Tuneable synthetic reduced graphene oxide scaffolds elicit high levels of three-dimensional glioblastoma interconnectivity in vitro

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S1 | Thermal Reduction



S1) Thermal conductivity over reduction of graphene oxide foam sample. Conductivity of the sample was taken via a transmission line method (inset) over increasing amount of time at 200 °C and 1 mBar pressure in the vacuum oven. Over 5 hours, conductivity increased from 10⁻⁴ S/m to 10⁻¹ S/m, reaching a plateau of the final value from around 3 hours of reduction. After this time, the sample also changed from a dull grey-brown to a more metallic lustrous silvery grey, consitent with expected changes in metallic properties.

S2 | Comparison with tissue measurements and existing scaffolds

S2) Table of mechanical properties. Tabulated data of mechanical properties for brain tissues of different species, and of other tissue scaffolds designed for brain cell research, as reported in literature. Also included is a much stiffer scaffold designed for use in vascular tissue engineering, for comparison.

*Where the modulus was not explicitly provided, these values were calculated by computing the ratio of stress to strain in the linear region of the data values as quoted in the literature.

Scaffold/Tissue	Young's modulus	Ref.
Brain tissue (Bovine – cerebrum)	(0.68 ± 0.20) kPa (Grey matter)	52
	(1.33 ± 0.63) kPa (White matter)	
Brain tissue (Porcine)	3.24 kPa (Indentation in vivo)	53
Brain Tissue (Rat)	(2.6 ± 0.3) kPa (Neonatal)	44
	(5.5 ± 0.7) kPa (Adolescent)	
	(5.7 ± 0.6 kPa) kPa (Adult)	
Brain tissue (Human)	8.67 kPa - 10.0 kPa (Grey matter)*	50
	10.8 kPa - 20.0 kPa (White matter)*	
Hyaluronic acid–collagen (HA–Coll) sponge, freeze	Variable with hyaluronic acid	45
dried and crosslinked with water-soluble	content, from (1.33 ± 0.20) kPa to	
carbodiimide - for brain tissue engineering	(6.31 ± 0.33) kPa	
Methacrylic anhydride hyaluronic acid (MA HA)	Variable with MA content and	44
photocrosslinked hydrogels - for investigating	photocrosslinking time, from (3.0 ±	
neural progenitor cell differentiation	0.4) kPa to (5.1 ± 0.4) kPa	
Range of 52 tuneable hydrogels made up of	Widely variable, from 100 Pa to 20	46
poly(ethylene glycol)/poly(L-lysine) – for	kPa, with different material	
investigating neural stem cell differentiation	properties yielding different cell	
	effects	
Nanofibrous, chemically crosslinked gelatine	Variable with gelatine content, with	47
scaffolds integrated with nerve growth factor - for	an 'optimal' value at 7.5% gelatine of	
brain tissue engineering	(1.2 ± 0.4) kPa	
Dual physically crosslinked sulfated	Variable over samples, from (0.90 ±	48
alginate-based polyurethane elastomers - for	0.20) MPa to (12.50 ± 1.95) MPa	
vascular tissue engineering		
This work (lyophilised reduced graphene oxide	Tuneable, in the region of 2 kPa to	-
foam scaffolds)	20 kPa	

S3 | Additional images for cell-scaffold coverage



S3) Additional images for cell-scaffold coverage and biocompatability. a) and b) SEM images showing widespread coverage of GS090, and HUVEC cells on the rGO scaffold respectively. These images have been fasle-coloured for clarity, and the raw image is supplied in Supplementary Figure S4 a. c) Optical micrograph of a large mass of glioblastoma cells as grown on our rGO scaffolds, with the foam having been broken up and mostly removed. The white arrow demarks cell mass, and the blue arrow highlights remaining scaffold material.

S4 | Scanning Electron Microscope images without false-colour



S4 i) Raw SEM images for Figure 4 c, and Supplementary Figure S3 a, b. a) b) and c) are SEM images as in Figure 4 c of the manuscript, and S3 a, b of the Supplementary Information respectively; without the addition of false-colouring.



S4 *ii*) **Raw SEM images for Figure 5** a) b) and c) are SEM images as in Figure 5 of the manuscript, without the addition of false-colouring. d) is as b), but with the addition of an outline of an individual cell body. Due to the presence of many cells, as well as extracellular matrix, it is not easy to distinguish individual cell bodies, locations of nuclei and extended parts of the cell bodies are clear.



S4 iii) Raw SEM images for Figure 6 SEM images as in Figure 6 of the manuscript, without the addition of false-colouring.