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Reversible surface functionalisation of emulsion-templated porous polymers using dithiophenol maleimide functional macromolecules

A. M. Eissa,[a,b,c]* P. Wilson,[d] C. Chen,[a] J. Collins,[d] M. Walker,[e] D. M. Haddleton[d] and N. R. Cameron[a,b]

A new facile and efficient route for the chemical functionalisation of thiol-acrylate polyHIPE materials with responsive macromolecules using the highly emissive dithiomaleimide (DTM) linker is demonstrated. Functionalisation is found to be reversible upon addition of thiol-containing compound, glutathione, resulting in switchable surface properties including fluorescence and wettability, hence broadening the scope of applications.

Macroporous polymers are increasingly used in a wide range of applications including reaction supports,[1-3] separation processes[4] and tissue engineering scaffolds.[5-7] Highly porous polymers with a well-defined morphology and a fully interconnected network of pores can be prepared by emulsion-templating whereby a high internal phase emulsion (HIPE) is created in which the major, "internal" phase, usually defined as constituting more than 74% of the volume, is dispersed within the continuous, minor, "external" phase. Most polyHIPE materials are prepared by radical polymerisation, initiated either thermally or photochemically; however, other methods have been reported.[8, 9] Nevertheless, a lack of functionality on the surface of polyHIPE materials can be a limitation in expanding their applications. For example, it has been shown that a galactose-functionalised styrene-based polyHIPE material surpassed the unfunctionalised material in its performance as a scaffold for 3D culture of mammalian hepatocytes.[10] Thus, the next step towards creating 'smart' polyHIPE materials that can respond to an external stimulus in the surrounding environment is to identify potential strategies for surface modification.[11]

Efforts have been made previously to introduce functionalities to the surface of polyHIPE materials.[9, 12-14] Two main routes have been suggested: either incorporation into the HIPE of a comonomer bearing the desired functionality or post-polymerisation functionalisation. As a result of the inherent instability of HIPEs, incorporation of a comonomer into the HIPE may destabilise the emulsion and ultimately lead to phase separation. In this sense, post-polymerisation functionalisation is a more attractive approach to produce functional polyHIPE materials without deteriorating their original open cell morphology with an interconnected network of pores.

Recently, polyHIPE materials made from thiol-acrylate photopolymerisation, employing commercially available multifunctional thiols and acrylates, have been found to feature unreacted thiols on their surfaces even when the used thiol : acrylate molar ratio in their preparation was 1 : 1.[15, 16] The unreacted thiols can be exploited as reactive 'handles' with which to introduce functionality onto the polyHIPE surface using a wide range of chemical transformations *via* the thiol-Michael addition click reaction.[17]

Recent studies have shown that dibromomaleimides (DBMs) are able to conjugate to cysteine residues[18] and to re-bridge peptides containing disulfide bonds following *in situ* reduction.[19] Dithiophenol maleimides (DTMs) have also been demonstrated as highly efficient and rapid re-bridging agents for the disulfide containing peptides.[20] Reaction with thiols proceeds *via* an addition, elimination mechanism resulting in the retention of the maleimide functionality, which lends itself to reversibility or further modification.[21] Furthermore, whereas substitution with aryl thiols such as thiophenol yields non- or weakly fluorescent products, alkyl thiols furnish highly fluorescent products which provides a convenient spectroscopic handle for the reaction and further applications. It is possible to readily synthesise well-defined α-functional DBM and DTM polymers, even without the need for protecting group chemistry.[22, 23] Synthesis of α-functional DTM polymers has been previously reported using reversible-addition fragmentation transfer (RAFT) polymerisation,[24] atom transfer radical polymerisation (ATRP)[25] and single electron transfer living radical polymerisation (SET-LRP).[26] Such polymers have been employed as functional handles for the preparation of

a. *School of Engineering, University of Warwick, Coventry, CV4 7AL, U.K.*
b. *Department of Materials Science and Engineering, Monash University, Clayton, 3800, Victoria, Australia.*
c. *Department of Polymers, Chemical Industries Research Division, National Research Centre (NRC), El-Bohoos Street, Dokki, Cairo, 12311, Egypt.*
d. *Department of Chemistry, University of Warwick, Coventry, CV4 7AL, U.K.*
e. *Department of Physics, University of Warwick, Coventry, CV4 7AL, U.K.*
† Footnotes relating to the title and/or authors should appear here.
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complex polymer architectures,[27] protein-polymer conjugates,[24] and polymeric nanoparticles,[28] all of which retain the fluorescent properties exhibited by DBMs/DTMs.

Herein, for the first time we describe the conjugation of DTMs to thiol-acrylate polyHIPE materials as a facile route to polyHIPE surface modification with responsive polymers, poly(ethylene glycol) (PEG) and poly(N-isopropylacrylamide) (pNIPAM). Conjugation of DTM end-capped polymers (PEG, poly(PEG acrylate) (pPEGA) and pNIPAM) to polyHIPE surfaces did not influence the morphology of the polyHIPEs after the conjugation reaction, retaining their original open-cell structure, as revealed by scanning electron microscopy (SEM). More interestingly, we observed that the polyHIPE materials became fluorescent after reaction with DTMs. The conjugation reaction was also found to be reversible in the presence of excess free thiol containing compounds such as glutathione which is known to regulate many biological processes including cell growth and division. This finding opens up the prospect of their use as fluorescent sensor matrices for cell culture to easily monitor cell physiology while growing on the scaffolds.

In this study, commercially-available trimethylolpropane tris(3-mercaptopropionate) (TMPTMP) and dipentaerythritol penta/hexa-acrylate (DPEHA) were utilised to produce polyHIPE materials with a well-defined and interconnected porosity. A nominal porosity which is defined by the HIPE aqueous phase content of 80% was used. SEM confirmed the interconnected, open-cell foam morphology of these materials and by analysis of SEM images, the average pore diameter was found to be between 20 and 30 μm, Fig. 1.
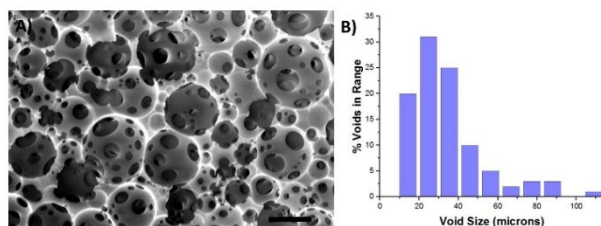
Figure 1. SEM showing the morphology of thiol-acrylate polyHIPE material (A) and void diameter distribution as determined by analysis of SEM images (B)

During the network formation, two simultaneous reactions take place upon UV irradiation of the HIPEs: the reaction between the thiol and the acrylate and the homopolymerisation of the acrylate. As a result of the occurrence of the competing homopolymerisation reaction, the number of acrylate groups available for reaction with thiols decreases. The final material therefore possess unreacted residual thiols, even when the thiol to acrylate ratio in the HIPE is kept constant at 1:1. The residual thiol content in the polyHIPEs can be quantified by a colourimetric (Ellman's) assay.[29] Residual thiols on the thiol-acrylate polyHIPE materials have been previously shown to be reactive towards a range of (meth)acrylates using thiol-ene "click" reactions.[15] This work therefore focused on the use of functional maleimides for the chemical functionalisation of thiol–acrylate polyHIPEs. Initially, DTM was allowed to react with a swollen polyHIPE disc in THF for 30 minutes. Following extensive washing in THF to remove any unreacted DTM, the polyHIPE material displayed a distinct colour change from white to yellow and, when the material was illuminated under UV light, bright fluorescence was observed. This is indicative of successful substitution at the polyHIPE surface,

which is in line with previous work that shows that the exchange of the thiophenol groups on the maleimide leads to bright emissive properties (~500 nm) in a range of solvents,[24] Fig. 2. However, as yet no attempt has been made to exploit the emissive property of dithiomaleimides on surfaces. Addition of an excess of competitive thiol-containing compound, glutathione, resulted in detachment of DTM from the polyHIPE *via* a second addition-elimination reaction. This coincided with the expected loss of fluorescence. The reversibility of this reaction imparts potential for a wide range of applications where protein or even cell attachment and detachment to and from surfaces, can be monitored by an on-off fluorescence switch.
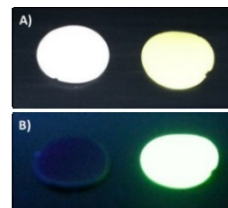
Figure 2. Photographs of unfunctionalised (left) and DTM-functionalised (right) polyHIPE materials under A) normal light and B) illumination by UV light (λ = 254 nm)

The results obtained with DTM inspired investigation of its potential to act as both a linker and a fluorophore for the conjugation of polymers to polyHIPE surfaces. Three representative DTM-functional polymers were synthesised for subsequent functionalisation of the thiol-acrylate polyHIPEs: linear DTM-PEG$_{100}$, DTM-pPEGA$_{100}$ and DTM-pNIPAm$_{100}$, Fig. 3. The DTM-PEG$_{100}$ was synthesised in a two-step reaction from DTM (ESI, Scheme SI-1). Successful addition of DTM to the chain-end of PEG was confirmed by ^{1}H NMR spectroscopy (ESI, Fig. SI-1). DTM-pPEGA$_{100}$ was synthesised using aqueous SET-LRP[30, 31] (M_n = 36000 g.mol^{-1}, Đ = 1.36).[26] Due to the hydrophobic nature of the DTM-initiator (DTM-Br) the polymerisation conditions were adapted to ensure a homogeneous system. Crucially, the initial disproportionation step was conducted in pure water, prior to addition of the NIPAM/DTM-Br solution ([DTM-Br] : [NIPAM] = [1] : [100]) in ethanol. Polymerisation was stopped at 98% conversion and dialysed against water to remove residual monomer yielding DTM-pNIPAM$_{100}$ (M_n = 9200 g.mol^{-1}, Đ = 1.12) (ESI, Scheme SI-2). The presence of the DTM group was confirmed by ^{1}H NMR spectroscopy and size exclusion chromatography (SEC) revealed a narrow molecular weight distribution consistent with the polymerisation of NIPAM *via* aqueous SET-LRP (ESI, Fig. SI-2,3). Ellman's assay was used to quantify the amount of free thiols on the polyHIPE materials before and after functionalisation (ESI, Table SI-1). The non-functionalised polyHIPEs possessed 0.125 mmol.g^{-1} of thiol groups. Upon addition of DTM the detectable level of thiol reduced > 10 fold to 0.010 mmol.g^{-1} which was consistent with the observed surface colour change and fluorescence. Addition of the DTM functional polymers proceeded with a 2-3 fold reduction in the level of detectable thiol (0.038-0.050 mmol.g^{-1}) which in the case of linear polymers DTM-PEG$_{100}$ and DTM-pNIPAM$_{100}$ can be attributed to the random coil nature of the polymer projecting from the polyHIPE surface that can potentially obstruct further

modification at nearby thiol groups. Conversely, the DTM-pPEGA$_{100}$ polymers would adopt a more comb, or rigid rod like conformation in solution. Though this would result in less blocking of neighbouring thiol groups when attached to the polyHIPE surface, the size of the PEGA side in close proximity to the functional end-group imposes steric hindrance and limits the amount of conjugation, akin the observations made during protein-polymer conjugation.[32]
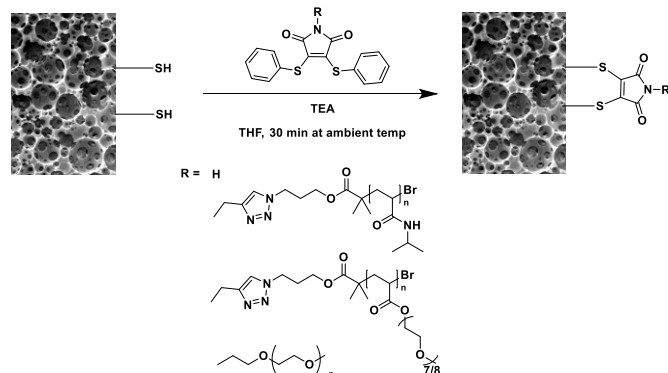
Figure 3. Schematic showing the functionalisation of thiol-acrylate polyHIPEs using DTM and functional polymers

As a result of their extreme surface roughness and highly porous nature, contact angle measurements of polyHIPE surfaces cannot be related to surface tension and therefore produce unreliable values. We alternatively investigated significant wettability changes of thiol-acrylate polyHIPE surfaces upon functionalisation with pPEGA$_{100}$ by depositing a 10 μL drop of deionised water containing a red food dye onto a dry disc of the polyHIPE material. Within a few minutes of application, it was clear that the water droplet spread on the surface of the pPEGA$_{100}$-functionalised polyHIPE, indicating that the material is sufficiently hydrophilic to allow absorption of the water droplet, Fig. 4A&B. In contrast, the water droplet remained nearly spherical on the surface of the unfunctionalised polyHIPE disc which reflects the hydrophobicity of the unfunctionalised polyHIPE. The pNIPAM$_{100}$-functionalised polyHIPE material showed a responsive wettability behaviour depending on the ambient temperature. pNIPAM exhibits hydrophilic-hydrophobic changes with lower critical solution temperature (LCST) in aqueous solution at around 32 °C. We therfore studied the wettability of the material below and above the LCST. At room temperature (ca. 25 °C), the material absorbed the water droplet within 1 hr of application; while at 40 °C, the water droplet remained almost spherical, indicating the complete transformation into hydrophobic surface, Fig. 4C&D.
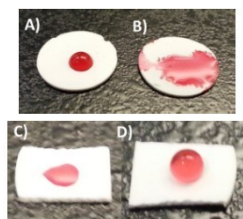
Figure 4. Surface wettability of thiol-acrylate polyHIPEs before (A) and after functionalisation with pPEGA$_{100}$ (B) and after functionalisation with pNIPAM$_{100}$ at room temperature (ca. 25 °C) (C) and at 40 °C (D).

Finally, the functionalised materials were analysed by X-ray photoelectron spectroscopy (XPS) to determine the chemical environment of atoms present at the surface. Table 1 lists the calculated atomic composition of DTM, pNIPAM$_{100}$, pPEGA$_{100}$ and PEG$_{100}$ functionalised as well as unfunctionalised polyHIPE materials. The peak-fitted high resolution C 1s spectra for all materials are shown in ESI, Fig. SI-4. In the unfunctionalised polyHIPE spectrum, there are three distinct chemical environments which comprise the C 1s peak. C1 component (284.6 eV) is assigned to C–C and C–H bonds; C2 (285.8 eV) is assigned to C–O bonds and C3 (288.6 eV) to (meth)acrylate ester bonds (O=C–O). Peak assignment and C 1s components fitting in all spectra shows clearly the introduction of the O=C-N bonding environment at 288.1 eV upon surface functionalisation with DTM, with this environment accounting for 8.2 % of the total C 1s intensity (ESI, Table SI-2). The intensity of this bonding environment increases further upon the conjugation of pNIPAM$_{100}$-functionalised polyHIPE to 11.8 %. Conversely, conjugation with pPEGA$_{100}$-functionalised polyHIPE leads to a relative increase in the O=C-O peak at 288.4 eV. In this case, the N 1s signal was no longer observed due to no N atoms in the repeat unit of the polymer chain and subsequent attenuation of the signal from the N atoms nearer the scaffold. Attenuation of the C 1s signal is comparatively less due to the longer inelastic mean free path at lower binding energies, and hence a small contribution from O=C-N bonding is still observed in the C 1s spectrum. In the case of conjugation with the PEG-functionalised polyHIPE, only C-C, C-H and C-O-C bonds are introduced in to the system and hence little change in the ratio of the O=C-O and O=C-N peaks is observed. Again attenuation of the DTM by the polymer chain leads to a N 1s intensity below the detection limit of the experiment.

Table 1. XPS data of unfunctionalised and functionalised polyHIPE materials

Sample	Overall Composition (Atomic %)			
	C 1s	O 1s	S 2p	N 1s
Thiol-Acrylate polyHIPE	81.57	15.99	2.45	0
DTM-functionalised polyHIPE	72.04	23.58	4.08	0.3
pNIPAM$_{100}$-functionalised polyHIPE	71.12	23.47	4.31	1.09
pPEGA$_{100}$-functionalised polyHIPE	68.3	27.12	4.58	0
PEG$_{100}$-functionalised polyHIPE	71.15	24.66	4.19	0

Further evidence for the successful conjugation of DTM and the pNIPAM$_{100}$-functionalised polyHIPE is found in the N 1s spectra shown in Fig. 5. As expected, the unfunctionalised scaffold exhibits no N 1s intensity and a small signal is detected upon the introduction of the DTM. A significant increase in the intensity of the component at 399.3 eV is observed upon conjugation to the pNIPAM$_{100}$-functionalised polyHIPE, indicating that this peak corresponds to the O=C-N-C bonding environment in the polymer. The spectra collected in the S 2p region exhibit no significant apparent changes before and after polymer conjugation. This is as expected due to the polymer chains being sulfur-free.
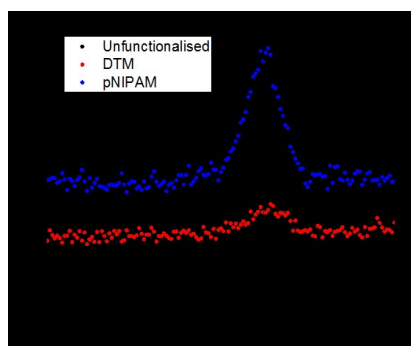
Figure 5. Overlaid high-resolution N 1s spectra for unfunctionalised and DTM and pNIPAM functionalised polyHIPE surfaces

In summary, we report for the first time a simple tailored surface functionalisation strategy for thiol-acrylate polyHIPE materials based on the rapid and mild reactions between the residual thiols on the polyHIPE surface and DTM functional polymers made by SET-LRP. A simple two-step reaction was used to end-cap PEG_{100} chains with DTM whereas the direct polymerisation of PEGA and NIPAM using a rationally prepared DTM-Br initiator by SET-LRP yielded DTM end-capped $pPEGA_{100}$ and $pNIPAM_{100}$, respectively. A colourimetric (Ellman's) assay was used to quantify the residual thiols on the surface before and after functionalisation. XPS was also used to determine the atomic composition of the functionalised and unfunctionalised polyHIPE materials. Not only are DTMs efficient conjugating agents but also they are fluorophores, allowing simultaneous polymer conjugation and fluorescent labelling of the polyHIPE surface. A key advantage of these functionalisation reactions is their reversibility upon addition of free thiol-containing molecules (such as glutathione) as indicated by an on-off fluorescent switch. We believe that the scope of this functionalisation approach could be expanded to conjugate a wide range of biologically relevant molecules/macromolecules including peptides, enzymes and catalysts to the surface of thiol-acrylate polyHIPE scaffolds. Future studies will focus on the application of these functional materials.

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Reversible surface functionalisation of emulsion-templated porous polymers using dithiophenol maleimide functional macromolecules

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Reversible, easy-to-monitor approach to the surface functionalisation of thiol-acrylate polyHIPEs that can be utilised in a wide range of applications.