Effect of Vitamin-C on the Growth of Endothelial Cells for Stent and Vascular Graft Applications

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Statement of Purpose: Endothelialization is vital for preventing thrombosis on cardiovascular medical devices such as coronary stents and vascular grafts. The anti-proliferative drugs such as sirolimus (SIR) and paclitaxel (PAT) are currently released from stents and vascular grafts to inhibit the growth of smooth muscle cells (SMCs) and thereby preventing neointimal hyperplasia [1]. However, these drugs delay or impair the growth of endothelial cells (ECs) on implant surfaces causing late thrombosis [2] – a serious condition which results in heart attack or death. Hence, a drug which inhibits the growth of SMCs and concurrently encourages the EC growth is currently needed for these implants. Vitamin-C has been shown to inhibit SMC growth and promotes EC growth when orally administered in patients [3]. Hence, the research goal of this study is to investigate the effect of L-AA on the growth of ECs when the drug is directly added to the cells for potential applications in locally drug delivering stents and vascular grafts. For comparison purposes, the effect of SIR and PAT on the growth of ECs was also investigated in this study.

Methods: A density of 15×10^3 human aortic endothelial cells (HAECs) was seeded in a 24-well plate. A dose of 100µg/mL of L-AA in phosphate buffered saline (PBS) was added to the cells and incubated for up to 24 or 48 hours. Similarly, a dose of 100µg/mL of SIR and PAT in 0.5% ethanol was also added to the cells and incubated for the time period mentioned above. Three different control samples were also used in the study: (a) control # 1 – cells with no drugs; (b) cells with only PBS; (c) cells with only 0.5% ethanol. The viability and proliferation of ECs were investigated at 1, 3, 5, and 7 days using a Resazurin fluorometric assay. The live cells were stained with fluorescein diacetate and imaged using fluorescence microscopy. The morphology of cells was investigated using phase contrast microscopy. The expression of surface adhesion molecule, platelet endothelial cell adhesion molecules (PECAM-1), was investigated using immunofluorescent microscopy. One-way ANOVA was used to determine the statistical significance at $p < 0.05$.

Results: L-AA and the three controls exhibited a significant increase in cell viability and proliferation from day-1 to day-7 while SIR and PAT showed very limited viability and proliferation of ECs (Fig 1). L-AA showed the presence of maximum EC number even greater than that of controls at different time points (Fig 1). The viability and proliferation of ECs increased in the following order SIR < PAT < Control #1 < Control #2 = Control #3 < L-AA. These results showed that L-AA strongly favored the growth of ECs while SIR and PAT inhibited the EC growth. A spreading of ECs with typical polygonal shape was observed for L-AA and controls while an elongated oval shape was observed for SIR and PAT (Figs 2 and 3). The expression of PECAM-1 was stronger on L-AA and controls while a weaker expression was observed for SIR and PAT (Fig 4).

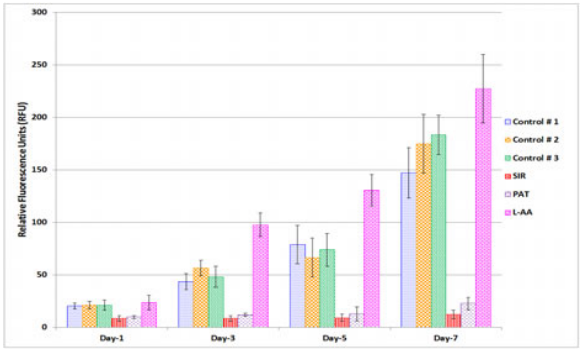

Fig 1. Quantitative EC viability and proliferation

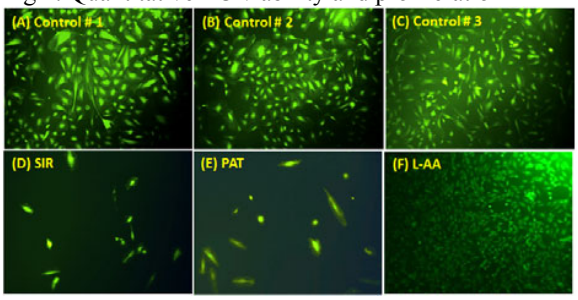

Fig 2. Fluorescence microscopy images of ECs

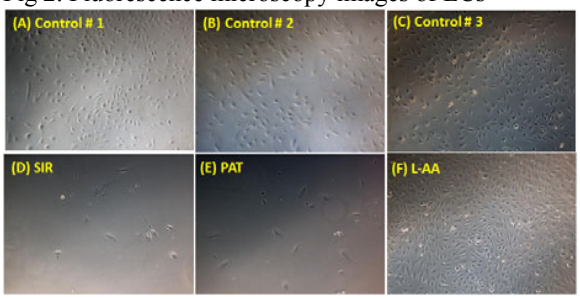

Fig 3. Phase contrast microscopy images of ECs

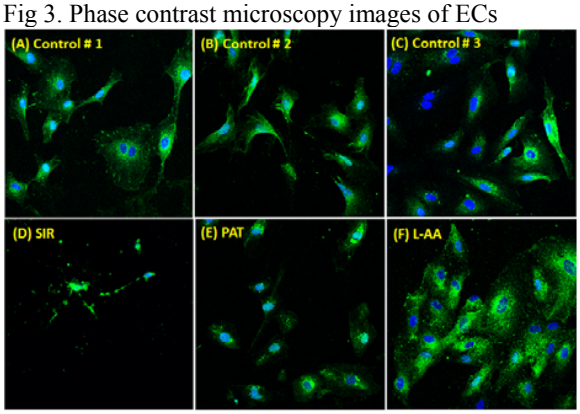

Fig 4. Immunofluorescent microscopy images of ECs

Conclusions: L-ascorbic acid strongly encouraged the growth of endothelial cells in *in vitro* conditions. Hence, L-AA is a promising drug for local delivery from stents and vascular grafts to promote endothelialization.

References: (1) Mani G. Biomaterials 2007; 28: 1689-1710; (2) Finn AV. Circulation 2007; 115: 2435-2441; (3) Aguirre R. Pharmacol Ther 2008; 119: 96-103.