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Published in:
Journal of Biomedical Materials Research. Part B: Applied Biomaterials

DOI:
10.1002/jbm.b.35251

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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Damen, A. H. A., van Donkelaar, C. C., Sharma, P. K., Wan, H., Cardinaels, R., Schmidt, T. A., & Ito, K. (2023). Friction reducing ability of a poly-l-lysine and dopamine modified hyaluronan coating for polycaprolactone cartilage resurfacing implants. *Journal of Biomedical Materials Research. Part B: Applied Biomaterials*, *111*(8), 1523-1532. Advance online publication. https://doi.org/10.1002/jbm.b.35251

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Received: 3 May 2022 | Revised: 5 January 2023 | Accepted: 12 March 2023

DOI: 10.1002/jbm.b.35251

RESEARCH ARTICLE

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Friction reducing ability of a poly-l-lysine and dopamine modified hyaluronan coating for polycaprolactone cartilage resurfacing implants

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Funding information
Chemelot InSciTe; Regenerative Medicine Crossing Borders (RegMedXB)

Abstract

Frictional properties of cartilage resurfacing implants should be sufficiently low to limit damaging of the opposing cartilage during articulation. The present study determines if native lubricious molecule proteoglycan 4 (PRG4) can adsorb onto a layer-by-layer bioinspired coating composed of poly-l-lysine (PLL) and dopamine modified hyaluronic acid (HADN) and thereby can reduce the friction between implant and articular cartilage. An ELISA was developed to quantify the amount of immobilized human recombinant (rh)PRG4 after exposure to the PLL-HADN coating. The effect on lubrication was evaluated by comparing the coefficient of friction (CoF) of bare polycaprolactone (PCL) disks to that of PLL-HADN coated PCL disks while articulated against cartilage using a ring-on-disk geometry and a lubricant solution consisting of native synovial fluid components including rhPRG4. The PLL-HADN coating effectively immobilized rhPRG4. The surface roughness of PCL disks significantly increased while the water contact angle significantly decreased after application of the coating. The average CoF measured during the first minute of bare PCL against cartilage exceeded twice the CoF of the PLL-HADN coated PCL against cartilage. After 60 min, the CoF reached equilibrium values which were still significantly higher for bare PCL compared to coated PCL. The present study demonstrated that PCL can effectively be coated with PLL-HADN. Additionally, this coating reduces the friction between PCL and cartilage when a PRG4-rich lubricant is used, similar to the lubricating surface of native cartilage. This makes PLL-HADN coating a promising application to improve the clinical success of PCL-based cartilage resurfacing implants.

KEYWORDS
articular cartilage, biolubrication, boundary lubrication, coating, lubricin

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1 | INTRODUCTION

Ultralow friction between opposing articular cartilage layers is an essential feature to maintain the physiological function of a healthy knee joint. One of the mechanisms which is thought to be responsible for this remarkable biotribological system is boundary lubrication. During boundary lubrication, direct contact between the molecular layers of the two opposing cartilage bearing surfaces occurs.[1,2] Previous research has shown that the mucin-like glycoprotein, proteoglycan 4 (PRG4) also known as lubricin, plays an important friction-reducing role in this mechanism.[3–9] In contrast to fluid-film lubrication, not only the composition of the lubricant is of importance, but also the biochemical properties, macromolecular composition and structure of the articulating surfaces.[10,11]

When focal cartilage defects are treated with artificial biomaterials, the natural lubrication mechanism is disturbed. Frictional properties of these focal knee resurfacing implants (FKRIs) need to be sufficiently low to ensure that the opposing cartilage is not damaged during articulation. Nowadays, various FKRIs are developed made out of metals or polymers.[12] Previous in vitro studies have shown that these types of materials exhibit suboptimal surface properties and generally lack sufficient lubrication, which can lead to tissue degradation and decreased lifespan post-implantation in vivo.[4,13–19]

Surface modifications like coatings are often used to enhance lubrication of synthetic systems. Also for medical devices, including orthopedic implants, coatings can be used to improve lubrication and wear resistance.[20–22] Ideally, the native lubricious molecules present in the surrounding synovial fluid should functionally adsorb to the artificial implant surface, mimicking the lamina splendens of the native cartilage that is being replaced. A biomolecule which was found to tether various molecules on various substrates including metals and polymers is dopamine.[23,24] Therefore, bioinspired coatings using dopamine conjugates and polysaccharides such as hyaluronic acid (HA) are getting more attention for use in biomedical applications.[25–27] This type of coating seems a promising lubricating candidate for FKRIs.[28–30] Wan et al. (2020) showed that friction can be reduced between polycarbonate urethane (PCU) and opposing cartilage by applying a layer-by-layer (LbL) biocompatible coating composed of poly-l-lysine (PLL) and hyaluronic acid – dopamine conjugate (HADN) on the biomaterial.[28] The cationic PLL showed attractive electrostatic interaction with the negatively charged surface of PCU while HADN (anionic polymer) interacts with PLL through both electrostatic interactions and covalent bonds. Subsequently, part of the HA and dopamine groups of the upper HADN layer that stay freely available were expected to interact with the molecules present in the synovial fluid.[28,31] Previous studies have shown that the mucin domains of PRG4 are able to reduce friction between two opposing (synthetic) articulating layers.[4,5,7] Therefore, the present study specifically focusses on in whether PRG4 binds to this LbL PLL-HADN coating.

To overcome the drawbacks associated with the use of non-degradable materials such as PCU for permanent implants (e.g., limited lifespan and mechanical mismatch), biodegradable polymers are gaining interest as material for regenerative FKRIs.[16,32] One such biomaterial that can be used as scaffold for cartilage tissue engineering is polycaprolactone (PCL).[33–36] Therefore, the aim of the present study was to determine if PCL can be coated with a LbL PLL-HADN coating and whether PRG4 can adsorb onto this coating and thereby reduce the friction when articulating against articular cartilage. If successful, PLL-HADN coating may be used to develop functionalized surfaces for PCL-based FKRIs.

A custom sandwich enzyme linked immunosorbent assay (ELISA) was developed to quantify the amount of immobilized human recombinant (rh)PRG4 after exposure to a PLL-HADN coating. The effect on lubrication was evaluated by comparing the coefficient of friction (CoF) of bare and PLL-HADN coated PCL against cartilage using a lubricant solution consisting of native synovial fluid components. Roughness and water contact angle (WCA) measurements were used to characterize the surface with and without the coating and/or synovial fluid components.

2 | MATERIALS AND METHODS

2.1 | Coating and lubricant preparation

Dopamine modification of HA to obtain HADN was described previously by Wan et al. (2020).[28] Briefly, 600 kDa HA (Kraeber & Co GmbH, Ellerbek, Germany) was coupled to dopamine hydrochloride (Sigma-Aldrich, St. Louis, MO) by the active agent N-(3-Dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC, Sigma-Aldrich). HADN was stored at −20°C until further use.

Lubricant solution was prepared fresh by adding in 10 mg/mL bovine serum albumin (BSA) (Roche, Mannheim, Germany), 3 mg/mL HA sodium salt (Streptococcus equi, 91%, M_w ≥ 1 MDa) (Alfa Aesar, Ward Hill, MA), and 200 μg/mL rhPRG4 (Lμbris Biopharma, LLC, Naples, FL) to phosphate buffered saline (PBS) (Sigma-Aldrich) to isolate the effect of these specific components.[17,37]

2.2 | Enzyme linked immunosorbent assay (ELISA)

A custom sandwich ELISA was created to measure the amount of immobilized rhPRG4 after exposure to a single-bilayer PLL-HADN coating. Briefly, high binding 96 well plates (96 Nunc maxisorp, Thermo Scientific, Denmark) were coated with 0.5 mg/mL PLL hydrobromide (M_w 30–70 kDa) (Sigma-Aldrich) followed by 0.5 mg/mL HADN in PBS for 10 min with intermediate rinsing with PBS. Both coated and uncoated wells (serving as negative control for comparison) were blocked with 5% milk powder (Elk, FrieslandCampina, The Netherlands) in PBS for 1 h at 37°C. After the block was removed, 100 μL of serial dilutions of rhPRG4 in PBS (range 3–200 ng/mL) were loaded on naked wells, and diluted lubricant samples (1:200 and 1:2000, range of detection limit) together with a negative control lacking rhPRG4 were loaded on both the coated and uncoated wells and incubated overnight at 4°C, all in duplicate. The plates were then

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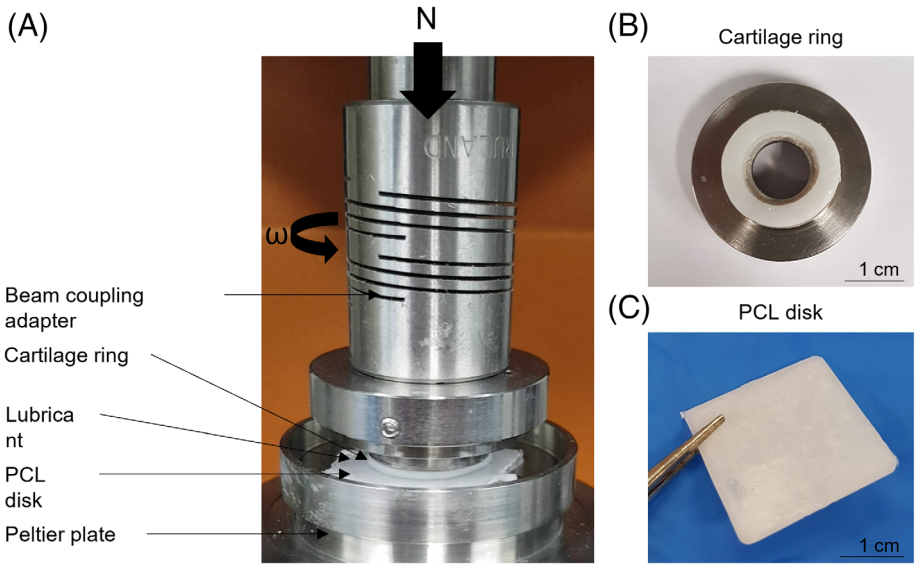

FIGURE 1 (A) The ring-on-disk friction system. (B) Top view of a cartilage ring glued to the upper holder and (C) a PCL disk.

blocked again and thereafter incubated with 2 µg/mL anti-PRG4 mouse monoclonal antibody (mAb) 9G3 (EMD Millipore, Billerica, MA) in antibody diluent (PBS + 0.1% BSA) for 1 h. Subsequently, stabilized peroxidase conjugated goat-anti mouse (Invitrogen, USA) at 126 ng/mL in antibody diluent was incubated for 1 h. Finally, plates were developed with 1 mg/mL TMB staining (Sigma-Aldrich), and the development was stopped with 0.8 M sulfuric acid (Sigma-Aldrich) in PBS. Absorbance of the samples and standards was measured at 450 nm in a Synergy HTX multimode reader (BioTek). The linear range was used for further calculations.

2.3 | Sample preparation

Square shaped PCL disks (25 × 25 mm, h = 1 mm) were obtained by melting 700 µg medical grade PCL granules (Purasorb PC12, Corbion Inc, The Netherlands) in a Teflon mold at 60°C for 15 min. Subsequently, a glass slide was placed on top to ensure a smooth surface and the PCL was cooled to room temperature (Figure 1C).

Skeletally mature bovine patella (3–6 years old) were collected soon after slaughter and stored at −20°C. For sample preparation, patellae were completely immersed in PBS at 4°C for 24 h. Cartilage quality was judged by visible inspection. Patellae which showed fissures or roughening of the surface were excluded. Full thickness cartilage was isolated from the underlying bone of the distal-lateral quadrant of the patella using a razor blade, and converted into a ring shape ($Ø_{in}$ = 13 mm, $Ø_{out}$ = 20 mm) using a custom made ring-punch (Figure 1B). The punched-out core of each ring was stored in PBS and used as control for surface changes due to the friction experiment.

2.4 | PLL-HADN coating on PCL disks

All PCL disks (n = 12) were degreased with N-hexane (Acros organics, Geel, Belgium) and rinsed extensively with milli-Q water, both for 10 min. Subsequently, half of the total number of PCL disks (n = 6) were alternately incubated with 625 µL of 0.5 mg/mL PLL in PBS and 0.5 mg/mL HADN in PBS for 10 min with intermediate rinsing with PBS. This procedure was performed four times to create an eight-layer deposition (coating thickness ∼ 78 nm[28]), ending with an HADN layer on top. The other PCL disks (n = 6) were stored in PBS.

2.5 | Water contact angle (WCA) measurements

Water contact angle measurements were performed at room temperature using an OCA 30 contact angle goniometer (DataPhysics Instruments) with the sessile drop method using 2 µL ultrapure water for each measurement. The average of five measurements on each sample was used for further analysis.

2.6 | Roughness measurements

The surface roughness of both cartilage and the PCL disks was measured in dry condition using a 20× objective of an optical profiler (PLµ 2300 Sensofar). For each measurement, an area covering approximately 640 × 480 µm^2 was imaged and corrected for tilt using a third-order polynomial fit. The arithmetic average of the roughness profile, Ra, was determined as $a = \frac{1}{n}\sum_{i=1}^{n}|y_i|$, with y being the deviation from the average sample height at a given point. The average of five measurements at different locations on each sample was taken for further analysis.

2.7 | Friction measurements

The CoF was measured using a Discovery Hybrid Rheometer (DHR-3, TA instruments) expanded with a ring on disk accessory as previously described by Damen et al. (2020) (Figure 1A). The cartilage ring was

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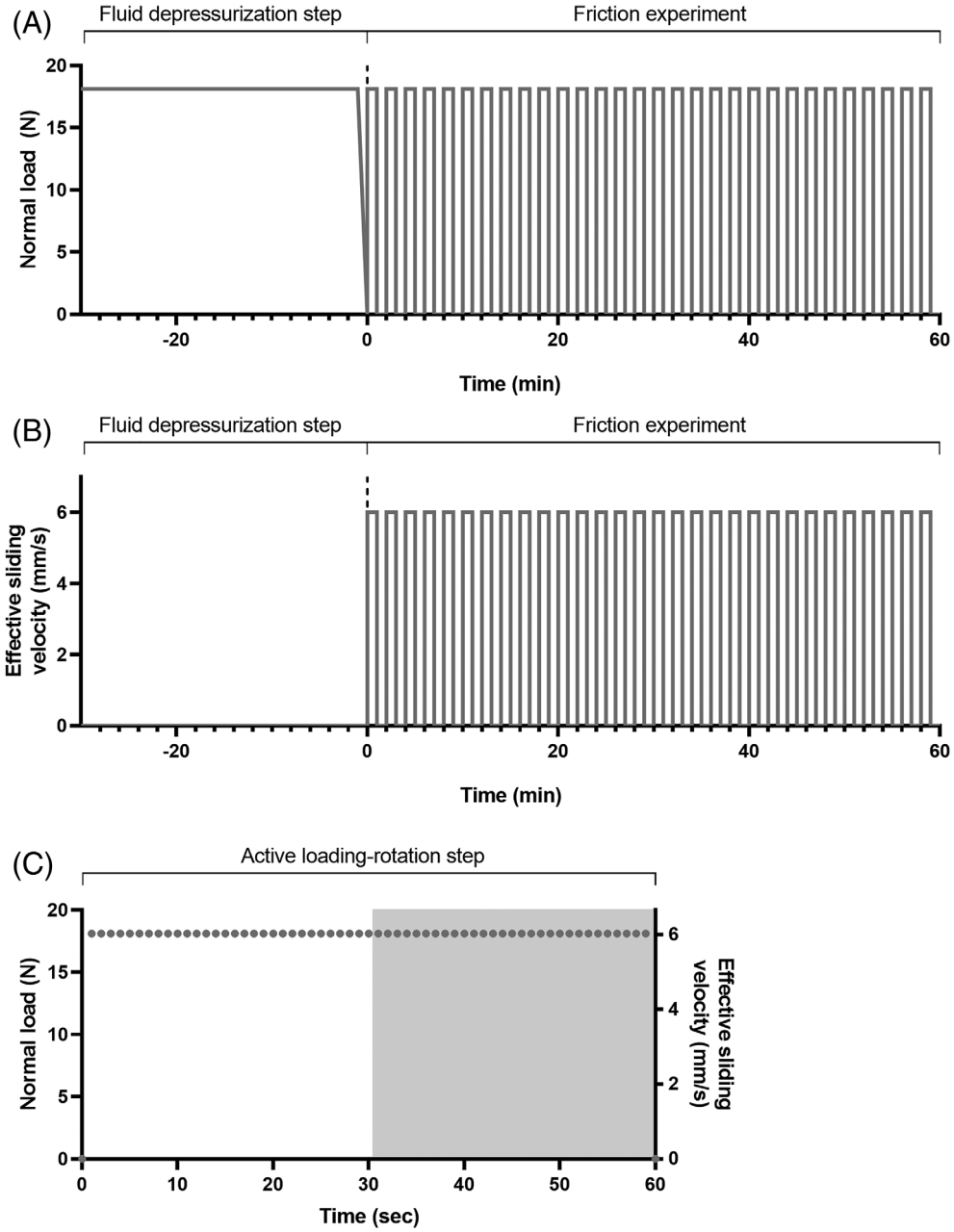

FIGURE 2 Schematic of the 30-minute fluid depressurization step followed by a 60-minute intermitted loading-rotation protocol depicting the (A) normal applied load and (B) effective sliding velocity. (C) Within each one-minute active loading-rotation step, the gray area indicates the duration in which the data was collected (30 data points, 1/sec) and averaged for further use.

glued with cyanoacrylate to the upper geometry holder and the PCL disk to the lower cup. The surface of the disk was wetted with 0.5 mL of lubricant solution to ensure lubricant presence on the contact interface. The temperature of the lubricant was kept constant during experiments at 32°C.[38] Before each friction test, samples were compressed and depressurized using a normal load of 18.1 N (0.1 MPa) for 30 min. The CoF was determined using an intermitted loading-rotation protocol with one-minute steps (Figure 2A). The normal load and the effective sliding velocity ($v_{eff} = \omega \times R_{mean}$, where ω is angular velocity and R_{mean} is average radius [=8.25 mm]) were set at respectively 18.1 N and 6 mm/s during the one-minute measuring steps. No load or rotation was applied during the one-minute rest in between. CoF was calculated by dividing the measured frictional force by the applied normal force for each second. The average CoF of the last 30 seconds of each active loading-rotation step was taken for further analysis (Figure 2B). Paired measurements were performed using the same cartilage ring for first a bare and subsequently a PLL-HADN coated PCL disk.

2.8 | Statistical analysis

Statistical analyses were performed with GraphPad Prism 8.02. Data are presented as mean ± standard deviation (*SD*). Two-tailed Mann Whitney test was used to confirm equality between the two groups of PCL disks prior to coating. One-tailed Mann–Whitney test was used to assess differences in surface roughness and WCA after half of the PCL disks were coated. The one-tailed Wilcoxon matched-pairs

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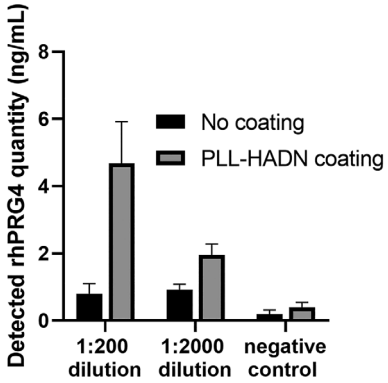

FIGURE 3 Determination of immobilized rhPRG4 quantity on naked (black bars) wells and PLL-HADN coated (gray bars) wells.

signed rank test was used to assess whether the CoF of the coated PCL disks was lower than that of the bare PCL disks measured using the same cartilage ring. For comparing the surface roughness and WCA of loaded and unloaded areas of cartilage and PCL after the friction experiment, two-tailed Wilcoxon matched-pairs signed rank tests were performed. *P*-values lower than 0.05 were considered significant.

3 | RESULTS

3.1 | Immobilized rhPRG4 quantification

The amount of immobilized rhPRG4 was two-fold and six-fold more on the PLL-HADN coated wells compared to the uncoated wells for respectively the 1:2000 and 1:200 lubricant solution (1:2000: 2.0 ± 0.3 ng/mL versus 0.9 ± 0.2 ng/mL and 1:200: 4.7 ± 1.2 ng/mL versus 0.8 ± 0.3 ng/mL, Figure 3). A rhPRG4-poor lubricant solution was used as negative control and showed negligible rhPRG4 quantity for both groups (on average 0.3 ± 0.2 ng/mL).

3.2 | Sample characterization

The average surface roughness and WCA of all 12 PCL disks prior to the experiment were 50.9 ± 9.2 nm and 74.7 ± 4.4°, respectively. There were no differences in surface roughness ($P = .49$) and WCA ($P = .82$) between the two groups of PCL disks prior to applying the coating.

3.3 | Effect of coating on surface characterization

The average surface roughness was significantly higher for the PLL-HADN coated PCL disks compared to the bare PCL disks, 83.0 ± 24.0 nm and 54.9 ± 6.8 nm, respectively ($P = .02$) (Figure 4). The average WCA was significantly lower for the PLL-HADN coated PCL disks compared to the bare PCL disks, 53.5 ± 3.5° and 69.5 ± 4.8°, respectively ($P = .001$) indicating that the coated disks were more hydrophilic. Brightfield microscopy showed a non-confluent layer of a bottlebrush structure on the coated disk whereas the appearance of the bare disk was rather smooth (Figure 4).

3.4 | Friction reducing effect of PLL-HADN coating

Figure 5 shows that the average CoF measured during the first minute of the friction experiment of the bare PCL against cartilage exceeded twice the CoF of the PLL-HADN coated PCL against cartilage, 0.196 ± 0.067 and 0.095 ± 0.028, respectively ($P = .016$). For both the bare and the PLL-HADN coated group, the CoF decreased over time. After 60 minutes, the CoF reached equilibrium values of 0.097 ± 0.002 for the bare PCL disks which was still significantly higher compared to 0.071 ± 0.002 for the coated PCL disks ($P = .047$).

3.5 | Cartilage surface changes due to friction experiment

No significant differences were found for both the average surface roughness and WCA of the cartilage rings after the friction experiment compared to the paired central core that was punched out during preparation and used as control (respectively $P = .16$ and $P = .44$) (Figure 6).

3.6 | Surface changes of PCL disks

Figure 7 shows that no significant differences were found in average surface roughness or WCA between the surface of the PCL disks prior to the friction experiment (Before) and the surface after the friction experiment (Loaded, ring). Further, no differences were found between the part of the PCL disk that was in contact with the opposing cartilage during the friction experiment (Loaded, ring) and the middle part that did not experience any contact (Unloaded, core), for both the bare and the PLL-HADN coated disks.

4 | DISCUSSION

The present study demonstrated that a single PLL layer overlaid with a layer of HADN enabled adsorption of rhPRG4 from the surrounding lubricant despite the presence of BSA and HA. In addition, it was demonstrated that friction between PCL implant material and cartilage was significantly lower for LbL PLL-HADN coated PCL disks compared to bare disks, presumably because the coating was able to bind rhPRG4 from of the lubricating fluid.

The present study examined a bioinspired PLL-HADN coating. Comparable to literature in which different coatings have been

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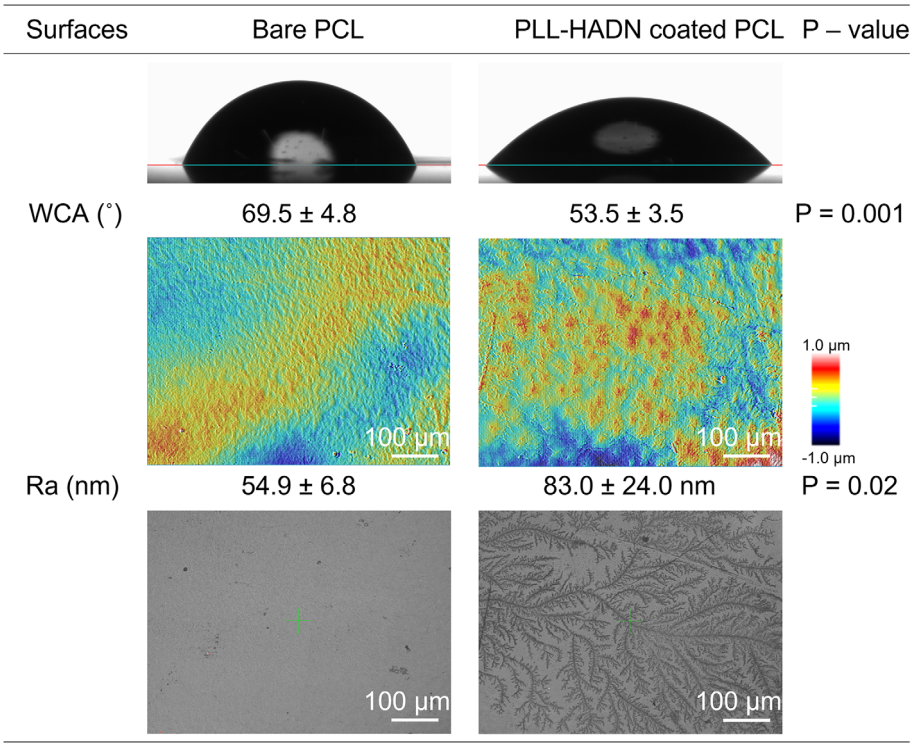

FIGURE 4 Representative images, average value ± *SD* and *P*-value of static water contact angle (WCA) measurement and average surface roughness (Ra) of both a bare (left) and a PLL-HADN coated (right) PCL disk. The bottom row shows the corresponding brightfield image of the PCL surface in the middle row.

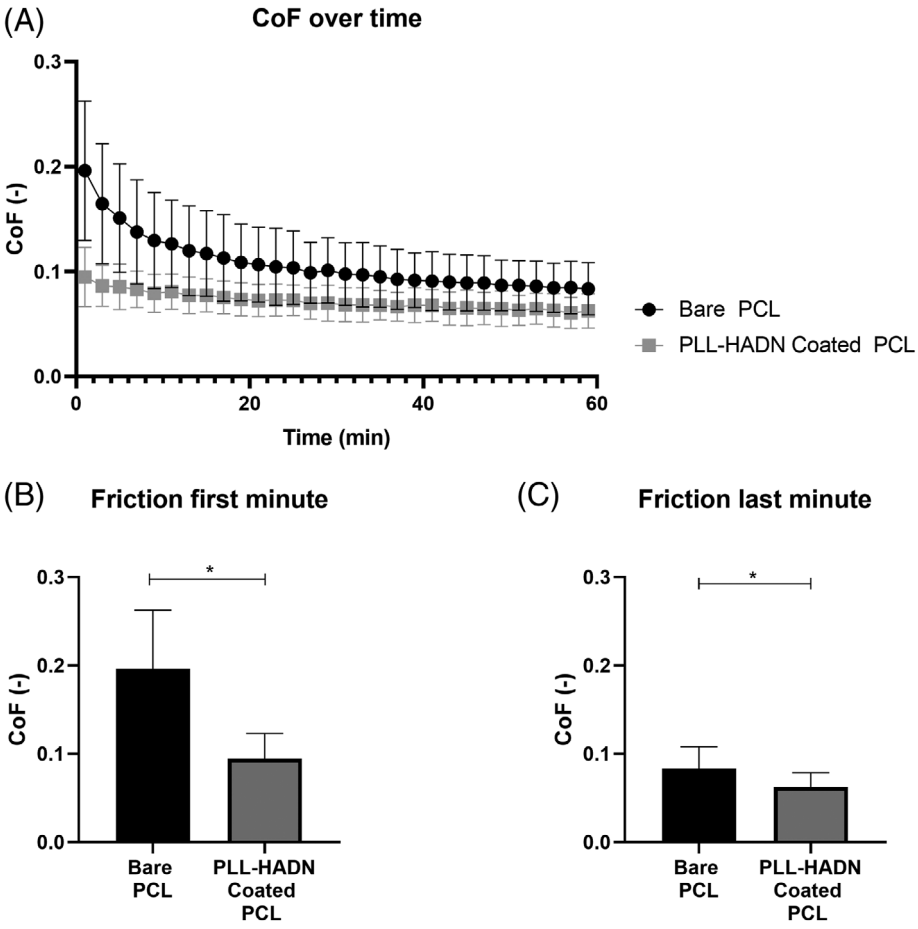

FIGURE 5 (A) The average ± *SD* CoF acquired over time with bare PCL (black) and PLL-HADN coated PCL (gray) disks against cartilage. (B) Average CoF measured during the first minute of the experiment. (C) Average CoF measured during the last minute of the experiment. $n = 6$, $^*P < .05$.

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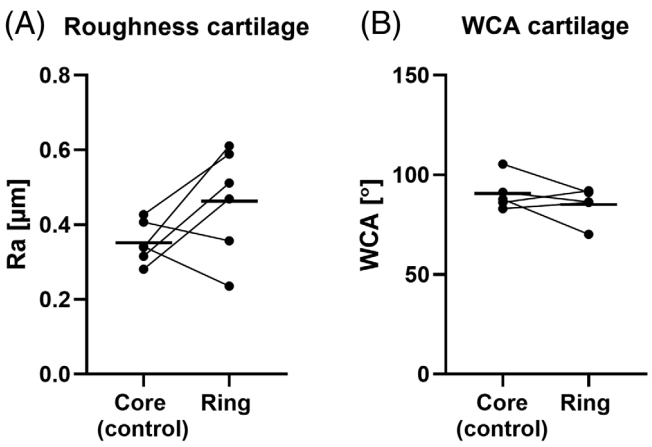

FIGURE 6 Individual paired values for (A) surface roughness and (B) water contact angle of the cartilage ring after the friction experiment compared to the middle core which was used as control. Connected datapoints represent the same sample. Horizontal bars indicate the average values.

studied before, the average surface roughness increases after applying the coating.[21,25,27–29] This is probably a result of the adhesion of PLL and HADN to the underlaying smooth PCL surface. Increased hydrophilicity due to the PLL-HADN coating observed by Wan et al. 2020 was also confirmed in the present study and in line with other studies using dopamine-conjugate coatings.[26–29] The comparable changes in surface roughness and wettability, together with the visible change using brightfield microscopy, provided evidence for the presence of the PLL-HADN coating on the PCL disks.

Previous literature reported divergent conclusions regarding PRG4 binding in the presence of albumin, the most abundant and surface-active protein of synovial fluid. It was stated that albumin blocked the interaction of PRG4 with HA and adsorption of PRG4 on PCU surfaces, but not that of PRG4 on collagen II fibrils.[17,39] This is one of the reasons why, in addition to using the lubricating components HA and PRG4, this study also added BSA to the lubricant solution. From the results of the present study, it was clear that, despite the presence of albumin, rhPRG4 attached to the PLL-HADN coating. The double amount of immobilized rhPRG4 on the coated compared to the uncoated surfaces gives evidence that rhPRG4 is deposited on the upper HADN layer (Figure 3). It has previously been suggested that the dopamine modification of HA prevents albumin blockade and therefore allows PRG4 interaction.[28] The findings of the present study are in agreement with and extend previous findings of Wan et al. (2020).[28]

Interestingly, directly after coating, a non-confluent bottlebrush structure was visible on the PLL-HADN coated PCL disks which was not shown before (Figure 4). The lack of confluence may be the reason for the wide spread in surface roughness directly after coating. Brightfield observations after the friction experiment showed that this structure was still present on both the loaded and unloaded part of the PLL-HADN coated PCL disks, whereas the bare disks remained equally smooth. It is uncertain why this bottlebrush structure was present, but it may be a product of the dry state in which the surfaces were observed, and it is uncertain whether this structure exists when the surface is wet. This may also explain why, despite the higher surface roughness (when dry), the PLL-HADN coated disks still result in a lower CoF compared to the bare PCL disks in wet condition.

After modification of the PCL disks with a PLL-HADN coating, the improved lubrication yielded a lower constant CoF (Figure 5). The PLL-HADN coated PCL reached equilibrium values after 20 min while the bare PCL only reached an equilibrium after 60 min. It was suggested that the amount of immobilized PRG4 on the contacting surfaces was saturated at that stage. A similar decrease over time was seen by Wan et al. (2020).[28] For the present study, a division could be made between the six paired samples. For four out of six PLL-HADN coated PCL disks, the CoF value measured in the first minute of the experiment was less than half the CoF value obtained with the bare PCL disk while using the same cartilage ring (an average CoF reduction of 60%). The other two paired samples showed an average reduction in CoF of only 26%. Remarkably, these two pairs were responsible for the lowest two CoF values of all bare PCL disks. Since there were no measurable differences prior to the experiment between these two bare PCL disks (circle and upwards triangle in Figure 7) and the others, it was suggested that this was a result of the biological differences between the cartilage rings. Altered cartilage tissue properties like proteoglycan or collagen loss and reduced function of the lamina splendens are common features of cartilage degeneration and make that the superficial layer is less able to absorb and obtain PRG4 to the surface. These features are not always captured using surface roughness and wettability measurements. It is suggested that the lower the quality of the cartilage, the more beneficial the effect of the coating on the opposing articulating surface.

The average WCA of 88° for both the cartilage rings and inner cores was comparable to earlier reported contact angles measured with deionized water on bovine cartilage (Figure 6B).[40] Similar to Wan et al. (2020), who measured a comparable roughness of 0.33 µm for fresh bovine cartilage, no significant differences in cartilage surface roughness were found after rubbing against biomaterial with a slightly higher contact pressure (0.4 MPa) (Figure 6A).[28] The fact that no significant differences were found between the loaded cartilage rings and the paired unloaded punched-out cartilage core suggests that the cartilage surface was not altered and that the observed reduction in friction can be attributed to the presence of the coating and not to alterations of the cartilage ring surface during the first measurement. Additionally, no significant differences were found in average surface roughness and WCA after the friction experiment between the loaded and unloaded area of both the coated and the bare PCL disks indicating that the coating is resistant over mechanical loading and that there was no debris. This finding is in agreement with Wan et al. (2020) who saw that the PLL-HADN remained tightly attached to the PCU.[28] Noteworthy is the sample that is depicted as (filled and unfilled) upward triangle in Figure 7. The loaded area of both the bare and the PLL-HADN coated PCL sample showed an increased surface roughness. This pair experienced the least decrease in CoF between the bare and the coated PCL disk. Surprisingly, the average roughness of

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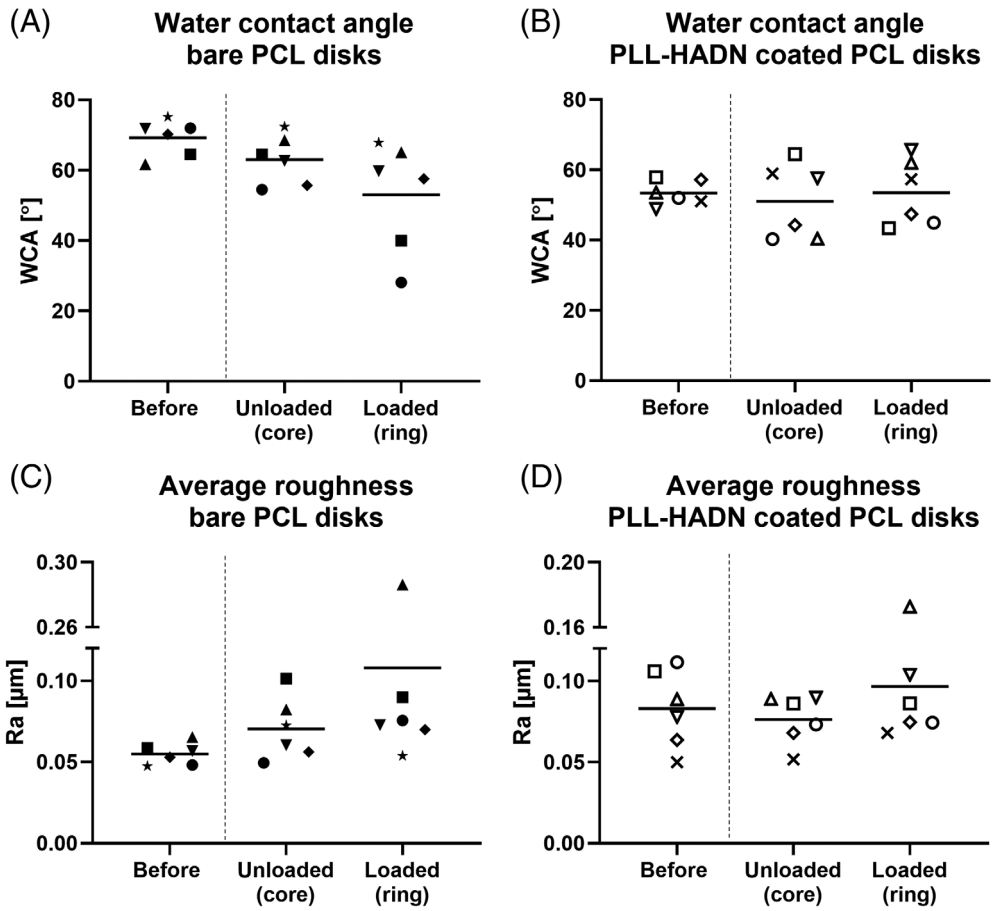

FIGURE 7 Average (A, B) water contact angle and (C, D) surface roughness for the (A, C) bare and the (B, D) PLL-HADN coated PCL disks. Disks were measured before and after the friction experiment in which for the later a division was made between the area that was loaded against the opposing cartilage ring and the area that was not (core). Same samples represent the same symbol (note, open and closed symbols are different PCL samples but were articulated against the same cartilage ring). Horizontal bars indicate the average values.

this specific cartilage ring was lower than that of the punched-out core which was used as control. This indicates that the cartilage ring did not become rougher due to rotation against this relatively rough surface. This study was performed to demonstrate the potential of PLL-HADN coating for friction reduction in PCL samples. To prevent secondary effects by e.g. wear, the pressures used were lower compared to the eventual in vivo application (two orders of magnitude). Future studies should investigate the effect on altered WCA and surface roughness when cartilage articulares against these biomaterials under physiologically relevant pressures.

Although the lubrication mode that was explored in this study is believed to be the dominating one, it should be noted that friction under in vivo conditions contains more complex modes and higher pressures than what can be simulated with this in vitro test setup. The present study added the loading-unloading phases of the gait cycle in an in vitro study to the friction behavior of a cartilage implant in the knee, using an intermitted loading protocol which allowed for fluid replenishment onto the articulating surfaces. The cartilage ring might also absorb fluid in this system, but there were no indications that fluid pressure in the cartilage ring was affected during the experiment, or that such effect would be different between the bare and the coated group.

More variance in CoF was observed for the bare PCL disks compared to the coated PCL disks against cartilage. The present study used a conservative method in which the bare PCL disk was used first, meaning that the cartilage ring was also subjected to rotation for the first time in this group. A pilot study prior to this experiment showed no difference in CoF obtained for repeated measurements using the same cartilage and PCL pair. Although all patellae were thawed for 24 h in PBS and equilibrated in the lubricant solution during static compression, more biolubricant residuals may have been present on the surface of one cartilage ring compared to the other, which might have resulted in the wider spread for the bare PCL CoF data. If the lamina splendens would have been affected by the first friction experiments against bare PCL, the CoF data of cartilage against PLL-HADN coated PCL is overestimated. The latter value would be even lower when used against intact cartilage in vivo.

The present study used an artificial synovial fluid to ensure a certain concentration of albumin, HA and PRG4 in the lubricant to isolate the effect of these specific important components on the coating. The lubricant was based on physiological concentrations found in healthy human knee joints.[37] A previous study using the same setup showed that CoF values using the present lubricant solution were close to values obtained with bovine synovial fluid for a cartilage-on-cartilage interface.[4] It is known that synovial fluid components and concentrations can change during the progression of osteoarthritis.[8,37] Future research should determine the effect of altered concentrations of lubricant components and the addition of phospholipids on the lubricating ability of the PLL-HADN coating.[41] In addition to friction, there are other factors that determine the clinical performance of materials

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during articulation. These may become apparent under higher pressures or other velocity profiles. Therefore, before translating this application to the clinic, further evaluation under clinically relevant conditions of load and kinematics is required. In addition, future research should determine the degradation time and durability of the PLL-HADN coating over a longer period of time. Furthermore, it may be interesting to see whether this friction-reducing coating can also be applied to materials other than PCU and PCL.

5 | CONCLUSION

In conclusion, the current study used a LbL self-assembly approach to functionalize the articulating surface of FKRI material to reduce the friction experienced when articulating against opposing cartilage. The present study showed, while using PLL as an adhesive, that the exposed dopamine modified hyaluronan was able to immobilize rhPRG4 despite the presence of other large biomolecules as albumin and HA in the solution. Furthermore, a significant difference in surface roughness and WCA together with a visible difference using brightfield microscopy showed that PCL can effectively be coated with PLL-HADN. Additionally, the friction reducing effect of this LbL PLL-HADN coating was demonstrated under conditions that are close to the in vivo situation. All together these results show that a coating can be used to further develop functionalized self-lubricating surfaces for osteochondral implants. Specifically, this PLL-HADN coating is a promising application to improve the clinical success of PCL FKRIs, mimicking the lubricating surface of native cartilage.

AUTHOR CONTRIBUTIONS

A. H. A. Damen, C. C. van Donkelaar, P. K. Sharma, T. A. Schmidt and K. Ito conceived and designed the study. H. Wan produced and consulted on the HADN. R. Cardinaels helped design the friction experiments. A. H. A. Damen conducted the experiments. A. H. A. Damen, C. C. van Donkelaar, P. K. Sharma, T. A. Schmidt and K. Ito examined and helped to interpret the data. A. H. A. Damen drafted the article. Critical revising of the article was done by all co-authors and all authors have given approval to the final version of the manuscript.

ACKNOWLEDGMENTS

This research was performed under the framework of Chemelot InSciTe, supported by the partners of Regenerative Medicine Crossing Borders and powered by Health~Holland, Top Sector Life Sciences & Health. We acknowledge Hans J. Kaper (University Medicial Center Gronginen) for his help regarding the PLL-HADN coating. We are grateful to TA instruments for providing the DHR-3 rheometer under the Young Distinguished Rheologist Award and Instrument Grant and to Lμbris BioPharma for the kind donation of rhPRG4.

CONFLICT OF INTEREST STATEMENT

TAS has authored patents on rhPRG4 and holds equity in Lubris LLC, FL, USA. TAS is also a paid consultant for Lubris LLC, FL, USA.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ROLE OF THE FUNDING SOURCE

The funding source has no role in the study design, collection, analysis and interpretation of data, nor in the writing of the manuscript and the decision to submit the manuscript for publication.

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REFERENCES

1. Jahn S, Seror J, Klein J. Lubrication of articular cartilage. *Annu Rev Biomed Eng*. 2016;18(1):235-258.
2. Wright V, Dowson D. Lubrication and cartilage. *J Anat*. 1976;121(Pt 1):107-118.
3. Jay GD, Torres JR, Rhee DK, et al. Association between friction and wear in diarthrodial joints lacking lubricin. *Arthritis Rheum*. 2007;56(11):3662-3669.
4. Damen AHA, van Donkelaar CC, Cardinaels RM, Brandt JM, Schmidt TA, Ito K. Proteoglycan 4 reduces friction more than other synovial fluid components for both cartilage-cartilage and cartilage-metal articulation. *Osteoarthr Cartil*. 2021;29(6):894-904.
5. Schmidt TA, Gastelum NS, Nguyen QT, Schumacher BL, Sah RL. Boundary lubrication of articular cartilage: role of synovial fluid constituents. *Arthritis Rheum*. 2007;56(3):882-891.
6. Gleghorn JP, Jones ARC, Flannery CR, Bonassar LJ. Boundary mode lubrication of articular cartilage by recombinant human lubricin. *J Orthop Res*. 2009;27(6):771-777.
7. Jay GD, Waller KA. The biology of lubricin: near frictionless joint motion. *Matrix Biol*. 2014;39:17-24.
8. Ludwig TE, McAllister JR, Lun V, Wiley JP, Schmidt TA. Diminished cartilage-lubricating ability of human osteoarthritic synovial fluid deficient in proteoglycan 4: restoration through proteoglycan 4 supplementation. *Arthritis Rheum*. 2012;64(12):3963-3971.
9. Ludwig TE, Hunter MM, Schmidt TA. Cartilage boundary lubrication synergism is mediated by hyaluronan concentration and PRG4 concentration and structure. *BMC Musculoskelet Disord*. 2015;16(1):386.
10. Klein J. Molecular mechanisms of synovial joint lubrication. *Proc IMechE Part J J Eng Tribol*. 2006;220:691-710.
11. Chan SMT, Neu CP, DuRaine G, Komvopoulos K, Reddi AH. Atomic force microscope investigation of the boundary-lubricant layer in articular cartilage. *Osteoarthr Cartil*. 2010;18(7):956-963.
12. Jeuken RM, Roth AK, Peters MJM, et al. In vitro and in vivo study on the osseointegration of BCP-coated versus uncoated nondegradable thermoplastic polyurethane focal knee resurfacing implants. *J Biomed Mater Res Part B Appl Biomater*. 2020;108(8):3370-3382.
13. Oungoulian SR, Durney KM, Jones BK, Ahmad CS, Hung CT, Ateshian GA. Wear and damage of articular cartilage with friction against orthopedic implant materials. *J Biomech*. 2015;48(10):1957-1964.
14. Stotter C, Stojanović B, Bauer C, et al. Effects of loading conditions on articular cartilage in a metal-on-cartilage pairing. *J Orthop Res*. 2019;37(12):2531-2539.
15. Li F, Su Y, Wang J, Wu G, Wang C. Influence of dynamic load on friction behavior of human articular cartilage, stainless steel and polyvinyl alcohol hydrogel as artificial cartilage. *J Mater Sci Mater Med*. 2010;21(1):147-154.
16. Majd SE, Rizqy AI, Kaper HJ, Schmidt TA, Kuijer R, Sharma PK. An in vitro study of cartilage–meniscus tribology to understand the

15524981, 2023, 8, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/jbm.b.35251 by Bibliotheek Rijksuniversiteit, Wiley Online Library on [10/07/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

changes caused by a meniscus implant. *Colloids Surf B Biointerfaces*. 2017;155:294-303.
17. Majd SE, Kuijer R, Schmidt TA, Sharma PK. Role of hydrophobicity on the adsorption of synovial fluid proteins and biolubrication of polycarbonate urethanes: materials for permanent meniscus implants. *Mater Des*. 2015;83:514-521.
18. Custers RJH, Dhert WJA, Saris DBF, et al. Cartilage degeneration in the goat knee caused by treating localized cartilage defects with metal implants. *Osteoarthr Cartil*. 2010;18(3):377-388.
19. Martinez-Carranza N, Ryd L, Hultenby K, et al. Treatment of full thickness focal cartilage lesions with a metallic resurfacing implant in a sheep animal model, 1 year evaluation. *Osteoarthr Cartil*. 2016;24(3):484-493.
20. Choudhury D, Lackner J, Fleming RA, Goss J, Chen J, Zou M. Diamond-like carbon coatings with zirconium-containing interlayers for orthopedic implants. *J Mech Behav Biomed Mater*. 2017;68:51-61.
21. Döring J, Crackau M, Nestler C, Welzel F, Bertrand J, Lohmann CH. Characteristics of different cathodic arc deposition coatings on CoCrMo for biomedical applications. *J Mech Behav Biomed Mater*. 2019;97:212-221.
22. Liu S, Zhang Q, Han Y, Sun Y, Zhang Y, Zhang H. Bioinspired surface functionalization of titanium alloy for enhanced lubrication and bacterial resistance. *Langmuir*. 2019;35(40):13189-13195.
23. Lee H, Dellatore SM, Miller WM, Messersmith PB. Mussel-inspired surface chemistry for multifunctional coatings. *Science*. 2007;318(5849):426-430.
24. Lee H, Rho J, Messersmith PB. Facile conjugation of biomolecu les onto surfaces via mussel adhesive protein inspired coatings. *Adv Mater*. 2009;21(4):431-434.
25. Wu F, Li J, Zhang K, et al. Multifunctional coating based on hyaluronic acid and dopamine conjugate for potential application on surface modification of cardiovascular implanted devices. *ACS Appl Mater Interfaces*. 2016;8(1):109-121.
26. Lee S, Kim S, Park J, Lee JY. Universal surface modification using dopamine-hyaluronic acid conjugates for anti-biofouling. *Int J Biol Macromol*. 2020;151:1314-1321.
27. Neto AI, Cibrão AC, Correia CR, et al. Nanostructured polymeric coatings based on chitosan and dopamine-modified hyaluronic acid for biomedical applications. *Small*. 2014;10(12):2459-2469.
28. Wan H, Zhao X, Lin C, Kaper HJ, Sharma PK. Nanostructured coating for biomaterial lubrication through biomacromolecular recruitment. *ACS Appl Mater Interfaces*. 2020;12(21):23726-23736.
29. Wan H, Ren K, Kaper HJ, Sharma PK. Cartilage lamina splendens inspired nanostructured coating for biomaterial lubrication. *J Colloid Interface Sci*. 2021;594:435-445.
30. Han L, Xiang L, Zhang J, et al. Biomimetic lubrication and surface interactions of dopamine-assisted zwitterionic polyelectrolyte coatings. *Langmuir*. 2018;34(38):11593-11601.
31. S D, X B, B Z, GW G, GD J, JN I. Synergistic interactions between grafted hyaluronic acid and lubricin provide enhanced wear protection and lubrication. *Biomacromolecules*. 2013;14(5):1669-1677.
32. Christenson EM, Dadsetan M, Wiggins M, Anderson JM, Hiltner A. Poly(carbonate urethane) and poly(ether urethane) biodegradation: in vivo studies. *J Biomed Mater Res Part A*. 2004;69A(3):407-416.
33. Moutos FT, Guilak F. Functional properties of cell-seeded three-dimensionally woven poly(ε-caprolactone) scaffolds for cartilage tissue engineering. *Tissue Eng Part A*. 2010;16(4):1291-1301.
34. Liao I, Moutos F, Estes B, Zhao X, Guilak F. Composite three-dimensional woven scaffolds with interpenetrating network hydrogels to create functional synthetic articular cartilage. *Adv Funct Mater*. 2013;23(47):5833-5839.
35. Moutos FT, Glass KA, Compton SA. Anatomically shaped tissue-engineered cartilage with tunable and inducible anticytokine delivery for biological joint resurfacing. *Proc Natl Acad Sci*. 2016;113(31):E4513-E4522.
36. Manavitehrani I, Fathi A, Badr H, Daly S, Shirazi AN, Dehghani F. Biomedical applications of biodegradable polyesters. *Polymers (Basel)*. 2016;8(1):20-52.
37. Hui AY, McCarty WJ, Masuda K, Firestein GS, Sah RL. A systems biology approach to synovial joint lubrication in health, injury, and disease. *Wiley Interdiscip Rev Syst Biol Med*. 2012;4(1):15-37.
38. Becher C, Springer J, Feil S, Cerulli G, Paessler HH. Intra-articular temperatures of the knee in sports – an in-vivo study of jogging and alpine skiing. *BMC Musculoskelet Disord*. 2008;9:46.
39. Majd SE, Kuijer R, Köwitsch A, Groth T, Schmidt TA, Sharma PK. Both hyaluronan and collagen type II keep proteoglycan 4 (lubricin) at the cartilage surface in a condition that provides low friction during boundary lubrication. *Langmuir*. 2014;30(48):14566-14572.
40. Thomas BH, Craig Fryman J, Liu K, Mason J. Hydrophilic–hydrophobic hydrogels for cartilage replacement. *J Mech Behav Biomed Mater*. 2009;2(6):588-595.
41. Lin W, Mashiah R, Seror J, et al. Lipid-hyaluronan synergy strongly reduces intrasynovial tissue boundary friction. *Acta Biomater*. 2019;83:314-321.

How to cite this article: Damen AHA, van Donkelaar CC, Sharma PK, et al. Friction reducing ability of a poly-l-lysine and dopamine modified hyaluronan coating for polycaprolactone cartilage resurfacing implants. *J Biomed Mater Res*. 2023;111(8):1523-1532. doi:10.1002/jbm.b.35251

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