Electronic Supplementary Information for

Peptide modification of purified gellan gum

C. J. Ferris,[† a,b] L. R. Stevens,[† a,b] K. J. Gilmore,[a] E. Mume,[c] I. Greguric,[c] D. M. Kirchmajer,[a,b] G. G. Wallace[a,*] and M. in het Panhuis[a,b] *

† Co-first authorship: C. J. Ferris and L. R. Stevens contributed equally to this work

[a]Intelligent Polymer Research Institute, ARC Centre of Excellence for Electromaterials Science, University of Wollongong, NSW 2522, Australia.

[b]Soft Materials Group, School of Chemistry, University of Wollongong, Wollongong, NSW 2522, Australia.

[c]Australian Nuclear Science and Technology Organisation, Lucas Heights, NSW 2234, Australia.

* Authors to whom correspondence should be addressed. E-mail: gwallace@uow.edu.au, panhuis@uow.edu.au,

S1. Peptide conjugation to commercial GG

Initial attempts to couple RGD peptides were conducted on solutions of as-received (commercial) GG. These reactions were conducted in 50 mM MES buffer at pH 6.5, and required reducing the GG concentration to 0.1% w/v to prevent premature gelation. Conjugation of radiolabelled peptide to as received GG, followed by removal of unbound peptide by dialysis yielded a conjugation efficiency <5%. Numerous adjustments to conjugation conditions (including temperature, peptide and EDC concentrations, reaction time and buffer) were made, however none resulted in significant improvements in yield. We hypothesised that these yields may have been limited by the presence of divalent cations, which are known to significantly impact the properties of GG, particularly gelation, by binding to the carboxylate residue on the GG backbone.

S2. Flow curves

As-received GG, purified NaGG and peptide modified RGD-GG were all observed to be shear thinning, typical of polymer dispersions (Figure S1). Both purification and modification of GG reduced the apparent viscosity of resulting solutions. We attribute these changes to a reduced concentration of divalent cations after purification, and a disruption of the carboxylate binding sites as a result of covalent modification with peptide.

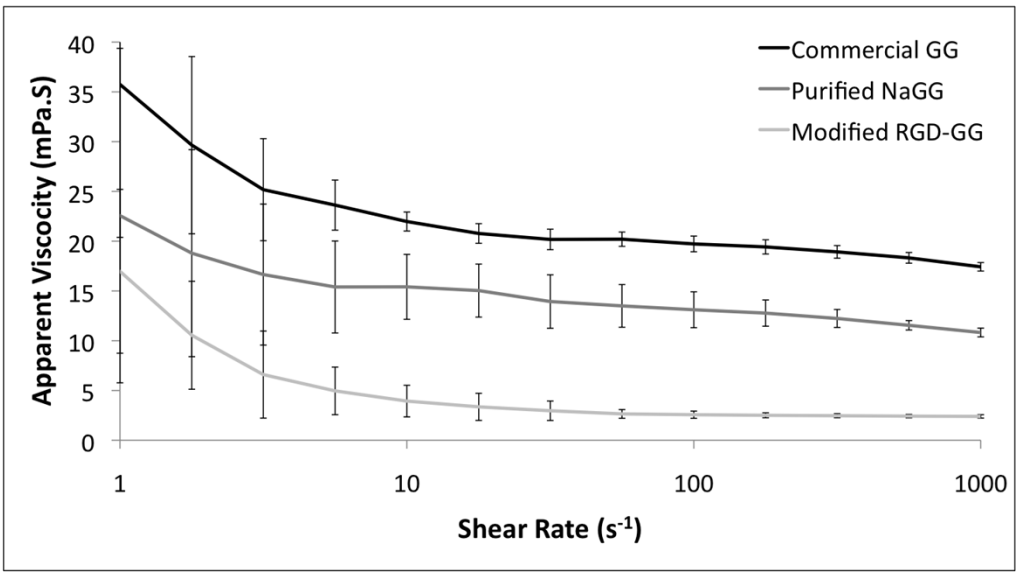

Figure S1: The shear dependant apparent viscosities of 1% (w/v) solutions of gellan gum (GG) in Milli-Q H_2O.

S3. Cell imaging

Cells under high serum (growth) conditions tended to form large cell aggregates even in RGD-GG hydrogels (Figure S2A, B). Under low serum (differentiation) conditions, single cells or those in small clusters differentiated when encapsulated in 0.15% (w/v) RGD-GG(1.0%) hydrogels (Figure S2B, C). Cells in the large clusters exhibited similar morphology to those cells maintained in growth media, attributed to be due the cell-cell interactions in the large clusters minimising the exposure of cells to the RGD peptide. PC12 cells encapsulated in 0.15% (w/v) NaGG hydrogels (Figure S2E, F) exhibited high rates of clustering and minimal differentiation even after 7 days in differentiation media.

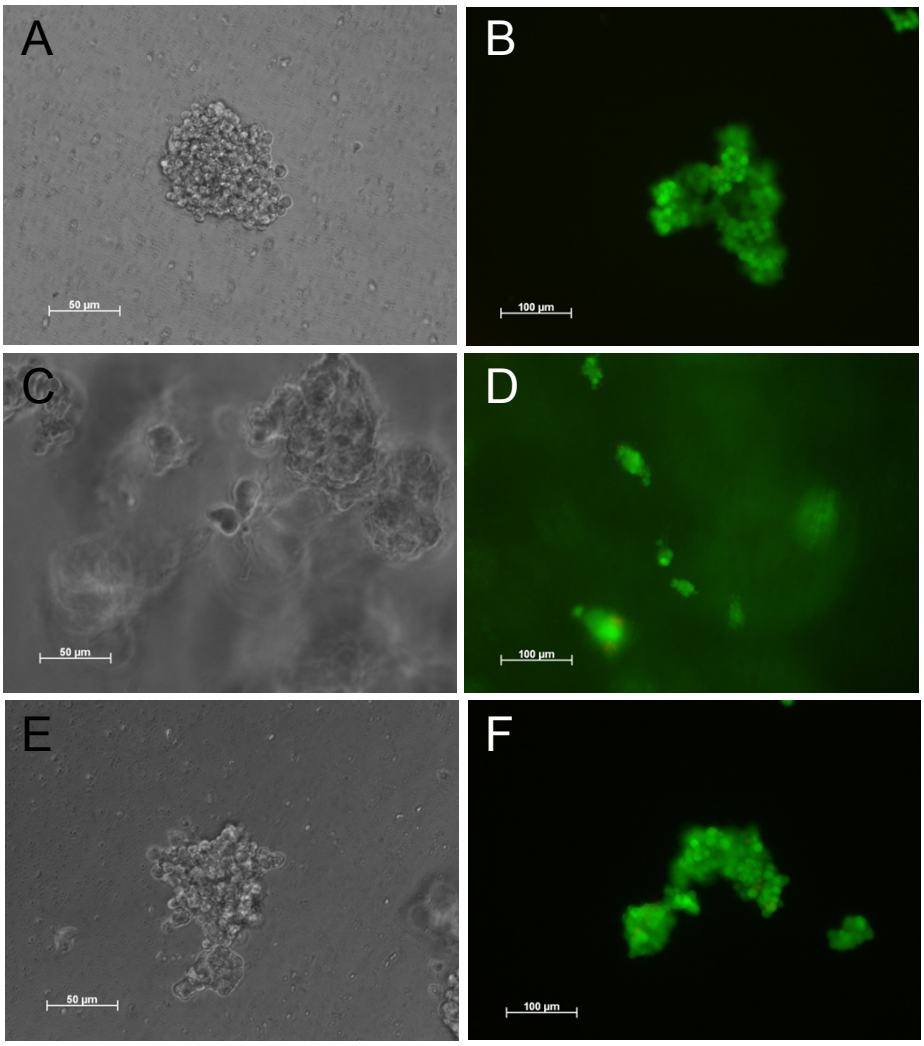

Figure S2: Unstained (A, C, E) and calcein stained (B, D, F) PC12 cells encapsulated in 0.15% (w/v) RGD-GG(1.0%) hydrogels for 6-7 days.