Microvascular Transplantation of Epiphyseal Plate Allografts Following ex vivo Storage in University of Wisconsin Preservation Solution

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INTRODUCTION
An effective strategy to address issues of epiphyseal growth plate dysfunction would be the microvascular transplantation of a donor epiphyseal plate allograft. If clinical utilization of cadaveric allografts is to be considered, then a reliable procedure for temporary storage that preserves cellular viability and function must be demonstrated. The goal of this study is to determine a suitable time frame that donor epiphyseal plate allografts can be stored ex vivo University of Wisconsin Storage Solution (UWSS) such that functional biological activity will be maintained in vivo. Our data indicate that storage of rabbit proximal tibial epiphyseal growth plate allografts for at least 3 days in UWSS preserves chondrocyte viability, proliferation and biosynthetic activity to permit new bone growth in vivo following short-term microvascular transplantation.

METHODS
Animals: Pathogen-free non-sibling female New Zealand White (NZW) rabbits at 10-12 weeks of age (Myrtle’s Rabbity Incorporated, TN) were used as donors and recipients of epiphyseal plate allografts in these studies. Epiphyseal Plate Harvest and Storage: Proximal tibial epiphyseal plates with intact vascular pedicles were harvested from anaesthetized donor rabbits as described previously (1). Donor tissue was perfused with and stored in cold UWSS (4°C) over set periods of time (Day 0, 4, 7, 14; n=3 per time point) to study the effects of storage on chondrocyte viability, proliferation and biosynthetic activity. Based on preliminary cell viability data, growth plate allografts were perfused with, and stored in, cold UWSS for 3 days at 4°C before microvascular anastomoses were performed. Control (Day 0 storage) allografts were stored up to 30min in saline solution until microvascular transplantation was done. Microvascular Transplantation Procedure: For short-term studies, growth plate allografts (with or without prior storage in UWSS; n=3 per time point) were transplanted to a subcutaneous region, proximal to the iliac crest, in age-matched recipient rabbits. Microvascular anastomoses were performed using the deep circumflex iliac vessels as donors. Post-operatively, antimicrobial chemoprophylaxis (cephazolin; 15mg/kg subcutaneous injection) was administered twice a day for 3 days, then once a day until sacrifice. Low dose immunosuppressant (cyclosporine A; 10mg/kg) was administered subcutaneously every day until sacrifice. Intravenous injection of calcine (10mg/kg) and BrdU (40mg/kg) was carried out 2 days and 1 day before sacrifice, respectively. A second dose of calcine was administered 1h before sacrifice. Analysis of epiphyseal plates: Tissue was harvested, bisected in the sagittal plane and sections (150-200μm) were incubated in a calcine AM/ethidium homodimer solution (Live/Dead Viability assay kit; Invitrogen) for 45min. Cell viability was then monitored by confocal microscopy. Remaining tissue was processed and embedded in paraffin or methyl methacrylate (MMA) to permit micro-thin sectioning (10μm). Immunohistochemistry was done on paraffin sections to detect BrdU incorporation or expression of the extracellular matrix protein, Type X collagen. MMA-embedded sections were analyzed by fluorescence microscopy to detect calcine incorporation into newly synthesized bone.

RESULTS
Ex vivo storage of epiphyseal plate allografts and effect on cell viability: Figure 1 shows that up to 4 days of storage in UWSS, the majority of chondrocytes in the growth plate are still viable as shown by uptake of calcine (green cells). By 7 days, live chondrocytes are still present, but an increase in cell death is evident by the presence of red-fluorescent cells due to incorporation of ethidium homodimer into DNA. By day 14, the majority of cells are not viable (results not shown).

Microvascular transplantation of epiphyseal plate allografts: Success of growth plate transplantation into the subcutaneous region of recipient rabbits was monitored by BrdU and calcine incorporation into viable proliferating chondrocytes and newly synthesized bone, respectively. We were able to demonstrate BrdU and calcine incorporation in allografts that were not stored prior to transplantation and harvested either 4 days or 7 days after surgery (results not shown). Figure 2 shows calcine incorporation into newly synthesized bone and metaphyseal bone in an allograft that had been stored for 3 days in UWSS prior to transplantation. BrdU-positive cells were also found in this growth plate allograft (results not shown). Compared to the native specimen (Fig. 2A), the growth plate area of the stored allograft appears wider which is likely due to thickening of the hypertrophic region as a result of deficient blood supply to the metaphyseal side during the 3 day storage period and following revascularization due to the absence of direct metaphyseal perfusion. This phenomenon has been reported previously (2). However, the cells in the thickened hypertrophic area of this pre-stored transplanted allograft are likely viable since we found intense Type X collagen staining in the extracellular matrix, similar to the native sample (results not shown).

DISCUSSION
We have presented promising data to support the use of cold storage in UWSS as a means to preserve epiphyseal plate allografts prior to microvascular transplantation. Specifically, a period of 3 days in UWSS was found to preserve cell viability and permit new bone growth. These experiments are currently being repeated and we also plan to analyze the effect of storing tissue for 7 days in UWSS prior to transplantation. Future work will involve establishing a transplantation model creating bone contact within the iliac crest region of NZW rabbits (3) which will enable longer-term follow up analyses of pre-stored allografts in vivo. Importantly, this model system will also allow us to monitor the effects of removing immunosuppression as well.

REFERENCES