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Bio-inspired adhesive porous particles with human MSCs encapsulation for systemic lupus erythematosus treatment

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ABSTRACT

Mesenchymal stem cells (MSCs) therapy is a promising treatment for Systemic lupus erythematosus (SLE) patients. However, this method is encumbered by suboptimal phenotype of MSCs used in clinical settings, and a short *in vivo* persistence time. Herein, inspired by the natural microstructure of the sand tower worm nest, we proposed novel adhesive porous particles with human MSCs encapsulation via microfluidic electrospray technology for SLE treatment. The porous microparticles were formed by immediate gelation reaction between sodium alginate (ALG) and poly-D-lysine (PDL), and then sacrificed polyethylene oxide (PEO) to form the pores. The resultant microparticles could protect MSCs from immune cells while maintain their immune modulating functions, and achieve rapid exchange of nutrients from the body. In addition, owing to the electrostatic adsorption and covalent bonding between PDL and tissues, the porous microparticles could adhere to the bowel surfaces tightly after intraperitoneal injection. Through *in vivo* imaging system (IVIS) methods and *in vivo* study, it was demonstrated that the MSCs-encapsulated porous adhesive microparticles could significantly increase the cellular half-life, turn activated inflammatory macrophages into an anti-inflammatory profile, and ameliorate disease progression in MRL/*lpr* mice. Thus, the MSCs-encapsulated porous microparticles showed distinctive functions in chronic SLE treatment, with additional potential to be used in a variety of biomedical applications.

1. Introduction

Systemic lupus erythematosus (SLE) is a common and potentially fatal autoimmune disease that affects almost all organs and tissues [1]. It is characterized by deposition of massive immune complexes, production of abundant autoantibodies, upregulation of inflammatory responses, and damage of different tissues [2]. Current effective treatment is combination drug therapy, including glucocorticoids, immunosuppressive agents, anti-malarial drugs and non-steroidal anti-inflammatory drug [3–5]. Although these clinical drugs can control clinical symptom to a degree, many patients experience significant side effects, especially infections, rivaling the manifestations of disease itself in severity [6,7]. Alternatively, multi-potent mesenchymal stem cells (MSCs) therapy has gradually emerged, with significant improvement in both disease activity and autoantibody levels. MSCs can modulate the immune system by exerting regulatory effects on various immune cells such as T and B-lymphocytes, natural killer cells, dendritic cells and macrophages, addressing the immune imbalance of SLE [8–11]. The abilities of MSCs to secrete anti-inflammatory cytokines expand regulatory T cells and down-regulate costimulatory molecules on antigen presenting cells has been well established [12–16]. However, the clinical efficacy of MSCs therapies has greatly been challenged by suboptimal phenotype of MSCs. In addition, the transplanted MSCs will be immediately recognized and killed by the immune system [17]. Therefore, novel MSCs therapies with increased persistence, fitness and curative potentials are yet to be developed.

In this study, inspired by the natural microstructure of the sand tower worm nest, we proposed novel adhesive porous particles with human MSCs encapsulation via microfluidic electrospray technology for systemic lupus erythematosus (SLE) treatment, as schemed in Fig. 1. Sandcastle worms could build porous mound-like reefs by sticking together large numbers of sand grains with cement secreted from the

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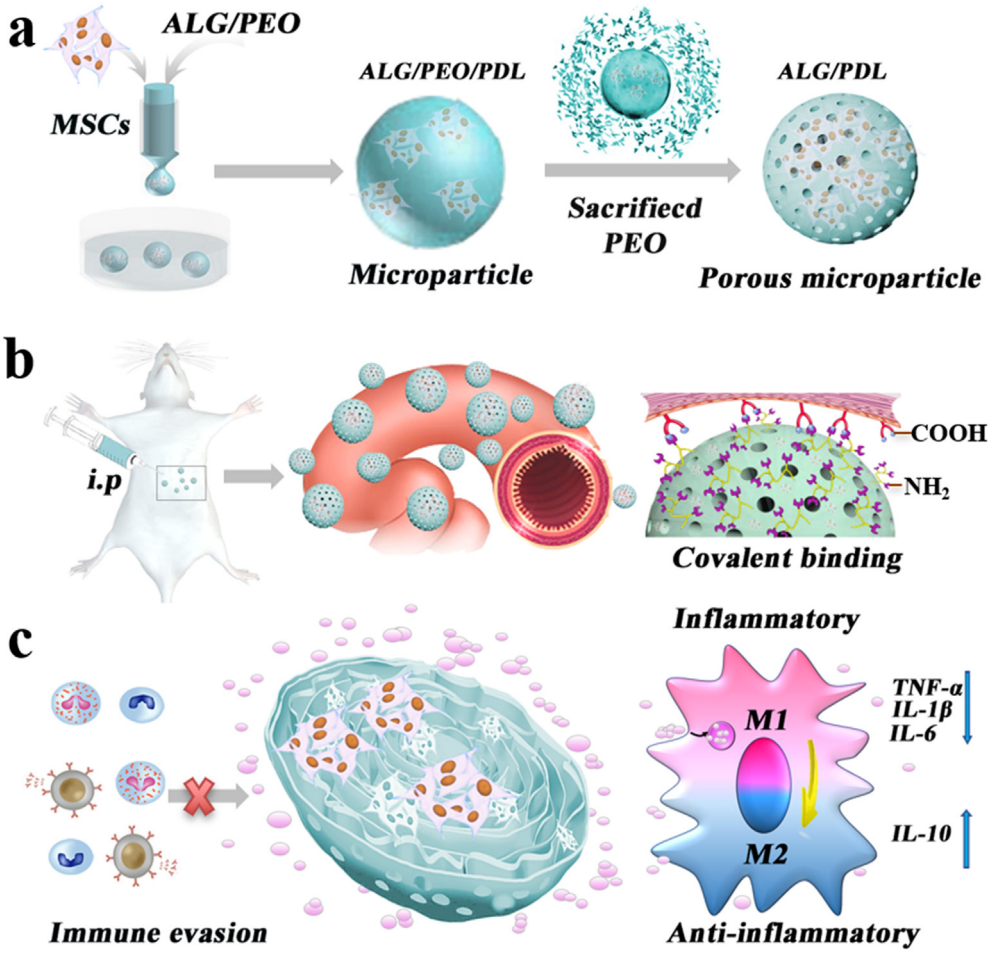

Fig. 1. Schematic demonstrating the fabrication of adhesive porous particles with MSCs encapsulation for immunomodulation after intraperitoneal transplantation in MRL/*lpr* mice. (a) Porous microparticles generation process from the microfluidic electrospray. (b)The MSCs encapsulated porous microparticles applied to SLE treatment by intraperitoneal injection. Porous microparticles could adhere to the bowel surfaces tightly according to the electrostatic adsorption and covalent bonding between PDL and tissues. (c) The porous microparticles protect MSCs from immune cells while maintain their immune modulating functions.

building organ under the water, protecting them from predator while acquiring nutrients, which could be imitated with designing microparticles [17–21]. For the generation of functional microparticles, a number of techniques, including electrospray, microfluidics, polymerization and complex emulsification have been explored [22–26]. Among them, microfluidic technology is most attractive as proved to show advance in morphologies, precisely tuning sizes and compartments within integrated flow [27,28]. However, most of the MSCs encapsulations require the addition of oil phase in the microfluidic emulsification processes, which is not friendly to MSCs. In addition, owing to inadequate design and innovation in hydrogel composition, the MSCs-loaded microparticles are usually lack of porous structure, which causes ineffective to ensure cell viability and functional factors transportation [29,30]. Moreover, it is still challenging to adhere MSCs-based microparticles on target site to ensure effective therapeutic effects.

Thus, we herein employed a simple microfluidic electrospray method to encapsulate MSCs into porous bioadhesive hydrogel microparticles for intraperitoneal injection and disease treatment. Microfluidic electrospray could encapsulate the cells in water phases, retaining bioactivity of the cells. The porous microparticles were formed by immediate gelation reaction between sodium alginate (ALG) and poly-D-lysine (PDL), and then polyethylene oxide (PEO) dissolved to form the pores. Size of the pores could be precisely controlled through adjusting the concentration of PEO, protecting MSCs from immune cells while maintain their immune modulating functions and rapid exchange of nutrients from the body. In addition, on according to the electrostatic adsorption and covalent bonding between PDL and tissues, the porous microparticles could adhere to the bowel surfaces tightly after intraperitoneal injection. It was demonstrated that the MSCs encapsulated porous adhesive microparticles could significantly increase the cellular half-life, turn activated inflammatory macrophages into an anti-inflammatory profile, and ameliorate disease progression in MRL/*lpr* mice. Thus, the MSCs encapsulated porous microparticles showed distinctive functions in chronic SLE treatment.

2. Experimental section

2.1. Materials

Sodium alginate, poly (ethylene oxide) (PEO) (average Mw = 900000 Da), Poly (D-lysine) (PDL, Mw 30–70 kDa), and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich. 1-ethyl- 3-[3-(dimethylamino) propyl] carbodiimide (EDC) and N-hydrosuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd., China. Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F-12), fetal bovine serum (FBS), penicillin/streptomycin (P/S), and Trypsin – EDTA solution were purchased from Gibco (Grand Island, NY). Cell counting kit-8 (CCK8) assay kit was obtained from KeyGEN BioTECH Co., Ltd (Nanjing, China). FITC-conjugated anti-human CD14, CD19, CD45, CD34, PE-conjugated anti-human CD73, CD105, CD90, HLA-DR antibodies were obtained from BD Biosciences (San Jose, CA).

All of the antibodies used for immunostaining were used according to the respective manufacturers' instructions.

2.2. Preparation of cell-laden porous microparticles

ALG (0.2 g) was dissolved in 10 mL of ddH_2O and was then stirred at 37 ˚C for 2 h to ensure full dissolution. Next, a PEO solution was added in a dropwise manner into the ALG solution with a mate ratio of 1:10. The MSC cells were cultured and mixed with PEO/ALG solution at 1×10^6/mL. The cell-laden mixture was then dispensed using a microfluidic electrospray system to generate cell-laden droplets. Next, the resultant droplets were immersed into a 10% PDL solution mixed EDC and NHS solution were prepared at 35 mg/mL and 17.5 mg/mL to generate cell-laden microparticles. The dispersive PEO solution was sacrificed to fabricate porous microparticles.

2.3. Biocompatibility of adhesive porous particles

The resultant encapsulated microparticles with different concentrations of precursor solution were added to each well containing 1 mL culture medium and incubated for 24h. The MSCs were plated in 96-well cell culture dishes with 4000 cells per well (100 μL) for 72 h. Following the incubation, 10 μL of CCK8 working solution was added in the cell culture media and then incubated for 1 h at 37 ˚C. Then, the optical density (OD) value was measured using a plate reader at 450 nm.

2.4. Isolation and culture of peripheral blood mononuclear cells and culture

Peripheral blood mononuclear cells (PBMCs) were isolated from lupus patients and healthy controls at the same time point, and then were co-cultured with MSCs for different hours (24,48,72 h) at ratio of 10:1, cells were harvested for examining by qRT-PCR.

2.5. Quantitative real-time polymerase chain reaction

Total RNA isolation was performed using TRIzol reagent (Vazyme Biotech Co., China). Reverse transcription was conducted using a commercial One Step RT-PCR kit (Vazyme Biotech Co., China), and qRT-PCR analysis was performed using FastStart Universal SYBR Green Master Mix (Vazyme Biotech Co., China) in triplicate for each condition. The housekeeping gene GAPDH was used as the internal control for gene expression normalization. The sequences of all the primers used for qRT-PCR are listed in Table 1.

2.6. In vivo residence time

In experiments to assess bioluminescence at multiple time points, mice were divided into two cohorts, injected with equal numbers of luciferase-expressing MSCs, and imaged at 2 h, 1 day, 3 days and 7 days. For imaging, 3 mg D-luciferin (Promega, Fitchburg, WI, USA) was injected intraperitoneally followed by luminescence imaging with the bioluminescence system (Caliper IVIS Lumina XR), luminescent signal analysis was performed as described previously [31].

2.7. Mouse macrophage isolation and culture

Briefly,1.0 mL of 3% brewer thioglycolate medium was injected into peritoneum of female B6 mice once a day for three days. After 3 days, mice were sacrificed and peritoneal exudate cells were harvested by peritoneal lavage using 5 mL RPMI 1640 medium. Cells were plated in 12-well flat-bottom culture plates in RPMI 1640 medium supplemented with 10% FBS and 100 U/mL penicillin/streptomycin. After 2 h of incubation at 37 ˚C, nonadherent cells were removed. The adherent macrophages were cultured in the presence of lipopolysaccharide (LPS,100 mg/mL) for 3 h and then further cultured with/without MSCs.

2.8. Enzyme-linked immunosorbent assay (ELISA)

Human TGF-β enzyme-linked immunosorbent assay (ELISA) was performed on the co-cultured supernatants. ELISA kit was obtained from FcMACS (Nanjing, China). Tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6 and IL-10 levels in supernatant were measured by ELISA kit (Shibayagi, Gunma, Japan), Blood urea nitrogen (BUN) and urinary creatinine concentrations were detected with a Thermo Autoanalyzer. Serum anti-dsDNA antibodies, TNF-α, TGF-β, IL-6 and IL-10 levels were measured by ELISA, Serum anti-dsDNA ELISA kit was from FUJIFILM Wako Pure Chemical Corporation, TNF-α, TGF-β, IL-6 ELISA kits were from BioLegend (San Diego, CA, USA), IL-10 was purchased from 4A Biotech Co., Ltd (Beijing, China), respectively, according to the manufacturer's instructions.

2.9. Animals and treatment

MRL/*lpr* mice lack Fas and spontaneously develop an SLE-like disease. Similar to SLE patients, MRL/*lpr* mice have a marked increase in anti-dsDNA antibodies in their blood and develop severe nephritis. MRL/*lpr* mice were purchased from the Shanghai SLAC Laboratory Animal Co., Ltd (Shanghai, P.R. China). Mice were housed under specific pathogen-free conditions in the animal center of the Affiliated Drum Tower Hospital of Nanjing University Medical School. All animal experiments were approved by the Committee of Experimental Animal Administration of the Affiliated Drum Tower Hospital of Nanjing University Medical School (ethics number 2019AE01012). In each experiment, female mice were divided into four groups (4 mice per group): PBS, microparticles, MSCs, Microparticles-MSCs (1×10^6 cells/injection), MSCs were injected intraperitoneal once at the age of 12 weeks.

2.10. H&E and IHC staining

The kidney was harvested and then snap frozen in liquid nitrogen and placed in optimal cutting temperature embedding matrix (Leica Biosystems, Nussloch, Germany). Frozen sections were cut at a thickness of 7 μm and stained with fluorescein isothiocyanate (FITC)-anti-mouse IgG or FITC-anti-mouse C3 (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The immunostaining was developed using diaminobenzidine tetrahydrochloride, followed by counterstain with Mayer's Hematoxylin.

2.11. Statistical analysis

All of the data were statistically analyzed using Origin 9.1 software wherever appropriate. The data from each group are presented as mean ± SD, and the comparison was made between the groups with the aid of a one-way analysis of variance test. A p-value of less than 0.05 was used to determine the statistical significance.

3. Results and discussion

In a typical experiment, ALG was mixed with PEO as the precursor solution for generation of porous microparticles. During the electrospray process, the precursor solution was sprayed through a simple microfluidic device. Under the electric field generated by a high voltage direct current (HVDC) equipment, the precursor solution could form “Taylor cone” at the outlet and turned into droplets while falling (Fig. 2 a). The droplets were then rapidly immersed in the gelling pool, which contained poly-D-lysine (PDL), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) and N-hydrosuccinimide (NHS). The porous microparticles were formed by immediate gelation reaction between ALG and PDL, and sacrifice of the dispersive PEO component. These pores could endow the microparticles with improved oxygen and nutrient exchange efficiency and thus assist cell survival and promote cell

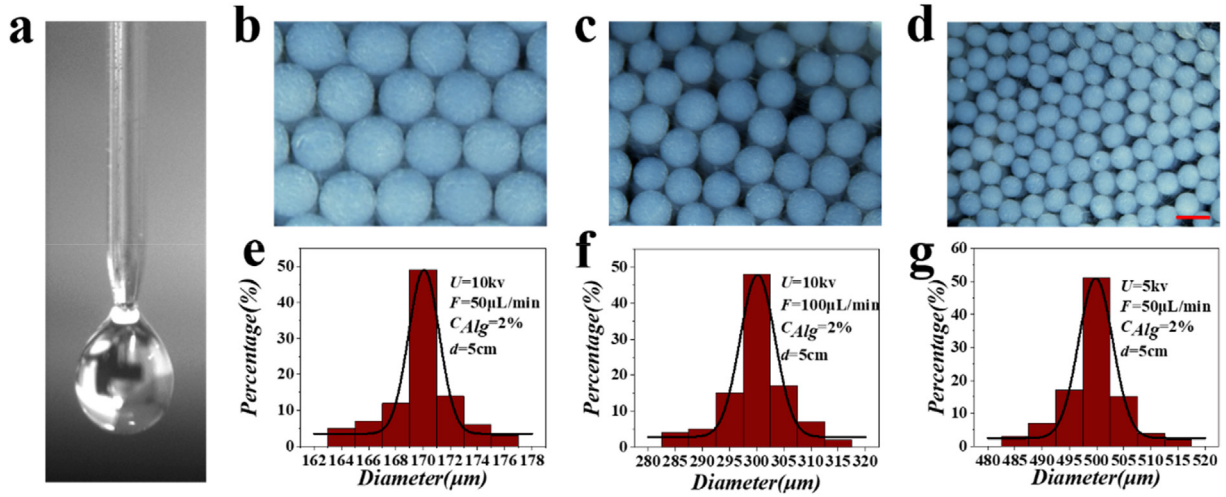

Fig. 2. Generation of PEO/ALG microparticles via microfluidic electrospray. (a) The formation of PEO/ALG droplets during the process of microfluidic electrospray. (b–d) Bright-field microscopic images of PEO/ALG porous microparticles. Scale bar is 200 μm. (e–f) Diameter distribution of PEO/ALG porous microparticles, 100 microparticles were measured for each map.

proliferation. Furthermore, the porous microparticles could adhere to the bowel surfaces tightly due to the electrostatic adsorption and covalent bonding between PDL and tissues with the existence of EDC and NHS (Fig. S1). In addition, by applying different parameters, microparticles with three different size distributions were formed and analyzed. As the cell survival play an essential role in the function of MSCs in particles, viability of MSCs incubated with three different size porous microspheres was measured. The cell viability was better in small size than big ones after 72 h incubation. There was no significant difference between diameters of 170 μm and 300 μm groups in cell survival. In our study, we applied the microparticle with diameter of 300 μm. (Fig. S2, Fig. S3, Fig. 2b–g).

The MSCs encapsulated porous adhesive microparticles were used for SLE treatment. The MSCs were obtained from human umbilical cord and identified by flow cytometric analysis, as the results showed that the expression of distinctive cell surface markers CD105, CD90 and CD73 was > 95%, in parallel with CD14, CD19, CD34, CD45 and HLA-DR expression < 2% (Fig. S4). In addition, cell viability and proliferation are vital in the efficacy of their therapeutic effect, thus, biocompatibility of the porous microparticles was investigated through the extract solution method. As shown in Fig. S5, the cell viability was over 80% in different concentration even after 72 h incubation compared with the control group, indicating that the porous microparticles were biocompatible with the MSCs. Furthermore, MSCs-laden porous microparticles generated by microfluidic electrospray were cultured in cell medium for 14 days to test cell proliferation capability (Fig. 3a). Viability of the encapsulated MSCs within 14 days was also analyzed in Fig. 3b. The MSCs were observed to proliferate well in the porous microparticles. When the culture continued, clusters could be observed by a large portion of proliferating cells, indicating the applicability of porous microparticles for long-term encapsulation of MSCs. The good proliferation may be ascribed to the biocompatible microenvironment of the porous microparticles, which could allow for the incorporation of extracellular matrix proteins, peptides, growth factors that support the growth of MSCs. Gene expression of MSCs with or without encapsulation was also detected to evaluate the effects of microparticles on immunomodulatory function of MSCs. A set of genes related to immunomodulatory factors associated with MSCs was chosen (CCL2, CCL5, CSF1, IL-6, IL-10, Nos2, Cox-2, TGF-β, TSG-6, PGE-2, HMOX). It could be observed that porous microparticles encapsulation led to differences in gene expression compared with control cells grown on tissue culture polystyrene. After encapsulation, CCL2 and CCL5 was expressed at lower level, whereas increased levels of CSF-1, IL-6, IL-10, Nos2, Cox-2, TGF-β, TSG-6, PGE-2, HMOX and HGF (Fig. 3c), indicating an enhanced immunomodulatory function.

To evaluate the therapeutic efficacy of porous MSCs-encapsulated microparticles in SLE treatment, their immunomodulatory function under SLE inflammatory environment was tested. Serum from both active lupus patients and healthy subjects was used to treat MSCs, and FBS was used as a control. After 48 h treatment, TGF-β, IL-6, and Cox-2 mRNA levels were detected using quantitative real-time PCR. Lupus serum promoted MSCs to secrete higher levels of TGF-β, IL-6, and Cox-2 compared with FBS, and TGF-β, IL-6, and Cox-2 significantly increased when stimulated by lupus serum compared with healthy serum (Fig. 4a and b; Fig. S6). In addition, the regulatory effects of MSCs on PBMCs were evaluated. PBMCs were isolated and co-cultured with MSCs or Microparticle-MSCs (Mi-MSCs) *in vitro* for different hours (24,48,72 h). The results showed that MSCs could significantly up-regulate the TGF-β level in the culture supernatant (Fig. 4c and d), which in turn led to the immune tolerance.

As survival of the porous microparticles encapsulated MSCs after intraperitoneal injection is very important, IVIS was employed to test the *in vivo* persistence time of MSCs. MSCs were transduced with a firefly luciferase reporter to image, and then simple MSCs and MSCs-laden porous microparticles were injected into the mice. Recipient animals were imaged at 2 h, 1 day, 3 days and 7 days after injection (Fig. 5a), and half-life of luminescent signal was calculated from an exponential decay curve fit (Fig. S7). Encapsulation led to longer *in vivo* persistence of MSCs relative to unencapsulated MSCs, which in turn led to the enhancement of immunomodulatory function *in vivo*. Furthermore, large number of macrophages exist in the abdominal cavity and distinct macrophage phenotypes contribute to tissue injury and repair. Thus, effects of MSCs on macrophages were investigated. Mouse peritoneal macrophages were activated with LPS, and then co-culture with MSCs. At mRNA level, activated macrophages co-cultured with MSCs showed lower expression of proinflammatory cytokines including TNF-α, IL-1β and IL-6, and higher expression of anti-inflammatory IL-10 (Fig. 5b). In addition, the levels of TNF-α, IL-1β and IL-6 in the co-cultured supernatants were also significantly decreased, whereas IL-10 levels were increased (Fig. 5c). Collectively, the results indicated that MSCs turned activated macrophages into an anti-inflammatory profile, which could enhance the resolution of inflammation.

The therapeutic effects of the MSCs-encapsulated microparticles were investigated by intraperitoneal injection of MRL/*lpr* mice which are the universal model of SLE. After successful model establishment, the mice were treated with PBS, Microparticles (Mi), MSCs, and Mi-

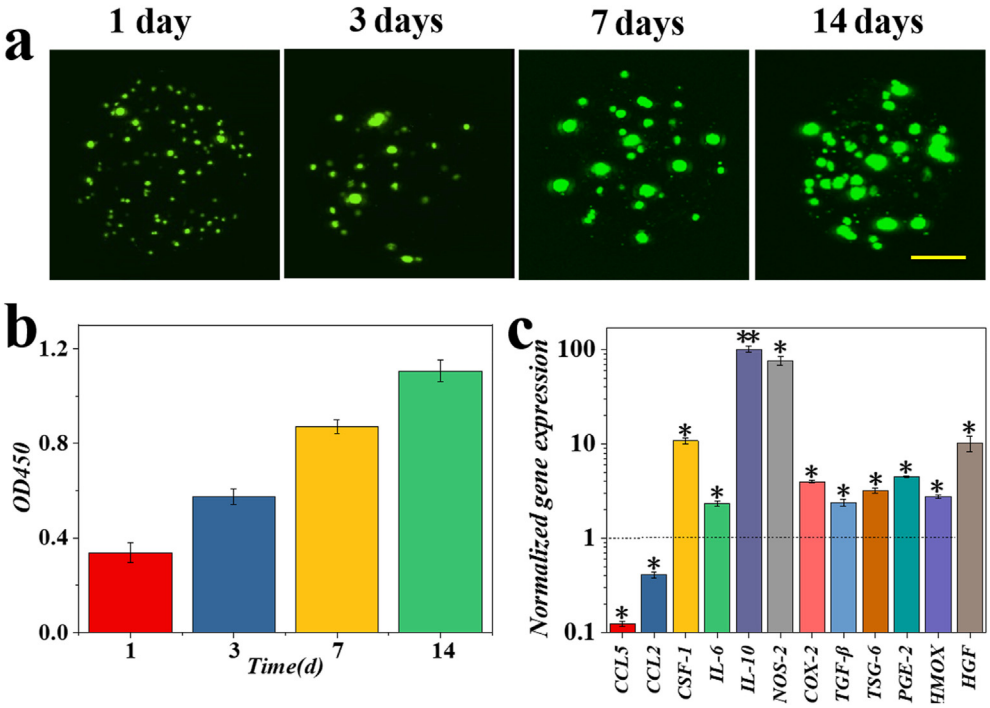

Fig. 3. Proliferation and immunomodulatory function of MSCs encapsulated in porous microparticles. (a) Representative images of MSCs in porous microparticles at day 1, 3, 7, and 14. Scale bar, 100 μm. (b) The viability of MSCs encapsulated in porous microparticles. (c) Gene expression in encapsulated MSCs normalized to MSCs on tissue culture plastic. $^{*}P < 0.05$, $^{**}P < 0.01$.

MSCs, respectively. The most apparent clinical sign of SLE was splenomegaly. There was a significant decrease in the spleen weight of MSCs and MSCs microparticles treated groups in comparison to the PBS and Mi groups (Fig. 6b). In addition, auto-antibodies, specifically anti-dsDNA antibodies in serum, play a crucial role in multiple organ impairment in SLE patients. The PBS treated MRL/*lpr* mice showed a remarkable increased level of anti-dsDNA antibodies, while that was found to decrease in MSCs and Mi-MSCs groups (Fig. 6c).

The MRL/*lpr* mice usually show renal disorders such as nephritis with glomerular basal membrane disorder, mesangial cell over-growth, and deposition of complement components (C3) and IgG. Deposition of IgG and C3 in the glomeruli was evaluated through immunostaining. It was found that compared with either PBS or Mi groups, the MSCs and Mi-MSCs groups could significantly decrease the deposition of IgG and C3 in the glomeruli of MRL/*lpr* mice. Thus, the MSCs treatment suppressed the complement activation through decreasing glomerular

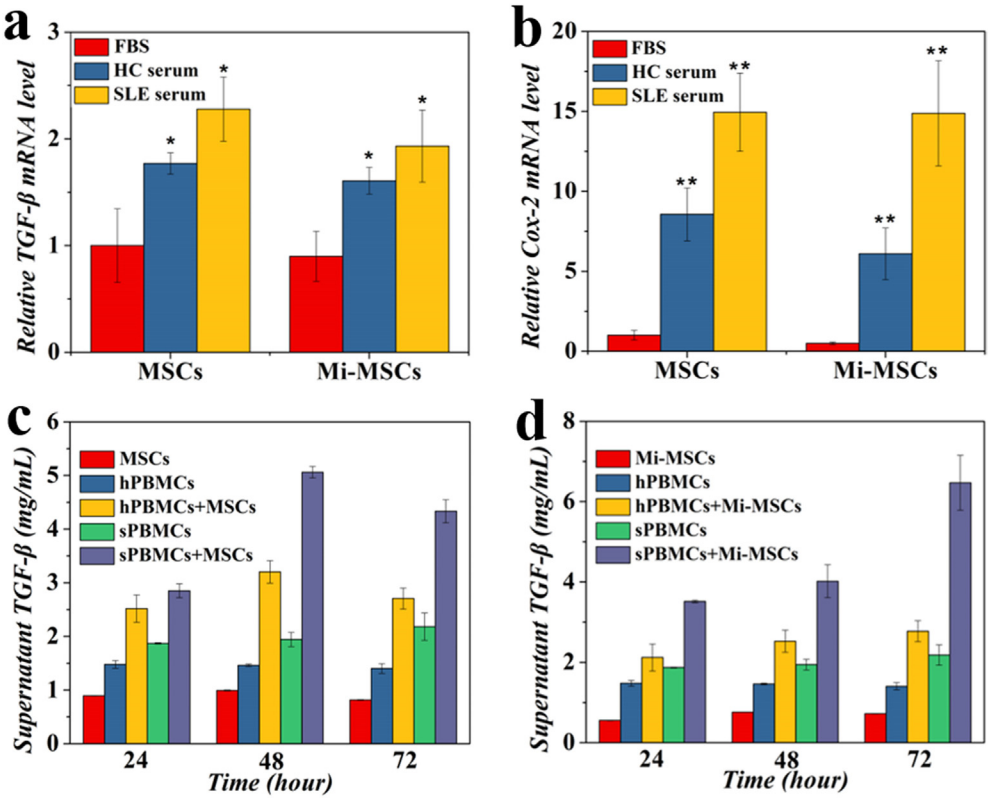

Fig. 4. Effect of encapsulation on MSCs function. (a) Serum from healthy controls and active SLE patients were used to stimulate MSCs and Mi-MSCs *in vitro*. The mRNA levels of TGF-β (a) and Cox-2 (b) were analyzed by real-time PCR. $^{*}P < 0.05$, $^{**}P < 0.01$. PBMC from active lupus patients (sPBMC) and healthy individuals (hPBMC) were co-cultured with MSCs (c) and Mi-MSCs (d) 24, 48,72 h later, the culture supernatants were collected and the level of TGF-β was assessed by ELISA.

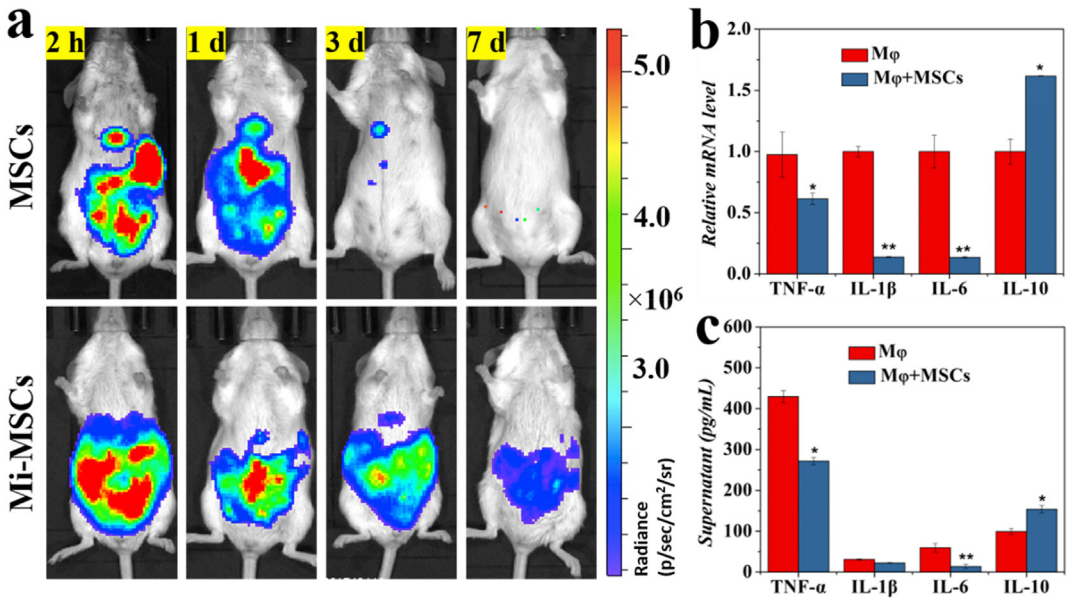

Fig. 5. MSCs polarized macrophages into an anti-inflammatory phenotype. (a) Luminescent signal of bare and encapsulated MSCs in B6 recipient mice after intraperitoneal transplantation. (b) Gene expression of TNF-a, IL-1β, IL-6 and IL-10 in macrophages by RT-qPCR. (c) Supernatant levels of TNF-a, IL-1β, IL-6 and IL-10 in the culture media were assessed by ELISA. *$P < 0.05$, **$P < 0.01$.

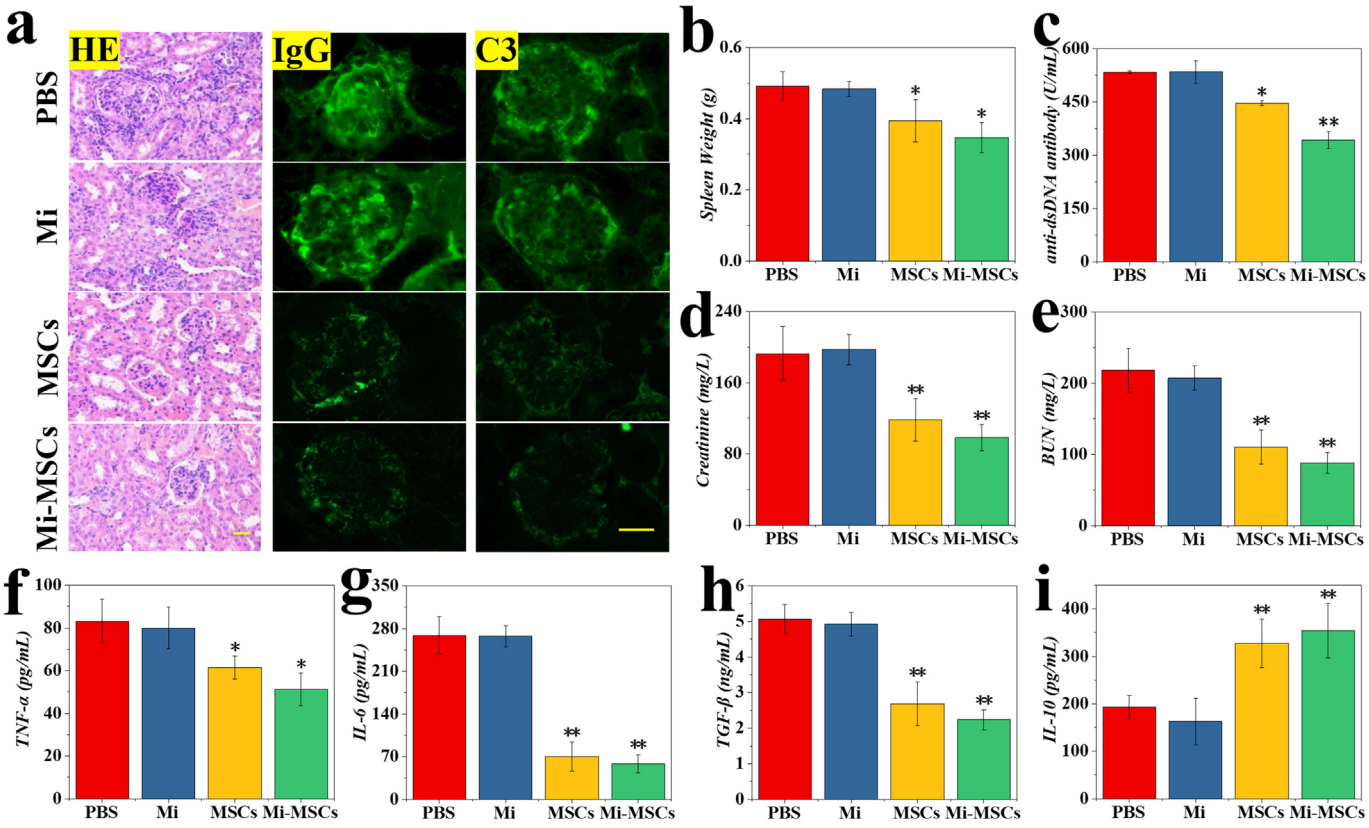

Fig. 6. Effect of MSCs in porous microparticles in MRL/*lpr* mice. MRL/*lpr* mice were treated with PBS, microparticles, MSCs or Mi-MSCs and were sacrificed 4 weeks after treatment. (a) Representative images of kidney hemotoxylin and eosin (H&E) and IgG and C3 immunostaining in glomeruli. Scale bar, 30 μm (b) Spleen weights of MRL/*lpr* mice at time of sacrifice. (c) anti-dsDNA antibodies level was measured by ELISA. (d, e) Serum creatinine and blood urea nitrogen (BUN) were measured in serum. (f, g, h, i) Cytokines TNF-α, IL-6, TGF-β and IL-10 were measured by ELISA. *$P < 0.05$, **$P < 0.01$.

immunocomplexes and C3 deposition. Furthermore, the histological analysis indicated that MSCs treatment markedly improved the kidney pathology in MRL/*lpr* mice, as shown by less mesangial cell proliferation and mesangial matrix deposition in glomeruli (Fig. 6a). Serum creatinine and blood urea nitrogen (BUN) were used as another important index of renal disease that reflected glomerular filtration which was distinct from glomeruli inflammatory. PBS or Mi groups had a progressive rise in creatinine and BUN levels compared with MSCs and Mi-MSCs groups (Fig. 6d and e). In addition, the production of inflammatory cytokines was measured through ELISA. A significant

reduction of TNF-α, IL-6 and TGF-β could be observed in MSCs and Mi-MSCs groups compared with controls, indicating a relatively lower inflammatory level (Fig. 6f, g, h), whereas the Anti-inflammatory factor IL-10 was detected, compared with controls, MSCs and Mi-MSCs groups had a progressive rise (Fig. 6i). These experimental evidences indicated that MSCs-encapsulated porous adhesive microparticles are ideal for treating SLE and versatile clinical diseases.

4. Conclusion

In conclusion, we reported the generation of bio-inspired adhesive porous particles with MSCs encapsulation for SLE treatment via microfluidic electrospray method. This technique could encapsulate the cells in water phases and retain bioactivity of the cells. The porous microparticles were formed by immediate gelation reaction between ALG and PDL, and then sacrificed PEO to form the pores. The porous structure could realize rapid exchange of nutrients from the body, protect MSCs from immune cells, and still maintain their immune modulating function. In addition, on according to the electrostatic adsorption and covalent bonding between PDL and tissues with the existence of EDC and NHS, the porous microparticles could tightly adhere onto the bowel surfaces after intraperitoneal injection. It was demonstrated that the resultant MSCs-encapsulated porous adhesive microparticles could effectively treat SLE through significantly increase the cellular half-life, turn activated inflammatory macrophages into an anti-inflammatory profile, and ameliorate disease progression. Therefore, the MSCs-encapsulated porous adhesive microparticles showed distinctive functions in chronic SLE treatment, and thus we believe that they will be widely employed in clinics.

Declaration of competing interestCOI

The authors declared that they have no conflicts of interest to this work.

CRediT authorship contribution statement

Min Nie: contributed equally to this work, Data curation, Writing - original draft. **Guopu Chen:** contributed equally to this work, Writing - review & editing. **Cheng Zhao:** Validation. **Jingjing Gan:** Validation. **Mihribangvl Alip:** Validation. **Yuanjin Zhao:** Conceptualization, Methodology. **Lingyun Sun:** Conceptualization, Methodology.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2020.07.018.

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