THE IN VITRO ANALYSIS OF SCAFFOLDS WITH GLYCOSAMINOGLYCANS ADDITION

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Introduction

Glycosaminoglycans are a group of polysaccharides which can be isolated from natural sources such as fish eyeballs or skin [1]. They are non-toxic and biocompatible, which is beneficial for their application in biomaterials science [2]. The use of a natural polymers combination to obtain scaffolds improves their properties such as stability in aqueous environment and mechanical parameters [3].

Materials and Methods

Collagen (Coll) was isolated from rat tail tendons under laboratory conditions. Chitosan (CTS) was purchased from Sigma-Aldrich company (Germany). Glycosaminoglycans (GAGs) were isolated from Salmo salar fish skin (from Koral s. c., Tychy, Poland) with the procedure reported previously [4]. Obtained glycosaminoglycans mixture content was identified by spectrophotometric method. The presence of hyaluronic acid (1.26%) and chondroitin sulfate (2.03%) was calculated based on the standard curves [4].
In the presented study the cytotoxicity test following the cells viability on the experimental materials was carried.

Results and Discussion

Composites (0.5 cm height, 0.12 mm diameter) were soaked in 70% EtOH (water solution) and washed in sterile phosphate buffer solution (PBS; pH = 7.4). For the studies human osteosarcoma cell line SaOS-2 was used. Cells were seeded at the density of 15 × 10^4 cells/composite and cultured for total of 4 days in alpha-MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics. The culture for 4 days was assumed as optimal and sufficient to compare the cells proliferation degree on the composites. Cell-seeded composites were examined with the CellTiter96Aqueous One Solution Cell Proliferation Assay (MTS, Promega, Poland). MTS solution was diluted 10× in phenol-free alpha-MEM and 400 µl aliquots were added per well per sample. The absorbance at 490 nm was measured after 30 min incubation at 37°C in the dark [5].
Results were expressed as% change in cell viability compared to results obtained for unmodified composites. For statistical analysis, the value of P < 0.05 was considered significant.

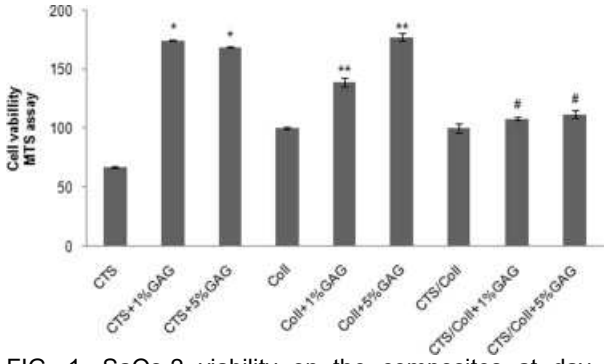

FIG. 1. SaOs-2 viability on the composites at day 4 culture *p < 0.05 vs. CTS; **p < 0.05 vs. Coll; #p < 0.05 vs. CTS/Coll; Student t-test.

The addition of glycosaminoglycans to the composites obtained with the use of chitosan, collagen or their mixture increased cell viability (FIG. 1) and this depended on GAGs amounts. It is plausible that increasing GAGs amounts in polymers led to substantial structural changes of the composites. These changes enhanced the biocompatibility of materials.

Conclusions

Glycosaminoglycans mixture was isolated from fish skin. The obtained porous collagen/chitosan based materials supplemented with GAGs demonstrated higher biocompatibility compared to composites without glycosaminoglycans. The increase of cells viability on composites with GAGs was observed. The study showed that the food industry wastes as fish skin can be used as the natural source of a compound that can be successfully used to prepare biocompatible materials for tissue engineering.

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