Preparation and characteristic of nanogel-cross-linked microsphere

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ABSTRACT SUMMARY
Nanogel consisting of self-assembled polysaccharides is one of the most attractive carriers for drug delivery systems. In the present study, nanogel-cross-linked microsphere was prepared by bottom-up approach using nanogels as building units. The size of nanogel-cross-linked microsphere was controlled in micrometer range and degradation was observed after subcutaneous injection in vivo.

INTRODUCTION
Bottom-up approach expanding attractive characters of nano-sized drug carriers into larger scale-materials is important for the next generation of drug delivery systems. Nano-sized carriers have been at the forefront of material-based drug delivery systems for the past few decades. Self-assembled nanogels have been known as one of the most beneficial drug carriers since hydrophobically modified polysaccharide such as cholesterol-bearing pullulan was firstly reported to form nanogels by physically cross-linking through hydrophobic groups. In comparison with polymeric micro/nanospheres, nanogels enable to contain a large amount of water, and incorporate bioactive drugs and proteins within the nanoscale cross-linked polymer networks [1].

Recently nanogel tectonic engineering, in which nanogels were used as building blocks to control nano-structure of macro-sized gels, have been developed [2]. In drug delivery or biomaterial field, the intelligent macrogels, which have designable cross-linking points and nano-domains, are still needed. In this study, nanogel-cross-linked microsphere was prepared through cross-linking of nanogels in water-in-oil emulsion.

EXPERIMENTAL METHODS
Acryloyl group and rhodamine dye was modified with cholesterol-bearing pullulan (CHP) by isocyanate or isothiocyanate chemistry using di-n-butyltin (IV) dilaurate as catalyst.

Acryloyl-bearing CHP (CHPOA) and 4-branched polyethylene glycols terminated with thiol groups (PEGSH) was mixed and added to cyclohexane containing phosphatidylcholine to form water-in-oil emulsion. After sonication freeze dyeing and centrifugation with sucrose, nanogel-cross-linked microsphere was yielded.

The basic characters of nanogel-cross-linked microspheres were evaluated by confocal laser scanning microscopy and laser diffraction particle size analysis. After subcutaneous injection to mice in vivo for 4 months, degradation of nanogel-cross-linked microspheres was observed by in vivo imaging using FITC-dextran as staining of blood vessel.
RESULTS AND DISCUSSION

Figure 2a shows the material formed spherical shape and nanogel-cross-linked microsphere was successfully prepared. Average diameter was determined as 8.1 µm through laser diffraction particle size analysis (Figure 2c and Table 1). Furthermore, preparation method of emulsion was changed and nanogel-cross-linked microsphere was controlled to over 50 µm (Figure 2b).

Figure 3 shows degradation behavior of nanogel-cross-linked microspheres after subcutaneous injection. While nanogel-cross-linked microspheres was still embedded in the skin resulting from 20×-lens observation, several microspheres were degraded by cells around edge of administration site.

<table>
<thead>
<tr>
<th></th>
<th>Median diameter</th>
<th>Mode diameter</th>
<th>Average diameter</th>
<th>Standard deviation</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>6.7 µm</td>
<td>8.1 µm</td>
<td>6.4 µm</td>
<td>0.2 (4.3 ~ 9.6 µm)</td>
</tr>
<tr>
<td>B</td>
<td>56 µm</td>
<td>64 µm</td>
<td>41 µm</td>
<td>0.4 (16 ~ 110 µm)</td>
</tr>
</tbody>
</table>

Table 1. Particle size of nanogel-cross-linked microspheres.

Figure 3. Degradation of nanogel-cross-linked microspheres in vivo (red: rhodamine-labeled CHPOA and green: FITC-dextran in blood vessel).

CONCLUSION

Nanogel-cross-linked microspheres, which were 8.1 µm or over 50 µm in size, were prepared by bottom-up approach using nanogels as building units. After subcutaneous injection, the nanogel-cross-linked microsphere was degraded in vivo. These findings suggested that nanogel-cross-linked microsphere enables to be used as carrier for a novel drug delivery system.

REFERENCES