BIOCOMATIBILITY STUDY OF BOC POLYMER MESH ENRICHED WITH HAP AND TCP COVERED BY PCL FIBRES

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Abstract

The study was conducted in order to determine the biocomatibility of polimer mesh based on BOC and enriched with HAp or TCP coverd by PCL submicrometric fibres. Human osteoblast cell line NHOst was cultured in standard conditions on disk-shaped polymer samples. Interactions between materials and cells were examined through microscopic observation of cells' adhesion and morphology, and tests of viability/proliferation and cytotoxicity. The study proved the biocompatibility of all examined materials, though the surface of TCP enriched polymer didn't promote the adhesion of cells.

***Key words**: polymer mesh, HAp, TCP, osteoblasts NHOst*

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Introduction

Polymers are widely used as a material for implants because of their flexibility, plasticity and possibility of modification. Natural biopolymers such as chitin, alginate and cellulose were used to obtain the new implant materials. These polymers in form of fibres allow the preparation of various types of composites which have features (e.g. anisotropy) different from those characteristic for typical natural tissues [1]. Many efforts have been made to improve the biocompatibility of different polymer materials, mainly by modifying the topography and physicochemical properties to promote cell activity at the surface of implants. There is a growing interest in search of new materials that allows air, liquids or cells to pass through. The objective of the study was to estimate the effect of such permeable polymer meshes and their surface modification achieved by the addition of HAp or TCP on adherence and viability of osteoblasts. Biopolymer fibres based on chitin butyric-acetic copolyesters (BOC) are characterized by the presence of variuos side groups. These active chemical groups could entrap nanoparticles such as TCP, HA into the polymer chain [2]. High wettability of BOC fibres could be modified by covering biopolymer mesh by submicometric hydrofobic fibres with PCL (poly-e-caprolactone). Domein microstructure of fibrous composiste could be siutable to adhesion and proliferation of cells contacted with materials [3]. The aim of this work was to check the biocompatibility of composite materials in in vitro conditions using as a biocompatilibity marker NHOst line (Normal Human Osteoblasts).

Materials and methods

Materials

BOC fibres were formed by a wet process from solution using ethyl alcohol as a solvent. A large laboratory spinning machine with exchangeable modules was used. As nanoadditives commercial available (Sigma-Aldrich) TCP (>100 nm) and HAp (60-80 nm) were used. Thin layer of fibres with PLC were covered using two-step procedure: melt blow method and pressing (50°C). Unmodified and modified BOC mesh enriched with HAp or TCP and covered with PCL fibres, in shape of 13 mm in diameter and 1 mm in thickness disks, were used in the study. The disks were sterylised by immersing in ethanol and by UV radiation, and placed in 24-well culture plates (Nunclon, Denmark).

Cell culture

The biocompatibility of biomaterials was compared using normal human osteoblasts NHOst (Lonza, USA). The cells were routinely grown in 75 mL flasks in OGM Bullet Kit (Lonza, USA) suitable for osteoblasts, in a 5% CO_2 and 95% air atmosphere at 37°C. A flask of cells was brought into suspension after incubating for 5 min in 0.5% trypsin plus EDTA (PAA, Austria). Following trypsinization, cells were washed by centrifugation at 400g for 5 min to give a pellet that was resuspended in fresh supplemented medium to a concentration of $3 \cdot 10^4$ cells/mL. Next, 1 mL of cell suspension was added to each well of 24-well plates containing sterile polymer samples. Tissue culture polystyrene (TCPS) bottom of empty wells served as a positive control. Cultures were performed for 3 and 7 days.

Viability/proliferation and cytotoxicity tests

At the selected time points (3 and 7 days), half of the volume of supernatants from above cells cultured on biomaterials was collected for cytotoxicity test (ToxiLight, Lonza, USA). The viability/proliferation of osteoblasts was achieved by ViaLight test (Lonza, USA) according to the producent's protocol. Results were obtained with the aid of luminescence microplate reader PolarStar Omega (BMG Labtech, Germany).

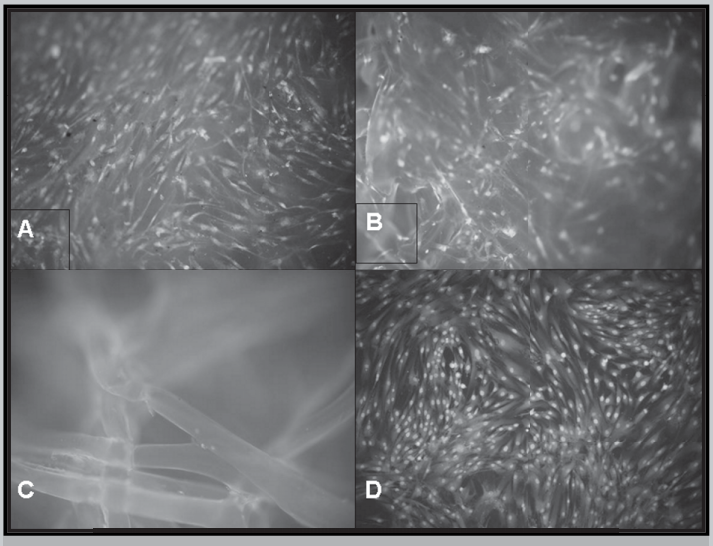

FIG. 1. The image of NHOst cells grown on the biomaterials surface after 7 days in culture. A) BOC, B) BOC+HAp, C) BOC+TCP, D)TCPS. Org. magn. 10x.

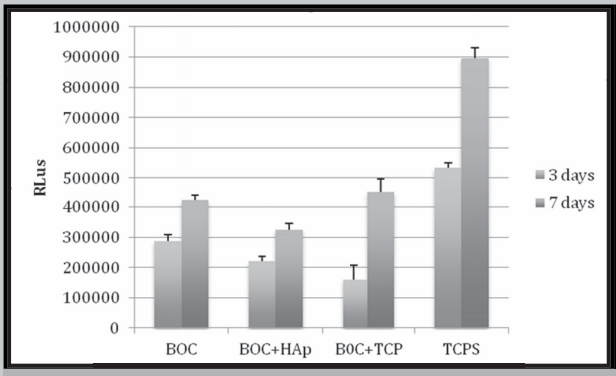

FIG. 2. The viability of osteoblasts cultured for 3 and 7 days in contact with biomaterials. RLus – Relative Luminescent units. All differences from control statistically significant when tested by means of the T-Tukey's test ($p<0.05$).

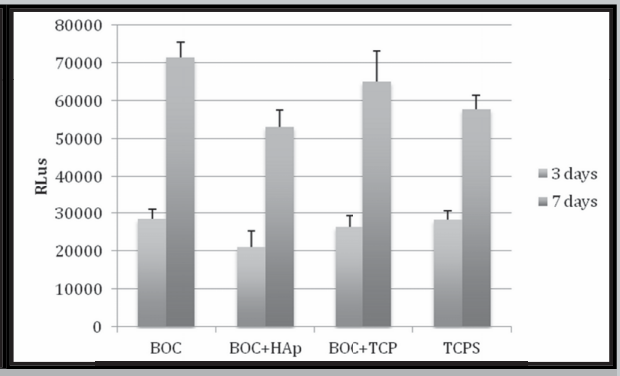

FIG. 3. The cytotoxicity of studied biomaterials after 3 and 7 days in culture with osteoblasts. RLus – Relative Luminescent units.

Cell morphology and adhesion

NHOst cells grown on biomaterials were observed under fluorescent microscopy (Olympus, Japan). Prior to observation the cells were stained with acridine orange for 30 sec.

Statistical analysis

At each time point four replicates were tested for the experimental and control samples, and four measurements on each sample were performed. The results were reported as mean values plus/minus the standard deviation (SD). Statistical analyses were performed using the T-Tukey's test. The statistical significance of differences was set at $p<0.05$.

Results and conclusions

All studied biomaterials are biocompatibile and promote the proliferation of NH osteoblasts (an evident increase of the cell number after 7 days of culture in comparison to the 3-day culture can be observed). It should be stressed that although the values of viability/proliferation test are much lower than in the case of control TCPS, the smooth surface of culture plate creates the best conditions for adhesion, spreading and proliferation of cells. However, although biocompatible, not all tested materials support the adhesion of NHOst cells. In the 7-day culture, very few cells adhered to the surface of BOC+TCP can be observed. High value of the viability test the material the BOC+TCP material owes to cells that are spread on the surface of TCPS, beneath the polymer mesh.

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