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Carcass Compound Materials Base on Fluoropolymer for Tissue Engineering in Orthopedics

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Abstract: In this work new type of composite materials for application as coatings for intramedullary implants in the field of orthopaedics and traumatology is offered. Method is based on ability of fluoropolymers to act as biologically inert binding agent and ability of fine-dyspersated hydroxyapatite powders to act as biologically active filling agent providing osteoinduction and osteoconduction processes. Results of investigations of adhesion, elastic and morphometric characteristics of offered composite were presented; chemical composition was determined. Estimation of toxicological properties, locally irritant action and hemolytic activity of offered composites was done according to GOST R ISO 10993. In vivo tests were carried out; it was shown that offered composites didn't cause any negative tissue reactions and stimulated osteogenesis processes in ectopic bone formation test.

Key words: Carcass Compound Materials; fluoropolymers; orthopaedic

INTRODUCTION

Modern orthopedics increasingly faces to such multifactor pathology like phosphate diabetes and Oilier disease. One of the most promising methods of solution of this problem is tissue engineering. Engineering should be understood to mean growing tissue like construct under laboratory conditions, preparing this construct for implantation, and implantation in order to regenerate tissue damaged by disease into its initial condition for the purpose of function rehabilitation (Hench et al., 2007). This multidisciplinary area touches on cell biology, chemistry, physics, material science, and surgery. This article will be focused on description of elastic carcass materials designed for bone tissue engineering on surface of intramedullary implants.

Calcium-phosphate (CP) coatings for intramedullary implants are to have group of physical-mechanical, chemical and biomimetic properties which determines complexity of problem. Such coatings are to have

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three-dimensional architectonics stimulating processes of osteogenesis for which purpose we need thickness of coating of more than 150 μm and high porosity with the size of pores of more than 100 μm. At the same time taking into account medico-technical requirements coatings are to have high elasticity and adequate adhesion to material of substrate.

One way to solve the problem of obtaining elastic porous coatings of required thickness is application of polymer composites (Barinov & Komlev, 2005) consisting of at least two phases: 1) organic phase in the form of bioinert poly-mer bonding agent possessing elasticity, adhesion, bioinertness and ability for sterilization; 2) inorganic phase in the form of bioactive filling consisting of fine-dispersed calcium-phosphate powders of differ-ent chemical composition which stimulate biological processes of osteoinduction and osteoconduction (Shutov, 2009).

In addition, application of biopolymer composites as implant coatings is prospective (Hench et al., 2007; Karlov & Shakhov, 2001) as technology of their production and covering allows regulation of basic coating characteristics providing their biological compatibility as follows: 1) regulation of mechanical characteristics and biological activity right up to bio-inertness by means of change of mass fraction of in-organic filling; 2) control of elemental composition and rate of biological degradation of composite by means of selection of correspondent forms of calcium-phosphate compounds; 3) regulation of morphometric characteristics of composite by means of change of filling dispersity. Coatings with thickness of up to 150…200 μm with developed three-dimensional architectonics on macro and micro level result from multilayer coating of biopolymer composites.

The main problem of this work was investigation of opportunity of obtaining of composite material on the base of tetrafluorethylene copolymer with vinyliden fluoride filled with hydroxyapatite (TFE/VDF-HA) meeting following demands:

1) thickness of composite material coating is nor less than 150 μm,
2) elasticity is nor less than 3-4 mm according to ISO 1519,
3) adhesion to substrate is nor less than 10x106 kg/m2,
4) porosity is nor less than 30%,
5) absence of toxicity

METHODS OF INVESTIGATION

Obtained TFE/VDF-HA composites were investigated by means of following methods:

1) thickness of one-layer coatings was measured by means of apparatus *Konstanta K5*,

2) elasticity of films was determined ” according to *ISO 1519* by means of apparatus *“Izgib*,

3) adhesion on normal detach was determined according to *ISO 4624* by means of apparatus *Adgeziometr OP*,

4) surface morphology for the purpose of determination of sizes, perimeter of pores and superficial porosity was investigated by means of method of scanning electron microscopy (SEM) by means of unit *Philips SEM 515*,

5) cytotoxicity of composites sterilized with ethylene oxide was estimated in accordance with GOST R ISO 10993.

6) Biological compatibility was tasted in vivo on 30 male mice (line Balb/c, weight 18-21 g). Mice were implanted with calcium-phosphate discs with marrow column extracted from animal femur (cellularity – 1.5x106) in medium DI-MEM (ISN) with 10% embryonic calf serum (“Vector”, Novosibirsk). Animals were taken out of the experiment after 1.5 months by ether narcosis; disks were extracted and hystologically analyzed.

RESULTS AND DISCUSSION

Three types of TFE/VDF-HA composite materials with different mass content of HA 30, 50, and 70 mass % were produced for investigations and symbolically divided on groups I, II, and III respectively.

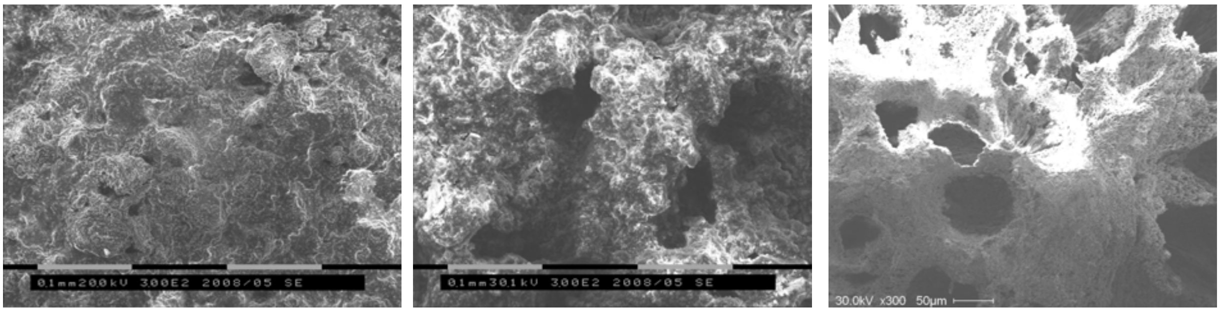
It has been determined that thickness (*h*) of one-layer coatings of composite material tends to increase with increase of mass fraction of hydroxyapatite in composite material. Minimal thickness is observed in least filled composites of the I group h = 122±2.4 µm (number of measurements n = 5) and maximal thickness – in the most filled composites of the III group h= 187±3.74 µm (n = 5), at that thickness of composites of the II group h = 146±2.92 µm (n = 5).

Results of measurement of flexibility ζ and adhesion power on normal detach ψ of coatings of composite materials tend to decrease with increase of quantity of HA introduced into TFE/VDF-HA co-polymer. Maximal ζ and ψ values are in composite of the I group: ζ = 2 mm (n = 3) and ψ = 12.1×106 kg/m2 (n = 5), lesser ζ and ψ values are in composite of the II group: ζ = 3 mm (n = 3) and ψ = 9.2×106 kg/m2 (n = 5), and minimal ζ and ψ values are in composite of the III group: ζ = 3.5 mm (n = 3) and ψ = 7.1×106 kg/m2 (n = 5). Obtained results can be explained with increase of porosity of composite coatings with increase of quantity of hydroxyapatite introduced into polymer leading to decrease of flexibility and adhesion power.

In figure 1 show images of carcass compound materials TFE/VDF-HA produced by methods of scanning electron microscopy by means of unit *Philips SEM 515*.

By sufficient increasing it clearly show that macrostructure of biopolymer composites is porous multilevel structure, where particles HA are connected among themselves by polymer binder.

As indicated earlier the summary porous is increases and significant quantity of capillary are appear with increase of HA content. Thus pours are become open and interpenetrating, specific surface of biopolymer composite are increased.



I II III

Fig. 1: Pours on surface of biopolymer composites (scanning electron microscope «Philips SEM 515», by increasing 300)

Structure of biopolymer composite became similar to structure of spongiform bone. It is additional factor promote to the bony tissue growth in pours, with bone block formation "biopolymer composite - bony tissue". It is shall in result the increase of rigidity fixing of intramedullary implants in medullar canal.

Results of investigation of basic morphometric characteristics of biocomposite surface are shown in table 1.

Table 1: Basic morphometric characteristics of biocomposite surface

Group of biocomposite	Total porosity, %	Pore square, µm^2			Pore perimeter, µm		
		Min.	Max.	Average	Min.	Max.	Average
I	9.63	1.4	5473.4	44.8	3.9	825.2	17.2
II	19.29	0.8	9464.0	34.5	4.2	1118.0	13.8
III	37.79	0.8	25379.6	90.0	5.2	3393.1	22.7

Toxicity, apyrogenicity, and hemolytic activity of TFE/VDF-HA biocomposite with minimal contain of HA (the I group) obtained in accordance with GOST R ISO 10993 are shown in table 2.

Table 2: Results of investigation of hemolytic activity, apyrogenicity and toxicity index of biocomposite of the I group

Index	Valid values	Results of investigation	Conformance
Hemolytic	No more than 2 %	0.7	Conf.
Toxicity index	70…120 %	88.4 %	Conf.
Pyrogenicity	Increase of temperature no more than 3°C	0.4° C	Conf.

Toxicological investigations of TFE/VDF-HA composite material carried out on laboratory animals didn't lead to animals' death and didn't reveal any macroscopic changes of organs and tissues, changes of weighting coefficients of internal organs. Extracts of TFE/VDF-HA composite materials didn't have any local and general irritation effect on skin and mucous membranes of laboratory animals.

It has been shown that gas sterilization by means of ethylene oxide may be used for composite sterilization.

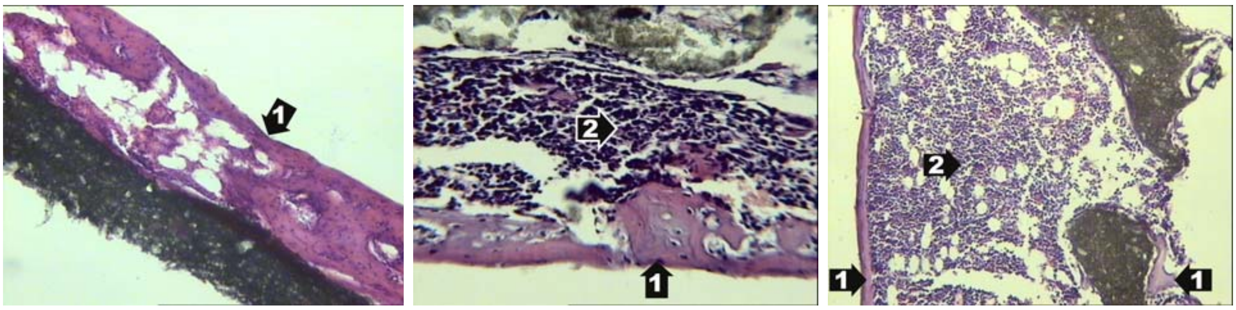
The results of investigation of biopolymer composite in vivo are presented in Table 3.

Table 3: The quantitative assessment of bioactivity of biopolymer composite TFE/VDF-HA

Group, composite	Inflammation on location of implantation	Encapsulation of implant	Probability of tissue plate formation, %	Histologic analysis	Efficiency of bone tissue grow, %
I group, fig. 2a	nonresponse	weak response	100	bone	73
II group, fig. 2б	nonresponse	weak response	100	bone with bone marrow	84
III group, fig. 2в	nonresponse	weak response	100	bone with bone marrow	93

In experiment in vivo it notes that the animals accept operation easy. In experiment there not are natural deaths of animals, also as local or total inflammatory and toxic responses on implant. Thus bio compatibility of all implants with biopolymer composite coating is satisfactory. The surface of implant have thin stomas' capsule.

Microscopic section of obtain preparation are presented on figure 2.



I II III

Fig. 2: Microscopic sections of preparation glowed on biopolymer composites of various groups

Microscopic sections (fig.2) are showing the bone tissue (1) and the medullar cavities filled by the bone marrow (2). On most porous materials (II— III groups) observe the bone ingrowth to pours of coating (fig.2, II, III). At the same time on least porous materials (I group) the bone apgrowth takes place on the surface of biopolymer composite formed the dense bone without the bone marrow (fig.2, I).

The quantitative assessment of the bone growth efficiency on biopolymer composites are presented in Table 5 and as a whole it coincide to the results obtained on other types of biocoatings (Rogero et al., 2003).

The decrease of percent of bone tissue formation with decrease mass fraction of hydroxyapatite relate to the decrease of porosity and the increase of hydrophoby of materials (Correa et al., 2007) and as consequence the decrease "availability hydroxyapatite" for medullar cells.

Thus the results of biopolymer composite TFE/VDF-HA research are show the perceptivity of application of proposes material as carcass for the bone engineering on the intramedullary implant surface.

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