Development and characterization of PLGA impregnated vascular graft for endovascular abdominal aortic aneurysm (AAA) repair

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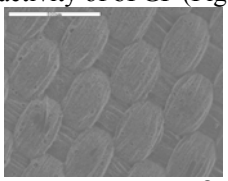
Introduction: Endovascular aneurysm repair (EVAR) is a minimally invasive procedure that has been shown to prevent aneurysm rupture, and to reduce patient morbidity and mortality compared to open surgical repair [1-3]. However, endoleak and migration of the stent-graft are two major problems encountered after EVAR. Progression of these two problems can cause aneurysm rupture or graft occlusion [4]. 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 [4, 5]. This can be achieved by modifying the aortic devices currently used for AAA repair. In this study we present a modified vascular graft that delivers a growth factor to increase cellular proliferation. Dacron vascular graft was impregnated with basic fibroblast growth factor (bFGF) and 50/50 Poly (DL-lactide-co-glycolide) acid. It is hypothesized that as PLGA degrades, the growth factor released will promote local proliferation of fibroblast, vascular smooth muscle cells and endothelial cells around the coated graft. The development of the modified graft, weight loss of the coated graft, controlled release of a protein (bovine serum albumin, i.e. BSA) and cellular bioactivity of the supernatant released on rabbit vascular smooth muscle cells (RVSMC), will be addressed.

Methods: 2 cm by 0.5 cm samples of vascular grafts were cut from the 4" by 4" low porosity woven vascular graft (Cooley Verisoft, Boston Scientific, and Natick, MA). The uncoated weight for each graft was recorded before coating. Coating solution for the grafts was prepared by an established solvent evaporation method used for the production of 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 ml solution of 5% 50/50 PLGA (inherent viscosity 0.89 dl/g, DURECT Co.) was prepared in methylene chloride. The aqueous protein solution consisted of 0.00025% bFGF, 0.00025% heparin, 3% magnesium hydroxide($Mg(OH)_2$), 1.2% sucrose, and 0.01%EDTA and 15% BSA. The aqueous solution was prepared with phosphate buffer saline solution (PBS). 500 µl of the aqueous protein solution was emulsified in the PLGA solution. The Dacron samples were then dip coated five times in the emulsion at room temperature. The 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 coated grafts in PBS buffer solution was evaluated at 6 hr, 1 day, 7 days, 14 days, 21 days and 28 days. Release kinetics study was performed in a hybridized incubator set at 12 rpm and 37°C. Furthermore, the percent weight loss, concentration of BSA released, surface morphology of the coated grafts and cellular bioactivity of the release medium were evaluated. The amount of BSA release was analyzed using QuantiPro™ BCA Assay Kit. Cellular bioactivity of the release medium on RVSMC proliferation was assayed using a MTT reagent kit.

Results / Discussion: The average amount coated on this graft after dip coating in the protein emulsion was 3.61± 0.12 mg. Uniform coating of the grafts with some porosity was observed (Figure 1a). Increase in porosity of the coating of the grafts was observed after 1 day of degradation studies. After 7 days, the smoother morphology of the graft was lost. Coating was only observed within the interstices of the graft. In terms of weight loss, an insignificant amount of the coating was lost within the first week while the graft was undergoing degradation studies. However, after 7 days, up to 24 % of the coating within graft has degraded.

The release of BSA encapsulated within the impregnated graft was monitored in the supernatant collected. Within the first 6 hours, 30% of BSA used in the preparation of the impregnated graft was released. By 28 days, most of the initial encapsulated BSA (>80%) was detected in PBS. The slowed controlled release of the protein (BSA) was due to the presence of $Mg(OH)_2$ and sucrose. $Mg(OH)_2$, an antacid, stabilizes the ortho ester bond and extends the release time of drugs [6].

The effect of encapsulated protein and released growth factor to induce cell proliferation was also examined. The encapsulation procedure did not affect the biological activity of bFGF (Figure1b).



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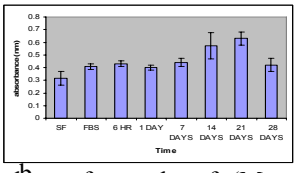

b

Figure 1a: Surface morphology of coated graft (Mag. 500X); **1b**: Effect of supernatant on RVSMC proliferation. (SF-Serum-Free, FBS-Fetal Bovine Serum)

Conclusions: In conclusion, the solvent evaporation technique was successful in impregnating Dacron vascular grafts with PLGA and bFGF. The growth factor released within the impregnated graft was able to retain its activity. Further analysis of the amount of bFGF released over the course of this study is underway. Future works include bioactivity of the supernatant released on endothelial and fibroblast cell proliferation.

References:

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