Enzyme triggered RGD-incorporated Tetronic tyramine hyrogels for the controlled myoblast function

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Statement of purpose : Cell adhesion on biomaterial substrates is an important process in the field of tissue engineering because the formation of robust cell adhesive structure on the substrate generally regulates other critical cellular activities such as proliferation and differentiation. Tetronic tyramine (Tet-TA) is a synthetic polymer that is capable of undergoing *in situ* polymerization in the presence of H_2O_2 and HRP enzyme resulting in a crosslinked hydrogel. This reaction utilizes phenol moieties in Tet-TA, which enables it to incorporate additional bioactive molecules, particularly polypeptides containing tyrosine into the hydrogel, during the polymerization. In this study, we used a Arg-Gly-Asp (RGD) containing peptide with a tyrosine end-group to prepare bio-functional Tet-TA hydrogels for the control of cell adhesion and differentiation. The objective of the present study was to investigate the effect of the concentration of the incorporated-peptide on adhesion, proliferation, and differentiaiton of myoblasts.

Methods : To synthesize Tet-TA hydrogels, we prepared two solutions; (1) 0.0625 wt% of H_2O_2 in PBS with Tet-TA, and (2) 0.0625 mg/ml of HRP in PBS with Tet-TA and various concentrations of GRGDGGGGGY. The mixing of these solutions spontaneously formed the Tet-TA hydrogels within 20 sec (Tet-R-0.5, Tet-R-1.0 and Tet-R-2.0 with the final peptide concentration of 0.5, 1.0 and 2.0 mg/ml, respectively). For the study, the hydrogels were prepared as a circular disk with 1mm thickness. C2C12 myoblasts were used as a model cell. For attachment study, cells were cultured on the hydrogels without serum condition for 4hr, and the adherent cells were analyzed. Proliferation of the myoblasts cultured on the hydrogles was evaluated using WST-1 assay. Myoblast differentiation was investigated using Real-time RT-PCR and immunstaining after 5 days of culture. MyoD, myogenin, and MHC were used as myogenic differentiation markers. At the same time, cells were immunostained for sarcomeric myosin using anti-MF20 antibody

Results : As shown in Fig. 1, there was gross difference in initial morphology of adherent cells, and projected cell area was increased with increasing the incorporated RGD concentration. The cells cultured on the hydrogels without the peptide maintained circular shape with limited spreading while extended significantly on the Tet-R-2.0 hydrogels. Proliferation of cultured myoblasts on the hydrogels showed significant difference in initial cell adhesion. The myoblasts cultured on the Tet-R-0 hydrogels showed very low absorbance value during 5 days of the assay, indicating that hydrogel itself without modification allowed limited cell compatibility. On the Tet-R-0.5, -1.0 and -2.0 hydrogels, the cells were continuously proliferating in a input RGD concentration dependent manner. There was significant difference in the level of the expression of the myoD, myogenin and MHC, which is normally up-regulated at very early, intermediate, and fully differentiated stage of myogenic development, respectively.

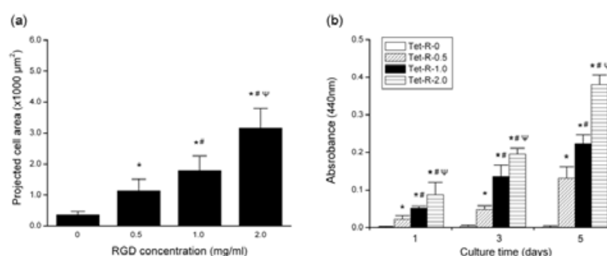

Figure. 1 (a) Projected area of the adherent myoblasts on the hydrogels after 4hr of culture and (b) proliferation of the cells on each hydrogels for 5 days.

We showed the expression of MHC, in which the expression was significantly increased as the peptide concentration within the hydrogels was increased. In addition we observed the formation of myotubes on the Tet-R-2.0 hydrogels with clear indication of multinucleated fused myotubes, resulting in the highly organized structure (Fig.2).

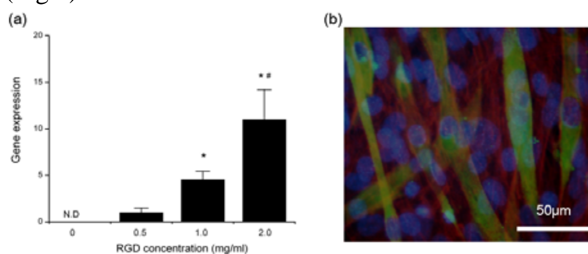

Figure. 2 (a) Relative exprssion of MHC from the myoblasts cultured on the Tet-TA hydrogels using Real-time RT-PCR and (b) an immunofluorescent image of myoblasts cultured on the Tet-R-2.0 hydrogels for 5 days, stained for sarcomeric myosin heavy chain

Conclusions : In this study, we prepared a enzyme triggered RGD-incorporated hydrogels and investigated the effect of the peptide concentration on the adhesion, proliferation and differentiation of C2C12 myoblasts. Analysis of the RGD-incorporated hydrogels demonstrated increase in RGD amount as the induced RGD concentration increased. In addition, adhesion, proliferation and differentiation of the myoblasts on the Tet-TA hydrogels can be controlled by the optimization of the peptide concentrations. Taken together, our results indicated that Tet-TA hydrogels can potentially be used as a bioactive substrate to regulate cell adhesion and other critical cell activities.

References:

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