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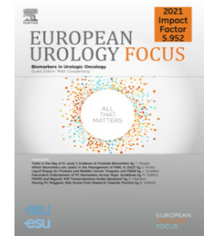

Grey Zone

Spermatogonial Stem Cells and In Vitro Spermatogenesis: How Far Are We from a Human Testis on a Chip?

Christian K. Ramsoomair [a,†], *Charles G. Alver* [a,b,†], *Ryan Flannigan* [c], *Ranjith Ramasamy* [a], *Ashutosh Agarwal* [a,b,*]

[a] *Desai Sethi Urology Institute, University of Miami Miller School of Medicine, Miami, FL, USA;* [b] *Department of Biomedical Engineering, University of Miami, Coral Gables, FL, USA;* [c] *Department of Urologic Sciences, The University of British Columbia, Vancouver, BC, Canada*

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1. Introduction

Spermatogenesis is the complex process whereby spermatogonial stem cells divide and differentiate into sperm. Spermatogenesis is regulated by somatic cells in the testes—including Sertoli, Leydig, and macrophage cells—that produce essential autocrine, paracrine, and endocrine factors. Accurate in vitro reproductions of the testis structure and function would improve developmental biology studies and facilitate research on the testis microenvironment and the molecular mechanisms affecting male fertility. Moreover, adaption of in vitro testis models to produce in vitro spermatogenesis (IVS) platforms promises reproductive hope for children who were sterilized by aggressive gonadotoxic cancer therapies. Current sperm preservation and extraction methods such as cryopreservation and testicular sperm extraction (TESE)/microTESE require functional sperm and are limited to the number of sperm collected, excluding prepubescent patients. IVS would allow continued maturation of immature spermatogenic tissue and eventual sperm production. Despite this promise, current culture systems fail to recapitulate the complexity of the human testis, so further development is necessary. Here we discuss the current state of IVS and human testis-on-a-chip (ToC) models, and suggest improvements to make human ToCs more robust for research and therapeutics.

2. State-of-the-art testis models

There are two general IVS approaches: organotypic cultures and "bottom-up" organoids. Organotypic cultures consist of testicular tissue cultured in vitro for induction and maintenance of spermatogenesis [1]. "Bottom-up" organoid cultures are formed using dissociated testicular cells and microfabricated structures. This method is clinically relevant for patients with damaged somatic testicular microenvironments. Despite this promise, limited practical knowledge of tissue maturation and the surrounding testis microenvironment hampers de novo organoid development. While these methods do not reflect the entire pathway for sperm maturation (eg, the epididymis is excluded), the aim is to produce sperm like those used for fertilization after TESE.

3. Challenges for human IVS

Despite experimental achievement of in vitro mammalian spermatogenic meiosis, current efforts have resulted in no live births in any species other than mice [2]. The most advanced human IVS models have produced undifferentiated postmeiotic cells. Studies have found that culture conditions are exceedingly complex and specific to species and maturity. Furthermore, static cultures do not recapitulate

* Corresponding author. Desai Sethi Urology Institute, University of Miami School of Medicine, Miami, FL, USA.
E-mail address: a.agarwal2@miami.edu (A. Agarwal).
† These authors contributed equally to this work.

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the hormonal and nutrient delivery paradigms required. There are major differences between human and mouse testes in germ cell composition (22% vs 0.3%) and the number of cell divisions (5 vs 12 divisions) [3]. With fewer divisions, a human ToC should prioritize the physiologic microenvironment and hormonal conditioning for successful production of sperm.

4. ToC models

Organ-on-a-chip (OoC) microfluidic devices provide a more dynamic physiomimetic human testis environment [4]. The general design, fabrication, and function of OoCs are shown in Figure 1. OoCs efficiently introduce hormones and nutrients while flushing cellular waste, limiting undesired paracrine feedback loops and allowing more physiologic, cyclic introduction of hormones than in static cultures. In addition, OoCs are easily integrated with imaging and assay equipment for continuous monitoring of spermatogenesis.

The Ogawa laboratory developed the gold-standard murine ToC, which sustained spermatogenesis over 6 months and produced sperm that resulted in healthy embryos after fertilization [5]. This ToC demonstrated significantly more efficient spermatogenesis in comparison to traditional cultures. Two major factors for successful IVS were identified: (1) continuous nutrient delivery and waste removal via limited direct flow; and (2) the use of intact tubules to yield more physiologically accurate cultures. Despite murine success, no study has reported a successful human organotypic ToC, and static IVS cultures have been limited and are not reproducible. These methods have failed in part because

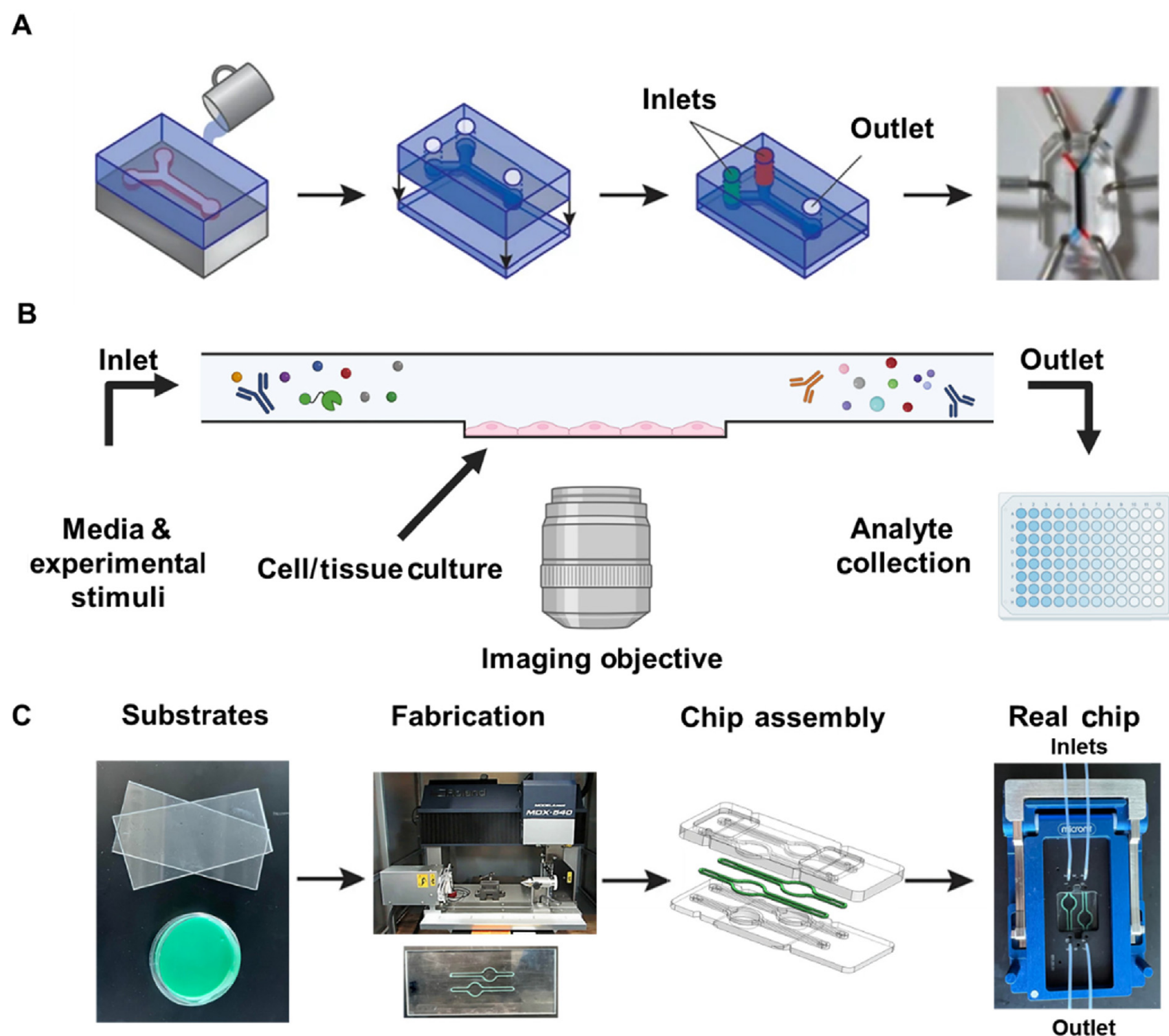

Fig. 1 – Fabrication and structure of an organ on a chip. (A) PDMS is poured over a negative mold of the desired microfluidic channels. The final channels are formed by sealing the molded PDMS to a glass cover slip or another PDMS chip. Inlet and outlet ports are placed into the PDMS chip for fluidic flow. Adapted from [9]. (B) Fluid entering the inlet passes over a culture of cells or tissues, providing fresh media and applying a physiological shear. Fluid continues towards the outlet, removing cellular waste, byproducts, and any excess substances introduced. Imaging platforms can be used to visualize cells inside the chip. Methods such as enzyme-linked immunosorbent assay can be used to analyze the chip effluent. (C) PMMA substrates are milled from computer-assisted design models using a CNC mill. Gaskets are made from flexible silicone molded in a CNC-fabricated mold. The final chip is assembled by sandwiching a top and a bottom PMMA chip around silicone gaskets to form resealable microfluidic channels. Cells can be seeded directly onto the bottom chip during this stage. A clamp applies pressure to maintain the microfluidic seal and has adapters for the inlet and outlet tubing. CNC = computer numerical control; PDMS = poly(dimethylpolysiloxane); PMMA = poly(methyl methacrylate).

of both a knowledge gap regarding the growth conditions for human organotypic testes and the longer timeline for human versus murine spermatogenesis [2].

Although organoid ToC models have been developed, complete spermatogenesis has not been reported for any of them. AbuMadighem et al [6] created a murine organoid ToC that replicates up to meiosis II and results in haploid spermatids with greater cell production than static cultures. Although this chip design offers superior control of specific cell-cell interactions and the surrounding cellular microenvironment, for human cells it often results in simplified models that focus on specific aspects of organs [4]. For successful IVS, maintenance of the seminiferous tubule and complex testis architecture are vital [1,5,7].

5. Ethics

Any ToC work must consider ethical standards: appropriate consent from donors and proper management of germ cells are mandatory. Patients must understand the full scope of the experimentation to consent to collection of human samples for ToCs. Patients—whether adults or prepubescent individuals and their guardians—must be informed of the research process and that the goal of the experiment is to produce viable sperm. If research might result in fertilization of an ovum, which is a common test of viability, researchers must inform the donor about any use, maintenance, or destruction of the resultant embryos. Moreover, researchers must caution patients that results are not guaranteed.

6. Chip material

Current ToC models are made of poly(dimethylsiloxane) (PDMS); while they efficiently deliver oxygen into OoCs, they also face several disadvantages. The permeability of the silicone polymer means that it is prone to absorption of small, hydrophobic molecules that may be re-released into the culture medium, especially after a controlled drug or hormone delivery regimen. In addition, PDMS-based chips are typically permanently sealed, so the researcher must flow cells into the device, limiting tissue removal after experimentation and thus chip reusability [4]. Because hormonal regulation and potential drug testing applications are important for spermatogenic devices, we propose the use of thermoplastics such as poly(methylmethacrylate) (PMMA) for ToC construction (Fig. 1C). PMMA-based chips offer low surface absorption, reusability, and greater cost-effectiveness [8]. Unlike PDMS, PMMA-based devices can be easily opened and resealed, allowing implantation and removal of larger tissue samples with minimal damage. Sample recovery is critical if nonfluidic collection of mature sperm, spermatogenic stem cells, or whole tissues is necessary. Therefore, thermoplastic-based microfluidic organotypic cultures may prove superior to current ToCs.

7. Conclusions

This discussion highlights the importance of maintaining the seminiferous tubules and precise user control over media flow in cultures and hormone introduction rates. While more studies are necessary to produce viable human sperm, our laboratory is working towards an organotypic, PMMA-based ToC to provide the most physiologically accurate microenvironment for IVS. On the basis of the current state of ToCs and the development timeline, we hope to see a functioning ToC within the next 20 years [1,5–7].

Conflicts of interest: The authors have nothing to disclose.

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