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A low friction, biphasic and boundary lubricating hydrogel for cartilage replacement

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ABSTRACT

Partial joint repair is a surgical procedure where an artificial material is used to replace localised chondral damage. These artificial bearing surfaces must articulate against cartilage, but current materials do not replicate both the biphasic and boundary lubrication mechanisms of cartilage. A research challenge therefore exists to provide a material that mimics both boundary and biphasic lubrication mechanisms of cartilage.

In this work a polymeric network of a biomimetic boundary lubricant, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), was incorporated into an ultra-tough double network (DN) biphasic (water phase + polymer phase) gel, to form a PMPC triple network (PMPC TN) hydrogel with boundary and biphasic lubrication capability. The presence of this third network of MPC was confirmed using ATR-FTIR. The PMPC TN hydrogel had a yield stress of 26 MPa, which is an order of magnitude higher than the peak stresses found in the native human knee. A preliminary pin on plate tribology study was performed where both the DN and PMPC TN hydrogels experienced a reduction in friction with increasing sliding speed which is consistent with biphasic lubrication. In the physiological sliding speed range, the PMPC TN hydrogel halved the friction compared to the DN hydrogel indicating the boundary lubricating PMPC network was working.

A biocompatible, tough, strong and chondral lubrication imitating PMPC TN hydrogel was synthesised in this work. By complementing the biphasic and boundary lubrication mechanisms of cartilage, PMPC TN hydrogel could reduce the reported incidence of chondral damage opposite partial joint repair implants, and therefore increase the clinical efficacy of partial joint repair.

Statement of Significance

This paper presents the synthesis, characterisation and preliminary tribological testing of a new biomaterial that aims to recreate the primary chondral lubrication mechanisms: boundary and biphasic lubrication. This work has demonstrated that the introduction of an established zwitterionic, biomimetic boundary lubricant can improve the frictional properties of an ultra-tough hydrogel. This new biomaterial, when used as a partial joint replacement bearing material, may help avoid damage to the opposing chondral surface—which has been reported as an issue for other non-biomimetic partial joint replacement materials.

Statement of Significance: Alongside the synthesis of a novel biomaterial focused on complementing the lubrication mechanisms of cartilage, your readership will gain insights into effective mechanical and tribological testing methods and materials characterisation methods for their own biomaterials.

1. Introduction

Human diarthroidal joint surfaces are covered in hyaline cartilage to enable low friction movement under the high loads

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experienced during routine activities [1]. Hyaline cartilage enables low friction via two primary mechanisms: boundary lubrication [2] and biphasic lubrication [3]. Boundary lubricants are molecules found on the surface of cartilage that form a protective molecular film [2]. Biphasic lubrication is defined by the load induced pressurisation of the chondral interstitial fluid that helps to support this load [3]. Any disruption to these lubricating mechanisms, for example chondral injury or osteoarthritis (OA), leads to the deterioration of the cartilage and ultimately joint pain. Indeed, current projections estimate that 8.3 million people in the UK will suffer from OA of the knee by 2035 [4].

Partial joint repair, which is an early intervention treatment for OA where an artificial material is fixed in a local chondral defect as to articulate against the opposing chondral surface, is an emerging technology within orthopaedics [5]. Several materials have been developed for partial joint repair, but none of them replicate both the biphasic and boundary lubrication mechanisms of cartilage. Materials currently being used include pyrocarbon (Moirai Orthopaedics PIR knee system) [6], Cobalt Chrome alloy (CoCr, Arthrosurface UniCap and HemiCap Systems) [7], polyvinyl alcohol (PVA) hydrogel (Cartiva SCI) [5] and a polyethylene—hyaluronic acid micro-composite (BioPoly) [8]. Solid materials, such as CoCr and pyrocarbon have neither boundary nor biphasic lubricating potential, and thus do not actively contribute to the lubrication process. Instead, they rely on the opposing surface to provide the lubrication mechanism. PVA hydrogels, being biphasic (solid phase + water phase) materials, may be a closer mimic to cartilage by enabling biphasic lubrication, but not boundary lubrication [5]; whereas the polyethylene—hyaluronic acid micro composite enables boundary lubrication but not biphasic lubrication [8]. A clear research challenge therefore exists to provide a material that imitates both boundary and biphasic lubrication mechanisms of cartilage.

Double network (DN) hydrogels are a class of ultra-tough, biphasic (water phase + solid phase) gels, which consist of a stiff and brittle polyelectrolyte gel, poly(2-Acrylamido-2-methylpropane sulfonic acid) (PAMPS), combined with a soft and ductile neutral polymer gel, Polyacrylamide (PAAm) [9]. The yield strength and energy to fracture of the DN hydrogels are greater than the sum of their two parts [9]. The high strength and toughness of these hydrogels are required so that the material can withstanding the demanding loads *in vivo*. The DN hydrogels also exhibit favourable coefficients of friction, but suffer from high wear [10]. The tribological properties of DN hydrogels can be modified through the addition of a third network, and this third network dominates the tribological behaviour of the material [11]. This third network would therefore provide an opportunity for introducing a boundary lubricating mechanism to the hydrogel.

Zwitterionic phospholipids, such as phosphatidylcholine, have been postulated to have a key role as a boundary lubricant in hyaline cartilage [12]. Indeed, surfaces bearing a zwitterionic, phosphatidylcholine mimicking polymer, poly(2-methacryloxyethyl phosphorylcholine) (PMPC) [13], have produced extremely low coefficients of friction. For example, two mica surfaces bearing PMPC articulated against each other with coefficient of friction values on the order of 10^{-4} at a contact pressure of 10.6 MPa [14]. Moreover, in the context of a functional engineering material, extremely low coefficients of friction and decreased macroscopic wear have been measured in tribological studies of PMPC surface modified cross linked polyethylene [15] and PMPC surface modified poly(ether ether ketone) [16]. The lubrication mechanism that governs such low coefficients of friction has been theorised as hydration lubrication [17]. Under shear, water molecules in the hydration shell of PMPC rapidly exchange with surrounding water molecules, allowing a fluid-like response to shear [17].

This study aims to synthesise, characterise and test a material that imitates both the biphasic and boundary lubrication mechanisms of cartilage, using a DN hydrogel to achieve the biphasic lubricating phase and polymerising a third network of a boundary lubricant mimicking polymer, PMPC, throughout the material to create the boundary lubricating phase. By imitating two of the primary chondral lubrication mechanisms, this material would be an ideal candidate material for early repair of localised cartilage defects in human joints.

2. Materials and methods

2.1. Materials

2-Acrylamido-2-methylpropane sulfonic acid (AMPS, 99%), acrylamide (AAm, ≥99%), methylenebisacrylamide (MBAA, 99%) and α-ketoglutaric acid (α-KA, >99.0%) (all Sigma-Aldrich, Poole, UK) were used to synthesise the hydrogels, and used without further purification. 2-methacryloyloxyethyl phosphorylcholine (MPC, 97.2%) (TCI UK Ltd, Oxford, UK) was used for zwitterionic third network and used without further purification. All water used was of ultra-pure grade with a resistivity of 18.2 MΩ cm. All solvents were of extra pure reagent grade and used without further purification.

2.2. Hydrogel synthesis

2.2.1. PAMPS single network hydrogel [9]

A 1 M aqueous solution of AMPS, with 4 mol% MBAA and 0.1 mol% α-KA, was degassed under argon bubbling for 30 min. The degassed monomer solution was then injected into a 40 mm × 80 mm void cut into a 1 mm butyl rubber sheet sandwiched between two borosilicate plates. The solution was polymerised under UV light (365 nm ± 50 nm) with a power density of 7.5 mW cm^{-2} for 24 h. The resulting single network PAMPS SN hydrogel sheet was immersed in a deionised water bath (replacing the water every day) for 7 days to remove any unreacted chemicals.

2.2.2. PAMPS-PAAm double network hydrogel [9]

The resulting swollen PAMPS single network (SN) hydrogel sheet was cut into multiple rounded edge rectangular samples of 15.5 mm × 21 mm. These samples were immersed for 24 h in an aqueous solution of 2 M AAm, with 0.1 mol% MBAA and 0.1 mol% α-KA. Each sample was press fit into a void cut from 3 mm thick butyl rubber sandwiched between two borosilicate plates and exposed to UV light under the same conditions as before. The resulting PAMPS-PAAm DN hydrogels, known from here on in as DN hydrogels, were immersed in a deionised water batch for 6 days in deionised water, as to remove any residual chemicals. The surfaces of the samples were gently wiped with damp lint free tissue to remove any non-crosslinked material from the sample surface.

2.2.3. PMPC triple network hydrogel

To create the PMPC TN hydrogels, the DN hydrogels were immersed in an aqueous solution of 1 M MPC, with 0.1 mol% MBAA and 0.1 mol% α-KA for 24 h. The UV polymerisation reaction from Section 2.2.1 was repeated and the samples were washed, as described in Section 2.2.2.

2.3. ATR-FTIR analysis

A Fourier transform infrared (FTIR) Spectrometer (Spectrum 100, Perkin Elmer, USA) equipped with a germanium single bounce micro-attenuated total reflection (ATR) objective was used to

collect all IR spectra. IR spectra were obtained from 75 scans between 800 cm^{-1} and 1800 cm^{-1} at a wavenumber resolution of 4 cm^{-1}. The presented IR absorbance spectrum for each hydrogel is the average of spectra obtained from three location of three independent samples. These spectra were not ATR corrected as these scans were only used for qualitative analysis.

2.4. Water content analysis

The water content of the hydrogels was measured using ISO 18369-4, which defines a gravimetric method to calculate the water content of hydrogels used for contact lenses [18]. SN, DN and TN hydrogel specimens that had been immersed in ringer's solution for 24 h, were dry blotted and weighed using a microscale with a precision of 0.01 mg (Sartorius AG, Germany). The samples were subsequently dried in a lab oven at 105 °C ± 5 °C until they reached a constant weight; after which they were weighed again.

The water content (ω_{H_2O}) is defined by Eq. (1):

$$\omega_{H_2O} = \frac{m_{hydrated} - m_{dry}}{m_{hydrated}} \cdot 100 \quad (1)$$

where $m_{hydrated}$ is the mass of the hydrated material and m_{dry} is the mass of the dehydrated material. The water content was measured for three independent repeats per condition.

2.5. Contact angle measurements

The contact angle of the hydrogels was measured using the captive bubble technique, in which a bubble of air is deposited on a substrate immersed in distilled water [19]. The captive bubble technique was used as to avoid drying effects of the hydrogel [20]. A bench top drop shape analyser (DSA25, Krüss GmbH, Germany) was used to collect all contact angle measurements. A 5 µl air bubble was dispensed onto the substrate by a software controlled dosing system. As the air bubble is the hydrophobic phase, materials with a smaller contact angle are considered more hydrophilic. The static contact angle of the air bubble was measured for three independent samples of the SN, DN and PMPC TN samples.

2.6. Surface topology

The surface topology of the samples was measured using a white light interferometer (WLI) (Wyko NT9100 Surface Profiler, Veeco, UK). The hydrogel surfaces were washed with water and wiped with a dry lint free wipe. Samples were measured at 20x effective magnification, stitching 4 measurements to image a 0.8 × 0.8 mm area at three locations on three independently synthesised samples for each condition. The average roughness (R_a) and the root mean square roughness (R_q) for each sample condition was presented.

2.7. Unconfined compressive yield

Measurements of compressive yield were undertaken on a screw-driven mechanical testing machine (5565, Instron, UK). Samples were cut using a 6 mm rotating cutting tool, creating cylindrical samples with a nominal thickness of 3.5 mm and nominal diameter of 6 mm. Due to the soft nature of the specimens, physical measurement of the sample dimensions, using Vernier callipers for example, was avoided due to deformation induced measurement error. Therefore, sample dimensions were measured using a in house validated, non-contact method, in which samples were digitally imaged (EOS 100D, Canon, Japan) and their dimensions were quantified using ImageJ (NIH, USA) [21]. These dimensions were then used to calculate the engineering stress at any given load. The yield forces of SN hydrogels were measured using a 100 N load cell (2525 Series, Instron, UK), and DN and TN yield forces were measured using a 5 kN load cell (2525 Series, Instron, UK). Accuracy of both load cells was 0.25% of indicated load over a range of 100–0.4% of load cell rating. The load cells were changed as to ensure accurate measurement of the yield force, which for SN was in the range of 1–10 N and for DN and TN was in the range of 800–1100 N. Samples were tested at room temperature in a bath of Ringer's solutions, as to approximate the salt content of the synovial fluid [22]. All tests were conducted by compressing the samples to failure between two impermeable stainless steel platens while the samples were free to expand radially. To minimise friction effects during testing, a thin film of silicone oil with a kinematic viscosity of 5×10^5 m^2 s^{-1} was applied to the faces of the compression platens [23]. The current height of the sample, h, and the original sample height, H, was used to calculate the true (Hencky) strain, ε_T, using Eq. (2):

$$\varepsilon_T = -\ln\frac{h}{H} \quad (2)$$

which for large deformations is a better estimate of the real strain in the sample than engineering strain [23]. For ease of comparison with the other literature on this subject, the compressive extension was also expressed as engineering strain, ε, using Eq. (3):

$$\varepsilon = \frac{h}{H} \quad (3)$$

The samples were compressed to failure at 1000% strain per second, with an exponentially decaying compressive extension rate as to maintain constant true strain rate. Due to the high loads during testing, the extension data was compliance corrected using a standard protocol, in which the frame compliance was determined by running a compression test minus the sample [24]. The yield stress and true strain was measured for five independently synthesised repeats for each condition.

2.8. Tribology testing

DN or TN samples were fixed to the base of small petri dishes with cyanoacrylate glue. A CoCr alloy ball with radius 9.5 mm and an arithmetic average roughness (R_a) of 0.01 µm was used as the countersurface. A CoCr ball was used to ensure the counterface was consistent in all tests. The hydrogel sample was held fixed whilst the CoCr alloy ball was reciprocated over a stroke length of 10 mm. Sliding friction was measured over a range of sliding speeds, V = 0.5–40 mm s^{-1} for 600 s at each speed. The average normal force for all cycles was 1 N ± 0.02 N, resulting in a contact radius of 3 mm and a peak contact pressure of 0.05 MPa. This low load was used to ensure the maximum shear stresses were confined in the hydrogel and reduce substrate effects. The reported friction coefficients are the average of the last 5 cycles as measured in the middle 60% of the free sliding portions of the lateral forces, both forward and reverse. Sliding tests were conducted with an average normal load applied by a PID control system. Surfaces were lubricated with phosphate buffered saline (PBS) at pH 7.4 at room temperature (20 °C). Six repeats were performed for each set of conditions.

2.9. Cytotoxicity study

2.9.1. Chondrocyte isolation

Chondrocytes were isolated as previously described [25,26]. Briefly, articular cartilage was harvested aseptically from the femoral condyles of freshly slaughtered 2–6 week old bovine obtained from a local slaughterhouse. The tissue was diced and digested overnight in 0.2% collagenase (type II, Gibco, Thermo Fisher Scientific, MA, USA), 100 µg/ml streptomycin, and 100 µg/ml

penicillin in Dulbecco's modified Eagle's medium (DMEM; 4.5 g/L glucose; Invitrogen, CA, USA). Chondrocytes from 4 separate calves were pooled together to limit biological variance. The next day cells were filtered, washed, counted and frozen at 6 million cells/ml for storage.

2.9.2. Scaffold sterilisation

DN and TN hydrogels were cut with a 4 mm rotating biopsy punch to create cylindrical samples 3 mm thick and 4 mm in diameter. Samples were then washed 3 times in sterile phosphate-buffered saline (PBS), sterilized under UV light for 2 h, and washed 3 more times in PBS. The sterilized hydrogels were stored overnight at 37 °C in cell culture media composed of DMEM, 10% (v/v) fetal bovine serum (Life Technologies, MA, USA), 100 μg/ml streptomycin (Gibco), 100 μg/ml penicillin (Gibco), and 50 μg/ml L-ascorbic acid (Sigma Aldrich).

2.9.3. Viability assessment

Chondrocytes (passage 1, pooled from 4 donors) were seeded at 100,000 cells/well in a 24 well plate and left overnight at 37 °C and 5% CO_2 in 2 ml of culture media composed of DMEM (4.5 g/L glucose; Invitrogen) supplemented with 10% (v/v) fetal bovine serum, 100 μg/ml streptomycin, 100 μg/ml penicillin, and 50 μg/ml L-ascorbic acid. The next day wells were ~80% confluent. Media was removed and transwells (8 μm pore polycarbonate membrane; Costar, Corning, USA) containing sterilized hydrogels where added to each well with 2 ml of fresh culture media. Transwells were used to prevent the hydrogels from disrupting the underlying cell layer while still allowing the hydrogels to be present in the same media as the cells. Chondrocytes were also cultured without hydrogels as blank negative controls and with autoclaved implant grade GUR 1020 UHMWPE as a negative material control. Media was changed every 3 days.

Cell viability was assessed using a LIVE/DEAD Cell Viability assay (Invitrogen) according to the manufacture's protocol at 1, 3 and 7 days of culture (n = 3 wells per time point per condition). Briefly, chondrocytes were rinsed twice with phenol-free DMEM (4.5 g/L glucose, 25 mM HEPES) and incubated with 2 μM Calcein AM and 4 μM ethidium homodimer-1 in phenol-free DMEM, staining live cells with green fluorescence and dead cells with red fluorescence. After 20 min, chondrocytes where imaged using an epifluorescence microscope (lX51, Olympus, Tokyo, Japan). At each time point at least 3–4 representative areas were imaged per well using 4× and 10× objectives.

2.10. Statistical analysis

All statistical analysis was performed using SPSS (v24, IBM, USA).

2.10.1. Mechanical test data

Mechanical test data were tested for normality using a Shapiro-Wilk test and tested for homogeneity of variances using Levene's Test of homogeneity of variance. The data were normally distributed in all cases, with no outliers. The assumption of homogeneity of variances was violated in all cases; therefore, statistical analysis was performed with a Welch's one way analysis of variance (ANOVA). When differences across tests were found, post hoc testing was performed using Games-Howell post hoc analysis. The significance level was set to $p < .05$.

2.10.2. Tribology test data

A two-way ANOVA was used to test for statistical significance. There were six outliers (out of a total number of 120 values), as assessed by visual inspection of a boxplot. Data were normally distributed, as assessed by Shapiro-Wilk's test ($p > .05$). The assumption of homogeneity of variances was violated, as assessed by Levene's test for equality of variances ($p < .001$). However, two-way ANOVA are relatively robust to violation of variances in this case, where the group sample sizes are equal, there is normality, and the ratio of the largest group variance to the smallest group variance is less than 3 [27].There was no significant interaction between the sample and sliding speed for the coefficient of friction ($p = .662$). The significance level was set to $p < .05$. The p values were adjusted for multiple comparisons (Bonferroni Correction). Statistical analysis was repeated with outliers removed, but this resulted in no change in the statistical conclusions.

3. Results

3.1. Hydrogel synthesis

Initial evidence of successful hydrogel formation was provided by ATR-FTIR. Absorption peaks at 1450 cm^{-1} and 1350 cm^{-1} were observed in the PAMPS-PAAm DN hydrogels (Fig. 1) and these were attributed to the amide groups in the PAAm. Following polymerization of the PMPC third network, new IR peaks at 1200 cm^{-1}, 1050 cm^{-1} and 950 cm^{-1}, corresponding to the phosphate and tertiary amine groups in the PMPC, appeared; thereby providing good evidence for the successful incorporation of this polymer into the hydrogel.

3.2. Water content and contact angle of hydrogels

As shown in Table 1, with each additional network added the water content of the sample fell and the DN and PMPC TN hydrogels both exhibited superhydrophilicity. The superhydrophilic nature of these samples was evident from the complete repulsion of the air droplet from the surface, which is typical of superhydrophilic surfaces.

3.3. Surface topography

Fig. 4 shows a representative surface roughness measurement of a DN hydrogel and PMPC TN hydrogel. The DN hydrogel had an R_a of 103 nm and an R_q of 136 nm, and the PMPC TN hydrogel had a R_a of 441 nm and an R_q of 612 nm.

3.4. Mechanical properties of hydrogels

All three hydrogels demonstrated a strongly non-linear stress/strain relationship (Fig. 2A). As shown in Fig. 3C, the yield strain of the SN was lower than either the DN or PMPC TN hydrogels ($p < .001$), but no difference between DN and PMPC TN was detected ($p = .282$). The SN yielded at 50% engineering strain while the DN and PMPC TN yielded at 98% and 94% engineering strain, respectively (Fig. 2b). As shown in Fig. 3D, yield stress was also greater for the DN and PMPC TN compared to the SN ($p < .001$), but a difference was also detected between the DN and PMPC TN ($p < .001$). The SN yielded at 0.5 MPa, the DN at 40 MPa and the PMPC TN yielded at 26 MPa.

3.5. Tribological properties of hydrogels

Fig. 4 shows the average friction coefficient for DN and PMPC TN hydrogels at each speed. Error bars represent the standard deviation of 6 measurements. With increasing sliding speed both types of hydrogel show a trend of decreasing friction which reduces towards a steady value. At all sliding speeds a lower friction coefficient was measured for TN hydrogels, although this was only

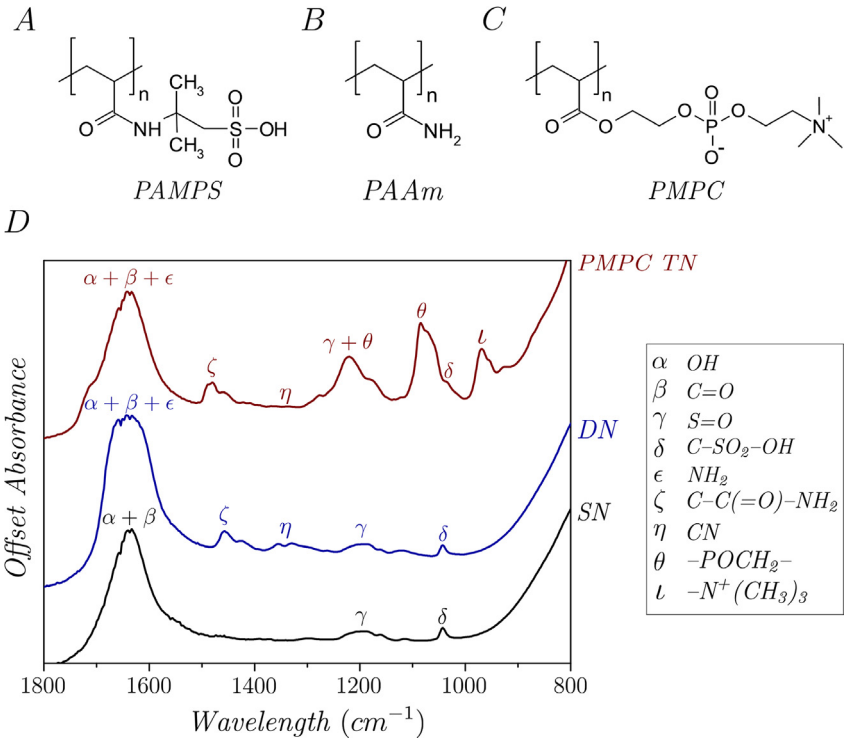

Fig. 1. Chemical structure of PAMPS (A), PAAm (B) and PMPC (C). As shown in D, after each polymerisation new FTIR peaks attributed to the chemical bonds of the intended polymeric network appear in the ATR-FTIR spectra.

Table 1
Water content (WC), and captive bubble contact angle (θ_c, air). The error values represent standard deviations from three independent samples.

Sample	WC (%)	$\theta_{c,\ air}$ (°)
SN	95.06 ± 0.08	Superhydrophilic[a]
DN	90.69 ± 1.12	Superhydrophilic[a]
PMPC TN	86.66 ± 0.12	Superhydrophilic[a]

[a] Air droplet repulsed by surface, so no measurement could be taken. This behaviour is typical of a superhydrophilic surface.

statistically significant ($p < .05$) between 3 mm s^{-1} and 15 mm s^{-1}. The maximum reduction in friction coefficient was 47% at 11.5 mm s^{-1}.

3.6. Cytotoxicity study

The LIVE/DEAD cell viability assay demonstrated high cell viability and proliferation throughout 1 week of culture for all conditions (see Fig. 5). In all conditions chondrocytes proliferated to 100% confluence by 3 days and by 1 week were over confluent and contracting away from the wells. Further, all conditions had very few dead cells present at 1 day, with a slight increase as cultures became confluent. However, all conditions were predominantly viable throughout culture with approximately > 90% viability at all time points and no visible different in cell morphology, proliferation, or cell death between treatment groups.

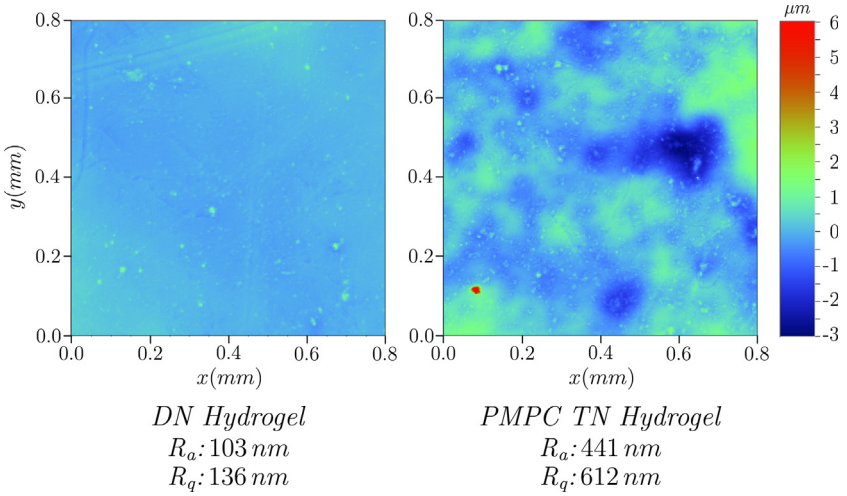

Fig. 2. Surface topography contour plots of 8 mm × 8 mm representative regions of a DN hydrogel (left) and PMPC TN hydrogel (right). The z-value magnitudes (height) for both images are given by the spectral plot to the far right.

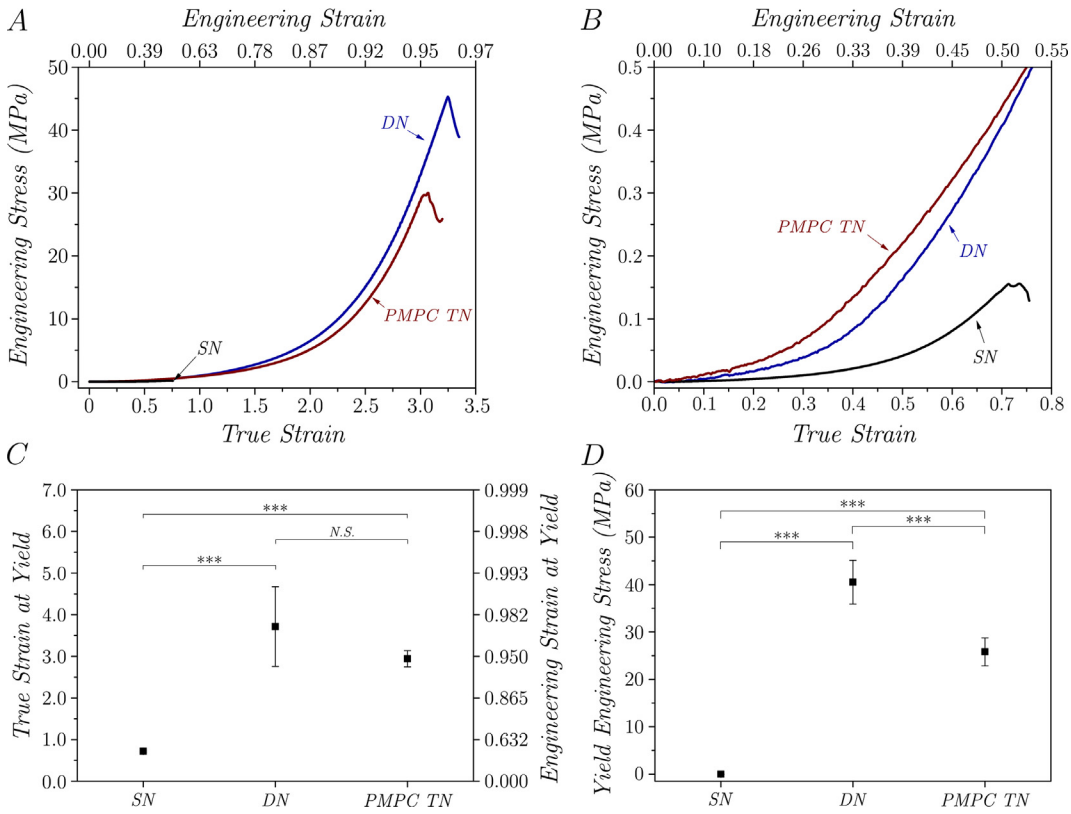

Fig. 3. Mechanical behaviour of the SN, DN and PMPC TN hydrogels. A: A typical stress-strain plot of each hydrogel. B: Magnification of the stress-strain plot in the region where the SN hydrogel yielded. C: The true/engineering strain at yield of the SN, DN and PMPC TN hydrogels. D: The yield stress of the SN, DN and PMPC TN hydrogels. The mean values are plotted with error bars representing the standard deviations of 5 independent repeats. N.S defined as $p > .05$ and ***represents any significance $p \leq .001$.

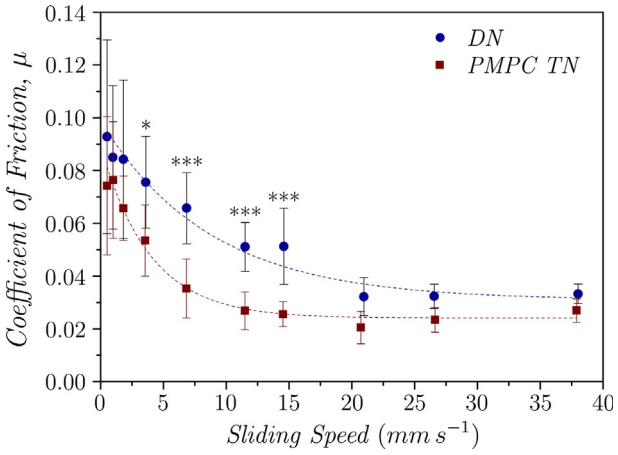

Fig. 4. Coefficient of friction for the DN and PMPC TN hydrogels at different sliding speeds. Dotted lines are added to guide the reader's eyes. Each point is the average COF for 6 independently synthesised samples, and the error bars represent standard deviations. Statistical analysis was performed for each sample at a given speed, where *represents $p \leq .05$, **represents $p \leq .01$, ***represents $p \leq .001$, and any pair of points that don't have stars are not significant ($p > .05$).

4. Discussion

This work presents a new material that imitates both the biphasic and boundary lubrication capability of articular cartilage. The incorporation of the PMPC boundary lubricant lowered the coefficient of friction of the material across a range of sliding speeds. As the PMPC network was polymerised throughout the volume of the porous hydrogel, the boundary lubrication will always be available, even if the material experiences surface wear. The triple network hydrogel had a yield strain of 94% and yield strength of 26 MPa, indicating this class of synthetic material could withstand the harsh loading environment of load bearing human joints.

The DN hydrogels synthesised in this study were stronger than those reported in the literature. The DN gels yielded at 98% engineering strain at a yield stress of 40 MPa, while Gong et al. reported a failure stress of 17 MPa at 92% strain [9]. Possible explanations for the improved yield stress and strain lie in differences between the experimental protocols used. To approximate the ionic conditions of the synovial fluid [22], our compression tests were conducted in a Ringer's solution bath, whereas the prior work was performed in a non-aqueous environment [9]. The ionic environment has been found to have affect the swelling and mechanical properties of single network polyelectrolyte gels, such as PAMPS [28]. However, Tanaka et al. found that the elastic modulus of DN hydrogels was quite insensitive to salt content [28], proposing that the strong osmotic pressure of the PAAm second network counteracted any reduction in osmotic pressure from salt screening of the ionic charges of the PAMPS primary network. The group, however, did not investigate the effects of salt concentration on yield stress. Another possibility could be that in this study the compression platens were lubricated. This was done to prevent bowing, which would generate an inhomogeneous stress-strain state within the sample, resulting in premature failure [29]. A third reason for differences could be the strain rate applied during testing. Gong et al. compressed their samples at a strain rate of 0.1% min^{-1} (0.00167% s^{-1}) [9], while in this study the samples were compressed at a strain rate of 1000% min^{-1} (16.67% s^{-1}). Biphasic materials are strain rate dependent, this can be flow dependent (based on the interaction of fluid with the matrix) [3] or flow rate independent (based on the viscoelastic properties of the matrix)

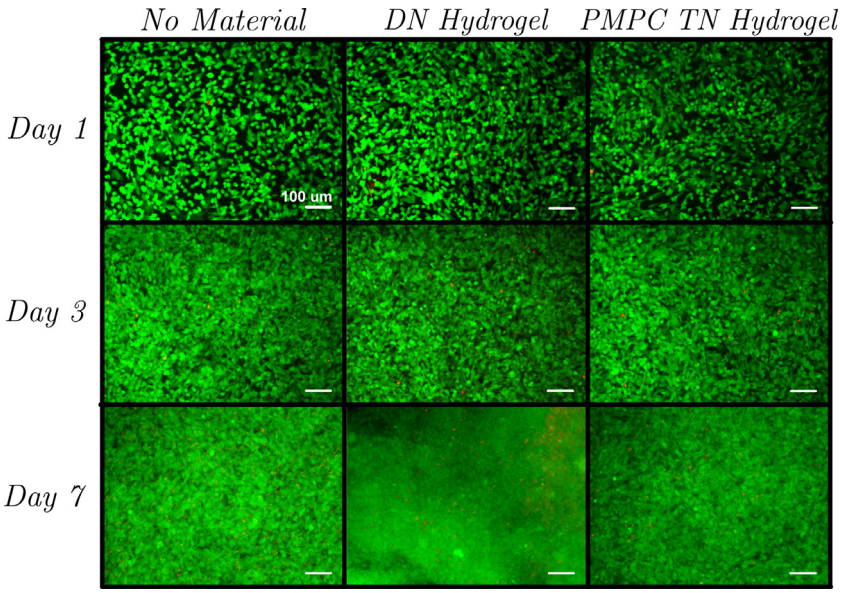

Fig. 5. Representative images from the LIVE/DEAD strain of chondrocytes from elution cytotoxicity study. Green cells are live and red cells are dead. Both the DN hydrogel and PMPC TN hydrogel exhibited approximately >90% viability at all time points. Therefore, the hydrogels in this study do not exhibit any pronounced cytotoxicity.

[30]. Therefore, at high strains the sample would have greater capacity to carry load. The high strain rate used in this study (16.66% s^{-1}) was chosen as to better approximate the strain rates expected *in vivo*. A recent MRI study measured an average chondral strain of approximately 2% and a maximum chondral strain of 8% upon application of a load equal to 50% bodyweight [31]. Combining this with the high rate of loading that has been measured using instrumented knee implants [1] (where during walking a load of 50% bodyweight is applied in approximately 0.2 s), the chondral strain rates in the knee would be between 10% s^{-1} and 100% s^{-1}, which is on the order of the strain rate used in this study (16.67% s^{-1}).

The PMPC TN retained much of the toughening effect that is endowed by the PAAm second network [9]. The yield stress of PMPC TN hydrogel was 25.8 MPa, only 36% less than the DN hydrogel. This yield stress is still well above the peak contact pressures found in the native knee, which have been reported as anywhere between 1.5 MPa and 8 MPa [32]. The reduction in yield strength could be due to two reasons: increased crosslinking density effecting the DN toughening mechanism or altered water content of the hydrogel. It is well documented that loose crosslinking of the second network is necessary for the toughening effect to occur and that residual cross linking sites are left over from each polymerisation step [33]. Therefore, polymerisation of MPC may have acted to further crosslink the second network; thereby reducing the yield stress of the hydrogel. An alternate reason for the slight reduction in yield stress could be changes in water content of the PMPC TN hydrogel. This decreased water content would act to increase the effective number of crosslinks per unit volume, which could disrupt the double network toughening mechanism [33].

Due to MPC's superhydrophilicity there is a large change in the water contact angle of PMPC modified substrates [17]. Using the captive bubble technique it was found that the DN hydrogel was superhydrophilic. This result is in contrast to sessile contact angle measurements reported in the literature, where contact angles of around 30° for DN hydrogels have been measured [34]. The difference in these results likely originates in the mode of testing. Taking sessile water contact angle measurement from a hydrogel will cause local dehydration of the sample, and therefore sessile contact angle measurements of hydrogels will not truly reflect their properties when completely hydrated [19]. Despite the reduced water content of the PMPC TN hydrogel, the PMPC TN hydrogels retained this superhydrophilicity.

There was a change in the surface topography between the DN and PMPC TN hydrogels. As shown in Fig. 2, the DN hydrogel had a R_a of 103 nm, while the R_a of PMPC TN was 441 nm. To the author's knowledge no surface topography measurements of double network hydrogels have been undertaken. It is unknown whether this increased roughness is specific to PMPC or if any third network would create a similar change in surface topography. The SN hydrogel has a low concentration per unit volume of PAMPS [9]; therefore, the network structure may be relatively unaffected by the addition of a second network. However, due to the relatively high concentration of PAAm in the DN hydrogel [9], the third network may act to deform the network structure. Alternatively, as these measurements were taken in a dry environment, these results could be due to differences in drying out of the hydrogel surface. Therefore, these results may not be representative of the surface *in situ*. Nonetheless, the R_a of both of the hydrogels are below that of cartilage, which has a reported R_a of 0.8 μm ± 0.3 μm [35].

The measured trend in friction coefficient is consistent with that found by other authors investigating biphasic lubrication [36–38]. The CoCr alloy ball used as a counterface in this work forms a circular contact area that moves across the hydrogel surface. Under the contact the fluid component of the hydrogel is pressurised and so will flow away from the contact. As the contact moves across the material surface the fluid can flow back into the area allowing it to rehydrate. A lower friction force is related to a higher level of hydration, so by moving the contact and allowing the area to rehydrate a low friction coefficient can be sustained. This is equivalent to the migrating contact defined by Caligaris and Ateshian [38] when studying cartilage lubrication. Extension of this theory by Moore and Burris [36] and by Reale and Dunn [37] has proposed that the measured friction coefficient is determined by the ratio of sliding speed to fluid flow in a biphasic material. They postulate that when sliding speed exceeds the rate at which fluid can drain from the contact the friction attains a minimum. Our results are consistent with this model, with both hydrogels showing a decreasing friction with sliding speed that reduces

to a steady minimum value. Indeed, a similar decrease in friction with sliding speed has been experimentally shown for other hydrogel systems [37,39]. An alternative testing configuration would be to use a hydrogel pin on a CoCr alloy plate resulting in a fixed point of contact on the hydrogel surface, referred to as a non-migrating contact. In this configuration the contact area of the hydrogel becomes dehydrated with time which results in a higher friction. Here a migrating contact testing configuration was chosen, as this is more relevant to the sliding conditions in the knee where the contact area moves with time and so the hydrogel would have chance to stay hydrated.

The PMPC TN hydrogel exhibited a reduced friction compared to the DN hydrogel over a range of sliding velocities. This reduction was most significant in the range of 3–15 mm/s which is within the 0–100 mm/s sliding speeds experienced by cartilage during gait [40]. The relationship between coefficient of friction and sliding velocity was consistent with the enhanced boundary lubrication observed for this material elsewhere [41]. PMPC has a strong affinity to water that may reduce adhesion between the hydrogel and counter surface as the PMPC would preferentially hold onto the water rather than make bonds with the CoCr alloy surface [42]. The water bonded to the PMPC can then be exchanged with surrounding water molecules during shear resulting in improved boundary lubrication. The introduction of the third PMPC network will also impact the mesh size of the hydrogel, which is known to affect diffusion rates and so would reduce the permeability of the hydrogel to water [43]. This would have the added effect of improving the biphasic mechanism of lubrication. To determine the relative contribution of each mechanism further studies are needed.

A limitation of the work is that all tests were conducted at room temperature. Temperature of testing can have a large effect on the material properties of non-linear biphasic materials [44]; therefore, future tests should investigate the mechanical properties at body temperature. A limitation of this material is its large stress-strain non-linearity. At low strains in unconfined compression the DN hydrogel has low resistance to deformation. *In vivo* the material will be semi-confined by cartilage, which would inhibit its radial expansion, and thus its resistance to deformation would be increased. Therefore, in future work a more physiological loading regime should be simulated. Due to the high strains experienced by the samples, true stress would a more accurate estimate of the stresses in the sample; however, due to the biphasic nature of these samples the constant volume approximation often used to calculate true stress would not be valid. Therefore, to measure the true stress the increase in sample surface area with strain over the duration of the test would need to be measured. Fixation of hydrogels in the defect site has been a case for concern. DN hydrogels, due to their ultra-high toughness relative to other hydrogels, have been shown to have single suture tear out strengths similar to cartilage and also a high strength of attachment to cartilage with acrylic tissue adhesive [45]. Future studies will aim to characterise the suture tear out strengths of PMPC TN.

The preliminary tribological study also had some limitations. CoCr alloy ball was used as a counterface to ensure the counterface was consistent in all tests. Cartilage would be the ideal counterface; however, the geometry and material properties vary between samples and with time during friction tests [38]. To simplify the tribological system—and thus ensure differences in friction coefficient was solely due to the incorporation of PMPC, rather than some other factor—PBS was used as a lubricating fluid. *In vivo*, the lubricating fluid would be synovial fluid, which contains numerous biomolecules that have been implicated in the lubrication of cartilage [2,38]. Therefore, future tests will be conducted in a more biologically relevant lubricant, such as bovine calf serum or human synovial fluid. This work only considered one load magnitude, which was lower than that experience by cartilage *in vivo*, and no cross-shear. Gong et al. have shown that load can influence measured friction coefficient with a tendency for reduced friction at higher loads [46]. This may mean that at a higher load the reduction in friction force between the hydrogels is less apparent. Similarly cross-shear has been shown to impact wear resistance and friction for biphasic materials [47], and may alter the results seen here. Due to the short duration of testing, no wear measurements were taken during this work. PMPC has shown to grant enhanced wear resistance to other materials [16]; therefore, future tribology testing will aim to quantify the wear of the PMPC TN and the counter surface. However, the current study was conducted in order to determine if there was a benefit to the development of TN hydrogels with a PMPC network, which our data supports.

PMPC-TN hydrogel has been proposed as an acellular partial joint replacement bearing material. *In situ*, only the edges of the PMPC TN hydrogel would be in direct contact with cells. However, any extractable chemicals from the hydrogel could cause systemic toxicity. As such, an elution cytoxicity study was deemed as a crucial initial test for the biocompatibility of PMPC TN hydrogels. Indeed, other partial joint replacement devices, such as Cartiva, have made use of elution testing to test the biocompatibility of their devices [48]. As shown in Fig. 5, the DN and PMPC TN hydrogels showed no signs of elution cytotoxicity with chondrocytes through one week of culture. The chondrocytes maintained high viability and proliferation throughout culture.

This is the first published work investigating the elution cytotoxicity of DN and TN based hydrogels. Previous work has only investigated the cytotoxicity of cells encapsulated in DN hydrogels. In one such study, DN hydrogel encapsulated NIH-3T3 fibroblasts exhibited a reduction in viability (71% after 3 days of culture) [49]. Due to this possible evidence of direct cell contact cytotoxicity of DN hydrogel, the precursor to the PMPC TN hydrogel, and that chondrocytes around the edge of the defect may be in direct contact with the hydrogel *in situ*, future work should aim to characterise the direct contact cytotoxicity of the PMPC TN hydrogels.

There were some limitations to the biocompatibility study. Due to the significant proliferation of chondrocytes this study could not be carried out past 1 week. The elution of cytotoxic components may occur over longer periods of time. Therefore, these tests should be conducted over a longer period of time, as to get a better idea of the long term elution cytotoxicity of these implants. Also, this testing does not provide any information about the potential cytoxicity of PMPC TN hydrogel wear particles. Therefore, the wear debris from future tribology testing should also be tested for any potential local or systemic toxicity.

Due to the unproven clinical efficacy of biological chondral repair therapies, such as debridement, microfracture, moscaicplasty and autologous chondrocyte implantation [50], non-biological strategies for chondral replacement are still investigated. A primary concern with this approach is the effect on the opposing cartilage surface because the materials currently used do not replicate its lubrication mechanism and may damage it [51]. Candidate materials for chondral replacement should ideally not only mimic the stiffness and strength of cartilage, but should also mimic its mechanisms for lubrication, as this is the primary function of the material. Indeed, the hydrogel synthesised in this study imitates both the biphasic and boundary lubrication mechanisms of cartilage, and may therefore provide a material that prevents opposing chondral damage, and improves clinical efficacy of the non-biological chondral replacement approach.

5. Conclusion

Synthesis of a material that exhibits the right combination of toughness, wear resistance and low friction needed to repair articular cartilage has long been the aim of many researchers. Here we have demonstrated that the introduction of an established biomimetic boundary lubricant can improve the frictional properties of an ultra-tough hydrogel whilst still providing the mechanical strength required for cartilage repair applications. This new biomaterial moves us one step closer to producing a viable replacement for damaged or absent cartilage in human joints.

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Declarations

There are no conflicts of interest relating to this work.

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