Biosynthetic PEG-MAL Hydrogel Stimulates Angiogenic Sprouting in Mouse Aortic Rings

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Statement of Purpose: Diabetes mellitus produces abnormalities of angiogenesis that may cause or contribute to many of the clinical manifestations of diabetes[1]. As vascularization is an essential design goal for successful cell-based therapies, we developed an *in vitro* model to study angiogenesis. In our research, we are studying vascular-inductive (*in vivo*) polyethylene glycol maleimide (PEG-MAL) hydrogel as a supportive islet delivery matrix for angiogenic therapies in type I diabetes and chronic limb ischemia[2]. We characterized the 3D spreading and migration behavior of encapsulated cells and tissue sections in PEG-MAL hydrogel towards development of an *in vitro* angiogenic analysis assay. While the majority of published research using PEG-diacrylate and PEG-vinyl sulfone matrices evaluate angiogenesis *in vitro* with 10% or higher (wt/vol) hydrogel[3], we notably found that a much lower wt/vol percentage hydrogel was necessary to achieve cell spreading or angiogenic sprouting in 3D in our hydrogel system.

Methods: NIH-3T3 fibroblast cells were embedded in matrigel and 5% and 2.5% (wt/vol) hydrogels to determine a wt/vol % material threshold for cell spreading and migration. Then, aortic cross sections were obtained from the thoracic aorta of healthy mice[4]. The aortic sections were encapsulated in either 5% or 2.5%(wt/vol) PEG-MAL hydrogels with GCRDVPMSMRGGDRCG (VPM) or GCRDGPQGIWGQKDRCG (GPQ), known fast-cleaving and slow-cleaving degradable peptides sensitive to collagenase respectively[5]. The gels were synthesized via a Michael reaction where the thiol on a cysteine residue of the cross-linking peptide conjugated (or PEGylated) the terminal amine on an arm of a multiarm PEG-MAL eventually linking arms of different PEG-MAL groups, resulting in a hydrogel structure[6].We embedded the aortic sections in these gels and kept them in an incubator. We checked daily for sprouts.

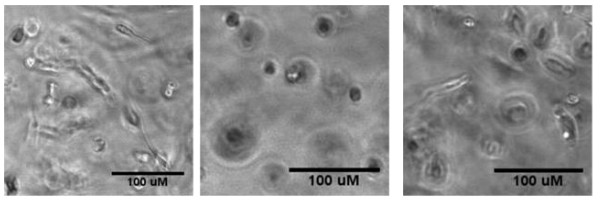

Figure 1. NIH-3T3 cell seeding, PEG-MAL hydrogel (24 hours)

Results: Fibroblast cells seeded in matrigel adhered and spread as expected. Cells in 5% PEG-MAL hydrogel gel did not spread and remained rounded. Cells in the 2.5% gels adhered and spread similar to matrigel (figure 1). Aortic rings in matrigel (control) began to sprout 4 days after embedding. The 2.5% PEG-MAL hydrogels showed sprouting after 4 days (figure 2) while the 5% gels did not show any signs of sprouting. It was determined that VPM was the better crosslinking peptide with an average sprouting length of 262 µM ± 50.5 µM at day 5. GPQ had an average of 171.2 µM ± 61.8 µM (figure 3) at day 5.

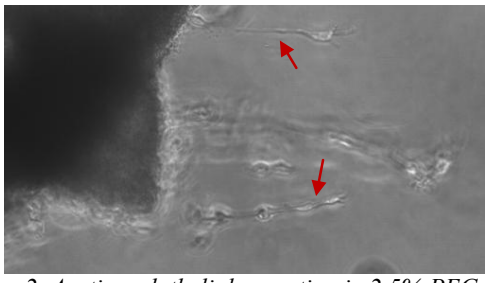

Figure 2. Aortic endothelial sprouting in 2.5% PEG-MAL gels with VPM crosslinker at day 4.

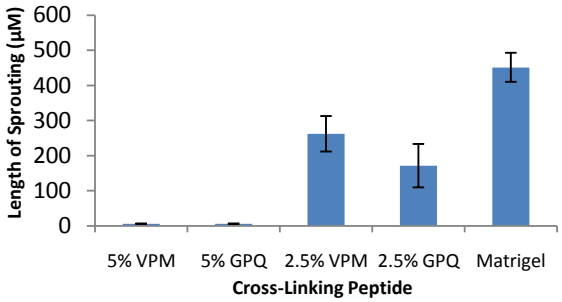

Figure 3. Average Sprouting Data, day 5

Conclusions: We found that 2.5% PEG-MAL hydrogel cross-linked with VPM and GPQ supported cell spreading and migration in 3D. Based on these preliminary results, VPM is a better candidate peptide for the *in vitro* angiogenesis model. Future research hopes to add growth factors such as VEGF and FGF to increase the aortic angiogenic sprouting[7] in the hydrogels and provide a better environment to study therapies for vascularization. In particular we hope to utilize this *in vitro* assay to characterize difference in angiogenesis between diabetic and healthy tissue using aortas harvested from diabetic mice.

References:

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