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Wear and Damage of Articular Cartilage with Friction Against Orthopaedic Implant Materials

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Abstract

The objective of this study was to measure the wear response of immature bovine articular cartilage tested against glass or alloys used in hemiarthroplasties. Two cobalt chromium alloys and a stainless steel alloy were selected for these investigations. The surface roughness of one of the cobalt chromium alloys was also varied within the range considered acceptable by regulatory agencies. Cartilage disks were tested in a configuration that promoted loss of interstitial fluid pressurization to accelerate conditions believed to occur in hemiarthroplasties. Results showed that considerably more damage occurred in cartilage samples tested against stainless steel (10 nm roughness) and low carbon cobalt chromium alloy (27 nm roughness) compared to glass (10 nm) and smoother low or high carbon cobalt chromium (10 nm). The two materials producing the greatest damage also exhibited higher equilibrium friction coefficients. Cartilage damage occurred primarily in the form of delamination at the interface between the superficial tangential zone and the transitional middle zone, with much less evidence of abrasive wear at the articular surface. These results suggest that cartilage damage from frictional loading occurs as a result of subsurface fatigue failure leading to the delamination. Surface chemistry and surface roughness of implant materials can have a significant influence on tissue damage, even when using materials and roughness values that satisfy regulatory requirements.

Keywords

Cartilage; friction; wear; damage; degeneration; orthopaedic implants; hemiarthroplasty

INTRODUCTION

The objective of this study was to investigate the wear mechanism of articular cartilage sliding against non-biological materials typically used in orthopaedic implants. The clinical

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Conflict of Interest

The authors have no conflict of interest to report for this study.

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motivation for this investigation is the observation that the native cartilage layer in a hemiarthroplasty appears to undergo accelerated cartilage wear. The mechanism causing this accelerated wear remains unclear, therefore this study proposed a specific mechanism for damage and wear and tested it experimentally.

Hemiarthroplasty is a surgical procedure that replaces one half of a diarthrodial joint with an artificial bearing surface, leaving the opposing articular layer intact. According to the U.S. Centers for Disease Control, there are approximately 360,000 hemiarthroplasty operations performed each year in the US (CDC/NCHS, 2013). Common indications for hemiarthroplasty include acute bone fracture or failed internal fixation, such as proximal femoral fractures of the hip (Giliberty, 1983), and proximal humeral fractures of the shoulder (Connor and D'Alessandro, 1995; Loew et al., 2006); osteonecrosis of the femoral or humeral head (Barnes et al., 1991; Baumgarten et al., 2004; Grecula et al., 1995); and low activity expectations (Cuckler and Tamarapalli, 1994). In all cases, hemiarthroplasty is indicated only if the non-resurfaced native articular layer shows no significant signs of disease, including osteoarthritis, rheumatoid arthritis, or Paget's disease (Rodriguez-Merchan, 2002).

There remains much controversy about the value of hemiarthroplasty over total joint arthroplasty (Baumgarten et al., 2004; Lindstrand et al., 2001; Orfaly et al., 2003), most notably for displaced femoral neck fractures (Blomfeldt et al., 2007; Cuckler and Tamarapalli, 1994; Rodriguez-Merchan, 2002), and the treatment of primary shoulder osteoarthritis (Boileau et al., 2006; Gartsman et al., 2000; Smith and Matsen, 1998). Hemiarthroplasties are less invasive, require less surgical time and result in lower blood loss than total arthroplasties (Blomfeldt et al., 2007; Gartsman et al., 2000). However, patient outcomes are sometimes shown to be less successful than with total arthroplasties, and often require revision, due in part to erosion of the articular surface (Champion and McNally, 2004; Haidukewych et al., 2002; Hasan et al., 2002; Hedtmann and Werner, 2007; Parsons et al., 2004; Sperling et al., 2002). There is a general uncertainty about the cause of seemingly accelerated cartilage wear in hemiarthroplasties.

In this study, it was hypothesized that accelerated wear occurs when the friction coefficient rises significantly above the typical value achieved under normal joint function. The friction coefficient of articular cartilage has been shown to vary over a relatively wide range (from as low as 0.002 to as high as 0.5) depending on the loading configuration (Forster and Fisher, 1999; McCutchen, 1962). However, based on prior studies, it has been shown that the friction coefficient can remain very low (between 0.002 and 0.01) as long as the interstitial fluid load support within cartilage remains elevated (Caligaris and Ateshian, 2008). These low values are believed to represent the functional range of the friction coefficient under physiological loading conditions of cartilage against cartilage (Caligaris and Ateshian, 2008; Caligaris et al., 2009). Loss of interstitial fluid load support, and the concomitant increase in friction, has been shown to occur when cartilage is in contact with an impermeable surface and the contact area remains stationary on the articular layer (Krishnan et al., 2004). This configuration allows depressurization of the cartilage interstitial fluid as it flows away from the loaded region.

In a preliminary finite element study (Rajan et al., 2010), we have shown that a hemiarthroplasty in a ball-and-socket joint, such as the shoulder or hip, where the ball is replaced with an impermeable artificial surface, may defeat the sustained interstitial fluid pressurization mechanism normally maintained by a migrating contact area between two native articular layers, by producing a relatively stationary contact area on the apposing native articular layer. Therefore, a primary factor for increased wear in hemiarthroplasties may be the increase in the friction coefficient due to loss of interstitial fluid pressurization.

In addition to interstitial fluid load support, another factor that influences the frictional response is the magnitude of the equilibrium friction coefficient, achieved when the fluid load support subsides to zero (Krishnan et al., 2004), which may depend significantly on properties of the artificial bearing material, including surface chemistry and surface roughness. Indeed, some investigators have suggested that using different materials for resurfacing the joint might produce less wear (Chan et al., 2011; Clarke, 1982; Foy et al., 1999; Maistrelli et al., 1991; Patel and Spector, 1997). However, a comprehensive set of quantitative data is lacking, as many of the previous in-vitro wear studies have employed artificial material counterfaces that do not meet guidelines of the U.S. Food & Drug Administration (FDA) for surface roughness of artificial joint components in contact with native tissue (Chan et al., 2011; Patel and Spector, 1997).

Therefore, the aim of this study was to measure the wear response of immature bovine articular cartilage against alloys used in hemiarthroplasties, or glass (which is commonly used in in-vitro friction studies). Two different cobalt chromium alloys, along with a stainless steel alloy, acceptable by the FDA for use as bearing counterfaces, were selected for these investigations. Material surface preparation was conducted and tested in accordance to FDA guidelines to ensure that surface roughness would be comparable to implanted joint components. A test configuration that promotes loss of fluid pressurization over time was specifically selected to determine whether the magnitude of the equilibrium friction coefficient was responsible for wear and damage of cartilage. The hypothesis tested in this study is that, for a given contact load and duration of loading, wear and damage of cartilage is greater in those materials exhibiting a higher equilibrium friction coefficient.

METHODS

Material Preparation

Cobalt-chromium low carbon (CoCrLC), cobalt-chromium high carbon (CoCrHC), and 316 low vacuum melt stainless steel (316SS) orthopaedic materials were obtained as cylindrical rod sections to be used as friction platens (M. Vincent & Associates, Bloomington, MN). Platen samples were ground flat and parallel using a surface grinder (Acer #AGS-1020AHD, Anaheim, CA). Platen samples were held in a custom jig to maintain surface flatness, then ground and polished using successively finer silicon carbide abrasives and final alumina oxide slurry polish steps. A subset of CoCrLC platen samples was withheld from the final polishing step to create a group with a rougher surface (CoCrLC-Ra25nm). Uncoated optical borosilicate glass discs were used as the glass platen group (Edmund Optics #43-892, Barrington NJ). Surface roughness measurements were performed before and after cartilage friction testing with an optical profilometer to ensure surface finish compliance to both ISO

21534:2007, and ASTM F2033 standards with average roughness R_a less than 50 nm for all platen samples. Six measurements were taken on each surface.

Sample Harvest

Experiments were conducted using articular cartilage explants harvested from matched left-right pairs of healthy tibial plateaus and opposing femoral condyles of 2–3 months old calf knee joints obtained from a local abattoir. Full thickness cartilage discs (Ø4 mm) were excised from the tibial plateaus of eight joints, placed with the articular surface down on a freezing stage microtome (Leica Instruments #SM2400, Nusslock, Germany), embedded and frozen in a water soluble sectioning gel (Thermo Scientific #1310APD, Rockford, IL), then trimmed of bone and deep zone tissue to obtain samples inclusive of an intact superficial zone, with a final thickness of 1.6 ± 0.05 mm (Figure 1). Samples were then randomized into various treatment groups and test type. Samples were rinsed with and transferred to filtered phosphate buffered saline (PBS) supplemented with an isothiazolone based biocide (Sigma-Aldrich #46878-U, St. Louis, MO) and protease inhibitor (0.05 M Ethylenediaminetetraacetic acid) (Oungoulian et al., 2013).

Mechanical Testing

A custom two-axis loading device equipped with a six-degrees-of-freedom load cell (JR3 Inc. #20E12A4, Woodland, CA) was used for all mechanical testing. The device was controlled with custom LabVIEW software (National Instruments Corporation #LabVIEW 2010, Austin, TX) and an associated motion controller (National Instruments Corporation #7354, Austin, TX) to provide continuous measures of specimen thickness, position, applied loads, and reaction loads.

Friction measurements against all platens were performed on cartilage disks ($n=8$ per treatment), using an unconfined compression creep loading configuration (2.22 N, equivalent to 0.18 MPa contact stress) with reciprocal sliding motion (± 5.0 mm at 1 mm/s) (Figure 1) (Krishnan et al., 2004). Sample solutions were collected for subsequent wear particulate measurements (see below).

The temporal response of the friction coefficient μ was evaluated from the normal and frictional load responses (Krishnan et al., 2004) over the 4 h testing duration. The creep deformation u was also recorded over the same duration. Three measures of μ were extracted from these responses: the minimum friction coefficient $\mu_{\min}$, measured from the first full cycle of sliding following load application; the friction coefficient $\mu_{98\%}$, achieved when the creep displacement u was at 98% of its equilibrium value; and the friction coefficient μ_{4h} , measured at the final time point. A two-way ANOVA was performed on these three measures of μ , for the effects of treatment and test time, followed by post-hoc testing using Tukey correction.

Cartilage samples were submerged in PBS throughout all tests and allowed to recover for a duration equal to or greater than the test time. Before and after testing, sample wet weights and macroscopic images were acquired. A swelling ratio was calculated as the wet weight ratio after and before testing.

Subsequent to recovery after friction testing, unconfined compression stress-relaxation tests were performed on samples to evaluate the equilibrium compressive modulus (E_Y). For each sample, a tare load was initially applied (0.025N with 360 s relaxation time), followed by the stress relaxation test (to 5% strain from pre-tare reference configuration, and 1200 s relaxation time).

A one-way ANOVA was performed on the swelling ratio and equilibrium compressive modulus for the effect of treatment, followed by post-hoc testing using Tukey correction.

Particulate Testing

Friction test bath solutions were analyzed with a particle sizing and counting analyzer (Multisizer 4, Beckman Coulter, Brea, CA) configured to measure particulates from 0.6 to 60 μm in diameter, using two separate aperture tubes (30 μm and 100 μm) with a 300 μL total aspiration volume. Counting analyzer calibration verification for both particulate size and number was performed per manufacturer specifications. Solution samples were placed in clean containers for testing, and agitated throughout test duration to keep particulates in suspension during characterization. Solution preparation and sample rinsing procedures were performed as described previously (Oungoulian et al., 2013). The volumetric wear rate (Moore, 1975) was evaluated as $K_V = (\text{total particulate volume})/(\text{sliding distance} \times \text{apparent area})$, where sliding distance = 10 mm and apparent area = 12.6 mm^2 . A one-way ANOVA was performed on K_V , for the effect of treatment, followed by post-hoc testing using Tukey correction.

Biochemical Analysis

After mechanical testing, samples were bisected and weighed, and half of each construct was lyophilized and digested in 0.5 mg/mL proteinase K solution (Fisher Scientific) overnight at 56 °C (Hollander et al., 1994). Sulfated GAG content was determined by dimethylmethylene blue spectrophotometric assay (Farndale et al., 1986; Kelly et al., 2006). Collagen content was assessed by orthohydroxyproline assay of acid-hydrolysates of proteinase K digests (Kelly et al., 2006) assuming a 1:7.64 OHP-to-collagen mass ratio (Hollander et al., 1994). Biochemical contents were normalized to tissue sample pre-test weights. A one-way ANOVA was performed on GAG and collagen contents, for the effect of treatment, followed by post-hoc testing using Tukey correction.

Histological Staining

The remaining bisected samples were randomized and divided into two groups, one group for histology and another for polarized light microscopy. Samples for histology ($n = 4$ per treatment) were fixed, sectioned, and stained for collagen with safranin-o (Kelly et al., 2006). Samples for polarized light microscopy ($n = 4$ per treatment) were sectioned (without fixation) and imaged (Olympus BX60 microscope, drop-in U-POT polarizer, Olympus DP72 camera) (Canal et al., 2008).

RESULTS

Material Roughness Testing

The measured roughness of platens (Table 1) demonstrated that there were no significant differences among glass and the three alloys subjected to a final polishing step ($p = 0.73$), all of which exhibited a roughness of approximately 10 nm. No differences were observed in surface roughness before and after testing ($p = 0.94$, Table 1). As intended, the CoCrLC-Ra25nm group exhibited significantly higher roughness compared to other platen groups ($p = 0.003$, Table 1).

Mechanical Testing

No significant difference was detected in $\mu_{\min}$ among any of the tested counterfaces (Figure 2A, $p = 0.72$). Differences were found in $\mu_{98\%}$ and μ_{4h} between the 316SS group and [Glass, CoCrHC, CoCrLC] ($p = 0.01$); and between CoCrLC-Ra25nm and [Glass, CoCrHC, CoCrLC] (Figure 2B, $p = 0.005$). No differences were detected between Glass, CoCrHC, and CoCrLC (Figure 2B, $p = 0.27$) or between 316SS and CoCrLC-Ra 25nm (Figure 2B, $p = 0.57$).

After friction testing, gross swelling on the articular side of the cartilage disk was evident for many of the samples in the 316SS and CoCrLC-Ra25nm groups (Figure 3).

Quantitatively, significant differences were detected in the swelling ratio between 316SS and [Glass, CoCrHC, CoCrLC] (Figure 4A $p = 0.01$), and between CoCrLC-Ra25nm and [Glass, CoCrHC, CoCrLC] (Figure 4A $p = 0.003$).

Significant differences were detected in the equilibrium modulus between glass and [CoCrLC-Ra25nm, 316SS] (Figure 4B $p = 0.013$), with no differences detected between other groups ($p = 0.14$).

Particulate Testing

Differences between Glass and CoCrLC-Ra25nm, and Glass and 316SS, were detected for the volumetric wear rate K_V (Figure 4C $p = 0.043$). However no differences were detected between any other groups ($p = 0.27$).

Biochemical Analysis

Post friction analysis of GAG content showed significant differences between CoCrLC-Ra25nm and [CoCrLC, 316SS] (Figure 5A, $p = 0.024$), although no differences were detected between other groups (Figure 5, $p = 0.099$). No differences were detected in collagen content between groups (Figure 5B $p = 0.35$).

Histological Staining

After sectioning and staining, nearly all samples exhibited swelling of the top layer (Figure 6). Several samples in groups exhibiting high swelling ratio (316SS and CoCrLC-Ra25nm) showed clear evidence of delamination of the top layer (Figure 6B), which had remained hidden on gross examination (Figure 3). In remaining groups, several samples hinted at initiation of delamination in the form of a darker narrow band at a subsurface location

coinciding with the demarcation line in fully delaminated samples (Figure 6). A qualitative comparison between untested (Figure 7A) and tested samples (Figure 7B) using polarized light microscopy showed that delamination occurred in the middle zone of the articular layer, where collagen fibrils exhibit a random orientation.

DISCUSSION

The results of this study demonstrated significant damage to articular cartilage when subjected to sustained frictional loading with accelerated loss of interstitial fluid pressurization. This damage was most evident through gross examination (Figure 3), quantitative measures of swelling and compressive modulus (Figure 4A & B), and histology (Figure 6). Two test groups, 316SS and CoCrLC-Ra25nm, exhibited significantly greater damage than all others. These two materials also produced statistically higher friction coefficients than the others (Figure 2B), whether measured at the onset of loss of fluid pressurization (98% creep deformation) or at the completion of friction testing (4 h).

The appearance of delamination at the juncture between the superficial zone and the middle zone (Figure 6, Figure 7) was a very notable finding of this study because it indicated that wear damage was primarily initiated by fatigue failure of the extracellular matrix in a subsurface region, thus leading to delamination, rather than abrasive wear of the articular surface. Indeed, histological sections showed no clear evidence of pervasive articular surface roughening (Figure 6), as would otherwise occur in abrasive wear. Furthermore, though particulate measurements exhibited significantly higher volumetric wear rate in one of the treatment groups (Figure 4C), the values of K_V remained very small across all treatments.

The fact that delamination initiated below the superficial zone is consistent with the findings of Buckley et al. (Buckley et al., 2010; Buckley et al., 2008), who reported that the layer just beneath the superficial zone of cartilage, both neonatal bovine and adult human, has the lowest shear modulus across the entire thickness of the articular layer. Those authors' in vitro measurements of cartilage deformation under the action of a tangential force clearly demonstrated that the greatest amount of shear strain occurs in this region. Prior investigations have also shown that proteoglycan content is lower in that zone, compared to the deeper zone (Maroudas, 1979; Oswald et al., 2008). This compositional parameter correlates with the depth-dependent equilibrium compressive Young's modulus (Schinagl et al., 1997; Wang et al., 2002), consistent with the Donnan osmotic pressure resulting from the fixed charge density of proteoglycans (Canal Guterl et al., 2010; Chahine et al., 2004; Maroudas, 1979). It is also well established that the collagen matrix in the middle zone exhibits a more random orientation than the highly organized superficial zone fibrils, oriented tangential to the articular surface, and deep zone fibrils, oriented perpendicular to the cartilage-bone interface (Clarke, 1971; Xia, 2008) (Figure 7). This ultrastructure is a likely reason that the middle zone exhibits the least resistance to shear, as may be examined more closely in future modeling investigations.

It follows from these combined findings that the amount of shear deformation occurring underneath the superficial zone increased in proportion to the magnitude of the tangential force, and thus the magnitude of the friction coefficient, since the normal force applied in all

the test groups was the same. Consequently, those counterface materials that produced the greatest friction coefficient would have subjected the cartilage to the greatest shear strain in that region, leading to the fatigue failure manifested as delamination.

Evidently, both surface chemistry and surface roughness influenced the magnitude of the friction coefficient: 316SS exhibited a higher friction coefficient than either cobalt chromium alloy (CoCrLC and CoCrHC), when the surfaces were equally smooth, suggesting that surface chemistry must influence the friction coefficient. However, when using the same material, roughness also influenced the value of the friction coefficient, as was evident from the CoCrLC and CoCrLC-Ra25nm groups (Figure 2B). In tribology, it is well established that both of these factors influence the friction coefficient between bearing surfaces [REF]. However, as long as the interstitial fluid load support was high, the friction coefficient remained comparably low across all counterface materials (Figure 2A), producing negligible shear deformation under those conditions.

Though 316SS and CoCrLC-Ra25nm showed the most damage to cartilage, the other impermeable counterface materials were not harmless. In preliminary experiments leading to the testing protocol of this study, a testing duration of 8 h was also investigated. For that duration, even glass exhibited gross damage on the articular side of the tissue sample, in some instances causing the top layer to shear off completely (Figure 8). This finding is consistent with the concept that damage is caused by fatigue failure: Even when the friction coefficient is lower, a longer testing duration (more cycles of loading) may accumulate damage until it becomes as extensive as higher friction materials tested for shorter durations (fewer cycles of loading).

The testing configuration of this study purposefully accelerated the loss of fluid load support over a relatively short period of time. This condition was achieved by testing cartilage under a stationary contact area and selecting a relatively small sample diameter (since the characteristic time to reach creep equilibrium is proportional to the square of the diameter (Carter et al., 2007)). Under in situ physiological conditions, where the contact region is much larger than the diameter of the samples tested in this study and a certain amount of contact area migration occurs, it is very unlikely that the fluid load support would subside to this extent. Therefore, the friction coefficient will rise by a much smaller amount than the range spanned between $\mu_{\min}$ and $\mu_{98\%}$ in Figure 2. It follows that cumulative fatigue damage under in situ conditions would likely occur much more slowly than in the accelerated testing conditions of this in vitro study, and partially offset by the tissue's limited ability to repair itself.

Clinical evidence of cartilage delamination has been reported previously (Pritzker et al., 2006), although some prior reports may also conflate subsurface cartilage delamination with delamination at the cartilage-bone interface. Thus, the results of this study provide significant in vitro evidence in support of this damage mechanism.

From a practical perspective, the results of this study suggest that 316SS, when compared to CoCr, should not be used as a bearing surface against articular cartilage, contrary to previous reports (Chan et al., 2011). Furthermore, though regulatory guidelines set the

maximum roughness of orthopaedic implant surfaces at 50 nm, this study demonstrates that a roughness of 25 nm can nevertheless be quite harmful, significantly more so than 10 nm. Therefore, tighter guidelines for surface roughness may have to be adopted for orthopaedic implants in the future.

The friction tests of this study were conducted in saline. Since synovial fluid contains boundary lubricants that further reduce the equilibrium friction coefficient relative to saline (Caligaris and Ateshian, 2008), the onset of significant fatigue damage would likely occur at a later time point in the presence of those lubricants. Similarly, adopting a testing configuration that can sustain interstitial fluid pressurization over longer durations would also delay the onset of damage. A direct verification of these hypothesized outcomes may be investigated in future studies.

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Figure 1.

Schematic representation of bovine calf articular cartilage harvest location and friction testing schematic. Articular cartilage plugs were harvested from the tibial plateau, trimmed of subchondral bone, and tested against flat counterfaces of different material composition and roughness.

Figure 2. Frictional properties of cartilage against different counterface materials. (A) Minimum friction coefficient (μ_{min} , no differences across counterface materials, $p = 0.72$). (B) Comparison of friction coefficient at time of 98% creep displacement and final time at 4h (* $p = 0.01$ for comparison of counterface materials).

Figure 3.

Representative qualitative macroscopic comparison of cartilage tissue samples before and after friction testing for glass and 316SS counterface materials. Top, front, and side views are shown, displaying cartilage plug resting on orange rubber base for better contrast. After friction testing, swelling of the upper region is evident and highlighted by arrows in the 316SS after-test images.

Figure 4. (A) Swelling ratio (* $p < 0.01$), (B) equilibrium compressive Young's modulus E_Y (* $p < 0.013$), and (C) volumetric wear rate K_V (* $p < 0.043$).

Figure 5. Summary of cartilage biochemical properties. (A) GAG content (* $p = 0.024$), and (B) collagen (COL) content ($p = 0.35$), normalized to sample initial wet weight.

Figure 6.

Representative histological images of samples after testing. Cartilage tissue samples after friction testing for glass, CoCr HC, CoCr LC, CoCr LC-Ra 25nm, and 316SS counterface materials stained with safranin-o for GAG showing damage and delamination of top layer for 316SS tested sample. (*) cartilage canals (typical in juvenile tissue).

Figure 7.

Representative polarized light microscopy images of (A) untested (undamaged) samples, and (B) friction tested damaged samples. Untested samples were prepared using the same methods as tested samples; they display a thin superficial zone with bright intensity indicating tangential orientation of collagen fibrils, a middle zone with dark intensity where collagen fibrils are randomly oriented, and a deep zone with bright intensity where collagen fibrils are oriented at 90° to the articular surface. Friction-tested damaged samples show a relatively swollen middle zone, indicative of extracellular matrix degradation consistent with the location of damage in samples showing delamination in Figure 6. The increased intensity inside the otherwise dark middle zone of tested samples suggests partial realignment of collagen fibrils due to swelling of the damaged matrix.

Figure 8.

Representative macroscopic image of cartilage tissue sample tested for 8 h against a smooth glass counterface. Top, and side views are shown. At the end of testing, complete delamination of the top tissue layer was seen. The location of layer delamination along the tissue depth is consistent with the location of the swollen middle zone and site of delamination initiation seen during shorter testing conditions.

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Roughness measurements for orthopaedic material platens before and after tissue friction testing. No differences were detected on a per material basis before and after testing ($p = 0.94$). No differences were detected across smooth materials (Glass, CoCrHC, CoCrLC, and 316SS, $p = 0.73$). CoCrLC-Ra25nm group was significantly rougher than all others ($p = 0.003$).

Table 1

Roughness - Ra	Glass	CoCrHC	CoCrLC	CoCrLC-Ra25nm	316SS
Before Test	[nm] 8.4 ± 4.6	11.3 ± 4.1	12.8 ± 3.3	26.9 ± 9.6	10.4 ± 3.7
After Test	[nm] 9.4 ± 3.4	11.9 ± 3.4	12.3 ± 2.1	25.4 ± 8.4	11.6 ± 4.9