Vascular growth is promoted by an ECM mimic and monitored by ultrasound and photoacoustic imaging technique

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Introduction

Angiogenesis, the development of blood vessels from the pre-existing vasculature, is a key component of tissue regeneration. Therapeutic angiogenesis aims to increase blood flow in ischemic tissues by stimulating the patient's endogenous capacity to develop new blood vessels. Previously, strategies have focused on the delivery, either directly or through gene therapy of angiogenic agents to induce formation of vasculature from existing vessels. Unfortunately, these strategies have met with limited clinical success. Recently, a significant amount of attention has been devoted to the developmental plasticity of stem cells, both *in vitro* and *in vivo*. Multiple types of stem cells have been shown the potential to differentiate into vascular cell types and secrete angiogenic and anti-apoptotic growth factors as well as increase vascularization in ischemic tissues after *in vivo* implantation. Due to poor integration efficiency of the stem cells to sites within the injured tissue and loss of cell viability there is a demand for 3-D scaffolds which can efficiently deliver cells to desired sites and promote vascular growth.

Materials and Methods

The PEGylated fibrin biomatrix was cast as previously described.[1] Implantation of PEGylated fibrin gels was performed in the subcutaneous space of Lewis rats under anesthesia. A 1 cm longitudinal incision was made through the skin of the ventral midline. Individual "pockets" for each gel were prepared in the subcutaneous space of both flanks by blunt dissection. The thin fascia covering the back musculature was removed carefully, exposing a uniform muscle surface. Gels (0.5ml in volume) were inserted into each pocket. After one week, rats were euthanized and the gel implants were excised and positioned in water cuvettes for imaging.

Simultaneous ultrasound and photoacoustic images were obtained using a 48 MHz focused ultrasound transducer interfaced with a nanosecond pulsed laser source operating at 532 nm wavelength. The 3D ultrasound and photoacoustic images were acquired by mechanically scanning the transducer over the region of interest and capturing spatially co-registered and temporally consecutive photoacoustic transients and ultrasound pulse-echo signals.

Results

Seven days after implantation the PEGylated fibrin biomatrix induced significant vascular growth in the implants. The ultrasound and photoacoustic images agree well with vascular structure in the gel samples. The results also suggest that the photoacoustic and ultrasound imaging can be used to sequentially monitor the growth of neovasculature *in vivo*.

Discussion

Quantitative and qualitative monitoring of neovascular growth is required in many vascular tissue engineering applications. For example, the contribution of progenitor cells in growing microvasculature has been demonstrated; however, the process of vascularization from progenitor cells is not well understood partially related to the absence of an imaging technique that is consistent, easy to use, and can quantitatively assess the dynamics of vascular growth or regression in a three-dimensional environment.

Our group has developed an ECM mimic by modifying fibrinogen with PEG derivatives. We have shown that our PEGylated fibrin biomatrix can increase entrapped stem cell viability and direct them to differentiate towards vascular cells without any angiogenic growth factors. [1] Furthermore, we have measured the extent of vascular growth as induced by our PEGylated fibrin biomatrix *in vivo* using a novel ultrasound and photoacoustic imaging technique.

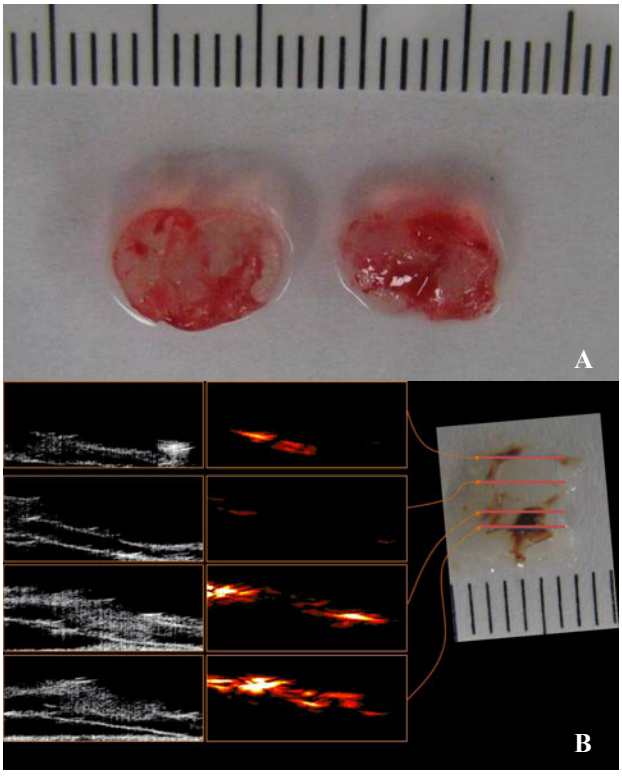

Figure 1 A: vascular ingrowth in the PEGylated fibrin biomatrix **B**: Ultrasound (left) and photoacoustic (middle) images of the dried explant (right).

References

1. G. Zhang, X. Wang, Z. Wang, J. Zhang, L. Suggs. Tissue Engineering, 2006, 12(1): 9-19.