³. Compared to scaffolds made of pure PLA, the nanocomposite scaffolds were found to have high cell affinity and biocompatibility. A PLGA/HA composite has been used for cartilage regeneration²⁴.

3.3.3 Nanofibre Scaffolds

Novel nanobiomaterials and the associated triggering mechanisms have the ability to mimic the ECM, the complex structure which enables tissue regeneration. Nanofibres, made of synthetic or natural materials, have been proposed as one of the most promising materials to develop scaffolds. These fibres have a high surface area and very good porosity which makes them suitable for enhanced cell colonisation and efficient nutrient exchange between cells and the external environment. In order to be used for scaffolding purposes the material should be able to interact with the cells in three dimensions and facilitate the communication between cells and the stimulants²⁵.

Proteins that make up the natural ECM have sizes in the nanometre range, making it vital when mimicking this synthetically to create a matrix that has dimensions in the nanoscale. The use of polymers in tissue engineering and gene delivery is well known. However, the problem with conventional methods in polymer processing is that it is not possible to achieve fibres less than 10 μm . This makes it undesirable for creating a perfect ECM that can replace the natural one. However, three methods have been found to be useful for producing fibres in the nanometre scale. They are self assembly, phase separation and electrospinning.

Self-assembly is the process in which molecules and supramolecular aggregates organise themselves into an ordered structure through weak and non-covalent bonds²⁶. The challenge is controlling this spontaneous organisation of components to form structures that can dynamically change and reorganise as required. Self-assembly creates the smallest nanofibres, the low productivity and scale-up difficulty of this method make it currently undesirable for commercial applications.

pH-induced self-assembly of a peptide-amphiphile was used to produce nanofibres of 5-8 nm diameter²⁷. The nanofibres were then cross linked and used to form an HA composite material which mimicked the matrix arrangement that exists between collagen fibrils and HA crystals in bone. A self-assembled peptide hydrogel as a scaffold to encapsulate chondrocytes for cartilage tissue repair and regeneration has also been reported²⁸. Recent developments in peptide self assembly with potential applications in nanobiotechnology have been reviewed by Lu *et al.*²⁹.

The second method used for the production of nanofibres is phase separation. It is the thermodynamic separation of a polymer solution into a polymer-rich component and a polymer-poor/solvent-rich component³⁰. The polymer is dissolved in a solution and is induced thermally to create a gel. Non-solvent is also used for phase separation. The solvent is then extracted from the gel by adding water and the gel is cooled and freeze dried to produce nanofibres, typically from 50-500 nm³¹. Biodegradable aliphatic polymers are normally used in this technique.

This method allows control of the pore size and scaffold structure for various applications by the addition of various porogens or by varying polymer concentrations. Additionally, this method doesn't need specialised equipment and is easy to carry out. However, similar to self assembly, this process is restricted to laboratory use and can only be used with a limited number of polymers.

The third and the most widely used method for the preparation of nanofibres is electrospinning. One of the oldest methods, it uses an electric field to create nanofibres. An electrostatic force is applied between the positively charged polymeric solution in a capillary tube and the substrate. When the charge becomes higher than the surface tension of the capillary tip, a fine polymer jet is created which is deposited on the substrate. One of the advantages of this process is that a variety of synthetic and natural polymers can be used. It is also possible to mix different types of polymers to create nanofibres and the thickness of the fibres can be controlled by changing the amount of polymer in the solution. By controlling the mechanical characteristics and scaffold geometry, this method can be used to produce fibres for a variety of applications. A wide range of polymer thickness from several microns to less than 100 nm can be produced with this method. One of the biggest advantages of this technology is that it is simple and scalable. However, it has been argued that the technique creates random voids in the scaffold and could result in micron sized scaffolds, depending on the type of polymer used. Due to this there is no suitable way of making a uniform, controlled, 3D pore structure in scaffolds created through this method³². However, several studies suggest that both the phase separation and electrospinning can be used to produce fibres of less than 100 nm³³.

Living cells have been incorporated in cellular matrices using electrospinning. Simultaneous electrospinning and electrospinning has been used to incorporate living cells in extra cellular matrices in a one step process³⁴. Vascular smooth muscle cells (SMCs) were electrospayed while electrospinning poly (ester urethane) urea (PEUU) to incorporate them into the scaffolds. This method offers a quick alternative to create tissues and other structures which takes long time to develop in the traditional bioreactor based methods. There was no decrease in cell viability and proliferation was similar to the cultures without SMCs. Muscle cell integrated PEUU fibres were found to be stronger and flexible with high tensile strength.

3.3.3.1 *Polymers used in Nanofibre Scaffolds*

Different types of polymers, both synthetic and natural, have been used to create nanofibres. The advantage of natural polymers is that they can contain several growth factors and biosignalling molecules that can trigger cell growth³⁵. The polymers that are used to make nanofibres and their potential uses are reviewed in this section.

PGA (polyglycolic acid) and PLA (polylactic acid) are two of the most commonly used polymers. One of the advantages of PGA is that it has a predictable bioabsorption³⁶. However, it has high degradability which may change the pH, creating unwanted tissue responses. Using PLA is advantageous because it is highly soluble in organic solvents, reduces hydrolysis and increases the time before degradation. A PLA scaffold for nerve tissue regeneration has been reported³⁷. A mixture of both polymers has been proposed to obtain desirable scaffold qualities.

The polymer PLGA has been widely used for tissue engineering purposes. Although the fibre dimensions of nanofibres created through this process are similar to PGA, the ability to control the mechanical properties gives it an advantage over solely PGA based nanofibres. PLGA-based nanofibres have been used for cardiac tissue engineering applications^{38,39}.

Polydioxanone (PDO or PDS) is a colourless, crystalline, biodegradable polymer used mainly in the preparation of sutures. It has been proposed as a suitable material for tissue regeneration and particularly for engineering vascular grafts⁴⁰. The mechanical properties of nanofibres of PDO resemble that of natural soft tissues like collagen and elastin⁴¹.

Polycaprolactone (PCL) is another polymer which has been successfully electrospun and useful for scaffolding. This elastic polymer has low toxicity and slow degradation, and has been used to create scaffolds for bone regeneration⁴² as well as cardiac graft engineering⁴³. PCL is also blended with other polymers, such as PGA and PLA, to incorporate the desirable qualities of both the polymers. For example, PCL mixed with PLA has shown increased flexibility and elasticity while maintaining the tensile strength of PLA fibres⁴⁴. This mixture is ideal for creating scaffolds for vascular grafts as these require both these qualities to withstand pressures created by blood flows.

Natural polymers have also been used to create nanofibres that mimic the ECM. Elastin has been electrospun to produce nanofibre matrices. Being one of the most important proteins that constitute artery walls, elastin has huge potential applications in vascular tissue engineering. Nanofibres of PDO and elastin were used to mimic cardiovascular grafts⁴⁵. The grafts had mechanical properties similar to that of native arteries and the bioactive nature of the elastin allowed the cells to migrate the full thickness in 7 days. Nanofibres and fibre networks of elastin-mimetic peptide polymers have also been reported⁴⁶.

Fibres of globular proteins like fibrinogen, haemoglobin and myoglobin have also been reported for applications in wound dressings, hemostatic products and scaffolding. The high surface area offered by these fibres make them ideal for hemostatic applications, particularly in enhancing resistance to infection in wounds. Electrospun fibrogenin fibre mats have been used as scaffolds for growing human bladder smooth muscle cells⁴⁷.

Collagens and derived materials e.g. gelatins, have also been used to form fibres to produce matrixes for various applications⁴⁸. Electrospun collagen was used to produce scaffolds for cartilage tissue engineering⁴⁹. Collagen nanofibres have also been used to grow mesenchymal stem cells with high amounts of osteogenic gene expression⁵⁰. Scaffolds made from electrospun fibres of gelatin have shown similar properties (cell adhesion, proliferation, attachment etc.) to that of nanofibre scaffolds made of other proteins like collagen, solubilised alpha-elastin, and recombinant human tropoelastin⁵¹.

Collagen blends, created by mixing collagen with other polymers, have also been reported to produce fibres for scaffolds. Nanofibres and non-woven fibre networks made from a mix of type I collagen and PEO (polyethylene oxide) have been reported for applications in tissue engineering, wound healing and as hemostatic agents⁵². The tensile strength and elasticity of the fabrics was dependent upon the weight ratio of the collagen-PEO blend. A mix of collagen and elastin was used to produce fibres for creating three layered vascular tubes. Studies showed that seeded endothelial cells, fibroblasts and muscle cells tended to grow and proliferate⁵³.

3.3.4 Bioactive Scaffolds

As mentioned earlier in this report, the topography of the scaffolds plays an important role in determining the cellular growth and other properties such as cell adhesion and proliferation. The use of polymers to create nanosized fibres help the process by making the scaffolds more hydrophilic and providing larger surface areas to grow and penetrate. Several techniques like soft lithography, polymer etching, demixing, dip-pen lithography, colloidal lithography, electron beam lithography etc. have been used for nanopatterning the surfaces to make them more bioactive. In addition to the above mentioned processes, functionalisation of the polymers with biomolecules and nanoparticles has been proposed to improve cell adhesion, migration and differentiation. Attaching growth factors to the surface of polymers helps to increase the cellular growth properties by triggering signals at the correct time. The bulk modification of the surfaces can also be achieved by co-polymerisation of the polymer chains.

Cross linked hydrophilic polymers that can absorb water without dissolving are widely used to create matrixes for tissue engineering. The porosity, easy modification and biocompatibility make these hydrogels attractive in regenerative medicine. Addition of certain ligands on the polymer surface has been shown to offer better control over cell behaviour in the ECM.

Lindermann *et al.*⁵⁴ have shown that coupling of the ligand arginine-glycine-aspartic acid (RGD) as high density islands can be used to alter cell behaviour in tissue culture. In their study, they controlled cell spreading, osteogenic differentiation and focal adhesion kinase (FAK) Y397 phosphorylation of MC3T3-E1 preosteoblasts by spacing the ligand islands in the hydrogel. If the ligand islands were closely distributed, it favoured FAK Y397 phosphorylation and cell spreading. But, when they were more widely spaced, it favoured differentiation while proliferation depended on the RGD bulk density. Functionalised peptides on nanocomposites can also enhance cell adhesion and regeneration⁵⁵.

Albumin nanoparticles functionalised with fibronectin were found to enhance cell secreted protein deposition on extracellular matrix fibrils⁵⁶. Antibody CD34 coated polytetrafluoroethylene (ePTFE) grafts have been shown to increase endothelialisation compared with bare grafts⁵⁷.

Another novel strategy used in tissue engineering is the use of magnetic nanoparticles to control the development of multilayered cell sheet-like structures, ECM and tubular structures. Liposomes can be used as carriers. The magnetic nanoparticles can be attached to the cells by endocytosis. The magnetically labelled cells can be then controlled externally using magnetic forces. It is also possible to conjugate different types of biomolecules like antibodies with magnetic nanoparticles to target specific cells. This method of manipulating the cell functions using magnetic force is called magnetic force-based tissue engineering (Mag-TE)⁵⁸. It has been found that the presence of magnetic force enhances cell adhesion, seeding and proliferation. Stem cells and progenitor cells can be magnetically tracked by labelling with iron oxide nanoparticles.

Bulte *et al.*⁵⁹ report the use of iron oxide nanoparticles to develop magnetodendrimers that can be used to label human neural stem cells (NSCs) and mesenchymal stem cells (MSCs) through nonspecific membrane adsorption processes. Sasaki *et al.*⁶⁰ report that coating magnetic nanoparticles with chitosan enhanced cell invasion efficiency and propose this method as a useful strategy to avoid tissue necrosis. The degree of magnetic force determined the efficacy of invasion. The system was found to enhance the cell seeding, cell-cell interactions and shortened the cell proliferation time. Perea *et al.*⁶¹ seeded human smooth muscle cells labelled with magnetic nanoparticles onto the luminal surface of a tubular shaped collagen membrane to create vascular grafts. Vascular grafts are multilayered structures and their tubular geometry makes conventional engineering practices almost ineffective. After 5 hrs they seeded human umbilical vein endothelial cells labelled with magnetic particles. Using a magnetic force for cell seeding (20-40 min) improved the seeding efficiency to 90%. Histological examination after five days of incubation have revealed densely packed multilayers of smooth muscle cells covered by a monolayer of endothelial cells.

However, one of the issues of using magnetic force in cell functional enhancement is that it may trigger the undesirable growth of endothelial and smooth muscle cells creating intimal thickening. The labelling technique has also been used to visualise and track cell migration after implanting. Noth *et al.*⁶² report the use of super paramagnetic iron oxide particles to label human mesenchymal stem cells to track their migration using MRI after transplanting it for cartilage repair.

3.3.5 Carbon Nanotubes

The unique capabilities and properties of carbon nanotubes (CNTs) make them potentially useful for various applications from drug delivery to sensing. These capabilities can also be used for tissue engineering. The sensing capabilities of CNTs can be used to monitor and evaluate the cellular interactions and the environmental changes once the tissues have been implanted on the body. The large surface area of CNTs makes them suitable for immobilising a variety of biomolecules and the small size makes allows for cellular sensing, tracking and labelling. The optical labelling of cells with CNTs helps in tracking cellular migration pathways, understanding the cell biodistribution as well as the evaluation of engineered tissues⁶³. Studies have shown that nanotubes can remain in the cells for a prolonged time during cell division implying that they can be used for studying cell proliferation and stem cell differentiation⁶⁴. They can also be functionalised to monitor cell functions using MRI. Functionalised nanotubes have been used for delivering drugs and nucleic acids. This capability can be utilised to deliver growth factors to cells to manipulate the cell growth.

However the most important use of nanotubes is in enhancing the capabilities of extracellular matrixes. Carbon nanotubes blended with polymers can be used to grow cells. A nanocomposite formed by blending collagen with SWNT has been used to grow living smooth muscle cells⁶⁵. A nanocomposite made of ultra short SWCNTs and poly propylene fumarate (PPF) has been used for bone tissue scaffold⁶⁶. Studies on a rabbit model using porous nanocomposite and comparing it with PPF polymer control scaffolds have shown that the nanocomposite enhanced bone tissue growth three times greater than the control. It also increased connective tissue organisation and reduced inflammatory cell density while the biodegradability was found to be similar to that of PPF.

Nanotubes can also be used to provide structural support to matrices made of polymers such as PLA, PGA or chitosan⁶⁷. Carbon nanotubes have also been cross-linked in hydrogels to increase the rigidity and to enhance cell growth. Alginate is a viscous gum used for mould-making in dentistry, prosthetics and lifecastingⁱ. CNTs have been used to increase the mechanical strength of alginate hydrogel⁶⁸. The CNT-Alg gel also displayed faster gelling while the saline sorption was comparable to that of conventional gel.

ⁱ <http://www.wordwebonline.com/search.pl?ww=5&w=alginate>

Polymer-functionalised CNTs have been used to grow neurons^{69,70}. Sheets and yarns derived from MWCNTs have been used to grow skin fibroblasts and Schwann cells. Carbon nanotube fibres prepared by a particle-coagulation spinning process have also been shown to promote mammalian and neuron cell growth⁷¹.

It was recently reported that coating CNTs onto electrical devices implanted in the nervous system improved electrical stimulation in rats and monkeys⁷². Conventional electrodes coated with CNTs showed increased charge transfer and decreased electrical impedance.

CNTs have been proposed as an excellent material in tissue engineering. Their rigidity and strength make them appealing in bone regeneration and their electrical properties are attractive for neuronal growth. However, the risks associated with CNTs are the subject of debate. The toxicity of nanotubes was found to be reduced or eliminated to a large extent by functionalising them with various polymers, chemicals and molecules. However, the biodegradability of the materials still remains an issue. The ideal situation is that these novel materials are removed by the body through normal metabolic processes. However, this has not yet been confirmed. While nanotubes promise a wealth of possibilities in tissue engineering, the risk of these materials should be assessed thoroughly and comprehensively before purposefully introducing them into the body.

3.3.6 Cell Sheet Engineering

In tissue engineering, the cultured cells are detached from the ECM using proteolytic enzymes such as trypsin. However, in this process cell-cell junctions are broken and cells are harvested as single cells. There is also the possibility of damaging cell membrane proteins. These single cell suspensions injected into animals for the transplantation of tissues may not work in large or hard tissues. To address these issues, temperature sensitive polymers have been proposed as an alternative to harvest the cultured cells⁷³. A poly (*N*-isopropylacrylamide) sheet (~ 20nm thick) was covalently attached to the surface of the culture dish. Cells were allowed to grow, migrate and proliferate at 37°C. At this temperature the polymer is hydrophobic, enhancing cell regeneration. However, when the temperature was reduced below 32°C, the polymer became hydrophilic, spontaneously lifting the cells from the surface as a single sheet, without the use of any proteolytic enzymes.

The method has been used for various tissue engineering applications including corneal reconstruction⁷⁴, periodontal regeneration, bladder augmentation, and cardiac patches.

However, one of the problems with this method is that the amount of ECM attached to the cell sheet is very low compared to other harvesting methods, so it is not ideal for creating cell-sparse tissues like bone or cartilage. But cell sheets composed of periosteum or perichondrium can be used for developing hard tissues⁷⁴.

3.3.7 Stem Cells

Nanotechnology can be used to encourage the growth and influence the differentiation of stem cells. As mentioned in section 3.3.4, magnetic nanoparticles can be used to label stem cells, as can quantum dots. Nanotechnology-based delivery systems could also be used to delivery of biomolecules required for differentiation.

Cells can respond to the shape of their environment. Mesenchymal stem cells (MSCs) can be grown on surfaces with nanoscale topographical features. Changes in these features can alter the differentiation pathway that stem cells can take by mimicking the complex structure of natural ECM. Initially, surfaces were roughened at random in an irreproducible fashion. This led to conflicting results due to the lack of consistency between studies. The development of new methods for nanopatterning surfaces have led to ordered, reproducible nanotopography being studied. For example, MSCs from bone marrow usually produce soft tissue, rather than bone, on metal implants. However, nanopatterned polymer surfaces created using electron beam lithography have been used to stimulate MSCs to produce bone mineral *in vitro*, in the absence of osteogenic supplements⁷⁵. MSCs grown on polymer nanogratings have been induced to follow a neuronal lineage⁷⁶.

Other uses of stem cells are covered in the Surgery, Implants and Coatings subsector report. A recent review also covers this area in further detail⁷⁷.

3.3.8 Bioreactors, Biocapsules and Biochips

Bioreactors are conventionally used to grow tissues *in vitro* by providing optimum conditions like temperature, pH, pressure etc. The reactors currently used in tissue engineering are mostly on the μl scale and are called micro bioreactors. However, cellular actions take place at the nano or pico level. Scaling down reactors will allow understanding of the cellular operations at their functional level and enable the creation of an optimum environment to speed up cell regeneration. Nanobiosensors can be used in these bioreactors to provide information on the changes taking place. This can be utilised to create a feedback mechanism to change the conditions automatically to suit the cell growth. These sensors can also be integrated with 'Lab on a chip' devices to monitor and manipulate the conditions inside the reactor to maximise cell adhesion, migration and proliferation. Additionally, the presence of nanosensors can reduce assay response times, reduce the analyte volume and allow parallel assay operation.

Prokop *et al.*⁷⁸ have developed such a system for cellular bioanalysis. They designed three types of bioreactors each having a culture chamber, inlet and outlet ports and microfluidic passages. The incorporation of nanosensors allowed the *in situ* measurement of physiological events in real time, opening up possibilities to create better bioreactors. The system they have developed has been applied to measure the reactions of three different cell lines in parallel which are of particular significance to pharmaceutical industry.

Microfluidic devices which can mimic the functions of several organs and the creation of an *in vivo* environment on a chip are becoming one of the most studied areas. These devices will reduce the complexity and huge expenses associated with *in vivo* testing. These microfluidic devices contain microchannels and microwells which mimic the complex tissue architecture. The micro chambers inside the cell biochips contain cell cultures or engineered tissues which are connected through a microfluidic network that transport nutrients and remove waste similar to in the body. Shuler *et al.*⁷⁹ reported the development and use of such a biochip system for the *in vitro* analysis of chronic liver toxicity of engineered tissues. The system they have developed is called microscale cell culture analog (μCCA) and consists of four chambers which mimic the pharmacokinetic model of a rat. L2 was selected to mimic lung, HepG2/C3A for liver while differentiated 3T3-L1 adipocytes were selected to mimic the functions of fat. Studies were conducted to analyse the bioaccumulation, distribution and toxicity of various compounds like fluoranthene, naphthalene etc.

The system is a starting point in developing microfluidic devices that can be used to mimic the *in vivo* functionalities *in vitro* and as an alternative to conducting expensive and complicated tests prior to animal trials. One of the additional benefits of a cell biochip is that it helps to preserve the cellular functions over a long term. This solves the problems with the use of Petri-dish methods for toxicology studies that need a long time frame for experiments. In Petri-dishes it is virtually impossible to keep their specific activity over a longer term due to the dedifferentiation of the cell types⁸⁰.

In cell based therapies, immunoisolation of implanted cells are very important for sustained release of therapeutics. However, the rejection of implanted cells has been high in past experiments conducted in this area. This may be due to many reasons including the biomechanical instability, anomalies in the pore size distribution, mechanical rupture of the membrane or due to the compatibility issues of the cell islets⁸¹. In addition to that, failures have also been attributed to the increased reaction of the grafted cells with cytokines and the leakage of antigens out of the biocapsules stimulating macrophage activation⁸². However, the key in the success of such implanted cells for sustained and controlled drug release is that it should be designed in such a way to allow the transfer of essential nutrients in and prevent the movement of macrophages, cytotoxic cells and antibodies into the system.

Desai *et al.*⁸³ report the development of such a biocapsule which allows the movement of molecules less than 7 nm and prevents those larger than 15 nm. Based on BioMEMS fabrication methods, they created membrane surfaces with uniform pore sizes as low as 7 nm. The membrane thickness was in the microscale (100-200 μm) to improve the dynamic responses. Perhaps one of the most important features of this novel approach is that it allows the replacement of islets even after implanting it on the body. This is particularly useful if the cells are required for a long term. The system was successfully tested for cell delivery in diabetic rats⁸⁴. The insulinoma cells encapsulated in the capsule were implanted into rats and diabetes was reverted from day 1. They also showed that a pore size of less than 20 nm is capable of retaining the insulin stimulation capability of the islets *in vivo*⁸⁵. The technology, which is capable of immunoisolation and biomolecular separation, also offers future potential in terms of incorporating nanosensors into the system for better control over release and real time monitoring, localised release of drugs and the immobilisation of growth factors.

3.4 Additional Demand for Research

- Design and development of novel nanophase materials which can enhance tissue engineering properties utilising nanotopography
- Methods to control and manipulate the nanomaterial topography for specific requirements.
- Development of intelligent polymers which can respond to changes in the environment
- Development of novel biomaterials and polymers which can be used as coatings to improve biocompatibility.
- Design and development of nanocomposite scaffolds that promote cell growth and proliferation.
- Scaffolds that can recruit cells instead of being seeded.
- Methods to create uniform, controlled, 3D pore structure in scaffolds
- New methods to incorporate living cells and other growth factors in scaffolds to trigger growth signals in a timely fashion.
- Techniques for the controlled release of growth factors
- Study into the potential of natural polymers as scaffolds.
- Potential of carbon nanofibres and nanotubes in neural engineering.

- Development of nanosensors which can be incorporated into nanobioreactors.
- Methods for ensuring their stability in the system
- Development of nanosensors which can monitor the tissue regeneration process *in situ*
- Development of novel materials which can self assemble *in situ* to form ECMs
- Suitable encapsulation techniques for the immunoisolation of implanted cells.

3.5 Applications and Perspectives

The growth of nanotechnology and associated characterisation techniques has increased the hope that tissues which can mimic the cellular functions can be regenerated more efficiently and be grafted into the body without rejection. Nanomaterials, due to their larger surface area and smaller thickness, offer the possibility of enhancing cell adhesion, migration and proliferation. Advances in various methods of nanopatterning polymers to make them suitable to grow different cells played an important role in the current growth of tissue engineering. It has been reported that nanotopography plays an important role in the functional behaviour of scaffolds and affects the various properties like cell adhesion and cell differentiation. The scaffolds that are made out of nanophase composites or from nanofibres are promising biodegradable platforms which can be used to grow cells *in vivo* or *in vitro*. Functionalising these scaffolds with several biomolecules can be used to trigger the cellular activities at the right time to enhance cellular regeneration. Cell sheet engineering, which allows grafting the cells along with the scaffold without the loss of cell-cell junctions using temperature sensitive polymers are particularly useful in cardiac engineering and corneal tissue regeneration.

Carbon nanotubes and carbon nanofibres have also been found to be extremely useful in creating neural networks. The unique properties of nanotubes have also been used to offer increased structural strength to the scaffolds. The application of alternating current on MWCNT composites can increase the osteoblastic proliferation by over 45 % and calcium deposition by over 300 %⁸⁶. Several different methods and materials based on nanotechnology offers hope that creating an artificial organ which can be similar in function to the natural organs are not too far away. Readers are advised to read this report along with the Implants, Surgery and Coatings subsector report, to gain an understanding of the role of nanotechnology in tissue engineering, novel implants and wound care management.

As a final note it is very important to consider the toxicity of many of these novel biomaterials as there is no universal agreement in the scientific world about the potential hazards of many nanomaterials.

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