Core/Shell Nanofibers with Embedded Liposomes as a Drug Delivery System

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Introduction
Liposomes are promising drug carriers. Their broader application to drug delivery systems is hampered because of their short half life and inefficient retention at the site of application. These disadvantages could be significantly reduced in combination with nanofibers. Different nanofiber liposome systems are produced: liposomes co-electrospun with nanofibers and core/shell nanofibers with embedded liposomes. Herein, we demonstrate that co-electrospinning does not conserve intact liposomes. However, intact liposomes incorporated into nanofibers by coaxial electrospinning show the greatest promise. We report polyvinyl alcohol (PVA)-core/poly-e-caprolactone (PCL) shell nanofibers with embedded liposomes and show that they preserve the enzymatic activity of encapsulated horseradish peroxidase (HRP).

Materials and Methods

1. Unilamellar liposomes were prepared from soybean-derived L-α-phosphatidylcholine with encapsulated 2mg/ml horseradish peroxidase (HRP), 25mg/ml fluoresceine or 5mg/ml FITC-dextran using the extrusion method.

2. Blend electrospinning of nanofibers-A mixture of 12% (w/v) polyvinyl alcohol (PVA) with 271 μg/ml HRP and 5mg/ml FITC-dextran encapsulated into liposomes were prepared.

3. Coaxial electrospinning of PCL/PVA nanofibers- 10% (w/v) PCL solution was used as a shell solution. The core solution consisted of either liposomes with encapsulated fluorescein dissolved in 5% (w/v) PVA or fluorescein dissolved in 5% (w/v) PVA.

4. Cryo field emission scanning microscopy (FESEM) and Confocal microscopy were used for liposomes visualization.

5. Determination of horseradish peroxidase activity-Enzyme activity was determined by the conversion of TMB substrate and correlated with the protein concentration (450 nm, ELISA reader).

6. Water content determination was determined by coulometric Karl Fischer titration. Overall water retention (%) was calculated as a ratio of the measured water content (mg) to the theoretical overall water content in liposomes (mg).

Results and Discussion

Figure 1. SEM and confocal microscopy analysis of blend electrospun PVA nanofibers with liposomes. (A)SEM analysis of nanofibers with incorporated liposomes shows a large number of non-homogeneous areas. (B) PVA nanofibers without liposomes as a control. (C) Visualization of liposomes containing FITC-dextran by confocal microscopy. Scale bars indicate(A) 50μm, (B) 20μm, and (C)20μm.

Figure 2. Cryo-field emission scanning electron microscopy (FESEM) of coaxially electrospun PVA-core/PCL-shell nanofibers with encapsulated liposomes. (A)FESEM of liposomes embedded within PVA-core/PCL-shell nanofibers. (Insert) Pure PVA-core/PCL-shell nanofiber mesh without liposomes as a control. (B) Details of an incorporated intact liposome in a coaxially prepared nanofiber with a smaller nanofiber attached to the surface of the PCL nanofiber. Scale bars indicate(A,B)100 μm and (Insert) 2μm.

Figure 3. Confocal microscopy of PVA-core/PCL-shell nanofibers prepared by coaxial electrospinning. Dry coaxially electrospun PVA-core/PCL-shell nanofiber mesh with encapsulated liposomes containing (A) fluorescein with signals distributed inside the intact liposomes visualized by confocal microscopy. Coaxial nanofibers prepared without liposomes but with the addition of (B) fluorescein show signals distributed throughout the entire sample. Scale bars indicate(A,B)20 μm.

Conclusion

Coaxial electrospinning enable incorporation of intact liposomes into nanofibers. Blend electrospinning did not conserve intact liposomes. Enzymes encapsulated in liposomes can better survive the electrospinning process, probably because of the shielding effect of the lipid sphere. Results indicate the preservation of intact liposomes filled with water even in a dry state. Liposomes entrapped in the nanofiber core maintained a water environment after several weeks of shelf storage.


Figure 5. Incorporation ratio and enzymatic activity of HRP

Table1. Water content of nanofibers

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample weight (mg)</th>
<th>Maximal water content (μg water/mg sample)</th>
<th>Measured water content (μg water/mg sample)</th>
<th>Incorporation ratio(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-LIP</td>
<td>129</td>
<td>2,759</td>
<td>1.8</td>
<td>65.23±0.95</td>
</tr>
<tr>
<td>B-LIP</td>
<td>121</td>
<td>4,413</td>
<td>1.6</td>
<td>36.26±0.78</td>
</tr>
</tbody>
</table>

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