A PEGylated Fibrin-Based Matrix Induce Vascularization of Human Adipose Derived Stem Cells
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Introduction: The bioengineering of autologous vascular networks is of extreme importance in wound healing and tissue regeneration. There is mounting evidence that adipose-derived stem cells (ASC) have plasticity towards differentiation into vascular cell types (1). ASC have been shown to assemble into networks with the addition of soluble cytokines, hormones and growth factors to induce cell differentiation (2). We hypothesize that three dimensional matrices, alone, can be used to direct ASC progenitor cells to differentiate towards vascular cell types. The present strategy allows for the formation of robust blood vessel formation using PEGylated fibrin as a structural ECM mimetic. Also, we show that the approximation of PEGylated fibrin gel to the native tissue environment controls and maintains the vascular phenotype in vivo.

Materials and Methods: ASC were isolated from human subcutaneous adipose tissue using methods previously described (3) and passage 2 cells were characterized for their immunophenotype. The PEGylated fibrin was prepared by adding SG-PEG-SG (Succinimidylglutarate modified polyethylene glycol) to a fibrinogen solution at a molar ratio of 10:1, followed by gelation using an equal volume of thrombin solution (4). ASC (25000 and 50000 cells/2 ml) were mixed immediately before gelation and allowed to proliferate and differentiate in the gel by culturing using α-MEM with 10% FBS. The morphology of the ASC vascular networks was observed by light microscope. Immunocytochemistry and RT-PCR were performed to analyze vascular (CD31, vWF) and perivascular (NG2, PDGFRβ and α-SMA) specific markers expressed by ASC in PEGylated fibrin gel. Further, engraftment and effect of ASC in inducing vascularization in vivo was assessed using an excision wound model in athymic rats.

Results: ASC formed dense vascular tube-like networks in the PEGylated fibrin gel, in the absence of additional soluble cytokines, by 7 days (Figure 1A). The amount of vascularization was related to the initial cell number plating density. RT-PCR and immunocytochemical analysis showed that there was significant increase in vascular cell specific markers; CD31 and vWF and perivascular specific markers, NG2 and PDGFRβ by day 11. Further in vivo, human ASC in PEGylated fibrin scaffold (25,000 cells/2ml, Figure 1B) integrated with newly formed host tissue and histological analysis showed increased vascularization in the newly formed tissue.

Discussion and Conclusion: The present study shows that autologous human ASC in PEGylated fibrin gel can differentiate into pericytes and endothelial phenotypes without addition of exogenous growth factors in vitro. Further we have demonstrated that human ASC have the ability to integrate into an excision wound and are capable of inducing vascular networks. Therefore, ASC can be combined with the currently developed PEGylated fibrin gel to develop tissue engineered vascular composites which can be potentially used to induce vascularization in extensive combat related wounds.

References:

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