Sequential Delivery of $VEGF_{165}$ and PDGF-BB to Repair Myocardial Infarction

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Statement of Purpose: Ischemic heart disease remains a worldwide medical problem. Protein-based therapies that promote angiogenesis have yet to provide satisfactory outcomes in clinical trials. The controlled spatiotemporal delivery of key growth factors (GFs) such as vascular endothelial growth factor ($VEGF_{165}$) and platelet-derived growth factor (PDGF-BB) holds great potential for the proper revascularization and repair of the infarcted myocardium (1). $VEGF_{165}$ triggers angiogenesis followed by PDGF-BB to stabilize nascent blood vessels (1). We utilize a delivery vehicle of a fibrin gel and a polyvalent coacervate of a polycation, poly(ethylene arginyl aspartate diglyceride) (PEAD), and heparin which controls the release of heparin-binding GFs (2). The objective of this study was to test the effect of controlled sequential delivery of $VEGF_{165}$ and PDGF-BB on the revascularization and cardiac function in a rat myocardial infraction (MI) model.

Methods: Coacervate was made by mixing heparin and 1.5µg PDGF first, then with PEAD at mass ratio of 50:10:1 PEAD:Heparin:GF. Fibrin gel was made by mixing 87µL fibrinogen (20mg/ml) with 1.5µg free VEGF, 7µl PDGF coacervate, 5µl Aprotonin (1mg/ml) and 1µl thrombin (1mg/ml). Release of VEGF and PDGF from delivery vehicle was determined by ELISA (PeproTech). A rat aortic ring assay was performed to test the angiogenic effects of release kinetics on the formation of microvasculature and its maintenance. 1.5 mm ring segments were embedded in 3D fibrin matrices and media was treated with different groups after overnight serum starvation. After 6 days incubation, microvasculature formation from rings was observed under microscope and quantified using ImageJ (NIH, Bethesda, MD). An in vivo rat MI model was induced by ligation of the left anterior descending coronary artery followed by intramyocardial injection of different treatments (n=7 per group). Echocardiography using micro-Imaging system (Visual Sonics, Ontario, Canada) was performed presurgery, and at 2, 12, and 28 days after MI. Histological and immunohistochemical analyses were performed at 4 weeks. Angiogenesis was assessed by staining 6µm heart sections for endothelilal marker VWF (US Abcam, Cambridge, MA) and smooth muscle cell marker α-SMA (Sigma Aldrich, St. Louis, MO), picosirius red staining for collagen deposition, and hematoxylin and eosin (H&E) staining for general observation. Quantification was performed using ImageJ. Group means were compared by ANOVA with Tukey's post hoc test (GraphPad Prism).

Results: A delivery system of a fibrin gel containing unbound $VEGF_{165}$ and PDGF-BB coacervate achieved sequential release kinetics, where nearly 95% of $VEGF_{165}$ was released by one week compared to 40% of PDGF-BB which continued its sustained release to approximately 75% by 3 weeks (Figure 1A). The sequential delivery system induced significantly more microvessel outgrowth and longer sprouts from rat aortic ring segments than all other groups. It showed 1.97 fold greater sprouting area over free VEGF+PDGF, and 6.3 fold over basal media. In vivo, as early as 2 weeks after MI, the delivery group significantly improved cardiac function with 45% fractional area change (FAC) value compared to 36% for free VEGF+PDGF and 32% for saline groups (Figure 1B). Pre-MI FAC levels were approximately 53% for all groups. Sequential delivery also significantly reduced fibrosis as shown by less collagen deposition compared to other groups (Figure 1C). It also improved angiogenesis showing greater number of neovessels using the co-staining of vWF and α-SMA.

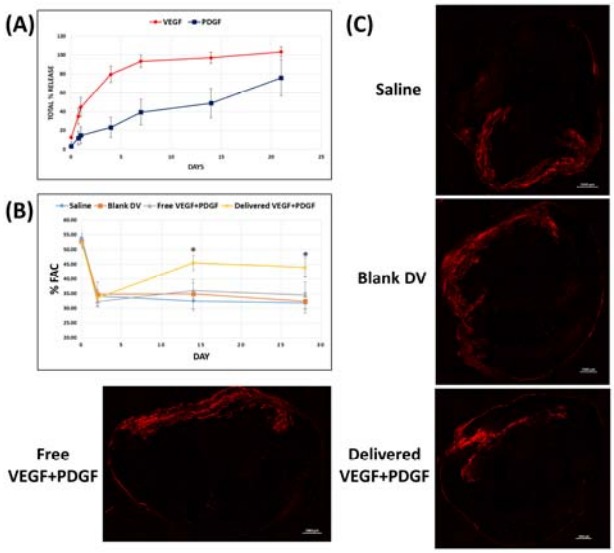

Figure 1. (A) Release profile of VEGF and PDGF from the fibrin-coacervate system. (B) Functional cardiac contractility measured using % fractional area change. (C) Representative images of picosirius red staining for assessment of fibrosis and collagen deposition. $P < 0.05$ between free and delivered groups.

Conclusions: The delivery system of a fibrin gel containing unbound VEGF and PDGF-loaded coacervates displayed desired sequential release of the 2 GFs. Results show the importance of spatiotemporal GF delivery to induce and maintain a robust angiogenesis process for mature and functional neovessel formation around the infarct zone. This system also reduced collagen deposition, fibrosis, and infract size. These improvements reflected at a functional level with a significant increase in cardiac contractility after its initial drop because of myocardial infarction. This system could prove beneficial in the clinical treatment of ischemic heart disease.

References:

1- Formiga F. Heart Fail Rev. 2012; 17:449–473
2- Chu H. J Control Release. 2011; 150:157-63