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Recent Microfluidic Devices for Studying Gamete and Embryo Biomechanics

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24 Abstract

25 The technical challenges of biomechanic research such as single cell analysis at a high monetary cost, labor, and
26 time for just a small number of measurements is a good match to the strengths of microfluidic devices. New
27 scientific discoveries in the fertilization and embryo development process, of which biomechanics is a major subset
28 of interest, is crucial to fuel the continual improvement of clinical practice in assisted reproduction. The following
29 review will highlight some recent microfluidic devices tailored for gamete and embryo biomechanics where
30 biomimicry arises as a major theme of microfluidic device design and function, and the application of fundamental
31 biomechanic principles are used to improve outcomes of cryopreservation.

32

1. Introduction

Biomechanical studies of gametes and embryos are critical to understanding the process before fertilization where the gametes need to physically find each other, the fertilization process, and how physical stimuli affect embryo development. Recently there have been large leaps of knowledge added to mechanics by which sperm exhibit rheotaxis phenomena (Miki and Clapham, 2013). On the other hand, the material property of the zona pellucida of the oocyte and its seemingly instantaneous change during zona hardening is of high biomechanical interest (Braden et al., 1954). It is also thought that mechanics play a role in development, where applied mechanical forces provides a feedback to embryo development by mimicking the fallopian tube's physical stimulation of the embryo as it peristaltically pumps the embryo into the uterus (Eytan et al., 2001; Kim et al., 2009).

Classical methods of biomechanical studies involve the use of micromechanical testing, optical tweezers, micropipette aspiration or atomic force microscopy (Thoumine et al., 1999; Hochmuth, 2000; Guck et al., 2001; Lulevich et al., 2006). Such techniques are normally of high cost and labor for single cell analysis. Biomechanics in general is therefore well suited for the incorporation of microfluidic tools to decrease cost, introduce high-throughput screening to reduce labor while simultaneously increasing the accuracy of measurement. Recent applications of microfluidics in biomechanical studies of gametes and embryos have revealed structural mechanisms of sperm that allows swimming with relative ease against direction of fluid flow and discoveries of never-before seen rheotaxis behavior that increases the sperm's chances of encountering an oocyte in its journey through the female reproductive tract (Su et al., 2012; Kantsler et al., 2014). In oocytes, microfluidic tools suggest that the zona pellucida is even stiffer than what was measured previously; further providing evidence that there is more to penetrating the glycoprotein membrane than physical force (Murayama et al., 2004). Microfluidics has shown that mechanical stimuli enhances embryo development and has provided evidence to advance current embryo culture techniques beyond the static petri dish (Kim et al., 2009). Lastly, microfluidic tools have also been applied to reduce osmotic and mechanical stress on oocyte and embryo during cryopreservation with vitrification: a now widespread component of infertility treatment and fertility preservation (Lai et al., 2015).

2. Sperm biomechanic studies under microfluidic shear

The studying of sperm mechanical behavior is key to understanding underlying mechanisms of male-derived infertility as the inability to physically reach the egg is one key element of failure to conceive. The journey spermatozoa undergo between ejaculation and fertilization spans a distance on the order of 10 centimeters: a perilous journey through diverse microenvironments in the cervix, uterus and oviduct where the sperm must contend with high viscosity and shear flows. Such a long journey requires strong navigational mechanisms to direct the sperm to the oocyte. Such mechanisms for navigation include chemotaxis (Inamdar et al., 2007; Kaupp et al., 2008), thermotaxis (Bahat et al., 2003), and rheotaxis (Miki and Clapham, 2013). While chemotaxis is a strong navigational mechanism for the sperm to reach the oocyte, it is likely only for short-distances as the convection within the female reproductive tract from muscle contractions are thought to disrupt the chemo-gradients over long-distances (Eisenbach, 1999). Thermotaxis is also thought to be based on ovulation-dependent temperature gradients between the isthmus and ampulla (Bahat et al., 2003), leaving rheotaxis as the universal mechanism for sperm navigation that is effective throughout all parts of the female reproductive tract.

2.1. Sperm motion mechanics

Sperm motility is critical to successful fertilization in normal reproduction. The mechanism for male infertility can often be attributed to the inability for the sperm to reach the egg. Using resistive force theory (RFT) and modeling the sperm with a rigid spherical head attached to a thin elastic flagellum swimming in shear flow (Marcos et al., 2014) it was discovered that the sperm number (Sp ; Eq. 1) of the flagellum is key to influencing the swimming sperm's tolerance to fluid shear rate. It is worthy to note that Sp in this biomechanical study is not the number of sperm or sperm concentration as is often used but rather a physical dimensionless parameter characterizing the period of traveling wave of the sperm flagellum to its bending relaxation time (Lauga and Powers, 2009). Sperm flagellum with low Sp behave like rigid rods which are almost completely free from the influence of shear flow regardless of shear direction (towards or against swimming direction) and shear rate. However, if the sperm has a higher Sp , its flagellum behaves more flexibly and the shear flow starts to affect the sperm's swimming velocity where the sperm swims faster in the forward direction at high shear conditions regardless of shear direction. At conditions with very high Sp , there is an even more favorable response to forward sperm progression when the

shear flow is against that of the swimming direction due to the advantageous shape change of the beating flagellum against shear flow.

$$Sp = L(\frac{\omega k_N}{EI})^{1/4} \quad (1)$$

Where L is the tail length, ω is the beating frequency, k_N is the resistive coefficient in the perpendicular axis which positively correlates with viscosity, and EI is the bending stiffness where E is the elastic modulus and I is the area moment of inertia. Based on the definition of Sp , it will increase as the beating frequency increases, rigidity decreases, or viscosity increases although it is noteworthy that the increased viscosity would also decrease beating frequency and thus by extension decrease Sp as well. The RFT model designed for enhancing motile sperm purification by microfluidics is a thorough and fascinating biomechanic basis for the sperm's ability and favoritism to swimming against fluid flow direction commonly known as rheotaxis.

2.2. Sperm rheotaxis

The biophysical microenvironment of the mammalian sperm dictates its movement inside the female reproductive system. A combination of cell secretions, ciliary beating, and muscle contractions within the female reproductive tract produce a fluid flowing opposite of the swimming direction of sperm. Microgrooves on the periphery walls of the female reproductive tract provide a safe-haven as the high surface boundary decreases fluid velocity. Sperm trajectory studies demonstrate that there is a clear directional bias towards the sidewalls and once entrance inside microgrooves are gained, a persistence to stay within the grooves. Such swimming behavior has been attributed to hydrodynamic interactions with the flow profile near the sidewall as well as rotational Brownian motion (Drescher et al., 2011; Li and Tang, 2009).

A major microfluidic strength is that it provides well-defined experimental parameters. By adjusting the volumetric flow rate in a single microfluidic device, a range of swimming behavior can be observed with random swimming patterns (1 $\mu\text{L}/\text{min}$ or less), swimming against the flow (3-5 $\mu\text{L}/\text{min}$) and simply being swept away by the flow (5 $\mu\text{L}/\text{min}$ or higher) (Tung et al., 2014). The authors also note that the grooves slow sperm swim speed due to the sidewalls physically restricting the flagellum beating amplitude but the Poiseuille flow profile reduces speed by a power of two over radius from the centerline of the cylindrical female reproductive tract (Eq. 2). Despite the

slowed swim speed, the large reduction in fluid velocity near the sidewalls makes swimming in the grooves a successful biophysical mechanism for reaching the oocyte (Fig. 1A-B).

$$u_z = -\frac{1}{4\mu} \frac{\delta p}{\delta z} (R^2 - r^2) \quad (2)$$

Where u_z is the velocity of fluid down the cylindrical tube, μ is dynamic viscosity, p is pressure, z is distance down the cylindrical tube, R is the cylinder radius and r is the radius of interest as quantified by distance from centerline between 0 and R .

A recent study investigated the swimming strategies of human and bull sperm under well-controlled flow conditions defined by microfluidic devices. It was discovered that human and bull sperm do not just exhibit rheotaxis, but also swim upstream in spiral trajectory along the sidewalls of the cylindrical channel (Kantsler et al., 2014) (Fig. 1C). This traverse velocity component is stronger in the human sperm than the bull sperm in fluid of seminal fluid viscosities. The spiraling trajectory of the sperm increases its fertilization potential as it increases its area coverage in the oviduct.

In well-defined microfluidic flow reversal experiments, the sperm cell typically performs a U-turn in response to the flow reversal with a typical response time of 5 to 10 seconds. This response time is interestingly in the same order as typical cervical contractions that generate peristaltic flow: suggesting an adaptation between sperm cells and the muscles of the uterus to produce momentary increases in transport velocity for each uterine muscle contraction (Kantsler et al., 2014). The quantitative characterizations provided by microfluidic tools suggest that sperm swimming behavior is not as simple as previously thought and that mammalian sperm have evolved to adapt to the conditions of the female reproductive tract during its most fertile phase and after intercourse. Such studies can have a clinical impact by guiding the optimization of fertilization medium in terms of viscosity and chemical composition as well as injection techniques for improved artificial insemination strategies.

2.3. Fluid condition changes in the female reproductive tract to accommodate sperm travel

The knowledge of sperm biomechanics has deepened in the last couple years to go beyond just rheotaxis. As microfluidic design is highly biologically inspired, it is now of high interest to definitively characterize changes in female reproductive tract fluid conditions during phases of heightened fertility. Near the time of ovulation in the estrous cycle, the isthmus epithelium is covered in a dense mucus that disappears after estrogen triggers a substantial

2-3 fold increase in oviductal fluid secretions at estrus (Gott, 1988; Jansen, 1978). It was recently discovered in female mice that the oviductal epithelia actively secretes substantial fluid that flow down the oviductal lumen and that this flow is generated within 4 hours of sexual stimulation and coitus (Miki and Clapham, 2013). This coitus-triggered flow is prolactin-triggered and serves to clear oviduct debris, lowers viscosity, and generates a directional flow to direct sperm rheotaxis. These recent studies in sperm biomechanics have deepened understanding of the swimming mechanics of sperm and the synchronous changes to the female reproductive tract fluid conditions during fertile phases of the estrous cycle and after coitus to assist the sperm in its trek to the egg. Such information provides knowledge needed to design more sophisticated microfluidic devices for more efficient sperm sorting or deeper experimentation of sperm behavior.

3. High accuracy and sensitivity in microfluidic analysis of oocyte biomechanical properties

There has been a recent surge of interest in the mechanical properties of oocytes, particularly in the changes to the zona pellucida during fertilization (Braden et al., 1954). There is however a large variability amongst oocytes, therefore there is a need for a high throughput and high sensitivity measurement methodology to more easily measure high numbers of oocytes individually and quickly.

3.1. Oocyte cellular force measurements by mechanical probe and cantilevers

Early sensitive methods for estimation of the oocyte zona pellucida local stiffness was achieved by mechanical probe indentation (Green, 1987) however the methodology is expensive in time, labor, and cost. Nevertheless, it demonstrated that the forces necessary to penetrate the zona pellucida is much higher than what is thought capable for sperm: implying that the spermatozoa cannot penetrate zona pellucida by force alone. Due to the significant deformation needed (Fig 2A) and the assumption that the specimen is isotropic and homogenous, it is thought to be underestimated due to the large depression dissipates much of the force precluding a true measurement of the local compression: adding further support for the inability for sperm to penetrate the zona pellucida solely by force. A much more recent micropipette-indentation microfluidic device was developed to measure the force and cell deformation using a vision-based method of tracking deflections of elastic, low-stiffness supporting posts holding

the oocyte (Liu et al., 2010). The device demonstrated the capability of mechanically distinguishing healthy young mouse oocytes and defective old oocytes and provided some evidence in differences in zona pellucida or cytoskeletal structure that may be reasons for this difference in mechanical properties. Another device made with an epoxy-based viscous polymer (SU-8) also used a visual-based approach of cantilever deflection to measure human oocyte mechanical behavior (Wacogne et al., 2008), and another device using a micropipette to push the oocyte against a magnetic spring capable of hypersensitive force measurements (Abadie et al., 2014). These devices however also suffer, as do all indentation-based analysis of oocytes, from the assumption of isotropic and homogenous material and large physical depressions of the oocyte.

3.2. Micromechanical sensing platform

Still plagued with a relatively low-throughput, and assumptions of homogeneity and isotropy, Murayama and coworkers used a tactile sensor based on resonance vibration to indirectly measure the zona pellucida's material properties. This measurement is based on the shift in resonance frequency of the sensor when it is in contact with another object and the shift in frequency is dependent on the stiffness of the contacted material (Murayama et al., 2004). The authors built a system with two, three dimensional (3D) micro-manipulators: One equipped with a holding pipette for the oocyte, and another equipped with the piezoelectric transducer as the sensor element (Fig. 2B). The sensor and oocyte come into contact in a chamber sandwiched by a glass slide and cover glass. With the system calibrated, the authors reported the Young's modulus of the zona pellucida to be 25.3 ± 7.9 kPa, which highlights the large variation of mechanical properties even amongst oocytes measured in the same conditions. This variation necessitates large sample sizes for confident measurements. As the device only requires contact and not large deformations to obtain a stiffness measurement, this technique is thought to be more accurate and does not require the assumptions of specimen isotropy and homogeneity (Murayama et al., 2004). Furthermore, the measured Young's modulus is several-fold higher than previously reported, further providing evidence that sperm cannot penetrate the zona pellucida by force alone.

3.3. Mechanical impedance measurement for cellular mechanical parameters

Mechanical studies of oocytes require precise parameters that normally require significant efforts in manipulating and analyzing a single cell. In response to the need to measure large population sizes due to the large variation in mechanical properties, lab-on-a-chip tools were recently used for high throughput measurements, of conventionally laborious parameters, on oocytes. Sakuma and colleagues developed an analyzer for the measurement of oocytes with built-in magnetically driven microtools (MMTs) for automating cell manipulation (Sakuma et al., 2012). The device consisted of a tunable wall and force sensor. This proof of concept device was programmed to deform the oocyte at 15%, 20% and 25% strain, which is relatively high thus suffering from the same assumptions and underestimation as early mechanical probing. However, the device demonstrated the ability to quickly and repeatedly deform oocytes at different strains, as well as an increase in Young's modulus in older oocytes. It is likely that the development of a high-throughput device that is also highly sensitive for oocyte mechanical analysis is soon to come.

It is worthy of note that the use of optical tools have achieved a high level of throughput and sensitivity at accessing oocyte maturation and quality (Valley et al., 2010; Hwang et al., 2009; Zeggari et al., 2006). Such non-destructive assessments of maturation and quality, by whichever means, has high clinical relevance to improving assisted reproductive outcomes and improves the depth of experimentation capable for fundamental basic research purposes.

4. Improvements in embryo development under microfluidic mechanical stimulation

The advantages afforded by the use of microfluidics is also well suited for the study of embryogenesis particularly in already well-studied embryogenesis models such as zebrafish or drosophila as well as early embryo development in mammals. The literature for the use of microfluidics for embryos is vast and span temperature, chemical and mechanical stimulation. There is much excitement in this area of study as evidenced by the daunting number of studies published in only the last five years: over 10,000 publications by the author's own literature search. In the interest of remaining within the scope, this review will focus on some select devices that are mechanically oriented and demonstrate proof-of-concept large-scale experimentation. The interested reader is encouraged to review the following articles (Beebe et al., 2002; Wheeler et al., 2007; Krisher and Wheeler, 2010;

Swain and Smith, 2011; Lai et al., 2012; Swain et al., 2013; Hwang and Lu, 2013) for thorough discussions of microfluidic experimentation and potential utility with mammalian embryos.

4.1. Mechanical stimulation improving embryo development

Mechanical stimulation improving mammalian embryo development is well documented within the literature. Current and future microfluidic devices for enhanced embryo developments owe much of its success to the early studies of laboratory tilters, shaker and rocker systems and devices (Hoppe and Pitts, 1972; Isachenko et al., 2006; Koike et al., 2010; Matsuura et al., 2010) which demonstrated significant enhancements to development despite the lack of well-defined flow conditions and fluid-mechanical stresses induced by the tilters, shakers, and rockers. When well-defined flow conditions were studied, simple one-dimensional fluid-mechanical stimulation of embryos was shown to result in poor embryo development across a range of flow rates (Hickman et al., 2002). These authors suggested much more studies are needed to explore dynamic medium stimulation and its effects on embryo development. Subsequently, a microfluidic device with channel constrictions and direct mechanical stimulation of bovine embryos during preimplantation development resulted in significantly more 2-cell embryos developing into 8-cell embryos (Kim et al., 2009) (Fig. 3A-B). This study was one of the first publications to demonstrate the precision afforded by microfluidics as a strength in an effort to gain insight into mechanisms for enhancement of assisted reproduction. A funnel-shaped microfluidic device was designed that combined fluid-mechanical stimulations to the embryo with the beneficial effects of media and autocrine retention to suggest that the previously reported poor embryo developments for microfluidics devices may be due to the removal of fluid containing important growth-promoting autocrine factors (Heo et al., 2010) (Fig. 3C). This study demonstrated a significant increase of later stage murine blastocysts and a greater percentage of embryo implantation and on-going pregnancy. Esteves and coworkers advanced these observations by using a microfluidic device to study the relationships of embryo culture chamber size, embryo number per chamber, and static versus dynamic media conditions. If the first two variables were identical, static versus dynamic culture did not significantly influence blastocyst development. Static culture, low chamber size (30 nL) and high embryo number (20 embryos/chamber) was detrimental for blastocyst development compared to all other nanoliter culture conditions. Finally, this report demonstrated that live mice could be obtained under all conditions. Again, when chamber size and embryo number/chamber variables were

the same, live birth rates were not significantly different between static and dynamic culture. Under static culture conditions, and within the same chamber size (both 30 nL and 270 nL), birth rates were significantly higher with 20 embryos/chamber versus 5 embryos/chamber (Esteves et al., 2013). This report emphasizes considerations that warrant exploration in the future: i) the definition and relationship of chamber size as described by volume when media is actually moving through a chamber, and ii) does this impact the description and interpretation of embryo number/chamber and embryo density.

Over time, the direct physical mechanical stimulation has increased dramatically in sophistication. An example is the use of microfluidic membrane deflection as a tool to mechanically stimulate embryos (Bae et al., 2011). The characterization study included a cautionary tale of the effects of excessive time duration of mechanical stimulus where embryos were underdeveloped in areas where there was over-mechanical stimulation. Although this study did not show significant improvement of embryo development, it provides direction for future developments of microfluidic devices that mimic peristaltic constricting stimulations *in vivo*.

4.2. Zebrafish microfluidic study of mechanical morphogenesis

The zebrafish embryo model is an ideal model for morphogenesis study within a microfluidic chip as the embryo undergoes dramatic morphological changes in only 4 days, is small in size, can be raised at low cost, and can be raised in large numbers. It has been used successfully as an intermediate model between cell-based assays and biological validation in whole animals. The model takes advantage of a microfluidic chip's ability to provide continuous and well-regulated experimental stimulations in both temperature and flow rate (Wielhouwer et al., 2011) (Fig. 3D). Zebrafish assays are usually imaged in multi-well plates which suffer from distortions in image capturing with phase-contrast and bright-field microscopy due to the large depth in the buffer solution and curvature of the meniscus. The use of microfluidics also improves upon this weakness as the geometries of the microfluidic devices allow for thin chambers as well as parallel top and bottoms of the chamber without a meniscus. The microfluidic device allowed for the introduction of aqueous compounds for experimental purposes. The authors demonstrate the usability of the device to characterize embryo viability over time as well as the types and severities of abnormalities from zebrafish embryo morphogenesis for pectoral fin hypoplasia, bent tails, yolk sac oedemas, pericardial oedema, and Meckel's cartilage hypoplasia (Wielhouwer et al., 2011). In addition to the ability of the

device to generate temperature gradients and study the effects of chemical exposures to ethanol, the device also demonstrated an effect of constrained spaces on a significant decrease in embryo body length and an increase in minor developmental malformations.

4.3. Microfluidic device for large-scale high-throughput morphogenesis study

To demonstrate the possibilities of microfluidic tools for embryogenesis study, a microfluidic device was designed to monitor hundreds of drosophila embryos in an up-right position within minutes: a difficult orientation to examine by conventional means (Fig. 3E-H) (Chung et al., 2011). This now enables extensive quantitative study of the dorsoventral system. Although this study was not specifically biomechanical, the authors demonstrate the capability of studying real-time dynamics of embryonic development and serves as a proof-of-concept for the extent of high-throughput capabilities designed with microfluidic tools to match that of the animal model. Another hydrodynamic trap device was designed to load, visualize and experiment on individual oocytes and embryos with no loss of cells while simultaneously allowing for high resolution imaging and extended culture (Angione et al., 2015).

5. Enhanced oocyte and embryo cryopreservation with a biomechanic and microfluidic approach of dehydration

Cryopreservation by vitrification is a thermodynamic phenomenon whereby the cell undergoes a phase change from liquid (water) to glass instead of liquid to solid (ice). The elimination of ice formation inside the cell is critical to safe and effective cryopreservation as the cold temperatures decreases the rate of metabolism and decay but the phase change to solid causes the cell to erupt due to the increase in volume from water inside the cell turning into ice. To necessitate vitrification, the cell must first be exposed to a high concentrations of cryoprotectant agents (CPA) and thus be subject to high osmolalities (Rall and Fahy, 1985). The cell shrinks in response to the high osmolalities by the osmotic water loss through the cell membrane and early studies have shown that there exists a threshold minimum cell volume where if the cell shrinks past, would cause lethal damage to membrane integrity

(Meryman, 1971). Such a concept can be mechanically appreciated as strain whereby the cell membrane will mechanically fail at a critical strain at fracture.

5.1. History of cryopreservation on minimum cell volume and strain

The current most commonly practiced method for oocyte and zygote vitrification involves a multi-step equilibration process (Smith et al., 2010) where the cells are manually pipetted into intermediate equilibrium drops where the cell is allowed to equilibrate to intermediate levels of CPA before exposed to the final levels of CPA for vitrification. The cell is allowed to shrink in stages, and sometimes to re-expand, to avoid that critical strain to the cell membrane. Multiple groups have demonstrated that the use of higher number of equilibration steps increases the cryosurvival and developmental competence outcomes (Kuwayama et al., 1992; Otoi et al., 1998). However these high numbers of equilibration steps were not clinically adopted due to their impracticality.

5.2. Mechanical measurements of cells typically viscoelastic and importance of strain rate

Recent biomechanical studies of the cell membrane have discovered that the membrane behaves as a viscoelastic sheet (Ragoonanan et al., 2010; Fu and Zhang, 2012). Viscoelasticity is a time-dependent molecular rearrangement of the material in response to an applied stress and one of the characteristics of viscoelasticity is that it will experience less stress due to a lower applied strain rate (Peeters et al., 2005). Meanwhile, it has been well documented that the osmotic shrinkage of the cell, and thereby its strain over time, in response to CPA exposure can be accurately predicted using the Kedem-Katchalsky equations (Kedem and Katchalsky, 1958; Pfaff, 1998; Paynter et al., 1999). However while the effects of strain during the CPA exposure on vitrification outcome is known, it was until recently unknown if there were any measurable effects of strain rate on vitrification outcomes.

5.3. Automated microfluidic devices for keeping cells at maximal volume for as long as possible.

Several lab-on-a-chip research groups have taken interest in cryopreservation using lab-on-a-chip microfluidic tools. Song and colleagues have demonstrated the use of a microfluidic device for slow-rate freezing (Song et al.,

2009) which uses less concentration solutions of CPAs (Fig. 4A). More recently, Heo and coworkers have demonstrated that a microfluidic device is capable of controlling precise customized CPA exposure profiles that essentially expose the oocytes to an unlimited amount of equilibration steps thus overcoming the limitations of manual pipetting and showed that experimental observations could be accurately predicted using Kedem-Katchalsky equations (Heo et al., 2011) (Fig. 4B). However, the study did not incorporate impermeable CPAs highly recommended for embryo and oocyte vitrification (Kuleshova et al., 1999), nor was it designed for cell removal after the CPA exposure precluding actual oocyte cooling and vitrification. The focus up until Heo's work has been to decrease the strain imposed upon the cells, and the effects of strain rate on the cell had been unexplored.

5.4. The importance of exposure to impermeable solutes and microfluidic enabled reduction of shrinkage or strain rate.

Most recently, Lai and colleagues used the Kedem-Katchalsky equations to model for cell shrinkage exposed to vitrification solutions containing impermeable CPAs using microfluidics, manual pipetting using equilibration steps, and manual pipetting with no equilibration steps which has long been known to be detrimental to cryosurvival. It was discovered through mathematical modelling that when impermeable CPAs were used, the strain and strain rate of the cell during CPA exposure can be independently controlled in a fixed amount of time depending on how the cell was exposed to CPA (Lai et al., 2015). Using a microfluidic device designed for delivery of the mathematically modelled CPA exposure profiles, the authors demonstrate that for a defined strain, the decrease of strain rate leads to improved outcomes. The enhanced outcome can be immediately visualized by a striking improvement in morphometrics: elimination of mechanical buckling of the viscoelastic membrane (permanent cell membrane deformations) as a direct consequence of less applied stress from decreased strain rate (Fig. 4C-E). Zygotes vitrified using the low strain rate microfluidic method also had a higher retention of lipid content, lower degree of transient membrane perforations during CPA exposure, and when cultured grew into blastomeres with higher developmental competence (Lai et al., 2015), where competence is measured by the cell number of each blastocyst. Such an increase in developmental competence has previously been shown to have increased implantation and ongoing pregnancy rates (Heo et al., 2010).

6. Conclusions

Microfluidics provide the user with an unprecedented precision in analysis and control in physical and fluid conditions which are applied to mechanical strain or fluid shear stress. These strengths are well suited for the biomechanical study of gametes and embryos and may have future clinical implementations. Microfluidics, for embryo development in particular, has generated a significant amount of scientific discovery and bioengineering designs that have heavy biomimicry elements with clinical applications starting to emerge with success in embryo culture and diagnostics by non-destructive analytical methods. Microfluidic sperm sorting has had success and clinical applicability for severe oligozoospermia cases. Future growth in knowledge of factors influencing gamete developmental competence and fertilization will drive even more sophisticated microfluidic devices that will likely enhance gamete research capabilities and clinical applicability. One may envision a microfluidic device capable of label-free, non-destructive, and high-throughput sperm sorting for normalcy of genetic content and morphology. It is also expected that the definitive and sensitive control of the microenvironment by microfluidics directed by biomimicry using basic knowledge of oocyte biology will improve oocyte culture and maturation as well as the fertilization event itself. Microfluidic tools in general, while versatile and powerful in enhancing effectiveness and sensitivity of biological assays, has often been criticized for being a lab-on-a-chip-in-a-lab of supporting equipment to drive the fluidics and analytics, which has impeded the widespread use of microfluidic tools. As there begins to be appreciation and balance between what can be done, and what needs to be accomplished for improving clinical care, the integration of microfluidics and other engineering technologies, such as optofluidics, into assisted reproductive technology applications will gain more widespread acceptance and use.

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Figure Legends

Fig. 1. (A) Microfluidic devices designed to mimic the microgrooves of (B) the female reproductive tract. Newly discovered spiral trajectory of sperm by microfluidic devices. Reproduced with permission from (A) (Tung et al., 2014) and (B) (Kantsler et al., 2014) respectively.

Fig. 2. (A) Zona pellucida measurements by quart fiber needle. (B) Piezoelectric transducer sensor for zona pellucida mechanical measurement based on resonance vibration technology. Reproduced with permission from (A) (Green, 1987) and (B) (Murayama et al., 2004) respectively.

Fig. 3. (A-B) Mechanical stimulation by channel constrictions encourages better embryo development. (C) Funnel-shaped microfluidic chamber for mechanical stimulation and autocrine factor retainment. (D) Mechanical membrane deflections for mechanical stimulation of embryos and temperature gradient generation within a microfluidic device. (E-H) High-throughput embryogenesis study with microfluidic devices. The microfluidic geometry allows for accurate studies of the dorsoventral system. Reproduced with permission from (A-B) (Kim et al., 2009), (C) (Heo et al., 2010), (D) (Wielhouwer et al., 2011), and (E-H) (Chung et al., 2011) respectively.

Fig. 4. (A) Microfluidic device for slow-rate freezing. (B) Microfluidic exposure of oocytes to a continuously changing permeable CPA solution. (C) Microfluidic device exposing zygotes to continuously changing solutions of permeable and impermeable CPA. The associated changes in morphology from (D) conventionally CPA exposed zygotes is significantly different from (E) microfluidic CPA exposed zygotes: demonstrating the effects of strain rate on the morphology and osmotic / mechanical stress to the zygote. Reproduced with permission from (A) (Song et al., 2009), (B) (Heo et al., 2011), and (C-E) (Lai et al., 2015) respectively.

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Figure 3

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