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Metal–Organic Framework Coatings as Cytoprotective Exoskeletons for Living Cells

Kang Liang, Joseph J. Richardson, Jiwei Cui, Frank Caruso, Christian J. Doonan,* and Paolo Falcaro**

Studying isolated living cell systems is essential to understanding complex biological functions,[1] and has led to their application in cell therapy, diagnostics, drug screening, and the dairy and beverage industries.[2–4] Cells are typically encased by a lipid bilayer membrane that offers limited protection ex vivo, thus long-term protection and preservation strategies are often required.[5,6] To overcome this inherent fragility to environmental stress (e.g., ultraviolet radiation, malnutrition, dehydration, and elevated temperatures) some organisms, such as *Bacillus subtilis,* form a robust multilayer coating that impedes cell division.[7] However, when stress conditions are alleviated the protective shell is rapidly dissolved and the cells are able to germinate and resume vegetative growth.[7] Such biological preservation mechanisms have inspired artificial strategies aimed at the synthesis of mechanically durable yet degradable individual cell coatings[8,9] to enable the exploitation of cells for unprecedented technological applications.[4,10,11] Recent studies have demonstrated that fabricating durable, synthetic coatings around living cells affords prolonged cellular viability to environmental stresses.[10] To this end various protective coatings have been explored, including silica,[12–14] silica-titania,[15] graphene,[16] polydopamine,[17] and an iron-tannate coordination complex.[8] Although these cell coatings offer a degree of protection from the external environment, controlled diffusion of molecules through the shell has not been demonstrated. However, an ideal protective shell should permit the transport of nutrients or chemical stimulants necessary for cell viability while preventing access of larger cytotoxic molecules, such as lytic enzymes, from accessing the cells. Accordingly, selectively controlling molecular transport through coatings for cells remains an important challenge.[18,19] In this study, we demonstrate that metal–organic frameworks can be effectively used for the production of porous cytoprotective exoskeletons for living cells.

Metal–organic frameworks (MOFs), are an emerging class of porous materials,[20–22] that can be constructed from biofriendly building blocks,[23] under physiological conditions.[24] Furthermore, they are thermally and chemically robust[23,24] and synthesized via a modular approach that facilitates fine-tuning of their pore shape,[25] chemical functionality,[26] and size.[27] This unique collection of properties points toward MOFs as potential candidate materials for artificial cell exoskeletons (**Scheme 1**).[18] Indeed, this concept is supported by recent studies that illustrated how biomolecules ranging from amino acids to enzymes can directly trigger the formation of MOF crystals via a biomimetic mineralization process,[24,28] overcoming the initial need for non-biological crystallization agents.[29,30] Additionally, this strategy was also successfully applied to thin films and patterned surfaces of proteins.[31] We hypothesized that the biomolecule-rich cell membranes and walls[32,33] could concentrate MOF precursors providing an effective interface for the crystallization of MOFs thus leading to a porous exoskeleton encasing living cells. A recent study illustrated that yeast cell walls can attract metal ions, which could serve as a reservoir for the formation of MOF crystals.[34] However, in this case the synthetic conditions employed were incompatible with living cells, thus the yeast cells were merely employed as templating agents for the fabrication of MOF capsules. Although this work highlighted a new method for the preparation of complex MOF architectures, the potential use of MOFs for the protection of living cells remains unexplored. Here, we demonstrate for the first time that a MOF material (zeolitic imidazolate framework-8, or ZIF-8[35]) can be crystallized on the surface of the living organisms *Saccharomyces cerevisiae* (baker's yeast, domain: eukariote, kingdom: fungi) and the bacterium *Micrococcus luteus* (domain: bacteria, kingdom: bacteria). In both cases, the MOF forms a protective coating on the respective cell walls that protect the organisms from the external environment while, remarkably, preserving cell life.

ZIF-8 coated yeast cells were fabricated by dispersing living cells in an aqueous solution of 2-methylimidazole (HmIm), followed by the addition of an aqueous solution of zinc acetate. After 10 min, the yeast cells were removed from solution and washed with water to remove the excess ZIF-8 precursors (Figure S1, Supporting Information). To assess cell viability before and after application of the MOF coating, we performed two standard assays that employ the indicators fluorescein diacetate (FDA) and resazurin, respectively;[36,37] in both cases

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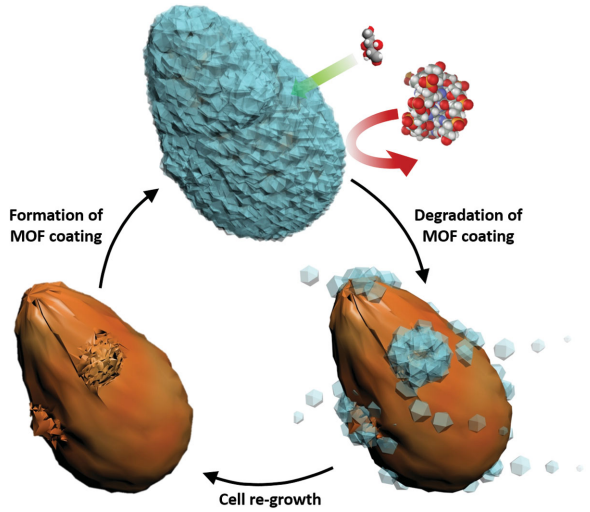

Scheme 1. Schematic illustration of biomimetic crystallization of cytoprotective metal–organic framework coatings on living cells.

the fluorescent intensity remained unchanged before and after formation of the MOF coating, indicating that the ZIF-8 coating is essentially non-toxic to yeast cells (Figure S2, Supporting Information). To confirm that ZIF-8 is non-toxic to yeast cells we performed a control test, where free ZIF-8 particles (average diameter ≈500 nm) and living yeast cells were combined in solution. After a 24 h incubation period the viability of the ZIF-8 treated yeast cells was found to be essentially identical to that of untreated cells (Figure S3, Supporting Information). These data confirm that ZIF-8 particles do not adversely affect yeast cells. We then analyzed the structure of the MOF exoskeleton by synchrotron small-angle X-ray scattering (SAXS). The resulting scattering pattern was comprised of peaks that were analogous in position and relative intensity to pure ZIF-8 (Figure S4, Supporting Information), thus confirming the structure and crystallinity of the coating. We also assessed the morphology and elemental distribution of the ZIF-8 coating using scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS), respectively. Analysis of c.a. 500 ZIF-8 coated yeast cells from SEM images revealed that all individual cells were homogeneously coated (i.e., partially naked cells or coatings on aggregates of cells were not observed). The elemental maps along with high-magnification SEM images of the ZIF-8 coated cells are shown in **Figure 1**a–e. Close analysis of the data clearly indicates a homogeneous distribution of Zn, O, and C on the cell surface, which strongly supports the formation of a continuous ZIF-8 coating on individual yeast cells. Furthermore, SEM measurements performed on cross sections of a crushed ZIF-8-coated yeast cell revealed an average ZIF-8 shell thickness of ≈100 nm (Figure S5, Supporting Information). We note that the thickness of the ZIF-8 coatings could be tuned in the 100–250 nm range by carrying out sequential ZIF-8 coating steps (Figure S6, Supporting Information) thus demonstrating the precise control of this approach. Confocal scanning laser microscopy (CLSM) was also employed to assess the homogeneity of the ZIF-8 coating (Figure 1f–h). For this experiment we labeled the yeast cells and ZIF-8 coating with FDA (green, Figure 1f) and Alexa Fluor 647 (red, Figure 1g), respectively. The high-resolution optical cross section shown in Figure 1h is consistent with the SEM/EDS data and provides further evidence of a continuous coating of ZIF-8 over the entire cell. In order to assess the generality of this approach, we examined the formation of ZIF-8 coatings on the bacterium *Micrococcus luteus* that possess a peptidoglycan-based outer membrane. *Micrococcus luteus* can survive in oligotrophic (nutrient deficient) environments and is of interest for biotechnological applications (e.g., terpenes biosynthesis[38]). Based on our investigation, a ZIF-8 coating was successfully formed on these Gram-positive cocci without affecting cell viability (Figure S7, Supporting Information). This highlights the potential of employing ZIF-8 as an exoskeletal coating for a variety of basic functional biological units.

We next determined whether the ZIF-8 coating forms a selectively permeable exoskeleton. Cellular functions are highly dependent on their microenvironment, which typically contain nutrients, neighboring cells, soluble factors (e.g., growth

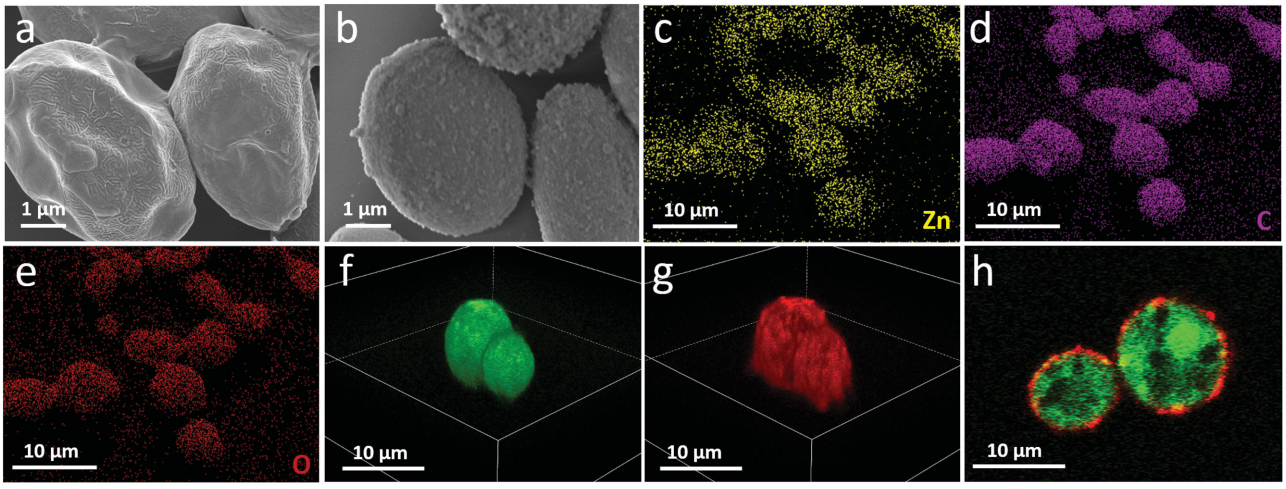

Figure 1. SEM images of a) native yeast and b) ZIF-8 coated yeast. c–e) EDS elemental mappings of ZIF-8 coated yeast. f,g) 3D cellular reconstruction of CLSM images of ZIF-8 coated yeast cells, and h) cellular cross section. The living yeast cells were labeled with FDA (green) and the ZIF-8 coatings were labeled by infiltration of Alexa Fluor 647 fluorescent dyes (red).

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factors), and cytokines.[4] Accordingly, the formation of an artificial coating around a living cell is expected to affect its behavior by regulating molecular transport to and from the cytoplasm.[39–41] To investigate the selective permeability of the MOF shell, ZIF-8 coated yeast cells were cultured in media containing yeast extract and glucose, an essential nutrient for maintaining cell health. Lyticase (M_W = 54.6 kDa, roughly 5.3 nm), a cytotoxic enzyme,[42] was then added to the culturing media. The cell viability was monitored for 24 h using FDA, a fluorescent indicator that enables the detection of living cells. Over the course of the experiment the ZIF-8 coated yeast did not give rise to a significant change in the fluorescent emission (5.3% and 19% loss in 3 and 24 h, respectively), while for the unprotected, naked, yeast a 95% reduction in fluorescence was detected within 3 h (**Figure 2**a and Figure S8, Supporting Information). This result suggests that lyticase, as expected, catalyzes cell lysis of the naked yeast cells and demonstrates that the ZIF-8 coating functions as a protective shell against this cytotoxic enzymatic agent. To further assess the cytoprotective capabilities of ZIF-8 coating on yeast cells, a small anti-fungal drug, filipin (M_W = 655 Da, 1 × 1.3 × 1.9 nm) was selected (Figure S9, Supporting Information). This antifungal drug was selected as the molecule is slightly bigger (three- to five-fold) than the ZIF-8 interconnecting micropores.[43] FDA was used as a live cell indicator to assess the antifungal effects of filipin. After 24 h incubation in the presence of filipin, the control sample (naked yeast) showed almost 100% mortality, while ZIF-8 coated yeast cells showed a minimal reduction in cell viability with less than 10% of cells killed by the anti-fungal drug (Figure S10, Supporting Information). These results indicate that the ZIF-8 coating is homogenous and can protect living cells from both relatively small molecules, such as anti-fungal chemicals and large cytotoxic proteins.

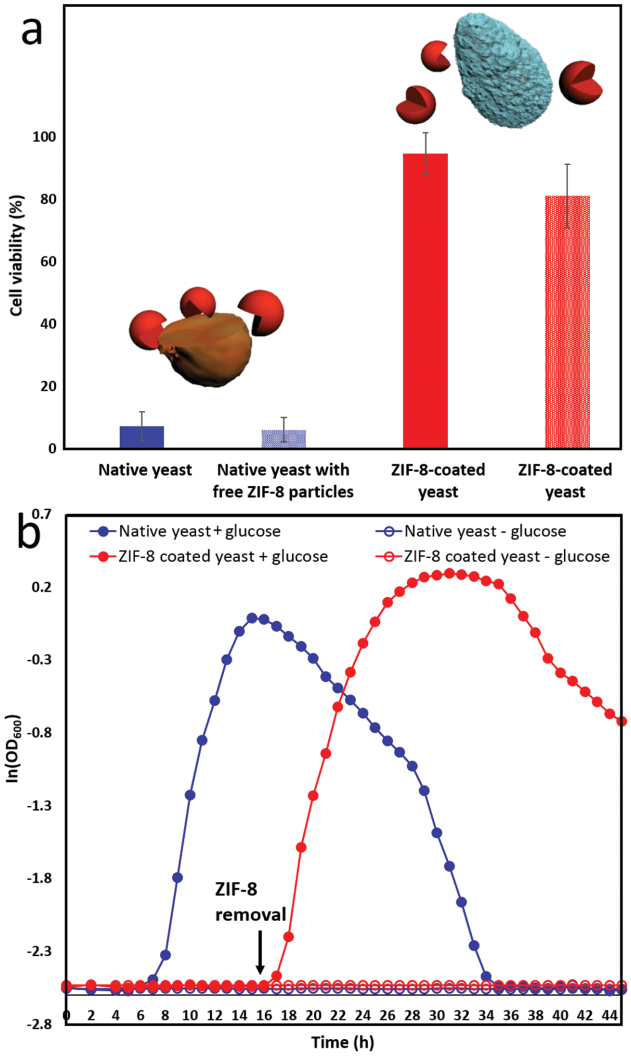

Figure 2. a) Cell viability (%) of native yeast (blue) and native yeast with free ZIF-8 particles (patterned blue) in the presence of cell lysis enzyme lyticase for 3 h, and ZIF-8 coated yeast in the presence of lyticase for 3 h (red) and 24 h (patterned red). b) Yeast growth measurement (OD_{600}) for native (blue circles) and ZIF-8 coated yeast (red circles) before and after the removal of MOF coatings by EDTA. Experiments were performed in triplicates.

Artificially induced cell hibernation engenders extended cell lifetimes and therefore underpins the advancement of engineering strategies toward governing cell division, proliferation, and differentiation.[8,12,44,45] Thus, we were motivated to investigate the time-dependent cellular responses of ZIF-8 coated yeast cells. First, we carried out optical density measurements at 600 nm (OD_{600}) for both native yeast and ZIF-8 coated yeast in culturing media containing glucose as a nutrient at 30 °C. OD_{600} experiments quantitatively measure cell proliferation by determining turbidity increases after division and are widely used to study the stage of cultured cells,[46,47] i.e., whether they are in a lag phase, growth phase, stationary phase, or death phase. For native yeast, the OD_{600} remained stable for 6 h before exponential growth was observed (Figure 2b). These data indicate that the yeast cells were initially in a dormant/hibernation state (lag phase for 6 h) before entering a rapid cell proliferation (budding) state. In contrast, the OD_{600} measurements of ZIF-8 coated yeast showed no obvious growth even after 6 h. Accordingly, it can be concluded that the ZIF-8 shell maintained the yeast in an extended lag phase. We postulate that ZIF-8 exoskeleton acts as a physical restriction suppressing the yeast cells from budding, as proposed for metal–polyphenol cell coatings.[8] Given that EDTA can degrade ZIF-8 films[31] we explored its potential to "switch-off" the artificially induced cell dormancy state. Figure 2b shows that subsequent to the addition of EDTA the yeast cells quickly moved to growth and germination states (EDTA was found having a negligible impact on yeast growth). We note that when compared to the incubation period of the naked cells (≈6 h), the division of ZIF-8 protected yeast is essentially instantaneous (approximately after a few minutes). This suggests that glucose, an essential nutrient to maintain the cell life, was able to diffuse through the ZIF-8 coating (Figure S11, Supporting Information). Furthermore, our data are supported by previous reports which demonstrate that glucose can be adsorbed by ZIF-8 crystals.[48,49] Furthermore, the OD_{600} data also showed that the growth rate and final cell number of the yeast, after the removal of the ZIF-8 shell, reached a similar level to native yeast (Figure 2b and Figure S12, Supporting Information). Thus, it can be concluded that the ZIF-8 coatings have no measurably negative impact on the yeast cells. In summary, our results show that a ZIF-8 shell

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can extend the cell's lifetime, by artificially suppressing cell division, without significantly affecting the activity of cells in the growth state. Based on our previous report which demonstrates that a variety of MOFs can be crystallized by biomacromolecules,[24,31] we anticipate that other MOF systems could be employed for cell coating applications.

In conclusion, we have reported for the first time the preparation of crystalline MOF protective coatings for living cells. Based on previous work,[24,28,31] we postulate that the formation of a robust ZIF-8 exoskeleton under physiological conditions is triggered by the biomolecule-rich surface of living yeast cells and bacteria. We posit that glycoproteins and peptidoglycans locally concentrate MOF precursors playing a role similar to the one disclosed for the bovine serum albumin (BSA).[24,31] Remarkably, the MOF coating controlled molecular trafficking to the cell cytoplasm and prevented cell division by inducing an artificial hibernation state. However, after removal of the ZIF-8 shell the cells immediately regained full functionality. We note that such precise control over cellular behavior is analogous to that observed in some unicellular organisms, which construct and dissolve exterior coatings as a protective mechanism. Accordingly, employing MOFs as a strategy to mimic natural protective mechanisms provides a new promising tool for the further progress of single cell in medicine and biotechnology.

Experimental Section

Experimental details including materials, formation of ZIF-8 coatings on cell surfaces, cell viability test, lyticase assay, cell division experiment, and instrumentation are documented in the Supporting Information.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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