

REVIEW

**Application of Nanoparticles of
Ceramics, Peptides, Silicon, Carbon,
and Diamonds in Tissue
Engineering**

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Application of Nanoparticles of Ceramics, Peptides, Silicon, Carbon, and Diamonds in Tissue Engineering

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Abstract

This review purports to fill the gap in knowledge that exists about the role of nanoparticles in Tissue Engineering. In this review, the use of the term nanoparticles will be exclusively confined to those materials or composites with grain sizes ranging from 100-500 nm. First, a brief description of the field will be provided. Next, research findings on the use of cells on ceramics, peptides, carbon, silicon, and diamond, will be scrutinized. Then, future applications and directions will be outlined. Finally, a succinct account will summarize some of the developments in the field of Tissue Engineering involving nanoparticles.

Keywords: CEP68; carbon nanotubes; confocal laser scanning microscopy; COX 2 (cyclooxygenase 2); CTGF (connective tissue growth factor), focal adhesion kinase; fluorenylmethoxy carbonyl; hydroxyapatite; MTT; MTS; nanocrystalline diamond; PLA (poly lactic acid); PGA (polyglycolic acid); Phytohemagglutinin; lipopolysaccharide; Poly (3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV); peripheral blood mononuclear cells; PMMA (poly (methyl) methacrylate); scanning electron microscopy; Saos-2 (Human osteoblast-like cells); SLE (systemic lupus erythematosus); TNF (tumor necrosis factor); single-walled carbon nanotubes; zirconium; WST.

Abbreviations

AFM (Atomic force microscopy); ALP (Alkaline phosphatase); CNT (Carbon nanotubes); CLSM (Confocal laser scanning microscopy); DNA (Deoxyribonucleic acid); DAPI; FAK (focal adhesion kinases); Fmoc (fluorenylmethoxy carbonyl); HA (hydroxyapatite); IL-8 (Interleukin8); LPS (Lipopolysaccharide); MTS; MTT; PBMCs (Peripheral blood mononuclear cells); PHA (Phytohemagglutinin); Phe (Phenylalanine); BMP-2 (Bone matrix protein-2); NCD (Nanocrystalline diamond); RMS (Root mean squared); SEM (Scanning electron microscopy); SWCNT (single-walled carbon nanotubes); (SPRI) Surface plasmon resonance imaging.

1. Introduction

Over the last decade, the scientific community has been abuzz with the excitement generated by nanoparticles, which promise to advance cell transplantation in human patients. In biomedicine, nanoparticles are being used as the ideal form in drug delivery and as scaffolds to support cell growth. A scaffold is defined as a non-toxic and biodegradable material that can support cell growth [1]. Cells are seeded on these scaffolds, which can aid and abet tissue regeneration by supporting cell proliferation on their surface, and cell characteristics such as proliferation, adhesion, morphology are examined with the ultimate aim of implanting the cell-bearing scaffolds in human patients to replace or regenerate cells in the knee, the inter-vertebral discs, the skull, and other areas of the body (Figure 1). To buttress cell growth, signaling moieties or growth factors, encapsulated in nanoparticles or microparticles, to afford sustained release, can further be integrated in the scaffold material [1]. Advances in nanotechnology have made possible the generation of materials and surfaces on a nanoscale level - a dimension that can recapitulate the *in vivo* environment of the extracellular matrix, and thus offer an environment conducive to cell adhesion, cell migration, and cell differentiation [1]. Scaffolds provide structural and mechanical support for tissue rebuilding and act as a substratum for cellular attachment, proliferation, differentiation, and migration [2]. Furthermore, since the surface roughness of cells approximates that of materials having nanometer dimensions, and since most proteins, which facilitate cell adhesion also measure in nanometers - better cell adhesion can be achieved on nanomaterials [3]. Some other features of a biomaterial surface that impact its biological performance include surface energy, wettability, chemistry, and topography [4].

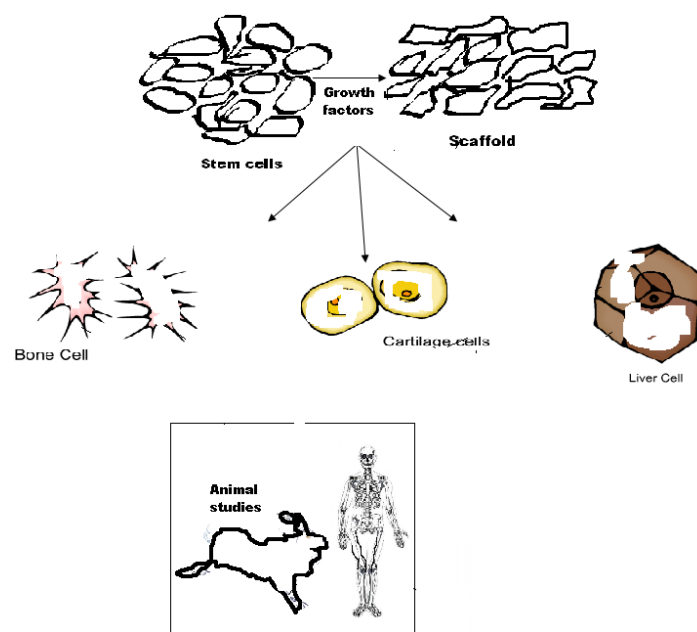


Figure 1: Schematic depiction of the steps involved in stem cell growth, differentiation, and transplantation.

Cells adhere, proliferate, and differentiate better on rough surfaces compared to smooth surfaces [4]. The primary step in cell-implant interaction is the adsorption of serum proteins, followed by the attachment of cellular integrins (receptors) to extracellular ligands in the protein layer formed by serum proteins, by a remodeling of cytoskeletal proteins, by the formation of focal adhesion contacts, by the activation of FAK, and by the induction of signaling pathways [4, 5]. Since most of the cell-implant interactions involve structures with nanometer to micrometer dimensions, a broad variety of nanoparticles has been utilized as scaffolds for tissue engineering applications; some examples include PLLA (50-350 nm), PLGA (500-800nm), PCL (500-900nm), chitosan/PEO (38-62nm), fibrinogen (320-600nm), alginate, hydroxyapatite, carbon nanotubes, nanodiamonds, titanium and aluminum, and zirconium [6-8]. Most of the knowledge gained so far has come from *in vitro* studies while *in vivo* studies have also been conducted. Encouraging results have come from bench studies that have used nanoparticles as cellular scaffolds; some animal studies look promising as well [9, 10]. Thus, one has every reason to be sanguine about the possibility of making the transition from bench to bedside in a significant fashion. As a caveat, however, it will be important to emphasize early on that an ideal tissue engineering scaffold will promote the proliferation, adhesion, migration, and differentiation of any desired cell type but hinder the growth or adhesion of competitive cell types, such as fibroblasts, which tend to compete with osteoblasts, thus leading to implant failure [11]. It is also important to mention that an ideal scaffold will have a rough surface, a pore size ranging from 50-450 μm , a porosity of between 60-70%, and a degraded particle size of smaller than 5 μm -particles less than 5 μm in size can go through blood vessels [12]. Other attributes critical to the success of a scaffold include proper tensile and mechanical strength, and low protein adsorption [8]. Moreover, with some exceptions to cartilage, an implanted scaffold will also have to quickly establish a blood supply since many cells in the center of a scaffold do not survive; to this effect, some scaffolds have been designed with co-culture of endothelial cells [8]. An ideal scaffold will also have very little effects on the immune system of the recipient. A critical but often overlooked factor in biomaterial studies in Tissue Engineering is the ability of a biomaterial to support the growth of more than one type of cells, while retarding fibroblast adhesion [8, 11]. A comprehensive review of studies performed on cell behavior on nano-scale biomaterials is not up to date, thus creating a need for this article.

2. Ceramics

Ceramics have been used in bone tissue engineering due to their osteoinductive and biocompatible properties [13]. Ceramics are inorganic, crystalline, non-metallic solids made by the heating and subsequent cooling of the material [14]. Since natural bone is a nano-composite comprising 35% organic protein and 65% inorganic ceramic (mostly hydroxyapatite), a fair amount of work has been conducted on conventional ceramics such as alumina, titania, zirconia, and hydroxyapatite [11, 15]. Nanophase ceramics - sans immobilized peptides - seeded with osteoblasts, as osteointegrative devices designed to merge with apposed bone, have come to the fore as well [14, 16]. The absence of peptides on the nanophase ceramics would circumvent the problems which could arise from the interactions of the peptides with the biomaterials. For instance, nanophase alumina, titania, zirconia, and hydroxyapatite (HA), with grain sizes less than 100nm, were compared with their conventional counterparts in regards to cell proliferation, cell adhesion, matrix formation, cellular migration and cell differentiation [7, 14-16]. Calcium deposition in the extracellular matrix of cells was statistically significantly greater, after 28 days of culture, on alumina, titania, and hydroxyapatite nanoceramics as opposed to that obtained from cells seeded on conventional ceramics (particle size greater than 100nm). Alkaline phosphatase (ALP) synthesis in osteoblasts cultured on nanophase alumina, titania, and HA was much higher after 21 and 28 days of culture - titania giving the highest ALP activity [16]. The proliferation of osteoblasts was significantly enhanced on nanophase alumina and titania versus conventional alumina and titania after 1, 3, and 5 days of culture, whereas statistically significant osteoblastic proliferation was observed in nanophase HA after day 3 and 5 of culture [14, 16]. Surface occupancy by cells serves as an indicator of cell motility: cell migration depends on gradual, continuous formation and severing of focal points by the proximal and distal ends, respectively, of cells and surfaces, which promote cell adhesion, curtailing cell migration [17]. In the context of surface occupancy, osteoblasts grown on nanophase alumina, titania, and HA particles covered half as much area as on conventional alumina, titania, and HA after 6 days of culture [16]. The authors claimed that the decreased surface occupancy seen on nanophase ceramics in their study accorded well with earlier results obtained by their group showing enhanced osteoblast adhesion on nanoceramics. It must be noted that decreased surface occupancy was observed at 6 days of culture: therefore, these cells were likely still migrating - protruding lamellipodia and filopodia - actin bundles, the former being dense, flat meshwork of actin, the later being bundled actin guiding cells by detecting environmental cues, trying to establish contacts using focal adhesions (large protein complexes that link the cell cytoskeleton to the extracellular matrix) - and would adhere to the surface with time [9]. Webster *et al.* [14] made a significant contribution to our knowledge of biomaterials that can be utilized in orthopedics and dentistry to realize better bonding between the implant material and the adjoining bone.

Yamashita *et al.* [18] investigated the effects of rough and smooth surfaces of ceria and yttria stabilized zirconium dioxide - another ceramic material that holds promise as a dental implant material on the growth and attachment of murine osteoblast-like MC3T3-E1 cells and compared their results to those obtained with the same cell line grown on pure alumina oxide and titanium [18]. Significantly greater cell attachment was seen at 1, 3, 6, and 24 hours of incubation, on rough specimens versus smooth specimens with no significant differences among the surface groups. The cell morphology, as observed by SEM (scanning electron microscopy), was normal on both types of surfaces. The SEM micrographs captured the transformation of the cells from an initial spherical shape to more progressively spread out morphology at 3, 6, and 24 hours of incubation. After 3 hours of incubation on the rough and smooth surfaces of titania, alumina, and the two kinds of zirconia, higher fluorescence intensities for $\alpha 5$ and $\beta 1$ integrins were obtained from cells grown on materials with rough surfaces. Within the specimens, the intensity of fluorescence did not vary. Finally, the staining pattern of the actin cytoskeleton as visualized by the fluorescence emission of Alexa-conjugated phalloidin was identical on both the smooth and rough surfaces. The study by Yamashita *et al.* [18] yielded valuable information on the effects of surface roughness on the behavior of cells, especially on zirconia (Figure 2 clearly shows actin filaments as seen by phalloidin staining on human umbilical cord mesenchymal stem cells; in Figure 3, DAPI-stained nuclei are visible in human umbilical cord mesenchymal stem cells: shared from the author's research). Quan *et al.* [19] prepared a zirconium oxide/hydroxyapatite composite and evaluated the viability and inflammatory propensity of immune cells on this biomaterial [19]. Ultrafine particles of zirconium (10-20nm diameter) and of hydroxyapatite (10-40nm diameter) were prepared and used to make simplex hydroxyapatite-zirconia composites and graded hydroxyapatite-zirconia composites. Simple composites were obtained by direct deposition of hydroxyapatite on zirconium oxide surface while varying amounts of hydroxyapatite and zirconium oxide were mixed and deposited on zirconium oxide to yield graded composites.

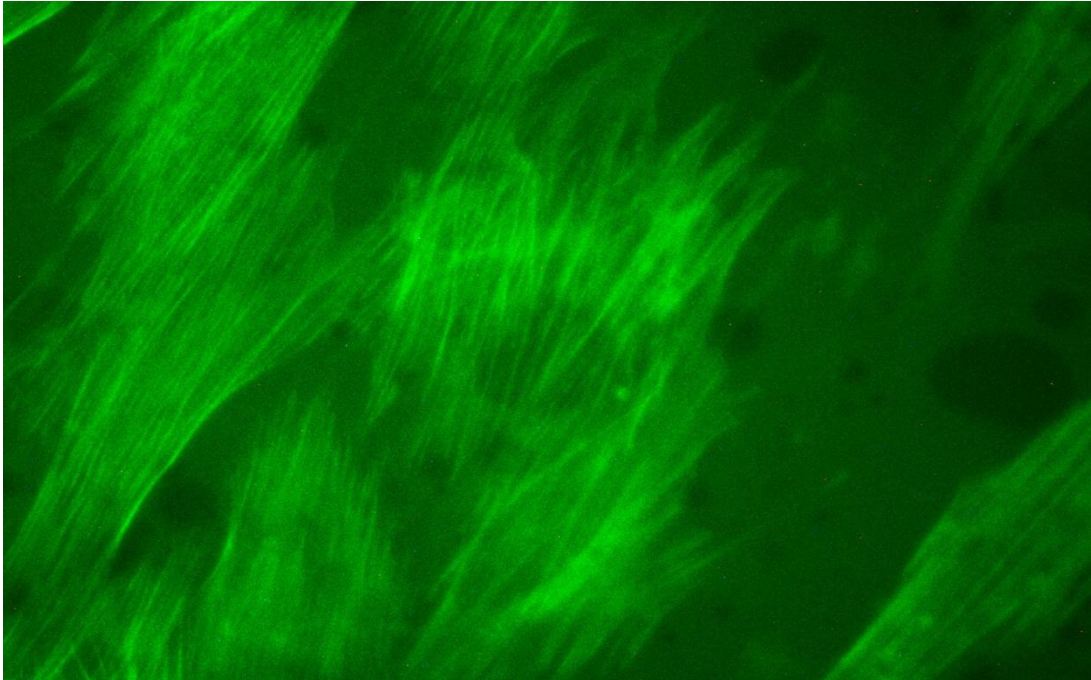


Figure 2: Actin filaments as visualized in human cells derived from Wharton's jelly by using fluorescently-labeled phalloidin (Courtesy: Author).

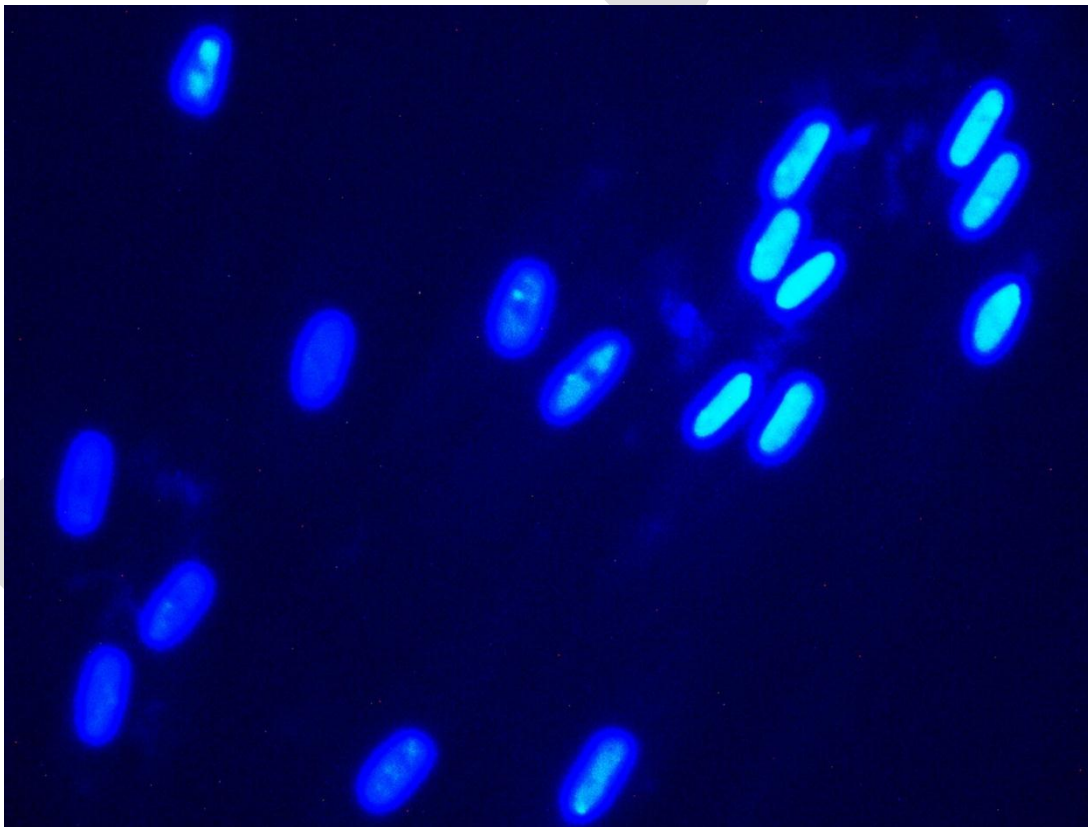


Figure 3: DAPI fluorescence staining can be used to visualize nuclei in cells. In this picture, cells from the Wharton's jelly were used and some photo-bleaching is visible (Courtesy: Author).

Phytohemagglutinin (PHA), a legume-derived lectin, that spurs T-cell division, and the bacterial endotoxin lipopolysaccharide (LPS), were used in these experiments [20-24]. Peripheral blood mononuclear cells, both PHA stimulated and unstimulated, were exposed to extracts of the biomaterials and proliferative capacity, cell death and apoptosis, release of pro-inflammatory cytokines, and expression of CD69 - a T cell activation marker - were examined. The MTT assay showed that the proliferation of PBMCs, cultured with the extracts from the composites, was not different from controls. Hardly any cell death was observed in lymphocytes incubated with extracts of the composites regardless of PHA stimulation. However, there was a significant increase in the number of apoptotic cells in PHA-stimulated lymphocytes after 24 hours of stimulation. Cement extracts did not inhibit the stimulatory effects of PHA on T lymphocytes since enhanced CD69 expression was observed by flow cytometry. Moreover, there was no detectable induction of the pro-inflammatory cytokines TNF-alpha and IL-6 unless the bacterial endotoxin LPS or the mitogen PHA was added to the PBMC culture.

The above study by Quan *et al.* was a well-designed study that plugged an existing gap in the literature about the effects of biomaterials on immune cells. Barring the up-regulation in apoptosis in the presence of PHA stimulation, most results comported well with previous findings. This study was noteworthy because it also included *in vivo* toxicity results from animal tests as well. Mice were injected with 50ml/kg of the zirconia/hydroxyapatite or normal saline and weight gain, death, and macroscopic parameters such as anorexia, diarrhea, lethargy, and chronic inflammation were examined at 21 days. The treatment group performed as well as the controls. Other studies should be conducted to rule out any deleterious effects that biomaterials can have on the immune system.

3. Nano-sized Peptides

The self-assembling nature and nano-dimensional diameters of FMOC (fluorenylmethoxy carbonyl)-dipeptides were utilized by Jayawarna *et al.* [25] to observe peptide assembly and cell behavior on scaffolds made from a combination of the amino acids glycine, alanine, leucine, and phenylalanine [25]. Fluorenylmethoxy carbonyl is a chemical group which is added to peptides during protein synthesis to prevent the amino group of the peptide from participating in promiscuous reactions [25]. Interactions between aromatic fluorenyl electron and hydrogen bonding mediate the assembly of FMOC peptides [26, 27].

While glycine-glycine dipeptide did not form a gel at the broad pH range tried, a 50:50 mixture of glycine-glycine and phenylalanine-phenylalanine dipeptide assembled into a gel at a pH of less than 7. Cryo-SEM examination of the morphology of these peptides revealed the diameter of the fibers to be 18-46 nm - comfortably in the range of the diameter of the extracellular matrix. Bovine chondrocytes were seeded on and encapsulated in 50:50 mixtures of FMOC-glycine-glycine and FMOC-phenylalanine-phenylalanine, and a 50:50 mixture of FMOC-lysine and FMOC-phenylalanine-phenylalanine scaffolds. Cell proliferation, cell morphology, and nuclear shape were evaluated. MTS assay confirmed the ability of the scaffolds to support cell growth; DAPI staining showed cells distributed throughout the matrix; and ESEM (environmental scanning electron microscope) micrographs had round chondrocytes clearly visible (Figure 4 shows results of MTS assay as performed by the author).

Jayawarna *et al.* [25] used a method that could be instrumental in cell transplantation and drug delivery. Injectable scaffolds made from dipeptides that could gel with changes in pH could simplify cell delivery. The approaches used to achieve gelation, including pH changes and mixing of various peptides could be replicated and adapted by the research community. While Jayawarna *et al.* did observe cell growth on peptide hydrogels compared to tissue culture polystyrene, it appeared that cell numbers were significantly lower on all three peptide scaffolds compared to tissue culture polystyrene. Lack of statistical analysis would weaken the results of the MTS assay. The other concern with FMOC-peptides would be cell toxicity – long-term toxicity from FMOC or the degraded products from FMOC. While FMOC-peptide derivatives have been shown to have anti-cancer activity, and FMOC-dipeptides have been shown to have anti-inflammatory actions, the toxicity of FMOC-dipeptides needs to be evaluated before widespread use [27, 28].

4. Carbon, Silicon, and Diamond

4.1. Carbon nanotubes

Carbon nanotubes came to the fore in the early 1990s when Iijima and Ichihashi announced their discovery [35].

Since then, many groups have tried to assess their suitability as biomaterials due largely to their electrical and mechanical properties. However, due to contradictory reports emanating from different laboratories, a consensus is yet to emerge on their utility as biomaterials [36].

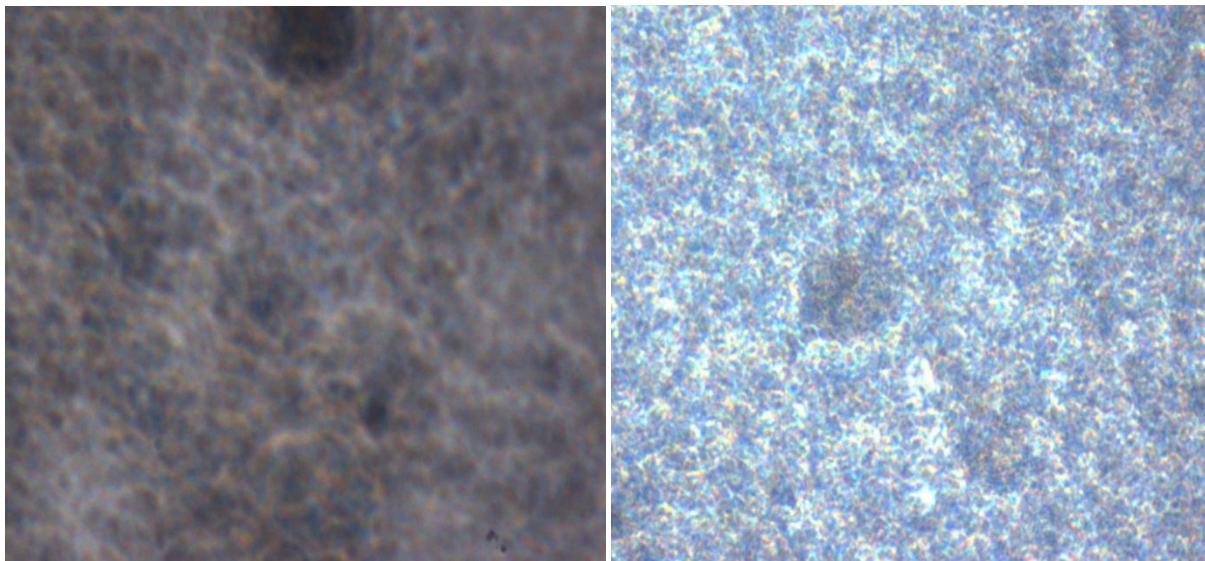


Figure 4: A three-dimensional MTS assay can be performed by using the MTS reagent to test for cell viability when cells are grown in or on the biomaterial. The control on left side lacks the deep-blue purple color observed in the image on right side (Courtesy: Author).

Human osteoblast-like cells (Saos-2) were cultured on uncoated porous polycarbonate membranes (PCMs; pore size of 0.8 μm and diameter of 47mm) and on PCMs coated with carbon nanotubes (CNTs; 5-20nm diameter and 20-40 μm length) in order to evaluate cell morphology [36]. As revealed by CLSM, cells grown on PCMs showed unilateral elongation whereas cells on CNTs extended in all directions, displaying ideal proliferation [36]. Aoki *et al.* made another striking example concerning the morphology of the peripheral parts of the cell: these osteoblast-like cells had achieved conspicuously flattened shapes; they had sent out many 10-20 μm long filopodia which were making contacts with the reticular network of CNTs at much smaller angles as compared to cells grown on PCMs.

While the above mentioned study addressed some important parameters regarding the biological application of carbon nanotubes, it fell short of adequately answering some important questions such as cell viability and toxicity, etc. The use of SEM to probe cell morphology, especially of the peripheral regions of cells such as filopodia, would raise a question as to the integrity of the cytoskeleton. For proper osseointegration, an ideal implant ought to ward off the overtures of fibroblasts as fibroblast attachment to biomaterials impairs osteoblast integration and leads to implantation failure [11]. Therefore, Aoki *et al.* [36] should have also examined whether carbon nanotubes would discourage fibroblastic adhesion.

4.2. Nano-textured silicon grooves

Tsai *et al.* studied cell morphology and cytoskeletal arrangement in osteoblast-like MG-63 cells on nano-textured silicon substrates with a surface pattern of grooves and ridges separated by smooth areas. The ridges and grooves had widths of 90, 250, and 500 nm, and the grooves had a depth of 215 nm [37]. The dimensions of the silicon grooves were chosen to parallel that of native collagen fibrils. Silicon chips were immersed in fibronectin - a major cell adhesion protein - to spur initial cell spreading. After verification of fibronectin absorption on the silicon surfaces, experiments were conducted to examine the effects of surface topography and surface chemical cue on cellular morphology of the cells, cellular alignment, changes in nuclear orientation, and on the development of the cytoskeleton. Cells cultured on nano-grooved surfaces had more elongated nuclei than cells cultured on flat surfaces. Cell spreading at 2 hours was higher on nano-grooved surfaces than on flat controls and fibronectin

coating seemed to promote cell spreading on both nano-grooved and flat surfaces. Fibronectin coating, regardless of the time of incubation, did not have any noticeable effect on cell elongation, which was defined as the ratio of cell length to cell width. At 8 hours of incubation, cells had spread on all types of surfaces used. However, cells cultured on flat surfaces were randomly oriented whereas cells on nano-grooves were more elongated and had aligned themselves with the direction of the ridges. Although average cellular orientation did not change on flat surfaces with fibronectin coating, at 24 hours, nearly 70% cells had aligned themselves along the ridges of the 500nm and 250nm nano-grooved materials in comparison to 50% of the cells on the 90nm grooved materials. At 24 hours, normal actin filament formation and focal contacts were seen in cells growing on both the flat, smooth surfaces and the patterned surfaces with one exception: cells grown on the 90nm grooved surfaces thinner had thinner actin filaments but proper alignment.

Silicon is a relatively new entrant as a biomaterial in tissue engineering. Thus, it is important that silicon's biocompatibility and suitability be evaluated in good detail. To this end, the experiments above by Tsai *et al.* yielded some exciting finds. It is well known that cells grow better on rough surfaces and, in this regard, the salient feature of the study by Tsai *et al.* was the fact that they also observed some additive effects with fibronectin absorption on the nanomaterials. It has been found that upright silicon oxide nanorods retard the adhesion of fibroblasts and endothelial cells [38]. However, since Tsai *et al.* used a special method to remove oxide contamination from the silicon nanotubes, there should not be any apprehensions about problems with cell adhesion. The results from the experiments above reaffirm earlier studies showing that surface chemistry drives initial cellular attachment whereas surface features help determine cell orientation and morphology.

4.3. Nanocrystalline diamonds

Nanocrystalline diamond presents with a distinctive set of attributes such as biocompatibility, mechanical stability, resistance to corrosion and bacterial colonization, high wear resistance, and an ability to indulge in covalent surface functionalization with proteins (BMP-2 and antibodies) and DNA [2, 39-43]. Kalbacova *et al.* [4] have characterized the performance of osteoblasts on nanocrystalline diamond (NCD) films - coated on silicon substrates with differing roughness - of nano-rough (RMS 20nm) and nano/micro rough (RMS 270 and 500nm) surface roughness [4]. In these experiments, tissue culture polystyrene was used as control. Using the techniques of SEM and immunofluorescence to scrutinize cell morphology, Kalbacova *et al.* [4] demonstrated a good degree of cell spreading on NCD films with RMS values of 20nm, 270nm, and 500nm, with greater filopodial extensions on RMS of 500nm. However, cell adhesion results showed that the highest number of cells adhered to diamond films with RMS of 20nm as compared to films with RMS of 270nm and 500nm, which attracted more cells than polystyrene. Interestingly, cells which adhered to the nano-rough surface displayed a cylindrical shape in the cell center and contained an actin ring on the edge of the cell. Using confocal microscopy for immunofluorescence staining of the cytoskeleton, Kalbacova *et al.* [4] found intense staining for vinculin and phosphorylated-FAK, under the cell center, in cells seeded on nano-rough (RMS 20nm) diamond surfaces: this staining pattern was typical of well-adhered and stabilized cells on a surface. Contrastingly, cells plated on rougher diamond surfaces were more spread out and had vinculin at the edge. The metabolic activity of osteoblasts, as measured by active cellular dehydrogenases, cultured on nano-rough diamond was comparable to that of cells plated on tissue culture polystyrene, and alkaline phosphatase activity – a measure of early marker of differentiation in osteoblasts - was significantly higher in cells cultivated for 11 days on nanocrystalline diamond of 20nm RMS as compared to that in control cells. The afore-mentioned study was well-designed and well-executed. Most results rarely deviated from those obtained from earlier experiments. Still, some results invite further investigation. For instance, results from the cell adhesion experiments showed faster and stronger cell adhesion to the roughest surfaces, to paraphrase the authors; however, that might be problematic if cells demonstrated such propinquity for the scaffolds [8]. The study utilized a novel concept in terms of geometric variation of the biomaterial surface and added a biological flavor to the work by immuno-staining and by quantifying bone mineralization on NCDs. Furthermore, surface morphology was seen to be a function of nano-dimensional scale; the most acceptable results were obtained with surface roughness around 20 nm. The roughest surface also had the most active FAK. Metabolic activity of the cells on NCD surface was nearly identical to that on polystyrene surfaces. ALP formation - none of the early markers of osteoblastic differentiation - was also higher on the nano-surfaces with the roughest dimensions, i.e., 20nm. The above study will help facilitate the use of nanoscale diamond into tissue engineering and regenerative medicine.

In a comparison study of osteoblast proliferation on carbon nanotubes, single-walled and multi-walled, and graphite - both isomorphs of carbon - cell proliferation of Saos-2 cells was greatest on single-walled carbon nanotubes (SWCNTs) at 7 days [14, 16, 44]. Greater cell adhesion of Saos-2 cells was achieved on carbon nanotubes compared to polycarbonate while a preponderantly higher number of filopodia extended from these osteoblast-like cells on carbon nanotubes as compared to polycarbonate, both observations aided by SEM. Protein adsorption was the highest on single-walled carbon nanotube, followed by multi-walled carbon nanotubes. A similar trend was observed in ALP (alkaline phosphatase) activity. The performance of graphite lagged behind polycarbonate in all the above experiments.

The above study utilized an ingenious approach - removing catalysts and amorphous carbon - to impart some hydrophilicity to the carbon nanotubes, which must have facilitated cell adhesion. Watari *et al.* [44] used a slightly questionable method to arrive at cell proliferation numbers. Counting cell numbers from SEM micrographs can be fraught with the dangers of inaccuracy. However, due to the nature of the biomaterials used, the choice of proliferation tests was limited since trypsinization or other procedures to detach cells would not have been applicable here.

Since the use of carbon nanotubes in tissue engineering is still in its infancy, the study by Watari *et al.* will definitely add to our scant knowledge about cell behavior on carbon surfaces. However, a more detailed toxicological characterization of carbon nanotubes must be undertaken before widespread use in the field. The existing data show that inhalation of ultrafine carbon particles produces pulmonary toxicity in mice [45]. Similarly, exposure to graphite has been a well-known etiological factor in pneumoconiosis or worker's lung disease, in which shortness of breath and coughing are the primary symptoms [45]. Moreover, multi-walled carbon nanotubes have been found to enter human keratinocytes and to trigger the release of IL-8 from these keratinocytes [10, 46]. Furthermore, it has also been known that intra-tracheal administration of single-walled carbon nanotubes leads to granuloma formation - an aggregation of macrophage-like giant cells encircling a bolus of carbon nanoparticles [45, 47]. Therefore, more studies are needed to assess the potential of carbon nanoparticles in tissue engineering.

5. Conclusion

In the preceding pages, a framework has been sketched to view recent developments made in the use of nanoparticles in tissue engineering. We have also outlined recent progress in the use of nanoparticles in tissue engineering. The emphasis has been on the variety of materials in nanometer dimensions that has found use in tissue engineering: these include ceramics, biological and synthetic polymers, inorganic materials such as hydroxyapatite that mimic bone, minerals such as carbon and diamond, and composites of nanomaterials. Great strides have been made in the synthesis and deposition of these nanoparticles in order to obtain biocompatible scaffolds that degrade at a controlled rate. Likewise, the safety profile of many nanoparticles used in tissue engineering has been examined, but with one notable exception: the effects nanoparticles have on the immune system. Apart from immune-compatibility studies, there is a cornucopia of information on properties of nanoparticles in regards to cell adhesion, biodegradability, cell spreading, formation of focal adhesions, etc. Most of these results have largely been obtained through *in vitro* studies and have been encouraging. However, a cautionary note must be appended: one must carefully evaluate the enormous amount of data obtained from *in vitro* studies. Thus, there also is a need for more *in vivo* studies before the use of nanoparticles becomes more widespread. Furthermore, there is a dire need to complete some comprehensive and major studies - both *in vitro* and *in vivo* - on the effect nanoparticles can have on both the innate and acquired components of the immune system: especially, effects such as hemolysis, thrombogenicity, and complement activation have to be examined. From a different perspective, optimal design and synthesis of nanoparticles will critically influence the field of tissue engineering.

Fears aside, the use of nanoparticles in tissue engineering is definitely going to expand what with the ongoing research efforts investigating nanoparticle toxicity, nanoparticle distribution and handling by the cells of the body, and with the ever-increasing use of imaging techniques such as atomic force microscopy (AFM), infrared and Raman microscopy, and surface plasmon resonance imaging (SPRI). Tissue engineering is a truly multidisciplinary field located at the crossroads of disciplines as diverse as biology, physics, chemistry, surgery, immunology, and physiology. Integration of knowledge gained in many of the above-mentioned fields will lead to more optimized synthesis/design of nanoparticles, a more lucid picture of nanoparticle toxicity a broader understanding of the effects of nanoparticles on the immune system that would lead to enhanced safety profiles

of nanoparticles, lest any safety concerns arise, and broadened use of nanoparticles in tissue engineering.

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