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Smart Microrobots for Mechanical Cell Characterization and Cell Convoying

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Abstract—This paper deals with the effective design of smart microrobots for both mechanical cell characterization and cell convoying for in vitro fertilization. The first microrobotic device was developed to evaluate oocyte mechanical behavior in order to sort oocytes. A multi-axial micro-force sensor based on a frictionless magnetic bearing was developed. The second microrobotic device presented is a cell convoying device consisting of a wireless micropusher based on magnetic actuation. As wireless capabilities are supported by this microrobotic system, no power supply connections to the micropusher are needed. Preliminary experiments have been performed regarding both cell transporting and biomechanical characterization capabilities under in vitro conditions on human oocytes so as to demonstrate the viability and effectiveness of the proposed setups.

Index Terms—Microrobotics, in vitro fertilization process, mechanical cell characterization, cell convoying.

SORTING fertilized oocytes (embryos) in order to select a good pattern for the transfer process is an important issue. The quality of the transferred embryos and the quality of the oocytes is a crucial parameter of the resulting embryo quality. To date, fertilized oocytes are sorted based on visual optical microscope information relayed to the operator for evaluation. Experimental observations have shown that cell morphological transformation is observed from 48 to 72 hours after the fertilization process. Thus, fertilization failures are usually detected only at an advanced stage when the fertilized oocytes fail to divide or stop their development. Some recent studies [1][2] have shown that strong mechanical behavior modifications are observed a few hours after the fertilization process. Using a new criterion for oocyte sorting associated with visual information should improve oocyte sorting for earlier failure detection procedures.

Repeated micromanipulation tasks required in the in vitro fertilization process are commonly carried out by highly skilled operators. Since these repeated manipulation are important, low efficiency tasks may be achieved even by experienced human operators. Oocytes are usually manipulated during the in vitro fertilization process using a micro-pipette based on suction method. Human oocytes are fragile and must be manipulated carefully in order to reduce damage to their external or internal membranes. The development of an automated and supervised system reducing involvement of human operators based on a minimally invasive approach during the crucial steps of the fertilization process is a suitable solution.

In the recent years, the robotics and microrobotics fields have played an important role in the development of a dedicated systems for microbiology applications. Many efforts have been devoted to development towards a high efficiency artificial fertilization process [3-11]. Despite these research efforts, studies focus mainly on a single step of the in vitro fertilization process. Furthermore, experimentation are seldom conducted on human oocytes. Developments including more than one accurate system performing the different crucial tasks needed are rare. This issue is addressed in this paper by the development of smart microrobots. The first device performs mechanical cell characterization for oocyte sorting while the second performs cell convoying for non-invasive cell transporting.

I. MECHANICAL CELL CHARACTERIZATION DEVICE

The micro-force sensor (figure 1) consists of a cylindrical glass tip (120 mm long and 500 µm) which levitates in a magnetic field produced by four NdFeB magnets (called $M_1$). Two cylindrical NdFeB magnets (ForceField), called $M_2$, are fitted to the glass tip. The force sensor configuration can be considered as two frictionless magnetic bearings called $L_1$ and $L_2$ respectively (cf. figure 1). To ensure stable levitation and overcome unstable forces, the cylindrical magnets as well as...
Table 1
Components of the force sensing device.

<table>
<thead>
<tr>
<th>Component</th>
<th>Material</th>
<th>Magnetic property</th>
<th>Dimension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnet M1</td>
<td>NdFeB</td>
<td>$B_r=1.3$ T</td>
<td>10 mm x 10 mm x 10 mm</td>
</tr>
<tr>
<td>Magnet M2</td>
<td>NdFeB</td>
<td>$B_r=0.95$ T</td>
<td>$\phi$ 1.63 mm x 2.34 mm</td>
</tr>
<tr>
<td>Levitating part</td>
<td>Glass</td>
<td></td>
<td>$\phi$ 0.5 mm x 95 mm</td>
</tr>
<tr>
<td>Tip of the endeffector</td>
<td>Glass</td>
<td></td>
<td>5 mm x $\phi$ 0.02 mm</td>
</tr>
<tr>
<td>Diamagnetic material</td>
<td>Graphite</td>
<td>$\chi_m=-12 e^{-5}$</td>
<td>40 mm x 40 mm x 10 mm</td>
</tr>
</tbody>
</table>

A. Force measurements model

The attractive magnetic forces $\vec{F}^m_u$ (cf. figure 2) along a direction $\vec{u}$ ($\vec{u} \in \{\vec{x}, \vec{y}, \vec{z}\}$) can be expressed in cartesian coordinates according to both remanent magnetic induction $B_r$ of the magnet $M_2$ and the magnetic induction $B_1$ produced by the magnets $M_1$ as [12]

$$\vec{F}^m_u = \frac{V B_r}{2\mu_0} \vec{\nabla} \cdot \vec{B}_1(G) \cdot \|\vec{B}_1(G)\|^2$$

(1)

Where $\vec{B}_1(G)$ is the magnetic induction produced by $M_1$ at the center of gravity $G$ of $M_2$, $\vec{\nabla}$ is the nablal operator ($\vec{\nabla} = (\frac{\partial}{\partial x}, \frac{\partial}{\partial y}, \frac{\partial}{\partial z})^T$), $\mu_0$ the vacuum permeability and $V$ the volume of $M_2$.

The repulsive diamagnetic forces $\vec{F}^d_u$ (cf. figure 2) along a direction $\vec{u}$ produced by a small element $P$ of the graphite on $M_2$ can be expressed in cartesian coordinates according to both dimensionless scalar magnetic susceptibility $\chi_m$ and the magnetic induction $B_2(P)$ produced by the magnets $M_2$ as [12]

$$\vec{F}^d_u = \frac{\chi m}{2\mu_0} \iint_{v'} \vec{\nabla} \cdot \vec{B}_2(p) \|\vec{B}_2(p)\|^2 dv'$$

(2)

B. Magnetic model validation

Since the magnetic and diamagnetic forces are expressed as a function of the magnetic induction produced by the magnets configuration $M_1$ as well as the magnets $M_2$, the determination of $\vec{B}_1$ and $\vec{B}_2$ are crucial. Hence, the more accurate the determination of the $\vec{B}_1$ and $\vec{B}_2$ the more accurate is the force measurements process. For this reason, we used a finite element analysis method achieved with Flux3D software. Figure 3 shows the simulated magnetic induction produced by a single magnet $M_1$ at different heights $z$ and compared to the averaged experimental measurements achieved by means of a calibrated Hall sensor (F.W. BELL Teslameter). The experimental measurements are performed by means of a servomotor.
which accurately move the probe above the magnet. In the present validation study, the travel range and the resolution step measurements are 20 mm and 1 µm respectively. We observed a small deviation between the simulated and the experimental curves (a relative error of less than 1%). The results show good agreement between the simulated and the experimental curves (a relative error of less than 1%).

The micropusher behavior is divided into two types: rotation around the contact point and translation of the contact point. As the angular behavior is a keypoint which allows precise positioning [14], this paper focuses on the angular model.

The magnet position is characterized by the reference (O, x, y, z) defined in figure 4. The pusher position is defined by the point I, center of the contact line between the pusher and the wall. Micropusher orientation is defined by the angles α and β respectively around z and x.

A. Angular Behavior Modeling

Angular behavior is given by the magnetic efforts applied by the magnet on the micropusher. The magnetic torque $d\vec{T}_m(P)$ and magnetic force $d\vec{F}_m(P)$ applied on an elementary volume $dv$ in point $P$ is considered as

$$\begin{align*}
\int d\vec{T}_m(P) &= \overline{\vec{M}} \land \overline{\vec{B}_0}.dv \\
\int d\vec{F}_m(P) &= \nabla(\overline{\vec{M}} \times \overline{\vec{B}_0}).dv
\end{align*}$$

where $\overline{\vec{B}_0}$ is the magnetic field of the permanent magnet calculated by the Finite Element Model FLUX3D and $\overline{\vec{M}}$ is the internal magnetization which represents the magnetic behavior of the soft ferromagnetic micro-pusher. To determine the magnetization, two hypotheses are assumed: The micropusher is a flat surface $S$; the magnetization module reached the magnetic saturation $M_{sat} = 5.1 \times 10^5 A.m^{-1}$. Magnetization orientation can be calculated classically by computing the well-known continuity relation of the magnetic field on the surface $S$ as described in [15].

Considering the very low inertia of micro-objects, the angular dynamic time constant of the micro-pusher is in the order of 10 µs, thus we consider only the static position given by

$$\int V d\vec{T}_m(P) = -\int V d\vec{F}_m(P) \land \overline{\vec{P}}$$
B. Experimental Validation

Orientation is determined by the equality between both terms of (4). These two strains induce two different behaviors. The magnetic torque \( \Gamma_m \) consequence is the alignment of the micropusher on the external magnetic field \( \mathbf{B}_0 \). In contrast, the magnetic force torque \( \mathbf{F}_m \wedge \mathbf{P} \) makes the micropusher lie flat on the wall. Both phenomena have the same value order and micropusher orientation is defined by the equilibrium of both physical effects.

The experimental measurements and simulations results are presented in figure 5. The experimental conditions are \( \mathbf{O}_I. \mathbf{\vec{x}} \in [-500; 500] \mu m, \mathbf{O}_I. \mathbf{\vec{y}} = 0, \mathbf{O}_I. \mathbf{\vec{z}} = 200 \mu m \). As presented in figure 5, experimental measurements and the model are similar.

![Fig. 5. Experimental and Simulated Micropusher Angles \( \beta \): Orientation \( \beta \) is presented in function of the relative position between the micropusher and the magnet \( \mathbf{O}_I \). Experimental and simulated orientations are relatively near. The micropusher orientation is different from the orientation of the magnetic field \( \mathbf{B}_0 \) around the micropusher: The micropusher is not aligned on magnetic field lines.](image)

The alignment of the ferromagnetic objects on the magnetic field is a specificity of the microworld. In fact, the scale effect on both phenomena is different: magnetic torque \( \Gamma_{m\perp} \) is a function of \( l^3 \) while magnetic force torque \( \mathbf{F}_m \wedge \mathbf{PI} \) is a function of \( l^4 \) (with \( l \) the micropusher characteristic size). Consequently, the smaller the micropusher the closer its orientation is to the magnetic field line.

III. BIOLOGICAL EXPERIMENTATIONS

A. Cell culture

The oocytes are prepared on Petri dishes with specific culture medium formed by Dulbecco’s Modified Eagle’s Medium (DMEM) with high glucose and L-glutamine components and 10 \% of foetal bovine serum. The oocytes can be assimilated morphologically to spherical cells with a thin surrounding biomembrane (100 - 150 \( \mu m \) radius). The cell observation is performed by means of an inverted microscope (Nikon) with 60x magnification lens.

In the present study, the experiments are conducted at ambient conditions on non-fertilized human oocytes which have not been selected for ICSI process. The oocytes are from the research group on genetics and reproduction at the Besançon Hospital Center (France).

B. Oocyte mechanical characterization experiments

Figure 6 shows the overview of the developed non-invasive process for mechanical cell characterization. Instead of moving the force sensing device, we choose to keep it immobile. Accordingly, oocytes assigned for mechanical characterization study are arranged on Petri dishes with a glass head. The latter are positioned above the micropositioning stage. No holding pipette is used for the mechanical characterization process in order to reduce tensile strain. Hence, the oocytes are squeezed against a glass slide. Figure 6 shows the mechanical characterization process.

Preliminary mechanical experiments are conducted on human oocytes not selected for \textit{in vitro} fertilization process. First mechanical characterization experimentation is focused on the estimation of the stiffness \( K \) of the biological sample as well as on Young’s Modulus \( E \). Since Young’s modulus can be used to predict the elongation or compression of elastic samples as long as the stress is less than the yield strength of the sample, mechanical characterization experiments on the biological sample are restricted to mechanical behavior where elastic linear properties are satisfied. Figure 7(A) shows experimental measurements of the oocyte deformation \( \delta \) as a function of the applied force \( F \). According to figure 7(A) the linear elastic behavior is satisfied for forces less then 0.2 \( \mu N \). Based on these assumptions, the stiffness \( K \) of the biological sample can be expressed by a linear analytical formula \( (F = K \delta) \). Hence, the stiffness of the biological sample is found in order of \( K = 0.015 N/m \). Young’s Modulus \( E \) is estimated using Hook’s law \( (\sigma=E \varepsilon) \). Figure 7(B) shows the stress \( \sigma = \frac{F}{a} \) as a function of the dimensionless strain \( \varepsilon = \frac{\delta}{R} \). Using linear interpolation function, Young’s Modulus is found equal to \( E = 0.14 M Pa \).

![Fig. 6. Overview of the developed non-invasive process which squeeze the oocyte against a glass slide. No holding pipette is used for the mechanical characterization process in order to reduce tensile strain. The displacement of the sensing part is monitored by means of the laser sensor.](image)

C. Oocyte pushing operation

Some open loop pushing operations were undertaken with the micropusher without force feedback. However, the force...
the cell is transferred in the channel. A magnet induces the displacement of the micro-pusher in the channel. Thus, the operation of a human oocyte: experimental validation of the concept of a new magnetic cell transport system.

An example of human oocyte micromanipulation is presented in figure 8. The oocyte is pushed from one workstation to another through the channel (horizontal motion in figure 8). The maximal velocity is 100 μm·s⁻¹. During the pushing operations, we never observe sticking effects between the micropusher and the oocyte. At the present time, the micropusher is made of nickel material, which is not biocompatible. However, these experiments on biological cells validate our concept of a new magnetic cell transport system.

[Image 7](7x7) Fig. 8. Pushing Operation of a Human Oocyte: Experimental validation of the micropusher glass sample support. The authors thank the LCEP-Besançon for collaboration on human oocyte micromanipulations. The authors thank the LCEP-Besançon for the fabrication of the micropusher glass sample support.

CONCLUSION

The paper has presented the description of two microrobot devices based on passive magnetic foundations: the mechanical cell characterization device based on forces sensing and the oocyte conveyance device based on non-invasive transporting approach. The design, calibration and the mechanical behavior of these devices are detailed. The results show good agreement between the simulated and the experimental data. The efficiency of the microrobotics systems is proved by successful in vitro experiments on human oocytes. Hence, accurate mechanical characterization for oocyte sorting criterion investigation is achieved as well as non-invasive transporting and positioning tasks.

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REFERENCES


