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The effect of FGF-1 loaded alginate microbeads on neovascularization and adipogenesis in a vascular pedicle model of adipose tissue engineering

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ABSTRACT

Engineered vascularized adipose tissue could serve as an alternative to traditional tissue reconstruction procedures. Adipose formation occurs in a coordinated fashion with neovascularization. Previous studies have shown that extracellular matrix-based materials supplemented with factors that stimulate neovascularization promote adipogenesis in a number of animal models. The present study examines the ability of fibroblast growth factor (FGF-1) delivered from alginate microbeads to induce neovascularization and adipogenesis in type I collagen gels in a vascular pedicle model of adipose tissue engineering. FGF-1 loaded microbeads stimulated greater vascular network formation in an *in vitro* 3D co-culture model than a single bolus of FGF-1. In *in vivo* studies, FGF-1 loaded beads suspended in collagen and implanted in a chamber surrounding the exposed femoral pedicle of a rat resulted in a significant increase in vascular density at 1 and 6 weeks in comparison to bolus administration of FGF-1. Staining for smooth muscle actin showed that over 48% of vessels had associated mural cells. While an increase in neovascularization was achieved, there was less than 3% adipose under any condition. These results show that delivery of FGF-1 from alginate beads stimulated a more persistent neovascularization response than bolus FGF-1 both *in vitro* and *in vivo*. However, unlike previous studies, this increased neovascularization did not result in adipogenesis. Future studies need to provide a better understanding of the relationship between neovascularization and adipogenesis in order to design advanced tissue engineering therapies.

1. Introduction

A significant challenge to the creation of clinically functional tissue engineering products is appropriate vascularization that provides oxygen and nutrients and promotes integration of the tissue constructs [1,2]. Despite almost 2 decades of extensive research in tissue engineering, clinical successes consist primarily of tissues which are thin enough that the normal neovascularization process is sufficient or tissues that can largely survive via diffusion of nutrients form existing vasculature [3,4]. One strategy that has been explored to improve the function of engineered tissue is to stimulate neovascularization through the use of soluble, naturally-occurring growth factors. Stimulating or accelerating vascular formation using growth factors is a promising solution; however therapeutic effect of the proteins can be enhanced through use of an appropriate delivery method. The delivery of a single, high concentration of proteins can stimulate a response, but sustained delivery of proteins has been demonstrated to be the most effective use of these proteins for stimulating a persistent neovascularization response [5–7].

Engineered adipose tissue could eventually serve as a material for use in the replacement of lost or damaged tissue due to tumor removal, trauma, lipoatrophy, or congenital defects. However, the growth of adipose tissue is tightly coupled to neovascularization. In adulthood, increases in adipose tissue are accompanied by increases in the microcirculation [8]. The concomitant relationship between angiogenesis and adipogenesis is not fully understood, but the dependency of adipogenesis on neovascularization been observed during embryonic development [9,10] and in wound healing where

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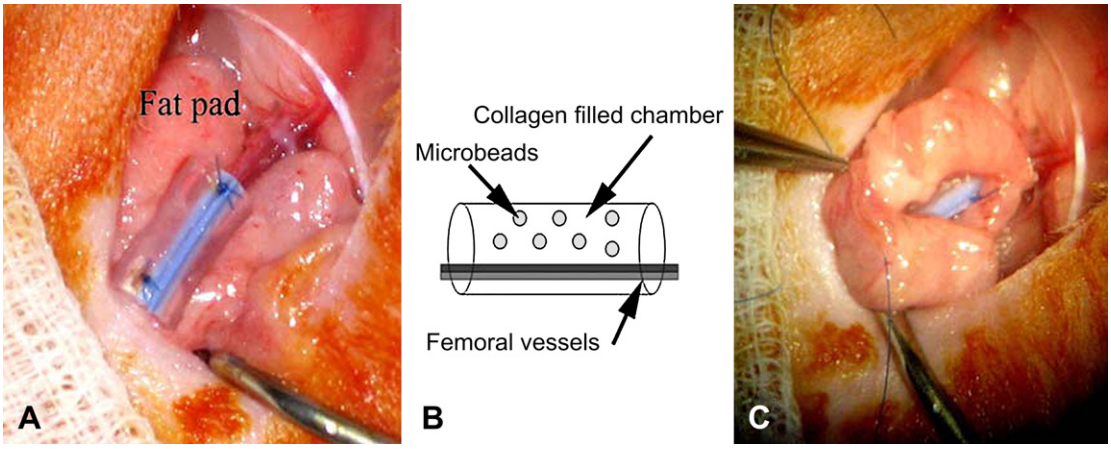

Fig. 1. Model of vascularized adipose formation. A silicone tube is cut open and wrapped around exposed femoral artery and vein (A). The tube is then filled with a collagen gel containing alginate microbeads and sutured closed. (B). A local fat pad is wrapped around the tube to seal the ends (C).

vascular formation precedes adipogenesis [1]. This information has been used to design approaches to adipose tissue engineering that incorporate factors that stimulate neovascularization.

Previous studies have demonstrated that Matrigel, a laminin-111 rich extract from a mouse sarcoma, induces adipogenesis when supplemented with fibroblast growth factor 2 [11,12], vascular endothelial growth factor-120 ($VEGF_{120}$,) and platelet derived growth factor (PDGF-BB) [13]. The adipogenic properties of these materials are thought to primarily result from their ability to induce neovascularization. Similarly type 1 collagen supplemented with FGF-2 generates vascularized adipose tissue in fat pads defects [14] or when implanted around the epigastric vein and artery in a rodent [15]. Without exogenous growth factors adipose formation was not achieved in either Matrigel or collagen. It has been suggested that supplementing these matrices with growth factors which promote angiogenesis leads to an environment rich in nutrients and oxygen which fosters the development and survival of adipose tissue.

Fibroblast growth factor 1 (FGF-1) is a potent modulator of a variety of cells [16–18]. It induces formation of vessels *in vitro* as well as *in vivo* [19–21] and has been shown to increase pre-adipocyte differentiation and proliferation [10]. We have previously shown that delivering FGF-1 from alginate microbeads increases vessel development in an experimental model useful for islet transplantation [7,22]. Delivering angiogens using alginate microbeads not only aids in containing the protein to the targeted area but, more importantly, allows for sustained delivery of proteins in order to induce persistent vascularization. The goal of the present studies was to investigate the effects of sustained local delivery of FGF-1 from alginate microbeads on neovascularization and subsequent adipogenesis in an *in vivo* model of vascularized adipose formation.

2. Materials and methods

2.1. Fabrication of microbeads

Microbeads for experiments were fabricated as previously described [7,22] based on a modification of a technique developed for islet encapsulation [23]. Briefly, 2% (w/v) low viscosity alginate of high guluronic acid content (LVG, G:M ratio > 1.5) was loaded into a custom-made air droplet microencapsulator and extruded through a 25 gauge needle (at air jacket pressure of 10 pounds per square inch (psi) and air jacket pressure of 15 psi) into a 1.1% $CaCl_2$ solution resulting in gelled spherical microbeads. For some studies, heparin (5U/ml) was added to the LVG solution prior to gelation. For all *in vitro* and *in vivo*, studies microbeads were sterilized by autoclaving the beads at 110 °C for 20 min. Microbeads were then allowed to re-swell in sterile saline for 2 days prior to incubation with FGF-1.

2.2. FGF-1 activity assay

Alginate microbeads of 2% LVG with and without heparin were incubated in FGF-1 solution (10 ng/μl) for 2 days. FGF-1 loaded microbeads were placed in a solution of sterile saline with physiological levels of calcium in a humid incubator at 37 °C and samples of release were taken at 4 and 8 h. Activity of released FGF-1 was tested using an enzyme immunoassay for quantification of proliferating cells. Human umbilical vein endothelial cells (HUVEC) were seeded in 96-well-plates (7000 cells/well) and grown in endothelial basal media with supplements (2% fetal bovine serum, bovine brain extract, gentamicin, hydrocortisone, EGF) for 3 days to approximately 80% confluence. The media was replaced with 0.5% serum in basal media with gentamicin for 24 h before stimulation with the test samples. All groups were studied in quadruplicate, with negative control of stimulation with PBS and positive controls of 20% serum and FGF-1 not released from the microbeads.

After 24 h of stimulation, wells were incubated with bromodeoxyuridine (BrdU). Twenty-four hours after addition of BrdU, the proliferative response was determined by measuring BrdU incorporation using a BrdU cell proliferation assay kit

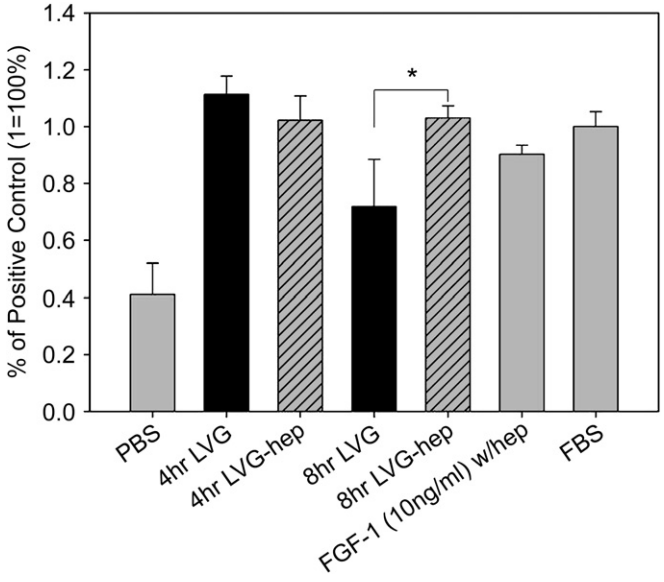

Fig. 2. FGF-1 released from alginate microbeads at 4 and 8 h significantly stimulated greater proliferation than negative control cells (PBS) ($p < 0.05$, $n = 4$). Black bars are FGF-1 released from alginate in the absence of heparin. Hashed bars are FGF-1 release from microbeads containing heparin. At 8 h FGF-1 released from alginate beads containing heparin caused a significantly greater proliferation (*) than FGF-1 released from beads without heparin.