Book of Abstracts

BBCE Summer School 2022

10-14 October 2022

Erlangen, Germany

Organized by Zoya Hadzhieva, Marcela Arango-Ospina and Prof. Aldo R. Boccaccini

(Institute of Biomaterials, FAU)

Herbal fingerprints – a tool for herbal extract identification and characterization

A.Bārzdiņa[1,2], A.Paulausks[1,2], D.Bandere[1,2], A.Brangule[1,2]

[1]Department of Pharmaceutical Chemistry, Riga Stradiņš University, 16 Dzirciema Str., LV-1007 Riga, Latvia
[2]Baltic Biomaterials Centre of Excellence, Riga Technical University, 1 Kalku Str., LV-1658 Riga, Latvia

With the growing demand for herbal medicines, the increase in international trade of medicinal plant materials, and growing costs, the search for analytical techniques that are quick and accessible by the industry has become one of the main tasks for researchers worldwide [1]. In this study, high-performance liquid chromatography (HPLC) and thin layer chromatography (TLC) were used to obtain chemical fingerprints of four taxonomically and evolutionary different medicinal plants (*Hibiscus sabdariffa* L., *Calendula officinalis* L., *Matricaria recutita* L., *Achillea millefolium* L.). Retention time shifting, principal component analysis (PCA), hierarchical cluster analysis (HCA), and orthogonal projections to latent structures (OPLS) analysis were applied to analyze and improve obtained fingerprints. Used analytical methods provided separate, compact clusters in PCA, and the results correlated with the evolutionary relationships between these plants. Obtained fingerprints allowed to identify chosen medicinal plants from extracts with several components and the data alignment technique greatly improved the quality of fingerprints. Chromatographic techniques combined with chemometric methods are suitable, accessible, and reliable for obtaining herbal fingerprints and are giving profound knowledge of the chemical composition of medicinal plants.

References

1. Suroowan S, Mahomoodally MF (2019). "Herbal Medicine of the 21st Century: A Focus on the Chemistry, Pharmacokinetics and Toxicity of Five Widely Advocated Phytotherapies". Curr Top Med Chem. 19(29):2718-2738. doi: 10.2174/1568026619666191112121330.

Development of Functional Composite Hydrogels for Bone Regeneration

Anna Rubina[1,2] *, Inta Kreicberga[1,2], Artemijs Sceglovs[1,2], Kristine Salma-Ancane[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Riga Technical University, Riga, Latvia
[2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

There is a critical need for new therapeutic strategies to repair bone damage caused by osteoporosis, therefore, the aim of this study was to develop and investigate bioactive composite hydrogels based on natural biopolymers - hyaluronic acid (HA) and ε-polylysine (ε-PL), as well as strontium ranelate loaded nanosized hydroxyapatite (SrRAN-nHAp).

Bioactive composite hydrogel systems were synthesized by chemically crosslinking ε-PL and HA at a mass ratio of 50:50 wt% using EDC and NHS. SrRAN-nHAp was synthesized by wet chemical precipitation (with 10 wt% Sr) and incorporated into composite hydrogels systems. Composite hydrogels with SrRAN-nHAp to ε-PL-HA mass ratios of 60:40 wt%, 50:50 wt%, 40:60 wt% and 70:30 wt% were synthesized and investigated.

XRD, SEM, μ-CT and FTIR analysis of the composites revealed a homogenous and porous chemically crosslinked structure, homogenous distribution of SrRan-nHAp powder particles, existence of hydroxyapatite crystalline phase, and high swelling degree. In the first 30 days the SrRAN-nHAp/ε-PL-HA composite hydrogels with 70:30 wt% showed the highest release of drug - 36% and composite hydrogels with 40:60 wt% showed the least release of drug - 27%.

In conclusion, the developed composite hydrogels based on ε-PL, HA and SrRAN-nHAp showed promising functionalities for bone regeneration application.

Mechanism postulated for biomineralization of bone

Abhishek Indurkar[1,2*], Rajan Choudhary[1,2], Kristaps Rubenis[1,2], Janis Locs[1,2*]

[1] Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of RTU, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Pulka Street 3, LV-1007 Riga, Latvia

[2] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Kalku Street 1, LV-1658 Riga, Latvia

Bone is a heterogenous and anisotropic nanocomposite, the components are arranged hierarchically into several structural levels. Calcium phosphates (Ca-P) majorly accounts for the inorganic counterpart whereas collagen was embraced as major organic component coupled with minor components such as non-collagenous proteins, hormones, small organic moieties, enzymes, etc. Currently only the materials representing the inorganic constituents of bone (Ca-P) are utilised as a bone substitute material. Moreover, the bone is a composite tissue therefore the presence an organic component cannot be neglected in development of biomimetic bone substitutes. It is evident that the gap zone of collagen provides site for nucleation and grown in a c-axis orientation. However, the factors which governed the Ca-P and collagen interaction remains unknown. The is a major obstacle in depicting the biomineralization process in human bones. Therefore, numerous postulations representing the role of different small organic moieties in regulating the biomineralization process are presented in last century. Majority of them stressed on the organic compounds containing carboxylic groups present in the organic counterparts governing biomineralization. This stipulates that the biomimetic bone substitute should reinforced with carboxylates to impart physiochemical properties of natural bone. Recent NMR studies of natural bone provides evidence of association of carboxylate compounds in mineral plates of bone. This review is focused on the different mechanism postulated for biomineralization of bone.

References

1. Lehninger, A. L. Mitochondria and Calcium Ion Transport THE FIFTH JUBILEE LECTURE. *Biochem. J.* **119**, 129–138 (1970).
2. Posner, A. S. Crystal chemistry of bone mineral. *https://doi.org/10.1152/physrev.1969.49.4.760* **49**, 760–792 (1969).
3. Glimcher, M. J., Bonar, L. C., Grynpas, M. D., Landis, W. J. & Roufosse, A. H. Recent studies of bone mineral: Is the amorphous calcium phosphate theory valid? *J. Cryst. Growth* **53**, 100–119 (1981).

Influence of polymer reinforcement on calcium phosphate bone cement properties

E. Rieksta[1,2], D. Loca[1,2*]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Institute of General Chemical Engineering, Faculty of Materials Science and Applied Chemistry, Riga Technical University, Riga, Latvia
[2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Calcium phosphate bone cements (CPCs) are widely known for their bioactivity, osteoconductivity, low temperature hardening ability and injectability. However, so far CPCs have insufficient mechanical properties limiting their use in the load bearing applications. Furthermore, to be used for minimally invasive surgeries cements should set in appropriate time window (~15 min), allowing surgeon to prepare the cement paste directly in the operating room and inject it into the necessary site. Therefore, in last decades researchers have focussed on the development of CPCs reinforced with biocompatible polymers that could enhance their mechanical properties as well as change their setting time.

The aim of the current research was to study the influence of ε-poly-L-lysine (ε-PL) reinforcement on the setting time of CPCs. Different α-TCP/ε-PL ratios (100/0 - 50/50wt%) as a solid phase and phosphate salt solution as a liquid phase were used in the study. Both the initial and final setting times were determined using Gilmore needle.

Obtained results revealed that the increase of ε-PL content in samples from 0wt% - 50wt%, significantly changed the initial setting time of CPCs (from 9.45 min - 48 min). Influence of ε-PL addition on CPCs mechanical properties will be assessed in the follow up studies.

Acknowledgment

The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No. 857287 (BBCE).

Effect of molecules initially released from damaged murine bones on MG63 and NIH3T3 cells

Anastasija Metlova[1], Irina Česnokova[1], Antons Sizovs[1]

[1]Latvian Institute of Organic Synthesis, anastasija.metlova@farm.osi.lv

Stimulation of bone fracture healing in many cases is essential for complete patient recovery. Understanding of the bone healing process could yield novel targets for pharmacological intervention in non-union cases. We report that extracts from damaged murine bones increase proliferation of osteoblast-like cells that are responsible for the rebuilding of bone tissue without affecting their metabolic activity. We propose further analysis of damaged bone extracts to identify molecules that stimulate proliferation of bone forming cells as potential drug candidates to be used in biomaterials.

Bone extracts were prepared from isolated femurs and tibias of mice. Isolated bones were cleaned, crushed or left intact, incubated in serum-free cell growth medium for 1 h, filtered and applied to MG63 and NIH3T3 cell lines. Quantification of the cell number and MTT assays were performed after 1, 3 and 7 days of incubation.

During 7 days extracts from damaged bones positively influenced proliferation of MG63 cells. Extracts from fractured femurs significantly increased proliferation of NIH3T3 cells within 7 days. No significant changes in metabolic activity were observed in either cell line.

LC–MS/MS analysis of polyunsaturated fatty acids and their acylcarnitines in blood plasma

Gukālova Baiba[1], Grīnberga Solveiga[1], Križanovska Valērija[1,2], Sevostjanovs Eduards [1], Dambrova Maija[1,2], Liepiņš Edgars[1]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia
[2]Department of Pharmaceutical Chemistry, Riga Stradins University, Latvia

The health benefits of omega-3 polyunsaturated fatty acids (PUFAs) are widely recognized in cardiometabolic disease management. The aim of this study was quantitative determination of PUFAs (free acids) and PUFAC (respective acylcarnitines) in mouse and healthy volunteer blood plasma using LC-MS/MS method.

A simple protein precipitation extraction with ACN/MeOH (3:1, v/v) was used to extract plasma samples and the resulting extracts were analyzed using reversed phase UPLC-MS/MS. The mobile phase consisted of gradient elution of 0.1% formic acid in water and ACN at a flow rate at 0.4 ml/min. Separation was achieved on an Acquity UPLC BEH C18 column. Detection was performed in the multiple reaction monitoring mode, using an electrospray ion source.

PUFA-rich diet significantly increased DHA and EPA, respective acylcarnitine levels and prevented the development of atherosclerosis in mice. In healthy volunteers after 5 days of fish oil intake, PUFAC levels increased only 1.1–2.5 times. After 10 years of regular fish oil intake PUFAC levels were 6 times higher than the baseline level of other volunteers. Novel methods of PUFAs and PUFAC quantitative determination could be used in clinical studies to validate PUFA and PUFAC plasma levels as promising markers of PUFA intake and cardiac content.

Calcium phosphate bone cements as anticancer drug delivery systems

E.Oselska[1], A.Pylostomou[1,2], D.Loca[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University, Pulka 3, LV-1007 Riga, Latvia
[2] Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

Today the number of patients who suffer from musculoskeletal disorders, especially bone cancer is rapidly increasing. Long-term treatment is needed and after extensive tumour resection a large void remains at the bone site, which favourably must be filled with biomaterials. Furthermore, recurrence of the cancer after surgical interventions is highly possible, thus biomaterials able to regenerate the natural bone tissues at the same time releasing anticancer drugs is of paramount importance. Thus the aim of the current study was to prepare doxorubicin (DOX) loaded calcium phosphate bone cements (CPC) and to analyze the effect of solid and liquid phase influence on such properties as cement setting time, phase composition, porosity, specific surface area, density, morphology and DOX release kinetics. Obtained results indicated that the setting time of CPC highly depends on both molarity of the liquid phase (0.5M and 1M) as well as the sintering temperature of the solid phase (700 ^{o}C and 750 ^{o}C). It was found that the release rate of doxorubicin can be controlled not only by changing the solid phase sintering temperature but also by changing the molarity of the liquid phase. Prepared CPCs were able to release the DOX for more than 40 days.

Acknowledgement: The authors acknowledge financial support from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 857287 (BBCE).

Volumetric analysis of HAP/collagen malar implants over a 1-year period.

Martins Lauskis[1], Girts Salms[1], Oskars Radzins[1]

[1]Riga Stradins University Institute of Stomatology

High and symmetrical cheekbones is considered a beauty standard in many cultures. Many patients with maxillary anteroposterior deficiency have an alteration of the cheekbone contour that can be corrected by performing a malar augmentation.

46 patients, who underwent orthognathic surgery combined with malar zone augmentation, were included in this retrospective study.

Preformed HA/collagen implants were then used to achieve the desired volume and shape of malar areas.

Four CBCT examinations were performed as part of standard orthognathic surgery protocol- 1 week before surgery (T0), 1 week after surgery (T1), 4 months after surgery (T2), 12 months after surgery (T3).

After superimposition of all CBCT examinations, data analysis was performed to evaluate the volumetric changes in malar region. Median volume gain initially after surgery was 5693 mm^3, after 4 months the median volume loss was 1445 mm^3, and after that from 4 months to 12 months the median volume loss was 36 mm^3. The volume lost during first 4 months after surgery was 25% of initial volume.

HA/collagen implants used for malar zone augmentation show good volumetric stability after initial compaction period, but overcorrection is needed initially.

Calcium Phosphate Biomaterials Influence Cell Metabolism

Jingzhi Fan[1,2], Theresa Schiemer[3], Antons Sizovs[2,4], Janis Locs[1,2], Kristaps Klavins[1,2]

[1]Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga Technical University, Riga, Latvia
[2]Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Latvia
[3]Medical University of Innsbruck, Innsbruck, Austria
[4]Latvian Institute of Organic Synthesis, Riga, Latvia

The interaction between cells and materials is fundamental in biomaterial development. Metabolite measurement can provide clues to cellular metabolism changes and explain and predict cell phenotype changes. The gained information can be used to develop new biomaterials-based treatment strategies. This work investigated calcium phosphate (CaP) with different compositions influencing cell metabolism. Specifically, hydroxyapatite (HAP), β-tricalcium phosphate (β-TCP), and their composites with different ratios (HAP/β-TCP at 95/5 and 58/42) were prepared. The calcium phosphate materials were characterized by XRD, and in vitro experiments with NIH/3T3 cells were carried out. Cells were harvested at different time points, and targeted quantitative metabolite analysis was performed. Totally 58 metabolites of cells were measured and analysed. PCA and PLS-DA between CaPs and control groups showed there were many metabolites dramatically affected by ceramic materials. ANOVA indicated that the metabolite level fluctuations between different CaPs groups were not remarkable. Pathway analysis identified aromatic amino acid metabolisms and energy metabolisms were significantly perturbed by CaPs. The obtained results demonstrated that metabolite differences were changing dynamically through time. This study showed the ability of metabolomics to identify the biomaterial chemical composition influence on cellular metabolism.

The impact of printed replicas on the surgical procedure of immature third molar autotransplantation. (Case Control study)

Miks Lejnieks[1,2], Ilze Akota[1], Gundega Jakobsone[1] Oskars Radzins[1,2], Uldis Spulle[1], Laura Neimane[1,2]

[1]Riga Stradins University Institute of Stomatology, Riga, Latvia
[2]Baltic Biomaterials Centre of Excellence, Riga, Latvia

OBJECTIVES:

The aim of the study was to compare surgical results of immature third molar autotransplantation without and with use of 3- dimensional printed donor tooth replicas.

METHODS:

The replica was created based on the cone-beam computed tomography data of the patient and subsequently 3- dimensional printed. Two groups of immature third molar autotransplantation were formed, one conventional and one using a printed donor tooth replicas.

Total surgery time from the first incision to the last suture, donor tooth extra- alveolar time and the number of donor tooth fitting attempts were monitored.

RESULTS:

Group A consisted of 22 patients, (6 males, 18 females, mean age 17.81, range 13-22).

The average total surgery time was 65.91 minutes, donor tooth extra-alveolar time was 63.63 seconds, and the average number of fitting attempts was 2.00.

Group B consisted of 19 patients, (8 males, 11 females, mean age 16.10, range 14-19).

The average total surgery time was 45.00 minutes, donor tooth extra-alveolar time was 61.32 seconds, and the average number of fitting attempts was 1.53.

CONCLUSIONS:

The use of a replica made the procedure less traumatic, reduced average total surgery time the donor extra-alveolar and the number of fitting attempts of the donor tooth.

ACKNOWLEDGMENTS: The authors acknowledge financial support from the European Union's Horizon 2020 research and inovation programme under the grant agreement No 857287

Presenting author's e-mail address: M.lejnieks@gmail.com