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Engineering a Nanoscale Al-MOF-Armored Antigen Carried by a "Trojan Horse"-Like Platform for Oral Vaccination to Induce Potent and Long-Lasting Immunity

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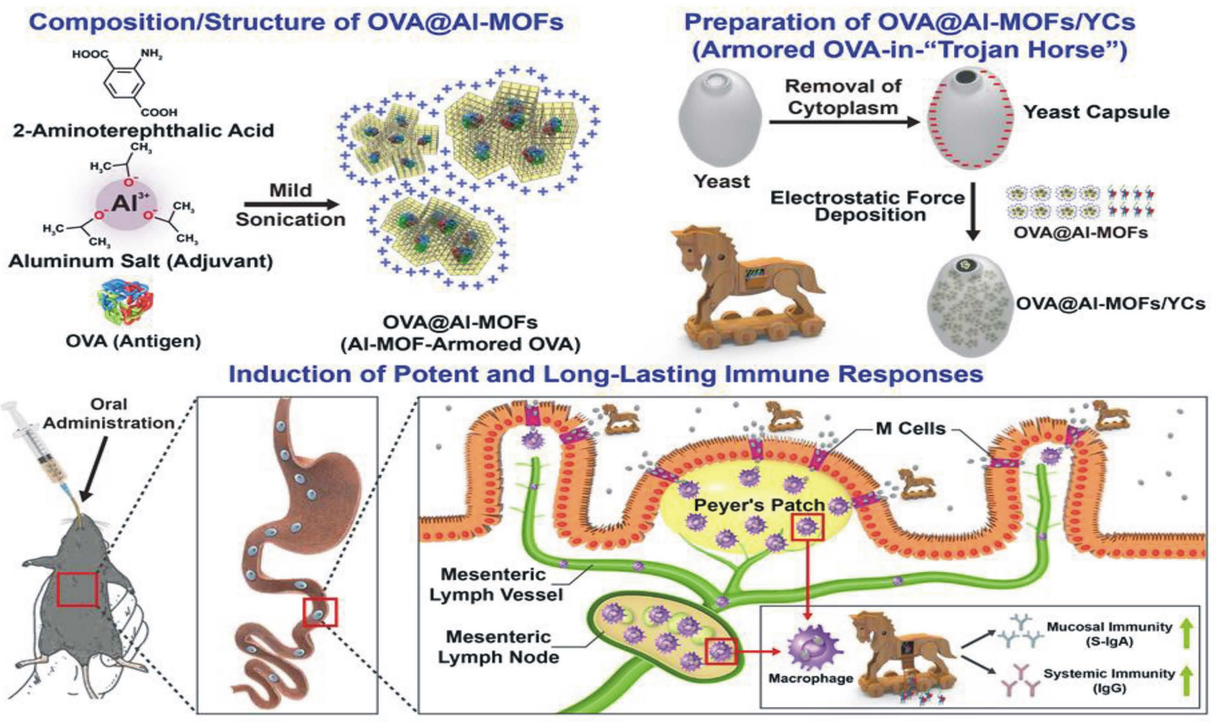
Abstract

Vaccination by the oral administration of an antigen faces many challenges, including gastrointestinal (GI) proteolysis and mucosal barriers. To limit GI proteolysis, a biomimetically mineralized aluminum-based metal–organic framework (Al-MOF) system that is resistant to ambient temperature and pH and can act synergistically as a delivery vehicle and an adjuvant is synthesized over a model antigen ovalbumin (OVA) to act as armor. To overcome mucosal barriers, a yeast-derived capsule is used to carry the Al-MOF-armored OVA as a "Trojan Horse"-like transport platform. In vitro experiments reveal that the mineralization of Al-MOFs forms armor on OVA that protects against highly acidic and degradative GI conditions. However, the mineralized Al-MOFs can gradually disintegrate in a phosphate ion-containing simulated intracellular fluid, slowly releasing their encapsulated OVA. In vivo studies reveal that the "Trojan Horse"-like transport platform specifically targets intestinal M cells, favoring the transepithelial transport of the Al-MOF-armored OVA, followed by subsequent endocytosis in local macrophages, ultimately accumulating in mesenteric lymph nodes, yielding long-lasting, high-levels of mucosal S-IgA and serum IgG antibodies. Such an engineered delivery platform may represent a promising strategy for the oral administration of prophylactic or therapeutic antigens for vaccination.



Schematic. Composition/structure of immune-activating Al-MOF-armored OVA (OVA@Al-MOFs) and preparation of "Trojan Horse"-like transport platform (OVA@Al-MOFs/YCs). Transport of OVA@Al-MOFs/YCs across tightly packed mucosal epithelium via intestinal M cells, followed by subsequent endocytosis in local macrophages, and their ultimate accumulation in mesenteric lymph nodes, inducing potent and long-lasting immune responses.

Results

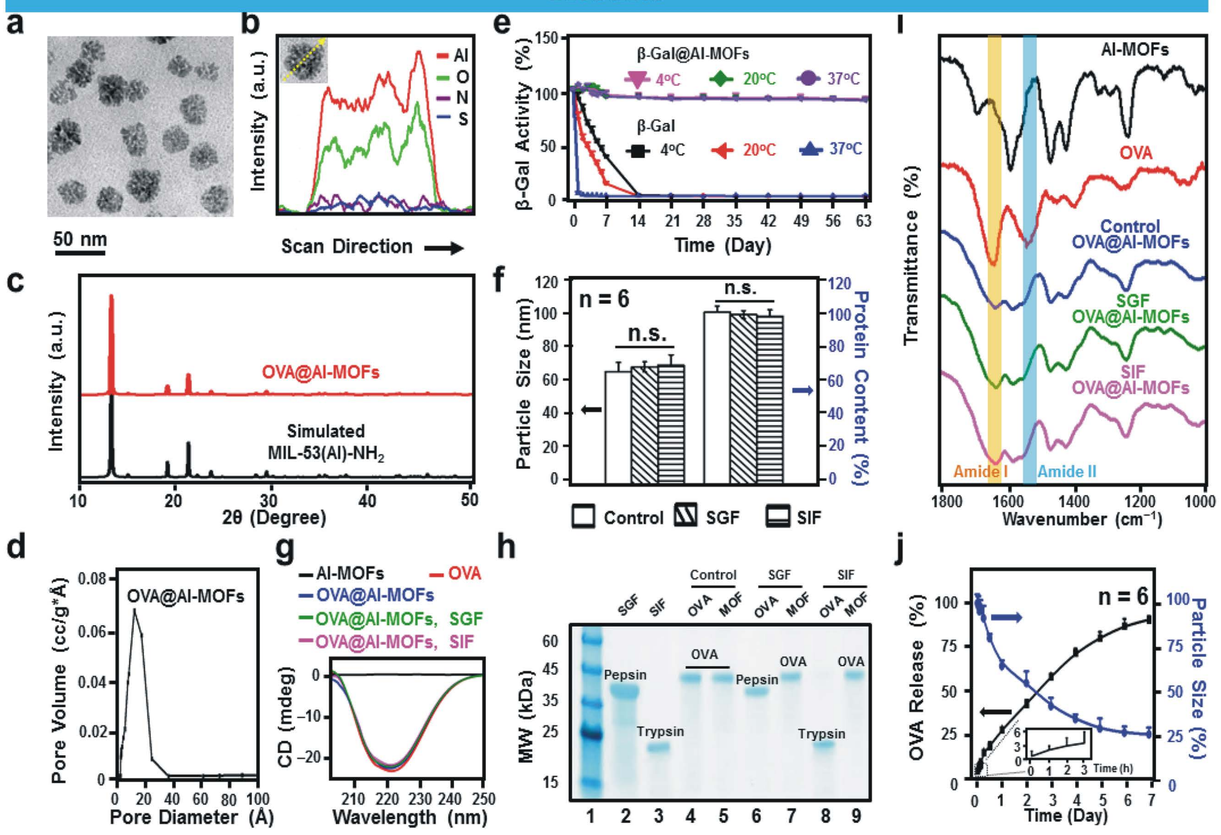


Figure 1. a) TEM micrograph and b) elemental linescan of OVA@Al-MOFs. c) PXRD patterns of OVA@Al-MOFs and simulated MIL-53(Al)-NH2. d) Distribution of pore sizes in OVA@Al-MOFs. e) Normalized enzymatic activities of free β-Gal and Al-MOF-armored β-Gal after storage in normal saline at various ambient temperatures for predetermined intervals. f) Particle size and protein content of OVA@Al-MOFs before (Control) and after treatment with SGF and SIF. g) CD spectra; h) SDS-PAGE results and i) FT−IR spectra of free OVA and Al-MOF-armored OVA before (Control) and after treatment with SGF and SIF. j) Changes in particle size and OVA release profile of OVA@Al-MOFs in PBS.

Results

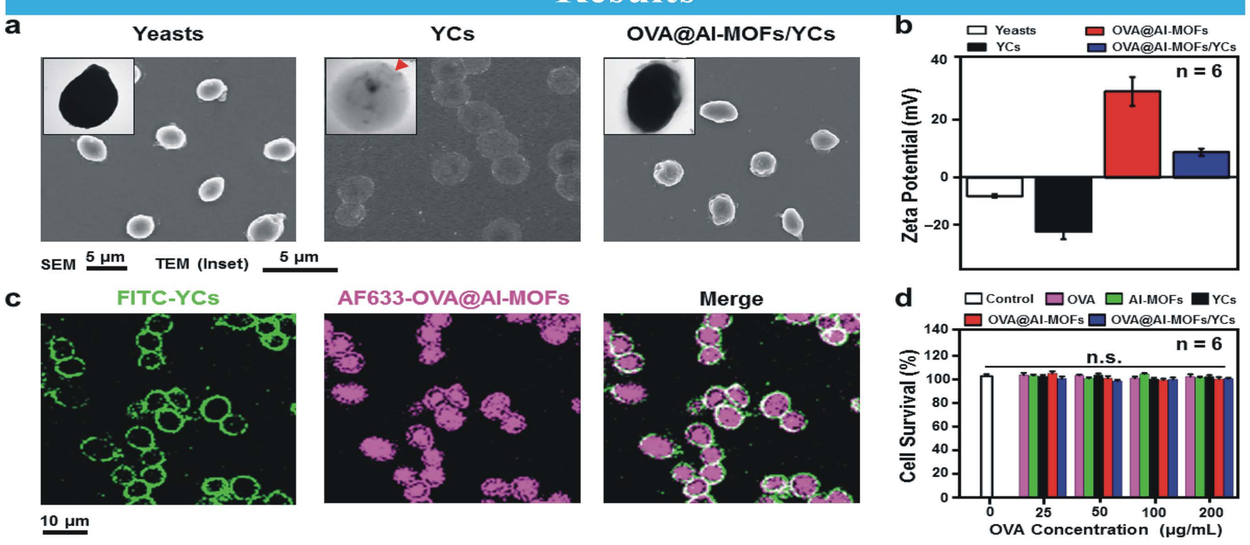


Figure 2. a) SEM micrographs and TEM micrographs (insert) of yeasts, YCs, and OVA@Al-MOF/YCs. b) Zeta potentials of yeasts, YCs, OVA@Al-MOFs, and OVA@Al-MOFs/YCs. c) CLSM images of FITC-YCs and AF633-OVA@Al-MOFs. d) Cytotoxicities of OVA@Al-MOFs/YCs at various concentrations of encapsulated OVA and of their components (free OVA, Al-MOFs, OVA@Al-MOFs, and YCs) as suspensions on Caco-2 cell monolayers. n.s.: not significant ($P > 0.05$).

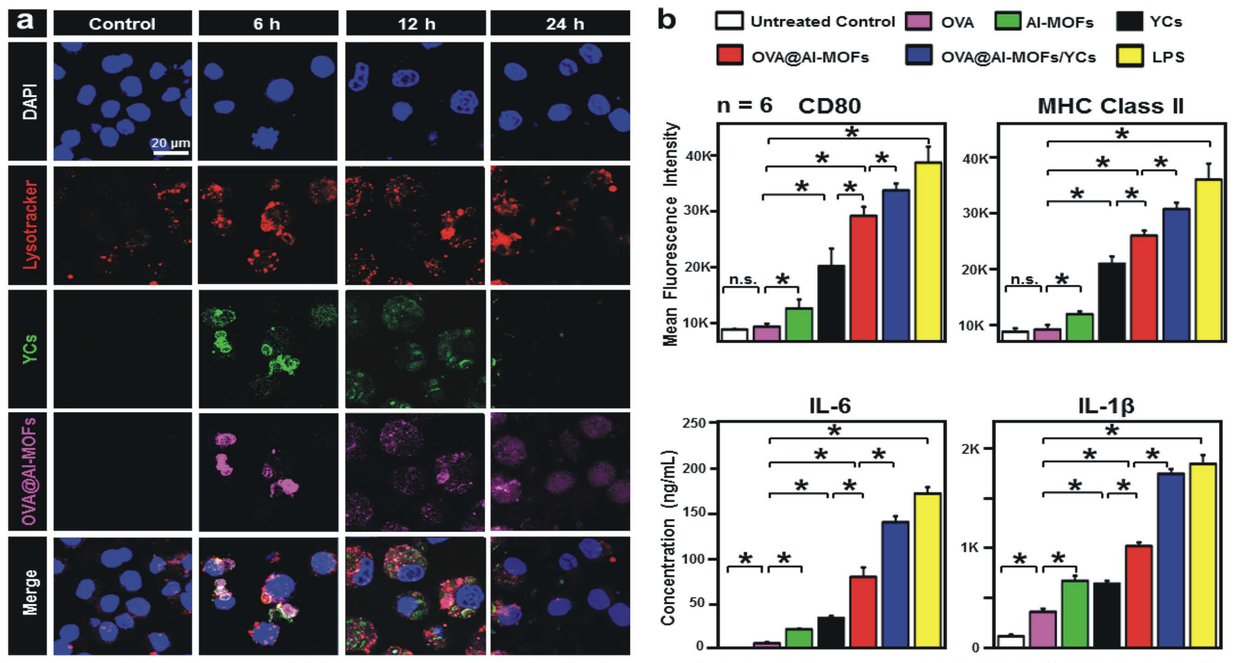


Figure 3. a) CLSM images of RAW264.7 macrophages that had been incubated with fluorescence-labeled OVA@Al-MOFs/YCs for predetermined intervals. b) Expression levels of CD80 and MHC class II on RAW264.7 macrophages and amounts of secreted IL-6 and IL-1β in culture supernatants after treatment with medium alone (Untreated Control), OVA, Al-MOFs, YCs, OVA@Al-MOFs, OVA@Al-MOFs/YCs, or LPS for 24 h. *:statistically significant ($P < 0.05$); n.s.: not significant ($P > 0.05$).

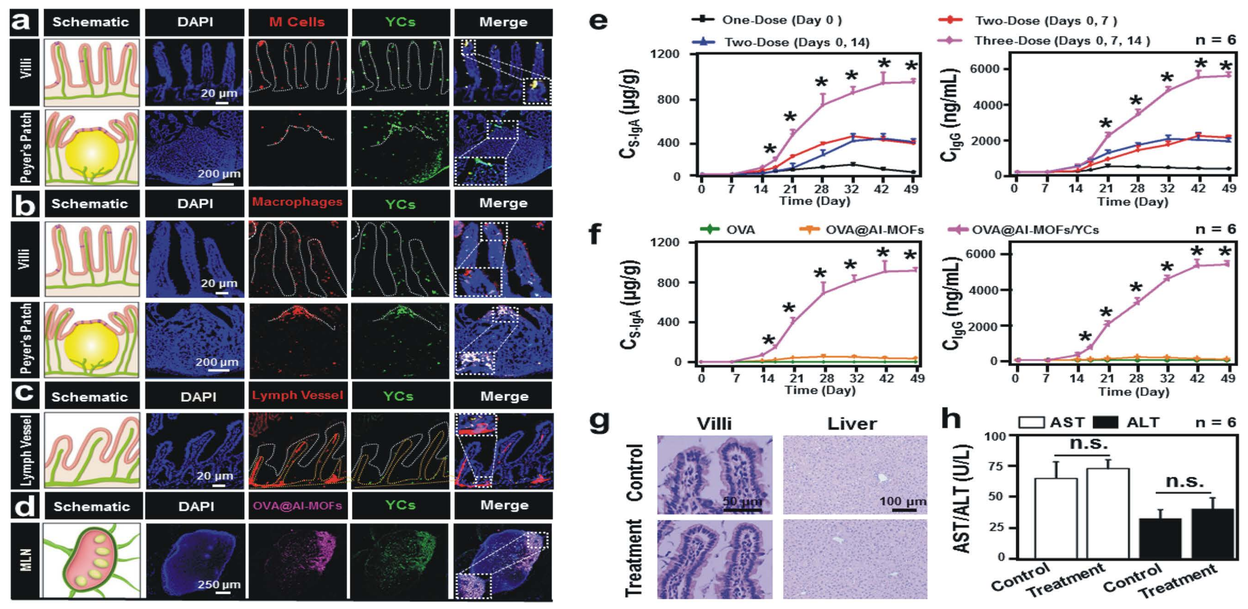


Figure 4. Route of transport of OVA@Al-MOFs/FITC-YCs following their oral administration in mice: schematic depictions and CLSM images of a) M cells and b) macrophages in intestinal tract, and c) lymph vessels, and d) mesenteric lymph node (MLN). OVA-specific S-IgA and IgG concentrations following oral treatment e) with OVA@Al-MOFs/YCs under various dosing regimens and f) with OVA, OVA@Al-MOFs, or OVA@Al-MOFs/YCs using a three-dose oral immunization schedule. g) Histological photomicrographs of intestinal villi and liver sections h) AST and ALT enzyme levels in plasma.

Conclusions

In conclusion, biomimetically mineralized Al-based MOFs can be used to preserve a protein antigen as protective armor at ambient temperature, and function both as an antigen delivery vehicle in highly degradative GI environments and an adjuvant to promote immune reactions. Furthermore, "Trojan Horse"-like YCs can effectively carry the Al-MOF-armored antigen to target intestinal M cells and convey them through the main gateway of the mucosal epithelium, inducing potent and long-lasting immunity, facilitating the application of this approach for oral vaccination.