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Initiation of puberty in mice following decellularized ovary transplant

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ABSTRACT

Clinical interventions to preserve fertility and restore hormone levels in female patients with therapy-induced ovarian failure are insufficient, particularly for pediatric cancer patients. Laparoscopic isolation of cortical ovarian tissue followed by cryopreservation with subsequent autotransplantation has temporarily restored fertility in at least 27 women who survived cancer, and aided in pubertal transition for one pediatric patient. However, reintroducing cancer cells through ovarian transplantation has been a major concern. Decellularization is a process of removing cellular material, while maintaining the organ skeleton of extracellular matrices (ECM). The ECM that remains could be stripped of cancer cells and reseeded with healthy ovarian cells. We tested whether a decellularized ovarian scaffold could be created, recellularized and transplanted to initiate puberty in mice. Bovine and human ovaries were decellularized, and the ovarian skeleton microstructures were characterized. Primary ovarian cells seeded onto decellularized scaffolds produced estradiol in vitro. Moreover, the recellularized grafts initiated puberty in mice that had been ovariectomized, providing data that could be used to drive future human transplants and have broader implications on the bioengineering of other organs with endocrine function.

1. Introduction

Eighty percent of children with cancer in the United States will survive for 5 or more years [1], and by 2015, 1 in 250 adults will have survived childhood cancer [2,3]. Although survival rates are increasing due to a variety of improving diagnostics and therapeutics, childhood cancer survivors have a variety of chronic health conditions. For example, adult survivors of childhood cancer are significantly more likely to be infertile or have difficulty becoming pregnant than their siblings [4]. This is due in part to the gonadotoxic effects of many chemotherapeutic and radiation therapies used to treat cancer [5–7]. In addition to the iatrogenic effects of radiation and chemotherapy, girls and young women can lose ovarian function due to a genetic predisposition to premature ovarian failure (e.g. fragile X syndrome and Turner's syndrome) or early onset familial cancer (BRCA1/2) [8]; or to treatments for rheumatologic disease [9], chronic kidney disease [10,11] and HIV [12,13]. The ovary is the endocrine organ of the female reproductive system producing steroid and peptide hormones necessary for the onset and progression through puberty and entrance into the normal, recurrent menstrual cycle. Ovarian hormones also significantly influence bone, skin, breast, vessels and additional endocrine tissues [14–18]. Ovarian hormones are produced by the somatic cells, granulosa and theca, that surround the female gamete, which combined make the ovarian follicle unit. Chemo- and radiation treatment destroy the follicles with their enclosed oocyte, reducing fertility and causing premature menopause [19]. The ability to develop a tissue-engineered ovarian system to sense, replace and titrate sex hormone production would more fully restore systemic functions of the

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ovarian endocrine system than conventional exogenous hormone replacement therapy.

There are limited hormone and fertility restoration options for patients with hormone responsive cancers and pre-pubertal patients. A method that is still considered experimental involves removal of an ovary or ovarian biopsy prior to cancer treatment, and storage of that tissue for later use. Cryobanked ovarian cortical tissue transplants have resulted in 27 reported live births, including a live birth obtained from egg retrieval following xenotransplant to a peritoneal pocket after bilateral oophorectomy [20,21]. Additionally, a pre-pubertal girl, sterilized by her cancer treatment, underwent transplant surgery to initiate puberty with ovarian tissue preserved prior to treatment [22]. However, there is a limited window during which these transplants allow recipients to maintain normal hormonal regulation with regular menstrual cycles and produce offspring [23]. There is also a risk of reintroducing cancer cells into the patient from the transplanted tissue, especially when the primary disease is disseminated, is a blood cancer [23–25], or is a soft tissue malignancy such as Ewing's Sarcoma [26]. Because of the increase in young female cancer survivors and inherent risk associated with direct transplantation of cryobanked tissues, there is an urgent need for safe and more sustainable alternatives.

One such alternative would be to implement an engineered ovary that provides hormones and an appropriate follicle niche while eliminating the risk of reintroducing cancerous cells. In this study, we focus on recapitulating the endocrine function of the ovary in a tissue-engineering mimic. We describe steps taken toward this goal by establishing an effective decellularization technique for human ovaries, and utilizing model organisms to provide demonstrable support for a transplantable endocrine organ. Within the fields of tissue engineering and regenerative medicine, decellularized organs and tissues have shown significant promise for restoring structure and function in such systems as lung, liver, kidney and heart [27–29]. Building upon previous successes of regenerating transplants from decellularized organs and tissues, we created human and bovine decellularized ovarian scaffolds as a proof-of-concept to show that ovarian cells retain viability and endocrine function in a natural three-dimensional scaffold, to induce a pubertal transition in ovariectomized mice after transplant.

2. Materials and methods

2.1. Human ovarian tissue acquisition and transport

Human ovarian tissue was obtained from participants following informed consent under Institutional Review Board (IRB)-approved protocols. These participants were undergoing ovarian tissue removal and cryopreservation for fertility preservation at National Physicians Cooperative (NPC) sites that are part of the Oncofertility Consortium (oncofertility.northwestern.edu). As part of this investigational protocol, 80% of participant ovarian tissue was cryopreserved for their future clinical use and up to 20% of the tissue was designated for research that would support future use of the cryopreserved portion. Most tissue can be carefully divided and utilized for several experiments. The representative native images (Figs. 1B, 2A–H) are from archival blocks for the same participant and are established from every sample received under this protocol. All participants were enrolled in 2013 and ranged in age from 6 to 34 years old. All participants had a cancer diagnosis and 4 out of 5 had a previous history of therapy (radiation, chemotherapy, immunosuppression) prior to ovarian tissue removal (Fig. 1A). The research tissue was transported to the laboratory in SAGE OTC Holding Media (Copper Surgical, Trumball, CT) at 4 °C for 14–24 h. A portion of the tissue was fixed in Modified Davidson's Fixative (Electron Microscopy Sciences). The remaining tissue was allowed to equilibrate to room temperature upon arrival and then processed for decellularization as described below.

2.2. Bovine ovarian tissue acquisition and decellularization

Bovine ovaries were collected from Aurora Packing Company (Aurora, IL) from young cows and transported to the lab in BoviPro Oocyte Holding Medium (1182/1210). Upon arrival the ovaries are rinsed in fresh medium and the excess fat is

a

Tissue ID	Age (YOB)	Diagnosis	Previous Cancer Treatment
A	6 (2007)	B-Cell precursor ALL	includes multi-agent chemotherapy and cranial radiation
B	14 (1998)	very high risk ALL (hypodiploid)	multi-agent induction chemotherapy
C	17 (1996)	very high risk B-cell precursor ALL	yes, details not available
D	31 (1981)	Pancreatic cancer	none
E	34 (1979)	ALL	yes, details not available

ALL - acute lymphoblastic leukemia

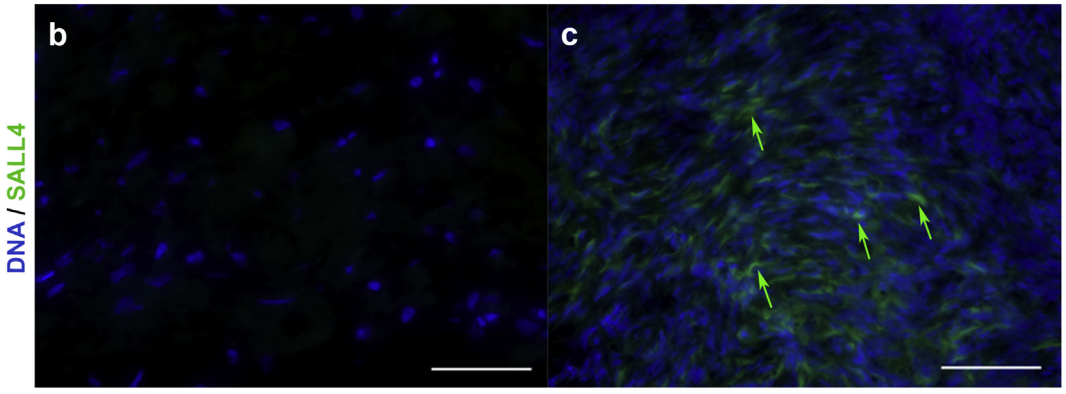

Fig. 1. Human ovarian tissue information and SALL4 staining. Table of age, diagnosis and treatment information for participants in this study (a). ALL: acute lymphoblastic leukemia. Representative images of native ovarian cortical tissue stained with an oncogene expressed in ALL patients. Participant D was diagnosed with pancreatic cancer and the participant's ovarian cortex contained no SALL4-positive cells (b, DNA, blue), while participant E was diagnosed with ALL and the ovarian tissue did contain SALL4-positive cells (c, SALL4, green, arrows). Scale bar: 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)