Optimization of biodegradable microparticles loaded with TRAP-6 for tissue engineering

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INTRODUCTION AND OJECTIVES

Bioencapsulation is widely used for tissue engineering, namely for local delivery of growth factors to injury site (Jaklenec 2008). Fibroblast growth factor (FGF) is involved in wound healing promoting angiogenesis, regulating cell proliferation, migration, and ECM metabolism. However, effects of direct administration of a protein were found to be insignificant because of its proteolysis. Therefore, encapsulation of FGF and other growth factors in polymer microparticles could be promising for tissue engineering (Liu 2007). However, high cost and possible viral contamination makes FGF rather difficult for this application. Recently, thrombin receptor agonist peptide (TRAP-6) was found to promote cell growth and proliferation due to its ability to mimic thrombin activity at wound healing (Rusanova 2006). The main advantage of the peptide over growth factors, in particular FGF, is its low cost and rather simple chemical synthesis. So, the use of TRAP-6 for wound healing seems to be promising.

Microparticles can play a dual role, namely they serve both as delivery systems of bioactive factors and as scaffolds providing cell attachment and growth (Zhu 2008). As well known, cell adhesion and spreading on microparticles is highly influenced by the microparticle surface chemistry. In order to enhance cell attachment, microparticle surface can be modified with positively charged polymers (Rainaldi 1998). The aim of the study was to develop biodegradable poly(D,L-lactide) (PDLLA) microparticles with improved surface chemistry loaded with TRAP-6 for tissue engineering.

MATERIALS AND METHODS

Materials

PDLLA (Mw 135 kDa), chitosan (Mw 60 kDa, DD 90), polyvinyl alcohol (Mw 18 kDa) and graft-copolymers of chitosan and PLA were used in this study. Methylene chloride, ethyl acetate, acetone were from Merck (Germany). Cultivation medium DMEM, Trypsin/EDTA and from PanEco (Russia), fetal bovine serum (FBS) from HyClone (USA), Trypan Blue dye from Gibco (USA) were used in the research. TRAP-6 (SFLLRN, Mw 980 Da) was synthesized at Shemyakin-Ovchinnikov Institute, Rus Acad. Sci.

Synthesis of chitosan-PLA copolymers

Graft-copolymers of chitosan and polylactide with various polyester chain lengths were produced by Solid-State Reactive Blending (SSRB) technique under conditions of shear deformation using a twin-screw extruder (Akopova 2012). The graft-copolymers with high length of polylactide chains containing gelatin (Chit-Gel-PLA) and were able to form colloidal solutions in organic medium while chitosan derivatives with short chains of oligo(L,D-lactide) (Chit-LA) were soluble in water medium.

Microparticle preparation

PDLLA microparticles were prepared by a simple oil-in-water (O/W) emulsion technique. Microparticles with theoretical TRAP-6 loading of 2.5% (wt/wt) were prepared by adding the peptide (as a powder) in PDLLA solution in ethyl acetate or methylene chloride/acetone (90/10% wt).

Microparticle surface modification

Surface modification was carried out either by physical sorption of chitosan onto the surface of prepared PDLLA microparticle by their incubation in chitosan solution (acetic buffer pH 4.5) or by introduction of Chit-LA and Chit-Gel-PLA copolymers either in water or in oil phase, respectively, at the microparticle preparation. The prepared microparticles were sterilized with UV-irradiation.

In vitro cell cultivation

The mouse fibroblast cell line L929 was cultivated in DMEM supplemented with 10% FBS in flasks (25 cm^2) in a CO_2 incubator in 5% CO_2 humidified atmosphere at 37°C. The medium was replaced and the cells were reseeded every 2–4 days.

Cell cultivation with microparticles was performed in non-adhesive 96-well plates. For cell cultivation sterile microparticles were washed three times with PBS and once with DMEM supplemented with 10% FBS. To each well 20 µg of microparticles and 200 µl of cell suspension ($5x10^4$ cells/ml) was added. The plates were placed into CO_2-incubator and agitation (150 rpm) of the plates was performed during 1 hour. The medium was changed each 2-3 days during the cultivation. Cell cultivation with TRAP-loaded PDLLA microparticles was performed in 96-well plates. Initial cell concentration was $5x10^4$ cells/ml.

Evaluation of cell viability

To determine cell growth kinetics, suspension of microparticles with attached cells from each well was taken at different time points (in 2, 4, and 7 days) and

a number of viable cells was calculated by Trypan Blue assay.

RESULTS AND DISCUSSION

TRAP-6 was encapsulated into PDLLA microparticles and the effect of the peptide on cell growth was evaluated. As can be seen from Figure 1 (A), TRAP-6 significantly increased cell growth and proliferation. However, microparticles prepared using this method had rather poor cell affinity on the microparticles surface. In order to promote attachment and growth of fibroblasts, surface properties of these microparticles have been modified by their incubation in chitosan solution. The obtained results showed that the modification of microparticles surface indeed enhanced cell growth (Figure 1 B). However, this modification can not be used for preparation of TRAP-6-loaded microparticles, since the low molecular weight (Mw 980 Da) peptide can be completely released from the microparticles during their incubation. Therefore it is necessary to develop one-step technique for preparation of TRAP-6 loaded microparticles with improved surfaced properties for cell adhesion. Such one-step technique has been recently proposed (Demina 2012). The cell growth on microparticles prepared using this method is shown on Figure 2.

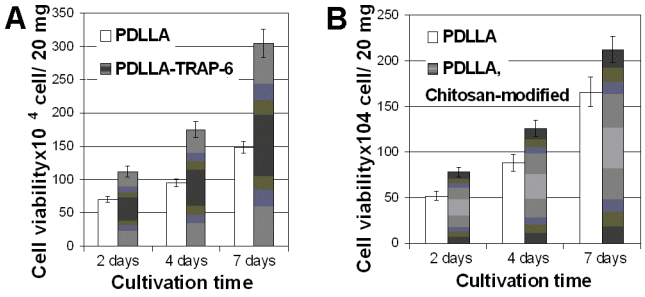

Figure 1 : Cultivation of L929 cells on PDLLA microparticles (A) loaded with TRAP-6; (B) coated with chitosan.

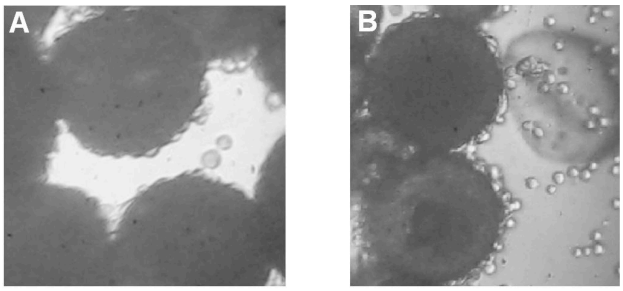

Figure 2 : L929 cells growth on (A) Chit-Gel-PLA and (B) Chit-LA copolymer microparticles after 7 days of cultivation.

CONCLUSIONS

In this study, PDLLA microparticles were studied for biocapsulation TRAP-6 to culture L929 cells. As compared to cells cultured on non-modified PDLLA microparticles cells grown on microparticles loaded with TRAP-6 showed much higher proliferation. Additionally, in order to enhance cell attachment and spreading, PDLLA microparticles were coated with chitosan by physical sorption. More over, a new one-step technique for modification of microparticle surface with usage of chitosan-based copolymers was developed. The cells also showed higher proliferation when cultured on surface of the modified microparticles by both techniques. The future research will be focused on TRAP-6 entrapment into surface modified microparticles, in order to enhance cell growth and to use these optimized microparticles for tissue engineering.

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