Soft Matter

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Interfacing biodegradable molecular hydrogels with liquid crystals†

Cite this: *Soft Matter*, 2013, **9**, 1188

I-Hsin Lin,‡[a] Louise S. Birchall,§[b] Nigel Hodson,¶[c] Rein V. Ulijn*[b] and Simon J. Webb*[a]

A self-assembled Fmoc-peptide hydrogel has been interfaced with a liquid crystal (LC) display to give an optical sensor for enzyme activity. An Fmoc-TL-OMe hydrogel was selected as it can be formed *in situ* by enzyme-mediated assembly with thermolysin, and undergoes enzyme-mediated diassembly upon subtilisin addition. This enzyme-responsive hydrogel provides a semi-rigid, highly hydrated and biocompatible environment that also holds the LC display in place. A dual layer design was developed, where a phospholipid-loaded upper gel layer was separated from the LC display by a phospholipid free lower layer. Subtilisin (0.15 μM) digested both layers to give a gel-to-sol transition after several hours that liberated the phospholipid and produced a light-to-dark optical change in the LC display. The optical response was dependent upon the gel-to-sol transition; elastase or common components of serum did not disassemble the Fmoc-TL-OMe hydrogel and did not give an optical response.

Received 19th September 2012
Accepted 12th November 2012

DOI: 10.1039/c2sm27160e

www.rsc.org/softmatter

Introduction

Gel-phase nanomaterials are predicted to have a significant impact on next-generation healthcare and energy related technologies.[1] The production of these novel materials *via* molecular self-assembly is becoming increasingly sophisticated as design rules emerge.[1a,1e,1f,2] Due to their similarities with the gel matrices of living systems, nanofibrous, highly hydrated gels provide excellent environments for biological assays involving proteins or whole cells.[3] Self-assembled molecular gels are also increasingly studied in the context of technological applications,[1e] such as electroactive and photoactive liquid crystal (LC) physical gels.[4]

Supramolecular gelation is triggered by stimuli that change the strength of interactions between gelators and with the surrounding solvent, enhancing self-recognition and leading to self-assembly. These triggers may include bulk changes of conditions such as pH, temperature, solvent polarity, or localised triggers such as light[5] or the catalytic action of enzymes.[6] The latter type utilise the catalytic ability of enzymes to induce molecular self-assembly (or disassembly), as demonstrated with proteases, esterases, phosphatases, β-lactamases and DNA ligases amongst others.[1e,7]

The development of these 3D supramolecular gels has been complemented by the use of LCs to detect and amplify dynamic supramolecular events. For example, the rotation of molecular motors causes co-operative changes in LC alignment,[8] whilst electro-optical switching has been visualised in LC physical gels.[9] Recently LCDs have been applied to the detection and amplification of biological events like biocatalysis,[10] molecular recognition[11] and cellular differentiation.[12] While mixed LC–gel systems have been used to report phase transitions, these rely on molecular compatibility between LCs and gelators, to produce hybrid materials.[4] Direct observation of macroscopic phase transitions by interfacial contact with LCs has not yet been reported.

We sought to bring these areas of materials science together and hypothesised that self-assembled hydrogels could be designed to fulfil multiple roles, providing a rigid, transparent, biosensitive and biocompatible environment around the LCD. The hydrogel should stabilise an LCD within an open chamber by holding the liquid crystal in place, and it was hoped the hydrogel would also prevent dehydration and dewetting. LC dewetting can easily occur within closed chambers in the presence of Fmoc-peptides and was observed during enzymatic Fmoc-TL-OMe digestion in closed chambers; we believe dewetting facilitated by released Fmoc-peptides was responsible for light-to-dark optical transitions we reported previously.[13] The gel could act as a substrate for added enzymes, producing a gel-to-sol transition that could release an entrapped surfactant. In effect this macroscopic gel-to-sol phase transition would be

[a]*Manchester Institute of Biotechnology (MIB) and School of Chemistry, University of Manchester, 131 Princess St., Manchester, UK, M1 7DN. E-mail: S.Webb@manchester.ac.uk*

[b]*WestCHEM/Department of Pure & Applied Chemistry, University of Strathclyde, 295 Cathedral St., Glasgow, UK, G1 1XL. E-mail: rein.ulijn@strath.ac.uk*

[c]*BioAFM Facility, Faculty of Medical and Human Sciences, University of Manchester, Oxford Road, Manchester, M13 9PT, UK*

† Electronic supplementary information (ESI) available: Experimental details and chamber design; AFM and HPLC procedures; further LC observations. See DOI: 10.1039/c2sm27160e

‡ IHL contributed optical studies on liquid crystal–hydrogel composites.

§ LSB contributed fluorescence, AFM and HPLC analysis on hydrogels.

¶ NH helped with AFM analysis.

translated and amplified, as the released surfactant induces cooperative realignment of the LCs to give easily visualised optical changes when the chamber is viewed between crossed polarisers (Fig. 1(a)). We hope this design will open a path to new types of cheap, power-free and portable biosensors in the future.

Results and discussion

Our initial sensor design was simple (Fig. 1(a)), being comprised of a surfactant-loaded self-assembled hydrogel directly overlaying the LCD. The chamber was designed to be open on the upper face, as light-to-dark optical changes due to LC dewetting were observed during soluble Fmoc-peptide release in enclosed chambers, which we previously interpreted as Fmoc-peptide induced homeotropic anchoring. We now believe that Fmoc-peptides, although they are clearly amphiphilic in molecular design, are not effective at realigning liquid crystals.

We chose the Fmoc-TL-OMe gel as the model self-assembled hydrogel, as this hydrogel undergoes both enzyme mediated association and disassociation.[14] We have previously shown that the action of thermolysin converts the non-assembling precursor molecules Fmoc-T and L-OMe into the gelator Fmoc-TL-OMe *via* a reverse hydrolysis/condensation reaction.[14,15] Fmoc-TL-OMe forms a relatively stiff (G' in the region of 9×10^4 Pa) and transparent hydrogel that can be digested by subtilisin, a broad spectrum serine endopeptidase that can also cleave ester bonds.[16] The surfactant selected to be contained within the hydrogel was dilauroyl phosphatidylcholine (DLPC), which has been shown to cause optical transitions in thin LC films within 120 minutes at concentrations as low as 10 μM.[17]

Optical sensor cells were designed based on previous work carried out by Brake and Abbott.[18] In brief, octadecyltrichlorosilane (OTS)-coated cover slips were placed in the centre of a glass-based Petri dish (containing a well of diameter 12 mm) with copper TEM grids containing the LC thin films placed on top of the OTS-coated cover slips. The hydrogel was then formed above the LC layer within the well of the Petri dish. Once the hydrogel had formed, enzyme/biomolecule solutions were added to the cell. Silicone isolators (2.5 mm thick) with central holes (diameter = 20 mm) were used to retain aqueous solutions (750 μL), such as added enzyme solution, over the top of the gel blocks (which were up to 250 μL in volume). The optical sensor cells were kept at room temperature and the appearance of the LCs was monitored using optical microscopy between crossed polarisers.

During *in situ* thermolysin-catalysed formation of the Fmoc-TL-OMe hydrogel from Fmoc-T and L-OMe in the absence of surfactant, the solution-to-gel phase transition at the LC interface did not result in an LC anchoring transition; planar anchoring was preserved throughout the gelation process (Fig. 1(b and e)) and the LC appeared bright. This indicates that neither Fmoc-T, L-OMe, enzyme nor Fmoc-TL-OMe fibres were able to trigger an LC planar-to-homeotropic anchoring transition, and the Fmoc-TL-OMe hydrogel/LC is suitable for use as an optical sensor.

To test if DLPC vesicles mixed with this Fmoc-peptide solution could induce a transition in nematic LCs, 5CB hosted within grids was contacted with an Fmoc-T, L-OMe and thermolysin mixture containing 500 μM DLPC vesicular solution at room temperature. Within two minutes, the optical appearance of the LCs was colourful/bright and followed the birefringence colour change from high to low interference colour order continuously (as defined on the Michel-Levy birefringence chart) over the next 20 minutes. Unfortunately gelation of Fmoc-peptide samples with DLPC present that were also in contact with LCs was slow and incomplete in most cases, although in those samples where the hydrogel had fully formed overnight the LC appeared uniformly bright with fibres present at the interface under crossed polarisers. In contrast, Fmoc-TL-OMe hydrogel samples with incorporated DLPC that had been gelled in the absence of LC were found to induce homeotropic ordering in the LCDs upon contact (Fig. 1(a)). Nonetheless incubation of these pre-gelled DLPC-laden gel blocks with buffer did not liberate sufficient DLPC from the hydrogel to produce LC optical transitions, suggesting that any DLPC which leaches from the gel will not produce a false reading.

However to reproducibly fabricate hydrogel/LC sensor chambers that did not display homeotropic ordering of the LC, a layered hydrogel–LC composite was designed (Fig. 1(c)). The DLPC-containing hydrogels were assembled over a blocking hydrogel layer that preserved LC planar anchoring and was designed to decrease LC dewetting upon contact with aqueous solutions. The LC beneath the first Fmoc-peptide hydrogel layer preserved its initial planar anchoring after the formation of the second hydrogel layer. This layered Fmoc-TL-OMe hydrogel structure in the chamber was translucent, but polarized microscopy through the gel showed measurement of changes in LC optical appearance would be feasible (Fig. 1(f)). During sample preparation and before the formation of the layer, the optical appearance of the LCs under crossed polarisers was determined to ensure even planar anchoring. This double layer

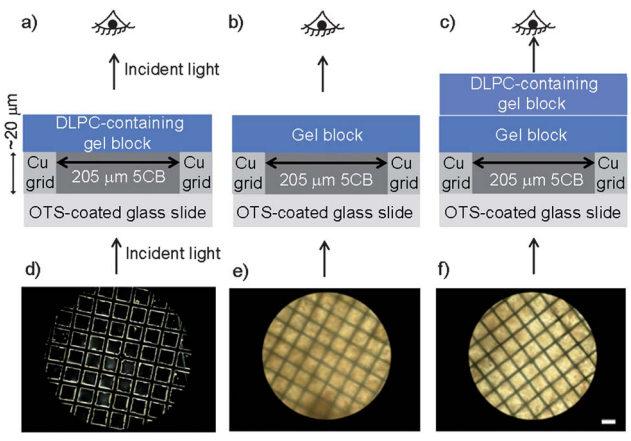

Fig. 1 Schematic illustrations and optical images of the hydrogel/LC chamber. (a–f) Schematic illustration of different chamber designs and corresponding optical polarized images of the LCD. The images are taken after (d) 1 h after contact with a DLPC-containing gel block; (e) 24 h after contact with a gel block and (f) overnight (~20 h) after contact with a gel block. Scale bar is 200 μm.

structure meant that aqueous solutions containing enzymes or other biomolecules could be easily added to the optical cell without disturbing the LC thin film.

To demonstrate enzymatic digestion of the Fmoc-TL-OMe hydrogel, the enzyme subtilisin was selected as a test case.[14] Enzyme-triggered gel-to-sol transitions were first monitored macroscopically by using the inversion test on vials filled with gel, then molecular changes studied by fluorescence spectroscopy and HPLC (Fig. 2). Typically the addition of subtilisin (0.3 mL, 0.15 μM, 100 mM, pH 8 phosphate buffer) to Fmoc-TL-OMe hydrogel (1 mL) resulted in a gel-to-sol transition within 3 h, even at this low ratio of enzyme solution to gel (33% v/v). Fluorescence spectroscopy of the fluorenyl components confirmed that a change in the supramolecular environment of the Fmoc-peptides corresponded to the gel-to-sol transition. After hydrolysis (24 h), the peaks characteristic of Fmoc-peptide gelation[15] disappeared and the emission spectra became that of an Fmoc-peptide in solution, with a single peak at ~320 nm. HPLC analysis during the gel digestion showed production of hydrolysis products; after 3 h all the Fmoc-TL-OMe was lost and after 6 h Fmoc-TL-OH began being converted to Fmoc-T-OH. Gels incubated with pH 8 buffer alone remained intact over a 24 h period. Atomic force microscopy (AFM) showed that the Fmoc-TL-OMe hydrogel was comprised of short twisted fibres with an average diameter of 78 nm. Subsequent imaging of the gels incubated with subtilisin showed that these gel fibrils were completely digested through the action of the enzyme (Fig. 2(e)).

These tests confirmed that subtilisin will cleave the target hydrogelator Fmoc-TL-OMe and should produce a gel-to-sol transition within 3 h. If this enzymatic digestion can be replicated within the layered hydrogel/LC sensor, the destruction of the gel structure should liberate the DLPC vesicles from the gel block into the aqueous solution, whereby spontaneous DLPC

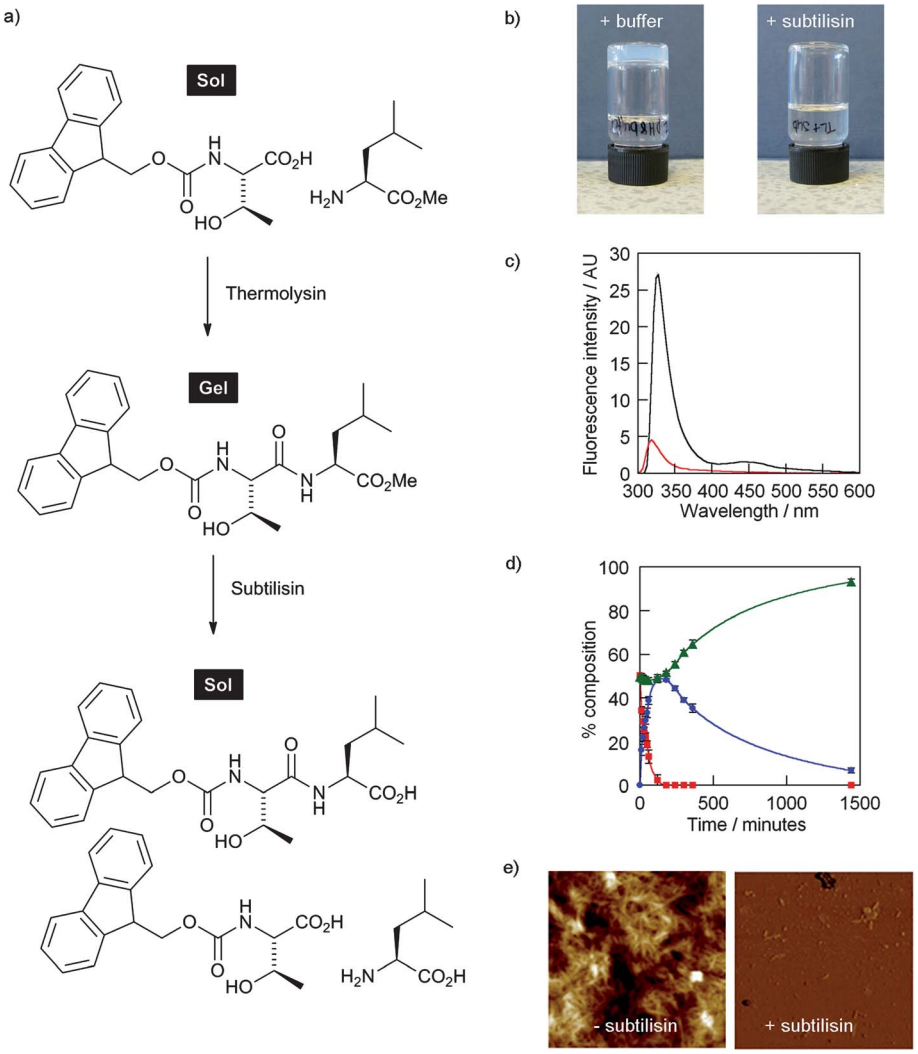

Fig. 2 (a) Scheme showing the major molecular components formed during thermolysin-catalysed formation of the Fmoc-TL-OMe hydrogel and subtilisin-catalysed digestion of the hydrogel. (b) Images of vial inversion tests showing the gel-to-sol transition of an Fmoc-TL-OMe hydrogel under the action of subtilisin. (c) Changes in the fluorescence spectrum of Fmoc-TL-OMe hydrogels both before (—) and after the action of subtilisin (—). (d) HPLC analysis of the products from the digestion of Fmoc-TL-OMe hydrogel by subtilisin: Fmoc-TL-OMe (■); Fmoc-TL-OH (●); Fmoc-T-OH (▲). Curve fits are to guide the eye. (e) AFM images (5 × 5 μm) of Fmoc-TL-OMe hydrogel fibres incubated with pH 8 buffer for 4 h (left) and after 4 h incubation with subtilisin (right).

adsorption at the aqueous–LC interface would result in an LC planar-to-homeotropic anchoring transition, *e.g.* a bright-to-black optical read-out.[10a] Layered Fmoc-TL-OMe hydrogels without DLPC (lower layer) and with DLPC incorporation (upper layer) were assembled at the LC interface; planar anchoring was preserved over each gelation step (Fig. 3(a)) and remained planar for over 24 h (Fig. 1(f)). Upon subtilisin (0.15 μM, 0.03 units per mL in 100 mM pH 8 phosphate buffer, 750 μL total volume, an enzyme–gel volume ratio of 3 : 1) addition to the hydrogel (lower 125 μL Fmoc-TL-OMe gel, upper 125 μL Fmoc-TL-OMe/DLPC gel), the LC anchoring remained planar up to ~6 h of incubation. Within the chamber significant hydrogel degradation was observed after 4 h incubation and apparently complete hydrogel degradation was visible to the naked eye after 6 h incubation. However, despite the observed digestion of the hydrogel, the LC specimen did not reveal 100% planar-to-homeotropic anchoring transitions, and a substantial area of the specimen remained with planar/tilted anchoring (Fig. 3(b)). Closer inspection under the optical microscope revealed trace amounts of Fmoc-TL-OMe fibrils covering the LC specimen in the area where LC planar/tilted anchoring remained, clearly illustrating the protective role of the hydrogel layer. However after 7 h, the fibrils had become fully digested/displaced from the LC interface and the LC adopted homeotropic anchoring, revealed by a uniform black hue under crossed polarisers (Fig. 3(c)).

Most Fmoc-TL-OMe/LC composites responded to subtilisin enzymatic activity by producing a bright-to-black optical read-out transition within 11 h of enzyme addition. Good batch-to-batch reproducibility was observed with >80% of samples undergoing the bright-to-black transition; unsuccessful assays were caused by hydrogel fibres obscuring the LC or LC dewetting, which occasionally occurred in some wells despite the overlaying hydrogel layer. In the absence of added subtilisin, all Fmoc-TL-OMe/LC composites preserved the gel structure and the LC retained planar anchoring after >24 h.[19] For different batches of Fmoc-TL-OMe/LC composite, we noted time differences for the appearance of the initial LC planar-to-homeotropic anchoring transition, ranging from ~7 h to ~11 h. The difference in these transition times appeared to be due to differences in the thickness of the gel layer and the morphology of the gel fibrils; nonetheless the rheological properties of these gels were reproducible.[15] Control LC/hydrogel optical cells incubated with buffer alone preserved their planar anchoring over the duration of the experiment, while separate addition of Fmoc-T, L-OMe or subtilisin to LCs did not produce a bright-to-black optical read-out. Adding pre-digested subtilisin–Fmoc-TL-OMe–Fmoc-TL-OMe-DLPC mixture in pH 8 phosphate buffer gave an LC planar-to-homeotropic anchoring transition, revealed during 30 minutes incubation with the mixture; we noted the transition time was comparable to that with pure DLPC dispersion in pH 8 phosphate buffer or in Tris-buffered saline (aqueous 10 mM Tris, 100 mM NaCl; pH 8.9) as reported by Brake *et al.*[10a] These results indicate that LCs can be used as optical indicators to report upon enzyme-induced hydrogel digestion in aqueous solution without using complicated instrumentation.

Next we investigated the specificity of the Fmoc-TL-OMe/LC composite by incubating the optical cell with elastase, an enzyme that should not hydrolyse Fmoc-TL-OMe, as well as other common components of physiological fluids. Elastase cleaves amide bonds flanked by small amino acid residues, such as the sequence AA.[16] Direct observations as well as HPLC analysis and fluorescence spectroscopy revealed that elastase did not digest the Fmoc-TL-OMe hydrogel under these conditions (Fig. 4(a and b)), and therefore should not lead to an anchoring transition in the LCs within an optical cell. Within the hydrogel/LC optical cells, incubation with elastase alone (1 μM elastase, 0.11 unit per mL, 750 μL total volume used in 100 mM pH 6 phosphate buffer[20]) did not cause hydrogel degradation and no optical response in the LCDs was observed (Fig. 4(c)). Even after 96 h, all of the Fmoc-TL-OMe/LC composites preserved LC planar anchoring. However, mixing subtilisin with elastase led to hydrogel degradation and DLPC liberation, which adsorbed onto the aqueous–LC interface to produce the LC anchoring transition (Fig. 4(d)); 80% of these mixed samples revealed the LC anchoring transition. As at pH 8, incubating the hydrogel with buffer at pH 6 did not cause a gel-to-sol transition.

We next investigated whether our LC–hydrogel composite was affected by high concentrations of glucose. The normal physiological concentration of glucose in serum is in the range of 3.6 to 5.8 mM,[21] so we incubated the Fmoc-TL-OMe/LC composite with 10 mM glucose in pH 8 phosphate buffer. All LCs within optical cells that were incubated with glucose alone retained their initial planar anchoring (Fig. 5(a)) and no hydrogel degradation was observed. As with elastase, adding

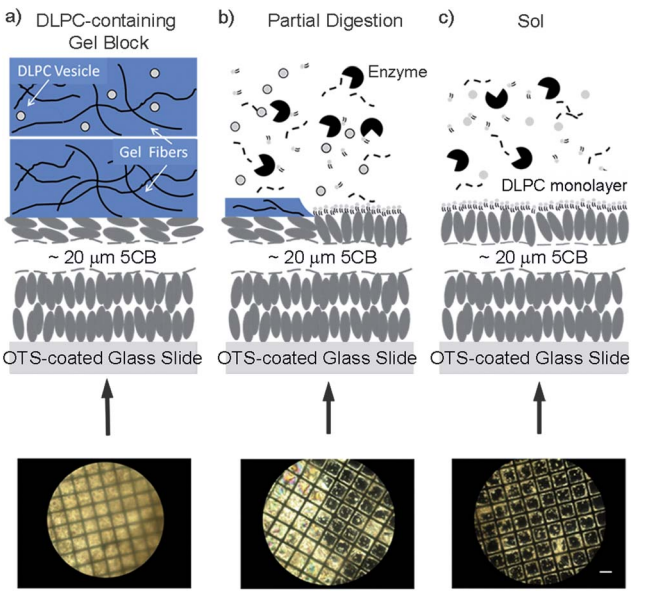

Fig. 3 Cartoon representations of the 5CB anchoring transition upon *in situ* subtilisin-induced Fmoc-TL-OMe hydrogel degradation at 5CB-gel interfaces. Optical images of 5CB anchoring at the 5CB-gel interface upon 0.15 μM subtilisin addition after (a) 0 h, (b) 6 h, and (c) 7 h at room temperature. The 5CB reveals dynamic anchoring transitions from planar to homeotropic alignment in parallel to the subtilisin-induced Fmoc-TL-OMe degradation at interfaces. Scale bar is 200 μm.

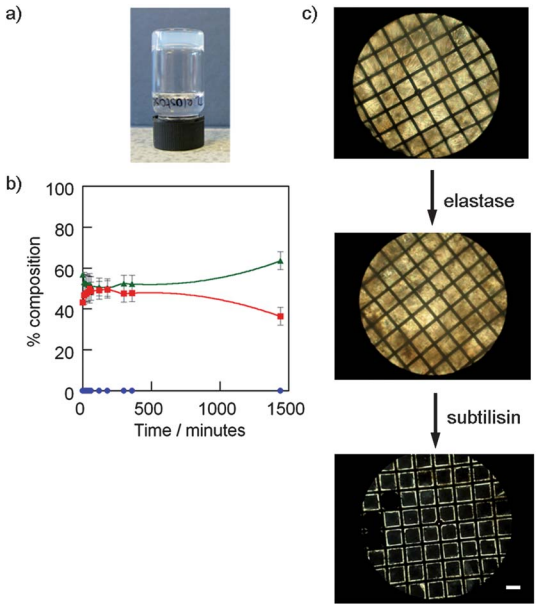

Fig. 4 Enzyme specificity testing with Fmoc-TL-OMe/LC composites. (a) Inset vial inversion test showing the gel remains intact after mixing with elastase. (b) HPLC analysis of the mixture of Fmoc-TL-OMe hydrogel and elastase: Fmoc-TL-OMe (■); Fmoc-TL-OH (●); Fmoc-T-OH (▲). (c) Optical images of 5CB anchoring at 5CB-gel interfaces after elastase addition (1 μM in 100 mM pH 6 phosphate buffer) and incubation for 24 h at room temperature, followed by mixing with 0.15 μM subtilisin; the LC planar-to-homeotropic anchoring started between 6 and 11 h after contact with subtilisin. Scale bar is 200 μm.

subtilisin to the glucose solution led to degradation of the hydrogel, with the subsequent release of DLPC producing a bright-to-black optical readout (Fig. 5(c)) in all of the specimens tested.

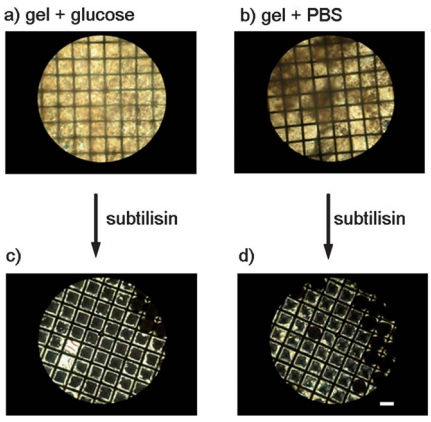

Fig. 5 Interferant testing with Fmoc-TL-OMe/LC composites. (a and b) Optical images of 5CB anchoring at 5CB-gel interfaces after interferant solution addition and incubation for 24 h at room temperature. Interferant solutions were: (a) 10 mM glucose in 100 mM pH 8 phosphate buffer (b) pH 7.4 PBS buffer. (c and d) Optical images of 5CB anchoring at the 5CB-gel interface for each interferant solution (above) after mixing with 0.15 μM subtilisin. Images taken after 24 h incubation at room temperature; LC planar-to-homeotropic anchoring started between 6 and 11 h upon contact with the interferant–subtilisin mixture. Scale bar is 200 μm.

Finally the effect of common physiological buffer components on Fmoc-TL-OMe/LC digestion by subtilisin was investigated. PBS buffer at pH 7.4 was used as this has Na^+, Cl^-, and K^+ at physiological ionic strengths,[22] namely 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4. Under these conditions the Fmoc-TL-OMe/LC composite retained its hydrogel structure and planar anchoring of the LCs was preserved in all five samples after incubation in PBS buffer for 24 h (Fig. 5(b)). However the addition of subtilisin to the PBS buffer resulted in the degradation of the hydrogel, liberating DLPC which induced an LC anchoring transition (Fig. 5(d)) with 100% batch-to-batch reproducibility.

Conclusions

This is the first time that an enzyme-digestible hydrogel has been interfaced with an LC display. The enzyme-sensing composite was designed to have complex hierarchical self-assembly, with self-assembled phospholipid vesicles entangled with self-assembled peptide nanofibres,[3c,23] only to be liberated after enzymatic digestion of these fibrils. Migration of the vesicles to the LC interface and subsequent lipid assembly at the aqueous–LC interface then induces cooperative reorientation of the LCs and the production of a visible optical signal.

Enzymes or molecules that do not initiate hydrogel degradation cannot liberate the phospholipid vesicles, and the LCs maintain planar anchoring under the robust hydrogel layers. Although subtilisin is not an analyte of widespread interest, it provided a useful proof-of-principle case for other peptide hydrogels that are disassembled by kinases[24] and medically important proteases.[25] Such enzyme-sensitive hydrogel–vesicle composites may have general utility for the screening of hydrolytic enzymes in biological samples, with the hydrogel layers providing a measure of protection against disruption of the LC display and preventing interference by non-analyte substances in the biological sample. There are also fundamental questions raised by this work; changes in LC orientation during *in situ* gelation of DLPC–Fmoc-peptide mixtures suggest that rather than assemble at the LC–aqueous interface, phospholipids may associate with the hydrogel fibrils during fibrilisation. Understanding such fibril–lipid interactions and developing other rigid, transparent and enzyme-digestible hydrogels will be key steps towards the elaboration of these composites into portable, robust and power-free enzyme sensors.

Acknowledgements

The authors gratefully acknowledge the BBSRC for financial support. We thank Ms L. Ballamy and Dr P. King for additional LC images.

References

1 (*a*) S. Zhang, *Nat. Biotechnol.*, 2003, **21**, 1171–1180; (*b*) A. M. Kloxin, A. M. Kasko, C. N. Salinas and K. S. Anseth,

Science, 2009, **324**, 59–63; (*c*) J. S. Mohammed and W. L. Murphy, *Adv. Mater.*, 2009, **21**, 2361–2374; (*d*) S. Kiyonaka, K. Sada, I. Yoshimura, S. Shinkai, N. Kato and I. Hamachi, *Nat. Mater.*, 2004, **3**, 58–64; (*e*) A. R. Hirst, B. Escuder, J. F. Miravet and D. K. Smith, *Angew. Chem., Int. Ed.*, 2008, **47**, 8002–8018; (*f*) W. Edwards, C. A. Lagadec and David K. Smith, *Soft Matter*, 2011, **7**, 110–117; (*g*) G. O. Lloyd and J. W. Steed, *Nat. Chem.*, 2009, **1**, 437–442; (*h*) K. A. Houton, K. L. Morris, L. Chen, M. Schmidtmann, J. T. A. Jones, L. C. Serpell, G. O. Lloyd and D. J. Adams, *Langmuir*, 2012, **28**, 9797–9806.

2 (*a*) L. C. Palmer and S. I. Stupp, *Acc. Chem. Res.*, 2008, **41**, 1674–1684; (*b*) E. F. Banwell, E. S. Abelardo, D. J. Adams, M. A. Birchall, A. Corrigan, A. M. Donald, M. Kirkland, L. C. Serpell, M. F. Butler and D. N. Woolfson, *Nat. Mater.*, 2009, **8**, 596–600.

3 (*a*) M. Zhou, A. M. Smith, A. K. Das, N. W. Hodson, R. F. Collins, R. V. Ulijn and J. E. Gough, *Biomaterials*, 2009, **30**, 2523–2530; (*b*) V. Jayawarna, S. M. Richardson, A. Hirst, N. W. Hodson, A. Saiani, J. E. Gough and R. V. Ulijn, *Acta Biomater.*, 2009, **5**, 934–943; (*c*) F. de Cogan, A. Booth, J. E. Gough and S. J. Webb, *Angew. Chem., Int. Ed.*, 2011, **50**, 12280–12284; (*d*) M. P. Lutolf and J. A. Hubbell, *Nat. Biotechnol.*, 2005, **23**, 47–55.

4 T. Kato and K. Tanabe, *Chem. Lett.*, 2009, **38**, 634–639.

5 T. Muraoka, C.-Y. Koh, H. Cui and S. I. Stupp, *Angew. Chem., Int. Ed.*, 2009, **48**, 5946–5949.

6 (*a*) Z. Yang, G. Liang and B. Xu, *Acc. Chem. Res.*, 2008, **41**, 315–326; (*b*) R. J. Williams, R. J. Mart and R. V. Ulijn, *Pept. Sci.*, 2010, **94**, 107–117; (*c*) J. Gao, H. Wang, L. Wang, J. Wang, D. Kong and Z. Yang, *J. Am. Chem. Soc.*, 2009, **131**, 11286–11287.

7 (*a*) Z. M. Yang and B. Xu, *Chem. Commun.*, 2004, 2424–2425; (*b*) R. J. Williams, A. M. Smith, R. Collins, N. Hodson, A. K. Das and R. V. Ulijn, *Nat. Nanotechnol.*, 2009, **4**, 19–24; (*c*) S. Winkler, D. Wilson and D. L. Kaplan, *Biochemistry*, 2000, **39**, 12739–12746; (*d*) Z. Yang, P.-L. Ho, G. Liang, K. H. Chow, Q. Wang, Y. Cao, Z. Guo and B. Xu, *J. Am. Chem. Soc.*, 2007, **129**, 266–267; (*e*) S. H. Um, J. B. Lee, N. Park, S. Y. Kwon, C. C. Umbach and D. Luo, *Nat. Mater.*, 2006, **5**, 797–801; (*f*) Z. Yang, H. Gu, D. Fu, P. Gao, J. K. Lam and B. Xu, *Adv. Mater.*, 2004, **16**, 1440–1444.

8 R. Eelkema, M. M. Pollard, J. Vicario, N. Katsonis, B. Serrano Ramon, C. W. M. Bastiaansen, D. J. Broer and B. L. Feringa, *Nature*, 2006, **440**, 163.

9 Y. Hirai, N. Mizoshita, M. Moriyama and T. Kato, *Langmuir*, 2009, **25**, 8423–8427.

10 (*a*) J. M. Brake, M. K. Daschner, Y.-Y. Luk and N. L. Abbott, *Science*, 2003, **302**, 2094–2097; (*b*) J. Hoogboom, K. Velonia, T. Rasing, A. E. Rowan and R. J. M. Nolte, *Chem. Commun.*, 2006, 434–435; (*c*) J.-S. Park, S. Teren, W. H. Tepp, D. J. Beebe, E. A. Johnson and N. L. Abbott, *Chem. Mater.*, 2006, **18**, 6147–6151; (*d*) J. S. Park and N. L. Abbott, *Adv. Mater.*, 2008, **20**, 1185–1190; (*e*) X. Bi, D. Hartono and K.-L. Yang, *Adv. Funct. Mater.*, 2009, **19**, 3760–3765; (*f*) Y. Liu, D. Cheng, I.-H. Lin, N. L. Abbott and H. Jiang, *Lab Chip*, 2012, **12**, 3746–3753.

11 (*a*) B. H. Clare and N. L. Abbott, *Langmuir*, 2005, **21**, 6451–6461; (*b*) C.-H. Jang, M. L. Tingey, N. L. Korpi, G. J. Wiepz, J. H. Schiller, P. J. Bertics and N. L. Abbott, *J. Am. Chem. Soc.*, 2005, **127**, 8912–8913; (*c*) A. D. Price and D. K. Schwartz, *J. Am. Chem. Soc.*, 2008, **130**, 8188–8194.

12 (*a*) C.-H. Jang, L.-L. Cheng, C. W. Olsen and N. L. Abbott, *Nano Lett.*, 2006, **6**, 1053–1058; (*b*) N. A. Lockwood, J. C. Mohr, L. Ji, C. J. Murphy, S. P. Palecek, J. J. de Pablo and N. L. Abbott, *Adv. Funct. Mater.*, 2006, **16**, 618–624.

13 L. S. Birchall, R. V. Ulijn and S. J. Webb, *Chem. Commun.*, 2008, 2861–2863.

14 A. K. Das, R. Collins and R. V. Ulijn, *Small*, 2008, **4**, 279–287.

15 M. Hughes, P. W. J. M. Frederix, J. Raeburn, L. S. Birchall, J. Sadownik, F. C. Coomer, I.-H. Lin, E. J. Cussen, N. T. Hunt, T. Tuttle, S. J. Webb, D. J. Adams and R. V. Ulijn, *Soft Matter*, 2012, **8**, 5595–5602.

16 A. J. Barret, N. D. Rawlings and J. F. Woessner, *Handbook of Proteolytic Enzymes*, Elsevier Academic Press, London, 2nd edn, 2004, vol. 2, p. 1786.

17 J. M. Brake, M. K. Daschner and N. L. Abbott, *Langmuir*, 2005, **21**, 2218–2228.

18 J. M. Brake and N. L. Abbott, *Langmuir*, 2002, **18**, 6101–6109.

19 No LC response was observed after 72 h, but if the lower LC layer were to be thinner or softer then a false positive might be possible after a shorter incubation time.

20 Porcine pancreatic elastase is stable and active at pH 6.0, with activity one third that at the optimum pH 8.0. See G. Feinstein, A. Kupfer and M. Sokolovsky, *Biochem. Biophys. Res. Commun.*, 1973, **50**, 1020–1026.

21 R. A. Shaw and H. H. Mantsch, Infrared Spectroscopy in Clinical and Diagnostic Analysis, in *Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation*, ed. R. A. Meyers, John Wiley & Sons Ltd., Chichester, 2001, p. 7.

22 L. Sherwood, *Essentials of Physiology*, International edn, Cengage Learning, Inc., Florence USA, 2012, p. 445.

23 (*a*) J. Boekhoven, M. Koot, T. A. Wezendonk, R. Eelkema and J. H. van Esch, *J. Am. Chem. Soc.*, 2012, **134**, 12908–12911; (*b*) A. Heeres, C. van der Pol, M. Stuart, A. Friggeri, B. L. Feringa and J. van Esch, *J. Am. Chem. Soc.*, 2003, **125**, 14252–14253.

24 Z. M. Yang, G. L. Liang, L. Wang and B. Xu, *J. Am. Chem. Soc.*, 2006, **128**, 3038–3043.

25 Y. Chau, Y. Luo, A. C. Y. Cheung, Y. Nagai, S. Zhang, J. B. Kobler, S. M. Zeitels and R. Langer, *Biomaterials*, 2008, **29**, 1713–1719.