Microdynamics of the Piezo-Driven Pipettes in ICSI

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***Abstract*—Undesirably low success rates have been reported in the intracytoplasmic sperm injection procedure. Recently a method using piezo-driven pipettes with a very small mercury column contributed substantial improvements in this process. Despite the toxicity of mercury, this new procedure is commonly utilized in many laboratories. However, there is no study available to date on the micromechanics of this procedure. The underlying principles of piercing are not clear for both cases, with and without the mercury. Presently, the pressure burst, which is caused by the abrupt axial motion of the mercury column, is attributed to this effect. Here, we take the mercury-filled pipettes and try to understand the governing physics. The findings point out the occurrence of considerable lateral tip oscillations of the injection pipette as the piezoelectric pulse train is introduced. We claim that the lateral dynamics play an important role in the piercing and should be considered to enlighten the process and the effects of the mercury. These claims are analytically studied and experimentally verified.**

***Index Terms*—Intracytoplasmic sperm injection (ICSI), microinjection, piezo drive, pipette dynamics.**

I. Introduction

MICROINJECTION operations are becoming increasingly important in molecular biology. These operations include the micromanipulation of cellular subjects, such as extremely delicate removal and transfer of the nucleus from a cell to an already enucleated recipient cell in cloning or piercing procedures through the zona pellucida and the membrane in intracytoplasmic sperm injection (ICSI).

ICSI is a method of fertilization for mammals. This procedure is successfully used on variety of species like mice, rats, and cattle [4], [7], [10], [17], [19]. Also, it is a very effective method in treating male factor infertility [1], [5], [14], [15], [18], [20]. The very first application of ICSI, which is named conventional ICSI, is conducted manually [2], [12], [18]. In this technique, the tip of the injection pipette is pushed gently about halfway through the oocyte. Then it is pushed forward swiftly until it penetrates the zona and the membrane. Afterward, the sperm is injected into the ooplasm. Conventional ICSI in most of the species including cattle, rats, and mice have proven mostly unsuccessful (failure rate $>90\%$) [2], [3]. The reason for the failure is the damage caused by the injection pipette to the membrane or the zona during the piercing process. Obviously, if this damage does not heal effectively, an abnormal growth occurs in the future stages of the development. Therefore, the piercing and the microinjection are vitally important for the success of the ICSI procedure.

A recently introduced method, piezo-assisted ICSI, seems to improve this process [6], [7], [10], [13], [18]. It uses piezo electrically activated impact devices, known as the piezodrill. In this method, a piezo actuator exerts an axial pulse train on the pipette at adjustable frequencies, amplitudes, and durations. The device results in higher efficiency of microinjection, particularly in the piercing phase. It is observed by the practicing experts that once these parameters are properly set the mission can be repeated successfully on most of the oocytes of the same species. There are still two sets of difficulties facing this microinjection procedure. First, the three parametric settings mentioned are currently determined using trial and error methods and without scientific reasons supporting them. Second, even with this method of microinjection, for some particular applications (for instance in mouse oocyte) the yield of the piercing process is still undesirably low [10], [21]. This is of particular interest to the cell biologists as the ICSI into mice is practiced very commonly.

Lately, several groups working in the field have confirmed an interesting outcome that a very small amount of mercury column in the pipette improves the success rate substantially [8], [10], [11], [20]. This procedure yields 80% fertilization for humans and 68% for mice at the blastocyst stage [10], [20]. These rates display significant improvement over the conventional ICSI. On the other hand, the mercury is a toxic substance and its usage is extremely restricted. Despite this constraint, the procedure is adapted for most of the ICSI applications [9]. At the present, however, there is still a mystery surrounding the underlying scientific reasons leading to the favorable property of the mercury. This lack of understanding prevents further developments of the alternative methods.

In this paper, we focus on the micro-dynamics of the pipettes during the zona pellucida piercing. Our experimental efforts show that the tip of the pipette holder (i.e., the base of the glass pipette) has transverse displacements that are comparable to (and even more than) the axial motion along the pipette. Obviously these movements are transmitted and expectedly further exaggerated through the flexible pipette tip. This observation suggests a closer look at the lateral oscillations of the drawn section of the glass injection pipette, which is extremely flimsy. First, we study analytically and numerically the transverse oscillations of this section departing from an impulsive base excitation. Then, we attempt to verify the findings using digital imaging techniques. Our simulation results coincide with the experimental observations and validate the earlier expressed claim that the glass pipette experiences substantial transverse oscillations.

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