

# NEWGEN Zlín

## Workshop and WG Meeting

On

# Hydrogel/ Biomaterialized Biomaterial for Bone Tissue Regeneration



15th - 16th November 2016

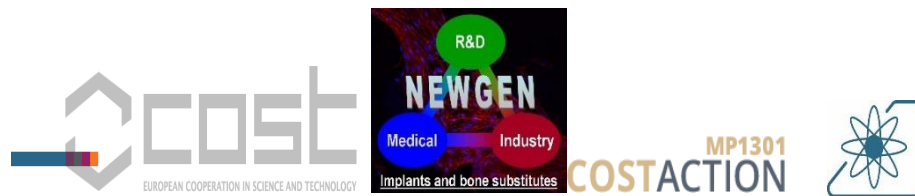
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Tomas Bata University in Zlín

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# **COST Actions Materials, Physics and Nanosciences (MPNS) MP1301 (MPNS COST Action MP1301)**

## **New Generation Biomimetic and Customized Implants for Bone Engineering**

### **General Information**

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[Dr Paola PALMERO](#) (IT)

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[Dr Mónica PÉREZ-CABERO](#)

#### **Administrative officer of the Action:**

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# Scientific Working Group

## Working Group 1 (WG-1)

Hydrogel Scaffolds / Biomaterials

## Working Group 2 (WG-2)

Bone Tissue Regeneration / Engineering

## Working Group 3 (WG-3)

Drug Delivery

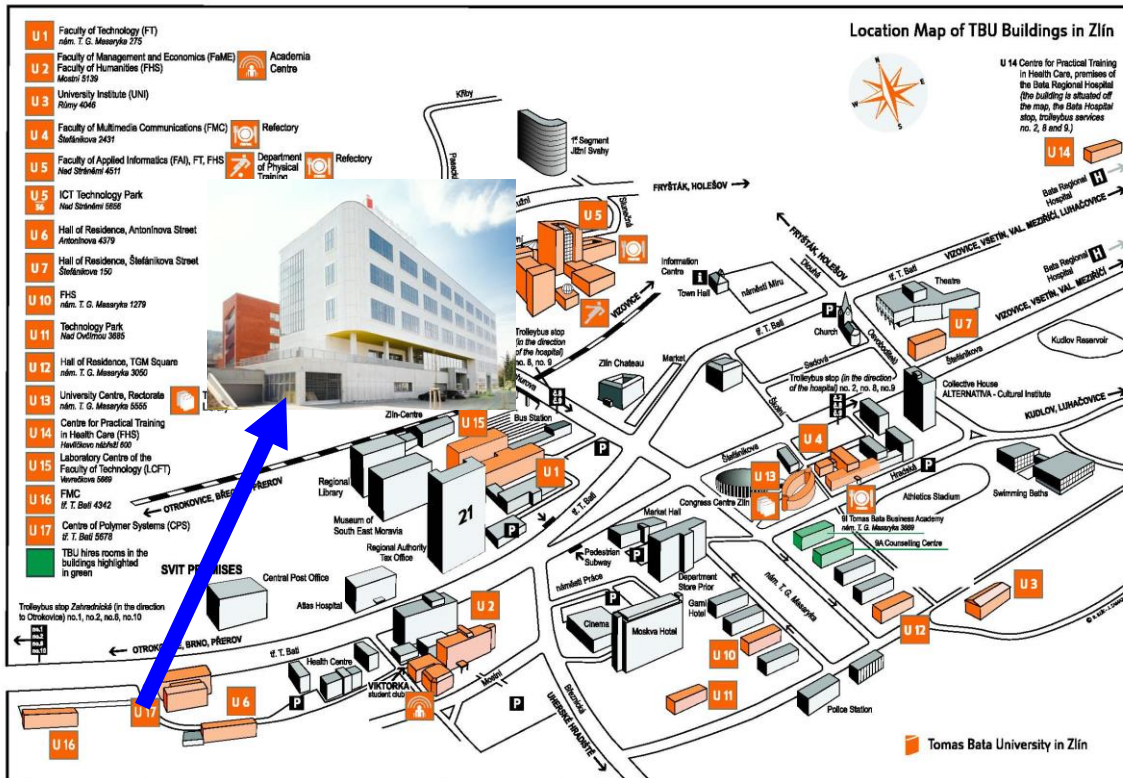
## Working Group 4 (WG-4)

In vitro and in vivo tests

# Workshop Venue

## Address

Centre of Polymer Systems  
 Trida Tomase Bati 5678  
 760 01 Zlín  
<http://newgen.utb.cz/>



## Hydrogel/Biomaterialized Biomaterial for Bone Tissue Regeneration

### Scientific Program

The preliminary schedule for MP-1301-Workshop and WG meeting:

#### 1st Day - 15 November 2016, Tuesday

- 08.30-09.00: Registration of the participants in the Workshop  
 09.00-09.05: Welcome from Rector, TBU in Zlín (Prof. Petr Saha)  
 09.05-09.30: Welcome from the MP-1301\_COST Action coordinator (Dr. Francis Cambier)  
 09.30-10.00: Invited Talk (Assoc. Prof. Radostina Alexandrova, Bulgaria)  
 10.00-10.30: **Coffee break and POSTER Session**  
 10.30-11.00: Invited Talk (Prof. Yannis Missirlis, Greece)  
**11.00-12.00: Technical Session**  
 11.00-11.20: David C Bassett (Norway)  
 11.20-11.40: Hai jun Xiao (Czech Republic)  
 11.40-12.00: Smarak Bandopadhyay (Czech Republic)  
 12.00-12.15: NEWGEN meeting in Greece in 2017 (Prof. Yannis Missirlis)  
 12.30-13.30: **Lunch**  
 13.30-14.00: CPS laboratory visit  
 14.00-14.30: Planery Talk (Prof. Jaroslav Cihlar, Brno, Czech Republic)  
**14.00-15.00: Technical Session**  
 14.00-14.20: Karine Salim (France)  
 14.20-14.40: Antonio Di Martino (Czech Republic)  
 14.40-15.00: Probal Basu (Czech Republic)  
 15.00-15:40: **Coffee break and POSTER Session [Technical Poster Session]**  
**15.40-16.20: Technical Session**  
 15.40-16.00: Bojana Obradovic (Serbia)  
 16.00-16.20: Alena Pavelkova (Czech Republic)  
 16.20-17.00: Working Group meeting (WG-1, WG-2, WG-3 and WG-4)  
 17.00-19.00: end of 1st day [Resting period]  
 19.00h: **Workshop Dinner**





## Hydrogel/Biomaterialized Biomaterial for Bone Tissue Regeneration

### Scientific Program

The preliminary schedule for MP-1301-Workshop and WG meeting:

#### 2nd Day – 16 November 2016, Wednesday

08.30-09.00 : Registration of the participants in the Workshop

09.00- 09.30: Invited Talk (Prof. Martijn van Grienvan, Germany)

09.30-10.00: Invited Talk (Dr. Stephe Scheiner, Austria)

10.00-10.30: **Coffee break and POSTER Session**

#### **10.30-11.30: Technical Session**

10.30-10.50: Silvia Ramirez (France)

10.50-11.10: Filatova Katerina (Czech Republic)

11.10-11.30: Ayan Ray (Czech Republic)

11.30-11.45: NEWGEN meeting in Vienna in 2017 (Dr. Stephan Scheiner)

11.45-12.00: Vote of Thanks (Assoc. Prof. Vladimír Sedlařík)

12.30-13.30: **Lunch**

#### **13.30-15:30: Visit of Bata Museum and Skyscraper (21) Building**

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	Oral Presentation
	Poster Presentation

## SEARCHING FOR NEW MATERIALS FOR BONE IMPLANTS: CHALLENGES AND SOLUTIONS OF IN VITRO BIOCOMPATIBILITY ASSESSMENT

Radostina Alexandrova<sup>1\*</sup>, Tanya Zhivkova<sup>1</sup>, Lora Dyakova<sup>2</sup>, Boyka-Andonova-Lilova<sup>1</sup>, Abedulkadir Abudalleh<sup>1</sup>, Milena Georgieva<sup>3</sup>, Georgi Miloshev<sup>3</sup>, Nabanita Saha<sup>4</sup>, Diana Rabadjieva<sup>5</sup>, Stefka Tepavitcharova<sup>5</sup>

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### Abstract

Improving the quality of life is a basic priority in EU policies. Among the diseases with most serious clinical consequences and economic burden are these associated with bone defects.

Due to the disadvantages and limitations of autografts (e.g. limited availability, additional surgical trauma) that are still considered as clinical "gold" standard and allografts (limited availability, foreign body immune reactions, risk of infections) there is a growing need for the development of new artificial bone substitute materials with improved physicochemical, mechanical and biological properties as close as possible to those of natural bone, that has to be substituted.

The aim of our study was to evaluate the influence of ion-modified calcium phosphates and brushite bone cements based on amorphous calcium phosphate and tartaric acid on cell viability and proliferation.

The following cell cultures were used as model systems: i) human (Lep3, MRC5, BJ) and mouse (BALB/c 3T3, L929) fibroblasts; ii) mouse bone marrow cells; iii) primary cultures from mouse bone explants; iv) some human permanent cancer cell lines. The investigations were performed by direct (the cells were cultured on the material's surface) and indirect (cells cultured in material's "extracts") experiments using methods such as MTT test (the "Gold" standard for cytotoxicity evaluation), neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique; double staining with acridine orange and propidium iodide; single cell gel electrophoresis (Comet assay), light and electron microscopy.

The results obtained reveal that the application of the above mentioned complex approach based on methods with different molecular/cellular targets and mechanisms of action and various experimental models (each one with its own advantages and limitations) is suitable and highly informative for in vitro biocompatibility assessment of new materials for bone implants. Although the preliminary data obtained for some of the examined materials are promising, additional investigations are required to clarify better their biocompatibility, osteoconductivity and osteoinductivity

### Acknowledgement

This work was supported by Fund "Scientific Research", Bulgarian Ministry of Education and Science (Grunt № T 02 5/12.12.2014 and Grant № B 02 30/12.12.2014) and by COST Action MP 1301.



## BIOMATERIALS' ELICITED MESSAGES INFLUENCE EPIGENETICS

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### Abstract

All living entities, including cells, respond to their environmental cues in ways that we only slightly know. In tissue engineering applications cells interrogate their environment, consisting of chemical, topographical and other physical cues, like mechanical signals. Evidence is being built up slowly that external mechanical signalling to cells are transmitted all the way to the chromatin, where they deconvolute the highly convoluted structure to facilitate relevant biochemical reactions that result in changes of the cell's epigenome. Scant evidence to this phenomenon will be presented.

## STRUCTURALLY AND FUNCTIONALLY GRADED BIOCOMPOSITES AND SCAFFOLDS BASED ON CA-PHOSPHATES FOR BONE TISSUE ENGINEERING

Cihlar J. \*, Drdlik D., Novotna L., Castkova K., Cihlar J. jr,  
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### Abstract

This contribution presents some results of a study of phase composition, morphology, mechanical and biological properties of functionally gradient composites and scaffolds in particular based on Ca-phosphate modified by doped zirconia or silica obtained during solution the project LD14072 (MEYS CZ) involved in the COST Action MP1301.

- A. The influence of the grain size of alumina and zirconia ceramics on the spreading and adhesion of MG63 cell lines was first investigated. The highest cell spreading and adhesion was obtained for ZrO<sub>2</sub> ceramics with an average grain size of 100 nm. The cell selection was observed on layered ZrO<sub>2</sub>/Al<sub>2</sub>O<sub>3</sub> composites. The cells predominantly adhered to ZrO<sub>2</sub> layers. The results showed a positive influence of nanostructured ceramic surfaces on biological behaviour of MG63 cells.
- B. Mixtures of nanoparticles of hydroxyapatite nanoparticles ZrO<sub>2</sub> doped with CaO and Y<sub>2</sub>O<sub>3</sub> were prepared by precipitation synthesis and hydrothermal treatment. Analysis of phase composition of the sintered ceramic composites showed decomposition of hydroxyapatite to tricalcium phosphates and partial transformation of ZrO<sub>2</sub> to CaZrO<sub>3</sub>. Modulus of elasticity and strength of sintered HA and ZrO<sub>2</sub> composites were affected by porosity. The bioactivity of HA/Ca-ZrO<sub>2</sub> composites was higher than HA/Y-ZrO<sub>2</sub> ones.
- C. Electrophoretic deposition was used for preparation of composite layer containing HA and microfibers of tetragonal ZrO<sub>2</sub>. During sintering occurred decay of HA to  $\alpha$ -TCP,  $\beta$ -TCP and calcium phosphate partially substituted with Li<sup>+</sup> cations. The fracture toughness of the composites was twice as high as toughness of pure HA due to oriented t-ZrO<sub>2</sub> microfibers, finer microstructure, tough  $\beta$ -TCP phase and pores presented in the composites. Composites showed bioactive behavior.
- D. Porous composite scaffolds consisted of bioinert alumina toughened zirconia (ATZ) core and bioactive surface layer based on Ca-phosphates. The core was prepared by replication technique and the surface layer by dip-coating. Maximum strength had scaffolds with the core from 95 wt.% ZrO<sub>2</sub> and 5 wt.% Al<sub>2</sub>O<sub>3</sub>. When tested in simulated body fluid an apatite layer formed on coated scaffold surfaces and none of composite materials had cytotoxic behavior when tested with a cell line MG63.
- E. The effect of silica on the mechanical and biological properties of the macroporous bioceramic scaffolds based on calcium phosphate and SiO<sub>2</sub> prepared by replication technique was studied. During sintering was observed a decomposition of hydroxyapatite to alpha and beta tricalcium phosphates partially substituted by silicate ions. The presence of cristobalite SiO<sub>2</sub> phase had positive effect on the compressive strength of scaffolds, which reached up to 30 MPa. In the course of test of scaffolds in simulated body fluid the apatite layer formed on the scaffold surfaces containing 0 to 20% cristobalite. In vitro tests using stem cells (ADSC) indicated that cells were viable and TCP/SiO<sub>2</sub> composite was bioactive.
- F. When solving the project the methods of shaping of bioceramic layers and scaffolds were inovated: EPD method, template method and the method of in situ foaming. Currently, was launched research into 3D printing of bioceramic scaffolds using LCM technology on the device CeraFab 7500 (Lithoz), into which we invite our colleagues from Action NewGen.



## **(PRE-)CLINICAL BONE ENGINEERING ALSO TAKING CO-MORBIDITIES INTO ACCOUNT**

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### **Abstract**

For bone engineering, constructs of biomaterials, cells and growth factors are most promising. Biomaterials can be of natural sources, synthetic sources, and composites. The cells used should be autologous, have the potential to proliferate and to differentiate. Stem cells of different sources can be obtained, such as from adipose tissue, bone marrow, umbilical cord and amniotic membrane. The age of the patient plays an important role for the healing success. Moreover, underlying diseases such as osteoporosis also influence the outcome. Stem cells from different sources were tested. Importantly, stem cells from young versus old donors were tested regarding their viability, ability to adhere to different biomaterials and ability to differentiate. Stem cells from young donors have a higher proliferation rate, are more viable and show more pronounced osteogenic differentiation as measured by alkaline phosphatase activity and mineral deposition. qPCR measurements partly confirmed these findings. Differentiation can be influenced by pulsed electromagnetic fields applied for short time during several days in an interval manner. Specific pulsed electromagnetic fields were able to enhance proliferation, alkaline phosphatase activity and mineral deposition of mesenchymal stem cells, thereby enhancing the chance of success of osteogenic constructs. Another possibility for influencing osteogenesis may be miRNA. miRNA signatures from osteoporotic patients were determined using miRNA arrays and validated using specific primers. Osteoporotic patients showed 11 miRNA that were upregulated in serum and 5 of them also in bone tissue. Modifying miRNA expression with antagomirs influences the osteogenic potential of stem cells positively. Thus, blocking specific miRNAs may enhance osteogenic differentiation of stem cells on biomaterials. Underlying facts such as age, gender and disease specific miRNA signatures may influence the interaction of stem cells with biomaterials and their ability to differentiate. First clinical cases showed enhanced bone healing. This urges us to continue our efforts in the field of bone engineering in order to improve the quality of care for patients

## STIFFNESS AND STRENGTH PREDICTION FOR A HYDROXYAPATITE-BASED BIOMATERIAL, CONSIDERING BONE REGENERATION

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### Abstract

Bone replacement materials must fulfill various requirements, such as biocompatibility and appropriate mechanical properties. In particular, the latter include suitable stiffness and sufficient strength. Finding such so-called biomaterials with adequate mechanical properties based on experimental trial-and-error approaches is cumbersome and ineffective, thus mathematical is considered a promising complement. Here, a hydroxyapatite-based, granular biomaterial, developed as bone replacement material with the human mandible as targeted application region, is studied. This material exhibits a distinctive hierarchical organization, with different types of pore spaces of characteristic lengths ranging from nanometers to millimeters. Additionally, once exposed to the targeted physiological environment (i.e. the immediate vicinity of mandibular bone tissue), new bone tissue forms around the biomaterial granules, whereas the hydroxyapatite crystals undergo resorption. The presented work aims at estimation of both stiffness and strength of this material, taking into account the compositional changes due to bone ingrowth and hydroxyapatite resorption. For this purpose, micromechanical homogenization techniques are employed, yielding a three-step stiffness homogenization scheme, giving access to the macroscopic stiffness tensor of the scaffold material. On the other hand, the micromechanical model is extended by a failure criterion relating to the hydroxyapatite needles, based on which the loading type-specific macroscopic strength of the scaffold material can be deduced. The above-sketched micromechanical model gives access to stiffness and strength estimates for the scaffold material, as functions of various material and design parameters of the material, including the macroporosity, the granule size, the density of cracks, as well as the rates of bone formation and scaffold resorption. The presented modelling approach provides insights beneficial both for the scaffold material design process and for a clinician actually applying such material. Namely, the aforementioned material and design parameters could be tuned based on model predictions, to optimize the mechanical properties of the scaffold material. Complementing this way the experimental trial-and-error strategy that, in this respect, still is the gold standard, would lead to a significant improvement of the design process.

## CONTROL OF GELLING KINETICS IN IONOTROPIC POLYMERS APPLIED TO 3D CELL FRIENDLY ENCAPSULATION

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### Abstract

Hydrogels are a hugely important class of biomaterial. Interest from the biomedical materials field originates from the ability of hydrogels, derived from both natural and synthetic origins, to inherently or be engineered to mimic physicochemical aspects of the extracellular matrix.

Polymers forming hydrogels must be stabilised in a continuous aqueous phase using physical and/or chemical crosslinks. This may be achieved via strong covalent crosslinks or physical networks containing transient, reversible junctions or entanglements based on e.g. ionic and hydrogen bonding or hydrophobic interactions. Of these types, ionic crosslinking offers a convenient route to gelation, without recourse to covalently modify the polymer, and many biocompatible polyelectrolyte systems, such as alginate, pectin, gellan and carrageenan, can be cross-linked under physiological or near physiological conditions. However, ionic gelling kinetics are difficult to control since they are governed by the intrinsic interactions between the ionotropic polymer and the gelling ion and ionic diffusion in water. Attempts to slow down the crosslinking process using retardants, or solid reservoirs of ionic crosslinking agents have been shown to be effective for limited applications, but problems such as inappropriate gelation times or non-cytocompatible conditions often arise.<sup>[1]</sup>

In this presentation, control of ionic crosslinking via a novel approach based on tuning the availability of the gelling ions will be described. This approach enables the preparation of two stable polymer solutions which upon mixing react to form a crosslinked hydrogel.<sup>[1]</sup> The time course over which this process occurs can be tuned from a few seconds to tens of minutes, thus representing a highly versatile system. All reactants are contained in the aqueous state and cell toxicity studies indicate that this approach is highly cytocompatible. Therefore this has particular utility to otherwise challenging applications in biocompatible 3D soft material structuring using, for example, microfluidics and additive manufacturing. By way of demonstration of this, high viability cell encapsulation using this novel method via microfluidic generated droplets will be presented.<sup>[2]</sup>

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## **EFFECTS OF X-SHAPED REDUCTION-SENSITIVE AMPHIPHILIC BLOCK COPOLYMER ON DRUG DELIVERY**

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### **Abstract**

To study the effects of X-shaped amphiphilic block copolymers on delivery and the reduction-sensitive property on drug release, a novel reduction-sensitive amphiphilic copolymer with a Gemini-like X shape, (PLGA)<sub>2</sub>-SS-4-arm-PEG2000, was successfully synthesised. The formation of micelles was proved with respect to the blue shift of the emission fluorescence as well as the increase of fluorescent intensity of coumarin 6 loaded particles. Smaller critical micellar concentration (CMC), smaller size and higher micellar stability were observed on X-shaped polymer nanomicelles (XNMs). The reduction sensitivity of polymers was confirmed by the increase of micellar sizes as well as the in vitro drug release profile. Cytotoxicity assays in vitro revealed that the blank XNMs were nontoxic against A2780 cells up to a concentration of 50 µg/mL, displaying good biocompatibility. DTX loaded X-shaped polymer nanomicelles (DTX/XNMs) were more toxic against A2780 cells than other formulations in both dose- and time-dependent manners. Cellular uptake assay displayed a higher intracellular drug delivery efficiency of XNMs than that of nanomicelles prepared with linear polymers. Besides, the promotion of tubulin polymerisation induced by DTX was visualised by immunofluorescence analysis. Therefore, this X-shaped reduction-sensitive block copolymer based on PLGA and PEG could effectively improve the micellar stability and significantly enhance the therapeutic efficacy of DTX by increasing the cellular uptake and selectively accelerating the drug release inside cancer cells.

## “BACTERIAL CELLULOSE” FROM APPLE JUICE MEDIUM: A SUSTAINABLE RAW MATERIAL FOR HYDROGEL

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### Abstract

In this study, our approach is to establish an inexpensive nutrient medium for optimal production of bacterial cellulose<sup>1</sup>. In the Europe, apple production is usually high and was the highest produced fruit in 2015. But, enormous amount of non-edible apples are wasted every year in Czech Republic and Europe annually due to sour taste or high acidic content<sup>2</sup>. Hence, emphasis has been given on “Apple” to launch it as one of the cheap resource of nutritive medium for the production of Bacterial Cellulose (BC); a sustainable raw material for Hydrogel which has synergistic effect in Hydrogel formation. BC production was conducted at 30°C, incubated for 15 days under static state using 250 ml conical flask where, 100 ml sterile apple juice (pH 3.8-4.3), was used to supply nutrient for bacterial culture *Gluconobacter xylinum* (CCM 3611T) and production of BC. This nature based bio-synthesized biopolymer (BC) was harvested after 15 days and washed with 0.5N NaOH following the standard method. A sustainable amount of BC was achieved from the Apple Juice gala medium (AJM). About 4.741gm in weight and 3.3mm in thickness, white; BC membrane was obtained. Moreover, the amount of production is better in comparison to the HS medium where the mat has an average weight of 1.590gm. It is observed that the cellulose mat has a uniform physical appearance in both “HS medium” and “AJM”. In conclusion, “AJM” can be considered as a value added and inexpensive substrate for BC production. The achieved BC can be considered as a cost-effective raw material/bio-material for the preparation of “Hydrogel”, an elixir medical device / scaffold for bone tissue regeneration.

### Acknowledgements

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## SYNTHETIC COPOLYMERS FOR BIOMEDICAL NEEDS: FROM RESORBABLE TO PERMANENT BIOMATERIALS

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### Abstract

Poly(lactic) acids and poly(lactic-co-glycolic) acids have been of increased interest during the past two decades as materials for biomedical applications. PLGA are biocompatible and biodegradable, and exhibit a wide range of degradation timeframes allowing sustained drug release. They also have tunable mechanical properties for implant applications (tissue engineering and regenerative medicine ) and, most importantly, are FDA approved polymers.

Moreover, several studies<sup>a</sup> has been reported the use of PLGA alone or in combination with other composite, i.e Hydroxyapatite (HA) material, in nerve & vascular regeneration, cartilage and bone tissue engineering. The scaffolds combined the degradability of the PLGA with the mechanical support of HA to form a tissue engineering replacement for bone defects.

In contrast to PLA/PLGA, PEKK (Poly-Ether-Ketone-Ketone) are resistant polymers and non-biodegradable. PEKK high-performance thermoplastic and biostable copolymers are currently used for medical applications including permanent surgical implants, dental applications...

Compared to the PEEK (polyether Ether Ketone), the PEKK shows better biocompatibility, wetting, adhesion and resistance which are major challenges in medical use, laser sintering processes and in industry (aerospace, automotive, sports, and equipment)<sup>b</sup>

PCAS proposes a comprehensive spectrum of standards PLA/PLGA, PEKK polymers as well as custom polymers for the most sophisticated applications

Regulatory aspect (Master File and ISO 10993 guidelines) and quality requirement will also be discussed.

PCAS, 53 years old is a leading contract manufacturing company in API, Pharmaceutical excipient and medical device materials. PCAS is a reliable supplier of these biomaterials with over 18 years experience in producing PLA/PLGA polymers under GMP quality system for parenteral formulations on the world market.

PCAS can support projects from early phase (R&D) to industrial scale production for marketed product (multi-kilograms to tons scale), along with worldwide regulatory affairs services.

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<sup>a</sup>Oya KERİMOĞLU et al, ANKEM Derg 2012;26(2)

<sup>b</sup><http://www.arkema.com>

For more information: [www.pcas.com](http://www.pcas.com).

## CHITOSAN : A VERSATILE TOOL FOR DRUG AND GENE DELIVERY

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### Abstract

Chitosan is a deacetylate derivative of chitin, composed of glucosamine and N-acetyl-glucosamine units[1]. Its properties, like solubility, biodegradability, biocompatibility, mucoadhesivity and antibacterial are directly affected by the degree of deacetylation (DD) and the molecular weight ( $M_w$ ) [2,3].

Since the early 1990s a great interest in chitosan for biomedical application and in particular in drug delivery has been grown [1]. In contrast to other biodegradable polymers, chitosan exhibits a cationic character which make it unique. This cationic character is responsible for its success as drug delivery carrier. Several bioactive compounds which range from anticancer drugs, peptides, antibiotics to analgesic have been encapsulated, grafted or adsorbed on chitosan particles to control or prolong the release but also to protect the drug from the degradation, reduce side effects and improve the therapeutic efficiency in vivo. However, physical and chemical modifications of chitosan, in case of neutral environment and loading of hydrophobic drugs, are necessary to improve the performances. Two chitosan derivatives are mainly obtained; i) hydrophilic, to improve the solubility in neutral and alkaline media and ii) amphiphilic-hydrophobic, to enhance the encapsulation and release properties. Next to the delivery of low molecular weight bioactive molecules a great interest to use chitosan as carrier for polynucleic acids for gene therapy application has been grown and positive results have been reported in vitro and in vivo [4]. Nucleic acids (DNA and RNA) with any bp number and conformation can be easily complexed or entrapped in chitosan particles with high loading capacity. The transfection efficiency depends on formulation factors such as DD and  $M_w$  of chitosan, amino-to-phosphate group (N/P) charge ratio, pH of medium, plasmid, and serum effect [5]. It has been reported that chitosan-DNA (RNA) polyplexes can be successfully administered by parenteral, oral, intranasal, pulmonary, and dermal routes.

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## FUNCTIONAL SIGNIFICANCE OF THREE DIMENSIONAL HYDROGEL SCAFFOLDS IN BONE TISSUE ENGINEERING – A REVIEW

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### Abstract

Bone is extremely specialized structural framework of human body. One of the exclusive property of bone tissue is power of regeneration. In past few decades, the bone related disorders has been increased notably. One of the common bone related disorder is osteoporosis which causes significant bone fracture. Nearly 75 million people has been affected by osteoporosis in Europe, USA and Japan<sup>[1]</sup>. The current treatment protocol involves the application of autograft and allograft or the administration of inert metallic/ceramic implants. But these methods have been found to insufficient during severe fracture situation or severe osteoporosis situation. The sole focus of tissue engineering is the development of three dimensional polymer scaffold which can mimic the extra cellular matrix (ECM) and facilitate the formation of bone. Hydrogels are three dimensional polymeric cross-linked network structures which can absorb and retain significant amount of water. The principle function of the three dimensional hydrogel scaffold material is the appropriate delivery of the seeded cells to the desired sites, promote cell adhesion, to facilitate the cell differentiation and proliferation. Hydrogel based biomaterials could efficiently act as a matrices which facilitates tissue regeneration<sup>[2]</sup>. Composite materials generally mimics the human bone and facilitate the bone development. The composites have the necessary potential to elevate the efficiency of a bone implant performance. Research reported that bio-mineralized (CaCO<sub>3</sub>) PVP-CMC hydrogel could be applied as an extracellular matrix and might be an efficient approach in bone tissue engineering. This review is an endeavour to ascertain the functional significance of three dimensional hydrogel scaffold in bone tissue engineering.

### Acknowledgements

This work was conducted within the framework of COST Action MP1301 “New Generation Biomimetic and Customized Implants for Bone Engineering” (www.cost.eu). The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic - NPU Program I (LO1504).

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## UTILIZATION OF BIOMIMETIC BIOREACTORS FOR BIOMATERIAL EVALUATION: GELLAN-GUM HYDROGELS WITH NANO-PARTICULATE BIOACTIVE-GLASS

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### Abstract

Biomimetic bioreactors are primarily designed to promote *in vitro* regeneration of functional tissue equivalents for tissue engineering (TE) applications by mimicking native cell environments. Yet, another important role of these bioreactors is to provide reliable characterization of new biomaterials under physiologically relevant conditions with the ultimate aim to decrease the extent of necessary animal studies. However, each tissue and organ in the body is exposed to different physical and biochemical signals, which compels customized approach to the bioreactor design. Hydrodynamic shear stresses were indicated as a key physical signal enhancing growth of tissue engineered bone *in vitro*, while dynamic compressive loadings were shown to affect cell metabolism, structure and biomechanical properties of articular cartilage both *in vitro* and *in vivo*. In the present study, we describe design and application of two biomimetic bioreactors relevant for bone and cartilage TE. The bioreactor with medium perfusion is constructed to direct the medium flow directly through the investigated specimen at physiological velocities while the custom-made bioreactor with dynamic compression provides dynamic compression of specimens in the physiological regime in conjunction with medium perfusion. The bioreactors were used to study hydroxyapatite (HAp) formation in gellan gum (GG) scaffolds with dispersed nano-particulate bioactive-glass (BAG) attractive for use in bone TE. Rehydrated porous scaffolds (discs 10 mm diameter, 5 mm thick) contained 2 % w/w GG and 2 % w/w BAG (composition: 70 n/n % SiO<sub>2</sub>, 30 n/n % CaO) while 2 % w/w GG samples served as a control. Samples were investigated over 14 days in a simulated body fluid (SBF) under (i) continuous perfusion (1.1 ml min<sup>-1</sup>), (ii) dynamic compression (5 % deformation, 0.68 Hz frequency, 337.5 μm s<sup>-1</sup> loading rate, 1 h on/ 1 h off) and continuous perfusion (1.1 ml min<sup>-1</sup>), and (iii) static conditions as a control. HAp formation was examined at the end of experiments by FEG-SEM and RAMAN analyses. Furthermore, mechanical properties of GG-BAG samples were monitored in the bioreactor with dynamic compression over time. Results of these studies have shown HAp formation in GG-BAG samples under all conditions, implying that this process is kinetically controlled. Furthermore, GG-BAG samples preserved structural integrity over 14 days under physiologically relevant dynamic compression indicating potentials for use as bone scaffolds.

## NON-TOXIC BIODEGRADABLE POLYESTER URETHANES BASED ON PLA

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### Abstract

Poly(lactide (PLA)-based polymers possess indisputable beneficial properties and they are perfectly convenient for temporary medical applications as drug carriers or resorbable supporting devices. Nevertheless, PLA is also known for its rigidity and low mechanical properties. On this view, the fabrication of non-toxic biodegradable material with enhanced mechanical performance is of particular interest. In the present study, the chain extending reaction using OH- functionalized PLA and biocompatible lysine diisocyanate can bring an option in the improving of the flexibility of PLA-based material with a maintenance of biodegradability.

In this research, firstly, the polyethylene glycol (PEG) of various molecular weight is used for the synthesis of functionalized PLA. Contrary to the different highly demanding synthesis techniques, a polycondensation reaction is performed, which is economically and environmentally beneficial – no need for solvents. In the second step, the polymer chains are linked by lysine ethyl ester diisocyanate to create high molecular weight polyester urethanes. The main objective of this study is to investigate physico-chemical properties, biodegradability *in vitro* and cytotoxicity. The results showed that the molecular weights of the products reached about 50,000 g·mol<sup>-1</sup> and the hydrolytic progress was rapid in the first 2 weeks; the drop in M<sub>n</sub> equaled approximately 70%. Tensile strength testing revealed that ductility increased alongside reduced molecular weight of PEG. Cytotoxicity tests carried out according to the EN ISO 10993-5 standard revealed a moderate cytotoxic effect of polymers.

## MINERALIZATION PROCESS OF CHITOSAN HYDROGELS BY CALCIUM PHOSPHATE APATITE

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### Abstract

Bone healing is a field of never ending demand for people of all ages. A bioinspired composite made of a physical hydrogel of chitosan (CS) mineralized by hydroxyapatite would combine the necessary healing and angiogenic properties of CS hydrogels [1] with the bioactivity and osteoconductivity of apatite [2], and present adaptable chemical, architectural, mechanical and biological properties.

Therefore, the objective of this work is to set up a protocol for the mineralization of CS physical hydrogels and to understand how the precipitation of apatite is influenced by the surrounding organic phase.

Hydrogels were prepared by dissolution of CS powder in acetic acid and then direct contact with a NaOH solution, while apatite was precipitated from  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{HPO}_4$  salts. The experimental set up developed in this Ph.D. work permitted to investigate two mineralization routes:

- precipitation of an apatite phase inside a preformed hydrogel,
- concomitant apatite precipitation and CS gelation.

X-Ray diffraction, optical and confocal microscopy observations showed the presence of apatite as a mineral phase on the CS hydrogel surface. Apatite crystals, which were formed, had an imperfect structure, a low crystalline order similar to the inorganic part of bone tissue.

This work helped to better understand the physico-chemical interactions between CS hydrogel and calcium phosphate salts. Strategies such as *in-situ* apatite precipitation and CS gelation are feasible, opening the way for a gradient of composition and structural properties of the mineral/hydrogel associations and a platform for additive manufacturing techniques.

### Acknowledgement

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## POLYMER-COATED SILICA PARTICLES FOR CONTROLLED DRUG DELIVERY

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### Abstract

In this work, we have successfully designed and prepared a new stimuli-responsive system that uses mesoporous silica particles as carriers and polymers as stimuli-responsive coating agents. The combination of the advantageous structural properties of mesoporous silica particles with provides an efficient strategy for the preparation of stable and safe drug delivery system. Mesoporous silica particles, with a MCM-41 type structure, diameter of 187 nm and 4 nm mesopores were synthesized by a modification of Stöber method. Pristine pH-responsive polymer as well as polymer with polylactic acid were successfully grafted to the surface of mesoporous silica particles to close the pore entrance avoiding premature release of the loaded cargo and to provide extended and sustained liberation. The successful grafting of polymer to Si to form hybrid-MSPs was confirmed by different characterization techniques (TGA, DLS, FTIR, SEM, TEM).

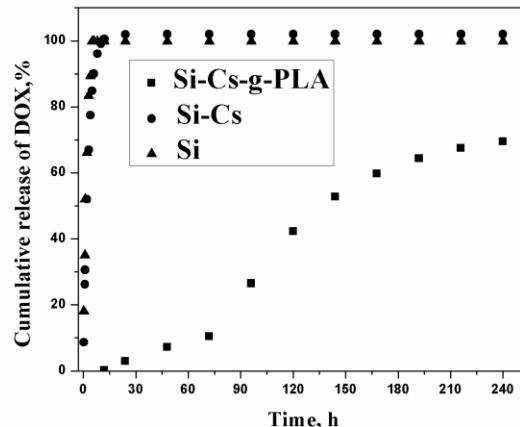
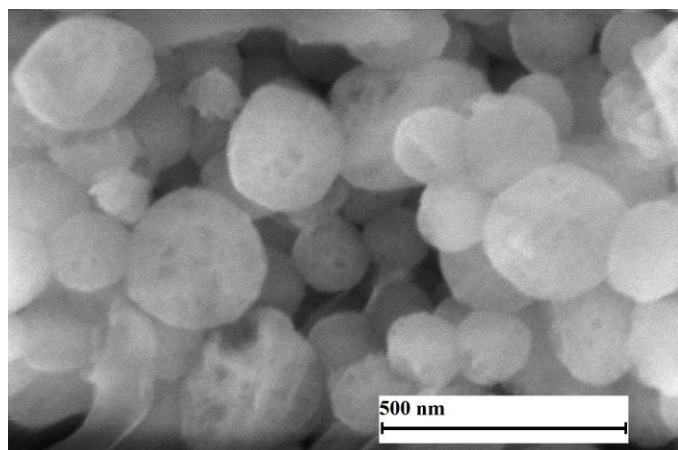


Figure 1. SEM-image of Si carrier and cumulative release of DOX from Si-based carriers, pH=7.

Results showed, that two obvious release stages, a sharp initial burst lasting for 3 hours and a slow release over a period of 10 days, were observed for the Si without any coating. The hybrid microspheres demonstrated two distinct drug release stages, a lag stage with no release rate, followed by a sustained release stage, which was controlled by the degradation of PLA encapsulant. The hybrid structure can deliver a release period of as long as 5 weeks. More importantly, the initial release burst of the hybrid structure was reduced in comparison with that of the Si.

### Acknowledgements

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## BIOMINERALIZED MAGNETIC HYDROGEL: AN APPLICATION TOWARDS BIOMEDICINE

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### Abstract

Magnetic nanoparticles (MNPs) are used in different biomedical and biomedicine applications, whereby each application requires specific particle properties. To fulfill these requirements, particle properties have to be optimized by means of variation of crystal structure, particle size, and size distribution<sup>1,2</sup>. To this aim, a novel aqueous coprecipitation method was implemented within the hydrogel matrix for the formation of MNPs and to achieve biomineralised magnetic hydrogel. It is an emerging and promising concept of utilizing hydrogel because of its 3 Dimensional, crosslinked internal structural properties. In this work, “PVP-CMC Hydrogel”<sup>3</sup> is used as matrix for synthesis for the biomineralized MNP, where the *unique porous networks structure* exhibited at freeze dried state. This porous structure embedded within the hydrogel which function as a chemical reactor, where inorganic salts ( $\text{FeCl}_2$  and  $\text{FeCl}_3$ ) are reacting with precipitating agents ammonium hydroxide ( $\text{NH}_4\text{OH}$ ) to generate MNP ( $\text{Fe}_3\text{O}_4$ ). Moreover, as the said hydrogel is prepared with biopolymer, biodegradable polymers and/or co-polymer like: Polyvinylpyrrolidone (PVP), carboxymethyl cellulose (CMC), polyethylene glycol (PEG), agar etc. that can be considered as an alternative eco-friendly biomaterial and can be used for versatile medical application such as a medical device for biomedicine, drug delivery etc. This hydrogel based in vitro synthesis process can be used to synthesize “bare” MNP, magnetic hydrogel<sup>4</sup> and extended to other oxides by changing the salts for the biomedical applications. Finally, this biomineralized ( $\text{Fe}_3\text{O}_4$ ) magnetic hydrogel can be recommended for its use as shoe sole for the treatment of foot aimed at diabetic patient.

### Acknowledgement

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## HYALURONAN HYDROGEL/CALCIUM PHOSPHATES COMPOSITES FOR MEDICAL APPLICATION

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### Abstract

In last decade, fabrication and investigation of inorganic-organic composites based on calcium phosphates (CaP) and biocompatible polymers has attracted research interest in the field of bone tissue engineering. Mechanical properties, degradation behaviour, and cell-material interaction are among the most dominantly affected characteristics resulting from the addition of inorganic material to hydrogel networks. Hyaluronic acid (HA) is a naturally derived polymer as a major component of the extracellular matrix, mediating various cellular activities, and has been widely used as tissue engineering scaffolds. The aim of the current research was to obtain HA/CaP hydrogels using three-dimensional network formation in inorganic particle suspension and to investigate the influence of inorganic part on the hydrogel formation and swelling properties.

A homogenous suspension of CaP particles for the preparation of HA and CaP hydrogels (HA/CaP) was obtained via wet chemical precipitation synthesis.

Hydrogels with HA concentration from 7 – 10 wt% were made under alkaline conditions (0.25 M NaOH), with 1.4-butanediol diglycidyl ether (BDDE) as crosslinking agent. CaP suspension was added at HA/CaP (w/w) ratios 2.33, 1 and 0.43, and mixed for 2 hours. Sol-gel transition underwent at 45 °C for 3 h, followed by sample neutralization with 0.25 M HCl. Swelling behavior of prepared hydrogels was evaluated in phosphate buffered saline (PBS) at 37 °C.

The effect of HA concentration and HA/CaP ratio on the swelling behavior of the prepared hydrogels were evaluated. Swelling kinetic studies revealed that the hydrogels swelled rapidly reaching equilibrium state within 48 hours. Swelling ratio values at equilibrium state in PBS are shown in Fig. 1. Increasing polymer concentration from 7wt% to 10wt%, the swelling ratios decreased twofold, except for the sample 0.43 HA/CaP, where hydrogel failed to form with 10wt% HA.

BDDE cross-linked HA/CaP hybrid hydrogels were successfully fabricated with HA/CaP ratio from 2.33 – 0.43. Samples were immersed in PBS solution and it was observed that HA hydrogels maintained their stable structure for 14 days and HA/CaP hydrogels for 36 days. Furthermore, the amount of CaP influenced the swelling behavior of obtained hydrogels. Addition of CaP particles to HA hydrogel reduced swelling ratio for two times.



## THE EFFECT OF ANTIMICROBIAL AGENTS ON THE STABILITY OF HYDROGELS

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### Abstract

This work is focused on the effect of antimicrobial agents on the stability of hydrogels. The aim of the study was the production of new hydrophilic gelling polymer compositions based on acrylic acid copolymer for application in cosmetology and pharmacy, respectively. The effect of the hydrogel modification with bioactive compounds, such as nisin (originating from whey fermentation products) and essential oils of citrus fruit, on physico-chemical and antibacterial properties of the resulting system was investigated. Prepared hydrogels were subjected to accelerated stress test, during which the pH, the viscosity and the concentration of nisin (by RP-HPLC) was measured. The antimicrobial efficacy test was also carried out. Results showed the addition of nisin decrease pH and viscosity and vice versa the essential oils do not influence these properties. During the stress test the concentration of nisin was decreased similarly in all prepared samples. All tested systems proved significant reduction of microorganisms growth but synergy of nisin and essential oils was found out and it was found to be promising preservative system.

### Acknowledgement

This work was funded by Ministry of Agriculture of the Czech Republic (Project no. QJ1310254) and the Ministry of Education, Youth and Sports of the Czech Republic (Project no. LO1504).

## THE DEGRADATION TESTS OF CERAMIC-POLYMER COMPOSITES WITH HYDROXYAPATITE FOR ORTHOPEDIC IMPLANTS

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### Abstract

Bioactive materials which can support bone ingrowth and osseointegration are common used in orthopedic applications. Bioactive hydroxyapatite (HAP) is one of the inorganic component of hard tissues, which is manufactured in The Institute of High Pressure Physics of the Polish Academy of Sciences (IHPP) and it is called GoHAP<sup>TM</sup>. The morphology, grain size and specific surface area of the nanopowder can be controlled by the microwave reactor and the high pressure consolidation technology for ceramic materials [1].

The aim of the GoIMPLANT project was to develop resorbable, tough, strong and biocompatible hybrid composite implants in according to patient's needs. To get better mechanical properties in our laboratory we used combination of GoHAP<sup>TM</sup> and biocompatible polymer like polylactic acid (PLA). We used isostatic pressing and uniaxial pressing to form our hybrid composite in form of cylinder or wedge. Biodegradable composites can be decomposed naturally after a certain period of implantation with degraded products, which will stay inside the body. Mechanical and biological performance of composites for implantation depends on the degradation rate. Used degradation medium, pH and temperature like inside human body can determine the test. The degradation study of composites was performed according to the ISO standards of medical devices, using PBS and TRIS-HCL solutions. Testing the changes of Ca<sup>2+</sup> concentration, the conductivity and pH under equilibrium conditions at 37°C PBS was checked once in a week.

Research was realized by the "GoIMPLANT" Project and it is founded by M-Era.Net program of The National Centre for Research and Development, co-financed from the European Union, Regional Development Fund.

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## MECHANICAL AND BIOLOGICAL EVALUATION OF CALCIUM PHOSPHATE / SILICA BASED MACROPOROUS SCAFFOLDS

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### Abstract

The aim of this work was to prepare sufficient strong and bioactive scaffolds based on calcium phosphates and silica. It is generally believed that incorporation of small amount of silica into the lattice of calcium phosphates has positive effect on both the proliferation of human osteoblasts and the rate of bone apposition. The effect of crystallized silica on mechanical and biological properties of scaffolds was therefore studied. Calcium phosphate / silica macroporous scaffolds were prepared by the polymer replica technique. Scaffolds with different amount of silica (up to 20 wt%) exhibited well-interconnected open pore structure with spherical pores exceeding 250  $\mu\text{m}$ , the size that allow cells to penetrate easily through the scaffold. The compressive strength of scaffolds containing silica was higher than the strength of pure calcium phosphate scaffolds of the same porosity; the strength was similar to the cancellous bone. The biological evaluations of scaffolds were done by in vitro testing methods. The apatite formation on the surface of coated scaffolds after immersion in MEM solution indicated a bioactive behaviour of prepared samples. Neither of tested ceramic material was cytotoxic, thus scaffolds containing silica could be suitable candidates for clinical applications.

## DEVELOPMENT OF INJECTABLE BONE SUBSTITUTE MATERIALS BASED ON CALCIUM PHOSPHATES AND REVERSE THERMORESPONSIVE POLYMER

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### Abstract

Fully injectable polymers, suitable to be implanted into the human body without surgical procedure may serve as scaffolds for guided bone regeneration. Among different types of polymers, reverse thermoresponsive polymer attract special attention due to their ability to form low viscous solutions at lower temperature, while at higher, i.e. body, temperature they form gel. Bone induction properties of such materials could be greatly improved by incorporation of appropriate calcium phosphate crystals. So far this was attempted by mixing pre-prepared calcium phosphate particles with injectable polymers. The aim of this investigation was to prepare *in situ* generated organic-inorganic composites consisting of reverse thermoresponsive pluronic F127-DMA and *in situ* precipitated calcium phosphates.

*In situ* calcium phosphate precipitation was initiated by fast mixing of calcium and phosphate polymer containing solutions, in water and phosphate buffer. Gel was formed within 5 minutes by raising temperature to 37°C. Calcium phosphates grown in gel were characterized by environmental (E-SEM) and high resolution scanning electron microscopy (HR SEM), electron dispersive spectrophotometry (EDS) and X-ray powder diffraction (XRD).

Results have shown that finely dispersed slightly aggregated crystalline calcium phosphate particles were formed, their composition depending on the medium used. In water, calcium dihydrogenphosphatedihydrate was formed, while in phosphate buffer poorly crystalline apatitic phase was formed. This points to the simple mode of controlling calcium phosphate phase formed within the gel.

## THE ROLE OF CHITIN IN THE BIOMINERALIZATION OF THE MOLLUSKS AND ITS POTENTIAL AS A BONE TISSUE REPLACEMENT BIOMATERIAL

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### Abstract

Functionalized hydrogels have a great potential as a biomaterial for bone tissue replacement. They can be used as matrices for bone tissue ingrowth, as a therapeutics (by increasing disk hydrations) or/and as a drug delivery systems. The challenge is to develop hydrogel scaffolds that are highly porous, but strong enough to withstand diverse mechanical strains, while mimicking the natural extracellular matrix (NEM) structure. Integration of natural extracellular organic matrix as a part of a biomaterial provides simple and efficient way to obtain scaffolds with enhanced framework structure. In this sense, marine organisms attract special attention as NEM sources. Their extracellular organic matrix is rich with diverse proteins and chitinous fibers. Potential of chitin is already known in the biomimetics<sup>1</sup>, but most research is conducted on the chitin isolated from arthropods, i.e. on  $\alpha$ -chitin. In comparison,  $\beta$ -chitin is more suitable to serve as a biomaterial since it has better water retention possibilities, but it is also more difficult to extract.<sup>2</sup> For example, it can be found in most biominerals of mollusks, but they are more mineralized than arthropods, so its extraction can be demanding and detrimental to chitin.<sup>3,4</sup>

The aim of this research was to determine which mollusk, the cuttlefish (*Sepia officinalis*, L.) or the shellfish (*Arca noae*, L.), is more suitable source of chitin. Structure – function relationships of organic matrix and biomineral structures were examined from both, morphological and structural perspective with the use of Fourier transmission spectroscopy (FTIR), atomic force microscopy (AFM) and field emission scanning electron microscopy (FESEM). Obtained results indicate that the process of isolation is much more feasible in the cuttlefish and that isolated chitin has a good structure and properties to be used as scaffold for functionalized hydrogels.

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## **BACTERIOPHAGE LOADING OF CALCIUM PHOSPHATE BIO-CERAMICS: A TOOL AGAINST INFECTIONS IN BONE SURGERY**

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### **Abstract**

Calcium phosphate ceramics, particularly hydroxyapatite and tri-calcium phosphate, have been used for several decades as choice materials for bone filling in bone and joint surgery, because of their mineral composition, and very good bio-activity.

Bacterial infections are a major complication of these surgical procedures, and their treatment with antibiotics is frequently inefficient because of their poor diffusion in the bone.

These nosocomial infections have become more and more frequent in recent years, because the number of patients requiring such procedures is on a constant rise (as the population gets older), but also because of the increase in the apparition of antibiotic-resistant bacterial strains.

The aim of this project is to address both of these issues, by using ceramics with porous structures, combined or not with organic polymers, in order to load them with antibacterial agents and to ensure their controlled release over time. Moreover, as the use of antibiotics has shown some limitations, using some other form of antibacterial therapy is becoming essential. Phage therapy has the advantages of having a fast and discriminating action on specific bacterial strains, which reduces the occurrence of side effects, and dramatically slows down the apparition of new resistant strains. It can be used with relative ease as a prophylactic.

In this context, the LMCPA as demonstrated the natural capacity of calcium phosphate ceramics to retain and release bacteriophages for up to 6 days. Their ability to retain the phages, and their release behavior varies with the amount of micropores in the ceramic. The overall goal of this project is to develop new devices that can administer phage based drugs locally, to prevent and cure infections related to bone and joint surgery.

## FORMATION OF POLYMER PARTICLES WITH SUPERCRITICAL CO<sub>2</sub>: A REVIEW

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### Abstract

Recently some particle formation processes and micronization has been increased due to growing concerns regarding health, safety and the environment. The particle formation processes are either solvent-less or use environmentally acceptable solvents such as carbon dioxide have come into favor. Supercritical CO<sub>2</sub> (sc CO<sub>2</sub>) (T > 31.1 °C, P > 7.3 MPa) has been used in pharmaceutical industries to minimize the use of organic solvents and micronize (0.1–5 mm) pharmaceuticals. Control of particle size increases the dissolution rate of drugs into the body. The low viscosity and high diffusivity of scCO<sub>2</sub> offer the possibility of novel processing routes for drug delivery. There are several techniques for the particle formation which is used supercritical CO<sub>2</sub> either as a solvent, and solution is rapidly expanded (rapid expansion of supercritical solutions, RESS), or as a solute leading to the formation of particles from gas-saturated solutions (PGSS), or as an anti-solvent leading precipitation induced particle formation as in (supercritical anti-solvent (SAS), gas anti-solvent (GAS) solution enhanced dispersion by supercritical fluid (SEDS) and aerosol solvent extraction systems (ASES)).

## THE OPTIMIZATION OF METHOD FOR PREPARING MICROPARTICLES

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### Abstract

Alginate is a natural polysaccharide which is obtained from brown algae. Alginates are widely used in food industries. It is also used in pharmaceutical industries as a matrix for the entrapment or encapsulated drugs. Alginate particles can be produced in several ways. One of them is a spray-coagulation method. The particles produced by this method are porous. Calcium chloride solution is used as gelation agent in various concentrations. This study is focused on optimalization of method for preparing alginate particles mainly on their size and shape. Conditions that are changed are flow rate, air pressure, height of needle, concentration of alginate and  $\text{CaCl}_2$  solution. The shape and a surface of particles is identified from scanning electron microscopy (SEM). Their size is measured by Mastersizer 3000 laser particle size analyzer. Results showed that changed conditions have an effect on physical properties of particles. The size of particles is decreased with decreasing flow rate, increasing pressure of air and also with more concentrated solution of calcium chloride.

### Acknowledgement

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## THE EFFECT OF PLASMA HYDROPHOBIZATION ON THE REALASE OF TEMOZOLOMIDE FROM POLYESTER AND POLYLACTIC ACID

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### Abstract

In the study, the effect of plasma deposited siloxane coating on the release kinetics of drug Temozolomide (TMZ) from the thin biodegradable polyesters films: polylactic acid (PLA) and polyester urethane (PEU) were investigated. The changes in surface properties were studied by the method of water contact angle measurements, nanoindentation and X-ray photoelectron spectroscopy. The hydrolytic degradation of materials as well as release kinetics of TMZ was investigated by using chromatographic and spectroscopic techniques, respectively. The plasma deposited hydrophobic siloxane coating significantly modified the release kinetics of TMZ from both polymers especially from PLA. The initial burst effect and the release rate of TMZ was significantly reduced. The hydrolytic degradability of materials were slightly reduced after hydrophobization especially for PLA as an effect of wettability modification.

### Acknowledgements

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## STRONTIUM SUBSTITUTED AND ALENDRONATE FUNCTIONALIZED HYDROXYAPATITE NANOCRYSTALS IN AN OVARECTOMIZED RAT SPINAL ARTHRODESIS MODEL

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### Abstract

Advanced age, osteoporosis and unhealthy life-style diminish bone formation and remodeling rate so fusion enhancement techniques could be beneficial for patients with osteoporosis who need spinal arthrodesis. Herein we investigated the posterolateral fusion rate in ovariectomized (OVX) rats using two new materials: strontium substituted hydroxyapatite (SrHA) nanocrystals and alendronate functionalized HA (HA-AL) nanocrystals. HA was synthesized at increasing Sr concentrations (SrHA5; SrHA10) and alendronate content (HA-AL7; HA-AL28). A posterolateral spinal fusion model in twenty-five Sham-Operated and in twenty-five OVX female rats was used. Materials were bilaterally implanted between transverse processes of lumbar vertebrae. Sham and OVX animals were divided in five groups: HA, SrHA5, SrHA10, HA-AL7 and HA-AL28. The assessment of bone fusion was carried out by micro-CT, histology and histomorphometry. Some gaps between the transverse processes were observed by micro-CT in OVX HA group, while they were not present in the other groups. These results were consistent with histological and histomorphometrical analyses showing that in OVX animals SrHA and HA-AL materials displayed significantly higher BV/TV and Tb.Th and significantly lower Tb.N and Tb.Sp in comparison with HA alone. Results suggest that in spinal fusion the incorporation of Sr, as well of AL, improves the biological performance of HA in a dose dependent way, and represents a promising strategy especially in osteoporosis patients with high risks of spinal fusion failure. Results also suggest that HA-AL28 could be the candidate biomaterial for spinal fusion in osteoporotic subjects.



## DEVELOPMENT OF NANO-HYDROXYAPATITE/POLY(VINYL ALCOHOL) HYBRID SYSTEMS FOR PROSPECT DRUG DELIVERY APPLICATIONS

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### Abstract

Fabrication and investigation of inorganic-organic composites based on calcium phosphates (CaP) and biocompatible polymers has attracted research interest in the field of bone tissue engineering for potential application in developing of controlled release drug delivery systems. Hydroxyapatite (HAp) is used for bone-tissue engineering, due to its bioactivity and osteoconductivity. Poly(vinyl alcohol) (PVA) is a hydrophilic polymer possessing biocompatibility. In order to achieve the synergic effect of bioactivity of HAp and adjustable biodegradability of PVA, PVA/HAp microgranules were formed by spray drying process of the polymeric suspensions containing HAp particles synthesized *in situ*. Moreover, PVA was modified with succinic anhydride to introduce carboxylic acid residues that either can be modified with organic biologically active compounds or can chelate bioactive metallic ions on the surface of the HAp particles.

PVA was modified to introduce –COOH groups by reaction between the –OH groups and succinic anhydride. The composites were obtained using two different preparation techniques: (a) aqueous solutions of the pure or modified PVA (M-PVA) and as-synthesized HAp suspension were mixed; (b) HAp was synthesized *in situ*, *i.e.*, in the presence of aqueous solutions of the PVA or M-PVA. In both cases, HAp were synthesized from Ca(OH)<sub>2</sub> and H<sub>3</sub>PO<sub>4</sub> through aqueous precipitation. In order to yield moldable composite powders, the suspensions were spray dried at temperature under boiling point of PVA. The characteristics of the products such as morphology, chemical and phase compositions *etc.* were studied.

The feasibility of producing microgranules (~2-10 μm) composed of nanosized HAp crystallites homogeneously embedded in the polymer matrix opens the possibility of developing injectable bone fillers or formulation of bone cements with hydraulic setting. The innovative biomaterial could be employed for controlled release of therapeutic agents at wound sites. This preliminary research contributes to further biomedical applications.

### Acknowledgements

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## NISIN IMMOBILIZATION INTO POLYESTER BASED MICROSPHERES

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### Abstract

The present study deals with the development of the polyvinyl alcohol (PVA) cross-linked with glutaric acid (GA) core and polylactic acid (PLA) multi-hollow shell encapsulation systems that could provide the effective encapsulation of hydrophilic active agents in the hydrophobic polymer matrix with controlled morphology. The microsphere systems were used as delivery systems for immobilization of model antibacterial agent – peptide nisin (NIS). The effect of cross-linking and the NIS amount on their morphology was investigated by using scanning electron microscopy (Figure 1). Encapsulation efficiency and release profile of nisin from the microspheres were studied by high performance liquid chromatography. Antibacterial activity of the prepared systems was tested by dilution and spread plate technique. Results showed the microspheres in the size range of 9-16  $\mu\text{m}$  in diameter with the spherical multi-hollow core-shell structure. The presence of cross-linking agent GA influences the release profile of the NIS and has synergistic effect on *Listeria monocytogenes* grown reduction.

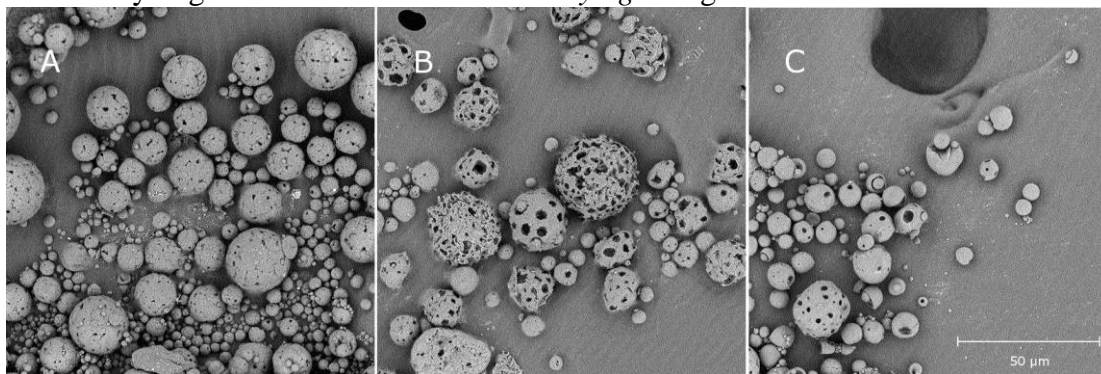


Figure 1 SEM pictures of PLA/PVA (A), PLA/PVA/NIS (B), PLA/PVA/GA/NIS (C)

### Acknowledgements

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## POLYANHYDRIDE / WAX SYSTEM FOR CONTROLLE CO-RELEASE OF BIOACTIVE COMPOUNDS

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### Abstract

Polyanhydrides are less known types of biodegradable polymers in contrast with e.g. polylactides. Due to the presence of unstable anhydride bonds these polymers are highly susceptible to hydrolysis and consequently fast degradation. In combination with, in most cases, very hydrophobic nature, majority of polyanhydrides exhibit so called “surface erosion” degradation pattern. This is highly desirable in drug release applications because zero order release kinetic can be achieved. On the other hand, their fast degradation can be problematic in cases in which long term application is required.

This work describes application of biodegradable poly(sebacic anhydride) (PSA) based matrix combined with biocompatible bees wax for co-release of two model drugs acetaminophen (AAP) and ibuprofen (IBU). The studied blend system consist between 0-75% of wax and fixed drug in concentration 5 mg.g<sup>-1</sup>. The wax in these systems should work as water repelling agent to slow degradation rate and prolong drug release. The blends were prepared by mixing all components in chloroform followed solvent evaporation and preparation of tablets (13mm diameter, 2mm thickness) by cold press moulding. The release experiment was performed into phosphate buffer (pH 7.4) at 37°C and amount of released drug was detected by HPLC with UV detection.

It was found that presence of wax in PSA matrix can significantly slow down the release rate of both model drugs as well as the initial “burst” effect phenomenon.

## THE ANALYSIS OF STRUCTURE OF ELECTROSPUN NONWOVEN MATERIAL WITH HYDROXYAPATITE PARTICLES

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### Abstract

Synthetic biodegradable polymers attracted special attention in electrospinning due to the elimination of a second surgery to remove the implanted carrier. Electrospinning is a process by which ultrafine fibers with nanometer or submicrometer diameters from different polymers (polyvinyl alcohol, polycaprolactone, polylactide acid, chitosan, etc.) can be produced. Electrospun fibers exhibit several unique characteristics, such as large surface area to mass or volume ratio, small pore size between depositing fibers of the electrospun mat.

Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) (HA) is a biomimetic material and are widely used in biomedical applications in combination with different materials since it is an inorganic component of bones and teeth. Due to its poor mechanical properties it cannot be used alone as bone implant material.

The aim of this study was to estimate the influence of HA particles surface treatment on the diameter of electrospun polyvinyl alcohol fibers. Additionally, the possibility to use methacrylic acid/methyl methacrylate copolymer for electrospinning process and fibres structure was investigated.

Two kinds of electrospun mats from polyvinylalcohol (PVA) and biodegradable methacrylic acid–methyl methacrylate (MA/MMA) copolymers were used for investigations. Electrospinning solution of 12% concentration were prepared by dissolving of PVA ( $M_w = 72000$  g/mol) in distilled water at 80 °C temperature. Electrospinning solution of 5–10% concentration was prepared by dissolving MA/MMA (Eudragit E100) in ethanol. Undoped HA or boron doped HA (BHA) powders were added (5 wt% by weight) to PVA solution.

It was obtained that HA particles treatment procedure influenced on the diameter of electrospun PVA nano-microfibers. The doping of HA particles by boron decrease amount of nanofibers with diameter of less than 100 nm.

Preliminary investigations show that biodegradable MA/MMA copolymer can be used as support (delivery) material for HA particles.

## NANOSTRUCTURED COMPOSITES BASED ON $\epsilon$ -POLYLYSINE AND HYDROXYAPATITE FOR BONE TISSUE REPAIR

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### Abstract

The development of biomaterials for tissue engineering is to create surfaces which can provoke specific cellular responses and direct new tissue regeneration. Thus, inorganic/organic systems such as calcium phosphate/natural biopolymers, have attracted a great deal of attention over the past few years as a carrier for the delivery of antibiotics in the treatment of bone tissues. Nanocrystalline hydroxyapatite (HAp) is a major inorganic component of bone with excellent biocompatibility and bioactivity and thereby considered to be promising bone replacement material.  $\epsilon$ -Polylysine ( $\epsilon$ -PL) is non-toxic, biodegradable, water soluble natural homopolymer with potential applications in medicine. In this study, novel bioactive nanostructured composites for bone tissue engineering were developed based on  $\epsilon$ -PL and HAp. This composite biomaterial would combine the chemical and biological functionality of  $\epsilon$ -PL and bioactivity of HAp.

Nanostructured composites based on HAp and  $\epsilon$ -PL were processed by spray drying technique using slurry of *in situ* synthesized HAp in 5% (w/v)  $\epsilon$ -PL aqueous solution. *In situ* synthesis of HAp was realized by precipitation method using  $\text{Ca}^{2+}$  and  $\text{PO}_4^{3-}$  ions sources as starting reagents with molar ratio of  $\text{Ca/P} = 1.67$  in order to obtain spray-dried products containing HAp and  $\epsilon$ -PL at the 50/50, 70/30 and 90/10 wt%. The obtained slurry was spray dried by using a tabletop spray dryer with nozzle of 1.4 mm diameter at a temperature  $\sim 190^\circ\text{C}$ . The collected spray-dried composite powders were structurally characterized using different analysis techniques.

SEM studies of obtained spray-dried products confirmed the formation of spherical microgranules with diameter in the range 1-25  $\mu\text{m}$ . Intimate interaction between HAp and  $\epsilon$ -PL was evident from FT-IR and SEM results. XRD patterns of fabricated composites revealed that nanocrystalline HAp was synthesized in the presence of  $\epsilon$ -PL. Accordingly,  $\epsilon$ -PL influences HAp crystal nucleation and growth, allow for chemical functionalization of the inorganic phase.

Novel composites based on HAp and  $\epsilon$ -PL were fabricated using a combination of processing routes - *in situ* precipitation and spray drying. Intimate interaction – adsorption of  $\epsilon$ -polylysine on hydroxyapatite crystallite surface is related with cationic nature of polymer.

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## THE ALLOCATION OF BONE FLUID IN BOVINE CORTICAL BONE UTILIZING A MULTI-TECHNIQUE ANALYSIS

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### Abstract

The mechanical properties of bone depend crucially on the materials composition in terms of mineral and collagen content, as well as the hierarchical organization of those components. What is less known, is the role of the third major structural component in bone, which is bone fluid. It is partitioned in three levels of pore spaces, ranging from the nanometer to the millimeter level. The porosity of cortical bone includes the vascular porosity, the lacunae porosity, and the ultra-structure porosity. To determine the partition of the major components, and specifically the fluid component, along the hierarchical structure of bone, 24 bone samples were cut using a diamond band saw and low speed saw from a bovine femur. The samples were polished in order to obtain images under a light microscope. The different porosities were computed from the images. To calculate the major components in the samples, the bovine bone samples were taken through a series of states, i.e. dehydrated state, rehydrated state with HBSS or xylene, and demineralized state using a 0.5 M EDTA solution. The mass of the bone components are normally given in terms of the content weight fraction, and for the samples rehydrated with HBSS the mean weight fraction of the organic component is 0.21, of the mineral component 0.67, and of the fluid component 0.12. The mean weight fraction of the fluid component within the vascular pores is 0.0161, within the lacunae pores 0.0098, and within the ultra-structure pores 0.0870. For the samples rehydrated with xylene, the mean weight fraction of the organic component is 0.20, of the mineral component 0.67, and of the bone fluid component 0.13. The mean weight fraction of the fluid component within the vascular pores is 0.02, within the lacunae pores 0.01, and within the ultra-structure pores 0.10. The mean volume fraction for the samples rehydrated with HBSS given in term of the organic component is 0.33, of the mineral component 0.4510, and of the fluid component 0.22. The mean volume fraction for the samples rehydrated with xylene given in term of the organic component is 0.29, of the mineral component 0.42, and of the fluid component 0.29. The mean volume fraction of fluid within the vascular pores is 0.03, within the lacunae pores 0.02, and within the ultra-structure pores 0.24.

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