Chitosan Magnetic Nanoparticles for Delivery of Urokinase-Type Plasminogen Activator

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Lysis of the fibrin clot by tissue plasminogen activator is currently the only approved therapy for treatment of acute ischemic stroke. Delivery of urokinase-type plasminogen activator (uPA) by binding the thrombolytic drug to a magnetic nanoparticle (MNP) as a drug carrier will ensure the drug to be delivered under magnetic guidance and retained in a local area in circulation, which is potentially useful for targeting fibrin clot in vivo. MNP as a drug carrier is usually composed of a magnetite core with superparamagnetic characteristics, and a polymer coating layer providing functional groups for drug binding, inhibiting aggregation, and increasing colloidal stability. Chitosan is a highly cytocompatible polysaccharide coating material for MNP. It stabilizes the nanoparticle colloid by its positively charged amine groups, thus creating a low-fouling surface-coating layer to limit nonspecific protein adsorption, and providing abundant anchor functional groups on particle surface for covalent binding with drug molecules.

In this study, we examine the preparation of chitosan-MNP and the feasibility to use it as a magnetic nanocarrier for delivery of uPA. Chitosan-MNP was synthesized and characterized by Fourier transform infrared spectroscopy, transmission electron microscopy, atomic force microscopy, superconducting quantum interference device, dynamic light-scattering, thermogravimetric analysis, differential scanning calorimetry, and X-ray diffraction. Chitosan coating on the particle surface provides abundant –NH2 functional groups for conjugating with uPA through glutaraldehyde or carbodiimide-mediated covalent bond formation. In vitro protease and thrombolytic activities of bound uPA were determined by chromogenic substrate and fibrin clot assays, respectively. The optimum drug loading is reached when 50000 IU/ml uPA is conjugated with 10 mg chitosan-MNP where 98% drug is attached to the carrier with full retention of its thrombolytic activity. Effective thrombolysis with uPA bound to chitosan-MNP under magnetic guidance is demonstrated in an ex vivo intravascular thrombolysis model where substantial reduction in blood clot lysis time was observed compared with runs without magnetic targeting or with free uPA using the same drug dosage.

Fig. 1. TEM images of chitosan-MNP (left, bar = 200 nm) and uPA bound to chitosan-MNP (right, bar = 100 nm) after staining with phosphotungstic acid.
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Abstract

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**RESULTS**

Table 1: Characteristics of Chitosan and Chitosan-Nanoparticles

**CONCLUSIONS**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time (h)</th>
<th>Activity (U/mL)</th>
<th>Nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>2</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>4</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Nanoparticles</td>
<td>6</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

**EXPERIMENTAL FLOWCHART**

For correlation binding with DNA molecules, protein function was monitored on a protein matrix. The protein matrix is used to immobilize the protein of interest. The immobilization procedure involves functionalization of the surface of the protein matrix. The immobilization process is optimized using various conditions to achieve the best results. The immobilized protein is then used for various applications such as detection of target molecules, purification of proteins, and as a reagent in various biochemical assays. The immobilized protein is also used as a coating material for various surfaces to improve their functionality.

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