HYALURONIC ELECTROSPUN MEMBRANES AS ACTIVE SCAFFOLDS FOR BONE AND CARTILAGE TISSUE

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Introduction

Biomimetic fibrous structures are the subject of scientific interest due to their presence in many human tissues. Thanks to the electrospinning method it is possible to obtain fibers with a submicrometric and nanometric diameter. Hyaluronic acid (HA) is a biopolymer present in many tissues in a fibrous form. It is one of the main components of the extracellular matrix (ECM), contributing to the growth of bone, cartilage and skin tissues [1]. Its physicochemical properties assure high absorbability, thus HA supports the barrier function against outside factors and it contributes to tissue hydrodynamics (firming effect) [2-3]. According to the literature, the morphology of the fibrous substrate depends on the forming conditions (i.e. on solvents) [3]. Considering application of the material as a biomimetic substrate, the best diameter of fibers is 50-250 nm. Unfortunately, in many cases the literature does not present the data concerning biocompatibility of the substrate, whereas bioactivity is a key factor in medical applications. The aim of this work is to select conditions for biological tests on fibrous substrates. Biochemical monitoring was conducted to assess cytotoxicity of the materials. The L929 cells growing in contact with the extracts of the materials underwent the tests of cytotoxicity and proliferation.

Materials and Methods

Commercially-available biopolymer Centiprio HA of molecular weight 1.8-2.0kDa was used in the study. Three types of HA fibers were obtained by means of electrospinning and then examined: pure HA fibers - 12% HA 2:1 WAM:DMF (Avantor), fibers with medication (abf) - 12% HA 2:1 WAM:DMF+3% Biofuroksym (Polpharma) and fibers with HAp - 11% HA 2:1 WAM:DMF+1% HAp (Sigma Aldrich). The morphology of fibrous materials was assessed by means of SEM (Nova NanoSEM). The presence of additives (abf, HAp) was established during EDS analysis (Genesis). Biological tests were conducted on fibroblast cell line L929, cultured in EMEM medium with 10% of fetal bovine serum at 5% CO_2 /37°C). The reference was the surface of cell culture plate wells (TCPS). TCPS was a negative control for cytotoxicity tests. Cell tests were run on 3. and 7. day of cell cultures in the presence of extracts of the tested materials. The primary biochemical analysis was cytotoxicity assay (ToxiLight, Lonza) and the test of total number of cells in the culture (ToxiLight 100% Lysis Control, Lonza). For each of the conducted tests the statistical analysis was run as well (t-student test, $p < 0.05$).

Results and Discussion

Both the biopolymer pure HA fibers and the modified ones (HA/HAp, HA/abf) display a similar range of diameter: 50-150nm. Extracts formed above all the materials (HA, HA/Hap, HA/abf) amounting to 1 ml of the tested material/1ml medium (called 0.5) or 0.2ml/1ml medium (called 0.2) showed cytotoxic effect on cell cultures. Hyaluronan expands in hydro-environment (it is a hydrogel) and at higher concentrations it prevents free gas exchange. Introducing an adequate amount of hyaluronan *in vivo* will probably result in colonisation of the outer layer of biomaterial and next metabolizing HA by surrounding cells. In the case of extracts of lower concentrations (i.e. 0.1ml/1ml medium, 0.05ml/1ml medium and 0.025/1ml medium) the cytotoxicity is disappearing gradually and there are smaller differences between the materials modified with hydroxyapatite or antibiotics (FIG. 1). For all the tested materials at concentrations of 0.1, 0.05, 0.025, it is a rule that the cell count at least doubles in a 7-day culture as compared to the 3-day one (FIG. 2), while relative cytotoxicity halves. It proves advantageous conditions of cell cultures that support adaptation and proliferation of cells.

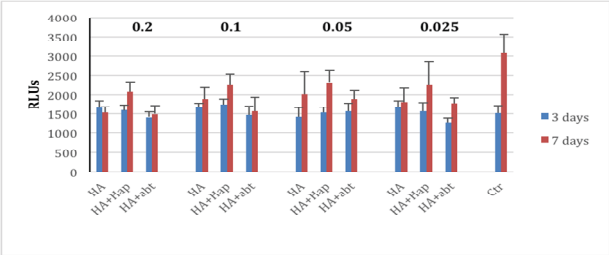

FIG. 1. Cytotoxicity of consequtive concentrations of studied materials after 3 and 7 days in *in vitro* cell culture conditions. RLUs – relative luminescence units.

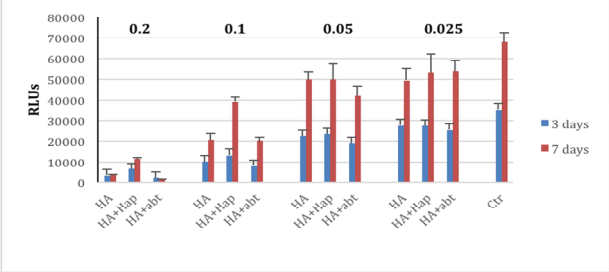

FIG. 2. Relative number of fibroblast cells after 3 and 7 days of *in vitro* culture with examined materials. RLUs – relative luminescence units.

Conclusions

Effectiveness of electrospinning as a method to obtain fibers depends on the ratio of solvents (WAM:DMF). Biological tests conducted at low concentrations of HA - considering its hydrogel characteristics - give credible results. The material itself is biocompatible: it does not cause cytotoxicity and it facilitates adaptation and proliferation of cells. However, it is necessary to carry out further research to assess how the materials behave in prolonged contact with other cell lines e.g. chondrocytes and osteoblasts.

Acknowledgments

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