Assessment of protective effect of polycation on plasmid DNA attached on the metal surface by SPR

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Abstract: Surface modification of the metal is a critical procedure for gene therapy on the metal implants, such as stent and artificial joint. Polycations can condense with DNA by self-assembly, form polyplexes spontaneously as a result of electrostatic interactions and protect DNA from degradation by DNase. The ability of chitosan, polyethyleneimine (PEI), and poly(amideamine) (PAMAM) dendrimers to condense plasmid DNA(pDNA) was determined by electrophoresis, X-ray photoelectron spectroscopy (XPS) and surface plasmon resonance (SPR). Polycation's attachment of pDNA to the metal surface was confirmed by XPS which showed no phosphorus peaks on the interface showing the protective effect on pDNA from DNase degradation. Polycations with higher molecular weight or hyperbranched or dendrimer structures can fulfill many of the requirements for effective gene protection from DNase degradation. Chitosan with higher molecular weight (>= 200 kDa) has superior efficiency to protect pDNA against DNase degradation on the surface of the gold chip. Hyperbranched PEIs and PAMAM dendrimers, even with lower molecular weight (for example, 20kDa for PEI, 50kDa for G5) can protect pDNA against DNase degradation. The results of this study present a platform for further optimization studies of polycation-based gene delivery systems.

Introduction

Surface modification of the metal is a critical procedure for gene delivery on the stent and artificial joint for gene therapy on the metal implants [1-4]. Polycations (cationic polymers) such as chitosan [5-8], PEI 9, 10 and PAMAM dendrimers [11, 12] can form complexes with pDNA (polyplexes) which is regarded as some of the most effective nonviral gene delivery systems. Polycations can condense with DNA by self-assembly, form polyplexes spontaneously as a result of electrostatic interactions and protect DNA from degradation by DNase (Lee et. al. [13], Lentacker et. al. [14]). We tested the ability of the polycation to protect pDNA against degradation by DNase based on the hypothesis that the in vivo efficiency was related to the protective effect. There are many articles concerning the protection of DNA in polyplex particles, but few approaches to assay the protection on the metal were reported. In this paper, we confirmed the association of pDNA and chitosan on the Au chip by XPS, and monitored the surface changes of pDNA, chitosan, PEI, and PAMAM dendrimers in presence of DNase, which reflected the protective effects of pDNA by polycations from DNase degradation. Since SPR chip has a metal surface (Au), its property is almost the same as the Au-electroplated metal implant.
Results and discussion

pDNA and chitosan attached on the Au chip was confirmed by XPS. The polycations protective effects on pDNA and the activities of DNase I was tested by electrophoresis analyses. Figure 1 shows the XPS characterization of the pDNA and chitosan layer on the Au chip. The very weak N1s peak at 399 eV was difficult to observe for both the chitosan layer and DNA layer, since N abundance is lower than C, O, H at the surface. However, P2s and P2p peaks can be obviously detected in the DNA layers.

Fig. 1. XPS results of the pDNA and chitosan layers on the Au chip.

![XPS results](image1)

Fig. 2. Electrophoresis photos of the pDNA, polycations with DNase I. (a) Lanes from bottom to top: pDNA (1%), pDNA in 1xPBS, pDNA + alkyl-modified chitosan(10mg/ml), pDNA + chitosan (Mw=22kDa, 10mg/ml), pDNA + chitosan (Mw=6k, 10mg/ml), pDNA + alkyl-modified chitosan(1mg/ml), pDNA + chitosan (Mw=22k,1mg/ml), pDNA + chitosan (Mw=6kDa, 1mg/ml). (b) Lanes from bottom to top: pDNA(1/100), pDNA(1/100) in 1 x PBS + DNase I (1/1000), DNA(1/20) + PEI(20k 1%) + DNase I(1/1000), DNA(1/20) + PEI(30kDa, 1%) + DNase I(1/1000), DNA(1/20) + PEI(50kDa, 1%) + DNase I(1/1000), DNA(1/20) + CHI(20W, 1%) + DNase I(1/1000), DNA(1/20) + CHI(30W, 1%) + DNase I(1/1000), DNA(1/20) + CHI(40W, 1%) + DNase I(1/1000).

Electrophoresis analyses were done to assess the protection from the insertion of ethidium bromide and degradation of DNase I by the various polycations.
Weak bands in lane 5 and lane 8 in Figure 2a can be found, which illustrated that chitosan with molecular weight less than 10kDa cannot protect pDNA from the insertion of ethidium bromide. Lanes in Figure 2b except lane 1 has any bands, which illustrated that pDNA was degraded by DNase I or protected by polycations. A new method with precise determination as described later in this paper was needed to evaluate the protective effect of polycations. From figure 2b, 5U/μl DNase I solution had enough activity for degradation of pDNA.

The real-time sensorgram of the interaction of chitosan and pDNA with DNase I by SPR is shown in Figure 3a.

From Figure 3a, pDNA (red line) linked onto the test chip (chitosan surface), and baseline raised about 1192 RU (SPR Response Unit), from 18945RU to 20137 RU, after injection of the DNase I, its RU value dropped down to 18569 RU. The difference between the RU value after DNase I and the RU value after the pDNA is 18569-20137=1568 RU (as listed in column “pDNA only” of Table 1). The chitosan (blue line) had no association onto the test chip (chitosan surface), and baseline remained at 18944 RU, after injection of the DNase I, its RU value changes little. The average difference of six tests between the RU value after DNase I and the RU value after the chitosan is about 0.6 RU (as listed in column “chitosan only” of Table 1). Other polycations have also tested by DNase I, and RU value has almost no changes.

In Figure 3b, pDNA was passed over the sensor surface, the sensorgram shows an increasing response as pDNA interact. Then polycations were passed over the pDNA surface individually, the sensorgram increases as polycations attached and covered the pDNA surface. At last, DNase was passed over the sensor surface. If a polycation has a protective effect from DNase degradation, the difference between the RU value after DNase I and the RU value after the polycation will have no change (such as CHI200k, PEI20k, G5 in Figure 3b).

**Fig. 3.** The sensorgram of pDNA, polycations and DNase I on the surface of SPR chip (a) Interaction of pDNA, chitosan and DNase I; (b) The real-time sensorgram of the protection of different polycations on pDNA from degradation of DNase I by SPR.

However, if a polycation has no protective effect, the difference will decrease remarkably (CHI22k in Figure 3b). Table 1 listed the differences between the RU value after DNase I and the RU value after the other polycations. Figure 4 was drawn according to the value of Table 1. The pDNA only and chitosan only are used as references. All the values in Table 1 were tested six times. The ± values in Table 1...
and the rods on the shadow bars in Figure 4 represented the standard errors of the six tests. Much less negative ΔRU of chitosan of Mw=22kDa than that of Mw=100kDa perhaps was caused by less pDNA attached on the surface of the chip.

Polycations with higher molecular weight exhibited better protection from DNase, which showed the dependency of the molecular weight of polycations on the protection from DNase I.

**Tab. 1.** The difference between the RU value after DNase I and the RU value after the pDNA.

<table>
<thead>
<tr>
<th>Polycation</th>
<th>pDNA only</th>
<th>Chitosan only</th>
<th>Chitosan 22 K</th>
<th>Chitosan 100 K</th>
<th>Chitosan 200 K</th>
<th>Chitosan 400 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔRU(4-2)</td>
<td>-1569.3±0.3</td>
<td>0.6±0.9</td>
<td>-535.7±26.6</td>
<td>-3456.5±9.0</td>
<td>274.5±4.8</td>
<td>136.3±7.0</td>
</tr>
<tr>
<td>Polycation</td>
<td>PEI 20k</td>
<td>PEI 50</td>
<td>G5</td>
<td>G6</td>
<td>G9</td>
<td>G10</td>
</tr>
<tr>
<td>ΔRU(4-2)</td>
<td>289.6±18.9</td>
<td>1365.4±6.3</td>
<td>2614.7±5.7</td>
<td>2282.1±7.7</td>
<td>2433.8±3.6</td>
<td>3206.4±4.4</td>
</tr>
</tbody>
</table>

Note that there were some differences between the electrophoresis and SPR assays for the protection of the polycations on pDNA from degradation of DNase I. Figure 2a shows that when the molecular weight of chitosan was greater than 10kDa, it could protect pDNA from the insertion of Ethidium Bromide (EB). In Figure 3b, Figure 4 and Table 1, chitosan with molecular weight less than 100kDa could not protect pDNA from degradation of DNase I. This is perhaps caused by the morphological differences between the particle state in solution and the surface state on the surface of metal, and the differences between physical interaction of electrophoresis and association/dissociation of SPR.

**Fig. 4.** A comparison of protective effect of polycations with different molecular weight on pDNA from DNase degradation. Bars show the difference between the RU value after DNase I and the RU value after the pDNA.

**Experimental part**

**Materials**

Chitosan, with molecular weight of 22 kDa (batch number D041215266), 100 kDa (batch number D070917169), 200 kDa (batch number D070917170), and 400 kDa (batch number D070917171), was obtained from Golden-shell Biochemical Co., Ltd, Zhejiang, China. PEI (20 kDa and 50kDa) was obtained from Qianglong Chemicals, Wuhan, China.
PAMAM was synthesised by Dr. Wang Yanming, State Key Laboratory of Functional Polymer Materials for Adsorption and Separation, Nankai University. Molecular weight of the fifth, sixth, ninth, and tenth generation of PAMAM (G5, G6, G9, and G10) is 50 kDa, 80 kDa, 640 kDa, and 1200 kDa, respectively. DNase I was obtained from Takara Biotechnology (Dalian) Co., Ltd. China.

**Formulation of polycations**

Chitosan stock solutions (1 mg/ml) were prepared by dissolving chitosan in 0.2M acetate buffer, pH 4.5, and then filtering the solutions under sterile conditions.

PEI (20 kDa and 50 kDa, 1 mg/ml) stock solutions were prepared by dissolving PEI in sterile deionized water.

PAMAM (G5, G6, G9, and G10) stock solutions (1 mg/ml) were prepared by dissolving 1mg PAMAM in 10 µl methanol, then dissolved in 990 µl sterile deionized water.

Plasmid DNA stock solution, 0.01mg/ml. The pDNA was 10 times diluted in 1x PBS buffer, the concentration was about 1μg/ml. DNase I was 1000 times diluted in buffer, the concentration was about 3U/μl.

**Characterization of polycations**

Agarose gel electrophoresis was performed for 5 hours at 25°C using 90 V to check the combination of pDNA and polycations and the activity of DNase I, and the protection effects of the polycations from DNase I degradation in solution.

The surface composition of the polycation layer and DNA layer on Au chip were determined from the intensities of the XPS (PHI-1600, Perkin-Elmer) N 1s, P 2p, and P 2s. The protection effects of the polycations on Au chip from DNase I degradation were investigated by SPR analysis (Biocore AB, Uppsala, Sweden). SPR chip was self-assembled by 11-Mercaptoundecanoic acid on Au chip, which has almost the same properties with metal surface of the Au-electroplated metal implant. Then polycation (chitosan, PEI, PAMAM) was coupled onto the chip by NHS/EDC chemistry to get polycation chip. pDNA, polycations, and DNase were passed over the sensor surface in sequence.

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**References**