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Vitrification of kidney precursors as a new source for organ transplantation ☆

Francisco Marco-Jiménez [a,*], Ximo Garcia-Dominguez [a], Estrella Jimenez-Trigos [a], Cesar D. Vera-Donoso [b], Jose S. Vicente [a]

[a] Instituto de Ciencia y Tecnología Animal, Universitat Politècnica de València, 46022 Valencia, Spain
[b] Servicio de Urología, Hospital Universitari i Politècnic La Fe, 46026 Valencia, Spain

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ABSTRACT

Kidney transplantation from deceased or living human donors has been limited by donor availability as opposed to the increasing demand, and by the risk of allograft loss rejection and immunosuppressive therapy toxicity. In recent years, xenotransplantation of developed kidney precursor cells has offered a novel solution for the unlimited supply of human donor organs. Specifically, transplantation of kidney precursors in adult hosts showed that intact embryonic kidneys underwent maturation, exhibiting functional properties, and averted humoural rejection post-transplantation from non-immunosuppressed hosts. Even if supply and demand could be balanced using xenotransplants or lab-grown organs from regenerative medicine, the future of these treatments would still be compromised by the ability to physically distribute the organs to patients in need and to produce these products in a way that allows adequate inventory control and quality assurance. Kidney precursors originating from fifteen-day old rabbit embryos were vitrified using Cryotop® as a device and VM3 as vitrification solution. After 3 months of storage in liquid nitrogen, 18 kidney precursors were transplanted into non-immunosuppressed adult hosts by laparoscopy surgery. Twenty-one days after allotransplantation, 9 new kidneys were recovered. All the new kidneys recovered exhibited significant growth and mature glomeruli. Having achieved these encouraging results, we report, for the first time, that it is possible to create a long-term biobank of kidney precursors as an unlimited source of organs for transplantation, facilitating the inventory control and distribution of organs.

Introduction

Kidney transplantation from deceased or living human donors has been limited by donor availability as opposed to the increasing demand, and by the risks of allograft loss rejection and immunosuppressive therapy toxicity [4]. In recent years, xenotransplantation of developed kidney precursor cells has provided a novel solution for the unlimited supply of human donor organs [6,10]. Specifically, transplantation of kidney precursors in adult hosts showed that intact embryonic kidneys underwent maturation, exhibiting functional properties, and averted post-transplant cellular rejection from non-immunosuppressed hosts [10]. Even if supply and demand could be balanced using xenotransplants or lab-grown organs from regenerative medicine, the future of these treatments would still be compromised by the ability to physically distribute the organs to patients in need and produce these products in a way that allows adequate inventory control and quality assurance [2,8]. To this end, organ cryopreservation will be indispensable.

The long-term banking of human organs or their engineered substitutes [8] for subsequent transplantation is a long-sought [13,23,25] and important goal [1,8,7,11,12,14,24,25,29]. Storage below the critical temperature of −130 °C allows the preservation of cells and tissues after a long storage in liquid nitrogen [16,22]. To date, small ovaries, blood vessels, heart valves, corneas and similar structures are the only macroscopic structures having the capacity to recover, at least in part, after vitrification [9]. Kidneys and hearts have been the most widely studied organs, but neither has been reproducibly recovered after cooling to temperatures lower than about −45 °C, evidently due at least in part to

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* Corresponding author at: Reproductive Biotechnology Laboratory, Institute of Science and Animal Technology (ICTA) at the Polytechnic University of Valencia, C/ Camino de Vera s/n, 46022 Valencia, Spain. Fax: +34 96 3877439.
E-mail address: fmarco@dca.upv.es (F. Marco-Jiménez).

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mechanical damage from ice itself, although in the case of kidneys at least, sporadic survival has sometimes been claimed after freezing to about −40° to −80 °C [9,7]. Fahy et al. Fahy et al. [9] reported a case history of one rabbit kidney that survived vitrification and supported the life of a recipient animal for an indefinite period of time. To our best knowledge, only Bottomley et al. Bottomley et al. [2] evaluated the cryopreservation of metanephroi immediately after thawing, but only under *in vitro* conditions.

In an effort to advance in organ cryopreservation, this study was conducted to evaluate the developed morphologically normal glomeruli of vitrified kidney precursors after their allotransplant in non immunosuppressive rabbits.

Materials and methods

Chemicals

All chemicals and reagents were purchased from the Sigma–Aldrich Corporation (St. Louis, MO, USA) unless otherwise stated.

Animals and ethical clearances

All animals were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (BOE, 2013; BOE = Official Spanish State Gazette). Ethical approval for this study was obtained from the Universidad Politécnica de Valencia Ethics Committee. New Zealand white females, 5 months old, were used as embryo donors and metanephroi recipients. The animals used came from the experimental farm of the Universidad Politécnica de Valencia. The rabbits were kept in conventional housing (with light alternating cycle of 16 light hours and eight dark hours, and under controlled environmental conditions: average daily minimum and maximum temperature of 17.5 and 25.5 °C, respectively). All rabbits had free access to fresh food and water.

Metanephroi recovery

Donor does were artificially inseminated with 0.5 ml of fresh heterospermic pool semen from fertile males at a rate of 40×10^6 - spermatozoa/mL in Tris-citric-glucose extender [28]. Immediately after insemination, ovulation was induced by an intramuscular injection of 1 μg buserelin acetate and the females were euthanised at day 15 post-insemination. Recovered 15 day old embryos (E15) were placed in Dulbecco's phosphate-buffered saline (DPBS) supplemented with 0.2% of bovine serum albumin (BSA) at 38.5 °C. Metanephroi were surgically dissected under a dissecting microscope using previously described techniques [21]. Some of the recovered embryos were placed in Bouin's solution to fix. They were then dehydrated through ethanol series, cleaned with xylol and embedded in paraffin. Next, 5–7 μm sections were cut for hematoxylin–eosin staining and the slides were studied by light microscopy in order to identify the position and the size of the metanephroi (Fig. 1).

Vitrification procedure

Vitrification was performed within 1 h after recovery following the minimum essential volume (MEV) method, using Cryotop® as device [17] (Kitazato-dibimed, Valencia, Spain) and VM3 as vitrification solution [7] (21st Century Medicine, Fontana, CA, EEUU). Cryotop® is the special container, consisting of a fine thin film strip attached to a hard handle. This allows us to minimise the volume of vitrification easily. All manipulations were performed at room temperature (25 ± 1 °C) and all the media were used at room temperature, except for the first warming solution, which was used at 37.5 °C.

Metanephroi were first submerged into 2.5 ml of equilibration solution that containing 1.7% w/v ethylene glycol (EG), 1.3% w/v formamide, 2.2% w/v dimethyl sulphoxide (DMSO), 0.7% w/v PVP K12 (polyvinylpyrrolidone of Mr 5000 Da) and 0.1% w/v final concentrations of commercially available SuperCool X-1000 and SuperCool Z-1000 (ice blockers) in base medium (BM: DPBS + 20% foetal bovine serum, FBS) for 3 min. Then, the metanephroi were submerged into 2.5 ml of solution containing 4.7% w/v EG, 3.6% w/v formamide, 6.2% w/v DMSO, 1.9% w/v PVP K12 and 0.3% w/v final concentrations of ice blockers in BM for 1 min. Finally, the metanephroi were submerged into 2.5 ml of vitrification solution consisting of 16.84% w/v EG, 12.86% w/v formamide, 22.3% w/v DMSO, 7% w/v PVP K12 and 1% w/v final concentrations of ice blockers in BM before being loaded into Cryotop® devices (Fig. 1) and directly plunged into liquid nitrogen (LN2) within 1 min.

For warming, metanephroi were submerged into 2.5 ml of a solution containing 1.25 M sucrose in BM for 1 min and later transferred stepwise into decreasing sucrose solutions (0.6, 0.3 and 0.15 M sucrose in BM) for 30 s before and then washed twice in BM for 5 min.

Metanephroi transplantation

After 3 months of storage in liquid nitrogen, the metanephroi were transplanted into recipients. Metanephroi were transplanted within 45 min after warming or collected (fresh). Recipients were sedated by intramuscular injection of 5 mg/kg of xylazine (Rompun, Bayer AG, Leverkusen, Germany) and anaesthetised by intravenous injection of 15 mg/kg ketamine hydrochloride (Imalgene®, Merial, S.A., Lyon, France) into the marginal ear vein. During laparoscopy, 3 mg/kg of morphine hydrochloride (Morfina, B. Braun, Barcelona, Spain) was administered intramuscularly. Abdominal laparoscopy was performed with two ports (one for the camera and one for dissecting forceps, Image). Metanephroi were aspirated in an epidural catheter (Vygon corporate, Paterna, Valencia, Spain), introduced into the inguinal region with an epidural needle and then transplanted into a pouch created by epidural needle in the retroperitoneal fat, adjacent to the renal vessels. Four metanephroi were transplanted into each host. After surgery, analgesia was administered for 3 days (0.03 mg/kg of buprenorphine hydrochloride, Buprex®, Esteve, Barcelona, Spain, each 12 h, and 0.2 mg/kg of meloxicam, Metacam®, 5 mg/mL; Norvet; Barcelona, Spain, every 24 h). In addition, all the recipients were treated with antibiotics (4 mg/kg of gentamicin [10% Ganadexil, Invesa, Barcelona, Spain) every 24 h for 3 days]. No immunosuppression was given to recipients. Metanephroi transplantation was assessed in three sessions.

Histomorphometry of the renal corpuscle and growth of transplanted kidney precursors

Twenty-one days after transplantation, hosts having received an allograft were euthanised and the new kidneys were removed (Fig. 2). The new kidneys were individually weighed, fixed in Bouin's solution and embedded in paraffin wax and stained, as previously described. The stained sections were examined with light microscopy for histological and histomorphometric analysis (Fig. 3). In the histomorphometric measurements, 20 renal corpuscle and glomeruli on each sample were measured (area and perimeter) in each of the groups – control and experimental. Photomicrographs were taken at total magnification of ×1000. In addition, the glomerular tuft cellularity was estimated by counting the total number of nuclei of each glomerulus. Photomicrographs were measured using ImageJ analysis software (public domain http://rsb.info.nih.gov/ij/). Kidneys originating from a 5-week-old rabbit (coeval with the metanephroi age) were used as controls.