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Nano-regenerative medicine towards clinical outcome of stem cell and tissue engineering in humans

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Abstract

Nanotechnology is a fast growing area of research that aims to create nanomaterials or nanostructures development in stem cell and tissue-based therapies. Concepts and discoveries from the fields of bio nano research provide exciting opportunities of using stem cells for regeneration of tissues and organs. The application of nanotechnology to stem-cell biology would be able to address the challenges of disease therapeutics. This review covers the potential of nanotechnology approaches towards regenerative medicine. Furthermore, it focuses on current aspects of stem- and tissue-cell engineering. The magnetic nanoparticles-based applications in stem-cell research open new frontiers in cell and tissue engineering.

Keywords: nanotechnology • magnetic nanoparticles • nanomaterials • regenerative medicine • stem cells • tissue engineering

Introduction

Nanotechnology and nanoengineering are the science and engineering involved in the design, synthesis, characterization, and application of materials and devices. Its smallest functional organization (in at least one dimension) on the nanometre scale ranges from a few to several hundred nanometres. The bulk of nanoengineered substrates are designed with very specific and controlled chemical and physical properties. It further results in strong control over the molecular synthesis and assembly designs. These materials and devices with a high degree of functional specificity interact with cells and tissues at a molecular (*i.e.* subcellular) level, thereby allowing a degree of integration of technology with medicine and physiology that was at all not previously attainable. The significance of nanoscience and latest nanotechnologies for human health and the associated opportunities, and developments is well addressed [1].

In all tissue types, a specific three-dimensional microenvironment surrounds every cell. This microenvironment comprises several other cells, extracellular matrix (ECM), proteins and a range of soluble as well as ECM-bound factors. The ECM is composed of a variety of molecules (ranging several hundred nanometres) that include collagens, glycoproteins, glycosaminoglycans and proteoglycans [2] and its primary function is to provide structural support to the composing cells. It also includes several distinctive constitutive structures in the basement membrane like pores, fibres and ridges of nanometre dimensions. The topographies present in the surrounding environ-

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ment of cell directly affect the cellular behaviour such as cell adhesion, migration, proliferation and differentiation. The fundamental understandings of biological and physical interactions of cells with their surrounding environment are the key to excel in the field of regenerative medicines [3]. Nanotechnological principles support the creation of these smart materials, but this approach is still much far from being achieved. A technique to replace/repair diseased tissue or organs by *in vitro* and *in vivo* pathways is regenerative medicine. These medicines have potential to restore the function of lost, damaged cells or ageing cells by replacing them with new ones in human body. Cells usually need some scaffold material for their proliferation. The surrounding environment is composed of nanometre-scaled particles that provide distinctive biological signals, which finally decide the reaction and behaviour of the cell [3]. Regeneration can be achieved with living cells, which are capable of division on some material that acts as scaffolds and must produce correct signals to get the desired cell behaviour. The commercialized relevance of nanotechnology is as effective as biological ones; in addition, it produces the supporting material for technological advancements.

Along with tissue engineering, nanotechnology provides the basic grounds for the development of regenerative medicines. Nanotechnological elements used for regenerative medicine include nanoparticles, nanofibres and nanodevices. Nanoparticles were used for specific and controlled delivery of growth factors, drugs and DNA molecules to the target site, whereas, nanofibres are used for preparing tissue scaffolds and for modifying the surface of implantable materials, nanodevices such as biosensors [3]. Embryonic, foetal, amniotic, umbilical cord and adult stem cells are capable of generating multiple therapeutically useful cell types. The generated cells are used for the treatment of various genetic and degenerative disorders such as age-related functional defects, osteoporosis, spine injuries, haematopoietic and immune system disorders, heart failures, chronic liver injuries, diabetes, arthritis, muscular, skin, lung, eye and digestive disorders, Parkinson's and Alzheimer's diseases, and aggressive and recurrent cancers [4]. Stem cells are the ideal raw materials for regenerative medicines, as these are capable of generating all types of cells, tissues, and shows unlimited growth morphology. These cells have revolutionized the field of regenerative medicine as well as cancer therapies. Main target of regenerative medicine is the *in vivo* regeneration. In few cases, *in vitro* regeneration has also been performed for some complex functional tissues. Both *in vivo* and *in vitro* regeneration strategies involve the use of porous scaffold on which stem cells are loaded. These scaffolds can be natural or may be synthesized manually. Depending upon the cells that need to be targeted, functionalization of scaffold is done accordingly with a variety of biological molecules. In addition to functionalization, entrapment of growth factors, drugs or genes, peptide sequences, such as arginine–glycine–aspartic acid or proteins in nanoparticles for continued release in controlled manner will boost the success rate of regeneration [5]. The *in vitro* tissue and cell regeneration is carried out in bioreactors under controlled conditions. In addition, for commercial large-scale industrial applications, these devices proved very beneficial, as these are integrated with a variety of BioMEMS (Micro electromachanical devices) for optimizing and controlling the specific operational conditions needed for tissue regeneration [5]. For real time monitoring and detection of specific cellular processes, several biosensors and laboratory-on-a-chip are integrated inside the bioreactors. The *in vitro* cell and tissue regeneration stem cells from the patients are first harvested, and then finally being seeded on the 3D scaffolds within a bioreactor. The hybrid construct in the form of tissue matrix is thus formed and implanted back into the patient's body. Both harvesting and stem-cell expansion need great efficacy and efficiency for the regeneration operation to be successful. Major problems concerned with the *in vitro* regeneration include isolation of stem cells from the patient, proliferation of stem cells outside the body through *in vitro* systems, process of culturing stem cells in bioreactor and the time lapse during implanting the engineered hybrid construct in patient's body [3]. Another strategy for tissue regeneration makes use of some intelligent materials capable of sending signals to the stem cells present inside the body. Stem cells receive the signal surrounding the damaged or diseased tissue of the body and then perceive the signals from these smart materials and trigger the regeneration process substantially. For the first time, In 1997, Whithman *et al.* [6] had initiated integrated platelet rich plasma (PRP) in fibrin glue. Further studies [7] reported that PRP was able to induce bone regeneration of the jaw. The study on regeneration confirms the fact that stem cells of bone marrow origin are responsible for repair of mesenchymal organs. Therefore, the stem cells with multipotent differentiating potential and biological products (PRP or its gel formulation Platelet Gel, PG) with stimulating proliferation efficiency aid in tissue repair and regenerative therapeutics. Addition of biomaterials acts as potential support in proper functioning of stem cells and PRP in repair particularly in bone repair system. A complex biological phenomenon of tissue repair is affected by several factors such as age, site depth of the lesion and co-morbidity. In the regeneration process, cells produce growth factors (GF), which crucially help in exchange of biochemical information to stimulate the regeneration process [8]. The combination of clinical applications employing cell infusions, PG or both, sometimes in combination with biomaterials, is now called regenerative medicine. It is currently applied to situations where no other therapy is available. The clinical application with the best results recorded as yet includes vascular surgery, maxillo-facial surgery, orthopaedic surgery and aesthetic medicine.

A meeting of traditional sciences such as chemistry, physics, materials science and biology together generates an emerging scientific discipline of nanotechnology. Figure 1 demonstrates schematic illustration of bio & nano technology in medical sciences. The present review explores the significance of nanoscience and latest nanotechnologies for regenerative medicinal therapeutics. Addressing the collective expertise, the review also suggests how to manage far-reaching developments in these novel technologies. The successful implementation of nano-regenerative medicine will definitely be a boon to the field of clinical therapeutics in future.

Stem-cell nano-engineering

A key challenge towards regenerative medicine and cell-based therapy is to expand and derive stem cells into a specific alignment by

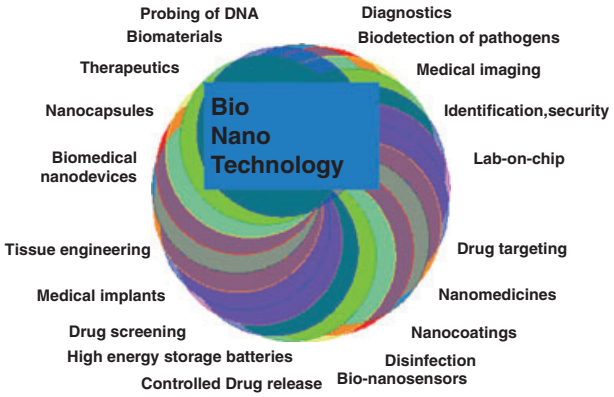

Fig. 1 Schematic illustration of bio & nano technology in medical sciences.

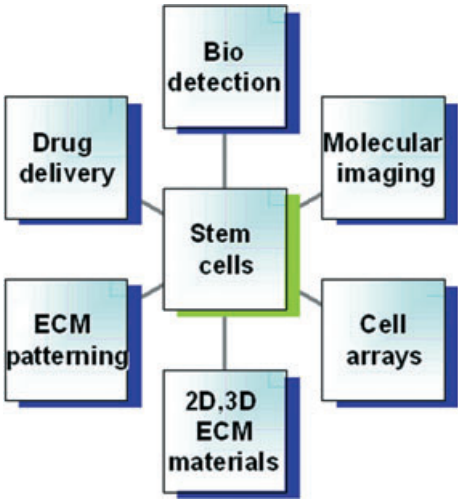

Fig. 2 Role of stem cell in biological sciences.

engineering advances in scaffold design with micro/nanotechnology. Modified nanoscale surfaces are designed for aligning a variety of stem cells including embryonic stem cells [9], mesenchymal stem cells (MSCs), [10–12] haematopoietic stem cells [13, 14] and NSC60, [15–17]. Park *et al.*, [10] had studied the effect of TiO_2 nanotube surfaces on rat MSCs and found that a spacing of 15–30 nm provides the optimum length scale for integrin clustering, focal contact formation, inducing cell proliferation, migration and differentiation into osteogenic lineages. The cellular behaviour of rat indicated that cell adhesion, spreading and growth were severely impaired on nanotubes of more than 50 nm sizes, and the cells showed apoptosis on at the level of 100 nm sizes of nanotubes [10]. A nanoisland of PS/PnBMA and PnBMA-coating was used to culture human MSCs (hMSCs). The cells cultured on the PnBMA-coating formed large dash-shaped spreader effects throughout the cell body with an organized actin cytoskeleton. However, the cells on the PS/PnBMA were smaller with marked reduction in cell spreading, and had stellate cell morphology with punctuate actin throughout the cell body along with the lack of focal contact formation [18]. Stem-cell nanotechnology is developing rapidly towards molecular imaging, and controlled proliferation and differentiation of stem cells. A very good explanation of nanomaterials for successful stem-cell labelling, tracking, gene delivery, differentiation, transplantation and their cytotoxic potential has been enlightened in nanotechnological manipulative review [19]. Figure 2 clearly depicts role of stem cells in biological sciences. The great challenge towards mechanism of interaction, function and metabolism between nanomaterials and stem cells is still the matter of future research [20]. As in Cellular cardiomyoplasty, the cell therapy-based action mechanism diminishes the size and fibrosis of infarct scars, improves viability of myocardium, induces positive remodelling (confines global ventricular dilation), stimulates ventricular and diastolic functions and induces panacrine effects [21]. Recently, to examine more about the investigative challenges, a micro- and nanotechnology-driven modern toolkit has been designed specifically for stem-cell biologists. The kit will further help in designing experiments, and their standardization in diverse physiological microenvironments [22]. The review of synergism between stem-cell biology and biomaterial technology recapitulates the molecular events involved in the production, clearance and interaction of molecules involved in regeneration/replacements of tissue/organs for novel clinical therapeutics [23].

Molecular imaging

The genetically encoded fluorescent and bioluminescent tags offer surplus information towards the living body images at the molecular level to enhance our understanding of human biology. In addition to the progress that has so far been made with the molecular agents, SPECT/CT hybrid systems capture functional information on molecular and cellular levels. They often provide anatomical detail of a targeted molecular structure more quickly, efficiently and clearly than standard imaging devices. The resultant images help in multi-functional applications such as the rapid identification of tumours, analysis of appropriate treatment, delivery of targeted therapy to precisely destroy target cells, and follow-up to assess treatment effectiveness. The novel light-producing transgenic animal model (GFAP-luc) Xenon (Society for Molecular Imaging's 3rd Annual Meeting) aids in tracking damage and repair in chronic neurological conditions such as post-ischaemic stroke or Parkinson's disease. To analyse the functioning of the heart muscles, clinicians usually recommend ultrasound-contrasting agent composed of tiny microbubbles that scatter light at specific organ of the body. The transient effect of microbubbles imaging discriminates it as one of the crucial sensitive and flexible method. It easily disrupts the pattern by adopting finer incident and reflective lights. One such example, Definity®, commonly known as Sonolysis™, are gas-filled microbubbles for novel therapeutic applications. An intravenous injection of microbubbles or local administration in vascular graft dissolves vascular thrombosis [24].

The targeted action of ultrasound is applied externally or internally (catheter) over the area of the blood clot to provide localized

prognosis of the infected patient. The microbubbles act on the principle of micromechanical device to defuse the clot. For blood clot dissolution, ultrasound pulses and blows the bubbles in the field leading to sound diffusion. Sonolysis nanosurgery is one amongst the best locally targeted nanoinvasive therapy for treatment of vascular thrombosis. It affords potential merits over the alternative therapeutic approach of mechanical thrombectomy and is faster than the conventional drug therapy with less risk of bleeding for treating thrombosis effectively. A new radio diagnostic agent, NeutroSpec^{TM}, has eliminated the need for removal and re-injection of blood into the patients. It directly labels white blood cells and myeloid precursors in less time. NeutroSpec^{TM} is available for more than 5-year-old patients with equivocal signs of appendicitis. In addition, NeutroSpec facilitates visualization of the gamma camera-generated images, thereby, allowing the physicians to locate the sites of infection [25]. It reduces the time delays and risks normally associated with alternative white blood cell labelling processes. A first class volumetric CT system, eXplore Locus Ultra, is capable of quantifying physiological measurements, elaborate anatomy of tissues, tumours and organ perfusion. The Locus Ultra also performs dynamic imaging by image acquisition within subseconds. For the coupled and functionalized approach of nanoparticles with biomolecules, an enhanced conjuctive methodology of molecular biology, bioorganic chemistry, bioinorganic chemistry and surface chemistry are required. Nanomaterials can be synthesized to desired sizes, shapes with controlled physicochemical properties [24, 25].

Nanomaterials for regenerative medicines

Nanoparticles

Nanoparticle synthesis for regenerative medicines mainly focuses on development of entrapment and delivery systems for genetic material, biomolecules (growth and differentiation factors), bone morphogenetic proteins and for reinforcing the bioactivity of 3D scaffolds for tissue engineering. Microspheres, microcapsules, liposomes, micelles and dendrimers are the nanoparticles mainly used in delivery systems. As per the requirements, solid, hollow or porous nanoparticles are synthesized through molecular self-assembly, nanomanipulation, bioaggregation and photochemical patterning [26, 27]. For stem-cell regeneration, disease therapeutics and targeted drug delivery, the biodegradable nanoparticle carries immense potential for future endeavour.

Magnetic nanomaterials: iron oxide NPs

Simple synthesis methods and universal availability make iron oxide like inorganic Nps as the most promising candidate for stem-cell research. Iron oxide Nps have the tendency to bind on either external cell membrane or pierces into the cytoplasm. Particles bound on the surface may interfere with cell-surface interactions or at times get detached from the membrane without affecting the motility of the cell [28]. However, iron oxide Nps inside the cytoplasm modify their surface to increase the uptake efficiency with very less deleterious effect [28]. Polymer coating like dextran enhances the stability and solubility of superparamagnetic iron oxide nps (SPIONs) and prevents it from forming aggregates [29, 30]. The polymer-coated SPIONs help in tracing stem/progenitor cells with MRI. Magnetic iron oxide nps and their composites are sensitive and are emerging tools for MRI as compared with the conventional gadolinium-based approach [31]. SPIONs are now frequently used as *in vivo* cellular-imaging agent. The Nps label stem cells through endocytosis or pinocytosis route [32–34]. Dextran-coated SPIONs reduce the labelling efficiency of stem cells and therefore are unfavourable for endocytosis. Moreover, iron oxide nps, when dissolved in cells, may lead to elevation of free hydroxyl radicals and reactive oxygen species. These may cause toxic effects such as increased apoptosis or alteration in cellular metabolism [35]. Dissolved Fe^{2+} ions released from the iron oxide Nps exhibit toxic effect on cells. To protect stem cells substantially from the toxic effect, the SPIONs were initiated with gold coating. Gold coating provides the inert shell around NPs and protects them from rapid dissolution within the cytoplasmic endosomes [36]. In addition, the gold-coated inert shell clarifies MRI contrast drastically. The convenient coupling surface chemistry of gold with thiol or amine moieties allows SPIONs to interact with biomolecules more easily [37]. The stem cell labelled with SPIONs can be detected with MRI even after implantation within the body. The magnetic sorting techniques thus allow quick and easier retrieval of stem cells from the spleen and bone marrow proficiently [38].

Magnetic NPs for *in vivo* stem-cell tracking

Tissue regeneration through transplanting progenitor/stem cells is an emerging era of therapeutic research. For *in vivo* cellular imaging, various techniques such as MRI, bioluminescence, positron emission tomography and multiple photon microscopy are widely used. Amongst all, MRI offers high resolution, speed, easy accessibility and 3D capabilities with additional information regarding the surrounding tissues [31, 39, 40]. In this regard, magnetic iron oxide Nps with controlled size offer great potential towards MRI applications. The nuclear spins couple to create a large magnetic domain in the magnetic nanocrystals. At certain circumstances of fluctuating temperature and variable crystal sizes, these particles randomly become locked in one direction, making the material wisely ferromagnetic [41]. The SPIONs surface coated with dextrans are now commercially available as Endorem (Geurbet, France) [42]. Transfection agents are usually required to facilitate cellular uptake, which may potentially damage the stem cells. Higher concentration of transfection agents causes toxicity, whilst lower concentration is not sufficient for cellular uptake [28]. Therefore, such agents can be reduced by employing SPIONs, which are widely tagged for labelling human MSCs (hMSCs) and ESCs (hESCs). The other dextran-coated SPIONs such as Feridex and Sinerem are now being combined with commercially available transfection agents such as, Fungene, Superfect, Lipofectamine [28,

42]. The SPIONs surface modification with internalized ligands enhances stem/progenitor cells labelling like fluorescein isothiocyanate-derivatized HIV-Tat peptide, dendrimers and polycationic transfection agents. Nowadays isotope and fluorescent labels are used for functionalization of SPIONs. A study by Weissleder and coworkers reported that NPs-tagged MRI optical and nuclear imaging helps in validation of cellular behaviour *in vivo* [31]. A collection of MRI contrast agents with different nanoparticle coatings on rodent and human mesenchymal and neural stem cells in different environmental conditions would definitely help investigators to access future research in a concrete direction [43]. Despite the enormous therapeutic potential of stem cell, many challenges, including the monitoring of cell fate *in vivo*, remain to be expounded in near future. The application of magnetic techniques offers great potential for tissue repair and regenerative medicine. However, much progress still remains to be made to answer the rejection of transplanted stem cells.

Nanocarriers

Controlled delivery of biomolecules is the critical point of concern in the support and enhancement of tissue growth during regeneration. Nanoscale carriers are capable of reaching the targets, which are otherwise inaccessible, like blood-brain barrier, tight junctions and capillaries. Nanocarriers are generated through a combination of various polymers (polylactic acid, polyglycolic acid, polyethylene glycol) with hydrogels. These nanocarriers possess different release properties for the entrapped molecules. The properties of polymers act as a key for fabricating nanoparticles. Some polymers are capable of 'stimuli-response', with change in temperature, pH or magnetic field, which further results in conformational changes such as swelling or shrinkage. Polyelectrolyte can act as efficient drug carriers because these undergo ionization due to pH changes in surrounding environment [44]. Solid nanoparticles having surface modifications also find applications in regenerative medicines. Bone regeneration and osteoblast adhesion are found to be enhanced with hydroxyapatite nanoparticles functionalized with specific biomolecules [45]. The multifunctional ability of nanocarriers makes them ideal vehicles for treating multi-drug resistance (MDR) cancer. The nanocarriers improve the therapeutic index of drugs by diverting the effects of ABC transporter-mediated drug efflux, which is the primary mechanism of MDR, thereby breaching towards therapeutics by overcoming drug resistance [46].

Nanoparticle scaffold

Scaffolds are the 3D constructions that are capable of mimicking the structure of the tissue that requires repair [47]. These scaffolds are porous biodegradable structures that provide a suitable environment for host-cell colonization. Nanofibres can be assembled to form porous scaffold that supports the tissue regeneration. These can be synthesized artificially or at times may be naturally synthesized. Nanofibres successfully replace the natural extracellular matrix because of large surface area and aids in cell colonization as well as in efficient exchange of nutrients and metabolic waste between scaffold and its environment. In a recent breakthrough of bone marrow modelling, a spatial distribution of the different cells with 3D scaffold culture system resulted in better structural organization as compared with 2D culture systems [48]. Nanomaterial shows a new hope and avenue towards controlled drug release properties. Organ decellularization for bioscaffolds fabrication is a novel concept of future research. In addition, the electrophysiological properties of bioartificial myocardium, along with it associative multi-electrode network, could provide electrical stimulation for the improved coupling of grafted cells and scaffolds with host cardiomyocytes for the treatment of myocardial diseases [21].

Nanodevices

Advancements in microfabrication technology for microelectronic applications also led to the invention of nanodevices. These nanodevices include biocapsules, bioreactors, biosensors and laboratory-on-a-chip. Biocapsules are the shelled nanodevices used for storage and transport of molecules to be delivered or collected in a controlled way. Fabrication of biocapsules can be carried out for selectively isolating specific molecules inside them. Smart capsules can be synthesized that carry nanodevices inside them for analysing entrapped molecules, and thus can acts as local, sensitive, and real-time diagnostic tool in disease detection. Bioreactors provide controlled set of conditions for cell and tissue regeneration for *in vitro* systems. Temperature, pH, pressure, nutrient supply and waste control are controlled on large scale for industrial purpose by integrating BioMEMS with these bioreactors [4]. Biosensors are used to monitor the changes occurring in the specific conditions inside the bioreactor. Nanosensor is a sensor capable of detecting biological, chemical, mechanical or electrical reactions in the local environment. Generally, used nanosensor includes quantum dots, fluorescent nanoparticles, metallic nanoparticles, carbon nanotubes [49], pH sensors or molecule-release sensor [50]. Motor proteins are integrated with the nanosensors as a source of energy [51].

Regenerative medicine in tissue engineering

The novel nano-textured biomaterial surface feature enables increased tissue regeneration with substantial proportional increase of immune responses [52]. Tissue-cell reactions depend on implantable surface structures and functions [53]. The major source of conventional biomaterials includes micron scale or larger surface features [54]. The nanometre scale includes the surface features found prominent in and on natural tissues; therefore, adding nanotopographies to the surfaces of conventional biomaterials may improve the functions of various cell types. In this radiance, many bio-inspired nanostructured materials have been designed [world scientific 2007, 55]. For example, an improved bone-cell response case has been reported while inducing nanostructured titanium implant

surfaces, leading to accelerated calcium deposition and improvement in integration with surrounding bones as compared with core conventional titanium surfaces [56–59]. In another application of regenerative medicine which includes cartilage, nano-structured polylactic-co-glycolic acid (PLGA) surface induction has been shown to stimulate chondrocyte adhesion and proliferation, as well as extracellular matrix production tremendously [60–62]. In addition, vascular graft (PLGA) and stent titanium surfaces with nanometre surface roughness values drastically improve inner endothelial vessel cell functions as compared with naked nanosmooth polymer and titanium surfaces [63–67].

Furthermore, with conventional biomaterials, various intrinsic nano-sized materials such as hydrophobic carbon nanotubes [68–72] and hydrophilic helical rosette nanotubes [72, 73] are still under keen investigation in regenerative medicine. These novel carbon-based nanomaterials speculate vigorous cellular interactions over currently implanted materials.

Contemporary strategies for the development of novel biomaterials in medicine can be grouped into two chief categories. The first strategy is all the way through altered chemistry; for example, titanium use is much better as compared with stainless steel for orthopaedic applications or using controlled drug release to form implant surfaces [74–77]. The second strategy uptakes amendment in the physical implant properties like surface roughness for generating nanometer surface features. For these reasons, selectively chosen biomaterials can be tailored to stimulate favourable cellular interactions by varying both chemical and physical factors. This review also discusses the importance of nanotechnology in active and passive implants of tissue engineering.

Passive implants and tissue engineering

The life span of passive implantation such as artificial joints, artificial hips, finishes normally after ~15 years leading to wear or implant loosening and therefore further surgeries are required to re-cope with the common human life [78]. Nanotechnologies could help reduce such kind of problems. To reduce these problems, a thin layer coating of a nanocrystalline structure over the implants made of titanium, cobalt or chromium alloys can be provided to make it harder, smoother, and consequently, more resistant to wear. This results in less wear of the polyethylene artificial socket. Moreover, the coated over-layer ensures better biocompatibility of the implant. The other suitable coating materials include diamond, metal ceramic and hydroxyapatite [79]. A natural component of the bone hydroxyapatite constitutes 70% of the mineral component, with the remaining 30% constituent of organic collagen fibres. New coating production methods in implants have now overshadowed hydroxyapatite to apply layers with a grain size of less than 50 nm, rather than the micrometre scale. The applied layer helps in biocompatibility by encouraging the growth and bonding of the bony tissue in the surrounding environment. *In vitro* studies have proved that the osteoblasts bone-forming cells deposit more calcium on materials with a grain size in the nanometre range than on conventional materials with a grain size in the micrometre range [79–81]. It might be the higher absorption of proteins that stimulate cell adhesion [82]. The bone resorbent cells, osteoclasts also function better when come into contact with these nanomaterials. Cell-to-cell functional coordination helps in the formation and maintenance of healthy bony tissue and therefore contributes to strong bonding between the implant and the surrounding bone, which are attached without the use of bone cement. [78, 83, 84]. The first successful implant with a hydroxyapatite layer enclosed with a nanostructure-coated artificial hip was achieved in 2000, on a patient in the Maastricht University Hospital. For the repair of bony tissue, accelerated nanoparticles of hydroxyapatite can also be introduced directly into the damaged bones. Implant coatings with various nanostructures based on diamond and metal ceramic are still under research due to their chief properties of hardness, smoothness, corrosion resistance and good bonding to the implant [79]. The implanting material improves the mechanical properties and biocompatibility with a nanostructure. This is made possible by coating the thin over layer of titanium dioxide with nanopores. An additional advantage of this approach relies on modulating the layer in such a way that metal ions with antiseptic effects, such as copper ions, are slowly released. It also reduces the likelihood of bacterial infections, which are a frequent complication with implants [85]. Another approach includes the fabrication of implants from nanopowders of titanium dioxide or aluminium oxide using a sinter process. Other alternative materials include nanostructure and composite of organic polymers into which nanoparticles of titanium, aluminium, or hydroxyapatite have been mixed [60, 78, 83] to form the complex. The key advantage of the modified organic polymers is that they dissolve slowly at the same time when the new bony tissue is being formed. Studies to generate bone with the help of scaffolds of carbon nanotubes are also underway to explore the novel cause [86]. A new and diminished type of implant stent is made of small tubes of woven thread used in dilating blood vessels. The inflammatory reactions often hinder and block the blood vessels. A key to solve such problem through stent is provided with nanopores coating of aluminium oxide. In addition, the radioactive substances adhered with them prevents the clogging of stent. The pores ensure sufficient induction and controlled release of radioactive material. The authenticity of stents still needs confirmation in animal trials [87]. Nowadays, cell-based tissue-engineered procedures are employed for the development of bioartificial myocardial regeneration. Myocardial regeneration is now possible with the help of different types of stem-cell transplantation. It includes autologous myoblasts [88], bone marrow stem cells [89], peripheral blood stem cells [90], vascular endothelial cells [91], mesothelial cells (biopsy of the omentum) [92], adipose tissue stem cells [93], umbilical cord cells, induced pluripotent stem cells (iPSCs) and embryonic pluripotent cells [94]. Tissue engineering and cell therapy-based electrostimulation (cardiac resynchronization therapy) are used with stem-cell transplantation associated clinical trials for MAGNUM (Myocardial Assistance by Grafting a New Upgraded bioartificial Myocardium). It is a recent advancement reported towards the treatment of myocardial diseases in ischaemic hearts [95]. Although in ischaemic disease, the

extracellular matrix is pathologically and deeply altered. Therefore, an associative procedure for regenerating both myocardial cells and the extracellular matrix is a mandatory condition for the cure. The improved efficiency of cellular cardiomyoplasty *via* tissue engineering is a feasible and safe approach reported for intra infarct cell therapy associated with a cell-seeded collagen scaffold grafted onto infarcted ventricles [95]. A new method of tissue engineering was proposed to improve the contractile function of engineered cardiac tissue cardiomyocyte viability, differentiation and their surrounding three-dimensional collagen environment. A novel *in vitro* method that couples arginine–glycine–aspartic acid–serine (RGD+) peptides on cross-linking with a collagen matrix drastically improves the performance of collagen-cell scaffolds. This newly designed scaffold might also serve as a potential platform for improving/engineering cellular transplantation in a true myocardium [96]. Towards this investigative challenge, a biodegradable tri-dimensional matrix seeded with cells and grafted onto the infracted ventricle is currently under research [21, 97].

Active implants and tissue engineering

An active implant has a chief and rich source of energy. Based on their functional role, active implants are categorized into two groups. The first group with long life span contains administering medicines, which include insulin, and morphine pumps. Recent research is underway for the long-term storage and controlled release of active substances on implantable microchips [98, 99]. The potential merit of administering medicine is their target and controlled drug delivery; it goes directly to the location where they are needed at varying rates. The controlled release could also be detected by a biosensor that responds to variable physiological parameters [100].The second group neural prostheses aid in repair or take over nerve functional responses. They are potentially used to bridge damaged nerve paths, induce muscular impulses and at times employed to replace senses. This group includes cochlear implants (for restoring hearing), pacemakers and defibrillators (for regulating the heart beat), bladder stimulators (for controlled emptying of the urinary bladder by spinal cord lesion patients), deep-brain stimulators (to combat tremor in patients with Parkinson's disease), as well as peroneus stimulators (to combat drop foot)-based responses.

For some years now, to enable thought-oriented devices, various research groups in the United States have also been working on neuroprostheses [101–106]. For instance, electrode-adhered microchips are fitted to the motor cerebral cortex, which record the electric signals associated with the thoughts commonly referred to as brain-machine interfaces. A major success has been achieved in enabling rats to operate handles by 'brain power' and in monkeys to operate the cursor of a computer or a robot arm by their thoughts [103, 104]. A few years ago, an electrode has been implanted in the cerebral cortex to enable the patient to operate a computer device in an amyotrophic lateral sclerosis patient [107]. According to the findings presented at the annual meeting of the American Academy of Physical Medicine and Rehabilitation, in Phoenix in October 2004 [108], a neuroprosthesis has been fitted to a paralyzed man. It enables him to operate the cursor of a computer device by thought, play video games, operate a light switch, and select a television channel. The ultimate goal, which is still far-off and under research, is to enable patients to operate arm, leg prostheses, and controlled restoration of their paralyzed limbs [102, 105].

Conclusion

Nanobiological research is a speculating area of interest in many countries and its relevance within physical sciences, molecular engineering, biology, biotechnology, and medicine is expected to increase in the future. In recent years, studies of interaction between nanomaterials, nanostructures and stem-cell nanotechnology has emerged as a new exciting field. The theoretical and experimental potential of nanotechnology to the fundamental developments in regenerative medicines for treatment of injuries and degenerative diseases has been widely speculated. In particular, a new interdisciplinary frontier in regeneration medicine is the effect of nanoparticles in molecular imaging. Nanomaterials such as fluorescent magnetic nanoparticles have been used for molecular imaging, scaffolds for tissue engineering, and designed nanostructures have been used in stem-cell treatments and tissue implants. The multidisciplinary applications of nanotechnologies for discovering new molecules and tailoring those could be incredible in its potential to improve human health. In the future, we could envision a world where nanodevices would be routinely getting implantation to participate in the repair of cells that deviate from the normal routine of mechanism. The successful development and implementation of nanotechnology with regenerative medicine foster a global perspective on research and bring together the spin-off benefits to human diseases in general. This broad sweep of knowledge traditional sciences like chemistry, physics, biology and materials science aids to bring together the required collective knowledge and expertise for the development of these novel nano-technologies. Current trends in nanotechnology have evolved hopeful revolution in medicine to increase the quality of human life, and to increase the initial formation of tissue necessary to prolong implant lifetime. Incorporating tissue cell–biomaterial interactions and cell-nanotopography interactions at the nanoscale and develop methods to create unique nanoscale surface features applicable to numerous medical fields is a major clinical goal of nanomedicine. Thus, it has become evident that nanotechnology will become a critical tool in the fight to resolve eventual medical issues. Other critical future challenge includes catalysing the development of biologically inspired nano biomaterials whose functions can stimulate the capabilities of natural organs and tissues.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. **Shrivastava S, Dash D.** Nanotechnology in agriculture. *J Nanotechnol.* 2009; 184702: 1–14.
2. **Engel E, Michiardi A, Navarro M, *et al.*** Nanotechnology in regenerative medicine: the materials side. *Cell Press.* 2007; 26: 39–47.
3. **Yang YK, Leong W.** Nanoscale surfacing for regenerative medicine. *WIREs Nanomed Nanobiotechnol.* 2010; 2: 478–95.
4. **Mimeault M, Hauke R, Batra SK.** Stem cells: a revolution in therapeutics–recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies. *Clin Pharmacol Ther.* 2007; 2: 252–64.
5. **Martin I, Wendt D, Heberer M.** The role of bioreactors in tissue engineering. *Trends Biotechnol.* 2004; 22: 80–6.
6. **Whitman DH, Berry RL, Green DM.** Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *J Oral Maxillofac Surg.* 1997; 55: 1294–9.
7. **Marx RE.** Platelet rich plasma: evidence to support its use. *J Oral Maxillofac Surg.* 2004; 62: 489–96.
8. **Porcellini A.** Regenerative medicine: a review. *Rev Bras Hematol Hemoter.* 2009; 3: 63–6.
9. **Kang X, Xie Y, Powell HM, *et al.*** Adipogenesis of murine embryonic stem cells in a three-dimensional culture system using electrospun polymer scaffolds. *Biomaterials.* 2007; 28: 450–8.
10. **Park J, Bauer S, Von MK, *et al.*** Nanosize and vitality: TiO_2 nanotube diameter directs cell fate. *Nano Lett.* 2007; 7: 1686–91.
11. **Dalby MJ, Gadegaard N, Tare R, *et al.*** The interaction of human bone marrow cells with nanotopographical features in three-dimensional constructs. *J Biomed Mater Res.* 2007; 79: 431–9.
12. **Yim EKF, Pang SW, Leong KW.** Synthetic nanostructures inducing differentiation of human mesenchymal stem cells into neuronal lineage. *Exp Cell Res.* 2007; 313: 1820–9.
13. **Chua KN, Chai C, Lee PC, *et al.*** Functional nanofiber scaffolds with different spacers modulate adhesion and expansion of cryopreserved umbilical cord blood hematopoietic stem/progenitor cells. *Exp Hematol.* 2007; 35: 771–81.
14. **Chua KN, Chai C, Lee PC, *et al.*** Surface-aminated electrospun nanofibers enhance adhesion and expansion of human umbilical cord blood hematopoietic stem/progenitor cells. *Biomaterials.* 2006; 27: 6043–51.
15. **Yang F, Murugan R, Ramakrishna S, *et al.*** Fabrication of ano-structured porous PLLA scaffold intended for nerve tissue engineering. *Biomaterials.* 2004; 25: 1891–900.
16. **Yang F, Murugan R, Wang S, *et al.*** Electrospinning of nano/micro scale poly(*l-lactic acid*) aligned fibers and their potential in neural tissue engineering. *Biomaterials.* 2005; 26: 2603–10.
17. **Guo J, Su H, Zeng Y, *et al.*** Reknitting the injured spinal cord by self assembling peptide nanofiber scaffold. *Nanomedicine.* 2007; 3: 311–21.
18. **Berry CC, Dalby MJ, Oreffo ROC, *et al.*** The interaction of human bone marrow cells with nanotopographical features in three-dimensional constructs. *J Biomed Mater Res A.* 2006; 79: 431–6.
19. **Ferreira L, Karp JM, Nobre L, *et al.*** New opportunities: the use of nanotechnologies to manipulate and track stem cells. *Cell Stem Cell.* 2008; 3: 136–46.
20. **Wang Z, Ruan J, Cui D.** Advances and prospect of nanotechnology in stem cells. *Res Lett.* 2009; 4: 593–605.
21. **Chachques JC.** Development of bioartificial myocardium using stem cells and nanobiotechnology templates. *Car Res Prac.* 2011; doi: 10.4061/2011/806795.
22. **Kshitiz K, Kim DH, Beebe DJ, *et al.*** Micro- and nanoengineering for stem cell biology: the promise with a caution *Trend Biotechnol.* 2011; 29: 399–408.
23. **Martino S, D'Angelo F, Armentano I, *et al.*** Stem cell-biomaterial interactions for regenerative medicine. *Biotechnol Adv.* 2011; doi: 10.1016/j.biotechadv.2011.06.015.
24. **Chan WC, Maxwell DJ, Gao X, *et al.*** Luminescent quantum dots for multiplexed biological detection and imaging. *Curr Opin Biotechnol.* 2002; 13: 40–6.
25. **Moriarty P.** Nanostructured materials. *Rep Prog Phys.* 2001; 64: 297–381.
26. **Allemann E, Gurny R, Doelker E.** Drug loaded nanopaticles–methods and drug targeting issues. *Eur J Pharm Biopharm.* 1993; 39: 173–91.
27. **Cade D, Ramus E, Rinaudo M, *et al.*** Tailoring of biocompatible polymers for elaboration of sugar-functionalized nanoparticles. *Biomacromolecules.* 2004; 5: 922–7.
28. **Bulte JW, Kraitchman DL.** Iron oxide MR contrast agents for molecular and cellular imaging. *NMR Biomedicine.* 2004; 17: 484–99.
29. **Dutz S, Wilfried A, Rudolf H, *et al.*** Influence of dextran coating on the magnetic behaviour of iron oxide nanoparticles. *J Magn Magn Mater.* 2007; 311: 51–4.
30. **Lawaczeck R, Menzel M, Pietsch H.** Superparamagnetic iron oxide particles: contrast media for magnetic resonance imaging. *Appl Organomet Chem.* 2004; 18: 506–13.
31. **Chemaly ER, Yoneyama R, Frangioni JV, *et al.*** Tracking stem cells in the cardiovascular system. *Trends Cardiovasc Med.* 2005; 15: 297–302.
32. **Lu CW, Hung Y, Hsiao JK, *et al.*** Bifunctional magnetic silica nanoparticles for highly efficient human stem cell labeling. *Nano Lett.* 2007; 7: 149–54.
33. **Rogers WJ, Meyer CH, Kramer CM.** Technology insight: *in vivo* cell tracking by use of MRI. *Nat Clin Pract Cardiovasc Med.* 2006; 3: 554–62.
34. **Gupta AK, Curtis ASG.** Lactoferrin and ceruloplasmin derivatized super paramagnetic iron oxide nanoparticles for targeting cell surface receptors. *Biomaterials.* 2004; 25: 3029–40.
35. **Emerit JC, Beaumont C, Trivin F.** Iron metabolism, free radicals, and oxidative injury. *Biomed Pharmacother.* 2001; 55: 333–9.
36. **Wang FH, Lee IH, Holmström N, *et al.*** Magnetic resonance tracking of nanoparticle labelled neural stem cells in a rats spinal cord. *Nanotechnology.* 2006; 17: 1911–5.
37. **Niemeyer CM, Ceyhan B.** DNA-directed functionalization of colloidal gold with proteins. *Angew Chem.* 2001; 40: 3685–8.
38. **Lewin M, Carlesso N, Tung CH.** Tat peptide-deri- vatized magnetic nanoparticles allow *in vivo* tracking and recovery of progenitor cells. *Nat Biotechnol.* 2000; 18: 410–4.
39. **Bulte JW, Douglas T, Witwer B, *et al.*** Magneto dendrimers allow endosomal magnetic labeling and *in vivo* tracking of stem cells. *Nat Biotechnol.* 2001; 19: 1141–7.
40. **Syková E, Jendelová P.** Migration, fate and *in vivo* imaging of adult stem cells in the CNS. *Cell Death Differ.* 2007; 14: 1336–42.
41. **Thorek DL, Chen AK, Czupryna J, *et al.*** Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann Biomed Eng.* 2006; 34: 23–38.

42. **Corot C, Robert P, Idée JM, *et al.*** Recent advances in iron oxide nanocrystal technology for medical imaging. *Adv Drug Deliv Rev.* 2006; 58: 1471–504.
43. **Mahmoudi M, Hosseinkhani H, Hosseinkhani M, *et al.*** Magnetic resonance imaging tracking of stem cells *in vivo* using iron oxide nanoparticles as a tool for the advancement of clinical regenerative medicine. *Chem Rev.* 2011; 111: 253–80.
44. **Murthy N, Campbell J, Fausto N, *et al.*** pH responsive polymeric carriers that target uptake and enhance the intracellular delivery of oligonucleotides. *J Cont Rel.* 2003; 89: 365–74.
45. **Liu H, Webster TJ.** Nanomedicine for implants: a review of studies and necessary experimental tools. *Biomaterials.* 2007; 2: 354–69.
46. **Milane L, Ganesh S, Shah S, *et al.*** Multimodal strategies for overcoming tumour drug resistance: Hypoxia, the Warburg effect, stem cells, and multifunctional nanotechnology. *J Cont Rel.* 2011; 155: 237–47.
47. **Lanza RP, Langer R, Vacanti J.** *Principles of Tissue Engineering* (2nd ed.) San Diego: Academic Press; 2000: p. 331–50.
48. **Di Maggio N, Piccinini E, Jaworski M, *et al.*** Toward modeling the bone marrow niche using scaffold-based 3D culture systems. *Biomaterials.* 2011; 32: 321–9.
49. **Liu H, Schmidt JJ, Bachand GD, *et al.*** Control of a biomolecular motor-powered nanodevice with an engineered chemical switch. *Nat Mater.* 2002; 1: 173–7.
50. **Harrison BS, Atala A.** A stem cell-based tissue engineering with silk biomaterials. *Biomaterials.* 2007; 28: 344–53.
51. **Bishnoi SW, Rozell CJ, Levin CS, *et al.*** An all-optical nanoscale pH meter. *Nano Lett.* 2006; 6: 1687–92.
52. **Khang D, Carpenter J, Chun YW, *et al.*** Nanotechnology for regenerative medicine. *Biomed Microdev.* 2010; 12: 575–87.
53. **Stevens MM, George JH.** Exploring and engineering the cell surface interface. *Science.* 2005; 310: 1135–8.
54. **Webster TJ.** Nanophase ceramics as improved bone tissue engineering materials. *Am Ceram Soc Bull.* 2003; 82: 23–8.
55. **Sato M, Webster TJ.** Nanobiotechnology: implications for the future of nanotechnology in orthopaedic applications. *Expert Rev Med Device.* 2004; 1: 105–14.
56. **Ergun C, Liu HN, Webster TJ, *et al.*** Increased osteoblast adhesion on nanoparticulate calcium. phosphates with higher Ca/P ratios. *J Biomed Mater Res Part A.* 2008; 85: 236–41.
57. **Webster TJ, Ergun C, Doremus RH, *et al.*** Enhanced functions of osteoblasts on nanophase. *Ceramics Biomater.* 2000; 21: 1803–10.
58. **Webster TJ, Siegel RW, Bizios R.** Osteoblast adhesion on nanophase ceramics. *Biomaterials.* 1999; 20: 1221–7.
59. **Yao C, Slamovich EB, Webster TJ.** Enhanced osteoblast functions on anodized titanium with nanotube-like structures. *J Biomed Mater Res Part A.* 2008; 85A: 157–66.
60. **Kay S, Thapa A, Haberstroh KM, *et al.*** Nanostructured polymer/nanophase ceramic composites enhance osteoblast and chondrocyte adhesion. *Tissue Eng.* 2002; 8: 753–61.
61. **Park GE, Pattison MA, Park K, *et al.*** Accelerated chondrocyte functions on NaOH-treated PLGA scaffolds. *Biomaterials.* 2005; 26: 3075–82.
62. **Savaiano JK, Webster TJ.** Altered responses of chondrocytes to nanophase PLGA/nanophase titania composites. *Biomaterials.* 2004; 25: 1205–13.
63. **Lu J, Rao MP, MacDonald NC, *et al.*** Improved endothelial cell adhesion and proliferation on patterned titanium surfaces with rationally designed, micrometer to nanometer features. *Acta Biomater.* 2008; 4: 192–201.
64. **Choudhary S, Haberstroh KM, Webster TJ.** Enhanced functions of vascular cells on nanostructured Ti for improved stent applications. *Tissue Eng.* 2007; 13: 1421–30.
65. **Miller DC, Haberstroh KM, Webster TJ.** PLGA nanometer surface features manipulate fibronectin inter-actions for improved vascular cell adhesion. *J Biomed Mater Res Part A.* 2007; 81: 678–84.
66. **McCann-Brown JA, Webster TJ, Haberstroh KM.** Vascular cells respond to endothelial cell flow-and pressure-released soluble proteins. *Chem Eng Commun.* 2007; 194: 309–21.
67. **Miller DC, Haberstroh KM, Webster TJ.** Fine particles, thin films and exchange anisotropy. *J Biomed Mater Res Part A.* 2005; 73: 476–84.
68. **Khang D, Sato M, Price RL, *et al.*** Selective adhesion and mineral deposition by osteoblasts on carbon nanofiber patterns. *Int J Nanomed.* 2006; 1: 65–72.
69. **Khang D, Kim SY, Liu-Snyder P, *et al.*** Enhanced fibronectin adsorption on carbon nanotube/poly(carbonate) urethane: independent role of surface nano-roughness and associated surface energy. *Biomaterials.* 2007; 28: 4756–68.
70. **Webster TJ, Waid MC, McKenzie JL, *et al.*** Nano-biotechnology: carbon nanofibers as improved neural and orthopedic implants. *Nanotechology.* 2004; 15: 48–54.
71. **Elias KL, Price RL, Webster TJ.** Enhanced functions of osteoblasts on nanometer diameter carbon fibers. *Biomaterials.* 2002; 23: 3279–87.
72. **Chun AL, Moralez JG, Fenniri H, *et al.*** Helical rosette nanotubes: a more effective orthopaedic implant material. *Nanotechnology.* 2004; 15: 234–9.
73. **Chun AL, Moralez JG, Webster TJ, *et al.*** Helical rosette nanotubes: a biomimetic coating for orthopaedics. *Biomaterials.* 2005; 26: 7304–9.
74. **Allen C, Maysinger D, Eisenberg A.** Nano-engineering block copolymer aggregates for drug delivery. *Colloids Surf B Biointer.* 1999; 16: 3–27.
75. **Langer R.** Drug delivery and targeting. *Nature.* 1998; 392: 5–10.
76. **Langer R.** Perspectives: drug delivery – drugs on target. *Science.* 2001; 293: 58–9.
77. **Svenson S, Tomalia DA.** Dendrimers in biomedical applications–reflections on the field. *Adv Drug Deliv Rev.* 2005; 57: 2106–29.
78. **Park GE, Webster TJ.** A review of nanotechnology for the development of better orthopedic implants. *J Biomed Nanotechnol.* 2005; 1: 18–29.
79. **Catledge SA, Fries MD, Vohra YK, *et al.*** Nanostructured ceramics for biomedical implants. *J Nanosci Nanotechnol.* 2002; 2: 293–312.
80. **Webster TJ, Ejiofor JU.** Increased osteoblast adhesion on nanophase metals: Ti, Ti6Al4V, and CoCrMo. *Biomaterials.* 2004; 25: 4731–9.
81. **Webster TJ, Hellenmeyer EL, Price RL.** Increased osteoblast functions on theta +delta nanofiber alumina. *Biomaterials.* 2005; 26: 953–60.
82. **Woo KM, Chen VJ, Ma PX.** Nanofibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment. *J Biomed Mater Res A.* 2003; 67: 531–7.
83. **Price RL, Waid MC, Haberstroh KM, *et al.*** Selective bone cell adhesion on formulations containing carbon nanofibers. *Biomaterials.* 2003; 24: 1877–87.
84. **Salata OV.** Applications of nanoparticles in biology and medicine. *J Nanobiotechnol.* 2004; 2: 3–9.
85. **Heidenau F, Mittelmeier W, Detsch R, *et al.*** A novel antibacterial titania coating: metal ion toxicity and *in vitro* surface colonization. *J Mater Sci.* 2005; 16: 883–8.

86. **Zhao B, Hu H, Mandal SK, *et al.*** A bone mimic based on the self-assembly of hydroxyapatite on single-walled carbon nanotubes. *Chem Mater.* 2005; 17: 3235–41.
87. **Huang N, Yang P, Leng YX, *et al.*** Hemocompatibility of titanium oxide films. *Biomaterials.* 2003; 24: 2177–87.
88. **Chachques JC, Herreros J, Trainini J, *et al.*** Autologous human serum for cell culture avoids the implantation of cardioverter-defibrillators in cellular cardiomyoplasty. *Int J Card.* 2004; 95: 29–33.
89. **Stamm C, Westphal B, Kleine HD, *et al.*** Autologous bone marrow stem-cell transplantation for myocardial regeneration. *Lancet.* 2003; 361: 45–6.
90. **Schachinger V, Assmus B, Britten MB, *et al.*** Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI trial. *J Am Col Card.* 2004; 44: 1690–9.
91. **Narmoneva DA, Vukmirovic R, Davis ME, *et al.*** Endothelial cells promote cardiac myocyte survival and spatial reorganization: implications for cardiac regeneration. *Circulation.* 2004; 110: 962–8.
92. **Bourahla B, Shafy A, Meilhac O, *et al.*** Mesothelial cells *versus* skeletal myoblasts for myocardial infarction *Asi Cardiovas Thorac Ann.* 2010; 18: 153–60.
93. **Planat-B'enard V, Menard C, Andr'e M, *et al.*** Spontaneous cardiomyocyte differentiation from adipose tissue stroma cells. *Circ Res.* 2004; 94: 223–9.
94. **Kehat I, Khimovich L, Caspi O, *et al.*** Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol.* 2004; 22: 1282–9.
95. **Chachques JC, Jorge C, *et al.*** Myocardial assistance by grafting a new bioartificial upgraded myocardium (MAGNUM trial): clinical feasibility study. *Ann Thorac Surg.* 2008; 85: 901–8.
96. **Schussler O, Coirault C, Tisserand ML, *et al.*** Use of arginine–glycine–aspartic acid adhesion peptides coupled with a new collagen scaffold to engineer a myocardium-like tissue graft. *Nat Clin Prac Cardiovas Med.* 2009; 6: 240–9.
97. **Cortes-Morichetti M, Frati G, Schussler O, *et al.*** Association between a cell-seeded collagen matrix and cellular cardiomyoplasty for myocardial support and regeneration. *Tissue Eng.* 2007; 13: 2681–7.
98. **Santini JT, Richards AC, Scheidt RA, *et al.*** Microchip technology in drug delivery. *Ann Med.* 2000; 32: 377–9.
99. **Santini JT, Cima MJ, Langer RA.** controlled-release microchip. *Nature.* 1999; 397: 335–8.
100. **LaVan DA, McGuire T, Langer R.** Small-scale systems for *in vivo* drug delivery. *Nat Biotechnol.* 2003; 21: 1184–91.
101. **Nicolelis MAL.** Action from thoughts. *Nature.* 2001; 409: 403–7.
102. **Nicolelis MAL.** Brain-machine interfaces to restore motor function and probe neural circuits. *Nat Rev Neurosci.* 2003; 4: 417–22.
103. **Nicolelis MAL, Chapin JK.** Controlling robots with the mind. *Sci Am.* 2002; 287: 46–53.
104. **Serruya MD, Hatsopoulos NG, Paninski L, *et al.*** Brain-machine interface: Instant neural control of a movement signal. *Nature.* 2002; 416: 141–2.
105. **Donoghue JP.** Connecting cortex to machines: Recent advances in brain interfaces. *Nat Neurosci.* 2002; 5: 1085–8.
106. **Schwartz AB.** Cortical neural prostheses. *Annu Rev Neurosci.* 2004; 27: 487–507.
107. **Kennedy PR, Bakay RAE, Moore MM, *et al.*** Transactions on rehabilitation engineering. *IEEE.* 2000; 8: 198–202.
108. **Press report of Brown University**, "Pilot study of mind-to-movement device shows early promise" 2008, http://www.news-medical.net/news/2004/10/10/5440.aspx.