Stacked collagen film enabled engineered small vascular graft

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Statement of Purpose: Vascular grafting is a surgical treatment that replaces a significantly or completely blocked blood vessel with a healthy blood vessel. The gold standard for grafting involves the use of a patient's own blood vessel, but suitable autologous veins or arteries are not always available for patients. Under this circumstance, synthetic vascular grafts made of expanded PTFE, PET, and polyurethane perform well in large diameter vessels (>6mm), but are not suitable for small diameter vessels due to thrombus formation. Therefore, much research effort has been focused on developing small-diameter vascular grafts.

Collagen is the most abundant protein in the human body. Since collagen possesses a major advantage in being biocompatible, cell-growth supportive, and controllable biodegradable, collagen is most extensively used in both research and medical applications. However, unlike the collagens in our body, the mechanical strength of purified collagen is relatively low, and non-water soluble collagen easily denatures to water-soluble gelatin during the engineering process. Thus, a harmful cross-linking process (glutaraldehyde) is required to increase the mechanical strength of the construct. Moreover, it is difficult to precisely control the material properties of the collagen based construct (including vascular graft) due to the lack of means to tune its properties.

To address these difficulties, the PI's lab has recently developed an engineered collagen film, which allows precise control of collagen fiber alignment, permeability, flexibility, biodegradation, thickness and transparency. More importantly, the mechanical strength (e.g., suture retention strength and burst pressure) of the engineered collagen based construct can be precisely controlled by varying the number of the stacked collagen films using the "wet and dry" method. The "wet and dry" method only requires repeated treatment with water followed by air drying in order to create permanent bonds between collagen film layers. Increasing the number of layers increases the mechanical strength of the collagen film based construct. In this study, we have developed a stacked collagen film based vascular graft for replacing the injured

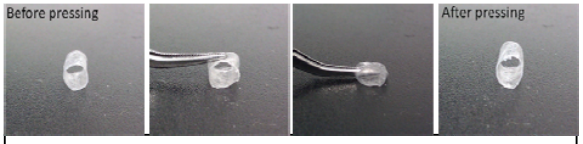

Figure 1. Very flexible vascular graft (5 stacked, collagen only, 5mm ID).

Methods: Fabrication of vascular graft using stacked collagen films: The collagen films were prepared by solvent casting on PDMS mold. Rat tail tendon derived collagen solution (6mg/ml) was pipetted onto a PDMS mold and dried under unidirectional air flow. Once completely dried, the film was peeled off, floated on 1xPBS and rolled around a 2mm mandrel. The rolled wet collagen film was air dried, and the second collagen film was rolled on the top of the first rolled collagen film, and air dried for permanent bonding without glue.

Mechanical characterization of graft: Suture retention strength (Instron mechanical tester) and burst pressure (manometer) were measured and

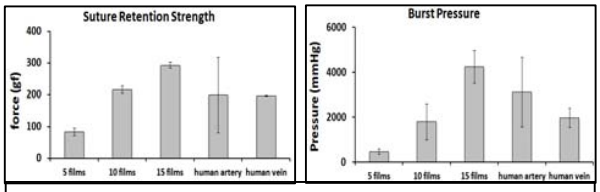

Figure 2. Controllable mechanical strength of vascular graft. Stack of 5, 10 and 15 collagen films (n=3/condition). Values for human artery and vein from[1]

compared those of human artery and vein.

Addition of Elastin for further mechanical property tuning: Collagen and elastin were mixed a 9:1 ratio by weight to prepare 6mg/ml solution and the films were fabricated as described above. The mechanical properties (suture retention strength and burst pressure) of the vascular graft made of the mixture of collagen and elastin was measured and compared with the collagen only graft.

Endothelial cell growth and migration toward collagen film: Human umbilical vein endothelial cells (HUVEC) were seeded on the collagen

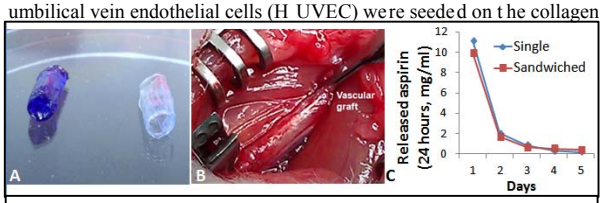

Figure 3. A) Toluidine blue stained heparin immobilized graft (left) and graft without heparin (right). B) Vascular graft implanted at rat carotid artery. C) Aspirin release profile for 5 days. Sandwiched: drug embedded collagen film was sandwiched by two additional collagen films.

film and allowed to grow for 3 days. In addition, HUVEC cells were exclusively seeded on the outside of the collagen film and allowed to migrate toward the collagen film.

Heparin immobilization on collagen film: Heparin was immobilized on collagen film to reduce the thrombogenic properties of collagen. The vascular graft was fabricated and then heparin solution (via NHS and EDC mediated coupling) was introduced to the graft. The presence of heparin on the graft was confirmed by Toluidine blue staining.

Controlled drug release from graft: Anti-platelet drug, Acetylsalicylic acid (Aspirin) was embedded on the graft, and the controlled drug release profile was examined.

Implantation of graft to rat carotid artery: The heparin immobilized graft (7mm long, 0.7mm ID) was implanted at the rat carotid artery (Fig. 3B).

Results: Controllable mechanical strength: Figure 1 visually demonstrates the very flexible nature of the vascular graft. After complete pressing with forceps, the vascular graft recovered its original tubular shape. The mechanical strength (e.g., suture retention strength and burst pressure) of the engineered collagen film based vascular graft can be controlled by the numbers of the stacked collagen films: When stacking more collagen films, the mechanical strength increases (Figure 2). The suture retention strength and burst pressure of the graft are 1.5 and 1.3 times stronger than that of human artery. In addition, the burst pressure of collagen/elastic vascular graft is 2.2 times higher than collagen only vascular graft. These results clearly demonstrate that the material/physical properties of the collagen film based vascular graft can be precisely tuned for perfect fit to the vascular implantation.

Supporting the vigorous growth and migration of vascular endothelial cells toward collagen film: The HUVECs (vWF immunostained) vigorously grew on the collagen film and formed a tightly packed monolayer. Also, the HUVECs exclusively seeded on the outside of the collagen film were completely covered with the migrated HUVECs within 2 weeks after seeding. These results demonstrate that once the vascular graft is implanted at injured site, the inner most surface of the vascular graft can be completely covered with the migrated endothelial cells from surrounding blood vessel to minimize the thrombus formation.

Improvement of hemocompatibility of the graft: The weak antigenicity of collagen makes it an attractive material for vascular graft, but the thrombogenic nature of collagen has be reduced. In order to address this, the thromboresistance drug heparin was successfully immobilized on the graft (Fig. 3A), and the anti-platelet drug Aspirin was embedded and continuously released up to 5 days (Fig. 3C). Furthermore, the release profiles of Aspirin can be further controlled by the placement of the Aspirin embedded collagen film (e.g., double amount released from sandwiched at 4th and 5th day as compared to single, Fig. 3C): collagen film acts as a drug diffusive barrier to control the amount and duration of released drug. These results indicate that the hemocompatibility of the graft can be further improved by addition of molecules and/or drugs.

Conclusions: In this study, we have developed a mechanically strong, cell growth supportive, and improved hemocompatible vascular graft for replacing the injured small diameter blood vessel based up on the engineered collagen film and a novel collagen film stacking method (wet & dry).

[1]L'Heureux, N., et al. Nature medicine 12, 361-365 (2006)