Cell recruitment strategies for in vivo vascular tissue engineering

Aditee Kurane, Naren Vyavahare, PhD.
Cardiovascular Implant Research Laboratory, Clemson University

Statement of purpose:

In vivo tissue engineering has been explored as a means to create autologous vascular replacements. Most strategies have utilized the idea of implanting inert materials in various locations to allow the formation of a capsule that is used as the replacement tissue. These ''biotubes'' are rich only in collagen which provides tensile strength to arteries however, in order to sustain continual pulsatile flow and prevent dilatation, elastin is a necessary component [1] Unfortunately, elastogenesis in tissue engineered constructs has been very limited [2]. We have pursued a novel in vivo method to recruit vascular cells into tubular elastin scaffolds. On implanting these repopulated scaffolds into vasculature, elastin would provide the necessary elasticity to the graft.

Methods:

Scaffold preparation: porcine carotid arteries were treated with 50mg/ml cyanogen bromide in 70% formic acid [3] to remove cells and all proteins and leave behind pure, mature elastin

Preparation of bFGF/SDF loaded elastin tubes: 2% solution of agarose prepared in TBS was diluted 1:1 with 6.6µg/ml solution of human recombinant bFGF in TBS or 3.33µg/ml solution of human recombinant SDF-1a to form 1% agarose gel with 3.3µg/ml bFGF or 1.66µg/ml SDF respectively. Four groups (depending on cytokine and location) were formed: FGF-subQ, FGF-Adi, SDF-subQ and SDF-Adi. For each group, 500µl of corresponding cytokine-gel solution was pipetted into tubular elastin scaffolds.

Subdermal implantation of tubular elastin scaffolds: Samples (n=6/group/location) were implanted into subdermal and adipose pouches in New Zealand White rabbits. After 28 days implants and surrounding capsules were retrieved for analysis.

Histology and Immunohistochemistry: Six micrometer thick cryosections were evaluated using routine histology - H&E for general morphology and Gomori's trichrome for new collagen formation. Immunostaining using anti-vimentin mAb (1:200 dilution) for fibroblasts, anti-CD146 (1:100) for endothelial cells, anti-α-smooth muscle actin (1:500) for myofibroblasts and anti-caldesmon (1:500) for smooth muscle cells was also performed.

Calcium assay: Quantitative calcium content in explanted scaffolds was determined using an atomic absorption spectrophotometer

Results:

None of the samples in any of the groups calcified (at the most 1µg Ca/mg dry tissue weight) - Figure 1.

Immunohistochemical staining (Figure 2) indicated that most of the infiltrating cells in all groups (both locations and both cytokines) were fibroblasts (2A) – a number of which also stained positive for smooth muscle α-actin (2B,D,E). Vascularization was seen in both the SDF-Adi as well as FGF-Adi (2E). There was positive staining for ECs only in the SDF-Adi group (2C) and positive staining for caldesmon only in the FGF-Adi group (2F).

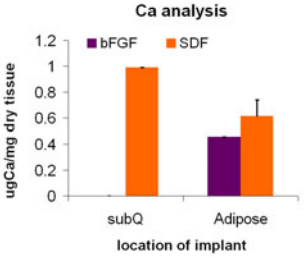

Figure 1. Quantitative calcium analysis in explanted tissue.

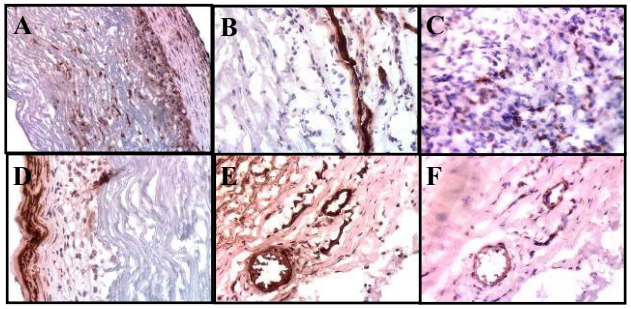

Figure 2. Immunohistochemical characterization of infiltrating cells in elastin scaffolds: (A) immunostaining for fibroblasts in SDF-subQ; (B,D,E) immunostaining for smooth muscle a-actin in SDF-Adipose, FGF-subQ and FGF-Adipose respectively; (C) immunostaining for endothelial cells in SDF-Adipose and (F) immunostaining for caldesmon (smooth muscle cells) in FGF-Adipose

Conclusions:

A novel in vivo cell recruitment method utilizing an agarose gel - growth factor system with tubular elastin scaffolds was used to study the effect of cytokine and/or implant location on the type of cells recruited. Previous studies of bFGF loaded tubes in rat subcutaneous pouches showed recruitment of fibroblasts and some myofibroblasts. Since stromal derived factor (SDF-1a) has been shown to cause the homing of ECs [4], we wanted to investigate the ability of SDF to recruit endothelial cells to the scaffold by our method. We implanted SDF loaded and bFGF loaded tubes into adipose tissue (since it is a well known source of stem/progenitor cells) as well as subcutaneous pouches. We observed a difference in the types of cells recruited – endothelial cells were recruited only by SDF in the adipose tissue whereas actual smooth muscle cells (stained by caldesmon) were found in the FGF-Adi group. Although the cellular infiltration into the scaffold was not complete in any group and finer aspects such as dosage of cytokines and duration of implant needs optimization, this study provides preliminary results in the use of specific cytokines and locations as a means for tissue specific autologous cell recruitment.

References:

1. Li DY. J Clin Invest 1998;102(10):1783–7.
2. Opitz F. Cardiovasc Res 2004;63(4):719–30.
3. Lu Q. Biomaterials 2004;25(22):5227–37.
4. Sengenes C. Stem Cells 2007; 25:2269-2276