patients of the group II (p<0,02).

Significant difference was fixed by the 7 day in the comparing groups according to the indices of Shiller-Pissarev tests and iodine number by Svrakov as well as to the PCI indices (p<0,05) showing advantages of the results of the 2nd group patients. The same results (p<0,05) were fixed on the 14 days results (FIGs. 1 and 2).

We did not fixed significant difference by Shiller-Pissarev tests indices and iodine number by Svrakov in comparing groups by the 21 days. At the same time, difference between average indices of SIP (p<0,01) was fixed what confirmed superiority of the 2nd group results. Examinations made 1 and 2 months later, had the same results. No significant difference fixed 2 months after the operation in the groups of examination. We also obtained positive results according to the X-ray methods of examination. Results we achieved demonstrate change of the oral fluid microcrystallization indices after the dental implantation 2,4±0,07 in respect of indices of control 1,55±0,05 (p<0,001). After the postoperative treatment finished, biophysical test indices were equal to 1,9±0,09 and showed authentically positive changes in respect of the initial indices (p<0,001). Authentic difference of the microcrystallization indices was fixed between the group II and group of control (p<0,01). These indices confirmed that process of osteointegration last in the system jaw – dental implant. Patients of the group of examination had no complications in the future and had successful orthopedic treatment.

## Conclusion

According to the results we achieved we could conclude that positive advantage of the dental implantation for the patients of the group II was achieved due to acupuncture application in the complex treatment. So, acupuncture treatment combined with therapy and rehabilitation procedures for patients underwent dental implantation operations, should be considered as expedient.

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# BIOINSPIRED NANOCOMPOSITE STRUCTURES FOR BONE TISSUE REGENERATION BASED ON COLLAGEN, GELATIN, POLYAMIDE AND HYDROXYAPATITE


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## Introduction

Numerous synthetic bone replacement materials are nowadays available. These both single- and multi-phase (i.e., composite) materials combine the advantages exhibited by each component of the material, with a structure and composition similar to that of natural bone [1,2]. Bone as a natural composite involves two main components, i.e. organic and inorganic materials. The organic portion of the bone comprises cells as well as the fibrous and amorphous part of the extracellular matrix. The fibrous part is formed by collagen (COL) fibres and the amorphous part by various glycoproteins or glycosaminoglycans that play important roles in controlling the function of osteoblasts as well as bone tissue mineralization [1,3]. The inorganic component comprises minerals, particularly hydroxyapatite (HA) and calcium phosphates. Another important feature of natural bone tissue is its nanoarchitecture. It has been observed that nanocrystalline HA promotes the adhesion, proliferation and differentiation of osteoblasts. Moreover, the deposition of calcium-containing minerals on nanocrystalline HA was higher than on microcrystalline HA [4]. The mechanical properties of the natural bone should also be taken into account when designing an artificial bone implant. Suitable mechanical properties of the artificial material, similar to those of the natural bone, can induce the differentiation of stem cells toward osteoblasts when this cell type is lacking [5]. The aims of this work are the preparation of bioinspired composite materials composed of collagen and gelatin matrix, gelatin and polyamide nanofibers and hydroxyapaptite powder, determination of their mechanical properties and preparation verification.

## Materials and methods

Three types of biocomposites have been prepared. The first type (GELHA) has been prepared by introduction of hydroxyapaptite (HA) powder (particle size 20-70nm, supplied by Berkeley Advanced Biomaterials, Inc., San Leandro, CA, USA) into porcine gelatin (GEL) matrix (Fluka) and mixed by kneading machine (HAAKE, Thermo Electron Corporation, USA) at room temperature at a rotation speed of 15min$^{-1}$ for 24 hours (FIG.1a). Mixture has been formed followed by drying at ambient atmosphere, pressure and humidity.

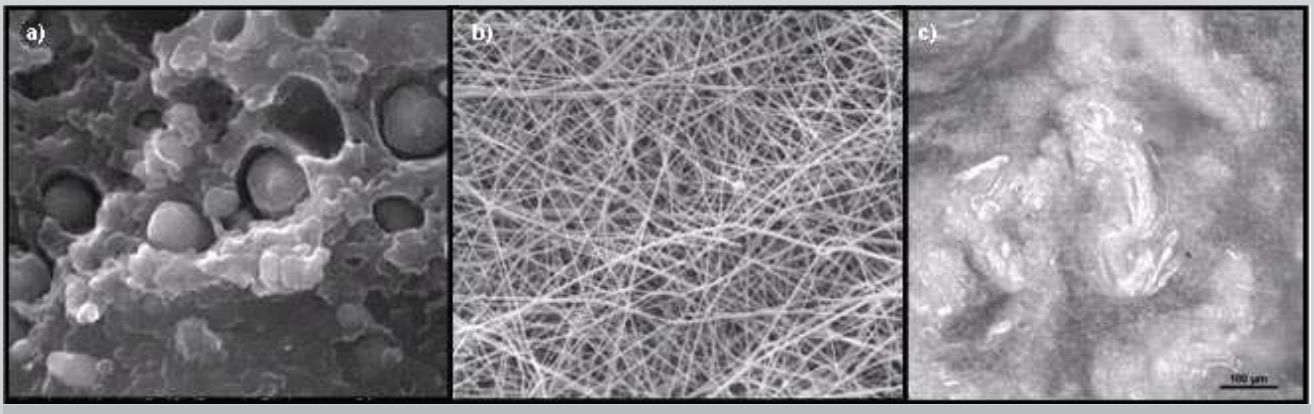


**FIG.1. SEM images of GELHA (a) composite (magnification 10000x) and NF-GELHA (b) composite (magnification 5000x). Micrograph of polished section of PAA-COLHA composite (c).**

Dried composite sample (75wt.% GEL and 25wt.% HA) was cut into rectangle-shaped pieces for testing of mechanical properties.

A basic material of the second type has been provided by Elmarco Ltd. Gelatin nanofibers loaded by HA (NF-GELHA) and has been prepared by followed procedure. Porcine gelatin was dissolved in organic solvent. Nanoparticles of hydroxyapatite (20wt.% to dry matter) were mixed into the solution. In an effort to avoid clumps creation, this mixture was 5 minutes in an ultrasonic bath. Basis weights for gelatine and nHA mixture was app.6 gsm. Nanofibers (FIG.1b) had to be crosslinked (48 hours by glutaraldehyde vapours) due to water solubility. Sixty four layers of NF-GELHA have been placed into the form and pressed at 40°C under the pressure of 35MPa for 5 minutes (Pracovní stroje Teplice, Czech Republic, type HLV 5.1).

The third type (PAA-COLHA) (FIG. 1c) has been prepared by introducing of HA powder (paricle size 20-70nm, supplied by Berkeley Advanced Biomaterials, Inc., San Leandro, CA, USA) into porcine collagen (VUP Brno, CR) and mixed by kneading machine at room temperature at a rotation speed of 20min$^{-1}$ for 24 hours (75wt.% COL and 25wt.% HA). After this procedure, polyamide nanofibers (PAA; 1,5g/m$^2$, Elmarco Ltd., CR) were added into the mixture (6 wt.% PAA and 94wt.% COLHA) and mixed by kneading machine at a room temperature at a rotation speed of 35min$^{-1}$ for 3 hours. The mixture have been placed into the form and pressed at 37°C under the pressure of 0.1MPa (Pracovní stroje Teplice, Czech Republic, type HLV 5.1). Approximate volume fractions of components were 46 vol.% COL, 25 ol.% HA and 29vol.% PAA.

The ultimate tensile strength and Young's modulus NF-GELHA and GELHA composites were determined with Inspekt 100 HT material tester (Hagewald & Peschke, Germany) and in the case of PAA-COLHA with testing system MTS 858.2 Mini Bionix (MTS Systems Corporation, Minnesota, USA), both experiments were provided with respect to ISO 527. Differences in HA concentration in matrices has been analyzed by Raman microscopy (Jobin Yvon, Labram HR, equipped with confocal microscope Olympus, exciting source-laser 780nm, step 2μm).

| Material | Tensile strength [MPa] | Young's modulus [GPa] |
|---|---|---|
| GELHA | 29.9 ± 1.3 | 2.2 ± 0.3 |
| NF-GELHA | 50.0 ± 2.1 | 1.2 ± 0.1 |
| PAA-COLHA | 10.2 ± 1.1 | 0.7 ± 0.1 |
| Cortical bone | 50–150 | 14-20 |
| Cancellous bone | 10–20 | 0.05-0.5 |

**TABLE 1. Results of the mechanical tests in comparison with mechanical properties of human bone [1].**

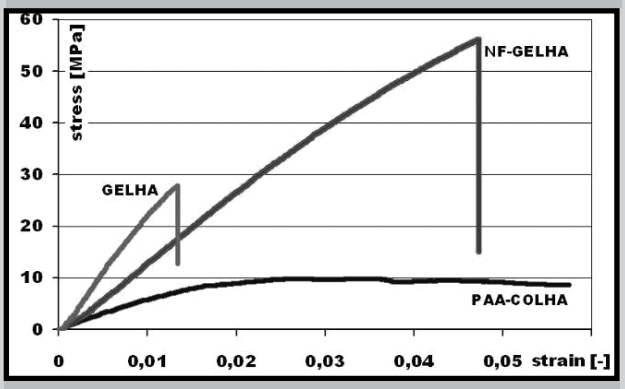


**FIG.2. Typical stress-strain curves of examined composites (tensile tests).**

## Results

The purpose of the mechanical testing was to determine the behavior of the composite with regard to the future potential application in bone tissue engineering. The ultimate tensile strength and Young's modulus for all types of composites were determined (see TABLE 1).

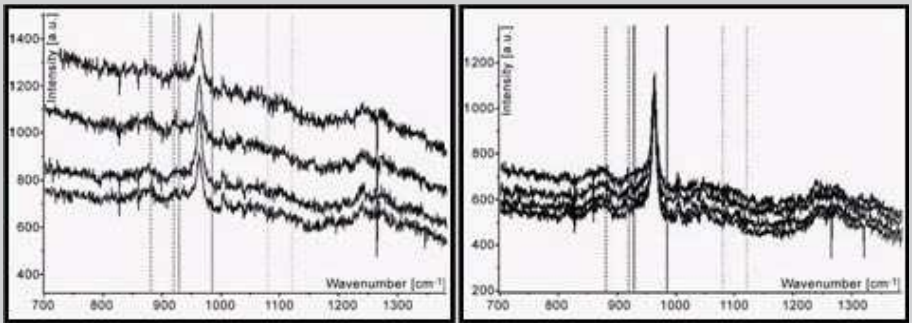


**FIG.3. An example of HA concentration mapping in GELHA (left) and NF-GELHA (right) composites.**

The behavior of the both GELHA and NF-GELHA composites at mechanical tests are similar, distinct brittle cracks appeared at specific load value (FIG.2). In the case of PAA-COLHA,

measured samples showed elastic behavior without presence of brittle cracks. This fact should be caused by nonhomogenous distribution of polyamide nanofibers and HA particles, respectively nonhomogenous structure caused by preparation (for ilustration see FIG.1). The brittle behavior of GELHA and NF-GELHA composites (in comparison with PAA-COLHA) is also illustrated by higher values of Young's modulus. Based on these results, further optimization of mechanical properties will be carried out by selecting the volume ratio of the fiber reinforcement to the matrix and also reinforcement suitable orientation and layering.

For HA concentration determination the automatically mapping (software Labspec ver. 2.08) has been used. Selected spectrum and collection of measured Raman spectra are depicted in FIG.3. Concentration maps were calculated on the base of band belongs to $PO_4^{3-}$ group at 964cm$^{-1}$. Mapping of HA concentration in examined composites showed a sufficient and comparable HA dispersion in all cases. Better HA dispersion was showed in the case of NF-GELHA composite. This homogeneity can be connected and probably influence the mechanical properties.

## Conclusions

This study has investigated the possibilities of preparation of composite materials based on various reinforcing materials and matrices. The influence of different composite constituents on mechanical properties of composites mainly based on the biodegradable materials was verified. Further optimization of mechanical properties and verification of proper structure composition are subjects of the future research.

## Acknowledgements

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# DISORDER OF THE MINERAL METABOLISM OF THE ORAL CAVITY FOR PATIENTS WITH ODONTOGENIC ABSCESES IN MAXILLOFACIAL AREA AND WAYS FOR ITS CORRECTION WITH STANDARD METHODS OF REHABILITATION

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## Introduction

Examination of the oral fluid (OF) has great potential in different diseases diagnostics now. Its indices describe oral cavity homeostasis as well as human body state in general [6].

Bone is a calcified tissue consisted of cells put into the main hard substance. Inorganic components make 70% of this substance and the principal of them is hydroxyapatite [3]. Due to this, great attention is paid to the ions calcium content and its compounds into the fluid systems of the human body for patients with pathological processes of the bone tissue as well as with pyoinflammatory complications of osteomyelitis presented by abscesses and phlegmons [2,4]. Pyoinflammatory process of any etiology is accompanied by morphological and functional disorders. It means that many enzymatic reactions are involved into the pathological process. Hydrolytic enzyme – acid phosphatase (AP) – should be considered as marker of this process. Many works give up to the examination of AP activity into the biological environment of the human body [1,5]. Last time, more attention is paid for micromorphological indices of the OF and its fractions examination [3]. Indices of the OF microcrystallization describe correctly processes of mineralization of maxillofacial area. But there are few works about changes of the OF microcrystallization indices for patients with pyoinflammatory diseases in maxillofacial area during the treatment. All this confirms the urgency of this work.

## Aim of work

is to study mineral metabolism of the oral cavity for patients with odontogenic abscesses in the maxillofacial area and ways of its correction with standard complex of rehabilitation methods.

## Objects and methods

We examined 30 patients with odontogenic abscesses in maxillofacial area. 15 patients had abscesses of mylohyoideus area and 15 patients with abscesses of pterygoid mandibular area. When patients went for medical care, doctors used intraoral approach method for the primary surgical d-bridement (PSD) of the suppurative focus and patients were instituted for the standard course of complex antiphlogistic therapy. Bandages were changed every day.