Comparison of Adsorption and Encapsulation Methods in Preparation of rSAG1-loaded PLGA Nanospheres as Particulate Vaccine against *Toxoplasma gondii* Infection

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**Abstract**

**Introduction:**
Despite all progress in vaccine research against toxoplasmosis, subunit vaccines still deal with poor immunogenicity, which could be overcome by using efficient delivery vehicles like PLGA. The proteinaceous nature of antigens makes the loading of protein more challenging than chemical medicines. Here, we prepared rSAG1-PLGA by adsorption and encapsulation methods and compared their characterizations.

**Methods and Results:**
Blank PLGA and rSAG1-encapsulated PLGA nanospheres were prepared using double emulsion solvent evaporation technique at room temperature. rSAG1-adsorbed PLGA nanospheres were prepared by incubating a suspension of freeze-dried blank PLGA with rSAG1 in PBS (pH 7.4) and it was mixed at 4°C overnight. Size, PDI, zeta potential, preparation yield, and adsorption/encapsulation efficiency of all prepared PLGA nanospheres were characterized and summarized in table below:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Size (nm)</th>
<th>PDI*</th>
<th>Zeta potential (mV)</th>
<th>A/E efficiency (%)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank PLGA</td>
<td>438 ± 11</td>
<td>0.12 ± 0.01</td>
<td>-5.56 ± 0.68</td>
<td>-</td>
<td>86.8 ± 3.56</td>
</tr>
<tr>
<td>rSAG1-adsorbed PLGA</td>
<td>486 ± 9.9</td>
<td>0.14 ± 0.02</td>
<td>-1.00 ± 0.33</td>
<td>69.73 ± 3.05</td>
<td>87.4 ± 2.7</td>
</tr>
<tr>
<td>rSAG1-encapsulated PLGA</td>
<td>471 ± 8.5</td>
<td>0.20 ± 0.04</td>
<td>-4.66 ± 0.6</td>
<td>46.93 ± 2.51</td>
<td>86.8 ± 2.86</td>
</tr>
</tbody>
</table>

*Poly Dispersity Index, A; adsorption, E; encapsulation

Moreover, in vitro release profile of both PLGA nanospheres during 4 weeks demonstrated more or less similar release pattern (zero-order release patterns). However, rSAG1 release in rSAG1-encapsulated PLGA happened slower than release in rSAG1-adsorbed one.

**Conclusions:**
Based on obtained size, both rSAG1-adsorbed and rSAG1-encapsulated particles could be efficiently taken up by presenting cells. Higher efficiency of adsorption than encapsulation makes adsorption method more economic in large scale. Protein during encapsulation process faces some stability problems due to exposure to harsh mechanical thermal and chemical stresses affecting protein integrity and immunogenicity. Therefore, protein adsorption would be applied as a suitable method for protein loading. We are going to evaluate the efficiency of both particles in eliciting immune responses in BALB/c.

**Keywords:**
adsorption, encapsulation, PLGA, rSAG1, *Toxoplasma gondii*

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