IN VITRO BIOCOMPATIBILITY STUDY OF BIODEGRADABLE HYBRID COMPOSITES BASED ON POLYLACTIDENANOFIBRES AND CALCIUM ALGINATE NANOCOMPOSITE FIBRES

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Introduction

Bone tissue engineering requires a suitable biomaterial scaffold that provides an environment for cell growth and bone tissue remodelling [1]. By combining properties of biodegradable fibrous polymers with high surface area and porosity with the bioactive properties of hydroxyapatite and magnetite nanoparticles, the temporary environment can be tailored to enhance the cell migration, proliferation and differentiation [2,3]. The aim of the study was to evaluate the biocompatibility of biodegradable hybrid composites based on polylactidenanofibres and calcium alginatenanocomposite fibres.

Materials and methods

Three-dimensional biomaterials were prepared fromcalcium alginate (CA) fabrics covered witha thin layer of synthetic fibrous polylactide (PLA). Various spinning methods were used in order to obtain composite materials of different composition and structure. MicrometricCA fibres (12-15 μm) were obtainedby the traditional spinning method (wet forming from solution). The fibres were modified by nanoadditives: hydroxyapatite and magnetite in different ratio (TABLE 1).SubmicrometricPLA fibres (4-6 μm) were obtained by the electrospinning method.

TABLE 1. Composition of materials.

Symbol	Composition	BET [g/m^2]
W1	CA+HAp CA+Fe_3O_4 90%:10%	91,26
W2	CA+Hap CA+Fe_3O_4 85%:15%	93,18
W3	CA+Hap CA+Fe_3O_4 50%:50%	75,58
W4	CA+Hap CA+Fe_3O_4 20%:80%	106

Samples of biomaterials were examined in vitro. The biocompatibility of biomaterials was compared using normal human osteoblasts NHOst and murine macrophages RAW 264.7. The cells were cultured in media optimized for their growth, in standard conditions (5% CO_2 and 95% air atmosphere, 37°C). After three passages the cells were brought into suspension and seeded onto sterile biomaterial samples fitted to the size of culture-plate wells. Tissue culture polystyrene bottom of empty wells served as a positive control. After 3 and 7 days' culture viability/proliferation and cytotoxicity tests were performed. Morphology and adhesion of cells grown on biomaterials were observed under SEM and fluorescent microscopy.

Results and conlusions

Results of tests evaluating the viability and proliferation of cells cultured on biomaterials and cytotoxic effects of the materials, indicate the biocompatibility of these materials. Of the four tested materials only W4 is characterized by high cytotoxicity and low viability of both cell lines used in the experiment, so this material should be excluded from further study.

The proper interpretation of the results of cytotoxicity tests requires their reference to the number of cells grown on the materials, expressed as the total level of the adenylate kinase activity (FIG.1a,b). Presentation of a number of dead cells as a% of total cell number shows that the cytotoxic effect of biomaterials on cell cultures is similar to the positive control. The number of cells growing on biomaterials W1, W2, W3 in a 7-day series is also similar

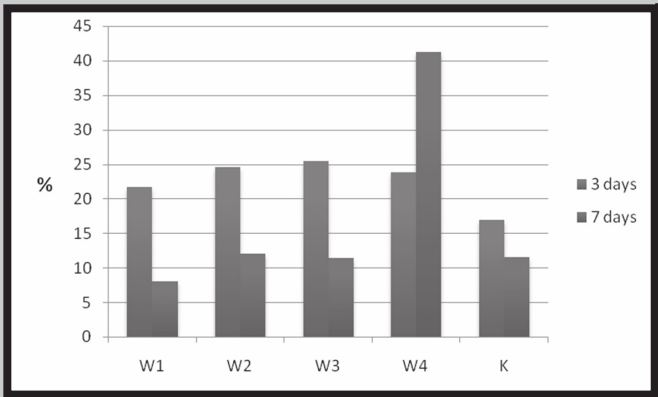

FIG.1a. Cytotoxicity of biomaterials expressed as a percentage of dead cells to the total NHOst cells number estimated with ToxiLight 100% lysis reagent and ToxiLight Bioassay kit.

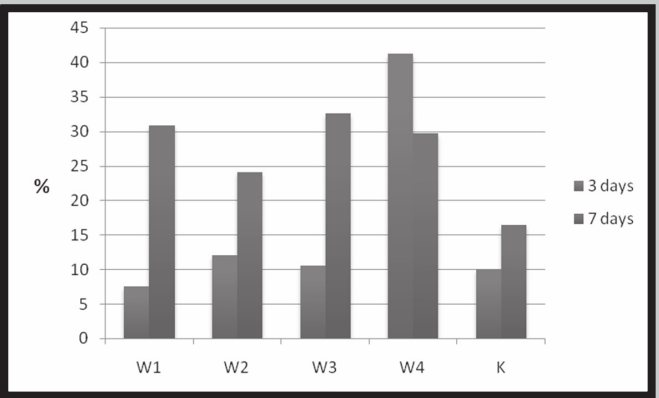

FIG.1b. Cytotoxicity of biomaterials expressed as a percentage of dead cells to the total RAW 264.7 cells numberestimated with ToxiLight 100% lysis reagent and ToxiLight Bioassay kit.

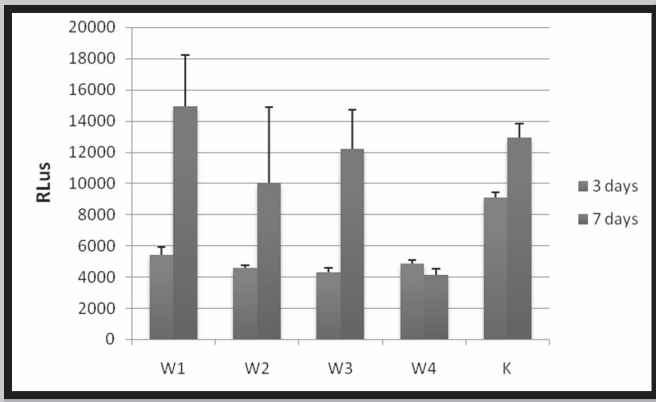

FIG. 2a. Proliferation of NHOst grown on the biomaterials for 3 and 7 days estimated with ToxiLight 100% lysis reagent and ToxiLight Bioassay kit. RLus – relative luminescence units.

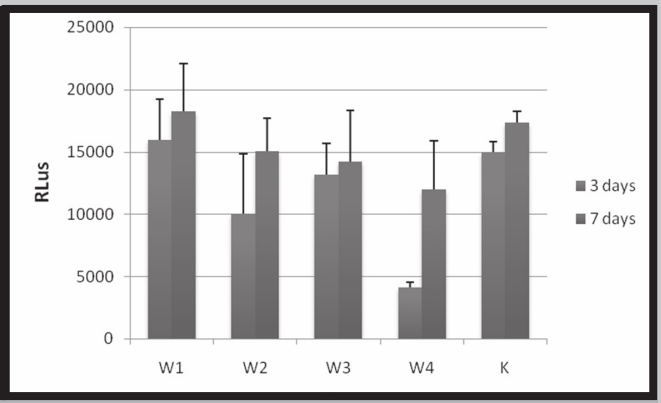

FIG. 2b. Proliferation of RAW 264.7grown on the biomaterials for 3 and 7 days estimated with ToxiLight 100% lysis reagent and ToxiLight Bioassay kit. RLus – relative luminescence units.

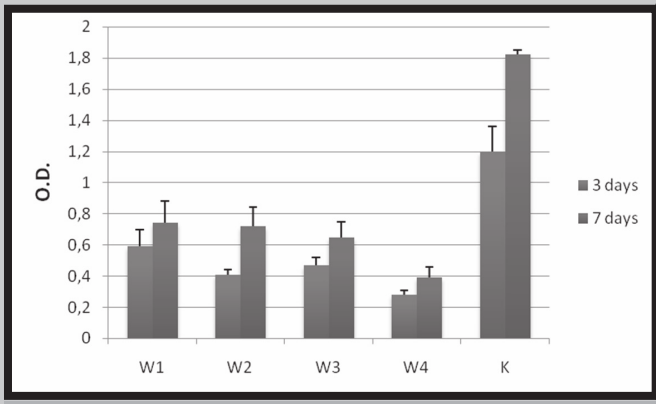

FIG.3a. Viability of NHOst grown on the biomaterials for 3 and 7 days measured by XTT test. O.D – optical density.

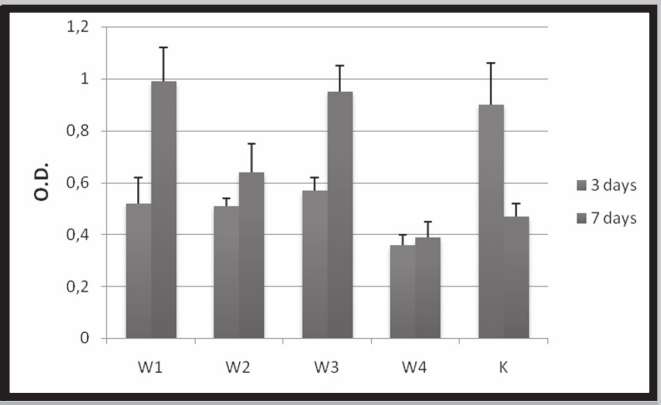

FIG.3b. Viability of RAW 264.7grown on the biomaterials for 3 and 7 days measured by XTT test. O.D – optical density.

to the control, indicating stabilization of the culture after 3 days of experiment (FIG.2a, 2b). Although in the case of NH osteoblastsa decrease of viabilitycompared to the control is observed (FIG.3a), which is probably due to the difficulty in adhesion and flattening of the large cells on the fibrous structure of the biomaterial, the proliferation of smaller RAW 264.7 macrophages, grown on biomaterials W1, W2 and W3,is similar, and viability on W1 and W2 is significantly higher (FIG.3b)

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References

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