Adhesion and Secretory Profile of Mesenchymal Stem Cells Upon Contact with Some Biomaterials

CARMEN SOFICA TATU[1,2#], CARMEN PANAITESCU[1,2#], LAURA MARUSCIAC[1#], ALINA MARIA SISU[3#], MIRABELA CRISTEA[2], DANIELA ANCA PUSCASIU[4*], GABRIELA TANASIE[1,2]

[1]Victor Babes University of Medicine and Pharmacy Timisoara, Functional Sciences Department, Physiology Discipline, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[2]OncoGen Centre, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[3]Victor Babes University of Medicine and Pharmacy Timisoara, Anatomy and Embryology Department, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

[4]Victor Babes University of Medicine and Pharmacy Timisoara, Microscopic Morphology Department, Cellular and Molecular Biology Discipline, 2 Eftimie Murgu Sq., 300041, Timisoara, Romania

The aim of this study was to investigate the adhesion and secretory profile of mesenchymal stem cells in contact with biomaterials used in the field of tissue engineering. Mesenchymal stem cells obtained from lipoaspirate and two biomaterials were used: anodized titanium plates coated with hydroxyapatite crystals and collagen meshes crosslinked with formic anhydride combined with poly(ε-caprolactone). Cell adhesion capacity of the two types of biomaterials was assessed by counting non-adhering cells in the supernatant, and the secretory profile by ELISA dosing of a series of cytokines and growth factors released by the cells upon contact with the two biomaterials. Faster fixation of mesenchymal stem cells on titanium matrices compared to the collagen ones was observed. In contact with the materials, the mesenchymal stem cells alter their secretory phenotype, with a slight increase in their pro-inflammatory potential.

Keywords: mesenchymal stem cells, biomaterials, adherence, secretory profile

Mesenchymal stem cells (MSCs) are promising candidates for cell therapy due to their self-renewal, multipotency and autologous transplant capacity. There are several *in vivo* and *in vitro* studies and even clinical trials regarding MSC involvement in tissue repair, but the precise mechanisms by which this is done are not clearly stated. Among the hypotheses taken into consideration for tissue repair is the supposition that they secrete a number of biologically active molecules such as cytokines and growth factors, which have the ability to modify the local microenvironment at the lesion level, thereby favoring the repair / restoration process [1]. In the case of larger lesions, especially concerning bone, it is not sufficient to use only the cell suspension, but these cells need to be placed on a support [2]. Biomaterials designed to mimic natural extracellular matrix conditions that favor stem cell adhesion, proliferation, and differentiation are a major area of biotechnology research [20].

In order for cells placed on biomaterials to survive, it is essential that they adhere to the surface of the material. Advances in biotechnology research offer new materials with good surfaces and suitable properties [3, 16, 17, 22, 24]. On the one hand, the chemical and physical properties of biomaterial surfaces influence the adhesion, proliferation, migration and differentiation of cells. On the other hand, biomaterials are useful in fundamental research of cell biology and tissue engineering [4, 19, 24].

This study aims to observe whether the MSC-biomaterial interaction leads to a change in the secretory profile of the cells. Also, in order for this assembly to work, the MSCs must adhere efficiently to the surface of the biomaterial. Therefore an efficient colonization of three-dimensional supports by the MSCs depends on the good adherence of these cells to the surface of the used material, this being the second goal of the study.

Experimental part

Materials and methods

MCSs from liposuction were used, which after isolation were expanded up to the 8th passage and then kept under cryoconservation conditions in liquid nitrogen.

The two properties of MSCs (adherence and secretory capacity) were studied using two biomaterials: anodized titanium plates coated with hydroxyapatite crystals (Ti-HA) that are generally used for bone reconstruction (squares with sides of 8 - 10 mm) and collagen meshes crosslinked with formic anhydride combined with poly(ε-caprolactone) (C-PCL), collagen : PCL ratio = 1:2, used for soft tissue reconstruction (5 mm diameter and 2 mm height). Glass slides with a diameter of 12 mm were used as control.

Study of MSC adhesion in vitro

The biomaterial samples were plated on 24-well plates. Cells were added on top of the biomaterials in each well, at a concentration of 5000 cells / well. Samples were incubated at 37 ° C in humid atmosphere with 5% CO_2. At 2 and 24 h, the cells in the supernatant were counted in the three batches: the batch used with the Ti-HA biomaterial, the batch used with the C-PCL biomaterial and a control batch for which glass slides were used as inert biomaterial.

Study of the MSC secretory profile in contact with biomaterials

Substances secreted by MSCs in contact with the biomaterials were evaluated using the ELISA method. The secretion of cytokines and growth factors (IL-13, IL-4, TGF-β, VEGF, IL-2, IL-10, IL-12, TNF-α, IFN-γ) in the culture supernatant was measured for five batches: control batch, represented by MSCs cultured under standard conditions, without any other addition or contact, two batches represented by MSCs cultured in direct contact with the two types of materials under study (Ti-HA and C-PCL) and

* email: danielapuscasiu@yahoo.com; Phone: +40724568494 #Authors contributed equally to the article

two batches of MSCs cultured in the presence of eluate of the same two materials.

Preparing samples for direct contact

The cells were seeded into well-plates and left in the incubator for 24 hours at 37 °C in a 5% CO_2 atmosphere in order to adhere to the well. The tested materials were placed in the center of each well over the adherent cells. Samples were then incubated for 5 days at 37 °C in a humid 5% CO_2 atmosphere.

Preparation of eluted samples

Samples of the described biomaterials were plated on culture plates containing MSC-specific culture medium, which fully covered the biomaterials. They were incubated for 7 days at 37°C. After removing the biomaterial from the culture medium, the eluate sample was centrifuged at 1000 rpm for 10 min and the supernatant was stored. A 6-well plate seeded with 10^5 cells / well was incubated with MSC culture medium for 24 h at 37 °C in 5% CO_2 atmosphere. After 24 h the culture medium was replaced with 3 mL of the eluate obtained by the protocol described above. The supernatant of an MSC culture without eluate or biomaterial was used as positive control, and culture medium with 15% DMSO as negative control.

Results and discussions

Cell adhesion to biomaterials

It was observed that after 2h of contact there was better cell adherence to Ti-HA than to the C-PCL matrix, however, the values were lower than those in the control group, while after 24 h, similar MSC adherence occurred for all types of biomaterial (fig. 1) as has been shown in other studies [5].

Moreover, the presence of the C-PCL matrix changed the *p*H of the culture medium, its color turning yellow a few minutes after contact. It was concluded that the C-PCL matrix releases in the medium substances that affect cells and, implicitly, their adhesion. Perhaps a change in the proportion of component elements and possibly its synthesis as a hydrogel would improve the properties of the C-PCL matrix [6]. Another aspect observed during the experiment was that the matrix tends to disintegrate rapidly in contact with the culture medium. Thus, the C-PCL matrix did not constitute a solid and stable substrate for MSCs to adhere to it, unlike the Ti-HA plates, which have proved to be a very good substrate for MSC adhesion. Cell adhesion is influenced by the surface of the biomaterial because all

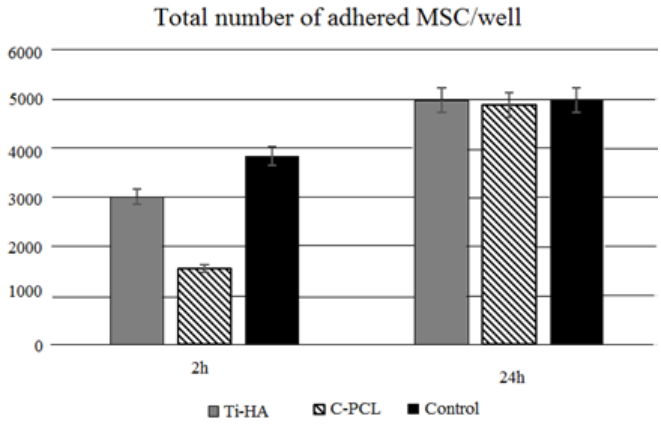

Fig. 1 Cell adherence to biomaterial
The graph shows the total number of adhered cells in each well

cell adhesion processes occur at the cell-biomaterial interface [7]. Studies by Silveiraet et al. show that the surface roughness of biomaterials influences cell adhesion so that the finer the surface, the better the adherence to it [8]. Therefore, because the Ti-HA plates have a much more stable and flatter surface than the C-PCL matrix, the cells adhere much better to the titanium plates than to the matrix. This is also confirmed by cell counts in the supernatant in the adhesion tests. It has been already proven that the coating of titanium matrices with hydroxyapatite improves the properties of this biomaterial [9].

The secretory profile

After 5 days of culturing MSCs in the normal culture medium, in direct contact with the biomaterials or in contact with their eluate, the supernatant was harvested and the concentration of a series of cytokines and growth factors was determined by ELISA. The results are shown in the table below (table 1).

It was found that, following contact with the study materials, the MSCs decreased the secretion of the immunoregulatory type of cytokines - IL-10, IL-4, IL-12 - while increasing release of proinflammatory factors such as TNF-α in the medium. In parallel, there was also a moderate increase in IL-13 and IFN-γ secretion, which are known to be involved in immunoregulatory mechanisms (figs. 2 and 3). MCSs have been shown to possess

Table 1
CONCENTRATION OF DIFFERENT CYTOKINES (pg/mL) IN THE CULTURE SUPERNATANT

	IL-13	IL-4	TGF-β	VEGF	IL-2	IL-10	IL-12	TNF-α	INF-γ
CSM Control	0.179	0.235	2394.633	129.32	1.854	1.124	0.345	6.955	4.568
C-PCL eluate	0.2195	0.168	2780.4005	228.395	1.8055	1.052	0.207	8.099	4.546
Ti-HA eluate	0.1975	0.1445	2652.602	235.115	2.19	0.8635	0.394	8.2905	5.044
C-PCL contact	0.234	0.0445	2463.891	174.415	2.052	0.6845	0.271	8.237	5.0965
Ti-HA contact	0.1975	0.1315	2418.842	169.9195	2.0655	0.81	0.311	7.427	4.67

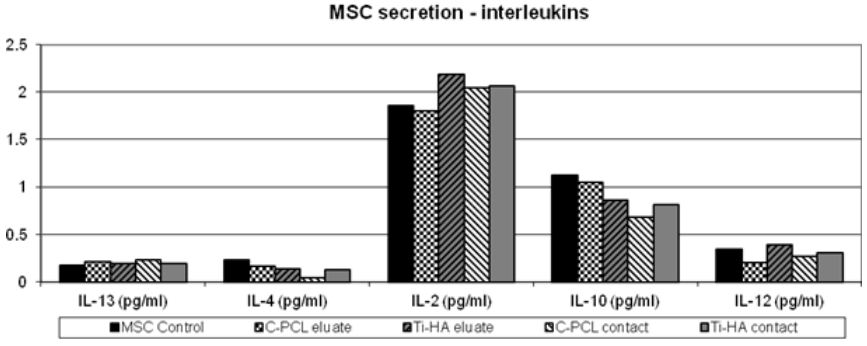

Fig. 2 Comparative interleukins concentration in MSC culture supernatant

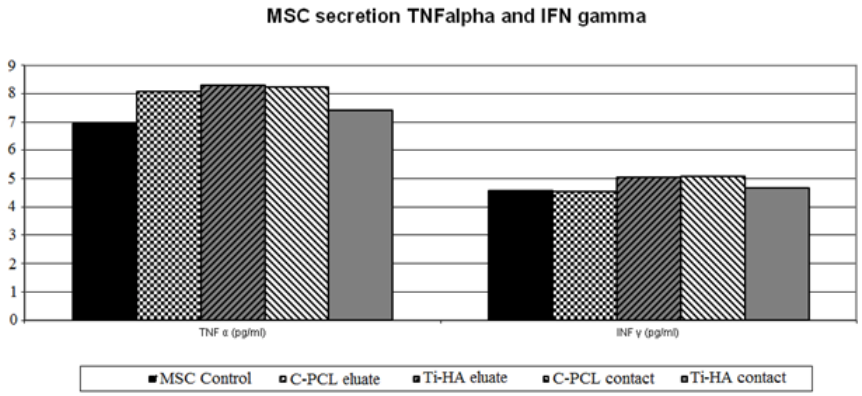

Fig. 3 TNF-α and IFN-γ concentration in MSC culture supernatant

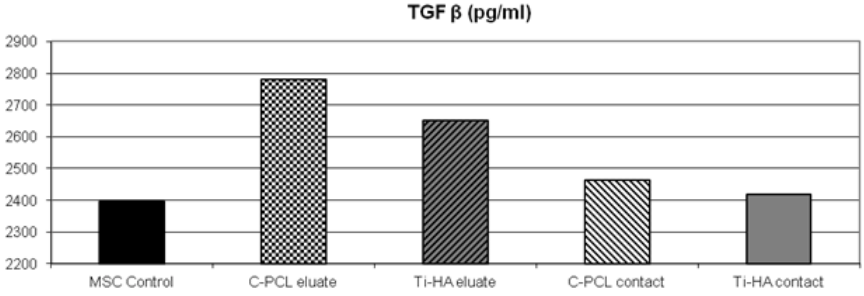

Fig. 4 MSC secretion of TGF-β in contact with materials or eluate

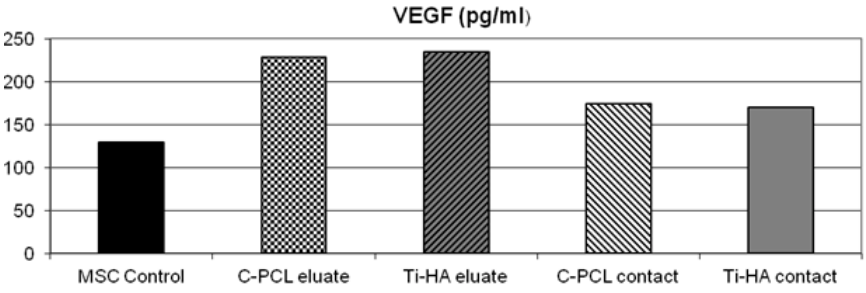

Fig. 5 MSC secretion of VEGF in contact with materials or eluate

immunomodulatory characteristics by inhibiting T cell proliferation *in vitro* [10, 23]. These observations have raised a particular interest in understanding the immunomodulatory effects of MSCs. Moreover, it was observed that MSC activating stimuli *in vitro* appear to induce the secretion of pro-inflammatory cytokines in the medium [11, 21].

TGF-β is a multifunctional peptide that controls proliferation, differentiation and other cellular functions in many tissue types. TGF-β has autocrine and paracrine activity that promotes cellular migration, differentiation into the osteogenic and chondrocyte lines and regulates angiogenesis. The concentrations of TGF-β secreted by the MSCs in the different experimental conditions are shown in figure 4.

VEGF is known to be a pro-angiogenic factor, and its production appears to be stimulated by MSC contact with the materials and, in particular, with the eluate (fig. 5). This finding is beneficial because the angiogenesis process is indispensable for tissue repair and reconstruction processes, and augmentation of VEGF secretion could stimulate the integration of MSC-biomaterial constructs in the respective tissues, while preserving the viability of the implanted cells. On the other hand, there are studies that show the necessity of secretion of pro-angiogenic growth factors at the implant site, in order for the implanted materials to be integrated into the body and to perform well [12,13,20]. It appears that the materials used as *in vivo* cellular supports need to be further improved in terms of porosity and access of cells inside them, so as to allow for optimal vascularisation, as well as regarding physical-mechanical properties corresponding to the tissue [14].

It appears that MSCs change their secretory phenotype in contact with *foreign* materials, and the studied materials seem to induce a slight increase in their pro-inflammatory potential. This finding contradicts various studies that highlight an anti-inflammatory MSC secretion profile that allows them to be used in repair processes associated with autoimmune disorders such as diabetes mellitus [15]. It is interesting to note that the eluate-induced changes have a greater amplitude than those induced by direct contact for both types of materials. It seems that the materials in this study require further improvements in order to avoid releasing in the medium molecules with the ability to activate the inflammatory response, especially since their *in vivo* use will also involve contact with cells of the immune system, which are more responsive to this type of signals than MSCs. It can also be speculated that the eluate is an *extract* of potentially active compounds that ensure their uniform distribution in the medium and non-discriminatory contact with all the cells in the culture, whereas in the case of direct contact, the cells in the immediate vicinity are more exposed, but there remains a larger percentage of cells that do not come into contact with the material or the soluble products released from it, so they retain their initial secretory profile for a longer duration.

Conclusions

In vitro adhesion assays reveal a faster fixation of mesenchymal stem cells on Ti-HA matrices compared to C-PCL, but after 24 h these differences fade away. In contact with the materials, MSCs change their secretory phenotype, with a slight increase in pro-inflammatory potential. Eluate-induced changes have a greater amplitude than those triggered by direct contact, probably because the eluate ensures the uniform distribution of active compounds in

the medium and their contact with all cells in the culture. The materials in the study require further improvements in order to avoid releasing in the medium molecules with the ability to activate the inflammatory response, especially as their *in vivo* use will also involve contact with cells of the immune system, which are more responsive to this type of signal.

Acknowledgement: This work was supported by the UMFT grant – PIII-C4-PCFI-2016/2017-03 (acronym NANOCEL)

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Manuscript received: 13.03.2017