In Situ Vascularization of Injectable Fibrin/Poly(Ethylene Glycol) Hydrogels by Human Amniotic Fluid Stem Cells

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Statement of Purpose: One of the greatest challenges in regenerative medicine is generating clinically relevant engineered tissues with functional blood vessels. Vascularization is a key hurdle faces in designing tissue constructs larger than the *in vivo* limit of oxygen diffusion. In this study, we present a proof of concept vascularized tissue using only cells derived from human amniotic fluid, which is an ideal stem cell source for therapies correcting birth defets. We utilized fibrin-based hydrogels as a foundation for vascular fomration, poly(ethlyene glycol) (PEG) to modify fibrinogen and increase scaffold longevity, and human amniotic fluid-derived stem cells (AFSC) as a source of vascular cell types (AFSC-EC). We have shown previously that AFSC-seeded fibrin-PEG hydrogels have the potential to form three-dimensional vascular-like networks *in vitro*. We hypothesized that subcutaneously injecting these hydrogels in immunodeficient mice would both induce a fibrin-driven angiogenic host response and promote *in situ* AFSC-derived neovascularization.

Methods: Primary human amniotic fluid was obtained from patients in their second trimester undergoing planned amnioreduction as part of a therapeutic treatment for twin-twin transfusion syndrome, and differentiated into endothelial cells, as previously described (Benavides, 2012). Fibrin/PEG-based hydrogels were prepared using previously published methods (Zhang, 2006). The microstructure of fibrin/PEG gels was analyzed using scanning electron microscopy. To assess the effect of reaction time on PEGylation, 80mg/ml fibrinogen was combined with 8mg/ml PEG an incubated at 37°C for up to 4 hours. Samples were run in gel electrophoresis and stained with 0.125% Coomassie Brilliant Blue R-250. *In* vivo vascularization was assessed through subcutaneous implantation in athymic nude mice. At day 14, mice were euthanized and hydrogels were explanted, fixed in 4% paraformaldehyde, denatured in ethanol, cleared with xylenes, and embedded in paraffin. Slides were H&E stained, or labeled for α-SMA and CD31, then imaged. Experimental protocols were approved by the IACUC and IRB of Baylor College of Medicine and Rice University.

Results: The percentage of total protein per band in PEGylated fibrinogen was calculated as fractions of fibrinogen (40-70 kDa range), low-molecular weight PEGylated fibrinogen (70-250kDa), and high-molecular weight PEGylated fibrinogen (250+ kDa), all normalized to fibrinogen-only samples. For PEGylation times of 1min, 5min, 20min, 1hr, 3hr, 6hr, and 24hr, fractions of fibrinogen-only were (63.7%, 53.7%, 47.4%, 46.0%, 43.6%, 13.8%, 43.4%, respectively), fractions of low-MW PEGylated fibrinogen were (34.5%, 37.2%, 40.4%, 40.4%, 39.0%, 41.8%, 41.6%, respectively), and fractions of high-MW PEGylated fibrinogen were (1.9%, 9.1%, 12.2%, 13.6%, 17.5%, 14.4%, 15.0%, respectively). Hydrogels subcutaneously injected on the dorsal side of an athymic nude mouse were clearly intact two weeks post-implantation. Explanted hydrogels were fixed and paraffin sectioned, then stained with hematoxylin and eosin. The degree of lumen formation was determined by comparing the number of cell-lined vessels per mm^2 in sections of various cell-seeded hydrogels. The average number of cell-lined lumen per mm^2 was significantly higher in hydrogels seeded with stem cells or co-cultures containing stem cells versus endothelial cell types alone Fig 1. A subset of these lumen were characterized by the presence of red blood cells, but there was no significant difference between groups. In sections of hydrogel explanted at two weeks, no cell controls showed αSMA-positive host cells infiltrating the fibrin/PEG scaffold, while AFSC/AFSC-EC and MSC/HUVEC seeded hydrogels showed a cleared interaction between CD31-positive and αSMA-positive cells. Select areas of cell-seeded hydrogels contained dual-positive CD31/α-SMA lumen, whereas no cell control did not.

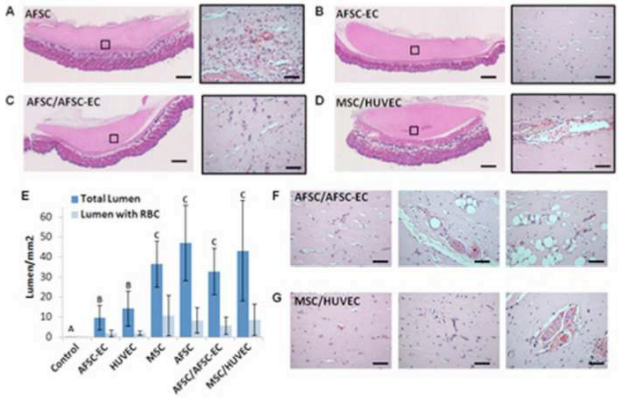

Figure 1. (A-D) Sections of hydrogels (2 weeks) were stained with H&E. Scale bars are 500μm for slide scans and 100μm for magnified images.(E) Lumen formation was determined in various gels. Bars which share a letter were not significantly different. A subset of this group was characterized by the presence of red blood cells. (F-G) Representative images. Scale bars100μm.

Conclusions: These results demonstrate the potential of cell from amniotic fluid to enhance vascularization of injectable fibrin/PEG hydrogels. *In situ* vascularization of subcutaneously injected fibrin/PEG hydrogels was correlated to the presence of a stem cell source, either AFSC or MSC.

References:

Benavides OM. *Tissue Eng. Part A*. 2012;18:1123-31.
Zhang G. A *Tissue Eng. Part A*. 2006;12:9-19.