MICROFLUIDIC PROTOCOL FOR PRE-IMPLANTATION CULTURE OF SINGLE MAMMALIAN EMBRYOS: TOWARDS AN OPTIMAL CULTURE PROTOCOL

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ABSTRACT
Microfluidics holds great potential for the field of assisted reproduction techniques (ART), to provide integrated platforms for combined embryo culture and characterization. The development of mouse embryos is not impaired in a microfluidic format: it proceeds faster during the pre-implantation period (< day 4), and gives similar birth rates. More importantly, single embryo culture is enhanced in a microfluidic environment.

KEYWORDS: microfluidics, assisted reproduction technology, mammalian embryo, embryo culture

INTRODUCTION

Assisted reproductive technologies (ART) which aim to help subfertile couples achieving successful pregnancy, still suffer from low success rates. Those are attributed not only to the underlying health problems, but also to weaknesses found in the complete procedure, and notably to suboptimal *in vitro* culture protocols employed during cell manipulation and prior to embryo transfer to the mother [1]. This is reflected by the fact that 50% of the embryos stop developing during this *in vitro* culture period. Last year, we reported the early development of a microdevice and protocol to address current issues in embryo handling and culture [2]. Microdevices are attractive in this context, as they provide integrated platforms, dynamic culture conditions can be implemented in a confined environment (< 500 nL), embryo manipulation is reduced and the embryo microenvironment can be tailored to resemble *in vivo* conditions.

EXPERIMENTAL

Device design and fabrication

The microfluidic devices are fabricated from PDMS using soft-lithography, and they are bonded to a glass substrate using plasma activation. The devices consist of a simple microchamber equipped with dedicated structures to retain embryos (see fig. 1 and 2); the size of the chamber is varied (30-480 nL) by altering its diameter (500-2000 µm) while keeping the height constant (150 µm). Culture experiments are carried out using chambers of 30 and 270 nL which are large enough to accommodate a large pool of embryos (up to 20) but small enough to enable culture at the single embryo level.

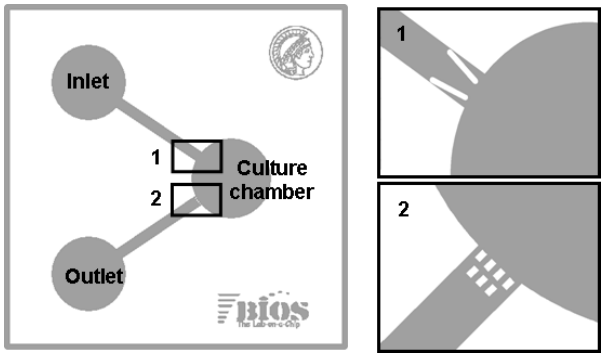

*Figure 1: Design of the microfluidic device for culture of embryos. The device includes a microfluidic chamber in which embryos are guided and trapped with the help of microstructures (*see right*).*

Figure 2: Picture of a microfluidic system made from PDMS, to be bonded on a glass substrate.

Embryo culture

Naturally fertilized mouse embryos are introduced in the devices using mild flow conditions (passive pumping) [3] and kept in place for 3.5-4.5 dpc (day post coitum). For full-term development experiments, embryos are retrieved out of the chamber and implanted in pseudo-pregnant mice at 3.5 dpc by creating a higher flow from the back reservoir to enable their passage through the V-shape structures (Fig. 1, top right). As previously described, we have optimized a protocol for medium

refreshment in the chamber using passive pumping [2, 3]. Here we compare different culture protocols in terms of embryo pre-implantation (blastocyst, 4.5 dpc) and full-term developments (birth rates): 1, 5 or 20 embryos, with/without medium refreshment, in 30 or 270 nL chambers or in 5 µL droplets (standard culture conditions).

RESULTS AND DISCUSSION

Culture experiment design

Embryo development is studied and compared using various culture conditions. First, the group size is changed: groups of 5 and 20 embryos are employed and single embryo culture is tested for both culture approaches. Next to this, two microfluidic volumes are tested (30 and 270 nL). Finally, a medium refreshment step is implemented at 3 dpc for one series of experiments (5 embryos), and this for various reasons. First, as microchambers present volumes 100-1000 times smaller than conventionally employed for embryo culture in droplets (5-20 µL), new nutrients are brought. Second, the toxic substances produced by the embryos (e.g., ammonia) that would accumulate in the nL environment are removed. Third, this mimics the medium replacement step which is required when culture protocols using 2 different media are employed.

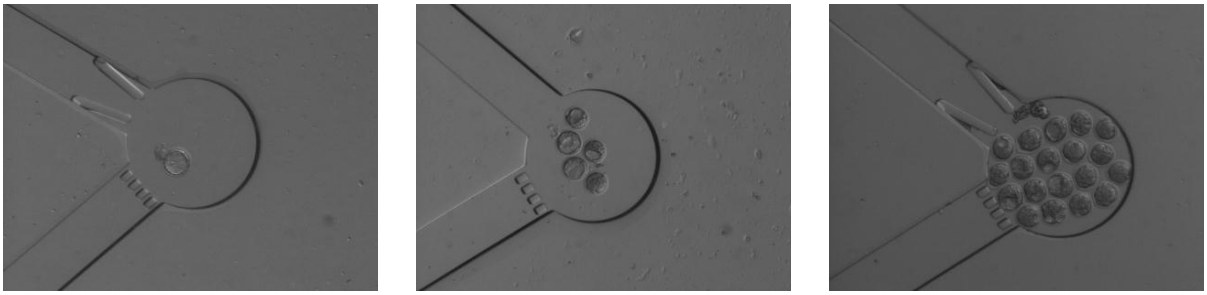

Figure 3: Culture of mouse embryos in microfluidic chamber of 30-nL volume. From left to right: single embryo, group of 5 embryos, and pool of 20 embryos. Pictures are taken at 3.5 dpc.

Embryo pre-implantation development

Figure 3 presents pictures of mouse embryos cultured in 30-nL microfluidic chambers at 3.5 dpc. As seen on these picture, for every group size, the embryo development is advanced at that time and the largest amount of embryos has already reached the blastocyst stage, which is detected as a formation of a cavity (blastocoel).

Specifically, when using microfluidic conditions, more than 70% of the embryos are blastocysts at 3.5 dpc when using the largest groups (20 embryos), while in droplets, no blastocyst is observed for single culture, and groups of 5 and 20 embryos only show 7 and 20% blastocyst rates at 4.5 dpc (figure 4, *left*). These results demonstrate that embryos develop much faster in a confined environment.

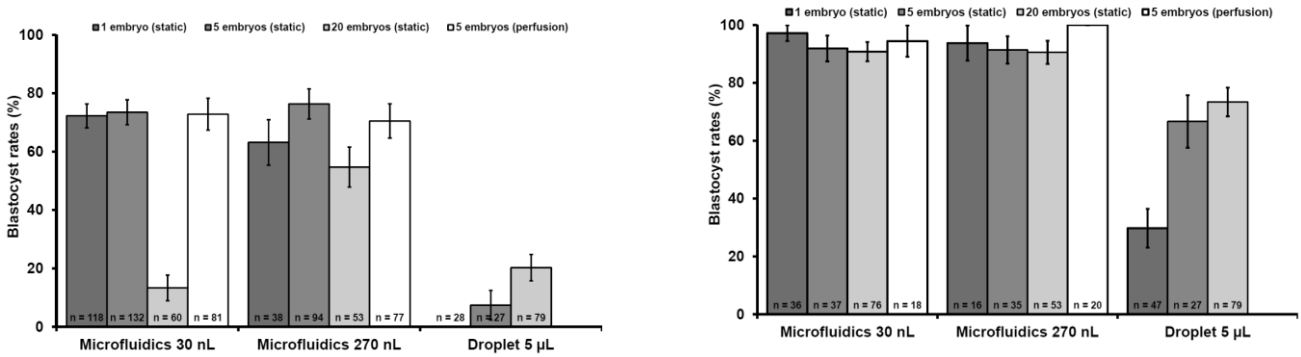

Figure 4: Blastocyst rates obtained for mouse embryos cultured in 30 and 270-nL microfluidic vessels and 5-µL droplets, as single embryos or groups of 5 or 20 embryos, at 3.5 (left) and 4.5 dpc (right), without and with perfusion.

In figure 4 (*right*), the pre-implantation rates are presented at 4.5 dpc. All microfluidic conditions yield >90% blastocyst rates, and this is independent of the group size and the volume of the microchamber. In contrary, droplet-based culture gives much lower development rate, ranging from 30 to 73% depending on the group size. These results notably suggest that single embryo development is impaired in a large volume of medium.

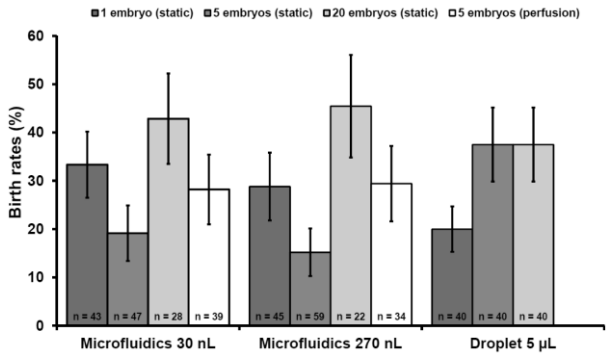

Figure 5: Full-term development rates obtained for mouse embryos cultured under the same conditions as in figure 4.

Embryo full-term development

The results on embryo full-term development are presented in figure 5 for all microfluidic conditions tested, as well as for various group sizes in droplets. The overall birth rate is comparable for all conditions, and no significant difference is found between microfluidics and a conventional approach. Still, the highest birth rates are observed for groups of 20 embryos cultured in a microvessel (43-45%) and the lowest for 5 embryos cultured in 270-nL chambers without any medium change (15%). More interestingly, the use of a confined environment (30 nL) benefits to a single embryo culture approach (33% *vs*. 20% in a droplet).

CONCLUSION

We demonstrate viable development of mouse embryos in microfluidic vessels (30-270 nL), with faster pre-implantation development than under standard conditions (5 µL droplets). The overall full-term development of mouse embryos in microfluidic devices is comparable to that observed for droplets of different sizes. However, a microfluidic culture environment yields higher birth rates compared to the conventional approach. In the next step, the potential of microfluidics will be investigated in the development of integrated platforms for *in situ* embryo characterization, in the frame of an ART procedure or for screening culture parameters in the purpose of improving the overall culture approach and protocol.

ACKNOWLEDGEMENTS

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