O1-1 **Inorganic-organic biomaterials as a substrate for cell immobilization**

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INTRODUCTION AND OBJECTIVES

Biological applications of the sol-gel chemistry are promising due to some advantages of the hybrid materials in comparison to organic polymer matrices, which have numerous applications in biotechnology (Migliaresi 2004). The biomolecules are entrapped in hard porous hybrids, which don't swell in water and protect the bioparticles from extreme conditions (Arcos D. 2010). Another advantage is that the encapsulated biomolecules preserve their activity. The immobilization of cells on porous hybrid matrices has been studied for more than 20 years (Salter 1991). In these processes the cells are covalently bound by suitable agents like γ-aminopropiltriethoxysilane. Such covalent bonding in some cases can block the access of the substrate towards the active site. That is the reason why big efforts are focused on the encapsulation of cells for preparation of bioreactors and biosensors (Maduraiveeran 2007).
Hybrid materials, synthesized by the sol-gel method are successfully used as matrices for immobilization of various cells, including osteoblast ones (Shirosaki 2005). The nanoporous hybrid matrix may serve as a barrier against external agents in the immune system and protects the implanted tissues from immune rejection (Xueen 2009).
The silicate and metal-silicate matrices permit the realization of effective encapsulation at appropriate conditions and shrinkage is reduced. Another feature is that these materials keep high cell activity, increase the stability against denaturation and operational stability of the immobilized cells (Meunier 2010).
The aim of the present work is the synthesis and structure investigation of the inorganic-organic hybrid biomaterials obtained, as well as the possibility of their application as matrices for immobilization of osteoblast cells.

MATERIALS AND METHODS

The synthetic process of the inorganic-organic hybrids is achieved by the sol-gel method, using a magnetic stirrer at room temperature. Vinyltrimetoxy silane (VTMS) and ethyltrimethoxy silanes (ETMS) were used as precursors and organic part - carrageenan. The ratio SiO_2-precursor:H_2O:HCl is 1:1:1.8x10^{-2}. 0.1 N HCl is added making the pH of the medium around 1-2.
Osteoblast cell line MG-63 was used in the experiments. These cells are known as human osteosarcome cell line, obtained from a 14 years old patient. They reveal a comparatively high degree of differentiation i.e. -they produce bone tissue *in vitro* and are a commonly accepted model for investigations in the field of bone and tissue engineering. Their human origin is a big advantage, having in mind the future application of the investigated materials in the human organism.
The cells were cultivated in a nutrient medium DMEM, (Dulbeco's Modified Eagles medium), enriched in potassium pyruvat, L-glutamin, antibiotics, 10mM HEPES buffer and 10% FBS incubated in a humid incubator at 37^o C in the presence of 5%CO_2. The medium is changed every 2 days. Passage of the culture is carried out when confluence is reached. Before the experiments the cells were treated with Tripsin/EDTA, the effect of Tripsin was blocked with FBS, followed by rinsing twice with nutrient medium. Their concentration was estimated using a hemocytometer of Burker.

RESULTS AND DISCUSSION

The synthesized samples were investigated by means of AFM for determination of the size of the structural aggregates and the dimensions of the structural units.
The investigations conveyed were on samples, prepared on the basis of precursors ETMS and VTMS with 5% replacement with an organic component. The influence of the type of precursor on the structure of synthesized hybrid materials was followed.

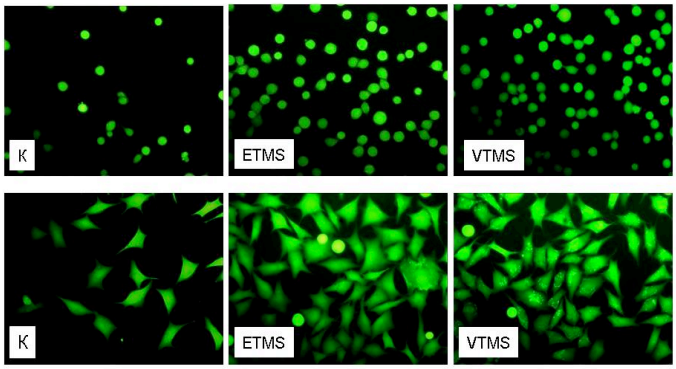

Figure 1: Morphology of osteoblast cells MG63, cultivated for 2 hours on pure (upper panel) and covered with fibronectin (lower panel)

The biocompatibility of the synthesized hybrid biomaterials was evaluated. Having in mind that the changes in the shape of the cells reflect the effectiveness of the interaction cell-material in our experiments, we have used the morphological approach for estimating the initial adhesion of the osteoblasts. To understand more about the interaction of osteoblasts with the investigated hybrid materials, we have studied the whole cell morphology and vitality of cells. To accomplish this task the osteoblasts were cultivated on the investigated matrices for 2 hours in a medium lacking serum to avoid the effect of the serum proteins. For comparison, part of the materials

was covered by fibronectin-one of the basic adhesive proteins of the extracellular matrix, which promotes the initial attachment of the cells to the material. We have established that on the pure materials, the cells are attached regularly and approximately to an equal extent to both hybrid materials (Fig. 1 upper panel). Their morphology is much rounded, showing a not good interaction with the material. One reason is that the gels are highly hydrated which makes them less susceptible for proteins and cells. The covering of the materials with fibronectin strongly increases their adhesive quality (Fig. 1, lower panel). Most of the cells are well spread, which fact indicates a higher affinity towards these surfaces because of the increased number of cell interactions with the surface. This means that the biomaterials absorb fibronectin in an appropriate conformation for the cells which leads to high initial adhesion and hence better growth. On the ETMS pattern, the osteoblasts appeared to be better spread compared to these with VTMS and the control.

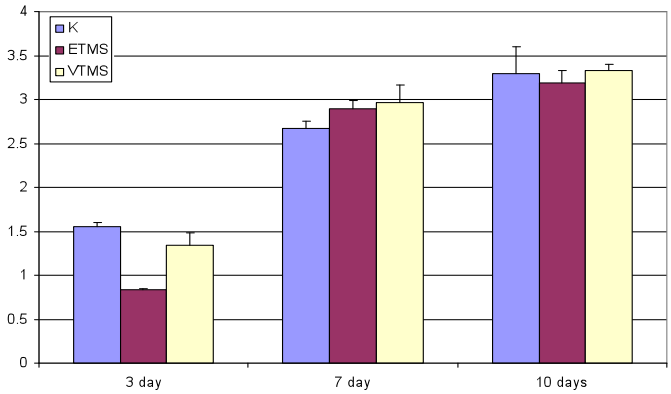

Figure 2: LDH-activity, as a function of optical density and incubation time

To follow further how the differences in the chemical composition of the hybrid materials affect the behavior of osteoblasts, we estimated the capability of these materials to keep cell growth for the period of 10 days by two methods-LDH-test- to evaluate cell proliferation and FDA - to prove cell vitality. The results obtained are presented on Fig. 2 and 3. It can be seen that a significant increase in the number of cells is established with prolonging of incubation time.

Most probably the diminishing of the differences is connected to the synthesis of adhesive proteins, which accumulate in the course of time on all surfaces. From there we can see that for all materials a significant increase in the number of cells is established with prolonging the time of incubation. When we compare the speed of osteoblast proliferation it is almost one and the same on both studied materials. Differences become significant only on the 3-rd day. Then on VTMS the cell growth is much better than on ETMS, but a little worse than the control. This tendency is kept during next days.

Analyzing the cell growth it was established that as a result of their slow initial adhesion, osteoblast cells reach confluence later – after the third day.

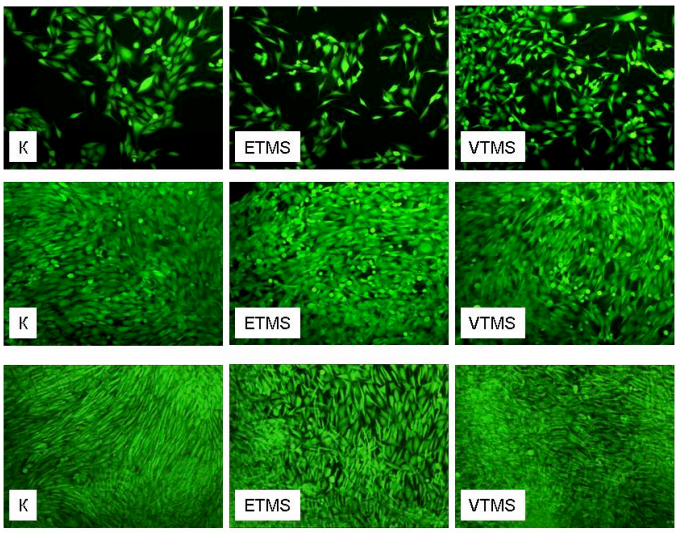

Figure 3: FDA studies of osteobalst cells MG63, cultivated for 3days (upper panel), 7days (middle panel) and 10 days (bottom panel)

The FDA analysis showed that cell shape is well spread and cells remain vital, revealing their good growth on the investigated materials.

CONCLUSIONS

Summarising the obtained results for the application of the synthesized materials, we can conclude that the hybrid with ETMS showed better initial adhesion, but in a long-term plan biomaterials on the base of VTMS are better for the immobilization of osteoblast cells. As a whole both materials appeared to be suitable substrates for immobilization of this type of stem cells. Future experiments on the cell adhesion and functional behavior of the osteoblast cells are forthcoming.

REFERENCES

- Migliaresi C. et al. (2004) *Composites.* in *Biomaterials Science: Materials in Medicine.* Eds. B. Rathner Elsivier Publishing Group, San Diego, California 181-197.
- Arcos D et al (2010) *Sol-gel silica-based biomaterials and bone tissue regeneration.* A. Biomat. 6 2874-2888.
- Salter G. et al. (1991) *New materials and technology for cell immobilization.* Cur. Biotech. 2 (3) 385-389.
- Maduraiveeran G. et al. (2007) *A facile electrochemical sensor designed from gold nanoparticles embedded in three-dimensional sol–gel network for concurrent detection of toxic chemicals.* Electr. Comm. 9 (8) 2051-2055.
- Shirosaki Y. et al. (2005) *In vitro cytocompatibility of MG63 cells on chitosan-organosiloxane hybrid membranes.* Biomaterials 26 (5) 485-493.
- Xueen J. et al. (2009) *Hydroxyapitite-multiwalled carbon nanotubes nanocomposite for adhesion and electrochemical study of human osteoblast-like cells (MG-63).* Electrochimica Acta 54 (13) 3611-3617.
- Meunier C. et al. (2010) *Encapsulation of cells within silica matrixes: Towards a new advance in the conception of living hybrid materials.* Journal of Colloid and Interface Science 342 (2) 211-224.