Biolistic Delivery of MOF-Protected Liposomes

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Abstract

Needle-and-syringe-based delivery has been the commercial standard for vaccine administration to date. With worsening medical personnel availability, increasing biohazard waste production, and the possibility of cross-contamination, we explore the possibility of biolistic delivery as an alternate skin-based delivery route. Delicate formulations like liposomes are inherently unsuitable for this delivery model as they are delicate biomaterials incapable of withstanding shear stress. When encapsulated within a crystalline and rigid coating made of zeolitic imidazolate frameworks, the liposomes are not only protected from thermal stress but also shear stress. The protection from shear stress is crucial, especially for formulations with cargo encapsulated inside the lumen of the liposomes. Moreover, the coating provides the liposomes a solid, rigid exterior which allows the particles to penetrate the tissue model and porcine tissue effectively.

Introduction

Biomaterials for healthcare applications have seen a paradigm shift over the last few decades. Targeting specific pathways to mitigate illness while minimizing side effects has prompted research to move toward developing more sophisticated delivery systems. Viral vectors for gene therapy and drug-loaded mesoporous silica are a few examples of compound therapeutics and prophylactics used in medicine today. Amongst these delivery systems, lipid-based platforms have stolen the limelight in the last few years, as lipid nanoparticle mRNA vaccines mRNA-1273 (Moderna) and BNT162b2 (BioNTech) hit the clinic to curb the spread of Covid-19. Liposomes are another example of lipid-based delivery vehicles often used to deliver immunostimulatory agents into the body and are used in commercially available vaccines like Cervavix, Epaxal, and Shingrix.

Lipid-based nanocarriers are not without issues, however. They are not stable for prolonged periods at room temperature and are susceptible to aggregation, leaky packing, or lipid fusion into multilamellar structures. They are shipped and stored within an expensive logistical network called “the cold chain”, which helps maintain strict environmental controls on the medication—some even requiring −80°C freezers for the duration of their trip from manufacturer to clinic. The cold chain not only significantly increases the cost of vaccines but is also a problematic barrier to accessibility in places with limited or fluctuating power supply. Developing a method to ship these vaccines without refrigeration can help combat the problem. One promising approach is coating easily degradable biomacromolecules in metal-organic frameworks (MOF). MOFs are coordination polymers that are stable at higher temperatures and have been reported to stabilize biomaterials in their matrix while preserving...
the integrity of the biological system. Zeolitic Imidazolate Frameworks (ZIF) are amongst the most well-studied MOFs for their potential in medicinal applications. They have bio-friendly synthetic conditions and have been reported to successfully encapsulate a wide array of biomaterials—ranging from small molecule drugs,\textsuperscript{7, 8} to nucleic acids,\textsuperscript{8, 9} and microorganisms.\textsuperscript{11, 12} They can be stored in a dry powder-like form, and the crystals can protect the encapsulated material against heat and other stress factors faced during long-distance transportation.\textsuperscript{13}

Another issue brought to the forefront in the last few years is the low healthcare personnel per capita numbers in highly populated areas of the world. Most vaccinations today are delivered via syringe and needle, requiring technical skills that cannot be transferred to the general population or volunteers quickly. Disposable needles result in biohazardous waste, and their accidental (or intentional) reuse spreads blood-borne pathogens worldwide. While intramuscular injections are more straightforward than intravenous injections, they are prone to neurovascular injuries. On the other hand, subcutaneous injections can result in the permeation of formulations parallel to the subcutaneous region instead of perpendicular, depending on the speed of administration.\textsuperscript{14} This can result in the formation of misshapen depots of drug or vaccine in the layers of skin, resulting in a different release pattern than anticipated. The general aversion to needles—especially in children—may deter vaccinating via both these administration routes. Attempting to mitigate these challenges makes investigating alternate vaccine administration methods an enticing research prospect.

Biolistic delivery is an alternative route of vaccine administration worthy of exploration, as its entry path is through the skin, mimicking a needle-syringe-based route. This technique involves firing micro-projectiles into a target using controlled mechanical force via a ballistic particle delivery system. Once optimized, biolistic delivery systems can deliver materials with the same speed and pressure in every repetition, offering an advantage over injection-based systems where the speed and accuracy rely on the medical professional. While the traditional use of biolistic delivery was aimed at plants for gene transfection,\textsuperscript{15-17} in the last couple of decades, several groups have shown that materials delivered biologically can penetrate the epidermis and lodge themselves in the dermis region effectively.\textsuperscript{18, 19} Gold or tungsten microparticles are commonly used for cargo loading in commercially available gene guns. The materials are loaded on the outside and hence exposed to shearing forces from the pressure of the gun. Lipid-based formulations tend to leak under shear stress, as previously demonstrated\textsuperscript{20} by Natsume and Yoshimoto—a finding we confirm in our investigation below. Without any protective outer coating, liposomes cannot be delivered via gene guns. Traditional gene guns also have the added financial burden of requiring a continuous supply of gold for preparing "bullets", which are both unsuitable for lipid-based formulations and very expensive to implement on a large scale.

Considering the above factors, we have developed an inexpensive biolistic delivery system called a "MOF-Jet" to deliver materials in solid powder and liquid suspension form.\textsuperscript{21} Although versatile, this system was initially designed to provide sensitive materials encapsulated in MOFs. In this work, we demonstrate that we can exploit biolistic routes of administration to deliver liposomal platforms in ZIF irrespective of surface charge, where ZIF provides biomaterial protection against shear forces and adequate mechanical strength to penetrate through the skin (Scheme 1). We hypothesized that liposomes might be challenging to deliver through the skin biologically due to their bubble-like construction. Their poor resistance against vibration,\textsuperscript{22} shear stress, and lack of rigidity might cause the liposomes to leak or not penetrate
the surface effectively. Liposomes of various surface charges were successfully protected in ZIF for biolistic delivery and shown that neither the processes of ZIF encapsulation nor the biolistic delivery disrupts the liposome's size or lamellarity. Furthermore, we show that the ZIF shells provide the liposomes with adequate rigidity to penetrate the tissue model and a significant population of the particles lodge themselves in the dermis level.

Scheme 1: Comparative studies between pristine and ZIF-coated liposomes demonstrate the mechanical stability of the ZIF coating. Pristine liposomes leak under the shear stress of the MOF-Jet, while ZIF-coated liposomes remain protected and negligible leaking is observed. The hard coating of the ZIF allows the coated liposomes to penetrate into the tissue model, while the pristine liposomes just pool on the surface.

Results and Discussion

To develop a protection mechanism that could be applied to various lipid-based vaccines, we aimed to test the consistency of ZIF coatings on different types of liposomes. A library of liposomes demonstrating a variety of surfaces was synthesized using a mixture of charged, uncharged, and PEG-conjugated lipids and were correspondingly named cationic (Cat), neutral (Neu), anionic (Ani), and PEGylated (PEG). The liposomes were characterized using Transmission Electron Microscopy (TEM) (Fig 1A-D) and Dynamic Light Scattering (DLS) (Fig 4F-I "pristine") to confirm their ~200 nm size range and unilamellar nature. ζ-potential (Fig 1G) of all liposomes was measured to ensure that varying the lipid compositions afforded us the variety in surface charge, which would help us test ZIF crystal growth on different nucleating surfaces. Cy5 encapsulated versions of all four liposomes were also synthesized (named Cy5@Cat/Neu/Ani/PEG), whose fluorescence spectra (Fig 1E) were recorded as well. Since Cy5@Lip was prepared by adding Cy5 to the buffer in which the liposomes were prepared, the excess unencapsulated dye was subsequently removed from the supernatant post ultracentrifugation at 160,000 ×g to obtain a light blue pellet (Fig 1F).
To demonstrate the versatility of ZIF encapsulation, all four liposomes were encapsulated in ZIF using four different ligand/metal (L/M) molar ratios that were varied in the synthetic conditions for encapsulation. The zinc acetate precursor’s final concentration was kept fixed at 20 mM, while the L/M ratios tested were 4, 8, 16 and 32. Each of these conditions were tested for a reaction time of 3 h and 24 h. The choice of optimal conditions aimed to maximize the liposomes’ encapsulation efficiency (Fig 2A-D) while minimizing adhesion of unencapsulated liposomes to free ZIF (Fig S1). At higher L/M ratios of 16 and 32—especially in a 24 h setup—we observe excellent encapsulation efficiencies regardless of the surface charge. We selected these two L/M ratios for further investigation and characterized them using Powder X-Ray Diffraction (PXRD) (Fig 2E) and Scanning Electron Microscopy (SEM) (Fig 2F). The PXRD of all liposomes encapsulated in L/M 16 and 32 indicate sodalite crystal structure of the products. While literature suggests that biomimetic mineralization is most successful when the nucleating surface has an anionic charge, our fine-tuned conditions allow the encapsulation of neutral and even cationic liposomes. When Cy5@Lip were encapsulated inside ZIF, the composite exhibited fluorescence as visualized by epifluorescence microscopy (Fig 2G).
Figure 2: (A-D) Encapsulation efficiencies of Cy5@Cat, Cy5@Neu, Cy5@Ani, and Cy5@PEG liposomes at various L/M ratios. Each of the liposomes were encapsulated in ZIF by adding ZnOAc and HMIM such that the L/M ratios were 32, 16, 8, and 4—as labeled on the x-axis. All conditions were measured for encapsulation efficiencies after 3 hrs (orange) and 24 hrs (yellow). Measurements were performed by quantifying the percentage of unencapsulated Cy5@Lip in the supernatant using fluorescence. Error bars are represented as ±SD. (E) PXRD pattern of Neu@Z-32 and simulated ZIF-8. (F) SEM micrograph of Neu@Z-32. Scale bar = 1 µm. (G) Epifluorescence micrograph of Neu@Z-32. Scale bar = 100 µm. No significant differences were observed in the PXRD patterns, SEM micrographs, and epifluorescence micrographs of Neu@Z-32, Cat@Z-32, Ani@Z-32, PEG@Z-32, Neu@Z-16, Cat@Z-16, Ani@Z-16, and PEG@Z-16.

Our investigation aimed to determine if the ZIF coating is necessary and beneficial in protecting the liposomes from shear stress damage. To test that, we compared the biolistic delivery of pristine and encapsulated liposomes delivered through the MOF-Jet (Fig S2). Initial attempts to biolistically deliver pristine liposomes into a vial showed no significant change in size upon applying mechanical pressure when measured through DLS (Fig S3). Next, we tested how the shear stress would affect leaking of components encapsulated inside the liposome (dye, in our case). A common approach in drug and vaccine development research is to encapsulate small molecule drugs or adjuvants inside the lumen of the liposomes; preventing leaking in such cases is crucial to delivering the expected dose. We used a cone-and-plate viscometer at four different shear rates to quantify the shear stress applied. As the shear stress was increased on the viscometer, we observed higher leaking of the encapsulated dye. This was done by encapsulating 5,6-carboxyfluorescein (CF)—a self-quenching dye—inside the liposome and quantifying the leaking using fluorescence intensities (Fig S4). The dye inside the liposomes is quenched owing to its high concentration, and as CF leaks out, it regains fluorescence. We observed that the relationship between shear stress and leaking was linear; thus, we used these measurements as a standard to quantify the shear stress applied by the MOF-Jet at various pressures on the delivered material (Fig S5). We replicated the experiment performed using the viscometer, but this time using the MOF-Jet. We not only observed the linear relationship between shear stress and leaking again, but also found that almost 35% of the encapsulated dye leaked at 4137 kPa. We encapsulated CF@Lip in ZIF using L/M 16 and 32 and compared their leaking at different pressures with that of unprotected CF@Lip. Both of the ZIF-encapsulated formulations showed negligible leaking, indicating that the coating was very effective in preventing escape of the encapsulated cargo (Fig S6).

It is worth noting that prevention of cargo leaking is not the only function the ZIF coating serves when used as a protective coating for biolistic delivery. The ZIF crystals provide the desired structural rigidity—absent in pristine liposomes—required for effective penetration of the
particles into the tissue. We performed preliminary experiments to qualitatively assess the difference between penetration profiles of Cy5@Lip and Cy5@Lip@Z. Upon delivering unencapsulated Cy5@Lip into a 2% agarose tissue model, the liposome suspension simply pooled on the agarose gel surface without any significant penetration (Fig S7). When repeated with Cy5@Lip@Z, particles embedded inside the gel were visible when cross-sectioned and imaged under the epifluorescence microscope (Fig S8). While this assessment indicates that particles penetrate well with the help of the ZIF coating, it is crucial to know how the payload would distribute across the epidermis and dermis when delivered into the skin. We repeated the biolistic delivery of Cy5@Lip@Z on the agarose tissue model (Fig 3A) and on porcine skin tissue (Fig 3B), but this time imaged the cross-sections and obtained a Z-stack on a confocal microscope. Even though 2% agarose is a known and published tissue model mimicking the density of soft tissue, its elasticity and toughness are poorly represented by the gel. There is a noticeable difference in penetration depth of both when compared side-by-side. The fluorescence of the Cy5@Lip@Z population was integrated at each depth to obtain a density plot for both samples (Fig 3C-D).

Figure 3: (A-B) Maximum intensity projection of the confocal Z-stack image of Cy5@Neu@Z-32 delivered biolistically into agarose tissue model and porcine skin tissue. Cy5@Neu@Z-32 was first delivered into a 2% agarose gel tissue model at 2068 kPa with a nozzle-to-gel distance of 2 cm, and into porcine skin tissue at 4826 kPa with a nozzle-to-tissue distance of 0.5 cm. The sample was then flipped onto a clean surface to avoid dragging the particles deeper into the gel, and cross-sectioned using a sharp blade. The cross-sectioned specimen was placed on a #0 coverslip with the top surface (depth = 0 mm, marked with a purple line) on the reader’s left, and confocal micrographs were captured. Scale bar = 1 mm. (C-D) 3-D population density plot representing the distribution of Cy5@Neu@Z-32 within the gel and tissue calculated from the overlay image shown in Fig 3A and 3B, respectively. The plots were generated using Fiji (image processing and analysis software).

There is extensive research on the use of ZIF coatings for biomaterials used in drug and vaccine delivery models since the kinetic lability of the ZIF coating makes it easy to remove in the presence of a chelating agent, acidic pH, or a phosphate-rich environment. As ZIF is known to degrade in tissues over several days to two weeks, there is promising application scope for this technique in pharmaceutics. To confirm that the process of encapsulation and removal of the ZIF coating does not damage the liposomes, we chemically "exfoliate" the ZIF coating from all the Lip@Z variants using EDTA as a chelating agent. The recovered liposomes were characterized using TEM and were all observed to be a similar size as
observed prior to encapsulation (Fig S9). When a ZIF-protected vaccine is to be delivered biolistically in a clinical setting, other stress factors are also at play. To simulate such a series of events, we (i) encapsulated our liposomes in ZIF, (ii) biolistically delivered them into 5% type A gelatin ballistic gel, (iii) melted the gel at physiological temperatures, and (iv) exfoliated the ZIF coating using EDTA to recover the liposomes back (Fig 4A). The choice of 5% gelatin A was based on its low melting temperature and its ability to withstand 2068 kPa pressure on the MOF-Jet. The recovered liposomes were characterized for their lamellarity and size range using TEM (Fig 4B-E) and DLS (Fig 4F-I). We were satisfied to observe that the liposomes remain unilamellar, and their final size was close to that of the pristine formulations. There were some very low intensity peaks under 50 nm observed at times with the DLS, but we suspect them to be small aggregates of salts and gelatin still remaining in solution. This once again demonstrates the outstanding preservation of delicate biomaterials that the exterior ZIF coating can provide—robust enough to even deliver through a pressurized biolistic delivery system.

**Figure 4:** (A) Experimental scheme illustrating the steps of the recovery experiment—(i) encapsulation of liposomes in ZIF, (ii) biolistic delivery of Lip@Z using MOF-Jet into 5% gelatin A ballistic gel at 2068 kPa with a nozzle-to-gel distance of 2 cm, (iii) removal of gelatin by melting, centrifuging at 37°C, washing twice, and (iv) chemical exfoliation of ZIF coating using 0.5M EDTA as a chelating agent at 4°C left overnight on a rotisserie. (B-E) TEM micrographs of Cat, Neu, Ani, and PEG liposomes after the recovery experiment were performed using Cat@Z-32, Neu@Z-32, Ani@Z-32, and PEG@Z-32, respectively. Scale bar = 200 nm for all micrographs. No significant differences were observed in the TEM micrographs when the recovery experiment was performed using Cat@Z-16, Neu@Z-16, Ani@Z-16, and PEG@Z-16. (F-I) DLS graphs of Cat, Neu, Ani, and PEG liposomes in pristine form (blue), after recovery from Lip@Z-16 (green) and Lip@Z-32 (magenta). Lip represents Cat/Neu/Ani/PEG in each respective graph. The X-axis of all graphs represents liposome size in nm.

**Conclusion**

In this work, we explored the mechanical protection ZIF provides to liposomes as a preliminary investigation for using biolistic delivery as an alternative to syringe-and-needle-based delivery of vaccines. We demonstrated that a wide variety of surfaces of delicate biomaterials could be coated using ZIF using the right conditions, and this coating can be just as easily removed—without causing any damage to the protected material. The protective coating
prevented the liposomes from leaking cargo and helped in effective penetration of the sample when delivered into the tissue model and porcine skin tissue. Together, all these conclusions lead us in a very promising direction of future research where the clinical relevance of biolistic delivery could be further investigated without much concern for damage caused to the biomaterials in the process.

**Materials and Methods**

**Chemicals**

18:0 PC (DSPC) 1,2-distearoyl-sn-glycero-3-phosphocholine, 18:1 TAP (DOTAP) 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt), 18:1 (Δ9-Cis) PE (DOPE) 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, 18:1 PA (DOPA) 1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt) and 18:0 PEG2000 PE 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy (polyethylene glycol)-2000] (ammonium salt) were purchased from Avanti Polar Lipids. MOPS 3-(4-morpholino) propane sulfonic acid, TCEP tris(2-carboxyethyl) phosphine, sodium chloride and chloroform were purchased from Fisher Scientific. Synthesis and characterization of cyanine-5-carboxylic acid used has been reported by Luzuriaga et al.\textsuperscript{11} Zinc acetate dihydrate, 2-methylimidazole, sodium hydroxide, 5(6)-carboxyfluorescein and gelatin type A (from porcine skin) were purchased from Millipore Sigma. Porcine tissue was purchased from Sierra for Medical Science. EDTA ethylenediaminetetraacetic acid was purchased from Cole-Parmer. Agarose (molecular biology grade) was purchased from Research Products International.

**Instruments**

Liposomes were extruded using Avanti Mini Extruder. Cy5@Lip was pelleted down using Sorvall MX-120 micro-ultracentrifuge. Fluorescence spectra of Cy5@Lip was recorded using Horiba Fluorolog fluorimeter. DLS measurements for size and zeta potential of liposomes were carried out using Malvern Analytical Zetasizer Nano ZS. TEM micrographs of pristine, exfoliated and recovered liposomes were taken using JEOL 1400 transmission electron microscope. Fluorescence intensity measurements for calculation of encapsulation efficiency, surface attachment and leaking of liposomes were carried out using Biotek Synergy H4 Hybrid microplate reader. SEM micrographs of Lip@Z were taken using Zeiss Supra 40. PXRD spectra was determined using Rigaku SmartLab X-ray diffractometer. Shear stress was applied on the CF@Lip using Anton Paar Modular Compact Rheometer 302. Epifluorescence images of Cy5@Lip in agarose, Cy5@Lip@Z both in solution and in agarose were taken on EVOS FL digital inverted fluorescence microscope. Z-stack penetration depth images were taken on Olympus FV3000 RS confocal microscope.

**Synthesis of liposomes**

Liposomes were synthesized by first preparing a thin film of 37.5 mg DSPC with 12.5 mg of DOTAP for cationic, DOPE for neutral, DOPA for anionic, and PEG-2000-PE for PEGylated. The lipids were dissolved in 2 ml chloroform and dried out in vacuum conditions. The lipid thin film was then resuspended in 2 ml M-buffer (100mM NaCl, 1mM TCEP, 20mM MOPS) and freeze-thawed 4-5 times. In the case of Cy5-encapsulated liposomes, the resuspension M-buffer also included 0.05 mg/ml Cy5-Carboxylate. In CF-encapsulated liposomes, the resuspension M-buffer had 7.525 mg/ml 5,6-carboxyfluorescein, and 4M NaOH was added dropwise to the resuspension M-buffer till the dye completely dissolved. The liposomes were then extruded serially through 1 um, 400 nm, and 200 nm membrane filters to obtain
monodisperse liposomal suspensions of around ~200 nm size. For the Cy5-encapsulated liposomes, the excess Cy5 in the supernatant was eliminated by two rounds of ultracentrifugation at 160,000 x g, 4°C, 45 mins, following which the supernatant was exchanged with fresh M-buffer each time.

Encapsulation in ZIF

Each of the liposome combinations were encapsulated in four different formulations of ZIF. The final concentration of reagents in each encapsulation is described in the table below. After adding reagents, the formulations were incubated for 24 h and then washed twice. For washing, the formulations were centrifuged at 4000 x g for 15 mins at RT, and the supernatant was exchanged with DI water. After washing, Lip@Z was dried under vacuum conditions overnight to obtain a crystalline solid. Encapsulation of Cy5@Lip followed the same procedure.

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<th>Lip@Z Formulation</th>
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<th>Concentration of Lipids (mg/ml)</th>
<th>Concentration of 2-Methylimidazole (mM)</th>
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Leaking studies and estimation of shear stress from MOF-Jet

200 µL of CF@Lip suspension was loaded onto the bullet of the MOF-Jet and delivered at 1379 kPa (200 PSI) into an empty scintillation vial. The process was repeated with 2758 kPa (400 PSI) and 4137 kPa (600 PSI). Each biolistically delivered sample was diluted 20× and measured for its fluorescence intensity against pristine CF@Lip of the same batch diluted 20×. To quantify the shear stress from the MOF-Jet, 200 µL of CF@Lip was sheared on a cone-and-plate viscometer at 350/s, 700/s, 1050/s, and 1400/s respectively for 60s at 25°C. All the samples were collected after shearing, diluted 20×, and their fluorescence intensity was quantified for leaked dye.

Estimation of encapsulated and surface-attached liposomes

The first supernatant obtained upon centrifugation after ZIF encapsulation was measured for its fluorescence intensity to calculate the encapsulation efficiency of the liposomes in ZIF. This was compared to the fluorescence intensity at the start to compare and estimate the percentage of liposomes that remained unencapsulated and were left behind in the supernatant. After two rounds of washing with DI water and drying the Lip@Z samples under vacuum overnight, they were resuspended in methanol to dissolve the surface-attached liposomes. The supernatant obtained upon centrifugation was measured for its fluorescence intensity. This was compared to the fluorescence intensity obtained by drying the same volume of liposome suspension added to the reaction mixture under vacuum and resuspending in methanol to compare and estimate the percentage of liposomes that electrostatically attached themselves to the ZIF crystals and could not be removed by washing with water.

Preparation of ballistic gels
For agarose gels, 2% agarose solution was heated and then cast in a cylindrical mold (diameter = 1 in, height = 0.5 in) and cooled at room temperature till solidified. 5% gelatin A solution was heated and cast in the same mold for gelatin gels. Both gels were stored in a closed container at 4°C for future use.

Penetration depth study

For the tissue model, 3 mg of Cy5@Lip@Z was loaded onto the bullet of the MOF-Jet and delivered into 2% agarose gel at 2068 kPa (300 PSI) and nozzle distance of 2 cm from the top gel surface. For porcine tissue, 3 mg of Cy5@Lip@ZIF was loaded onto the bullet of the MOF-Jet and delivered into porcine tissue 4826 kPa (700 PSI) and nozzle distance of 0.5 cm from the top tissue surface. Each respective sample was flipped upside down and longitudinally sectioned to avoid dragging particles along the blade. The section was laid flat on a #0 coverslip for confocal microscope imaging.

Exfoliation and recovery from ballistic gel

For the control exfoliation, Lip@ZIF obtained from 1 ml reaction volume was exfoliated using 2 ml of 0.25M EDTA at 4°C for 18h on a rotisserie.

For the recovery experiment, Lip@Z was loaded onto the bullet of the MOF-Jet and delivered into 5% gelatin A gel at 2068 kPa (300 PSI) and nozzle distance of 2 cm from the top gel surface. The gel was then cut into 2-3 pieces and carefully transferred to a 15 ml centrifuge tube. The samples were centrifuged for 15 mins at 4000 xg at 37°C, and the supernatant was exchanged with DI water. This washing process was repeated twice to ensure minimal gelatin remained in the vial. The extracted Lip@ZIF was exfoliated using the procedure above.

Author Contributions

S.K. optimized the synthesis of Lip@Z, executed the encapsulation efficiency, leaking, penetration depth and recovery experiments, and performed PXRD characterizations. Y.H.W. performed TEM characterizations and aided with penetration depth experiments. T.S.H. performed SEM characterizations. F.C.H. performed SEM characterizations and assisted with recovery experiments. A.R. and I.T. helped in the synthesis of liposomes and ZIF encapsulation. J.G. (under the guidance of N.J.D.) aided with obtaining confocal Z-stack images. S.K. and J.J.G. composed the manuscript. J.J.G. conceived the project. All authors have read and approved the final version of the manuscript.

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