

# Biomechanical and Physical Properties of Osteochondral Tissues after Prolonged Storage with the Missouri Osteochondral Allograft Preservation System

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**INTRODUCTION:** Osteochondral allograft (OCA) transplantation is a surgical approach for treating cartilage lesions which involves replacing damaged cartilage with grafts composed of healthy bone and cartilage obtained from a cadaveric donor.<sup>1,2</sup> Successful OCA transplantation relies on sufficient chondrocyte viability at the time of transplantation and the ability of the transplanted tissues to bear weight.<sup>3</sup> Storage in Lactated Ringer's solution (LRS) maintains viability up to 14 days and is used by Mount Sinai Allograft Technologies (Toronto, Canada) and others.<sup>2,4</sup> Certain culture media-based storage solutions are capable of maintaining viability up to 28 days while the Missouri Osteochondral Allograft Preservation System (MOPS), a proprietary serum-free media, maintains viability for 56 days.<sup>5,6</sup> While the effects of various storage media on chondrocyte viability is well studied, there are less data available on biomechanical properties of bone and cartilage. The effect of MOPS on the biomechanical properties of cartilage have been reported in one study<sup>2</sup> and showed no significant change with respect to storage time, while effects on bone properties have not been examined. Therefore, this study aimed to quantify biomechanical and physical properties of cartilage and bone in ovine femoral condyles stored in MOPS or LRS for up to 56 days.

**METHODS:** All procedures were performed with Institutional Animal Care Committee approval in accordance with the Canadian Council on Animal Care. Femoral condyles from 15 female Arcott sheep (4-6 years old, 70 ± 15 kg) were preserved in either MOPS media or LRS supplemented with antibiotics bacitracin (1 g/L) and cefazolin (50,000 U/L) and assigned to storage times of 0, 14, 28 or 56 days for right lateral, right medial, left medial and left lateral condyles, respectively. At the end of the storage times, 6 mm diameter osteochondral cores were harvested from each femoral condyle. Each core underwent Micro-CT scanning at 14.2 µm (Skyscan 1174) to quantify subchondral bone mineral density (BMD), subchondral bone thickness, trabecular BMD and trabecular percent bone volume (BV/TV). The subchondral bone region of interest (ROI) covered the central 2 mm of the core and was manually drawn slice by slice using the coronal plane images to capture the full thickness of subchondral bone (Fig.1). The trabecular bone ROI was chosen by selecting a region of 2.83 mm offset from the end of the subchondral bone, which was defined as the first slice where pores were visible across the width of the sample (Fig.1). Cartilage thickness was measured on a Mach-1 Micromechanical Tester using a 26-gauge needle probe to penetrate cartilage until it contacted calcified cartilage. Thickness was then calculated as the distance between the inflection points on the load-displacement curve. Then, the needle probe was replaced with a 1 mm diameter spherical indenter and cartilage indented to 10% strain at a rate of 0.2 mm/s to quantify Young's modulus. Statistical comparisons were made using a two-way ANOVA with treatment and storage duration as independent variables followed by LSD post-hoc tests (Statistica v.14).

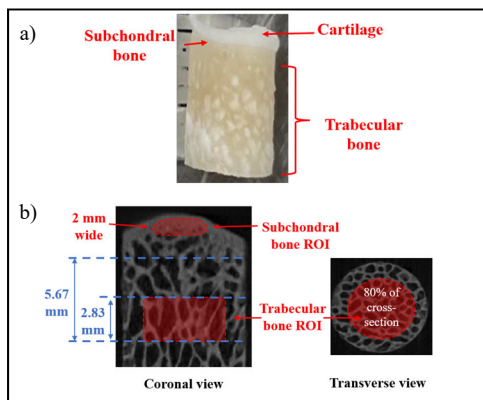
**RESULTS:** There were no significant differences between MOPS and LRS samples at any storage time for subchondral bone thickness, subchondral BMD, trabecular BMD or trabecular bone volume. After 28 days, LRS and MOPS stored samples had significantly lower ( $p=0.009$  and  $p=0.017$ ) subchondral bone mineral density ( $0.76 \pm 0.02 \text{ g/cm}^3$  and  $0.77 \pm 0.04 \text{ g/cm}^3$ ), respectively, than 14 day MOPS samples ( $0.87 \pm 0.06 \text{ g/cm}^3$ ). There were no significant differences in the Young's modulus between the MOPS-stored samples and LRS-stored samples at any storage time. Modulus measured after 28 days in LRS ( $2.27 \pm 0.51 \text{ MPa}$ ) was significantly lower ( $p=0.028$ ) than MOPS samples stored for 14 days ( $4.08 \pm 1.29 \text{ MPa}$ ).

**DISCUSSION:** Overall, the MOPS and LRS stored samples exhibited moduli and physical properties that were statistically the same at each storage time. This finding agrees with an earlier study that conducted unconfined compression tests and reported no significant difference in cartilage biomechanical properties in MOPS samples compared to those stored using standard tissue bank protocols.<sup>2</sup> The lack of significant differences in cartilage moduli may be due to the indentation test, which is sensitive to changes at the cartilage surface that is rich in collagen and may not query deeper zones of cartilage where proteoglycans are more abundant. LRS-stored ovine samples have previously shown proteoglycan diminish gradually beginning at the articular surface and progressing towards bone, while MOPS maintained proteoglycan levels over time.<sup>7</sup> The main limitation of this study is that longitudinal changes could not be inferred due to intrinsically different weight bearing properties between medial and lateral femoral condyle surfaces,<sup>8</sup> hence, statistical comparisons over time could only be made between day 0 and day 56 samples as well as day 14 and day 28 samples.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Ovine tissues stored in MOPS or LRS media exhibited equivalent physical properties of bone and biomechanical properties of cartilage. This work contributes to understanding the effects of prolonged storage on the weight-bearing properties of osteochondral tissues, which is critical for the success of these tissues once transplanted.

**REFERENCES:** [1] De Caro. *Arthroscopy*. 2015. 31(4):757-765. [2] Stoker. *Am J Sports Med*. 2018. 46(1):58-65. [3] Vilela. *Springer International Publishing*. 2017. 29-53. [4] Gross. *Clin Orthop Relat Res*. 2008. 466(8):466-1863. [5] Stoker. *J Knee Surg*. 2018. 31(8):772. [6] Kyle D. *J Knee Surg*. 2021 36(4):450-455. [7] Changoor. *Trans Orthop Res Soc*. 2022;0608. [8] Risch. *J Orthop Res*. 2021. 39(4):780.

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**Figure 1.** a) Image showing osteochondral core sample taken from ovine condyle. b) Micro-CT scans of osteochondral core.

	0 Days		14 Days		28 Days		56 Days	
	LRS	MOPS	LRS	MOPS	LRS	MOPS	LRS	MOPS
Cartilage Thickness (mm)	0.38 ± 0.16 (n=7)	0.62 ± 0.23 (n=8)	1.03 ± 0.43 (n=7)	0.93 ± 0.25 (n=6)	0.85 ± 0.35 (n=6)	1.04 ± 0.44 (n=7)	0.34 ± 0.08 (n=6)	0.53 ± 0.26 (n=6)
Cartilage Young's Modulus (Mpa)	6.97 ± 2.48 (n=7)	7.11 ± 2.92 (n=8)	3.10 ± 1.11 (n=5)	4.08 ± 1.29 (n=6)*	2.27 ± 0.51 (n=6)*	3.59 ± 1.53 (n=6)	6.24 ± 0.79 (n=5)	5.98 ± 3.16 (n=6)
Subchondral Thickness (mm)	0.46 ± 0.27 (n=7)	0.58 ± 0.12 (n=8)	0.93 ± 0.44 (n=7)	0.83 ± 0.12 (n=6)	0.88 ± 0.31 (n=6)	0.89 ± 0.20 (n=7)	0.45 ± 0.09 (n=6)	0.54 ± 0.14 (n=6)
Subchondral Bone Mineral Density (g/cm <sup>3</sup> )	0.82 ± 0.12 (n=7)	0.84 ± 0.08 (n=8)	0.84 ± 0.08 (n=7)	0.87 ± 0.06 (n=7)*	0.76 ± 0.02 (n=6)*	0.77 ± 0.04 (n=7)*	0.76 ± 0.08 (n=6)	0.79 ± 0.10 (n=6)
Trabecular Bone Mineral Density (g/cm <sup>3</sup> )	0.26 ± 0.08 (n=7)	0.30 ± 0.10 (n=8)	0.50 ± 0.08 (n=7)	0.46 ± 0.07 (n=6)	0.34 ± 0.16 (n=6)	0.47 ± 0.09 (n=7)	0.26 ± 0.07 (n=6)	0.34 ± 0.11 (n=6)
Percent Bone Volume (%)	27.64 ± 3.62 (n=7)	29.69 ± 4.79 (n=8)	39.35 ± 4.20 (n=7)	38.34 ± 4.37 (n=6)	37.50 ± 3.87 (n=6)	38.95 ± 5.27 (n=7)	28.72 ± 2.85 (n=6)	31.67 ± 4.71 (n=6)

**Table 1.** Cartilage and bone properties after storage in either MOPS or LRS media. (\*) denotes statistically significant differences ( $p<0.05$ ) among entries within the same row. Data reported as average ± standard deviation.