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Topographic Variations in Biomechanical and Biochemical Properties in the Ankle Joint: An In Vitro Bovine Study Evaluating Native and Engineered Cartilage

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Purpose: The purposes of this study were to identify differences in the biomechanical and biochemical properties among the articulating surfaces of the ankle joint and to evaluate the functional and biological properties of engineered neocartilage generated using chondrocytes from different locations in the ankle joint. **Methods:** The properties of the different topographies within the ankle joint (tibial plafond, talar dome, and distal fibula) were evaluated in 28 specimens using 7 bovine ankles; the femoral condyle was used as a control. Chondrocytes from the same locations were used to form 28 neocartilage constructs by tissue engineering using an additional 7 bovine ankles. The functional properties of neocartilage were compared with native tissue values. **Results:** Articular cartilage from the tibial plafond, distal fibula, talar dome, and femoral condyle exhibited Young modulus values of 4.8 ± 0.5 MPa, 3.9 ± 0.1 MPa, 1.7 ± 0.2 MPa, and 4.0 ± 0.5 MPa, respectively. The compressive properties of the corresponding tissues were 370 ± 22 kPa, 242 ± 18 kPa, 255 ± 26 kPa, and 274 ± 18 kPa, respectively. The tibial plafond exhibited 3-fold higher tensile properties and 2-fold higher compressive and shear moduli compared with its articulating talar dome; the same disparity was observed in neocartilage. Similar trends were detected in biochemical data for both native and engineered tissues. **Conclusions:** The cartilage properties of the various topographic locations within the ankle are significantly different. In particular, the opposing articulating surfaces of the ankle have significantly different biomechanical and biochemical properties. The disparity between tibial plafond and talar dome cartilage and chondrocytes warrants further evaluation in clinical studies to evaluate their exact role in the pathogenesis of ankle lesions. **Clinical Relevance:** Therapeutic modalities for cartilage lesions need to consider the exact topographic source of the cells or cartilage grafts used. Furthermore, the capacity of generating neocartilage implants from location-specific chondrocytes of the ankle joint may be used in the future as a tool for the treatment of chondral lesions.

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The ankle joint represents an increasingly common site for various chondral and osteochondral disorders. Most chondral lesions usually occur in young and active individuals as a result of ankle injury or fracture.[1-4] These lesions are severely debilitating as a result of both cartilage and bone degeneration and, eventually, may lead to the development of osteoarthritis. Interestingly, most of the articular cartilage lesions reported in the ankle joint involve the talus and, more specifically, the talar dome. In contrast, the tibial plafond represents an uncommon site of chondral lesions, with a ratio of 1 to 14 to 1 to 20 lesions of the talus.[5,6] The disparity in the incidence of chondral lesions between these 2 opposing cartilage surfaces in the ankle is surprising, especially when considering examples from the knee and hip joint, where cartilage lesions occur in a more comparable rate among the different articulating surfaces of the joint.[7,8]

The prevalence of cartilage lesions and degenerative changes is dramatically lower in the ankle joint in comparison with the knee.[9,10] Little is known regarding the pathogenic mechanisms responsible for this difference. A few reports describe the anatomic and biomechanical differences among the cartilage comprising different joints, which could be responsible for the disparity in the prevalence of osteoarthritis.[11,12] Significant topographic variations in the cartilage material properties have been described that may be related to the increased risk of degeneration.[13] In addition, variations in the biochemical properties of the extracellular matrix and the metabolic activity of the chondrocytes between the ankle and the knee joint have been reported.[14,15] This suggests that biomechanical and biological factors may be involved in the pathogenesis of chondral lesions. However, a comparison of the biomechanical and biological characteristics of cartilage in the opposing articulating surfaces of the ankle joint, in relation to the knee, has yet to be performed.

Several treatment modalities have been used for the management of chondral lesions at the ankle joint. Unfortunately, treatment is particularly challenging, especially in young patients; unsatisfactory outcomes are commonly reported because of the inability to repair the articular cartilage damage and restore normal joint function.[2,16,17] Tissue engineering opens a new field in the management of ankle lesions by developing tissues that can replace the damaged cartilage.[18,19] A technique for the development of engineered scaffold-free neocartilage has been described.[20] The self-assembling approach for neocartilage development is considered a representative model of native tissue morphogenesis, maturation, and maintenance.[21] Furthermore, such neotissues recapitulate the morphologic structure and organization of native tissues and, thus, present as a model that imitates the fundamental in vivo biological processes.[20,22] Self-assembly has been successful in engineering neocartilage tissue that exhibits biomechanical and functional properties that approach those of native tissue, representing a promising future treatment approach.[22] As such, self-assembled neocartilage is considered a suitable model for studying chondrocyte behavior during cartilage development, during maturation, and importantly, during tissue regeneration.

The aim of this study was 2-fold: (1) to identify potential differences in the biomechanical properties among the different articulating surfaces of the ankle joint and (2) to investigate the functional and biological properties of engineered neocartilage generated using chondrocytes from different locations in the ankle joint. Our hypothesis was that native articular cartilage from the tibia plafond would exhibit higher tensile, compressive, and shear moduli compared with the talar dome. Furthermore, we hypothesized that neocartilage generated using cells from the tibial plafond would show superior biomechanical properties and increased biosynthetic activity compared with the talar dome.

Methods

Chondrocyte Isolation and Construct Formation

The use of the specimens was evaluated and approved by the institutional review board. Articular cartilage was obtained from 14 fresh cadaveric healthy bovine knee and ankle joints, within 24 to 48 hours postmortem. Articular cartilage explants were harvested using 5-mm punches from the distal femur condyle and the tibia plafond, talar dome, and fibula of 7 knees and 7 ankles, respectively. All specimens were assayed biomechanically and biochemically to investigate joint- and location-dependent differences. Exclusion criteria were (1) evidence of cartilage degeneration, (2) presence of chondral or bone defects, or (3) any sign of joint injury or instability. The native tissue specimens were collected randomly from all topographic locations of the talar dome, tibial plafond, and fibula. By use of the 5-mm disks, 3-mm disks were punched out and used for creep indentation testing (7 for each location, 28 in total), as previously described.[23] For tensile testing, dog bone–shaped samples were created (7 from each location).[23] Tissue from the initial 5-mm disks was used for biochemical analysis as described later. This testing approach represents a validated method for evaluating biomechanical and biochemical properties of cartilage.[13,24,25]

For engineered neocartilage construct formation, chondrocytes were isolated from 7 cadaveric ankle and knee joints, as previously described.[23] In brief, tissue from the tibial plafond, distal fibula, and talar dome, using the femoral condyle as a control, was collected separately and minced into 1-mm pieces, followed by digestion in 0.2% type II collagenase (Worthington, Lakewood, NJ) for 18 hours, as previously described.[20,23] The digestion took place in cell culture medium made of Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Carlsbad, CA) containing 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). After the tissue had fully digested, the articular chondrocytes were washed 3 times in DMEM, including centrifugation and filtration through a 70-μm filter. Cells were then counted, and 4.5 million cells from each location separately were seeded into 2% agarose wells to form neocartilage constructs (7 per group, 28 in total).[20] Constructs were

Table 1. Growth Metrics of Neocartilage at End of 4-Week Culture

Location	Wet Weight (mg)	Hydration (%)	Diameter (mm)	Thickness (mm)
Tibial plafond	26.28 ± 4.36^{B}	84.12 ± 9.14^{B}	6.03 ± 0.48^{A}	1.07 ± 0.15^{B}
Fibula	25.09 ± 4.74^{B}	$86.81 \pm 9.03^{A,B}$	5.25 ± 0.39^{B}	1.02 ± 0.16^{B}
Talar dome	34.03 ± 7.21^{A}	90.51 ± 10.43^{A}	$5.66 \pm 0.53^{A,B}$	1.57 ± 0.19^{A}
Distal femur	21.74 ± 3.96^{B}	$88.45 \pm 9.94^{A,B}$	$5.65 \pm 0.57^{A,B}$	0.91 ± 0.12^{B}

NOTE. Values are presented as mean ± standard deviation. Groups marked with the same letter are not significantly different, whereas groups marked with different letters are statistically significant.

fed 500 μL of chondrogenic medium, consisting 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 μg/mL of L-proline, 50 μg/mL of ascorbate-2-phosphate, and 100 μg/mL of sodium pyruvate (Fisher Scientific, Pittsburgh, PA) daily for 10 days. At this time point, the constructs were unconfined from the agarose and transferred to 48-well plates, in which they were fed 1 mL of chondrogenic medium every other day until the end of culture at week 4.

Histology

Histologic evaluation of the tissue was performed as previously described.[26] Samples from both native and engineered tissue were cryoembedded and sectioned at 14 μm (7 for each group, 56 in total). Histology samples were fixed in formalin and then stained with safranin O/fast green and picrosirius red for glycosaminoglycan (GAG) and collagen staining, respectively.

Quantitative Biochemistry

Samples from both native and engineered tissue were frozen at −20°C for 24 hours and then lyophilized to determine their dry weights. A total of 28 samples of native tissue and 28 samples of engineered tissue were assayed. Subsequently, a pepsin digestion protocol was used to digest each sample.[26] A Blyscan Glycosaminoglycan dimethyl methylene blue assay kit (Accurate Chemical and Scientific, Westbury, NY) was used to quantify sulfated GAG content. Collagen abundance was determined using a chloramine-T hydroxyproline assay using a SIRCOL collagen standard (Biocolor, Carrickfergus, UK).

High-Performance Liquid Chromatography

Both native and engineered neocartilage samples (56 in total) were digested in 800 μL of 6N hydrochloric acid at 100°C for 18 hours, after which they were dried in a vacuum concentrator. Next, samples were suspended in 50 μL of 10-μmol/L pyridoxine and 2.4-mmol/L homoarginine and then diluted 5× in 0.5% heptafluorobutyric acid in 10% acetonitrile. For high-performance liquid chromatography, 10 μL of each sample was used as previously described,[27] by use of pyridinoline standards (Quidel, San Diego, CA), to quantify the cross-link content.

Biomechanical Testing

Samples were tested for tensile and compressive properties. For tensile testing, a uniaxial material testing system (Instron model 5565; Instron, Canton, MA) was used to apply uniaxial tension to dog bone–shaped samples created using a dermal punch.[26] Seven specimens were used from each location from both native and engineered tissue, resulting in 28 samples of native tissue and 28 specimens of engineered tissue. Samples were glued into paper frames and loaded into the machine grips. The gauge length was measured as the distance between the glued ends of the dog bone–shaped samples. On loading, a pull-to-failure test was run at a 1% gauge length per second until failure. The slope of the linear portion of each stress-strain curve was reported as the Young modulus (E_Y), whereas the peak of the curve was recorded as the ultimate tensile strength (UTS). For compressive and shear testing, a creep indentation apparatus was used.[28] Samples were tested as previously described[26] using a semi-analytical, semi-numeric, biphasic model[29] and finite-element optimization.[13] Each sample's aggregate modulus and shear modulus were analyzed. In choosing parameters for comparing different tissues, compression and tensile moduli were assayed because they are intrinsic mechanical properties. In contrast, the effect of shear forces on cartilage is mitigated by lubrication; the coefficient of friction is not an intrinsic property but, instead, depends on tissue curvature, surface roughness, and a multitude of other parameters including the lubrication regimen. As intrinsic properties, tensile and compressive moduli are commonly measured to inform the mechanical quality of cartilage. Thus both tensile and compressive tests were performed such that we would be able to compare our data with the literature values[13]; the value of the shear modulus was also calculated.

Statistical Analysis

All quantitative assessments in this study were performed using seven samples. To compare among different groups, 1-way analysis of variance was performed by use of JMP 9 software (SAS Institute, Cary, NC). If significance was identified ($P < .05$), a Tukey HSD (honestly significant difference) post hoc test was applied. In Tables 1-3, groups marked by different

Table 2. Biomechanical and Biochemical Properties of Native Articular Cartilage Harvested From Knee Joint and Different Topographic Locations of Ankle Joint

Native Tissue	E_Y (MPa)	Aggregate Modulus (kPa)	Collagen/WW (%)	GAG/WW (%)	Shear Modulus (kPa)
Distal femur	4.06 ± 0.54 (4.46, 3.66)^{B}	274 ± 18 (310, 239)^{B}	13.47 ± 3.04 (15.72, 11.22)^{B}	11.28 ± 0.88 (11.93, 10.63)^{B}	134 ± 9 (140, 127)^{B}
Tibia	4.82 ± 0.51 (5.20, 4.44)^{A}	370 ± 22 (415, 326)^{A}	20.47 ± 3.42 (23.01, 17.94)^{A}	17.95 ± 1.46 (19.03, 16.87)^{A}	180 ± 11 (188, 171)^{A}
Fibula	3.86 ± 0.12 (3.95, 3.77)^{B}	242 ± 18 (279, 205)^{B}	14.39 ± 2.76 (16.44, 12.35)^{B}	10.82 ± 1.99 (12.30, 9.34)^{B}	119 ± 8 (125, 113)^{C}
Talus	1.7 ± 0.24 (1.88, 1.52)^{C}	255 ± 26 (307, 203)^{B}	8.42 ± 1.79 (9.75, 7.10)^{C}	10.07 ± 1.36 (11.08, 9.06)^{B}	126 ± 12 (135, 117)^{B,C}

NOTE. Values are presented as mean ± standard deviation with confidence intervals of 95% included in parenthesis (+95% confidence interval, −95% confidence interval). Tukey post hoc testing was applied if $P < .05$. Groups marked with the same letter are not significantly different, whereas groups marked with different letters are statistically significant.

Table 3. Biomechanical and Biochemical Properties of Neocartilage Generated Using Chondrocytes From Knee Joint and Different Topographic Locations of Ankle Joint

Engineered Tissue	E_Y (MPa)	Aggregate Modulus (kPa)	Collagen/WW (%)	GAG/WW (%)	Shear Modulus (kPa)	Pyridinoline/WW (nmol/mg)	Pyridinoline/Collagen (nmol/mg)
Distal femur	0.65 ± 0.17 (0.77, 0.52)^{B}	97 ± 35 (123, 71)^{B}	1.67 ± 0.20 (1.82, 1.53)^{B}	4.03 ± 0.48 (4.38, 3.67)^{B}	47 ± 17 (60, 34)^{B}	0.05 ± 0.02 (0.06, 0.04)^{A,B}	0.03 ± 0.01 (0.04, 0.02)
Tibia	0.89 ± 0.23 (1.06, 0.72)^{A}	157 ± 52 (198, 118)^{A}	2.45 ± 0.35 (2.71, 2.18)^{A}	5.83 ± 0.46 (6.17, 5.49)^{A}	76 ± 24 (99, 58)^{A}	0.07 ± 0.04 (0.10, 0.04)^{A}	0.03 ± 0.02 (0.04, 0.01)
Fibula	0.64 ± 0.16 (0.77, 0.52)^{B}	104 ± 43 (136, 72)^{B}	1.64 ± 0.19 (1.78, 1.50)^{B}	4.02 ± 0.56 (4.43, 3.60)^{B}	50 ± 21 (66, 34)^{B}	0.03 ± 0.01 (0.04, 0.02)^{B}	0.02 ± 0.01 (0.03, 0.01)
Talus	0.24 ± 0.13 (0.34, 0.14)^{C}	92 ± 40 (122, 62)^{B}	1.12 ± 0.08 (1.18, 1.07)^{C}	4.28 ± 0.57 (4.70, 3.86)^{B}	45 ± 20 (60, 30)^{B}	0.03 ± 0.01 (0.04, 0.03)^{B}	0.03 ± 0.01 (0.04, 0.02)

NOTE. Values are presented as mean ± standard deviation with confidence intervals of 95% included in parenthesis (+95% confidence interval, −95% confidence interval). Tukey post hoc testing was applied if $P < .05$. Groups marked with the same letter are not significantly different, whereas groups marked with different letters are statistically significant.

letters represent significant differences. Values are presented as mean ± standard deviation with confidence intervals of 95% included in parenthesis (+95% confidence interval, −95% confidence interval). Positive correlations in regression analysis were considered present when a positive sloping regression line was depicted graphically, while negative correlations were considered present when a negative sloping regression line was depicted. A strong correlation was considered present when $1 > R^2 > 0.5$ and a weak correlation when $0.5 > R^2 > 0$.

Results

Gross Morphology and Histology

No significant differences were observed in the macroscopic appearance among cartilage explants taken from different topographic locations. At the end of a 4-week culture period, chondrocytes from all topographic locations formed disk-shaped constructs, having flat surfaces and no macroscopic deformities. In terms of the growth metrics of neocartilage (Table 1), neotissue from the talar dome had approximately 50% increased thickness compared with all other groups ($P = .0001$). The increased water content and wet weight reflect this difference. Neocartilage formed from cells of the tibial plafond, on the other hand, presented with significantly increased diameter ($P = .001$). Histologic evaluation of the neotissue was consistent among all groups (Fig 1). There were no technical failures in any sample, and no sample was excluded from the analysis.

Biochemical Analysis

Collagen, GAG, and pyridinoline content of native cartilage from different topographic locations of the ankle joint were evaluated. In terms of collagen content of native tissue, the tibial plafond cartilage exhibited a 3-fold increase in collagen content normalized to tissue wet weight in comparison with the talus cartilage, which exhibited the lowest amount of collagen ($P < .0001$) (Table 2); no significant differences were observed between distal femur and fibula cartilage. Regarding the GAG content of the tissues, tibial plafond cartilage presented with the highest amount of GAG per wet weight, whereas no significant differences were observed among the other groups (Table 2 and Fig 2). Regarding the amount of pyridinoline cross-links per wet weight and per collagen, no significant differences were detected among the groups (Table 2).

Similar trends were observed in the neocartilage constructs formed using cells from the corresponding locations. Specifically, tibial plafond neocartilage presented with the highest amount of collagen per wet weight among all groups, whereas the talus constructs presented the lowest ratio of collagen per wet weight ($P < .0001$) (Table 3 and Fig 3). No significant differences were detected between the femur and fibula constructs' collagen content. A strong positive correlation ($R^2 = 0.92$, $P < .05$) was detected between collagen content and E_Y. In terms of the GAG content of the neocartilage, tibial plafond constructs synthesized the highest GAG per wet weight, with an approximately 50% increase, compared with the other groups ($P < .0001$), whereas no significant differences were

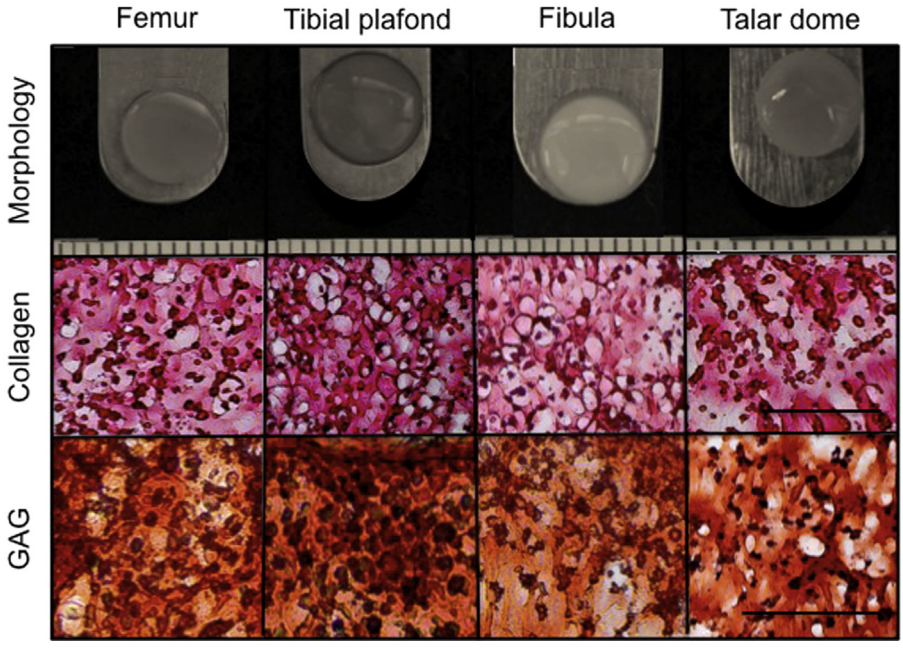

Fig 1. Gross morphology/histology of neocartilage, with picrosirius red staining for collagen and safranin O/fast-green staining for GAG. The histology scale bar represents 100 μm.

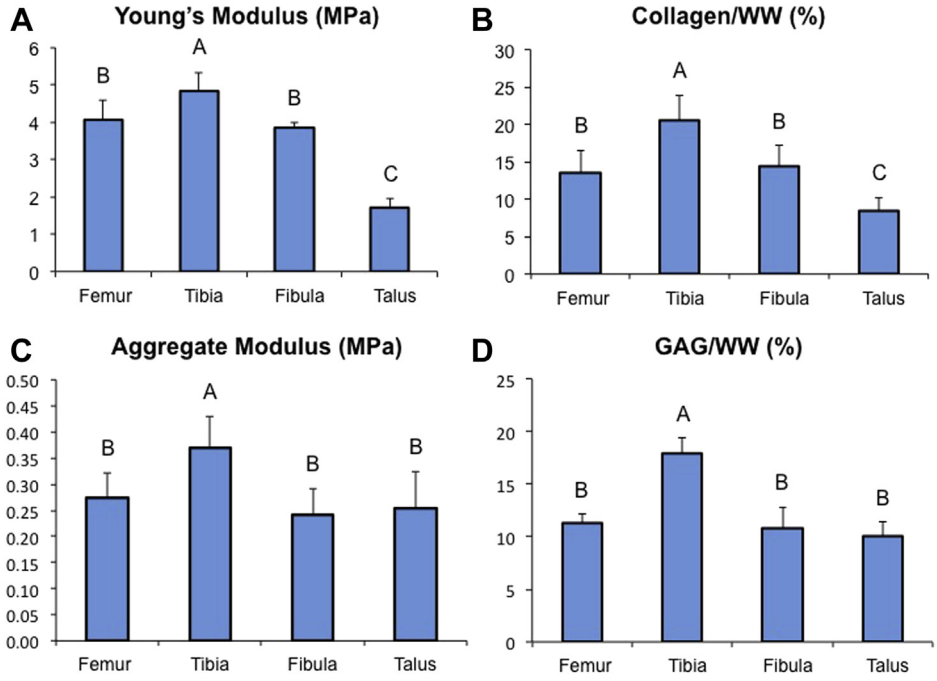

Fig 2. Biomechanical and biochemical properties of native articular cartilage from different topographic locations of ankle joint. Femoral condyle explants were used as a control. (WW, wet weight.)

observed among them. Evaluation of the pyridinoline content of neotissues showed tibial plafond neocartilage to exhibit the highest amount of pyridinoline per wet weight among all groups ($P = .02$). However, no significant differences were observed among groups regarding pyridinoline content per collagen. Finally, a positive correlation was detected between pyridinoline content and the tensile modulus of neocartilage (Fig 4).

Biomechanical Analysis

Biomechanical evaluation of the native articular cartilage compared the functional properties of the tissue harvested from different topographic locations of the ankle joint and the distal femur. Tensile testing showed the tibial plafond cartilage to exhibit the highest E_Y and UTS over the other regions, whereas talus cartilage presented with the lowest properties

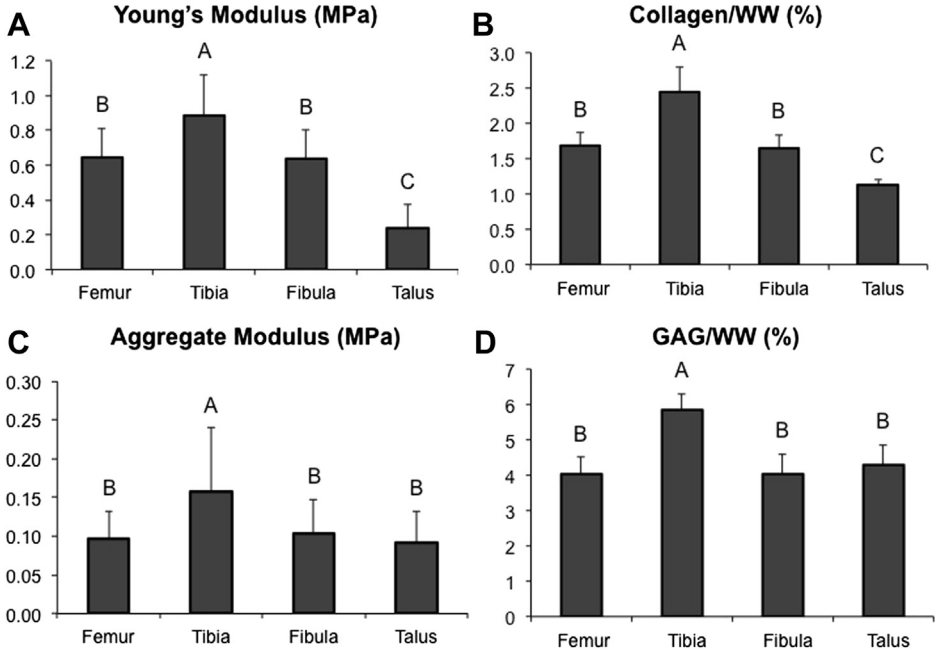

Fig 3. Biomechanical and biochemical properties of neocartilage generated using chondrocytes from different topographic locations of ankle joint. Femoral condyle chondrocytes were used as a control. (WW, wet weight.)

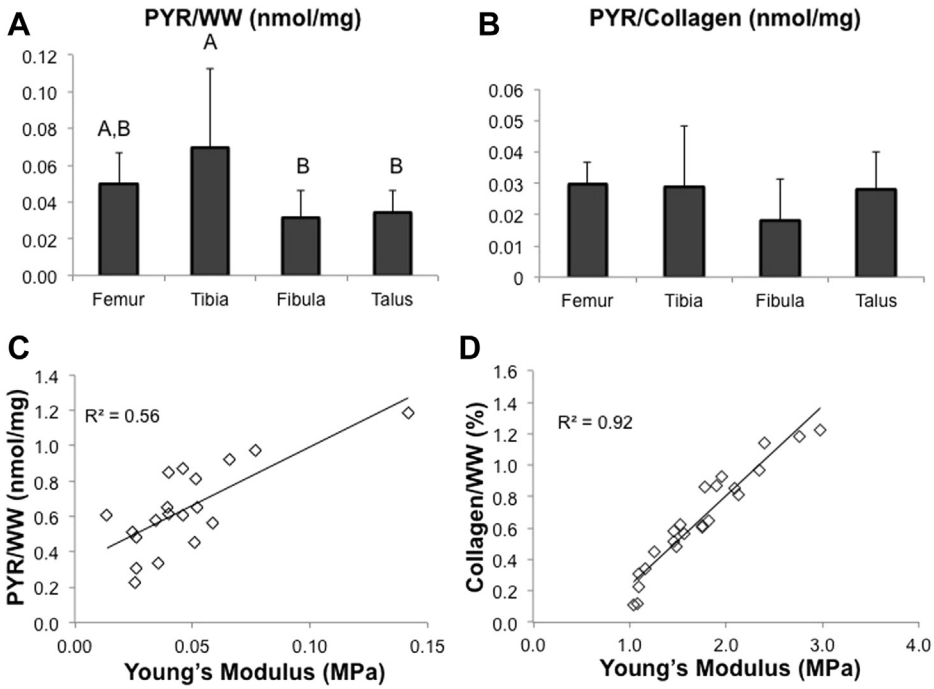

Fig 4. Pyridinoline (PYR) content of neocartilage per wet weight (WW) and per collagen. A positive correlation was detected between pyridinoline content and the tensile modulus of neocartilage, as well as between collagen content and the tensile modulus.

($P < .0001$). Specifically, tensile strength was 3.2 ± 0.3 MPa, 2.4 ± 0.2 MPa, 1.0 ± 0.1 MPa, and 2.3 ± 0.2 MPa for the tibial plafond, distal fibula, talar dome, and femoral condyle, respectively. No significant differences were observed between distal femur and distal fibula cartilage tensile properties. In terms of the compressive properties, tibial plafond cartilage presented with a significantly higher aggregate modulus and permeability comparatively ($P < .0001$); no significant differences were observed among the other groups. Shear modulus values were significantly higher in the tibial plafond in comparison with all other groups ($P < .0001$) (Table 2 and Fig 2).

Similar trends were observed when we evaluated the biomechanical properties of neocartilage formed using cells from different topographic locations of the ankle joint and distal femur. Tibial plafond neocartilage presented with significantly higher compressive moduli compared with all groups ($P = .02$); no significant differences were observed among the other groups. In terms of the tensile properties, tibial neocartilage yielded the highest E_Y and UTS, whereas no significant differences were observed among the other groups (Fig 3). Specifically, tibial plafond constructs exhibited 300% more tensile strength and 170% more compressive moduli compared with the talar dome ($P < .0001$). Neocartilage constructs from tibial plafond chondrocytes showed a significantly higher shear modulus compared with the other groups ($P = .02$) (Table 3 and Fig 3).

Discussion

This study shows that the biomechanical and biochemical properties of different topographic locations within the ankle joint exhibit significant variations. Specifically, tibial plafond articular cartilage exhibited significantly higher tensile properties in comparison with all other articular surfaces of the ankle joint. More importantly, the opposing articulating surfaces were found to have significantly different biomechanical and biochemical properties, predisposing the talar dome cartilage to degenerative pathologies. The tensile modulus and strength of talar dome cartilage were 3 times lower compared with tibial plafond cartilage. The compressive properties were also higher in the native tibial plafond compared with all other topographic areas. The disparity of tensile, shear, and compressive properties of opposing cartilage surfaces, which could potentially lead to degenerative changes of the biomechanically inferior articular surface, should be evaluated in clinical studies in an attempt to explain the increased occurrence of chondral lesions in the talus.

In addition to the mismatches detected in native cartilage, the findings of this study highlight the dissimilar biological behavior of cells isolated from different topographic regions within the ankle joint, as evaluated biomechanically, biochemically, and histologically. To assess cellular response, the self-assembling process was used because it has been shown to recapitulate the fundamental in vivo biological processes of articular cartilage generation and

development. The biomechanical properties of the neocartilage followed the same pattern with native tissue. For instance, constructs made of chondrocytes from the tibial plafond showed 4 times higher tensile properties compared with constructs engineered from talar dome chondrocytes. The compressive and shear moduli of the tibial plafond neocartilage were 2 times higher than those formed from talar dome cells. A strong positive correlation was detected between the biomechanical properties and biochemical profile of the neocartilage. The amount of collagen and GAG produced from tibial plafond cells was remarkably higher compared with other locations. The use of the self-assembling process toward neocartilage generation confirmed that the cells of the different topographies within the ankle joint exhibit differences in their synthetic and metabolic activity. The results of this study imply that the biological response to injury or other pathology of the different topographic areas may likewise vary. Ankle chondrocytes of the talar dome may exhibit a significantly lower potential to respond to injury and lower healing capacity in comparison with chondrocytes of the opposing tibial plafond. This study evaluated the biomechanical and biochemical characteristics of articular cartilage from areas of the ankle joint most commonly implicated in chondral lesions; it did not assess the biomechanical behavior of bone tissue. The propensity of each part of the ankle joint for fractures may be attributable to factors such as anatomic and biomechanical features of the subchondral bone and mechanism of injury. Different biological behavior at the cellular level might be an additional pathogenic mechanism that contributes to the increased occurrence of chondral lesions in the talar dome compared with the tibial plafond.

The ankle and knee are remarkably different joints in terms of their anatomic features, prevalence of chondral lesions, and chondrocyte characteristics, with the ankle joint exhibiting significantly lower susceptibility to osteoarthritis.[9] Factors that could potentially explain the dramatically different prevalence of degenerative changes between the 2 joints are the different biomechanical properties of articular cartilage between these joints,[11,13] differences in collagen and GAG content of the tissues,[14] variances in the thickness of the 4 zones of cartilage,[10] and different metabolic activity[15] of articular chondrocytes. Furthermore, differences between ankle and knee chondrocytes in their response to catabolic stimuli have been previously identified.[10] This study's findings are in accordance with the literature showing a potential disparity in biomechanical properties and biochemical content between knee and ankle articular cartilage and differences in the biological behavior of the knee and ankle chondrocytes. Future studies may shed more light on the potential mechanisms underlying the difference in the prevalence of osteoarthritis between these 2 joints.

The use of autologous chondrocytes and cartilaginous grafts is continuously increasing for the treatment of chondral lesions in the ankle joint. For instance, autologous chondrocyte implantation for the treatment of lesions located in the talus is gaining interest.[30,31] Traditionally, the cells were isolated from the ipsilateral knee joint. Some studies suggested the use of talus chondrocytes,[32,33] on the basis of the lower incidence of osteoarthritis in the ankle joint. Similarly, talar allografts have been used for large osteochondral defects of the talus.[34,35] Even though the efficiency of these procedures still needs to be evaluated with large randomized controlled trials, a potential limitation for the use of ankle chondrocytes is the potential loss of these favorable properties when expanded in monolayer culture.[36] In addition, there is no consensus in the literature regarding the proteoglycan and collagen synthesis characteristics between ankle and knee chondrocytes.[12,14,15,36] On the basis of the findings of this study, chondrocytes from the tibial plafond produced higher amounts of collagen and GAG and also had a superior capacity for generating new tissue, as compared with those isolated from the femur. In contrast, chondrocytes isolated from the talar dome appeared to have worse biomechanical properties and a worse biological profile than femur chondrocytes. The tensile, shear, and compressive properties of the native femoral cartilage were inferior only to those of the tibial plafond, with the talus exhibiting significantly lower biomechanical properties. As a consequence, the effectiveness of the use of ankle chondrocytes or allografts for these repairs could be potentially amplified when tibial plafond chondrocytes or grafts are used. Further research is required to identify the characteristics of chondrocytes and cartilage tissue in different topographic locations, suggesting that current therapeutic modalities need to consider the exact topographic source of the cells or cartilage grafts used.

An interesting finding of this study is the comparatively large difference observed between the tensile properties of the talar dome and those of the tibial plafond, in contrast to the much smaller difference between their compressive properties. Specifically, tibial plafond cartilage shows a 3-fold difference in tensile moduli and strength in comparison with the talar dome in native and engineered tissue. In contrast, the talar dome exhibits only 50% to 60% less compressive modulus in native and engineered tissue. In the literature, degeneration of cartilage is correlated with repetitive compressive forces.[10,37] However, various reports indicate that cartilage is more prone to injuries during shear loading of the joint.[38] Excessive shear strain causes abundant collagen fibril strain that has been shown to correspond accurately with areas of collagen destruction leading to

cartilage damage.[39] Our results showed that the tibial plafond exhibited 70% higher shear moduli in comparison with the talar dome. This study showed an important disparity in the ability of the tibial plafond and talus to withstand tensile, compressive, and shear forces, indicating the presence of a complex pathogenic mechanism responsible for cartilage degeneration. Future research should focus on evaluating the role of tensile and shear loading in the development of chondral lesions and degenerative osteoarthritis.

To our knowledge, this is the first study to use chondrocytes from the tibial plafond and other ankle locations to engineer articular cartilage without the use of scaffolds. Tissue engineering represents a promising technique for developing neocartilage that could be used in the treatment of chondral and osteochondral lesions. Different mechanical and biochemical stimuli can be combined to enhance the functional properties of the engineered tissue on par with native tissue values.[23,27,40,41] In this study the native tibial plafond exhibited significantly better properties in comparison with all other locations of the ankle joint. The measured differences of tibial plafond articular cartilage were also mirrored in the biomechanical and biochemical properties of neocartilage constructs generated using tibial plafond cells. These results may suggest that cells from a specific topographic source need to be tailored for use in particular indications.

Limitations

One limitation of this study is that the biomechanical and biochemical properties were evaluated in an in vitro model. It remains to be seen whether these in vitro results would be reflected by in vivo findings. Another limitation is that there was no separate analysis of the different topographic locations in each location. The limited amount of cartilage in each location along with the high number of chondrocytes needed for neocartilage generation prevented a separate analysis. Native tissue specimens were randomly collected from all different locations in each group. Similarly, to evaluate cellular behavior, no in vivo studies were used because the in vitro model of the self-assembling process was used. However, this model has been shown to be representative of the processes of tissue development and maturation.

Conclusions

The cartilage properties of the various topographic locations within the ankle are significantly different. In particular, the opposing articulating surfaces of the ankle have significantly different biomechanical and biochemical properties. The disparity between tibial plafond and talar dome cartilage and chondrocytes warrants further evaluation in clinical studies to evaluate their exact role in the pathogenesis of ankle lesions.

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