Osteoblast-like MG-63 cells behavior on poly-□-caprolactone/wollastonite nanocomposites surface

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Statement of purpose: At present, the golden standard used in bone surgery is still the application of autografts and allografts. These commonly applied methods, show a number of limitations, inter alia: lack of sufficient number of tissue donors, risk of the rejection of a transplanted grafts or transmission of infection and diseases from donors [1]. These drawbacks incline searching for alternative materials that could be applied in regenerative medicine. Among them bioresorbable polymer/bioceramics nanocomposites can be considered as a scaffolds for the bone cells growth. They are characterized by improving their mechanical properties and good osseointegration. They can also promote apatite nucleation and protein adsorption on their surface and therefore expect to provoke better adhesion of osteoblasts to the implanted material [2].
The aim of this work was to obtain bioresorbable poly-□-caprolactone/nanowollastonite films, with different bioceramic particles content and to evaluate the influence of as manufactured materials on the human osteoblast-like MG-63 cells behavior.
Materials and methods: Nanocomposites were prepared by a solvent evaporation technique. Briefly, wollastonite nanopowder was suspended in dichloromethane and the as received suspension was mixed with the 10% solution of poly-□-caprolactone (80000 Da) prepared in the same solvent. Samples were dried in the air for 24 hours, followed by 48 hours drying in a vacuum drier. For this study, nanocomposite samples containing 0.5wt. % (PCL/0.5WS) and 5wt% (PCL/5WS) of bioceramic filler were prepared. Film prepared, plus a solution of pure polymer in solvent was used as a reference sample.
To evaluate physicochemical properties, the obtained nanocomposites were characterized by FTIR-ATR, SEM, DSC, AFM, profilometry and contact angle measurement.
For biological evaluation, prepared samples were contacted with human osteoblast-like cells MG-63 obtained from ATCC (Rockville, MD, USA). At the selected time points (3 and 7 days), cell behavior on the studied materials was examined. Morphology of the cell cultures were observed under the fluorescence microscope. Adhesion of the cells was evaluated by crystal violet staining/extraction. The viability of cells was estimated by the MTT assay and total protein concentration. Alkaline phosphatase assessment was used to estimate differentiation of the cells.
Results: The results revealed that osteoblast-like cells adherence to the tested materials was significantly improved on PCL/5%WS, nanocomposites on day 3 of the experiment. After 7 days of culturing, cell adhesion measured in crystal violet assay was significantly improved on both nanocomposites in comparison to the unmodified polymer (Fig.1a).

The proliferation of MG-63 cells after 7 days was not altered when comparing behaviour of obtained nanocomposites and the polymer control (Fig.1b). Additionally, the lack of significant difference in proliferation rate may indicate that the cells have been translated to differentiation rather than division. This was further confirmed by the detection of higher levels of active alkaline phosphatase in supernatants of the cells cultured with PCL/0.5WS and PCL/5WS rather than the reference (Fig.1c). Both on days 3 and 7, cells co-cultured with polymer nanocomposite samples (PCL/0.5WS and PCL/5WS) released less proteins than the cells cultured on the PCL control (Fig.1d). This may imply that the osteoblast-like cells did not become (over) activated by the nanocomposites as they did not increase the release of mediators/factors. In fact they differentiated and possibly matured into osteocytes, forming bones.

Fluorescent microscopy observations show that significantly more cells were present on the surface of nanocomposites (PCL/5WS > PCL/0.5WS) than on the pure polymer.

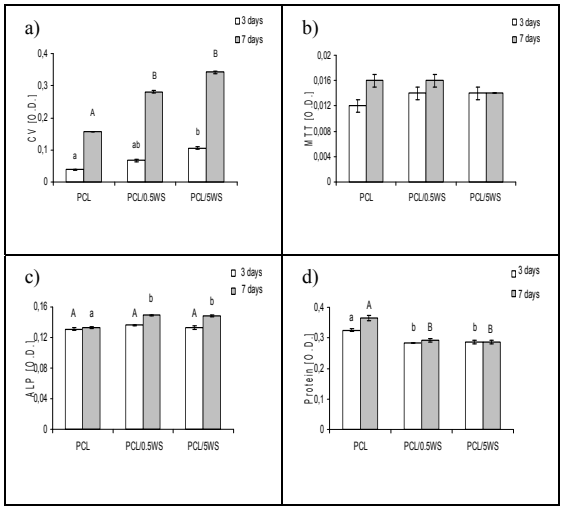

Fig.1. MG-63 cells behavior on the poly-□-caprolactone/ wollastonite nanocomposites and pure polymer after 3 and 7 days of culturing.

Conclusions: Obtained results indicate that investigated materials do not show cytotoxic properties after 3 and 7 days of culturing on MG-63 cells.

After further evaluation, as prepared composites may become good alternative material for culturing bone tissue cells, and may be potential candidates for preparation of bioactive bone substitutes.

References:

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