

## Effects of pulse-electric fields on tilapia's egg surfaces with rectangular and exponential decay waveforms

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In both electro-permeabilization and electrofusion, a high-intensity DC pulse electric field is employed to induced membrane breakdown and deliver macromolecules into living- targeted biological cells. The transient electric field is always applied in a pulsed form to prevent irreversible cell damage. The most commonly used waveforms are the rectangular pulse (square pulse, SP) and the exponential decay pulse (capacitor discharging, CD). The SP is usually generated by gating the output of a high-voltage power supply, while the CD is generated by discharging a capacitor that has been precharged at a high voltage. In the present study, the pulse width (time constant,  $\tau$ ) of the SP is characterized as a half of period of the waveform and is equivalent to the decay constant  $\tau$  of the CD. The electric field of the latter case is an exponential function of time which  $\tau$  is defined as the length of time at which the field strength is reduced to 0.368 times of the initial value. Electric field strengths of 50-100 kV/m generated for SP and CD with arbitrary mark-space ratio (pulse width: a half of period) were employed to observe the surface of Nile tilapia (*Oreochromis niloticus* L.) eggs in suspensions for electrical sex reversal propose. Pore densities and pore sizes appearing on the shell surface of the egg were measured from SEM micrographs. The local pores are minimum distance packed forming hexagonal patterns. The mean distance between pores is  $1.65 \pm 0.32$  micron (mean  $\pm$  SD). They are volcano-shaped pores with a mean diameter (DP) of about  $232 \pm 25$  nm and the pore density per square micron (PD) is  $0.73 \pm 0.02$ . Surprisingly, the eggs possesses a large single pore at the pole (the so called "polar pore") which diameter is  $11.1 \pm 0.4$  micron and is larger than the local pores. We found that 3-5 wave pulses, 50  $\mu$ s of  $\tau$  with 1:1 mark-space ratio were the optimized conditions for electro-permeabilization. Several deep, sharp pores and some partially open pores were easily observed through experiments. Pore densities of both cases of SP and CD were nearly equal (0.73). In the case of CD of five-square wave pulses, it was very interesting that the DP of CD was  $764 \pm 41$  nm was larger than that of the SP experiments ( $686 \pm 43$  nm). Pore densities for the SP and CD were not significantly different.

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