MRI contrast agent delivery using spore capsules: controlled release in blood plasma†

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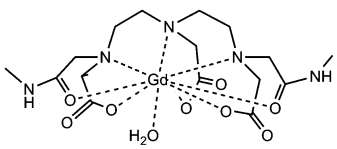
The exine coatings of spores can be used to encapsulate drug molecules. We have demonstrated that these microcapsules can be filled with a commercial gadolinium(III) MRI contrast agent (in this proof of concept study Gd–DTPA–BMA was used) which is slowly released in plasma due to enzymatic digestion of the capsule.

There is considerable interest in the development of technologies for the targeted delivery and slow release of drugs *in vivo*.[1,2] Sporopollenin forms a microcapsule which constitutes the outer layer of the pollen grains of mosses and ferns. The spores are treated to remove the inner layers of cellulose leaving a non-immunogenic exine composed of sporopollenin, see Fig. 1.[3–5] Facile methods have been developed for loading the internal cavity by either compressing the sporopollenin or using reduced pressure to fill them with a solution of the desired compound.[6,7]



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Although sporopollenin is extremely robust,[8] enzymes present in human plasma are thought to partially digest the capsule releasing the contents. These microcapsules could potentially have useful application in the encapsulation and release of magnetic resonance imaging (MRI) contrast agents (CAs). For example, in magnetic resonance angiography a longer retention time of the CA in the vasculature is required for equilibrium imaging. Such blood pool agents have been produced by structural modification for protein binding[9] or increasing the size to give macromolecular agents.[10] Another approach is the formation of small biodegradable polymeric particles containing CAs that are slowly released. They have been produced for general blood pool imaging or targeting artherosclerotic plaques.[11,12] Sporopollenin capsules could offer a cheap, readily available alternative to these methods. These are large particles for intravenous injection, see Fig. 1, however, although preliminary studies suggest no ill effects due to rapid digestion, they may be more applicable to oral delivery of the encapsulated agent where it has been shown that the particles can be dosed and transported intact into the blood.[13]

The aims of this work are to determine if sporopollenin can be loaded with a commercial CA, whether detection of individual particles is possible using MR microimaging experiments at 11.74 T and to characterise the release profile over time of the CA. Spores of *Lycopodium clavatum*, which have a diameter of *ca.* 25 μm, were chosen as they are the best characterised of the available sporopollenin. Metal salts and nanoparticles have previously been incorporated onto the inner surface of these spores.[6] There are also functional groups on the outside of the spore which can form complexes with metal ions. Our strategy is to encapsulate pre-formed metal complexes rather than bind 'free' metal ions, which will provide a non-toxic compound on release of the capsule contents. The commonly used clinical CA Omniscan™ was used as a representative example; it is a bis-amide DTPA chelated gadolinium(III) contrast agent that is a neutral complex.‡ The particles were immersed in a solution of Omniscan™ and a vacuum applied to load the particles with the contrast agent solution. In order to minimise the amount of complex on the surface, loaded sporopollenin exines were then washed repeatedly with deionised water. The loading level of the CA was determined by ICP-OES to be 0.80% by

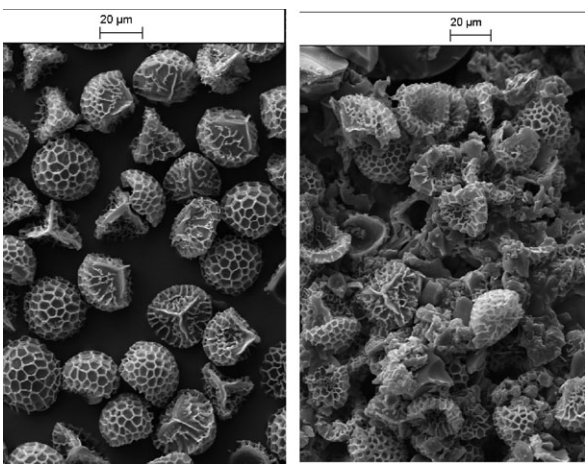

Fig. 1 SEM pictures of the sporopollenin before (left) and after (right) incubation in plasma for 30 min. The partial digestion can clearly be observed which would allow release of the capsule contents.

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† Electronic supplementary information (ESI) available: Movie of microimaging experiment, experimental data. See DOI: 10.1039/b909551a

mass of CA, which represents a 32 fold 'dilution' of the initial contrast agent solution.

To probe the loaded capsules, a microimaging experiment was carried out at 11.74 T (see movie in ESI†). The sporopollenin exines were suspended in agar, a gel monolith separated and 3D spin echo images acquired. The gel monolith was transferred to a 1 mm diameter capillary tube and then placed inside a solenoid coil. The water in the gel can be clearly seen against the empty background of the 2 mm coil and the bright spots within the gel represent the spores containing the contrast agent, see Fig. 2. The voxel size was 23 μm × 15 μm × 15 μm and the spore capsule diameter *ca.* 25 μm. The images show bright areas representing the contrast enhancement due to the shortening of relaxation time of protons on the water encapsulated in the spore along with the contrast agent. Some appear too large to be a single particle and so it is likely that many of the bright areas represent clumps of sporopollenin capsules that were not fully dispersed. The control imaging experiment was carried out with the sporopollenin treated in an identical manner with deionised water containing no contrast agent, and these capsules were not detectable.

Having demonstrated that the sporopollenin could be loaded with the contrast agent and detected using microimaging, the next step was to monitor CA release from the spores *in vitro* using human plasma. Firstly, we obtained further data on the digestion of the sporopollenin particles in plasma. Previous experiments have suggested that enzymatic digestion occurs in human blood despite the extreme physical robustness of these particles.[14] It is also proposed that the required enzymes are present in plasma but not serum. We monitored the effect of digestion of sporopollenin particles in human plasma for a period of 30 min by collecting SEM images before and after, see Fig. 1. It can clearly be seen from these images that some of the sporopollenin particles are partially digested on this timescale, offering a route to release of the spore contents. Further work is required to specifically identify which enzymes are responsible. Experiments were then carried out to monitor the release of the contrast agent in fresh human plasma.

The relaxivity of a solution containing sporopollenin was monitored by comparing the intensity of the MR image to a control. A two tube experiment was designed where the inner tube contained a layer of sporopollenin at the bottom (*ca.* 5 mg) and either plasma or a buffered aqueous solution (control) was added to both the inner and outer tubes. Cross sectional images of the solution were then recorded

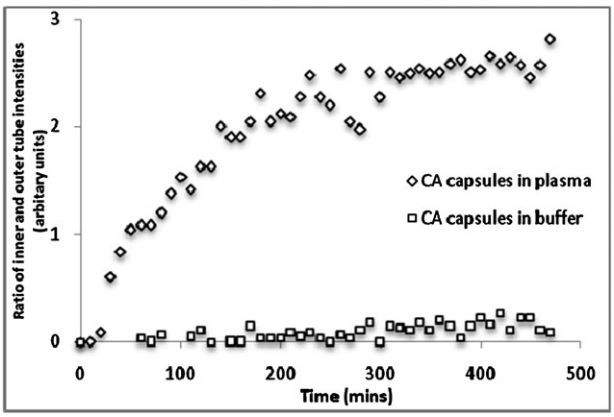

Fig. 3 A plot showing release of the contrast agent over 8 hours. A maximum is reached after *ca.* 4 hours for the sample in plasma and no significant release is observed for the control sample.

every 10 minutes over a period of 8 hours. Each image was analysed with identically sized 'regions of interest' in both the inner and outer tubes where an average voxel intensity was determined. The ratio of intensities between the inner and outer tube was plotted *vs.* time showing the release profile of the plasma sample, see Fig. 3.

A significant increase in contrast was observed for the CA in the plasma sample, with the majority of release occurring over *ca.* 4 hours. The samples in buffered solution showed only a small variation in the intensity ratio over the timescale of the experiment, suggesting little or no release of CA, and clearly demonstrates that plasma initiates the release of the encapsulated MR contrast agents. The best results were obtained after allowing the sporopollenin capsules to settle to the bottom of the tube.§ The *in vitro* release profile correlates well with biodegradable polymers that show complete release over *ca.* 5 hours.[10]

In conclusion, these compounds have considerable potential in the delivery and release of drugs. They offer a cheap and accessible alternative to more complex biodegradable polymers which show a similar release profile. We are currently investigating the mechanism of the sporopollenin decomposition to determine precisely which enzymes are involved. *In vivo* studies are being designed to determine the efficacy of the CA loaded spore capsules for imaging. We are also investigating the encapsulation of other metallodrugs and radiopharmaceutical imaging agents containing metal centres.[15–17]

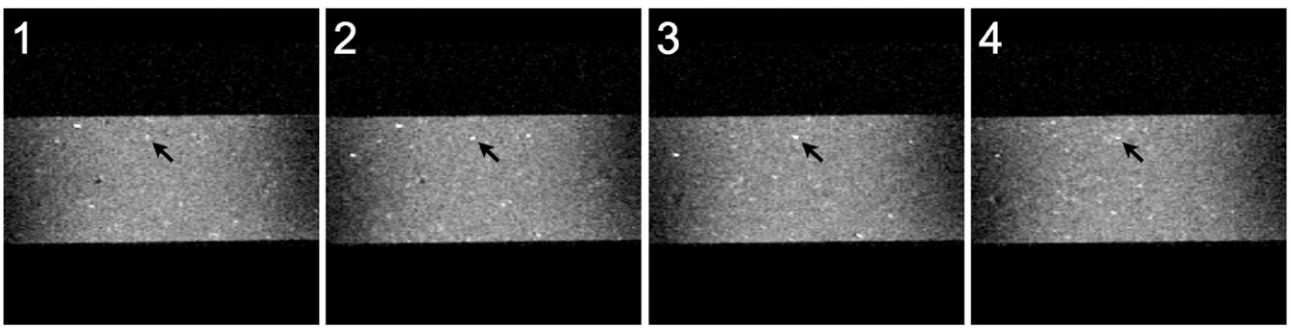

Fig. 2 Four consecutive slices through the agar gel monolith containing Omniscan™ encapsulated in spores. Each frame has dimensions of 3 × 3 mm. A spore capsule clump that appears in several consecutive slices is highlighted with arrows.

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Notes and references

‡ Omniscan was selected because it was the agent used clinically by the local hospital. The reported issues with systemic nephrogenic fibrosis and gadolinium CAs may be related to the lower stability of the acyclic chelator in this agent.[18] Although the agent has not been withdrawn, macrocyclic CAs are a more stable alternative. This study represents a proof of principle and any CA can be incorporated by the same methods.

§ A suspension of sporopollenin particles initially forms when either the plasma or phosphate buffer solution is added. To allow this to settle out without interfering with the image, the samples were allowed to stand at 4 °C for 30 min prior to the imaging experiment. The lowered temperature was sufficient to inhibit the digestion of the particles. To confirm that the CA was released intact, a sample was allowed to digest in plasma for a longer time period (48 hours), filtered and then left to stand at rt for one week prior to analysis by mass spectrometry.

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