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RESEARCH ARTICLE

Tribology of enzymatically degraded cartilage mimicking early osteoarthritis

Ke REN[1], Miguel Alejandro REINA MAHECHA[1], Maike HÜBNER[1], Zhiwei CUI[2], Hans J. KAPER[1], Hugo C. VAN DER VEEN[3], Prashant K. SHARMA[1,*]

[1] *Department of Biomedical Engineering, University Medical Centre Groningen, University of Groningen, Groningen 9713AV, the Netherlands*

[2] *Department of Mechanical Engineering, Eindhoven University of Technology, Eindhoven 5600MB, the Netherlands*

[3] *Department of Orthopedic Surgery, University Medical Centre Groningen, University of Groningen, Groningen 9700RB, the Netherlands*

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Abstract: Healthy cartilage is a water-filled super lubricious tissue. Collagen type II provides it structural stability, and proteoglycans absorb water to keep the cartilage in a swollen condition, providing it the ability to creep and provide weeping lubrication. Osteoarthritis (OA) is a degenerative and debilitating disorder of diarthrodial joints, where articular cartilage damage originates from enzymatic degradation and mechanical damage (wear). The objective of this research is to observe the level of cartilage damage present in knee arthroplasty patients and to understand the friction and creep behavior of enzymatically degraded bovine cartilage *in vitro*. Lateral (Lat) and medial (Med) condylar cartilages from OA patients undergoing total knee arthroplasty showed signs of enzymatic degradation and mechanical damage. Bovine cartilages were exposed to collagenase III and chondroitinase ABC to degrade collagen and proteoglycans, respectively. The loss of proteoglycans or collagen network and morphological changes were observed through histology and the atomic force microscope (AFM), respectively. A significant effect on creep due to enzymatic treatment was not observed. But the enzymatic treatment was found to significantly decrease the coefficient of friction (COF) at 4 N, while higher COF was shown from chondroitinase ABC degraded cartilage at 40 N. Collagenase III treatment leads to the release of intact proteoglycans at the sliding interface, while chondroitinase ABC treatment leads to the loss of chondroitin sulfate (CS) from the proteoglycans. Chondroitinase ABC-digested bovine cartilage mimicked patient samples the best because of the similar distributions of proteoglycans, collagen network, and friction behavior.

Keywords: lubrication; cartilage; boundary lubrication; osteoarthritis (OA)

1 Introduction

Hyaline cartilage is a biological material, which in combination with the synovial fluid and sliding against cartilage or meniscus is known to provide ultralow friction [1] and extraordinary load-bearing capability [2]. Its function ensures smooth movement and protects articulating bones from being worn off [3]. The cartilage's unique mechanical and tribological properties can be attributed to its composition and structure [4]. Cartilage is made up of a dense extracellular matrix (ECM > 95%) with a sparse distribution of chondrocyte cells (< 5% of the volume). The ECM consists mainly of water, which makes up to 60%–85% of the wet weight of cartilage. The solid part is composed of collagen (15%–22% wet weight, most are collagen type II) [5] and proteoglycans (4%–7% wet weight) [6], as well as elastin [7], hyaluronic

* Corresponding author: Prashant K. SHARMA, E-mail: pk.sharma@umcg.nl

acid (HA), phosphatidylcholine (PC) lipids, and other smaller proteins including lubricin and cartilage link protein [8].

Osteoarthritis (OA) is a degenerative and debilitating joint disease that affects an estimated 15% of the population worldwide [9]. Clinical symptoms include chronic pain, joint instability, stiffness, and radiographic joint space narrowing [10]. To the early signs of diseased belongs, cartilage fibrillation of the superficial layer as well as swelling increased permeability and decreased stiffness due to a loss of proteoglycans in the cartilage matrix [11, 12]. The swelling and increased water content of the cartilage are caused by the disruption of the collagen network since the network is not able to adequately counteract the swelling properties of proteoglycans [13]. The loss of proteoglycans diminishes the tissue's ability to retain water. Stiffness and compressive strength are thus reduced. Histologically, a loss of proteoglycan content in the upper layers and microscopic disruption of the superficial layer has been observed [14].

The early stages of OA are characterized by the progressive breakdown of the ECM [15, 16]. The molecular mechanisms and the order of progression of the disease on this level are to this day not entirely clear. There is mounting evidence however, that cartilage destruction happens due to inflammatory processes on the molecular level. Jahn et al. [17] hypothesized that cartilage degeneration in the early stage of OA might initiate due to high friction, e.g., caused by an initial trauma or injury, leading to increased shear strain of the cartilage, which may cause the chondrocytes to produce inflammatory mediators such as cytokines and chemokines (e.g., interleukin (IL)-1, IL-6, IL-8, IL-17, and IL-18). These factors increase chondrocyte catabolism and the production of proteolytic enzymes such as matrix metalloproteinase (MMP) and aggrecanase, leading to cartilage ECM destruction. Upon articulation, degraded tissue is then abraded, which results in a self-reinforcing cycle of higher friction, higher shear strains, and further abrasion [17].

At present, there is an enormous scientific effort [18–27] to develop strategies to enhance cartilage lubrication and chondroprotection in order to counteract OA development. Intra-articular injection of HA (called viscosupplementation) to improve joint lubrication, alleviate pain, and delay surgery is a modus operandi, but with limited pain relief [28, 29]. It is desired that adding functional macromolecules to the viscosupplements could enhance lubrication [22, 25, 30–32] and prevent further damage at an early stage of the disease. In order to optimize the macromolecules for maximum functionality, *in vitro* test methodologies are necessary, which are inexpensive and have lower approval hurdles at universities and companies worldwide compared to *in vivo* test models [33]. Besides rare studies, which use healthy human cartilage [20], healthy animal cartilage is a popular choice for such lubrication studies [25, 34–36]. But healthy animal cartilage is unable to mimic early OA correctly. Some studies do use enzymatically degraded animal cartilage, but various enzymes have been used in the past (Table 1) with differences in protocol, making it difficult to decide on the suitable model for use. The effect of enzymatic degradation on the tribological and biomechanical properties of cartilage is unclear. Furthermore, some enzymes (e.g., trypsin) non-specifically degrade both collagen and proteoglycans, making it challenging to understand the mechanisms.

Collagenase III (also known as MMP-13) plays an essential role in the natural processes occurring during OA. It is highly efficient in breaking down collagen type II [44]. In addition, chondroitinase ABC is used to break down glycosaminoglycans (GAG) side chains: chondroitin sulfates (CSs) [37].

The aims of this study are two folds. 1) To understand the changes in the tribological behavior including COF and dynamic creep of cartilage when collagen type II is degraded or proteoglycans are degraded individually; 2) to understand which of the two enzymes mimics early OA in an in vitro model using bovine cartilage. Collagenase III and chondroitinase ABC are utilized to degrade collagen and proteoglycans, respectively. The study uses histology, surface morphology, and biomechanical and tribological characteristics to compare OA patient cartilage to degraded bovine cartilage and arrives at a recommendation. Although both porcine [40] and bovine cartilage [30, 37–39, 41–43] have been used for cartilage tribology research (Table 1), bovine cartilage seems to be more popular due to its similarity with human articular cartilage in terms of tissue structure,

Table 1 Studies about effects of enzymatic degradation of cartilage on the coefficient of friction (COF).

Research group	Animal model	Ref.	Enzyme			Degradation			Friction model			Diameter bone plug (mm)	Lubricant				Normal pressure (MPa) Normal load (N)	Sliding speed (mm/s)	Sliding distance (mm)	Outcome
			Colla-genase	Trypsin	Chon-droitinase	Concen-tration (U/mL)	Volume (mL)	Time (h)	Cartilage–cartilage	Cartilage–glass	Cartilage–metal		Phosphate buffered saline (PBS)	Other saline	HA	Synovial fluid				
Basalo et al.	Bovine	[37]			×	0.1	3	24		×		4.78	×				0.5 MPa	1	4	Degraded cartilage increased COF
Katta et al.	Bovine	[38]			×	0.1		24	×			9	×				0.5, 2, and 3.15 MPa	4	4	Higher COF for low pressure (0.5 MPa), lower for high pressure (> 2 MPa)
Morgese et al.	Bovine	[30]			×	1		1	×			2				×	5 and 10 N	5		Degraded cartilage increased COF
Pickard et al.	Bovine	[39]			×	2.5	1				×	3		×			0.5 and 4 MPa	4	40	No difference
Kumar et al.	Porcine	[40]			×	1		12		×		8		×	×		27 N	1		Degraded cartilage increased COF
Lee et al.	Porcine	[41]	×		×				×			—	×			×		0.1	0.1	Digestion reduces stick–slip
Chan et al.	Bovine	[42]		×							×	—								Increased COF for degraded cartilage
Hills and Monds	Bovine	[43]		×												×				Trypsin digestion decreased COF

condyle size, thickness, and biomechanical properties [45–47]. And according to the theory from Moore and Burris [34], the diameter of cartilage plugs affects the lubricious behavior through rehydration. Thus, instead of osteochondral plugs, whole bovine femoral condyle was used to imitate the knee joint better and avoid cartilage depressurization [30, 37–39, 41–43].

2 Materials and methods

2.1 Preparation of OA human cartilage

Seven OA human knee joint cartilages from medial front and back (Med and Med B) and lateral front and back (Lat and Lat B) parts of condyles in two patients (namely Patient 1 and Patient 2), cut off during standard joint replacement surgery, were obtained from the department of orthopedics, University Medical Center Groningen (UMCG) under the METc approval of M20.251839 and research registry of UMCG 5649339. The samples were washed in sterile PBS to remove any remaining blood, and then kept in sterile phosphate PBS at 4 °C for 2 h, during which the measurements were performed.

2.2 Histology of OA human cartilage

The OA human cartilages were fixated in 10% formalin solution and stored at 4 °C for at least 4 d before decalcification. After fixation, the samples were placed in 10% ethylenediaminetetraacetic acid (EDTA) solution and decalcified at room temperature for 6–8 weeks [48] and exchanged 1–2 times a week. Following decalcification, the cartilage and a bone layer of about 1 mm were cut off with a scalpel, and pieces of around 8 mm × 2 mm × 2 mm were made. The samples were dehydrated, and paraffin embedded. The embedded samples were then manually sliced into 5 μm thickness using a microtome (RM2265, Leica, Germany). To visualize collagen II or proteoglycan content, cartilage sections were stained with Safranine O–Fast Green counterstain or Picrosirius Red, respectively [48].

2.3 Tribological testing of OA human cartilage

All tribological measurements were performed using a Universal Mechanical Tester (UMT) (UMT-3, Bruker, USA). The samples were mounted in the UMT and preloaded to the specified normal load, which was held constant for 5 s before starting the measurement. Frictional experiments were performed on cartilage samples (n = 3, n means the number of bovine cartilage samples) against borosilicate glass (Schott BK-7, Gerhard Menzel GmbH, Germany), using UMT with reciprocal sliding motion (±30 mm at 6 mm/s). In principle, borosilicate glass and stainless steel are widely applied as the primary counter substrate [49]. Here we chose the former because it is much more hydrophilic (the contact angles of both glass and steel are shown in Fig. S1 in the Electronic Supplementary Material (ESM)) and its ability to interact with macromolecules on cartilage surface (i.e., PC lipids) [50]. The experiments with a normal load of 4 N using the high performance force sensor (DFM-1.0, Bruker, USA) load cell were conducted to mimic the swing phase in the knee. 150 cycles were performed (back and forth), corresponding to a measurement duration of 1,500 s. The temperature during measurements was held constant at 33 °C, matching the intra-articular temperature in the knee joint during mild activity [51]. To avoid drying of the cartilage during the experiment, 5 mL of PBS was added at the cartilage glass interface. During the measurements, not only the COF, but also the dynamic creep (as a descent of the carriage position z) was determined. The procedure of COF and dynamic creep test in detail is shown in Fig. 1.

2.4 Preparation of the bovine femoral condyle for experiments

Fresh knee joints from approximately two-year-old bulls were obtained from the local abattoir (Kroon Vlees, the Netherlands) and processed within 24 h after slaughter. Flesh, ligaments, and joint capsule were carefully removed using a scalpel without touching the cartilage surface. Cartilage samples were taken from the central area of both femoral condyles. An oscillating electric saw (MultiTalent 250 SL, C. & E. Fein GmbH, Germany) with a wide blade (Starlock MB225, MultiBlade, the Netherlands) was used to dissect each condyle into a 2 cm × 4 cm piece of cartilage with approximately 1.5 cm underlying bone. During the cutting process, the cartilage was irrigated with sterile PBS. Until further processing, the samples

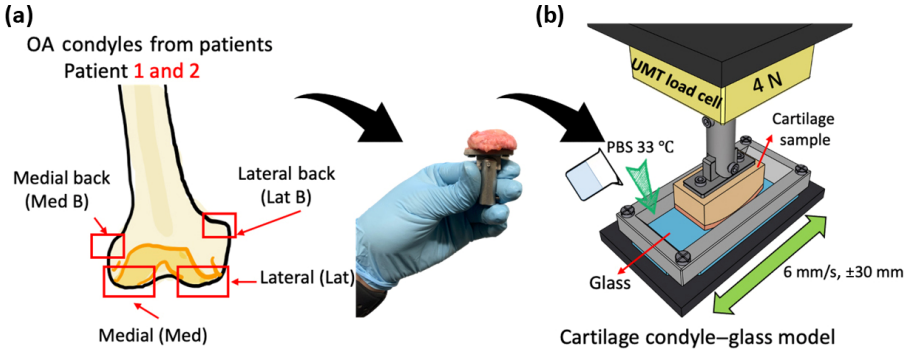

Fig. 1 Schematic of OA condyle cartilage treatment from patients: (a) OA human condyles obtained from four different parts (Lat, Lat B, Med, and Med B); (b) tribological test, where condyles were slid against glass in PBS solution at 4 and 40 N.

were kept in sterile PBS at 4 °C for approximately 0.5 h. The whole experiment processing of bovine cartilage enzymatic degradation is shown in Fig. 2.

2.5 Enzymatic degradation of bovine cartilage

Two different enzymes, namely chondroitinase ABC and collagenase III, were used to degrade various cartilage matrix components to achieve the digestion of ECM similar to the changes in early OA. An overview of all enzymes with reference to concentrations used in the previous digestion protocols can be found in Table 2. The concentration and optimum pH of the enzymes used in this study and their catalogue numbers (CAT) from the corresponding suppliers are specified in Table 2.

The tris buffer is recommended for enzymatic digestion with MMPs such as collagenase III and chondroitinase ABC [37, 44]. In order to maintain a standard protocol, the tris buffer at pH 7.5 was used for degradation with all enzymes. Sodium hydroxide was used to adjust the pH. For the solubility of chondroitinase ABC, 0.02% bovine serum albumin (BSA) had to be added to the buffer. To sterilize the buffer, it was syringe-filtered with a 0.22 μm filter (SLG033SB, Merck Millex, Germany).

The prepared bovine femoral condyles were placed in contact with a 5 mL tris buffer containing the enzyme and 1% antibiotics in such a way that the cartilage was completely submerged in the degradation solution. The samples were left to degrade in the

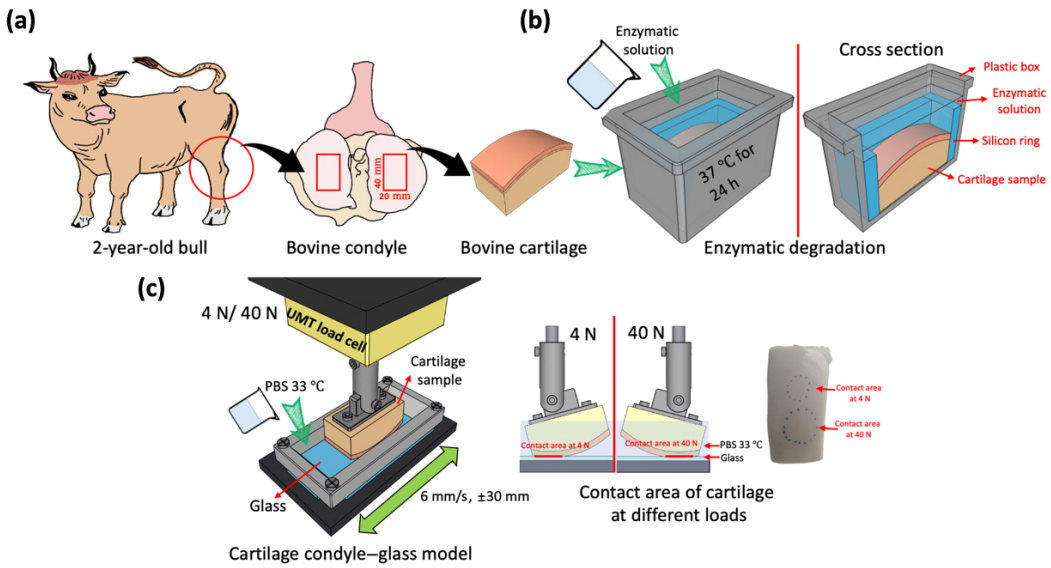

Fig. 2 Schematic of bovine condyle cartilage treatment: (a) bovine condyles harvested from a two-year-old bull; (b) digestion of bovine condyles; and (c) tribological test, where condyles were slid against glass in PBS solution at 4 and 40 N. The contact area was determined after rubbing tests.

Table 2 Specifications for all enzymes used in this study.

Enzyme	Concentration	Ref.	pH	Supplier	CAT
Collagenase III	50 μg/mL	[44]	7.5	STEMCELL Technologies	07422
Chondroitinase ABC	0.1 U/mL	[37]	7.5	Sigma Aldrich	C3667

incubator at 37 °C for 24 h. Untreated cartilage samples as a control group were just left in a 5 mL tris buffer in the same condition.

2.6 Bovine cartilage morphology and roughness measurement with the AFM

The samples were taken out of the enzyme solution for approximately 15 min before the measurement, and they were thoroughly rinsed, and then left to equilibrate in PBS. Before the test, the samples were fixated in 10% formalin solution (Chemical Abstracts Service (CAS) 50-00-0, VWR chemicals, USA) for at least 48 h. The surface roughness of the samples were measured by the AFM (Nanoscope IV Dimension 3100, Veeco, USA) equipped with a dimension hybrid *XYZ* scanning probe microscopy (SPM) head (Nanoscope IV Dimension 3100, Veeco, USA) on the differently cartilage faces with a scan area of 50 μm × 50 μm and a scanning frequency of 1 Hz. The surface topography was measured by the AFM operating with the contact mode in air using a non-conductive silicon nitride probe (DNP-10, Bruker, USA) at a constant normal force of 5 nN. Data analysis was performed on NanoScope Analysis software (Bruker, USA). The root mean squared (RMS) roughness ($R_q = \sqrt{\frac{1}{n}\sum_{i=1}^{n} y_i^2}$) was calculated from the average height at three locations per sample [42]. Due to the sample curvature, all images were flattened with a 1st or 2nd order before calculating the roughness.

2.7 Histology measurement of bovine cartilage

The treatment of histology measurement of bovine cartilage was the same as that of OA human cartilage in Section 2.2.

2.8 Mechanical and tribological testing with the UMT

All mechanical and tribological measurements were performed using UMT in the same way as human samples in Section 2.3.

2.8.1 *Stiffness and static creep*

Cartilage was pressed against borosilicate glass, and the deformation as a function of the applied load was measured and used as an indicator of stiffness. The cartilage was loaded with a linearly increasing force (2 N/min, max 4 N), while the deformation was registered by a capacitive sensor, attached to the side of the holder. Deformation was converted in strain using the cartilage thickness of 2 mm. Force (N) was converted into stress (Pa = N/mm^2) by dividing with the contact area. The slope of the stress vs. strain graph was taken as the stiffness of the cartilage. Following the stiffness measurement, static creep, i.e., gradual increase in deformation at a constant applied load of 4 N, was recorded for 700 s. After each measurement, the glass plate as a counter-surface was replaced and thoroughly cleaned to make sure that the measurements would not be influenced by residues from the previous sample.

2.8.2 *Tribological testing*

Frictional experiments were performed on cartilage samples (n = 3) against borosilicate glass, using UMT with reciprocal sliding motion (±30 mm at 6 mm/s), which was as same as the tribological test of OA human cartilage in Section 2.3. But the additional load cell of DFH-5.0 for 40 N was applied to mimic the stance phase of human gait cycle. The procedure of COF and dynamic creep test in detail is shown in Fig. 2(c).

2.8.3 *Determination of contact area*

The contact zone was clearly visible on the sample surface when left to dry for a few minutes. This zone was marked with a pen (Lumocolor permanent, blue, size S, STAEDTLER, Germany) manually (Fig. 2(c)). After that, the samples were placed on millimeter paper

and photographed. A custom-made MATLAB script was used to calibrate the pictures and determine the previously marked area.

2.9 Statistical analysis

All data are expressed as mean value±standard deviation, calculated from three independent experiments. Statistical analysis was performed with GraphPad Prism software (Version 8.0, GraphPad Software, USA). Significant differences among groups were determined by using one-way analysis of variance (ANOVA). Significance was defined as $p < 0.05$.

3 Results and discussion

3.1 Human osteoarthritic cartilage

3.1.1 Histology

The tissue structures of OA human cartilages in two patients are revealed in Fig. 3. Totally seven pieces of cartilage harvested from OA patients came from anterior and posterior parts of the Med and Lat femoral condyles. The samples were stained with Safranine O–Fast Green for proteoglycans, and Picrosirius Red for collagen. All cartilages had severe damage not only on the surface of the superficial zone but also in the middle and deep zones. In particular, the sample of Patient 1 Med missed a lot of tissues upon the surface (the black arrows). Safranine O staining showed mass proteoglycan depletion in superficial and middle zones of all OA samples. A more intense Picrosirius Red staining was observed in the superficial to middle region, where proteoglycan depletion with lower intense staining was observed. This artifact has been observed in Ref. [52]. In a nutshell, we can say that the level of cartilage degradation cannot be generalized. The degradation is patient and location-specific with regions of only enzymatic degradation and regions of enzymatic degradation coupled with clear signs of mechanical damage. The patient samples came from late OA; thus, we can expect predominantly enzymatic degradation and much less mechanical damage in early OA.

3.1.2 Tribological performance

The ribological properties of patient cartilage at 4 N including COF and dynamic creep are presented in Fig. 4. Wide variations in COF and dynamic creep were observed between the two patients and in different locations. Except for "Med" Patient 1, most locations showed creep and gradual increase in COF. The biphasic model [45–48] of cartilage lubrication relates increasing cartilage creep to a rise in a solid–solid contact at the cartilage–glass interface to explain the gradual increase in COF.

Med B in Patients 1 and 2 showed similar and the lowest COFs (i.e., 0.22±0.01 and 0.23±0.01, respectively,

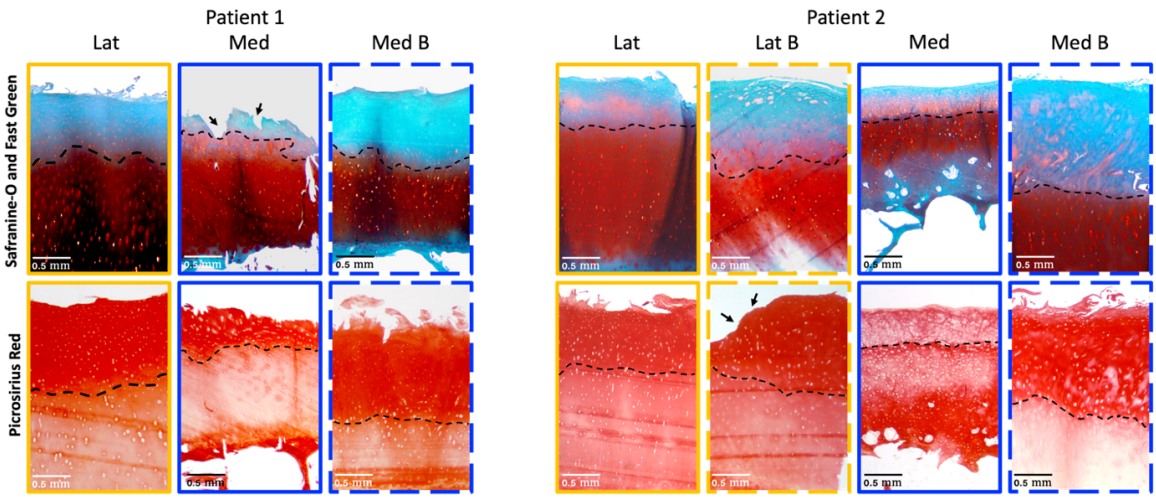

Fig. 3 Histology of OA condyle cartilages from patients. The dotted lines represent the border, where proteoglycan or collagen contents were depleted. The black arrows mean the huge matrix loss on cartilage surface. The blue area in Safranine O-stained cartilage indicates GAG depletion, whereas the red-colored region in Picrosirius Red cartilage shows a collagen network. The same artifact is observed by others [52], where collagen staining is deeper in GAG-depleted regions.

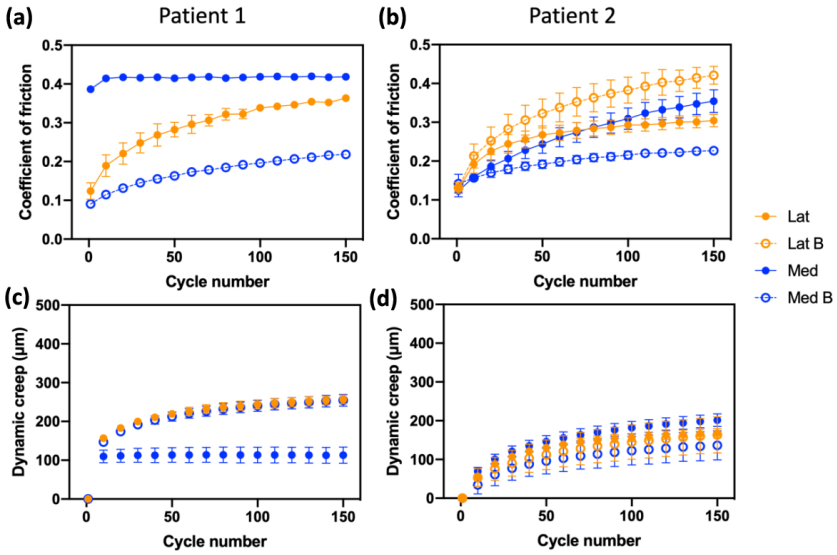

Fig. 4 Dynamic COFs of (a) Patient 1 and (b) Patient 2 and dynamic creeps of (c) Patient 1 and (d) Patient 2. The error bars represent the standard deviation (n = 3).

after 150 cycles) although the creeps of these specimens were very different, i.e., 254.2±15.0 μm and 136.5±65.1 μm, respectively. Med Patient 1 maintained the COF of 0.42±0.01 during the whole rubbing period due to severe morphology damage in the superficial zone of cartilage tissue according to histology results (Fig. 3). This severe damage also affected dynamic creep result, where Med of Patient 1 had a constant and the lowest creep of 113.1±20.8 μm (Fig. 4(c)). The sample of Lat Patient 1 had an increased value of COF to 0.36±0.01 and creep to 256.7±11.1 μm at the 150th cycle. As for Patient 2 (Figs. 4(b) and 4(d)), the COF and dynamic creep increased with the increasing cycles. The second lowest COF coming from Patient 2 was Lat with 0.31±0.02. In addition, Lat B had the highest COF of 0.42±0.02, while Med was 0.36±0.03 after 150 cycles. The range of dynamic creep of all samples from Patient 2 was from around 100–200 μm.

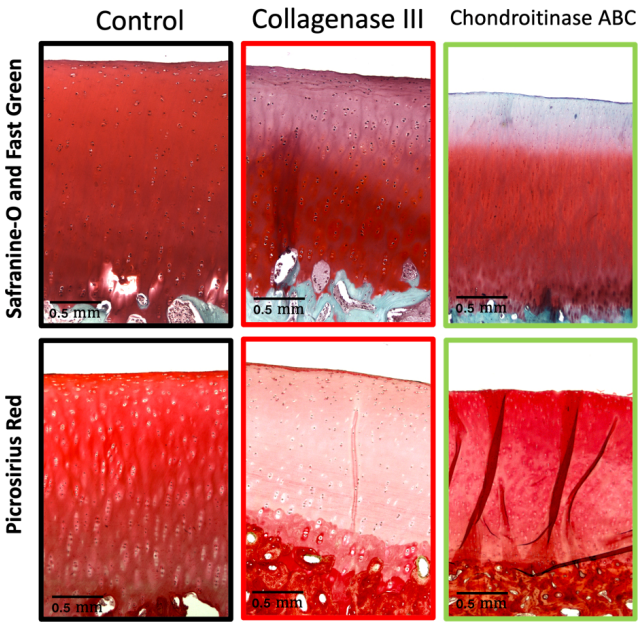

Fig. 5 Histology of healthy bovine cartilage and cartilage treated with collagenase III and chondroitinase ABC for 24 h.

3.2 Bovine cartilage

3.2.1 Histology structure

Figure 5 shows a selection of histology results of control cartilage and 24 h degraded samples with different enzymes. All enzymes showed a noticeable effect.

Collagenase III or MMP-13 is over expressed in cartilage tissue of OA patients and is known to degrade type II collagen [53], which is abundant in hyaline cartilage. Collagenase III-treated bovine cartilages generally showed lighter Picrosirius Red staining up to the deep zone, as compared to the control group and chondroitinase ABC-treated cartilage, indicating collagen degradation. However, the shown sample also had a noticeable loss of proteoglycans from the superficial to middle zone due to the loss of Safranine O staining, which was also observed in Refs. [54, 55].

The keratin sulfate region of aggrecan (a component of proteoglycans) is known to protect collagen type II [56] against proteolytic activity, and absence of proteoglycans in the superficial zone would mean that the collagen network would be more damaged in that region. The other possibility is that collagen type II degradation allows the proteoglycans to get released from the cartilage, showing lower Safranine O staining.

Chondroitinase ABC degradation showed a loss of proteoglycans until the middle zone, where the Safranine O staining was light in this region.

The overall results showed that cartilage digestive enzymes yield damage to specific components. For example, chondroitinase ABC cleaves proteoglycans, while collagenases III destroys collagen network in cartilage. And collagen degradation could also lead to the loss in proteoglycans.

Bovine cartilage after 24 h of chondroitinase ABC degradation showed the closest resemblance to human OA cartilage. According to histology results, chondroitinase ABC bovine cartilage had a comparable proteoglycan and collagen distribution compared to OA human cartilage specimens.

3.2.2 *Morphology and roughness*

Figure 6(a) shows the topographical images, and Fig. 6(d) shows the roughness data obtained by the AFM of cartilage samples treated with collagenase III and chondroitinase ABC, and healthy cartilage stored in a tris buffer for 24 h as the control group. Enzymatic treatments seem to have altered the cartilage surface and increased roughness, as compared to the control sample (333±50 nm). However, after treatment of collagenase III, the cartilages had some valleys with large areas on the surface, in which the roughness value was 496±67 nm. In addition, the cartilage treated with chondroitinase ABC had a roughness of 574±96 nm. These results imply that enzymatic degradation of cartilage with collagenase III and chondroitinase ABC gave rise to clear changes on the surface, which become evident as increased roughness.

3.2.3 *Mechanical measurement*

Figure 6(b) shows the stiffness, and Fig. 6(c) shows the static creeps after 700 s of healthy bovine cartilage and degraded cartilage treated with different enzymes. All enzymatic degradations significantly decreased

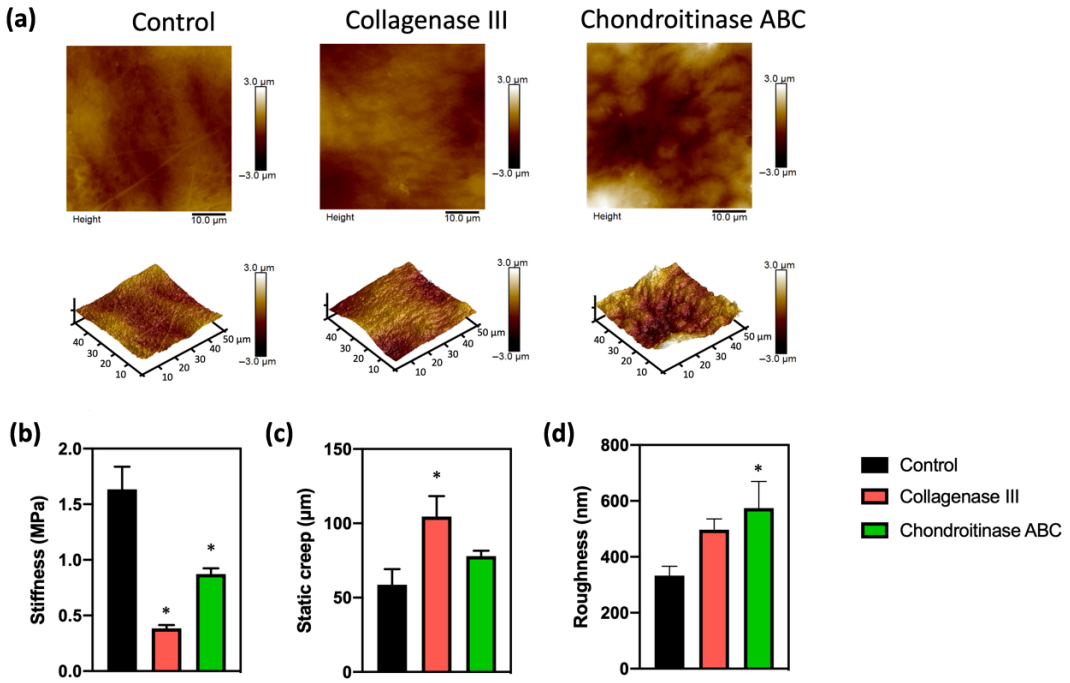

Fig. 6 (a) AFM images, (b) stiffness and (c) static creeps after 700 s, and (d) roughness of cartilage samples treated by collagenase III, chondroitinase ABC, and healthy cartilage. The error bars represent the standard deviation (n = 3). * p < 0.05: compared with the control group.

the stiffness, as compared to the control group. Healthy cartilage had the highest stiffness value of 1.63±0.21 MPa. Degradation with collagenase III considerably reduced the stiffness to 0.38±0.05 MPa. Chondroitinase ABC at the chosen concentrations yielded a lower decrease with 0.87±0.09 MPa.

As for the static creep data of various cartilage samples after 700 s, collagenase III-degraded cartilage had a higher static creep (104.0±14.0 μm) than that of the control group (58.0±11.0 μm). Static creep for cartilage degraded with chondroitinase ABC was not statistically different than that of the control group, yet showed a much higher value of 78.0±6.0 μm compared to that of the control group.

The stiffness and static creep results showed that enzymes cause a decrease in stiffness and an increase of creep on cartilage. In Ref. [11], OA always yields a reduction in cartilage stiffness and an increase in creep. Proteoglycans are responsible for holding the water inside the cartilage, and collagen arcades provide structural integrity to cartilage by holding proteoglycans in place and restricting excessive proteoglycan swelling. Chondroitinase ABC degraded the proteoglycans in the superficial zone (Fig. 5), decreasing the water holding capacity, resulting in a significant decrease in stiffness but not in static creep.

Cleaving collagen fibers will damage the arcade structure, allowing the proteoglycans to get released or swell without any resistance, leading to a decrease in stiffness and increase in creep. Collagenase III shows clear signs of collagen degradation (Fig. 5), which is reflected in a significant change in stiffness and creep after collagenase III treatment.

3.2.4 *Tribology properties*

COFs (Fig. 7(a) for 4 N and Fig. 7(c) for 40 N), dynamic creeps (Fig. 7(b) for 4 N and Fig. 7(d)) for 40 N), and contact areas (Fig. 7(e)) of control as well as enzymatically degraded bovine cartilage are shown in Fig. 7.

Because of high viscosity of the synovial fluid, the cartilage *in vivo* is known to get lubricated through

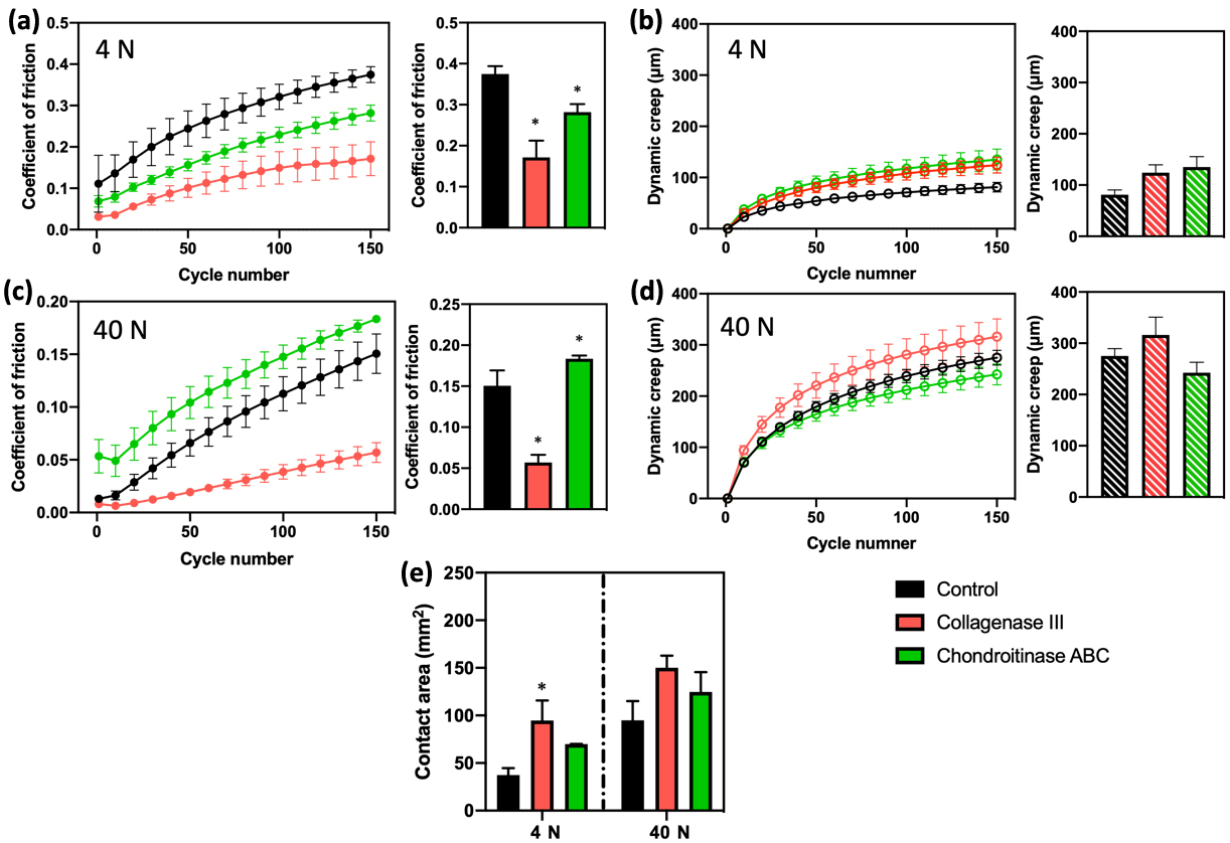

Fig. 7 Dynamic COFs ((a) 4 N and (c) 40 N), dynamic creeps ((b) 4 N and (d) 40 N), and (e) contact areas of bovine cartilage treated with and without enzymes. The error bars represent the standard deviation ($n = 3$). * $p < 0.05$: compared with the control group.

fluid film formation due to hydrodynamic [57] and fluid pressurization and weeping mechanisms [58]. In low velocity regions of the gait cycle however, cartilage surfaces come in contact, and then they are lubricated by boundary lubrication mechanism provided by the lamina splendens [59]. In our experiments, when healthy bovine cartilage (control) was rubbed against glass in PBS (low viscosity), the COF and dynamic creep of cartilage both increased with time. The observed time-dependent behavior of the COF has been previously described by many other authors and is attributed to a subsequent transfer of load bearing from the fluid to the solid phase (matrix) of cartilage [37, 60–62]. A low COF when most of the load is sustained by the fluid phase is also known as biphasic lubrication or interstitial fluid pressurization [51]. This explains the increase in creep and COF over time: With further compression and interstitial fluid migrating out of the contact zone of cartilage, more amount of load has to be supported by the cartilage matrix, leading to a higher COF.

The COFs for healthy cartilage were observed to be lower at 40 N, as compared to those at 4 N, but the creep values had an opposite trend. The reason is the fluid film lubrication at 40 N due to fluid pressurization and weeping mechanism proposed by McCutchen [58], which was absent at 4 N, and boundary lubrication predominates at the low load. The reason for this argument is the fact that at 40 N, the healthy cartilage dynamic creep was 275.0±15.0 μm, which was significantly around 3 times higher than that at 4 N, i.e., 81.2±16.2 μm, and creep is essential for the weeping lubrication to take place. The contact areas were also observed to be higher at 40 N, as compared to those at 4 N due to higher creeps (Figs. 7(b), 7(d), and 7(e)).

At the low load (4 N), enzymatic degradation of cartilage resulted in a significant decrease in COF and an insignificant increase in dynamic creep. The COF of healthy cartilage (control) was 0.37±0.02, which was significantly higher than those of cartilage treated with collagenase III (0.17±0.04) and chondroitinase ABC (0.28±0.02). In addition, no significant difference was observed in the dynamic creep behaviors of control, collagenase III-, and chondroitinase ABC-treated bovine cartilages, which were 81.2±16.2, 124.0±27.0, and 135.0±35.7 μm, respectively, after 150 cycles. After tribological experiment at 4 N, the contact area of cartilage was also obtained, as shown in Fig. 7(e). For chondroitinase ABC-degraded samples, the increase in contact area, which was 69.8±0.4 mm^2, was not significantly different, as compared to that of the control group (37.5±7.3 mm^2). But the contact area of collagenase III group was 94.7±36.6 mm^2, which is significantly higher than that of the control group.

In general, at the low load, i.e., 4 N, all cartilage samples had a dynamic creep of around 100 μm, meaning that the weeping lubrication hardly affected the fluid film formation. Boundary lubrication is thus the dominating lubrication mechanism. As for the collagenase III group, the destruction of the collagen network (Fig. 5) may allow intact proteoglycans, i.e., aggrecan macromolecule aggregates to move to the surface or even get released into the cartilage–glass interface, increasing the boundary lubrication. This has been previously shown that charged brush-like polymers like proteoglycans [63] are extremely efficient lubricants in aqueous media [64]. As for chondroitinase ABC-treated cartilage, the CS chains on the GAGs were cleaved, and these CS fragments may also get released into the cartilage–glass interface upon cartilage surface to enhance boundary lubrication [65]. Since CS are much smaller (molecular weight (M_W) ≈ 25 kDa) [66], as compared to proteoglycans (M_W ≈ 300 MDa) [66], the COF measured after collagenase III treatment was thus lower than that after chondroitinase ABC treatment.

At a high load of 40 N in PBS, the final COF of chondroitinase ABC-treated cartilage was 0.18±0.01, which was significantly higher, as compared to that of the control group with a final value of 0.15±0.02 (Fig. 7(c)). However, the COF for collagenase III-treated cartilage (0.06±0.01) was significantly lower (40%) than that of the control group. The dynamic creep values of different groups at 40 N were similar, i.e., 275.0±15.0 μm (control), 316.0±35.0 μm (collagenase III), as well as 243.0±20.0 μm (chondroitinase ABC). After 150 cycles, the contact area of control sample was 94.9±20.2 mm^2. In contrast, collagenase III digestive cartilage samples had a contact area of 150.2±21.9 mm^2, and the contact area of chondroitinase ABC was 124.6±36.0 mm^2.

Compared to 4 N, at a high load of 40 N, the predominant lubrication mechanism was weeping lubrication. Therefore, in Figs. 7(c) and 7(d), we can see a decrease in COF and an increase in dynamic creep at 40 N compared to that at 4 N (Figs. 7(a) and 7(b)). Collagenase III-degraded cartilage showed very low COFs, whereas the COFs of chondroitinase ABC-degraded cartilage were higher than those of the control group, despite having a similar creep. The combined release of the interstitial fluid due to weeping and proteoglycans into the cartilage glass interface could enhance the local viscosity and create a gel-like layer at the interface. This could improve fluid film lubrication for collagenase III-degraded cartilage. On the contrary, for chondroitinase ABC-degraded cartilage, small pieces of CS chains (M_W ≈ 25 KDa) [66] got release, which may not be enough to enhance the local viscosity; furthermore, the absence of proteoglycans inside the cartilage tissue caused that cartilage was not able to hold enough water to maintain more efficient weeping lubrication compared to healthy cartilage [11].

Similar research regarding cartilage friction behavior after digestion has been investigated in recent years. Caligaris et al. [67] investigated the frictional response of OA human tibiofemoral joints, where they showed that slight OA human samples had higher COFs than severe OA samples against glass both in PBS and synovial fluid. Finally, they concluded that the COF of human tibiofemoral cartilage does not necessarily increase with naturally increasing OA for visual stages ranging from 1 to 3. Katta et al. [38] treated bovine cartilage with chondroitinase ABC before a long-time dynamic tribology test against cartilage for 10 h. At relatively high pressures (2 and 3.15 MPa), higher COFs were observed in native bovine cartilage compared to digested samples. Both the above studies did not provide an explanation for this decrease in COF. Our results show a similar reduction in COF at the low load (4 N) after enzymatic degradation. Based on mechanical property, histology information, as well as the morphology of cartilage treated by enzymes, we made a hypothesis that explained such a phenomenon in the lubrication behavior (Fig. 8) [34].

3.3 Comparison of condyle cartilage between bovine and OA patient

The proteoglycans and collagen distributions of bovine cartilage digested by chondroitinase ABC (histology results in Fig. 5) are comparable with those of the patients' cartilage samples (histology results

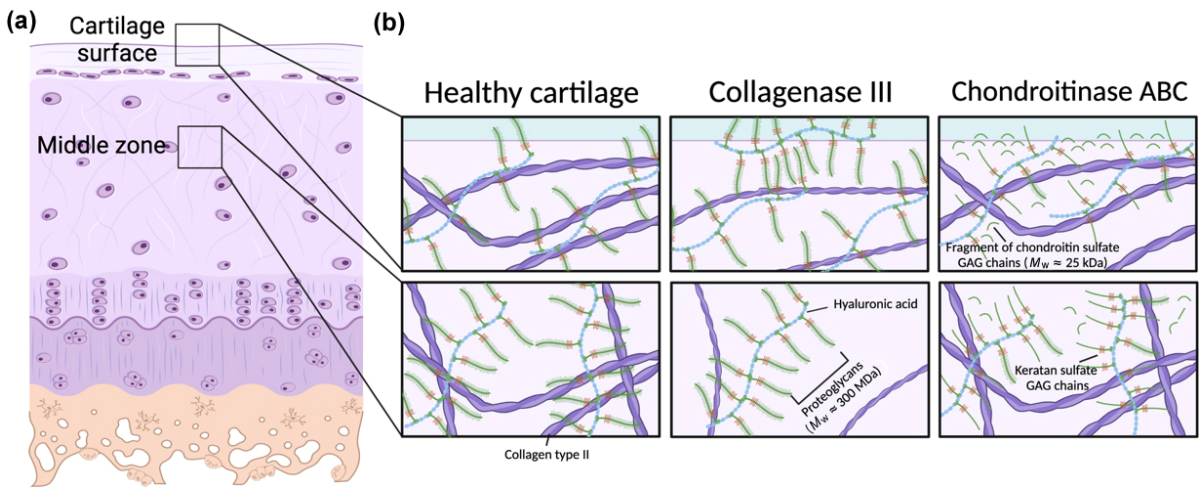

Fig. 8 Schematic showing the effect of enzymatic degradation on bovine cartilage sliding against glass: (a) cartilage basic structure; (b) distributions of collagen type II network and proteoglycans on bovine cartilage surface and in middle zone of healthy cartilage and cartilage treated by collagenase II and chondroitinase ABC. After the digestion from collagenase III, cartilage may allow the remaining intact proteoglycans to the surface of cartilage tissue: A part of released proteoglycans (M_W ≈ 300 MDa) may migrate to the solid–solid cartilage–glass interface to create a gel-like fluid layer to enhance fluid film lubrication. After the degradation of chondroitinase ABC, the cleavage of CS chains of proteoglycans results in these small fragments released upon cartilage surface to enhance the boundary at 4 N. But these fragments (M_W ≈ 25 kDa) can hardly affect the fluid film lubrication.

in Fig. 3): proteoglycan loss in the superficial and middle zones of both cartilages from bovine and patients. And the enhancement of staining intensity for collagen in this area after digesting by chondroitinase ABC gave rise to a similar histology structure compared to OA human cartilage. The tribological behavior of condyle cartilages from patients varied, although the COF may depend on the dynamic creep in this case. Whereas, compared with chondroitinase ABC sample and groups of Lat and Med B in Patient 2, the similarities in COF and dynamic creep were quite clear (Fig. 9).

4 Conclusions

In this research, the level of cartilage damage present in knee arthroplasty patients suffering from OA was first investigated, followed by measuring their friction and creep behavior. Later, collagenase III and chondroitinase ABC enzymes were used to degrade bovine cartilage to mimic patients' cartilage conditions. The conclusions obtained were as follows:

1) OA human cartilage shows proteoglycan depletion in superficial and middle zones in most of all cartilage samples. COF and creep increased with sliding time.

2) Collagenase III degraded collagen, and chondroitinase ABC degraded the proteoglycans. Both caused a reduction in COF at the low load (4 N) because of boundary lubrication enhancement, resulting from the released intact proteoglycans ($M_W \approx 300$ MDa) from the superficial and middle zones due to the collagen network disruption by collagenase III or release of fragments of CS ($M_W \approx 25$ kDa) after the cleavage of chondroitinase ABC.

3) At the high load (40 N), cartilage digested by collagenase III decreased the COF due to proteoglycan release and adequate weeping lubrication. Chondroitinase ABC digestion increased the COF because small fragments of CS were inefficient in providing boundary lubrication at the high load, and weeping lubrication was ineffective due to proteoglycan depletion.

4) Histology results indicate that proteoglycan removal by chondroitinase ABC from the superficial and middle zones was similar to that by OA human cartilage. The creep and friction behaviors of OA human cartilage and chondroitinase ABC-degraded cartilage were similar.

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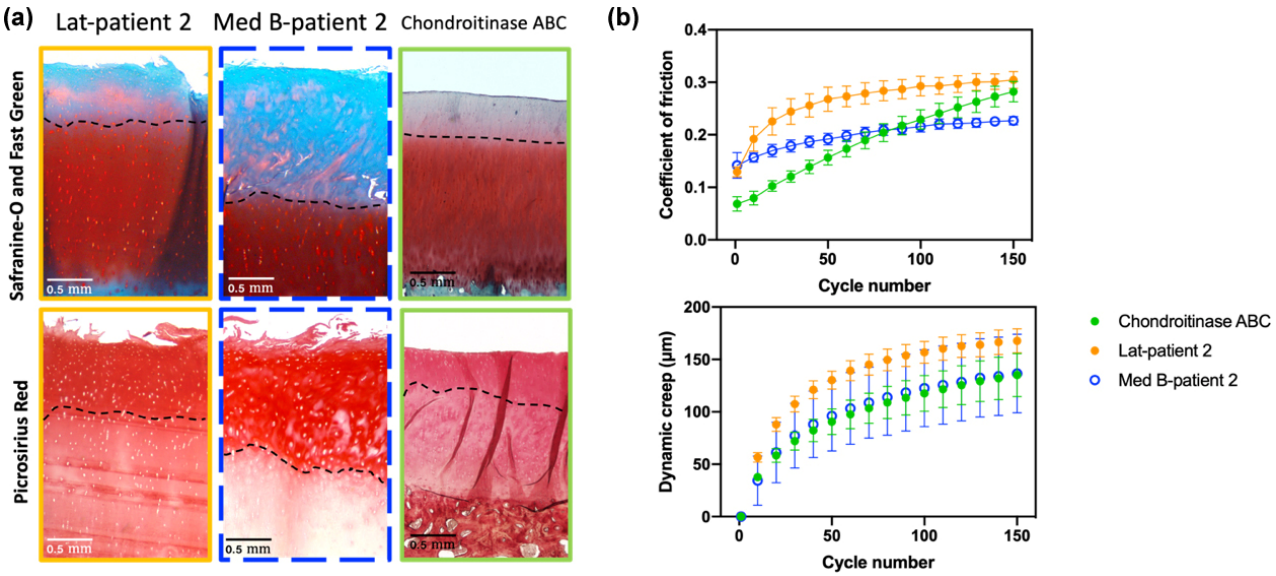

Fig. 9 Comparison between bovine cartilage treated with 0.1 U/mL chondroitinase ABC for 24 h and OA cartilage of Lat and Med B from Patient 2 in (a) histology and (b) tribological behavior. The error bars represent the standard deviation ($n = 3$).

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Declaration of competing interest

The authors have no competing interests to declare that are relevant to the content of this article.

Ethical approval

This study does not contain any studies with human or animal subjects performed by any of the authors.

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Ke REN. He received his B.S. degree in material processing and control engineering from Taiyuan University of Technology, China, in 2015 and got his M.S. degree in materials science engineering from China University of Geosciences (Beijing), China, in 2018. He is currently a Ph.D. candidate at Department of Biomedical Engineering, University of Groningen, the Netherlands. His research interests are mainly about cartilage lubrication and osteoarthritis and biotribology.

Prashant K. SHARMA. He was trained in metallurgy and materials science during his bachelor and master degrees from Visvesvaraya National Institute of Technology, India, and Indian Institute of Science, India, respectively. He obtained his Ph.D. degree from Luleå University of Technology, Sweden, in 2002. Since then, he has spent 21 years of research on the biotic–abiotic interface. At present, the research focus is on all aspects of biotribology and friction-related diseases including dry eye disease, dry mouth, osteoarthritis, and cardiovascular catheterization. Till now, he has earned a patent in the broad area of biotribology and in total 90 publications with an H-index of 33 (Google Scholar).