

# Supramolecular Approaches to Combining Membrane Transport with Adhesion

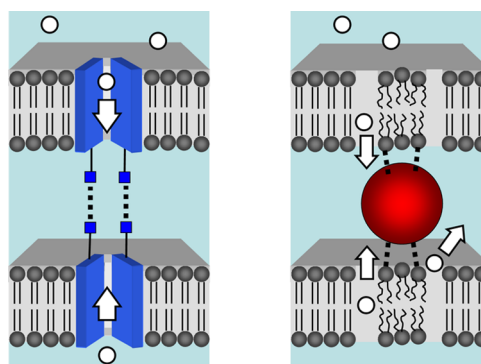
SIMON J. WEBB\*

*Manchester Institute of Biotechnology and the School of Chemistry, University of Manchester, 131 Princess St, Manchester M1 7DN, United Kingdom*

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## CONSPECTUS

Cells carefully control the transit of compounds through their membranes using “gated” protein channels that respond to chemical stimuli. Connexin gap junctions, which are high conductance cell-to-cell channels, are a remarkable class of “gated” channel with multiple levels of assembly. A gap junction between adhering cells comprises two half-channels in each cell membrane that adhere to each other to form a continuous cell-to-cell channel. Each half-channel is a hexameric assembly of six protein transmembrane subunits. These gap junctions display both intramembrane assembly and intermembrane assembly, making them an attractive target for biomimetic studies. Although many examples of self-assembled channels have been developed, few can also mediate intermembrane adhesion. Developing systems that combine membrane adhesion with controlled transit across the membrane would not only provide a better understanding of self-assembly in and around the membrane, but would also provide a route towards smart biomaterials, targeted drug delivery and an interface with nanotechnology.

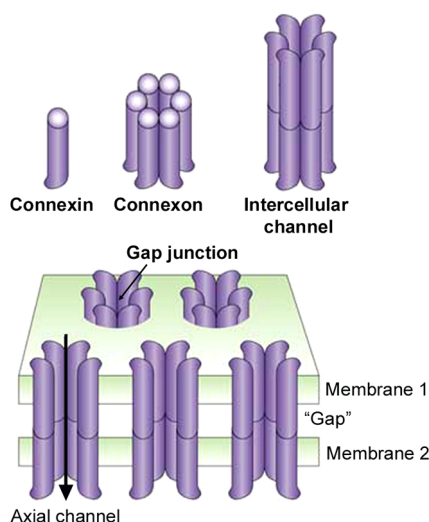


This Account describes our biomimetic approaches to combining membrane adhesion with membrane transport, using both self-assembled “sticky” pores and “sticky” nanoparticles to trigger transit across membranes. This combination links both fundamental and applied research, acting as a bridge between molecular level assembly and the formation of functional biomaterials. The ultimate goal is to create complex self-assembled systems in biological or biomimetic environments that can both interface with cells and transport compounds across bilayers in response to remote chemical or electromagnetic signals. Our research in this area started with fundamental studies of intramembrane and intermembrane self-assembly, building upon previously known channel-forming compounds to create self-assembled channels that were switchable or able to mediate vesicle–vesicle adhesion. Subsequently, nanoparticles with a “sticky” coating were used to mediate adhesion between vesicles. Combining these adhesive properties with the unique characteristics of nanosized magnetite allowed a noninvasive magnetic signal to trigger transport of compounds out of magnetic nanoparticle-vesicle assemblies. Adding an extravesicular matrix produced new responsive biomaterials for use in tissue engineering. These biomaterials can be magnetically patterned and can deliver drugs upon receipt of a magnetic signal, allowing spatiotemporal control over cellular responses.

## 1. Introduction

Transport across cellular membranes involves some of the most elegant structures in cells, the pores and channels within the phospholipid bilayer. To create structures of similar complexity that possess the same exquisite level of control over transport is a great challenge for supramolecular chemists. The formation of synthetic ion channels through self-assembly is subject to intensive research, driven both by the need to improve our fundamental understanding of intermolecular interactions within membranes

as well as a desire for real-world applications in sensing and antibiotic development.<sup>1,2</sup> The drive for real-world applications like drug delivery<sup>3</sup> has also produced other ways of facilitating the transit of hydrophilic compounds across phospholipid bilayers. These methods, such as membrane melting, appear to rely on different transport mechanisms compared to discrete channels, yet recent research suggests some similarity in the underlying physical processes. Nonetheless both channel self-assembly and membrane melting have been exploited to produce membrane transport,



**FIGURE 1.** Diagram showing the relationships between the connexin monomer, the hexameric connexon and the dodecameric intercellular channel known as a gap junction. Each intercellular channel interconnects the cytoplasm of the cells. Reproduced with permission from ref 4.

and several of these methods have proved themselves to be suitable for use either *in vitro* or *in vivo*.

Intermembrane assembly has only rarely been combined with controlled transport through self-assembled channels. This is despite gap junctions, which are important for cell-to-cell communication, displaying both inter- and intramembrane assembly (Figure 1). A connexin gap junction between adhering cells is composed of two abutting hemichannels (connexons) in each cell, which are themselves hexameric assemblies of six protein transmembrane subunits termed connexins. Docking between hemichannels gives a high conductance cell-to-cell channel that allows rapid intercellular communication.<sup>4</sup>

Combining controlled transit across vesicle bilayers with the controlled adhesion of these channels to other membranes will give several advantages. As well as providing drug delivery vehicles that will target particular cell types and deliver drugs on demand, there is the deeper understanding that will result from studying the interplay between intramembrane assembly and intermembrane adhesion, processes that require multivalent binding and the high effective concentrations produced in membranes.

**1.1. Self-Assembled Pores.** Classical methods for forming channels or pores in phospholipid bilayers use the assembly of molecular scaffolds through or across the membrane. Many elegant examples of self-assembled pores exist, with assembly across the membrane exemplified by the octiphenyl  $\beta$ -barrels of Matile and co-workers<sup>5</sup> and through-membrane assembly exemplified by the cyclic peptides of

Ghadiri and co-workers.<sup>6</sup> To add a switching mechanism, a soluble agent that will trigger pore assembly or block the pore entrance can be added, and some carefully designed recent examples have been developed into sensors.<sup>2</sup> However the chemical agent should be removable to “switch-off” compound release, which remains a challenge. Furthermore, to be applicable in biochemical and biomedical contexts, the chemical switching mechanism should be nontoxic to cells and orthogonal to most biological triggers.

**1.2. Thermal Pores.** Vesicles composed of saturated phospholipids like dimyristoyl phosphatidylcholine (DMPC) and dipalmitoyl phosphatidylcholine (DPPC) “melt” at a defined temperature (the  $T_m$ ), which is accompanied by a large increase in membrane permeability and rapid escape of the vesicle contents. Vesicle poration is ascribed to disorder at boundaries between coexisting solid ordered ( $s_o$ ) and liquid ordered ( $l_d$ ) phases allowing the passage of water-soluble compounds through the bilayer. Interestingly, recent studies have indicated that ion conduction events at these phase boundaries appear similar to those observed for membrane-embedded protein channels.<sup>7</sup> Such thermally sensitive vesicles were first used by Yatvin and co-workers to deliver neomycin to *E. coli* cells<sup>8</sup> and methotrexate to murine tumors,<sup>9</sup> while more recently Ca(II) release has been used to trigger biomaterial formation.<sup>10</sup> At first glance, thermal release appears to be less sophisticated than forming self-assembled pores, but this method of release does have some advantages. It does not require the time-consuming synthesis of complex pore-forming molecules, and nanoparticles can be used to noninvasively generate local hyperthermia. In particular, the unique characteristics of nanosized magnetite (superparamagnetism) and gold (plasmon resonance) can convert alternating magnetic fields or near-infrared irradiation into heat. This heat then permeabilizes membranes in proximity to the nanoparticles by membrane melting<sup>11</sup> or microbubble cavitation.<sup>12</sup>

**1.3. Membrane–Membrane Adhesion.** The cross-linking of phospholipid vesicles into conglomerates has been investigated both as a mimic of the cell adhesion process<sup>13</sup> and to produce *in vivo* drug delivery platforms that deliver drugs selectively to the lymph nodes.<sup>14</sup> Unmodified phospholipid vesicles are kinetically stable, although they will undergo fusion if the membranes are brought into close proximity; to successfully cross-link intact vesicles, the adhesive groups must project a sufficient distance from the vesicle surface. The interactions used to mediate adhesion range from the relatively weak, such as histidine-copper(II) coordination,<sup>15</sup> to the relatively strong, like biotin–avidin.<sup>16</sup>



compared to chloroform, which was ascribed to partitioning of **2** and geometric constraints exerted by the membrane. The large diameter of the desired membrane channels suggested small dyes might pass through them, such as 5/6-carboxyfluorescein (5/6-CF,  $10 \times 6.5$  Å) which can be easily measured through the alleviation of self-quenching. Addition of  $4 \mu\text{M}$   $\text{PdCl}_2(\text{PhCN})_2$  **2** to vesicles encapsulating 5/6-CF and doped with 0.5 mol % porphyrin **1** in the membrane ( $0.5 \mu\text{M}$ ) released 5/6-CF at a rate 12-fold greater than that from undoped vesicles. Unexpectedly, both lower and higher concentrations of **2** were less effective at triggering 5/6-CF release. Intramembrane speciation profiles were calculated from the **1** + **2** binding constants, which suggested that  $\sim 4 \mu\text{M}$  Pd(II) produced the highest fraction of Pd/porphyrin oligomer in the membrane, with higher concentrations dissociating these oligomers into monomers that were presumed to be ineffective transporters.<sup>20</sup>

Other researchers have found complex ionophoric behavior using “molecular squares”. Fyles and Tong found adding 4,4'-bipyridine to Pd(II)-capped lipids gave ion channel-like activity, but analysis of the conductance data suggested that the desired “molecular square” was not formed in the membrane. Instead, extended aggregates of Pd(II)-lipid and phospholipid were suggested to be responsible for the long-lived and highly conducting channel openings observed.<sup>21</sup> Instead of in situ assembly, Boccalon, Iengo, and Tecilla preassembled kinetically inert Re(I)-dipyridylporphyrin “molecular squares” in solution prior to addition to the membrane. These Re(I)-porphyrin squares displayed good ionophoric activity, with data analysis suggesting that dimerization of these tetramers produced membrane-spanning channels. Although assembly/disassembly of these Re(I) channels could not be used to modulate activity, ionophoric activity was halted by the addition of dendrimers of the right size to block the entrance of Re(I)-dipyridylporphyrin channels. This is an elegant combination of multiple assembly processes; assembly of tetramers in solution, through-membrane dimerization and inclusion of dendrimers into these membrane-bound channels.<sup>22</sup> Alternatively, rather than metal-mediated assembly of “molecular squares”, an interesting recent example by Gokel and co-workers used Cu(II)-mediated assembly to create “molecular capsules” in the membrane that acted as cation selective channels.<sup>23</sup>

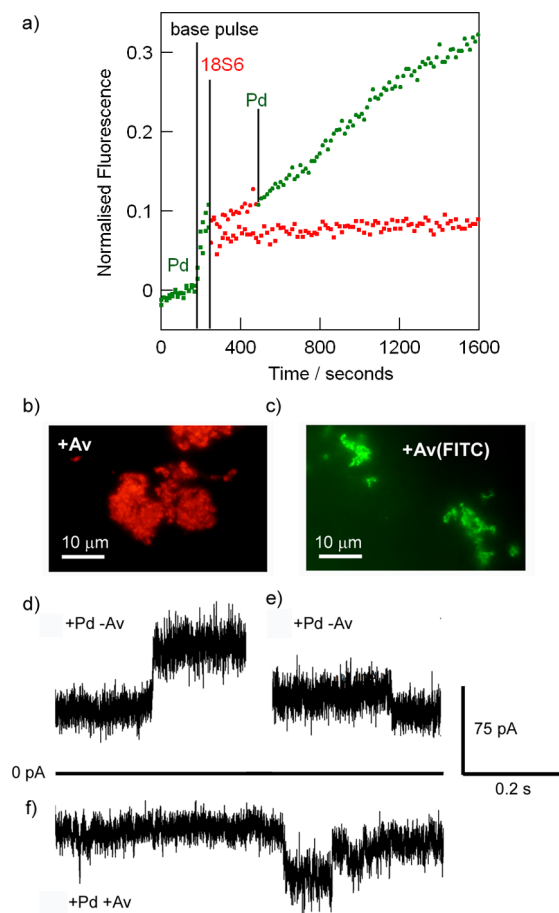
**3.2. Cholate Pores.** Due to the difficulty in synthesizing porphyrins like **1**, poor membrane incorporation and problems characterizing membrane-active species, our attention turned to developing the bischolate ion channels of

Kobuke et al. into self-assembled adhesive channels.<sup>24</sup> These membrane-spanning bischolates form  $\text{K}^+$  selective tri- or tetrameric ion channels in phospholipid bilayers. By splitting these dimers into pyridyl terminated cholates (Figure 2d), we hoped that palladium(II) would reversibly bring together the halves to create membrane-spanning ion channels. As with the porphyrins, there was the potential to functionalize the terminal carboxylates with adhesive groups.

The hydroxypyrene trisulfonate (HPTS) assay was used to measure  $\text{H}^+/\text{Na}^+$  antiport across the membrane, but this assay was incompatible with **2** and required  $\text{PdCl}_2$  **5** to be used. At a loading of 1 mol % ( $10 \mu\text{M}$  in solution), the HPTS assay showed that **3** was unable to transport sodium ions.<sup>25</sup> However the addition of  $5 \mu\text{M}$   $\text{PdCl}_2$  switched on a  $>9$ -fold increase in  $\text{Na}^+$  transport. As with porphyrin **1**, binding assays in solution gave estimates of the affinity of palladium(II) for membrane-bound **3**, confirming that membrane-spanning dimeric complexes should form at these concentrations. To dissociate the complex and switch off  $\text{Na}^+$  transport, nonbasic thia-crown 18S6 was carefully selected to only interact with Pd(II) and not otherwise perturb the system. Indeed the addition of an equivalent of the thia-crown stopped  $\text{Na}^+$  flow through the membrane, consistent with Pd(II) extraction and the channel disassembly (Figure 3a). This was reversed by the addition of further palladium(II) to reform the channels.

Having demonstrated intramembrane assembly/disassembly, the next step was to append adhesive groups to channel-forming lipid **3**. Elaboration of the terminal carboxylate of pyridyl cholate **3** with a short triethyleneglycol spacer terminated with biotin gave adhesive channel **4**.<sup>26</sup> As expected, the addition of avidin (0.5 equiv) to vesicles doped with **4** (1 mol %) rapidly produced vesicle aggregates (Figure 3b,c). The formation of these aggregates was insensitive to the presence of palladium(II), showing the orthogonality of the intra- and intermembrane assembly processes. Ion transport by **4** + Pd(II) was measured using both HPTS assays and planar bilayer conductances (PBC). Under the same conditions used with **3**, HPTS assays revealed that monomeric **4** did not significantly transport  $\text{Na}^+$  but adding Pd(II) to **4** gave a 4-fold increase in  $\text{Na}^+$  transport across the membrane. PBC measurements on the mixture of Pd(II) and **4** showed step-changes in conductance that are consistent with ion channel formation (Figure 3d–f). However, both HPTS assays and PBC measurements showed that avidin complexation to the termini of **4** + Pd(II) decreased ion flux. Subsequent studies suggested the decrease in net

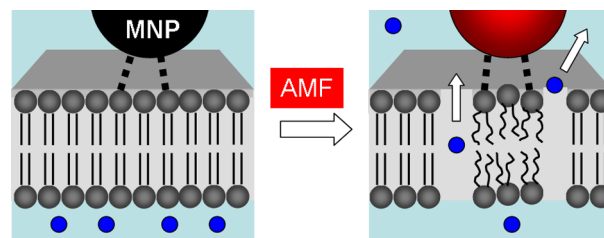




**FIGURE 3.** (a)  $\text{Na}^+$  transport after the addition of NaOH (base pulse at 180 s) to HPTS vesicles mixed with **3** and  $\text{PdCl}_2$  (solid green square). 18S6 addition gave closed channels (solid red square and circle, indicated by 18S6 at 240 s). To one sample, addition of further  $\text{PdCl}_2$  (at 480 s, indicated by Pd) reopened the channels (solid green circle). (b) Representative epi-fluorescence micrographs of aggregates formed by mixing rhodamine labeled vesicles doped with **4** with 0.5 equiv of avidin or (c) fluorescein labeled avidin. (d, e) Current–time recordings from PBC studies on **4** +  $\text{PdCl}_2$  that show (d) multiple channel openings and (e) channel closing. (f) PBC studies on **4** +  $\text{PdCl}_2$  + avidin show typical channel opening and closing. Adapted with permission from refs 25 and 26.

ion flow could result from several effects, such as avidin blocking the channel entrances or preventing the cholates from adopting conformations that permit ion transit (avidin binding sites are 30 Å apart).

Biotinylated pyridyl-cholate **4** exhibits multiple levels of orthogonal self-assembly, with through-membrane assembly mediated by  $\text{Pd(II)}$  and intermembrane assembly mediated by avidin. The challenges in using a modular approach to create self-assembled channels are also illustrated; as the precursors become more functionalized, the outcome of self-assembly becomes more uncertain. Linking vesicles together into aggregates should produce



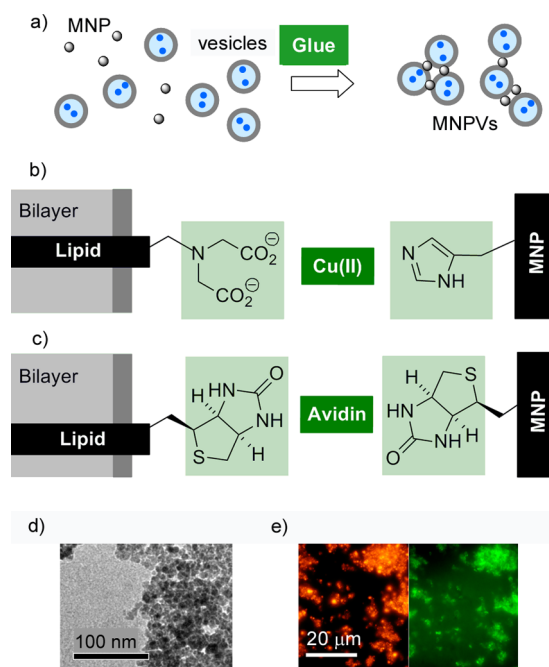
**FIGURE 4.** Schematic representation of magnetic nanoparticles interacting with thermally sensitive phospholipid bilayers. An AMF signal generates heat in the nanoparticles that melts the bilayers; disorder at the boundaries between  $s_0$  and  $l_d$  phases allows water-soluble compounds to pass through the bilayer.

new functional materials, yet maintaining control over ion channel activity will be a challenge. Furthermore in the case of **1**, **3**, and **4**, more biocompatible chemical switches than  $\text{Pd(II)}$  are required to interface these materials with the biological world.

## 4. Adhesive Nanoparticles: Magnetic Opening of Thermal Pores

**4.1. Magnetic Hyperthermia.** The heating of magnetic nanoparticles by an applied alternating magnetic field (AMF) is an exciting application that has been touted as a potential cancer treatment, with cancerous cells destroyed by magnetic nanoparticles within the tumors.<sup>27</sup> The appeal of magnetic hyperthermia lies in the noninvasive nature of the AMF and the transparency of biological materials to magnetic signals, although the amount of thermal energy required to kill all cancer cells remains a limitation. However the amount of heat required for drug release from thermally sensitive vesicles (Figure 4) is much lower, for example, DPPC bilayers have a  $T_m \sim 42^\circ\text{C}$ , just  $5^\circ\text{C}$  above body temperature. This was illustrated in 2002 by Babin-cova et al., who used an AMF to release doxorubicin from thermosensitive DPPC vesicles incorporating magnetic nanoparticles.<sup>28</sup>

Magnetoliposomes, phospholipid vesicles with incorporated magnetic nanoparticles, have also been applied as magnetically targeted drug delivery vehicles<sup>29,30</sup> and as imaging agents.<sup>31</sup> To create magnetoliposomes, magnetic nanoparticles can either be inserted into the vesicle membrane (e.g., using hydrophobic particles or naked nanoparticles) or they can be encapsulated within the vesicle lumen as a ferrofluid. Ménager and co-workers have exploited both methods to prepare vesicles for in vivo magnetic targeting and remote control of drug release.<sup>32,33</sup> The entrapment of magnetic nanoparticles in the lumen of vesicles appears to be the most popular



**FIGURE 5.** (a) Generalized scheme for the formation of MNPVs. (b,c) Adhesive interactions used to link magnetic nanoparticles (MNP) to vesicles. (d) TEM image of biotinylated MNP. (e) Fluorescence microscopy images of MNPVs cross-linked by FITC-avidin, vesicles are labeled with 0.1 mol % rhodamine lipid (left, rhodamine channel; right, fluorescein channel). Adapted with permission from ref 47.

method currently, and has been used to release 5/6-CF within rat forearm skeletal muscle<sup>34</sup> or deliver doxorubicin to cancer cells;<sup>35,36</sup> in both the latter cases, the vesicles were folate-labeled to target cells that overexpressed the folate receptor.

We wondered if adhesive nanoparticles attached to the bilayer could be used as pore-forming agents instead of adhesive lipids embedded in the bilayer. We chose to use reversible supramolecular interactions to link nanoparticles to membranes, in the hope that this link would be reversible. Furthermore these particles should act as cross-linkers and produce magnetically sensitive nanoparticle/vesicle conglomerates (Figure 5a).

#### 4.2. Magnetic Nanoparticle–Vesicle Assemblies (MNPVs).

Previously, we had mediated adhesion between vesicles through multiple weak interactions, specifically the coordination of polyhistidine<sup>37</sup> or histidine-capped lipids<sup>38</sup> to copper(iminodiacetate) (Cu(IDA))-capped lipids ( $K \sim 10^3 \text{ M}^{-1}$ ). Given that histidine-coated nanoparticles are also multivalent ligands, we believed that they would adhere to vesicles doped with Cu(IDA)-capped lipids (Figure 5b).

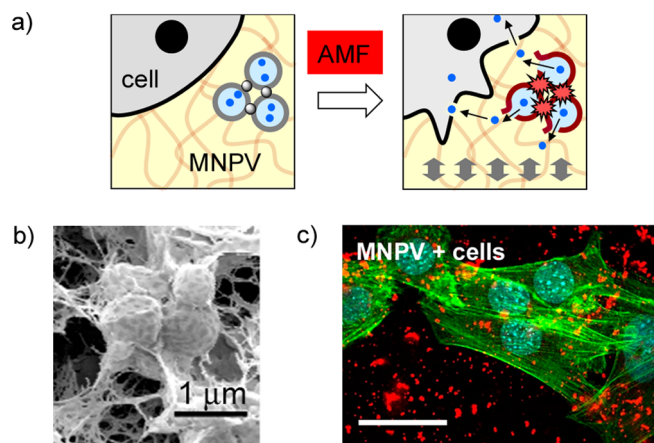
Magnetite nanoparticles around 10 nm diameter were selected (Figure 5d), as Fe<sub>3</sub>O<sub>4</sub> exists in the superparamagnetic regime in this size range and efficiently converts an AMF

(ca. 400 kHz) into heat. The strength of the catechol–iron oxide interaction is well-established, and a histidine-dopamine conjugate was used to coat ~80% of the magnetic nanoparticle surface.<sup>39</sup> Mixing these histidine-coated magnetic nanoparticles with vesicles bearing Cu(IDA)-capped lipids resulted in the formation of large (20–100 μm diameter) magnetic nanoparticle-vesicle aggregates (MNPVs). The weak adhesive interaction was reversible and the addition of EDTA extracted the copper(II) “glue” and dissociated MNPVs into their constituent parts. MNPVs could be magnetically manipulated with strong NdFeB magnets into patterns of condensed vesicle aggregates, mimicking the high densities of adhering cells in tissue,<sup>39</sup> while magnetic sedimentation could be used to purify MNPVs that encapsulated proteins like cytochrome *c*.<sup>40</sup>

Vesicles composed of 1:10 DMPC/DPPC were aggregated by magnetic nanoparticles to give thermally sensitive MNPVs. Tests showed complete release of stored 5/6-CF or the anticancer drug methotrexate occurred at the melting temperature of 37 °C. Similarly a fluorescently tagged polysaccharide (4 kDa FITC-dextran), a model for difficult-to-release small proteins and polysaccharides, was also released, albeit more slowly. The release of cytotoxic drugs like methotrexate highlights the potential of MNPVs as in vivo drug delivery vehicles, with the potential to utilize both magnetic positioning and AMF-induced release to noninvasively deliver drugs to selected regions in the body.<sup>32</sup>

**4.3. Applications of Magnetic Release: MNPVs in Cell Culture.** “Smart” biomaterials are another area of biomedical science where MNPVs should make an impact. If the cross-linking of vesicles in MNPVs is reminiscent of cellular adhesion in tissue, the addition of an extravesicular matrix is a further analogy that adds structural reinforcement and produces new biomaterials (Figure 6a).

Several biocompatible hydrogels were screened, although those requiring large changes in temperature, pH, or ionic strength to gel were excluded as they might compromise the vesicles. Calcium alginate was found to gel the surrounding solution without the gel fibrils penetrating and disrupting the vesicles excessively. The integrity of the MNPVs was verified by cryo-scanning electron microscopy (Figure 6b) and by encapsulating 5/6-CF; only 3% leakage was observed after 1 h. The alginate matrix did not significantly affect the  $T_m$  of the MNPVs, although the rate at which compounds escape from these MNPV-containing gels (MNPV-gels) was slowed due to diffusion through the hydrogel. Exposure to a 400 kHz AMF produced 100% release of



**FIGURE 6.** (a) Scheme showing cells and MNPVs coimmobilized in a hydrogel (yellow). Chemical messengers, such as drugs (blue), can be noninvasively released by an AMF to induce responses from cultured cells. (b) Representative cryo-ESEM micrograph of an MNPV embedded in alginate gel. (c) Confocal microscopy image of myoblasts and rhodamine-labeled MNPVs coimmobilized in alginate (scale bar 25  $\mu\text{m}$ ), with fluorescent staining of nuclei (blue) and f-actin (green). Adapted with permission from refs 44 and 47.

encapsulated 5/6-CF after 600 s, which was more effective than external heating; heating an MNPV-gel to 40 °C produced  $\sim 50\%$  5/6-CF release after 900 s.<sup>41</sup> The surrounding solution was only warmed by 2 °C after 600 s, suggesting that the thermal energy was localized around the MNPVs in the gel. An alginate gel containing separated thermally sensitive vesicles and magnetic nanoparticles did not release 5/6-CF upon exposure to an AMF, confirming the importance of the adhesive link.

Using MNPV-gels with cells required several modifications. Preliminary experiments indicated that Cu(IIA)-capped lipids had significant cell toxicity, while cell culture conditions (37 °C) melt MNPVs composed of 10:1 DPPC/DMPC. Using the stronger and more biocompatible biotin–avidin interaction (Figure 5c) to aggregate vesicles had good literature precedent,<sup>16</sup> while DPPC vesicles melt 5 °C above cell culture conditions. Coupling biotin to dopamine provided a coating agent that biotinylated magnetic nanoparticles with  $\sim 50\%$  efficiency, although these particles were more hydrophobic and more difficult to disperse in buffer. Biotinylated DPPC vesicles were created using low loadings (0.2 mol %) of commercially available biotinylated phospholipids. Interestingly, this biotin coating led to vesicle–fibroblast adhesion,<sup>42</sup> probably through biotin-recognizing receptors in the cell membranes.<sup>43</sup> Addition of the avidin “glue” to a mixture of biotinylated nanoparticles and vesicles produced large MNPVs, with colocalization of both vesicles and avidin (Figure 5e). The biotinylated MNPVs were mixed

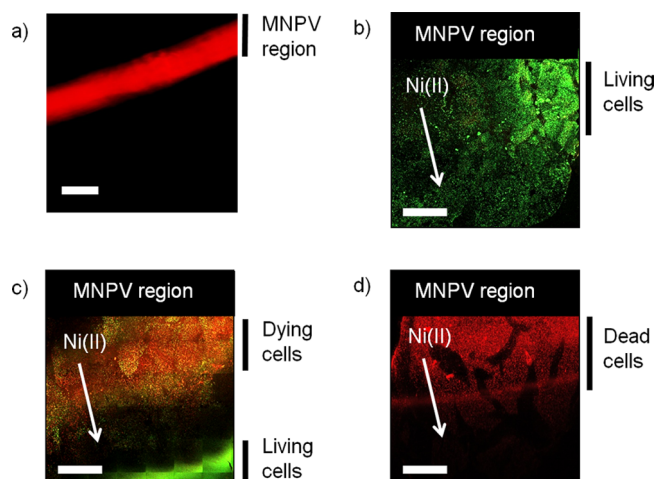
with cells (if required) and immobilized in calcium alginate to afford the prototype biomaterial (Figure 6c); biocompatibility with anchorage dependent cells like fibroblasts could be improved by adding fibronectin.<sup>44</sup>

Initially a simple chemical messenger/cellular response was sought. Cell death is an easy to measure cellular response, so we chose cytotoxic nickel(II) to induce apoptosis in a dose dependent manner.<sup>45</sup> As with 5/6-CF, nickel(II) was released from an MNPV-gel block by an AMF signal, albeit with lower efficiency. Nonetheless the concentration in the gel block was calculated to be at a level expected to induce apoptosis ( $\sim 300 \mu\text{M}$ ). Fibroblasts coimmobilized with Ni(II)-containing MNPVs in alginate supplemented with fibronectin were found to proliferate normally, with a 25% increase in cell number. However magnetic triggering by an AMF allowed stored Ni(II) to cross the MNPV membranes and diffuse through the surrounding gel, resulting in a dramatic decrease in cell viability. Cell number and metabolic activity both plummeted, and after 24 h more than 85% of the cells in the hydrogel had died. Imaging studies revealed cell degradation and confirmed magnetically induced apoptosis in the gel.<sup>44</sup>

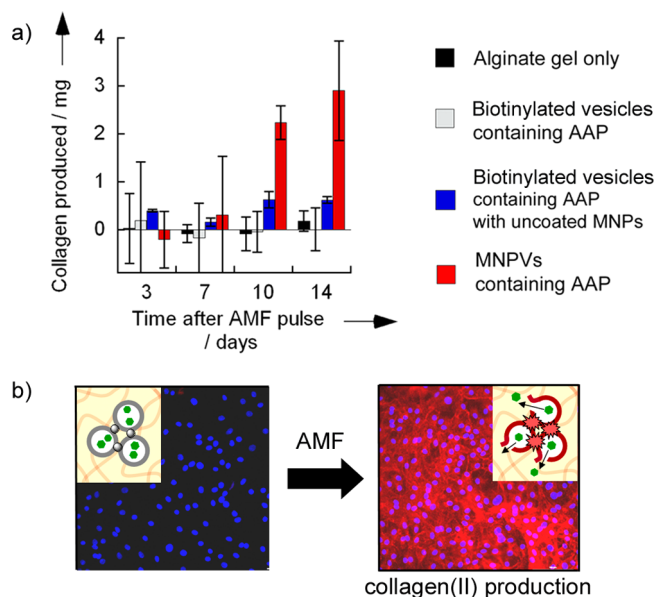
The slowed diffusion of nickel(II) ions through alginate gels was exploited to illustrate how magnetically patterned materials might facilitate spatiotemporal control over cellular responses. Patterns of Ni(II)-containing MNPVs in alginate were produced by placing a strong NdFeB permanent magnet at one end of the cell culture chamber before calcium(II) infusion. The patterned gels were then subjected to 400 kHz AMF pulses to release the Ni(II) ions from the MNPV region. Live/dead assays across the gel revealed a “zone of death” that progressed from the MNPV-containing region, with the closest cells dying within 12 h, followed by cell death throughout the gel after 24 h (Figure 7).<sup>44</sup> Such magnetic patterning might be generalized to other messenger/cell pairs, providing the released compounds only become uniformly distributed in the gel after a cell response has occurred.

The magnetic induction of cell death in these biomaterials shows the potential of MNPVs for delivering cell-killing drugs, but to achieve their potential in tissue engineering the magnetic signal should alter the actions of living cells. Chondrocytes encased within calcium alginate have shown promise as engineered scaffolds to replace articular cartilage, as they produce glycosaminoglycans and collagen II.<sup>46</sup> For chondrocytes to produce extracellular collagen, either ascorbic acid or ascorbic acid-2-phosphate (AAP) is an essential additive in the media.





**FIGURE 7.** (a) Magnetically patterned MNPVs (rhodamine labeled) in alginate. Scale bar 500  $\mu\text{m}$ . (b–d) Live–dead assays on fibroblasts cultured in alginate containing a pattern of Ni(II)-containing MNPVs (no fluorescent label). Images taken (b) 1 h, (c) 12 h, and (d) 24 h after exposure to an AMF. Scale bars 2.5 mm. Reproduced with permission from ref 44.



**FIGURE 8.** (a) AMF-induced production of collagen by chondrocytes: (solid black) alginate only; (solid gray) AAP-containing biotinylated vesicles in alginate; (solid blue) AAP-containing biotinylated vesicles with uncoated MNP in alginate; (solid red) AAP-containing MNPVs in alginate. (b) Confocal microscopy images of alginate gels containing chondrocytes and MNPVs, with fluorescent staining of cell nuclei (blue) and collagen II (red). Adapted with permission from ref 47.

The chondrocyte/AAP combination appeared to be well suited to our MNPV-gels, as chondrocytes show good tolerance for alginate hydrogels while AAP is a polar and air-insensitive precursor that can be encapsulated within MNPVs. As with Ni(II) and 5/6-CF, AAP was shown

to be magnetically released by an AMF, giving an AAP concentration in the gel block of  $\sim 400 \mu\text{M}$ ; high enough to induce a strong cellular response.<sup>47</sup>

Chondrocytes and AAP-containing MNPVs were immobilized within alginate in glass vials and exposed to a 400 kHz AMF pulse. Collagen production by the chondrocytes in the gel was measured over 14 days using the Sircol collagen assay, which showed that, after an initial lag period, the chondrocytes responded to the magnetic release of AAP by producing collagen at levels comparable to literature reports (Figure 8).<sup>48</sup> The magnetically induced generation of collagen by chondrocytes was also visualized using fluorescence microscopy, with large amounts of extracellular collagen I and collagen II visible in samples exposed to an AMF. In contrast, little cell response was observed without an adhesive link between nanoparticles and vesicles. The collagen penetrated the alginate gel to form a mesh around the chondrocytes, showing the potential of these MNPV-hydrogels for the noninvasive production of cartilage-like extracellular matrices.

## 5. Concluding Remarks

The combination of controlled release and intermembrane adhesion is likely to remain a fruitful area of research in the future. This Account describes our efforts to combine these processes, aiming not only to improve our understanding of inter- and intramembrane self-assembly in a biomimetic environment, but also to develop constructs that can be employed in a biomedical context.

Applying the inter- and intramembrane assembly of synthetic channels to cellular membranes may provide antibiotics able to combat pathogens using a synergistic combination of known processes; synthetic ion channels can be effective antibacterial agents,<sup>1</sup> while the aggregation of pathogens by multivalent ligands, like IgM, aids clearance by the reticuloendothelial system.<sup>49</sup> Recently, “sticky” magnetic nanoparticles adhering to targeted ion channel proteins have been shown to allow magnetic control over transit across cell membranes, with channels coaxed open by exposure to static<sup>50</sup> or alternating magnetic fields.<sup>51</sup> In future, thermosensitive semisynthetic or self-assembled pores could be appended to magnetic nanoparticles, which when combined with the remarkably efficient aggregation of cells by “sticky” magnetic nanoparticles<sup>52</sup> could produce magnetically controlled cell–cell communication.

Combining vesicle–cell intermembrane adhesion with controlled transport from vesicles into cells has great



biomedical prospects, yet the ability to recharge vesicles with encapsulated compounds is an important goal that is yet to be achieved. This would allow the delivery of compounds to cells on multiple occasions, without the repeated administration of drug-loaded vesicles. Such recharging will require the ingress of chemical precursors into the vesicles from the surrounding solution and the conversion of these precursors into impermeable species by vesicle-bound enzymes. Creating these vesicular “nanofactories” would be a key advance, an advance that requires synthetic pores able to tightly control entry and exit from vesicles.

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## BIOGRAPHICAL INFORMATION

**Simon Webb** is Senior Lecturer in Chemistry at the University of Manchester. Dr. Webb completed B.Sc. and M.Sc. degrees at the University of Auckland, before undertaking Ph.D. studies with Prof. J. K. M. Sanders at the University of Cambridge. Postdoctoral periods in Nijmegen (with Prof. R. J. M. Nolte) and Sheffield (with Prof. N. H. Williams and Prof. C. A. Hunter) followed, before he moved to Manchester in 2002. Dr. Webb's interests currently lie in the use of molecular self-assembly to create systems that mimic biological membranes and improve our understanding of important biological processes.

## FOOTNOTES

\*E-mail: S.Webb@manchester.ac.uk.

The author declares no competing financial interest.

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