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Communication

Synergistic effect of T80/B30 vesicles and T80/PN320 mixed micelles with Se/C on nasal mucosal immunity

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ABSTRACT

Selenium doped carbon (Se/C), an easily fabricated material, was found to be bio-active and it can serve as an adjuvant to enhance the immune effect of Tween 80/Brij 30 (T80/B30) vesicles and Tween 80/polymer cationic surfactant PN320 (T80/PN320) mixed micelles. The synergistic effect of the combination of T80/B30 vesicles and T80/PN320 mixed micelles with Se/C on nasal mucosal immunity was studied in this work, which might provide theoretical basis for developing the related new adjuvant for nasal immunization of recombinant protein, peptide and split protein vaccine. Since both selenium and carbon were bio-compatible elements, Se/C might be safe for practical applications, and this was also reflected by the low hemolytic activity of the materials. This work not only reports an efficient protocol for adjuvant development, but also significantly expands the application scope of selenium chemistry.

Selenium-containing compounds and materials have attracted much attention for their unique chemical- and bio-activities. They are employed as building blocks in organic synthesis [1], as catalysts in fine chemical production [2] and environment protection [3], and as the distinctive components in materials science [4]. They are also widely used in the medical field for their antibacterial [5], antifungal [6] and antiviral activities [7]. As a necessary trace element for human beings that can be metabolized in the body [8], selenium is relatively safer than many transition metals for both human health and ecological safety considerations. Moreover, China possesses very rich selenium resources, and it results in low cost of selenium for large-scale applications. Developing advanced selenium-containing materials not only brings additional opportunities for applications, but also obviously enhances the resource values and is of profound strategic significances.

During the past decade, we have focused on the investigations of selenium science. Carbon-based selenium materials, such as the selenized glucose, selenium-doped carbon (Se/C) and selenium-doped polymeric carbon nitride (Se/PCN) were invented and applied in many fields [9]. The biological applications of these materials are especially practical because both selenium and carbon are bio-compatible elements, and some of these materials have been successfully commercialized. For example, selenized glucose can now be produced at kilogram scale [10] and is sold as a kind of selenium fertilizer for crops. It is also effective for inhibiting mycotoxin deoxynivalenol generation and may be useful for the control of wheat scab disease [11]. The downstream product, Se/C, has been found to be an efficient antibacterial material against *Xanthomonas campestris* pv. *campestris* and may be used as the key component in related new biocide development for cabbage black rot disease prevention and cure [12]. Recently, we started our project on developing the selenium-involved immunologic agents, and it was found that the combination of Tween 80/Brij 30 (T80/B30) vesicles and Tween 80/polymer cationic surfactant PN320 (T80/PN320) mixed micelles with Se/C showed very good synergistic immune effect on nasal mucosa. Herein, we wish to report our findings.

Nasal vaccination is a promising alternative to traditional injectable vaccines, as it is comfortable in physical aspect & psychological aspect and capable of eliciting strong systemic and local immune responses [13]. On the other hand, Se/C was easily prepared by calcining the commercially available selenized glucose [10], and it was recently found to have antibacterial activity [12]. Thus, the vesicles of 2.0% T80/B30 (12:5) and the mixed micelles of 1.0% T80/PN320 (3:5) with good nasal mucosal immune activity and antibacterial activity were used with Se/C. It not only played a synergistic immune effect of vesicles, mixed micelles and Se/C to enhance the immune responses, but also exerted the antibacterial effect of Se/C to avoid the use of preservatives with strong side effects such as thiomersal.

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Physicochemical characterizations of vesicles of T80/B30 and mixed micelles of T80/PN320 were shown in Fig. 1. The mixed surfactant system can self-assemble to form molecular ordered assemblies such as mixed micelles, vesicles, gels and lyotropic liquid crystals in a solution above a certain concentration. The critical micelle concentration (cmc) of the T80/B30 (12:5) nonionic surfactant mixed system in 0.01 mol/L PBS was about (4.6×10^{-3})%. The results showed that the total concentration of T80/B30 (12:5) ($c_{T80/B30}$) increased from 0.01% to 0.04%, and the particle size of T80/B30 (12:5) raised rapidly from 8 nm to 25 nm, which indicated that large aggregates were formed in the system. After that, the particle size of mixed system increased slowly with the increase of $c_{T80/B30}$ (Fig. S1 in Supporting information). When $c_{T80/B30}$ was 2.0%, the particle size of the system was about 30 nm, and the solution changed from colorless to bluish (Figs. 1a and b). The existence of large aggregates was confirmed by freeze-fracture TEM (FF-TEM) (Fig. 1c). The critical packing parameter of B30 was 0.64, which could be mixed with the cationic surfactant didodecyldimethyl ammonium bromide and the anionic surfactant sodium dodecylsulfate in a certain proportion to form 16 nm to 25 nm small vesicles [14]. According to the appearance, particle size, FF-TEM images of 2.0% T80/B30 (12:5) and the self-assembly characteristics of B30, it suggested that with the increase of $c_{T80/B30}$, the transformation of the T80/B30 (12:5) mixed micelle to the mixed vesicle was generated. Therefore, the aggregation morphology of 2.0% T80/B30 (12:5) in solution was small vesicles.

The cmc of T80/PN320 (3:5) nonionic and cationic surfactant mixed system in 0.01 mol/L PBS was about (5.5×10^{-3})%. As the total concentration of T80/PN320 (3:5) increased, the particle size of T80/PN320 (3:5) solution raised slightly (Fig. S2 in Supporting information). 1.0% T80/PN320 (3:5) was a colorless and transparent solution with a particle size of about 12 nm, and FF-TEM showed that it was a typical spherical micelle system [15] (Figs. 1a–c).

After the model antigen ovalbumin (OVA) was formulated with T80/B30 (12:5) and T80/PN320 (3:5) systems, the appearance of the solution changed little (Fig. 1a). OVA might exist in the palisade layer of the mixed micelle and the small cistern of the vesicle. The average particle size of the mixed micelle system increased slightly despite the PDI magnified distinctly, and the average particle size and PDI of the vesicle system did not change significantly (Fig. 1b). The microstructure of T80/B30 (12:5) vesicles and T80/PN320 (3:5) mixed micelles remained stable after adding OVA (Fig. 1c).

Hemolytic activities of T80/B30 vesicles and T80/PN320 mixed micelles with Se/C were initially studied and the results were illustrated in Fig. 2. Due to the mechanical damage of the materials to erythrocytes, Se/C alone possessed some hemolytic activity. Mixed with 2.0% T80/B30 (12:5) vesicles and 1.0% T80/PN320 (3:5) mixed micelles, the hemolytic activities increased, but no significant differences were observed in comparison with that of the vesicles and mixed micelles without Se/C. The hemolytic activity of 2.0% T80/B30 (12:5) + Se/C was significantly higher than that of the control group of 2.0% T80 ($P < 0.001$), but that of 1.0% T80/PN320 (3:5) + Se/C was significantly lower than that of the control group of 2.0% T80 ($P < 0.01$), showing better safety for practical applications.

Synergistic immunities of T80/B30 vesicles and T80/PN320 mixed micelles with Se/C were then investigated and the results were summarized in Fig. 3. Se/C alone was difficult to enter the immune system through nasal epithelial cells, resulting in its very low immune activity in nasal mucosa. After antigens were formulated with T80/B30 and T80/PN320, the OVA specific serum IgG antibody titers significantly increased and were higher than the control group of OVA alone ($P < 0.001$). The antibody titer induced by 2.0% T80/B30 (12:5) + Se/C was slightly higher than that of 2.0% T80/B30 (12:5). The antibody titer induced by 1.0% T80/PN320 (3:5) + Se/C was significantly higher than that of 1.0% T80/PN320 (3:5) ($P < 0.05$), and was lower than that of the positive control group of cholera toxin B subunit (CTB) but had no statistically significant difference (Fig. 3a).

Induced by the T80/B30 vesicles and T80/PN320 mixed micelles with Se/C, the OVA specific sIgA levels in nasal cavity and lung were obviously higher than that of the control group with OVA alone ($P < 0.001$). The 2.0% T80/B30 (12:5) + Se/C induced OVA specific sIgA levels in nasal cavity and lung were slightly higher than that induced by 2.0% T80/B30 (12:5). The level of OVA-specific sIgA antibody induced by 1.0% T80/PN320 (3:5) + Se/C was significantly higher than 1.0% T80/PN320 (3:5) (nasal cavity: $P < 0.01$, lung: $P < 0.05$), and significantly better than 2.0% T80/B30 (12:5) + Se/C ($P < 0.001$), similar to CTB (Figs. 3b and c).

The particle sizes before and after 2.0% T80/B30 (12:5) vesicles load OVA were about 30 nm, which is an ideal particle size for excellent vaccine adjuvant. It can quickly enter lymphatic vessels, migrate to lymph nodes, and activate dendritic cells [16]. The mucin in nasal mucus contains a considerable proportion of sialic acid, which is negatively charged at physiological pH [17]. The novel polymer hyperbranched Gemini quaternary ammonium salt PN320 has the advantages of high cation charge density, low toxicity, no irritation and easy biodegradation. The zeta potential before and after 1.0% T80/PN320 (3:5) mixed micelles load OVA were 22.60 ± 0.54, 20.03 ± 0.93 mV, respectively (Table S2 in Supporting information), so it could have a strong electrostatic interaction with mucin, promoting its retention in nasal cavity and interaction with nasal epithelium. As surfactants that promote nasal administration, T80/B30 and T80/PN320 can increase the permeability of nasal mucosa, temporarily open the tight junction of mucosal epithelial cells, enhance the passage of antigen and Se/C

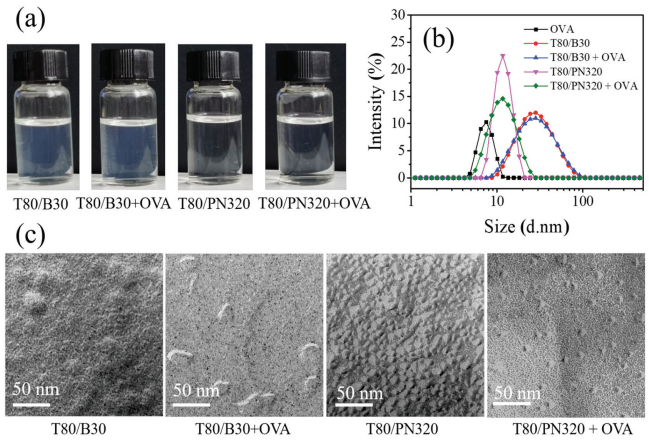

Fig. 1. Physicochemical characterizations of vesicles of T80/B30 and mixed micelles of T80/PN320: (a) Photographs of the appearance; (b) Particle size distribution (PSD); (c) Images of Freeze-fracture TEM (FF-TEM). The weight ratios of T80/B30 and T80/PN320 were 12:5 and 3:5, and their total concentrations were 2 wt% and 1 wt% respectively. All solutions were in 0.01 mol/L PBS.

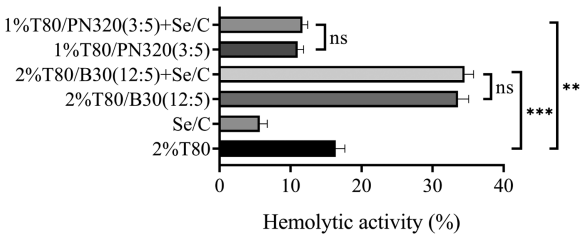

Fig. 2. Hemolytic activities of T80/B30 vesicles and T80/PN320 mixed micelles with Se/C at 37 °C. The data were expressed as mean ± SD ($n = 3$). *** $P < 0.001$, ** $P < 0.01$, ns no significant difference.

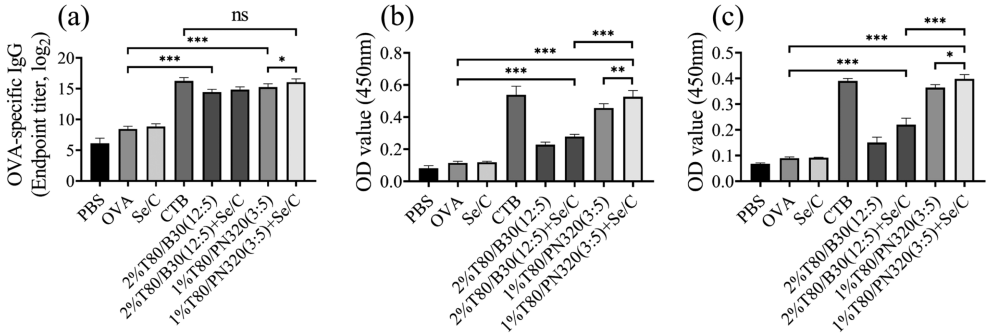

Fig. 3. OVA specific serum IgG and mucosal sIgA antibody levels synergistically induced by T80/B30 and T80/PN320 with Se/C: (a) OVA specific serum IgG antibody titer; (b) OVA specific sIgA level in nasal cavity; (c) OVA specific sIgA level in lung. The data were expressed as mean ± SD ($n = 5$). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

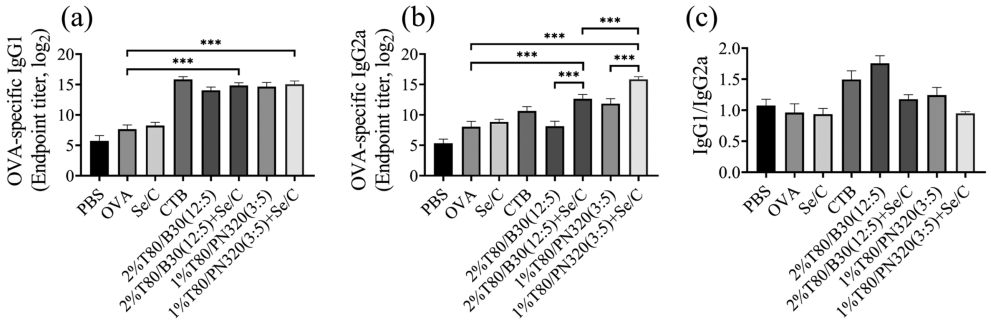

Fig. 4. The antibody titers of OVA specific serum IgG1 and IgG2a synergistically induced by T80/B30 and T80/PN320 with Se/C: (a) IgG1; (b) IgG2a; (c) IgG1/ IgG2a. The data were expressed as mean ± SD ($n = 5$). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

through the mucosa, reduce the activity of proteolytic enzyme of nasal mucosa, and protect the antigen from being destroyed [18].

The antibody titers of antigen specific serum IgG1 and IgG2a synergistically induced by T80/B30 and T80/PN320 with Se/C were analyzed and the results were summarized in Fig. 4. The antibody levels of IgG1 and IgG2a induced by 2.0% T80/B30 (12:5) + Se/C and 1.0% T80/PN320 (3:5) + Se/C were significantly higher than those induced by OVA alone (Figs. 4a and b). Being synergistically induced by 2.0% T80/B30 (12:5) + Se/C and 1.0% T80/PN320 (3:5) + Se/C, the OVA specific IgG2a antibody titer was greatly promoted, and the titer of 1.0% T80/PN320 (3:5) + Se/C was significantly higher than 2.0% T80/B30 (12:5) + Se/C ($P < 0.001$) (Fig. 4b). IgG2a is the most effective antibody subtype in host response to virus and bacterial infection [19]. Because IgG1 is induced by Th2 cytokines and IgG2a is induced by Th1 cytokines, the production of two IgG subclasses, IgG1 and IgG2a, is related to Th2 and Th1 immune responses, respectively [20]. The ratio of IgG1/IgG2a ranged from 0.5 to 2.0, showing that the Th1 and Th2 type immune responses were mixed or balanced [21]. As shown in Fig. 4, although the 2.0% T80/B30 (12:5) + Se/C induced OVA specific IgG2a antibody level was obviously enhanced, the immune response was still the mixed immune responses of Th1 and Th2, biased to the Th2 type response. 1.0% T80/PN320 (3:5) + Se/C could induce the mixed immune responses of Th1 and Th2, slightly biased to the Th1 type response (Fig. 4c). The above experimental results clearly showed that, T80/B30 vesicles and T80/PN320 mixed micelles with Se/C led to very good the synergistic immunities, and the addition of Se/C could significantly enhance the cellular immune response of immunized mice and it also promoted the humoral immune and mucosal immune responses. Selenium exerts its influence over immune responses involves balanced reactive oxygen species (ROS) and redox by glutathione peroxidase (GPX) and thioredoxin reductases (TR) enzymes, respectively [22]. IFN-γ is known as a Th1 network cytokine and plays a key role in promoting the T cell response. Selenium can enhance the activation of immune cells and increase the level of IFN-γ and its ratio to IL-4 [23].

In conclusion, the combination of Se/C with T80/B30 and T80/PN320 can improve the immune response level of mice, especially for the IgG2a antibody level, and effectively protect the host from virus and bacteria. T80/PN320 + Se/C has low hemolytic activity and is very safe. It can induce high levels of cellular immune, humoral immune and mucosal immune response. It is expected to be developed as a new adjuvant for nasal immunization of recombinant protein, peptide and split protein vaccine. Further investigations on the application of selenium-containing materials in medicinal chemistry are ongoing in our laboratory.

Declaration of competing interest

The authors report no declarations of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.cclet.2021.03.029.

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