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-o-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
building organ under the water, protecting them from predator while acquiring nutrients, which could be imitated with designing micro-particles [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-α-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 (α-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-hydroxysuccinimide (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 ddH2O 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 molar ratio of 1:10. The MSCs were cultured and mixed with PEO/ALG solution at 1 × 10^6/mL. The cell-laden mixture was then dispersed 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, 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 thioglycollate 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% PBS 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 μg/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 FeMACS (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 markedly 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 diamino benzidine 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-o-lysine (PDL), 1-ethyl-3-[3-(dimethylamino) propyl] carbodiimide (EDC) and N-hydroxysuccinimide (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 survival.
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 80% in different concentration even after 72 h incubation compared with FBS, and 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 Mi-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 MSC-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 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 pro-inflammatory 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-MSCs (Fig. 2b; 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.
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...
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.

4. Conclusion

In conclusion, we reported the generation of bio-inspired adhesive porous particles with MSC 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 interest

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. Mihrirangv1 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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