Charles University in Prague

Faculty of Science

Summary of Ph.D. Thesis

The Effect of Carbon Nanostructures on Human Cell Behavior and the Role of Fetal Bovine Serum in Cell Adhesion

Mgr. Martina Verdánová

Prague, 2016

Doctoral Study Programs in Biomedicine

Charles University in Prague and

Academy of Sciences of the Czech Republic

Study Program: Molecular and Cell Biology, Genetics and Virology

Chairperson of Subject Area Board: prof. RNDr. Stanislav Zadražil, DrSc.

Training Institute:
Institute of Inherited Metabolic Disorders, Charles University in Prague – First Faculty of Medicine and General University Hospital in Prague
Ke Karlovu 455/2
128 08 Prague 2

Author: Mgr. Martina Verdánová

Supervisor: doc. RNDr. Marie Hubálek Kalbáčová, Ph.D.

Advisor: RNDr. Ing. Kalbáč Martin, Ph.D.

The Ph.D. Thesis can be seen in the respective libraries of Faculty of Science, Charles University in Prague.

Abstract

Graphene (G) and nanocrystalline diamond (NCD) are carbon allotropes and promising nanomaterials with an excellent combination of their properties, such as high mechanical strength, electrical and thermal conductivity, possibility of functionalization and very high surface area to volume ratio. For these reasons, G and NCD are employed next to electronics in biomedical applications, including implant coating, drug and gene delivery and biosensing.

For a fundamental characterization of cell behavior on G and NCD, we studied osteoblast adhesion and proliferation on differently treated G and NCD. Generally, both G and NCD exhibited better properties for osteoblast cultivation than control tissue culture polystyrene. Better cell adhesion but lower cell proliferation were observed on NCD compared to G. The most surprising finding was that hydrophobic G with nanowrinkled topography enhanced cell proliferation extensively, in comparison to hydrophilic and flat G and both NCDs (hydrophobic and hydrophilic) with slightly higher roughness. Promoted cell proliferation enables faster cell colonization of G and NCD substrates, meaning faster new tissue formation which is beneficial in biomedical applications.

Furthermore, it was shown that osteoblast adhesion was promoted in the initial absence of fetal bovine serum (FBS); however, osteoblast proliferation was suppressed regardless of the material used. As a follow-up to this difference, we characterized cell adhesion to tissue culture polystyrene in the presence and absence of FBS with three different cell types. Consistently for all tested cell types, no classic focal adhesions were formed during cell adhesion in the absence of FBS proteins. Moreover, signaling within these cells proceeded in an unusual manner. In contrast, FBS absence affected cell shape, area and number variously in the tested cell types. For the first time, the cell-substrate contact in the absence of serum proteins for anchorage-dependent cells was described in detail.

In the last part of this thesis, the use of sericin (silk protein) as a replacement for FBS in freezing medium for osteosarcoma cell line and primary human mesenchymal stem cells (hMSCs) was evaluated. It was shown that 1 % sericin could substitute for 25 % FBS in the freezing medium for hMSCs, in contrast to osteosarcoma cell line. Moreover, hMSCs could be cryopreserved in a growth medium containing only 10 % DMSO with adequate results. Finally, different freezing formulas should be evaluated for different cell types to find the most satisfactory results.

Contents

1 Introduction

1.1 Graphene and nanocrystalline diamond

Graphene (G) and nanocrystalline diamond (NCD) are carbon allotropes and both of them used in this thesis are defined as nanomaterials. A nanomaterial is defined as a material smaller than 100 nm at least in one dimension. The greatest advantage of a nanomaterial is the very high surface area to volume ratio (1).

G is a single-atom-thick layer of sp^2-bonded carbon atoms arranged in a two-dimensional honeycomb structure. NCD is made up of tetrahedral clusters of sp^3-bonded carbon. Both nanomaterials exhibit an exceptional combination of their properties such as high mechanical strength, electrical and thermal conductivity, biocompatibility, possibility of functionalization and a very high surface area to volume ratio (2-5).

Besides electronics, G and NCD can be used in various biomedical applications, including drug and gene delivery, biosensing, bioimaging, and can be used as a substrate for cell culturing for tissue engineering purposes (6-10).

1.2 Material surface properties affecting cell behavior

Generally, the behavior of those cells cultivated on some surface is extensively affected by the underlying surface properties, such as surface chemistry, wettability, topography, charge/energy state and material stiffness (11, 12). It is important to mention that the proteins from the body fluids or culture medium adsorb on the surface faster than do the cells themselves. Consequently, the cell adhesion is affected to a large extent by the adsorbed protein layer. Therefore, the surface properties of various materials are important primarily for the protein adsorption (the amount and conformation of adsorbed proteins) (13).

1.3 Cell adhesion

Cells receive external signals in the form of soluble molecules (cytokines, hormones and growth factors), or by direct interaction with other cells (cell-cell contact), or by contact with the extracellular matrix (ECM). The information obtained from the cell surrounding is integrated in the cell and affects the cell migration, proliferation, differentiation and death through the cell signaling and changes in gene expression (14).

Cell adhesion to ECM is mediated by focal adhesions (FAs) that are micron-sized protein assemblies linking the ECM to the actin cytoskeleton in cells. Integrins are the leading

proteins out of approximately 230 proteins associated with FAs (15). Integrins are transmembrane heterodimeric receptors binding to various ligands in ECM, such as fibronectin, vitronectin, collagen, laminin and many others (16). The adaptor protein vinculin is a crucial regulator of FA formation and couples (together with talin) integrins to the actin cytoskeleton. It is often used as a marker of FAs. The FA architecture could be classified into several functional layers; from the exterior of the cell, these are the integrin extracellular domain layer (extracellular part of integrins), integrin signaling layer (e.g. intracellular part of integrins, paxillin, focal adhesion kinase – FAK), force transduction layer (e.g. talin and vinculin), actin regulatory layer (e.g. zyxin) and actin stress fibers (17).

1.4 Fetal bovine serum

Fetal bovine serum (FBS) is the blood fraction that remains after blood coagulation. It contains various proteins and factors important for cell adhesion, proliferation and survival (18). FBS often serves as an essential supplement of culture medium for *in vitro* cell cultivation and cryoprotection.

In biomedical applications, the usage of FBS is problematic due to its high batch-to-batch variability, the possible transmission of fungal, bacterial, viral or prion infection, and the possibility of anti-FBS antibody production (19).

Generally, it is believed that FBS proteins compete for adsorption to a surface and that cell adhesion is influenced by the balance between adhesion-promoting (e.g. fibronectin) and adhesion-inhibiting (e.g. bovine serum albumin) proteins. Finally, all factors such as the type of proteins present in the cell cultivation environment, their quantity and conformation significantly affect cell adhesion and subsequent cell behavior.

1.5 Sericin as a cryoprotectant

Sericin is an amorphous sticky glycoprotein, derived from the silkworm (Bombyx mori) cocoon (20). Sericin has a number of attractive properties that are the subject of current research, e.g. suppression of skin tumorigenesis (21) and the acceleration of mammalian cells' proliferation (22). Since usage of FBS is often undesirable primarily for clinical applications, alternatives for FBS are being explored. One promising possibility is sericin. It has been presented that sericin can be used as a cryoprotectant during freezing of various cell types, such as human adipose tissue-derived stem cells (23), myeloma cell lines, ovarian cells, fibroblasts, keratinocytes and insect cell lines (24), rat insulinoma cell lines, and mouse hybridoma cell lines (25).

2 Aims of the thesis

I. To characterize the behavior of osteoblasts on differently treated graphene and nanocrystalline diamond, with regard to cell adhesion and proliferation in the presence and the absence of FBS proteins.

II. To characterize an early phase of cell adhesion with respect to the presence or absence of FBS. To compare early cell adhesion of immortalized cell line and primary cells.

III. To evaluate sericin as a substitute for FBS in a freezing medium for immortalized cell line and primary cells.

3 Materials and methods

- **Tested materials** [nanocrystalline diamond treated with oxygen plasma (NCD-O), nanocrystalline diamond treated with hydrogen plasma (NCD-H), single-layer graphene treated with H_2/Ar (1-LG), single-layer graphene treated in an oxidizing atmosphere (1-LG-O)]
- **Cells used** (human osteoblast-like cell line SAOS-2, human dermal fibroblasts, human mesenchymal stem cells)
- **(Immuno)fluorescence staining of cells** (nuclei, actin stress fibers, vinculin, CD44, talin, pFAK-Y397, Rho-Y486, pERK1/2)
- **Cell imaging by fluorescence microscopy**
- **Advanced image analyses** (cell number and cell area determination, analysis of focal adhesions, measurement of fluorescence intensity)
- **Cell adhesion strength determination**
- **CFU-F assay** (colony-forming unit-fibroblast)
- **Protein pre-adsorption on glass surfaces**
- **Cryopreservation of cells**
- **Inhibition of integrins**
- **Quantitative real-time PCR**
- **Transcriptomic profiling** (using chip from Affymetrix)
- **Transfection of cells and production of stable cell line expressing vinculin gene**
- **Live-cell imaging**
- **Statistical analyses** (One-way ANOVA, nonparametric Mann-Whitney U test, Wilcoxon signed-rank test, Kruskal-Wallis ANOVA with subsequent post-hoc test based on pair-wise comparisons with the Bonferroni correction)

4 Results

4.1 Osteoblast behavior on graphene and nanocrystalline diamond (Publications A-C + unpublished results)

The first part of this thesis is focused on the behavior of human osteoblasts (SAOS-2 cells, cell line derived from the primary osteosarcoma) cultivated on large-scale graphene (G) or nanocrystalline diamond (NCD) for a short time (2 h) and a longer time (48 h). Bone implant coating or cellular sensors are the possible applications of these carbon nanomaterials in terms of the research in this thesis. For this reason, basic information on cell interactions with these exceptional and promising nanomaterials is essential and thus far only little explored.

Variously modified G and NCD resulting in their diverse properties were investigated in this thesis. Two hydrophobic samples, i.e. graphene treated with hydrogen (1-LG, roughness 9 ± 3 nm) and NCD treated with hydrogen (NCD-H, roughness 20 ± 3 nm) and two hydrophilic samples, i.e. graphene treated with oxygen (1-LG-O, roughness 5 ± 2 nm) and NCD treated with oxygen (NCD-O, roughness 20 ± 3 nm) were researched. These four tested samples vary at least in the carbon atom arrangement (G and NCD), degree of wettability and nanotopography.

Moreover, cell adhesion on G and NCD was characterized a) under standard conditions, i.e. in the presence of FBS (the cells adhere on pre-formed protein layer) and b) under non-standard conditions, i.e. in the absence of FBS (the cells adhere directly on the plain material). Immunofluorescence and advanced image analyses were the most frequently used biological methods in this section. Moreover, G and NCD were characterized using many physical methods by our colleagues from the institutes of the Czech Academy of Science. These results enabled us to interpret our biological observations accurately.

It was shown that the fewest cells adhered to G and these were the smallest cells. On the other hand, cells on G revealed superior proliferation in comparison to NCD and TCPS (summarized in Fig. 1). In more detail, it was demonstrated that, behind the accelerated cell proliferation on G, more properties of 1-LG were present than of 1-LG-O. However, 1-LG-O still promoted cell proliferation more than both NCD. Similar degrees of cell adhesion and proliferation were observed on hydrophilic NCD-O and hydrophobic NCD-H. It was demonstrated that the lack of FBS for the first 2 h of cultivation promoted cell adhesion; however, suppressed further cell proliferation.

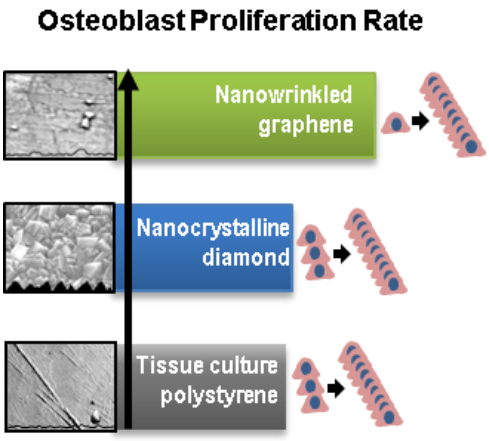

Figure 1: Schematic representation of osteoblast proliferation rate on graphene, nanocrystalline diamond and tissue culture polystyrene.

In addition, detailed characterization of osteoblast adhesion on NCD-O and NCD-H was performed, using the inhibition of integrins by antibodies and qRT-PCR method. It was shown that osteoblasts adhered to hydrophobic NCD-H mainly through fibronectin, while they adhered predominantly through vitronectin to hydrophilic NCD-O.

4.2 Effect of serum proteins on initial cell adhesion (Publication D + unpublished results)

Since we repeatedly found that a cell adhesion in the absence of FBS differs extensively from a cell adhesion in the presence of FBS, regardless of material used, the effect of FBS on cell adhesion was further studied in more detail with tissue culture polystyrene (TCPS).

Adhesion of human osteosarcoma cell line (SAOS-2), primary human fibroblasts and human mesenchymal stem cells (hMSCs) was studied at 2 h after cell seeding. Immunofluorescence and image analyses were the most frequently applied methods in this section. Moreover, real-time monitoring of osteoblast adhesion by fluorescence microscope using stable cell line expressing vinculin gene conjugated with GFP was performed. However, this method was not suitable for this purpose, since cells were dying early.

Osteoblasts and hMSCs demonstrated similar cell shape and area with respect to the presence of FBS (with FBS – round shape and smaller cell area; without FBS – ragged shape and larger cell area). On the other hand, fibroblasts and hMSCs showed a similar trend in cell number with regard to the presence of FBS (with FBS – more cells; without FBS – fewer cells). With regard to cell adhesion quality, all three cell types developed classic FAs with expression of vinculin, talin and pFAK in these FAs, in contrast to all cells that were seeded

without FBS that did not produce classic FAs. Moreover, signaling within these cells (adhered in FBS absence) proceeded in an unusual manner (lower expression of pFAK and pERK1/2 compared to those cells adhered in FBS presence). This observation was also confirmed by a transcriptomic profiling experiment that revealed downregulation of ERK and FAK in those osteoblasts adhered in FBS absence.

Since the most detailed characterization of cell adhesion was performed using osteoblasts, a schematic representation of osteoblast adhesion on TCPS in the presence of FBS and in its absence is shown in Fig. 2.

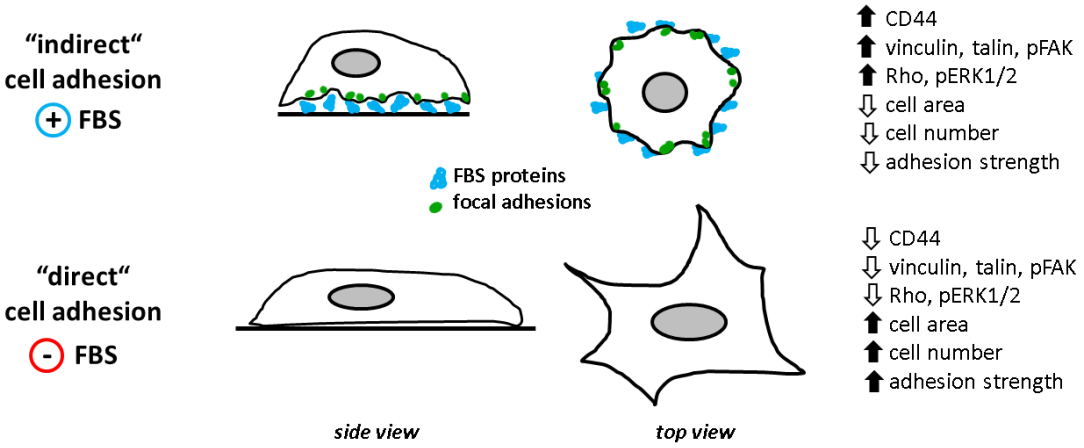

Figure 2: Schematic representation of osteoblast adhesion on tissue culture polystyrene in the presence and absence of FBS.

4.3 Evaluation of sericin as a fetal bovine serum-replacing cryoprotectant during freezing of cells (Publication E)

For the reason that the use of FBS in medical applications is problematic due to the danger of infection or allergic reaction, sericin was investigated as a replacement of FBS in freezing media for osteosarcoma cell line and primary hMSCs. Since DMSO is the most frequently used compound of freezing medium and its high concentrations are cytotoxic to eukaryotic cells (26, 27), DMSO-free or DMSO-low-concentration freezing media were also tested.

Cell viability (24 h after thawing, both hMSC and osteoblasts) and colony-forming ability (2 weeks after thawing, only for hMSCs) were determined. It was demonstrated that 1% sericin can substitute for 25 % FBS in the freezing medium for primary hMSCs, in contrast to osteosarcoma cell line, but cannot substitute for DMSO. Moreover, hMSCs could be cryopreserved in a growth medium containing only 10 % DMSO with adequate results.

5 Discussion

5.1 Osteoblast behavior on graphene and nanocrystalline diamond

The very important finding of these studies is accelerated osteoblast proliferation on G compared to NCD and TCPS. This enhanced osteoblast proliferation on G was caused mainly by hydrophobic 1-LG where cell proliferation was superior.

The reason for the accelerated cell proliferation rate on 1-LG compared to 1-LG-O and both NCDs could be the difference in "nano-roughness", in particular the wrinkled morphology of 1-LG in nanoscale. The positive effects of sub-100 nm structural features on the cells were previously reported (28, 29). The mechanism behind this phenomenon is probably the fact that nanotopography of materials greatly enhances the surface area, enabling a binding of specific proteins in particular amounts and conformations (30). Moreover, the cell membrane in contact with the nano-structured surface is exposed to various mechanical forces that can reorganize its components, and specific ion channels can open which can lead to changes in cell behavior (31).

The enhanced osteoblast proliferation on 1-LG was also detected in the initial FBS absence. Thus, the factor behind this superior proliferation rate on 1-LG cannot only be protein interactions with surface nanotopography. However, the accelerated cell proliferation on 1-LG is clearly visible under both conditions – in FBS presence and absence. This is a great advantage for the potential use of 1-LG as a bioelectronic sensor and actuator, where protein interlayers can cause electrode fouling and affect sensor performance (32).

5.2 Effect of FBS proteins on initial cell adhesion

Osteosarcoma cell line, primary fibroblasts and hMSCs partially demonstrated various adhesion reactions to the absence of FBS proteins. This could be connected to the origin of cells. Generally, tumor cells are characterized by changes in intercellular adhesion selectivity and also adhesion selectivity to ECM. Shifts in cell-cell and cell-ECM interactions (e.g. down- or up-regulation of integrin genes) are oncogene- and cell type-specific. However, cell adhesiveness is mostly reduced in cancer cells (33). This could explain the lower adhesiveness of osteosarcoma cell line in the presence of FBS, in contrast to primary fibroblasts and hMSCs from healthy donors, since FBS mimics the situation in the body where proteins are present.

The most distinct feature was no formation of classic FAs in cells that adhered in FBS absence, compared to the cells adhered in FBS presence. The cells that adhere without any

external proteins probably use a different mechanism to anchor themselves to the surface. This contact is probably mediated by non-specific physical interactions such as van der Waals bonding, hydrogen bonding or charged interactions between polar groups (e.g. hydroxyl) on the substrate and integrins on the cell surface (34).

Based on expression and localization of signaling proteins such as pFAK and ERK1/2, it could be said that cell signaling in the absence of FBS is transduced by an alternative signaling pathway compared to the standard cell signaling initiated by FBS proteins (182). Since a cell environment without FBS is poor in growth factors, a decreased level of activated, i.e. phosphorylated ERK1/2 was found in cells that adhered in the absence of FBS, in contrast to the cells that adhered on the FBS proteins. This is in correlation with the study by Chen *et al.* showing that the addition of growth factors to serum-deprived cells led to the increased phosphorylation of ERK proteins (183).

5.3 Evaluation of sericin as a fetal bovine serum-replacing cryoprotectant during freezing of cells

We showed that 1 % sericin could substitute for 25 % FBS in the freezing solution for primary hMSCs. Similar results were also published for rat insulinoma cell line, mouse hybridoma cell line (35) and for rat pancreatic islets (36). The novelty of our findings is that hMSCs could be cryopreserved in a growth medium containing only 10 % DMSO without any additional proteins (FBS or sericin), with satisfactory results. Interestingly, freezing medium with 1 % sericin instead of 25 % FBS or medium containing only 10 % DMSO was not beneficial for osteoblastic cell line.

It seems that primary hMSCs are more resistant to a heat stress (freezing and thawing) than the immortalized osteosarcoma cell line. A possible explanation could be the fact that hMSCs are less differentiated cells with more self-renewing ability compared to differentiated osteoblasts.

6 Conclusions

I. We characterized osteoblast behavior on differently treated graphene (G) and nanocrystalline diamond (NCD) in terms of cell adhesion and proliferation. Generally, both G and NCD exhibited better properties for osteoblast cultivation in comparison to control tissue culture polystyrene. Better cell adhesion but lower cell proliferation were observed on NCD compared to G. It was shown that osteoblasts adhered to hydrophobic NCD-H mainly through fibronectin, while they adhered to hydrophilic NCD-O predominantly through vitronectin. However, different wettability properties of topographically identical NCDs had no effect on osteoblast proliferation. In contrast, hydrophobic 1-LG with nanowrinkled topography enhanced cell proliferation extensively, in comparison to hydrophilic and flat 1-LG-O. Promoted cell proliferation enables faster cell colonization of G and NCD substrates, meaning faster new tissue formation which is beneficial in biomedical applications. Furthermore, it was shown that osteoblast adhesion was promoted in the initial absence of FBS; however, osteoblast proliferation was suppressed by these conditions.

II. We characterized cell adhesion of osteosarcoma cell line SAOS-2, primary human fibroblasts and human mesenchymal stem cells (hMSCs) in the presence and absence of FBS. For all three tested cell types, it was found that no classic focal adhesions were formed during cell adhesion in the absence of FBS proteins. Moreover, signaling within these cells proceeded in an unusual manner. In contrast, tested cell types differed in cell shape, area and number considering the adhesion in the presence or the absence of FBS. For the first time, the cell-substrate contact in the absence of serum proteins for anchorage-dependent cells was described in detail.

III. We evaluated the use of sericin as a replacement of FBS in freezing medium for osteosarcoma cell line and primary hMSCs from healthy donors. It was shown that 1 % sericin could substitute for 25 % FBS in the freezing medium for primary hMSCs in contrast to osteosarcoma cell line. Moreover, hMSCs could be cryopreserved in a growth medium containing only 10 % DMSO, with adequate results. Finally, different freezing formulas should be evaluated for different cell types to find the most satisfactory results.

7 References

1. Padmanabhan J, Kyriakides TR. Nanomaterials, Inflammation, and Tissue Engineering. Wires Nanomed Nanobi. 2015;7(3):355-70.
2. Sanchez VC, Jachak A, Hurt RH, Kane AB. Biological Interactions of Graphene-Family Nanomaterials: An Interdisciplinary Review. Chem Res Toxicol. 2012;25(1):15-34.
3. Mao HY, Laurent S, Chen W, Akhavan O, Imani M, Ashkarran AA, et al. Graphene: Promises, Facts, Opportunities, and Challenges in Nanomedicine. Chem Rev. 2013;113(5):3407-24.
4. Tang L, Tsai C, Gerberich WW, Kruckeberg L, Kania DR. Biocompatibility of Chemical-Vapor-Deposited Diamond. Biomaterials. 1995;16(6):483-8.
5. Fries MD, Vohra YK. Properties of nanocrystalline diamond thin films grown by MPCVD for biomedical implant purposes. Diam Relat Mat. 2004;13(9):1740-3.
6. Shen H, Zhang LM, Liu M, Zhang ZJ. Biomedical Applications of Graphene. Theranostics. 2012;2(3):283-94.
7. Zhang XQ, Chen M, Lam R, Xu XY, Osawa E, Ho D. Polymer-Functionalized Nanodiamond Platforms as Vehicles for Gene Delivery. Acs Nano. 2009;3(9):2609-16.
8. Lien ZY, Hsu TC, Liu KK, Liao WS, Hwang KC, Chao JI. Cancer cell labeling and tracking using fluorescent and magnetic nanodiamond. Biomaterials. 2012;33(26):6172-85.
9. Izak T, Novotna K, Kopova I, Bacakova L, Rezek B, Kromka A. H-terminated diamond as optically transparent impedance sensor for real-time monitoring of cell growth. Phys Status Solidi B-Basic Solid State Phys. 2013;250(12):2741-6.
10. Yang L, Sheldon BW, Webster TJ. The impact of diamond nanocrystallinity on osteoblast functions. Biomaterials. 2009;30(20):3458-65.
11. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126(4):677-89.
12. Thevenot P, Hu WJ, Tang LP. Surface chemistry influences implant biocompatibility. Curr Top Med Chem. 2008;8(4):270-80.
13. Anselme K. Biomaterials and interface with bone. Osteoporos Int. 2011;22(6):2037-42.
14. Geiger B, Spatz JP, Bershadsky AD. Environmental sensing through focal adhesions. Nature reviews Molecular cell biology. 2009;10(1):21-33.
15. Winograd-Katz SE, Fassler R, Geiger B, Legate KR. The integrin adhesome: from genes and proteins to human disease. Nat Rev Mol Cell Bio. 2014;15(4):273-88.

16. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002;110(6):673-87.
17. Kanchanawong P, Shtengel G, Pasapera AM, Ramko EB, Davidson MW, Hess HF, et al. Nanoscale architecture of integrin-based cell adhesions. Nature. 2010;468(7323):580-4.
18. Krebs HA. Chemical composition of blood plasma and serum. Annual review of biochemistry. 1950;19:409-30.
19. Sundin M, Ringden O, Sundberg B, Nava S, Gotherstrom C, Le Blanc K. No alloantibodies against mesenchymal stromal cells, but presence of anti-fetal calf serum antibodies, after transplantation in allogeneic hematopoietic stem cell recipients. Haematologica. 2007;92(9):1208-15.
20. Chen FJ, Porter D, Vollrath F. Morphology and structure of silkworm cocoons. Mat Sci Eng C-Mater. 2012;32(4):772-8.
21. Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H, Kato N. Silk protein, sericin, suppresses DMBA-TPA-induced mouse skin tumorigenesis by reducing oxidative stress, inflammatory responses and endogenous tumor promoter TNF-alpha. Oncol Rep. 2003;10(3):537-43.
22. Terada S, Takada N, Itoh K, Saitoh T, Sasaki M, Yamada H. Silk Protein Sericin Improves Mammalian Cell Culture. In: Smith R, editor. Cell Technology for Cell Products. 3: Springer Netherlands; 2007. p. 397-401.
23. Miyamoto Y, Oishi K, Yukawa H, Noguchi H, Sasaki M, Iwata H, et al. Cryopreservation of Human Adipose Tissue-Derived Stem/Progenitor Cells Using the Silk Protein Sericin. Cell Transplant. 2012;21(2-3):617-22.
24. Sasaki M, Kato Y, Yamada H, Terada S. Development of a novel serum-free freezing medium for mammalian cells using the silk protein sericin. Biotechnol Appl Bioc. 2005;42:183-8.
25. Toyosawa T, Oumi Y, Ogawa A, Sasaki M, Yamada H, Terada S. Novel Serum-Free Cryopreservation of Mammalian Cells Using Seric. In: Shirahata S, Ikura K, Nagao M, Ichikawa A, Teruya K, editors. Animal Cell Technology: Basic & Applied Aspects. Animal Cell Technology: Basic & Applied Aspects. 15: Springer Netherlands; 2009. p. 41-5.
26. Qi WD, Ding DL, Salvi RJ. Cytotoxic effects of dimethyl sulphoxide (DMSO) on cochlear organotypic cultures. Hearing Res. 2008;236(1-2):52-60.
27. Wang X, Hua TC, Sun DW, Liu BL, Yang GH, Cao YL. Cryopreservation of tissue-engineered dermal replacement in Me2SO: Toxicity study and effects of concentration and cooling rates on cell viability. Cryobiology. 2007;55(1):60-5.

28. Babchenko O, Kromka A, Hruska K, Kalbacova M, Broz A, Vanecek M. Fabrication of nano-structured diamond films for SAOS-2 cell cultivation. Phys Status Solidi A. 2009;206(9):2033-7.
29. Higgins AM, Banik BL, Brown JL. Nanotopography Sensing Through Intracellular Signaling and Mechanotransduction with an Emphasis on Bone. J Biomater Tiss Eng. 2013;3(4):396-408.
30. Curtis ASG, Dalby M, Gadegaard N. Cell signaling arising from nanotopography: implications for nanomedical devices. Nanomedicine-Uk. 2006;1(1):67-72.
31. Martinez E, Engel E, Planell JA, Samitier J. Effects of artificial micro- and nano-structured surfaces on cell behaviour. Ann Anat. 2009;191(1):126-35.
32. Rezek B, Kratka M, Kromka A, Kalbacova M. Effects of protein inter-layers on cell-diamond FET characteristics. Biosensors & bioelectronics. 2010;26(4):1307-12.
33. Khalili AA, Ahmad MR. A Review of Cell Adhesion Studies for Biomedical and Biological Applications. Int J Mol Sci. 2015;16(8):18149-84.
34. Audiffred JF, De Leo SE, Brown PK, Hale-Donze H, Monroe WT. Characterization and Applications of Serum-Free Induced Adhesion in Jurkat Suspension Cells. Biotechnol Bioeng. 2010;106(5):784-93.
35. Toyosawa T, Oumi Y, Ogawa A, Sasaki M, Yamada H, Terada S. Novel Serum-Free Cryopreservation of Mammalian Cells Using Sericin. Anima Cell Tech. 2009;15:41-5.
36. Ohnishi K, Murakami M, Morikawa M, Yamaguchi A. Effect of the silk protein sericin on cryopreserved rat islets. J Hepato-Bil-Pan Sci. 2012;19(4):354-60.

8 Curriculum vitae

Name: Martina Verdánová
Date of Birth: 19.5.1986
Place of Birth: Jindřichův Hradec, Czech Republic

Education:

2011 – present: PhD study, Faculty of Science, Charles University in Prague, Field of study: Molecular and Cellular Biology, Genetics and Virology, Group: Laboratory of Interaction of Cells with Nanomaterials (group leader: doc. RNDr. Marie Hubálek Kalbáčová, Ph.D)

2009-2011: Master study, Faculty of Science, Charles University in Prague, Field of study: Molecular and Cellular Biology, Genetics and Virology, Group: Laboratory of Virology (group leader: doc. RNDr. Jitka Forstová, CSc.)

Work experience:

2016 – present: Quality Control Specialist, SOTIO a.s.

2012 – present: Researcher in Molecular Genetics, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague

2011-2014: Researcher, J. Heyrovsky Institute of Physical Chemistry of the ASCR, v. v. i.

2009-2011: **Technician**, Faculty of Science, Charles University in Prague

Fellowships:

2012: **Fellowship at Technical University in Dresden**, Germany - Faculty of Medicine of Carl Gustav Carus, Institute of Physiological Chemistry, Bone group – Dr. Ute Hempel (2 weeks)

9 List of original publications used for Ph.D. Thesis

A) **Martina Verdanova**, Antonin Broz, Martin Kalbac, Marie Kalbacova (2012): Influence of oxygen and hydrogen treated graphene on cell adhesion in the presence or absence of fetal bovine serum. Phys. Status Solidi B 249, 12, 2503–2506. IF_{2012} = 1.489

B) Marie Hubalek Kalbacova, **Martina Verdanova**, Antonin Broz, Aliaksei Vetushka, Antonin Fejfar, Martin Kalbac (2014): Modulated surface of single-layer graphene controls cell behavior. Carbon 72, 207-214. IF_{2014} = 6.196

C) **Martina Verdanova**, Bohuslav Rezek, Antonin Broz, Egor Ukraintsev, Oleg Babchenko, Anna Artemenko, Tibor Izak, Alexander Kromka, Martin Kalbac, Marie Hubalek Kalbacova (2016): Nanocarbon Allotropes - Graphene and Nanocrystalline Diamond - Promote Cell Proliferation. Small 12, 18, 2499–2509. IF_{2014} = 8.368

D) **Martina Verdanova,** Pavla Sauerova, Ute Hempel, Marie Hubalek Kalbacova (2016): The effect of serum proteins on initial osteoblast adhesion. Cell Biology International - under review

E) **Martina Verdanova**, Robert Pytlik, Marie Hubalek Kalbacova (2014): Evaluation of Sericin as a Fetal Bovine Serum-Replacing Cryoprotectant During Freezing of Human Mesenchymal Stromal Cells and Human Osteoblast-Like Cells. Biopreservation and Biobanking 12, 2, 99-105. IF_{2014} = 1.340

10 List of publications that are not the basis of Ph.D. Thesis

Marie Kalbáčová, **Martina Verdánová**, Filip Mravec, Tereza Halasová, Miloslav Pekař (2014): Effect of CTAB and CTAB in the presence of hyaluronan on selected human cell types. Colloids and Surfaces A: Physicochem. Eng. Aspects 460, 204–208. IF_{2014} = 2.752

Tomáš Suchý, Monika Šupová, Pavla Sauerová, **Martina Verdánová**, Zbyněk Sucharda, Šárka Rýglová, Margit Žaloudková, Radek Sedláček, Marie Hubálek Kalbáčová (2015): The effects of different cross-linking conditions on collagen-based nanocomposite scaffolds - an in vitro evaluation using mesenchymal stem cells. Biomed. Mater. 10, 065008. IF_{2014} = 3.697

Pavla Sauerová, **Martina Verdánová**, Filip Mravec, Tereza Pilgrová, Tereza Venerová, Marie Hubálek Kalbáčová, Miloslav Pekař (2015): Hyaluronic acid as a modulator of the cytotoxic effects of cationic surfactants. Colloids and Surfaces A: Physicochem. Eng. Aspects 483, 155–161. IF_{2014} = 2.752