Preparation and Performance of Acrylate Hydrogel Nanocomposite Adjuvant

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Abstract. In order to meet the urgent need of developing novel vaccine adjuvants with rapid development in the field of biomedicine, a novel acrylate hydrogel nanocomposite adjuvant with biological safety and cell targeting was prepared by compounding polyacrylate nanoemulsion, which can form antigen reservoir, with TLR3 agonist Poly I:C. The microscopic morphology of the composite adjuvant was observed, and its particle size, zeta potential, and rheological properties were characterized and analyzed. The composite adjuvant exhibited a particle size of less than 50 nm and a cell viability of 75.59% at Poly I:C concentration of 10 μg/mL, showing good biocompatibility. Furthermore, the composite adjuvant showed significant cell targeting and enhanced antigen phagocytosis capabilities. This study provides a new approach for developing composite adjuvant systems with synergistic immune-enhancing functions, demonstrating promising application potential.

Keywords: Hydrogel; Nanocomposite Adjuvant; Agonist; Phagocytosis.

1. Introduction

In recent years, novel biomedical therapeutic strategies based on polymeric materials have attracted significant attention. Due to their high molecular weight, structural tunability, and multifunctionality, polymeric materials can be used to construct nano-structures or micro-structures with excellent drug-loading capacity and targeted delivery performance, making them indispensable tools for disease treatment and the regulation of biological systems[1, 2].

In the field of vaccine adjuvants, acrylic polymers have been widely used in biomedical fields such as drug delivery, sustained-release formulations and vaccine adjuvants due to their excellent biocompatibility and safety[3-5]. Hydrogels formed from acrylates can create an antigen depot at the injection site. The mesh structure of the hydrogel enables the slow and sustained release of antigens, prolonging the exposure time between the immune system and the antigens. Meanwhile, with the rapid advancement of modern biomedical technology, the efficacy of single adjuvants often falls short of requirements. As a result, adjuvant development is increasingly shifting toward composite formulations. Khandhar[6] successfully activated and induced a strong Th1-type cellular immune response by compounding anionic polyacrylic acid and alum as a compound adjuvant. Immunopotentiators are substances that can regulate and stimulate the immune system nonspecifically. In modern vaccinology, many scholars compounded CpG, QuilA or other immunopotentiators into compound adjuvants, and studied the effect of immunopotentiators on adjuvant performance. Chen T.H et al.[7] combined PELC, a novel nanoemulsion adjuvant composed of biodegradable PEG-b-PLACL, Span85 and squalene, with CpG, and found that PELC/CpG could induce potent immune response, induce more specific IFN-γ in spleen, and improve the immune capacity of the body. Polyinosinic (Poly I:C) is a synthetic dsRNA complex. As a TLR3 agonist, it binds to TLR3 receptors on cells, which are widely expressed on dendritic cells, T cells, natural killer cells, and some non-immune cells, thus mediating innate and adaptive immune responses. This indicates that Poly I:C has the potential as a vaccine adjuvant[8]. Sun et al.[9] formed the composite adjuvant Al-Poly I:C by covalently binding the phosphate group of Poly I:C to the hydroxyl group of

the traditional adjuvant aluminum oxyhydroxide (AlOOH). The results found that Al-Poly I:C in the HBV model can significantly increase the levels of IgG, IgG1 and IgG2c specific antibodies in serum, and the number of dendritic cells carrying the antigen also increased by 2.5% compared with the antigen group.

The incorporation of immunopotentiators into adjuvant systems can produce a synergistic effect, inducing a higher level of immune response in the body. Therefore, composite adjuvants prepared by combining polyacrylate-based materials with Poly I:C deserves in-depth exploration and development. In this project, a polyacrylate hydrogel nanocomposite adjuvant was fabricated by formulating Poly I:C with polyacrylate hydrogel and a lab-made nanoemulsion. The microstructure, particle size, rheological properties, cytotoxicity, and cellular uptake capability of the composite adjuvant were characterized.

2. Experimental

2.1 Materials

Dodecyl acrylate (LA) was purchased from RYOJI Chemical. Acrylic acid was purchased from Tianjin Damao Chemical Reagent Factory. PolyI:C was purchased from Kaipeng Biotechnology Co., Ltd. Azobisisobutyronitrile and allyl pentaerythritol (APE) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. Cyclohexane was purchased from Shanghai Maclin Biochemical Technology Co., Ltd. Ethyl acetate was purchased from Tianjin City Yongda Chemical Reagent Co., Ltd. Nanoemulsion was self-made by our research group.

2.2 Preparation of Acrylate Hydrogel Complex Adjuvant

AA, $NaHCO_3$, dodecyl acrylate, APE, AIBN, dispersant, cyclohexane and ethyl acetate were added into a four-necked flask, mechanically stirred for 20 min under N_2 atmosphere, then the temperature of the system was raised to 68℃, white particles appeared after a period of time, reacted at constant temperature for 4h, stopped heating, filtered and washed with ethyl acetate when the temperature dropped below 30℃. The white product was dried at 55℃ for 48 h in a vacuum oven, then swelled in pure water, and then adjusted to pH 7 with NaOH solution to form acrylate hydrogel. Polyacrylate nanoemulsion (PNOE) was prepared by mixing acrylate hydrogel with self-made nanoemulsion. Finally, acrylate hydrogel complex adjuvant (AHCP) was prepared by mixing PNOE with a certain amount of 10 mg/mL Poly I:C solution.

2.3 Morphology of AHCP

Transmission electron microscope (TEM, JEM-2100Plus, Nippon Electronics) was used to observe the micro-morphology of the AHCP.

2.4 Particle Size and Zeta Potential

The particle size and Zeta potential of acrylate hydrogel complex adjuvant were measured by dynamic light scattering particle size analyzer (Z3000, PSS, USA). Take the AHCP and dilute it with pure water to 15 wt% as the particle size sample to be tested, and dilute it to 2 wt% as the Zeta potential sample to be tested. Particle size test conditions: test temperature is 23℃, detection angle is 90°, each sample is tested 3 times in parallel. Zeta potential test conditions: the test temperature is 23℃, the temperature of the detection cell is balanced for 60s before detection, and each sample is measured for 3 times.

2.5 Rotational Viscosity

Flow scanning and oscillation amplitude testing of AHCP were performed using a rotational rheometer (DHR-1, TA Inc., USA). Flow scanning test conditions: 25 mm plate, shear rate: 1~100 s^{-}

[1], test temperature: 25℃. Oscillation amplitude test conditions: frequency: 10 rad/s, strain: 1~1000%, test temperature: 25℃.

2.6 Cytotoxicity

The macrophages were adjusted to a concentration of 1×10^5 cells/mL and seeded into a cell culture plate at 100 μL per well. The plate was placed in a CO_2 incubator to allow cell adhesion. PNOE and AHCP were added to the cell culture plate at concentrations of 2 mg/mL and 1 mg/mL, respectively, while Poly I:C was added at concentrations of 20 μg/mL and 10 μg/mL. Each drug concentration was tested in three times, with control wells (cell control) and blank wells set up simultaneously. After 12 hours of incubation, the cytotoxicity of the drugs on macrophages was assessed using the CCK-8 method. The absorbance at 450 nm was measured with a microplate reader, and the cell viability rate was calculated.

$$\text{Cell viability}=\left[\frac{As-Ab}{Ac-Ab}\right]\times100\% \quad (1)$$

As: Absorbance of experimental wells. Ac: Absorbance of control well. Ab: Absorbance of blank well.

2.7 Phagocytosis

Macrophages were adjusted to a concentration of 4×10^5 cells/mL, and the macrophage suspension was added to the cell culture plate at 1 mL/well to allow cell adhesion. PNOE and AHCP were complexed with FITC-OVA respectively and then added to the cell culture plate. Blank control and FITC-OVA control groups were also set up. The plate was incubated in a CO_2 incubator for 12 hours. After incubation, the excess liquid was aspirated and discarded. Each well was washed twice with PBS, followed by fixation with 4% paraformaldehyde fixative for 20 minutes. The wells were then washed three times with PBS. DAPI staining solution was added, and the plate was covered with tin foil to avoid light for 5 minutes. Afterward, the cell culture plate was washed three times with PBS. Finally, an antifade mounting agent was added, and the phagocytic ability of the macrophages was observed using an inverted fluorescence microscope. The concentrations of PNOE and AHCP in each well were 2 mg/mL, Poly I:C was 2 μg/mL and FITC OVA was 10 μg/mL.

3. Results and Discussion

3.1 Morphology

Dilute PNOE and AHCP to 15 wt% and add them into the test tube to observe the appearance, as shown in Figure 1. The results show that they are uniform and transparent to blue light. At the same time, dilute PNOE and AHCP to 0.1 wt% and observe their microscopic morphology at magnification of 20000 ×. It is found that the particle size is mostly about 50 nm, the particle structure is complete, and the dispersibility is relatively good.

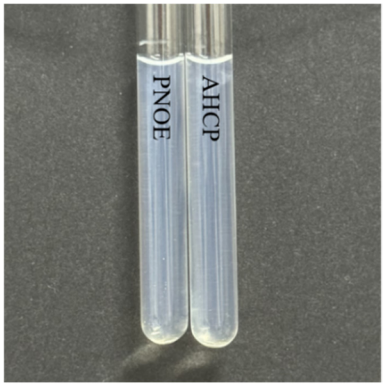

Figure 1. Appearance of PNOE and AHCP diluted to 15 wt%.

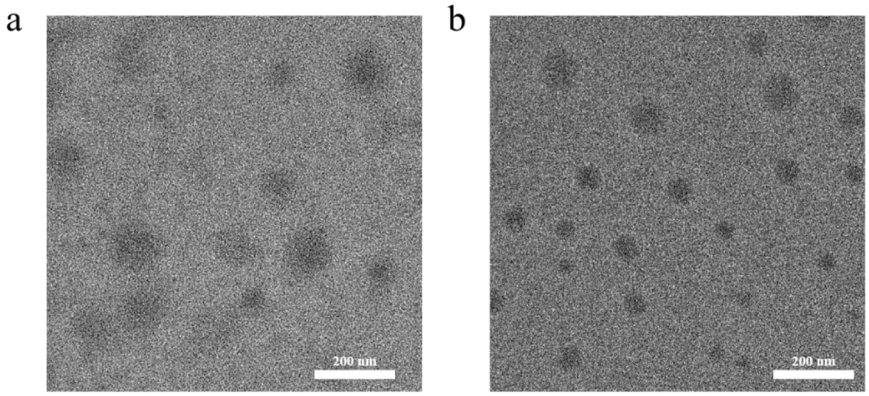

Figure 2. Microscopic morphology of (a) PNOE and (b) AHCP.

3.2 Particle Size and Zeta Potential

Nanoparticles have unique biological activity due to their large specific surface area due to their small size, and are efficiently delivered to antigen presenting cells (APCs) by coating antigen. The particle size, polydispersity index (P.I.) and Zeta potential of PNOE and AHCP were measured, and the results are shown in Table 1 and Figure 3.

Table 1 shows that the average particle size of PNOE and AHCP is 46.53 nm and 46.67 nm, respectively. And the average P.I. is 0.22 and 0.14, respectively. As shown in Figure 3, the Zeta potential of PNOE and AHCP were -37.44 mV and -42.05 mV, respectively. The higher absolute value of Zeta potential indicates that the system is stable and difficult to settle.

Table 1. Average particle size and P.I. of adjuvant.

Sample	Mean particle diameter/nm	SD	P.I.	SD
PNOE	46.53	0.602	0.22	0.06
AHCP	46.67	3.62	0.14	0.01

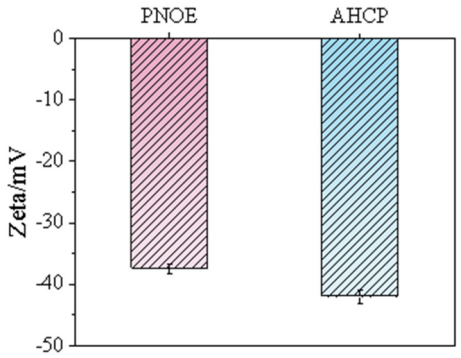

Figure 3. Zeta potential of PNOE and AHCP.

3.3 Rheological Property

The viscoelasticity of acrylate hydrogel complex adjuvant may affect its antigen release behavior after entering the body as vaccine adjuvant. The dynamic mechanical behavior of PNOE and AHCP was studied, and the rheological test curves are shown in Figure 4.

Figure 4a shows that shear thinning effect is observed with increasing shear rate of the complex adjuvant, indicating that acrylate hydrogel complex adjuvant has good injectability. In Figure 6b, G′ represents storage modulus, G′′ represents loss modulus, and with increasing strain, amplitude increases, and storage modulus decreases for PNOE and AHCP. When the critical strain is exceeded,

G′<G′′, the critical strain point of PNOE is 125.27%, which is less than 125.44% of AHCP, but there is no obvious difference, indicating that the introduction of Poly I:C has no effect on the dynamic mechanical behavior of the composite adjuvant.

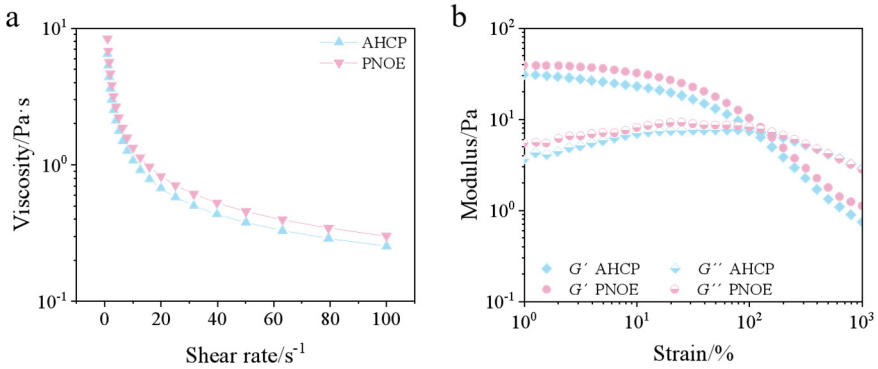

Figure 4. Rheological property: (a) Shear rate-viscosity. (b) Strain-modulus.

3.4 Cytotoxicity

The cytotoxicity of the composite adjuvants was evaluated using the CCK-8 assay, and the cell viability was calculated accordingly. The results are shown in Figure 5. At a drug concentration of 2 mg/mL, with Poly I:C at 20 μg/mL, the cell viability for PNOE and AHCP were 78.7% and 63.9%, respectively. At a drug concentration of 1 mg/mL, with Poly I:C at 10 μg/mL, the cell viability for PNOE and AHCP were 83.78% and 75.59%, respectively. The lower cell viability observed for AHCP compared to PNOE at the same drug concentration may be attributed to the indirect induction of apoptosis by Poly I:C after activating signaling pathways. When the concentration of Poly I:C was less than 10 μg/mL, the composite adjuvants incorporating Poly I:C exhibited good cell status and high biosafety in macrophages, laying the foundation for exploring drug concentrations in macrophage phagocytosis.

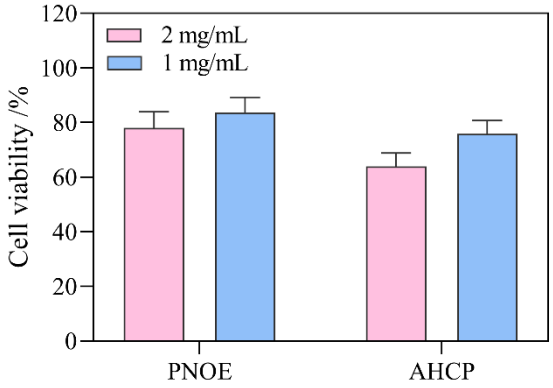

Figure 5. Cell viability of PNOE and AHCP.

3.5 Phagocytosis

Macrophages are professional phagocytes that non-specifically recognize and engulf nanoparticles when stimulated by external substances such as nanoparticles. The surface and interior of macrophages express various Toll-like receptors (TLR). Poly I:C is a TLR3 agonist, and macrophages are one of the key target cells of Poly I:C. Therefore, in theory, antigens encapsulated by the AHCP, which contains Poly I:C, are more likely to be endocytosed by macrophages. The results of macrophage phagocytosis are shown in Figure 6. As illustrated, the blank control group exhibited only blue nuclear fluorescence, while the pure antigen FITC-OVA group showed only very faint green fluorescence, indicating weak antigen uptake by macrophages in the pure antigen stimulation group. In both the PNOE/OVA and AHCP/OVA groups, green fluorescence increased, with the

AHCP/OVA group demonstrating a significant enhancement in both the intensity and area of green fluorescence. This suggests that the addition of Poly I:C markedly enhances the phagocytosis of antigens by macrophages, which is likely to elicit a more potent immune response.

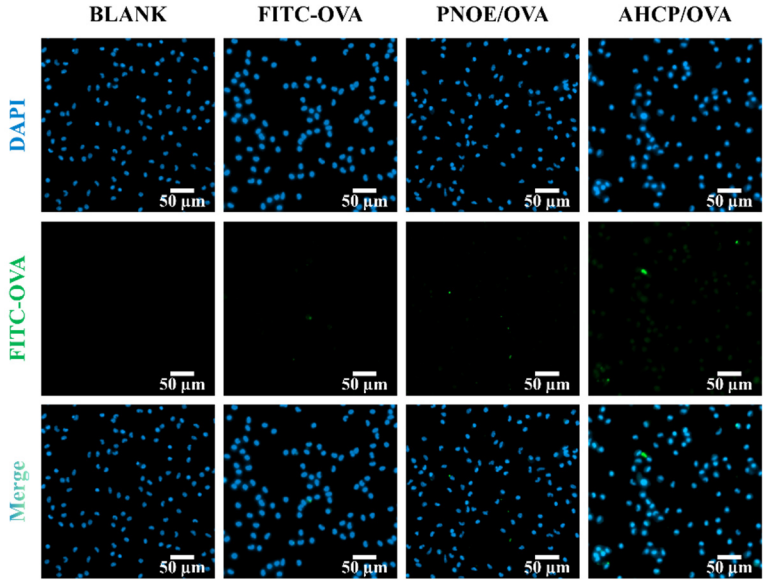

Figure 6. Phagocytic capacity of macrophage.

4. Conclusion

In this paper, acrylate hydrogel composite adjuvant was prepared by introducing Poly I:C into the mixture of acrylate hydrogel and nanoemulsion. The composite adjuvant was injectable, and the particle size was 46.67 nm. When the concentration of Poly I:C was 10 μg/mL, the cell viability could reach 75.59%. The AHCP had certain cell targeting, and the composite adjuvant with Poly I:C had stronger ability to promote endocytosis of antigen. The promising biosafety and targeting properties of this acrylate hydrogel nanocomposite adjuvant highlight its significant potential for future adjuvant development.

Acknowledgments

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