Calcium Phosphate Nanoparticle-Assisted Dissolving Microneedles for Transdermal DNA Delivery

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Introduction:

Most children develop a fear of needles at a young age. No procedure is more responsible for this than vaccinations. Vaccines keep children alive and healthy, yet ultimately cause fear and pain. While needles are necessary at the moment, the microneedle patch would eliminate the need for injections and visible needles, and therefore the fear and pain associated with them. The microneedle patch uses micron-scale needles to deliver the vaccine and causes virtually no pain to the recipient. However, it remains a challenge to find a safe and efficient delivery system to help genetic material target specific cells with microneedle patches as a delivery mechanism for vaccines. This study investigates the use of calcium phosphate (CaP) as the delivery agent loaded in a dissolving microneedle patch. CaP is the main mineral found in human bones and teeth and is considered as a novel non-viral vector for targeted gene delivery.

Materials and Methods:

Cytokine and Mechanical Strength Test. CaP nanoparticles were prepared using the coprecipitation method [1]. A total of 6.2 μL of 2M calcium chloride ($CaCl_2$) was mixed with 43.8 μL of deionized water. This was mixed, approximately one-eighth of the solution at a time, into 50 μL of 2x HBS solution. It was then allowed to evaporate to approximately 20 μL.

DOTAP was also prepared for this set of samples. Sixty micrograms of DOTAP was dissolved in 940 μg of chloroform and evaporated using a rotary evaporator at 40°C for two hours. The film was then evaporated overnight to ensure chloroform removal. It was then hydrated with 120 μL of deionized water, sonicated, and allowed to evaporate to approximately 20 μL of solution.

Lipofectamine© and the dsCPG-ODN were mixed at a 1:1 ratio in water and evaporated down to 20 μL.

Once all solutions were evaporated to ~ 20 μL, they were placed onto their respective silicon-negative molds. This was followed by centrifugation at 3000 rpm for 3 min. They were then dried. Pure gelatin solution (40% w/w) was prepared using an autoclave to completely dissolve it for 20 minutes at 120°C. This solution, once the temperature decreased below 50°C, was poured into the molds and then centrifuged at 3000 rpm for one hour. The samples were then dried overnight to ensure the gelatin was set.

For the cytokine induction test, the IFN-β1 and IL-12 mRNA level was determined after a 4 h treatment. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was utilized as an internal control. Briefly, raw 264.7 cells were cultured in a 96-well plate at a density of 1×10^5 cells/well over night. Microneedles patch containing 10 μg ODNs with ACP nanoparticles was placed on MEM medium (200 μl) and incubated at 37°C for 10 min. Then, cells were treated with the extracted solution (1:9 diluted with MEM medium), and followed 4 hr incubation at 37°C with 5% CO_2.

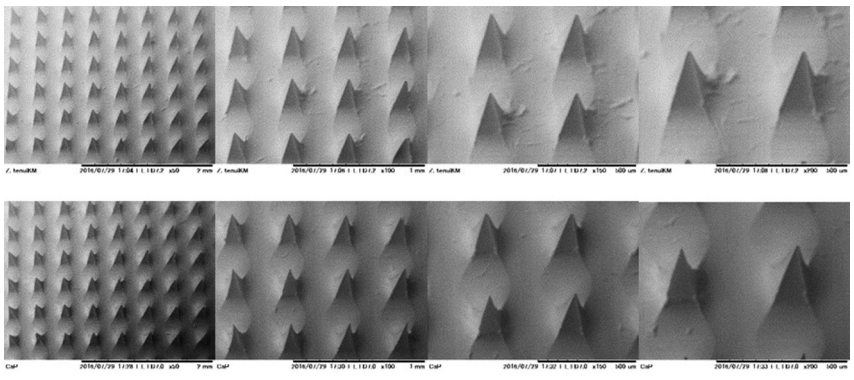
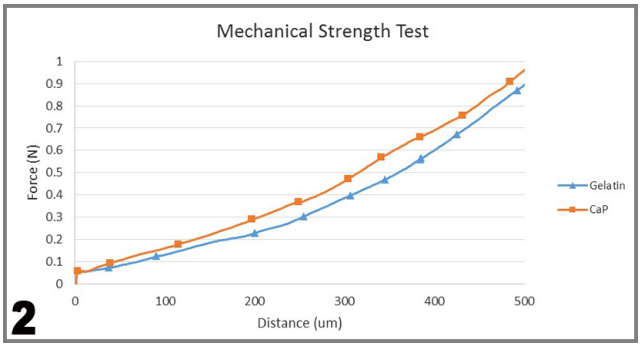
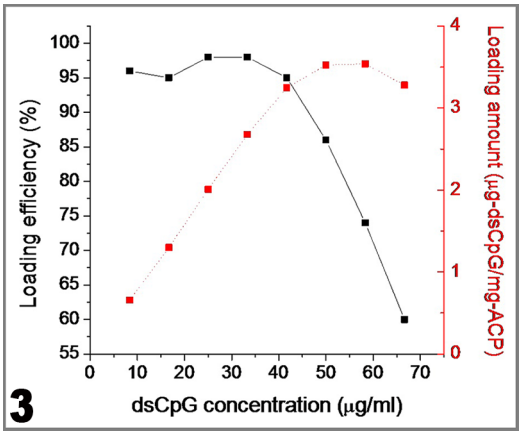


Figure 1: Gelatin patch on top, calcium phosphate patch on bottom; 50X, 100X, 150X, 200X left to right.

After the treatment, cells were washed with PBS, and total RNA was extracted using Isogen solution (Nippon, Japan) and treated with a DNase I digestion step according to the manufacturer's instructions. The obtained RNA was further purified by Agencourt RNAclean XP reagent and followed the reverse transcribed into complementary DNA (cDNA). cDNA was synthesized from total RNA (250 ng) with oligo dT primers (25 pmol) using a PrimeScript™ RT reagent kit (Takara, Japan). Quantitative real-time polymerase chain reaction (qPCR) was





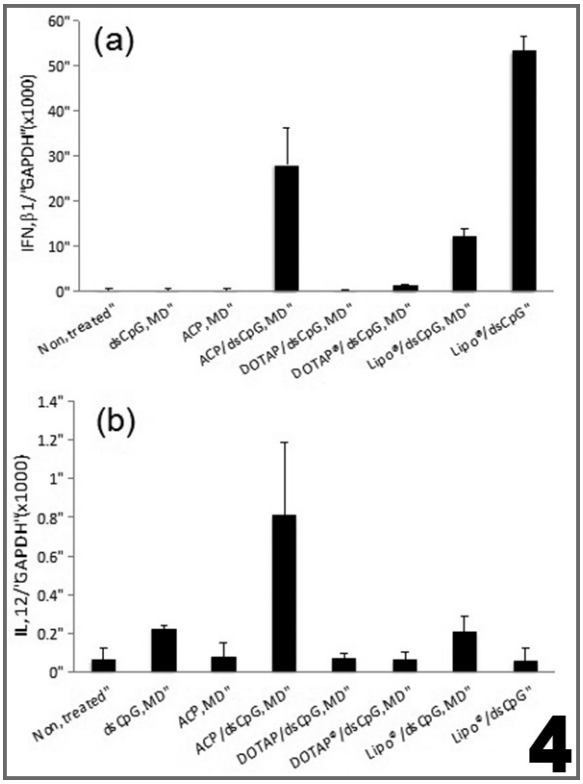


Figure 2: *Mechanical strength test.* ***Figure 3:*** *Loading efficiency of DNA into CaP.* ***Figure 4:*** *Cytokine induction test.*

performed using the LightCycler FastStart DNA Master SYBER Green I kit (Roche Appl. Sci. Japan) in a finial reaction volume of 15 μl. Each reaction consisted of 1 μl of first-strand reaction product, 0.75 μl each of upstream and downstream primers (10 .M), 3 μl of Enzyme Mix and 9.5 μl of diethylpyrocarbonate (DEPC)-water. All samples were pre-denatured for 5 min at 94°C, followed by 40 thermal cycles (94°C for 30 sec, 60°C for 30 sec, and 72°C for 1 min), and an additional extension at 72°C for 7 min.

Additional CaP samples were fabricated in order to determine the mechanical strength of the patches. These were compared to gelatin-only patches. The patches were tested using a universal testing machine.

Loading Capacity Test. All samples were created using 18.6 μL of $CaCl_2$, 150 μL of 2x HBS, and enough water to create a total of 300 μL. This created a total of approximately 3.66 mg of ACP in each solution. The amount of DNA added varied between 2.5 μg and 20 μg in increments of 2.5 μg. The solutions were allowed to settle at room temperature for 15 minutes and centrifuged before their concentration was measured. The control solutions were the amount of DNA being tested in water. This was used to measure the concentration and was the baseline for the initial concentration of samples prior to centrifugation. The efficiency of the loading was then calculated.

Results:

The mechanical strength indicates that the microneedle patch with calcium phosphate is stronger than the gelatin-only microneedle patch. The amount of CpG-ODN that can be loaded into calcium phosphate has been demonstrated to be 2.73 μg DNA/mg CaP. The cytokine induction results demonstrate that the CaP treatment has a much higher induction than the other treatments. The Lipfectamine® treatment that was placed directly into media has a higher induction for IFN-β1 because it did not have to be released by the microneedle patch.

Conclusions:

Based on these results, the calcium phosphate is a viable compound for the microneedles. It also enhances the mechanical strength of the microneedle patch when compared to gelatin. It is also an effective treatment based on the enhanced cytokine induction. This research demonstrates a proof of concept as well as a possible alternative to hypodermic needle vaccine delivery.

Acknowledgements:

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References:

[1] Min Jiang and G. Chen, High Ca2+-phosphate transfection efficiency in low-density neuronal cultures. Nat Protoc, 2006. 1(2): p. 695-700.