THE TOXICITY OF THE MATERIALS IN RELATION TO MESENCHYMAL STEM CELLS

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The cytotoxicity of the biomaterials "Collapan", "Osteomatrix" and the collagen sponge "Liostipte" in relation to mesenchymal stem cells (MSC) of the bone marrow was studied; optimal variants of bone replacement matrices were determined to be used as a biotransplant for spondylosyndesis.

Keywords: mesenchymal stem cells of the human bone marrow, collapan, osteomatrix, lyosten, biocompatibility.

An alternative to bone grafting for the regeneration of non-healing defects are cell transplants, the use of osteogenic growth factors and cell-free substrates or the implantation of bone equivalents. Necessary conditions for bone regeneration are osteoinductive signals, the matrix, which delays signals and has an adhesive surface, and osteogenic cells that attach to the matrix and differentiate into osteoblasts in response to osteoinductive signals [1–3].

"Collapan" materials in the form of plates, granules and gel, "Osteomatrix" and collagen sponge "Liostipte" were tested for toxicity at the direct contact of the carrier matrix with MSCs. Supernatants obtained after the materials were held in a complete nutrient medium for 24 hours were tested in relation to MSCs as well. Cytotoxicity was evaluated in the MTT test. Biomaterials and supernatants were introduced into the sample wells of a 24-well plate with MSC culture. The toxic effect of materials on MSCs was evaluated after 24 hours and 7 days (Table 1).

Table 1

Toxic effect of supernatants from biomaterials in relation to MSC *in vitro*

	Samples	Toxicity, % of dead cells	
		After 24 hours	After 7 days
1	Collapan plates	30,1 ± 0,05	9,1 ± 0,17
2	Collapan granules	17,3 ± 0,26	10,9 ± 0,34
3	Collapan Gel	32,8 ± 0,074	8,3 ± 0,094
4	Osteomatrix	15,7 ±0,47	8,7 ± 0,16
5	Collagen sponge	0,0 ± 0,032	0,0 ± 0,07

Note: The experimental data id presented as the mean ± standard deviation

As can be seen from the results presented in the table, supernatants did not have high toxicity in relation to MSCs. When assessing acute toxicity after 24 hours of MSC culturing, the viability of the cells ranged from 67,2 to 100 %. The supernatants of the material "Osteomatrix" (15,7 %) and collagen sponge (0 %) turned out to be the least toxic. After 7 days the cytotoxicity of all materials significantly reduced.

Biomaterials also had a slight toxic effect in direct contact with cells (Table 2).

Table 2

Toxicity of biomaterials in relation to MSCs at direct contact *in vitro*

	Samples	Toxicity, %	
		After 24 hours	After 7 days
1	Collapan plates	28,1 ± 0,04	11,2 ± 0,079
2	Collapan granules	12,4 ± 0,32	6,6 ± 0,024
3	Collapan Gel	28,2 ± 0,04	13,0 ± 0,083
4	Osteomatrix	11 ± 0,01	7,6 ± 0,148
5	Collagen sponge	11,3 ± 0,01	3,4 ± 0,166

Note: The experimental data of three tests is presented as the mean value ± standard deviation

Thus, all five materials studied had the cytotoxicity, which caused the death of no more than 30 % of the cells. When MSC were cultured with "Osteomatrix", "Collapan granules" and "Lyostipte" the lowest toxicity of these materials in relation to the cells was revealed.

While increasing the cultivation period up to 7 days, cells adapted and started to proliferate under experimental conditions, both at culturing with supernatants and in the direct contact with materials.

Therefore, the studied biocomposite materials have an inconciderable toxicity in relation to human mesenchymal stromal cells in vitro and can be used as a bone replacement matrix in spondylosyndesis.

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CHARACTERIZATION OF BIOCHEMICAL PROPERTIES AND BIOLOGICAL ACTIVITY OF COMPOUNDS OF A CARBOHYDRATE NATURE OF SOME OF BAZIDIOMYCETES

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The study of physico-chemical and biological properties of polysaccharides of basidiomycetes is the basis for the development of new therapeutic and prophylactic preparations. Biological action of medicinal mushrooms is largely determined polysaccharides.

Keywords: fungoterapy, basidiomycetous fungi, macromycetes, polysaccharides, phagocytic activity.

Traditional use of mushrooms for medical purposes has developed into a new close to medicine branch called fungoterapy. In the composition of mushrooms contain biologically active polysaccharides lentinan, lanostane, hantaran, lanofil, grifolan, which affect the human body much softer than the synthetic means, it is better tolerated and usually do not possess cumulative properties. Preparations based on mushrooms are characterized by a wide spectrum of biological activity: immunostimulate, antitumor, antioxidant, hepatoprotective, antimicrobial, etc.

Many species of wood-destroying fungi of the class Basidiomycetes are enough to grow well in artificial culture methods. Of particular interest are the representatives of the genera Schizophyllum, Trametes and Hericium.

Melanistic ordinary (Schizophyllum commune) has antitumor, antimicrobial, anti-inflammatory and antiviral properties. Melanistic recently attracted the attention of specialists of the pharmaceutical industry.

The prickly hericium (Hericium erinaceus) has antitumor activity, helps in chronic gastritis, ulcer and cancer of stomach and esophagus, chronic bronchitis, used to treat Alzheimer's disease, because it has the ability to repair the nerve cells.

Coriolus multicolored (Trametes versicolor) contain antibiotic and anti-cancer substances, strengthens the immune system, has antiviral, antibacterial properties, antioxidant. This fungus is used in various chronic diseases.

We used the fungi belonging to the division Basidiomycota, class Basidiomycetes, genera Schizophyllum, Hericium, Trametes.

Deep mushroom mycelium was grown in Erlenmeyer flasks on rocking (180 rpm) on the following media: glucose-peptone, the beer wort (70 Balling), whey. As inoculum used culture daily 10–12 mushrooms, grown deep in the beer wort. After the cultivation, the mycelium was separated from culture liquid by filtration through a thick cloth, was washed with distilled water and used to carry out the relevant tests.

The content of total protein in S. commune, H. erinaceus и T. versicolor ranged from 15,0 to 22,4 %, respectively, of the polysaccharides from 11,8 to 22,0 %, lipids – from 3,1 to 4,4 %.

The study of the carbohydrate composition of polysaccharides showed that they all were heteroglycans. In most of the polysaccharides was dominated by glucose (75,3–91,1 %), was also attended by galactose (5,6–13,4 %) and mannose (4,5 to 17,8 percent).