ISSN 1027-5495. Functional Materials, 26, No.3 (2019), p. 609-614.
doi:https://doi.org/10.15407/fm26.03.609

Functional hybrid organo-inorganic composite materials of the incorporative type for the recovery of articular cartilage defects

S. Krivileva*[1]*, A. Rassokha*[1]*, A. Zakovorotniy*[1]*,
***V. Moiseev*[1]*, V. Zhukov*[2]**

[1]National Technical University "Kharkiv Polytechnic Institute", Kyrpichova Str., 2, Kharkiv, 61000, Ukraine
[2]Kharkiv National Medical University, Nauky av. 4, Kharkiv, 61000, Ukraine

Received April 4, 2019

The concept of obtaining a functional hybrid organo-inorganic composite material of the incorporation type with a binder based on polyacrylic acid and a nanocrystalline filler of a frag- mentary structure has been developed. $Ca_3(PO_4)_2$ particles contain two phases with a semicohe-rent boundary between them. Structuring of the composite takes place at five levels: in the volume of the polymer binder, in the interfacial layer and the polymer matrix adjacent to it, and in the outer and inner layers of the calcium phosphate filler. The effects of composition, structure, and the ratio of the initial components on the properties of the composite were studied. The use of the composite causes the growth of young tissue of the articular cartilage with the restoration of its structures.
Keywords: hybrid organo-inorganic composites, coherent boundary, incorporation type, structuring mechanism, chelate complex.

Разработана концепция получения функционального гибридного органо-неорганического композиционного материала инкорпоративного типа со связующим на основе полиакриловой кислоты и нанокристаллическим наполнителем фрагментарной структуры. Структурирование композита происходит на пяти уровнях: в объеме полимерного связующего, в межфазном слое, полимерной матрице, примыкающей к нему, во внешнем и внутреннем слоях кальцийфосфатного наполнителя. Изучено влияние состава, структуры и соотношения компонентов на свойства композита. Использование композита обусловливает рост молодой ткани суставного хряща с восстановлением его исходных структур.

Функціональні гібридні органо-неорганічні композиційні матеріали інкорпоративного типу для відновлення дефектів суглобового хряща. *С.П.Кривільова, О.М.Рассоха, О.Ю.Заковоротний, В.Ф.Моисеев, В.І.Жуков.*
Розроблено концепцію отримання функціонального гібридного органо-неорганічного композиційного матеріалу інкорпоратівного типу зі зв'язуючим на основі поліакрилової кислоти і нанокристалічним наповнювачем фрагментарної структури, Структурування композиту відбувається на п'яти рівнях: в об'ємі полімерного зв'язуючого, в міжфазному шарі, полімерній матриці, що примикає до нього, у зовнішньому і внутрішньому шарах кальційфосфатного наповнювача. Вивчено вплив складу, структури і співвідношення компонентів на властивості композиту. Використання композиту обумовлює ріст молодої тканини суглобового хряща з відновленням його структур.

1. Introduction

Fundamentally different materials are necessary for the correction of structural violations of various parts of the skeleton [1, 2]. For engineering of articular cartilage, materials developed for other sites are not applicable. Known polymer composites and bioceramic materials do not cause regeneration of articular cartilage, but only lead to its punching [3-5].

A promising direction in modern medical materials science is the development of functional organo-inorganic hybrid composites based on hydroxylapatite, calcium phosphates, bioglasses and bio-metals a new class of nanomaterials that are formed by the incorporation of inorganic fillers into polymer matrices [6]. In these composites, the bioactive phase serves either as a matrix in which the polymer phase is dispersed, or as a filler distributed in the polymer matrix. In both cases, the properties of the phases are realized in the material: on the one hand, the ability to regenerate cartilage tissue (bioactive phase), and on the other hand, elasticity and osteoplasticity.

The aim of this work is to create a functional hybrid organo-inorganic composite material of an incorporated type to restore articular cartilage defects.

To achieve this goal it was necessary to solve the following tasks:

(i) to create a concept for producing a hybrid organic-inorganic composite material of an incorporated type with a binder based on polyacrylic acid and a nanocrystalline filler with particles of a fragmented structure; (ii) to study the features of the formation of a fragmented structure of a calcium phosphate filler and, based on the data obtained, to develop a simplified technology for the synthesis of bioceramic materials for use as organo-inorganic composite fillers; (iii) to study the effects of the composition, ratio of components and structure of the inorganic filler on the complex of deformation-strength and operational properties of the composite; (iv) to study the possibility of using the developed material for the regeneration of articular cartilage in vivo, and to conduct a full range of toxicological and hygienic tests.

2. Experimental

In the present study, high-purity $Ca(OH)_2$, CaF_2 and H_3PO_4 and magnesium stearate $Mg(C_{17}H_{35}COO)_2$ were used to obtain an inorganic filler. The filler was synthesized from pre-synthesized $Ca_3(PO_4)_2$, CaF_2 and $Mg(C_{17}H_{35}COO)_2$ by triple roasting of tablet mixtures at a temperature of 1150 - 1250 °C with holding for 2 hours.

As the polymer component, we used a mixture of aqueous solutions of polyacrylic acid with a molecular weight of 25000 and polyethyleneoxide with a molecular weight of 1400-1600, macromolecules of which interacts due to hydrogen bonds. The use of polyethylene oxide significantly improves the rheological characteristics of the organomineral system at the stage of formation of a functional hybrid material. The structure of nanoparticles of various $Ca_3(PO_4)_2$ polymorphic modifications, namely, the α and β-modifications with a semicoherent interface between them, was determined by high-temperature microscopy in situ [8] and X-ray diffraction (XRD) analysis.

Functional hybrid organo-mineral composite materials were prepared according to standard laboratory procedures. The mixing of the ingredients (dispersed and dispersion phases) was carried out under normal conditions for at least 60 seconds to obtain a homogeneous material; then it was transferred to the mold for the initial stage of structuring ("setting"). The deformation and strength (breaking stress under compression, impact strength, relative compressive deformation during failure), technological and other properties were determined by standard methods [9, 10].

The possibility of using the developed material for the regeneration of articular cartilage was investigated under specific surgical procedures for plastic surgery of traumatic defects of the articular cartilage, followed by the replacement of pathological foci with a developed biocomposite. For this purpose, samples of the materials were planted in articular cartilages of laboratory animals. Transcortical defects in the form of cylindrical holes were made in the area of the knee and hip joints with a 4-mm-diameter dental burr; then the holes were filled with samples of hybrid polymer-ceramic composites.

A full range of toxicological tests and assessment of the specificity of restructuring and mechanisms of tissue regeneration at different stages of the repair process was carried out on the basis of clinical, X-ray, morphological and biochemical analyzes performed at Kharkov Medical University.

3. Results and discussion

For the production of a hybrid organic-inorganic composite of an incorporated type, a concept has been developed based on the use an inorganic filler consisting simultaneously of fragmentary structured nanoparticles of two different $Ca_3(PO_4)_2$ phases with a semicoherent interface between them. As a result of the implementation of the developed concept, a functional hybrid nanocomposite material was obtained, purposefully designed to restore defects in articular cartilage.

The formation of the material with a unique structure occurs at five levels:

1) in the volume of the polymer binder;

2) in the interfacial layer at the interface between the filler and the polymer matrix;

3) in the areas of the polymer matrix adjacent to the interfacial layer;

4) in the outer part of the α-$Ca_3(PO_4)_2$ dispersed filler;

5) in the internal non-hydratable part of the inorganic calcium phosphate β-$Ca_3(PO_4)_2$ filler.

The model of the structure of the functional hybrid organo-inorganic composite of an incorporated type in vivo is shown in Fig. 1. The use of the specially developed inorganic filler with a specific fragmented structure of nanocrystalline particles allows the hybrid nanocomposite structure to be formed at five levels; its schematic image is shown in Fig. 2a.

The introduction of the fragmentary structured calcium-phosphate particles into an aqueous polymer solution allows for obtaining a durable composite that is highly stable due to the formation of hydroxylapatite (first non-stoichiometric $Ca_9\square(PO_4)_5(HPO_4)(OH)\square$, and then the stoichiometric structure - see Fig. 2b) which is confirmed by the results of the XRD.

As a result of hydration processes at the phase boundary in which α-$Ca_3(PO_4)_2$, water, and a portion of the cabroxyl groups of polyacrylic acid take part, an organomineral interphase region is formed; this is characterized by high strength and stability of properties and sizes (i.e., significant decrease in shrinkage during the formation of the composite). An equimolecular amount of water available in the system is involved in the hydration processes; as a result, the polymer binder is almost completely free of moisture. The flow rate and the completion degree for each of these processes depends on the ratio of ingredients in the composite material, the structure of the filler, the properties of the binder, temperature and other factors. Full stabilization of the strength and operational properties of the composite (which continues in the environment of a living organism) usually occurs only after 7-10 days.

It is advisable to use hydrophilic polymers as matrices in composites for regenerative medicine. These polymers are crosslinked by chemical and physical methods. This is the result of intermolecular interaction of the components of the polymer matrix-inorganic filler system (due to the formation of hydrogen bonds, hydration, etc.). In composites for regenerative medicine, it is advisable to use particles of various crystalline shapes, morphology and sizes as inorganic fillers, since it is these characteristics that determine the final properties of the materials (due to specific effects associated with the behavior of induction charges).

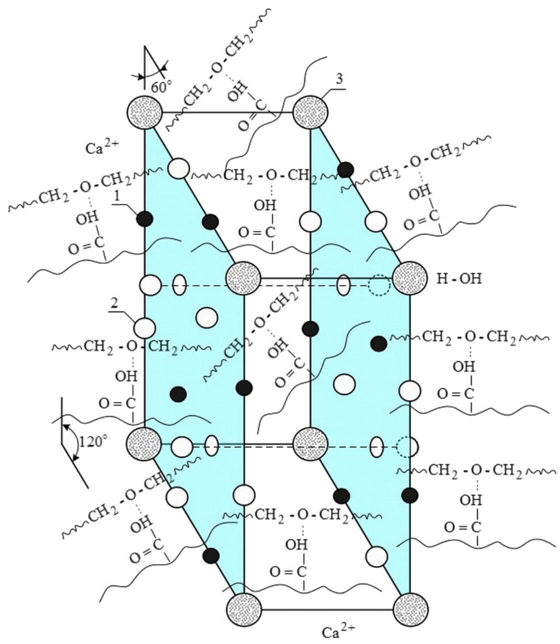

Fig. 1 - Schematic representation of the structure of an organo-inorganic hybrid nanocomposite material of an incorporated type *in vivo*: *1* - phosphate ions; *2* - calcium ions; *3* - hydroxyl groups.

Therefore, it seemed most appropriate to use an aqueous solution of polyacrylic acid and polyethylene oxide as a binder, and synthetic nanocrystalline powders of apatite composition as a filler [7]. These powders are spherical mesoporous polycrystals with biocompatibility and biomineralization potential; this is especially important for the regenerative approach in tissue engineering). The presence of MgO and $Ca_{10}(PO_4)_6F_2$ in the filler composition makes it possible to increase the strength of the composite and increase its stability in a chemically aggressive environment of a living organism.

We have revealed an improvement in mechanical properties due to the hybridization of the material - the combination of organic

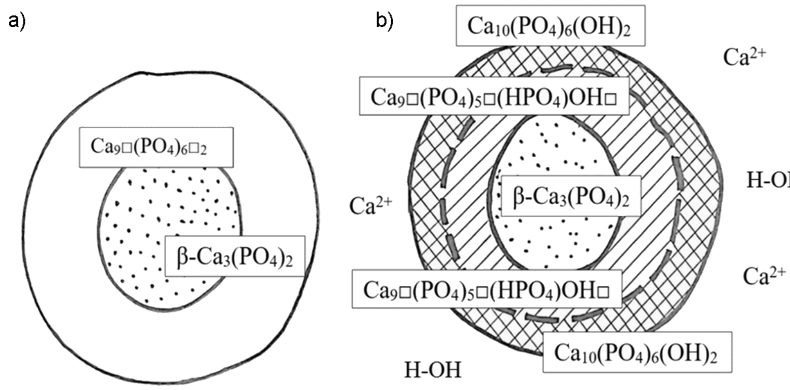

Fig. 2 - Schematic representation of the structure of a finely dispersed particle of an inorganic filler, consisting in the interior of a non-hydratable β-modification $Ca_3(PO_4)_2$ and the outer sphere from α-$Ca_3(PO_4)_2$: a - in the initial state; b- in the humid environment of a living organism *in vivo*.

molecules with inorganic nanoparticles (see Table 2). And this, in turn, increases cell adhesion and tissue regeneration (according to the results of biomedical research). Thus, by adjusting the concentration of the inorganic filler, it is possible to control the deformation-strength properties of the composite in accordance with the properties of articular cartilage cells.

On the basis of our previous researches [11,12], a synthesis technology of $Ca_3(PO_4)_2$ nanocrystals with a special structure (which we called fragmented) was developed. A single particle contains two different phases of α- and β-$Ca_3(PO_4)_2$ modifications with a semicoherent boundary between them. The authors of [13] called similar nanoscale ZrO_2 particles "centaurs". It was revealed that the optimal ratio of the components during the synthesis of calcium phosphates in the solid phase should be determined taking into account the volatility of phosphorus compounds used as precursors.

The XRD results confirmed that the synthesis products of $Ca_3(PO_4)_2$ and $Ca_{10}(PO_4)_6F_2$ are high purity nanocrystalline materials with a particle size of less than 80 nm. $Ca_3(PO_4)_2$ nanoparticles are crystals consisting of non-hydratable β-modification of tricalcium phosphate in the inner part, and hydratable α-form in the outer part. The α-$Ca_3(PO_4)_2$ modification differs from the β-$Ca_3(PO_4)_2$ in that the first is apatite in nature and its formula can be written as $Ca_9\square(PO_4)_6\square_2$, where □ is a vacancy. When a composite material is prepared in an aqueous medium, a partial hydrolysis of $Ca_3(PO_4)_2$ takes place and the substance becomes crystalline hydroxylapatite of non-stoichiometric composition $Ca_9\square(PO_4)_5(HPO_4)(OH)\square$ with channels only half filled with water. In a physiological environment (which contains H-OH and Ca^{2+}) for a long time, non-stoichiometric hydroxylapatite $Ca_9\square(PO_4)_5(HPO_4)(OH)\square$ is converted to crystalline $Ca_{10}(PO_4)_6(OH)_2$ with a stoichiometric composition.

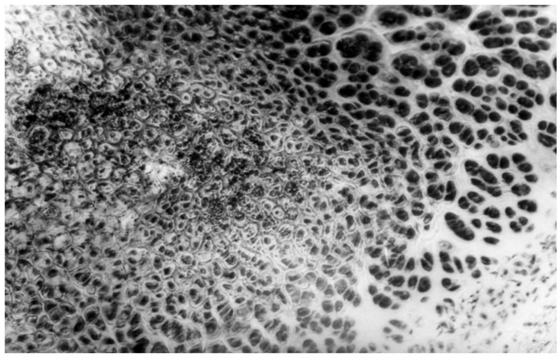

Fig. 3. - Formation of cartilaginous regenerate using a hybrid organo-inorganic composite based on a nanocrystalline powder in a composition with a polymeric binder. Fields of newly formed articular cartilage; cartilaginous cells of different degree of maturity (after 14 days). x80.

The influence of the synthesis conditions on the structure and properties of the filler was studied, also, the optimal ratio of the starting components and the heat treatment modes (1100 - 1250°C) were determined; this made it possible to obtain nanocrystalline powders with a fragmented particle structure (see Fig. 2). It is established that the use of $Mg(C_{17}H_{35}COO)_2$ in the charge and the content of $Ca_{10}(PO_4)_6F_2$ in the filler composition increase the mechanical properties and stability of the functional hybrid organo-mineral composite (see table 2, examples 1-3), which results in its rather high fatigue strength, including *in vivo*.

The effect of the composition of the composites on the complex of their deformation-

Table 1. The composition of functional hybrid organo-inorganic composites of an incorporated type

Component name	The content of components in a hybrid organo-inorganic composite, mass %									
	1	2	3	4	5	6	7	8	9	10
Polyacrylic acid (mol.mas. 25000)	18.0	16.5	14.0	20.0	12.0	-	-	16.5	16.5	17.00
Polyethylene oxide (mol.mass 1400-1600)	1.50	2.00	3.00	3.00	1.20	2.00	2.00	-	-	2.00
Nanocrystalline filler	62.5	65.0	69.0	5.0	74.8	65.0	65.0	65.0	65.0	64.0
Distilled water	18.0	16.5	14.0	14.0	19.0	16.5	16.5	16.5	16.5	17.0
Polyacrylic acid (mol.mass 23000)	-	-	-	-	-	16.5	-	-	-	-
Polyacrylic acid (mol.mass 28000)	-	-	-	-	-	-	16.5	-	-	-
Polyethylene oxide (mol.mass 1000)	-	-	-	-	-	-	-	2.00	-	-
Polyethylene oxide (mol.mass 1800)	-	-	-	-	-	-	-	-	2.00	-

strength and technological properties was studied. The compositions of hybrid organo-inorganic composites are shown in Table 1, and their properties are shown in Table 2. Analyzing the data obtained, we can conclude that both the content of the dispersed phase in the polymer matrix and a certain molecular weight of the polymers (25000 for polyacrylic acid and from 1400 to 1600 for polyethylene oxide) have a significant impact on the complex of properties of the composite and determine the area of its functional purpose. As can be seen from Table 2, for the restoration of defects in articular cartilage, the whole range of the studied compositions is promising. A method of sterilization of a hybrid organo-mineral composite has been developed. Also it has been determined that adding contrasting and antibiotic components to its composition does not result in significant deterioration of the final mechanical properties if the contrast medium is incorporated during structuring of the polymer matrix in situ [14].

The possibility of using the developed material for the regeneration of articular cartilage was assessed. Verification showed that during implantation of the developed hybrid composite, the articular cartilage was newly formed and the initial histological structures were restored. In this case, no signs of general intoxication were detected; local histological effect was not manifested. The material compensates for the damaged area of the articular cartilage and creates the necessary conditions for its regeneration. Fig. 3 shows the regeneration of the articular cartilage after 14 days of in vivo testing. In this case, an organo-inorganic composite material was used, purposefully developed by the authors for plastic surgery of defects in articular cartilage. The fields of newly formed cartilage and cartilage cells of various maturity are clearly visible on it.

A full range of biomedical and toxicological tests was carried out; the tests showed that the use of the developed material ensures the replacement of damaged cartilage tissue and the growth of young cartilage tissue. Further, biodegradation of the composite and restoration of the initial structures of the articular cartilage follow. In this case, the material does not have any toxic effect on the body. Implantation of the composite is quite favorable for the body, which will completely restore the lost function of the joint.

4. Conclusions

A concept has been developed for producing a hybrid organic-inorganic composite of an incorporated type; the concept is based on the use of a fragmentary structure of $Ca_3(PO_4)_2$ nanoparticles as an inorganic filler which consist simultaneously of two different $Ca_3(PO_4)_2$ phases with a semicoherent interface between them. As a result of the implementation of the developed concept, a functional hybrid nanocomposite material was produced, purposefully designed to restore defects in articular cartilage. A technology has been developed for the synthesis of $Ca_3(PO_4)_2$ nanocrystals with a fragmentary structure consisting of the non-hydratable β-modification of $Ca_3(PO_4)_2$ in the inner part, and of the hydratable α-form in the outer part of the same compound.

Table 2. Properties of hybrid organo-inorganic composites

Composite	Parameter name					
	Breaking compressive stress, MPa	Time recruitment strength, min	Relative deformation at fracture, %	Shaping time, min	Toughness, KJ/m^2	Open porosity of the composite, %
1	105	60	0,8	15	3,5	3
2	110	55	1,0	14	3,5	4
3	113	50	1,3	10	4,0	5
4	77	68	0,8	15	3,0	3
5	93	70	0,8	20	2,8	2
6	85	73	1,0	17	3,1	1
7	90	75	1,1	20	2,7	3
8	75	70	1,2	13	3,2	4
9	70	65	1,3	14	3,0	2
10	87	75	1,0	14	2,8	2

Note to table 2: compositions of composites 1-3 provides the formation of a functional material having a complex of high functional properties with an equimolecular ratio of water and carboxyl groups of polyacrylic acid; compositions of hybrid composites 4-5 with non-optimal ratio of ingredients; composites 6-9, illustrating the effect of the molecular weight of the polymer ingredient (polyacrylic acid and polyethylene oxide) on the complex of the studied properties of hybrid organo-mineral composites; in composite 10, hydroxylapatite with a non-optimal phase ratio in the external and internal spheres of the filler particles is used.

The introduction of such an inorganic filler into an aqueous solution of a polymeric material allows one to form a strong three-dimensional framework which has high mechanical strength due to the formation of hydroxylapatite; first, non-stoichiometric $Ca_9□(PO_4)_5(HPO_4)(OH)□$ is formed, and then the formation of hydroxylapatite stoichiometric structure takes place.

The effects of the composition, structure and the ratio of polymeric materials with optimal molecular weight and the dispersed filler with a certain structure on the complex of deformation-strength and technological properties of the composite were studied.

The possibility of using the developed material for the regeneration of articular cartilage in vivo was evaluated. It is shown that the developed hybrid organo-inorganic composite compensates for the lost portion of the articular cartilage and creates the necessary conditions for its regeneration. This makes its use promising in tissue engineering.

References

1. L.L.Hench, *Sci.*, **295**, 1014, (2002).
2. D.Logeart-Avramoglou, F.Anagnoston, R.Bizios, H.Petite, *J. Cell. Mol. Med.*, **9**, 72 (2005).
3. O.Sych, N.Pinchuk, A.Parkhomey et al., *Functional Materials*, **14**, 430 (2007).
4. S.Krivileva S., O.Zakovorotnyi O., V.Moiseev, N.Ponomareva, O.Rassokha, O.Zinchenko, *Functional Materials*, **26**, 347 (2019).
5. O.Otychenko, T.Babutina, O.Kuda et al., *Functional Materials*, **24**, 577 (2017).
6. A.Veresov, V.Pytliaev, Yr.Tretiakov, *Russ. Chem. J.*, **48**, 52 (2004).
7. S.Krivileva, A.Rassokha, Minerals and mountain breeds, Kharkiv, *Point Publ.*, 2014, 124 p.
8. A.N.Vinchell, G.Vinchell. Optical properties of artificial minerals, Moscow: Wold Publ., 1980, 526 p.
9. Ya.Rabek, Experimental methods in the chemistry of polymers, in 2 parts. Per. from English Part 1. - Moscow: Wold Publ., 1983, 384 p.
10. State Standard of the USSR 14359-69. Plastics. Testing methods. General requirements of the methods of mechanical testing, Moskov: publishing house of standards, 1979, 19 p.
11. Krivileva S., Bulletin of NTU "KhPI", 9 (1285), (2018), p. 128-133.
12. S.Krivileva, A.Rassokha, A. Zakovorotnyi, M. Zinchenko, N.Bukatenko, V.Zhukov, *Functional Materials*, **25**, 549 (2018).
13. V.Shevchenko, M.Balmakov, *Phys. Chem. Glass*, **28**, 631 (2002).
14. S.Krivileva, V.Moiseev, *Functional Materials*, **25**, 358 (2018).