Osteogenic Potential of Mesenchymal Stem Cells from Bone Marrow *in Situ*: Role of Physicochemical Properties of Artificial Surfaces

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Correlation analysis demonstrated the role of inorganic parameters of the surfaces of calcium phosphate materials in the regulation of osteogenic differentiation of mesenchymal precursors. The progenitor stromal cells were isolated from syngeneic bone marrow immobilized *in vitro* on calcium phosphate surfaces with different structure, phasic, and elemental composition. After 45 days of subcutaneous ectopic osteogenesis in BALB/c mice, the tissues grown on these matrixes were characterized histologically. It was found that adhesion of bone marrow cells is the initial stage determining their future proliferation (conduction) over the artificial surface and the area of formed tissue plate. The success of histogenesis depends on surface roughness. The optimal roughness class was 4-5 (Russian State Standards), which enables differentiation of progenitor stromal cells under the specific microenvironmental conditions into the connective and adipose tissue cells. Differentiation of the progenitor cells into the stromal cells producing the hemopoiesis-inducing microenvironment also takes place in the foci of active hemopoiesis. Induction of osteogenic potential of the stromal precursors (osteoinduction) is determined by the ratio between calcium and phosphate atoms in surface coatings. In our experimental system, osteogenic differentiation of stromal mechanocytes was blocked only at Ca/P<0.5.

Key Words: *mouse; ectopic osteogenesis; calcium phosphates*; *structure; composition*

Osteogenic properties of hydroxylapatite and tricalcium phosphate, the main components of bone mineral matrix, were clearly demonstrated by the phenomenon of ectopic osteogenesis, *i.e.* formation of the bone tissue on the surface of calcium phosphate (CP) materials. Prof. M. R. Urist was among the first investigators who described this phenomenon [15]. Realization of this phenomenon requires several prerequisites [7,9]: 1) a source of stromal precursor cells; 2) specific growth factor (morphogenetic bone proteins); 3) specific osseous microenvironment.

Mesenchymal stem cells (MSC) can differentiate into osteocytes, chondrocytes, fibrocytes, adipocytes, neural cells, myocytes, and stromal cells maintaining hemopoiesis [5,8,13,14]. However, in the multicellular system the choice of differentiation pathway remains poorly studied.

Depending on physicochemical properties (crystallinity, porosity, solubility, surface roughness *et al.*), the specimens of CP-materials have different ability to maintain osteogenesis [9,11,12]. At present, the key

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combination of their structure, thickness, and dilution rate needed to optimal realization of MSC osteogenic potential is unknown. Therefore, the study of the complex role of inorganic properties of CP-surfaces in the regulation of differentiation of the stromal precursors is interesting from theoretical and practical viewpoints.

MATERIALS AND METHODS

The phenomenon of ectopic osteogenesis (subcutaneous or intramuscular implantation of CP-materials into soft tissues) [12] is an adequate experimental approach for studying osteogenic properties in MSC pool.

The experiments were carried out on male BALB/c mice (n=62) from Collection Stock of Experimental Biomodeling (Research Institute of Pharmacology, Siberian Division of Russian Academy of Medical Sciences). The experimental animals (n=41) were narcotized with ether and injected with one implant, which had an aseptically introduced column of syngeneic bone marrow (mean area 7.5 mm^2) taken from mouse femoral bone. To provide cell adhesion, the organotypic bone marrow culture was cultured on a matrix for 45 min in a medium containing 95% RPMI and 5% FCS (both from ICN). The bone marrow was a source of MSC and growth factors. When the matrixes and bone marrow fragments were injected separately, the tissue lamellas were not formed.

After 45 days the implants were isolated and photographed in reflected light with fixed parameters. Morphometry of optical density (D), initial area of the bone marrow (prior to implantation), and tissue lamellas grown on the CP-surfaces was performed using PhotoShop 7.0 software [6]. The histological analysis was performed routinely (light microscopy of thin sections). After decalcification of the tissue lamellas grown on the implants, serial paraffin sections (parallel to the disc surface) were routinely stained with hematoxylin and eosin.

We used disks made of medical titanium (diameter 12 mm and thickness 1 mm). The artificial biocompatible (bioinert and bioactive) surfaces were formed on these disks by a modified method of anode spark-discharge (microarc) oxidation and by slip-casting technique. Different plating techniques produces implants with individual physicochemical surface properties (Table 1): 1) bioinert (cermet, CM) coating plated by anode-spark method in 10% phosphoric acid (n=5); 2) bioactive CP-dense (CPD) coating plated by anode-spark method in 10% phosphoric acid with hydroxylapatite (n=7); 3) bioactive CP-loose (CPL) coating plated by anode-spark method in 10% phosphoric acid with hydroxylapatite (n=10); 4) crystal CPD (n=2) and CPL (n=3) coating plated by annealing at 800°C; 5) bioactive CP-coating (CPM) plated by microarc method in 10% phosphoric acid with hydroxylapatite and calcium carbonate (n=6); and 6) bioactive CP-glass ceramic coating (CPGC) plated by slip-casting and annealed at 600°C (n=8).

Surface morphology of biocompatible coatings was examined by scanning electron microscopy (SEM) on a SEM 515 electron microscope (Phillips) with an EDAX head for spectrum analysis. The surface pictures were used to measure the diameter of pores. Quantitative elemental composition of coatings was determined by the analysis of characteristic X-ray spectra. Phase composition was established on a DRON-3 diffractometer by using X-ray phasic analysis with Co and Cu K_α-radiation. Thickness of the coatings was measured with a VT-1 vortex-current thickness meter.

The surface state was assessed on the basis of the parameters of vertical irregularities of the profile with the help of Talysurf 5-120 system (resolution 1 nm). The following parameters of surface roughness of the matrixes were determined: roughness class according to Russian State Standards 2789-73; the mean roughness within several lengths of the examined sites (Ra); difference between the mean heights measured for 5 most high ridges and 5 most low depressions of the surface over the distance of the examined site.

The data were processed statistically using nonparametrical Wilcoxon—Mann—Whitney U test. Correlation analysis was carried out with Spearman rang test.

RESULTS

Considerable progress was recently achieved in inducing *in vitro* differentiation of MSC towards different pathways, which opens new vistas for cell therapy. However, realization of stromal precursor cell potential *in vivo* is much more problematic. We suppose that in many cases this problem can be solved by the scaffold technology, which creates the microenvironment for MSC.

For osteogenic cell, such microenvironment can be created with the help of CP-materials.

Examination of 7 types of implants revealed pronounced difference in physicochemical properties of CP-surfaces and bioinert CM-layer or CPD matrix exhibiting weak biologic activity (Table 1).

Examination of artificial surfaces with SEM showed that except CPD layer, the anode spark-discharge CP-coatings had a clear surface microrelief. Basic elements of the coating (with certain variations for different surface types) were partially destructed spherulites 30 μ in diameter. These spherulites had internal cavities, which transformed into the deep spherical

TABLE 1. Mean Physicochemical Parameters of Artificial Surfaces and Tissue Lamellas Grown during Subcutaneous Osteogenesis in BALB/c Mice

Surface	Roughness class	Ra, μ	Rz, μ	Matrix thickness, μ	Pore diameter, μ	[Ca], atomic %	[P], atomic %	Ca/P	Probability of tissue lamella formation, %	Probability of bone formation, %	Production of lamella and bone formation, %	Area of tissue lamella, % bone marrow area
CM	8	0.535	3.592	3.95	1.0	0.93	16.83	0.055	0	0	0	0
		n_1=12	n_1=12	n_1=20	n_1=40				n_2=5			
CPD	6	1.431*	8.581*	18.46*	5.5*	3.0	9.4	0.32	29	0	0	
		n_1=6	n_1=6	n_1=13	n_1=40				n_2=7	0	0	
Crystalline CPD	5-6	2.264$^+$	10.951$^+$	16.69	1.62$^+$	2.8	7.7	0.36	50			
		n_1=6	n_1=6	n_1=13	n_1=80				n_2=2			41
CPL	4	6.476$^+$	33.110$^+$	135.85$^+$	21.0$^+$	5.7	11.5	0.50	90	100	90	168$^+$
		n_1=6	n_1=6	n_1=13	n_1=40					n_2=10		
Crystalline CPL	4	6.692$^+$	34.050$^+$	149.31$^+$	12.4$^+$	4.5	10.5	0.43	100	67	67	142$^+$
		n_1=6	n_1=6	n_1=13	n_1=45				n_2=3			
CPM	5	2.551$^+$	14.050$^+$	55.0$^+$	3.08	9.4	13.4	0.70	83	80	66	123$^+$
		n_1=9	n_1=9	n_1=10	n_1=110				n_2=6			
CPGC	5	2.952$^+$	15.792$^+$	219.1$^+$	211.0$^+$	20.9	11.5	1.81	75	100	75	71
		n_1=6	n_1=6	n_1=12	n_1=30				n_2=8			

Note. n_1 and n_2 are the numbers of measurements and the amount of specimens, respectively. *p<0.05 and ^+p<0.05 compared to CM-and CPD-surfaces, respectively (Wilcoxon—Mann—Whitney U test).

pores. The lamella-splinter elements were observed between the spherulites (Fig. 1).

Macrorelief of CPGC coating was formed by pores with depth and diameter up to 200 μ. The microrelief (Fig. 1) demonstrated spherical or ellipsoidal CP-particles (diameter 3-5 μ) and shallow pores of irregular shape between the macropores.

X-Ray phase analysis showed that bioinert CM-coating contained virtually no calcium. Addition of hydroxylapatite into suspension during coating of titanium with bioactive layers by anode spark-discharge technique considerably increased calcium level and Ca/P ratio (Table 1). This method increased the content of complex titanium and phosphorus compounds in the coatings. In addition, the following calcium compounds appeared: calcium titanate, calcium titanophosphate (CPD), and calcium phosphate (CPL). Further improvement of the anode spark-discharge technique to produce bioactive layers resulted in CPM-coating, in which the diffractometer revealed tricalcium phosphate.

CPGC-surface was characterized by maximum calcium content (Table 1). Total lack of titanium in the surface layer was probably caused by marked thickness of the coating (about 200 μ). Its phase composition (90% hydroxylapatite, 5% tricalcium phosphate) was most close to the mineral matrix of bone tissue characterized with significant biological activity.

Our data proved qualitative differences of bioactive implants from artificial bioinert substances by their chemical properties. Realization and potentiation of biological activity of their surface layer is caused by the appearance of calcium and further rise of its content in this layer, increase in atomic Ca/P ratio, formation of complex compounds of calcium, phosphate, and titanium (transition layer), and appearance of CP-compounds. By the degree of approximation to the properties of the natural mineral bone matrix the produced surfaces ranged as follows: CM<CPD<CPL<CPM<CPGC. The following groups of biocompatible surfaces result from these relationships: 1) CM, CPD-X-ray amorphous, and CPD-crystalline coatings can be termed as smooth (roughness class is 6-8), calcium-deficient (Ca/P<1.67, which is characteristic of stoichiometric hydroxylapatite), thin, and small-porous (pore diameter is less than 50 μ) surfaces; 2) CPL-X-ray amorphous, CPL-crystalline, and CPM coatings are characterized by roughness class is 4-5, calcium deficiency, thickness (>50 μ), and small pore diameter (<50 μ); 3) CPGC coatings are characterized by non-stoichiometric composition (Ca/P>1.67), thickness, and large pore diameter (about 200 μ).

X-ray-amorphous state of CP-coatings obtained by anode spark-discharge technique was characterized by submicrocrystalline structure with mean grain diameter of 460 nm (n=200). Annealing increased grain size, which is important for biological applications (Table 1).

The study of tissue reaction showed that 45 days after subcutaneous implantation of the examined matrixes carrying the syngeneic bone marrow, there were no signs of inflammatory reaction in each examined group. The implants were characterized by high biocompatibility indicating negligible response of the subcutaneous tissues to implantation.

The probability (P1) of tissue lamella formation from bone marrow on the biocompatible discs increased in the following order (Table 1): CM<CPD<CPD-crystalline<CPGC<CPM<CPL<CPL-crystalline. The number of cells adhered to the bioinert CM-implants was insufficient for the growth of tissue macrostructure: the presence of calcium ions is a prerequisite condition for this growth. The increase in the size of apatite crystals after annealing increased the probability of tissue lamella formation.

The presence of calcium ions is a necessary, but not the sufficient condition for successful histogenesis on the matrices. For example, efficiency of the forma-

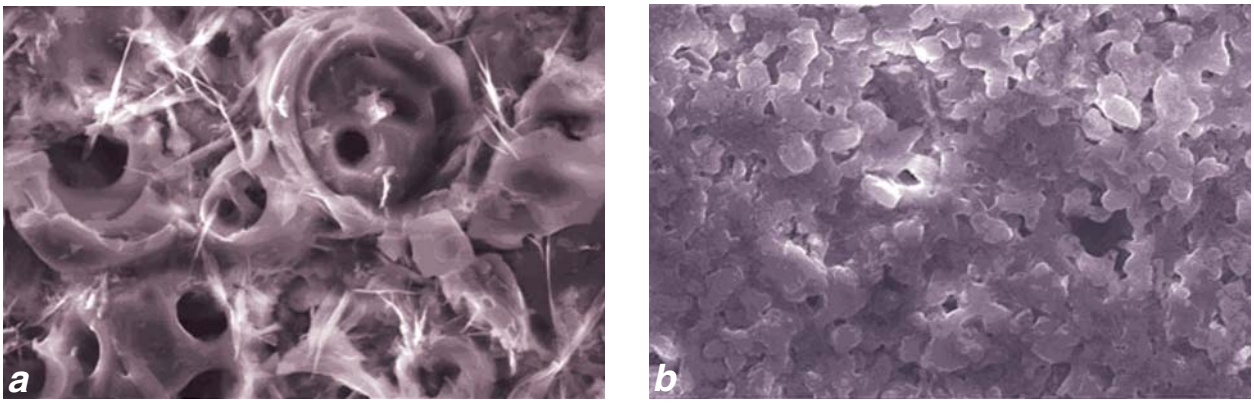

Fig. 1. Scanning electron microscopy of artificial surfaces. *a*) relief of loose calcium phosphate coating, ×1100; *b*) microrelief of calcium phosphate glass ceramic coating, ×2200.

tion of the tissues on CPD-layer was 29%, which can be explained by low structuredness of its surface in comparison with other implants (Table 1).

Adhesion of bone marrow cells is the initial stage, which determines their future conduction (migration) on the surface, which can be assessed by the area of the forming tissue lamella.

It should be emphasized that within the proposed interpretation of ectopic osteogenesis test, conduction on the surface of artificial material can result from mechanical (cell migration) and biological expansion due to cell proliferation and their enhanced synthetic activity (production of cell matrix). Cell proliferation should be proved by certain tests. In our experiments, young hemopoietic and stromal cells capable of division were seen on histological sections.

As a rule, the CP-surfaced promote migration of progenitor cells and their offspring in BALB/c mice. The exceptions are crystalline CPD-coatings. By the area of grown tissue lamellas, the coatings ranked as follows: CPD-crystalline<CPGC<CPM<CPL-crystalline<CPL (Table 1). Macroporosity of CPGC-matrixes could promote migration of the cell into the coating, which would impede surface conduction.

Further studies showed that adhesion of stromal cells (including osteogenic cells) and their migration along the implant surface were necessary, but not sufficient conditions for osteogenesis.

Changes in the tissue structure can be assessed by shifts in spectral parameters of its interaction with photons, specifically, by changes in absorption and reflection of visible light. According to computer morphometry data, optical density of tissue lamellas in tissue structures formed on the disks with different CP-coatings was significantly ($p<0.05$) lower than that of bone marrow (Table 2). This attests to a decrease in the total number of chromophores that intensively absorb photons in the forming tissue. In cells of the blood system (bone marrow and blood), hemoglobin is the substance that most intensively absorbs visible light [1]. Therefore, increased light reflection (in comparison with that of bone marrow) can attest to the formation of histological structures with decreased number of erythroid cells and erythrocytes. In ectopic osteogenesis test, the role of these structures can be played by the connective tissue and its derivatives (Table 2).

Thus, the microenvironment promotes differentiation of stromal precursors into connective tissue cells (fibroblasts and adipocytes). Probably, MSC comply the stochastic model and form the precursor cells for all differentiation pathways. However, specific microenvironment limits their osteogenic potential [10]. At the same time, certain CP-coatings demonstrated probabilistic differentiation of MCS into osteoblasts and possibly stromal cells capable to maintain hemopoiesis (Tables 1 and 2).

CPD-coating did not promote realization of the osteogenic potential of MSC pool. The specimens demonstrated calcified and encapsulated sites of red bone marrow with blast cells of various hemopoietic stems

TABLE 2. Optical Density (*D*) and Histological Composition of Some Tissue Lamellas Grown on Different CP-Matrixes during Ectopic Subcutaneous Osteogenesis Test in BALB/c Mice (*X*)

Surface sample (number)	Optical density (n=3)		Histological composition of series sections of tissue lamellas
	bone marrow, grey-scale values	tissue lamella, % bone marrow D	
CPM 1	20.153	56.9*	Lamellar bone, bone marrow, LSCT, adipose tissue
CPM 2	21.334	21.4*	LSCT, adipose tissue, bone marrow
CPM 3	19.583	3.5*	Lamellar bone
CPM 4	20.213	30.8*	Lamellar bone, bone marrow, LSCT sites
CPM 5	19.075	23.4*	Lamellar bone, small amount of bone marrow
CPL 1	14.586	61.9*	Lamellar bone, bone marrow, LSCT sites with ossification elements
CPL 2	21.057	4.05*	LSCT with ossification sites, small amount of lamellar bone
CPL 3	23.180	3.9*	Lamellar bone and LSCT are located layerwise, the signs of fibrosis, sites of adipose tissue, small amount of bone marrow
CPL 1 crystalline	29.908	3.7*	Adipose tissue, LSCT
CPL 2 crystalline	27.525	27.8*	Bone marrow with the sites of lamellar bone
CPL 3 crystalline	26.475	7.1*	Growing lamellar bone, bone marrow, LSCT sites
CPGC	18.832	9.4*	Lamellar bone, abundant bone marrow

Note. LSCT is loose connective tissue. *$p<0.05$ compared to bone marrow data (Wilcoxon—Mann—Whitney *U* test).

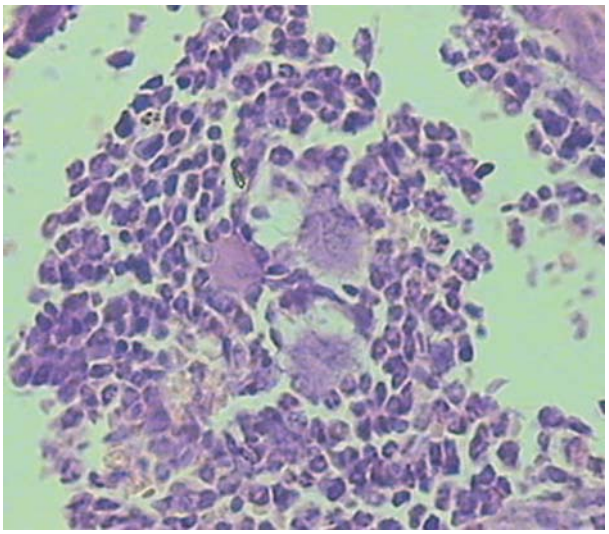

Fig. 2. Histological section of bone marrow grown on CP-surfaces in ectopic osteogenesis test. Eosin and hematoxylin staining, ×400.

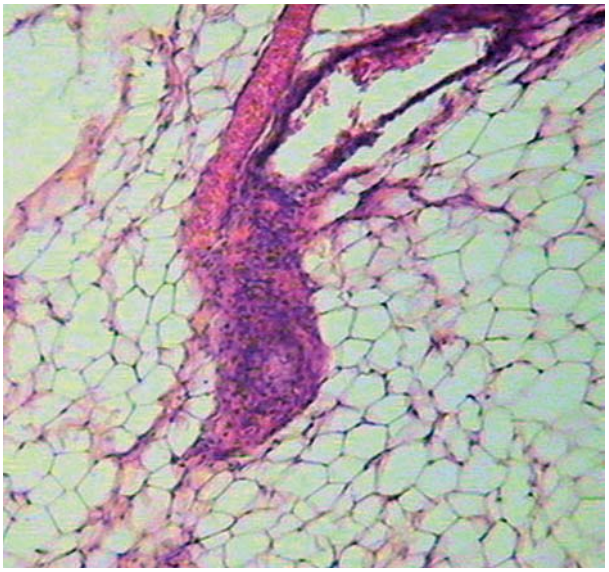

Fig. 3. Histological section of adipose tissue grown on CP-surfaces in ectopic osteogenesis test. Eosin and hematoxylin staining, ×100.

and a great number of megakaryocytes, and also capillaries filled with erythrocytes. In some specimens, sites of loose connective tissue were alternated with longitudinal fibrous bands. In peripheral sites we observed dense fibrillar elements (collagen fibers) forming a capsule. Typical fibrocytes and fibroblasts were seem between these fibers.

With the help of CP-materials having certain physicochemical elements, osteogenic differentiation of MSC could be triggered. Four of 6 examined CP-coatings had osteogenic potential (Table 1), and all these coatings were characterized by pronounced roughness or macroporosity of their surface.

Sections had the following sites of various sizes: bone marrow (Fig. 2), adipose tissue (Fig. 3), loose

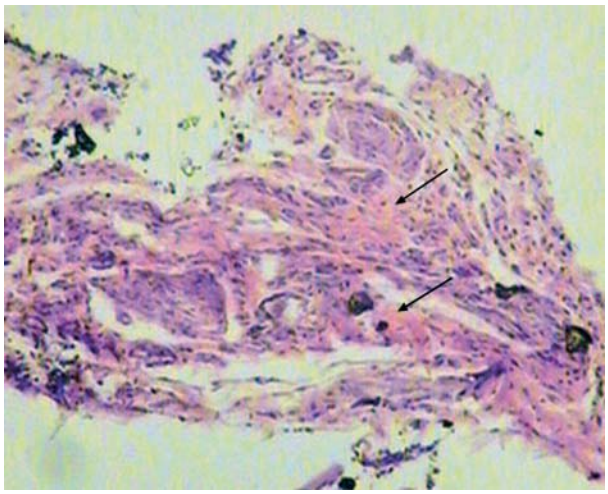

Fig. 4. Histological section of loose connective tissue grown on CP-surfaces in ectopic osteogenesis test. Eosin and hematoxylin staining, ×40. The arrows show the ossification sites.

connective tissue with ossification elements (Fig. 4), and lamellar bone (Fig 5). The presence of osteoblasts (Fig. 6) attested to intensive formation of the bone tissue. Artificial CPL and CPGC-coatings were characterized with maximum (100%) probability of osteogenesis induction. To a lesser degree, osteogenic potential was realized by CPM- (80%) and CPL-crystalline (67%) coatings.

Thus, the examined CP-surfaces with "primitive" physicochemical composition differing from that of the natural bone strongly affected functional properties of MSC pool.

We tried to assess statistically the surface properties that determine adhesion, migration, and osteogenic differentiation of the stromal precursors leading to metaplasia of the bone marrow and connective tissue into mature bone.

All parameters were subdivided into 3 groups: 1) physical properties of the surface (roughness class, Ra, Rz, coating thickness, and mean pore diameter); 2) chemical properties of the surface (atomic concentrations of phosphorus and calcium and their ratio); 3) parameters of tissue lamellas growing on artificial surfaces: probability of their formation, probability of osteogenesis, production of both probabilities, and the area of tissue lamellas.

Correlation analysis of physical and chemical parameters of the coatings showed that coating thickness directly correlated with calcium-to-phosphor ratio ($p<0.05$) and pore diameter ($p<0.01$).

In addition, this analysis showed that probability of tissue lamella formation and their area (parameters characterizing adhesion and conduction of stromal mechanocytes) directly depended on roughness class. A strict correlation was found between the probability of tissue lamella formation and their area (92%, $p<0.01$).

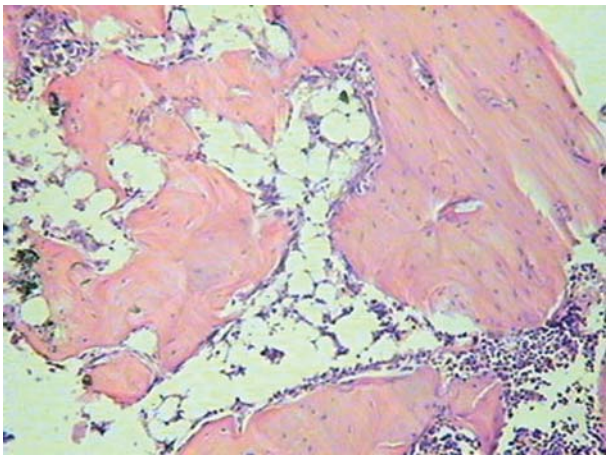

Fig. 5. Histological section of lamellar bone tissue grown on CP-surfaces in ectopic osteogenesis test. Eosin and hematoxylin staining, ×100.

The chain of events can be constructed as follows: roughness of the surface promoted adhesion of a large number of stromal mechanocytes (site of histogenesis) thereby enlarging the "conquered" territory for future expansion. In addition, surface roughness promoted conduction of the cells.

Since tissue lamellas were virtually absent on the bioinert CM- and CPD-surfaces, the indices of their roughness can be considered critical for cell adhesion under certain mechanical load (shear or breaking strengths in adjacent subcutaneous structures).

The probability that bone tissue would be formed in the tissue lamellas by 77% depended on Ca/P ratio in the coating: the higher this ratio, the more intensive differentiation of MSC into bone tissue (bone induction).

These data agree with the fact that probability of the formation of tissue lamella on the implant and the probability of the development of bone tissue on it are statistically independent (r=0.59, p=0.16). Adhesion and expansion of the stromal (including osteogenic) cells along the implant surface is the necessary, but not sufficient condition for their differentiation into bone tissue. These events are determined by different physicochemical surface factors.

Since the above events are statistically independent, their probabilities could by multiplied to obtain the probability of entire biological chain in the ectopic osteogenesis test: cell adhesion, conduction and bone induction. The group of these processes depended by 87% (p<0.02) on the initial stage (formation of tissue lamellas) and correlated by 90% (p<0.01) with their area.

Ectopic osteogenesis developed more successfully on "thick" matrixes (Tables 1 and 3), probably due to the fact that the thickness of the coating positively correlated with Ca/P ratio and pore diameter. The experiment showed that osteogenic potential can be realized if the thickness of the coating is not less than 50-80 μ. At the same time, bone tissue formed on both microporous (pore diameter less than 50 μ) and macroporous (pore diameter 150-250 μ) matrixes.

Therefore, correlation analysis demonstrated individual role of physical and chemical parameters of the artificial surfaces in the regulation of MSC *in situ* differentiation: 1) adhesion of bone marrow cells is the initial stage determining their further conduction along the artificial surface (the area of the forming tissue lamella). Success of histogenesis depends on roughness of the surface. The optimum is roughness class 4-5, which under specific microenvironmental conditions ensures differentiation of progenitor stromal cells into connective and adipose tissue cells. The foci of active hemopoiesis (Fig. 2) suggest additional differentiation of MSC into the stromal cells that form hemopoiesis-inducing microenvironment; 2) adhesion

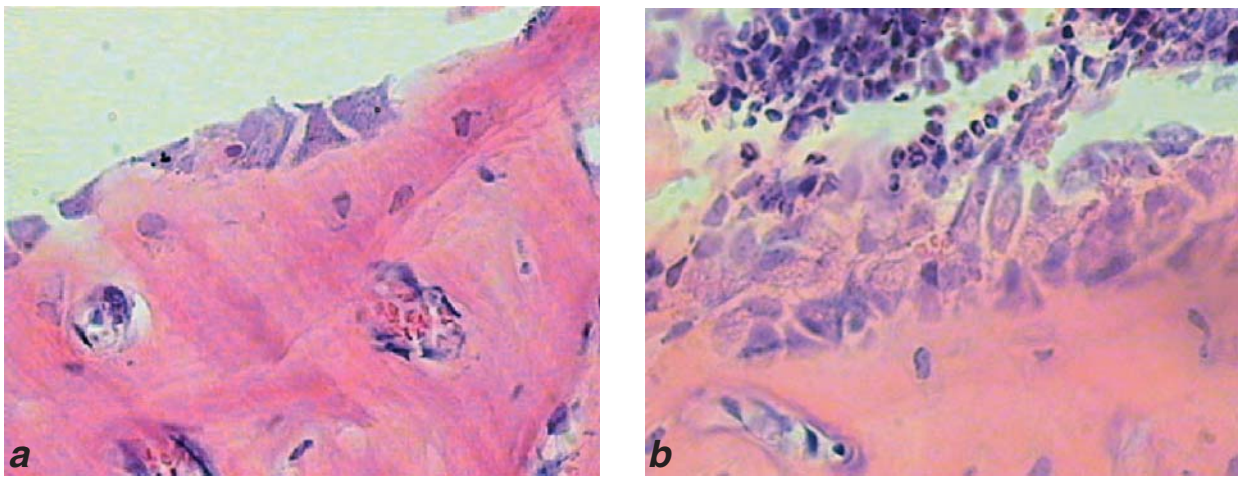

Fig. 6. The development of bone tissue in the tissue lamellas grown on CP-surfaces in ectopic osteogenesis test. Eosin and hematoxylin staining, ×400. *a*) free osteoblasts on the bone surface; *b*) invasion of osteoblasts into osseous matrix.

TABLE 3. Correlation between Physical, Chemical, and Biological Parameters (Spearman Test, *r*, *p*) during Subcutaneous Osteogenesis on CP-surfaces in BALB/c Mice

Parameter	Roughness class	Ra	Rz	Matrix thickness	Pore diameter	Ca/P	Probability of tissue lamella formation	Probability of bone formation	Production of lamella and bone formation	Area of tissue lamella
Physicochemical parameters										
Roughness class									*r*=-0.7856	*r*=-0.8614
									p=0.0363	*p*=0.0127
Ra									*r*=0.7629	*r*=0.8899
									p=0.0461	*p*=0.0073
Rz									*r*=0.7794	*r*=0.8948
									p=0.0388	*p*=0.0065
Matrix thickness					*r*=0.9286	*r*=0.7937			*r*=0.8496	
					p=0.0025	*p*=0.0331			*p*=0.0155	
Pore diameter										
Ca/P				*r*=0.7937				*r*=0.7724		
				p=0.0331				*p*=0.0418		
Parameters of tissue lamellas grown on CP-surfaces										
Probability of tissue lamella formation	*r*=-0.9627	*r*=0.8482	*r*=0.8514						*r*=0.8731	*r*=0.9214
	p=0.0005	*p*=0.0159	*p*=0.0151						*p*=0.0103	*p*=0.0032
Probability of bone formation						*r*=0.7724				
						p=0.0418				
Production of lamella and bone formation	*r*=-0.7856	*r*=0.7629	*r*=0.7794	*r*=0.8496	*r*=0.8524	*r*=0.8731		*r*=0.8977		
	p=0.0363	*p*=0.0461	*p*=0.0388	*p*=0.0155	*p*=0.0148		*p*=0.0103			*p*=0.0061
Area of tissue lamella	*r*=-0.8614	*r*=0.8899	*r*=0.8948				*r*=0.9214		*r*=0.8977	
	p=0.0127	*p*=0.0073	*p*=0.0065				*p*=0.0032		*p*=0.0061	

and conduction of the stromal cells along the implant surface is necessary, but not sufficient condition for their differentiation into bone tissue. Bone induction is determined by Ca/P ratio in the coating. The presence of hydroxylapatite is not prerequisite for osteogenesis; 3) ectopic osteogenesis develops more successfully on "thick" matrixes. The minimal thickness of CP-layer is 50-80 μ.

The pool of stem and committed cells is a heterogenic population controlled by the distant and local (short-ranged) regulatory mechanisms. All tissues have structural and functional units (microregions), which incorporates specialized cells, stromal elements, microcirculatory vessels, and nerve terminals [3]. The data showed that microenvironment of stromal cells is determined not only by organic, but also by certain physicochemical parameters, whose local or systemic variations can affect the fate of MSC pool. Specifically, the blood contains circulating hydroxylapatite microcrystals, which can provoke pathological calcification and ossification in tissues and implants (for example, cardiac valves) during alteration of their surface structure caused by inflammation or destruction [4]. In addition, implantation of CP-materials (with or without the cells) capable to biodegradation can be of importance for bone induction during many pathological states resisting therapy (false joints, ununited fractures, phosphate diabetes, *etc*.). The possibility of local or systemic reaction of the bone tissue during implantation of CP-materials was previously shown [2].

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