DNA complex release system by injectable auto-forming alginate gel including amorphous calcium phosphate

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Statement of Purpose: Plasmid DNA complexes with polycations or cationic lipids have been examined for non-viral gene therapy. The complexes usually have positive charge on their surface, and showed high gene expression in *in vitro.* However, gene expression of the cationic complexes in the target tissues is not satisfactory because of adverse interaction with blood components or cells.

We found that hyaluronic acid (HA) could deposit onto the pDNA/polycation (or cationic lipid) complexes to recharge their surface to negative. The ternary complex was highly biocompatible, and could be targeted to the malignant cells. Moreover, we found that the pDNA/PEI/HA ternary complex could be freeze-dried without any cryoprotectant maintaining their gene transfection activity under precise conditions. It enabled the preparation of very small (< 70 nm) plasmid DNA complex particles with relatively high concentration.[1] Injection of the finely dispersed suspension of the small pDNA/polycation/HA complexes strongly suppressed the tumor growth in mice, and the small tumors completely disappeared.

In those experiments, multiple injections of the complexes were required to achieve the satisfactory therapeutic effect, but single injection often leads to unsatisfactory efficacy owing to the short duration of the gene expression by such artificial vectors. However, multiple injections are sometimes difficult depending on the injection site. Slow release system would, thus, be desired to get high therapeutic effect by a single injection.

On the other hand, an alginate gel is known to degrade by dissociation of the ionic crosslinking under slightly acidic conditions. It is expected that the alginate gel would be gradually degraded under the low-pH environment in tumor tissue. We have reported that the alginate gel could be automatically formed in a living body by injection of alginate solution, probably due to the interaction with divalent cations such as Ca^{2+} ion in body fluids. Moreover, we found that the degradation rate of alginate gel could be controlled by addition of amorphous calcium phosphate (ACP) as buffering agent and supplier of Ca^{2+}.[2]

In this study, we prepared injectable auto-forming alginate gel including pDNA complexes and ACP as a durable gene transfection system, which would be slowly degraded, and release DNA complex in the body. The slow-releasing behavior of the system, and therapeutic effect were examined in tumor model mice.

Methods: Preparation of Alginate solution including DNA complex particles and ACP: A fine pDNA complex particles suspension was prepared by mixing pDNA, PEI, and HA at highly diluted concentrations. It was concentrated by lyophilizing-and-rehydration process to a required concentration.[1] ACP was synthesized from calcium chloride dehydrate and sodium dihydrogenphosphate in alkaline condition.[2] The ACP and the concentrated DNA complex suspension was added to sodium alginate solution.

DNA release from alginate gel: DNA complex particles were labeled by YOYO-1. The sodium alginate solution containing the DNA complex and ACP and was added to a simulated body fluid (SBF) to form a gel. The gel was then immersed in acetate buffer (pH 4.5) to examine the degradation and release profiles. The amount of the released DNA was determined by fluorescence intensity.

Measurement of the degradation rate of alginate gel in mice: Ba^{2+} was added to the alginate solution including ACP and DNA complexes as contrast media. It was injected around tumor in mice. The volume of the alginate gel formed in the body was measured by X-ray CT.

Therapeutic effect in tumor-bearing mice: B16 cells were subcutaneously inoculated into mice. When the tumor size increased to 5-7 mm, alginate solution including ACP and pDNA(GM-CSF) complexes was injected around the tumor just once. Tumor size was measured every 2 or 3 days for 100 days.

Results: The alginate gel without ACP showed initial burst of the DNA release, then soon stopped releasing. On the other hand, the alginate gel including ACP continuously released DNA complexes, and the rate was dependent on the amount of ACP. The released DNA complexes dispersed as small as original DNA complexes, and integrity of the DNA was confirmed by agarose gel electrophoresis.

When the alginate solution containing ACP and DNA complex was inoculated around tumor in the mice, a soft gel was soon formed, and then gradually degraded. The degradation rate could be controlled by amount of ACP. ACP-including alginate gel containing DNA/PEI/HA ternary complex prepared with plasmid encoding GM-CSF gene induced complete disappearance of the tumor in 60% of the mice by single administration.

Conclusions: The alginate solution containing ACP and DNA complex inoculated around tumor in mice formed a soft gel soon. It was gradually degraded to release the DNA complex continuously. The degradation rate of the alginate gel could be controlled by amount of ACP. The slow releasing system prepared with GM-CSF gene-encoding plasmid showed high therapeutic effect in tumor-bearing mice. Such injectable auto-forming alginate gel seems to be promising as a sustained-gene expression device.

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References:

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