

New Technology for cellular piercing: Rotationally Oscillating μ -injector, Description and Validation Tests

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Abstract

ICSI (intracytoplasmic sperm injection) procedure is one of the most commonly used cellular-injection processes. In ICSI a drawn glass pipette is pushed against the biological cell and a series of force impulses are exerted on it axially to achieve the piercing through the zona and the membrane in sequence for the ensuing injection. In most advanced applications a piezo actuator creates this impulsive forcing. This procedure presently requires a very small mercury column inside the glass pipette which is found to be helpful especially for minimizing the transverse oscillations. Despite the toxic mercury, the procedure is commonly utilized in many laboratories. Earlier investigations point out that considerable lateral tip oscillations of the injection pipette remain as the piezo-electric pulses are introduced. Such oscillations damage the cell membrane and impart adverse effects on the success rate of the injection. In this study, we introduce a novel microinjection procedure, which will remedy the shortfalls of the present technology. The highlight of this procedure is the introduction of rotational oscillations to the pipette during the drilling. These oscillations of small amplitudes (few degrees) and high enough frequencies (about 100 Hz) are shown to create very effective piercing. The so-called Ros-Drill[®] is a mercury-free and minimally invasive device of which the prototypes are designed and built including the relevant peripheral control hardware and software. Preliminary experimental results are presented on mouse oocytes and they are very encouraging. In the early trials on mouse oocytes, several blastocyst stage developments are reported using new drilling device. We also explain in this text the implementation protocols developed for the new technology.

I. Introduction and Problem Statement

In recent years, cellular piercing has become a crucial procedure in cellular biology, especially in various nuclear or subcellular transfer operations, DNA microinjection and Intracytoplasmic Sperm Injection (ICSI). This procedure is successfully used on variety of species like mouse, rat, and cattle [4,7,10,16,18]. It is also used very effectively in treating male factor infertility [1,5,14,15,17,19].

The very first application of ICSI, which is called “Conventional ICSI”, is conducted manually [2,12,17]. In this technique, the tip of the injection pipette is pushed gently about