OPTOELECTRONIC TWEEZERS FOR QUANTITATIVE ASSESSMENT OF EMBRYO DEVELOPMENTAL STAGE

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ABSTRACT

Successful *in vitro* fertilization (IVF) requires that only the top ~1-2 healthiest embryos are transferred to the uterus. The current gold standard is to select only the most developmentally advanced embryos for uterine transfer. This approach is solely *qualitative* and consequently has limited sensitivity and specificity. A quantitative approach, if more sensitive, would greatly improve IVF outcomes. We assess the response of mouse embryos to Optoelectronic Tweezers (OET) throughout key stages in their early development, and demonstrate that OET can serve as a means to quantitatively guide identification of the most developed (i.e. likeliest to be healthy) embryos, following IVF.

INTRODUCTION

In the United States alone, 1.2 million women (or 2% of women of reproductive age) visited their doctor for reproductive assistance [1]. Of those treated, approximately 30% will successfully deliver one or more children (in 2006) [1]. One of the major factors involved in successful birth outcomes is the selection of healthy embryos for implantation. Currently, embryos are selected by an experienced technician using solely qualitative observations. One of the major indicators of an embryo's health is its developmental maturity relative to its peers. Embryos which reach developmental stages faster than their peers have a greater chance of successful implantation into the mother. Therefore, a method to quantitatively discern embryos which reach certain developmental stages faster than others could greatly improve the success rate of *in vitro* fertilization (IVF).

During development there are vast changes in embryo morphology. These differences in morphology can manifest themselves as differences in electrical properties. Therefore, if one can systematically detect differences in electrical properties of different embryos, those embryos could be sorted by developmental stage. One such technique that can accomplish this is dielectrophoresis (DEP). DEP is the force exhibited on the induced dipole moments in a particle when subjected to a non-uniform electric field. The DEP force scales as [2]:

$$F_{DEP} \propto Re[K^*]\nabla E^2 \quad (1)$$

where E is the electric field and K* is the Clausius-Mosotti (CM) Function defined by [2] (pp. 5-33):

$$K^* = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

where ϵ_p^* and ϵ_m^* are the complex permittivity of the particle and medium respectively. The complex permittivity is equal to $\epsilon^* = \epsilon - j\sigma/\omega$, where ϵ, σ, and ω are the permittivity, conductivity, and electric field frequency, respectively. The CM factor can be either positive or negative meaning that the DEP force can cause particles to be either attracted to (pDEP) or repelled from (nDEP) electric field maxima, respectively. Thus, if the electrical properties of the embryos in different stages of development are unique, then their respective CM factor will be different manifesting itself as a difference in DEP response. While there has been prior work on using DEP to sort *unfertilized* oocytes [3] and *pre-cleavage* embryos [4], there has no work in the literature regarding the sorting of *post-cleavage* embryos via DEP.

In this paper, DEP forces are enacted on pre-implantation mouse embryos through use of a device known as Optoelectronic Tweezers (OET) [5]. In the device, low intensity (<1 W/cm^2), incoherent light interacts with a photosensitive substrate and, in conjunction with an externally applied electrical bias, creates localized DEP traps in the illuminated areas (Fig. 1). On-demand, parallel DEP trap generation is possible simply by altering the optical pattern.

EMBRYO CM FACTOR MODELING

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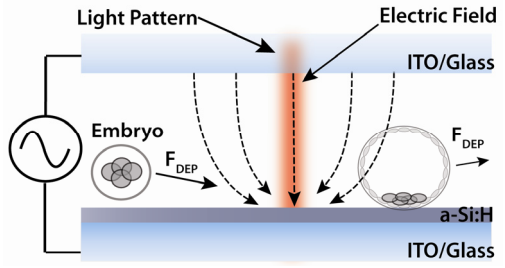

Fig. 1: Overview of OET device for embryo sorting. Incident light creates localized areas of high conductivity in a-Si:H layer. This causes an externally applied electric field to form gradients in the liquid layer. Particles in the vicinity of the gradients experience a DEP force.

To investigate the CM factor (and DEP force) for embryos we look at two critical stages of embryo development, the morula and blastocyst stage. These are the stages at which embryos are typically implanted at following IVF. At the morula stage, embryos consist of a dense clump of 16-32 cells encased in an electrically leaky membrane known as the *zona pellucida*. At the end of the morula stage, the cells in the morula differentiate for the first time to form an inner cell mass (which later becomes the fetus) and a membrane of cells (trophoblast) surrounding a fluid filled cavity (blastocoele). To electrically model these two stages of development, we model the 1-cell to morula stage as an insulating core surrounded by an electrically leaky membrane, and the blastocyst as an insulating shell. Using a multi-shelled model [2] and typical cell parameters [6, 7], we extract an effective complex permittivity for each. The resulting complex permittivity is then placed into the Eq. 2 assuming media with conductivity 10 mS/m and a relative permittivity of 78.

The predicated real part of the CM factor is plotted in Fig. 2 for both the morula (insulating core) and blastocyst (insulating shell) as a function of frequency. Below 100 kHz, there is a stark contrast between the two stages. Namely, the morula exhibits a strong pDEP response and the blastocyst exhibits a strong nDEP response. At earlier stages of development (1-cell through 4-to-16-cell/morula), the embryo possesses a greater electrical admittance, relative to the surrounding medium. This is likely due to the highly conductive space between the *zona pellucida* and interiorly-located embryonic cells. This results in a positive CM factor, and, therefore, a pDEP response. However, starting at the early-blastocyst stage, the admittance of the embryos becomes smaller than that of the media, resulting in a negative valued CM factor, and, thus, an nDEP response. This is likely due to the formation of the trophoectoderm epithelium which

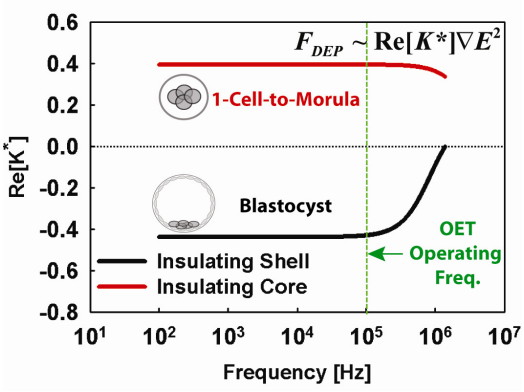

Fig. 2: CM factor versus frequency for insulating core (1-cell to morula) and insulating shell (blastocyst). The insulating core exhibits pDEP and the insulating shell exhibits nDEP. This suggests that embryos in the 1-cell to morula stage and blastocyst stage should experience a pDEP and nDEP response, respectively.

electrically screens the highly conductive interior (blastocoele). This decrease (~1000x) in admittance at the blastocyst stage has been confirmed by Benos *et al.*[8].

The response shown in Fig. 2 is predicated on the selection of a suitable medium. Specifically, the medium conductivity used must fall in between the low and high admittance states of the developing embryos. Media conductivities outside of this range will result in either a pDEP or nDEP response regardless of embryo morphology. Therefore, it can be presumed that optimized media conductivities for different strains of embryos will be necessary in order to produce the largest dichotomy in response. In the context of these experiments, it is important that the media conductivity remain relatively constant for all groups due the dependence of the DEP response on the electrical properties of the media. This sensitivity to media conductivity is most prevalent at the point where the two complex permittivities are nearly identical (i.e. where the developing embryo transitions from a pDEP response to a nDEP response). To maximize internal consistency and precision, medium conductivity must be carefully monitored. It is also important to note that only certain conductivities of media (~1mS/m – 100 mS/m) can be used in the OET device presented here due to the fact that the liquid layer is part of the electrical circuit pertaining to device operation. For higher liquid conductivities (~1 S/m), a different OET device has been developed [9]. However, as above, at these high conductivities the embryos are unlikely to exhibit the large full scale range of DEP responses observed here.

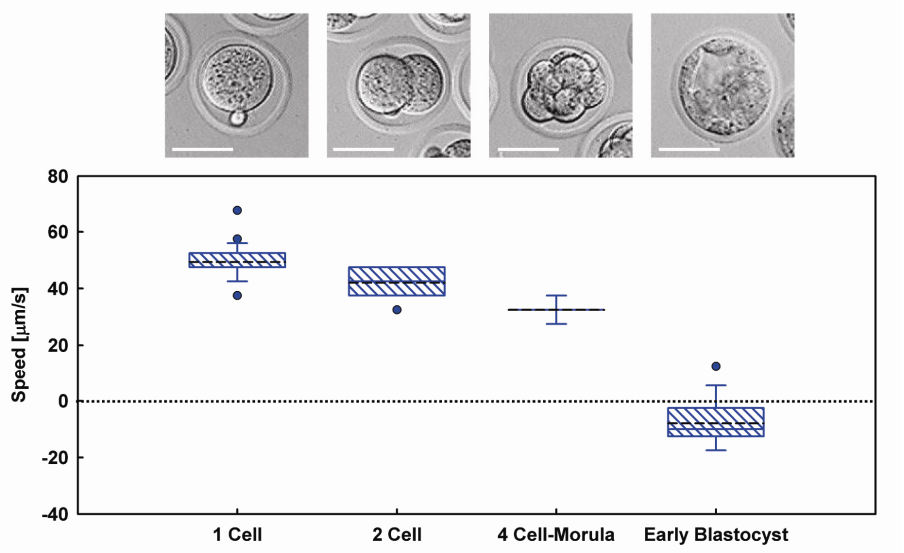

Fig. 3: Morphological dependence of Embryo DEP response. Box plot showing speeds of embryos at varying developmental stages (n = 30-35, for each stage) (black dashed line is mean speed). A typical brightfield image corresponding to the embryos at each stage is placed above each box. Mean values are all significantly different (two-tail alpha, $p<0.005$). Scale bar 50 µm.

EXPERIMENTAL

A 6" glass wafer with a 300 nm layer of sputtered indium tin oxide (ITO) (Thin Film Devices, USA) was coated with a 1 µm layer of hydrogenated amorphous silicon (a-Si:H) deposited via plasma-enhanced chemical vapor deposition (PECVD) (100 sccm 10% SiH_4:Ar, 400 sccm Ar, 900 mTorr, 350°C, 200 W). The a-Si:H coated ITO wafer, along with another 6" ITO-coated glass wafer, was then diced into 2x2 cm chips with a dicing saw (ESEC 8003) forming the bottom and top OET substrates, respectively. The bottom OET substrate (a-Si:H coated ITO) was then subjected to a brief oxygen plasma (51.1 sccm O_2, 300 W, 1 min.) and placed in a solution of 2-[Methoxy(polyethyleneoxy)propyl]trimethoxysilane (Gelest Inc., USA) for 2 hours. The immersed chips were then rinsed in ethanol and air dried. This resulted in a thin layer of poly-ethylene glycol (PEG) on the surface of the bottom substrate which aided in reducing adherence of the embryos to the surface. Electrical contacts were made to the ITO on both the top and bottom substrate using an electrically conductive silver epoxy. The top and bottom substrates are separated by a 200 µm layer of double sided tape.

A custom-built microscope was used for all experiments. The sample was placed on a stage connected to a mechanical drive (Newport LTA-HL and Newport ESP300-1NN111), which allowed the stage to be moved at a known rate. Viewing occurred from the topside via a 5x objective lens. The optical patterns used for manipulation were formed using a commercial data projector (2400MP, Dell, USA) controlled by an external computer running commercial presentation software (Microsoft Powerpoint 2003). Viewing and image capture occurred via a CCD camera (XCD-X710CR, Sony, USA) connected to an external computer. Electrical bias was applied using a standard function generator (33220A, Agilent, USA).

A total of 164 mouse zygotes were harvested at the 1-cell stage and cultured *ex vivo*, in KSOM+AA media. Groups of 30-35 embryos at the developmental stages shown in Fig. 3 were suspended in 10 mS/m buffer (Media T, Cytopulse Sciences) and placed in the OET device. The maximum induced speed (directly proportional to DEP force) associated with each embryo was recorded (20 Vppk, 100 kHz). pDEP and nDEP responses were associated with positive and negative speeds, respectively (Fig. 3). As is evident from Fig. 3 and 4, at the 1-cell stage, embryos exhibit a strong pDEP response. Progressing from the 1-cell stage onwards, the embryos experience a smaller and smaller pDEP response until the blastocyst stage, at which point the embryos experience a nDEP response. Mean values of response at the four developmental stages shown are significantly different ($p<0.005$). As expected from the CM factor modelling, the 1 cell to morula stage embryos experience decreasingly positive DEP, while at the early blastocyst stage, embryos experience negative DEP. This broad spectrum in DEP responses is attributed to the changing morphology, and concurrent changes in impedance, of the embryo relative to the suspension media.

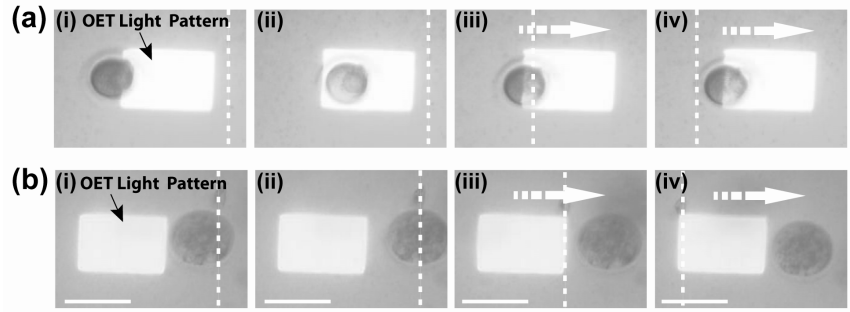

Fig. 4: White dotted line indicates a stationary point on the OET chip. (a) Sequence of images of a 1-cell embryo undergoing pDEP response. Embryo is spontaneously attracted to light pattern (i)-(ii). Stage is moved relative to light pattern resulting in movement of embryo (arrow) (iii)-(iv). (f) Sequence of images of a blastocyst undergoing nDEP response. Embryo is spontaneously repulsed from light pattern (i)-(ii). Stage is moved relative to light pattern resulting in movement of embryo (arrow) (iii)-(iv). Scale bar 100 μm.

CONCLUSION

The use of OET to quantitatively assess the developmental maturity of pre-implantation embryos has been demonstrated. This method removes the subjectivity associated with current state-of-the-art techniques. By providing a means of systematically and quantitatively assessing the developmental maturity of individual embryos, the success rate of IVF transfers can be enhanced and instances of adverse outcomes (maternal and fetal complications) will be reduced.

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