Amoeba-inspired magnetic microgel assembly assisted by engineered dextran-binding protein for vaccination against life-threatening systemic infection

Shuo Liu1,2,3, Yan Zhao1, Linpei Guo4, and Qilin Yu1,2

1 Key Laboratory of Molecular Microbiology and Technology, Ministry of Education, Department of Microbiology, College of Life Sciences, Nankai University, Tianjin 300071, China
2 Research Center for Infectious Diseases, Nankai University, Tianjin 300350, China
3 College of Environmental Science and Engineering, Nankai Key Laboratory of Environmental Remediation and Pollution Control, Nankai University, Tianjin 300350, China
4 Department of Urology, The Affiliated Wuxi No.2 People’s Hospital of Nanjing Medical University, Wuxi 214002, China

© Tsinghua University Press 2022
Received: 7 May 2022 / Revised: 25 July 2022 / Accepted: 25 July 2022

ABSTRACT
Vaccination is critical for population protection from pathogenic infections. However, its efficiency is frequently compromised by a failure of antigen retention and presentation. Herein, we designed a dextran-binding protein DexBP, which is composed of the carbohydrate-binding domains of Trichoderma reesei cellobiohydrolases Cel6A and Cel7A, together with the sequence of the fluorescent protein mCherry. DexBP was further prepared by engineered Escherichia coli cells and grafted to magnetic nanoparticles. The magnetic nanoparticles were integrated with a dextran/poly(vinyl alcohol) framework and a reactive oxygen species-responsive linker, obtaining magnetic polymeric microgels for carrying pathogen antigen. Similar to amoeba aggregation, the microgels self-assembled to form aggregates and further induced dendritic cell aggregation. This step-by-step assembly retained antigens at lymph nodes, promoted antigen presentation, stimulated humoral immunity, and protected the mice from life-threatening systemic infections. This study developed a magnetic microgel-assembling platform for dynamically regulating immune response during protection of the body from dangerous infections.

KEYWORDS
magnetic nanoparticle, engineered protein, dextran, microgel, vaccination, systemic infection

1 Introduction
Vaccination is one of the most effective strategies to prevent and eliminate life-threatening infectious diseases [1–4]. Successful vaccines could protect individuals by activation of the immune system with exogenous antigens, followed by generation of potent and durable antibodies to fight against invading pathogens. With the help of vaccination, a series of previous epidemics, such as smallpox and polio, have been nearly eradicated. Most recently, the rapid development of COVID-19 vaccines is expected to control this worldwide pandemic in the near future [5–7]. To realize efficient vaccination, adjuvants are indispensable to enhance antigen-specific immune responses [8–10]. However, there are only a few adjuvants with enough potency and low toxicity for production of clinical vaccine. Therefore, it is urgently needed to develop safe, versatile, and wide-spectrum adjuvants for vaccination with high efficiency against different kinds of infections.

Taking advantage of high surface-to-volume ratio and unique optical, electrical, and magnetic properties, nanomaterials are an ideal candidate of adjuvants. They could high-efficiently deliver antigens, release the cargos in a controlled manner, protect antigens from degradation, and facilitate antigen accumulation/presentation at lymph nodes [11–13]. Among them, microgels (MG) and magnetic nanoparticles (MNPs) are two important candidates of novel adjuvants. On the one hand, microgels (also known as hydrogel micro-particles) possess the merits of both hydrogels and nanoparticles, not only exhibiting good biocompatibility and high capacity to load hydrophilic antigens, but also enabling injection through small needles for vaccination [14–17]. On the other hand, MNPs have been widely applied in magnetic hyperthermia, magnetic resonance imaging, and magnetic field-directed drug delivery [18–20]. As a versatile biomedical platform, it was reported that MNPs can be used to targeted delivery of immunotherapeutic drugs or antigens for activation of potent immune response and to magnetic hyperthermia for antitumor and anti-infection therapy [21–24].

Based on the high loading capacity of microgels and the superparamagnetic property of MNPs, we hypothesized that the integration of microgels and MNPs can be used to develop novel adjuvants for eliciting potent and durable immune response. In this study, inspired by the foraging behavior of amoeba [25], we constructed a hybrid magnetic microgel assembly for improvement of vaccination efficiency. The magnetic microgel is composed of MNPs functionalized by genetically engineered
dextran-binding protein (DexBP, Scheme 1(a)), the poly(vinyl alcohol) (PVA)/dextran framework, and the reactive oxygen species (ROS)-responsive linker N-(4-borobenzenyl)-N-(4-boronophenyl)-N,N',N'-tetramethyipropane-1,3-diaminium (TSPBA, Scheme 1(b)). During incubation with DexBP-grafted MNPs, dextran, PVA-203, and TSPBA, DexBP could bind dextran, while TSPBA could link PVA and dextran via dynamic phenylborate ester bond. Therefore, the components rapidly self-assembled to form the magnetic microgel aggregates, and strongly induced aggregation of dendritic cells during microgel-cell interaction (Scheme 1(c)). This step-by-step assembly realized strong antigen retention at lymph nodes, enhancement of antigen uptake/presentation, activation of spleen T cells, promotion of specific antibody production, and efficient protection of the mice from life-threatening systemic infections. This study developed a magnetic microgel assembly platform for dynamic regulation of immune response and for enhanced vaccination against dangerous infections.

2 Experimental

2.1 Materials

4-(Bromomethyl) phenyboronic acid, N,N,N',N'-tetramethyl-1,3-propanediamine (TMPA), N,N-dimethylformamide (DMF), PVA, dextran (500 kD), tetraethylorthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), iron(III) acetylacetonate, manganese(II) acetylacetonate, 1,2-dodecanediol, oleic acid, oleylamine, benzyl ether, hexadecyltrimethylammonium bromide (CTAB), 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide (EDC), N-hydroxysulfo succinimide (NHS), 2-morpholinoethanesulfonic acid (MES), N-succinimidyl-3-[2-pyridyldithiol]propionate (SPDP), Hoechst 33342, Lyso-Tracker Red, fluorescein diacetate (FDA), and FITC-labeled dextran were purchased from Sigma, USA. Cyanine 5 carboxylic acid (Cy5-COOH) was purchased from AAT Bio, USA, Ovalbumin (OVA) was purchased from Damas-beta, China. DexBP and Candida albicans antigen (CaAg) were expressed and purified from the genetically engineered Escherichia coli strains EcDexBP and EcCaAg as described below, respectively.

2.2 Strain construction and protein preparation

The E. coli strain NKEc201 was constructed to produce the dextran-binding protein DexBP. Firstly, the DexBP-encoding sequence (named DexBP) was in silica designed by fusing the protease-recognizing Smt3 sequence, two carbohydrate-binding domains of Trichoderma reesei cellobiohydrolase Cel6A (CBD$_{CBD6A}$ CSSVWGGCGGGQWSSGTPCASCSTCVYSDYYSQCL), the sequence of the red fluorescence protein mCherry, two carbohydrate-binding domains of T. reesei cellobiohydrolase Cel7A (CBD$_{CBD7A}$ TQSHYGQCGGGYSGPTVCASGTTCVLYNPPYYSQCL), and the 6xHis tag for nickel column purification (Figs. 1(a) and 1(b)). The DexBP gene was de novo synthesized and cloned into the plasmid pET28a, obtaining the plasmid pDexBP. This plasmid was further transformed into E. coli BL21 (Ec0), obtaining the DexBP-expressing strain EcDexBP. As a control, the mCherry-encoding gene, named mCherry, was also designed, synthesized, and cloned into the plasmid pET-28a. The obtained plasmid pmCherry was transformed in BL21, obtaining the mCherry-expressing strain EcCherry.

To express the C. albicans protein antigen Eap1 (CaAg), the C. albicans EAP1 gene was amplified from the genome of C. albicans SC3514, and then cloned into the plasmid pET28a, obtaining the plasmid pEap1. This plasmid was further transformed into E. coli BL21 (Ec0), obtaining the CaAg-expressing strain EcEap1.

The expression of DexBP and CaAg was induced in LB medium containing 1 mM IPTG for 12 h, respectively. The aimed proteins were purified from the whole protein extracts by using

![Scheme 1](image_url)  
Scheme 1 Schematic illustration of magnetic microgel preparation and bioeffect. (a) Synthetic route of the genetically engineered dextran-binding protein DexBP based on genetically engineered E. coli cells (EcDexBP). (b) Chemical composition of the magnetic microgel. (c) Magnetic assembly of the magnetic microgel and its induction of dendritic cell aggregation. pDexBP, the plasmid carrying the expression element of DexBP; IPTG, isopropyl β-D-thiogalactoside; MG+CaAg, the CaAg-loading microgel; MG+MNP-DexBP+CaAg, the antigen-loading magnetic microgel.
nickel column.

2.3 Dextran-binding assays
To evaluate the capacity of DexBP and mCherry to bind dextran, starch particles and mesoporous silica nanoparticles (MSNs) grafted by DexBP or mCherry were used. In the starch particle-synthetic protein co-incubation experiment, the natural corn starch particles were suspended in phosphate buffered saline (PBS) buffer (5 mg/mL), and then the purified DexBP or mCherry was added into the suspensions to a concentration of 0.1 mg/L. The mixtures were incubated with gently shaking at 37 °C for 30 min. The starch particles were then harvested by centrifugation at 6,000 rpm for 2 min, washed twice by PBS, and observed by confocal microscopy (FV1000, Olympus). In the MN-Dextran co-incubation experiment, 20 mg of APTES-functionalized MSNs were activated by glutaraldehyde, and then reacted with 5 mg of DexBP or mCherry in PBS at 4 °C for 12 h. The nanoparticles were then centrifuged at 12,000 rpm for 5 min, washed twice by PBS, obtaining DexBP-grafted MSNs and mCherry-grafted MSNs, respectively. The grafted MSNs were suspended in PBS (1 mg/mL), and then FITC-labeled dextran was added into the MSN suspensions to a final concentration of 10, 50, 100, or 200 µg/mL. The mixtures were incubated at 37 °C for 30 min, followed by centrifugation at 12,000 rpm for 5 min. Fluorescence intensity of the supernatants was determined by a fluorescent microplate reader (Enspire, PerkinElmer). The percentage of adsorbed dextran was calculated by the fluorescence intensity of the supernatants divided by the initial fluorescence intensity of the FITC-labeled dextran without addition of the MSNs.

2.4 Synthesis of MNP and MNP-DexBP
The MnFe₂O₄ MNP cores were synthesized by previous methods. Briefly, 2 mmol iron(III) acetylacetonate, 1 mmol manganese(II) acetylacetonate, 10 mmol 1,2-dodecanedioic, 6 mmol oleic acid, and 6 mmol oleylamine were mixed with 20 mL benzyl ether in a three-neck flask. The flask was heated to 200 °C and incubated at the same temperature for 1 h, followed by further heating at 298 °C for 1 h. The reaction system was cooled to room temperature, and harvested by centrifugation. 180 mg of the obtained nanoparticles were further added into 20 mL of the same fresh reaction solution, heated at 200 °C for 1 h, and further at 298 °C for 1 h. The final pellets were harvested and washed by ethanol, obtaining large-size MnFe₂O₄ MNPs for efficiently inducing magnetic aggregation.

To modify the large-size MnFe₂O₄ MNPs by DexBP, the nanoparticles were firstly coated by a thin SiO₂ layer. The MNPs were suspended in distilled water containing 100 mg CTAB, followed by sonication. The suspension was further heated to 75 °C. Under magnetic stirring, 100 µL TEOS and 50 µL APTES were added. The mixture was further heated at 75 °C for 1 h. The SiO₂ layer-coated MNPs (MNP@SiO₂) exposing amino groups were harvested by centrifugation. To graft the MNPs by DexBP, 20 mg of the obtained MNPs were activated by 2 mg SPDP in dimethyl sulfoxide (DMSO) for 1 h, and reacted with 2 mg DexBP in PBS buffer (pH = 7.2). The MNPs were then centrifuged and washed by distilled water, obtaining DexBP-modified MNP, i.e., MNP-DexBP.

2.5 Synthesis of TSPBA
TSPBA was synthesized according to previous reference with slight modification. 4-(Bromomethyl) phenylboronic acid (1 g, 4.6 mmol) and N,N,N’,N”-tetramethyl-1,3-propanediamine (0.2 g, 1.5 mmol) were mixed in DMF (40 mL) and stirred at 60 °C for 24 h. Then, the clear solution was precipitated in tetrahydrofuran (THF) (100 mL) and centrifuged, and the white solid was further washed with THF and dried under vacuum overnight. Pure TSPBA was obtained as a white solid, which was then characterized by ‘H-NMR (Fig.S1 in the Electronic Supplementary Material (ESM)). ‘H-NMR (400 MHz, D₂O, δ): 7.77 (d, 4H), 7.50 (d, 4H), 4.53 (s, 4H), 3.35 (t, 4H), 3.06 (s, 12H), 2.77 (s, 2H).

2.6 Preparation of antigen-loaded ROS-responsive microgel
The MNP-free and antigen-loading microgel, named MG+CaAg, was prepared by mixing PVA, dextran (Mw = 500 kD), CaAg, and TSPBA in distilled water. The final concentrations of the reagents were 12.5, 7.5, 0.1, and 5 mg/mL, respectively, obtaining the antigen-free microgel at a concentration of 2.5% (w/v). The MNP-containing microgel, named MG+MNP-DexBP+CaAg, was prepared by mixing PVA, dextran, MNP-DexBP, CaAg, and TSPBA in distilled water. The final concentrations of the reagents were 12.5, 7.5, 0.2, 0.1, and 5 mg/mL, respectively.

2.7 Characterization
To observe the morphology of the microgels, the prepared MNP, MG+CaAg, and MG+MNP-DexBP+CaAg were dispersed in distilled water, dried at mica sheets, and then observed by atom force microscopy (AFM, Dimension Icon, Bruker). The microgels were also observed by confocal microscopy (A1+, NIKON, Japan), by using FITC-labeled CaAg and rhodamine B-labeled dextran. Functional groups of the microgels were detected by a Fourier transform infrared (FT-IR) spectrometer (System 2000, PerkinElmer, USA). The hydrodynamic sizes and zeta potentials of MG+CaAg, MG+MNP-DexBP+CaAg, and MNP-DexBP were measured by a dynamic light scattering (DLS, Malvern Panalytical, Zetasizer Nano ZS0303081003) after dispersed in water or complete cell culture media.

2.8 Antigen-releasing assays
To measure the antigen-releasing capacity, FITC-labeled CaAg and the microgels containing rhodamine B-labeled dextran were used to prepare MG+CaAg and MG+MNP-DexBP+CaAg, with the final volume of 200 µL. The microgels were added into cylinder capsules with a volume of 500 µL and with the membrane pore size of 0.22 µm. The capsules were suspended in PBS buffer and incubated at 37 °C for indicated time. The extra-capsule solutions sampled at different time points were used for measurement of FITC fluorescence intensity to reflect the content of released antigen. No fluorescence of rhodamine B was detected in extra-capsule solutions of both MG+CaAg and MG+MNP-DexBP+CaAg, indicating that no antigen-loaded microgels went directly through the pores.

2.9 Cell viability assays
The DC2.4 dendritic cells were cultured in a Dulbecco’s modified Eagle’s medium (DMEM) medium supplemented with 10% (v/v) fetal bovine serum (Gibco), 100 U/mL penicillin, and 100 U/mL streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. The cells were seeded in a 96-well plate and incubated 24 h. MG+CaAg and MG+MNP-DexBP+CaAg at different concentrations were added into the cell cultures, and the cells were incubated for another 24 h. Cell viability of the treated cells was detected by using the CCK-8 assay kits (Solarbio, China).

2.10 Cell uptake assays
To observe cellular uptake of FITC-labeled antigens (i.e., fOVA and fCaAg), the DC2.4 cells were incubated with MG+fOVA,
2.11 Cell aggregation assays

To investigate the effect of the microgels on cell aggregation, the overnight cultured dendritic cells were stained by Hoechst 33342 and digested from the microplates by using trypsin solution, washed twice and re-suspended in PBS. 100 μL of CaAg solution (100 mg/L) or the microgel solution (2.5%, containing 100 mg/L CaAg) was added into 2 mL of cell suspensions, and then the mixture was added into confocal dishes. After 1 h of incubation, the cells were observed by confocal microscopy. The average sizes of cell aggregates and the percent of free cells in each group were calculated. At least 20 observed fields were used for statistical analysis.

2.12 In vivo imaging of antigens and histological observation of lymph nodes

All animal experiments were approved by the Animal Care and Use Committee at Nankai University (Approval number 2021-SYDWLL-000023). To investigate the effect of the microgels on in vivo distribution of antigens, the Cy5-labeled antigen (Cy5-CaAg, 10 μg), MG+Cy5-CaAg (2.5 mg + 10 μg), or MG+MNPDexBP+Cy5-CaAg (2.5 mg + 10 μg) were subcutaneously injected into the hind footpad of 6-week-old Balb/c female mice (n = 6, Huafukang, China). At 1 and 24 h after injection, the Cy5 fluorescence intensity was detected by a small animal fluorescence imaging system (Xenogen, USA). To evaluate the effect of the microgels on lymph node dendritic cell activation, the mice were injected by label-free CaAg or CaAg-loading microgels. At 72 h after injection, the inguinal lymph nodes of three mice in each group were isolated and homogenized to obtain the lymph node cells. The cells were stained by FITC-conjugated anti-mouse CD11c antibody and APC-conjugated anti-mouse CD86 antibody, followed by flow cytometry to determine the proportion of CD11c+ cells in all lymph cells and the proportion of CD86+ cells in CD11c+ lymph cells. To observe the histological structures, the mice were vaccinated on day 0 (prime vaccination) and 7 (boost vaccination). The inguinal lymph nodes, together with the livers, spleens, lungs, kidneys, and hearts, were sampled on day 14, weighed, fixed by 4% formaldehyde, dehydrated by ethanol, embedded by paraffin, sectioned into slices, and stained by hematoxylin & eosin (H&E) for light microscopy (Olympus, Japan).

2.13 Activation of spleen T cells and antibody production

To investigate activation of spleen T cells by the microgels, the mice were immunized by the vaccines as described above on day 0 and 7. On day 14, the mice were sacrificed to collect the spleens. The spleens were homogenized to obtain single cell suspensions. The splenocytes were then stained by the FITC-conjugated anti-mouse CD4 antibody and the APC-conjugated anti-mouse IL-2, IL-4, INF-γ, or TNF-α antibody. The stained splenocytes were analyzed by flow cytometry.

To assess activation of CaAg-specific antibody production, the blood of immunized mice was sampled on day 14 and 21, followed by centrifugation to obtain mouse serum. The levels of CaAg-specific IgG were determined by enzyme-linked immunosorbent assay (ELISA) kits (Dingguo, China).

2.14 Systemic infection model of C. albicans

Balb/c female mice (n = 20) were pre-immunized by CaAg (10 μg), MG+CaAg (2.5 mg + 10 μg), or MG+MNPDexBP+CaAg (2.5 mg + 10 μg) as described above with the prime-boost procedure (day -14 and -7). C. albicans SC5314 cells (ATCC, USA) were cultured overnight in liquid YPD medium, and suspended in saline to the concentration of 5 × 10^7 cells/mL. 100 μL of fungal cell suspension per mouse was intravenously injected into the control mice (receiving no vaccination) and immunized mice by tail veins on day 0. Survival rate and body weight of the mice were recorded from day 0. On day 3, the kidneys were sampled from three mice per group, weighed, and homogenized in saline, and the number of fungal cells in the suspensions was measured by colony forming unit (CFU) assays in YPD plates. The kidneys were also fixed by 4% formaldehyde, embedded, and stained by periodic acid-schiff (PAS) staining for histopathological observation.

To detect the levels of blood serum and the cytokines TNF-α and G-CSF, the serum of the treated mice was sampled on day 3 after fungal infection. Serum urea levels were detected by a serum urea assay kit (Leagene, China). The serum levels of TNF-α and G-CSF were detected by a mouse TNF-α and G-CSF assay kits (Jianglaibio, China). The serum anti-CaAg levels were detected by ELISA assay kits (Dingguo, China).

2.15 Statistical analysis

Each experiment was performed with three replicates, and the values were shown with means ± SD. Difference between groups was compared by a one-way analysis of variance (ANOVA) test (P < 0.05). All statistical tests were performed using the SPSS software package (Version 20, IBM).

3 Results and discussion

3.1 Design and characterization of the dextran-binding protein

The dextran-binding protein, named DexBP, was designed by fusing two CBD_{CBD}A domains, one mCherry domain, and two CBD_{CBD}A domains (Figs. 1(a) and 1(b)) [26]. The protein-encoding gene, named DexBP, was then designed by using the optimized codons of C. elegans, de novo synthesized, and cloned into the plasmid pET-28a. The obtained plasmid pDexBP was further transformed into E. coli BL21, obtaining the DexBP-expressing strain EcDexBP. The control strain EcCherry was constructed by similar method to express the control protein mCherry. After IPTG incubation, both EcDexBP and EcCherry were observed by confocal microscopy to evaluate expression of the aimed proteins. As shown in Fig. 1(c), the two strains exhibited strong fluorescence of mCherry, indicating high expression levels of the synthetic proteins in the strains. The two proteins, i.e., mCherry and DexBP, were isolated from the two strains respectively by nickel columns for further experiments.

To investigate the capacity of the synthetic proteins to bind dextran, the starch particles were co-incubated with starch particles composed of dextran chains. Confocal microscopy showed that the starch particles strongly bound DexBP, but failed to bind the control mCherry (Fig. 1(d)), indicating that DexBP had much stronger capacity to bind starch. Similarly, during co-incubation of the MSNs grafted by the synthetic proteins and FITC-labeled dextran, the mCherry-grafted MSNs only adsorbed quite low levels of dextran (<5%). In contrast, the DexBP-grafted MSNs adsorbed >95% dextran at the initial dextran concentration of 10 mg/L, and adsorbed >55% dextran even at the
concentration of 200 mg/L (Fig. 1(e)). Together, these results revealed that DexBP had a high dextran-binding capability.

3.2 Characterization of MG-CaAg and MG-MNP-DexBP

To construct a microgel-based vaccination platform with potent and durable immune activation, a ROS-responsive MG was firstly constructed, which was composed of PVA, dextran, and TSPBA linker. AFM observation and zeta potential analysis showed that the spherical microgel was well-dispersed in distilled water, with the diameter of 0.2–0.7 μm (Fig. S2(a) in the ESM), and with the zeta potential at ~0 mV (Fig. S2(b) in the ESM). After inclusion of the C. albicans protein antigen (CaAg) Eap1, the microgel, named as MG+CaAg, maintained spherical morphology similar to the initial MG, with the stable size of 0.2–0.8 μm (Figs. 2(a) and 2(c)). The microgels and the antigen bound together by the N-B coordination between the boronobenzyl groups of TSPBA and the amino groups of the antigen, and by the interaction between the benzene rings of TSPBA and the guanidyl groups of the antigen [27].

Aggregation of particle-like adjuvants facilitates antigen presentation and activation of immune cells [28–30]. Magnetic assembly is a promising way to realize ordered aggregation of particles [31, 32]. To induce amoeba-mimic aggregation, the magnetic MNP-DexBP nanoparticles (i.e., the MNP’s grafted by DexBP, Fig. S3 in the ESM) were introduced to bind the microgel dextran for inducing magnetic aggregation of the microgels. The initial MnFe₂O₄ MNPs were firstly synthesized by a thermal deposition method, and then coated by a thin SiO₂ layer by using TEOS and APTES [21]. Transmission electron microscopy (TEM) observation showed that the initial MNPs had spherical morphology, with the diameter of 10–12 nm (Fig. S3(a) in the ESM), and the SiO₂-coated MNPs (MNP@SiO₂) had a very thin layer on the surface of MNPs. MNP@SiO₂ was further grafted by DexBP with the aid of the coupling agent SPDP through the Michael addition reaction, obtaining MNP-DexBP having similar morphology with MNP@SiO₂ (Fig. S3(a) in the ESM). FT-IR spectra showed the presence of –Si–O– group (adsorption peak at 1,080 cm⁻¹) in both MNP@SiO₂ and MNP-DexBP, and the presence of –NH–CO– group in MNP-DexBP (adsorption peak at 1,630 and 1,560 cm⁻¹) (Fig. S3(b) in the ESM). DLS analysis further showed that MNP-DexBP had a hydration diameter larger than MNP@SiO₂ (18–20 nm versus 10–14 nm, Fig. S3(c) in the ESM), indicating the presence of a DexBP-formed hydration layer on the surface of the nanoparticles. Moreover, MNP@SiO₂ exhibited a positive zeta potential at +37.8 mV, while MNP-DexBP had a negative zeta potential at −4.8 mV (Fig. S3(d) in the ESM). These results confirmed successful modification DexBP on the surface of MNPs.

The magnetic microgel MG+MNP-DexBP+CaAg was obtained by mixing the microgel components, CaAg, and MNP-DexBP in distilled water. As revealed by AFM observation and DLS analysis, MG+MNP-DexBP+CaAg formed obvious microgel aggregates with the size of 1–4 μm (Figs. 2(b) and 2(c)). Zeta potential analysis further revealed that MG+MNP-DexBP+CaAg and MG+CaAg had similar zeta potential at +3–5 mV (Fig. S4 in the ESM).
The stability of the magnetic microgels was further evaluated by DLS analysis under different pH and different temperatures. As shown in Fig. S5(a) in the ESM, the mean size of the magnetic microgels only slightly decreased after incubation at pH = 5 for 1 h as compared at pH = 7, but significantly decreased after incubation at pH = 3 (from 1,686 to 882.8 nm). Moreover, the magnetic microgels were stable at both 25 and 37 °C, as the mean size maintained > 1,500 nm (Fig. S5(b) in the ESM).

To visualize the three-dimensional structure of the microgels by confocal microscopy, FITC-labeled CaAg and rhodamine B-labeled dextran were used. As shown in Fig. 2(d), MG+CaAg microgels were well-dispersed, with CaAg mainly located at the surface of microgels (Fig. 2(d), top). Differently, MG+MNP-DexBP+CaAg exhibited the aggregate morphology, with the size larger than MG+CaAg (Fig. 2(d), down). Moreover, MG+MNP-DexBP+CaAg had the yellow color, indicating that CaAg was loaded into the magnetic microgel (Fig. 2(d), down). Antigen release assays further revealed that MG+MNP-DexBP+CaAg released much less contents of the CaAg antigen than MG+CaAg (9% versus 58% after 7 days, Fig. S6(a) in the ESM), indicating a high stability of the magnetic microgel in antigen capture under the stimuli-free condition. Using the rhodamine B-labeled dextran as a fluorescence indicator of the magnetic microgel, we further monitored dynamic change of MG+CaAg and MG+MNP-DexBP+CaAg by means of confocal microscopy. Upon addition of TSPBA into the mixture of MNP-DexBP, rhodamine B-labeled dextran, FITC-labeled CaAg, and PVA, the microgels immediately formed, and then slowly aggregated to form larger particles (Movie ESM1). As a control, the size of MG+CaAg remained unchanged (Movie ESM2). Taken together, the results confirmed that MNP-DexBP strongly induced assembly of MG+CaAg microgels to form amoeba-like aggregates.

3.3 ROS response of the MG+MNP-DexBP+CaAg assembly
As previously reported, TSPBA-PVA-based hydrogel exhibited ROS responsiveness due to oxidation and hydrolyzation of TSPBA, leading to the disintegration of the hydrogel and release of loaded cargo [33–35]. To verify this, the MG+MNP-Dex+CaAg aggregates were treated by H$_2$O$_2$, a common ROS produced by macrophages under pathogen infection or nanoparticle uptake [36].

As shown in Fig. 3(a), H$_2$O$_2$ addition led to remarkable disassembly of the aggregates, forming randomly distributed single microgels (Fig. 3(a)), with the sizes decreasing from 1–4 to 0.5–1.5 μm (Fig. 3(b)). Confocal observation further confirmed that H$_2$O$_2$ strongly reduced the size of the microgel aggregates, and led to exposure of the antigen on the surface of the microgels (Fig. 3(c)). Consistently, antigen release assays revealed that H$_2$O$_2$ addition led to drastic release of the antigen from the microgel into the solution (61% for 1 mM H$_2$O$_2$ and 49% for 0.5 mM H$_2$O$_2$ after 7 days, Fig. S6(b) in the ESM), confirming that ROS strongly induced antigen release from the microgel. These results revealed that the magnetic microgel sensitively responded to ROS for disassembling and antigen release, which may be attributed to the presence of TSPBA linker that could be cleaved by ROS [35].

3.4 Induction of dendritic cell aggregation by the magnetic microgel
Dendritic cells are critical for antigen presentation and induction of adaptive immune response [37–39]. Owing to the presence of dextran in the microgels that could be well recognized by dendritic cells [40, 41], we hypothesized that the magnetic microgels could recruit these cells and induce their aggregation. To test this, free DC2.4 dendritic cells were co-incubated with the magnetic microgels and control microgels for confocal observation. As shown in Fig. 4(a), while the cells treated by CaAg or MG+CaAg exhibited well-dispersed state, the cells treated by MG+MNP-DexBP+CaAg formed obvious aggregates, and the FDA fluorescence of the cells perfectly overlapped the rhodamine B fluorescence of the microgel. Statistical analysis further showed that the MG+MNP-DexBP+CaAg group had much higher
average size of cellular aggregates than the CaAg or MG+CaAg groups (180 μm versus 25–30 μm, Fig. 4(b)), with much less free cells than the other two groups (2% versus 50–95%, Fig. 4(c)). Therefore, the magnetic microgels, similar to the inducer (e.g., the cake) of amoeba aggregation, strongly induce the aggregation of dendritic cells (Fig. 4(d)).

3.5 Enhanced antigen uptake and antigen presentation by the magnetic microgel

To evaluate the effect of the magnetic microgel on antigen uptake and subsequent presentation, a model antigen OVA was used for assessing antigen uptake and presentation in DC2.4 dendritic cells. Initially, the cytotoxicity of OVA, MG+OVA, and MG+MNP-DexBP+OVA to DC2.4 dendritic cells was evaluated using CCK-8 kits. As shown in Fig. 5(a), cell viability after treated by the above agents is above 90%, even if the concentration of microgels increased to 200 mg/L. Therefore, these agents are suitable for further biomedical applications.

Foreign agents, such as pathogens, artificial nanoparticles, and vaccine adjuvants, may evoke ROS production in immune cells, which is required for further immune activation [42, 43]. We then detected intracellular ROS levels in the microgel-treated dendritic cells by using 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) staining and fluorescence quantification. Compared to free OVA, both kinds of OVA-loading microgels led to obvious increase in ROS levels in the treated cells. Especially, MG+MNP-DexBP+OVA rendered the highest ROS levels among the treatments (0.4-fold higher than MG+OVA, and 1.2-fold higher than OVA, Fig. 5(b)), which may be attributed to the ROS-stimulating effect of the MNPs in the microgels. In addition, after co-incubation of the magnetic microgel and the suspended DC2.4 cells for 12 h, the cell aggregates were disassembled to form free cells (Fig. S7 in the ESM). These results indicated that the magnetic microgel induced strong ROS production, which led to disassembly of the microgels and dendritic cell aggregates, and further to release of antigen for cellular internalization and processing.

Next, we used FITC-labeled OVA (fOVA) to evaluate internalization efficiency of fOVA, MG+fOVA, and MG+MNP-DexBP+fOVA in DC2.4 cells. As shown in Fig. 5(c), the group cultured with MG+MNP-DexBP+fOVA exhibited the highest fluorescence intensity after 12 h, and the group of free fOVA had the lowest fluorescence intensity, indicating that encapsulation of OVA in microgels, especially in magnetic microgels (MG+MNP-DexBP), significantly increased internalization of the antigen.

The uptake and release of fOVA were also monitored by confocal microscopy. As shown in Fig. 5(d), while OVA and Lyso-Tracker Red were well overlapped in the DC2.4 cells after 12 h of incubation in both the fOVA group and the MG+fOVA group, fOVA was abundantly not co-localized with Lyso-Tracker in the MG+MNP-DexBP+fOVA group, with the Pearson’s correlation coefficient (PCC) value of fOVA with Lyso-Tracker in the MG+MNP-DexBP+fOVA group lower than in the other two groups (Fig. 5(e)). This indicated that MG+MNP-DexBP+fOVA rendered the fOVA antigen efficiently escape from the lysosomes for further processing. Similar results were observed by using FITC-labeled CaAg (fCaAg). MG+MNP-DexBP+fCaAg led to a high level of the internalized fCaAg antigen into the dendritic cells (Fig. S8(a) in the ESM), and fCaAg was efficiently escaped from the lysosomes (PCC value < 0.5, Fig. S8(b) in the ESM).

Antigen presentation is a critical process in dendritic cells for transporting the antigen message to downstream T cells and consequent activation of humoral immunity [44, 45]. Flow cytometry was used to evaluate exposure of 25D1.16, an OVA-derived peptide reflecting the efficiency of antigen presentation.
3.6 Enhanced antigen retention in lymph nodes by the magnetic microgels

Encouraged by the in vitro characterization results of OVA-loading magnetic microgels, we turned to perform in vivo vaccination studies. Initially, Cy5-CaAg-loading microgels and magnetic microgels, or free Cy5-CaAg was injected subcutaneously into the hind footpad of mice, and antigen migration in mice was observed at 1 and 24 h after injection using the animal imaging system. As shown in Fig. 6(a), fluorescence signal of free Cy5-CaAg at lymph nodes was observed at 1 h after injection and attenuated significantly at 24 h, indicating that most of the free antigen was rapidly expelled from the body. In contrast, the magnetic microgels efficiently retained most of the antigens at the lymph node regions even after 24 h, while the MNP-free microgel only partially retained the antigens (Fig. 6(a)). Organ fluorescence quantification confirmed that the inguinal and axillary lymph nodes of the MG+MNP-DexBP+CaAg group had much higher Cy5 intensity than other organs at 1 h post-injection (Fig. S9(a) in the ESM). Besides lymph nodes, the microgel together with the antigen could be slightly delivered to the liver, heart, lung, spleen, and kidney, but could not be delivered to other organs, e.g., the brain. Flow cytometry further showed that the magnetic microgel led to the highest proportion of CD11c+ cells (i.e., dendritic cells) in all lymph cells (Fig. S9(b) in the ESM), together with the highest proportion of CD86+ cells in CD11c+ lymph cells (Fig. S9(c) in the ESM). These results indicated the strongest activity of the magnetic microgel in dendritic cell recruitment and activation. Therefore, the magnetic microgel drastically enhanced antigen retention for dendritic cell recruitment and activation in lymph nodes.

Enhanced antigen retention in the lymph nodes may facilitate lymph node development. As expected, on day 14 after prime-boost vaccination, the MG+MNP-DexBP+CaAg group exhibited the highest inguinal lymph node weight, which was 1.5-fold higher than the weight in the MG+CaAg group, and > 2-fold higher than the weight in the control and free CaAg group (Fig. 6(b)). Histological observation further showed that the magnetic microgel led to the highest cell density in the lymph nodes as compared to other treatments (Fig. 6(c)), confirming the strongest immune cell recruitment induced by this gel. Interestingly, the
3.7 Activation of spleen T cells and humoral immunity by the magnetic microgel

We further investigate the effect of the magnetic microgel on spleen T cells and humoral immunity. On day 0 and 7, the mice were subcutaneously immunized by CaAg, MG+CaAg, or MG+MNP-DexBP+CaAg for assays of activated T cells and serum CaAg-specific IgG levels (Fig. 7(a)). On day 14, the splenocytes of the vaccinated mice were sampled for staining by APC-conjugated cytokine antibodies and the FITC-conjugated CD4 antibody and further flow cytometry. As compared to free CaAg and MG+CaAg, MG+MNP-DexBP+CaAg led to the highest proportions of interleukin-2 (IL-2)-positive, interleukin-4 (IL-4)-positive, interferon-γ (IFN-γ)-positive, and tumor necrosis factor-α (TNF-α)-positive cells in CD4+ T cells (Figs. 7(b)–7(e)). This indicated that the spleen T cells were strongly activated by the magnetic microgels.

The specific anti-CaAg antibody was further detected using ELISA. As shown in Fig. 7(f), the CaAg-specific IgG levels of the MG+MNP-DexBP+CaAg group were the highest compared with those of other groups on both day 14 and 21, suggesting that encapsulation of the antigen into the magnetic microgels could strongly activate humoral immunity for production of specific antibodies. The enhanced antigen retention and lymph node development by the microgel was most likely associated with the strong activation of spleen T cells by the microgel and humoral immunity, which may further endow the mice efficiently tolerating to pathogen infections.

3.8 Prevention of systemic infection by magnetic microgel-mediated vaccination

A systemic infection model with the life-threatening fungal
Figure 6  In vivo imaging and biosafety of CaAg. (a) Distribution of CaAg in vivo after injection for 1 and 24 h. (b) Inguinal lymph node weight of mice. (c) H&E staining of lymph node on day 14 after prime-boost vaccination. (d) H&E staining of the liver, heart, lung, spleen, and kidney on day 14. Scale bar = 100 μm. The asterisks (*) indicates significant difference between the MG+MNP-DexBP+CaAg group and other groups (P < 0.05).

Figure 7  Activation of immune response in the mice by the magnetic microgel. (a) Procedure of mouse vaccination and design of immune activation assays. (b) Percentage of IL-2-producing CD4+ T cells in spleens, (c) percentage of IL-4-producing CD4+ T cells, (d) percentage of IFN-γ-producing CD4+ T cells, and (e) percentage of TNF-α-producing CD4+ T cells. (f) CaAg-specific IgG levels in the mouse serum revealed by ELISA. The asterisks (*) indicates significant difference between the MG+MNP-DexBP+CaAg group and other groups (P < 0.05).
pathogen *C. albicans* was used for evaluating the efficiency of the magnetic microgel-mediated vaccination. The mice were pre-immunized by CaAg, Mg+CaAg, or Mg+MNP-DexBP+CaAg with the normal prime-boost vaccination procedure on day −14 and −7, and then were infected by the pathogens on day 0 for assays of survival rate, antibody levels, kidney fungal burden, and cytokine production (Fig. 8(a)). While all of the mice in the control and free CaAg-immunized groups were dead in 3 days after pathogen infection, the mice in the microgel-immunized groups had longer survival time (Fig. 8(b)). Especially, while Mg+CaAg failed to protect the mice from death after 7 days, Mg+MNP-DexBP+CaAg prevented all of the mice from death in 7 days, and avoided the death of 60% mice even after 14 days (Fig. 8(b)). Moreover, while the body weights of control group and the groups treated by CaAg and Mg+CaAg rapidly decreased after fungal infection, the body weights of the Mg+MNP-DexBP+CaAg group slightly increased in the first 4 days, and then gradually increased (Fig. 8(c)), which may be attributed to body recovery from fungal infection. Consistently, the Mg+MNP-DexBP+CaAg group had the highest serum anti-CaAg antibody levels among the treatments on day 3 (Fig S10 in the ESM), indicating that the magnetic microgel maintained high antibody levels for preventing fungal infection and mouse death.

Generally, systemic *C. albicans* infections severely disrupted the structure and function of kidneys [46–48]. The weight, histopathological analysis, and fungal burden of kidneys were assessed on day 3 (Figs. 8(d)–8(f)). Definitely, the control group suffered from severe fungal invasion and inflammation, and exhibited the highest kidney weight and fungal burden. Vaccination by CaAg or Mg+CaAg slightly decreased the kidney weight and fungal burden, but the fungal invasion was still fatal as the *C. albicans* hyphae (dark pink filaments) could be clearly observed in the histopathological image of kidney (Fig. 8(e)). On the contrary, Mg+MNP-DexBP+CaAg efficiently prevented fungal infection, which was revealed the lowest kidney weight, neglectable fungal burden, and unobservable *C. albicans* hyphae in the histopathological image (Figs. 8(d)–8(f)). Moreover, the Mg+MNP-DexBP+CaAg group has the lowest level of blood urea, TNF-α, and G-CSF (Figs. 8(g)–8(i)), indicating the strongest activity of the magnetic microgel vaccination in protecting the kidney from inflammation. Taken together, these results revealed that the vaccination by Mg+MNP-DexBP+CaAg strongly attenuated the life-threatening fungal infection, and efficiently protected the mice from death caused by systemic infections, which may be associated with enhanced T cell activation and humoral immunity as shown in Fig. 7.

### 4 Conclusions

In summary, this study designed an amoeba-inspired microgel
assembly for efficient vaccination against life-threatening infections. The microgel was composed of the dextran/PVA framework, the ROS-responsive linker TSPB, and magnetic nanoparticles functionalized by a genetically engineered dextran-binding protein. Similar to the behavior of amoeba aggregation, the magnetic microgels self-assembled to form aggregates, and further induced aggregation of dendritic cells. After further incubation with the dendritic cells, the magnetic microgels evoked ROS production, leading to disassembly of the microgel, antigen release, and enhanced antigen presentation. In vivo experiments further showed that the step-by-step assembly and disassembly of magnetic microgels realized retention of antigens in lymph nodes, activation of spleen T cell, enhancement of humoral immunity, and protection of mice from life-threatening systemic infections. This study developed an amoeba-inspired assembly-disassembly strategy to regulate immune response for efficient vaccination against infectious diseases.

Acknowledgements
This work was supported by the National Natural Science Foundation of China (Nos. 3217010793 and 31870139), Tianjin Synthetic Bionotechnology Innovation Capacity Improvement Project (No. TSBCIP-KIIG-006), the Natural Science Foundation of Tianjin (No. 19JZDG33800), and the Fundamental Research Funds for the Central Universities.

Electronic Supplementary Material: Supplementary material (AFM image and zeta potential of MG; TEM, FT-IR, DLS, and zeta potential of MNP-DexBP; zeta potential of MG+CaAg and MG+MNP-DexBP+CaAg; antigen release profile of MG+CaAg and MG+MNP-DexBP+CaAg; aggregation and dispersion of dendritic cells induced by MG+MNP-DexBP+CaAg; uptake of FITC-labeled CaAg (iCaAg) and intracellular distribution of iCaAg in the dendritic cells; antigen retention and dendritic cell activation in lymph nodes; and serum anti-CaAg antibody levels on day 3 after C. albicans infection in the mice pre-immunized by PBS (control), CaAg, MG+CaAg, and MG+MNP-DexBP+CaAg) is available in the online version of this article at https://doi.org/10.1007/s12274-022-4809-1.

References
[1] Kollmann, T. R.; Marchant, A.; Way, S. S. Vaccination strategies to enhance immunity in neonates. Science 2020, 368, 612–615.
[2] Iwasaki, A.; Omer, S. B. Why and how vaccines work. Nat. Rev. Immunol. 2020, 20, 87–88.
[3] Laupèze, B.; Del Giudice, G.; Doherty, M. T.; Van Der Most, R.; Vaccination as a preventative measure contributing to immune fitness. npj Vaccines 2021, 6, 93.
[4] Mascola, J. R.; Fauci, A. S. Novel vaccine technologies for the 21st century. Nat. Rev. Immunol. 2020, 20, 87–88.
[5] Su, S.; Du, L. Y.; Jiang, S. B. Learning from the past: Development of safe and effective COVID19 vaccines. Nat. Rev. Microbiol. 2021, 19, 211–219.
[6] Sa-Ngumnoo, N.; Namdeo, K.; Khongkow, M.; Ruktanonchai, U.; Zhao, Y. X.; Liang, X. J. Review: Development of SARS-CoV-2 immuno-enhanced COVID-19 vaccines with nano-platform. Nano Res. 2022, 15, 2196–2225.
[7] Mendonça, S. A.; Lorincz, R.; Boucher, P.; Curiel, D. T. Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic. npj Vaccines 2021, 6, 97.
[8] Li, S. X.; Feng, X. R.; Wang, J. X.; He, L.; Wang, C. X.; Ding, J. X.; Chen, X. S. Polymer nanoparticles as adjuvants in cancer immunotherapy. Nano Res. 2018, 11, 5769–5786.
[9] Pulondran, B.; Arunachalam, P. S.; O’Hagan, D. T. Emerging concepts in the science of vaccine adjuvants. Nat. Rev. Drug Discov. 2021, 20, 454–475.
[10] Orr, M. T.; Khandhar, A. P.; Seydoux, E.; Liang, H.; Gage, E.; Mikasa, T.; Beebe, E. L.; Rintala, N. D.; Persson, K. H.; Ahniyaz, A. et al. Reprogramming the adjuvant properties of aluminum oxyhydroxide with nanoparticle technology. npj Vaccines 2019, 4, 1.
[11] He, X. D.; Zhou, S. Q.; Huang, W. C.; Seiffhou, A.; Mabrouk, M. T.; Morgan, M. T.; Ortega, J.; Abrams, S. I.; Lovell, J. F. A potent cancer vaccine adjuvant system for particleization of short, synthetic CD8 T cell epitopes. ACS Nano 2021, 15, 4357–4371.
[12] Li, X. P.; He, X. F.; He, D. R.; Liu, Y.; Chen, K.; Yin, P. C. A polymeric co-assembly of subunit vaccine with polyoxometalates induces enhanced immune responses. Nano Res. 2022, 15, 4175–4180.
[13] Fries, C. N.; Curvino, E. J.; Chen, J. L.; Parmar, S. R.; Fouda, G. G.; Collier, J. H. Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. Nat. Nanotechnol. 2020, 16, 1–14.
[14] Daly, A. C.; Riley, L.; Segura, T.; Burdick, J. A. Hydrogel microparticles for biomedical applications. Nat. Rev. Mater. 2020, 5, 20–43.
[15] Nguyen, T. P. T.; Li, F. Y.; Shrestha, S.; Tuan, R. S.; Thissen, H.; Forsythe, J. S.; Frith, J. E. Cell-laden injectable microgels: Current status and future prospects for cartilage regeneration. Biomaterials 2021, 279, 121214.
[16] Griffin, D. R. Archang, M. M.; Kuan, C. H.; Weaver, W. M.; Weinstein, J. S.; Feng, A. C.; Ruccia, A.; Sideris, E.; Ragkousis, V.; Koh, J. et al. Activating an adaptive immune response from a hydrogel scaffold imparts regenerative wound healing. Nat. Mater. 2021, 20, 560–569.
[17] Pan, Y.; Qi, X. Y.; Li, X. Y.; Luan, S. F.; Huang, Y. B. Application of mannosyl-functionalized microgel as a novel vaccine delivery platform for subunit vaccines. Adv. Funct. Mater. 2021, 31, 2105742.
[18] Gavilán, H.; Avugadda, S. K.; Fernández-Cabada, T.; Soni, N.; Cassani, M.; Mai, B. T.; Chantrell, R.; Pellegrino, T. Magnetic nanoparticles and clusters for magnetic hyperthermia: Optimizing their heat performance and developing combinatorial therapies to tackle cancer. Chem. Soc. Rev. 2021, 50, 11614–11667.
[19] Liu, X. L.; Zhang, Y. F.; Wang, Y. Y.; Zha, W. J.; Li, G. L.; Ma, X. W.; Zhang, Y. H.; Chen, S. Z.; Tiwari, S.; Shi, K. J. et al. Comprehensive understanding of magnetic hyperthermia for improving antitumor therapeutic efficacy. Theranostics 2020, 10, 3793–3815.
[20] Xiong, J. Q.; Wu, M.; Chen, J. L.; Liu, Y. F.; Chen, Y. R.; Fan, G. L.; Liu, Y. Y.; Cheng, J. J; Wang, Z. H.; Wang, S. X. et al. Cancer-erythrocyte hybrid membrane-camouflaged magnetic nanoparticles with enhanced photothermal-immunotherapy for ovarian cancer. ACS Nano 2021, 15, 19756–19770.
[21] Yu, Q. L.; Deng, T.; Lin, F. C.; Zhang, B.; Zink, J. I. Supramolecular assemblies of heterogeneous mesosporous silica nanoparticles to deliver antimicrobial peptides and antibiotics for synergistic eradication of pathogenic biofilms. ACS Nano 2020, 14, 5926–5937.
[22] Chiang, C. S.; Lin, Y. J.; Lee, R.; Lai, Y. H.; Cheng, H. W.; Hsieh, C. H.; Shyu, W. C.; Chen, S. Y. Combination of fusocoid-based magnetic nanoparticles and immunomodulators enhances tumour-localized immunotherapy. Nat. Nanotechnol. 2018, 13, 746–754.
[23] Chao, Y.; Chen, G. B.; Liang, C.; Xu, J.; Dong, Z. L.; Han, X.; Wang, C.; Liu, Z. Iron nanoparticles for low-power local magnetic hyperthermia in combination with immune checkpoint blockade for systemic antitumor therapy. Nano Lett. 2019, 19, 4287–4296.
[24] Yu, Q. L.; Zhang, Y. M.; Liu, Y. H.; Xu, X.; Liu, Y. Magnetism and photo dual-controlled supramolecular assembly for suppression of tumor invasion and metastasis. Sci. Adv. 2018, 4, eaat2297.
[25] Tero, A.; Takagi, S.; Saigusa, T.; Ito, K.; Bebber, D. P.; Fricker, M. D.; Yumiki, K.; Kobayashi, R.; Nakagaki, T. Rules for biologically inspired adaptive network design. Science 2010, 327, 439–442.
[26] Ott, W.; Jobst, M. A.; Bauer, M. S.; Durner, E.; Milles, L. F.; Nash, M. A.; Gaub, H. E. Elastin-like polypeptide linkers for single-molecule force spectroscopy. ACS Nano 2017, 11, 6346–6354.
[27] Zhao, Y.; Liu, S.; Shi, Z. S.; Zhu, H. Q.; Li, M. C.; Yu, Q. L. Pathogen infection-responsive nanoplatform targeting macrophage...
endoplasmic reticulum for treating life-threatening systemic infection. Nano Res. 2022, 15, 6243–6255.

[28] Wang, H.; Mooney, D. J. Biomaterial-assisted targeted modulation of immune cells in cancer treatment. Nat. Mat. 2018, 17, 761–772.

[29] Kim, J.; Li, W. A.; Choi, Y.; Lewin, S. A.; Verbeke, C. S.; Dranoff, G.; Mooney, D. J. Injectable, spontaneously assembling, inorganic scaffolds modulate immune cells in vivo and increase vaccine efficacy. Nat. Biotechnol. 2015, 33, 64–72.

[30] Zhou, W. X.; Chen, X. L.; Zhou, Y.; Shi, S.; Liang, C.; Yu, X. J.; Chen, H. Y.; Guo, Q.; Zhang, Y. W. et al. Exosomes derived from immunogenically dying tumor cells as a versatile tool for vaccination against pancreatic cancer. Biomaterials 2022, 280, 121306.

[31] Nicolas-Boluda, A.; Yang, Z. J.; Guilbert, T.; Fouassier, L.; Carn, F.; Gazeau, F.; Pileni, M. P. Self-assemblies of Fe_{3}O_{4} nanocrystals: Toward nanoscale precision of photothermal effects in the tumor microenvironment. Adv. Funct. Mater. 2021, 31, 2006824.

[32] Antman-Passig, M.; Giron, J.; Karni, M.; Motiei, M.; Schori, H.; Shefi, O. Magnetic assembly of a multifunctional guidance conduit for peripheral nerve repair. Adv. Funct. Mater. 2021, 31, 2010837.

[33] Zhao, H.; Huang, J.; Li, Y.; Lv, X. J.; Zhou, H. T.; Wang, H. R.; Xu, Y. Y.; Wang, C.; Wang, J.; Liu, Z. ROS-scavenging hydrogel to promote healing of bacteria infected diabetic wounds. Biomaterials 2020, 258, 120286.

[34] Li, Z. H.; Zhu, D. S.; Hui, Q.; Bi, J. N.; Yu, B. J.; Huang, Z.; Hu, S. Q.; Wang, Z. Z.; Caranasos, T.; Rossi, J. et al. Injection of ROS-responsive hydrogel loaded with basic fibroblast growth factor into the pericardial cavity for heart repair. Adv. Funct. Mater. 2021, 31, 2004377.

[35] Ruan, H. T.; Hu, Q. Y.; Wen, D.; Chen, Q.; Chen, G. J.; Lu, Y. F.; Wang, J. Q.; Cheng, H.; Lu, W. Y.; Gu, Z. A dual-bioresponsive drug-delivery depot for combination of epigenetic modulation and immune checkpoint blockade. Adv. Mater. 2019, 31, 1806957.

[36] Forman, H. J.; Torres, M. Reactive oxygen species and cell signaling: Respiratory burst in macrophage signaling. Am. J. Respir. Crit. Care Med. 2002, 166, S4–S8.

[37] Steinman, R. M. Decisions about dendritic cells: Past, present, and future. Annu. Rev. Immunol. 2012, 30, 1–22.

[38] Wculek, S. K.; Cueto, F. J.; Mujal, A. M.; Melero, I.; Krummel, M. F.; Sancho, D. Dendritic cells in cancer immunology and immunotherapy. Nat. Rev. Immunol. 2020, 20, 7–24.

[39] Yin, X. Y.; Chen, S. T.; Eisenbarth, S. C. Dendritic cell regulation of T Helper cells. Annu. Rev. Immunol. 2021, 39, 759–790.

[40] El-Sayed, N.; Korotchenko, E.; Scheibthofer, S.; Weiss, R.; Schneider, M. Functionalized multifunctional nanovaccine for targeting dendritic cells and modulation of immune response. Int. J. Pharm. 2021, 593, 120123.

[41] Vetvicka, V. Glucan-immunostimulant, adjuvant, potential drug. World J. Clin. Oncol. 2011, 2, 115–119.

[42] Nauseef, W. M. How human neutrophils kill and degrade microbes: An integrated view. Immunol. Rev. 2007, 219, 88–102.

[43] Germain, R. N. MHC-dependent antigen processing and peptide presentation: Providing ligands for T lymphocyte activation. Cell 1994, 76, 287–299.

[44] Itano, A. A.; Jenkins, M. K. Antigen presentation to naive CD4 T cells in the lymph node. Nat. Immunol. 2003, 4, 733–739.

[45] Jhunjhunwala, S.; Hammer, C.; Delamarre, L. Antigen presentation in cancer: Insights into tumour immunogenicity and immune evasion. Nat. Rev. Cancer 2021, 21, 298–312.

[46] Jawale, C. V.; Ramani, K.; Li, D. D.; Coleman, B. M.; Oberoi, R. S.; Kupul, S.; Lin, L.; Desai, J. V.; Delgoffe, G. M.; Lionakis, M. S. et al. Restoring glucose uptake rescues neutrophil dysfunction and protects against systemic fungal infection in mouse models of kidney disease. Sci. Transl. Med. 2020, 12, eaay5691.

[47] Peng, L. P.; Wei, H. A.; Tian, L.; Xu, J. C.; Li, M. C.; Yu, Q. L. Phospholipid/protein co-mediated assembly of Cu_{2}O nanoparticles for specific inhibition of growth and biofilm formation of pathogenic fungi. Sci. China Mater. 2021, 64, 759–768.

[48] Boniche, C.; Rossi, S. A.; Kischkel, B.; Barbalho, F. V.; Moura, A. N. D.; Nosanchuk, J. D.; Travassos, L. R.; Taborda, C. P. Immunotherapy against systemic fungal infections based on monoclonal antibodies. J. Fungi 2020, 6, 31.