

## MINERALIZED POLY(VINYL ALCOHOL) CRYOGELS FOR OSTEOINTEGRATION

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## МИНЕРАЛИЗОВАННЫЕ КРИОГЕЛИ ПОЛИВИНИЛОВОГО СПИРТА ДЛЯ ОСТЕОИНТЕГРАЦИИ

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**Аннотация.** Криогели поливинилового спирта, минерализованные фосфатами кальция были получены циклическим замораживанием-оттаиванием. Синтез фосфатов кальция проводился *in situ* под воздействием микроволнового излучения при нагревании и перемешивании. С использованием методов ИК-спектроскопии и сканирующей электронной микроскопии и микрорентгеноспектрального анализа был установлен состав композитов, а также их морфология и свойства поверхности. Биологическая совместимость материала была исследована *in vivo* на крысе линии Wistar. Оценка качества образования костной ткани между имплантантом на основе криогеля и поврежденной костью проводилась методом компьютерной томографии. В кости с введенным композитом наблюдается улучшение консолидации костного дефекта по сравнению с контрольной костью.

**Introduction.** Musculoskeletal conditions are the second greatest cause of disability globally. Every year over 2.2 million people worldwide require bone grafting surgery to repair large bone defects of critical size arising from accidents, trauma or diseases (like tumour resection). Hence, the demand for new technologies for orthopedic implants manufacturing increases.

Calcium phosphates (CaP) ceramics have been frequently used as bone substitutes due to the chemical similarity to mammalian bones and teeth. In addition to its potential to mimic the mineral phase of bone, CaP can be moulded into bone defects, then harden *in situ* to provide stability. CaP-based materials offer excellent biocompatibility and biodegradability that promote and foster their application in biomedicine. The examples include dental implants, percutaneous devices and use in periodontal treatment, treatment of bone defects, fracture treatment, total joint replacement (bone augmentation), orthopedics, cranio-maxillofacial reconstruction, otolaryngology and spinal surgery.

However, due to the ceramic origin, any bioceramics made of calcium orthophosphates possess poor mechanical properties that do not allow them to be used in load-bearing areas, such as artificial teeth or bones.

For instance, fracture toughness of hydroxyapatite bioceramics does not exceed the value of  $1.0 \text{ MPa m}^{1/2}$ . Meanwhile, for the human bone values of fracture toughness are  $2\text{--}12 \text{ MPa m}^{0.5}$ .

Polyvinyl alcohol (PVA) is one of the most widely used polymers in biomedical engineering and pharmaceutical technology due to its ability to form crosslinked structures without toxic additives along with a simple molecule structure, which can be tailored depending on the application purpose. PVA cryogels, known for its excellent biocompatibility and mechanical properties, has been extensively used in the development of artificial tissues. Particularly, PVA cryogels prepared by freezing and thawing techniques show enhanced mechanical strength and good viscoelasticity. They have physical properties more similar to those of human tissues and are easily attached onto the underlying bone.

Composites, especially mineralized hydrogels, partially exhibit the properties of their starting materials, yet they could be endowed with synergistic effects, overcoming the limitations of the individual components. As for Calcium phosphates/polyvinyl alcohol (CaP/PVA) composites, a soft elastic gel is combined with a bioactive inorganic phase, radically improving its mechanical properties.

The aim of the present paper is to describe synthesis of CaP/PVA hydrogels composite materials and their characteristics, including biocompatibility *in vivo*.

**Materials and methods.** CaP/PVA hydrogels with different ratio of the components were obtained by adding calcium hydrophosphate powders to PVA water solution. Mixture was subsequently stirred in a plastic mold for 4 hours and processed through three freezing-thawing cycles. Freezing was performed at  $-80^\circ\text{C}$  and thawing at  $25^\circ\text{C}$  for 24 hours per stage. Obtained hydrogels were sterilized, using 96% vol. ethanol solution. Sterilization time – 2 hours. Surface morphology was investigated by scanning electron microscope on a Hitachi TM-3000 instrument operated at an acceleration voltage of 15 kV with surface charge elimination, using a QUANTAX 70 energy dispersive spectrometer system for elemental analysis. IR-spectroscopy of samples was performed using the Agilent Cary 630 Fourier spectrophotometer. *In vivo* biocompatibility testing was carried out using a six-month old Wistar rat. The animal was anesthetized by “Sevoran” gas anesthesia with 8% vol. sevoflurane via a face mask during procedure. Bone fractures of 3 mm. in the proximal metaphysis of both tibia bones were formed. Anterior medial access to the left tibia was performed and CaP/PVA cryogel (50 wt. % CaP) was introduced into the (intra)medullary canal. Subsequently, the wound was sutured with Vicryl sutures. At 60 days after the surgery, a rat was sacrificed by a 100% vol. carbon dioxide inhalation. The experiment was carried out according to the Rules and regulations of laboratory work with experimental animals, the Federal Law of the Russian Federation "On the Protection of Animals from Cruel Treatment" of 01.01.1997. Formation of the bone tissue between inserted hydrogel and native bone was investigated computed tomography, using Optima CT 660 CT scanner.

### Results and discussion.

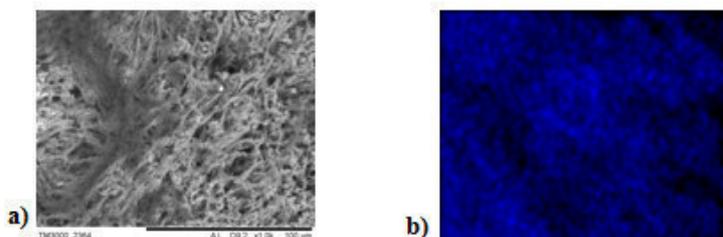


Fig. 1 SEM (a) and EMP (b) images of CaP/PVA hydrogel

Fig. 1 shows SEM and EMP images of the sample, introduced to the rat's tibia. The developed microporous surface with an average pores size up to  $50\ \mu$  and even distribution of CaP was observed. Hydrogel's surface appears to be rather smooth and crystals of calcium phosphate are accentuated.

IR-spectra reflects on composition of the sample, incorporated into the rat's bone. According to the spectra, absorbance bands at  $1300\text{--}1400\ \text{cm}^{-1}$  corresponded with bending modes of  $\text{--CH}$  and  $\text{--OH}$  groups in PVA matrix. Meanwhile, absorbance at  $1025\ \text{cm}^{-1}$  and  $560\ \text{cm}^{-1}$  was assigned to non-degenerate symmetric P–O stretching mode and triply degenerate O–P–O bending mode of  $\text{PO}_4^{3-}$  group.

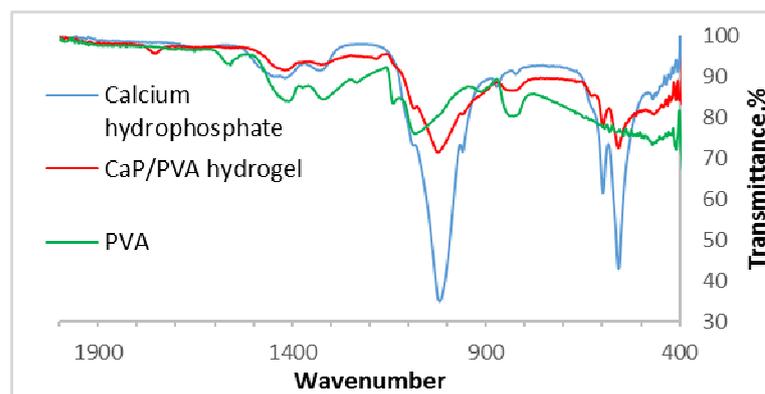


Fig. 2 IR-spectra of CaP/PVA hydrogel

The CT (Hounsfield units, HU) at the center of the bone defect in rat's tibia proximal metaphysis was evaluated at +674 HU. Coincidentally, a cloud-shaped hydrogel body with the +593 HU was observed within the (intra)medullary canal of hydrogel-introduced tibia. The size of an initial fracture was 3 mm. Following 60 days after the surgery, the 2,7 mm fracture with +344 HU was observed.

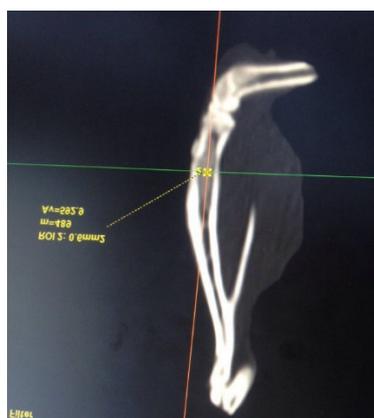


Fig. 3 CT observation of the partially consolidated rat's tibia fracture

## REFERENCES

1. Timofejeva A., D'Este M., Loca D. (2017) Calcium phosphate/polyvinyl alcohol composite hydrogels: A review on the freeze-thawing synthesis approach and applications in regenerative medicine [Electronic version]. European Polymer Journal, no.95, pp. 547–565.