Bioengineering an Artificial Ovary with 3D Printing

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Statement of Purpose: Women suffer harsh consequences of gonadal toxicity and dysfunction that arise from chemotherapy and radiation therapies and developmental disorders. Treatment options are limited for resulting infertility, hormone insufficiency, and inability to go through puberty. The female ovary consists of follicles, cell spheroids possessing the oocyte, and somatic cells responsible for hormone production and cycling. In humans, autotransplant of cryopreserved ovarian tissue has given patients short-term hormone cycling (<19 months) and live birth[1,2]. Autotransplants, which are only available for patients suffering gonadotoxicity from cancer therapies, pose a risk of cancer and possess a short life span[3]. Great advances in follicle culture and transplant, including live birth in mice, have been made with biomaterial strategies, most commonly hydrogel bead encapsulation[4].These strategies, however, do not permit advanced design to optimize transplant function of a sustaining follicle pool. Therefore, we are investigating 3D printing towards developing an artificial ovary for long-term fertility and hormone health options. 3D printing is a flexible design tool that provides on-demand scaffold design, material and cell placement within 3D constructs. In these studies, we are optimizing scaffold design for follicle culture that will pave the way for transplant studies.

Methods and Materials: Printing and material parameters were optimized to print well-defined gelatin scaffolds resulting in either a grid-like pattern (Fig 1A) or a tortuous pore network (Fig 1B) using a 3D-Bioplotter (Envisiontec). Scaffolds were EDC/NHS cross-linked and architecture was assessed by light microscopy. Murine ovarian somatic cells were isolated by plating ovarian enzymatic digestion to select for adherent cells. Optionally, somatic cells were seeded onto scaffolds one day prior to follicle seeding. Murine follicles were mechanically and enzymatically isolated from excised ovaries. Secondary follicles (150-200 µm diameter) were selected and seeded into gelatin scaffolds by mouth pipetting and cultured up to 8 days. Cell viability and arrangement were analyzed by confocal fluorescence microscopy using Live/Dead and CellTracker stains. Oocyte diameter was monitored by light microscopy and estradiol content of media was assessed by ELISA.

Results: The resulting 3D printed gelatin scaffolds provided adhesion of somatic cells on the struts as well as sufficient space for follicles to infiltrate and reside within the scaffold pores (Fig 1C). Somatic cells also started to surround follicles over time (Fig 1C inset). Although follicles are difficult to culture longer term, these follicles remained viable within the 3D printed gelatin scaffolds for 8 days. Furthermore, follicles (oocytes) displayed growth in the scaffolds, and somatic cells demonstrated normal function, continually producing estradiol over the culture period (Fig 1D, H). When pore geometries were compared, follicles made more intimate contacts with the scaffold in the tortuous pattern over the grid pattern (Fig 1E). Follicles with only 1 scaffold contact resulted in a 50% survival rate, but with 2 or more resulted in 82.8% survival. This was evident when follicles maintained spheroid-shape in scaffold pore corners, but dissociated when scaffold contact was limited (Fig 1E-G).

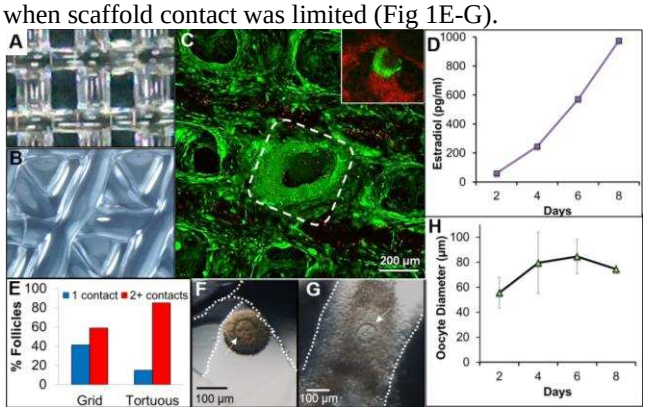

Figure 1: Gelatin scaffolds with (A) grid-like or (B) tortuous pattern (C) Fluorescence image of somatic cells coating scaffold struts, follicle seeded in pore (circled), Live/dead stain; inset: somatic cells (red) surrounding follicle (green) (D) Estradiol is produced over 8 days (E) Percentage of follicles making 1 or 2+ contacts with scaffold in both pore structures Light images of follicles in scaffolds after 6 days (struts dashed), a follicle is observed (F) intact in tortuous but (G) dissociated in grid pattern (H) Average oocyte diameter grows over 8 days

Conclusions: This work is the first to demonstrate well-defined 3D printed scaffolds as an artificial environment for supporting follicle health and growth. We leveraged the advantages of 3D printing to begin to understand the important effects of scaffold geometry on the behavior of follicles in vitro. Future studies will investigate mechanisms related to this geometry-dependent observation. Long-term goals include further optimizing scaffold architecture, biomaterial, and cell organization towards developing a functional and clinically translatable artificial ovary. Identifying important 3D printed scaffold design parameters and impact on cell behavior is not only needed in developing a fully functioning artificial ovary, but can also provide significant insight for 3D printing other tissues and organs.

References: (1) Jeruss J. N Engl J Med. 2009:360(9):902-11. (2) Ernst E. European Journal of Cancer. 2013;49 (4):911–914. (3) Bastings L. Hum Reprod Update. 2013;19(5):483–506. (4) Xu M. Tissue Engineering. 2006;12(10):2739–2746.