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Supporting Information

Processable Conducting Graphene/Chitosan Hydrogels for Tissue Engineering.

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Supporting Information

The effect of lactic acid on the composite preparation

Preliminary experiments were carried out to determine the optimum chitosan:lactic acid ratio for composite preparation. The following table demonstrates the effect of chitosan:lactic acid ratio on the mechanical properties of the films. Films with reduced lactic acid were found to display worse mechanical properties and based on the results 1:2 was found to be the optimum chitosan:lactic acid ratio for the process.

Table S1. Mechanical properties of chitosan composites at different chitosan/lactic acid ratios.

Chitosan/Lactic acid ratio	Tensile Strength [MPa]	Elongation at break [%]
1:0.5	18.2±0.9	3.5±0.7
1:1	17.99±1.1	4.4±0.5
1:1.5	17.35±1	8.9±0.4
1:2	21.1±1.5	12±0.3
1:3	20.1±1.2	11.2±0.5

Thermogravimetric analysis

Thermogravimetric analysis (TGA) can be used to assess the individual components of the chitosan/acid/graphene system. Reduced graphene alone shows little weight loss over the temperature range of 30-900 °C. In the TGA curves of the composites (Fig. S1) three regions of weight loss can be observed, 100-300 °C attributed to water and lactic acid (LA) loss, a major weight loss 300-375 °C and a slow weight loss to 900°C showing chitosan decomposition and a slow weight loss from 375-900 °C that is attributed to partial graphene decomposition. Although the decomposition temperature of lactic acid is as low as 100 °C,

the composites show good thermal stability up to 250 °C, indicating successful removal of the excess lactic acid. Composite with 3 wt% graphene content (CSG-3) shows slight increase in the decomposition temperature and slower degradation profile compared to that of chitosan/lactic acid materials (CSG-0).

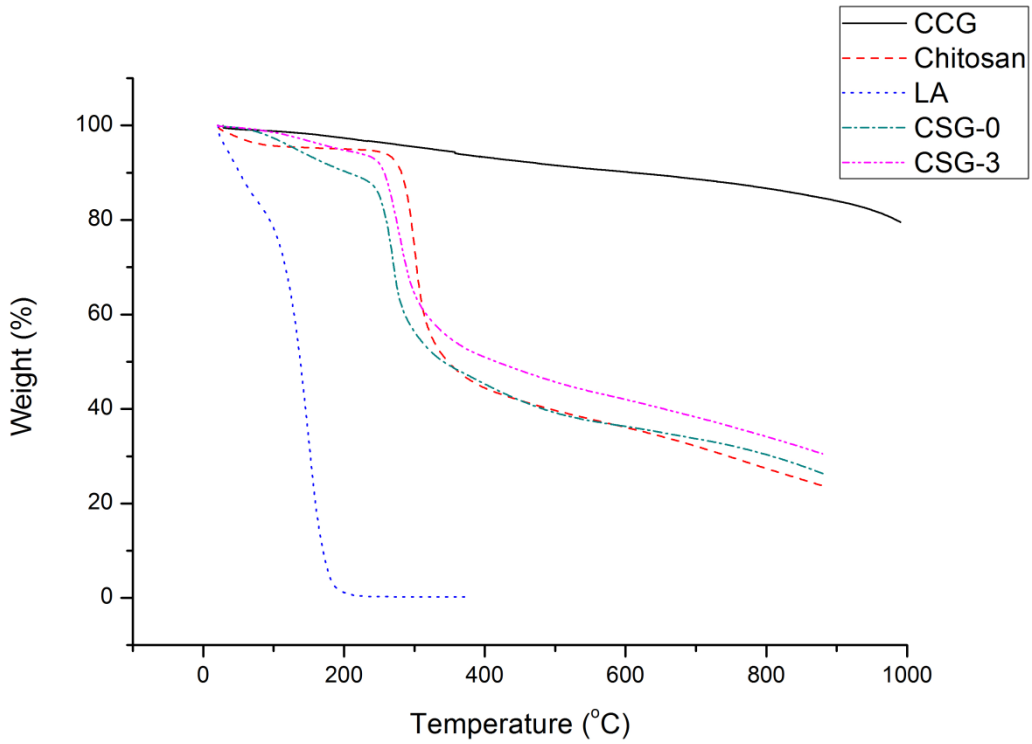


Fig. S1 Thermal gravimetry curves of CCG, pristine chitosan, lactic acid (LA) and graphene/chitosan composites CSG-0 and CSG-3 prepared with 0 and 3 wt% graphene respectively.

Freeze drying

Highly porous, three-dimensional materials with very low density could easily be produced using freeze drying.

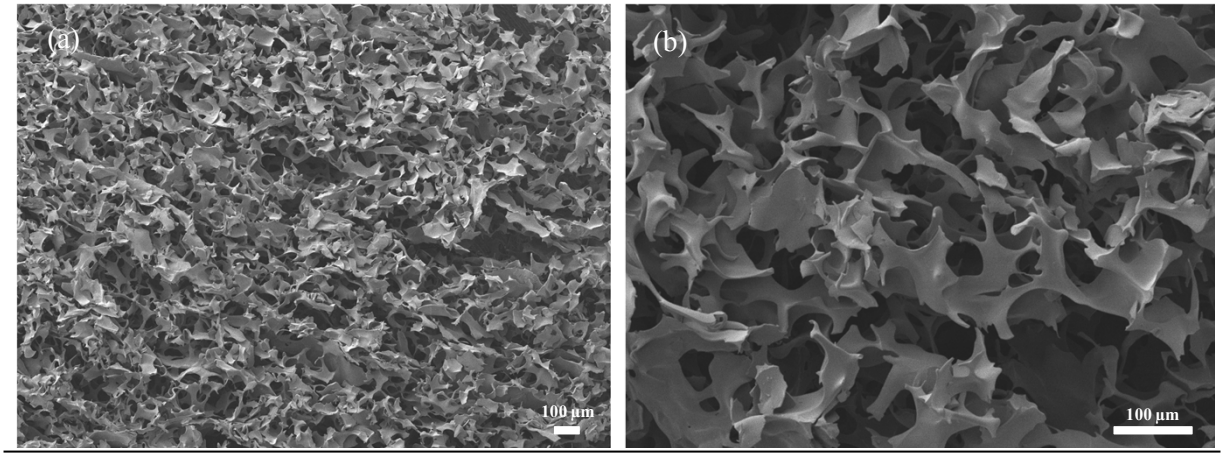
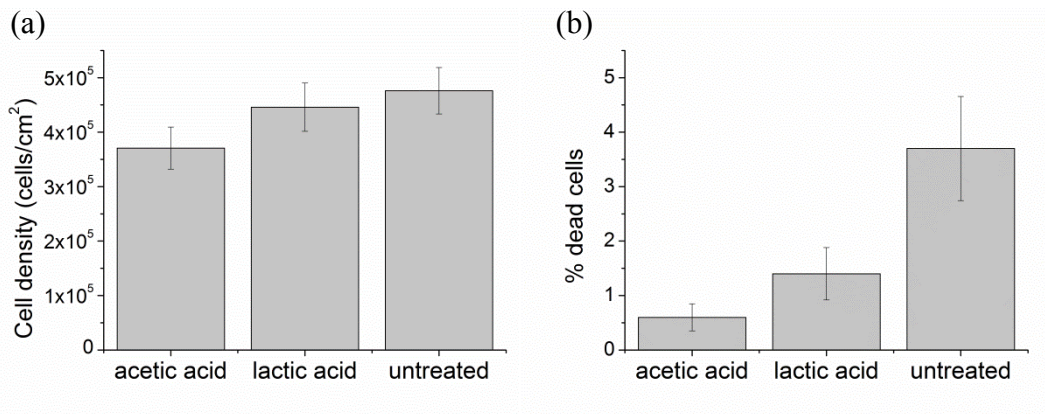


Fig. S2 Highly porous freeze dried graphene/chitosan composite.**Growth of mammalian cells in diluted CSG dispersions**

Prior to dilution in cell culture media, the pH of the dispersions were estimated at pH 4 using pH paper. The pH of cell media containing 5 % w/v of the dispersions was estimated at pH 7.0, which is within the range tolerated by fibroblast cells. The cells proliferated in the presence of the material, despite the slightly rounded appearance after 24 hours in the lactic acid solution. Fig. S3b shows the estimates for the cell density (measured by flow cytometry of trypsinised cells) after 5 days of exposure to the chitosan dispersions. All groups showed a 10-15 fold increase in cell number from the cell number seeded, with final densities of 450000 ± 40000 cells cm^{-2} for lactic acid stabilised CSG dispersions, 370000 ± 40000 cells cm^{-2} for acetic acid stabilised CSG dispersions, and 470000 ± 40000 cells cm^{-2} for the untreated controls (Fig. S3a). Higher cell number was determined to correlate with a slight increase in the percentage of dead cells in the cultures, as shown in S3b. The greatest proportion of dead cells was observed in the untreated group, suggesting that cell death was influenced more by high cell density than the CSG treatments. All cultures were observed to have less than 5 % dead cells, which our lab has found to be typical of L-929 cells grown at high cell density. Overall, the presence of the CSG dispersions in the growth media of L-929 cells had very little effect, slowing the proliferation to a minor degree, slightly reducing the proportion of dead cells, and allowing fibroblasts to adhere to tissue culture surfaces with typical fibroblast morphology.



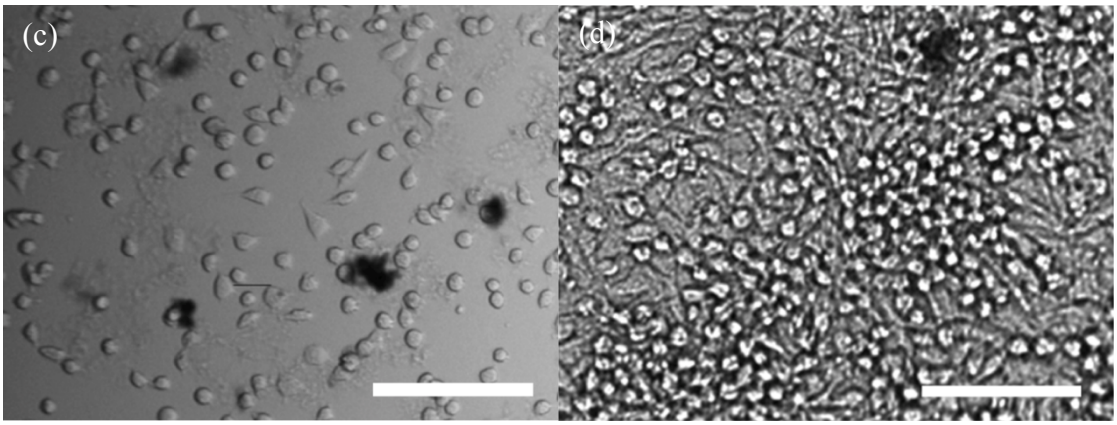


Fig. S3 Flow cytometric analysis of L-929 cells showing (a) cell density and (b) % dead cells after 5 days of exposure to CSG dispersions stabilised with either lactic acid or acetic acid. The percentage of dead cells was determined by propidium iodide staining of cells with compromised membrane activity. The measurements were performed in triplicate, and each error bar represents one standard deviation. Images (c) and (d) show bright field microscope images of L-929 cells grown with 5% w/v CSG-0.5 at 24 hours and 144 hours, respectively. Scale bars represent 100 μm.