P-046	**Entrapment of PDLLA microbeads loaded with TRAP-6 within modified macroporous PVA hydrogel for tissue engineering**

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INTRODUCTION

Tissue engineering aims to restore sick, injured or lost tissues and organs using biomaterials and cell technologies. The approach is based on use of biodegradable or semi-degradable polymer scaffolds which could be seeded with cells *in vitro,* and then implanted to support 3D cell growth *in vivo* leading to tissue repair. Among other biomaterials, macroporous polyvinyl alcohol hydrogels (PVA cryogels) prepared by polymerization in the presence of heterophase in water-frozen solutions are widely used for this purpose. However, disadvantages of commonly used physical (not cross-linked) PVA cryogels are their rather low mechanical and thermal stability. In the present study a novel approach based on PVA acrylic derivatives as a macromer for preparation of PVA cross-linked cryogels has been proposed.

Moreover, modification of scaffolds surface with charged molecules in order to enhance cell attachment is of great interest. Another promising tissue engineering strategy is based on the entrapment of biodegradable microspheres, e.g. PLGA microparticles loaded with growth factors within the scaffolds (Charlton 2008). Growth factors and other bioactive molecules are essential to promote tissue regeneration (Biondi 2009). In current research, we used a synthetic peptide, namely thrombin receptor agonist peptide (TRAP-6), since recently it was shown to accelerate wound healing (Markvicheva 2006).

In this research we aimed to study possibility of the entrapment of poly(D,L-lactide) (PDLLA**)** microparticles loaded with TRAP-6 in macroporous modified PVA cryogels which can serve as scaffolds for tissue engineering.

MATERIALS AND METHODS

Materials

Polyvinyl alcohol (PVA: BF-03 sort, Mw 12000) was from CCP (Taiwan). Glycidylmethacrylate and AA were from Sigma (USA). Poly(D,L-lactide) (Mw 135000) were synthesized in CEIB (Belgium). Cultivation medium DMEM, Trypsin/EDTA and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (for MTT assay) from PanEco (Russia), fetal bovine serum (FBS) from HyClone (USA), Trypan Blue dye from Gibco (USA) were used in the research. TRAP-6 was synthesized at Shemyakin-Ovchinnikov Institute.

Preparation of modified PVA cryogels

PVA modification with glycidylmethacrylate (fig. 1) was carried out, in order to introduce double bonds able to cross-linking as described earlier (Artyukhov 2010).

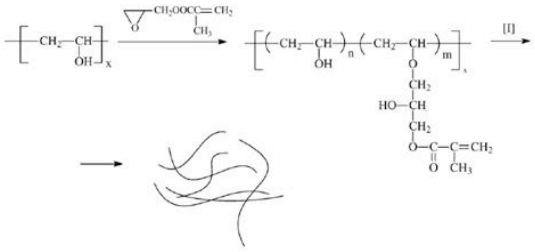

Figure 1 : Reaction of PVA modification

TRAP-6 sorption in the PVA scaffolds

The scaffold patterns were incubated in TRAP-6 solution (0.1 mM) in PBS (pH 7.4) at gentle stirring for 24 hours. TRAP-6 concentration was measured by spectrophotometer Beckman DU-70 (Beckman Instruments, USA) at a wavelength of 218 nm.

Preparation of PDLLA microparticles loaded with TRAP-6

Microparticles with theoretical TRAP-6 loading of 2.5% (wt/wt) were prepared using double w/o/w emulsion-evaporation technique as described earlier for PLGA microbeads (Stashevskaya 2007). However, instead of methylene chloride, we used the oil phase which was obtained by dissolving PDLLA (8% w/w) in a mixture of solvents (methylene chloride: acetone = 9:1 v/v).

Cell lines

Mouse fibroblasts L929 were used as a model cells. Cells were cultured in the DMEM medium supplemented with 10% FBS in a CO_2 incubator in 5% CO_2 humidified atmosphere at 37°C.

Cytotoxicity study

The cytotoxicity of the scaffolds was studied in extract-test and direct contact-test. For the extract-test, the extracts were prepared by incubating the scaffolds with cell cultivation media DMEM for 24 hours and the obtained extracts were then added to cell monolayer. Cell viability was measured at day 2 and 8 using MTT-assay. For the direct contact test, the scaffolds were kept in contact with the cell monolayer for 24 hours, after that MTT assay was performed. In order to study cell growth within the scaffolds, L929 cells were seeded upon the scaffold at cell density 5×10^5 cell/well and cultured in 24-

well plates for 1-10 days. After that cell viability was measured by MTT-assay.

Cultivation of cells in the presence of TRAP-6

In order to observe an effect of TRAP-6, L929 cells were seeded upon the scaffold (5×10^4 cell/well), and TRAP-6 (7 µM) was added to the scaffolds on day 1 and day 7. Cell viability was measured by MTT. Cell monolayer culture (5×10^5 cell/well) was used as a control. Cell cultivation with TRAP-loaded PDLLA microbeads was performed in 96-well plates. Cells were seeded in concentration $5x10^4$ cells/ml, considering microsphere concentration of 10 wt % in the culture medium. The number of viable cells was calculated by Trypan Blue assay.

RESULTS AND DISCUSSION

In order to promote cell attachment and growth, the scaffold surfaces were modified with negatively charged acrylic acid (AA) or positively charged 2-dimethylamino ethylmethacrylate) (DMAEMA). The cytotoxicity of the scaffolds was studied and the results confirmed that modification of the scaffolds neither with AA, nor with DMAEMA didn't lead to any cytotoxicity. Cell growth kinetics of mouse fibroblasts on the modified scaffold within 10 days is shown in Fig. 2. As can be seen, the addition of TRAP-6 in the culture medium promoted cell growth and proliferation within the scaffold. Since our attempt to adsorb TRAP-6 on the scaffold failed (<0.02 µmol in 1.5 hours), we decided to encapsulate it previously into microparticles which could be then entrapped in the scaffold. This approach was suggested to provide sustained TRAP-6 release in future study.

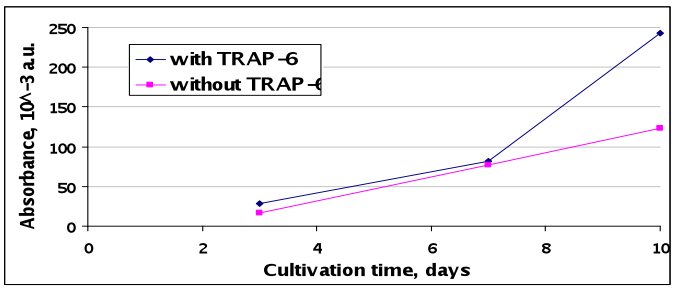

Figure 2 : Growth curve of L929 cells cultured on the PVA scaffolds modified with DMAEMA

The effect of TRAP-6 entrapped in PDLLA microbeads on cell proliferation is shown in Fig. 3.

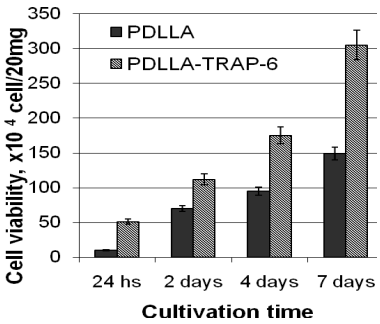

Figure 3 : Cultivation of L-929 mouse fibroblasts on PDLLA microbeads loaded with TRAP-6.

CONCLUSIONS

It was shown that modified macroporous PVA hydrogels (PVA cryogels) can be used as scaffolds to support 3D cell growth. TRAP-6 added in cultivation medium was demonstrated to promote cell growth and proliferation within the scaffolds. Microencapsulation of TRAP-6 in PDLLA microbeads was demonstrated also to enhance cell proliferation.

Thus, we consider that the entrapment of PDLLA microbeads loaded with TRAP-6 into macropores of the modified PVA hydrogel will allow to enhance 3D cell growth within this scaffold.

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