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Review Article

Addition of nanoscaled bioinspired surface features: A revolution for bone-related implants and scaffolds?

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Abstract: Our expanding ability to handle the "literally invisible" building blocks of our world has started to provoke a seismic shift on the technology, environment and health sectors of our society. During the last two decades, it has become increasingly evident that the "nanosized" subunits composing many materials—living, natural and synthetic—are becoming more and more accessible for predefined manipulations at the nanosize scale. The use of equally nanoscale sized or functionalized tools may, therefore, grant us unprecedented prospects to achieve many therapeutic aims. In the past decade, it has become clear that nano-scale surface topography significantly influences cell behaviour and may, potentially, be utilized as a powerful tool to enhance the bioactivity and/or integration of implanted devices. In this review, we briefly outline the state of the art and some of the current approaches and concepts for the future utilization of nanotechnology to create biomimetic implantable medical devices and scaffolds for *in vivo* and *in vitro* tissue engineering, with a focus on bone. Based on current knowledge, it must be concluded that not the materials and surfaces themselves but the systematic biological evaluation of these new material concepts represent the bottleneck for new biomedical product development based on nanotechnological principles. © 2013 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 102A: 275–294, 2014.

Key Words: nanotechnology, cell culture, surface design, bone, implant

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INTRODUCTION

Technological development within the last two decades brought into the spot light various research concepts for miniaturization of devices in all market segments. In particular, the potential ability to control and manipulate materials at an atomic level generated a torrent of new ideas and concepts for the redesign and development of new products for current and new fields of application. Besides the use in the fields of energy transport and storage, electronics, optics and environmental engineering, nanotechnology is also revolutionizing medicine. The new opportunities for the use of nanomaterials in various medical applications have recently been summarized under the term "Nanomedicine."[1] Further, it has been predicted that a dramatic growth in nanomedicine research will take place in the coming decades with an almost revolutionary large impact on all medical sectors such as diagnostics, drug discovery and delivery, tissue engineering, imaging agents and implantable devices.[2] The economic relevance of nanomedicine is obvious when patents and funding are taken as an index.[3,4] On the steadily expanding list of nanomedical products, we find different materials ranging from nanoscaled drug delivery systems, through nanofibers and three-dimensional (3D) scaffolds exhibiting nanocues for cells, degradable and nondegradable nanomaterials or nanocoatings, and last but not least therapeutic nanoparticles. To enable nanomedical innovations, it is crucial that the concurrent technology must undergo further development. It has been proposed that the nanotechnology improving today's implants may become one of the strongest commercial sectors in nanomedicine in the coming decade[5]. In this review, we report on the state of the art for the application of biomimetic principles for bone tissue

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regeneration and permanent implants using nanotechnological methods. In addition, we also highlight the progress limiting factors and the future concepts aiming at a real quantum leap in this area.

Definition of the nano-aspect in nanomedicine

The implementation of nanosized materials or units with totally new properties raised many questions for the definition of the term "Nanotechnology." An international group of scientists reviewed the published data in 2006 and came to the consensus that "*Nanotechnology comprises the emerging applications of Nanoscience. Nanoscience, on the other hand, deals with functional systems either based on the use of subunits with specific size-dependent properties or of individual or combined functionalized subunits.*"[6] ASTM 2456 refers nanotechnology as "*to a wide range of technologies that measure, manipulate, or incorporate materials and/or features with at least one dimension between approximately 1 and 100 nanometers (nm). Such applications exploit the properties, distinct from bulk/macroscopic systems, of nanoscale components.*"[7] Depending on the dimensions, with nanoscale as length scale, nano materials can be classified as: (a) materials which inside are composed of nanosized structural elements/units (bulk nanostructured materials), (b) nanostructured surfaces, layered or bearing lamellar structures including coatings, (c) nanosized filamentous structures, and (d) nanoparticles and nanodevices. Besides the definition of "nano," it is important to know which biological reactions are evoked by nanodimensional cues. Nanosizing of materials gives rise to new quantum mechanical effects modulating their physico-chemical properties such as optical, electrical, electronic, and mechanical characteristics, but how does this relate to new implantable materials? It is well accepted that nanoscale material features strongly influence the material—biology interactions [8,9] as will be discussed exemplarily in the following chapters.

The role of "nano" in the living organism

Living multicellular organisms are structured in their organization at each level. This is based on the interaction between cells and their surroundings including gradients in the noncellular environment [extracellular fluid and extracellular matrix (ECM)]. The ECM encompasses micro- and nanoscale aspects acting as cues for cells modulating their behavior and functionality. These aspects can be divided in structural, chemical, strain based and stiffness cues. The effects provoked by nanoscale cues may differ from those induced by microscale cues. Whereas microscale structural aspects predominantly affect cell functionality based on the change of the 3D cell shape,[10–13] the nanoscale features predominantly trigger their effects through components of the cell membrane such as integrins, receptor molecules and ion channels.[9,14–16] *In vivo*, the extracellular matrix nanoscale features are predominantly defined by the most abundant extracellular protein collagen in most tissues. Collagen fibrils are typically characterized by mean diameters of 45–110 nm depending on the specific tissue and species.[17] In bone apatite crystals of sizes of around 10–25 nm are deposited within the collagen matrix.[18,19] Also, in combination with other structural proteins, signalling factors are embedded within the ECM. Some become active if released from ECM molecules others are (also) active in their bound state.[20,21]

Biomimetic interfaces for bone injury repair

One of the most intriguing and probably the most important future approaches to design implantable materials in nanomedicine is to include concepts for biomimetic materials, for example, three-dimensionality, ligands for cell adhesion equivalent to those present in natural ECM of the target tissue, bioactive molecules, physical stiffness including local differences, fibrous aspects,[22] topography including nanostructures and microstructures and last but not least relevant surface chemistry modification. These biomimetic materials should neither induce prolonged inflammatory responses (relative to normal healing) nor other adverse side effects. Following implantation, recipient cells within the direct vincity will react with specific proteins that are immediately adsorbed to the implant surface upon contact with body fluids. Proteins, however, interact differently if a nanostructure is presented at the surface as shown in pilot studies by Webster et al.[9,23,24] Such studies concluded that the composition of the adsorbed proteins and their quaternary structure is dependent on the (nanometer) dimensions of the surface structures. Similarly, plane surfaces with different surface chemistry are also known to differ in their protein adsorption capacity. Thus both, the nanoscale surface structure and chemistry affect the protein adsorption potential as well as the types and composition of adsorbed proteins. This subsequently leads to the formation of specific protein interfaces between implanted material and cells. In the optimal configuration, the obtained protein layer shall not evoke an unusual intense and or prolonged inflammatory reaction triggered by changes in protein conformation of the adsorbed proteins but on the contrary, selectively stimulate the recruitement of appropriate cell types and the subsequent formation of the envisioned tissue. In some cases, the nature of the implant material chemistry or surface topography evokes suboptimal reactions (e.g., attachment of undesired cell types, adverse changes in cell functional state). To overcome these reactions, the implant material can be greatly modified by the use of biocompatible coatings isolating the implant from the biological environment ["protective layer," for example, diamonds like carbon (DLC) layer] and/or introducing a surface chemistry and structural modifications that are designed to evoke the correct body response. One possibility is to introduce features closely mimicking those of the target tissue. In the section "Nanostructured surfaces and inorganic coatings," a short overview will be given regarding this aspect by highlighting first, how surfaces can be structured and coated, and second, what kind of effects such types of structured and coated surfaces are able to evoke. A second way to produce a biomimetic surface which is less dependent on the adsorption of protein is to coat the surface with an artificial organic ECM. Such artificial ECM of which the composition

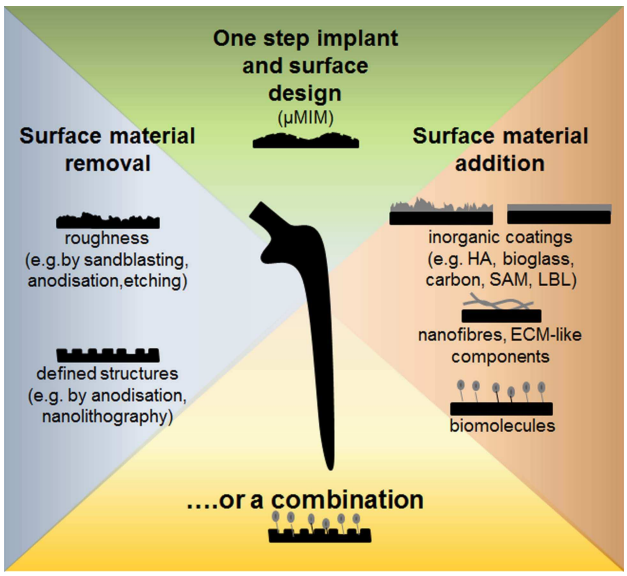

FIGURE 1. Schematic overview of various ways to design and chemically modify metallic implant surfaces. In brackets some examples of surface modifications are given. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

can be defined by the tissue aimed to be repaired can also be used as a stand-alone system for *in vivo* and *in vitro* tissue engineering. In the section, "Surface Biofunctionalization and artificial bioinspired ECM" examples of such ECM utilization will be addressed in detail.

NANOSTRUCTURED SURFACES AND INORGANIC COATINGS ON METALLIC IMPLANTS

As defined recently by Williams, the biocompatibility of a long term implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation in the host, without eliciting any undesirable local or systemic effects in that host.[25] It is clear that the host response represents not only a reaction to the material but to a large degree also to its surface properties.[26] The surface of any implantable material forms the first line of interaction with the host environment crucially paving the path to either, formation of the envisioned (successful integration) or the wrong tissue (failed integration, inflammatory response). In this respect, the surface physical characteristics of implantable devices may single-handedly dictate the route to clinical success.[27,28] The surface properties can be adapted by the material itself based on removal of a part of the surface or by addition of material onto the surface (surface coating; Fig. 1).

Nanostructuring of metallic implants

Cells recognize the micro and nanotopography of substrates and as a result may be affected in their performance. Such change in cell performance can appear for instance as a change of cell migration velocity or directionality,[29] promotion of the functional differentiation of progenitor cells and dynamic host tissue integration as in the case of orthopedic and dental implants[30,31] or reduction of cell adhesion.[32] Zhu et al.[33] found that rabbit osteoblast can sense grooves with dimensions as little as 70 nm as concluded by the observed cell elongation and alignment to the surface features. The team of Webster could show that by reducing the surface roughness to the nanoscale the macrophage response to alumina became reduced[34] and osteoblast cell adhesion increased.[9] Advances in biological sciences have shown that cells are sensitive to features as small as 5 nm in size and that cells continuously interact with the nanoscale features of the surrounding tissue.[35,36] The nanoscaled and microscaled cues may differently affect cells in terms of cell shape, proliferation, and differentiation.[10,23,37,38] These reactions may be determined by both cell type and surface features. Depending on the size range (microscale versus nanoscale) and the target tissue type, some of these topographical cues may be advantageous in promoting regenerative cell functions as suggested by effects on shape, proliferation, and differentiation of cells of the osteoblast lineage.[23,39,40] For example at the micrometer scale, Kunzler et al.[41] could show that fibroblast proliferation is stimulated by a smooth (arithmetic average of the absolute deviation of the roughness profile from the mean line, Ra $= 1\mu m$) but in contrast osteoblasts by a rough titanium coated surface (Ra $> 3.5\ \mu m$). At the nanometer scale, Webster et al.[23] investigated osteoblast and fibroblast cell adhesion on differently structured hydroxyapatite (HA) surfaces and found that by decreasing the HA grain size from 179 to 67 nm, osteoblast adhesion was increased but fibroblast adhesion decreased. Thus, the size specificity of such nanotopography features is of critical importance in defining the resulting bioactivity. As various cell types may populate an implanted surface, a randomized surface modification may result in areas promoting one cell type at the expense of another, whereas at other locations with a slightly different surface structure the other cell type may have advantages. Through controlled design, biomechanical cues may be provided to manipulate cell behavior at the implant–host tissue interface into a single-defined direction.[42]

Cells adhere to the implant surface through adsorbed proteins with accessible integrin binding motifs. Integrins are cell membrane proteins that mediate the contact of the cell with these proteins. Cells express specific sets of different types of integrins. The relative expression of these integrins is directly connected to the type of substrate[43] and probably defined by the type of accessible integrin binding motifs of the adsorbed proteins to which these integrins can adhere.[44] The integrin expression pattern is related to functional status of the cells.[45,46] Protein adsorption on substrates in terms of the amount and type of proteins is affected by surface charge and surface energy (observed as surface wetting capability).[47,48,49,50] By that the surface energy of an implanted material represents a key parameter with which cell behavior at the material interface can be altered. For example, Arima and Iwata[50] investigated the effects of surface charge and wettability on protein adsorption as well as endothelial and HeLa cell adhesion by using self-assembled monolayers, which were generated by mixing alkanotiols carrying different terminal groups (CH3, CH_3/OH,

CH_3/COOH, and CH_3/NH_2). In case of CH_3/COOH- and CH_3/NH_2-based surfaces protein adsorption was increased with increasing surface wettability (110° → 20°). The protein adsorption on CH_3/OH-based surfaces remained on the same level. Studying cell adhesion it was found that on hydrophobic surface (contact angle of around 110°) this was strongly reduced on all three, that is, CH_3/OH, CH_3/COOH and CH_3/NH_2, based surfaces. Maximal cell adhesion was seen in the range of 20–60° in case of negatively charged CH_3/COOH, and around 50–70° for positively charged CH_3/NH_2 based surfaces (at which similar quantity of attached cells were detected). A contact angle as low as 40° was needed to obtain similar cell densities on CH_3/OH based surfaces. Differences in cell adhesion on these three surfaces became even more prominent if albumin has been preadsorbed. Maximal differences were seen in the wettability range of 50–90°. Findings of Scotchford et al.[48] using similar surfaces with COOH, OH, and CH_3 terminated groups, suggest that fibronectin is especially adsorbed on COOH-containing surfaces whereas the adsorption of albumin which inhibits cell adhesion is more extensive on CH_3-based surfaces. Wei et al.[47] using differently oxidized hexamethyldisiloxone surfaces evaluated fibronectin and albumin binding under competitive conditions. They could prove that fibronectin selectively binds to surfaces with high wettability while albumin binds to surfaces with low wettability (e.g., four times more fibronectin on 0° contact angle surfaces than on 110° surfaces). Interestingly, no remarkable differences were found using single protein type solutions. Similarly, by conversion of hydrophobic microrough sandblasted and acid-etched Ti implants to superhydrophilic implants by alkali treatment Milleret et al.[51] showed that alkali treatment greatly affected the interaction of the surface with blood components. On untreated Ti surfaces blood clots remained thin, patchy and nonstructured lacking large fibrin fiber networks whereas blood clots on alkali treated surfaces assembled in an organized and layered architecture. Surface energy therefore represents one key factor that defines which proteins are preferentially adsorbed under competition and how. The selective binding of proteins may be due to the fact that proteins depending on their own charge preferentially adsorb to negatively, weakly charged or positively charged surfaces.[52,53] Not only *in vitro* but also *in vivo*, it was shown that the modification of surface chemistry greatly alters the biological response. For instance, Buser et al.[54] demonstrated that osseointegration was strongly enhanced if wettability of a structured etched titanium surface was increased from 138° to 0°.

Wettability of a surface is not only determined by the surface energy but also by the surface structure. With increasing roughness surfaces may become more hydrophobic[38,55] and together with surface area increases which may result in increased protein adsorption. It has been also hypothesized that the curvature of the surface structures additionally plays an important key role in protein adsorption.[56,57,55] Furthermore, the presence of nanoscale features may change the conformation of adsorbed integrin ligand motif containing proteins.[56,58] Webster et al.[24] reported that nanosized surfaces increased not only the total protein binding but also fibronectin and vitronectin binding by a factor of two without, however, affecting for instance albumin binding to the surface. These increased amounts of adsorbed cell adhesion proteins were found to correlate with increased osteoblast cell numbers adhering to these surfaces. Topography nanopatterning, as a another type of surface modification, may directly influence cell spreading and motility. It has been shown that even minor variations in surface nanofeature distribution lead, consequently, to different cell attachment, spreading and filopodia outgrowth patterns.[59,60] The latter may be based on the cell's ability to form focal adhesions—a process dependent on the intermolecular spacing distance of the surface bound integrin adhesion ligands.[61] In addition to *in vitro* studies the biological relevance of nanostructuring of implants has been shown in numerous *in vivo* studies.[62–64] In summary, wettability and nanostructuring affect cell adhesion and osseointegration. However, as certain studies suggest, the final *in vitro* and *in vivo* outcome cannot simply be deduced from individual effects that each surface parameter elicits.[63]

Nanostructured surfaces can be obtained by various approaches, each of them resulting in distinct surface features. These features may range from random roughness to defined nanostructures.

Random surface roughness. Surface roughness can be obtained by different techniques. Examples are:

a. *Controlled chemical oxidation.* This approach is seen as a straightforward way to produce nanoscale surface roughness of metal surfaces.[65,66] For instance, the team around de Olivierea oxidized TiO_2 surfaces using a mixture of H_2SO_4/H_2O_2 producing bioactive surfaces with pits in the nanometer range. This resulted in a nearly fivefold stimulation of calcium deposition on these surfaces in calvarial bone cell cultures without affting cell proliferation or viability as observed after 14 days in culture.[66] To evaluate the performance of these surfaces *in vivo* untreated and treated titanium screws were implanted in the mandibular bone of adult dogs.[65] In line with the *in vitro* data, and compared to untreated implants, a significant increase in bone to implant contact area after 8 weeks of implantation due to this treatment was detected around the treated screws.
b. *Sandblasting in combination with etching.* Similar surfaces to those previously mentioned have been produced by sandblasting combined with HCl/H_2SO_4 etching.[38] As demonstrated by using osteoblastic MG63 cells such kind of surface treatment enhanced the extent of cell differentiation as measured by osteocalcin release. Additionally, the total cell number was found to be reduced on these surfaces. A further treatment increasing the wettability strongly promoted osteoblast differentiation[38] and *in vivo* osseointegration[54] of these surfaces.
c. *Anodization*. Surfaces may be modified by using an electrochemical based anodization. By varying the anodization voltage potential, the size, and distribution of the

patterns generated by such procedure may be controlled. For example, the deposition of porous TiO_2/Ti_2O_3 layers using fast anodization produced random network-like surface roughness patterns similar to surface etching with pore size range variations of 10–300 nm.[67] As a result, osteoblastic cell adhesion increased by 50%.

d. *Layer-by-layer assembly*. This relatively new technique exploits the principle of electrostatic attraction between opposite charged species. Nanostructured coatings may be created based on an element deposition using colloidal suspensions thereof.[68,69] The adsorption rate/nature of such coating can be controlled with nanometer precision. So far this promising technique has been used for various applications including the production of microstructured surfaces using microparticles instead of nanoparticles[70,71] or to functionalize the implant surface with bioactive polyelectrolyte multilayers.[72] However, to our knowledge such approach has so far not been used for implant nanostructuring. To show the potential advantage for implant material surface design this technique has been applied for instance to produce TiO_2 particle films on glass substrates using 21 nm sized TiO_2 particles and the biological response of this coating was subsequently evaluated.[73] By increasing the number of deposited layers, a linear increase in nanoscale surface roughness (Ra) from ~20 to 140 nm was achieved. Enhanced initial attachment of mouse stromal cells took place on the functionalized surfaces using all nanometer roughness variations indicating noticeable surface bioactivity.
e. *Sintering techniques.* A new approach to obtain defined surface roughness in one step with implant production has recently been described, is micrometal injection moulding (microMIM) [74]. The sintering of a compacted mixture of micro and nanoparticles resulted not only in a nanostructured bulk material with outstanding positive mechanical characteristics but, in addition led to submicrometer scale surface roughness. This roughness is the result of the grain boundary grooves at the material surface which dimensions can be steered by particle size and sintering temperature.[74] *In vitro* these surfaces exhibited a good bioacceptance.[74] The biological effects *in vivo* still have to be evaluated.

The methods described above to obtain nano-to submicrometer roughness are relatively simple to apply. The application of all of these methods resulted in positive effects *in vitro* on osteoblasts performance and *in vivo* on osseointegration. Still, a systematic approach comparing all of aforementioned methods resulting in deeper understanding of the involved mechanisms is lacking. This lack of such knowledge limits the ranking of corresponding findings and subsequent, clear definition of further development steps.

Defined nanofeatures. The approaches discussed in the previous chapter aimed at producing randomized surfaces topography patterns. As mentioned in that chapter, the reaction to certain structures may be strongly cell type dependent. A random distribution of surface features in terms of conformation and spatial distribution may induce specific and desired but at the same time also undesired cell responses. To eliminate ineffective and/or adverse nanocues, efforts are currently made to produce more defined structures. Here, various techniques have been developed, partly only at the lab scale stage. These include:

a. *Anodization*. Besides generating surface roughness, defined geometrically complex nanofeatures can also be produced by anodization. The structures are, however, neither patterned, nor highly ordered. Nanotubes of various diameters (30, 50, 70, and 100 nm) have also been created using anodization in hydrofluoric and acetic acid.[75] As indicated by ALP, osteocalcin and osteopontin mRNA synthesis osteoblast differentiation of human mesenchymal stem cell was increased with increasing nanotubes diameter. Similarly, Sjöström et al.[76] used anodization to fabricate pillar-like nanostructured TiO_2 surfaces with reasonably reproducible heights (15, 55, and 100 nm), diameter (28, 41, and 55 nm) and center-to-center distances (40, 74, and 115 nm). Here, the 15 nm height pillar functionalized surface seems to give the best response regarding osteocalcin and osteopontin synthesis by human mesenchymal stem cells. Thus the anodization approach appears promising in terms to affect cell performance.
b. *Self-assembly–based surfaces (SAM).* SAM's are based on chemisorption of the hydrophilic head group followed by an outward alignment of the hydrophobic tail (e.g., asymmetric polystyrene-block-poly(methylmethacrylate). The advantage of SAM lies in generating a chemically, and structurally, well-defined surfaces using economically "cheap" approaches. Several SAM technologies are currently used which, partly, are still in the experimental stage. Examples include: phase-separated (di)block copolymer,[77,78] polymer mixture demixing,[79–81] colloidal surface structuring,[56,73] and nanoparticle containing micelle coating.[82] In the process of block-copolymer and polymer demixing (mixture of two immiscible polymers) a phase separation takes place resulting in highly patterned and ordered, self-organized nanotopography with controlled nanoscale roughness and structure following surface application and solvent evaporation. The polymer mixture demixing, resulted in structures are neither patterned nor highly ordered. Different structures can be obtained using polymer demixing as Lim et al., for example, dissolved Poly(L-lactic acid)(PLLA)/polystyrene (PS) in chloroform. After spin casting a nanostructred surface was created exhibiting nanopits.[79] By increasing the total polymer concentration in the solvent solution, nanopit sizes were created with increasing size (14, 29, and 45 nm deep nanopits using 0.5, 1.0, and 1.5% PLLA/PS, respectively) which covered the surface area homogeneously. Superior human foetal bone cell attachment and growth were reported on substrates with the 14 and 29 nm nanopits together with an upregulation of biochemical expression of several adhesion molecules. Dalby et al.[80] used a PS/polybromestyrene (PBrS) toluene mix

and were able to produce a nanometric 13 nm high (diameter range 0.05–0.5 μm) island surface topography. This topography was found to increase cell spreading area per cell and to modify the gene expression of fibroblasts as compared to nonstructured surfaces. In a follow-up study using a PS/polybutylmethacrylate mix, surfaces with 40 and 90 nm high islands (23 and 18% random surface area coverage by the pits, respectively) were created.[81] The team showed that fibroblast adhesion on both surfaces was reduced as indicated by an observed increased cell circularity, reduced cell spreading and poor actin organization.

In case of colloidal surface structuring the material surface is coated by diluted colloidal nanoparticles suspension. The adsorption of particles to the surface is kinetically controlled. For example the team of Kunzler[56] produced using this technique nanoparticle density gradients of average particle height of 64 nm. This was achieved by adsorbing silica nanoparticles with a diameter of 73 nm onto poly(ethylene imine) (PEI) coated surfaces, followed by sintering at 1125°C. It was shown that by increasing the nanoparticle density (from 0 to 21%) and concurrently decreasing the interparticle spacing osteoblast attachment, spreading and cell population size decreased particularly on the substrate with 21% surface coverage and ~130 nm particle spacing. Rice et al.[83] prepared nanostructured surfaces by colloidal adsorbtion of 107 nm sized latex particles at different densities on titanium films. Upon subsequent titanium coating surfaces with protrusions of 110 nm height and 160 nm diameter and defined surface coverages varying from 3 to 43% and concomitant mean interprotrusion spacing variations of 470–210 nm were produced. However, with the obtained surfaces induced no differences in osteoblast cell adhesion unlike the surfaces of the previous mentioned study of Kunzler et al.[56] By nanoparticle containing micelles coating a surface with regular distribution of nanoparticles could be obtained with a spacing which was defined by the micelle size. Arnold et al.[82] showed that after removal of the micelle components, a PEGylation of the remaining surfaces and functionalization of the the 8 nm sized gold nanoparticles with integrin binding entities cell adhesion could be dramatically influenced. They observed a strong reduction of osteoblast adhesion if the spacing between the nanoparticles was above 73 nm.[82]

c. *Nanolithography.* Nanolithography is highly interesting and versatile as a technology for surface structuring. Because of its high costs and technical challenges in translating such application from 2D test surfaces to 3D implants however, nanolithography remains poorly explored within the medical application remit. Surface nano to submicro structures can be produced using conventional lithographic techniques such as photo, electron, ion beam X-ray, reactive ion etching, and extreme ultraviolet lithography.[84,85] Hybrid nanotechnology techniques are often used integrating the above mentioned self-assembled materials as a first step to define the primary pattern (for an overview see Refs. 86 and 87; some examples: Refs. 88–90). Combining 2D colloidal self-assembly and 3D phase lithography Chang et al.[91] produced very impressive surfaces. With this method they were able to design complex 3D periodic structures with 80 nm minimum feature size, representing roughly one-fourth of the operating wavelength. To our knowledge, the biological responses to this kind of surface according has yet to be evaluated.

In the last decade, combined biological and engineering research efforts have been utilized aiming at nanotopography functionalization of material surfaces in order to induce optimal cell reactions at the implant interface. Such efforts have been advanced by our increased understanding and progress made in the field's cell biology and material science. It became evident that generally, the size variation of any given structural feature plays a major role for the associated bioactivity.[10,24,92] Moreover, periodicity variations of such structures selectively promoted or inhibited cell recruitment highlighting the potential of such technology in producing intelligent, tissue-specific, implant surfaces. As seen from Table I, a vast variety of nanotopography features has been investigated—each with partly well-defined geometrical characteristics and resulting bioactive response. The geometrical organization of the structures is found to be important for the extent of the effects, for example, in how far nanopits decrease cell adhesion and spreading.[93] However, it is still unclear how regular the geometrical organization of the obtained structures must be designed to obtain the envisioned biological reaction. Evidence was found that for osteoblastic differentiation not an exact order but rather a controlled disorder (described as controlled with random small variations) of nanocues is preferred and that a completely ordered distribution of these cues is even disadvantageous.[94]

Thus interesting findings regarding cellular responses on various nanostructured surfaces have been reported, but until today, such findings are mainly restricted to the descriptive research domain. The underlying mechanisms are not yet well understood even for a specific cellular response using cell cultures with only one cell type. Furthermore, it is not known what consequences these surfaces have on cells when the complexity of the *in vivo* system is taken into account such as, for example multiple cell types and the 3D dimensionality. The knowledge situation is even more dramatic in case of *in vivo* evaluation. As no biological parameters can be defined in this context, the interactions and mechanisms resulting in the observed outcome following implantation remain unidentified. Hence, except for some descriptive statements nearly no new mechanistic information may be obtained from *in vivo* tests.

Inorganic coatings

It has become evident that for applications in implant and scaffold surface design, it is necessary to mimic the physiological situation *in vivo* with precision with the main player

TABLE I. Some Examples of Surface Nanopatterning: The Resulting Nanotopography and the *In Vitro* Biological Impact

Technique	Created Surface. Feature and Composition	Nanofeature Characteristics (units in nanometer)			Proposed Application	Bioactivity and Maximal Effect Characteristics	Citation
		Height or Depth (nm)	Diameter or Cross-size (nm)	Distribution and/or Centre to Centre Distance (nm)			
Compaction of 32 nm TiO_2 nanoparticles	Particle grain boundaries; Ti			Random 39 and 4520	Orthopaedic and dental implants	↑rat OB differentiation, OB proliferation, OB adhesion, ↓ rat fibroblast adhesion All maximal with 39nm grain	23 95
Layer-by-Layer assembly using 21 nm TiO_2 NP	Roughness, Ti	Ra 5 to ~100	–	–	Orthopaedic implants	↑ MSC attachment after 4–24 h (Ra 100nm)	73
Controlled oxidation (using H_2SO_4/H_2O_2)	Nanopit network; Ti	roughness	≤ 50	Random	Orthopaedic implants	relative to untreated surface: (-) rat OB proliferation, viability ↑after 10 days: differentiation	66
Sandblasting plus controlled oxidation (SLA)	Nanopit network; Ti; (more structured as controlled oxidation)	roughness	nm-μm	Random	Orthopaedic implants	relative to controlled oxidation: ↓ MG63 OB adhesion ↑ MG63 OB differentiation	38
Anodisation	Nanopillars; Ti.	~15, ~55 and ~100	~28, ~14 and ~55	~40, ~74 and ~115	Orthopaedic implants	↑ hMSC cell spreading, differentiation All 28 nm diameter	76
	Nanopit network; Ti.	roughness	~10–300	Random	Orthopaedic implants	↑ adhesion U-2 OS OB	67
	Densely packed nanotubes; Ti	?	~30, ~50, ~70 & ~100	Random	Orthopaedic implants	↑ hMSC differentiation ↓ adhesion. All maximal on d = 100nM tubes	75
	Densely packed nanotubes; Ti	?	100	Random	Transcutaneous implants	Versus unmodified Ti: ↑Keratinocyte cell density	96
Polymer Demixing	Nanopits by PLLA/ PS	~14, 29 & 45	–	Random; Resp. ~300, ~500, ~1000	Orthopaedic implants	↑ hOB cell spreading area; adhesion; pY397, αv integrin and paxillin synthesis (max. nanopit depth: 14 nm) ↑ hOB circularity (45 nm depth pits)	79
	Nano-islands by PS/ PBrS	13	Range 50–500	Random; range 200–600 c-to-c	Tissue regeneration	Versus plane PS: ↑ fibroblast spreading area; modification gene expression of various genes	80
Colloidal particle adsorption.	Adsorbed latex particles+ Ti coating	~111	~159	~ 470, 320, 250 & 210 (3, 19, 30, 45% surface coverage)	Orthopaedic implants	No sign. effect on hOB adhesion and cytokine production	83
	SiO_2 nanoparticle adsorption+ sintering.	~63.7	80?	Random; 0–21 % coverage density gradient	Orthopaedic implants	↓ rat calvarial OB number (0 → 21% coverage)	56
	SiO_2 nanoparticle adsorption	7, 14 and 21	7, 14 and 21	random	–	↓ L929 fibroblast cell density (max. 7nm)	97
Electron beam nanolithography with hot-embossing	Nanopits in PMMA .	100	~120	Defined (300 c-to-c) to displaced ordered to random	Orthopaedic implants	from defined to disordered: ↑ hMCS osteoblast differentiation, number of cells	94
Interference UV laser nanolithography with deep reactive ion etching	Sharp-tip nanoposts and nanograte; SiO_2.	50–100, 200–300 and 500–600	–	230 c-to-c	Implants/ Tissue regeneration	↓ fibroblast attachment with increasing nanopost or nanograte height ↑ fibroblast elongation and orientation with increasing nanograte height	90

(-): no effect, ↑: promotion and ↓: inhibition. OB: osteoblasts, hMSC: human mesenchymal stem cells, PS: Polystyrene, NP: nanoparticle, c-to-c: centre to centre

being the natural extracellular matrix (ECM). The application of a coating aims at providing topography and chemotactic cues in order to promote the recruitment of target cell type(s) and/or to isolate a nonbiocompatible metal implant (component) from the biological environment. Currently, a variety of methods and coating materials are used. Of these, promising examples may be found such as hydroxyapatite, bioactive glasses and carbon based coatings. These are discussed in more detail in the following chapter.

Calcium phoshate based coatings. Besides organic components such as collagen and other proteins bone is mainly composed of nanocrystalline apatite with variable amounts of OH (hydroxyapatite) and CO_3 (carbonated apatite or dahllite)[98] hence, bone may be described as a natural nanostructured composite material.[99] Synthetic hydroxyapatite (HA) resembles the bone material to a high degree. HA is usually considered as being osteoconductive.[100] As biomimetic and bioactive material, HA is used in the form of interconnected and highly porous foams to treat various types of bone defects as bone substitutes,[101] drug delivery vehicles,[102] and as coating material.[103] Currently, calcium phosphate coatings are applied amongst other coating compounds to permanent titanium alloy bone implants to enhance osseointegration. Besides a stronger bonding between implant and bone also a decrease in the release of metal ions is achieved.[104] The dissolution rate of calcium phosphate coatings is vastly defined by their degree of crystalinity.[105,106] The method used to apply this kind of coating greatly affects the type of the resulting topography (surface structure and chemistry). Of such methods plasma spraying, solgel dip coating or mineral coating via incubation in calcium and phosphate containing fluids or electrochemical deposition are all commonly used in industry and research labs. Depending on the application procedure, a different calcium phosphate layer varying in composition, thickness (20 nm–100 μm) and surface structure is obtained resulting not only in differences in cell reactions but also in degradation behavior and adhesion of the coating layer to the substratum.[104,106–108] Optimal adhesion is currently achieved by reducing the layer thickness and by the introduction of interlayers such as a 1:1 mixture of TiO_2 and HA.[104]

Controversial findings regarding the extent of the positive effects of calcium phosphate coatings *in vitro* on osteoblast performance[109–111] and in animal and human studies on osseointegration (bone to implant contact) and long-term clinical success have been reported.[112–113] Some evidence exists that differences in cell/host repsonses might be due to surface structure with nanostructured surfaces promoting MSC proliferation and osteoblast adhesion in comparison to microstructured HA.[23,111] Some attempts have been made to improve this kind of coating by including osteogenic factors like bone morphogenetic proteins with the aim to increase the biomimetic nature of such coatings.[114] Calcium phosphate coatings possess the advantage that their characteristics can be additionally modified by the inclusion of other bioactive or layer stabilizing materials such as SiO_2, ZrO_2, TiO_2, fluorine, and magnesium.[115–118] However, also antibiotic compounds such as silver may be incorporated in the coating mixture[119] to overcome infections. In case of HA coated implants it has been reported that these infections are more prevalent compared to noncoated implants.[120] Further approaches are a subsequent specific chemical functionalization of the coating layer[121] or as mentioned above by adsorption of bioactive substances to the layer[122–124] resulting in a multifunctional HA coating. The long-term *in vivo* success of these multifunctional coatings still remains to be elucidated.

One potential interesting approach is combining calcium phosphate with bioglass. Bioglass coatings are known to promote *in vitro* cell proliferation and differentiation of osteoblastic cells as well as *in vivo* bone formation (e.g., bioactive glass coated surfaces versus noncoated Ti alloy surfaces[125,126]). Furthermore, evidence was found that angiogenesis is promoted by bioactive glasses.[127] Contradictory results have been published regarding the *in vitro* and *in vivo* performance of bioglass relative to those of hydroxyapatite coated surfaces.[126,128–130] However, overall a clear improvement in bioactivity resulting from the application of bioactive glasses may be perceived. Tan et al.[129] found that HA/bioglass composite coatings compared to pure HA and bioglass coatings were persistently superior with regard to both osteoblast response and biochemical stimulation.[129] Xie et al.[131] showed that in animal studies a similar nanocrystalline composite coating resulted in increased bone ongrowth after *in vivo* implantation in rabbit condylar bone in comparison to hydroxyapatite only coated titanium alloy.

Although the potential advantages of calcium phosphate based coatings for osseointegration have been recognized, a systematic evaluation regarding, the surface characteristics and the long-term effects is still missing. Additionally, the bioactivity of such coatings is primarily evlauated and optimized through *in vitro* tests whereby, and despite undisputed value, the findings of such tests may not be fully extrapolated to an *in vivo* situation.[132]

Carbon-based Coatings

C:H, DLC and NCD coatings. Amorphous hydrogenated carbon (a-C:H)(having no long range crystalline order), diamond-like carbon (DLC) and nano-crystalline diamond (NCD) are hard, untoxic and highly chemically inert materials suitable for coating of implant surfaces.[133] Upon deposition, DLC and a-C:H coatings can be present in uniform thickness thus maintaining the geometrical features of the underlying substrate. NCD coatings, on the other hand, may provide additional bioactive and nano-scale surface roughness due to the formation of nanopyramids on the surface during the deposition process. All three types of carbon coatings can be used as protective layer and to reduce the corrosion and wear formation at the articulating surfaces of implants. More importantly, these carbon based coatings may greatly enhance the bioactivity of the underlying non- or less biocompatible materials which, otherwise, possess superior long-term mechanical characteristics (e.g., cobalt–chromium–molybdenum).[133–136] Additionally, further enhancement of a-C:H and DLC coatings bioactivity has been

achieved by incorporating other components such as titanium and silicon into the coating.[134,137] To improve the adhesion of carbon based coatings, interlayers of silicon or of carbide-forming metals (e.g., Al, Ti, Cr, W) between the substrate and the carbon coating [138] can be introduced. However, these carbon based coatings have been shown to delaminate if a defect in the surface is present due to the long-term instability of the currently used metal-carbon interlayer thus limiting their immediate application for biomedical implants.[138,139] Partially delaminated surfaces may cause excessive wear in load bearing articulating implants. Therefore, large efforts were made to improve this interlayer. Recently, Falub et al.[140] developed and analyzed a new strategy to improve DLC coatings by integration of a 90 nm Si-DLC interlayer between the CoCrMo and DLC. This modification increased the threshold strain energy release rate G_{TH} of 60 J m^{-2} up to 470 J m^{-2}, which is an improvement by a factor of 8.[140]

Carbon nanotubes. Carbon nanotubes (CNT) exhibit a unique 1D hollow structure and extraordinary mechanical, electrical, thermal, and optical properties that make them one of the most promising engineered nanomaterials for a range of technical and biomedical applications. CNT coatings represent an example of noncrystalline carbon coatings with the main advantage of hardness and chemical stability. Since the description of CNTs in 1991 by Iiijma[141] the number of new material concepts including those for medical use is constantly increasing. CNT consist purely of carbon atoms arranged in a hexagonal lattice that is rolled up to a single cylinder of nanoscale dimensions (single-walled CNT, SWCNT) or a nanotube of multiple concentric graphene layers (multiwalled CNT, MWCNT). The length of the tubes may range from few nanometers to few millimeters. Both SWCNT and MWCNT types can be produced by electrical arc discharge, laser ablation or chemical vapor deposition (CVD) techniques with the latter allowing for the large-scale and low-cost synthesis of these materials, a prerequisite for their widespread application.[142]

Because of their fibrous structure, CNT coatings add a nanostructure to surfaces whereby the fibers may be randomly oriented or aligned. It has been shown that CNT fibers exhibit organizational patterns reminiscent of those seen in bone collagen matrix. This may explain the capacity of CNT coatings in supporting cell adhesion and, in the case of aligned CNT fibers, positively influencing cell orientation and outgrowth direction.[143–145] It has been shown that SWCNT functionalized with carboxyl groups can be readily incorporated into Type I collagen scaffolds without affecting cell viability or proliferation.[146] MWCNTs have been reported to accelerate *in vivo* ectopic bone formation by deposited rhBMP-2/collagen. MWCNT's adjoining bone induce little local inflammatory reaction, show high bone-tissue compatibility, permit bone repair and became integrated into new bone.[147]

The advantage of such composite materials lies in their improved mechanical properties and an enhanced substrate-native ECM interaction leading, ultimately, to a better cell recruitment and adhesion.[148] Balani et al.[149] used plasma spraying for distributing MWCNT within hydroxyapatite coatings. The presence of CNTs positively influenced the crystalinity of the hydroxyapatite, which according to the authors, indicates that CNTs promoted the nucleation/ precipitation of hydroxyapatite crystals. In a similar study chemical carboxy-functionalized SWCNT exhibiting negative groups at the surface attracted calcium and lead to self-assembly of hydroxyapatite [150] supporting the concept of using CNT based scaffolds for bone therapy. Layer-by-layer assembled CNT composite induced osteoblast differentiation and matrix mineralization compared to pure titanium or cell culture plastic as substrate.[151] In addition to applications in bone regeneration, scaffolds containing chemically functionalized CNTs have been shown to be promising substrates for neuronal growth.[152–155] Therefore, and based on promising findings, pure CNT and CNT containing coatings are currently evaluated as strong candidates for neural, orthopaedic implant, and tissue engineering scaffold functionalization.[156–159]

Especially due to the persistent nature of CNT's, there are still several important challenges and open questions that remain to be addressed carefully to allow the safe and successful use of CNT in biomedicine such as a detailed understanding of biocompatibility, biodistribution, and biodegradation of CNTs. For instance, for MWCNT-chitosan (CHI) scaffolds it was reported that disassembly of the scaffold structure resulted in dispersion away from the implanted scaffold of some clusters of MWCNT/CHI aggregates into the newly regenerated and small MWCNT/CHI aggregates in the surrounding tissue.[159] Most of MWCNT/CHI forming the scaffold structure migrated from the implant zone, most likely by transfer into the blood circulation system, which may induce adverse effects at remote locations. In the circulation, interactions of released CNTs with blood constituents may induce hypothetically opsonization, blood coagulation and activation of the complement system or immune competent cells.[160]

Various reports have highlighted the potential adverse health effects of free and respirable CNT's.[161–164] Such findings must, therefore, be taken into account with increased use of CNT species in the field of nanomedicine. We have shown that some CNTs may directly affect basic cell functions,[163,165–167] depending on the state of agglomeration of the CNTs[165] and the degree of CNTs contaminations.[168] However, recent studies on CNT cytotoxicity showed low or no acute effect on heart cells[169,170] or human Jurkat T cells being an accepted *in vitro* model for the immune system.[171]

In short, further evaluation of the long term effects together with the use of appropriate animal models remains a must to realize the full potential of CNTs in the field of nanomedicine.

BIOINSPIRED SURFACE MODIFICATION AND ARTIFICIAL ECM

After implantation an inflammatory response is a common feature of surgical trauma and is a part of the healing process. This response may be strongly intensified due to surface properties of the implanted biomaterial. Attempts have

TABLE II. Examples of Some Biodegradable Fiber Scaffolds and Their Possible Applications

Material	Fibers Diameter (nm)	Orientation	Type	Seeded Cells	Possible Application	Citation
Collagen I	50–300	Random/uniform	Biological	Rabbit conjunctiva fibroblasts	n.d.	176
Collagen I	30–50	Uniform	Biological	Rabbit corneal fibroblasts	Corneal tissue replacement	177
PLLA	500	Random/uniax	Synthetic	MSC	Vascular graft	178
Poly(ester urethane) Urea (PEUUR)	280–2300	Uniform	Synthetic	MSC	Ligament	179
PLC/collagen	300	Uniform	Blend	Human skeletal muscle cells	Muscle tissue	180
PLC/collagen	500–600	Uniform	Blend	Neurons, Schwann cells	Nerve implants	181
PLC/gelatin	400–600	Random	Blend	MSC	Bone	182
Poly(α-hydroxy esters)	300–1500	Random	Synthetic	Chondrocytes, MSC	Bone, cartilage	183
PLC	700	Random	Synthetic	MSC	Bone, cartilage	184–186
PLC/collagen/PES	200–1500	Random	Synthetic	MSC	Liver	187
Collagen I	140–700	Random	Biological	MSC	n.d.	188
[P(LLA-CL)]	200–700	Random	Synthetic	MSC	n.d.	188

Random: random oriented; Uniform: uniformly aligned; Uniax: uniaxially aligned. MSC: mesenchymal stem cells.

been made to prepare materials that mimic the natural ECM regarding its structural and/or biological properties in order to reduce the duration and magnitude of the inflammatory response to a more physiological extent and with this promoting optimal tissue integration. Numerous concepts have been evaluated in order to develop functional and instructive bioengineered cellular nano environments with controlled physical, mechanical, and chemical parameters for biomedical applications for different tissues including bone regeneration. A key characteristic of ECM is the 3D nanofibrilar architecture consisting of soluble and fibrous proteins, proteoglycans, glycoproteins, and in case of bone of the inorganic component hydroxyapatite. Within the body, the ECM may assume different characteristics depending on the organ or tissue function (cornea, tendon, cartilage, bone, etc.). These ECM types are characterized by different length scales, layers, and morphologies.[172] Therefore, the desirable characteristics of a scaffold are strongly dependent on their end use, that is, the type of target tissue. The most important criteria that a scaffold of interest should fulfil are: biocompatibility, biodegradability, 3D structure, nonimmunogenicity, noncorrosive properties, sterility, high surface to volume ratio, porosity with interconnected pores, modifiable surface and adequate mechanical properties[173] but also its bioactivity. Several methodologies have been introduced to produce biomimetic surfaces addressing different aspects of the natural counterpart such as nanofibrous/hydrogel and/or bioactive molecules coatings [e.g., growth factors, cell binding motifs such as arginine–glycine–aspartic acid (RGD) peptides]. Some of these technologies used for bone are described in the following sections.

Electrospun nanofibers

Electrospinning[174,175] has been shown to be a very simple, efficient and cost-effective method for producing continuous fibers on top of surfaces or as standalone cell scaffold. Electrospinning enables most, if not all of these requirements as a versatile method to fabricate nanofibers of various materials from polymers to ceramics, and in the range of fiber diameter from 3 nm to several micrometers. Electrospun nanofibers provide high surface area-to-volume ratio and can be used to produce high porosity scaffolds for tissue engineering. Such characteristics are critical in permitting the cellular colonization into the depth of the structure together with the efficient supply with oxygen and nutrients. For an overview of nano fibrous scaffold see Table II.

Nanofibrous structures produced by electrospinning provide attractive ECM conditions for the anchorage, migration and differentiation of tissue cells, including those responsible for the regeneration of bone.[189] As bone tissue is essentially composed of organic and inorganic nanocomposites, ECMs ought to be designed to have the mechanical properties needed to sustain loads and should be favorable for recruiting osteoblasts and/or mesenchymal stromal cells.[189]

Naturally derived as well as synthetic nondegradable polymers are primarily used for electro-spinning of nano-fibrous scaffolds. Current trends appear to favor the use of biodegradable polymers, such as collagen, allowing better infiltration of cells into the scaffold as opposed to using nondegradable polymeric base materials.[190] Additionally, the base material is eliminated through biodegradation after fulfilling its function thus eliminating potential foreign-body/device-dependent complications. In this respect, several in depth reviews highlighted the various polymer species used for the fabrication of nano-fibrous scaffolds for tissue engineering and drug release from electro-spun fibers.[174,175,190–196] To fine-tune the functional and mechanical properties of such scaffolds in relation to their intended application, different approaches have been developed. For example, electrospinning of polymeric blends consisting of two or more

biocomposites, and core–shell structures (multiaxial/ coaxial electro-spinning), and the addition of nanoparticles,[190,197,198] such as nanohydroxyapatite[199–204] and nanosilver[205–207] as well as SWCNTs.[208] Further functionalization of electrospun fibers by coating, incorporation of drugs and proteins, short amino acid sequences or growth factors was also reported.[191,209–217] 3D nano fibrous scaffolds with patterned micropores were also produced by using UV photolithography.[219]

Pure PCL nanofibrous scaffolds have been investigated for bone formation *in vivo* in a rat model.[186] Adhesion, growth and osteogenic differentiation of human mandible-derived mesenchymal stromal cells (MSC) could be further increased by incorporation of gelatine in the PLC nanofibers in comparison to PLC-only nanofibers.[182] Other approaches to increase cell adhesion have been shown by Chan et al.[188] When compared to 2-D plastic, glass or collagen and gelatine-coated glass substrates, the nanotexture and chemistry of the collagen and collagen-coated poly(L-lactic-co-ε-caprolactone) [P(LLA-CL)] nanofibers clearly influenced early (10-30 min) recruitment of MSC.[188]

As the ideal artificial ECM for bone tissue regeneration would typically form a nanoscale organized composite between the inorganic and organic ingredients, to combine both hydroxyapatite (HA) and fibrous organic constituents has been atempted in numerous studies.[189] For example composite nanofiber scaffolds PLA in combination with demineralized bone powders exhibited stronger osteoinductive effects *in vivo* in a rat model when compared to PLA-only scaffolds.[220]

Mechanically, the organic fibrous network provides resilience, while the inorganic crystals harden the matrix, in combination contributing to a strong and tough ECM.[221] The use of bioactive inorganics with natural or synthetic biopolymers is considered a promising strategy to develop artificial matrices for bone tissue regeneration.[189] Electrospinning of such composite solutions, however, has not been easily implemented in the production of nanofibrous structures. This process will likely gain momentum with improvement of preparation of fine nanocrystalline particles and subsequent homogeneous dispersion within the polymer solutions. When HA crystals of tens of nanometers in size were homogeneously dispersed in a hydrophobic PLA solution by using a surfactant that mediates the interface of the hydrophobic solution and the hydrophilic nanorcystals a homogeneous nanosized fibrous scaffold could be produced.[189,222] Ultrafine $CaCO_3$-particles have also been successfully incorporated within thr biopolymers composition to form electrospun fibers.[223] Employing similar strategies many articles have reported nanocomposite electrospinning using biodegradable synthetic polymers with bioactive inorganic nanoparticulates (e.g. HA, tricalcium phosphate, bioactive glass) and most of the nanocomposite fibers resulted in improvement in the mechanical properties and/ or bone cell function.[189] As an ideal biomimetic approach HA was precipitated *in situ* from Ca and P precursors within gelatin or collagen solutions and subsequently electrospun into a nanofibrous mesh.[204,222,224]

As a further approach, a silicon-based inorganic precursor, glycidoxypropyl trimethoxysilane, was homogenized with gelatin, which was then aged to form siloxane groups and linkages with the amino acids of gelatin to generate a hybridized structure. This material was used for electrospinning and resulting meshes showed an excellent ability to form bone mineral.[225]

Numerous other approaches for electrospinning of composite materials for bone tissue regeneration have been reviewed by Shin and coworkers.[189]

More recent advancements in the field of electrospinning of nanofibers for bone regeneration are: surface mineralization of nanofibers,[226–230] tethering of cell adhesion proteins or amino acid sequences to nanofibers[213,231–235] as well as functionalization of nanofibers with bone-promoting drugs such as BMP-2.[236,237] Further advancement of the electrospinning technology such as dual-source dual-power electrospinning,[238] fabrication of porous electrospun nanofibers[239] will allow the delevopment of new multifunctional scaffolds for bone regeneration.

Other nanofiber, ECM-mimicking composites

The most prominent examples of nanofiber composites not made by electrospinning include acellular tissue derived matrices,[240] natural hydrogels (like agarose and collagen) and artificial synthetic hydrogels. Artificial synthetic hydrogels have the advantage of comprising well-defined components. They are also synthesized from inert synthetic molecules such as poly(ethylene glycol) enriched with specific ligand molecules that have been designed to provide cues to the cell at the nanoscale.[241] For example, for growth factor administration, the growth factor can either be freely embedded in the hydrogel or bound to it.[242] Such smart hydrogels can be produced through highly controlled and selective reaction schemes, such as Click reactions (the chemical synthesis of compounds and combinatorial libraries through heteroatom linking) efficiently linking small molecular subunits,[243] and physical crosslinking (such as hydrogen bonding). Furthermore, protein folding and protein–protein interactions can be used to create well-ordered networks at the molecular scale. This approach has been used to create a synthetic hydrogels from the self-assembly of leucine zipper domains (a protein motif that facilitates protein–protein interactions) whereby the rate of gel degradation and mass loss can be precisely controlled.[244] In addition to controlling the structure and chemistry of synthetic hydrogels, advanced gel materials can respond to stimulation allowing the manipulation of the temporal and spatial availability of bioactive moieties in the nanoscale dimension within the cellular microenvironment. Novel concepts for tissue engineering also included proteolysis susceptible chemical crosslinks in poly(ethylene glycol) gels permitting cell-initiated proteolytic processes that occur in native tissues enhancing, subsequently, cell colonization of the synthetic network.[245–247] Calcium-sensitive protein building blocks[248]; fibrinogen[249]; single-stranded DNA components[250] or fibrin-analogues[251] can also be incorporated to expand or contract in response to external or cellular

triggers. With this for instance the release of biomolecules may be achieved on demand.

So far, positive effects on the biological environment have been proven mainly using *in vitro* systems. In the coming years it needs to be proven to what extend these surface modifications have an positive effect on osseointegration and long-term success rate of permanent implants.

Surface biofunctionalization by introducing biomolecules

A functionalization using specific bioactive biomolecules (proteins, peptides, DNA, etc.) represents the (with respect to size) smallest surface modification. One simple method to immobilize these molecules is by dip coating resulting in their adsorption. This method has, however, the disadvantage that after implantation these molecules may be replaced by other body fluid components competing for the implant surface space. This may be one of the reasons why for example Schliephake et al.[252] could not detect any effect of surface biofunctionalization by adsorbed collagen, rBMP and/or RGD peptide to dual acid-etched titanium screws in a dog model after 1 and 3 months of implantation. Thus a stable immobilization might be needed to achieve positive effects. The covalent binding of biomolecules to the surface using linker molecules such as 3-aminopropyltriethoxylsilane,[253] N-(2-aminoethyl)-3-aminopropyl-trimethoxysilane and 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC)[254] or, more recently, dopamine[255] would lead to a more stable immobilization/surface modification. In various *in vitro* experiments, a positive effect of this kind of coatings regarding cell adhesion and differentiation has been reported[254,256–260] proving that such molecules, if stably attached have a positive effect. Furthermore, these data suggest that these surfaces may have the potency to promote osseointegration *in vivo*. One of the disadvantages of this methodology is that in order to obtain the surfaces many steps are needed in which partly nonbiocompatible components are used. A solution could represent coupling molecules that do not covalently bind but have the characteristic to strongly interact with the implant surface and by that ensuring a stable binding. Several approaches have been proposed. One is the use of coatings with polymeric brushes such as poly(ethylene) glycol (PEG) as for instance examined by Park et al.[261] or of poly(L-Lysine)-*graft*-PEG-RGD peptide as evaluated by Germanier et al.[262] Both groups reported that the PEG based linkers increased the chemical stability of the coating and improved osseointegration as shown using animal models.

A very interesting and promising type of self-assembled coatings represents the coating with self-assembled peptide fibers which are functionalized with cell adhesive molecules. By that two aspects of biomimetic coatings are addressed (nanostructure and integration of bioactive molecules). *In vitro* tests revealed that this kind of coating had positive effects on osteoblastic cell adhesion and differentiation.[263]

Overall, the biological reaction as well as the stability and by that the applicability of this coating under *in vivo* conditions and their long-term effect on the clinical success of permanent bone implants has, however, still to be proven. Although a very high standard regarding the possibilities of surface nanofunctionalization could be achieved in the previous decades, a real progress in this field can really be achieved after a systematic *in vitro* and *in vivo* evaluation of these surfaces.

PROGRESS LIMITING FACTORS

The undisputed potency of nanotechnology to dramatically change our world is truly remarkable particularly when such technology is utilized to produce new materials in medicine. The brief state of the art and the examples mentioned in this overview clearly underline this. The emerging field of implantable materials in nanomedicine, however, is still in its infancy. Nearly, no systematic evaluation of the various materials is available, neither *in vitro* nor *in vivo*. The question is what impact the development of a new fancy surface for improved of implant and scaffold surface design really has, if no ranking in the biological performance is made relative to defined and commonly used reference surfaces and materials. Furthermore, the performed *in vivo* studies are in most cases especially focussed on the effects of the new surfaces on early stages of osseointegration and do not aim to make a prognosis on the long-term success. This certainly makes sense for degradable implants supporting bone regeneration, however, not for permanent implants. Unstable coatings may improve osseointegration of permanent implants without ensuring bone formation at the implant surface later on during the bone remodelling process at which time point the coating is degraded. For the long-term prognosis of the fate of the implant the tissue formed during late stage bone remodelling is by that certainly equally important. This fact is in our opinion often underestimated.

Current *in vitro* tests being used to select the best surfaces for further *in vivo* testing also have their clear drawbacks. Even by using the simplest set-up, that is, investigating the cell-surface interaction using solely one single cell type, a comprehensive insight into the exact mechanisms by which nanoscale surface topography regulates cell behavior is still lacking. So far it has only been shown how complex and manifold cell surface interactions are and that by minute variations in micro- and nanocues and their order different cell reactions may be evoked. Many steps still have to be undertaken to be able to steer the cell fate even in a mono cell type culture although some ideas in this regard have been developed.[75,82,264] The *in vivo* situation is more complex and characterized by the presence of more than one cell-type. Different cell types may interact and compete to colonize the implant surface.[265] Here, it is primarily not important that for instance cells of the osteoblast lineage can perfectly adhere, proliferate and differentiate on an implant that need to be osteointegrated (as currently is assumed and evaluated, e.g., Ref. 266) but that relative to the other competing cell types (like fibroblasts) osteoblastic lineage cells have a clear advantage. Only by including this population dynamic aspect one can make a prognosis regarding a material's biocompatibility according to the

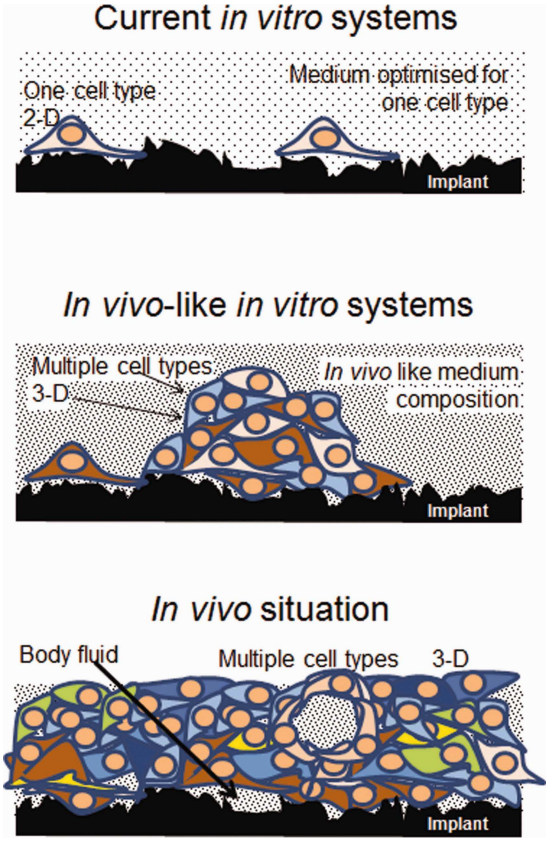

FIGURE 2. Schematic description of current and future *in vitro* test systems for evaluating implant devices in comparision to the *in vivo* situation after implantation. Different colors of the cells represent different cell types. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

definition of Williams referring to the ability to incorporate with the desired degree in the host.[25] Therefore, current *in vitro* cell culture tests are far from optimal and their prognostic value for animals and humans is highly questionable.[132] Besides this it is not known which *in vitro* parameters can be taken as predictive parameter regarding which tissue is formed after implantation at the tissue-implant surface interface. Here, new concepts have to be developed mimicking more appropriate the niche in which the implant is placed, for example, by using multiple cell types to include the interaction and competition between the cell types at the implant surface,[265,267,268] by adding the third dimension[269] and the *in vivo*-like composition of the extracellular fluid[51,270,271] (Fig. 2). These new concepts and parameters have to be evaluated and validated with respect to their prognostic value for the *in vivo* situation using animal models and by using clinical data of human studies and analysis of retrieved implants. At the first glance it leads to believe that focussing on the development of an improved *in vitro* evaluation set-up may retard further progress in nanotechnology. However, it finally—and only under this premise—will enable the superior benefit of the full potential and all possibilities that nanotechnology offer.

CONCLUSIONS

Further research in this area is still to be conducted to define optimal cues steering desired cell/host reactions at the osteo—implant interface. However, it is clear that nanoscale surface topography and functionalization significantly influences cell behavior *in vitro* and *in vivo* and may, potentially, be utilized as powerful tools to enhance the bioactivity and/ or integration of implanted devices. Nanoscaled surface design: a revolution for bone-related implants and scaffolds? This is still an open question that may not be answered yet even following a comprehensive literature review. The wealth of data nonetheless, provides enough evidence that this revolution may take place in the near future. The lack of systematic evaluation of the "fancy" nanotechnologically produced surfaces and materials remains a big hurdle, in fact a bottle neck, in developing nanoscaled biomimetic medical devices (for bone tissue regeneration and long-term implant osseointegration; for soft issue integration and inhibited tissue integration for short term implants) to their full potential. With respect to *in vitro* tests it must be concluded that such test after many years of research are still in their infanthood and a dramatic evolution is needed to enable prognostic statements with respect to the fate of an medical device after implantation. New *in vitro* configurations, taking the *in vivo* aspects of the target tissue into account may certainly boost the kind of statements that can be made. It is clear that only by improving *in vitro* test set-ups the foreseen quantum leap in implant and scaffold design-paradigm based on nanosized cues may materialize. Generally, improved biological testing may finally render it possible to abandon the road of empirical optimization and step over onto the track of knowledge-based implant and scaffold design.

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REFERENCES

1. Riehemann K, Schneider SW, Luger TA, Godin B, Ferrari M, Fuchs H. Nanomedicine--challenge and perspectives. Angew Chem Int Ed Engl 2009;48:872–897.
2. Visiongain. Nanomedicine 2006–2011. report. Volume 2009. http://www.visiongain.com/Report/198/Nanomedicine-2006–2011: Visiongain; 2006. Accessed on 7 May 2012.
3. Wolbring G. Scoping document on Nanotechnology and disabled people for the Center for Nanotechnology in Society at Arizona State University. Volume 2009; 2009.
4. Wagner V, Dullart A, Bock AK, Zweck A. The emerging nanomedicine landscape. Nat Biotechnol 2006;24:1211–1217.
5. Webster TJ. Projections for nanomedicine into the next decade: But is it all about pharmaceuticals? Int J Nanomed 2008;3:1.
6. Brune H, Ernst H, Grunwald A, Grünwald W, Hofmann H, Janich P, Krug HF, Mayor M, Schmid G, Simon U, Vogel, V. Nanotechnology—Assessment and Perspectives. Berlin: Springer; 2006.
7. ASTM E2456. E 2456-06 Terminology for Nanotechnology. West Conshohocken, PA 19428–2959, USA: ASTM international; 2006.
8. Tjong SC. Novel nanoparticle-reinforced metal matrix composites with enhanced mechanical properties. Adv Eng Mater 2007;9:639–652.
9. Webster TJ, Siegel RW, Bizios R. Osteoblast adhesion on nanophase ceramics. Biomaterials 1999;20:1221–1227.

10. Bruinink A, Kaiser J-P, Meyer DC. Effect of biomaterial surface morphologies on bone marrow cell performance. Adv Eng Mater 2005;7:411–418.
11. Born AK, Rottmar M, Lischer S, Pleskova M, Bruinink A, Maniura-Weber K. Correlating cell architecture with osteogenesis: Firsts steps towards live single cell monitoring. Eur Cell Mater 2009;18:49–62.
12. Curtis A. Correlating cell architecture with osteogenesis:firsts steps towards live single cell monitoring. Expert Rev Med Devices 2005;2:293–301.
13. Unadkat HV, Hulsman M, Cornelissen K, Papenburg BJ, Truckenmüller RK, Carpenter AE, Wessling M, Post GF, Uetz M, Reinders MJ, Stamatialis D, van Blitterswijk CA, de Boer J. An algorithm-based topographical biomaterials library to instruct cell fate. Proc Natl Acad Sci USA 2011;108:16565–16570.
14. Hamilton DW, Brunette DM. The effect of substratum topography on osteoblast adhesion mediated signal transduction and phosphorylation. Biomaterials 2007;28:1806–1819.
15. Aznavoorian S, Stracke ML, Krutzsch H, Schiffmann E, Liotta LA. Signal transduction for chemotaxis and haptotaxis by matrix molecules in tumor cells. J Cell Biol 1990;110:1427–1438.
16. Lim JY, Donahue HJ. Cell sensing and response to micro- and nanostructured surfaces produced by chemical and topographic patterning. Tissue Engin 2007;13:1879–1891.
17. Tzaphlidou M. The role of collagen in bone structure: an image processing approach. Micron 2005;36:593–601.
18. Vetter U, Eanes ED, Kopp JB, Termine JD, Robey GP. Changes in apatite crystal size in bones of patients with osteogenesis imperfecta. Calcif Tissue Int 1991;49:248–250.
19. Eppell SJ, Tong W, Katz JL, Kuhn L, Glimcher MJ. Shape and size of isolated bone mineralites measured using atomic force microscopy. J Orthop Res 2001;19:1027–1034.
20. Bergers G, Brekken R, McMahon G, Vu TH, Itoh T, Tamaki K, Tanzawa K, Thorpe P, Itohara S, Werb Z, Hanahan D. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2000;2:737–744.
21. Ker ED, Nain AS, Weiss LE, Wang J, Suhan J, Amon CH, Campbell PG. Bioprinting of growth factors onto aligned sub-micron fibrous scaffolds for simultaneous control of cell differentiation and alignment. Biomaterials 2011;32:8097–8107.
22. Vasita R, Katti D. Nanofibers and their applications in tissue engineering. Int J Nanomed 2006;1:15–30.
23. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. J Biomed Mater Res 2000;51:475–483.
24. Webster TJ, Schadler LS, Siegel RW, Bizios R. Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. Tissue Eng 2001;7:291–301.
25. Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941–2953.
26. Ratner BD. The biocompatibility manifesto: biocompatibility for the twenty-first century. J Cardiovasc Trans Res 2011;4:523–527.
27. Cooper LF. A role for surface topography in creating and maintaining bone at titanium endosseous implants. J Prosth Dent 2000;84:522–534.
28. Shalabi MM, Gortemaker A, van't Hof MA, Jansen JA, Creugers NHJ. Implant surface roughness and bone healing: A systematic review. J Dent Res 2006;85:496–500.
29. Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. Angew Chem Int Ed Engl 2009;48:5406–5415.
30. Mendonça G, Mendonça DBS, Aragão FJL, Cooper LF. Advancing dental implant surface technology—From micron—to nanotopography. Biomaterials 2008;29:3822–3835.
31. McNamara LE, McMurray RJ, Biggs MJP, Kantawong F, Oreffo ROC, Dalby JM. Nanotopographical control of stem cell differentiation. J Tissue Eng 2010;2010:120623.
32. Karagkiozaki VC, Logothetidis SD, Kassavetis SN, Giannoglou GD. Nanomedicine for the reduction of the thrombogenicity of stent coatings. Int J Nanomed 2010;5:239–248.
33. Zhu B, Lu Q, Yin J, Hu J, Wang Z. Alignment of osteoblast-like cells and cell-produced collagen matrix induced by nanogrooves. Tissue Eng 2005;11:825–834.
34. Khang D L-SP, Pareta R, Lu J, Webster TJ. Reduced responses of macrophages on nanometer surface features of altered alumina crystalline phases. Acta Biomater 2009;5:1425–1432.
35. Curtis A, Wilkinson C. Nanotechniques and approaches in biotechnology. Mater Today 2001;4:22–28.
36. Curtis ASG, Dalby MJ, Gadegaard N. Cell signaling arising from nanotopography: implications for nanomedical devices. Nanomed 2006;1:67–72.
37. Zinger O, Anselme K, Denzer A, Habersetzer P, Wieland M, Jeanfils J, Hardouin P, Landolt D. Time-dependent morphology and adhesion of osteoblastic cells on titanium model surfaces featuring scale-resolved topography. Biomaterials 2004;25:2695–2711.
38. Zhao G, Raines AL, Wieland M, Schwartz Z, Boyan BD. Requirement for both micron- and submicron scale structure for synergistic responses of osteoblasts to substrate surface energy and topography. Biomaterials 2007;28:2821–2829.
39. Smith LL, Niziolek PJ, Haberstroh KM, Nauman EA, Webster TJ. Decreased fi broblast and increased osteoblast adhesion on nanostructured NaOH-etched PLGA scaffolds. Int J Nanomed 2007;2:383–388.
40. Biela SA, Su Y, Spatz JP, Kemkener R. Different sensitivity of human endothelial cells, smooth muscle cells and fibroblasts to topography in the nano-micro range. Acta Biomater 2009;5: 2460–2466.
41. Kunzler TP, Drobek T, Schuler M, Spencer ND. Systematic study of osteoblast and fibroblast response to roughness by means of surface-morphology gradients. Biomaterials 2007;28:2175–2182.
42. Clark P, Connolly P, Curtis AS, Dow JA, Wilkinson CD. Topographical control of cell behaviour: I. Simple step cues. Development 1987;99:439–448.
43. Sinha RK, Tuan RS. Regulation of human osteoblast integrin expression by orthopedic implant materials. Bone 1996;5: 451–457.
44. Llopis-Hernández V, Rico P, Ballester-Beltrán J, Moratal D, Salmerón-Sánchez M. Role of surface chemistry in protein remodeling at the cell-material interface. Plos One 2011;6:epub-e19610.
45. Liu H, Niu A, Chen S-E, Li Y-P. β3-Integrin mediates satellite cell differentiation in regenerating mouse muscle. FASEB J 2011;25: 1914–1921.
46. Olivares-Navarrete R, Raz P, Zhao G, Chen J, Wieland M, Cochran DL, Chaudhri RA, Ornoy A, Boyan BD, Schwartz Z. Integrin alpha2beta1 plays a critical role in osteoblast response to micron-scale surface structure and surface energy of titanium substrates. Proc Nat Acad Sci USA 2008;105:15767–15772.
47. Wei J, Igarashi T, Okumori N, Igarashi T, Maetani T, Liu B, Yoahinari M. Influence of surface wettability on competitive protein adsorption and initial attachment of osteoblasts. Biomed Mat 2009;4:045002.
48. Scotchford CA, Gilmore CP, Cooper E, Leggett GJ, Downes S. Protein adsorption and human osteoblast-like cell attachment and growth on alkylthiol on gold self-assembled monolayers. J Biomed Mat Res 2001;59:84–99.
49. Michiardi A, Aparicio C, Buddy D. Ratner BD, Planell JA, Gil J. The influence of surface energy on competitive protein adsorption on oxidized NiTi surfaces. Biomaterials 2007;28:586–594.
50. Arima Y, Iwata H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. Biomaterials 2007;28: 3074–3082.
51. Milleret V, Tugulu S, Schlottig F, Hall H. Alkali treatment of microrough titanium surfaces affects macrophage/monocyte adhesion, platelet activation and architecture of blood clot formation. Eur Cell Mater 2011;21:430–444.
52. Pasche S, Vörös J, Griesser HJ, Spencer ND, Textor M. Effects of ionic strength and surface charge on protein adsorption at PEGylated surfaces. J Phys Chem B 2005;109:17545–17552.
53. Yamazaki K, Ikeda T, Isono T, Ogino T. Selective adsorption of protein molecules on phase-separated sapphire surfaces. J Colloid Interface Sci 2011;361:64–70.
54. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochron DL, Hoffmann B, Lussi A, Steinemann SG. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res 2004;83:529–533.

55. Ostrovskaya L, Podestà A, Milani P, Ralchenko V. Influence of surface morphology on the wettability of cluster-assembled carbon films. Europhys Lett 2003;63:410–407.
56. Kunzler TP, Huwiler C, Drobek T, Vörös J, Spencer ND. Systematic study of osteoblast response to nanotopography by means of nanoparticle-density gradients. Biomaterials 2007;28: 5000–5006.
57. 10993-5/TC194 I, CEN/TC206. Biologische Beurteilung von Medizinprodukten- Teil 5: Prüfung auf in vitro-Zytotoxizität (ISO 10993-5:2009). In: Standardization ECf, editor. B-100 Brussel: CEN; 2009.
58. Stevens MM, George JH. Exploring and engineering the cell surface interface. Science 2005;310:1135–1138.
59. Gadegaard N, Martines E, Riehle MO, Seunarine K, Wilkinson CDW. Applications of nano-patterning to tissue engineering. Microelectron Eng 2006;83:1577–1581.
60. Dalby MJ, Gadegaard N, Riehle MO, Wilkinson CD, Curtis AS. Investigating filopodia sensing using arrays of defined nano-pits down to 35 nm diameter in size. Int J Biochem Cell Biol 2004;36: 2005–2015.
61. Selhuber-Unkel C, Erdmann T, López-García M, Kessler H, Schwarz US, Spatz JP. Cell adhesion strength is controlled by intermolecular spacing of adhesion receptors. Biophys J 2010; 98:543–551.
62. Xiao J, Zhou H, Zhao L, Sun Y, Guan S, Liu B, Kong L. The effect of hierarchical micro/nanosurface titanium implant on osseointegration in ovariectomized sheep. Osteoporos Int 2011;22: 1907–1913.
63. Rani VVD, Vinoth-Kumar L, Anitha VC, Manzoor K, Deepthy M, Shantikumar VN. Osteointegration of titanium implant is sensitive to specific nanostructure morphology. Acta Biomater 2012;8: 1976–1989.
64. Ballo A, Agheli H, Lausmaa J, Thomsen P, Petronis S. Nanostructured model implants for in vivo studies: influence of well-defined nanotopography on de novo bone formation on titanium implants. Int J Nanomed 2011;6:3415–3428.
65. Tavares MG, de Oliveira PT, Nanci A, Hawthorne AC, Rosa AL, Xavier SP. Treatment of a commercial, machined surface titanium implant with H2SO4/ H2O2 enhances contact osteogenesis. Clin Oral Implants Res 2007;18:452–458.
66. de Oliveira PT, Zalzal SF, Beloti MM, Rosa AL, Nanci A. Enhancement of *in vitro* osteogenesis on titanium by chemically produced nanotopography. J Biomed Mater Res A 2007;80A: 554–564.
67. Huang H-H, Pan S-J, Lai Y-L, Lee T-H, Chen C-C, Lu F-H. Osteoblast-like cell initial adhesion onto a network-structured titanium oxide layer. Scr Mater 2004;51:1017–1021.
68. Mahlambi MM, Mishra AK, Mishra SB, Raichur AM, Mamba BB, Krause RW. Layer-by-Layer self-assembled metal-Ion- (Ag-, Co-, Ni-, and Pd-) doped TiO2 nanoparticles: synthesis, characterisation, and visible light degradation of rhodamine B. J Nanomater 2012;ID 302046.
69. Huh P, Kim S-C. Nanostructured ZnO arrays with self-ZnO layer created using simple electrostatic layer-by-layer assembly. J Nanomater 2012;ID 131672.
70. Sher P, Custódio CA, Mano J. Layer-by-layer technique for producing porous nanostructured 3D constructs using moldable freeform assembly of spherical templates. Small 2010;6: 2644–2648.
71. Zink C, Hall H, Brunette DM, Spencer ND. Orthogonal nanometer-micrometer roughness gradients probe morphological influences on cell behaviour. Biomaterials 2012;33:8055–8061.
72. Hu Y, Cai K, Luo Z, Zhang Y, Li L, Lai M, Hou Y, Huang Y, Li J, Ding X and others. Regulation of the differentiation of mesenchymal stem cells in vitro and osteogenesis in vivo by microenvironmental modification of titanium alloy surfaces. Biomaterials 2012;33:3515–3528.
73. Kommireddy DS, Sriram SM, Lvov YM, Mills DK. Stem cell attachment to layer-by-layer assembled TiO2 nanoparticle thin films. Biomaterials 2006;27:4296–4303.
74. Bitar M, Friederici V, Imgrund P, Brose C, Bruinink A. In vitro bioactivity of micro metal injection moulded stainless steel with defined surface features. Eur Cell Mater 2012;23:333–347.
75. Oh S, Brammer KS, Li YSJ, Teng D, Engler AJ, Chien S, Jin S. Stem cell fate dictated solely by altered nanotube dimension. Proc Nat Acad Sci USA 2009;106:2130–2135.
76. Sjöström T, Dalby MJ, Hart A, Tare R, Oreffo ROC, Su B. Fabrication of pillar-like titania nanostructures on titanium and their interactions with human skeletal stem cells. Acta Biomat 2009;5: 1433–1441.
77. Lohmüller T, Aydin D, Schwieder M, Morhard C, Louban I, Pacholski C, Spatz JP. Nanopatterning by block copolymer micelle nanolithography and bioinspired applications. Biointerphases 2011;6:MR1–12.
78. Spatz JP. Nano- and micropatterning by organic–inorganic templating of hierarchical self-assembled structures. Angew Chem Int Ed Engl 2002;41:3359–3362.
79. Lim JY, Dreiss AD, Zhou Z, Hansen JC, Siedlecki CA, Hengstebeck RW, Cheng J, Winograd N, Donahue HJ. The regulation of integrin-mediated osteoblast focal adhesion and focal adhesion kinase expression by nanoscale topography. Biomaterials 2007; 28:1787–1797.
80. Dalby MJ, Yarwood SJ, Riehle MO, Johnstone HJH, Affrossman S, Curtis ASG. Increasing fibroblast response to materials using nanotopography: morphological and genetic measurements of cell response to 13-nm-high polymer demixed islands. Exp Cell Res 2002;276:1–9.
81. Berry CC, Dalby MJ, McCloy D, Affrossman S. The fibroblast response to tubes exhibiting internal nanotopography. Biomaterials 2005;26:4985–4992.
82. Arnold M, Cavalcanti-Adam EA, Glass R, Blümmel J, Eck W, Kantlehner M, Kessler H, Spatz JP. Activation of integrin function by nanopatterned adhesive interfaces. Chemphyschem 2004;5: 383–388.
83. Rice JM, Hunt JA, Gallagher JA, Hanarp P, Sutherland DS, Gold J. Quantitative assessment of the response of primary derived human osteoblasts and macrophages to a range of nanotopography surfaces in a single culture model in vitro. Biomaterials 2003;24:4799–4818.
84. Lee JW, Lee KB, Jeon HS, Park HK. Effects of surface nano-topography on human osteoblast filopodia. Anal Sci 2011;27:369–374.
85. Dalby MJ, Gadegaard N, Wilkinson CD. The response of fibroblasts to hexagonal nanotopography fabricated by electron beam lithography. J Biomed Mater Res A 2008;84:973–979.
86. Wood MA. Colloidal lithography and current fabrication techniques producing in-plane nanotopography for biological applications. J R Soc Interface 2007;4:1–17.
87. Saavedra HM, Mullen TJ, Zhang P, Dewey DC, Claridge SA, Weiss PS. Hybrid strategies in nanolithography. Rep Prog Phys 2010;73:036501.
88. Arnold M, Schwieder M, Blümmel J, Cavalcanti-Adam EA, López-Garcia M, Kessler H, Geiger B, Spatz JP. Cell interactions with hierarchically structured nano-patterned adhesive surfaces. Soft Matter 2009;5:72–77.
89. Purwaningsih L, Schoen T, Wolfram T, Pacholski C, Spatz JP. Fabrication of multi-parametric platforms based on nanocone arrays for determination of cellular response. Beilstein J Nanotechnol 2011;2:545–551.
90. Choi CH, Hagvall SH, Wu BM, Dunn JCY, Beygui RE, Kim CJ. Cell interaction with three-dimensional sharp-tip nanotopography. Biomaterials 2007;28:1672–1679.
91. Chang CH, Tian L, Hesse WR, Gao H, Choi HJ, Kim JG, Siddiqui M, Barbastathis G. From two-dimensional colloidal self-assembly to three-dimensional nanolithography. Nano Lett 2011;11: 2533–2537.
92. Teixeira AI, McKie GA, Foley JD, Bertics PJ, Nealey PF, Murphy CJ. The effect of environmental factors on the response of human corneal epithelial cells to nanoscale substrate topography. Biomaterials 2006;27:3945–3954.
93. Curtis AS, Gadegaard N, Dalby MJ, Riehle MO, Wilkinson CD, Aitchison G. Cells react to nanoscale order and symmetry in their surroundings. IEEE Trans Nanobiosci 2004;3:61–65.
94. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, Wilkinson C, D., Oreffo RO. The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. Nat Mater 2007;6:997–1003.

95. Webster TJ, Ergun C, Doremus RH, Siegel RW, Bizios R. Enhanced functions of osteoblasts on nanophase ceramics. Biomaterials 2000;21:1803–1810.
96. Zile MA, Puckett S, Webster TJ. Nanostructured titanium promotes keratinocyte density. J Biomed Mater Res A 2011;97A:59–65.
97. Cousins BG, Doherty PJ, Williams RL, Fink J, Garvey MJ. The effect of silica nanoparticulate coatings on cellular response. J Mater Sci Mater Med 2004;15:355–359.
98. Wenk H-R, Heidelbach F. Crystal alignment of carbonated apatite in bone and calcified tendon: results from quantitative texture analysis. Bone 1999;24:361–369.
99. Chow LC, Sun L. Properties of nanostrucutred hydroxyapatite prepared by a spray drying technique. J Res Natl Inst Stand Technol 2004;109:543–551.
100. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J 2001;10 Suppl 2:S96–S101.
101. Kokubo T. Bioceramics and their Clinical Applications. Woodhead Publishing Ltd, Cambridge, UK; 2008. 784 p.
102. Shi X, Wang Y, Ren L, Gong Y, Wang DA. Enhancing alendronate release from a novel PLGA/hydroxyapatite microspheric system for bone repairing applications. Pharm Res 2009;26:422–430.
103. Tonino AJ, van der Wal BCH, Heyligers IC, Grimm B. Bone remodeling and hydroxyapatite resorption in coated primary hip prostheses. Clin Orthop Relat Res 2009;467:478–484.
104. Lu YP, Li MS, Li ST, Wang ZG, Zhu RF. Plasma-sprayed hydroxyapatite+titania composite bond coat for hydroxyapatite coating on titanium substrate. Biomaterials 2004;25:4393–4403.
105. Sun L, Berndt CC, Khor KA, Cheang HN, Gross KA. Surface characteristics and dissolution behavior of plasma-sprayed hydroxyapatite coating. J Biomed Mater Res 2002;62:228–236.
106. Wang J, Layrolle P, Stigter M, de Groot K. Biomimetic and electrolytic calcium phosphate coatings on titanium alloy: physicochemical characteristics and cell attachment. Biomaterials 2004; 25:583–592.
107. Lindgren M, Astrand M, Wiklund U, Engqvist H. Investigation of boundary conditions for biomimetic HA deposition on titanium oxide surfaces. J Mater Sci Mater Med 2009;20:1401–1408.
108. Massaro C, Baker MA, Cosentino F, Ramires PA, Klose S, Milella E. Surface and biological evaluation of hydroxyapatite-based coatings on titanium deposited by different techniques. J Biomed Mater Res 2001;58:651–657.
109. Verket A, Tiainen H, Haugen HJ, Lyngstadaas SP, Nilsen O, Reseland JE. Enhanced osteoblast differentiation on scaffolds coated with TiO2 compared to SiO2 and CaP coatings. Biointerphases 2012;7:36.
110. Wang J, de Boer J, de Groot K. Proliferation and differentiation of osteoblast-like MC3T3-E1 cells on biomimetically and electrolytically deposited calcium phosphate coatings. J Biomed Mater Res A 2008;90:664–670.
111. Chen F, Lam WM, Lin CJ, Qiu GX, Wu ZH, Luk KD, Lu WW. Biocompatibility of electrophoretical deposition of nanostructured hydroxyapatite coating on roughen titanium surface: in vitro evaluation using mesenchymal stem cells. J Biomed Mat Res B Appl Biomat 2007;82:183–191.
112. Epinette JA, Manley MT. Fifteen Years of Clinical Expierience with Hydroxyapatite Coatings in Joint Arthroplasty. Paris: Springer Verlag France; 2004.
113. Alghamdi HS, A J A van Oirschot B, Bosco R, van den Beucken JJ, Aldosari AA, Anil S, Jansen JA. Biological response to titanium implants coated with nanocrystals calcium phosphate or type 1 collagen in a dog model. Clin Oral Impl Res. 2012. doi: 10.1111/j.1600-0501.2011.02409.x. [Epub ahead of print].
114. Lu Y, Markel MD, Nemke B, Lee JS, Graf BK, Murphy WL. Influence of hydroxyapatite-coated and growth factor-releasing interference screws on tendon-bone healing in an ovine model. Arthroscopy 2009;25:1427–1434.
115. Cai Y, Zhang S, Zeng X, Sun D. Effect of fluorine incorporation on long-term stability of magnesium-containing hydroxyapatite coatings. J Mat Sci Mat Med 2011;22:1633–1638.
116. Dimitrievska S, Bureau MN, Antoniou J, Mwale F, Petit A, Lima RS, Marple BR. Titania-hydroxyapatite nanocomposite coatings support human mesenchymal stem cells osteogenic differentiation. J Biomed Mater Res A 2011;98:576–588.
117. Lee TM, Yang CY, Chang E, Tsai RS. Comparison of plasma-sprayed hydroxyapatite coatings and zirconia-reinforced hydroxyapatite composite coatings: in vivo study. J Biomed Mater Res A 2004;71:652–660.
118. Aniket, Young A, Marriott I, El-Ghannam A. Promotion of pro-osteogenic responses by a bioactive ceramic coating. J Biomed Mater Res A 2012;100:3314–3325.
119. Chen Y, Zheng X, Xie Y, Ding C, Ruan H, Fan C. Anti-bacterial and cytotoxic properties of plasma sprayed silver-containing HA coatings. J Mater Sci Mater Med 2008;19:3603–3609.
120. Oosterbos KJM, Vogely CH, Dhert WJA, Tonino AJ. Hydroxyapatite-coated Ti6Al4V implants and peri-implant infection. In: Epinette JA, Manley MT, editors. Fifteen Years of Clinical Experience with Hydroxyapatite Coatings in Joint Arthroplasty. Paris: Springer Verlag France; 2004. p177–189.
121. Nelson M, Balasundaram G, Webster TJ. Increased osteoblast adhesion on nanoparticulate crystalline hydroxyapatite functionalized with KRSR. Int J Nanomed 2006;1:339–349.
122. Autefage H, Briand-Mésange F, Cazalbou S, Drouet C, Fourmy D, Gonçalvès S, Salles JP, Combes C, Swider P, Rey C. Adsorption and release of BMP-2 on nanocrystalline apatite-coated and uncoated hydroxyapatite/beta-tricalcium phosphate porous ceramics. J Biomed Mater Res B Appl Biomater 2009;91:706–715.
123. dos Santos EA, Farina M, Soares GA, Anselme K. Surface energy of hydroxyapatite and beta-tricalcium phosphate ceramics driving serum protein adsorption and osteoblast adhesion. J Mater Sci Mater Med 2008;19:2307–2316.
124. Zhou H, Wu T, Dong X, Wamng Q, Shen J. Adsorption mechanism of BMP-7 on hydroxyapatite (001) surfaces. Biochem Biophys Res Commun 2007;361:91–96.
125. Foppiano S, Marshall SJ, Marshall GW, Saiz E, Tomsia AP. Bioactive glass coatings affect the behavior of osteoblast-like cells. Acta Biomater 2008;3:765–771.
126. Wheeler DL, Montfort MJ, McLoughlin SW. Differential healing response of bone adjacent to porous implants coated with hydroxyapatite and 45S5 bioactive glass. J Biomed Mater Res 2000;55:603–612.
127. Gorustovich AA, Roether JA, Boccaccini AR. Effect of bioactive glasses on angiogenesis: A review of in vitro and in vivo evidences. Tissue Eng B 2010;16:199–207.
128. Ducheyne P, Qiu Q. Bioactive ceramics: the effect of surface reactivity on bone formation and bone cell function. Biomaterials 1999;20:2287–2303.
129. Tan F, Naciri M, Al-Rubeai M. Osteoconductivity and growth factor production by MG63 osteoblastic cells on bioglass-coated orthopedic implants. Biotechol Bioeng 2011;108:454–464.
130. Lopez-Sastre A, Gonzalo-Orden JM, Altónaga JAR, Altónaga JR, Orden MA. Coating titanium implants with bioglass and with hydroxyapatite. Int Orthop 1998;22:380–383.
131. Xie XH, Yu XW, Zeng SX, Du RL, Hu YH, Z. Y, Lu EY, Dai KR, Tang TT. Enhanced osteointegration of orthopaedic implant gradient coating composed of bioactive glass and nanohydroxyapatite. J Mater Sci Mater Med 2010;21:2165–2173.
132. Bruinink A, Luginbuehl R. Evaluation of biocompatibility using in vitro methods: Interpretation and limitations. Adv Biochem Engin/Biotechnol 2012;126:117–152.
133. Chai F, Mathis N, Blanchemain N, Meunier C, Hildebrand HF. Osteoblast interaction with DLC-coated Si substrates. Acta Biomater 2008;4:1369–1381.
134. Schroeder A, Francz G, Bruinink A, Hauert R, Mayer J, Wintermantel E. Titanium containing amorphous hydrogenated carbon coatings (a-C:H/Ti): surface analysis and evaluation of cellular reactions using bone marrow cells in vitro. Biomaterials 2000;21:449–456.
135. Sabata M, Francesco DA, Ilaria A, Roberto T, Manuela P, Mariaserena D, Samantha M, Auro C, Giorgio CG, Maria KJ and others. Hydrogenated amourphous carbon nanopatterned film designs drive human bone marrow mesenchymal stem cell cytoskeleton architecture. Tissue Eng Part A 2009;15:3139–3149.
136. Yang L, Sheldon BW, Webster TJ. The impact of diamond nanocrystallinity on osteoblast functions. Biomaterials 2009;30:3458–3465.
137. Randeniya LK, Bendavid A, Martin PJ, Amin MS, Preston EW, Magdon Ismail FS, Coe S. Incorporation of Si and SiOx into

diamond-like carbon films: Impact on surface properties and osteoblast adhesion. Acta Biomater 2009;5:1791–1797.
138. Nöthe M, Buuron A, Koch HJ, Penkalla WP, Rehbach WP, Bolt H. Characterization of a-C:H films with metal interlayers and mixed interfaces. Surf Coatings Technol 1999;116–119:335–341.
139. Lee SH, Kim JG, VChoi HW, Lee KR. Microtensile strain on the corosion performance of diamond-like carbon coating. J Biomed Mater Res 2007;85A:808–814.
140. Falub CV, Thorwarth G, Affolter C, Müller U, Voisard C, Hauert R. A quantitative in-vitro method to predict the adhesion lifetime of diamond-like-carbon (DLC) thin films on biomedical implants. Acta Biomater 2009;5:3086–3097.
141. Iijima S. Helical microtubes of graphitic carbon. Nature 1991;354: 56–58.
142. Su DS. 20 years of carbon nanotubes. Chem Sus Chem 2011;4: 811–813.
143. Bajaj P, Khang D, Webster TJ. Control of spatial cell attachment on carbon nanofiber patterns on polycarbonate urethane. Int J Nanomed 2006;1:361–365.
144. Yuen FLY, Zak G, Waldman SD, Docoslis A. Morphology of fibroblasts grown on substrates formed by dielectrophoretically aligned carbon nanotubes. Cytotechnology 2008;56:9–17.
145. Khang D, Sato M, Price RL, Ribbe AE, Webster TJ. Selective adhesion and mineral deposition by osteoblasts on carbon nanofiber patterns. Int J Nanomed 2006;1:65–72.
146. MacDonald RA, Laurenzi BF, Viswanathan G, Ajayan PM, Stegemann JP. Collagen-carbon nanotube composite materials as scaffolds in tissue engineering. J Biomed Mater Res A 2005;74: 489–496.
147. Usui Y, Aoki K, Narita N, Murakami N, Nakamura I, Nakamura K, Ishigaki N, Yamazaki H, Horiuchi H, Kato H and others. Carbon nanotubes with high bone-tissue compatibility and bone-formation acceleration effects. Small 2008;4:240–246.
148. Correa-Duarte MA, Wagner N, Rojas-Chapana J, Morsczeck C, M. T, M. G. Fabrication and biocompatibility of carbon nanotube-based 3D networks as scaffolds for cell seeding and growth. Nano Lett 2004;11:2233–2236.
149. Balani K, Anderson R, Laha T, Andara M, Tercero J, Crumpler E, Agarwal A. Plasma-sprayed carbon nanotube reinforced hydroxyapatite coatings and their interaction with human osteoblasts in vitro. Biomaterials 2007;28:618–624.
150. Zhao B, Hu H, Swadhin K, Mandal K, Haddon RC. A bone mimic based on the self-assembly of hydroxyapatite on chemically functionalized single-walled carbon nanotubes. Chem Mater 2005;17:3235–3241.
151. Bhattacharya M, Wutticharoenmongkol-Thitiwongsawet P, Hamamoto DT, Lee D, Cui T, Prasad HS, Ahmad M. Bone formation on carbon nanotubes composite. J Biomed Mater Res A 2011;96:75–82.
152. Hu H, Ni Y, Mandal SK, Montana V, Zhao B, Haddon RC, Parpura V. Polyethyleneimine functionalized single-walled carbon nanotubes as a substrate for neuronal growth. J Phys Chem B 2005; 109:4285–4289.
153. Hu H, Ni Y, Montana V, Haddon RC, Parpura V. Chemically functionalized carbon nanotubes as substrates for neuronal growth. Nano Lett 2004;4:507–511.
154. Ni Y, Hu H, Malarkey EB, Zhao B, Montana V, Haddon RC, Parpura V. Chemically functionalized water soluble single-walled carbon nanotubes modulate neurite outgrowth. J Nanosci Nanotechnol 2005;5:1707–1712.
155. Lu Y, Li T, Zhao X, Li M, Cao Y, Yang H, Duan YY. Electrodeposited polypyrrole/carbon nanotubes composite films electrodes for neural interfaces. Biomaterials 2010;31:5169–5181.
156. Keefer EW, Botterman BR, Romero MI, Rossi AF, Gross GW. Carbon nanotube coating improves neuronal recordings. Nat Nanotechnol 2008;3:434–439.
157. Lin C, Han H, Zhang F, Li A. Electrophoretic deposition of HA/MWNTs composite coating for biomaterial applications. J Mater Sci Mater Med 2008;19:2569–2574.
158. Vandrovcová M, Bačáková L. Adhesion, growth and differentiation of osteoblasts on surface-modified materials developed for bone implants. Physiol Res 2011;60:403–417.
159. Abarrategi A, Gutiérrez MC, Moreno-Vicente C, Hortigüela MJ, Ramos V, López-Lacomba JL, Ferrer ML, del Monte F. Multiwall carbon nanotube scaffolds for tissue engineering purposes. Biomaterials 2008;29:94–102.
160. Dobrovolskaia MA, McNeil SE. Immunological properties of engineered nanomaterials. Nat Nanotechnol 2007;2:469–478.
161. Wick P, Clift MJD, Rösslein M, Rothen-Ruthishauser B. A brief summary of carbon nanotubes science and technology: a health and safety perspective. ChemSusChem 2011;18:905–911.
162. Hirsch C, Roesslein M, Krug HF, Wick P. Nanomaterial cell interactions: Are current in vitro tests reliable? Nanomed 2011;6: 837–847.
163. Kaiser JP, Rösslein M, Bürki-Thurnherr T, Wick P. Carbon nanotubes—Curse or blessing. Curr Med Chem 2011;18:2115–2128.
164. Krug HF, Wick P. Nanotoxicology: An interdisciplinary challenge. Angew Chem Int Ed Engl 2011.
165. Wick P, Manser P, Limbach LK, Dettlaff-Weglikowska U, Krumeich F, Roth S, Stark WJ, Bruinink A. The degree and kind of agglomeration affect carbon nanotube cytotoxicity. Toxicol Lett 2007;168:121–131.
166. Kaiser JP, Wick P, Manser P, Spohn P, Bruinink A. Single walled carbon nanotubes (SWCNT) affect cell physiology and cell architecture. J Mater Sci Mater Med 2008;19:1523–1527.
167. Bruinink A, Hasler S, Manser P. In vitro effects of SWCNT: Role of treatment duration. Phys Status Solidi B 2009;246:2423–2427.
168. Pulskamp K, Diabate S, Krug HF. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. Toxicol Lett 2007;168:58–74.
169. Helfenstein M, Miragoli M, Rohr S, Müller L, Wick P, Mohr M, Gehr P, Rothen-Rutishauser B. Effects of combustion-derived ultrafine particles and manufactured nanoparticles on heart cells in vitro. Toxicol 2008;253:70–78.
170. Thurnherr T, Brandenberger C, Fischer K, Diener L, Manser P, Maeder-Althaus X, Kaiser JP, Krug HF, Rothen-Rutishauser B, Wick P. A comparison of acute and long-term effects of industrial multiwalled carbon nanotubes on human lung and immune cells in vitro. Toxicol Lett 2011;200:176–186.
171. Thurnherr T, Su DS, Diener L, Weinberg G, Manser P, Pfänder N, Arrigo R, Schuster ME, Wick P, Krug HF. Comprehensive evaluation of in vitro toxicity of three large-scale produced carbon nanotubes on human Jurkat T cells and a comparison to crocidolite asbestos. Nanotoxicol 2009;3:319–338.
172. Griffith LG. Emerging design principles in biomaterials and scaffolds for tissue engineering. Ann N Y Acad Sci 2002;961:83–95.
173. Murugan R, Ramakrishna S. Design strategies of tissue engineering scaffolds with controlled fiber orientation. Tissue Eng 2007; 13:1845–1866.
174. Ashammakhi N, Ndreu A, Piras AM, Nikkola L, Sindelar T, Ylikauppila H, Harlin A, Gomes ME, Neves NM, Chiellini E and others. Biodegradable nanomats produced by electrospinning: expanding multifunctionality and potential for tissue engineering. J Nanosci Nanotechnol 2007;7:862–882.
175. Greiner A, Wendorff JH. Electrospinning: a fascinating method for the preparation of ultrathin fibers. Angew Chem Int Ed Engl 2007;46:5670–5703.
176. Zhong S, Teo WE, Zhu X, Beuerman RW, Ramakrishna S, Yung LY. An aligned nanofibrous collagen scaffold by electrospinning and its effects on in vitro fibroblast culture. J Biomed Mater Res A 2006;79:456–463.
177. Wray LS, Orwin EJ. Recreating the microenvironment of the native cornea for tissue engineering applications. Tissue Eng Part A 2009;15:1463–1472.
178. Hashi CK, Zhu Y, Yang GY, Young WL, Hsiao BS, Wang K, Chu B, Li S. Antithrombogenic property of bone marrow mesenchymal stem cells in nanofibrous vascular grafts. Proc Natl Acad Sci USA 2007;104:11915–11920.
179. Bashur CA, Shaffer RD, Dahlgren LA, Guelcher SA, Goldstein AS. Effect of fiber diameter and alignment of electrospun polyurethane meshes on mesenchymal progenitor cells. Tissue Eng Part A 2009:2435–2445.
180. Choi JS, Lee SJ, Christ GJ, Atala A, Yoo JJ. The influence of electrospun aligned poly(epsilon-caprolactone)/collagen

nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. Biomaterials 2008;29:2899–2906.
181. Schnell E, Klinkhammer K, Balzer S, Brook G, Klee D, Dalton P, Mey J. Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. Biomaterials 2007;28: 3012–3025.
182. Rim NG, Lee JH, Jung SI, Lee BK, Kim CH, Shin H. Modulation of osteogenic differentiation of human mesenchymal stem cells by poly[(L-lactide)-co-(epsilon-caprolactone)]/gelatin nanofibers. Macromol Biosci 2009;9:795–804.
183. Li WJ, Cooper JA, Jr., Mauck RL, Tuan RS. Fabrication and characterization of six electrospun poly(alpha-hydroxy ester)-based fibrous scaffolds for tissue engineering applications. Acta Biomater 2006;2:377–385
184. Li WJ, Tuli R, Huang X, Laquerriere P, Tuan RS. Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. Biomaterials 2005;26:5158–5166.
185. Yoshimoto H, Shin YM, Terai H, Vacanti JP. A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering. Biomaterials 2003;24:2077–2082.
186. Shin M, Yoshimoto H, Vacanti JP. In vivo bone tissue engineering using mesenchymal stem cells on a novel electrospun nanofibrous scaffold. Tissue Eng 2004;10:33–41.
187. Kazemnejad S, Allameh A, Soleimani M, Gharehbaghian A, Mohammadi Y, Amirizadeh N, Jazayery M. Biochemical and molecular characterization of hepatocyte-like cells derived from human bone marrow mesenchymal stem cells on a novel three-dimensional biocompatible nanofibrous scaffold. J Gastroenterol Hepatol 2009;24:278–287.
188. Chan CK, Liao S, Li B, Lareu RR, Larrick JW, Ramakrishna S, Raghunath M. Early adhesive behavior of bone-marrow-derived mesenchymal stem cells on collagen electrospun fibers. Biomed Mater 2009;4:035006.
189. Shin S-H, Purevdorj O, Castano O, Planell JA, Kim H-W. A short review: Recent advances in electrospinning for bone tissue regeneration. J Tissue Eng 2012;3:2041731412443530.
190. Venugopal J, Prabhakaran MP, Low S, Choon AT, Zhang YZ, Deepika G, Ramakrishna S. Nanotechnology for nanomedicine and delivery of drugs. Curr Pharm Des 2008;14:2184–2200.
191. Kriegel C, Arrechi A, Kit K, McClements DJ, Weiss J. Fabrication, functionalization, and application of electrospun biopolymer nanofibers. Crit Rev Food Sci Nutr 2008;48:775–797.
192. Kumbar SG, James R, Nukavarapu SP, Laurencin CT. Electrospun nanofiber scaffolds: engineering soft tissues. Biomed Mater 2008;3:034002.
193. Prabhakaran MP, Venugopal J, Ramakrishna S. Electrospun nano-structured scaffolds for bone tissue engineering. Acta Biomater 2009;5:2884–2893.
194. Venugopal J, Ramakrishna S. Applications of polymer nanofibers in biomedicine and biotechnology. Appl Biochem Biotechnol 2005;125:147–158.
195. Wang HS, Fu GD, Li XS. Functional polymeric nanofibers from electrospinning. Recent Pat Nanotechnol 2009;3:21–31.
196. Xu C, Inai R, Kotaki M, Ramakrishna S. Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. Tissue Eng 2004;10:1160–1168.
197. Zhang YZ, Su B, Venugopal J, Ramakrishna S, Lim CT. Biomimetic and bioactive nanofibrous scaffolds from electrospun composite nanofibers. Int J Nanomed 2007;2:623–638.
198. Venugopal J, Rajeswari R, Shayanti M, Low S, Bongso A, Dev VR, Deepika G, Choon AT, Ramakrishna S. Electrosprayed hydroxyapatite on polymer nanofibres to differentiate mesenchymal stem cells to osteogenesis. J Biomater Sci Polym Ed. 2012. [Epub ahead of print].
199. Thomas V, Dean DR, Jose MV, Mathew B, Chowdhury S, Vohra YK. Nanostructured biocomposite scaffolds based on collagen coelectrospun with nanohydroxyapatite. Biomacromolecules 2007;8:631–637.
200. Thomas V, Jagani S, Johnson K, Jose MV, Dean DR, Vohra YK, Nyairo E. Electrospun bioactive nanocomposite scaffolds of polycaprolactone and nanohydroxyapatite for bone tissue engineering. J Nanosci Nanotechnol 2006;6:487–493.
201. Ngiam M, Liao S, Patil AJ, Cheng Z, Chan CK, Ramakrishna S. The fabrication of nano-hydroxyapatite on PLGA and PLGA/collagen nanofibrous composite scaffolds and their effects in osteoblastic behavior for bone tissue engineering. Bone 2009;45:4–16.
202. Liao S, Ngiam M, Chan CK, Ramakrishna S. Fabrication of nano-hydroxyapatite/collagen/osteonectin composites for bone graft applications. Biomed Mater 2009;4:25019.
203. Ngiam M, Liao S, Patil AJ, Cheng Z, Yang F, Gubler MJ, Ramakrishna S, Chan CK. Fabrication of mineralized polymeric nanofibrous composites for bone graft materials. Tissue Eng Part A 2009;15:535–546.
204. Zhang Y, Venugopal JR, El-Turki A, Ramakrishna S, Su B, Lim CT. Electrospun biomimetic nanocomposite nanofibers of hydroxyapatite/chitosan for bone tissue engineering. Biomaterials 2008;29:4314–4322.
205. Kim J, Lee J, Kwon S, Jeong S. Preparation of biodegradable polymer/silver nanoparticles composite and its antibacterial efficacy. J Nanosci Nanotechnol 2009;9:1098–1102.
206. Xu X, Yang Q, Bai J, Lu T, Li Y, Jing X. Fabrication of biodegradable electrospun poly(L-lactide-co-glycolide) fibers with antimicrobial nanosilver particles. J Nanosci Nanotechnol 2008;8: 5066–5070.
207. Penchev H, Paneva D, Manolova N, Rashkov I. Electrospun hybrid nanofibers based on chitosan or N-carboxyethylchitosan and silver nanoparticles. Macromol Biosci 2009;9:884–894.
208. Kang MS, Shin MK, Ismail YA, Shin SR, Kim SI, Kim H, Lee H, Kim SJ. The fabrication of polyaniline/single-walled carbon nanotube fibers containing a highly-oriented filler. Nanotechnology 2009;20:085701.
209. Schofer M, Fuchs-Winkelmann S, Wack C, Rudisile M, Dersch R, Leifeld I, Wendorff J, Greiner A, Paletta JR, Boudriot U. Lack of obvious influence of PLLA nanofibers on the gene expression of BMP-2 and VEGF during growth and differentiation of human mesenchymal stem cells. Sci World J 2009;9:313–319.
210. Park YJ, Kim KH, Lee JY, Ku Y, Lee SJ, Min BM, Chung CP. Immobilization of bone morphogenetic protein-2 on a nanofibrous chitosan membrane for enhanced guided bone regeneration. Biotechnol Appl Biochem 2006;43(Pt 1):17–24.
211. Chew SY, Wen J, Yim EK, Leong KW. Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules 2005;6:2017–2024.
212. Schofer MD, Boudriot U, Bockelmann S, Walz A, Wendorff JH, Greiner A, Paletta JR, Fuchs-Winkelmann S. Effect of direct RGD incorporation in PLLA nanofibers on growth and osteogenic differentiation of human mesenchymal stem cells. J Mater Sci Mater Med 2009;20:1535–1540.
213. Ahmed I, Ponery AS, Nur EKA, Kamal J, Meshel AS, Sheetz MP, Schindler M, Meiners S. Morphology, cytoskeletal organization, and myosin dynamics of mouse embryonic fibroblasts cultured on nanofibrillar surfaces. Mol Cell Biochem 2007;301:241–249.
214. Koh HS, Yong T, Chan CK, Ramakrishna S. Enhancement of neurite outgrowth using nano-structured scaffolds coupled with laminin. Biomaterials 2008;29:3574–3582.
215. Luu YK, Kim K, Hsiao BS, Chu B, Hadjiargyrou M. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA-PEG block copolymers. J Control Release 2003;89:341–353.
216. Liang D, Luu YK, Kim K, Hsiao BS, Hadjiargyrou M, Chu B. In vitro non-viral gene delivery with nanofibrous scaffolds. Nucleic Acids Res 2005;33:e170.
217. Bellan LM, Cross JD, Strychalski EA, Moran-Mirabal J, Craighead HG. Individually resolved DNA molecules stretched and embedded in electrospun polymer nanofibers. Nano Lett 2006;6: 2526–2530.
218. Nie H, Wang CH. Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA. J Control Release 2007;120:111–121.
219. Yixiang D, Yong T, Liao S, Chan CK, Ramakrishna S. Degradation of electrospun nanofiber scaffold by short wave length ultraviolet radiation treatment and its potential applications in tissue engineering. Tissue Eng Part A 2008;14:1321–1329.
220. Ko EK, Jeong SI, Rim NG, Lee YM, Shin H, Lee BK. In vitro osteogenic differentiation of human mesenchymal stem cells

and in vivo bone formation in composite nanofiber meshes. Tissue Eng Part A 2008;14:2105–2119.
221. Bandyopadhyay-Ghosh S. Bone as a collagen-hydroxyapatite composite and its repair. Trends Biomater Artif Organs 2008;22: 116–124.
222. Kim HW, Lee HH, Knowles JC. Electrospinning biomedical nanocomposite fibres of hydroyapatite/poly(lactic acid) for bone regeneration. J Biomed Mater Res A 2006;79:643–649.
223. Fujihara K, Kotaki M, Ramakrishna S. Guided bone regeneration membrane made of polycaprolactone/calcium carbonate composite nano-fibers. Biomaterials 2005;26:4139–4147.
224. Song JH, Kim HE, Kim HW. Electrospun fibrous web of collagen-apatite precipitated nanocomposite for bone regeneration. J Mater Sci Mater Med. 2008;19:2925–2932.
225. Song J-H, Yoon B-H, Kim H-E, Kim H-W. Bioactive and degradable hybridized nanofibers of gelatin–siloxane for bone regeneration. J Biomed Mater Res A 2008;84A:875–884.
226. Yu H-S, Jang J-H, Kim T-I, Lee H-H, Kim H-W. Apatite-mineralized polycaprolactone nanofibrous web as a bone tissue regeneration substrate. J Biomed Mater Res A 2009;88A: 747–754.
227. Liao S, Murugan R, Chan CK, Ramakrishna S. Processing nanoengineered scaffolds through electrospinning and mineralization suitable for biomimetic bone tissue engineering. J Mech Behav Biomed Mater 2008;1:252–260.
228. Chen J, Chu B, Hsiao BS. Mineralization of hydroxyapatite in electrospun nanofibrous poly(L-lactic acid) scaffolds. J Biomed Mater Res A 2006;79A:307–317.
229. Cui W, Li X, Zhou S, Weng J. In situ growth of hydroxyapatite within electrospun poly(DL-lactide) fibers. J Biomed Mat Res Part A 2007;82A:831–841.
230. Ngiam M, Liao S, Patil AJ, Cheng Z, Yang F, Gubler MJ, Ramakrishna S, Chan CK. Fabrication of mineralized polymeric nanofibrous composites for bone graft materials. Tissue Eng Part A 2009;15:535–546.
231. Yoo HS, Kim TG, Park TG. Surface-functionalized electrospun nanofibers for tissue engineering and drug delivery. Adv Drug Delivery Rev 2009;61:1033–1042.
232. Kim TG, Park TG. Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly(D,L-lactic-co-glycolic acid) nanofiber mesh. Tissue Eng 2006;12:221–233.
233. Kwon IK, Matsuda T. Co-electrospun nanofiber fabrics of poly(L-lactide-co-epsilon-caprolactone) with type I collagen or heparin. Biomacromolecules 2005;6:2096–2105.
234. Kim J-E, Noh K-T, Yu H-S, Lee H-Y, Jang J-H, Kim H-W. A fibronectin peptide-coupled biopolymer nanofibrous matrix to speed up initial cellular events. Adv Eng Mater B 2010;12:94–100.
235. Kumbar SG, James R, Nukavarapu SP, Laurencin CT. Electrospun nanofiber scaffolds: engineering soft tissues. Biomed Mater 2008;3:034002.
236. Casper CL, Yamaguchi N, Kiick KL, Rabolt JF. Functionalizing electrospun fibers with biologically relevant macromolecules. Biomacromolecules 2005;6:1998–2007.
237. Li C, Vepari C, Jin HJ, Kim HJ, Kaplan DL. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. Biomaterials 2006;27: 3115–3124.
238. Wang C, Wang M. Dual-source dual-power electrospinning and characteristics of multifunctional scaffolds for bone tissue engineering. J Mater Sci Mater Med 2012;23:2381–2397.
239. Zhang YZ, Feng Y, Huang Z-M, Ramakrishna S, Lim CT. Fabrication of porous electrospun nanofibres. Nanotechnology 2006;17: 901–908.
240. Walles T, Lichtenberg A, Puschmann C, Leyh R, Wilhelmi M, Kallenbach K, Haverich A, Mertsching H. In vivo model for cross-species porcine endogenous retrovirus transmission using tissue engineered pulmonary arteries. Eur J Cardiothorac Surg 2003;24: 358–363.
241. Mahoney MJ, Anseth KS. Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. Biomaterials 2006;27:2265–2274.
242. Lienemann PS, Lutolf MP, Ehrbar M. Biomimetic hydrogels for controlled biomolecule delivery to augment bone regeneration. Adv Drug Delivery Rev. 2012;64:1078–1089.
243. Malkoch M, Vestberg R, Gupta N, Mespouille L, Dubois P, Mason AF, Hedrick JL, Liao Q, Frank CW, Kingsbury K, Hawker CJ. Synthesis of well-defined hydrogel networks using click chemistry. Chem Commun 2006:2774–2776.
244. Shen W, Zhang K, Kornfield JA, Tirrell DA. Tuning the erosion rate of artificial protein hydrogels through control of network topology. Nat Mater 2006;5:153–158.
245. Lutolf MP, Weber FE, Schmoekel HG, Schense JC, Kohler T, Muller R, Hubbell JA. Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. Nat Biotechnol 2003; 21:513–518.
246. Adelow C, Segura T, Hubbell JA, Frey P. The effect of enzymatically degradable poly(ethylene glycol) hydrogels on smooth muscle cell phenotype. Biomaterials 2008;29:314–326.
247. Kraehenbuehl TP, Zammaretti P, Van der Vlies AJ, Schoenmakers RG, Lutolf MP, Jaconi ME, Hubbell JA. Three-dimensional extracellular matrix-directed cardioprogenitor differentiation: systematic modulation of a synthetic cell-responsive PEG-hydrogel. Biomaterials 2008;29:2757–2766.
248. Ehrick JD, Deo SK, Browning TW, Bachas LG, Madou MJ, Daunert S. Genetically engineered protein in hydrogels tailors stimuli-responsive characteristics. Nat Mater 2005;4:298–302.
249. Peled E, Boss J, Bejar J, Zinman C, Seliktar D. A novel poly(-ethylene glycol)-fibrinogen hydrogel for tibial segmental defect repair in a rat model. J Biomed Mater Res A 2007;80: 874–884.
250. Murakami Y, Maeda M. DNA-responsive hydrogels that can shrink or swell. Biomacromolecules 2005;6:2927–2929.
251. Ehrbar M, Rizzi SC, Hlushchuk R, Djonov V, Zisch AH, Hubbell JA, Weber FE, Lutolf MP. Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering. Biomaterials 2007;28:3856–3866.
252. Schliephake H, Aref A, Scharnweber D, Bierbaum S, Sewing A. Effect of modifications of dual acid-etched implant surfaces on peri-implant bone formation. I. Organic coatings. Clin Oral Implants Res 2009;20:31–37.
253. Xiao SJ, Textor M, Spencer ND, Wieland M, Keller B, Sigrist H. Immobilization of the cell-adhesive peptide Arg-Gly-Asp-Cys (RGDC) on titanium surfaces by covalent chemical attachment. J Mater Sci Mater Med 1997;8:867–872.
254. Rezania A, Johnson R, Lefkow AR, Healy KE. Bioactivation of metal oxide surfaces. 1. Surface characterization and cell response. Langmuir 1999;15:6931–6939.
255. Lee H, Dellatore SM, Miller WM, Messersmith PB. Mussel-inspired surface chemistry for multifunctional coatings. Science 2007;318:426–430.
256. Kämmerer PW, Heller M, Brieger J, Klein MO, Al-Nawas B, Gabriel M. Immobilisation of linear and cyclic RGD-peptides on titanium surfaces and their impact on endothelial cell adhesion and proliferation. Eur Cell Mater 2011;21:364–372.
259. Cao X, Yu WQ, Qiu J, Zhao YF, Zhang YL, Zhang FQ. RGD peptide immobilized on TiO(2) nanotubes for increased bone marrow stromal cells adhesion and osteogenic gene expression. J Mater Sci Mater Med 2012;23:527–536.
258. Secchi AG, Grigoriou V, Shapiro IM, Cavalcanti-Adam EA, Composto RJ, Ducheyne P, Adams CS. RGDS peptides immobilized on titanium alloy stimulate bone cell attachment, differentiation and confer resistance to apoptosis. J Biomed Mater Res A 2007; 83:577–884.
259. Poh CK, Shi Z, Lim TY, Neoh KG, Wang W. The effect of VEGF functionalization of titanium on endothelial cells in vitro. Biomaterials 2010;31:1578–1585.
260. Shi Z, Neoh KG, Kang ET, Poh C, Wang W. Titanium with surface-grafted dextran and immobilized bone morphogenetic protein-2 for inhibition of bacterial adhesion and enhancement of osteoblast functions. Tissue Eng Part A 2009;15:417–426.
261. Park JW, Kurashima K, Tustusmi Y, C.H. A, Suh JY, Doi H, Nomura N, Noda K, Hanawa T. Bone healing of commercial oral implants with RGD immobilization through electrodeposited poly(ethylene glycol) in rabbit cancellous bone. Acta Biomater 2011;7:3222–3229.
262. Germanier Y, Tosatti S, Broggini N, Textor M, Buser D. Enhanced bone apposition around biofunctionalized sandblasted

and acid-etched titanium implant surfaces. A histomorphometric study in miniature pigs. Clin Oral Implants Res 2006;17:251–257.
263. Ceylan H, Kocabey S, Tekinay AB, Guler MO. Surface-adhesive and osteogenic self-assembled peptide nanofibers for bioinspired functionalization of titanium surfaces. Soft Matter 2012;8: 3929–3937.
264. Webster TJ, Ejiofor JU. Increased osteoblast adhesion on nanophase metals: Ti, Ti6Al4V, and CoCrMo. Biomaterials 2004;25: 4731–4739.
265. Burguera EF, Bitar M, Bruinink A. Novel in vitro co-culture methodology to investigate heterotypic cell-cell interactions. Eur Cell Mater 2010;19:166–179.
266. Lavenus S, Trichet V, Le Chevalier S, Hoornaert A, Louarn G, Layrolle P. Cell differentiation and osseointegration influenced by nanoscale anodized titanium surfaces. Nanomedicine 2012;7: 967–980.
267. Wein F, Bruinink A. Human triple cell co-culture for evaluation of bone implant materials. Integr Biol (Camb). 2013. [Epub ahead of print].
268. Zhang Y, Andrukhov O, Berner S, Matejka M, Wieland M, Rausch-Fan X, Schedle A. Osteogenic properties of hydrophilic and hydrophobic titanium surfaces evaluated with osteoblast-like cells (MG63) in coculture with human umbilical vein endothelial cells (HUVEC). Dent Mater 2010;26: 1043–1051.
269. Moczulska M, Bitar M, Święszkowski W, Bruinink A. Biological characterization of woven fabric using 2- and 3-dimensional cell cultures. J Biomed Mater Res A 2012;100:882–893.
270. Chen C, Loe F, Blocki A, Peng Y, Raghunath M. Applying macromolecular crowding to enhance extracellular matrix deposition and its remodeling in vitro for tissue engineering and cell-based therapies. Adv Drug Deliv Rev 2011;63: 277–290.
271. Zeiger AS, Loe FC, Li R, Raghunath M, Van Vliet KJ. Macromolecular crowding directs extracellular matrix organization and mesenchymal stem cell behavior. Plos One 2012;7: e37904.