Characterization and optimization of elastin based peptides for use in tissue engineered vascular grafts

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Statement of Purpose: The growing incidence of cardiovascular diseases in the US due to obesity and type II diabetes has increased the demand for bypass surgeries. Due to the limited supply of suitable donor arteries, there is a growing need for viable blood vessel substitutes. Synthetic alternatives made of Dacron and ePTFE have been successful as large diameter blood vessel replacements, but have failed when implemented as small diameter vessels. Along with poor compliance, this failure rate is largely due to the inability of the constructs to provide a suitable environment for appropriate vascular development.

The goal of this work is to engineer a vascular graft that would be able to promote a microenvironment suitable for vascular growth. As such, our strategy employs using a PEG-DA hydrogel tailored with an elastin mimetic peptide as a potential scaffold for tissue engineered vascular grafts.

Methods: A 23 amino acid (aa) amino acid sequence, AKAAKVGVAPGRGDSAAKAAKK, and a slight variation of the peptide, AKAAKVGVAPGAAKAAKK (19 aa) were first characterized in 2D cell culture for elastin production. Briefly, 30,000 human aortic smooth muscle cells (SMCs)/cm^2 (passage 4) were seeded onto a 24 well plate followed by the addition of either sterile filtered, using 0.2 µm pore size nylon filters, 23 aa or 19 aa peptide at varying concentrations. Elastin deposition was characterized after 48 hours by first removing old media from the well plates and washing with PBS. The well plate was then frozen and lyophilized. Each well was resuspended with 1 mL of 0.1 N NaOH and reacted in a hot water bath at 100 °C for 1 hour. The samples were then centrifuged (8000 g, 10 min) and the supernatant containing cell debris, collagen, and other ECM proteins were discarded. The centrifugate was resuspended in 1 mL of 0.25 N oxalic acid and samples were reacted first in a 100 °C water bath for 1 hour and then transferred to 100 °C oven to further react for 48 hours. After 48 hours, the samples were centrifuged using Amicon YM-3 filters and were resuspended in 250 µL of PBS. A Fastin assay was used to determine the elastin content in each sample.

Before a peptide can be incorporated into a PEG-DA hydrogel, it must first be conjugated to a mono acrylate PEG derivative, PEG-SVA. The 23 aa elastin mimetic peptide sequence was conjugated with PEG-SVA under basic conditions at 1:1 molar ratio. PEG-DA hydrogels were fabricated between two glass slides (using a 0.75 mm spacer) by first mixing a 10% solution of 10,000 MW PEG-DA in 10 mM HEPES with 2,2 dimethyl-2-phenyl-acetophenone, DMAP, and exposing to UV light for 10 seconds (Omnicure S2000 UV Lamp, Exfo, Toronto, Canada, 365 nm, 15 mW/cm2 intensity). The glass slide on top was then lifted and washed with PBS. A 500 µL solution of PEG- 23 aa peptide (1 nM) with DMAP was added and the glass slide was carefully placed on top resulting an equally distributed peptide solution over the surface. The "sandwiched" construct was exposed to UV light to induce crosslinking between PEG-DA and the monoacrylated PEG-23 aa peptide. Residual peptide solution and DMAP were washed off and 14.3 mm diameter samples were cut and carefully placed in a 24 well plate. The wells were then incubated with SMCs (passage 3) media for 2 hours and 30,000 cells/cm^2 were seeded on top of the surface. Cell adhesion was quantified after 48 hours.

Results: Elastin deposition pointed towards a competitive phenomenon with increasing peptide, either 23 or 19 aa, concentrations.

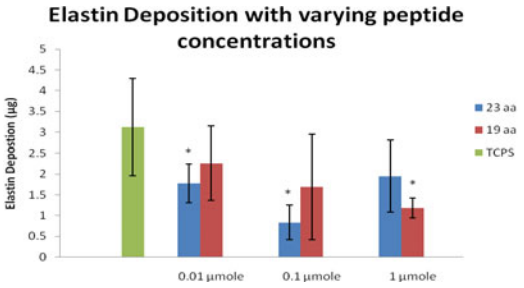

Figure 1. Elastin deposition after 48 hours. The decrease in elastin deposition with increasing peptide concentrations alludes to a competition phenomenon for elastin production. (* $p<0.05$ statistical difference compared to TCPS)

SMCs adhered on top of a PEG-DA hydrogel modified with the 23 aa elastin mimetic peptide sequence.

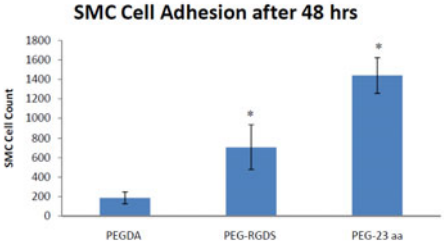

Figure 1. SMC Cell adhesion after 48 hours on PEG-23 aa. (* $p<0.01$ statistical difference compared to PEGDA)

Conclusions: Our modified hydrogels are promising scaffolds for vascular grafts as they have the potential to create a microenvironment for proper ECM deposition and growth. The 23 aa elastin mimetic peptide sequence is showing promise in our goal towards developing a viable scaffold. Further optimization of the sequences is under way to yield maximum amounts of cell adhesion and ECM deposition. Further studies will look at quantifying desmosine and collagen production in a 2D cell culture system *in vitro*.

References:

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(Campbell GR. Curr Pharm Biotech. 2007; 8(1): 43-50)