MICROFLUIDIC PROTOCOL FOR IN VITRO CULTURE OF HUMAN EMBRYOS

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ABSTRACT

In vitro culture of pre-implantation embryos is a key-step in Assisted Reproductive Technologies (ART) protocols. In present work we examine the potential of microfluidic devices for pre-implantation human embryo culture, in comparison with conventional droplet-based culture (control). Donated frozen day-4 embryos are cultured for 72 h after thawing in both formats, and graded at four different time points (24, 28, 48 & 72 h). Specifically, pre-implantation developmental rates (blastocyst rates) and embryo qualities are evaluated. Blastocyst rates show no statistical difference between the two culture groups, while the embryo distribution shifts towards higher quality blastocysts in the microfluidic group.

KEYWORDS: Human Embryo Culture, Blastocyst Rate, Embryo Quality, Prospective Randomized Controlled Trial, Microfluidics

INTRODUCTION

In vitro culture of pre-implantation embryos is widely employed every day in IVF centers, and consists of one of the key steps in ART protocol. Although it has been used in clinics since 1970s, the protocol for the culture of human pre-implantation embryos is still neither standardized nor optimized [1]. As an alternative approach to improve embryo culture, microfluidics has recently attracted much attention. Microfluidics provides high control at the micrometer scale; it is easily amenable to dynamic culture, while limiting embryo manipulation. Furthermore, embryo culture can easily be combined with embryo characterization in this format which lends itself well to the realization of integrated devices. Previous work from our group [2, 3] has proven that microfluidics supports single mouse embryo growth, with higher pre-implantation and full-term developmental rates compared to conventional droplet culture. Here, we report the results of a prospective randomized controlled *pseudo*-clinical trial where we examine the applicability of our microfluidic approach for the culture of human embryos. It is the first time to our best knowledge that human embryos are cultured in a complete microfluidic format.

EXPERIMENTAL

The design of the microfluidic device resembles that developed for mouse embryo culture [2] with minor adaptations. The embryo is cultured in a sub-microliter chamber (diameter 1500 µm, height 360 µm), which is connected to inlet and outlet microchannels via constrictions to retain the embryo inside the chamber during culture (See Fig. 1). The microfluidic structures are fabricated in polydimethylsiloxane (PDMS), which is bonded to a glass substrate. Embryos are introduced into pre-conditioned systems by passive pumping [4].

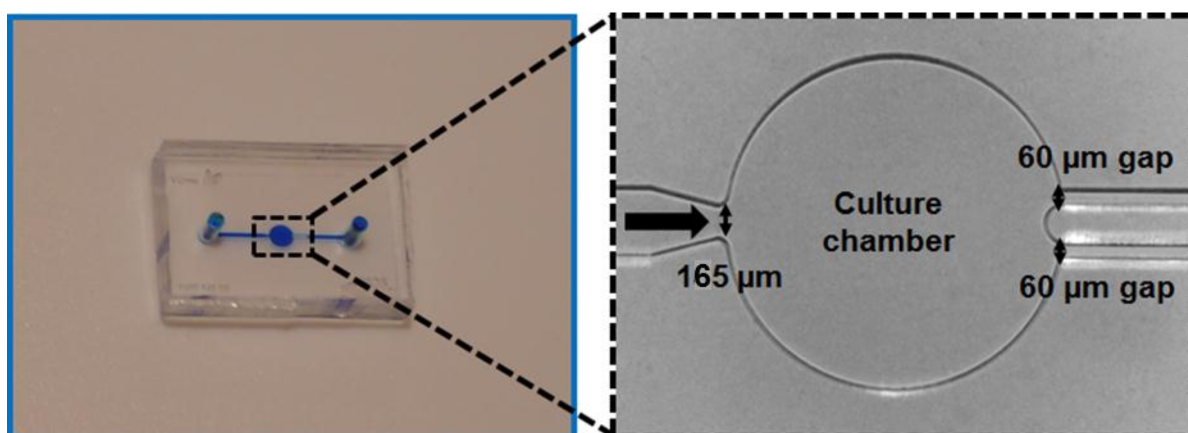

Figure 1: Microfluidic platform for human embryo culture fabricated from PDMS and glass. Left*: Picture of the device with channels filled by blue ink for visualization purposes;* Right*: Microscopic picture of the culture chamber showing the constricted areas at the inlet and outlet to retain embryos in the chamber.*

Donated human embryos which were frozen on day 4 are used in our experiments. After thawing, embryos are immediately graded, and only embryos with sufficient morphological quality are retained in our study. Specifically, embryos to be included should have at least 8 blastomeres, with maximum 20% fragmentation and no more than 25% atresia. Em-

bryos are then randomly allocated to microfluidic or droplet (25 µL) culture, and kept in a clinical incubator (36.8°C, 5% CO_2, ~20% O_2 , 95% humidity). Embryos are assessed at four different time points, after 24, 28, 48 and 72 h of culture, using the Gardner grading system [4], as detailed below.

RESULTS AND DISCUSSION

101 embryos (50 in microfluidic group and 51 as control) have been included in our experiments, which are spread over 8 independent days. The microfluidic platform supports the development of human embryos (See Fig. 2 & 3). Maximal blastocyst rates of 64.0% and 66.7% are reached for the microfluidic (at 28 h and 48 h) and control (at 72 h) groups, respectively (Fig. 2). Statistically, blastocyst rates of the two groups at all four different assessment points during culture are comparable: Fisher's Exact Test gives p = 0.55, 0.42, 1.00, 0.68, respectively for the four time points considered. However, the still increasing blastocyst number after 48 h culture (day 6 in clinical embryo developmental schedule) in the control group indicates that embryo development in that group is delayed with respect to the well-accepted embryo development timeline. This behind-schedule development situation is not observed in microfluidics. Figure 2 shows a fully hatched embryo in a microfluidic chamber after 3 days of culture.

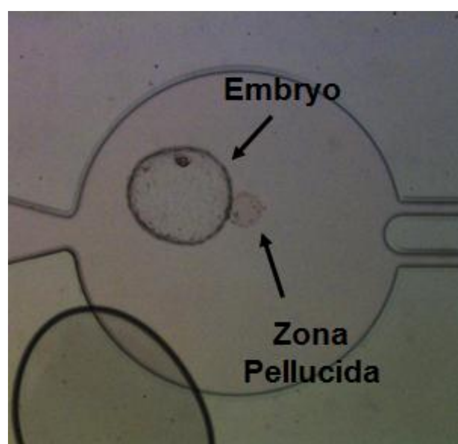

Figure 2: Microscopic picture of a hatched human embryo in a microchamber after 72 h of culture.

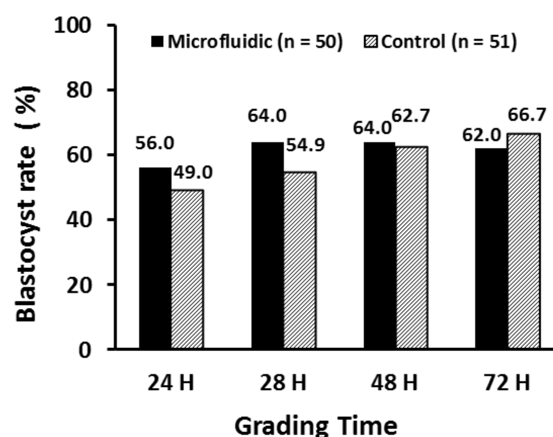

Figure 3: Blastocyst rates measured at different time points after starting of the in vitro culture in microfluidic devices and droplets, for 101 embryos, experiments being carried out in 8 independent weeks.

Embryos that have reached blastocyst stage are graded in terms, on one hand, of expansion level (from early blastocyst (B1) to fully hatched blastocyst (B6), using a 6 degree scale), and on the other hand, of inner cell mass (ICM) and trophectoderm (TE) quality (from 1 to 3, 1 corresponding to high quality and 3 to low quality, for full blastocysts). For further analysis, we classify the embryos in three groups of low quality (LQ), good quality (GQ) and high quality (HQ) embryos based on the Gardner grading, and as detailed in Table 1.

Table 1. Criteria taken into account for the classification of blastocysts into quality categories

Quality level	Expansion level	ICM/TE grade	Additional aspects [a]
HQ	3,4,5,6	1/1, 1/2, 2/1	
GQ	3,4,5,6	2/2	Expansion level 1, 2 at 24 hand 28h
LQ	3,4,5,6	2/3, 3/2, 3/3, (1/3, 3/1)	Expansion level 1 and 2 at 48 h and 72 h

[a.] *Assignment conditions of early blastocysts which are at expansion level 1 and 2 (no ICM and TE grades available for early blastocysts in Gardner grading).*

Classification of the blastocysts into these three quality categories shows that the overall embryo distribution shifts towards higher quality in the microfluidic group compared to the control group (Fig. 4). After 48 h culture, 66.7% of the blastocysts in the microfluidic group are of high quality against 44.4% only in the control group. The embryo quality is

the most important parameter besides the blastocyst rate for *in vitro* culture of pre-implantation embryos. Altogether, the higher embryo quality observed in the microfluidic group suggests that this format could offer improved pre-implantation embryo culture conditions, and subsequently higher success rates in ART treatments.

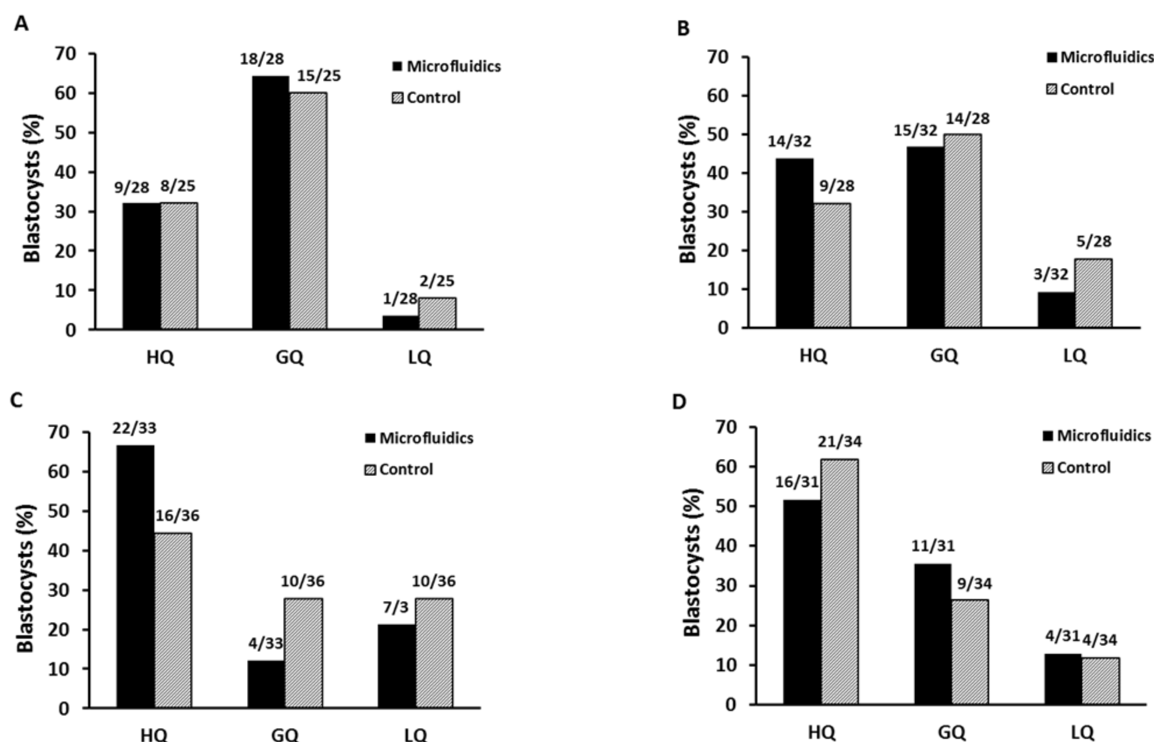

Figure 4: Blastocyst quality distribution after 24 h (A), 28 h (B), 48h (C), 72 h (D) culture. Blastocysts are classified as low quality (LQ), good quality (GQ) and high quality (HQ) embryos depending on their developmental stage and the quality of their inner cell mass (ICM) and trophectoderm (TE).

CONCLUSION

We report for the first time human embryo culture using a complete microfluidic protocol. Donated day 4 frozen human embryos have been cultured in either microfluidic platforms or conventional microliter droplets in a perspective randomized controlled trial to compare these two formats. Microfluidic protocol supports human embryo development in our hands, giving comparable blastocyst rates as in the control group at four different embryo quality assessment time points. Most interestingly, the embryo quality level in the microfluidic group is higher than in the control group. After 48 h culture, 66.7% of the blastocysts obtained in the microfluidic group are of high quality, against 44.4% in the droplet counterpart. Altogether, these results suggest that microfluidics could offer improved pre-implantation embryo culture conditions in ART treatments.

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REFERENCES

[1] Vatja et al., “Embryo culture: can we perform better than nature?”, Reproductive Biomedicine Online, 20, pp. 453-469 (2010).

[2] Esteves et al., “Microfluidic protocol for pre-implantation culture of single mammalian embryos towards an optimal culture”, Proceedings of MicroTAS 2011, Seattle, 2-6 October 2011, pp.1517- 1519.

[3] Esteves et al., “Full-term development of single mouse embryo cultured in a confined nanoliter environment”, under revision.

[4] Walker and Beebe, “A passive pumping method for microfluidic devices”, Lab Chip, 2, pp.131-134 (2002).

[5] Gardner and Schoolcraft, “In-vitro culture of human blastocysts”, Towards Reproductive Certainty: Fertility and Genetics Beyond 1999 (Eds) Jansen R & Mortimer D, Parthenon Press, Carnforth, pp. 378-388 (1999).

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