Dacron vascular graft impregnated with connective tissue growth factor

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Introduction: Abdominal aortic aneurysm is 13th leading cause of death in the United States. Endovascular aortic aneurysm repair (EVAR) is a minimally invasive procedure that has been shown to prevent aneurysm rupture, and to reduce patient morbidity and mortality compared to open surgical repair [1-2]. For an EVAR procedure, a stent-graft consisting of a vascular graft and stent is deployed to the aneurysm site to prevent further expansion. However, endoleak and migration of the stent-graft are two major problems encountered after EVAR. Endoleak is known as the persistent blood flow into the aneurysm sac after stent-graft repair [3]. The occurrence of endoleak or migration could cause the aneurysm to expand and rupture if left untreated. Lack of tissue ingrowth between the stent graft and the arterial vessels, especially tissue bonding to the vascular graft might be a reason why these problems occur. Therefore, a permanent tissue bonding or seal between the aorta and the implanted prosthetic device (stent-graft) is proposed to prevent proximal and distal migration of aortic devices [3, 4]. In this study we present a modified vascular graft that delivers a growth factor to increase cellular proliferation. Dacron vascular graft was impregnated with CTGF and 50/50 Poly (DL-lactide-co-glycolide) acid. CTGF is a cysteine rich growth factor that is induced by transforming growth factor beta. CTGF promotes cellular adhesion and proliferation of fibroblast cells, proliferation of vascular smooth muscle cells, increases the expression of type I and III collagen and induces fibrosis during wound healing [5].

It is hypothesized that as PLGA degrades, the growth factor released will promote local proliferation of fibroblast and vascular smooth muscle cells around the coated graft and it would also induce fibrosis to facilitate a biological incorporation between the stent graft and artery. The development of the modified graft, release of the protein and cellular bioactivity on vascular cells, will be addressed.

Methods: 2 cm by 0.5 cm samples woven Dacron vascular grafts were obtained from the 4" by 4" Cooley low porosity woven vascular graft obtained from Boston Scientific Co. The weight of the uncoated grafts was recorded before coating. Coating solution for the grafts was prepared by modifying an established solvent evaporation method used to prepare biodegradable microspheres [6]. The first step in the preparation of these microspheres, primary emulsion (water-in-oil emulsion) was used. This step emulsifies the aqueous protein solution in the polymer phase. Briefly, 5(w/v) % 50/50 PLGA (inherent viscosity 0.82 dl/g, DURECT Co.) was prepared in dichloromethane. The aqueous protein solution consisted of 0.00067(w/v)% CTGF, 3(w/v)% Magnesium hydroxide ($Mg(OH)_2$), 0.6 (w/v)% Sucrose, and 0.01(w/v) % EDTA and 15% BSA. The aqueous phase was prepared with phosphate buffer saline solution (PBS). The aqueous phase was then emulsified in the polymer phase to make the coating solution. The samples were dip coated five times. Coated samples were then placed in a Petri dish to air dry in a dust free environment for 24 hours and subsequently vacuumed dried for 24 hours. The release kinetics of the protein from coated grafts in PBS buffer solution was monitored at 12 hr, 1 day, 3 days, 7 days, 14 days, 21 days and 28 days. Release kinetics samples underwent constant rotation at 37°C. Furthermore, concentration of BSA and CTGF released, surface morphology of the coated grafts before and after release kinetics, and cellular bioactivity of the release medium were evaluated. The amount of BSA release was analyzed using QuantiPro™ BCA Assay kit. The amount of CTGF released was determined using a noncommericialized CTGF ELISA. Cellular bioactivity of the release medium on vascular cells (i.e. fibroblast and rabbit vascular smooth muscle cell) was assayed using a cell viability reagent kit (i.e. MTT reagent). In addition, vascular cells were seeded onto the modified grafts to determine qualitatively its effect on cellular proliferation.

Results / Discussion: The average amount coated on this graft after modification was 4.28 ± 0.45 mg. Uniform coating of the grafts with some porosity was observed. . An increase in porosity of the coated vascular graft was observed after the 7th day. This is might be due to the increase water uptake of the polymer that induces the release of protein impregnated within the vascular graft. According to release profile of CTGF from the impregnated vascular graft most of the protein within the graft released within 7 days (See Figure 1a

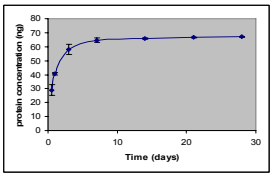

Figure 1: Release profile of CTGF from modified vascular graft (n=4)

Conclusions: In conclusion, the solvent evaporation technique was successful in impregnating Dacron vascular grafts with CTGF. Most of the growth factor released within 7 days. . Future works include bioactivity of the supernatant released on rabbit vascular smooth muscle cells and fibroblast cell proliferation.

References:

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