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# Nanocrystalline Diamond Films: Applications and Advances in Nanomedicine

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## 1. Introduction

Biomaterials play essential roles in modern strategies in regenerative medicine and tissue engineering by designable biophysical and biochemical cues that direct cellular behavior and function [1-4]. The guidance provided by biomaterials may improve restoration and function of damaged or nonfunctional tissues both in cell-based therapies, such as those where carriers deliver transplanted cells or matrices induce morphogenesis in bioengineered tissues constructed *ex vivo*, and in cellular therapies, such as those where materials induce growth and differentiation of cells from healthy residual tissues *in situ* [3, 5-7].

Stem cells are defined by their ability to self-renew and produce specialized progeny [8, 9]. Consequently, they are the most versatile and promising cell source for the regeneration of aged, injured and diseased tissues. According to their developmental status, stem cells can be classified into two categories: embryonic stem cells and adult stem cells. However, despite the remarkable potential clinical applications of each of these stem-cell populations, their use is currently limited. Thus, a major goal is to develop new culture based approaches, using advanced biomaterials, that more closely mimic what the body already does so well, to promote differentiation of pluripotent cells [3].

Nanomedicine, the application of nanotechnology for medical purpose, is emerging as a new interdisciplinary research field, cutting across biology, chemistry, engineering and medicine. It is expected to lead major advances in disease detection, diagnosis, treatment and further to replacement of damaged tissues and organs. Over the past two decades, there have been significant advances in disease diagnostics, drug delivery, stem cell therapy and tissue engineering. In parallel, nanotechnology has shown great potential for the creation of the next generation of new biomaterials.

Biomaterials that promote regeneration are important in both research and clinical applications [10]. However, current implants have a limited life-expectancy, and younger patients who receive them generally expect to endure revision surgeries to replace worn components. A primary problem with current designs is the generation of wear debris

particles at the articulating surface that causes local pain and inflammation. Large debris are normally sequestered by fibrous tissue, while small debris are taken up by macrophages and multinucleated giant cells which may release cytokines that result in inflammation. Thus, the proposed solution for the problem caused by wear debris is to develop durable materials for the articulating surfaces that are more wear resistant, which would reduce the generation of debris particles.

Recently, it was shown that diamond particles (NDs), the diamond structure at a nanometer scale (4-5 nm in size), appear to possess high bioactivity at the molecular level, presenting antioxidant and anticarcinogenic properties. Functionalization of NDs with biological molecules, such as peptides, proteins and nucleic acid, has led to practical significance for biomedical applications, covering their use for single particle imaging in cells, drug delivery and protein separation. For instance, carboxylated nanodiamond has been shown as a useful probe for detecting and labeling the interaction of nanoparticles and bio-objects such as cells and bacteria [11], because NDs can be easily functionalized to conjugate with bio-molecules and can emit bright fluorescence without photobleaching [12-15]. Moreover, the ND particles were phagocytosed into cells by macropinocytosis and clathrin-mediated endocytosis pathways during tracking of cells. However, cell growth ability such as cell division and differentiation were not altered after long-term cell culture for 10 days. Together, NDs are non-cytotoxic and with bright fluorescence, thus has served as a versatile tool in biosensing and bioimaging applications [12, 16].

Diamond has been one of the most desired and investigated materials in the past years. From an extensive list of superlative properties, the ultra-hardness, the chemical inertness, the high thermal conductivity, and the high optical transparency are just a few examples of its remarkable nature. Applications such as cutting tools, abrasives, structural components, heat sinks, bearings, and optical windows (X-ray, IR, and laser windows) are examples that diamond has a wide-ranging impact in many fields.

## 2. Nanodiamond films

In the late 1980s, polycrystalline diamond films with fine grains were grown for optical coatings [17, 18], wear resistant coatings [19], high-pressure synchrotron X-ray windows [20] and X-ray lithography masks [21]. The first reference to these materials as 'nanocrystalline' was at the Workshop on the Science and Technology of Diamond Films in 1990 [17]. Most of these materials would now be classified as forms of nanocrystalline diamond (NCD) and further characterized the presence of large intrinsic stress and non-diamond phases in these material [22-25]. These NCD materials were all grown in hydrogen-rich chemical vapour deposition (CVD) environments, with typically less than 2% methane (or hydrocarbon) in hydrogen as reactants, exhibiting clusters (cauliflower morphologies), limited surface smoothness, high compressive stress, delamination, and high content of non-diamond phase. NCD was deposited on Si or other substrates which had been 'treated' or 'seeded' to increase the diamond nucleation density [26, 27]. This wet seeding process was in solution containing diamond powder for ultrasonication to create necessary nucleation sites and the process was varied among labs and individuals [28, 29]. By controlling nucleation density and growth conditions, grain sizes were usually 5-150 nm for films less than several  $\mu\text{m}$  thick. In 1994, Gruen and coworkers [30-32] developed the growth of nanocrystalline diamond films by CVD under hydrogen-poor and carbon-containing argon gas plasmas conditions. In 1999 [33], this new material was reviewed under the label of 'Nanocrystalline

Diamond Films'. In 2001 [34], the label ultra-nanocrystalline diamond films (UNCD) came to be applied to these materials in order to distinguish them from the more traditional NCD films discussed above [35]. The nanocrystallinity is the result of new growth and nucleation mechanisms, which involve the insertion of C<sub>2</sub>, carbon dimer, into carbon-carbon and carbon-hydrogen bonds, resulting in heterogeneous nucleation rates on the order 10<sup>10</sup> cm<sup>2</sup> s<sup>-1</sup> [26, 29]. Detailed investigations using synchrotron-based, near-edge X-ray absorption fine-structure spectroscopy (NEXAFS) showed that UNCD films grown using this seeding approach and growth chemistry are of very high quality, with greater than 99% sp<sup>3</sup> bonding [33]. The UNCD films, a form of NCD, has led to applications in micro-electromechanical systems (MEMS) and nano-electromechanical systems (NEMS) [36-38], corrosion resistance [39], biocompatible coatings [40-42], and biosensors [16, 43, 44].

Diamond coatings with nanosized crystallites, NCD, present a great potential in biomedicine and biotechnology. NCD combines surface smoothness with high corrosion resistance and biotolerance, which are ideal features for applications in medicine onto surgical tools and medical implants. For example, joint implants coated with NCD can take benefit of its protective character. The NCD coating acts as a selective protective barrier between the implant and the human environment, preventing the release of metallic ions into the body. NCD presents the highest resistance to bacterial colonization when compared to medical steel and titanium [45]. This property is very important since infection due to microbial colonization of the implant surface may lead to implant rejection. In addition, the high wear resistance and the low coefficient of friction of NCD allow the reduction of the amount of wear debris generated during the joint functioning, thus increasing the life of the prosthesis [46]. Further, the residues formed due to wear in this case are diamond particles, which are completely harmless, initiating little or no adverse reactions from human monocytes and polymorphonuclear leukocytes [47-49]. NCD is also included in this recent group of materials and can be used as a template for the immobilization of active molecules for biological applications or for biosensor applications [44, 50-52]. One example is the functionalization of NCD surface with bone morphogenetic protein-2 (BMP-2) creating a biomimetic coating that results in improved osseointegration, which is a powerful strategy in tissue engineering as well as in bone tissue regeneration [53]. The NCD surface can also be modified with the linking of antibody, human IgG, which provide biomolecular recognition capability and specificity characteristics, proving a biologically sensitive field-effect transistor (Bio-FET) [44].

This paper offers a review of present knowledge of the synthesis and characterization, cell behavior, focused on *in vitro* adhesion, proliferation and differentiation on nanodiamond films. The aim is to highlight nanodiamond films as new generation biomaterials for improving the future development on clinical transplantation and tissue engineering.

### 3. Surface modifications

Cellular adhesion is of fundamental importance in many biological processes as the adhered cells will sense, interpret, integrate, and then respond to the extracellular signals. Chemical and physical signals from the substrate such as surface energy, topography, electrostatic charge, and wettability play a vital role in stimulating cell adhesion and influencing cell growth behavior. The cellular adhesion properties of as-grown diamond surfaces or functionalized diamond surfaces have been studied recently. The as-grown diamond films were characterized as hydrophobic surfaces with abundance of C-C and C-H bonds [54].

The functionalized surface properties of diamond can be made hydrophobic or hydrophilic with hydrogen or oxygen termination, respectively, which have implications for cellular adhesion. The methods of surface modifications are summarized as following:

1. Hydrogen termination (hydrophobic surface):
2. Diamond films were treated in pure hydrogen plasma treatment at 300-800 W in the microwave plasma CVD system at 5 mTorr for 2-15 min. All freshly prepared hydrogen-terminated diamond samples were used immediately for cell culture.[55-58]
3. Oxygen termination (hydrophilic surface):
  - a. Diamond samples were exposed to UV irradiation (18 W, 254 nm) for 18 h in air. After UV functionalization, the samples were rinsed with ultrapure water, tetrahydrofuran, and finally with hexane.[55]
  - b. Diamond films were exposed to pure oxygen plasma CVD system at 800 W at 5 mTorr for 10-15 min.[56, 58]
  - c. Diamond films were oxidized in concentrated  $\text{HNO}_3$  at 60-70° C for 24 hours. This oxidation reaction transformed the face of the film from hydrophobic to hydrophilic surface by adding carboxylate groups to the films. [59, 60]
4. Bio-molecular conjugation [61-64]

#### 4. Nanodiamond-cell interaction: biological performance and response

Cell adhesion is involved in various natural phenomena such as embryogenesis, maintenance of tissue structure, wound healing, immune response, and tissue integration of biomaterial. The biocompatibility of biomaterials is very closely related to cell behavior on contact with them and particularly to cell adhesion to their surface. Surface characteristics of materials, such as their topography, chemistry, or surface energy, play an essential part in cell adhesion on biomaterials. Thus attachment, adhesion and spreading belong to the first phase of cell/material interactions and the quality of this phase will influence the cell's capacity to proliferate or to differentiate itself on contact with the implant. Material/cell interaction depends on the surface aspects of materials which may be described according to their wettability, topography, chemistry and surface energy. These surface characteristics determine how and what kinds of biological molecules will adhere to the surface and more particularly determine the orientation of adhered molecules, and also finally determine the cell behavior while in contact [3, 8, 65]. As previously shown, cells in contact with a surface will firstly attach, adhere and then spread. This first phase depends on specific adhesion proteins such as integrin and cadherin as demonstrated by Chen et al [66]. Thereafter, the quality of this adhesion will influence their morphology, and their capacity for proliferation and differentiation. Early *in vitro* biocompatibility and cytocompatibility studies focused on the morphology and growth capacity of cells on nanodiamond films with various chemical compositions and topographies [15, 53, 56, 58, 67, 68]. Recently, it was found that nanodiamond films further determine the differentiating stage in stem cells, which expands other possibilities for nanodiamond films into organ repair and tissue engineering.

##### 4.1 Biocompatibility tests: morphological aspect and growth capacity of cells on nanodiamond films

NCD films possess numerous valuable physical, chemical and mechanical properties, making NCD an excellent material for implantable biomedical devices. There is still one



very important property required for biomaterials, i.e., biocompatibility. The biocompatibility of a material is determined by *in vitro* and *in vivo* tests, involving the interaction of the material with cells.

*In vitro* studies of biocompatibility of UNCD coatings, produced by MPECVD using Ar/CH<sub>4</sub> as reactive gas, were carried out by Shi et al. [69]. They grew mouse embryonic fibroblasts (MEFs) on UNCD films up to 4 days and found that UNCD film coated substrates can dramatically promote the growth of MEFs, while the quartz substrates inhibit cell attachment. On growing human cervical carcinoma cell line (HeLa), neuronal cell line (PC12) and osteoblastic cells (MC3T3) on UNCD films, no toxicological effects on the cells in culture were observed. It was noted that maximum cell attachment, cell spreading and nuclear coverage were observed on UNCD films compared to two commonly used materials in MEMS platinum and silicon substrates [70]. Amaral et al performed bone marrow cell culture tests on NCD films, prepared by using a hot-filament chemical vapor deposition (HFCVD) technique in Ar-CH<sub>4</sub>-H<sub>2</sub> gas mixtures, to observe its effects on cellular reaction, osteoblast, and osteoblast activity [71]. The nanometric feature of NCD resulted in increased bone cell proliferation and minimized activity of osteoclast-like cells. Following previous study, Amaral and coworkers cultured primary human gingival fibroblast cell cultures on NCD films for 21 days and no damage to the cells was observed. On performing the cytotoxicity tests using a standard cell line, it was found out that NCD films promotes cell attachment and normal cell growth rates [72]. Several other studies were made on the morphological behavior of mesenchymal stem cells on NCD coating prepared by MPECVD method in hydrogen-rich gas mixtures, which revealed good surface biocompatibility of the coatings [58]. Their investigations indicated that NCD coatings were biocompatible to not only cell lines, but also primary stem cells.

All these *in vitro* studies showed that NCD films tended to promote the growth and adhesion of cells without any toxicological effect. There are other applications where it is desirable that there should not be any cell attachment to a surface, for example, in case of catheters and temporary implants. After getting a primary indication of the biocompatibility of NCD films through *in vitro* tests, several *in vivo* studies were initiated by implants with NCD coating in laboratory animals. An attempt was made to study the osseous healing at the implant sites by inserting implants into 4-year-old female sheep calvaria for 3 days, 1 week and 4 weeks intervals. It was observed that implant surfaces coating with NCD films and then conjugating with BMP-2 enhanced osseointegration *in vivo*. After implanting NCD coated implants in transplantation sites of sheep for different time periods, it has been observed that the NCD-coated implants did not show any significant toxicological effect and are well tolerated in the sheep body. Results further suggest that this technical advancement can be readily applied in clinical therapies with regard to bone healing, since primary human mesenchymal stromal cells strongly activated the expression of osteogenic markers when being cultivated on NCD absorbed with physiological amounts of BMP-2 [73].

The above *in vitro* and *in vivo* studies indicated the biocompatibility of NCD films prepared by a variety of techniques. The general finding so far is that control of cell adhesion and proliferation on NCD can be achieved by altering NCD surface chemistry and surface topography and wettability, probably due to the correlation between these surface properties and the adsorption of endogenous proteins that regulate cell behavior. Adsorbed proteins can be detected on biomaterials within seconds of exposure to the blood, and a monolayer of adsorbed proteins forms in seconds to minutes. Fibronectin, vitronectin and laminin are pro-adhesive proteins, with relatively high concentration in blood, that are

recognized by various cellular integrin receptors [74]. It has been observed that fibronectin governs the adhesion and spreading of cells on a material surface [75]. These plasma proteins play an important role in the initial recruitment of cells to the biomaterial surface. The glycoprotein fibronectin consists of multiple specific binding sites and is capable of interacting with a wide variety of other biomaterials, through the formation of fibrillar extracellular matrix or fibrils. So, the specific surface of a biomaterial plays a key role in adsorption of fibronectin or other pro-adhesive proteins and hence better proliferation of cells. The interaction of neural stem cells with UNCD films and the consequent cellular signaling processes are schematized in Figure 1. Some studies revealed that the adhesion and spreading of cells on NCD surfaces is related to the bonding structure present on the surface and the ratio of  $sp^2/sp^3$  [76]. It has also been observed that the microstructure of the NCD films and the kind of treatments seemed to influence the biological effects of cells. However, the correlation between these surface properties (chemistry, topography and wettability) and cell responses is complicated and not clearly understood.

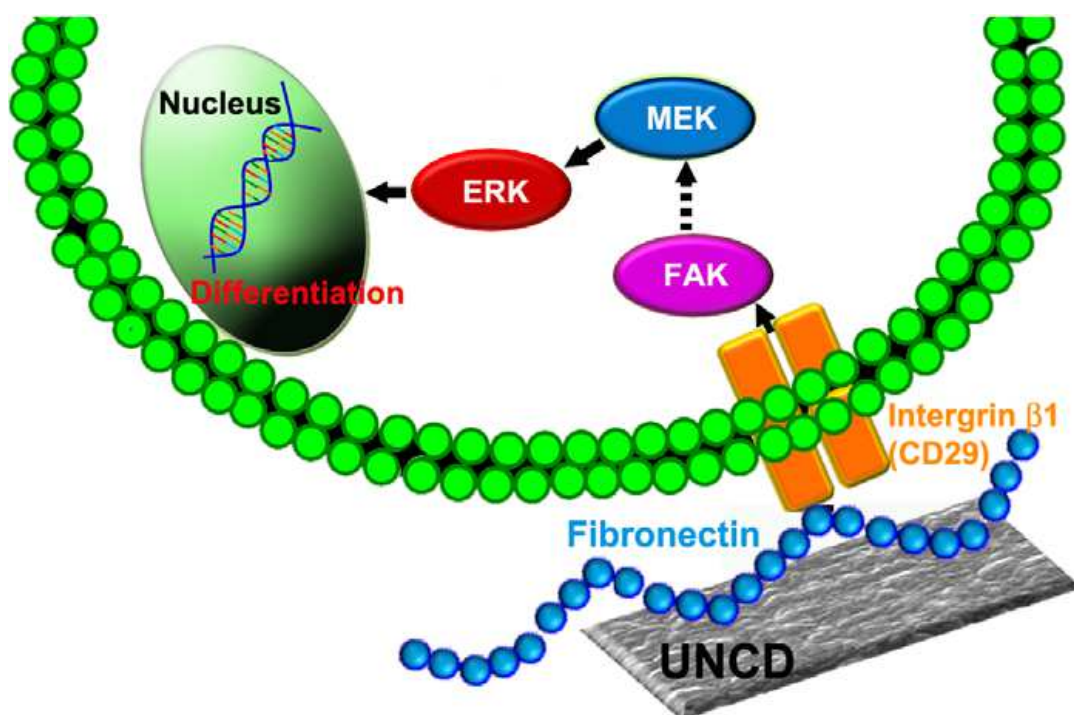


Fig. 1. Schematic drawing summarizes the role of H-UNCD films in mediating differentiation from neural stem cells. Absorbed fibronectin on H-UNCD surface activates integrin  $\beta 1$  (CD29), focal adhesion kinase (FAK) and (extracellular signaling kinase) ERK1/2 pathways and, in turn, leads to an ultimate and specification of neuronal differentiation from NSC.

#### 4.2 Topography effects of nanodiamond films on cells

The comparison of the behavior of different cell types on nanodiamond films shows that they react differently according to surface smoothness [55, 57, 60, 68, 77, 78]. Scanning electron microscopy (SEM) and immunofluorescence staining examinations of osteoblast on nanodiamond films with various surface roughness (nanometer and micrometer) generally demonstrated that enhanced osteoblast functions (including adhesion, proliferation,

intracellular protein synthesis, alkaline phosphatase activity and extracellular calcium deposition) on nanocrystalline diamond (RMS~20 nm) compared to submicron diamond grain size films and control for all time periods tested up to 21 days [57, 60]. In addition, an SEM study of osteoblast attachment on NCD films explains the topographical impact diamond had on osteoblast functions by showing complex and longer filopodia extensions. To investigate the adhesion of normal human dermal fibroblast cells grown on NCD films with various surface smoothnesses, atomic force microscopy were performed. The examination demonstrated that cell viability and adhesion force was better on smooth surfaces (UNCD films) compared to micron diamond grain size films, no matter the terminations of diamond films [55]. Although mesenchymal stem cells and non-differentiated cells adhere similarly on all NCD surfaces with different roughness (20, 270, and 500 nm) and control polystyrene, their metabolic activity on NCD surfaces is increased. On the other hand, osteoblasts adhere on NCD significantly more than on polystyrene, and their metabolic activity is decreased on nano/microrough NCD surfaces in contrast to mesenchymal stem cells. These differences could be attributed to the distinct properties of the two cell types in the human body. Alternatively, the different response of osteoblasts could be attributed to the specific surface topography as well as to the biocompatible properties of diamond. [79]. Hence the controlled topographically structured NCD coatings on various substrates is promising for preparation of better implants, which offer faster colonization by specific cells as well as longer-term stability.

#### **4.3 Surface chemistry effects of nanodiamond films on cells**

The bio-compatibility and resistance to chemical corrosion of diamond may increase lifetime of stents, joints, and other implants in the human body. It is also possible to make a chemical functionalization of diamond surface and create bio-passive or bio-active patterns. Kalbacova et al [80] showed that viability and adhesion of human osteoblasts (SAOS-2) cultured on NCD films are predominantly determined by NCD surface termination. Increasing surface nano-roughness plays a secondary yet positive role. Hydrophilic surface of NCD films (O-terminated surface) provides good conditions for osteoblast adhesion and spreading and consequently on their viability (metabolic activity and proliferation). It was shown that hydrophobic H-terminated diamond surfaces are less favorable for osteoblast-like cell adhesion and growth than hydrophilic O-terminated surfaces [80, 81]. This is in agreement with observations on other materials and cells, such as Ti6Al4V titanium alloy [82, 83] and human dermal fibroblast [55]. In addition to cells lines, different kinds of stem cells have also been studied and the results show difference on cell lines and stem cells. Chen et al [56] cultured neural stem cells on different functionalized diamond films in low serum and without any differentiation factors to investigate the biological effects on NSCs. We found that H-terminated UNCD films spontaneously induced cell proliferation and neuronal differentiation and O-terminated UNCD films were also shown to further improve neural differentiation, with a preference to differentiate into oligodendrocytes. Clem [58] reported that H-terminated ultra-smooth nanostructured diamond surfaces supported robust adhesion and survival of mesenchymal stem cells, while oxygen (O)- and fluorine (F)-terminated surfaces resisted cell adhesion. Thalhammer [84] used four different materials (glass, PCD, NCD and Si) coated with monolayers nanodiamonds and displayed promising similarity to the protein-coated materials regarding neuronal cell attachment,



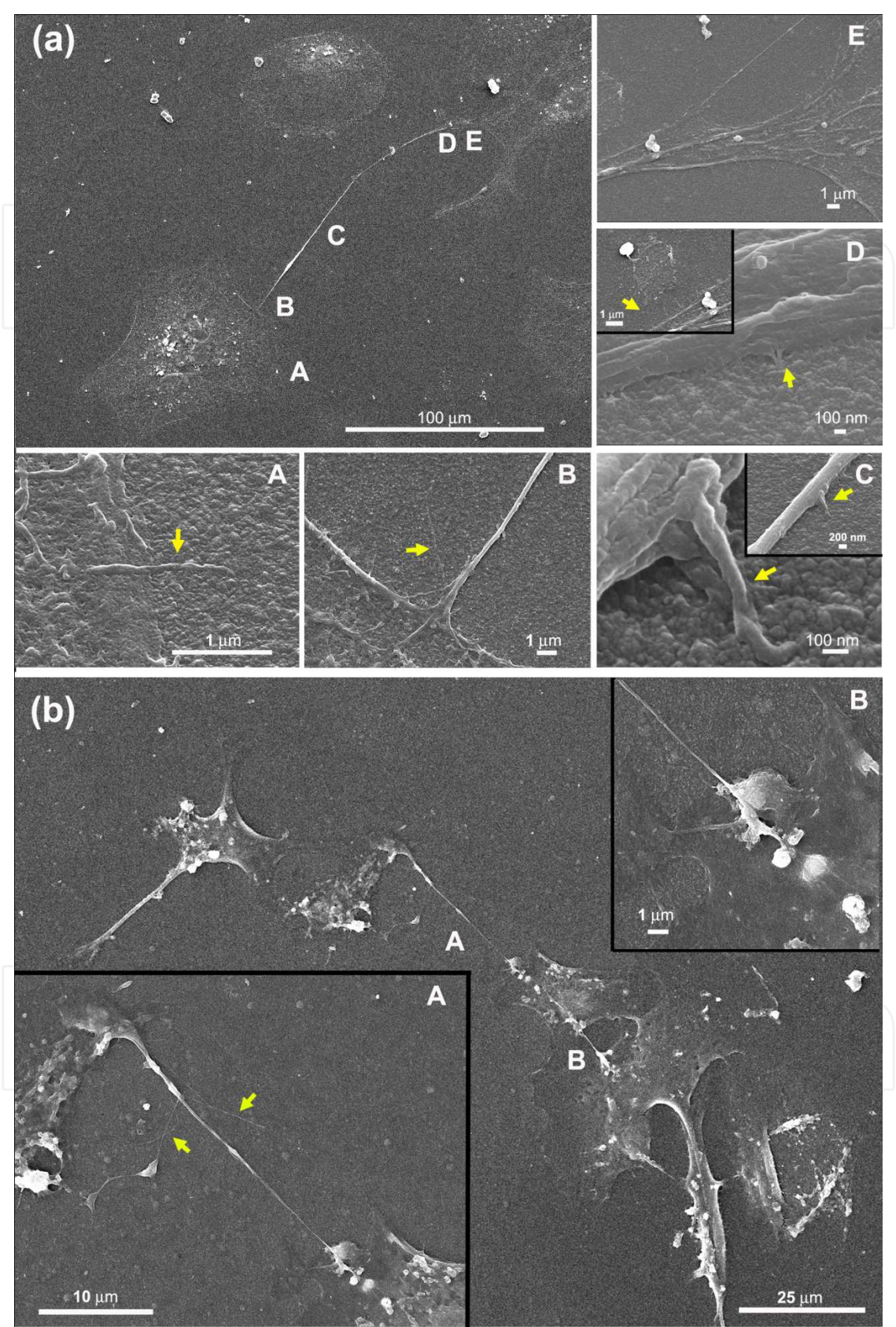


Fig. 2. Scanning electron photomicrographs of neural stem cells cultured on H-UNCD films in regular medium without any differentiating reagents for seven days. Higher

magnification scanning electron microscopy was performed to enlarge different areas (A-E) in graph (a) and (A-B) in graph (b). Yellow arrows show the filopodia at higher magnifications.

neurite outgrowth and functional network formation. Importantly, the neurons were able to grow in direct contact with the NCD-coated material and could be easily maintained in culture for an extended period, equal to those on protein-coated substrates. To further investigate the interaction of cell to NCD film, Chen et al observed the morphology of cells cultured in H-terminated UNCD films and revealed that there were filopodia/nano-diamond interactions (Figure 2). Thus, NCD layering might prove a valuable material for implants on a wide range of substrates. These indicate that diamond films can be easily modified to either promote or prevent cell/biomaterial interactions. This is an interesting feature for tissue engineering and bio-electronics. A question remained though to what kinds of mechanism and key points to affect the degree of the cell adhesion and selectivity.

## 5. Molecular mechanisms of signaling transduction from UNCD films to nuclei

Cells do not interact with a naked material either *in vitro* or *in vivo*. At the beginning step, the material is conditioned by the biological fluid components. This is a complex process strongly dependent on the cell culture conditions including the underlying substrate and mediating medium/proteins. Surface energy may influence protein adsorption and the structural rearrangement of the proteins on positively and negatively charged substrates (hydrophilic/hydrophobic surface). Protein from serum containing media adsorbed on surfaces forming multiple molecular layers. Hydrophobic H-terminated surfaces were found less favorable for osteoblastic cell adhesion, spreading and viability than hydrophilic O-terminated surfaces [5]. Recently, it was shown that microscopic (30–200  $\mu\text{m}$ ) patterns of H- and O-terminated surface can lead to a selective adhesion and arrangement of osteoblasts [85]. This effect also works on human periodontal ligament fibroblast and human cervical carcinoma (HeLa) cells [85–87]. The differential adsorption of “serum proteins” on the negative or positive charged regions from medium with fetal bovine serum (FBS) was studied. It was proposed that the selectivity is due to the serum proteins, which are adsorbed in about the same monolayer thickness (2–4 nm) on both H and O-diamond surfaces, but in different composition and conformations of proteins [88]. When osteoblasts were placed on the diamond surface in McCoy's 5A medium without FBS, cell attachment on H/O-patterned diamond surfaces was not selective [85, 89]. This excluded a direct effect of diamond C-H and C-O surface dipoles on the cell selectivity. FBS adsorption to diamond proceeds in two stages. Formation of monolayer thickness (2–4 nm) FBS layer on both H- and O-diamond was observed within short period of time (<18 h) [86, 88]. AFM nanoshaving showed that this primary FBS layer is less adhesive to H-diamond than to O-diamond. After long time adsorption (6 days), formation of a thick FBS layer was observed on H-diamond (~35 nm) than on O-diamond (~17 nm) [86]. Moreover, it is clear that not only the nature of adsorbed biological molecules but also their conformation and composition will influence consequent cell adhesion. Changes in conformation of pre-adsorbed specific proteins, fibronectin, (not bovine serum albumin or vitronectin, which is abundant in FBS) were observed. These would affect cell binding domain conformations and then affect the affinity with its cell surface receptor [58, 86].



Osteoblast adhesion on materials may also be considered in relation to the expression of the various adhesion proteins and cell receptors. Numerous studies using immunofluorescent staining have shown the presence of vinculin and pY397 focal adhesion kinase (FAK) in cultured human osteoblasts on nanostructured diamond films [60, 78, 79]. The osteoblasts adhered on ultra nano-cones and nano-cones, showing large focal adhesions and relatively strong activation of FAK, are thus more predestined for successful colonization of the entire environment [60, 78]. Hamilton [90] suggested that osteoblast response to substrates with specific topographical features requires FAK-Y397-Src-Y416 complexes for ERK1/2 phosphorylation. Yet on smooth surfaces, Src-independent routes of ERK1/2 activation are present, which finally induce the differentiation of osteoblast further to promote bone formation. The same cell signaling pathway has been studied on other materials, such as titanium alloys [83]. According to published data, the contact of cell to fibronectin could be

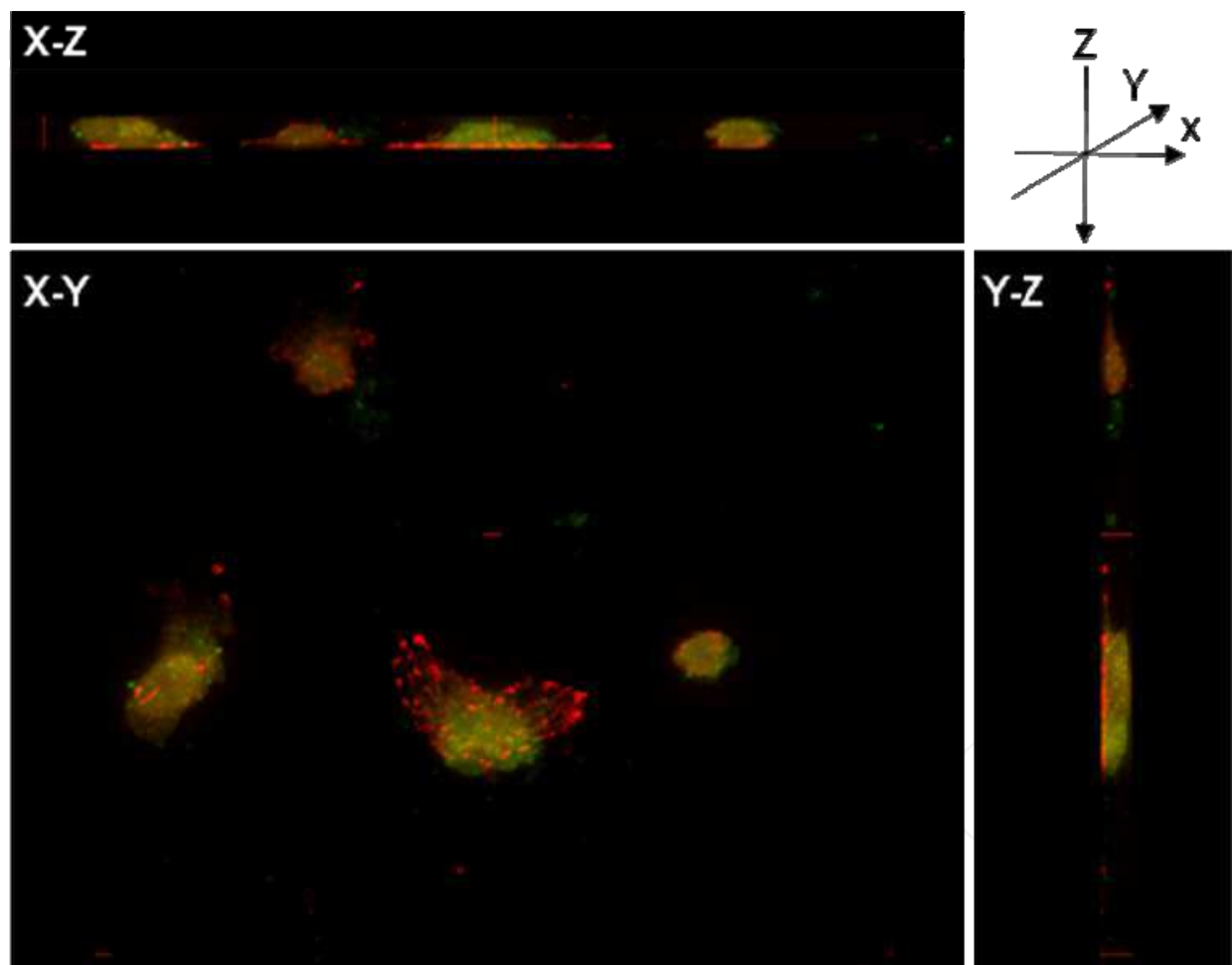


Fig. 3. The confocal immunofluorescence image of neural stem cells grown on the H-UNCD film in the regular medium without any differentiating reagents for 8 hours of culture. Alexafluor 594 labeled phospho-FAK (Red) and DyLight 488 labeled phospho-ERK (Green). The phospho-FAK and phospho-ERK were detected in the cells simultaneously and localized to their proper subcellular positions. In the quadrant of X-Z and Y-Z stacking images, phospho-FAK was observed in basal cell membrane adherent to H-UNCD films, while phosphor-ERK was shown assembled in the cell body.

mediated by integrin  $\beta$ 1 [91, 92]. Integrins are transmembrane protein family and composed of  $\alpha$  and  $\beta$  subunits as heterodimer. Functions of integrins were involved in the regulation of proliferation, survival, migration and differentiation. The high level of integrin  $\beta$ 1 expression has been used to enrich human epidermal and rodent neural stem cells from more restricted progenitor populations [58, 92]. Moreover, Chen et al [66] showed that increased levels of neuronal differentiation in neural stem cells grown on H-UNCD surfaces are due to absorbance of fibronectin from medium to H-terminated UNCD films, resulting in integrin  $\beta$ 1-FAK-ERK1/2 signaling (Figure 3) in conditions of low serum-growth factors and free of differentiating reagents.

| Number | Function             | gi number | Name  |
|--------|----------------------|-----------|---|
| 1      | Extracellular matrix | 224863    | Fibronectin   |
| 2      | Blood                | 78099200  | Hemoglobin subunit epsilon  |
| 3      |                      | 126022898 | Hemoglobin alpha subunit 1  |
| 4      |                      | 203283896 | Apolipoprotein A-I preproprotein  |
| 5      |                      | 3915607   | Apolipoprotein A-I  |
| 6      |                      | 77735387  | Fetuin B  |
| 7      |                      | 166159174 | Angiotensinogen (serpin peptidase inhibitor, clade A, member 8)                           |
| 8      |                      | 95147674  | Complement factor B   |
| 9      |                      | 2501351   | Transferrin   |
| 10     |                      | 27807209  | Alpha-2-macroglobulin   |
| 11     |                      | 78369364  | Group-specific component (vitamin D binding protein)                                      |
| 12     | Epithelium           | 16303309  | Type II keratin 5   |
| 13     |                      | 148747492 | Keratin 2   |
| 14     |                      | 73996312  | Similar to Keratin, type II cytoskeletal 5 (Cytokeratin 5) (58 kDa cytokeratin) isoform 3 |
| 15     |                      | 9910294   | Keratin 71  |
| 16     |                      | 4159806   | Type II keratin subunit protein   |
| 17     | Cytoskeleton         | 28336     | Mutant beta-actin   |
| 18     | Others               | 27806907  | Clusterin   |
| 19     |                      | 2232299   | IgM heavy chain constant region   |
| 20     |                      | 27806809  | Regucalcin  |

Table 1. Differential protein expression profile identified by LC-MS/MS, showing proteins preferentially absorbed on H-UNCD films, but not on Petri dish polystyrene surface.

6. Proteomic analysis of proteins that are adsorbed to UNCD films by using LC-MS/MS

We showed that the abundant fibronectin adsorbed onto the H-UNCD film formed locally dense and conformed layer that allows for the pro-adhesive motifs to be accessible by integrins and further activates the whole signaling pathway [66]. To further investigate what other serum proteins might be bound to UNCD films, we performed proteomic analysis,



using LC-MS/MS on serum proteins that are adsorbed to H-UNCD films. We demonstrated that H-UNCD films could adsorb proteins from culture medium more efficient than Petri dish's polystyrene surface could (Table 1). These proteins included not only fibronectin but also proteins that are known to be present in blood, epithelium, cytoskeleton, and others. It would be of interest to further explore the roles of these proteins in shaping the UNCD-cell interaction and the ultimate differentiation into desired cell types.

## 7. Conclusion

Highly intense research on biocompatibility of NCD films showed that it is a promising material for biomedical applications. NCD films possess easy surface functionalization and nano-topography, offering favorable condition for the growth of fibroblasts, osteoblasts and stem cells without inflammatory response and cytotoxicity. From published *in vitro* studies, NCD films elicited an improved proliferation and differentiation capacity for human osteoblasts and neural stem cells, compared to conventional polystyrene Petri dishes. The relevant mechanism of cellular signaling transduction has been investigated and shown to act through fibronectin-integrin-FAK-ERK pathway. These results suggest the potential usage of NCD films as novel medical devices and implants such as a coating for joint implant and nerve repair in tissue engineering. The delamination and corrosion of the NCD films during its long-term use in medical implants are to be carefully considered for its future biomedical applications. We performed proteomic analysis, using LC-MS/MS, to identify proteins that are adsorbed to UNCD films. We demonstrated proteins such as fibronectin, transferrin, and several keratin proteins that could be adsorbed more efficiently onto UNCD films than to Petri dish's polystyrene surface. It would be of interest to further explore the roles of these proteins in shaping the UNCD-cell interaction and the subsequent differentiation into desired cell types. Finally, more systematic studies *in vivo* are now warranted to confirm its use in biomedical devices for commercial applications.

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These contribution books collect reviews and original articles from eminent experts working in the interdisciplinary arena of biomaterial development and use. From their direct and recent experience, the readers can achieve a wide vision on the new and ongoing potentialities of different synthetic and engineered biomaterials. Contributions were selected not based on a direct market or clinical interest, but on results coming from a very fundamental studies. This too will allow to gain a more general view of what and how the various biomaterials can do and work for, along with the methodologies necessary to design, develop and characterize them, without the restrictions necessary imposed by industrial or profit concerns. Biomaterial constructs and supramolecular assemblies have been studied, for example, as drug and protein carriers, tissue scaffolds, or to manage the interactions between artificial devices and the body. In this volume of the biomaterial series have been gathered in particular reviews and papers focusing on the application of new and known macromolecular compounds to nanotechnology and nanomedicine, along with their chemical and mechanical engineering aimed to fit specific biomedical purposes.

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