Introduction: Efficacy of osteochondral (OC) allografts may be due in part to the presence of viable chondrocytes within graft cartilage.1,2 In OC allografts, chondrocytes, especially those at the articular surface, are susceptible to handling- and storage-associated death.3,4 Chondrocytes in the superficial zone normally secrete a lubricant molecule proteoglycan-4 (PRG4),5 which has a friction-lowering boundary lubricating ability.6,7 Death of chondrocytes in OC allografts may disrupt cartilage lubricant production. We hypothesized that loss of chondrocytes within OC grafts leads to decreased local PRG4 lubricant secretion, after graft storage and subsequent implant. To test this hypothesis, the effect of OC allograft treatment (FROZEN vs. FRESH) on secretion of functional PRG4 was analyzed (i) after storage, and (ii) after 6-months in vivo in adult goats.

Methods (Fig. 1): Studies were IACUC approved. Donor OC allografts were prepared from both knees of adult Boer goats (n=5, 2-4yo). Each knee was divided into medial (MFC) and lateral (LFC) sites with a trochlea fragment taken from each site. Contralateral knees were prepared from both knees of adult Boer goats (n=5, 2-4yo). Each knee was divided into medial (MFC) and lateral (LFC) sites with a trochlea fragment taken from each site.

Exp. 1: Stored OC Donors. The effect of storage on PRG4 release was determined for donor cartilage. Cumulative PRG4 release was determined during 15d incubation. To distinguish between release of existing and newly synthesized PRG4, cartilage was incubated ± 100 µg/mL cycloheximide (CX) to inhibit protein synthesis was blocked with CX, with most nascent PRG4 production. We hypothesized that loss of chondrocytes within OC grafts leads to decreased local PRG4 lubricant secretion, after graft storage and subsequent implant. To test this hypothesis, the effect of OC allograft treatment (FROZEN vs. FRESH) on secretion of functional PRG4 was analyzed (i) after storage, and (ii) after 6-months in vivo in adult goats.

Exp. 2: In Vivo Allograft Surgery. The effect of storage on subsequent in vivo PRG4-secreting chondrocyte function was determined for OC allografts transplanted into recipient goats and retrieved at 6 months. Adult Boer goats (n=7, 3yo) were operated (OP’d) in one knee, with one FROZEN and one FRESH site-matched OC allograft (d=8mm, h=5mm) implanted into alternating MFC and lateral trochlea (LT) sites of each knee. Contralateral knees were NON-OP’d controls. At 6 months, animals were euthanized and both knees analyzed. For MFC, LT, as well as LFC sites, portions of cartilage from OP’d and NON-OP’ed knees were analyzed for cellularity and PRG4 release, as well as functionality of the produced lubricant. For the latter, the cartilage-conditioned medium (CM) was concentrated (2x) and analyzed for friction-lowering function in a cartilage-on-cartilage friction test with PBS as a negative control, and synovial fluid (SF) as a positive control.1 SF was tested both diluted (3%SF) to match ~PRG4 concentration in 2x CM and full-strength (100%SF). Statistics. Effects of treatment and site were assessed by 2-way nested or repeated measures ANOVA & Tukey post-hoc. *p<0.05, **p<0.01, ***p<0.001.

Results: Exp. 1: PRG4 production by cartilage of donor allografts was reduced by freezing. Freezing reduced cumulative PRG4 production over 15d by ~85% (Fig. 2A). For FRESH cartilage, PRG4 secretion was sustained at high rates over 7d (Fig. 2B). The PRG4 released from FRESH cartilage was mostly newly synthesized, since that released from FROZEN was much lower. In addition, PRG4 release was similar when protein synthesis was blocked with CX, with most nascent PRG4 released during day 1. Exp. 2: After 6 months in vivo, the PRG4-secreting function of OC allografts was diminished by freezing. PRG4 release was markedly reduced by prior FROZEN storage (by ~4-8x vs. FRESH, p=0.06 and p=0.05 for MFC and LT, respectively, by ~4-13x vs. NON-OP, p=0.01 and p=0.05, Fig. 3). Concomitantly, cartilage cellularity was similarly higher in NON-OP and FRESH, and ~96% lower in FROZEN retrievals (Fig. 4). The lubricating function of 2x CM from FRESH and NON-OP retrievals was markedly better than PBS (p<0.001), and was similar to ~3%SF (Fig. 4B). In contrast, the lubricating function of 2x CM from FROZEN retrievals was markedly worse than ~3%SF (p=0.01), and not different from PBS (p=0.2).

Discussion: The results show that storage conditions of OC allografts (FROZEN) that decrease cell viability also reduce PRG4 secretion, both after storage and after implant and retrieval in goat knees. Thus, the PRG4-secreting function of grafts appears to be maintained in vivo based on its state after storage. They do not appear to be significantly repopulated by cells that subsequently produce PRG4. PRG4 secretion may not only be a useful marker of allograft performance, but also a mechanism by which such grafts protect their articular surface following cartilage repair. Both PRG4 secretion and chondrocyte viability may be useful predictors or markers of biological performance.

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