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# Functional Properties of Native and Tissue-Engineered Cartilage Toward Understanding the Pathogenesis of Chondral Lesions at the Knee: A Bovine Cadaveric Study

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**ABSTRACT:** Chondral lesions frequently occur in different topographic locations of the knee. This study evaluated the functional properties among the articulating surfaces of the tibiofemoral and patellofemoral joints, and whether neo-cartilage engineered using chondrocytes from different knee locations would reflect these differences. The biomechanical properties of bovine cartilage isolated from eight locations within the tibiofemoral (medial and lateral condyle, medial and lateral tibial plateau) and patellofemoral joints (medial and lateral trochlea, medial and lateral patella) were examined. Tensile Young's moduli (tensile moduli) and aggregate moduli of the medial condyle were lower than those of the medial tibial plateau ( $6.11 \pm 0.89$  MPa vs.  $7.19 \pm 1.05$  MPa,  $p = 0.04$  and  $354.4 \pm 38.3$  kPa vs.  $419.4 \pm 31.3$  kPa,  $p = 0.002$ , respectively). Patella tensile and compressive moduli were lower than the trochlea ( $4.79 \pm 2.01$  MPa vs.  $6.91 \pm 2.46$  MPa,  $p = 0.01$  and  $337.4 \pm 37.2$  kPa vs.  $389.1 \pm 38.3$  kPa,  $p = 0.0005$ , respectively). Furthermore, chondrocytes from the above locations were used to engineer neo-cartilage, and its respective properties were evaluated. In neo-cartilage, medial condyle tensile and aggregate moduli were lower than in the medial tibial plateau ( $0.96 \pm 0.23$  MPa vs.  $1.31 \pm 0.31$  MPa,  $p = 0.02$ , and  $115.8 \pm 26.0$  kPa vs.  $160.8 \pm 18.8$  kPa,  $p = 0.001$ , respectively). Compared to trochlear chondrocytes, neo-cartilage formed from patellar chondrocytes exhibited lower tensile and compressive moduli ( $1.16 \pm 0.27$  MPa vs.  $0.74 \pm 0.25$  MPa,  $p < 0.001$ , and  $109.1 \pm 24.0$  kPa vs.  $82.5 \pm 18.1$  kPa,  $p < 0.001$ ). A significant degree of disparity in biomechanical properties of the opposing articular surfaces was detected; the medial condyle and patella exhibited inferior properties compared to the opposing medial tibial plateau and trochlea, respectively. © 2017 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. *J Orthop Res* 35:2452–2464, 2017.

**Keywords:** cartilage; knee; tibiofemoral; patellofemoral; tissue engineering; biomechanics; neo-cartilage; pathogenesis

Chondral lesions at different locations within the knee joint represent a major source of pain and disability. Table 1 summarizes the findings of all large arthroscopic studies that report the incidence of chondral defects on the different topographies of the tibiofemoral and patellofemoral joints.<sup>1–6</sup> As shown, chondral lesions are present in approximately 60% of all patients undergoing arthroscopy.<sup>1,3,5,6</sup> Interestingly, in all the presented studies, patients were relatively young, with mean age ranging from 37 to 43 years old.<sup>1–6</sup> The incidence of isolated chondral lesions, that is, cartilage lesions that were the sole pathologic finding of knee arthroscopy, is approximately 30%.<sup>1,3,6</sup> In most cases, a single lesion is present; however, the largest arthroscopic study to date reports the presence of an average of 2.7 lesions per knee.<sup>3</sup> A remarkably high number of chondral lesions are seen in young

adults undergoing arthroscopic examination, suggesting impending osteoarthritis at a later age.

The incidence of chondral lesions among the topographic locations of the knee varies significantly. The tibiofemoral joint is more commonly affected in comparison to the patellofemoral joint. Specifically, the medial condyle was found to be involved more frequently compared to any other location within the knee joint (Table 1).<sup>1–6</sup> Chondral lesions located at the medial condyle are reported with an incidence ranging from 32.2% to 58.0% of cases.<sup>1–6</sup> This suggests that in approximately one-third to one-half of patients suffering from a knee chondral lesion, the defect is located at the medial condyle. In contrast, medial tibial plateau is involved less frequently, with the incidence ranging from only 2.6% to 9.0%.<sup>1–6</sup> At the lateral knee compartment, lateral condyle lesions are detected in an incidence ranging from 9.0% to 20.5%, while lateral tibial plateau is involved with a range of 5.7–11.0%.<sup>1–6</sup> A significant degree of disparity exists in incidence of chondral lesions between the opposing articular surfaces at the tibiofemoral joint, with the medial knee compartment exhibiting the higher degree of disparity.

An increased interest was recently expressed in the pathogenesis of chondral lesions affecting the patellofemoral joint. Patellar chondral lesions are more frequent compared to trochlear lesions. Specifically, the patella is affected in 11.0–37.5%, while the trochlea is involved in only 6.0–15.5% of the patients.<sup>1–6</sup> Patellofemoral joint biomechanics have

All authors substantially contributed to research design, interpretation of data. NKP and NL contributed to acquisition and data analysis, as well as drafting the initial manuscript. All authors revised the paper critically and approved the submitted and final version.

**Clinical Significance:** Identification of disparity in the functional properties between the opposing surfaces can assist in understanding the pathogenesis of knee cartilage degeneration.

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**Table 1.** Incidence of Chondral Defects on the Different Topographies of the Tibiofemoral and Patellofemoral Joints

<b>Curl, 1997</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
19,827 out of 31,516 knees with at least 1 chondral lesion (63%)			Medial Femoral Condyle	32.3%	Trochlea	15.5%
Mean age 43 yo 38.4% ♀ : 61.6% ♂	<u>59,569 lesions</u> • 74% - single • 2.7 lesions/knee	Grade* I – 9.7% Grade* II – 28.1% Grade* III – 41.0% Grade* IV – 19.2% (OCD or # - 2%)	Medial Tibial Plateau	4.5%	Patella	21.3%
			Lateral Femoral Condyle	20.5%		
			Lateral Tibial Plateau	5.7%		
<b>Hjelle, 2002</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
606 out of 1,000 knees with chondral lesions (61%) 193 of those were focal chondral lesions (19%)			Medial Femoral Condyle	58%	Trochlea	6%
Mean age 39 yo 37.7% ♀ : 62.3% ♂	<u>232 lesions</u> • 80% - single • 12% - double • 8% - 3 lesions	ICRS I – 14% ICRS II – 26% ICRS III – 55% ICRS IV – 5%	Medial Tibial Plateau	5%	Patella	11%
			Lateral Femoral Condyle	9%		
			Lateral Tibial Plateau	11%		
<b>Aroen, 2004</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
655 out of 993 knees with chondral lesions (66%) 203 of those were isolated cartilage lesions (20%)			Medial Femoral Condyle	43%	Trochlea	8%
Mean age 39 yo 34% ♀ : 66% ♂	<u>203 lesions</u> • “kissing” lesions in 5%	ICRS I&II – 89% ICRS III&IV – 11%	Medial Tibial Plateau	9%	Patella	23%
			Lateral Femoral Condyle	11%		
			Lateral Tibial Plateau	6%		
<b>Figueroa, 2007</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
82 out of 190 knees with at least 1 chondral lesion (43%)			Medial Femoral Condyle	32.2%	Trochlea	9.6%
Mean age 37 yo 48.8% ♀ : 51.2% ♂	<u>115 lesions</u> • 71.9% - single • 23.2% - double • 4.9% - >3 lesions	ICRS I – 13.9% ICRS II – 34.8% ICRS III – 39.1% ICRS IV – 12.2%	Medial Tibial Plateau	2.6%	Medial Patella	22.6%
			Lateral Femoral Condyle	14.8%	Lateral Patella	10.4%
			Lateral Tibial Plateau	7.8%		
<b>Widuchowski, 2007</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
15,074 out of 21,124 knees with chondral lesions (60%) 4,538 out of 15,074 - isolated cartilage lesions (30%)			Medial Femoral Condyle	34%	Trochlea	8%
Mean age 39 yo 34% ♀ : 66% ♂	<u>Focal lesions in 40%</u>	Grade* I – 23% Grade* II – 42% Grade* III – 24% Grade* IV – 12%	Medial Tibial Plateau	6%	Patella	36%
			Lateral Femoral Condyle	9%		
			Lateral Tibial Plateau	7%		
<b>Widuchowski, 2008</b>			<u>Tibiofemoral joint</u>		<u>Patellofemoral joint</u>	
2,931 out of 5,233 knees with chondral lesions (57%) 4,538 out of 15,074 - isolated cartilage lesions (30%)			Medial Femoral Condyle	32.2%	Trochlea	7.1%
Mean age 43 yo 38.4% ♀ : 61.6% ♂	<u>Focal lesions in 40%</u> • 68.5% - single	Grade* I&II - 94.8% Grade* III&IV - 5.2%	Medial Tibial Plateau	7.1%	Patella	37.5%
			Lateral Femoral Condyle	9.3%		
			Lateral Tibial Plateau	6.8%		

A systematic literature search in PubMed, EMBASE, and Scopus databases was performed using relevant key words (cartilage OR chondral, defect OR lesions, knee, arthroscopy) and their combinations. Only studies with arthroscopic evaluation of more than 100 patients were included.

been considered having an important role in cartilage pathology.<sup>7–11</sup> Indeed, the incidence of patellar chondral lesions with recurrent patellar dislocations is exceedingly high, ranging from 73.5% to 95.7% in MRI and arthroscopic studies.<sup>12–14</sup> Chondral lesions at the patella are seen in both acute and chronic patellofemoral dislocations, indicating that both acute injury and malalignment could be etiologically associated with cartilage degeneration.<sup>15,16</sup> It has been suggested that specific anatomic and kinematic characteristics could be involved in the development of chondral lesions at the patellofemoral joint; however, the exact pathogenetic process remains unexplored.

The exact mechanism that leads to an increased incidence of chondral lesions in specific locations within the normal knee is rather unexplored. Certain theories have been proposed to explain the difference seen among the various locations. In a study that evaluated the functional properties of various topographic locations in the bovine ankle joint, it was shown that the opposing surfaces of the ankle exhibited significant disparity in their biomechanical and biological properties that could serve as the basis for pathogenetic phenomena that lead to cartilage lesions.<sup>17</sup> Specifically, talar dome cartilage was shown to have significantly lower tensile and compressive properties compared to the tibial plafond. Interestingly, examining neo-cartilage engineered from these two locations suggested that analogously dissimilar biological behavior at the cellular level due to location could also contribute to the topographic variability of functional properties.<sup>17</sup> The biomechanically inferior articular surface could be vulnerable to damage, and this could initiate degenerative changes due to inadequate healing responses at the talus. This theory has been supported by studies that show that a better improvement in outcome after alignment procedures is expected when chondral lesions were located at specific topographies in both tibiofemoral and patellofemoral joints.<sup>13,18,19</sup> However, before testing this theory, additional basic research would be useful to further evaluate the biomechanical and biological properties of cartilage in specific topographic locations of the knee joint. Evidence from disparities in the ankle joint motivates similar examinations in the knee to determine topographic variations in chondrocyte activity toward understanding different incidences of knee chondral lesions.

The aims of this study were (i) to evaluate the biomechanical and biosynthetic properties among the different articulating surfaces of the tibiofemoral and patellofemoral joints and (ii) to evaluate whether engineered neo-cartilage generated using chondrocytes from different knee locations would retain these differences in functional properties. Our hypothesis was that significant disparity would be present in biomechanical and biological properties of articular cartilage among the different locations of the tibiofemoral

and patellofemoral joints. Secondly, we hypothesized that neo-cartilage generated using cells from these areas would exhibit higher biomechanical properties and increased biosynthetic activity, indicating that these differences also exist at the cellular activity level.

## METHODS

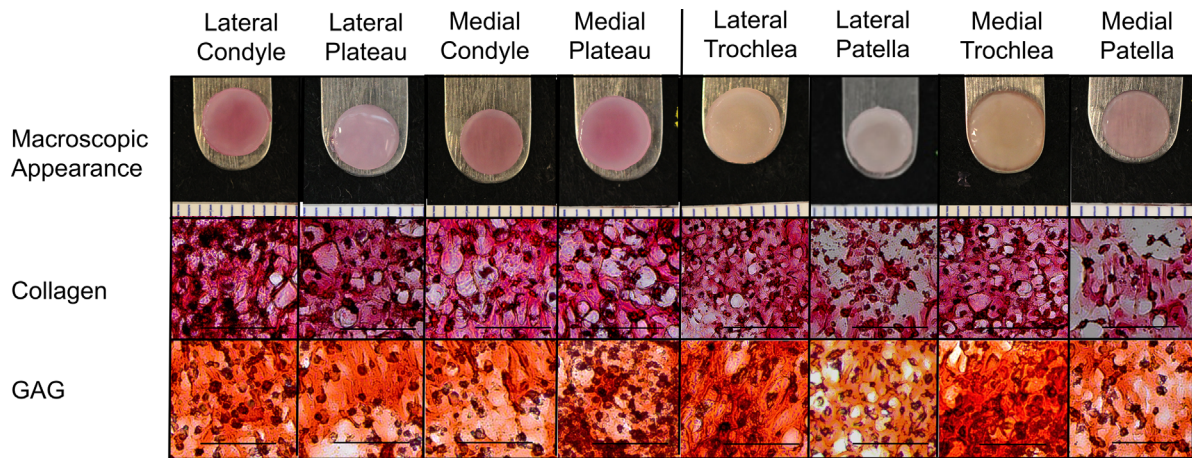
### Native Tissue Cartilage Isolation

Eight healthy cadaveric bovine knee joints of 4–8 week-old calves were obtained within 24–48 h post mortem. Joints were excluded if any degree of cartilage degeneration was present, or if the joint exhibited signs of injury or instability. Articular cartilage explants from the tibiofemoral and patellofemoral joints were harvested using 5 mm biopsy punches. Specifically, all samples were initially isolated as osteochondral cylindrical blocks using a biopsy punch, and the osseous part was excised using a blade. To ensure that all samples would have similar thickness, the superficial zone was removed as well as the deep zone leaving a cartilage explant of 1 mm thickness composed of the middle zone. Samples were randomly collected from each topographic location of the tibiofemoral and patellofemoral joints. Biomechanical and biochemical analyses were performed on all specimens. From the 5-mm disks, smaller concentric 3-mm disks were punched and used for creep indentation testing. Dog bone-shaped samples were made for tensile testing, as described below, and remaining tissue from the 5-mm disks was used for biochemical analysis.

### Chondrocyte Isolation and Construct Formation

To form engineered neo-cartilage constructs, chondrocytes were isolated from eight knees from 4 to 8 week-old calves, as previously described.<sup>17</sup> Tissue from the middle zone from each topographic location from tibiofemoral and patellofemoral joints was collected and minced into pieces of approximately 1 mm diameter and then digested in 0.2% type II collagenase (Worthington, Lakewood, NJ) cell culture medium composed of Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Carlsbad, CA) comprised of low glucose (1 g/L), 10% fetal bovine serum (Atlanta Biologicals, Flowery Branch, GA), 1% non-essential amino acids (Life Technologies), 25 mg of L-ascorbic acid (Sigma–Aldrich, St. Louis, MO), and 1% penicillin/streptomycin/amphotericin B (BioWhittaker, Walkersville, MD) for 18 h. Following digestion, chondrocytes were washed three times in DMEM, centrifuged, filtered through a 70- $\mu$ m filter, and counted. No differences were seen in cell isolation efficiency and cell viability among the different locations.

After cell isolation, engineering of neo-cartilage was performed. Based on previous work,<sup>17</sup> 4.5 million cells from each joint were seeded into 2% agarose wells to form 5 mm neo-cartilage constructs. These constructs were fed with 0.5 ml of chondrogenic medium daily for 10 days. The medium was made of DMEM, 1% penicillin/streptomycin/amphotericin B < 1% non-essential amino acids, 100-nmol/L dexamethasone (Sigma–Aldrich), 1% ITS+ (BD Scientific, Franklin Lakes, NJ), 40  $\mu$ g/ml of L-proline, 50  $\mu$ g/ml of ascorbate-2-phosphate, and 100  $\mu$ g/ml of sodium pyruvate (Fisher Scientific, Pittsburgh, PA). After the 10th day, the constructs were unconfined from the wells and moved to 48-well plates, where they were fed 1 ml of chondrogenic medium every other day for the 4-week duration of the culture (Fig. 1). No growth



**Figure 1.** Gross morphologic appearance and histology of neo-cartilage. Picosirius red staining was used for collagen and safranin O/fast-green staining for GAG. The histology scale bar represents 100  $\mu\text{m}$ .

factor or any physical stimulation was used in these constructs in order to eliminate any potential topographic variation in the response to these stimuli. The rationale of using the 4.5 million cell density, 4-week culture time, and medium composition was based on previous experimental work that showed that these conditions ensure the formation of neo-cartilage with substantial functional properties using the self-assembling process.<sup>17,20–22</sup>

#### Histology

Samples from the native and engineered tissue were cryo-embedded and sectioned at 14  $\mu\text{m}$ . Samples were formalin-fixed and subsequently stained with safranin-O/fast green and with picosirius red for glycosaminoglycan (GAG) and collagen analyses, respectively.

#### Quantitative Biochemistry

In total, eight samples of native tissue and eight samples of engineered tissue were assayed. To measure dry weights, samples from both native and engineered tissue were lyophilized. Next, the samples were digested using a pepsin digestion protocol.<sup>17</sup> To quantify sulfated GAG content, a Blyscan GAG dimethyl methylene blue assay kit was used (Accurate Chemical and Scientific, Westbury, NY), and to determine the amount of collagen, a chloramine-T hydroxyproline assay was used.<sup>17</sup> A SIRCOL collagen standard was used in the latter assay (Bicolor, Carrickfergus, UK).

#### High-Performance Liquid Chromatography (HPLC)

Samples were digested in 800  $\mu\text{l}$  of 6N hydrochloric acid at 100°C for 18h, and then dried in a vacuum concentrator. Subsequently, all samples were suspended in 50  $\mu\text{l}$  of 10- $\mu\text{mol/L}$  pyridoxine and 2.4- $\text{mmol/L}$  homoarginine. Then, the samples were diluted by a factor of 5 in 0.5% heptafluorobutyric acid in 10% acetonitrile. Ten microliter 10  $\mu\text{l}$  of each sample was used for HPLC, and pyridinoline standards (Quidel, San Diego, CA) were used to quantify the cross-link content.

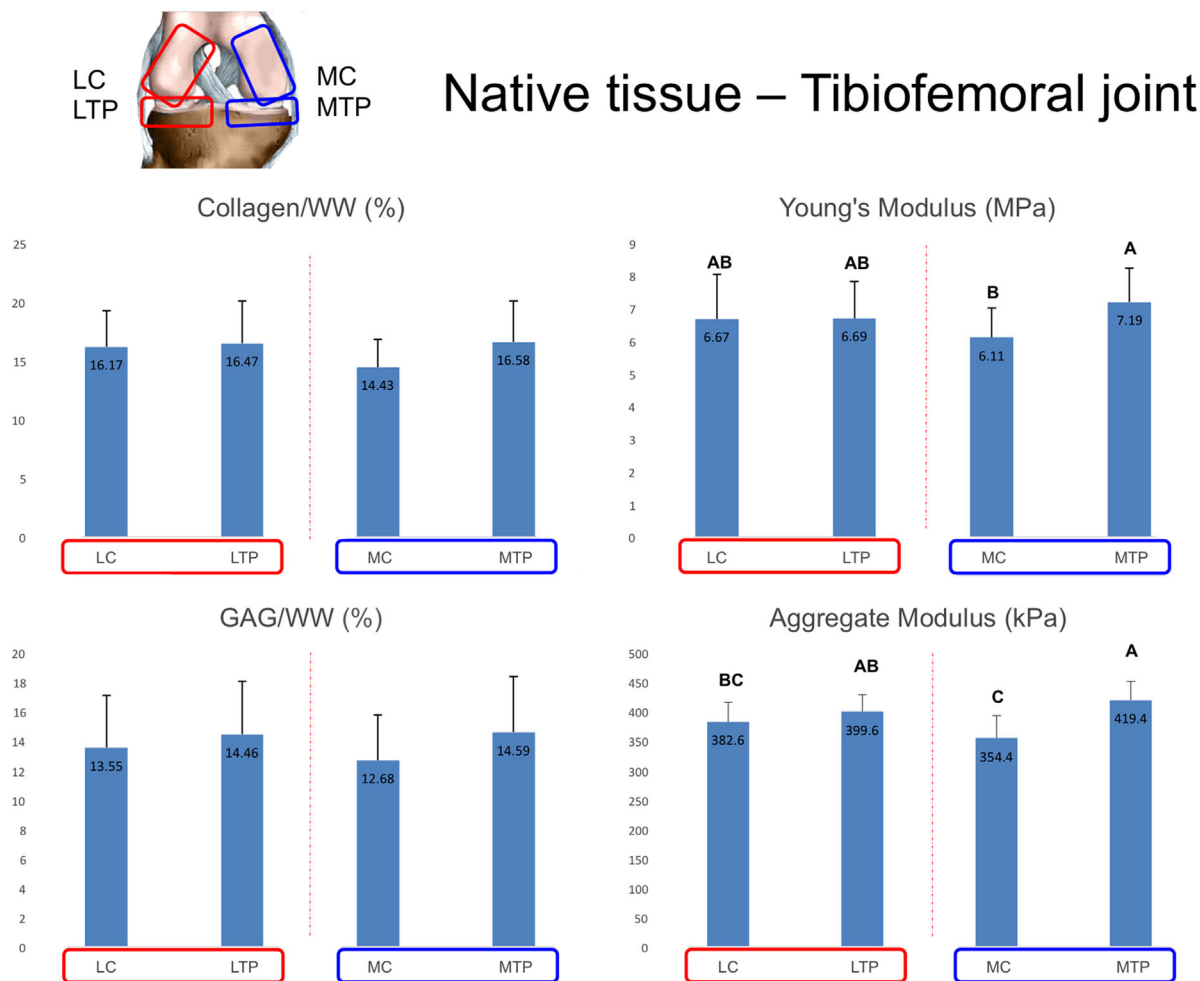
#### Biomechanical Testing

To determine biomechanical properties of the samples, tensile and compressive tests were performed. A uniaxial material testing system was used to apply uniaxial tension to

dog bone-shaped samples. The samples were prepared in a uniform manner so that gauge length for each construct was 1 mm, with thickness and width being 0.5 and 0.7 mm, respectively. Attention was also paid so that the dog bone shape would ensure that when the samples were loaded in the tensile testing machine the direction of testing would be parallel to the split lines of articular cartilage. A pull-to-failure test was run at a strain rate of 1% per second until failure. To test for compressive properties, a creep indentation apparatus was used. For indentation testing, a flat porous indenter with 0.4 mm radius was applied perpendicular to the articular surface so that the sample would experience approximately 15% strain. Prior to indentation, all samples were submerged in PBS until equilibrium. As previously described, samples were tested using a semi-analytical, semi-numerical, biphasic model and finite-element optimization.<sup>17,23,24</sup> The aggregate modulus was analyzed for each sample. Compression and tensile moduli were selected for comparison as intrinsic mechanical properties, while shear forces on cartilage are extrinsic properties that depend on factors such as lubrication, tissue geometry, and roughness. Therefore, the data obtained from compressive and tensile tests could be compared to literature values in order to assess the mechanical quality of our cartilage samples. Finally, in order to test our hypothesis that the functional properties of native tissue would be associated with those of the engineered neo-cartilage tissue, a correlation analysis was performed between the mechanical properties of native and engineered tissue, that is, tensile modulus and aggregate modulus. We also tested the correlation between tensile modulus and collagen content, as well as between aggregate modulus and GAG content in both native and engineered tissues.

#### Statistical Analysis

The data for this study were analyzed using JMP 12 software (SAS Institute, Cary, NC). With a sample size of 8, one-way analysis of variance (ANOVA) was used to compare results among different groups. If results were statistically significant ( $p < 0.05$ ), a Tukey HSD post hoc test was performed. In Figures 2–6, different letters symbolize significantly different groups. Values are shown as mean  $\pm$  standard deviation. A ratio has been calculated between the properties of the opposing articular surfaces in order to quantify disparity and



**Figure 2.** Biochemical and biomechanical properties of native articular cartilage from different topographic locations of the tibiofemoral joint (LC, lateral condyle; LTP, lateral tibial plateau; MC, medial condyle; MTP, medial tibial plateau; WW, wet weight).

it is presented as a percentage. For quantitative assessment of potential correlations, the coefficient of determination  $R^2$  and the resultant Pearson's  $R$ -value were calculated. Positive correlations depicted graphically as positive sloping regression lines. All correlations were considered significant when  $p < 0.05$ .

## RESULTS

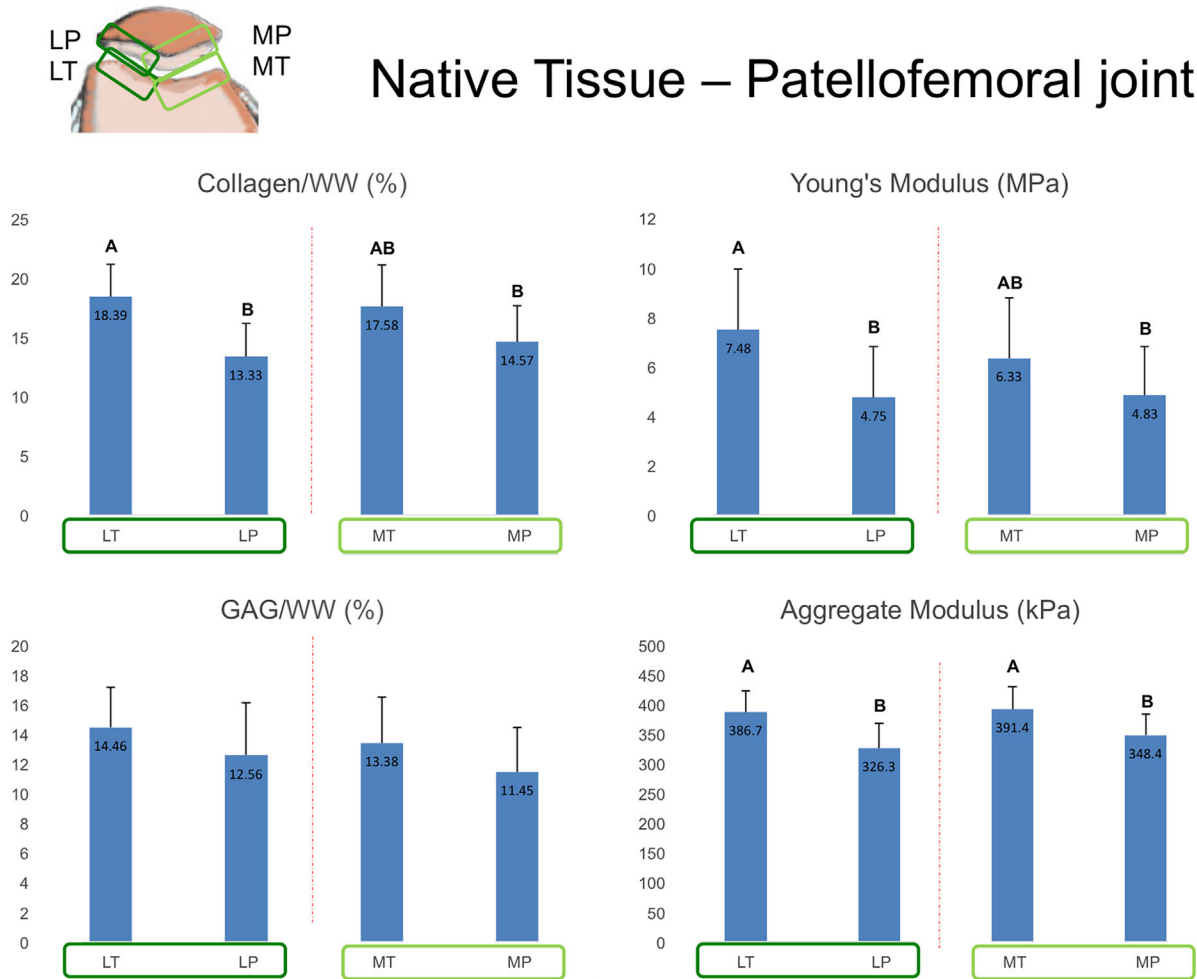
### Native Tissue

At the tibiofemoral joint, as shown in Figure 2, tensile modulus and aggregate modulus of the medial condyle were significantly lower than those of the medial tibial plateau ( $6.11 \pm 0.89$  MPa vs.  $7.19 \pm 1.05$  MPa,  $p = 0.04$  and  $354.4 \pm 38.3$  kPa vs.  $419.4 \pm 31.3$  kPa,  $p = 0.002$ , respectively). These significant discrepancies in both tensile and compressive moduli between the medial condyle and medial tibial plateau were noted to be at a ratio of approximately 0.85 for both comparisons. In contrast, no statistical significant difference was seen between the tensile and compressive moduli of the lateral condyle and lateral tibial plateau ( $6.67 \pm 1.37$  MPa vs.  $6.69 \pm 1.14$  MPa,  $p = 0.98$ , and

$382.6 \pm 32.6$  kPa vs.  $399.6 \pm 28.5$  kPa,  $p = 0.28$ , respectively). In biochemical properties, no difference was seen in collagen and GAG content among the different topographic locations of the tibiofemoral joint.

At the patellofemoral joint, aggregate modulus values of the medial patellar facet were significantly lower compared to those of the medial trochlea ( $348.4 \pm 35.2$  kPa vs.  $391.4 \pm 37.9$  kPa, respectively,  $p = 0.03$ ) (Fig. 3). No difference was detected in tensile modulus between medial patella and medial trochlea ( $4.83 \pm 2.00$  MPa vs.  $6.33 \pm 2.46$  MPa, respectively,  $p = 0.20$ ). At the lateral site, the lateral patella facet exhibited lower tensile ( $4.75 \pm 2.05$  MPa vs.  $7.48 \pm 2.45$  MPa,  $p = 0.03$ ), and compressive moduli ( $326.3 \pm 41.5$  kPa vs.  $386.7 \pm 36.4$  kPa,  $p = 0.008$ ). Significant discrepancies were demonstrated between the tensile moduli of the patella and the trochlea in both lateral and medial sites (ratio of 0.76 and 0.59, respectively). Similarly, collagen content was higher in the trochlea compared to the patella but this difference reached significance only in the lateral compartment ( $17.58 \pm 3.48\%$  Col/WW vs.  $14.57 \pm 3.04\%$  Col/WW,  $p = 0.08$  and  $13.33 \pm 2.84\%$  Col/WW vs.  $18.39 \pm 2.76\%$





**Figure 3.** Biochemical and biomechanical properties of native articular cartilage from different topographic locations of the patellofemoral joint (LT, lateral trochlea; LP, lateral patella; MT, medial trochlea; MP, medial patella; WW, wet weight).

Col/WW,  $p=0.002$ , for medial site and lateral site, respectively). No difference was seen in compressive modulus and GAG content among the different locations of the patellofemoral joint.

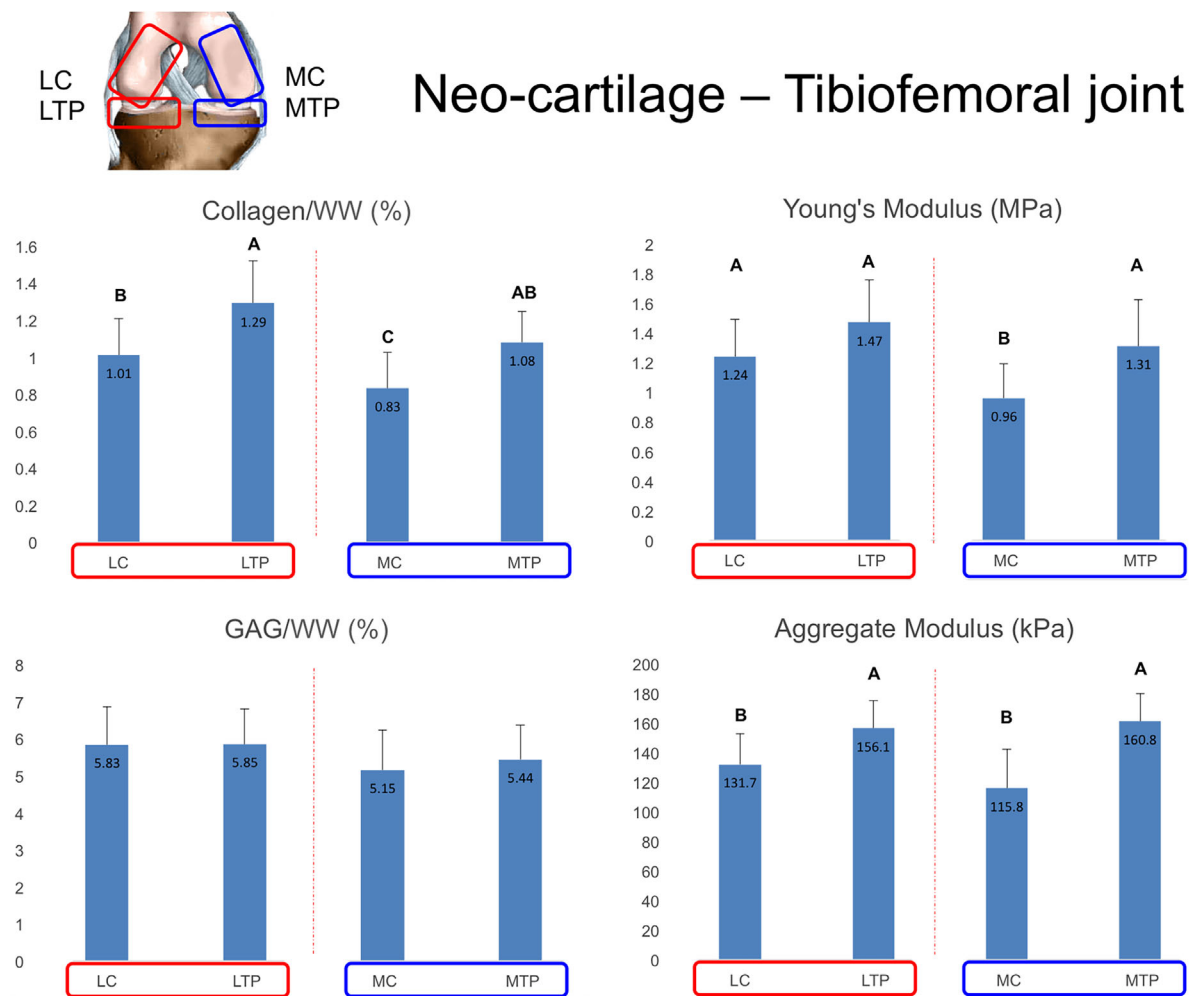
### Neo-Cartilage

A significant degree of discrepancy was seen in both tensile and compressive moduli between neo-cartilage from medial condyle chondrocytes and neo-cartilage from medial tibial plateau chondrocytes (Fig. 4). Specifically, tensile and aggregate moduli of neo-cartilage from the medial condyle were significantly lower than those of neo-cartilage from medial tibial plateau, ( $0.96 \pm 0.23$  MPa vs.  $1.31 \pm 0.31$  MPa,  $p=0.02$ , and  $115.8 \pm 26.0$  kPa vs.  $160.8 \pm 18.8$  kPa,  $p=0.001$ , respectively), with ratio values of 0.73 and 0.72, respectively. At the lateral compartment, no difference was seen in tensile moduli between lateral condyle and lateral tibial plateau ( $1.24 \pm 0.25$  MPa vs.  $1.47 \pm 0.28$  MPa, respectively,  $p=0.105$ ). However, a significantly lower aggregate modulus was shown in lateral condyle neo-cartilage compared to lateral tibial plateau neo-

cartilage ( $131.7 \pm 20.8$  kPa vs.  $156.1 \pm 18.9$  kPa, respectively,  $p=0.03$ ), with a discrepancy ratio of 0.84, which is higher compared to that seen in the medial compartment that reached 0.72.

As shown in Figure 5, there was no difference in the amount of cross-links detected between the neo-cartilage formed from lateral condyle and lateral tibial plateau chondrocytes ( $p=0.984$ ). In contrast, the neo-cartilage generated from medial condyle chondrocytes demonstrated significantly lower amount of cross-links ( $p=0.02$ ).

At the patellofemoral joint, neo-cartilage formed from patellar chondrocytes exhibited significantly lower tensile and compressive moduli compared to those of neo-cartilage formed from trochlear chondrocytes ( $1.16 \pm 0.27$  MPa vs.  $0.74 \pm 0.25$  MPa, and  $109.1 \pm 24.0$  kPa vs.  $82.5 \pm 18.1$  kPa,  $p < 0.001$ , for both comparisons) (Fig. 6). When comparing the discrepancy between the medial and the lateral sites, both locations exhibited a large degree of disparity; a higher degree of discrepancy was seen for tensile modulus at the medial site compared to the lateral



**Figure 4.** Biochemical and biomechanical properties of neo-cartilage generated using chondrocytes from different topographic locations of the tibiofemoral joint (LC, lateral condyle; LTP, lateral tibial plateau; MC, medial condyle; MTP, medial tibial plateau; WW, wet weight).

site (0.60 and 0.69, respectively). Collagen content was significantly lower in patellar neo-cartilage compared to trochlea. No difference was detected in the compressive moduli between the neo-cartilage formed from lateral patella and lateral trochlea ( $84.2 \pm 21.0$  kPa vs.  $104.7 \pm 22.6$  kPa, respectively,  $p=0.08$ ). At the medial site, a significant difference was shown ( $79.1 \pm 16.1$  kPa vs.  $112.4 \pm 27.3$  kPa, respectively,  $p=0.01$ ), with aggregate modulus of patellar neo-cartilage being 70% that of the trochlea. No differences were shown in GAG content. Regarding cross-links, lateral trochlear neo-cartilage exhibited the higher amount of cross-links and medial patella exhibited the lower amount, but no significant discrepancy between the opposing articulating surfaces was seen ( $p=0.07$  and  $p=0.38$ , for the medial and lateral site, respectively). Additional biomechanical data for native and engineered tissue are provided in Tables 2 and 3.

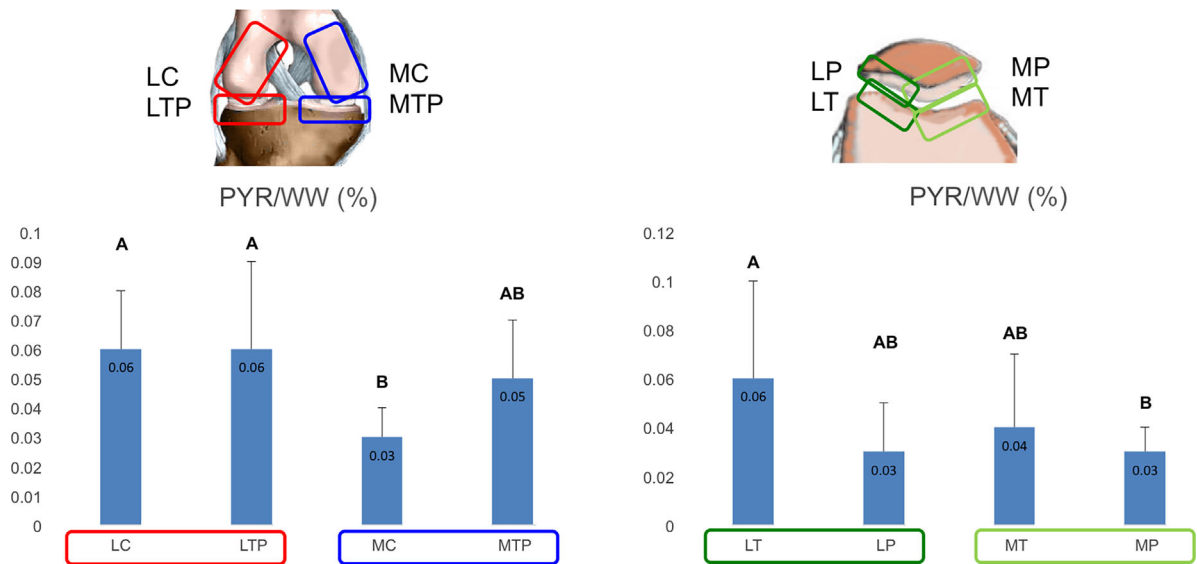
For evaluating the quantitative measurement of the correlations between the mechanical properties of the native and engineered cartilage, the Pearson's  $R$ -value

and coefficient of determination  $R^2$  were obtained (Fig. 7). As shown, the tensile modulus and aggregate modulus of native tissue were positively correlated with the tensile and aggregate moduli of engineered tissue, with  $R$ -values 0.77 ( $p=0.02$ ) and 0.84 ( $p=0.008$ ), respectively, that both represent a strong positive correlation. Regarding the correlations between the tensile properties and collagen, the  $R$ -values were 0.84 ( $p=0.008$ ) and 0.74 ( $p=0.03$ ) in native and engineered tissue, respectively. Finally, the correlation between compressive modulus and GAG content was also positive with  $R$  of 0.83 ( $p=0.009$ ) and 0.89 ( $p=0.002$ ) for native and engineered tissue, respectively.

## DISCUSSION

The findings of this study indicate that the tibiofemoral and patellofemoral joints exhibit significant topographic variability in cartilage functional properties. Specifically, our results demonstrate a significant disparity in biomechanical and biological properties of cartilage among the opposing





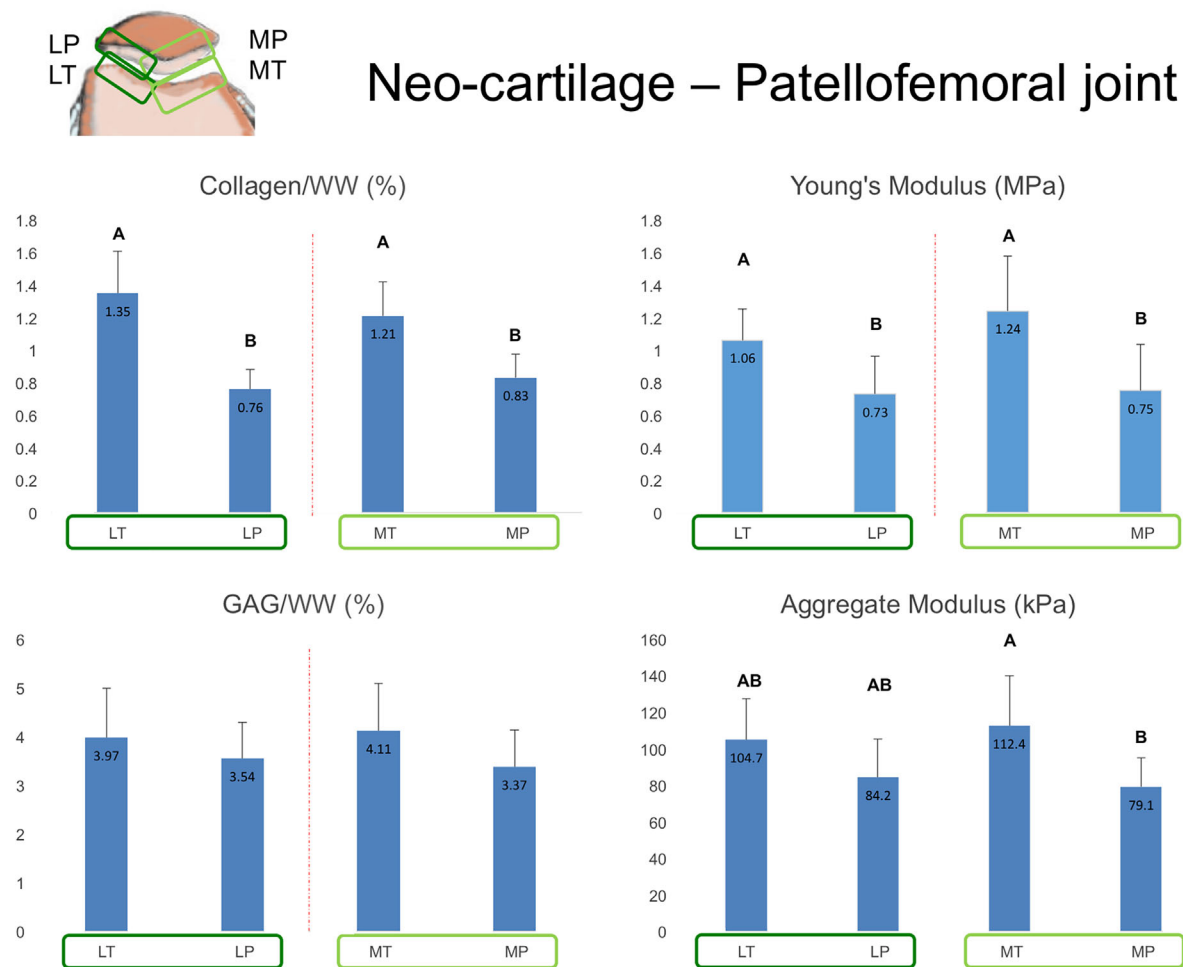
**Figure 5.** Pyridinoline (PYR) content of neo-cartilage per wet weight for the tibiofemoral and patellofemoral joints (LC, lateral condyle; LTP, lateral tibial plateau; MC, medial condyle; MTP, medial tibial plateau; LT, lateral trochlea; LP, lateral patella; MT, medial trochlea; MP, medial patella; WW, wet weight).

articular surfaces of the tibiofemoral and patellofemoral joints, confirming our first hypothesis. At the tibiofemoral joint, the medial condyle was found to have tensile and compressive moduli lower than those of the opposing medial plateau, while no such disparity was seen at the lateral compartment of the knee. At the patellofemoral joint, patellar cartilage was demonstrated to have inferior biomechanical and biological properties in comparison to the opposing trochlea. At the biological level, we found that neo-cartilage fabricated from chondrocytes isolated from the medial condyle and patella exhibited lower functional properties in comparison to those of the medial tibial plateau and trochlea, respectively, confirming our second hypothesis. The disparity detected among these locations could be potentially associated with cartilage degeneration and wear at the biomechanically inferior cartilage surface.

Some interesting observations can be made regarding the disparity seen between the biomechanical properties at the tibiofemoral joint in both native and engineered tissue. Specifically, the medial condyle exhibited the lowest biomechanical properties among the different locations, while the medial tibial plateau showed the highest properties. As a result, a high degree of disparity was seen at the medial compartment. As shown in Table 1, the medial condyle is the most common location of chondral injuries within the tibiofemoral joint with its incidence reported consistently above 32%, while the medial tibial plateau consistently has an incidence lower than 10%.<sup>1-6</sup> In contrast, at the lateral knee compartment, the condyle and plateau exhibit biomechanical properties that do not differ significantly, and the incidence of chondral lesions at both locations is comparable.<sup>1-6</sup> At the

patellofemoral joint, in both native and engineered tissue, patella cartilage exhibited lower biomechanical properties. Interestingly, patellar lesions are seen 1.4–5.2 times more often compared to the trochlea (Table 1).<sup>1,3,6</sup> Our findings confirm previous work that reported inferior compressive properties of the patellar cartilage in comparison to that of trochlea and also associated the disparity of the material properties as a potential etiological factor for early degenerative changes such as fibrillation.<sup>25</sup> Additional research is needed to evaluate whether these associations exist in human cartilage, and to further attempt to correlate them with the clinical incidence of chondral lesions.

A high degree of variability in the functional properties of articular cartilage and neo-cartilage was detected among the different locations in both tibiofemoral and patellofemoral joints. This confirms previous work that notes the existence of significant topographical variations in cartilage material properties and thickness at the ankle joint.<sup>23</sup> At the knee joint, it was also shown that significant differences are present in the biomechanical properties of cartilage from different sites of the distal femur.<sup>26</sup> In a study that evaluated the effect of meniscectomy in the non-operative controls, a certain degree of variability was also seen among the different topographic locations of the tibial plateau.<sup>27</sup> In two studies that found that the compressive and tensile properties of the patellofemoral groove and femoral condyles increase significantly during fetal and postnatal development, topographic differences were seen mainly in tensile properties of articular cartilage.<sup>28,29</sup> In all the above studies, no direct comparison and no associations were made with the opposing condyle articular surface. The novelty of the present study is the comparison of biomechanical and biochemical properties between the



**Figure 6.** Biochemical and biomechanical properties of neo-cartilage generated using chondrocytes from different topographic locations of the patellofemoral joint (LT, lateral trochlea; LP, lateral patella; MT, medial trochlea; MP, medial patella; WW, wet weight).

opposing articular surfaces among multiple locations in both tibiofemoral and patellofemoral joints.

The differences seen in the functional properties of native tibiofemoral and patellofemoral joints were also detected in the engineered tissue confirming the second hypothesis of this study. Using correlation analysis, it was shown that there was a strong positive correlation between the mechanical properties of the native and engineered tissue, but also between the tensile and compressive properties and the collagen and GAG content, respectively; this correlation was exhibited in both native and engineered tissue. Using the self-assembling process that recapitulates developmental milestones of articular cartilage formation,<sup>20,22,30</sup> this study was able to indirectly evaluate the biological behavior of cells. As shown, collagen biosynthesis as well as the amount of collagen cross-links at the medial compartment paralleled the tensile properties of neo-cartilage. At the lateral compartment, collagen synthesis was significantly lower in neo-cartilage from the lateral condyle compared to that of the lateral plateau, though no differences were found in the tensile properties. At the patellofemoral joint, our data showed

a significant difference in collagen content but not in GAG between the opposing surfaces of the lateral trochlea and patella in partial agreement with previous work.<sup>25</sup> Our results also identified a disparity of the biochemical synthetic capacity of patella chondrocytes using the self-assembling process. Poor biological response of the chondrocytes at certain locations may be implicated in the pathogenesis of chondral defects, as it would inadequately reverse the degeneration process of the knee.

Another important aspect is the potential role of chondrocytes in the superficial zones of the opposing articular surfaces that could play a dominant role in the biomechanical properties. Superficial chondrocytes are also unique in terms of their capacity to synthesize superficial zone protein.<sup>31</sup> Interestingly, the amount of SZP that exists among the different locations in the knee joint varies significantly which can be partially associated with the differences in the biomechanical properties.<sup>32</sup> Furthermore, the collagen fibers at the superficial zone have an orientation, which is parallel to the articular surface along the split line orientation, and this characteristic ensures higher tensile moduli

**Table 2.** Additional Mechanical Data for Native Tissue Topographic Locations, Represented as Mean  $\pm$  SD

	Ultimate Tensile Strength (MPa)	Shear Modulus (kPa)	Poisson's Ratio	Permeability ( $10^{-15}$ m <sup>4</sup> /N s)
<b>Tibiofemoral joint</b>				
Lateral condyle	3.71 $\pm$ 0.94	188.5 $\pm$ 24.5 <sup>AB</sup>	0.28 $\pm$ 0.08	0.63 $\pm$ 0.84
Lateral tibial plateau	3.58 $\pm$ 0.78	195.0 $\pm$ 18.4 <sup>AB</sup>	0.33 $\pm$ 0.10	0.58 $\pm$ 0.42
Medial condyle	3.34 $\pm$ 0.61	176.3 $\pm$ 22.6 <sup>B</sup>	0.26 $\pm$ 0.09	0.61 $\pm$ 0.67
Medial tibial plateau	3.78 $\pm$ 0.71	209.7 $\pm$ 21.5 <sup>A</sup>	0.31 $\pm$ 0.11	0.55 $\pm$ 0.39
<b>Patellofemoral joint</b>				
Lateral trochlea	3.20 $\pm$ 1.66 <sup>A</sup>	186.8 $\pm$ 20.8 <sup>A</sup>	0.25 $\pm$ 0.12	0.65 $\pm$ 0.68
Lateral patella	2.51 $\pm$ 1.38 <sup>B</sup>	159.9 $\pm$ 24.0 <sup>B</sup>	0.29 $\pm$ 0.09	0.77 $\pm$ 0.35
Medial trochlea	4.33 $\pm$ 1.66 <sup>AB</sup>	190.9 $\pm$ 19.9 <sup>A</sup>	0.30 $\pm$ 0.09	0.69 $\pm$ 0.58
Medial patella	2.43 $\pm$ 1.54 <sup>B</sup>	171.6 $\pm$ 25.8 <sup>AB</sup>	0.28 $\pm$ 0.11	0.71 $\pm$ 0.57

Letters indicate statistical significance between the groups marked with a different letter.

when tested along this orientation.<sup>33</sup> It is also suggested that chondrocytes of the superficial zone are more prone to deformation changes such as elongation and rotation under tensile loading and compression under axial compressive loading.<sup>34</sup> All the above characteristics of the chondrocytes in the superficial zone need to be further evaluated toward a more comprehensive understanding of the role in pathogenic phenomena at the knee joint.

The findings of this work indicate that the opposing articular surfaces in the knee joint exhibit distinct characteristics that could be responsible for their physiology and pathology. The study of lubrication or frictional characteristics of these surfaces could provide a more comprehensive explanation. Indeed, a topographic comparison of the frictional properties demonstrated significant variations in coefficients of friction across the knee joint.<sup>32,35</sup> Specifically, the medial posterior femoral condyle had a coefficient of friction three times higher than its opposing medial posterior tibial plateau.<sup>32</sup> Similarly, the coefficient of friction of the articular cartilage surface of the patella was significantly higher compared to that of the opposing trochlear groove.<sup>32</sup> It was suggested that the materials properties in conjunction with the lubrication properties could be used to highlight differences

between the medial and lateral compartments associated with osteoarthritis.<sup>35</sup> Based on the findings of this work that distinct characteristics exist in the compressive, tensile, and shear moduli, research should explore other characteristics, such as lubrication properties to identify their role in knee joint biomechanics and articular cartilage homeostasis.

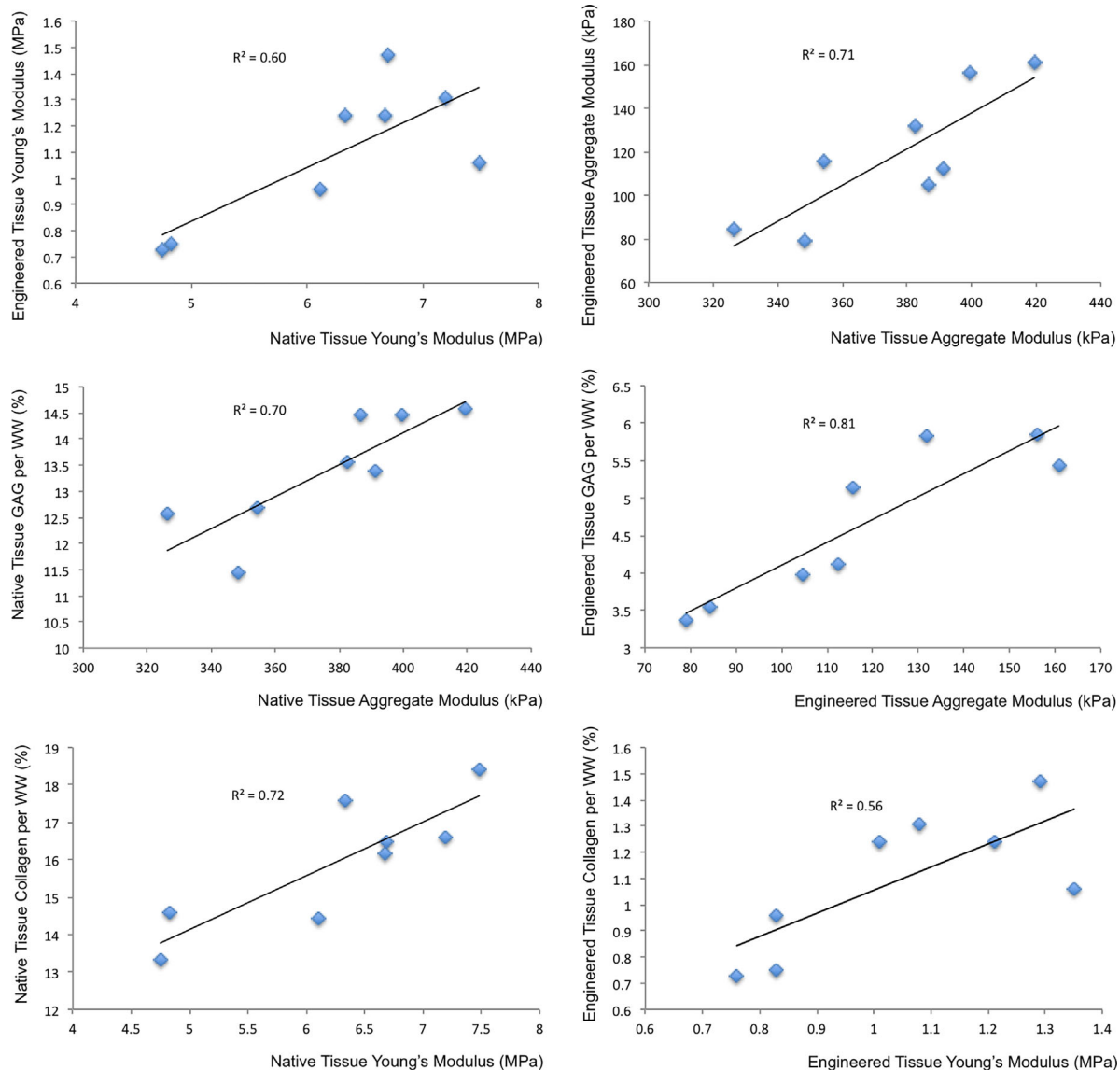
As shown in the ankle,<sup>17</sup> the present work highlights a potential association between the disparity in biomechanical properties of the opposing knee articular surfaces and the incidence of chondral lesions. The concept of equitable stress distribution has been followed in osteotomies, where the aim is to distribute loading more equally between the knee compartments.<sup>36</sup> Indeed, high tibial osteotomies have been shown to reduce contact pressure and contact area at the medial compartment, and therefore, is considered a viable solution for patients with isolated chondral lesions.<sup>18</sup>

With the use of tissue engineering techniques, neo-cartilage without the use of scaffold was successfully created. Using the self-assembling process, engineered neo-cartilage was generated using chondrocytes originating from the specific topographic locations of the knee.<sup>20,30</sup> Interestingly, the biomechanical and biochemical properties of the engi-

**Table 3.** Additional Mechanical Data for Neo-Cartilage Topographic Locations, Represented as Mean  $\pm$  SD

	Ultimate Tensile Strength (MPa)	Shear Modulus (kPa)	Poisson's Ratio	Permeability ( $10^{-15}$ m <sup>4</sup> /N s)
<b>Tibiofemoral joint</b>				
Lateral condyle	0.67 $\pm$ 0.18 <sup>A</sup>	64.5 $\pm$ 14.2 <sup>A</sup>	0.18 $\pm$ 0.2	7.2 $\pm$ 5.8
Lateral tibial plateau	0.79 $\pm$ 0.15 <sup>A</sup>	77.4 $\pm$ 11.5 <sup>A</sup>	0.23 $\pm$ 0.18	5.3 $\pm$ 7.1
Medial condyle	0.48 $\pm$ 0.11 <sup>B</sup>	57.9 $\pm$ 16.3 <sup>A</sup>	0.22 $\pm$ 0.25	8.3 $\pm$ 11.5
Medial tibial plateau	0.65 $\pm$ 0.12 <sup>A</sup>	70.0 $\pm$ 12.1 <sup>A</sup>	0.19 $\pm$ 0.31	9.1 $\pm$ 10.1
<b>Patellofemoral joint</b>				
Lateral trochlea	0.56 $\pm$ 0.17 <sup>A</sup>	52.9 $\pm$ 15.8	0.24 $\pm$ 0.22	9.8 $\pm$ 11.2
Lateral patella	0.39 $\pm$ 0.12 <sup>B</sup>	40.8 $\pm$ 16.8	0.20 $\pm$ 0.26	11.4 $\pm$ 6.9
Medial trochlea	0.64 $\pm$ 0.21 <sup>A</sup>	55.9 $\pm$ 17.3	0.31 $\pm$ 0.16	6.8 $\pm$ 8.4
Medial patella	0.36 $\pm$ 0.14 <sup>B</sup>	39.5 $\pm$ 13.6	0.25 $\pm$ 0.21	8.9 $\pm$ 9.1

Letters indicate statistical significance between the groups marked with a different letter.



**Figure 7.** A strong positive correlation ( $R^2 > 0.5$ ) was found between the mechanical properties of native articular cartilage and neo-cartilage. In addition, a strong positive correlation ( $R^2 \geq 0.72$ ,  $R^2 \geq 0.56$ , and  $R^2 \geq 0.70$ ,  $R^2 \geq 0.81$ ) was found between the tensile or Young's modulus/collagen and aggregate modulus/GAG in both native and engineered neo-cartilage, respectively.

neered tissues were found to correspond to those of the native tissues, indicating that the chondrocytes retain their inherent properties. This observation is clinically important as it suggests that the properties of the engineered tissue could be associated with the topographic source of the isolated chondrocytes. As tissue engineering is emerging as a powerful tool to successfully develop neo-cartilage, the concept of location-dependent properties of chondrocytes should be considered.

#### Limitations

This study has certain limitations. First, this is an in vitro evaluation of bovine native and engineered tissues. It was difficult to evaluate separately various different topographies for each location (e.g., to test

eight different topographies within the medial condyle), due to the high number of specimens needed for this analysis. To address this, randomly selected specimens from all topographies within location were used. Bovine source was used due to the limited number of cells isolated from human knee, and the differences seen in this study are representative for human knee.<sup>23,26</sup> However, careful interpretation of our results should be made, since there are differences in joint biomechanics, kinetics, and contact areas between bovine and human knee. This study could serve as the initial step toward identifying potential pathogenetic mechanisms for cartilage degeneration. Another limitation of the study is that it did not evaluate the specifics of the exact mechanism that could lead to cartilage degeneration. However, the

hypothesis that the disparity in the biomechanical properties is responsible for cartilage degeneration is well supported in the literature. This study focused on the characterization of the biomechanical properties of the tibiofemoral and patellofemoral joints. Furthermore, this study evaluated indirectly cellular response using the self-assembling process that is shown to be representative of the phenomena occurring during cartilage development. The advantage of this approach was that it also evaluated the feasibility of developing neo-cartilage with specific characteristics from each location, as part of the inherent cellular characteristics of the chondrocytes. An important finding of this work is that different locations of the knee joint exhibit different biomechanical properties. This may reflect the different physiological demands in that specific region. Additional research is required to specify the specific load demands for each location within the knee.

## CONCLUSIONS

This study demonstrated that the biomechanical and biological properties of the opposing articulating surfaces exhibit a high degree of disparity that may predispose to cartilage degeneration in specific locations. The medial condyle has inferior properties compared to the opposing medial tibial plateau, while at the patellofemoral joint the patella had inferior functional properties compared to the trochlea. These differences may also exist at the cellular level, as shown by the corresponding properties of neo-cartilage created from the respective locations.

## ACKNOWLEDGMENT

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