Formation of Endothelial Cell Networks in Hydrogel Scaffolds Assembled from Modular Collagen-Fibrin Microenvironments

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Statement of Purpose: A current key challenge in tissue engineering is the creation of a vasculature within larger constructs to allow transport of nutrients and waste products to and from resident cells. One method to create a functional vasculature is to recapitulate aspects of vasculogenesis. This can be achieved in vitro by co-cultures of endothelial cells (EC) and mesenchymal stem cells (MSC) in three-dimensional protein hydrogels. The concentration and ratio of both cells and proteins modulates the extent of cord formation. One major limitation of this method, however, is that when vessel-like structures are pre-formed in bulk gels in vitro, the ability to shape the gels to injury sites is diminished. This issue can be overcome by the creation of defined hydrogel microenvironments (microbeads) that allow patterning of pre-formed structures at the time of implantation. In this study, we seeded EC and MSC into microbeads composed of collagen (COL) and fibrin (FIB), and assessed the development of vessel-like structures in vitro.

Methods: Human bone marrow-derived MSC and human umbilical vein EC in a 1:1 ratio were embedded in 3D hydrogels composed of 40/60 (mass ratio) COL/FIB. The hydrogel mixture was used to create microbeads via a water-in-oil emulsion process. Hydrogel and microbead morphology, as well as average microbead diameter and size distribution, were assessed using acellular constructs. To visualize cell morphology and vessel formation, EC were labeled with fluorescent protein (mCherry). Basement membrane deposition was assessed using immunofluroescence.

Results: Figure 1 shows the size, size distribution, and matrix architecture of acellular COL/FIB microbeads, as well as the matrix architecture of acellular COL/FIB bulk hydrogels. Acellular COL/FIB microbeads formed using the water-in-oil emulsification process were roughly spherical and were 205 ± 71 μm in diameter. The matrix architecture, shown using confocal reflectance microscopy, differed between microbeads and bulk gels. In particular, the density of collagen and fibrin fibers was higher in microbeads than in bulk gels of the same composition and total protein concentration.

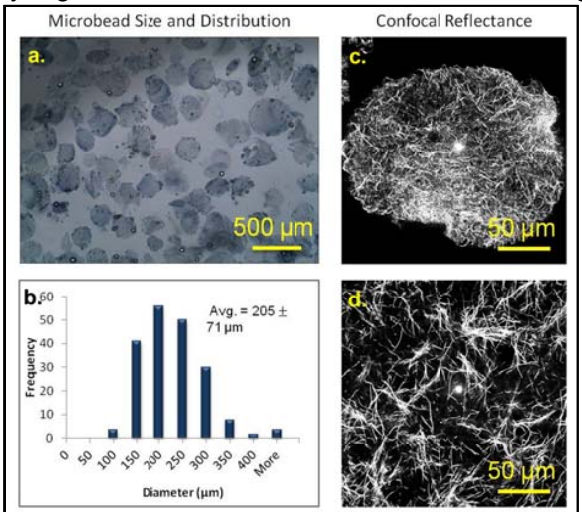

Fig. 1 - Acellular microbeads (a) and population size distribution (b). Laser scanning confocal reflectance images of acellular microbeads (c) and bulk gels (d).

Using fluorescently labeled EC, the formation of vessel-like structures was analyzed over time. EC formed into cords within two weeks in both microbeads and bulk gels. Representative images of microbeads and bulk gels in culture for 14 days are shown in Figure. 2 (a) and (b), respectively. COL/FIB microbeads stained for laminin at day 14 showed abundant basement membrane closely associated with embedded cells (Fig. 2c).

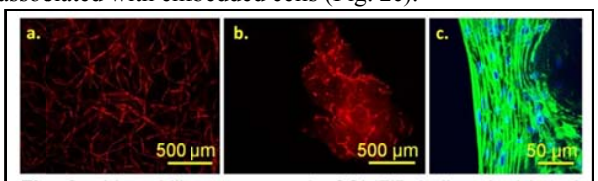

Fig. 2 - Vessel-like structures in COL/FIB bulk gels (a) and COL/FIB microbeads (b) at day 14. Basement membrane and nuclear staining of COL/FIB microbeads at day 14 (c). EC are shown in red, laminin in green, and nuclei in blue.

COL/FIB microbeads further embedded in acellular fibrin hydrogels supported formation of vessel-like structures (Fig. 3). MSC proliferated substantially into the fibrin hydrogels by day 7, and vessel-like structures also invaded into the surrounding matrix (Fig. 3b).

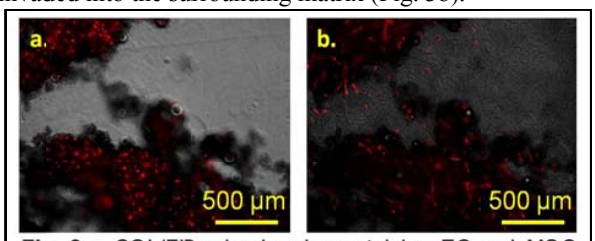

Fig. 3 – COL/FIB microbeads containing EC and MSC embedded in acellular fibrin hydrogels at day 1 (a) and day 7 (b). EC are shown in red.

Conclusions: Modular, spheroidal microenvironments were created using a water-in-oil emulsification process, and were shown to support vessel-like network formation in vitro. The COL/FIB and EC-MSC ratios used had previously been shown to support vessel formation in bulk gels[1]. In microbeads, the presence of a basement membrane by day 14 indicated maturation of the vessel-like structures. Microbeads embedded into acellular fibrin gels also supported formation of vascular cords within the beads, as well as into the surrounding fibrin matrix. This study suggests that modular, EC-MSC seeded protein microbeads can spatially and temporally guide vascular morphogenesis for regenerative applications.

References: [1]Rao RR. Angiogenesis. 2012;15(2):253-64.