Functionalized Gellan Gum Hydrogels Potentiate Endothelial Cell Performance

Lucília P. da Silva[1,2,3,4] Amit K. Jha[3,4], Vitor Correlo[1,2], Alexandra Marques[1,2], Rui L. Reis[1,2], and Kevin E. Healy[3,4]
[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Guimarães, Portugal; [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal; [3]Department of Bioengineering, University of California at Berkeley, Berkeley, California, USA; [4]Department of Materials Science and Engineering, University of California at Berkeley, Berkeley, California, USA

Statement of Purpose: Gellan Gum (GG) hydrogels have been proposed for regenerative medicine and tissue engineering applications since they can be easily prepared at room temperature, and by the addition of ions, being highly advantageous for the encapsulation of viable cells (Oliveira *et al*. 2010). However, GG hydrogels lack cell adhesive properties and are slowly degraded by hydrolysis (Oliveira *et al*. 2010). Considering these constrains, we propose a divinyl sulfone functionalized gellan gum (GG-DVS) hydrogel, crosslinked with a thiol crosslinker sensitive to human metalloproteinase 1 (MMP-1), and decorated with the T1 and C16 thiol terminated angiogenic peptides, (Grote *et al.*, 2007; Ponce *et al.*, 2001) to improve endothelial cell performance. These promising functionalized GG hydrogels, showing tunable physical properties and degradability, and improved endothelial performance, will ultimately promote *in vivo* angiogenesis via the direct interaction with endothelial progenitor cells either seeded or derived from the host after implantation.

Methods: GG polymer was chemically functionalized with 10 to 30 molar excess of divinyl sulfone (DVS) groups by a one-step reaction, followed by dialysis and diethyl ether precipitation, obtaining different degrees of substitution, as determined by ^{1}H NMR. GG-DVS functionalized polymer was reacted with thiol terminated peptides (e.g., C16 or T1) by a one-step reaction, followed by dialysis. The efficiency of the reaction was determined by ^{1}H NMR and tyrosine/tryptophan absorption. GG-DVS hydrogels were formed by Michael Type Reaction using different concentrations of GG-DVS (1 to 3 wt%) and a thiol crosslinker sensitive to MMP-1 (10 to 100 %), under physiological conditions. GG-DVS hydrogels were characterized in terms of rheology, swelling and MMP-1-driven degradation. Human Umbilical Cord Vein Endothelial cells (HUVECs) (Lonza, USA) were encapsulated within peptide functionalized GG-DVS hydrogels. After 3, 7 and 14 days, cell adhesion, spreading and viability was evaluated respectively by phalloidin, vinculin and calcein/PI staining. Cell proliferation was determined after DNA quantification and endothelial phenotype preservation analyzed by immunocytochemistry for CD31.

Results: GG functionalization with DVS was confirmed by ^{1}H NMR. The spectrum showed a GG characteristic peak (δ 1.32), and the DVS proton peaks (δ 6.1, 6.2 and 6.9), and a degree of substitution of 95 % (Fig. 1A). Likewise, the ^{1}H NMR spectrum to confirm the GG-DVS functionalization with thiol peptides shows the characteristic peptide peaks (δ 7.1 and 7.5), and quantified by tryphtophan/tyrosine absorption. GG-DVS hydrogels were formed after 1-3 min of MMP-1 sensitive crosslinker addition to GG-DVS solution. Hydrogels' viscoelastic properties were tuned by varying the amount of GG-DVS polymer and crosslinker (Fig. 1B). Accordingly, hydrogels showed elastic modulus ranging from 150Pa to 5.4 KPa, and high elasticity, as indicated by the low loss damping ratio (< 0.01) (Fig. 1B). HUVECs were encapsulated in 1 wt% GG-DVS hydrogels with variable crosslinking densities (10 to 100%), as well as with different amounts of T1 or C16 peptides (400-800 μM). After 3 days, cells were viable (Fig. 1C – top) and spread (Fig. 1C – bottom) within the hydrogels. Cell adhesion was improved by increasing the amount of peptide, but not by the crosslinking density.

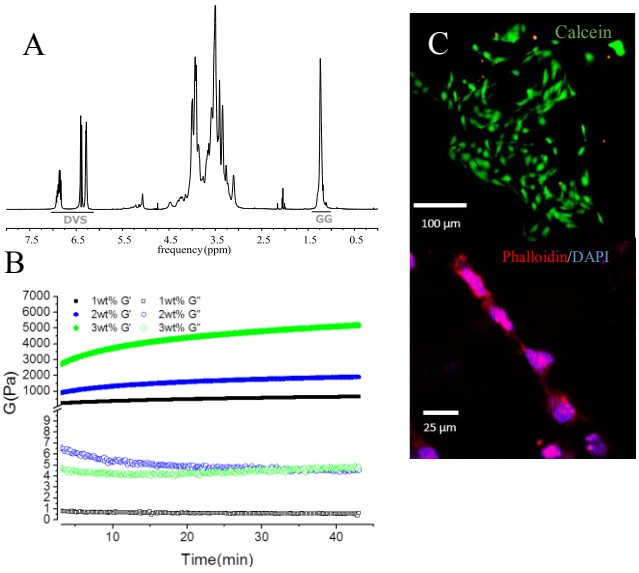

Fig. 1 - A) ^{1}H NMR spectrum of GG-DVS polymer. B) Viscoelastic properties of hydrogels with different crosslinking densities. C) Viability (top) and spreading (bottom) of HUVECs in hydrogels functionalized with C16 (top) and T1 (bottom) peptides.

Conclusions: Our modified GG-DVS hydrogels comprise physical and biological properties that turn them into promising materials for tissue regeneration purposes. These hydrogels have cell-driven degradation through the action of MMP-1, possess tunable viscoelastic properties and, essentially, have biological cues for endothelial cell survival, spreading and differentiation. Hence, these properties will potentially favor angiogenesis via the direct interaction with endothelial progenitor cells either seeded or derived from the host after implantation.

References:

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