Controlling the Delivery of Vascular Endothelial Growth Factor and Platelet Derived Growth Factor

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Statement of Purpose: Angiogenesis is the formation of new blood vessels from a pre-existing vascular network. It plays a vital role in the healing of tissues by establishing a blood supply. This process is stimulated by growth factors, particularly Vascular Endothelial Growth Factor (VEGF) and Platelet Derived Growth Factor (PDGF). These stimulate endothelial cell proliferation, migration and survival, along with recruitment of pericytes to stabilize and strengthen newly formed blood vessels.

Two sets of materials have been used to successfully release either $VEGF_{165}$ or PDGF-BB. These systems mimic heparin through electrostatically binding to the growth factor peptide sequences containing arginine and lysine amino acids. However, the aim is to produce more control over the availability of these growth factors than is possible with heparin functional materials.

Core-shell particles composed of polystyrene-co-divinyl benzene core surrounded by a polymer consisting of oleyl phenyl hydrogen phosphate (OPHP) shell crosslinked with ethylene glycol dimethacrylate (EGDMA) showed continuous release of $VEGF_{165}$ for 7 days. Polybutyl methacrylate (PBMA) particles were functionalized with either linear or hper-branched poly(2-acrylamido-2-methyl-1-propane sulfonic acid) (PAMPS) and had the ability to release both $VEGF_{165}$ and PDGF-BB for at least 7 days.

The overall aim of this project is to produce a material capable of releasing both $VEGF_{165}$ and PDGF-BB ina controlled way over time to stimulate angiogenesis. The final intent is to develop a system in which the patient's own growth factors can be taken up by a dressing type material and be released over time, removing the need for the delivery of external growth factors

Methods: OPHP was synthesized from oleyl alcohol (Sigma Aldrich UK) and phenyl phosphodichloridate (Sigma Aldrich UK). PS-co-DVB core OPHP-co-EGDMA shell latexes were produced in an emulsion reactor heated at 70^{o}C with stirring at 400rpm. The latexes containing glycerol methacrylate acetonide (GMAC) were produced in the same manner. PBMA latexes were made via surfactant free emulsion polymerization with PAMPS was added as a stabilizer. This produced latexes with PAMPS incorporated. Particle size, zeta potential measurements and TEM were done on all latex samples. To study protein release and binding 0.5ml 100ng/ml VEGF or PDGF solution containing 1% bovine serum albumin was added to each latex sample. The protein was left to bind for 12 hours at 4^{o}C. The protein solution was changed for PBS and samples placed in a 37^{o}C oven for varying time points. Protein release was analyzed using sandwich ELISA kits purchased from R&D systems (Minneapolis, MN 55413).

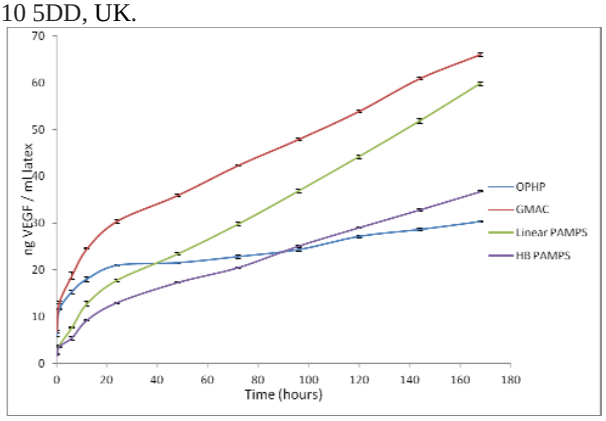

Figure 1. Release data of $VEGF_{165}$ from 50:50 OPHP:EGDMA, 50:50 GMAC:EGDMA, linear PAMPS and hyper-branched PAMPS. The phosphate based materials show a burst release profile and the sulfonic acid based materials do not. The branched PAMPS show the slowest release profile overall.

Results: OPHP and PAMPS based systems showed controlled release of pro-angiogenic growth factors over the period of 7 days. The ratio of OPHP to EGDMA can be altered to give varying release profiles of $VEGF_{165}$. The addition of GMAC to the OPHP system produces a slower release profile, without the plateau seen with OPHP-co-EGDMA. Upon deprotection of GMAC, the outer shell of the latex became larger, allowing $VEGF_{165}$ to bind deeper within the shell of the latex. This gives the steadier release profile seen with OPHP after the inclusion of GMAC. By maintaining the same quantity of OPHP the ratio of GMAC within the latex shell can be altered to give varying release profile. The most successful release profiles were seen with 50:50 OPHP:EGDMA and 50:50 GMAC:EGDMA.

PAMPS functionalized latexes show steady growth factor release over 7 days, with no burst release. The branched PAMPS have a slower release profile compared to linear PAMPS. This is due to branched PAMPS producing a larger network structure, allowing growth factors to bind deeper within the outer shell of the particle. In contrast, linear PAMPS produced a more open structure which allows for easier diffusion.

Conclusions: Two systems have been developed that can successfully bind and release $VEGF_{165}$ and PDGF-BB by electrostatic binding of the growth factors on to or within the outer layer of polymer particles. It is next important to assess the release of $VEGF_{165}$ and PDGF-BB simultaneously along with looking at the effect on endothelial cells.

References: Gilmore L. ChemBioChem. 2009:10:2165-2170.