Development of hydrogels functionalized with cell adhesive peptide and growth factors for control of endothelial cell activities for therapeutic angiogenesis

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Statement of Purpose: Peripheral arterial disease (PAD) is one of the most prevailing medical conditions in US which are generally caused by atherosclerosis, stenosis, and embolism. Although many therapeutic options are available for the treatment of PAD, several limitations related with operative risk, reoccurrence, and infection still exist. A cell-based therapy may be a potential strategy for the promotion of neovascularization, however, poor survival rate of transplanted cells, inflammation and low engraftment efficiency leave significant room for improvement. The ultimate goal of our project is to develop a cell-interactive hydrogel as a substrate to modulate the formation of an endothelial cell sheet that can be directly transferred to an ischemic region for the treatment of PAD. Given that, we here developed multifunctional hydrogels, based on Tetronic®-tyramine polymer, immobilized with both cell-adhesive peptide (RGD) and growth factor (bFGF) to control the adhesion and proliferation of endothelial cells, which can generate confluent layer. We then used these hydrogels for transplantation of endothelial cell sheet to hindlimb ischemia which was induced by femoral artery excision in nude mice, evaluated the efficacy in terms of therapeutic angiogenesis.

Methods: Functionalized Tetronic®-tyramine hydrogels were prepared by an enzymatically activated process. Rheological properties of hydrogels were investigated using an Advanced Rheometer. The structure of the hydrogels was examined using a scanning electron microscope (SEM) after lyophilization. Fluorescent dyes (NHS-rhodamine solution for the analysis of cell-adhesive peptide and FITC for bFGF) were used to study the immobilization efficiency. The release of un-reacted bioactive molecules was monitored for over a week after hydrogel formation. Human umbilical vein endothelial cells (HUVECs) at passage number from 4 to 6 were used. Cell adhesion on the hydrogel was examined after 12 hr of cell culture and by counting after fixation with 4% paraformaldehyde in PBS. The HUVEC sheet cultured on the hydrogels was directly transplanted on the ischemic regions created on the proximal femoral vessels of the right hindlimb on nude mice (BklNbt:BALB/c/nu/nu, female). All of the ischemic hindlimb tissues were harvested 14 days after treatment. The fate of the transplanted cells was visualized by using an optical imaging apparatus. We compared the therapeutic angiogenesis between cell sheet and the group treated by intramuscular injection with the same number of trypsinzed cells

Results: As shown in Fig. 1(a), the hydrogels immobilized with bFGF were successfully prepared and the un-reacted bFGF was released over 5 days, however, the residual amount was less than 5%, indicating that immobilization yield was approximately 95%. We then transplanted a HUVEC sheet in a nude mouse model with induced hindlimb ischemia to study its therapeutic efficacy for the treatment of PAD. The no treatment group showed rapid necrosis of the ischemic hindlimb tissues, and resulted in complete limb loss after 14 days. However, the necrosis rate was significantly reduced in mice treated with HUVEC sheet, with approximately 50% foot necrosis and 30% limb salvage after 14 days. We then tracked the transplanted cells using fluorescent techniques. As shown in Figs. 1(b) and 1(c), cells that were intramuscularly injected were diffused quickly and the fluorescence was detected throughout the whole body after implantation while the localized fluorescence intensity was observed in the group treated with HUVEC sheet. The fluorescence of the HUVEC layer minimally changed at the implant site over 10 days, while it gradually faded away in the group treated with intramuscularly injection of trypsinized cells.

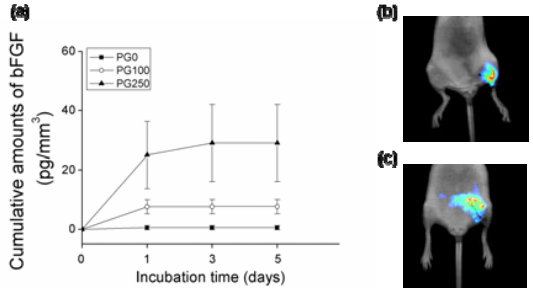

Figure 1. (a) Cumulative amount of bFGF released from the hydrogels for over 5 days as un-reacted form. The fluorescence images after transplantation of (b) trypsinized cells and (c) HUVEC sheet.

Conclusions: In this study, we prepared hydrogels *in situ* immobilized with a RGD peptide and bFGF, and found that they significantly facilitated the adhesion and proliferation of HUVECs, enabling us to generate a confluent monolayer. The harvested cell layer was then directly transplanted from the hydrogels to ischemic hindlimbs of nude mice, which enhanced therapeutic angiogenesis for over 14 days. In vivo tracking demonstrated prolonged retention of the transplanted HUVEC layer at the injury site.

References: Park KM. Acta Biomaterialia. 2009. 5(6):1956-1965. Fadini GP. Atherosclerosis. 2010. 209(1): 10-17. Nakagami H. Arteriosclerosis Thrombosis and Vascular Biology, 2005. 25(12): 2542-2547.