# Needle-free Skin Immunization using Low Frequency Ultrasound

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Comment [c1]:

Needle-free immunization is desirable due to inherent needle-associated problems and alternative ways of vaccination are sought. One technique is the application of low-frequency ultrasound to the skin to permeabilize the latter before vaccine application. Indeed low-frequency ultrasound has been shown to assist transcutaneous vaccination (Tezel et al, 2005).

In this abstract, we report our study on the influence of the various experimental parameters on the immune responses in an attempt to optimize the protocol. In vitro results have shown a marked dependence of the extent of antigen permeation on experimental conditions such as ultrasound protocol, nature and volume of coupling medium and distance of probe from skin (Dahlan et al, 2005).

Pulses of ultrasound (20 kHz) were applied to the shaved abdominal skin of anesthetized Balb/c mice via a coupling medium (20 ml; water or sodium dodecyl sulphate (SDS) solution) for a total sonication time of 45s using a probe at 7.5 mm from the skin. After sonication, vaccine (tetanus toxoid) solution was applied to the treated skin for 1h. Two booster doses were given on days 15 and 46. Animals were bled on days 14, 45 and 60 and the serum was analyzed for antibody levels using ELISA. Intramuscular (IM) (±Alum) injections and topical applications (±SDS 1%w/v) without US were the controls. The influence of SDS concentration (0, 0.5 & 1 %w/v) in the coupling medium and of ultrasound duty cycle (0.1s ON, 0.9s OFF vs 0.2s ON, 0.8s OFF in every second until total 'on' time was 45s) on antibody titres was determined. Statistical tests (Kruskal-Wallis test followed by Nemenyi test) were used.

The mean antibody titres (±SD, n=4-5) are shown in Table 1. As expected, the negative controls and the positive controls showed low and high immune responses respectively. Ultrasound (US) treatment alone only resulted in increased antibody titres after the second boost when it was statistically the same as the IM response ($p>0.05$). Combination of SDS 1%w/v and US resulted in increased immune responses after first dose ($p<0.05$) and antibody levels for all combinations of US and SDS increased with boosting. After the first dose, no difference was found between 0.5 and 1 %w/v SDS concentrations ($p>0.05$). The lower SDS concentration has advantage that SDS-associated skin irritancy is expected to be less. 10% duty cycle yielded similar immune response as 20% US duty cycle after the first 2 doses, but was surprisingly higher after the third dose.

To conclude, we have shown that the extent of the immune response can be modulated by the ultrasound experimental parameters.

Table 1 IgG levels of animal groups.
Numbers represent log serum dilution that gave an OD of 0.2 are shown.

| Treatment Group | Day 14 | Day 45 | Day 60 |
|---|---|---|---|
| IM injection | 3.3±0.9 | 3.9±0.3 | 4.5±0 |
| IM injection + Alum | 3.6±0.2 | 3.9±0.1 | 5.4±0.3 |
| Topical application | 1.3±0.1 | 1.2±0 | 1.3±0.1 |
| SDS (1%w/v) only | 1.2±0 | 1.2±0 | 1.2±0 |
| US(20%) alone | 1.4±0.1 | 1.4±0.4 | 3.7±0.7 |
| US(20%) + SDS (0.5%w/v) | 1.6±0.3 | 2.5±0.6 | 4.8±0.5 |
| US(20%) + SDS (1%w/v) | 2.1±0.2 | 2.4±0.5 | 3.7±1.1 |
| US(10%) + SDS (1%w/v) | 2.2±0.7 | 2.8±0.5 | 4.2±0.5 |

Tezel, A. et al. Vaccine (2005) **23**:3800-3807
Dahlan, A. et al, S. J. Pharm. Pharmacol (2005) **57**:S92