

Nanotechnology for regenerative medicine

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Abstract Future biomaterials must simultaneously enhance tissue regeneration while minimizing immune responses and inhibiting infection. While the field of tissue engineering has promised to develop materials that can promote tissue regeneration for the entire body, such promises have not become reality. However, tissue engineering has experienced great progress due to the recent emergence of nanotechnology. Specifically, it has now been well established that increased tissue regeneration can be achieved on almost any surface by employing novel nano-textured surface features. Numerous studies have reported that nanotechnology accelerates various regenerative therapies, such as those for the bone, vascular, heart, cartilage, bladder and brain tissue. Various nano-structured polymers and metals (alloys) have been investigated for their bio (and cyto) compatibility properties. This review paper discusses several of the latest nanotechnology findings in regenerative medicine (also now called nanomedicine) as well as their relative levels of success.

Keywords Nanotechnology · Tissue engineering · Orthopedic · Vascular · Neural · Skin · Cellular and protein interactions

1 Introduction

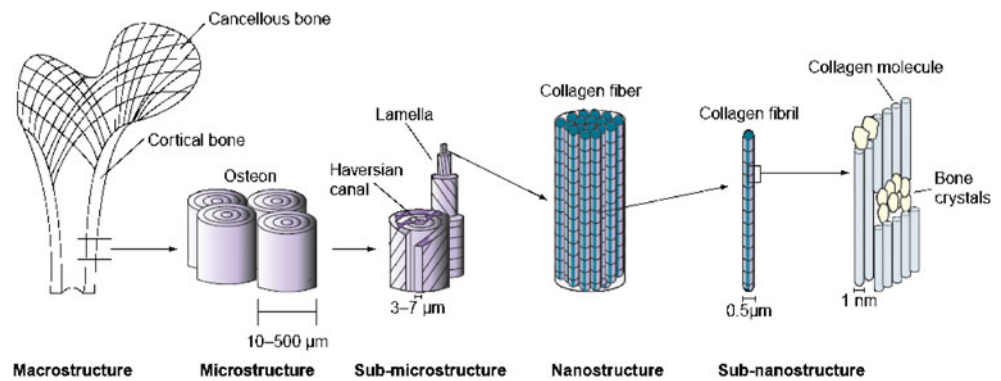
Tissue cells elicit specific, distinct reactions depending on implant surface structures (Stevens and George 2005). The majority of conventional biomaterials possess micron scale or larger surface features (Webster 2003). Since most of the surface features found in and on natural tissues are on the nanometer scale (Fig. 1), it has been widely speculated that adding nano-topographies to the surfaces of conventional biomaterials may promote the functions of various cell types. In this light, many nanostructured materials have been called bio-inspired nanomaterials (World Scientific 2007; Sato and Webster 2004). For example, nano-structured titanium implant surfaces promote bone cell responses leading to accelerated calcium deposition improving integration with surrounding bone compared to conventional titanium surfaces (Ergun et al. 2008; Webster et al. 2000, 1999; Yao et al. 2008). But bone is not the only application of nanomaterials in regenerative medicine. For cartilage applications, nano-structured poly-lactic-co-glycolic acid (PLGA) surfaces have been shown to accelerate chondrocyte adhesion and proliferation, as well as extracellular matrix production (Kay et al. 2002; Park et al. 2005; Savaiano and Webster 2004). Furthermore, vascular graft (PLGA) and stent (titanium) surfaces with nanometer surface roughness values improve endothelial (inner vessel cells) cell functions as compared to nano-smooth polymer and titanium surfaces (Lu et al. 2008; Choudhary et al. 2007; Miller et al. 2007; McCann-Brown et al. 2007; Miller et al. 2005).

In addition to the incorporation of nanometer surface features on conventional biomaterials, intrinsic nano-sized materials such as carbon nanotubes (hydrophobic) (Khang et al. 2006, 2007; Webster et al. 2004; Elias et al. 2002) and helical rosette nanotubes (hydrophilic) (Chun et al. 2005,

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Fig. 1 Macro-nano structures of Bone. Image adopted from Sato and Webster (2004)



2004) are under intense investigation in regenerative medicine. Both types of these novel carbon based nano-materials promote cellular interactions over currently implanted materials. Such data strongly supports the hypothesis that nano surface topography, in addition to surface chemistry, dictate initial protein adsorption and bioactivity (since initial protein interactions mediate cellular adhesion) as well as subsequent cellular adhesion (Fig. 2).

Current strategies for the development of improved biomaterials can be grouped into two main categories. The first strategy is through altered chemistry (such as using titanium compared to stainless steel for orthopedic applications or using controlled drug release from implant surfaces) (Allen et al. 1999; Langer 1998, 2001; Svenson and Tomalia 2005). The second strategy involves changing physical implant properties such as generating nanometer surface features and, thus, changing surface roughness. For these reasons, biomaterials can be tailored by selectively varying both chemical and physical factors in order to maximize favorable cellular interactions (i.e., increasing functions of tissue-forming cells but decreasing immune cell and bacteria functions).

This review paper discusses the importance of nanotechnology in various tissue engineering applications. Specifically, vascular, bone, skin, cell encapsulation and

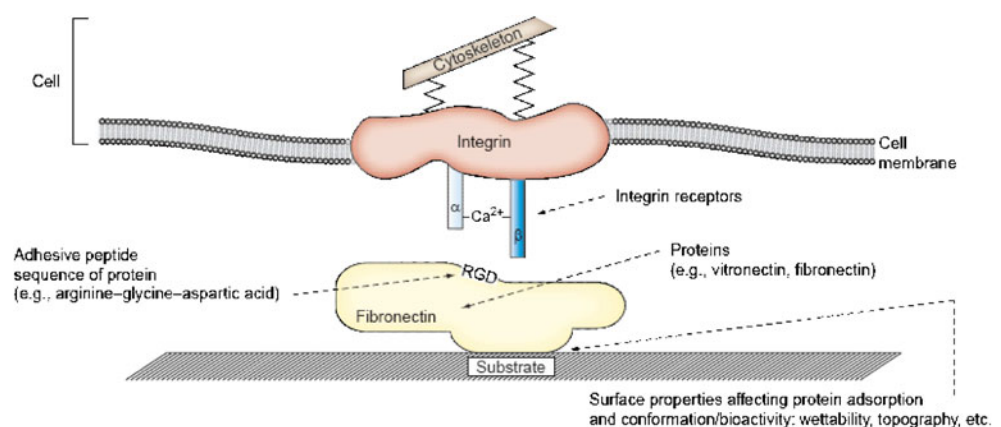
bladder system therapies will be discussed (Fig. 3), each having already seen promising results.

2 Nanotechnology for regenerative medicine applications

2.1 Bone regeneration

Previous studies demonstrated greater bone-forming cell (osteoblast) functions on various nano-materials, such as nano-hydroxyapatite (Sato et al. 2005), electro-spun silk (Jin et al. 2004), anodized titanium (Yao et al. 2008) and nano-structured titanium surfaces as compared to conventional orthopedic implant materials (Khang et al. 2008; Webster et al. 1999). As a next step, efforts have focused on understanding the mechanisms of enhanced bone cell functions on these materials and these studies have provided clear evidence that altered amounts and bioactivity of adsorbed proteins (such as vitronectin, fibronectin and collagen) were responsible due to increased surface energy of nanostructured surfaces (Khang et al. 2007; Webster et al. 2001) (Fig. 2). In addition to these observed greater initial responses of osteoblasts, longer term functions of bone cells (such as calcium and phosphate mineral

Fig. 2 Initial protein interactions leading to cell recognition of implants. Adopted from Sato and Webster (2004)



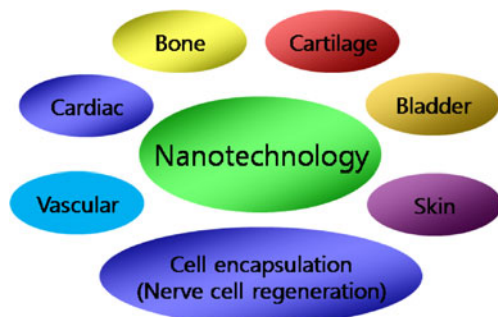


Fig. 3 Numerous applications of nanotechnology in tissue engineering

deposition (CaP) crucial for osseointegration of orthopedic implants are promoted on nanostructured biomaterials (Ergun et al. 2008; Webster 2003). In this manner, nano-phase titanium, Ti6AlV4, and CoCrMo have all promoted greater calcium crystallization (CaP) compared to micro-phase samples of these same materials (Ward and Webster 2006). Recent studies have also shown that bone cells respond differently on submicron and nanometer scale titanium surfaces, despite the minimal size difference between these two surface topographies (Khang et al. 2008). In fact, it has been demonstrated that small changes in nanometer surface features can have larger consequences towards bone regeneration (Khang et al. 2008). Another design parameter, using aligned nanometer surface features on metals, has also proven to further mimic the natural anisotropy of natural bone to promote bone cell functions (Khang et al. 2008).

Each of these studies provided clear evidence that bone cell behavior is strongly dependent upon the size of surface features where nanometer and submicron sized surface features (Fig. 4) can substantially improve long term functions of bone cells. For these reasons, in the near future, it is strongly believed that these optimized nano-textured implant materials will enter commercialization in the orthopedic as well as dental markets; some nano-materials have already received FDA approval for human implantation (Sato and Webster 2004).

2.2 Skin

Replacement and re-growth of damaged skin has been considered one of the first successful tissue engineering applications. Several tissue engineering (TE) products have been approved by the FDA for use as a skin graft material, aimed towards patients suffering from severe burns or diabetic ulcers. A well known version of these products, Apligraf[®], is produced and marketed by Organogenesis. The graft is composed of a layer of human keratinocytes cultured on top of a matrix of bovine type I collagen and human dermal fibroblasts (Eaglstein and Falanga 1997). Primarily used in the treatment of venous leg ulcers and

diabetic foot ulcers, it has been used in over 200,000 patients to date (I. Organogenesis 2008). Unfortunately, all current TE skin products are extremely expensive, largely because of the extensive *in vitro* cell culture time required before the graft fully matures and can be used clinically. However, the use of a nanostructured TE scaffold may significantly lower this culture time, and would, therefore, be advantageous in the production of artificial skin grafts.

For example, Chung et al. (Chung et al. 2006) explored the use of poly(ϵ -caprolactone) (PCL) grafted with nano-structured chitosan (CS) as a TE scaffold for the growth of human dermal fibroblasts. To create nano-structured CS, they first cast PCL as a flat surface, then applied acetone to the surface at 40°C while spinning at 80 rpm. The resultant nano-structured PCL surface was used as a mold, over which a crosslinked form of CS was cast, followed by an additional layer of PCL over the top smooth side of the CS film. This yielded an approximately 106 nm thick nano-structured CS film on top of a 100 μ m thick PCL film. Resultant nano-CS/PCL surfaces exhibited significantly higher surface roughness values as compared to smooth CS/PCL surfaces: 106.0 nm compared to 3.6 nm, respectively (Fig. 5). Furthermore, these nano-CS/PCL constructs exhibited significantly ($p < 0.001$) higher rates of fibroblast proliferation and viability as compared to smooth CS/PCL surfaces or nano-rough PCL surfaces. As such, the technique of solvent spin-etching for polymers may represent an inexpensive means to prepare nanoscale TE scaffolds as improved artificial skin grafts.

Recently, a group based in Singapore developed an alternative approach to wound healing, which they termed autologous layered dermal reconstitution (ALDR) (Chong et al. 2007). This technique relies upon novel TE scaffolds which consist of electrospun fibers made of PCL and gelatin, between 300 and 600 nm in diameter, with a total thickness of only 28 μ m (Fig. 6). The scaffolds were seeded with human dermal fibroblasts, which remained viable in the scaffold for all time points tested (up to 2 weeks) and doubled in population approximately every 3 days. Although no *in vivo* results are currently available, ALDR using electrospun scaffolds should offer a distinct advantage over traditional techniques. Namely, ALDR will allow for a rapid, layer-by-layer buildup of tissue in deep wounds, with dermal fibroblasts distributed throughout. This can occur because the electrospinning process takes place on top of a commercially available polyurethane wound dressing. As little as 48–72 h after implantation, the wound dressing can be removed, and another scaffold/wound dressing construct placed in the wound site. This is repeated until the wound area is fully repaired. Since each scaffold will be individually seeded with dermal fibroblasts prior to implantation, this layer by layer technique eliminates the long *in vitro* culture times otherwise needed for cellular

infiltration and growth within larger, single-layer scaffolds. The end result is a continuous layer of tissue, wherein the use of a porous, nanostructured scaffolds allows for rapid cellular proliferation and integration between layers.

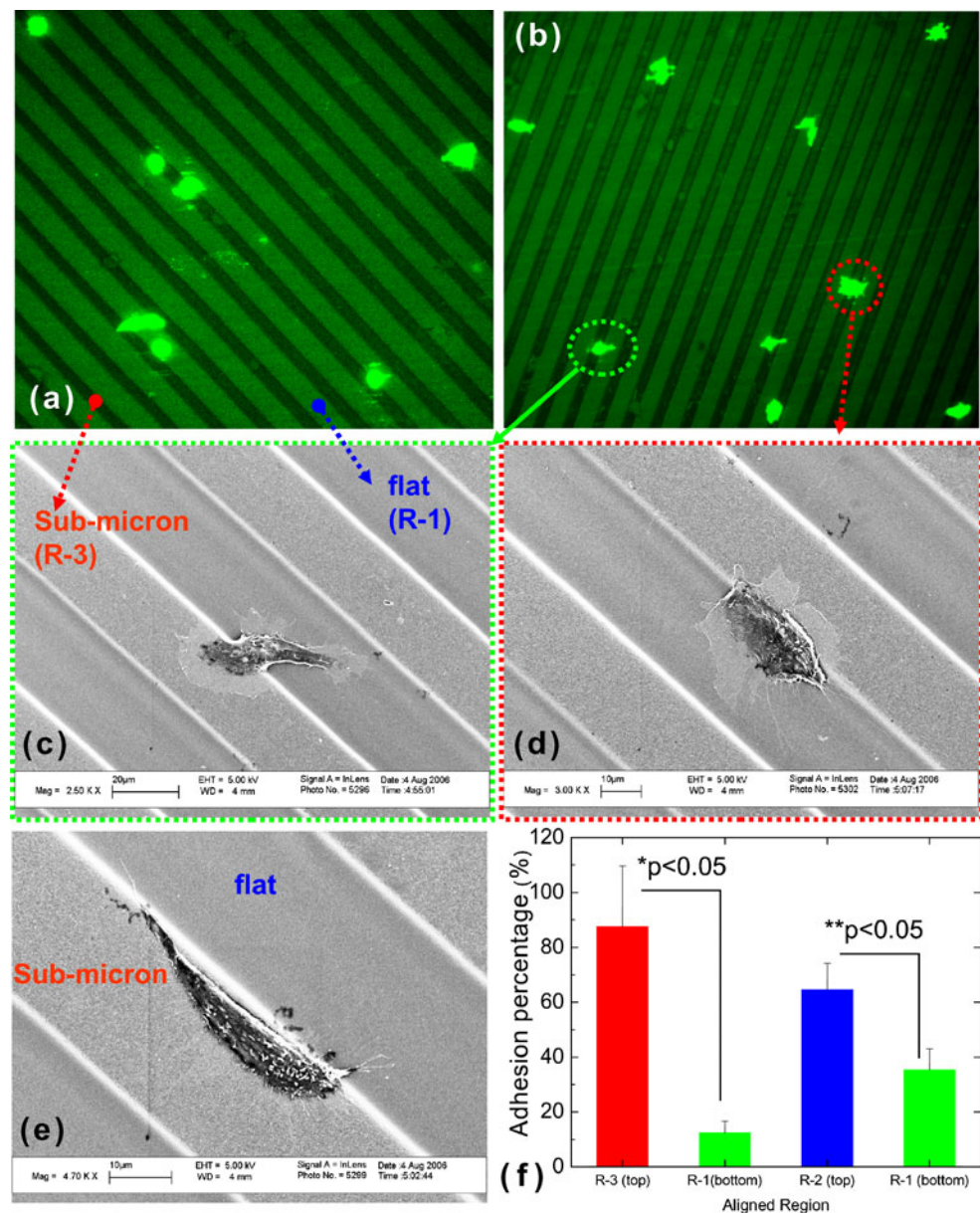
In another example, Sun et al. (Sun et al. 2005) fabricated electrospun polystyrene scaffolds, and tested the responses of skin fibroblasts, keratinocytes, and endothelial cells in both individual and co-culture systems using serum-free media. In individual cultures, no cells remained viable more than 3–4 days after cell seeding. Nonetheless, when cultured together, cells became integrated into the scaffold and even showed distinct regions of organization, with keratinocytes present in a continuous layer on the scaffold's upper, outer surface, while fibroblasts and endothelial cells were concentrated below that layer. Additionally, the

authors hypothesized that a nanoscale, electrospun scaffold is better suited to TE applications than decellularized human dermis, as the porosity of the former promotes rapid vascularization, whereas the latter consists of tightly woven proteins with sparse, tortuous voids.

2.3 Cardiac tissue

Many research groups have made significant contributions in recent years towards the development of functional heart tissue constructs. Their efforts have been plagued by the notoriously demanding cell culture requirements of cardiac myocytes. Zong et al. were one of the first groups to report positive results of the use of cardiac myocytes for developing cardiac tissue. They did so by creating

Fig. 4 Bone cell adhesion on aligned nanostructured titanium patterns. Fluorescence live cell images, (a) and (b), showed that osteoblasts adhered more on sub-micron structures than flat titanium. The SEM images (c)–(e) indicated different cell adhesion. (f) Bone cell adhesion density analysis showed that both nanometer and sub-micron surface features led to selective adhesion compared to flat surfaces. Greater adhesion probability on sub-micron roughness compared to nanometer surfaces was observed (*R3* submicron, *R2* nano and *R1* flat). Image was adopted from Khang et al. (2008)



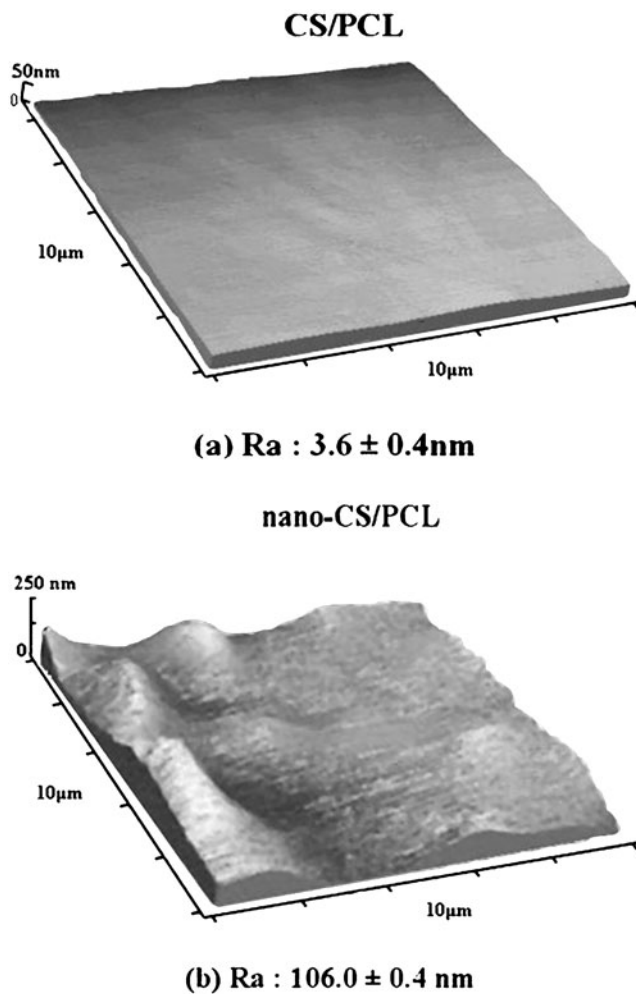
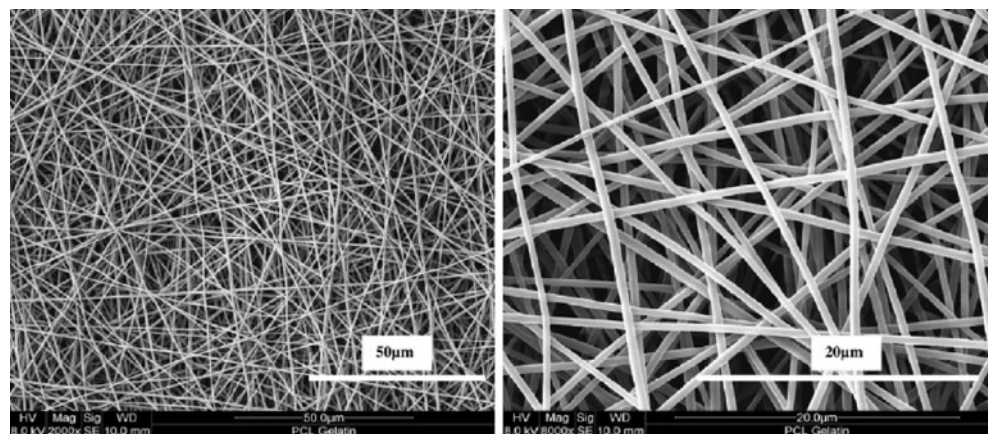


Fig. 5 Representative AFM images and surface roughness values of: (a) smooth CS/PCL surfaces and (b) Nano-CS/PCL surfaces created by Chung et al. Image adopted from Chung et al. (2006)

submicron structured scaffolds consisting of electrospun poly(L-lactide) (PLLA) and poly(lactic-co-glycolic) acid (PLGA) fibers (Zong et al. 2005). They produced scaffolds consisting of randomly oriented fibers, then subjected some to a uniaxial stretching and heating process in order to

Fig. 6 PCL-Gelatin hybrid scaffolds created via electrospinning, consisting of randomly oriented fibers between 300 and 600 nm in diameter. Image adopted from Chong et al. (2007)



achieve a higher degree of fiber alignment. Not only did cardiac myocytes adhere to and grow on the surface of the scaffolds, they actually ‘crawled’ inside and aligned with the local orientation of the fibers (Fig. 7). Cells showed a preference for pure PLLA scaffolds as compared to PLGA scaffolds, likely due to the decreased hydrophilicity of such materials. One of the most remarkable results was that only 7 days after cell seeding onto PLLA scaffolds, cells had established a cohesive electrical unit and were able to respond to external pacing stimuli at rates up to 6 Hz.

Beyond the regeneration of the functional myocardium, a substantial amount of recent research has focused on developing and growing *in vitro* TE heart valves. With over 85,000 artificial or animal-derived replacement valves implanted in the U.S. each year (Neidert and Tranquillo 2006), there is a distinct need for such products. Of the replacements currently available, artificial valves require the use of chronic anticoagulant therapy, while animal-derived valves often calcify, harden, and must be replaced after several years. A successfully developed TE valve would not possess any such shortcomings. As an added advantage, it could grow to accommodate enlargement of the heart as pediatric patients mature. Recently, Brody et al. (Brody et al. 2006) characterized the decellularized basement membrane of porcine aortic valves, finding specific nanoscale surface feature dimensions in all regions of the valve. Specifically, analysis using both SEM and AFM (Fig. 8) revealed a matrix of fibers possessing diameters of approximately 30 nm. In addition, data analysis indicated that the matrix had an average pore diameter of approximately 30 nm, with a typical pore depth of 22 nm. Interestingly, there were no significant differences in feature size between measurements performed at the base of the valve or those performed at the apex. Clearly, this membrane would be termed a nanoscale substrate. In fact, feature sizes of the aortic basement membranes examined in this study were significantly smaller than those of the endothelial basement membrane found in both the cornea and bladder of various mammals. As such, it would

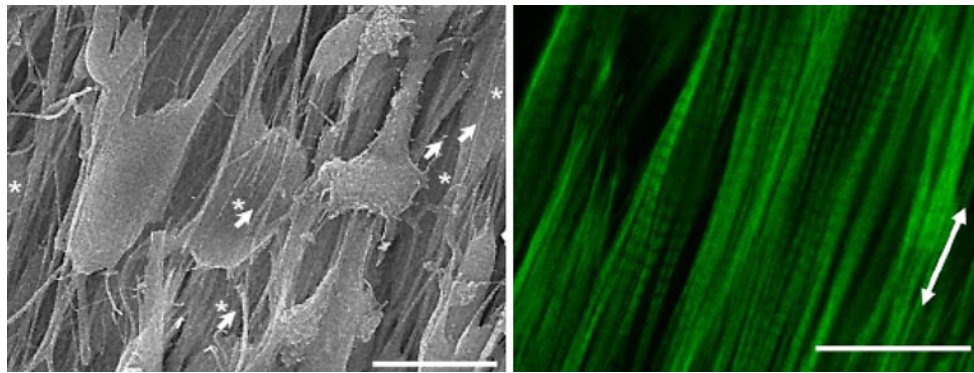


Fig. 7 *Left* SEM image of cardiac myocytes growing on uniaxially stretched, aligned electrospun PLLA scaffolds. Stars indicate cells which have ‘crawled’ beneath PLLA fibers, arrows denote these

fibers. Scale bar=40 μm . *Right* Confocal laser microscopy image of actin fibers within cardiac myocytes growing on the same scaffolds. Scale bar=20 μm . Image adopted from Zong et al. (2005)

certainly follow that an optimum TE scaffold should possess similar scale surface features in order to optimize its interactions with endothelial cells, thus, ensuring a confluent and healthy endothelium across the valve surface.

2.4 Vascular tissue

Finding surfaces that will promote the adhesion, proliferation, and proper function of endothelial and vascular smooth muscle cells is a rapidly expanding sector of tissue engineering. There are two main applications, one aimed towards developing small diameter vascular grafts and the second aimed towards creating the next generation of vascular stent materials. In both cases, several groups have focused their efforts on ‘tuning’ the surface’s topography at the submicron and nanoscale level in order to optimize vascular cell interactions. Our research group has reported a number of findings in recent years, generally pertaining to

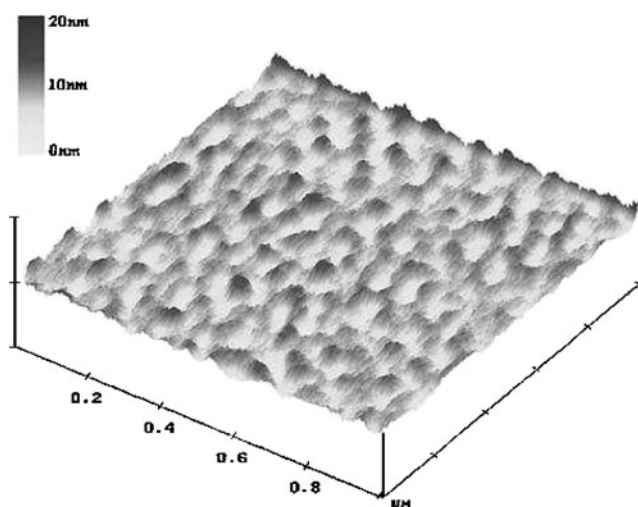


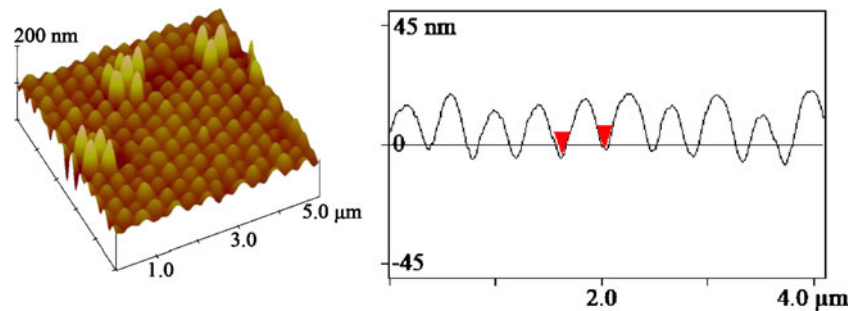
Fig. 8 AFM image of aortic valve basement membrane, demonstrating nanoscale topography in all dimensions. Image adopted from Brody et al. (2006)

vascular cell function on nanostructured PLGA surfaces, as well as titanium and nitinol (Samaroo et al. 2008).

Specifically, in 2004, Miller et al. (2004) first reported enhanced endothelial and vascular smooth muscle cell adhesion and proliferation on nanostructured as compared to smooth PLGA surfaces. He later fabricated a series of PLGA surfaces with identical chemistry composed of submicron scale spherical surface features (Miller et al. 2007), finding that surfaces with 200 nm lateral diameter spherical surface features exhibited significantly ($p < 0.05$) increased endothelial and vascular smooth muscle cell adhesion as compared to smooth surfaces or those with 100 nm or 500 nm lateral diameter spherical surface features. It was not until recently, however, that we were able to observe the mechanisms behind increased vascular cell adhesion to these surfaces (Carpenter et al. 2008). To do so, Carpenter et al. fabricated a series of PLGA surfaces with spherical surface features with diameters of 190, 300, 400, or 950 nm (Fig. 9). The water contact angle and surface free energy, relative affinity for adsorption of fibronectin and collagen type IV, and quantity of endothelial cell adhesion was determined on each type of surface. Specifically, endothelial cell adhesion to submicron scale PLGA surfaces was directly proportional to the quantity of fibronectin and collagen type IV present on those surfaces. In addition, all of the same-chemistry PLGA surfaces, regardless of surface topography, exhibited statistically equivalent endothelial cell adhesion in the absence of proteins. Furthermore, it was observed that fibronectin and collagen type IV adsorption, as well as endothelial cell adhesion, were more dependent on the vertical dimension of surface features than the lateral dimension, and that the optimum vertical surface feature dimension was approximately 20 nm.

Lu et al. also recently reported increased adhesion and proliferation levels of endothelial cells on titanium (Ti) stent surfaces patterned with nanometer as opposed to micron scale surface features (Lu et al. 2008). These surfaces consisted of periodic arrays of linear, rectangular

Fig. 9 Representative AFM image and height profile of a PLGA surface created using a template of PS nanobeads with a diameter of 400 nm. Horizontal distance between *red arrows*, 410.16 nm. Image adopted from Carpenter et al. (2008)



cross-section grooves with width and spacings between 750 nm and 100 μm . Such grooves were produced using a novel plasma-etching technique, known as titanium inductively coupled plasma deep etching (TIDE), a process that allows for extremely high resolution coupled with relatively low costs. Endothelial cell proliferation studies yielded a surprising result: although random nanostructured Ti surfaces exhibited higher initial endothelial cell adhesion than smooth Ti surfaces, those surfaces with linear nanoscale surface patterns exhibited endothelial cell adhesion which was higher. Furthermore, whereas endothelial cells reached confluence after 5 days in culture on surfaces with linear nanoscale surface patterns, they were less than 20% confluent on surfaces with random nanoscale surface patterns after that same time interval. For all time points, as the width

and spacing between grooves decreased, the alignment of endothelial cells increased, with cells exhibiting a more linear, rather than rounded, morphology.

He et al. (He et al. 2006) evaluated human coronary artery endothelial cell adhesion and gene expression on aligned and randomly oriented electrospun poly(L-lactic acid)-co-poly(ϵ -caprolactone) (P(LLA-CL)) coated with collagen type I. Aligned scaffolds were created using a sharpened rotating disk collector. Randomly oriented fibers had an average diameter of 470 nm, while aligned fibers had an average diameter of 406 nm, with the decrease likely due to stretching caused by the rotating disk collector. Scaffolds were approximately 25 μm thick, with porosity between 64% and 67% for randomly oriented fibers, or between 69% and 72% for aligned fibers (Fig. 10). Fibers

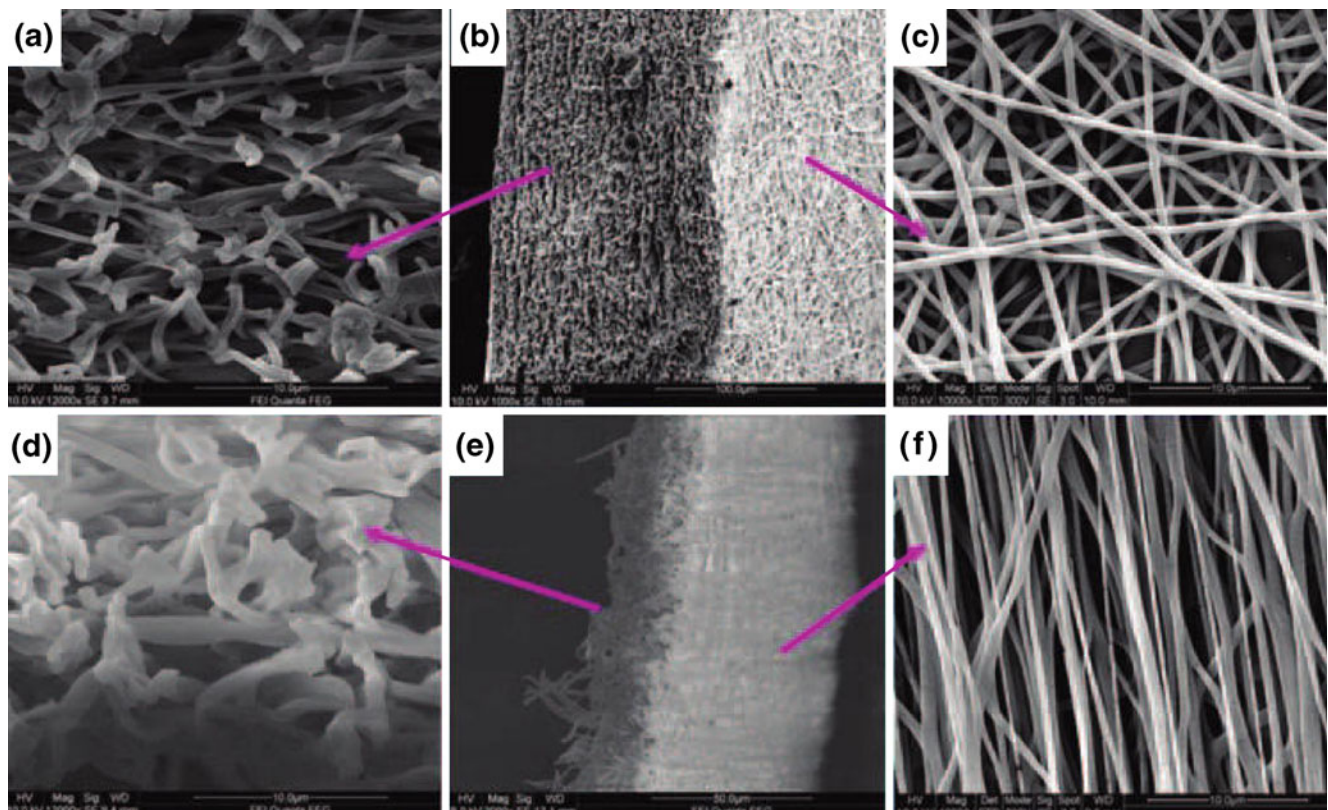


Fig. 10 SEM images of a cross-section (**a** and **d**), 3-dimensional structure (**b** and **e**), and top view (**c** and **f**) of random (**a–c**) and aligned (**d–f**) P(LLA-CL) nanofiber scaffolds. Image adopted from He et al. (2006)

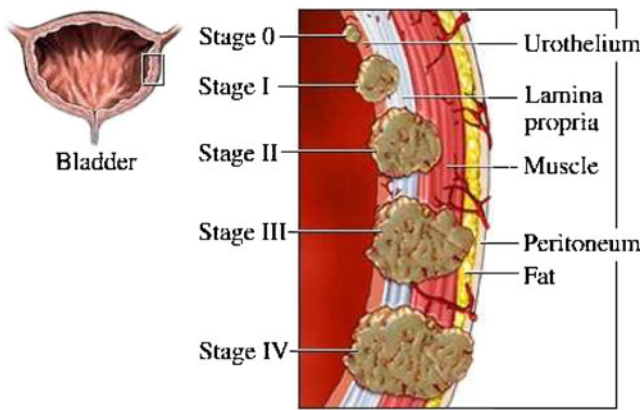


Fig. 11 Image of the urinary bladder and urothelial carcinoma of the bladder. *Stage 0* flat carcinoma (epithelium confined), *stage I* lamina propria invasion, *stage II* invasive into muscle layer, *stage III* extension into fat area, and *stage IV* extension to fat area

were coated with collagen by first subjecting scaffolds to a plasma treatment to increase hydrophilicity, then soaking overnight in a 290 $\mu\text{g/ml}$ solution of collagen. Mechanical testing indicated that scaffolds consisting of aligned fibers exhibited increased tensile modulus and tensile strength but lower ultimate tensile strain as compared to scaffolds consisting of randomly oriented fibers. Endothelial cell culture results demonstrated that scaffolds consisting of randomly oriented and aligned fibers both supported cell adhesion and resulted in nearly equivalent levels of gene expression, however, scaffolds consisting of aligned fibers induced cellular alignment and an elongated morphology. Furthermore, as compared to P(LLA-CL) scaffolds which were not coated with collagen, both types of P(LLA-CL) scaffolds coated with collagen (randomly oriented and aligned fibers) demonstrated increased endothelial cell adhesion, spreading, and proliferation.

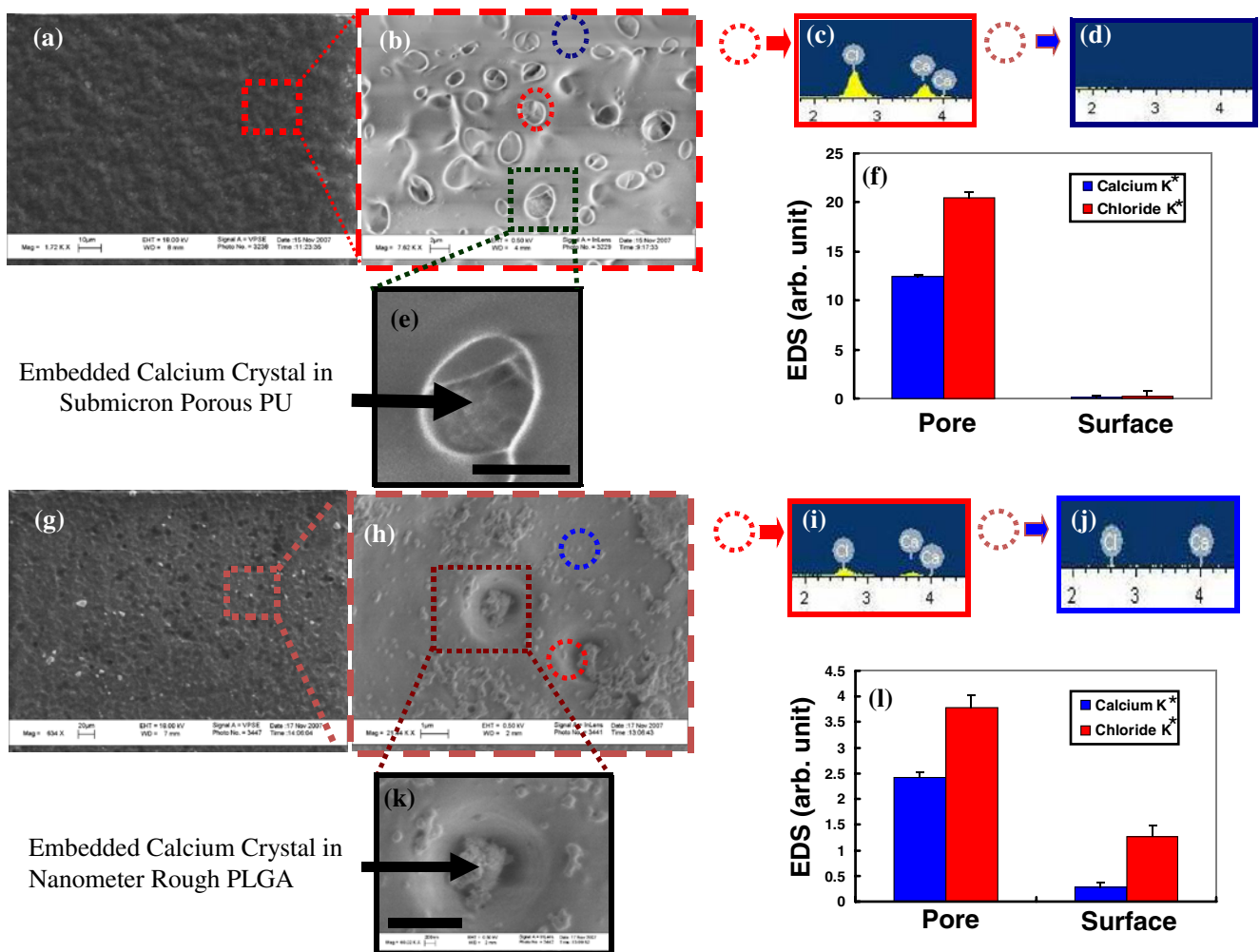


Fig. 12 Conformation of calcium stones on nanometer rough PU and PLGA. SEM image after 4 h of calcium oxalate soaking on conventional (a and g) and nanometer rough PU and PLGA (b and h), respectively. Some of the pores were observed with embedded calcium crystals. (e and k) and (c and i) showed EDS signals from the

pores of PU and PLGA, respectively. It was distinct that the non-embedded surface (d and j) showed negligible Cl and Ca signals by EDS although Ca and Cl signals were detected in pores on PU and PLGA (f and l). * mark is K orbital

2.5 Bladder tissue

There are numerous clinical trials underway which employ tissue engineering in order to address problems such as the recurrence of bladder cancer and the eventual need for the transplantation of donor organs (Fig. 11). Several such trials focus upon bladder disease, applying novel concepts of tissue engineering in order to return diseased tissue to a healthy, functional state. To that end, several bladder substitutes have been developed which utilize various biomaterials, both natural and synthetic (Atala 2000).

Poly-*dl*-lactide-*co*-glycolide (PLGA, 50:50) has successfully promoted the growth of bladder smooth muscle and urothelial cells *in vitro* (Atala et al. 1992). Unfortunately, however, many conventional synthetic biodegradable polymers and natural scaffolds investigated to date have shown limited success in bladder tissue regeneration applications due to their poor mechanical stability, adverse tissue and immune responses, etc. (Agrawal et al. 1997; Kambic et al. 1992). Thus, it is necessary to develop bladder organ replacement design strategies not with conventional biodegradable polymers, but rather with advanced novel biodegradable polymers in which the synthetic material is quickly and efficiently replaced by healthy host tissue. It is known that bladder cells respond differently to nano-dimensional, micron, and flat surface features; this could be because the surfaces of these nano materials mimic their natural environment (i.e., extracellular matrix proteins in bladder tissue are nano-dimensional) (Ayad et al. 1994). Recently, it was reported that PLGA and polyurethane (PU) with nanoscale surface features promoted the growth of bladder smooth muscle cells more than conventional PLGA and PU (Thapa et al. 2003).

Interestingly, tissue engineered bladders often form a small number of calcium oxalate stones even after successful implantation. Such idiopathic calcium oxalate urolithiasis is a frequent and recurrent multifactorial disease, occurring even after the removal of calcium oxalate stones. A study of calcium stone recurrence showed a 14% return after 1 year, 35% after 5 years, and 52% after

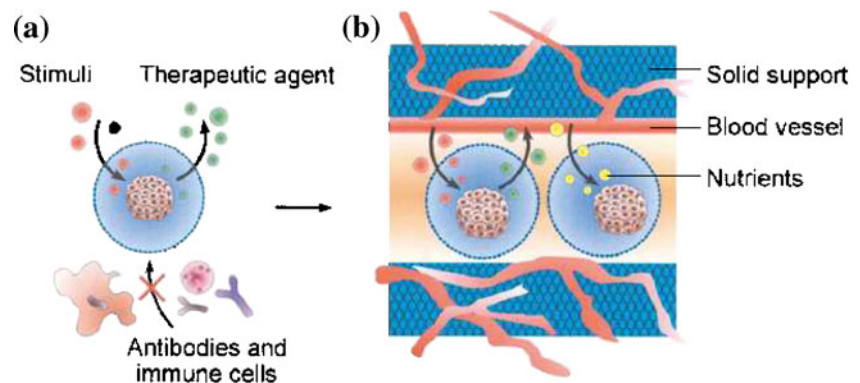
10 years (Uribarri et al. 1989). Recent data (Chun et al. 2008) indicated that submicron pored, nanometer rough PU not only enhanced the adhesion and proliferation of bladder urothelial cells but also led to the formation of fewer calcium oxalate stones than conventional polymers. The pores in the surface of advanced polymers created using nanotechnology functioned to absorb calcium and oxalate separately, thus, preventing interactions which would otherwise lead to the formation of calcium oxalate stones (Fig. 12).

In addition, Pattison et al (Pattison et al. 2005) showed that novel, three-dimensional, porous PLGA scaffolds promote increased adhesion, growth, and protein production by bladder smooth muscle cells as compared to what occurred on conventional, micro-dimensional scaffolds. Moreover, experiments provided evidence that, in general, scaffolds and resident cells experiencing a sustained pressure stimulus functioned similarly to those experiencing atmospheric pressures. Thus, these engineered scaffolds using nanotechnology hold great promise towards the development of *in vivo* replacements for the urinary bladder wall.

2.6 Cell encapsulation

In the few last decades, researchers involved in the development of pharmaceuticals have understood that drug delivery is a fundamental part of drug development. One of the emerging technologies that has gained the attention of the scientific community is cell encapsulation because of its wide range of possible applications in nano-biotechnology, medicine and ecology. Microencapsulation of living genetically engineered cells may be used as drug delivery vehicles, immunotherapies and engineered tissues (Dove 2002). People envisage the use of encapsulated cells in clinical treatments, like type I diabetes, central nervous system regeneration, and tumor therapy. Implantation of microencapsulated recombinant cells has great potential for gene therapy where therapeutic proteins can be sustained and, in the long-term, delivered by microencapsulated cells (Wang et al. 2006).

Fig. 13 Cell microencapsulation. (a) Nutrients, oxygen and stimuli diffuse across the membrane, whereas antibodies and immune cells are excluded. (b) Pre-vascularized solid support system to facilitate optimal nutrition of the enclosed cells. Image adopted from Orive et al. (2003)



The technology of cell microencapsulation represents a strategy in which cells that secrete therapeutic products are immobilized and immunoprotected within polymeric and biocompatible devices (Orive et al. 2003). Based on this concept, a wide spectrum of cells and tissues may be immobilized, enhancing the potential applicability of this strategy to the treatment of numerous diseases (Chang 2005). One potential impact of this drug delivery approach is that the administration of immunosuppressants and implementation of strict immunosuppressive protocols can be eliminated, therefore the serious risks associated with these drugs can also be avoided. Figure 13 shows the concept of cell encapsulation.

Various materials including microspheres and hydrogels have been employed as injectable scaffolds in these approaches. Hydrogels have a similar microstructure to extracellular matrix (ECM) proteins and can undergo a sol–gel transition in very mild conditions. The hydrogel precursor loaded with targeted cells can be injected into the damaged site and experience a gel transition in situ due to physical or chemical stimuli. The encapsulated cells grow within the hydrogel and secrete their own ECM to reestablish the damaged tissue.

This strategy has provided a wide range of promising therapeutic treatments for central nervous system diseases (Borlongan et al. 2004), diabetes (Korbitt et al. 2004), hemophilia (Hortelano et al. 1996), and anemia (Rinsch et al. 2002) among others. This technology offers a safe and manufacturable method for the local and systemic delivery of therapeutic molecules from the enclosed cells. It can be considered as a “living drug delivery system” where the transplanted cells provide an unlimited drug source. As long as the cells are viable and functional, they are able to release the desired products in a more physiological manner. The membrane of the microcapsule can serve as an immunoisolation barrier to keep the host’s immune system away from the living cells, but at the same time, it allows nutrients, oxygen, waste, and cell products to pass through without much difficulty.

2.6.1 Micro-encapsulation of cells

Possible micro-encapsulation techniques include droplet generation, emulsion formation, polyelectrolyte multilayering, and direct polymerization from a surface-adsorbed initiator. In many applications, it is desirable to use scaffolds prepared from polymers with material properties that resemble those of the native ECM, a soft, tough, and elastomeric network that provides mechanical stability and structural integrity to cells.

For instance, in the transplant therapy for type I diabetes, an approach to overcome the use of immunosuppressive drugs is the immunoprotection of pancreatic islets by

alginate microencapsulation in order to prevent graft rejection (Soonshiong et al. 1994; de Vos et al. 2003). Alginate–poly-L-lysine–alginate (APA) microcapsules are one of the most widely studied microcapsules and they provide a special three-dimensional microenvironment in which cell growth and metabolism are greatly affected (such as cell proliferation, cell differentiation and metabolic function change). Alginates are linear polysaccharides of 1–4 linked β -D-mannuronic acid and its C-5 epimer, α -D-guluronic acid with varying proportions. Two guluronate blocks of adjacent polymer chains can be cross-linked with multivalent cations (Ca^{2+} , Cu^{2+} , Ba^{2+}) through interactions with the carboxylic groups in the sugars, which led to the formation of a gel network for use in cell microencapsulation by many researchers (Joki et al. 2001; Cruise et al. 1999). Commonly, alginates with high guluronic acid content generate hard porous gels which can retain their integrity for the long term. The excessive swelling and shrinking of these alginates do not occur. Hence, they can maintain their physical form during cross-linking. In contrast, alginates with high mannuronic acid content develop softer, less porous beads which tend to disintegrate earlier than beads made with alginates rich in guluronic acid. Furthermore, alginates with high mannuronic acid content readily undergo a high degree of swelling and shrinking during Ca^{2+} cross-linking, and cells encapsulated in these soft alginates tend to proliferate without inhibition (Simpson et al. 2003).

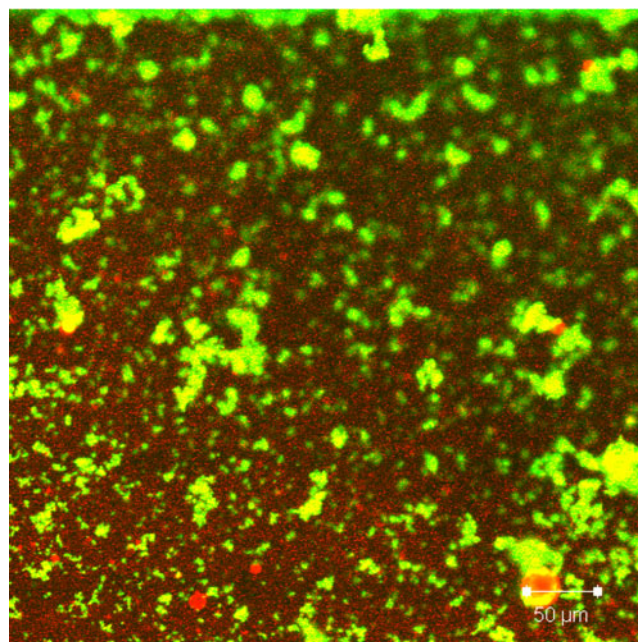


Fig. 14 PC12 cells observed under a confocal microscope (stained with live (green)/dead cell (red) assay). Image adopted from Pareta and Webster (2007)

2.6.2 Cell encapsulation using nanotechnology

Instead of using capsule technology as used in microencapsulation, layer by layer nanoscale coatings can be applied directly to the surface of the cell(s) to be encapsulated. These biocompatible nano-structured coatings serve in a similar fashion as the micro-capsules but have the added advantage of easy availability of diffusion of vital oxygen and essential nutrients. The encapsulation using nanotechnology also significantly reduces the volume of the cells to be encapsulated, thus, reducing clotting.

One of such techniques used for nano-structured cell encapsulation is co-axial electrospinning. In this technique, the cells are mixed with a nano-structured hydrogel and are forced through the inner capillary while another biodegradable polymer is forced through the outer co-axial capillary under the effect of electro-hydrodynamic forces (Pareta and Edirisinghe 2006), thus producing an intimate coating of a biodegradable polymer over the cells embedded in a nano-structured hydrogel. Figure 14 shows encapsulated PC12 cells (a model nerve cell) in a nanostructured PLGA matrix after 3 days showing very high viability.

3 Conclusions

Incorporating nanotechnology into medicine promises to greatly increase the quality of human life, because all tissues have a finite functional lifetime. Nanotechnology is being used to inhibit infection, decrease inflammation, and overall increase the initial formation of tissue necessary to prolong implant lifetime. Current trends in nanotechnology have evolved very systematically, with direct applications to the current limitations of medicine (such as for cancer and heart disease). To achieve this goal, an extremely broad, interdisciplinary research field is needed. Such a research field is required which will both unravel the mechanisms of tissue cell-biomaterial interactions at the nanoscale and develop methods to create unique nanoscale surface features applicable to numerous medical fields. For example, stem and cancer cell therapeutics incorporating nanoscale biomaterials represent a major clinical goal of nanomedicine. Thus, it has become evident that nanotechnology will be a critical tool in the fight to resolve current critical medical issues. It will catalyze the development of biologically inspired nano biomaterials whose functions can meet or even exceed the capabilities of natural organs and tissues in the human body.

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References

- C.M. Agrawal, A. Pennick, X. Wang, R.C. Schenck, Porous-coated titanium implant impregnated with a biodegradable protein delivery system *J. Biomed. Mater. Res.* **36**(4), 516–521 (1997)
- C. Allen, D. Maysinger, A. Eisenberg, Nano-engineering block copolymer aggregates for drug delivery *Colloids Surf., B Biointerfaces* **16**(1–4), 3–27 (1999)
- A. Atala, Tissue engineering of artificial organs *J. Endourol.* **14**(1), 49–57 (2000)
- A. Atala, J.P. Vacanti, C.A. Peters, J. Mandell, A.B. Retik, M.R. Freeman, Formation of urothelial structures *in vivo* from dissociated cells attached to biodegradable polymer scaffolds *in vitro* *J Urol* **148**(2), 658–662 (1992)
- S. Ayad, R. Boot-handford, M. Humpries, K. Kadler, A. Shuttleworth, *The Extracellular Matrix Facts Book* (Academic, San Diego, 1994)
- C.V. Borlongan, S.J.M. Skinner, M. Geaney, A.V. Vasconcellos, R.B. Elliott, D.F. Emerich, Neuroprotection by encapsulated choroid plexus in a rodent model of huntington's disease *Neuroreport* **15** (16), 2521–2525 (2004)
- S. Brody, T. Anilkumar, S. Liliensiek, J.A. Last, C.J. Murphy, A. Pandit, Characterizing nanoscale topography of the aortic heart valve basement membrane for tissue engineering heart valve scaffold design *Tissue Eng.* **12**(2), 413–421 (2006)
- J. Carpenter, D. Khang, T.J. Webster, Nanometer polymer surface features: the influence on surface energy, protein adsorption and endothelial cell adhesion *Nanotechnology* **19** (2008) 505103 (8 pp), doi:10.1088/0957-4484/19/50/505103
- T.M.S. Chang, Therapeutic applications of polymeric artificial cells *Nat. Rev. Drug Discov.* **4**(3), 221–235 (2005)
- E.J. Chong, T.T. Phan, I.J. Lim, Y.Z. Zhang, B.H. Bay, S. Ramakrishna, C.T. Lim, Evaluation of electrospun pcl/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution *Acta Biomaterialia* **3**(3), 321–330 (2007)
- S. Choudhary, K.M. Haberstroh, T.J. Webster, Enhanced functions of vascular cells on nanostructured ti for improved stent applications *Tissue Eng.* **13**(7), 1421–1430 (2007)
- A.L. Chun, J.G. Moralez, H. Fenniri, T.J. Webster, Helical rosette nanotubes: a more effective orthopaedic implant material *Nanotechnology* **15**(4), S234–S239 (2004)
- A.L. Chun, J.G. Moralez, T.J. Webster, H. Fenniri, Helical rosette nanotubes: a biomimetic coating for orthopedics? *Biomaterials* **26**(35), 7304–7309 (2005)
- Y.W. Chun, D. Khang, K.M. Haberstroh, K.M.T.J. Webster, The role of polymer nano surface roughness and submicron pores for improving bladder urothelial cell density and inhibiting calcium oxalate stone formation *Nanotechnology*, in press (2008)
- T.-W. Chung, Y.-Z. Wang, Y.-Y. Huang, C.-I. Pan, S.-S. Wang, Poly (ε-caprolactone) grafted with nano-structured chitosan enhances growth of human dermal fibroblasts *Artif. Organs* **30**(1), 35–41 (2006)
- G.M. Cruise, O.D. Hegre, F.V. Lamberti, S.R. Hager, R. Hill, D.S. Scharp, J.A. Hubbell, *In vitro* and *in vivo* performance of porcine islets encapsulated in interfacially photopolymerized poly(ethylene glycol) diacrylate membranes *Cell Transplant* **8** (3), 293–306 (1999)
- P. de Vos, C.G. van Hoogmoed, J. van Zanten, S. Netter, J.H. Strubbe, H.J. Busscher, Long-term biocompatibility, chemistry, and function of microencapsulated pancreatic islets *Biomaterials* **24** (2), 305–312 (2003)
- A. Dove, Cell-based therapies go live *Nat. Biotechnol.* **20**(4), 339–343 (2002)
- W.H. Eaglstein, V. Falanga, Tissue engineering and the development of apligraf(r), a human skin equivalent *Clin. Ther.* **19**(5), 894–905 (1997)

- K.L. Elias, R.L. Price, T.J. Webster, Enhanced functions of osteoblasts on nanometer diameter carbon fibers *Biomaterials* **23**(15), 3279–3287 (2002)
- C. Ergun, H.N. Liu, T.J. Webster, E. Olcay, S. Yilmaz, F.C. Sahin, Increased osteoblast adhesion on nanoparticulate calcium phosphates with higher ca/p ratios *J. Biomed. Mater. Res. Part A* **85A**(1), 236–241 (2008)
- W. He, T. Yong, Z.W. Ma, R. Inai, W.E. Teo, S. Ramakrishna, Biodegradable polymer nanofiber mesh to maintain functions of endothelial cells *Tissue Eng.* **12**(9), 2457–2466 (2006)
- G. Hortelano, A. AlHendy, F.A. Ofosu, P.L. Chang, Delivery of human factor ix in mice by encapsulated recombinant myoblasts: a novel approach towards allogeneic gene therapy of hemophilia b *Blood* **87**(12), 5095–5103 (1996)
- I. Organogenesis, Organogenesis debuts educational campaign for sufferers of chronic leg and foot ulcers. Canton, (2008)
- H.J. Jin, J.S. Chen, V. Karageorgiou, G.H. Altman, D.L. Kaplan, Human bone marrow stromal cell responses on electrospun silk fibroin mats *Biomaterials* **25**(6), 1039–1047 (2004)
- T. Joki, M. Machluf, A. Atala, J.H. Zhu, N.T. Seyfried, I.F. Dunn, T. Abe, R.S. Carroll, P.M. Black, Continuous release of endostatin from microencapsulated engineered cells for tumor therapy *Nat. Biotechnol.* **19**(1), 35–39 (2001)
- H. Kambic, R. Kay, J.F. Chen, M. Matsushita, H. Harasaki, S. Zilber, Biodegradable pericardial implants for bladder augmentation: a 2.5-year study in dogs *J. Urol.* **148**(2), 539–543 (1992)
- S. Kay, A. Thapa, K.M. Haberstroh, T.J. Webster, Nanostructured polymer/nanophase ceramic composites enhance osteoblast and chondrocyte adhesion *Tissue Eng.* **8**(5), 753–761 (2002)
- D. Khang, M. Sato, R.L. Price, A.E. Ribbe, T.J. Webster, Selective adhesion and mineral deposition by osteoblasts on carbon nanofiber patterns *Int. J. Nanomedicine* **1**(1), 65–72 (2006)
- D. Khang, S.Y. Kim, P. Liu-Snyder, G.T.R. Palmore, S.M. Durbin, T.J. Webster, Enhanced fibronectin adsorption on carbon nanotube/poly(carbonate) urethane: independent role of surface nano-roughness and associated surface energy *Biomaterials* **28**(32), 4756–4768 (2007)
- D. Khang, J. Lu, C. Yao, K.M. Haberstroh, T.J. Webster, The role of nanometer and sub-micron surface features on vascular and bone cell adhesion on titanium *Biomaterials* **29**(8), 970–983 (2008)
- G.S. Korbitt, A.G. Mallett, Z. Ao, M. Flashner, R.V. Rajotte, Improved survival of microencapsulated islets during *in vitro* culture and enhanced metabolic function following transplantation *Diabetologia* **47**(10), 1810–1818 (2004)
- R. Langer, Drug delivery and targeting *Nature* **392**(6679), 5–10 (1998)
- R. Langer, Perspectives: drug delivery—drugs on target *Science* **293**(5527), 58–59 (2001)
- J. Lu, M.P. Rao, N.C. MacDonald, D. Khang, T.J. Webster, Improved endothelial cell adhesion and proliferation on patterned titanium surfaces with rationally designed, micrometer to nanometer features *Acta Biomaterialia* **4**(1), 192–201 (2008)
- J.A. McCann-Brown, T.J. Webster, K.M. Haberstroh, Vascular cells respond to endothelial cell flow- and pressure-released soluble proteins *Chem. Eng. Commun.* **194**(3), 309–321 (2007)
- D.C. Miller, A. Thapa, K.M. Haberstroh, T.J. Webster, Endothelial and vascular smooth muscle cell function on poly(lactic-co-glycolic acid) with nano-structured surface features *Biomaterials* **25**(1), 53–61 (2004)
- D.C. Miller, K.M. Haberstroh, T.J. Webster, Mechanism(s) of increased vascular cell adhesion on nanostructured poly(lactic-co-glycolic acid) films *J. Biomed. Mater. Res. Part A* **73A**(4), 476–484 (2005)
- D.C. Miller, K.M. Haberstroh, T.J. Webster, PLGA nanometer surface features manipulate fibronectin interactions for improved vascular cell adhesion *J. Biomed. Mater. Res. Part A* **81A**(3), 678–684 (2007)
- T.J. Webster (ed.), *Nanotechnology for the regeneration of hard and soft tissues* World Scientific, Danvers, MA, 2007.
- M.R. Neidert, R.T. Tranquillo, Tissue-engineered valves with commissural alignment *Tissue Eng.* **12**(4), 891–903 (2006)
- G. Orive, R. Hernández, A. Gascón, R. Calafiore, T. Chang, P. Vos, Cell encapsulation: promise and progress *Nat. Med.* **9**, 104–109 (2003) doi:10.1038/nm0103-104
- R. Pareta, M.J. Edirisinghe, A novel method for the preparation of biodegradable microspheres for protein drug delivery *J. R. Soc. Interface* **3**(9), 573–582 (2006)
- R. Pareta, T. Webster, Encapsulated neural cells in nano-featured polymer scaffolds, *MRS*, Boston, MA, (2007)
- G.E. Park, M.A. Pattison, K. Park, T.J. Webster, Accelerated chondrocyte functions on naoh-treated plga scaffolds *Biomaterials* **26**(16), 3075–3082 (2005)
- M.A. Pattison, S. Wurster, T.J. Webster, K.M. Haberstroh, Three-dimensional, nano-structured plga scaffolds for bladder tissue replacement applications *Biomaterials* **26**(15), 2491–2500 (2005)
- C. Rinsch, P. Dupraz, B.L. Schneider, N. Deglon, P.H. Maxwell, P.J. Ratcliffe, P. Aebischer, Delivery of erythropoietin by encapsulated myoblasts in a genetic model of severe anemia *Kidney Int.* **62**(4), 1395–1401 (2002)
- H.D. Samaroo, J. Lu, T.J. Webster, Enhanced endothelial cell density on niti surfaces with sub-micron to nanometer roughness. *Int. J. Nanomedicine* (2008) (in press)
- M. Sato, T.J. Webster, Nanobiotechnology: implications for the future of nanotechnology in orthopedic applications *Expert. Rev. Med. Devices* **1**(1), 105–114 (2004)
- M. Sato, E.B. Slamovich, T.J. Webster, Enhanced osteoblast adhesion on hydrothermally treated hydroxyapatite/titania/poly(lactide-co-glycolide) sol-gel titanium coatings *Biomaterials* **26**(12), 1349–1357 (2005)
- J.K. Savaiano, T.J. Webster, Altered responses of chondrocytes to nanophase plga/nanophase titania composites *Biomaterials* **25**(7-8), 1205–1213 (2004)
- N.E. Simpson, S.C. Grant, S.J. Blackband, I. Constantinidis, Nmr properties of alginate microbeads *Biomaterials* **24**(27), 4941–4948 (2003)
- P. Soonshiong, R.E. Heintz, N. Merideth, Q.X. Yao, Z.W. Yao, T.L. Zheng, M. Murphy, M.K. Moloney, M. Schmehl, M. Harris, R. Mendez, R. Mendez, P.A. Sandford, Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation *Lancet* **343**(8903), 950–951 (1994)
- M.M. Stevens, J.H. George, Exploring and engineering the cell surface interface *Science* **310**(5751), 1135–1138 (2005)
- T. Sun, S. Mai, D. Norton, J.W. Haycock, A.J. Ryan, S. Macneil, Self-organization of skin cells in three-dimensional electrospun polystyrene scaffolds *Tissue Eng.* **11**(7-8), 1023–1033 (2005)
- S. Svenson, D.A. Tomalia, Commentary—dendrimers in biomedical applications—reflections on the field *Adv. Drug Deliv. Rev.* **57**(15), 2106–2129 (2005)
- A. Thapa, D.C. Miller, T.J. Webster, K.M. Haberstroh, Nano-structured polymers enhance bladder smooth muscle cell function *Biomaterials* **24**(17), 2915–2926 (2003)
- J. Uribarri, H. Carroll, M. Oh, The first kidney stone *Ann. Intern. Med.* **111**(12), 1006–1009 (1989)
- W. Wang, X.D. Liu, Y.B. Xie, H. Zhang, W.T. Yu, Y. Xiong, W.Y. Xie, X.J. Ma, Microencapsulation using natural polysaccharides for drug delivery and cell implantation *J. Mater. Chem.* **16**(32), 3252–3267 (2006)
- B.C. Ward, T.J. Webster, The effect of nanotopography on calcium and phosphorus deposition on metallic materials *in vitro* *Biomaterials* **27**(16), 3064–3074 (2006)
- T.J. Webster, Nanophase ceramics as improved bone tissue engineering materials *Am. Ceram. Soc. Bull.* **82**(6), 23–28B (2003)
- T.J. Webster, R.W. Siegel, R. Bizios, Osteoblast adhesion on nanophase ceramics *Biomaterials* **20**(13), 1221–1227 (1999)

- T.J. Webster, C. Ergun, R.H. Doremus, R.W. Siegel, R. Bizios, Enhanced functions of osteoblasts on nanophase ceramics *Biomaterials* **21**(17), 1803–1810 (2000)
- T.J. Webster, L.S. Schadler, R.W. Siegel, R. Bizios, Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin *Tissue Eng* **7**(3), 291–301 (2001)
- T.J. Webster, M.C. Waid, J.L. McKenzie, R.L. Price, J.U. Ejiogor, Nano-biotechnology: carbon nanofibres as improved neural and orthopaedic implants *Nanotechnology* **15**(1), 48–54 (2004)
- C. Yao, E.B. Slamovich, T.J. Webster, Enhanced osteoblast functions on anodized titanium with nanotube-like structures *J. Biomed. Mater. Res. Part A* **85A**(1), 157–166 (2008)
- X. Zong, H. Bien, C.-Y. Chung, L. Yin, D. Fang, B.S. Hsiao, B. Chu, E. Entcheva, Electrospun fine-textured scaffolds for heart tissue constructs *Biomaterials* **26**(26), 5330–5338 (2005)