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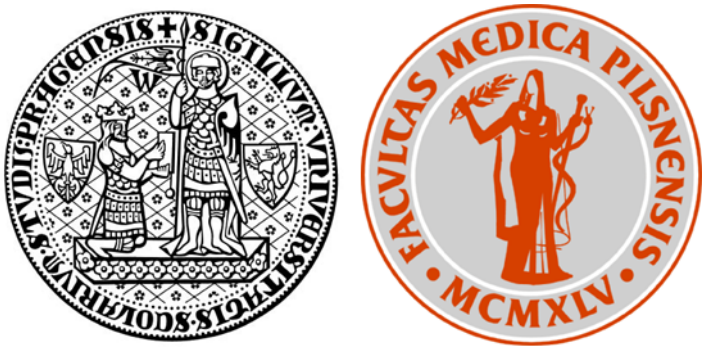

STUDY OF CELL INTERACTIONS WITH BIOMIMETIC MATERIAL AND ITS APPLICATION IN BIOMEDICINE

The Self Report of the Ph.D. Thesis

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ABSTRACT

Biomaterials are considered as very promising tools for regenerative medicine. They have compensatory or supporting function in organism and they are often developed to support specific conventional medical procedures. So-called biomimetic materials are developed to imitate natural environment of organism and to induce positive innate responses of organism. An essential part of biomaterial development is *in vitro* biological evaluation, which characterizes (often for the first time) the potential of developed material for its clinical application. This Ph.D. thesis deals with *in vitro* biological evaluation of three different biomimetic materials. In all three cases, the comprehensive evaluation was an integral part of the material development and optimization processes. Each material was *in vitro* characterized at the level of cell-material interactions with respect to its intended specific application. In the first part, cell response to potential drug delivery system based on colloidal complexes of cationic surfactants with hyaluronic acid (HyA) was characterized. HyA protection ability and its limits were described; also the role of fetal bovine serum (FBS) in cell response to the stress stimuli was confirmed. Results considered surfactant-HyA complexes as promising system for drug delivery. In the second part, cell carriers (scaffolds) based on collagen with application potential for bone surgery were evaluated. We proved the impact of crosslinking process of scaffold on adhesion of human cells and benefits and potential of dynamic cultivation for cell culturing on biodegradable scaffolds. Moreover, we selected the optimal biodegradable scaffold suitable for cell cultivation. In the third part, local drug delivery system based on collagen/hydroxyapatite nano-/micro-structured resorbable layers with controlled elution of antibiotics was evaluated *in vitro*. The positive effect of hydroxyapatite content on cells and its limits in relation to the tested antibiotic type were emphasized. Layers were recommended for clinical application as bioactive interfaces that can not only support new formation of bone but also can serve as local drug delivery system. The last part of this thesis focuses on general description of cell adhesion process as a fundamental point of cell-surface interaction. For the first time, the difference in the early cell adhesion in the presence and absence of serum proteins was described in detail. Expression and localization of various proteins involved in cell adhesion and signaling were evaluated as being dependent on the presence or absence of serum proteins. Taken together, results of this thesis helped to evaluate the developed biomaterials under *in vitro* conditions. It was shown that every tested material has potential to support established medical procedures or to become the new alternative of treatment in the regenerative medicine. Our results also demonstrated the importance of *in vitro* biological evaluation in biomaterial development.

ABSTRAKT

Biomateriály jsou považovány za velmi slibné nástroje pro regenerativní medicínu. Mají kompenzační nebo podpůrnou funkci v organismu a jsou často vyvíjeny k podpoře specifických konvenčních léčebných postupů. Takzvané biomimetické materiály jsou vyvíjeny tak, aby napodobily přirozené prostředí organismu a indukovaly pozitivní přirozené reakce vlastní organismu. Podstatnou součástí vývoje biomateriálu je *in vitro* biologické hodnocení, které charakterizuje (často poprvé) potenciál vytvořeného materiálu pro klinické využití. Tato Ph.D. práce se zabývá *in vitro* biologickým hodnocením tří různých biomimetických materiálů. Ve všech třech případech bylo komplexní hodnocení nedílnou součástí procesů vývoje a optimalizace materiálu. Každý materiál byl *in vitro* charakterizována na úrovni interakce buňka-materiál s ohledem na zamýšlenou specifickou aplikaci. V první části práce je charakterizována reakce buněk na potenciální „drug delivery" systém založený na bázi koloidních komplexů kationických surfaktantů s kyselinou hyaluronovou (HyA). Práce popisuje protektivní účinek HyA, ale také jeho limity, a potvrzuje úlohu fetálního bovinního séra (FBS) v buněčné odezvě na stresové stimuly. Výsledky ukázaly, že komplexy surfaktantů a HyA jsou slibné „drug delivery" systémy. Ve druhé části práce jsou hodnoceny kolagenové buněčné nosiče („scaffoldy") s potenciálem pro aplikaci v kostní chirurgii. Je popsán vliv procesu zesíťování nosiče na adhezi lidských buněk, výhody a potenciál dynamické kultivace pro kultivaci buněk na biodegradabilních nosičích. Na základě výsledků je v závěru vybrán optimální biodegradabilní nosič použitelný pro kultivaci buněk. Ve třetí části práce je v podmínkách *in vitro* hodnocen lokální nosič léčiv založený na nano/mikro-strukturovaných resorbovatelných vrstvách kolagenu / hydroxyapatitu s řízenou elucí antibiotik. Jsou zde popsány pozitivní účinky hydroxyapatitu na buňky a jeho limity vůči testovanému typu antibiotika. Vrstvy byly doporučeny pro klinickou aplikaci jako bioaktivní rozhraní, která mohou podporovat kostní novotvorbu, ale také sloužit jako lokální nosič antibiotik. Poslední část práce je zaměřena na obecný popis procesu buněčné adheze jako základního bodu interakce mezi buňkami a povrchem. Rozdíl v časné buněčné adhezi za přítomnosti a nepřítomnosti sérových proteinů byl vůbec poprvé detailně popsán v publikaci této práce. Exprese a lokalizace různých proteinů zapojených do buněčné adheze a signalizace byla vyhodnocena jako závislá na přítomnosti nebo nepřítomnosti sérových proteinů. Celkově vzato, výsledky této práce pomohly vyhodnotit biomateriály připravené za *in vitr*o podmínek a byly nedílnou součástí jejich vývoje a optimalizace. Bylo ukázáno, že každý testovaný materiál má potenciál podpořit zavedené léčebné postupy nebo se stát novou léčebnou alternativou regenerativní medicíny. Naše výsledky rovněž prokázaly důležitost *in vitro* biologického hodnocení ve vývoji biomateriálů.

CONTENT

1 INTRODUCTION

1.1 Biomaterials, Composite Materials, Biomimetic Materials and Biocompatibility

Nowadays, biomaterials science represents a very dynamic field of regenerative medicine. Compensation or support of specific functions of biological system are common reasons of development of new biomaterials. Biomaterials can be derived from nature or products of living system (e.g. biopolymers) or they can be also derived from various synthetic components (e.g. synthetic polymers such as poly(glycolic acid) and poly(lactic acid)). With the respect to complexity and wide range of requirements of living system, biomaterials are often designated on principle of composite material (Wang, 2003). In addition, they are often based on principle of biomimetic materials that are able to induce specific cellular responses and direct new tissue formation mediated by biomolecular recognition, which can be manipulated by altering different parameters of the biomaterial (Shin et al., 2003). Biomimetic materials imitate the function or structure, properties of natural materials, or are developed in the same way as is common in nature.

The crucial phenomenon of biomaterial science is "biocompatibility", which terms function and interactions of biomaterial with living system (Williams, 2003). Biomaterial compatibility with the organism, as fundamental step of biomaterials development, is ordinarily determined firstly at the level of cells under *in vitro* conditions and secondly it is determined at the level of tissues and organisms under *in vivo* conditions.

1.2 Application of Biomimetic Materials

Biomimetic material interferes are applied in a wide range of medical areas with various requirements. Based on these more or less specific requirements, biomimetic materials are developed and tailored with the respect of defined future applications. In result, composition of biomimetic material should allow specific structural desing to ensure maximal imitation of extracellular matrix (ECM) of typical tissue (Anselme et al., 2012; Shin et al., 2003).

In the frame of this thesis, we can divide biomaterials into two groups according to the purpose of material-induced imitation: i) the imitation of specific mechanism (e.g. drug delivery systems or depots of therapeutic substances) and ii) the imitation of specific structure (e.g. scaffold as a cell carrier or a structural substitution) respectively.

Drug delivery systems are generally developed to maximize a therapeutic effect of delivered agents (e.g. drugs, genes, various contrast agents etc) or to maximize target tissue

concentration of these agents, while minimizing the risk of negative effects, e.g. the risk of systemic toxicity. Those systems are often nanostructured with high surface-area-to-volume ratio and also with high porosity and 3D open porous structure supporting efficient drug delivery control (Rogina, 2014). Drug delivery systems typically work on a glaze of micelles, polymers, nanoparticles or nanotubes (Cheung et al., 2012; Prestwich, 2011; Safari and Zarnegar, 2014). Various systems are also presently developed in combination of various antibiotics (e.g. frequently used gentamicin or vancomycin) loaded to different materials in various forms (fibres, powders, solutions, hydrogels) (Bertazzoni Minelli et al., 2004; Chen et al., 2012).

Artificial structural substitutes, so called scaffolds, are often developed to compensate lost or irreversible damaged tissues caused by tissue trauma, inflammation or tumor occurence. Appropriate scaffold should offer enough interactions for cells and ECM and thus to support effective tissue renewal. Therapeutic potential of scaffolds can be significantly elevated by combination with cell therapy, which can be further enhanced by application of stem cells (Anselme et al., 2012; Caplan and Dennis, 2006). In general, ideal scaffold should allow infiltration and colonization of cells (artificially added or naturally occurred) and serve for good tissue regeneration and recovery. As various results show, design of the ideal scaffold should respect many parameters, thus its development is still a high challenge. Currently, it seems that level of permeability, 3D interconnectivity of pores, elasticity, stiffness, stability, surface topography and pore size are crutial scaffold parameters with significant impact on cell ingrowth, migration and scaffold colonization (Karp et al., 2003). Nowadays, dynamic cultivation has been considered as the most effective way for suitable collonization of cells before scaffold implantation (Zhang et al., 2010).

1.3 Native and Artificial Extracellular Matrix

Apart from soluble factors provided by environment, cells are also fundamentally affected by chemical and physical interactions with non-soluble components of their environment - extracellular matrix (ECM). Composition and structure of ECM are essential factors for determination of cell survival and behaviour, thus, these are also key points in the development of an articifial ECM such as biomimetic material.

Native ECM is a complex and highly dynamic network of macromolecules, which occur in all tissues. ECM is three-dimensional non-cellular structure, which serves as unique substrate for every tissue and which is continually (not only in organism development) modified and remodelled (Bonnans et al., 2014; Gattazzo et al., 2014). Major components of

ECM are fibrous proteins (collagens and elastin), glycoproteins (fibronectin (FN), vitronectin (VN), laminin and fibrilin), glykosaminoglycans (GAGs) (hyaluronic acid (HyA)) and proteoglycans (PGs) (heparan, chondroitin and keratin sulfates) (Hynes and Naba, 2012; Walters and Gentleman, 2015). Collagen is the main structural protein of the most of ECMs (hard and soft tissues). It secures extracellular scaffolding and stiffness and binds inorganic compounds, proteoglycans, glycoproteins or growth factors to ECM network (Dong and Lv, 2016; Salasznyk et al., 2004). Glycoproteins, FN and VN, fundamentally participate in cell adhesion process. Extremely widely distributed GAG is HyA. HyA is connected with a wide spectrum of functions at various organism levels. It participates in maintaining of tissue homeostasis, hydration, wound healing, cell migration, proliferation and signalization (Dicker et al., 2014). Also inorganic compounds play important role in ECM. The unique component is calcium phosphate (CaP), often called as bioapatite (bCaP), in bone ECM (not chemically pure hydroxyapatite (HA)) that provides rigidity, roughness of matrix surface and protein availability for cells. It significantly participates on cell adhesion and differentiation (Novotna et al., 2014). Every type of ECM exhibits a combination of properties that have individual and overall impact on cell behaviour and final cell interactions. Besides chemical surface properties, the key properties seems to be roughness, surface topography, stiffness, porosity and pore size (Dohan Ehrenfest et al., 2010).

The biomimetic material should achieve above mentioned ECM properties and simultaneously it should allow natural and gradual remodellation to regenerate the tissue and to be integrated to organism (Swinehart and Badylak, 2016). Besides the maximal structure imitation based on compounds of native ECM, artificial conditions often need alternative compounds for perfect imitation of the native state (BaoLin and MA, 2014). Such compounds are biodegradable polymers - syntetic or natural biodegradable polymers (e.g. polylactide, polycaprolactone, collagen, chitosan, HyA). Their application in tissue engineering arises from the knowledge of native ECM (Chen et al., 2002). Polymers can additionally provide biocompatibility and biodegradability without release of toxic degradation products. In result, synthetic biodegradable polymers associated with naturally derived polymers are widely used (Tian et al., 2012; Venugopal and Ramakrishna, 2005). Moreover, „three-dimensionality“ (3D) of artificial ECM is currently often provided by electrospinning method, which seems to be, together with various combinantions of polymers and their derivates, the dominant and the most perspective scaffold-preparing method in tissue engineering (Raiskup and Spoerl, 2013; Wollensak and Spoerl, 2004).

1.4 Cell - Extracellular Matrix Interactions

Cell-ECM interaction essentialy contributes to the cellular adhesion, migration and differentiation. Interactions between cell and ECM are bidirectional and take place in highly specific microenvironment.

Cell adhesion is the process allowing cells to interact with each other and with ECM. It reflects a condition of cell and cell environment resulting in cell fate direction (proliferation, differentiation or migration). It is crutial factor based on bidirectional communication, which can dynamically react on specific situation - it can be adapted by surrounding requirements and simultaneously it can adapt its own surrounding as well (e.g. bidirectionality via remodelation). For anchorage-dependent cells, cell adhesion is a matter of survival and growth. Adhesion structures can be highly specialized in specialized cell contacts due to securing of specific physiological function of tissues. Typical representatives of specialized contacts are focal adhesions (FAs) of migrating cells (Sergé, 2016). It is considered that FAs play a central role in adhesion process (Zaidel-Bar et al., 2003) and serve as adhesive signalling centres for cells. FA is a network of integrins and other cytoplasmic proteins, which interconnects actin cytoskeleton to surrounding ECM (integrins interact with FN, collagen, laminin or VN of ECM) (Kim et al., 1992; Ruoslahti, 1996). Dynamics and turnover of FAs are key points of FAs role – it directs cell-ECM interactions, adhesion signalization and cell migration.

With respect to cell adhesion process and its requirement, fetal bovine serum (FBS; the blood fraction after clotting, free of blood cell elements) is the standard supplement of *in vitro* cultivation medium. It mimics chemical composition of *in vivo* environment via wide variety of proteins and factors important for cell survival, adhesion and proliferation (growth factors, adhesion-mediating factors such as FN, VN, hormones, nutrients and transport factors (e.g. albumin, transferrin)) (Krebs, 1950). However, FBS cannot be used for clinical applications due to its many potential health risks (especially viral contaminants or the possibility of anti-FBS antibody production) (Ga et al., 1991; Sundin et al., 2007). Moreover, certain proteins of FBS can negatively interact with various molecules, thus some experimental systems (nanoparticles, gene delivery systems, methods based on transfection principles etc.) should use FBS alternatives (Fasano et al., 2005; Little et al., 2009).

1.5 Stem Cells and Stemness

Embryonic and adult stem cells exist in organism. While embryonic stem cells direct development of whole organism, adult stem cells repair and renew tissues with the aim of maintenance and restoration of the organism. Stem cells are cells that exhibit two main properties together: 1) self- renewal ability that secures undifferentiated state of cell and thus maintains the stemness in organism; 2) differentiation ability that specialized cell types (tissue cells).

Mesenchymal stem cells (MSC) can be described as a specific group of stromal cells with multipotent differentiation ability at the level of mesodermal lineage (they can differentiate to osteocytes, adipocytes and chondrocytes) (Uccelli et al., 2008). MSCs are widely used for tissue engineering and development of cell therapy because of their high plasticity in culture with ability to migrate to sites of injury. Besides differentiation potential, other essencial feature of MSCs is production of bioactive molecules that support regenerative environment and repair (see 1.5.2). Just these findings predict MSCs as an efficient tool with high potential for future therapy (Camassola et al., 2012; Meirelles and Nardi, 2009).

Cell differentiation includes a number of essential changes in cell size and shape, metabolic activity, cell signal transduction activity, membrane potential etc. It depends on number of external factors such as growth factors, hormones or various signal molecules. With respect to gene expression, the pool of three specific transcription factors (Oct4, Sox2 or Nanog) is critical in the stemness maintenance. Disbalance in their expression leads to differentiatiated state (Christophersen and Helin, 2010). Differentiation into different lineages is directed by other transcriptional factors – (e.g. osteoblasts differentiation is regulated by Runx2, Osterix and β-catenin (Komori, 2006; Nishio et al., 2006); adipocyte differentiation is regulated by C/EBP family and PPARγ (Hu et al., 1995). Cell differentiation is directed also by various external processes and factors (e.g. growth factors). Wide variety of effects induced by growth factors on cell behaviour and function are known. The key growth factors are for example BMPs (bone morphogenetic proteins), FGFs (fibroblasts growth factors) and VEGF (vascular endothelial growth factos).

Many cell processes (e.g. cell adhesion, proliferation, differentiation and self-renewal) were recently found to be crutially affected by the way of cell cultivation. Thus, 3D culturing (e.g. cultivation of cells on scaffolds) together with dynamic cultivation (flow of medium) seems to be key parameters that mimic structural and dynamic conditions of organism and which can support more natural way of cell behaviour (Zhang et al., 2010).

2 AIMS OF THE THESIS

This PhD thesis is composed of four parts (I-IV). The first three parts (I-III) deals with various biomimetic materials – every part describes one material type and its impact on cells. The last part (IV) is focused on general description of cell adhesion process onto standard cultivation surface i.e. it describes fundamental point of cell-surface interaction.

I To characterize cell response to **colloidal complexes of CTAB or Septonex with hyaluronic acid** - direct cell interactions with potential drug/nuclei acid/diagnostic dye delivery system or cosmetic agent with the respect to fetal bovine serum presence or absence during cultivation.

II To evaluate *in vitro* **collagen based scaffolds** and their suitability for stem cell therapy in bone surgery application by using mesenchymal stem cells derived from different organisms with respect to different cultivation conditions (static and dynamic).

III To evaluate *in vitro* **collagen/hydroxyapatite nano/micro structured resorbable layers with controlled elution of antibiotics** for pro-osteointegration support.

IV To describe an **early phase of cell adhesion of different cell types** in context of fetal bovine serum presence or absence under tissue culture conditions.

3 MATERIALS AND METHODS

Tested Materials:

- CTAB-HyA and Septonex-HyA complexes (in publications A-B)
- Collagen based scaffolds consisted of: poly(DL-lactide) electrospun nano/sub-micronfibres (PDLLA), a natural collagen (type I) matrix supplemented with HyA and natural calcium phosphate nano-particles (bCaP) (in publications C-D)
- Collagen/hydroxyapatite/vancomycin, collagen/hydroxyapatite/gentamicin and collagen/hydroxyapatite/vanco-gentamicin electrospun nanostructured layers (in publications E-F)

Culture of Cells:

- Human osteoblast-like cell line (SAOS-2)
- Spontaneously immortalised human keratinocyte cell line HaCaT
- Human dermal fibroblasts
- Human mesenchymal stem (stromal) cells
- Porcine mesenchymal stem (stromal) cells

Cell Seeding onto Scaffolds and Cultivation

- static (standard) cultivation conditions or dynamic cultivation conditions (bioreactor cultivation with flow rate 30 µl/ min; (Piola et al., 2013))

Determination of DNA content (proteinase K, Quant-iT™ PicoGreen® dsDNA Assay Kit)

Gene Expression Analyses - qRT-PCR (Quantitative Polymerase Chain Reaction with Reverse Transcription)

Determination of Metalloproteinase Activity by Zymography

(Immuno)Fluorescence Staining of Cells

- Nuclei, actin stress fibers, CD44, talin, vinculin, pERK1/2, pFAK, etc.

Cell Imaging by Light Microscopy

Cell Imaging by Fluorescence Wide-Field Microscopy

Cell Imaging by Fluorescence Confocal Microscopy

Advanced Image Analyses:

(ImageJ (Rasband, W.S., ImageJ, U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016) and Cell Profiler (Broad Institute, USA) softwares were used)

- Cell number determination
- Cell area determination
- Measurement of fluorescence intensity
- 3D reconstruction of confocal microscope images

Western blot analysis

Enzyme-linked immunosorbent assay (ELISA)

Statistical Analyses (STATISTICA Software, StatSoft, Czech Republic was used):

- Nonparametric Mann-Whitney U test; Nonparametric Kruskal-Wallis ANOVA with a subsequent post-hoc Multiple comparison test, Wilcoxon signed-rank test

4 RESULTS

4.1 PART I: HyA-Surfactant Complexes (publications A - B)

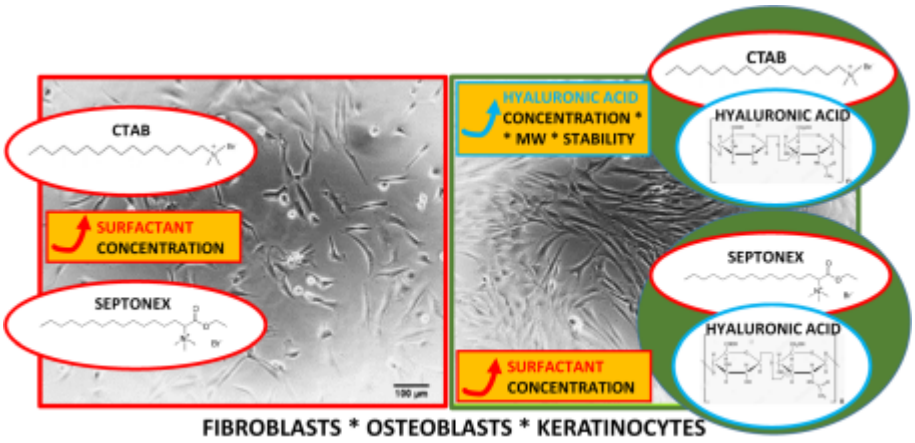

Figure 1: Graphical abstract of first part of PhD thesis. Schematic representation of colloidal complexes of CTAB or Septonex with hyaluronic acid.

Part I of thesis is focused on characterization of cell behaviour affected by direct interactions of cells with **colloidal complexes of CTAB (**Cetrimonium bromide**) or Septonex with hyaluronic acid (HyA)** for 24 h (Figure 1). CTAB-HyA and Septonex-HyA complexes were prepared as possible drug/gene delivery system (Halasová et al., 2011). Function of developed complexes is based on possibility of cationic surfactants (CTAB and Septonex) to form micelles and to interact with negatively charged substances (some kinds of drugs, nucleic acids etc.). Thus, surfactant micelles can be ideal carriers of solubilized drugs or other "cargo" and they can serve as an interesting tool in drug or gene cell delivery, for study of cell trafficking process or for cell structures visualisation. Additionally, thanks to their antiseptic properties they can be used as antimicrobial agents for topical applications (cosmetic or pharmaceutic industry) (Nakata et al., 2011). However, surfactants alone are cytotoxic (Grant et al., 1992), thus, hyaluronic acid seems to ideal biocompatibile and naturally occurring molecule for reduction of surfactant induced cytotoxicity (Halasová et al., 2013; Kalbáčová et al., 2014) or for complex entry to cell (Torchilin, 2001).

Surfactants' cytotoxicity and the ability of HyA bound in the complex with these surfactants to reduce their cytotoxicity (Figure 1) were determined. Different cell types were treated with all the prepared CTAB-HyA or Septonex-HyA complexes and controls under standard (FBS presence) and non-standard conditions (FBS absence for 4h) for 24 h. After this time, cell metabolic activity was measured by MTS assay and microscopy analysis was performed.

Firstly (publication A), only human osteoblasts were used for cytotoxicity evaluation. Under standard conditions, metabolic activity of cells treated with all of the used free CTAB or Septonex concentrations was significantly decreased in comparison to control untreated cells. Nevertheless, cytotoxicity (drop of cell metabolic activity under 75 % (Flahaut et al., 2006)) was detected only at the highest concentrations used. HyA in the complex with surfactants was able to significantly reduce their negative effect on cells. Under non-standard conditions, the trend of metabolic activity was similar but reduced in total. HyA showed highly positive effect on cell metabolic activity - this effect rose proportionally with increasing of CTAB or Septonex concentration. Also changes in cell morphology of treated cells observed by light microscopy were in agreement with metabolic activity results.

Secondly (publication B), other cell types were used – cell line of osteoblasts (SAOS-2), cell line of keratinocytes and primary fibroblasts. Additionally, the concentrations of the surfactants and HyA in complexes were unified to be able to compare the effects directly. The treatment with surfactants or surfactant-HyA complexes exhibited a similar trend in all the three cell types tested, also concentration of 6 μM (and above) of both free surfactants led to cytotoxicity in all the cell types tested. Similarly to results in publication A, the increase in viability and thus in the level of HyA protection was more apparent in cases of higher surfactant concentrations. Surprisingly, HyA protection was observed only in complexes with a lower HyA concentration (5 mg/l), whereas a higher HyA concentration (500 mg/l) had minimal or no protective effect when compared to treatment with free surfactants. Also changes in cell morphology of treated cells observed by light microscopy confiremed cell metabolic activity results.

Interestingly, the higher sensitivity to Septonex (than to CTAB) was evident in fibroblasts and osteoblasts, while keratinocytes were extremly sensitive to CTAB (not to Septonex). It was shown indirectly (by nuclei number determination) that CTAB combines induction in cell metabolism with lethal toxicity, while Septonex predominantly causes lethal toxicity in case of fibroblasts.

Under non-standard conditions, the trend was similar but cytotoxic effect was stronger. Stability of the prepared complexes was also determined and no differences were found out. Finally, complexes of surfactant with high molecular weight HyA (ca 900 kDa) and lower molecular weight HyA (ca 600 kDa) were studied on osteoblasts under standard conditions because it was already demonstrated on similar system that the biological function of HyA depends on its molecular weight (Mizrahy et al., 2011a). However, our results did not confirm this observation.

4.2 PART II: Collagen-Based Scaffolds as Cell Carriers (publications C, manuscript in preparation D)

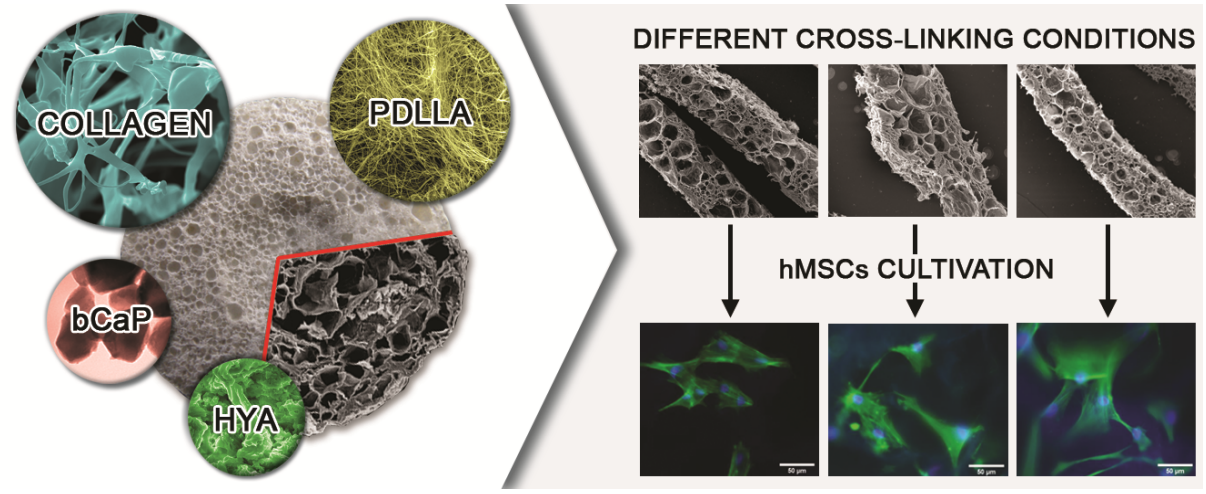

Figure 2: Graphical abstract of second part of PhD thesis (publication C)

The part II of this thesis is focused on *in vitro* evaluation of **collagen based scaffolds** (fish or calf collagen type I matrix reinforced with poly(DL-lactide) sub-micron fibres and supplemented with bioapatite (bCaP) nano-particles and hyaluronic acid) by means of different types of cells (MSCs of different origin and cell line of osteoblasts). Scaffold composition imitates the structure and composition of bone ECM.

While the mechanical stability of collagen scaffolds can be enhanced by crosslinking, the impact of various crosslinking agents (genipin, EDC/NHS/EtOH or EDC/NHS/PBS (Chang et al., 2007; Chen et al., 2005; Ma et al., 2004)) on scaffold based on collagen isolated form fish skin and its colonization by cells was determined *in vitro* (publication C, Figure 2). Initially, hMSCs were cultivated in the 2- and 7- days infusions of the tested scaffolds in order to check the possible release of cytotoxic agents from the individual scaffolds into the cultivation medium. The metabolic activity of the cells treated with these infusions slightly decreased but not bellow the cytotoxic level. Thus, all the infusions were non-cytotoxic after short and long incubation. Then the cells were seeded directly on the scaffolds for 2 and 7 days and their amount was found comparable for all three scaffolds tested at both time points (cell amount reached approximately 50% of control cell amount). The best morphological appearance and symmetrical cell distribution was observed on the genipin cross-linked scaffold. The cells on the EDC/NHS/EtOH and EDC/NHS/PBS crosslinked scaffolds were smaller and had spread to lower extent. However, after 7 days, the cells on all the scaffolds were similarly organised and displayed a comparable appearance. The majority of adhered

cells was observed on the surface of the scaffold and up to a depth of 200 μm (i.e. 10–15% of scaffold depth). In summary, genipin was selected as the best crosslinking agent for developed type of scaffold.

In next step (manuscript in preparation D), two types of scaffolds (S4 and S6, differing in their composition; Figure 3) based on collagen isolated form calf skin were developed. The main focus of their evalution was seeding efficiency of porcine MSCs (pMSCs) with respect to static cultivation conditions (i.e. standardly used way of cultivation) and dynamic cultivation conditions (i.e. cultivation under constant flow of medium).

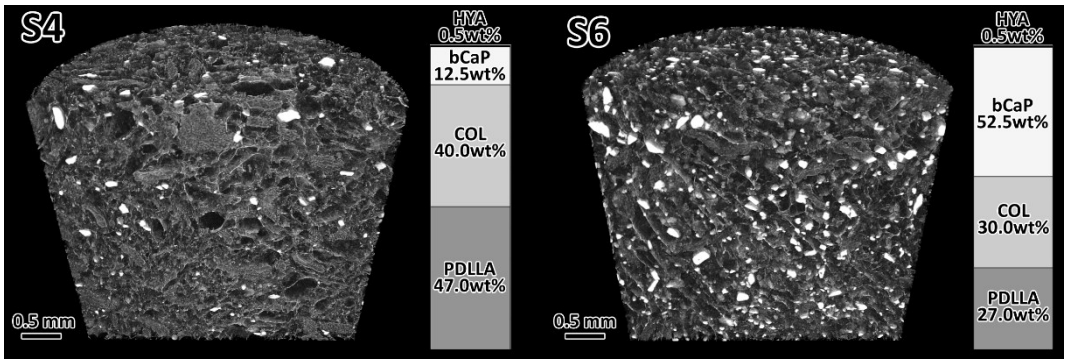

Figure 3: MicroCT images and composition scaffolds S4 and S6. Collagen matrix (COL), PDLLA sub-micron fibres, bCaP (bioapatite) nanoparticles and HyA powder (manuscript in preparation D).

Cell ability to penetrate through the scaffold up to a maximall depth of 300 μm which was observed in samples after dynamic cultivation either in S4 and S6 after 2 days, or in S6 after 7 days. After 7 days of cultivation, we determined general loss of cells in both scaffold in comparison to situation after 2 days. Comparison of both cutivation conditions determined relatively narrow layer of cells on surface of scaffolds cultivated under static conditions and wider layer on surface of scaffolds cultivated under dynamic conditions. Moreover, higher DNA content (but insignificantly) was detected on S4 scaffold in comparison to S6. After 7 days, we detected dramatic decreas of DNA content on both scaffolds (similarly to microscopy observation). More marked decrease was detected in both scaffolds cultivated under static cultivation conditions.

In general, no relevant expression profiles (up-regulation or down- regulation) of cells cultivated on scaffolds were detected by qRT-PC, however we can follow some trend. After 2 days, cells on S4 were up-regulated in expression of most of selected genes in comparison to S6. Interestingly, after 14 days, the situation was opposite. With the respect to osteodifferentiation markers, S4 and S6 were comparable in their expression after 14 days.

Finally, scaffold matrix remodelation by seeded cells after 2 days of cultivation (determination of activity of matrix metalloproteinases (MMP2 and MMP9) by zymography) was examined. In general, the higher activity of MMPs in case of S4 in contrast to S6 was observed.

4.3 PART III: Collagen/Hydroxyapatite Nano-/Micro-structured Resorbable Layers Impregnated by Antibiotics (publications E, manuscript in preparation F)

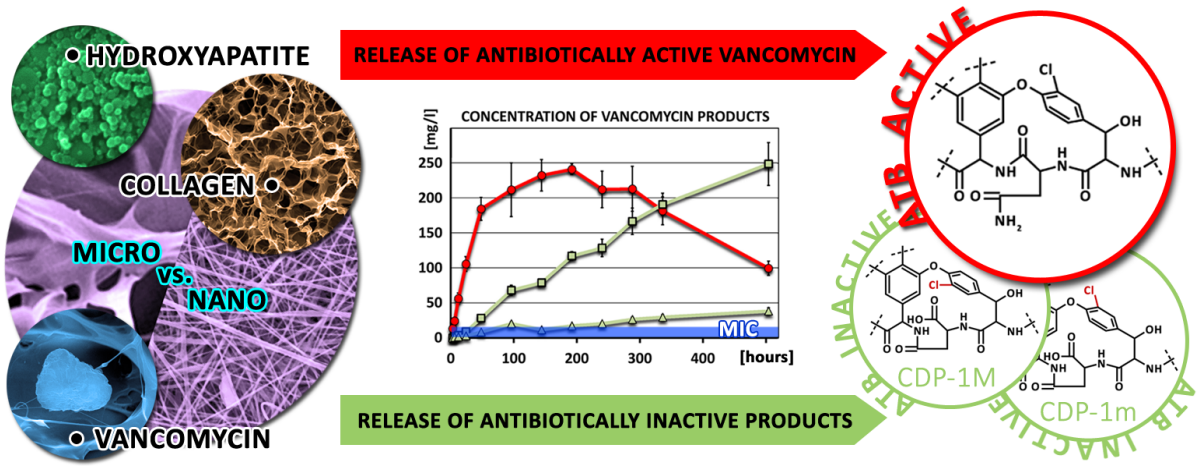

Figure 4: Graphical abstract of third part of PhD thesis

The part III of this thesis focuses on *in vitro* evaluation of collagen/hydroxyapatite nano/micro-structured resorbable layers impregnated by antibiotics (Figure 4). Besides profylaxis of osteomyelitis or its treatment, layers should also play a role as a bone/implant bioactive interface. The composition and structure of layers should mimic bone matrix (collagen I and HA). Electrospun impregnated layers with three various contents of HA (0, 5 and 15 %wt) with 1) 10 %wt of vancomycin; 2) 10 %wt of gentamicin; 3) 10 %wt of vancomycin with gentamicin (1/1 w/w) were analyzed. The *in vitro* evaluation was conducted using osteoblasts (SAOS-2) in direct contact with the layers (for 2 or 8 days) or in their 1-day infusions.

Firstly, metabolic activity of cells incubated in all infusions (1-day) was determined as comparable to control cells. Thus, the cytocompatibility of all the tested infusions was confirmed.

Then the cells were seeded on the layers and after 2 days their amount was found decreased to 1/3 or less of the cell amount on polystyrene control. Simultaneously, values of metabolic activity of all the controls (layers with antibiotic absence) were the highest within every group of layers with the same HA content. Furthermore, increased HA content

proportionaly elevated cell number in all the tested layers. After 8 days of cultivation, the trend similar to 2-days situation was detected; however, the values were generally elevated. Interestingly, values measured after 2 days and 8 days showed limits of HA positive effect in relation to the antibiotic type. While vancomycin seemed to be cytotoxic the least if it was combined with layers containing 15 %wt of HA, gentamicin and vancomycin-gentamicin were cytotoxic the least in layers containing only 5 %wt of HA. These results were confirmed by fluorescence microscopy.

4.4 PART IV: Initial Cell Adhesion of Three Cell Types in the Presence and Absence of Serum Proteins (publication G)

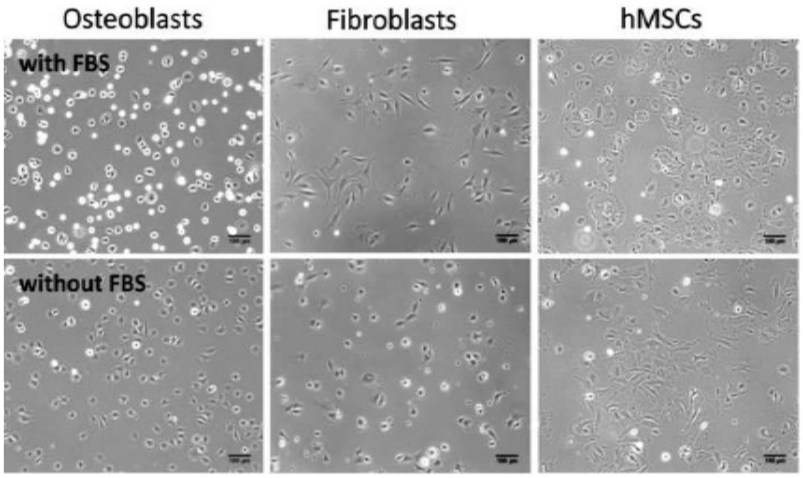

Figure 5: Phase contrast images of osteoblasts, fibroblasts and human mesenchymal stem cells (hMSCs). These cells were cultivated in FBS presence (with FBS) or in FBS absence (without FBS) on tissue culture polystyrene for 2 h.

The last part of the thesis is focused on early phase of adhesion (2 h after cell seeding) of three selected cell types - osteoblastic cell line (SAOS-2), primary human fibroblasts and human mesenchymal stem cells (hMSCs). Differences in adhesion under standard cultivation conditions (FBS presence in medium) and non-standard cultivation conditions (FBS absence in medium) were compared. It was observed that these tested conditions induce significantly different reactions of cell to the substrate. Changes were detected at the morphological level (cell shape and area; Figure 5), proliferation level (cell number) and at the level of expression and localization of various proteins participating in cell adhesion.

With respect to FBS presence, osteoblasts and hMSCs demonstrated similar cell shape and area (round shape and smaller cell area in FBS presence contrary to ragged shape and larger cell area in FBS absence). Interestingly, fibroblasts and hMSCs showed a similar trend in cell number under different FBS conditions (more cells under FBS presence). Moreover, it

was shown that all of three cell types developed classical FAs in FBS presence. In contrast, no classical focal adhesions were observed in FBS absence in all the tested cell types. Moreover, significantly lower level of activated kinases pFAK and pERK1/2 was observed under these conditions. Thus, the development of a compensating signalling pathway in cell adhesion process under FBS absence was indicated.

In addition, the response of osteoblasts cultivated on the surface pre-treated with fibronectin (FN) and vitronectin (VN) in comparison to the surface pre-treated with complete FBS or the surface without proteins was demonstrated. After 2 h, osteoblasts cultivated on proteins showed comparable morphology, while morphology of osteoblasts adhered on naked surface (without proteins) was totally different. Similar situation was observed with FAs presence, those were well developed in protein presence; however, they were very poor in the protein absence. Results strongly reflected the impact of tested proteins on cell behaviour.

5 DISCUSSION

5.1 HyA- surfactant complexes

The key finding of experiments with **colloidal complexes of CTAB or Septonex with hyaluronic acid (HyA)** was the confirmation of predicted HyA protectivity when present in the form of a pre-prepared surfactant-HyA complex. This protectivity phenomenon is well supported by two different studies preformed in the past. The first study confirms our finding by demonstration of HyA protectivity against surfactants, despite the experiments were performed with separated addition of HyA and surfactants to cells (Kalbáčová et al., 2014). The second study shows CD44 mediated entry of HyA coated particles into the cells (Mizrahy et al., 2014).

Next finding was that HyA protectivity is limited by its concentration. Protection was defined only with a lower HyA concentration (5 mg/l), whereas a higher HyA concentration (500 mg/l) had minimal or no protective effect when compared to treatment with free surfactants. The reduction in HyA protection is directly connected to surfactant presence and its concentration, because the treatment with both HyA concentrations in a free form had no effect on cell viability. This finding is supported by Mizrahy, who demonstrated that the quality of HyA and its ability to interact with cells may be significantly altered if it is not free and interacts with other components (e.g. particles coated by HyA)(Mizrahy et al., 2011b).

Moreover, FBS plays a positive role under the stress conditions induced by the presence of surfactant. Simultaneously metabolic activity reflected that FBS absence is generally stressful for cells. These results are in agreement with those from previous studies of our laboratory or other observations (Kalbáčová et al., 2014; Verdánová et al., 2012).

The cell reactions to free surfactants or complexes with HyA were directed by a combination of cell origin and cell sensitivity to the structure of the surfactant and complexes. The dependence of toxicity only on the structure of the surfactant and its subsequent impact on different cell types has already been well described in the past by other authors (Inácio et al., 2011) (Cornelis et al., 1992) (Lee et al., 2000) (Bigliardi et al., 1994). Additionally, the potential of developed complexes also in serum-free systems and the long stability of complexes under *in vitro* conditions was confirmed.

Finally, the impact of molecular weight of HyA on its protectivity was analysed. While high-molecular weight of HyA (10^7 Da) is known as an iniciator of immunosuppressive effects, lower-molecular weight of HyA is highly angiogenic and pro-inflammatory (Aya and Stern, 2014). In general, no observed significant differences in

protectivity between 600 kDa and 900 kDa HyA indicates that HyA protection is determined solely by its concentration and not by its molecular weight in our system. This presumption is supported by Mizrahy et al. (Mizrahy et al., 2011b), who demonstrated a linear relationship between the molecular weight of free HyA and its affinity to CD44 receptor (it means that molecular weight can trigger "HyA-cell" interactions and subsequent transport to the cell).

All results strongly indicate that the system of cationic surfactant with HyA in the complex can be employed for "delivery system" purposes in various biomedical applications.

5.2 Collagen-Based Scaffolds as Cell Carriers

As for the evaluation of composite scaffolds based on collagen isolated form fish skin and crosslinked by various crosslinking agents, the best appearance and symmetrical distribution of cells was obtained on the genipin crosslinked scaffold after 2 days, while cell morphology and organization were comparable on all the scaffolds used after 7 days. The cause of the observed difference after 2 days was most likely due to higher swelling ratio of scaffolds crosslinked with EDC/NHS/EtOH and EDC/NHS/PBS in comparison to genipin crosslinked scaffold. This observation is supported by previous finding that the higher scaffold stiffness is proportional to the higher cell number and more homogenous cellular distribution (Haugh et al., 2011). In addition, material rigidity was shown to determine stem cell fate and cell differentiation (Engler et al., 2006).

Observed disability of the cells to freely penetrate into the scaffold (max. penetration to depth of 200μm) was probably result of the unevenness of the crosslinking, pore size variability (O'Brien et al., 2005), specific collagen sequence/motif accessibility (Xu et al., 2012) or nano-spacing of cell-attractive motifs (Wang et al., 2013). Also low availability of nutrients and gases in depth of the scaffold probably reduced penetration of cells to the scaffold volume (McCoy et al., 2012).

With respect to type of developed scaffold, genipin was selected as the best crosslinking agent and thus was recommended for further advanced *in vitro* (dynamic cultivation) and subsequent *in vivo* studies.

Further, two types of scaffolds (S4 and S6 differing in their composition) based on collagen type I isolated from calf skin cross-linked by EDC/NHS/EtOH were evaluated under static and dynamic conditions. With respect to contemporary trend of 3D/dynamic cultivation conditions in a bioreactor system (allows the control of pH, oxygen content, and temperature (Diederichs et al., 2009)), biological evaluation was primary focused on scaffold colonization cultivation conditions – static versus dynamic.

The majority of adhered cells was observed on the surface of both scaffolds cultivated under both conditions and up to a depth of 300 μm (i.e. 6 % of scaffold depth). These results reflect relatively low ability of the majority of cells to penetrate into scaffold. If we take into account observed reduction of cell amount on both scaffold (provided by DNA content analysis) in time, poor penetration will be probably connected to poor nutrition availability for cells in scaffold volume. Inhomogenity of scaffold often causes hypoxic conditions, which lead to cell death in scaffold volume (Volkmer et al., 2008).

The higher effectivity of scaffold colonization by cells using dynamic cultivation contrary to standard static cultivation was demonstrated in compliance with findings already described by others (e.g. Diederichs et al., 2009; Gerecht-Nir et al., 2004; Schumacher et al., 2010). We also observed positive effect of dynamic cultivation on cell amount that is also in agreement with Schumacher, who detected cell number decrease in the case of static conditions, while dynamic cultivation allowed homogeneous cell growth in scaffold (Schumacher et al., 2010). Results also reflected that S6 compostion supports better cell migration and better nutrient supplement to deeper structures, which can be affected just by higher bCaP content in S6 (Deligianni et al., 2001). The high bCaP content in S6 can also contributes to S6 suitability for cells by reduction of collagen swelling and increase in its compactness and stability caused by CaP-collagen interactions (Huang et al., 2012).

Despite any observed up-regulation of expression in genes involved in differentiation or stemmnes, interesting trends in expression of selected markers were detected. Comparison of 2- and 14-days expression trends indicates that S6 supports up-regulation (statitically insignificant) of expression of all the tested markers with increasing time contrary to S4. It predicts S6 as more suitable scaffold for cells in comparison to S4. Additionaly, S6 suitability is also supported by its high bCaP content, which is known for its osteoinductive effects (Deligianni et al., 2001; Novotna et al., 2014; Prosecká et al., 2015). Moreover, the up-regulation of signaling molecules (ERK1/2 and p38) predicts S6 for long-term suitability. Elevated activity of ERK1/2 signalling was in the past connected to elevated concentration of growth factors (Chen et al., 1992), thus elevated expression of these genese in cells seeded on S6 can reflect that cells are less deprivated on S6 with increasing time in comparsion to S4.

Finally, elevated activity of MMPs detected in supernatants from cells cultivated in static conditions on S4 can reflect impaired penetration of cells to this scaffold under this conditions in contrast to dynamic conditions and S6. This could be explained by typical phenomenon observed in cancer cells that degrade ECM by MMPs with the aim to prepare

the path for tumor cells to migrate, invade and spread to distant areas (Bourboulia and Stetler-Stevenson, 2010).

In summary, with the respect to cell penetration, osteodifferentiation and adaptability, results indicated S6 scaffold as the one more suitable for cell application in bone surgery. In addtition, based on *in vitro* results, the efficiency of dynamic cultivation method for scaffold colonization with cells was confirmed.

5.3 Collagen/Hydroxyapatite Nano/Micro-Structured Resorbable Layers Impregnated by Antibiotics

Tests of infusions of layers impregnated with antibiotics confirmed that these layers are cytocompatible despite the fact that they release ca 800mg/l of vancomycin in a day, which is a lethal dose for tested bacteria strains (publication E). This is in agreement with data describing the local administration of vancomycin to other osteoblastic cells (MG-63), when concentrations of 1 g/l and less had no negative effect on these cells (Edin et al., 1996). In addition, relative safety of vancomycin and its potential for local administration was also confirmed by testing of the wide range of vancomycin doses (0 - 5000 mg/l) in the presence of pre-seeded osteoblasts by Rathbone et al.(Rathbone et al., 2011)

Contrary to infusions, cells seeded directly on layers showed the clear difference in cytotoxicity of both tested antibiotics. While gentamicin impregnated layers dramatically reduced cell metabolic activity and cell amounts at both incubation times, vancomycin impregnated layers seemed to be markedly less cytotoxic. Dramatic cytotoxicity of gentamicin releasing layers can be caused by higher amount of released gentamicin from the layer in comparison to vancomycin (supporting results of colleagues are still in process). Significant cytotoxicity of gentamicin was already described (Negrette-Guzmán et al., 2015; Rathbone et al., 2011).

The positive effect of HA content in a layer on cells and thus HA-induced reduction of cytotoxicity of antibiotics-impregnated layers was demonstrated. HA regulation of cell adhesion, cell spreading morphology and migration was demonstrated in compliance of other authors (Deligianni et al., 2001; Novotna et al., 2014). Moreover, limits of HA positive effects were demonstrated also in relation to the antibiotic type. Whereas, gentamicin and vancomycin-gentamicin impregnated layers provided the best conditions for cells in 5 %wt HA presence, only vancomycin impregnated layers provided the best conditions in the presence of 15 %wt HA.

The cell recovery on layers after 8 days was also demonstrated. This is the next important indicator of cytocompatibility which can predict the osteo-inductive potential of layers.

In summary, it was demonstrated that bone-producing cells can survive on these layers (with exception of gentamicin impregnated layers with 0 %wt and 15 %wt HA content) and that their behaviour depends on the type of antibiotic used together with the degree of layer mineralisation. From the obtained results layer impregnated with vancomycin containing 15 %wt of HA was recommended as the best candidate for subsequent *in vivo* evaluation and for bone surgery application.

5.4 Initial Cell Adhesion of Three Cell Types in the Presence and Absence of Serum Proteins

Osteoblasts, primary fibroblasts and hMSCs confirmed that the various cells can react differently to the presence or absence of FBS on the substrate surface with respect to cell shape, area and number. It was concluded that the differing cell reactions were connected to the origin of the cells. Osteosarcoma cell line (SAOS-2) is generally used model (Rodan et al., 1987) and possesses certain characteristic features (Pautke et al., 2004) that may differ from those of primary and healthy cells such as primary human dermal fibroblasts and hMSCs.

In contrast to changes in morphology and cell amount, the expression and localization of proteins of FAs didn´t show significant differences at the level of cell types. Determining are only various cultivation conditions. In comparison to situation in FBS presence, no classical FAs were observed under non-standard conditions (FBS absence). Moreover, lower levels of signalling molecules (activated kinases pFAK and pERK1/2) were observed under FBS absence. With respect to decrease in expression and localization of both kinases (Saoncella et al., 1999), we suggest that cells use alternative signalling pathway for adhesion process under stress conditions (such as FBS absence). Thus in agreement with research of others, we showed the key signaling role of ERK1/2 and FAK kinases in standard cell adhesion process.

Moreover, we compared cell adhesion on FBS, preadsorbed FN and VN and naked surfaces and found out important participation of FN and VN in adhesion process and thus the impact of protein presence on the rate of cell survival and morphology.

In summary, these results demonstrated the importance of initial protein layer in cell adhesion process (Anselme et al., 2012) and also crutial role of matrix composition and its

dominant impact on cell morphology (Kim et al., 1992; Salasznyk et al., 2004; Walters and Gentleman, 2015).

6 CONCLUSION

I. The response of cells to **colloidal complexes of CTAB or Septonex with hyaluronic acid** with the respect to fetal bovine serum presence or absence during cultivation was characterized. In general, HyA protection ability with respect to cationic surfactant-induced cytotoxicity was confirmed. A detailed definition of the limits of HyA protection in general was provided. In addition, the positive effect of FBS on cells under stress conditions (surfactant presence) was revealed. The cell protective function of HyA in complexes containing surfactants under non-standard conditions was also confirmed, thus the potential of the use of the complexes in serum-free systems was verified. These results strongly indicated the potential of cationic surfactant-HyA complex systems as delivery system in various biomedical applications.

II. **Collagen based scaffolds** with suitability for stem cell therapy in bone surgery application *in vitro* by using MSCs derived from different organisms were evaluated.

Firstly, different crosslinking agents were evaluated aiming at determination of their effect on cytotoxicity and adhesion, proliferation and penetration of cells into scaffolds. Genipin was found to be the most effective crosslinking agent improving biocompatible properties of scaffolds based on collagen isolated from fish skin.

Secondly, two types of developed collagen-based scaffolds (based on calf skin collagen and with different composition) were evaluated by advanced methods with the focus on static and dynamic cultivation of the cells. Effectivity of both cultivation conditions was determined with the respect to cell colonization and penetration into the scaffold depth and also with respect to potential application of scaffolds for cell therapy. The higher effectivity of dynamic cultivation in comparison to static cultivation was confirmed. The scaffold with the higher content of bCaP evinced better cell colonization and penetration into scaffold depth, and thus it was selected for futher biological *in vivo* evaluation.

III. C**ollagen/hydroxyapatite nano/micro-structured resorbable layers with controlled elution of antibiotics** (vancomycin, gentamicin and mixture of both) were evaluated under *in vitro* conditions. Vancomycin impregnated layer with 15 %wt content of HA, vancomycin/gentamicin impregnated layer with 5 %wt content of HA and

gentamicin impregnated layer with 5 %wt content of HA evinced the highest rates of cytocompatibility, metabolic activity and the best morphological parameters. It was shown that bone-producing cells can survive on all the layers used with exception of some gentamicin impregnated layer layers, which were apparently cytotoxic for osteoblasts. Interestingly, it was demonstrated that antibiotic impregnation together with the degree of HA content participate in resulting cell number and affect cell metabolic activity. Vancomycin impregnated layer with 15 %wt HA provided such doses of vancomycin that exerted the lowest negative effect on bone-like cell behaviour and on the other hand ensured sufficient antibacterial activity. Thus, this layer was recommended as the best candidate for subsequent *in vivo* evaluation.

IV. An **early phase of cell adhesion** of osteosarcoma cell line SAOS-2, primary human fibroblasts and hMSCs in the context of FBS presence or absence in the cultivation medium was described. Generally, it was demonstrated that cell adhesion mediated by FBS proteins differs greatly from "direct" cell adhesion on protein-free surface. The diverse intensity and localization of membrane, signalling and FAs structural proteins involved in cell adhesion were observed. With the respect to all three tested cell types, it was found out that no classic focal adhesions were formed during cell adhesion in the absence of FBS proteins. The alternative signalisation of cells under stress conditions (FBS absence) was further outlined. For the first time, the cell-substrate contact in the absence of serum proteins for anchorage-dependent cells was described in detail.

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8 COMPLETE LIST OF AUTHOR'S PUBLICATIONS AND MANUSCRIPTS IN PREPARATION

PART I

A. **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 = 2.752

B. **Pavla Sauerová,** Tereza Pilgrová, Miloslav Pekař and Marie Hubálek Kalbáčová (2017): **Hyaluronic Acid in Complexes with Surfactants: The Efficient Tool for Reduction of the Cytotoxic Effect of Surfactants on Human Cell Types.** Journal of Biological Macromolecules – accepted for publication. IF = 3.138

PART II

C. Tomáš Suchý, Monika Šupová, **Pavla Sauerová**, Martina Verdánová, Zbyněk Sucharda, Šárka Rýglová, Margit Žaloudková, Radek Sedláček and 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 = 3.697

D. **Pavla Sauerová**, Tomáš Suchý, Monika Šupová, Zbyněk Sucharda, Šárka Rýglová, Margit Žaloudková, Tereza Kubíková, Zbyněk Tonar, Martin Bartoš, Jana Juhásová, Štefan Juhás, Jiří Klíma and Marie Hubálek Kalbáčová (2017): **Comparison of Seeding Efficiency on the Biodegradable Scaffolds of Different Composition and Cultivation Conditions.** Manuscript in preparation

PART III

E. Tomáš Suchý, Monika Šupová, Eva Klapková; Václava Adámková, Jan Závora,Margit Žaloudková, Šárka Rýglová, Rastislav Ballay, František Denk, Marek Pokorný, **Pavla Sauerová**, Marie Hubálek Kalbáčová, Lukáš Horný, Jan Veselý, Tereza Voňavková, Richard Průša (2017): **The Release Kinetics, Antimicrobial Activity and Cytocompatibility of Differently Prepared Collagen/Hydroxyapatite/Vancomycin Layers: Microstructure vs. Nanostructure.** European Journal of Pharmaceutical Sciences 100, 219-229. IF = 3.77

F. Tomáš Suchý, Monika Šupová; Eva Klapková; Václava Adámková; Jan Závora; Margit Žaloudková; Šárka Rýglová; Rastislav Ballay; František Denk; Marek Pokorný; **Pavla Sauerová**; Marie Hubálek Kalbáčová; Lukáš Horný; Jan Veselý; Tereza Voňavková; Richard Průša (2017): **Evaluation of Collagen/Hydroxyapatite Layers Impregnated by Different Antibiotics – Novel Potential Local Drug Delivery for Bone Surgery**. Manuscript in preparation

PART IV

G. Martina Verdánová, **Pavla Sauerová**, Ute Hempel, Marie Hubálek Kalbáčová (2017): **Initial Cell Adhesion of Three Cell Types in the Presence and Absence of Serum Proteins.** Histochemistry and Cell biology 147 (5), published online. IF = 2.78

Mentioned accepted publication are included in full form in this thesis.

Tomáš Suchý, Monika Šupová, Martin Bartoš, Radek Sedláček, Marco Piola, Monica Soncini, Gianfranco Beniamino Fiore, **Pavla Sauerová**, Marie Hubálek Kalbáčová (2017): **Dry versus hydrated collagen scaffolds: are dry states representative of hydrated states. Biomedical Materials** – submitted.

9 CURRICULUM VITAE

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2013 – present: PhD study, Faculty of Medicine in Pilsen, Charles University, Field of study: Anatomy, Histology and Embryology, Group: Laboratory of Interaction of Cells with Nanomaterials (group leader: doc. RNDr. Marie Hubálek Kalbáčová, Ph.D)

2011-2013: 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:

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2013 – present: Researcher, Biomedical Center of Faculty of Medicine in Pilsen, Charles University and Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University in Prague

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10 LIST OF ATTENDED CONFERENCES WITH CONTRIBUTIONS

Studentská vědecká konference LF v Plzni 2014, Plzeň, Czech Republic

„Kyselina hyaluronová jako užitečný nástroj pro zmírnění cytotoxického vlivu surfaktantů“
P. Sauerová , M. Verdánová , F. Mravec, T. Pilgrová , T. venerová, M. Pekař a M. Hubálek Kalbáčová
LECTURE

Biomateriály a jejich povrchy VII, 16.-19. září 2014, Herbertov – Horní Mlýn, Czech Republic

Lecture: 1.část: **“Kyselina hyaluronová a její různé využití v biomedicínských aplikacích“**
P. Sauerová , M. Verdánová , F. Mravec, T. Pilgrová , T. Venerová, M. Pekař a M. Hubálek Kalbáčová
LECTURE
2.část: **“Biologické testovánínanokompozitních nosičů pro inženýrství kostní tkáně (pilotní studie)“**
P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
LECTURE

Stem Cells and Cell therapy: From research to modern clinical application, 22. - 24.října 2014, Černá Hora, Brno, Czech Republic

„Hyaluronic acid can reduce surfactant cytotoxocity“
P. Sauerová , M. Verdánová , F. Mravec, T. Pilgrová , T. Venerová, M. Pekař a M. Hubálek Kalbáčová
POSTER

Studentská vědecká konference LF v Plzni 2015, Plzeň, Czech Republic

„Interakce mezenchymálních kmenových buněk s kolagenovými nosiči síťovanými různými látkami“
P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
LECTURE

5th International Symposium Interface Biology of Implants 2015, 6.-8.5. 2015, Warnemünde, Germany

„Interactions of Mesenchymal Stem Cells with Collagen-based Scaffolds Cross-linked by EDC/NHS or Genipin“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
POSTER

27th European Conference on Biomaterials, 30.8.-4.92015, Kraków, Poland

„Cultivation of Mesenchymal Stem Cells on Collagen-based Scaffolds Cross-linked by EDC/NHS or Genipin“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
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Biomateriály a jejich povrchy VIII, 15.-18.9. září 2015, Herbertov – Horní Mlýn, Czech Republic

„Interakce mezenchymálních kmenových buněk s kolagenovými nosiči síťovanými různými látkami“

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LECTURE

12th International Medical Postgraduate Conference, 26.-28.11. 2015 Hradec Králové, Czech Republic

„**Development and *in vitro* testing of new composite materials for bone surgery applications“**

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
LECTURE

Studentská vědecká konference LF v Plzni 2016, Plzeň, Czech Republic

„Regulace cytotoxického vlivu surfaktantů pomocí kyseliny hyaluronové“

P. Sauerová, M. Verdánová, F. Mravec, T. Pilgrová, M. Pekař a M. Hubálek Kalbáčová

LECTURE

BioNanoMed 2016, 6.-8-4.2016, Krems, Austria

„Analysis of Mesenchymal Stem Cell Interactions with Cross-Linked Collagen-Based Scaffolds“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, **M. Hubálek Kalbáčová**
LECTURE

Termis EU 2016, 27.6. – 1.7.2016, Uppsala, Sweden

„In vitro analysis of mesenchymal stem cell interactions with collagen-based scaffolds cross-linked by EDC/NHS or genipin“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Hubálek Kalbáčová
LECTURE

Biomateriály a jejich povrchy IX, 20..-23.9.2016, Herbertov – Horní Mlýn, Czech Republic
„Studium interakce buněk s biomateriály na bázi kolagenu“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Bartoš, M. Hubálek Kalbáčová
LECTURE

Studentská vědecká konference LF v Plzni 2017, Plzeň, Czech Republic

„ In vitro hodnocení resorbovatelné kolagenové nanovrstvy s řízenou elucí antibiotik“

P. Sauerova, M. Verdánová, T. Suchý, M. Šupová, S. Rýglová, M. Žaloudková, Z. Sucharda, M. Bartoš, M. Hubálek Kalbáčová
LECTURE