A Pro-Angiogenic Peptide-Tethering Platform Using Biotinylated Tyrosine-Derived Polymeric Fiber Mats

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Statement of purpose: Angiogenesis, or the formation of new blood vessels from existing ones, is essential for the transport of nutrients and overall healing. Critical sized defects necessitate the use of synthetic scaffolds that must recapitulate the pro-angiogenic response. Therefore, incorporating bioactivity into synthetic polymers is necessary to elicit proper cellular responses and tissue regeneration. Biotinylated polymers provide an efficient tethering platform on which to modify a scaffold with bioactive cues. Both the polymer used to tether bioactive cues as well as the cues themselves can be optimized for the tissue application. Previous work has demonstrated that the non-cytotoxic, biodegradable tyrosine-derived polymer E0500 allowed for the gradual replacement of the polymer network with cell-derived extracellular matrix (ECM) while maintaining the polymer network support.[1] E0500 was biotinylated through carboxylic acid groups (BN-E0500). In order to elicit a pro-angiogenic response in endothelial cells, the pro-angiogenic peptide SDKP[2] was tethered to BN-E0500 via the biotin-streptavidin affinity interaction.

Methods: E0500, a tyrosine-derived polymer containing 5 mol percent of desaminotyrosyl tyrosine was synthesized using a previously published procedure.[3] E0500 was biotinylated through carboiimidine chemistry.[4] Peptides were prepared by solid-phase peptide synthesis as previously described.[5] Electrospun fiber mats were hydrated in PBS at 37°C for 1 hour, incubated with 20 μg/ml streptavidin at 37°C for 30 minutes, washed twice with PBS, and then incubated with 10 mg/ml biotinylated peptide at 37°C for 30 minutes, washed twice with PBS, and finally plated with Human Dermal Microvascular Endothelial Cells (HDMECs). Quantitative PCR (qPCR) was performed according to established protocol.[6]

Results: BN-E0500 had similar molecular weight retention values and mechanical properties as E0500, demonstrating that there was no significant effect of biotin on polymer stability or mechanics (Figure 1). A biotinylated pro-angiogenic peptide (BN-SDKP) was synthesized and tethered to an electrospun mat of BN-E0500. HDMECs were then grown on fiber mats in the presence or absence of BN-SDKP for 24 hours. Increased mRNA levels of VEGF, PDGF, and FGF were observed by qPCR when the peptide was attached through biotin-streptavidin binding as compared to adsorbed peptide and the fiber mat alone (Figure 2). This suggests that cellular behavior can be altered in response to interactions with biotinylated peptides in this system, as these growth factors are closely associated with angiogenesis.

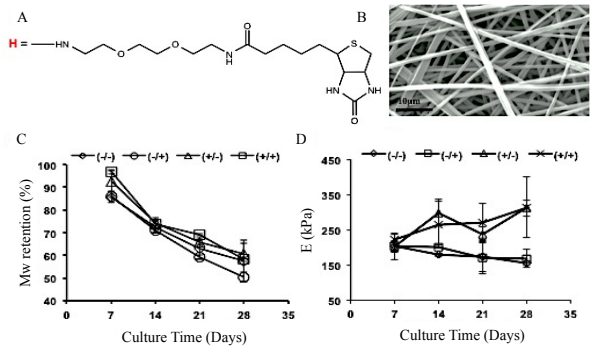

Figure 1. (A) Amine-PEG_2-biotin was conjugated to the carboxylic acid in E0500 to give BN-E0500. (B) SEM micrograph of electrospun BN-E0500. NIH 3T3s were grown on the fiber mats and were decellularized (+/+) or not (+/-). Control conditions were performed in which the mats were kept in medium without cells and either decellularized (-/+) or not (-/-). (C) Graphs show percent Mw retention and (D) Young's modulus as a function of time ± standard deviation (n=3).

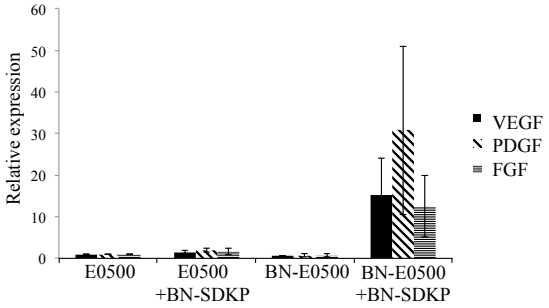

Figure 2. Real time PCR of pro-angiogenic growth factors. E0500 and BN-E0500 fiber mats were treated with BN-SDKP as indicated. Cells were grown upon the fiber mats for 24 hours, RNA was isolated, and RT-qPCR was performed. Graph shows results normalized against GAPDH ± standard error (n=2).

Conclusions: The results demonstrate a novel approach to functionalize synthetic scaffolds with pro-angiogenic peptides using the biotin-streptavidin affinity interaction. Single or multiple peptides could be tethered via this mechanism, with the added potential to form spatial gradients in a single scaffold.

References:

1. Goyal, R *et al*. J. Funct. Biomater. 2017; 8(1).
2. Wag, D et al. Am. J. Physiol. Heart Circ. Physiol. 2004;287(5): H2099-105.
3. Ertel, SI and Kohn JK. J. Biomed. Mater. Res. 1994; 28:919-930.
4. Bushman, JS, Raynor, JE, and Kohn, JK (Google patents, 2015).
5. Guler MO *et al*. Biomacromolecules. 2006;7:1855-63.
6. Mao, Y et al. J. Mater. Sci: Mater. Med. 2017;28:100