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Developing Customizable Scaffolds With Antimicrobial Properties for Vascular Tissue Regeneration Using Low Temperature Plasma

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Abstract : Bypass surgery, using the autologous vein has been one of the most effective treatments for cardiovascular diseases (CVD). More recently tissue engineering including engineered vascular grafts to synthesize blood vessels is gaining usage. Dacron and ePTFE has been employed for vascular grafts, however, these does not work well for small diameter grafts (<6 mm) due to intimal hyperplasia and thrombosis. In the present study PTFE was treated with LTP to improve the endothelialization of intimal surface of graft. Scaffolds were also modified with polyvinylpyrrolidone coated silver nanoparticles (Ag-PVP) and the antimicrobial peptides, p753 and p359. Human umbilical vein endothelial cells (HUVEC) were plated on the developed scaffolds and cell proliferation was determined by the MTT assay. Cells attachment on scaffolds was visualized by microscopy. mRNA expressions levels of different cell markers were investigated using quantitative real-time PCR (qPCR). X ray photoelectron spectroscopic confirmed the introduction of oxygenated functionalities from LTP air plasma. Microscopic and MTT assays indicated increase in cell viability in LTP treated scaffolds. Gene expression studies shows enhanced expression of cell adhesion marker Integrin- α 5 gene after LTP treatment. The KB test displayed a zone of inhibition for Ag-PVP, p753 and p359 of 19mm, 14mm, and 12mm respectively. To determine toxicity of antimicrobial agents to cells, MTT Assay was performed using HEK293 cells. MTT Assay exhibited that Ag-PVP and the peptides were non-toxic to cells at 100µg/mL and 50µg/mL, respectively. Live/dead analysis and plate count of treated bacteria exhibited bacterial inhibition on develop scaffold compared to non-treated scaffold. SEM was performed to analyze the structural changes of bacteria after treatment with antimicrobial agents. Gene expression studies were conducted on RNA from bacteria treated with Ag-PVP and peptides using qRT-PCR. Based on our initial results, more scaffolds alternatives will be developed and investigated for cell growth and vascularization studies.

Keywords : low temperature plasma, vascular graft, HUVEC cells, antimicrobial
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