A photolabile hydrogel for guided three-dimensional cell growth and migration

YING LUO[1,2] AND MOLLY S. SHOICHET[1,2,3]

[1]Department of Chemical Engineering and Applied Chemistry, University of Toronto, 200 College Street, Toronto, Ontario M5S 3E5, Canada
[2]Institute of Biomaterials and Biomedical Engineering, University of Toronto, 4 Taddle Creek Road, Toronto, Ontario M5S 3G9, Canada
[3]Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario M5S 3H6, Canada
*e-mail: molly@chem-eng.utoronto.ca

Published online: 21 March 2004; doi:10.1038/nmat1092

Tissue engineering aims to replace, repair or regenerate tissue/organ function, by delivering signalling molecules and cells on a three-dimensional (3D) biomaterials scaffold that supports cell infiltration and tissue organization[1,2]. To control cell behaviour and ultimately induce structural and functional tissue formation on surfaces, planar substrates have been patterned with adhesion signals that mimic the spatial cues to guide cell attachment and function[3–5]. The objective of this study is to create biochemical channels in 3D hydrogel matrices for guided axonal growth. An agarose hydrogel modified with a cysteine compound containing a sulphydryl protecting group provides a photolabile substrate that can be patterned with biochemical cues. In this transparent hydrogel we immobilized the adhesive fibronectin peptide fragment, glycine–arginine–glycine–aspartic acid–serine (GRGDS), in selected volumes of the matrix using a focused laser. We verified *in vitro* the guidance effects of GRGDS oligopeptide-modified channels on the 3D cell migration and neurite outgrowth. This method for immobilizing biomolecules in 3D matrices can generally be applied to any optically clear hydrogel, offering a solution to construct scaffolds with programmed spatial features for tissue engineering applications.

Hydrogels have been widely studied as tissue scaffolds because they are biocompatible and non-adhesive to cells, allowing cell adhesion to be programmed in[6–8]. Current microfabrication methods for 3D hydrogel matrices with controlled intrinsic structure mainly include photolithographic patterning[9–11], microfluidic patterning[12], electrochemical deposition[13] and 3D printing[14]. Notably, although these layering techniques can conveniently shape the hydrogel on X–Y planes, they have limited control over both the coherence of the layers along the *z* direction and the local chemistry. Combining photolabile hydrogel matrices with focused light provides the possibility of eliminating the layering process and directly modifying the local physical or chemical properties in 3D. This results in a promising (and perhaps facile) way to fabricate novel tissue constructs[15,16], as is described herein to control cell behaviour by controlling the local chemical properties of gels.

Reconstituting adhesive biomolecules into biomaterials is of great importance to understanding cell–substrate interactions that can be translated to tissue-regeneration designs. Using 2D lithographic techniques, adhesive biomolecules can be localized in arbitrary shapes and sizes[17,18]. For example, patterning narrow strips of the extracellular matrix (ECM) adhesion protein, laminin, on non-cell-adhesive 2D substrates elicited oriented neurite growth through integrin-receptor mediated processes[19,20]. Similar results were obtained using oligopeptides derived from laminin–integrin active sites, such as YIGSR, IKVAV and RGD (the letters are the standard amino acid symbols), among others[4,21]. The fidelity of neurons to the peptide regions was attributed to both cell-adhesive and non-adhesive regions that mimic the *in vivo* attractive and repulsive haptotactic (that is, contact-mediated) cues, respectively, present during development[22]. This inspired us to investigate ways to create isolated biomolecular channels in cell invasive, yet non-adhesive, matrices, thereby extending 2D to 3D patterning, and better approximating functional ECM analogues.

In this study, we used a soft thermosensitive agarose hydrogel as a model material because it is optically transparent, facilitating bulk modification using light. Dilute low-gelling-temperature agarose hydrogel is permissive to 3D neurite outgrowth and has been used as spinal cord surrogates[23,24]. A general scheme for producing oligopeptide channels in the agarose gel matrices is illustrated in Fig. 1. We hypothesized that the spatial resolution between adhesive and non-adhesive volumes in the agarose matrix would promote guidance within the peptide-modified channels.

To prepare a photolabile matrix for 3D photo-fabrication, the photosensitive S-2-nitrobenzyl-cysteine (S-NBC), which can be efficiently cleaved in aqueous environments to release free nucleophile on irradiation by ultraviolet (UV) light[25,26], was first bound to the dissolved agarose polymer through a 1,1′-carbonyldiimidazole (CDI) activation process. There was a direct correlation between the amount of S-NBC-modified agarose and the amount of CDI activation. Moderately modified agarose (that is, up to 5 wt%) remained thermally reversible, easily dissolving at high temperatures, and forming optically transparent hydrogels on cooling. S-NBC-modified agarose had a UV absorption peak at 266 nm, which was attributed to the 2-nitrobenzyl groups in the gel (Supplementary Information, S1). Photo-irradiating S-NBC-modified agarose hydrogel matrices (using a UV lamp) resulted in free sulphydryl groups, which were then available to react with sulphydryl-reactive biomolecules. The S-NBC-modified hydrogel matrices were proven to be safe cell substrates, showing no toxicity or

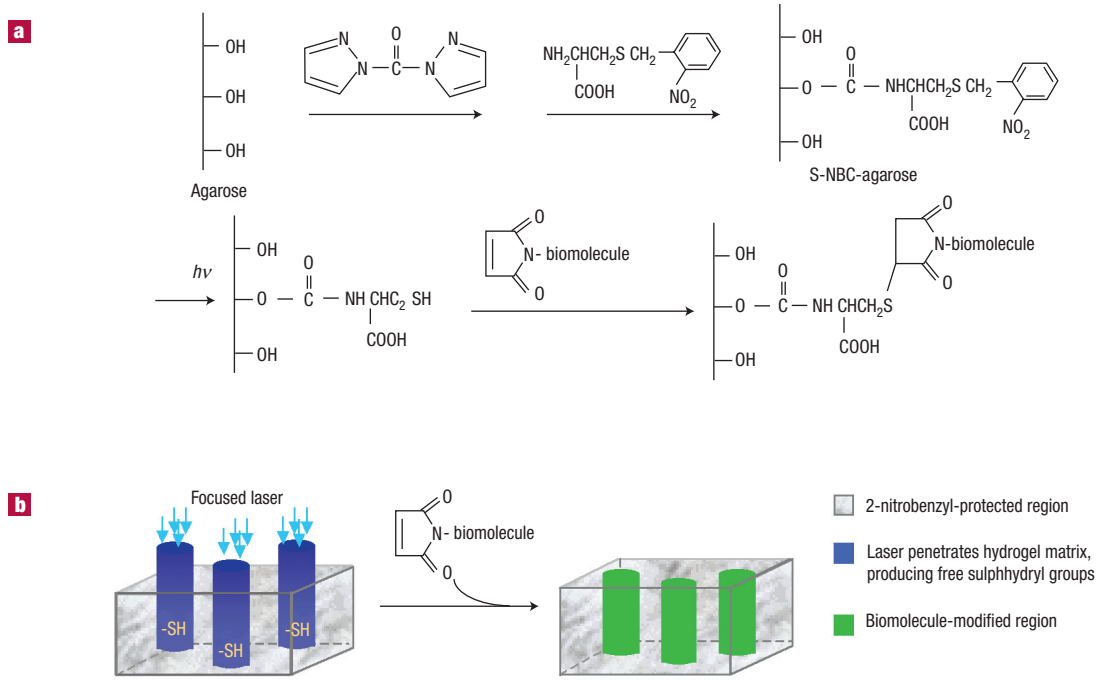

Figure 1 The general strategy used to create adhesive biochemical channels in agarose hydrogel matrices relies on modifying agarose with photolabile groups, focused laser light sources and biomolecule coupling. Specifically, **a**, bulk agarose is first activated with CDI followed by reaction with 2-nitrobenzyl-protected cysteine. On exposure to UV light, the 2-nitrobnezyl group is cleaved leaving free sulphydryl groups that react with maleimido-terminated biomolecules. **b**, The strategy to create biomolecular channels in agarose hydrogel matrices uses the chemical modification strategy in (**a**) and a focused laser, resulting in alternating volumes of cell-adhesive (peptide) channels separated by non-adhesive (agarose) volumes.

inhibitory effects on cell adhesion or neurite growth (Supplementary Information, S2).

To fabricate the free sulphydryl channels in the gel, the S-NBC-modified agarose gel was irradiated with a He–Cd 325 nm laser (4.0 mW). A 0.3-mm-diameter laser beam, focused by a convex lens with a focal length of 10 cm, was used to irradiate a 1.5-mm-thick, 0.5 wt% agarose gel sample for 1 s. Free sulphydryl functional channels were created on irradiation, providing the template for biomolecule immobilization.

To demonstrate the universality of this technique, we coupled agarose with the GRGDS sequence, an oligopeptide capable of promoting integrin-mediated cell adhesion[27,28]. A maleimide-terminated GRGDS, (*N*-α-(3-maleimidopropionyl)-*N*-ε-fluorescein) lysine-GRGDS, was prepared by solid-state peptide synthesis, with the fluorescein added simply to facilitate the visualization of the oligopeptide. The distribution of the peptide in the gel after laser fabrication was analysed by confocal microscopy and the peptide channels were observed penetrating the 1.5-mm-thick sample. Figure 2a shows a typical XY cross-section micrograph of the oligopeptide distribution at a given depth within the agarose matrix: GRGDS peptides were conjugated to the gel in isolated circular channels defined by the shape of the laser beam. These circular peptide domains were observed continuously at all depths in the gel. The diameter of the channels was between 150 and 170 μm over a 1 mm depth, corresponding to the focal spot size of the focused laser beam of 160 μm. The XY cross-section micrographs at all levels were reconstructed by confocal microscopy to provide the 3D stereo channel image (Fig. 2b).

The fluorescence intensity profile of the cross-sections of two oligopeptide channels in the bulk hydrogel was analysed (Fig. 2c). The intensity increased sharply at the irradiated regions, demonstrating that homogenous agarose hydrogels cause minimal lateral scattering during irradiation and such gel matrices are suitable for 3D photo-fabrication.

The transverse diameter of the biochemical channels is determined by the focal spot size of the focused laser, but their longitudinal dimensions are affected by two factors: (i) the depth of focus of the focused beam (usually defined as the distance over which the focal spot size changes ±5%)[29]; and (ii) the transmittance of light through the hydrogel material, which depends on the concentration of 2-nitrobenzyl photolabile moieties and not on agarose, which absorbs only 1.2% of the incident 325-nm light across a 1.5-mm gel. The theoretical depth of focus using the convex lens with a focal length of 10 cm is 7 cm (ref. 29; see Supplementary Information, S3). Thus the longitudinal dimensions of our channels are mainly limited by the concentration of the photolabile moiety, S-NBC. The variation in longitudinal fluorescent intensity in the channel is shown in Fig. 2d, where the relative fluorescence intensity decreases with depth of fluorescein-labelled GRGDS as a result of the S-NBC absorption of light travelling through the 3D hydrogel matrix. The transmittance of light through S-NBC-modified agarose hydrogels decreases with gel thickness and S-NBC concentration (Fig. 3). The light intensity at different depths can be calculated using the Beer–Lambert law with the corresponding extinction coefficient for the 2-nitrobenzyl moiety of 1,409 L mol^{-1} cm^{-1}. Generally, the lower the concentration of biomolecular ligands (or photolabile moiety) required, the thicker the photofabricated gel.

We found that irradiating agarose hydrogel matrices with the laser beam for more than 1 s did not further increase the immobilization of peptides at the gel surface region, which received the highest dose of energy longitudinally[30]. We approximated the GRGDS concentration at

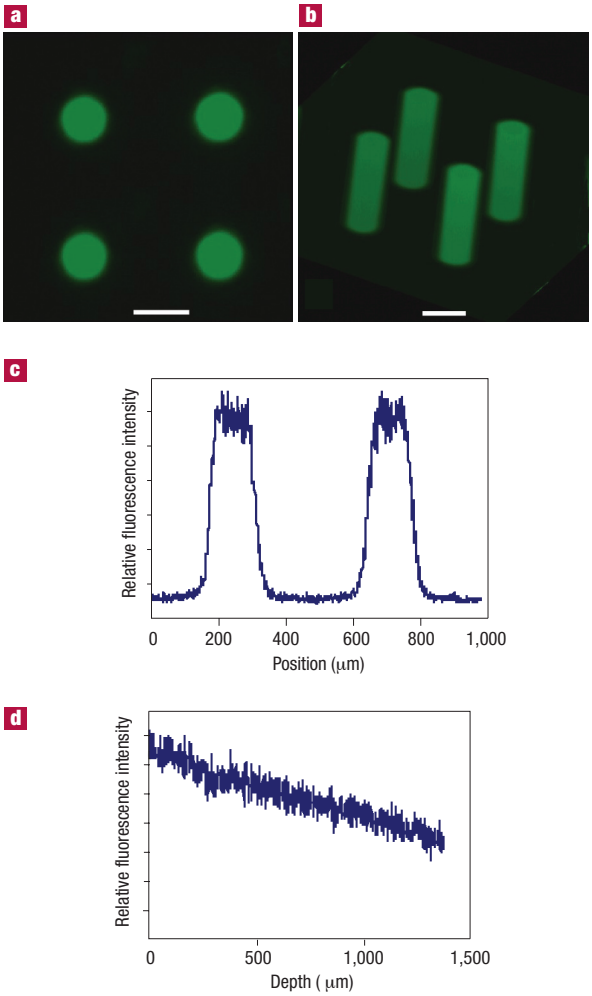

Figure 2 Biochemical channels synthesized in agarose hydrogels were characterized with a fluorescein-tagged GRGDS peptide. a, A representative XY cross-section image of green fluorescently labelled oligopeptide channels (scale bar: 200 μm); **b**, A representative image of the green fluorescently labelled oligopeptide channels constructed from a series of XY cross-sectional micrographs over a 0.5 mm depth (scale bar: 200 μm); **c**, The relative fluorescence intensity profile of a line pass through the centre of the cross-sections of two oligopeptide channels shows intensity contrast between the peptide modified and non-modified regions; **d**, The longitudinal fluorescence intensity profile along the central axis of the channel shows a decrease in fluorescent intensity with depth, indicating a concentration gradient of oligopeptide.

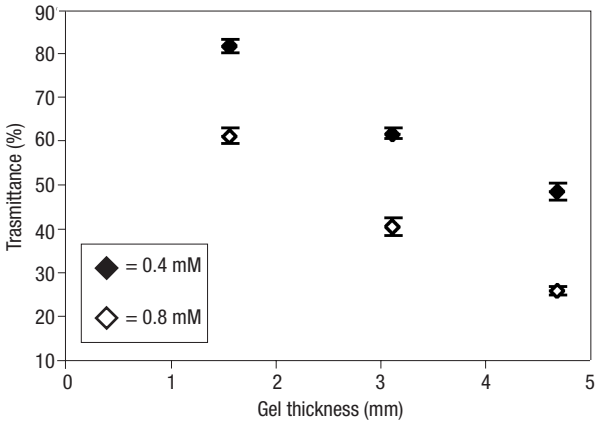

Figure 3 The transmittance of laser light is affected by both the thicknesses of the S-NBC-modified agarose hydrogel matrices and the concentration of S-NBC of 0.4 mM and 0.8 mM (mean ± standard deviation, $n = 5$). The data indicate that the concentration of the S-NBC in the matrix affects the depth (or length) of modified channels. In those channels used for guided cell growth (0.4 mM S-NBC, 1.5 mm), the concentration of peptide on the bottom surface may decrease to 80% of what it was on the top (laser exposed) surface.

the top surface of the channel at 41.5 ± 2.2 μM (0.5 wt% gel, 0.4 mM S-NBC) (Supplementary Information, S4). By comparing the local irradiation intensity on the surface to that within the channel (Fig. 3) and assuming a linear relationship between the irradiation intensity and photochemical yield[31], we estimated GRGDS concentration to vary from 41.5 ± 2.2 μM at the gel surface to 33.0 ± 2.0 μM at 1.5 mm depth. This chemical gradient may be useful for guided regeneration[3]; alternatively, it could be minimized by irradiating samples from both top and bottom.

To determine how neural cells responded to the GRGDS adhesion domains in the hydrogel, we used agarose matrices (0.5 wt%, 0.4 mM S-NBC), which have been shown to permit neurite growth[21]. Dissociated cells from explants of E9 chick dorsal root ganglia were plated on top (that is, the same side that had been irradiated) of the hydrogel matrices containing biochemical channels. Owing to generally poor adhesion properties of the agarose surfaces, cells formed clusters on top of the oligopeptide channels. Intriguingly (from day three onwards after cell plating), cell clusters that aggregated on oligopeptide channels were observed to extend thick processes into the biochemical channels; the migration of cells and elongation of cell processes were often found to turn at the edge of the adhesive biochemical domain, indicating the guidance effect of the channel chemistry. Representative images of cell behaviour inside the hydrogel matrices show that the GRGDS channel promoted neurite extension and cell migration (Fig. 4): Fig. 4a (optical image) and 4b (fluorescent image of green fluorescein-tagged peptide channel and red F-actin rhodamine–phalloidin cytoskeletal staining) show a single process emerging from a cell cluster. In Fig. 4c, the blue DAPI nuclear staining shows that multiple cells migrated into the GRGDS channel. At day six of cell culture, a majority of the channels (76 ± 16%, mean ± standard deviation, $n = 50$ channels) were observed to contain neurites and cellular assemblies, having an average migration distance of 633 ± 181 μm (mean ± standard deviation, $n = 50$ channels).

Cell invasion into non-degradable physically crosslinked matrices are probably affected by both the mechanical and biochemical properties of the hydrogel material. (Supplementary Information, S5). By dynamic rheological analysis, we found that there was a small difference in the local mechanical properties of modified (storage modulus, $G' = 17.0 \pm 0.7$ Pa, $n = 4$) and un-modified ($G' = 14.8 \pm 0.6$ Pa) agarose due to laser-activated chemistry. Despite a slightly higher G' for the modified agarose, this difference did not affect cell penetration, confirming that the chemical differences between the channels and the surrounding volumes are responsible for the observed cellular response.

To understand further the interaction between the GRGDS oligopeptide channels and cells, neural cells were cultured on matrices modified with scrambled oligopeptide channels containing the inactive sequence GRDGS. Neither neurite outgrowth nor cell migration was observed inside the scrambled oligopeptide channels. Therefore, the guided axonal growth and cell migration observed in

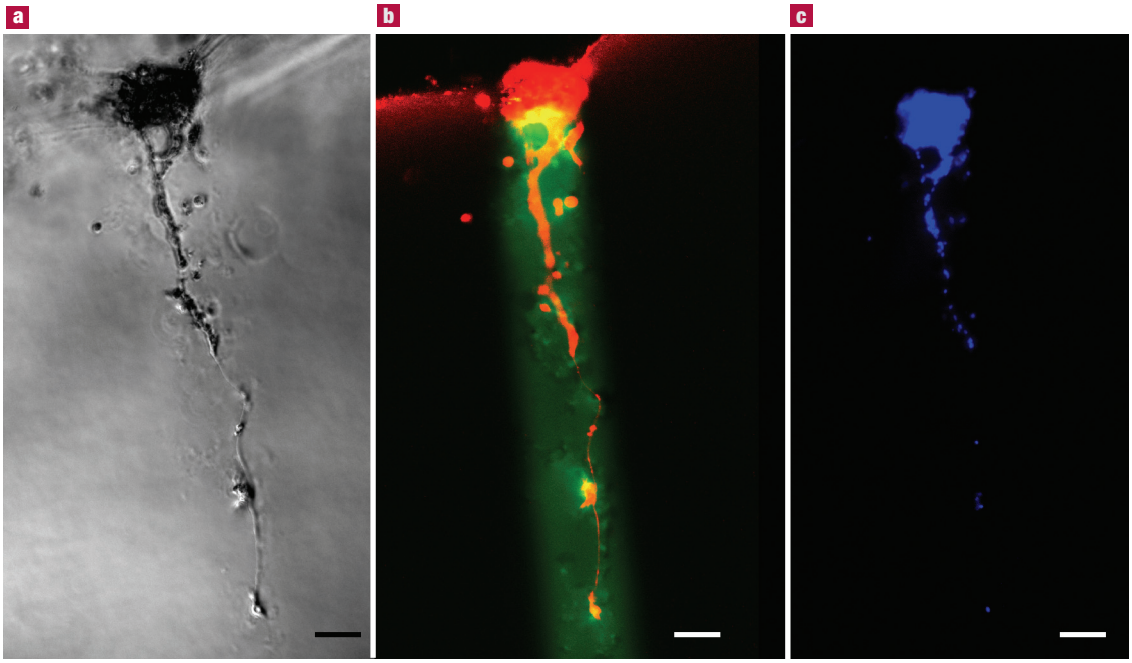

Figure 4 Primary rat dorsal root ganglia cells were plated on 3D patterned GRGDS oligopeptide-modified, 0.5 wt% agarose gels. Three days after plating, DRG cells grew within GRGDS-oligopeptide-modified agarose channels only, and not in surrounding volumes. A cell cluster on top of a GRGDS channel shows cell migration into the channel and extension of a process into the oligopeptide-modified channel as viewed by: **a**, optical microscopy; **b**, confocal fluorescent microscopy, where the channel is green (due to a fluorescein-labelled oligopeptide) and the cells are red (due to the cytoskeletal F-actin rhodamine–phalloidin stain); and **c**, fluorescent microscopy, where a nuclear DAPI stain (blue) confirms cell migration into the oligopeptide-modified channel. (Scale bars: 100 μm)

GRGDS channels probably resulted from the activation of integrin receptors in the cell membrane and not from local mechanical property variations or non-specific depositions of proteins in the oligopeptide-modified regions. The unreacted S-NBC-modified agarose (found between the biochemical channels) is non-adhesive and non-cytotoxic (Supplementary Information, S2).

Neurons are able to respond to a wide range of adhesion ligand concentrations on substrates by regulating the expression of integrin receptors[32]. We observed a similar cellular response from DRG cells, with respect to the neurite number and length, when the GRGDS concentration was varied from 13 μM to 70 μM on agarose surfaces[30]. This suggests that the ligand concentration variation in chemical channels (of 41.5 μM to 33 μM) observed within the 1.5-mm-thick channels probably had a minimal (if any) effect on cell behaviour. The axonal guidance that we observed in our GRGDS channels may result from either direct interaction between neurons and GRGDS, or the integrated crosstalk of multiple cell types found in the DRG explants. Additional experiments are necessary to identify the cellular components inside the GRGDS channels to reveal the mechanisms leading to the oriented cellular assemblies.

In summary, we demonstrate a paradigm to immobilize biomolecules in selected volumes in a 3D hydrogel matrix using laser fabrication techniques and photochemistry. The cellular guidance observed is achieved by chemical (and not physical) channels. Provided that the hydrogel materials are cell invasive, matrices with heterogeneous biochemical domains are novel platforms to facilitate the elucidation of fundamental cell–substrate interactions. Adhesion channels elicited oriented axonal growth in hydrogels, suggesting that the incorporation of the biochemical channels into biodegradable hydrogel matrices may find clinical applications for guided nerve regeneration or be extended to other tissues.

METHODS

All chemicals were purchased from Sigma and used as received unless otherwise specified.

MODIFICATION OF ULTRA-LOW-GELLING-TEMPERATURE (ULGT) AGAROSE WITH S-NBC

S-NBC was synthesized by reacting L-cysteine and 2-nitrobenzyl bromide (1:1 molar ratio) in NaOH solution at room temperature for 2 h. The pure product was obtained by recrystallization in water. To conjugate S-NBC to the agarose polymer, the ULGT agarose polymer was first dissolved in dimethyl sulphoxide (DMSO). Under an inert nitrogen environment, CDI was added to partially activate the hydroxyl groups in agarose polymer. After 1 h activation, a solution of S-NBC in DMSO was added. The mixture was kept at room temperature overnight before it was extensively dialysed against water to remove the unreacted S-NBC. The substitution level of S-NBC in the resulting agarose was determined by measuring the absorbance at 266 nm using an ultraviolet spectrometer (Ultraspec 4000, Biopharmacia).

CREATING PHYSIOCHEMICAL CHANNELS IN ULGT AGAROSE HYDROGEL MATRIX

(*N*-α-(3-maleimidopropionyl)-*N*-ε-fluorescein)-lysine-GRGDS was prepared by solid-state peptide synthesis based on Fmoc chemistry. *N*-ε-fluorescein-lysine-GRGDS was first synthesised without cleaving the side-chain protecting groups on a peptide synthesizer (Pioneer, BioApplied Systems). 3-Maleimidopropionic acid was activated using dicyclohexyl carbodiimide in dichloromethane and reacted with the amine terminal of the peptide on the resin. The maleimide-activated peptide was de-protected and cleaved from the resin using 95% aqueous trifluoroacetic acid and lyophilised. Scrambled maleimide-activated oligopeptide, (*N*-α-(3-maleimidopropionyl)-*N*-ε-fluorescein)-lysine-GRDGS, was synthesised using the same method described to synthesize activated GRGDS.

Irradiation of the hydrogel was performed using a 325 nm He-Cd laser (Omnichrome 3074R-S-A03, Melles Griot). The laser was focused by a convex fused-silica lens. 0.5 wt% S-NBC agarose solution prepared in phosphate buffer (pH 7.4) was refrigerated at 4 °C for 3 h to form a hydrogel sample of ~1–5 mm thick. The gel was placed at the focal spot of the focused laser for irradiation and moved using an XY stage. To obtain the transmittance of light through the hydrogel matrices, the laser-beam energy was measured before and after it penetrated through the sample volume. The irradiated sample was immersed and shaken in phosphate buffer containing maleimide-activated GRGDS oligopeptide for 8 h. Unreacted GRGDS peptide was removed by washing the gel for 2 days. Peptide distribution in the hydrogel matrix was imaged using a laser scanning confocal microscope (FV300, Olympus).

RHEOLOGICAL CHARACTERIZATION

To investigate how the photo-modification affected the mechanical stiffness of the hydrogel, dynamic rheological tests were performed to measure the shear modulus of the modified and unmodified agarose hydrogels. S-NBC agarose solutions (0.5 wt%), irradiated (1 min, 0.4 W, X-cite, EFOS) or non-irradiated, were mixed with maleimide-GRGDS at 1 mg per ml for 1 h at room temperature. To measure the complex shear moduli of the modified and un-modified agarose hydrogel, 0.5 ml of solution was loaded between the cone and plate geometry on a dynamic rheometer (AR 2000, TA Instruments) and cooled down at

4 °C for 90 min to solidify. The temperature was raised to 37 °C (to simulate the cell-culture temperature) and equilibrated for 5 min. The frequency sweep was performed from 0.1 to 100 rad s^{-1} with a 1% shear strain applied to each sample.

NEURAL CELL RESPONSE TO BIOCHEMICAL CHANNELS OF GRGDS

To study the guidance effect of GRGDS oligopeptide channels on axonal growth, the cells dissociated from the dorsal root ganglia explanted from E9 chicks, were plated on top of the hydrogel matrices containing either active GRGDS or scrambled GRDGS oligopeptide channels. After culturing the cells in the media containing 10% horse serum and 50 ng per ml nerve growth factor for 6 days (37 °C, 5% CO_2), the samples were imaged on their side by phase contrast/fluorescence microscopy (Zeiss LM410) and confocal microscopy (FV300, Olympus) so as to observe the cell behaviour inside the hydrogel matrices. Rhodamine–phalloidin (red) and DAPI (blue) fluorescent markers (Molecular Probes) were used to identify cellular skeletons and nuclei, respectively, in the gel using standard methods.

Received 2 July 2003; accepted 3 February 2004; published 21 March 2004.

References

1. Griffith, L. G. & Naughton, G. Tissue engineering—current challenges and expanding opportunities. *Science* **295,** 1009–1014 (2002).
2. Sipe, J. D. Tissue engineering and reparative medicine. *Ann. NY Acad. Sci.* **961,** 1–9 (2002).
3. Dertinger, S. K., Jiang, X., Li, Z., Murthy, V. N. & Whitesides, G. M. Gradients of substrate-bound laminin orient axonal specification of neurons. *Proc. Natl Acad. Sci. USA* **99,** 12542–12547 (2002).
4. Saneinejad, S. & Shoichet, M. S. Patterned poly(chlorotrifluoroethylene) guides primary nerve cell adhesion and neurite outgrowth. *J. Biomed. Mater. Res.* **50,** 465–474 (2000).
5. Herbert, C. B. *et al.* Micropatterning gradients and controlling surface densities of photoactivatable biomolecules on self-assembled monolayers of oligo(ethylene glycol) alkanethiolates. *Chem. Biol.* **4,** 731–737 (1997).
6. Halstenberg, S., Panitch, A., Rizzi, S., Hall, H. & Hubbell, J. A. Biologically engineered protein-graft-poly(ethylene glycol) hydrogels: a cell adhesive and plasmin-degradable biosynthetic material for tissue repair. *Biomacromolecules* **3,** 710–723 (2002).
7. Behravesh, E., Jo, S., Zygourakis, K. & Mikos, A. G. Synthesis of in situ cross-linkable macroporous biodegradable poly(propylene fumarate-co-ethylene glycol) hydrogels. *Biomacromolecules* **3,** 374–381 (2002).
8. Alsberg, E., Anderson, K. W., Albeiruti, A., Rowley, J. A. & Mooney, D. J. Engineering growing tissues. *Proc. Natl Acad. Sci. USA* **99,** 12025–12030 (2002).
9. Liu, V. A. & Bhatia, S. N. Three-dimensional photopatterning of hydrogels containing living cells. *Biomed. Microdevices* **4,** 257–266 (2002).
10. Ward, J. H., Bashir, R. & Peppas, N. A. Micropatterning of biomedical polymer surfaces by novel UV polymerization techniques. *J. Biomed. Mater. Res.* **56,** 351–360 (2001).
11. Yu, T. & Ober, C. K. Methods for the topographical patterning and patterned surface modification of hydrogels based on hydroxyethyl methacrylate. *Biomacromolecules* **4,** 1126–1131 (2003).
12. Tan, W. & Desai, T. A. Microfluidic patterning of cellular biopolymer matrices for biomimetic 3D structures. *Biomed. Microdevices* **5,** 235–244 (2003).
13. Fernandes, R. *et al.* Electrochemically induced deposition of a polysaccharide hydrogel onto a patterned surface. *Langmuir* **19,** 4058–4062 (2003).
14. Mironov, V., Boland, T., Trusk, T., Forgacs, G. & Markwald, R. R. Organ printing: computer-aided jet-based 3D tissue engineering. *Trends Biotechnol.* **21,** 157–161 (2003).
15. Borkenhagen, M., Clemence, J. F., Sigrist, H. & Aebischer, P. Three-dimensional extracellular matrix engineering in the nervous system. *J. Biomed. Mater. Res.* **40,** 392–400 (1998).
16. Luo, N., Metters, A. T., Hutchison, J. B., Bowman, C. N. & Anseth, K. S. A methacrylated photoiniferter as a chemical basis for microlithography: Micropatterning based on photografting polymerization. *Macromolecules* **36,** 6739–6745 (2003).
17. Blawas, A. S. & Reichert, W. M. Protein patterning. *Biomaterials* **19,** 595–609 (1998).
18. Chen, C. S., Mrksich, M., Huang, S., Whitesides, G. M. & Ingber, D. E. Micropatterned surfaces for control of cell shape, position, and function. *Biotechnol. Prog.* **14,** 356–363 (1998).
19. Clark, P., Britland, S. & Connolly, P. Growth cone guidance and neuron morphology on micropatterned laminin surfaces. *J. Cell Sci.* **105,** 203–212 (1993).
20. Hammarback, J. A., Palm, S. L., Furcht, L. T. & Letourneau, P. C. Guidance of neurite outgrowth by pathways of substratum-adsorbed laminin. *J. Neurosci. Res.* **13,** 213–220 (1985).
21. Ranieri, J. P. *et al.* Spatial control of neuronal cell attachment and differentiation on covalently patterned laminin oligopeptide substrates. *Intl J. Dev. Neurosci.* **12,** 725–735 (1994).
22. Tessier-Lavigne, M. & Goodman, C. S. The molecular biology of axon guidance. *Science* **274,** 1123–1133 (1996).
23. Bellamkonda, R., Ranieri, J. P. & Aebischer, P. Laminin oligopeptide derivatized agarose gels allow three-dimensional neurite extension in vitro. *J. Neurosci. Res.* **41,** 501–509 (1995).
24. Gillies, G. T. *et al.* A spinal cord surrogate with nanoscale porosity for in vitro simulations of restorative neurosurgical techniques. *Nanotechnology* **13,** 587–591 (2002).
25. Adams, S. R. & Tsien, R. Y. Controlling cell chemistry with caged compounds. *Annu. Rev. Physiol.* **55,** 755–784 (1993).
26. McCray, J. A. & Trentham, D. R. Properties and uses of photoreactive caged compounds. *Annu. Rev. Biophys. Biophys. Chem.* **18,** 239–270 (1989).
27. Yamada, Y. & Kleinman, H. K. Functional domains of cell adhesion molecules. *Curr. Opin. Cell Biol.* **4,** 819–823 (1992).
28. Ruoslahti, E. RGD and other recognition sequences for integrins. *Annu. Rev. Cell. Dev. Biol.* **12,** 697–715 (1996).
29. Siegman, A. E. *Lasers* (University Science Books, Mill Valley, California, 1986).
30. Luo, Y. in *Department of Chemical Engineering and Applied Chemistry* 95–96, 129–132 (University of Toronto, Toronto, Ontario, Canada, 2003).
31. Silbey, R. J. & Alberty, R. A. *Physical chemistry* (Wiley, New York, 2001).
32. Condic, M. L. & Letourneau, P. C. Ligand-induced changes in integrin expression regulate neuronal adhesion and neurite outgrowth. *Nature* **389,** 852–856 (1997).

Acknowledgements

We are grateful to the Natural Sciences and Engineering Research Council of Canada, Ontario Graduate Scholarship and Connaught for funding and thank Ying-Fang Chen, Patricia Musoke-Zawedde and David Martens for their assistance.
Correspondence and requests for materials should be addressed to M.S.
Supplementary Information accompanies the paper on www.nature.com/naturematerials

Competing financial interests

The authors declare that they have no competing financial interests.