The Roles of Mercury in ICSI

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Abstract

Intracytoplasmic sperm injection (ICSI), microinjection of a single spermatozoon into an oocyte, is a routine procedure in assisted reproduction programs. This procedure uses fine control of small bore microinjection needles and precise volume control via hydraulic syringe pumps. In many early experiments mercury is placed within the injection system because its high surface tension in the system facilitates the injection procedure. However, mercury is cytotoxic and therefore alternative fluids or approaches are needed. Here we examine the main properties of mercury and their impact on the various aspects of ICSI. We conclude that the small momentum diffusivity of mercury is the most important contributing factor that facilitates the ICSI procedures.

Keywords: Intracytoplasmic Sperm Injection, Piezoelectric actuator, momentum diffusion

1. Introduction

Intracytoplasmic Sperm Injection (ICSI) is a common assisted reproductive technology (ART) method in which a single spermatozoon is mechanically deposited into an oocyte using a fine glass injection pipette. During ICSI, two crucial mechanical steps are involved. First, the tail of
the spermatozoon is severed from its head and only the sperm head which contains the DNA is injected into the oocyte. Second, the sperm head must be injected into the oocyte without damaging the oocyte and causing cytoplasmic leakage which leads to rapid cell death. These two steps are microsurgery on an individual cell scale. Mechanically driven micropipettes are the tool used for both separating sperm tail from head and for sperm injection. Interestingly, a very small amount of mercury loaded inside the microinjection pipette has been found to enhance the success of the microinjection procedure. In certain species such as mice and rats, the ICSI process is almost impossible to conduct without the presence of mercury.

The toxicity of mercury makes it very undesirable in conducting ICSI. Currently efforts are underway to totally eliminate mercury from the ICSI process (Ergenic and Olgac 2007, Putra et al. 2007; Ergenc et al., 2008). However, to date these attempts only have resulted in limited success. Therefore, it is imperative to obtain a physical understanding of the role of mercury in the microinjection processes.

Mercury was introduced and used during the microinjection of the live spermatozoa into sea urchin eggs (Hiramoto 1962) because its large surface tension facilitates the precise control of the volume of the liquid to be aspirated into or expelled from the pipette. Subsequent injections have typically included mercury in the microinjection pipette, for example, the injections of sperm into hamster eggs (Uehara and Yanagimachi 1976). Later, mercury was used when piezo-driven micropipettes were used for mouse ICSI (Kimura and Yanagimachi 1995). In short, a historical account of mercury during ICSI procedure does not provide an understanding of the underlying cause of the beneficial effect in drastically increasing the ICSI success rate. Recently several investigators have initiated studies to elucidate the physical understanding of this phenomenon (Ediz and Olgac 2004, 2005; Fan et al., 2006).

Ediz and Olgac (2004, 2005) have examined the micro-dynamics of the piezo-driven pipettes. For an extremely flexible, drawn section of the injection pipette, both analytical and experimental results verify that the flexible pipette shows extensive lateral oscillations during the penetration process. From a theoretical point of view, mercury should have a strong effect on the lateral vibration of the pipette. Following their work, Fan et al. (2006) conducted a theoretical study of the dynamics of the piezo-driven pipette immersed in a viscous liquid. The model included the fluid dynamics force acting on the pipette by the surrounding liquid. It was found that surrounding liquid has a strong damping effect on the vibration of the microinjection pipette. Moreover, the damping effect is dependent on the oscillation frequency of the microinjection pipette. For a microinjection pipette containing mercury, its resonance frequency is lower than a pipette without mercury. Consequently, the damping on the pipette could actually be lower than in the absence of mercury. In experiments conducted with liquid surrounding the pipette, the lateral vibration of the pipette in a liquid is hardly noticeable, whether or not mercury is present in the microinjection pipette (Fan et al. 2006). In short, the effect of mercury on the pipette lateral vibration is not significant enough to account for its dramatically beneficial role in ICSI.

In this paper, we propose explanations for the role of mercury in ICSI procedure. We conduct theoretical study of transient responses of the liquid inside a pipette to show that kinematic viscosity of a liquid is the crucial parameter affecting the motion of the liquid when the pipette is
suddenly driven into motion. Mercury is found to have such a small kinematic viscosity unmatched by any known liquid.

2. The mechanics of sperm decapitation and microinjection

Figure 1 shows the schematic of sperm decapitation and oocyte penetration using microinjection pipette. Both the cells and the pipette are surrounded by a liquid (not shown). In both cases, the microinjection pipette is brought into close contact with the sperm or oocytes. The pipette is made to move along the axial direction by an impulsive force generated by a piezoelectric actuator. The relative motion between the pipette edge and the cell generates the cutting effect. In the case of the sperm cell, the cell is not anchored. In the case of the oocyte, the cell is clamped by a holding pipette opposing the pipette. However the cell is very compliant and the membrane easily deforms without any tearing or breakage. Therefore, generating relative motion between the pipette and the cell is possible only when the pipette moves very quickly; the inertia of the liquid surrounding the cell provides the forces to prevent the cell to move with the pipette. Thus the density of the liquid surrounding the cell is also an important parameter.

![Figure 1](image)

**Figure 1.** Microinjection pipette used for cell cutting and oocyte penetration.

Because of the liquid viscosity, the moving pipette will drag along the surrounding liquid which in turn diminishes the relative motion between the pipette and the oocyte. The liquid inertia becomes relatively significant only when the pipette moves sufficiently fast. Therefore, the cutting speed, the liquid viscosity and density are important parameters determining the effectiveness of the cell cutting.

To illustrate the liquid parameters that determine the dragging along effect, we first use a simplified model. Consider a flat plate driven into motion of speed \( U \). The fluid next to the plate will be dragged along as shown qualitatively in Figure 2. The equation describing the fluid velocity is given by (Pozrikidis 1997):

\[
\frac{\partial u}{\partial t} = \nu \frac{\partial^2 u}{\partial y^2}
\]

where \( u \) is the fluid velocity, \( \nu \) is called the kinematic viscosity of the fluid. It is the ratio of the fluid viscosity to its density. In this example, it is an indication of how fast linear momentum is diffused into the fluid. For this reason, it is called momentum diffusivity (Batchelor 1967).
Figure 2. Momentum diffusion from a flat plate driven into sudden motion into the surrounding fluid. Velocity distribution for fluid with (a) large momentum diffusion; (b) small momentum diffusion.

The analytical solution for the fluid flow velocity is given by

\[ u(y, t) = U \text{Erfc}(\frac{\eta}{2}) \]

where \( U \) is the speed of the plate, \( \text{Erfc} \) is the complementary error function, \( \eta = y / (\nu t)^{1/2} \).

<table>
<thead>
<tr>
<th>Table 1 Physical parameters of the fluids</th>
</tr>
</thead>
<tbody>
<tr>
<td>At 15°C and 1 atm</td>
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<tr>
<td>( \rho ) gm/cm(^3)</td>
</tr>
<tr>
<td>( \mu ) gm/cm sec</td>
</tr>
<tr>
<td>( \nu ) cm(^2) / sec</td>
</tr>
</tbody>
</table>

We examine the fluid velocity within 3 micrometers from the plate 30 microseconds after the plate started to move. Using the parameters in Table 1 (Batchelor 1967), we obtain the velocity distribution at the end of 30 microseconds in Figure 3.

Figure 3 Fluid velocity next to the moving plate for different fluids. The small momentum diffusivity of the mercury results in less “dragging along” effect.
The viscosity of the liquid overcomes the fluid inertia so that the liquid next to the pipette is dragged along. This “dragging along” effect increases with the momentum diffusivity. From Table 1, we see that mercury has a viscosity which is slightly above that of water but is more than ten times denser than water. Its momentum diffusivity is an order of magnitude smaller than that of water, smaller still than water-like fluid with higher viscosity. Therefore, if mercury surrounds the moving plate, it has far less dragging along effect than water. This provides an intuitive explanation why mercury helps.

Based on the above-mentioned reasons it becomes clear that if we wish to eliminate mercury in the ICSI process, we have to design fast moving pipette so that the liquid inertia becomes relatively more important. We obtain a quantitative analysis in the following section.

3. Fluid flow within the microinjection pipette

Consider the fluid flow inside a circular pipette under an impulsive motion. The microinjection pipette is very long, and the flow is assumed to be axisymmetric and one dimensional. The flow velocity field can be expressed as

\[ u(r,t) = u(r) e_x \]

where \( r \) is the radial position, \( t \) is time, and \( e_x \) is the unit vector along the axis of the pipette, as shown in Figure 4.

![Figure 4. A long hollow cylinder as a model of the pipette.](image)

The momentum equation for the flow reduces to:

\[ \frac{\partial u}{\partial t} = \nu \frac{1}{r} \frac{\partial}{\partial r} \left( r \frac{\partial u}{\partial r} \right) \]

where \( \nu \) is the kinematic viscosity of the fluid. In (2), we have assumed that there is no pressure gradient applied along the pipette.

The initial and boundary conditions for the flow field are:

\[ u(r,t=0) = 0 \quad \text{for all } r < R, \quad (3a) \]
\[ u(r=R,t) = U \quad \text{for all } t \geq 0, \quad (3b) \]
and
\[ u \text{ is finite at } r = 0. \quad (3c) \]

Using the technique of the separation of variables, we introduce

\[ u(r,t) = U - G(t)F(r) \]

Equation (2) reduces to
\[
\frac{G'(t)}{G(t)} = \nu \frac{1}{F(r)} \frac{1}{r} \frac{d}{dr} \left( r \frac{dF}{dr} \right) = -\lambda^2 ,
\]  
(5)

where \( \lambda \) is a constant to be determined later. The solution for \( G \) is
\[
G(t) = De^{-\lambda t} ,
\]  
(6)

where \( D \) is an arbitrary constant. The ODE for the function \( F \) reduces to
\[
r^2 F'' + r F' + \frac{\lambda^2}{\nu} r^2 F = 0 .
\]  
(7)

Introducing a new variable \( x = \frac{\lambda}{\sqrt{\nu}} r \), the equation (7) reduces to
\[
x^2 \frac{d^2 F}{dx^2} + x \frac{dF}{dx} + x^2 F = 0 .
\]  
(8)

The general solution to (8) can be written as
\[
F = C_1 J_0(x) + C_2 Y_0(x).
\]  
(9)

where \( J_0(x) \), \( Y_0(x) \) are the 0th order Bessel functions of the first and second kind, respectively; \( C_1 \) and \( C_2 \) are two constants. The plots of these two Bessel functions are shown in Figure 5. There are an infinite number of roots for \( J_0(x_k) = 0 \), the first ten of them are listed in Table 2.

Figure 5. Bessel functions of the first and second kind. Solid line is \( J_0(x) \), and the dashed line is \( Y_0(x) \).

Table 2. First ten zeros of the 0th order Bessel function of the first kind.

<table>
<thead>
<tr>
<th>( x )</th>
<th>( J_0(x) )</th>
</tr>
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<tbody>
<tr>
<td>( x_1 )</td>
<td>2.404825</td>
</tr>
<tr>
<td>( x_2 )</td>
<td>5.520078</td>
</tr>
<tr>
<td>( x_3 )</td>
<td>8.653727</td>
</tr>
<tr>
<td>( x_4 )</td>
<td>11.79153</td>
</tr>
<tr>
<td>( x_5 )</td>
<td>14.93092</td>
</tr>
<tr>
<td>( x_6 )</td>
<td>18.07106</td>
</tr>
<tr>
<td>( x_7 )</td>
<td>21.21164</td>
</tr>
<tr>
<td>( x_8 )</td>
<td>24.35247</td>
</tr>
<tr>
<td>( x_9 )</td>
<td>27.49348</td>
</tr>
<tr>
<td>( x_{10} )</td>
<td>30.63461</td>
</tr>
</tbody>
</table>
Since $Y_0(x) \to -\infty$ at $x = 0$, the condition (3c) determines that $C_2 = 0$. Furthermore, the boundary condition (3b) requires that:
\[
J_0\left(\frac{\lambda}{\sqrt{V}}R\right) = 0;
\]
which can be satisfied when the coefficient $\lambda$ is selected at the roots of the Bessel function,
\[
\frac{\lambda_k}{\sqrt{V}} R = x_k \text{ or } \frac{\sqrt{V}}{R} x_k,
\]
where the values of $x_k$ are listed in the Table 2. Merging (6) and (9), we find that the general solution for the velocity as
\[
u(r, t) = U - \sum_{k=1}^{\infty} A_k e^{-\nu x_k^2 t / R^2} J_0\left(\frac{x_k r}{R}\right).
\]
The initial condition (3a) is used to determined the coefficients $A_k$, that is
\[
U = \sum_{k=1}^{\infty} A_k J_0\left(\frac{x_k}{R}\right).
\]
Using the orthogonal conditions
\[
\int_0^1 J_0(x_m \xi) J_0(x_n \xi) d\xi = \frac{1}{2} \delta_{nm} J_1^2(x_n),
\]
the coefficients in (12) are found to be
\[
A_k = 2U \frac{\int_0^1 J_0(x_k \xi) \xi d\xi}{J_1^2(x_k)}.
\]
Therefore, the velocity field for the flow inside the pipette is given by
\[
u(r, t) = U \left[ 1 - 2 \sum_{k=1}^{\infty} \frac{\int_0^1 J_0(x_k \xi) \xi d\xi}{J_1^2(x_k)} J_0\left(\frac{x_k r}{R}\right) e^{-\nu x_k^2 t / R^2} \right].
\]
Consider the physical parameters, $\nu = 10^{-6} \text{ m}^2/\text{s}$, $R = 4 \mu m$, the normalized velocity profiles at different times are plotted in Figure 6. From the figure it can be seen that in this kind of small pipette, the velocity develops very quickly. After $10 \mu$sec, the velocity profile is almost uniform; that is, there is hardly any difference between the velocities of the pipette wall and the liquid inside.

This analysis shows that if a pipette is filled with water, it only takes about 10 microseconds for the liquid inside to move together with the pipette. Assuming the pipette develops a speed instantaneously, the velocity difference between the pipette and the internal liquid disappears about 10 microseconds. In reality, it takes a finite amount of time for the pipette to reach a certain velocity. The time it takes depends on the dynamic characteristics of the piezoelectric actuator and the pipette. We have estimated that the fastest commercially available piezoelectric actuators have response time around 10 microseconds based on the resonance frequencies provided by vendors. Therefore, these actuators are not capable of driving the pipette to its maximum velocity within 10 microseconds.
Figure 6. Velocity profiles at different time instants. x-axis is the dimensionless radial position $r/R$, and the y-axis is the dimensionless velocity $u/U$. Arranged from the bottom, the curves are at times of $10^{-7}, 5 \times 10^{-7}, 10^{-6}, 2 \times 10^{-6}, 5 \times 10^{-6}, 10^{-5}$ sec, respectively.

When mercury is inside the pipette, there is approximately a ten-fold decrease in the kinematic viscosity. According to (15), this corresponds to a ten-fold increase in the time it takes for the mercury to catch up with the pipette motion. Now consider the situation where the pipette is partially filled with water and partially with mercury, for example, the left side with water and the right side with mercury, and the pipette is suddenly accelerated to the left in the arrangement shown in Figure 1(a). The difference in the responding times for these two liquids will deform the interface between the two liquids and induce a negative (relative) pressure there. This pressure at the interface establishes a pressure gradient in the water, which conserves the constant flux over any cross-section along the pipette. The pressure gradient is also favorable in sucking in the sperm tail and helping the sperm decapitation. Even though the dynamic characteristics of the piezoelectric actuator and the pipette remain the same, placing mercury inside the pipette extended the time window to allow sufficient time to accelerate the pipette and to cut the sperm and the zona.

Conclusion and Recommendations

Mercury is unique in its large density (larger than many metals). Yet its viscosity is only slightly larger than that of water. There are no other liquids with properties close to those of mercury. It is therefore very difficult to find a replacement. But generally speaking, denser and less viscous liquids are desirable.

We have found there are no liquids with kinematic viscosity close to that of mercury. That is to say that there can be no liquid that has similar fluid dynamic properties. Therefore, if we were to remove mercury from ICSI procedures, we must design a piezopipette system with response time within 10 microseconds. We report that we have experimented with a new system which has demonstrated its ability to cut the zona successfully without the need for mercury. However, our new system was not successful in cutting sperm heads from their tails. It seems that cutting sperm is easier than cutting the zona. Several investigators have reported success in new methods
for cutting zona without the need for the mercury (Ergenc et al., 2008). It remains to be seen whether these new methods are effective in cutting the sperm.

References


