Acid-degradable solid-walled microcapsules for pH-responsive burst-release drug delivery†

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Received 1st October 2010, Accepted 8th November 2010
DOI: 10.1039/c0cc04190d

Acid-degradable microcapsules were prepared *via* an interfacial polymerization. Degradation of the thin wall of the capsules leads to all-or-nothing cargo release. The only byproducts of degradation are acetone, and a non-toxic triamide. Proof-of-concept experiments showed that cargo can be delivered to and released in cells.

Microspherical delivery vehicles have found use in applications as diverse as printing, agriculture, adhesives, immunology, and chemotherapy.[1] For biological delivery, ideal vehicles have small tunable sizes, high loadings of cargo, facile and scalable preparation, and good biocompatibility.[2] It is also important that delivery vehicles be responsive to their local environment such that they retain their cargo until they reach their target, whereupon complete release should take place. Acidic,[3–5] reducing,[6,7] and oxidizing[8,9] environments are commonly exploited as triggers for this kind of delivery.

Liquid-filled microcapsules (MCs) are a promising architecture for biological delivery. The mass of encapsulated material is very high relative to that of the thin wall material, allowing for high cargo loading. Additionally, "all-or-nothing" burst-release kinetics can be achieved if chemical responsiveness is built into the construction of the wall (Fig. 1a).[1,10] Many formulations of liquid-filled MCs have been studied but few, if any, combine favorable encapsulation properties, triggered cargo release, and biocompatibility.[1,11,12] There have been recent reports of environmentally responsive MCs, but in each case, complete biocompatibility cannot be assured because polymers remain after the capsules degrade.[7,13]

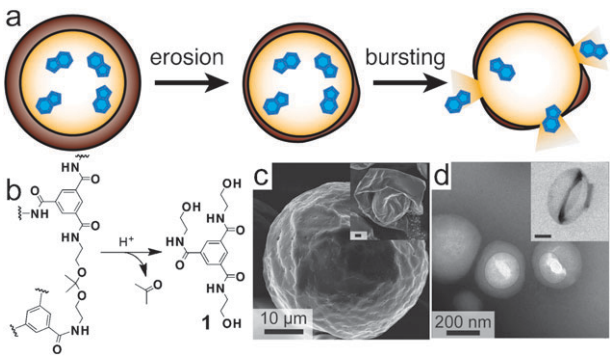

Fig. 1 (a) MC degradation leads to burst-release *via* wall erosion, which occurs by (b) acid-catalyzed ketal hydrolysis, generating only acetone and triamide **1** as byproducts. SEM (c) and TEM (d) images reveal smooth spherical capsules at two scales. Images of ruptured or folded capsules (insets) support a liquid-filled architecture. The thickness of MC walls is estimated to be 2–7% of the overall diameter.

In pursuit of the ideal MC material, we decided to work with polyamides formed by the reaction of acid chlorides and amines. We chose this reaction because it is fast, scalable, and its monomers are flexible enough to allow for the introduction of responsiveness. Acid-degradability was conferred to capsules using a ketal-containing diamine.[14] This made capsules that are stable under ordinary physiological conditions, but quickly release their cargo once inside the acidified endosome of a cell. Additionally, the ketal moiety has previously been shown to be biocompatible and useful for biological delivery applications.[4,15]

Millimetre-sized capsules were synthesized in accordance with the literature precedent.[16] An emulsion was formed by rapidly stirring toluene containing trimesoyl chloride (TM) and water with 0.3% polyvinyl alcohol (PVA) as a surfactant. Next, water containing an acid-sensitive diamine, diethylaminoketal (DEAK), was added.[14] Capsules formed instantly and could be isolated and purified by filtration and thorough rinsing with acetone and ether. The desired burst response was then tested by observing the capsules after the addition of acidic water (Movie S1, ESI†). As expected, rapid degradation and complete dispersal of encapsulated material occurs upon exposure to acidic environments.

Having proven the concept of acid-rupturable capsules, the next obstacle was to tune the capsules to a size appropriate for biological applications. Before optimizing other conditions, the internal phase of the capsules was changed from toluene to capric/caprylic triglyceride, an FDA-approved oil obtained from coconut oil.[17] Emulsifying by homogenization yielded capsules on the order of tens of microns (Fig. 1c). Making smaller capsules required increasing the concentration of PVA to 3% and using sonication in place of homogenization. This yielded capsules measuring *ca.* 300 nm (Fig. 1d and Fig. S1, ESI†). SEM and TEM images show smooth spherical capsules. Images of ruptured capsules support that they are thin-walled and hollow. These smaller capsules were used for all future biological assays.

The biocompatibility of any delivery vehicle intended for internal use is of critical importance to its eventual success. The cytotoxicity of the acid-degradable MCs was measured in HeLa epithelial cells and RAW 264.7 macrophage cells using a cellular viability assay.[18] It was found that the capsules were nontoxic up to concentrations of 1 mg mL^{-1} (Fig. S2, ESI†). This is comparable to other well-known biocompatible materials.[3] Importantly, the two products resulting from

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† Electronic supplementary information (ESI) available: Experimental procedures, cytotoxicity assays, and degradation plots. See DOI: 10.1039/c0cc04190d

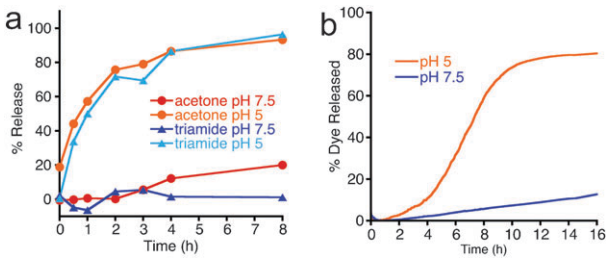

Fig. 2 (a) Normalized ^{1}H integrations of acetone and triamide **1** from MCs. Degradation occurs over the first 8 h at pH 5. (b) Release of an encapsulated dye from MCs at pH 5 features a lag from wall degradation associated with erosion occurring prior to burst release.

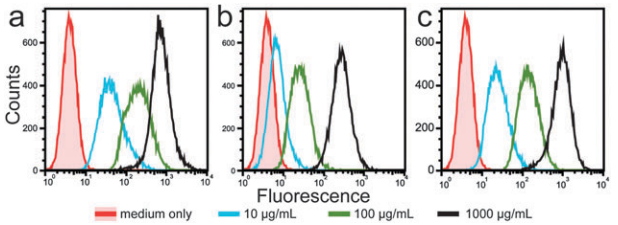

Fig. 3 Flow cytometry analysis of uptake of 1000, 100, and 10 μg mL^{-1} of emulsion (a), non-degradable MCs (b), and degradable MCs (c).

complete ketal hydrolysis—acetone and triamide **1** (Fig. 1b)—are also biocompatible. Acetone is a secondary metabolite and an authentic sample of triamide **1** was found to be nontoxic in the same two cell lines.

Next, the degradation behavior of capsule walls was measured under physiological conditions. Capsules were suspended in deuterated buffers at 25 °C at pH 5 or pH 7.5 and the evolution of their NMR spectra was observed (Fig. S3 and S4, ESI†). As the polymeric wall degraded, peaks corresponding to acetone and triamide **1** could clearly be seen to grow in the spectra. The integrals of these peaks were normalized to an internal standard and plotted *versus* time (Fig. 2a). At pH 5, the apparent half-life of degradation is roughly 1 h; at pH 7.5, it is roughly 25-fold longer (Fig. S5, ESI†). This may be ideal for physiological delivery because degradation should be rapid in endosomes while a half-life of 25 h might still avoid toxic bioaccumulation. The release of acetone appears to be at the same rate and stoichiometry as triamide **1**, in line with the putative structure of the polymer.

The kinetics of cargo release from these MCs might be expected to be different from that of the polymeric wall material. To assay this, capsules loaded with a hydrophobic fluorescent dye were suspended in a quartz cuvette containing $CHCl_3$ and water. Capsules selectively rested at the interface between phases and dye release could be determined by measuring the UV absorbance of the organic phase (Fig. 2b). After the experiment was concluded, the absorbance of complete dye release was determined by adding acid to fully destroy capsule walls. No absorbance increase could be found in the aqueous phase, indicating that all of the dye partitioned to the organic phase. Interestingly, at pH 5 the dye released from capsules after a lag phase of roughly 4 h. This corresponds to the point at which most of the wall material has been degraded. Once wall failure begins to occur, the dye is more rapidly released from the MCs. At pH 7.5, dye release was much slower and lacked a distinct lag phase over 2 d (Fig. S6, ESI†). This absence of a lag phase is presumably because as the capsule walls degrade they become more permeable and slow leaching occurs. Importantly, no dye release was observed from nondegradable MCs, indicating that observed release was, in fact, due to particle degradation, and not desorption or leaching.

Because the mechanism of payload delivery for these capsules is based on the acidification of cellular endosomes, it is important to confirm that they are taken up into cells in a predictable and uniform way. Degradable and non-degradable capsules containing a fluorescent dye were prepared. The non-degradable capsules were prepared using terephthaloyl chloride and diethylene triamine in place of TM and DEAK. Additionally, a fluorescent emulsion was prepared as a control using the same mixing method as the capsules, but without acid chloride or amine. This generated a stable emulsion that lacked the solid wall of the capsules. All samples were incubated with RAW 264.7 macrophage cells overnight. It was hypothesized that these cells would take up samples through phagocytosis. The cells were then trypsinized and washed by repeated cycles of centrifugation and resuspension in pH 7.4 PBS to remove any surface-bound material. After fixing cells, they were analyzed by flow cytometry (Fig. 3). Cells incubated with fluorescently labeled samples significantly increase in overall fluorescence, indicating that the capsules are taken up. Importantly, the increase is uniform throughout the population of cells. Also encouraging is the dose-dependence of the fluorescence increase in cells: a 10-fold decrease in concentration corresponds to a similar decrease in cell-associated fluorescence. This dependence, coupled with having trypsinized and repeatedly washed of cells, supports that MCs have been internalized into cells, rather than being adsorbed onto the surface of cells.

As a proof-of-concept experiment to demonstrate delivery and function of a model drug in cells, capsules containing paclitaxel were prepared and the *in vitro* cytotoxicities of loaded capsules were measured. Capsules were co-incubated with cells for 24 h and cellular viability assays were performed (Fig. 4). As expected, non-degradable capsules do not lead to significant cell death regardless of the encapsulant. Oil emulsified in water leads to significant toxicity also independently of the encapsulant, presumably due to disruption of cellular membranes. In contrast, acid-degradable capsules only lead to cytotoxicity when loaded with paclitaxel. This implies that capsules effectively deliver their payload into cells. If degradation were occurring before internalization, their toxicity would

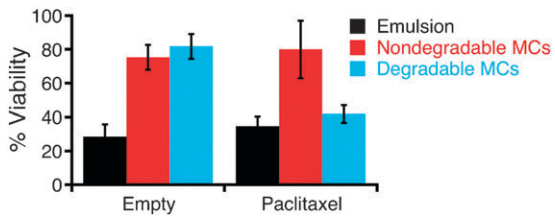

Fig. 4 Cellular viability assays measuring cytotoxicity of empty and paclitaxel-loaded emulsions, non-degradable MCs, and degradable MCs.

resemble that of the emulsion. Conversely, no toxicity would be observed if they were internalized but not released.

In conclusion, we have prepared acid-degradable microcapsules capable of delivering their cargo in an environmentally responsive manner. These capsules have a predictable pH-dependent degradation, and neither they nor their byproducts are inherently toxic. They are taken up well in cells and specifically deliver their payload. Based on these characteristics, these capsules may prove to be a useful tool to the growing field of drug delivery.

We thank U.S. Department of Energy BES (DE-AC02-05CH11231) for funding, Jungwon Park for assistance with TEM acquisition, and Ann Fisher at the UC Berkeley Cell Culture Facility.

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