Tribological evaluation of a novel hybrid for repair of articular cartilage defects

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Abstract

The friction and wear properties of silica/poly(tetrahydrofuran)/poly(ε-caprolactone) (SiO_2/PTHF/PCL-diCOOH) hybrid materials that are proposed as cartilage tissue engineering materials were investigated against living articular cartilage. A testing rig was designed to allow testing against fresh bovine cartilage. The friction force and wear were compared for five compositions of the hybrid biomaterial articulating against freshly harvested bovine cartilage in diluted bovine calf serum. Under a non-migrating contact, the friction force increased and hence shear force applied to the opposing articular cartilage also increased, resulting in minor damage to the cartilage surface. This worse case testing scenario was used to discriminate between material formulations and revealed the increase in friction and damaged area was lowest for the hybrid containing the most silica. Further friction and wear tests on one hybrid formulation with an elastic modulus closest to that of cartilage were then conducted in a custom incubator system. This demonstrated that over a five day period the friction force, cell viability and glucosaminoglycan (GAG) release into the lubricant were similar between a cartilage-cartilage interface and the hybrid-cartilage interface, supporting the use of these materials for cartilage repair. These results demonstrate how tribology testing can play a part in the development of new materials for chondral tissue engineering.

Statement of Significance

Designing materials that maintain the low friction and wear of articular cartilage whilst supporting the growth of new tissue is critical if further damage is to be avoided during repair of cartilage defects. This work examines the tribological performance of a SiO_2/PTHF/PCL-diCOOH hybrid material and demonstrates a testing protocol that could be applied to any proposed material for cartilage regeneration. Tribological tests demonstrated that changing the hybrid composition decreased friction and reduced damage to the cartilage counterface. This study demonstrates how tribological testing can be integrated into the design process to produce materials with a higher chance of clinical success.

1. Introduction

The regeneration of cartilage in osteochondral defects remains a challenge. Clinical treatments like Autologous Chondrocyte Implantation and microfracture often regenerate fibrocartilage rather than the desired hyaline cartilage. Scaffolds used in tissue engineering aim to solve this problem by providing the right conditions for hyaline cartilage to form. Such scaffolds must integrate with both the native cartilage and the underlying subchondral bone, retain their structural integrity long enough for cartilage to reform and not cause any damage to the opposing cartilage tissue [1]. An ideal device would stimulate regeneration of articular cartilage while also providing a surface that preserves the integrity of the opposing surface and also transmit load to the regenerating tissue before safely biodegrading. Current scaffold materials have been proposed, but biodegradable devices with a combination of suitable mechanical properties and integration of these materials with the surrounding healthy cartilage are yet to be developed [2].

Bioactive glasses bond with both hard [3] and soft tissues [4], but they are stiff, hard and brittle, making them unsuitable for implanting into the articulating surface, even as composites, as the particulates could cause wear [5]. Incorporating ceramics or glasses into polymer composite materials can lower stiffness, but composites often suffer from the polymer masking the bioactive

phase; a mismatch in the rate of biodegradation of the components and a lack of bonding between the components, which lowers the mechanical strength from the theoretical values [6]. To overcome these deficiencies, inorganic/organic hybrid materials have been developed. In Class II and Class IV hybrid materials, the inorganic glasses and organic polymers are nanoscale co-networks with covalent bonds (coupling) between the networks [7]. Mechanical strength and degradation rate can be adjusted to the desired values for a particular application by changing the ratio of inorganic to organic and amount of covalent coupling in the hybrid.

Tallia *et al.* recently presented the development of a novel hybrid based on silica (SiO_2), carboxylated poly(ε-caprolactone) (PCL-diCOOH) and poly(tetrahydrofuran) (PTHF) [8], as illustrated in Figure 1. The transparency (Figure 1), and TEM images in previous work, indicated that the material had a homogenous microstructure. By controlling the weight percent (wt%) of SiO_2 in the final material, the storage modulus in tension, *E'*, varied between 0.4-132 MPa [8] covering the reported range of moduli for articular cartilage [9]. When 3D printed into a scaffold with a grid-like structure of ~250 μm wide pore channels, the scaffold stimulated chondrocytes to produce matrix representative of articular cartilage matrix and provoked human mesenchymal stem cells (MSCs) down a chondrogenic path, when cultured with chondrogenic media [8].

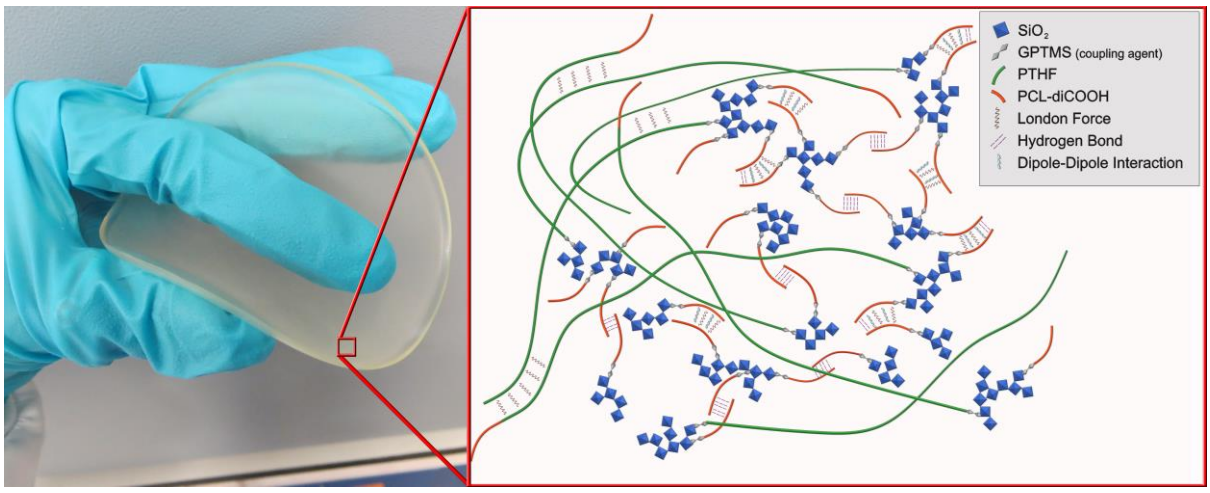


Figure 1. The transparent appearance of cast SiO_2/PTHF/PCL-diCOOH hybrid material with a schematic of the structure of the hybrid.

Although the characteristics of this material are promising in terms of cell regeneration and mechanical stiffness, the tribological properties have not yet been established. The material itself must be able to withstand damage under sliding or it will not survive long enough to support the new tissue growth. We will also compare the hybrid material to PCL alone, as it is a popular polymer for cartilage repair strategies, but can surface damage under shear [10]. Here, we investigate the tribological properties of the hybrid biomaterial for the first time, as a function of composition, and determine whether release of the biodegradable polymer from the hybrid affects wear. Importantly, we investigate the tribological properties of the hybrid sliding against "living" cartilage if damage to the healthy cartilage during defect repair, as seen previously [11, 12], is to be avoided.

Most *in vitro* testing of cartilage against implant materials has previously been short term, typically less than 24 hours, as the properties of the cartilage tissue begin to degrade with time. Often, in short term testing, a cartilage pin is loaded in such a way as to give a non-migrating contact, which reduces the fluid in the tissue and results in higher friction forces and increased wear [13]. A non-migrating contact is where the cartilage sample is loaded constantly throughout the test and thus the intrinsic fluid-load support mechanism is suppressed

. This testing configuration has been used to test chondroplasty and hemiarthoplasty materials [14-16] and although more likely to result in cartilage damage, it allows for greater discrimination between the effects of experimental variables [17].

Recent advances in cell culture techniques and bioreactor design mean it is now possible to consider longer term testing of cartilage tissue in a more physiological setup, bridging the gap between *in vitro* testing and animal studies [18]. Wimmer *et al.* developed a cartilage bioreactor that approximates the rolling-sliding kinetics of a knee joint which can be housed in an incubator [19, 20]. We made a single-station system, based on their design, with some modifications to allow friction measurements to be made during testing.

Here, five SiO_2/PTHF/PCL-diCOOH hybrid materials containing increasing wt% SiO_2 were investigated to determine their suitability as potential materials for cartilage regeneration, particularly as to whether they could take the role of articulating surface, in terms of friction and wear resistance. From these formulations, one material was taken forward for testing against cartilage explants maintained in cell culture conditions. This work will inform the continuing material design process to determine the best hybrid formulation for articular cartilage repair.

2. Materials and methods

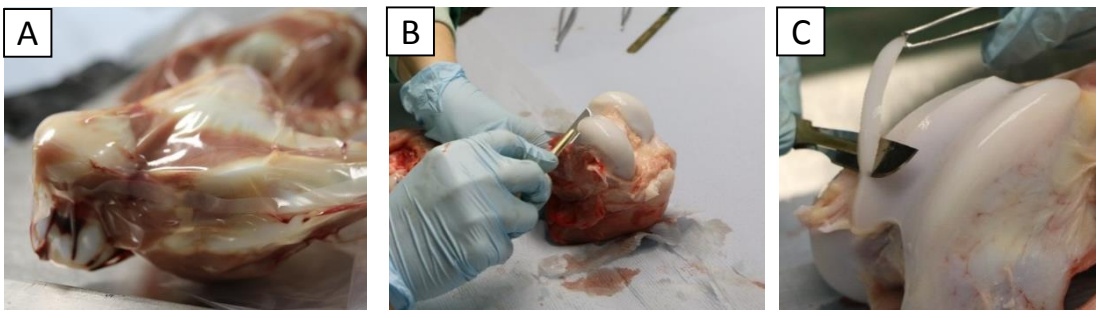
2.1 Materials

SiO_2/PTHF/PCL-diCOOH hybrid materials were prepared in a two-step procedure, as described in Tallia *et al*. [8]. First, TEMPO oxidation was applied to PCL-diol (average M_n = 530 Da), producing the dicarboxylic acid form (PCL-diCOOH), that was then introduced into the following sol-gel hybrid synthesis. An organic precursor solution of the PCL-diCOOH (1 mol), (3-glycidyloxypropyl)trimethoxysilane (GPTMS, 2 mol) and boron trifluoride diethyletherate ($BF_3 \cdot OEt_2$, 0.5 mol) was prepared in THF and stirred at room temperature for 1.5 hours, during which polymerisation of THF to pTHF occurred. At the same time, tetraethyl orthosilicate (TEOS), the silica precursor, was completely hydrolysed in a stoichiometric volume of deionised water by 1 M hydrochloric acid, at a ratio of 1:3 % v/v with respect to water. When the two separate solutions were fully reacted, the TEOS hydrolysis solution was added dropwise to the organic precursor solution and stirred at room temperature for 30 minutes to form the hybrid sol, that was cast in cylindrical polytetrafluoroethylene (PTFE) containers (internal Ø = 130 mm), which were placed at 40°C for ageing and drying. After drying, the shrinkage inherent in the sol-gel process determined the final dimensions of the samples in the shape of thin discs. Five different TEOS/PCL-diCOOH %w/w (0/100, 60/40, 70/30, 80/20, 90/10) were used in order to prepare the five hybrid compositions tested. All chemicals were purchased from Sigma-Aldrich and VWR, UK, unless

specified otherwise. At the end of the drying step, the SiO_2 content of the hybrid was determined by thermogravimetric analysis (TGA). The composition of the five formulations tested is given in Table 1. For all materials, the free surface (not in contact with the mould during casting) was tested.

For short term friction tests, cartilage pins of 8 mm diameter removed from the femoral condyle of an adult sheep using a biopsy punch and stored at -20°C in phosphate buffered saline (PBS). Before use, samples were defrosted overnight and then secured in an aluminium holder using bone cement.

For bioreactor testing, a similar protocol as described previously was followed [19]. Cartilage samples were removed from stifle joints of bovine calves obtained from a local abattoir within 48 hours of slaughter (Figure 2A). The joints were kept intact until dissection, at which time cartilage was removed from the medial and lateral femoral condyles (Figure 2B) and the rim of the trochlear groove using a scalpel (Figure 2C). Removed cartilage was stored in sterile PBS supplemented with 100 μg mL^{-1} streptomycin and 100 μg mL^{-1} penicillin (Figure 2D). Cartilage flats were prepared from the femoral condyle cartilage using a custom oval punch to give samples with length 20 mm and width 14 mm (Figure 2E). These were placed in sterile petri dishes and held in position by porous polyethylene (PE) wafers under semi-confined compression (Figure 2F). The resulting samples had a convex curvature with a typical radius 20 mm along the long axis of the sample. At the same time SiO_2/PTHF/PCL-diCOOH hybrid samples were cut using the same oval punch and secured using double sided tape on a PE wafer and secured in sterile petri dishes. Cartilage balls were prepared by taking the cartilage from the rim of the trochlear grove and cutting into strips 8 mm wide by 20 mm long. These were secured to poly(oxymethylene) copolymer (POM-C) balls using polyamdie-6 sutures (Figure 2G).



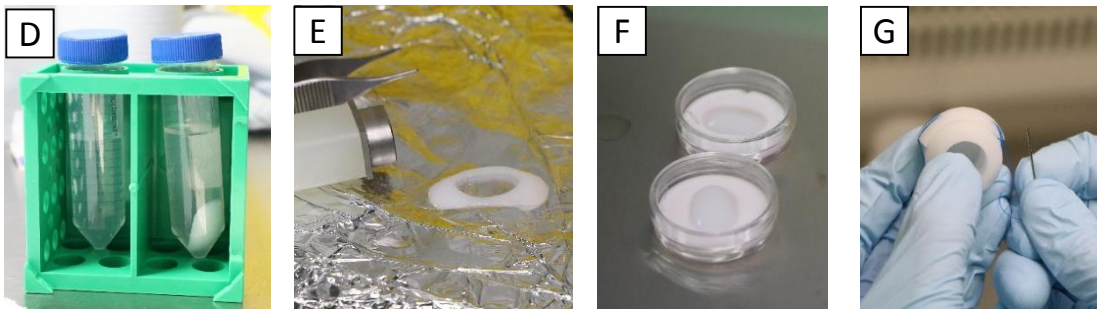


Figure 2 Retrieval and preparation of cartilage samples: (A) joints as received; (B) removing cartilage from femoral condyles; (C) removing cartilage from rim of trochlear grove; (D) storage in PBS supplemented with antibiotics; (E) removal of cartilage flat with custom punch; (F) samples confined in petri dishes; (G) samples secured on POM-C ball using surgical sutures.

Cartilage and hybrid samples were cultured at 37 °C and 5% CO_2 in culture media composed of DMEM (4.5 g L^{-1} glucose; Invitrogen) supplemented with 10% (v/v) fetal bovine serum, 100 µg mL^{-1} streptomycin, 100 µg mL^{-1} penicillin, and 50 µg mL^{-1} L-ascorbic acid. Media was changed daily. Samples were cultured for 24 hours before testing began.

2.2 Contact angle measurements

Static contact angles were determined using the sessile drop technique using pure water. A 5 µl drop was placed gently on the surface of the hybrid material and photographed. The shape of the drop was fitted using a sessile drop model (Krüss Surface Science, Hamburg, Germany). For each hybrid formulation measurements were taken in five different areas and averaged.

2.3 Atomic Force Microscopy measurements

Atomic Force Microscopy (AFM, Alpha 300 RA, WITec, Ulm, Germany) images were acquired at a scan rate of 1 Hz in contact mode. A silicon nitride tip with a nominal cantilever spring constant of 0.2 N/m (XVA3000, LOT-Quantum Design Ltd, Surrey, UK) was used.

2.4 Short term friction and wear tests

Short term friction and wear tests were carried out of all five hybrid compositions, in a monolithic form, in order to choose the best composition that would go forward for bioreactor testing.

2.4.1 Friction measurements

A custom tribometer was used to conduct friction tests under linear reciprocating motion (Figure 3). Hybrid material samples were clamped on a stainless steel holder. Cartilage pins were reciprocated with a stroke length of 5 mm at 1 Hz, giving an average speed of 10 mm s^{-1} for 1 hour. Tests were conducted in bovine calf serum (BCS) diluted to 20 g L^{-1} protein in distilled water. Cartilage flats and polished CoCrMo (ISO 5832-12) disks were used as positive and negative controls respectively. CoCrMo was chosen as a negative control as this is the material from which a medical device in current clinical use for partial resurfacing is made [21]. Tests were conducted at room temperature (20°C) with six repeats performed for each set of conditions. Normal loads and friction forces were measured by a 6-axis load cell (Gamma, ATI Industrial Automation, USA).

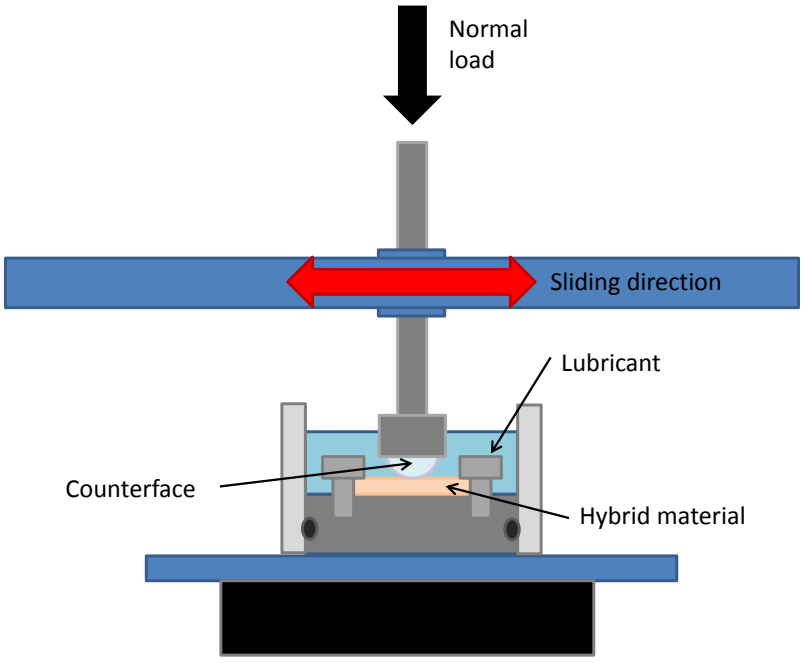


Figure 3 Schematic of custom tribometer used to measure friction.

All tests were conducted under a deadweight load of 1 N. Fuji pressure sensitive film (ultra-super low grade, Fuji Photo Film Co., Japan) was used to measure the contact area and the average contact pressure calculated for a deadweight load of 1 N. Contact pressures are given in Table 1 along with calculated compressive moduli for the hybrid material based on Hertzian contact theory (see supplementary material S1).

2.4.2 Wear measurements

Following testing, samples were rinsed with deionised water, gently cleaned with 2 % detergent solution and examined under an optical microscope for signs of surface damage. Macroscale wear was investigated using a NT9100 Veeco white light interferometer (WLI) with a magnification of 11x. Surface roughness, R_a, was calculated over an area of 430 µm x 547 µm. WLI was used rather than AFM as the wear track area was in the order of mm^2.

2.5 Bioreactor tests

From the short term tests, the hybrid with 25 wt% silica content was tested in a bioreactor test, which involved using rigs, such as that shown in Figure 3, inside a cell culture incubator at 37 °C with an atmosphere of 5% CO_2.

2.5.1 Friction measurements

Before testing, the culture media, in the petri dishes containing cartilage, was exchanged for fresh media. Petri dishes containing the cartilage or hybrid flat samples (Figure 2F) were fixed to a 6-axis load cell allowing simultaneous measurement of normal and horizontal forces. Cartilage balls (Figure

2G) were press fit onto a stainless steel shaft on a translation and rotation stage. A 10 N load was applied as a deadweight. In the bioreactor tests, contact pressures were not directly measured but can be calculated from Hertzian contact theory to be approximately 0.2 MPa for a cartilage-cartilage pairing, and 0.1 MPa for a cartilage-hybrid pairing. The cartilage ball was reciprocated with a translation of 10 mm combined with a rotation of 45° to give a rolling motion in which the point of contact would move on both cartilage surfaces (i.e. a migrating contact configuration in contrast to the non-migrating contact described in Section 2.4.1). Motion was applied at 0.5 Hz for 1 hour after which the test media was collected, fresh media added and the samples returned to the incubator. For each sample, 1 hour tests were performed on five sequential days. Four cartilage-cartilage and four hybrid-cartilage pairs were tested.

2.5.2 Cell Viability

Cell viability within the cartilage was assessed with a LIVE/DEAD cell assay (Invitrogen) according to the manufacturer's protocol. After the testing period, cartilage samples were removed from their holders and rinsed in sterile PBS. They were then bisected perpendicular to the reciprocating direction and thin slices were obtained using a scalpel and placed in 12 well plates. The tissues were incubated with 2 µM calcein AM and 4 µM ethidium homodimer-1 in sterile PBS, staining live cells with green fluorescence and dead cells with red fluorescence. After 40 min, chondrocytes were imaged using an epifluorescence microscope (Axio Observer, Zeiss) using 5x and 10× objectives.

2.5.3 Glycosaminoglycan release into media

Media from friction tests was collected and stored at -20°C. For analysis, 0.5 mL media was mixed with 0.5 mL papain digestion buffer (5 mM L-cysteine, 5 mM EDTA, pH 8.0 containing 125 mg mL^{-1} papain) overnight at 65°C. The dimethylmethylene blue (DMMB) dye reaction was used to quantify

glycosaminoglycans (GAGs) released into solution. 200 µL of digested media was added to 2 mL of DMMB reagent solution (40 mM NaCl; 40 mM glycine; 46 mM DMMB, 10 mM acetic acid, pH 3.0), mixed for 30 seconds and the absorbance read at 525 nm using UV-Vis spectrometer (Perkin-Elmer, UK). A calibration curve was compiled using shark cartilage chondroitin sulphate solutions with concentrations between 10 and 50 µg mL^{-1}. Calibration solutions were prepared in culture media and also digested with papain.

2.5.4. Hybrid wear measurements

As before, samples were rinsed with deionised water, gently cleaned with 2 % detergent solution and examined under an optical microscope before measurement of surface roughness using WLI.

Release of Si into the testing media was assessed using inductively coupled plasma – optical emission spectrometer (Thermo Scientific iCAP 6300 Duo ICP–OES) with a sensitivity of 1 ppb. Solutions were prepared by filtration of the media to 0.2 µm (Corning® syringe filters) and dilution by factor 2 in deionised water. Si concentration was measured in the radial direction of the flame and calibration with standards 0, 0.1, 0.2, 0.4, 0.8, 1, 2, 5, 10 µg mL^{-1} was carried out at the beginning of the sequence. The relative polymer content of the hybrid was assessed at day 5 by thermogravimetric analysis (TGA) at a heating rate of 10 K min^{-1} to 800°C and compared with non-loaded soaked samples.

2.6 Statistical analysis

Mean values are reported for all tests. Statistical analysis was performed using a one-way analysis of variance (ANOVA) using the software package SPSS. A posthoc Tukey test with statistical significance evaluated at $p<0.05$ was used as a comparison of means.

3. Results

3.1 Material characterisation

Contact angle measurements showed that at low silica content, samples were hydrophilic. As the wt% silica increased the samples became progressively more hydrophobic and the contact angle increased from 56 ± 3° for the 3 wt% silica to 94 ± 2° for the 39 wt% silica (Figure 4). This is in contrast to expectations as the pure silica is extremely hydrophilic and so it was expected that with increasing silica the hybrid material would also become more hydrophilic. It has previously been shown that the surface hydrophobicity of cast dry PCL films is determined by the solvent environment during drying [22], which may explain this unexpected result. Hybrid materials with a higher SiO_2 content are formed with more TEOS. This in turn increases the concentration of solvent in the cast drying atmosphere which may attract more of the hydrophobic segments of the PCL molecules to the surface.

Table 1. Hybrid formulations and properties. Actual wt% inorganic was determined previously [8].

Ratio by mass of TEOS:PCL-diCOOH	Actual wt% Inorganic	Calculated average contact pressure with cartilage pin, MPa (± S.D.)	Calculated range of compressive modulus, MPa
0:100	3	0.021 ± 0.002	0.09-0.14
60:40	12	0.032 ± 0.001	0.19-0.30
70:30	17	0.039 ± 0.006	0.22-0.45
80:20	25	0.071 ± 0.006	0.63-1.28
90:10	39	0.446 ± 0.102	2.12-208

The free surface of the 5 formulations was examined by optical microscopy and AFM. At low SiO_2 content, the hybrids had a significant number of surface features contributing to a high surface roughness. AFM images (Figure 4) revealed these features to be holes in the hybrid material. This could influence the contact angle. At low SiO_2 content, both the number of holes and the average size of the holes were larger (detected in the roughness data, Figure 5B). The holes are consistent with pockets of solvent that became trapped during the cast-drying process. For the high wt % SiO_2

hybrids, the drying process was longer, so the solvent is less likely to be trapped resulting in a smoother surface [23]. The behaviour of polymer and silica during drying may also play a role, with the polymer giving a more irregular surface, whereas the silica tends to give very smooth surfaces.

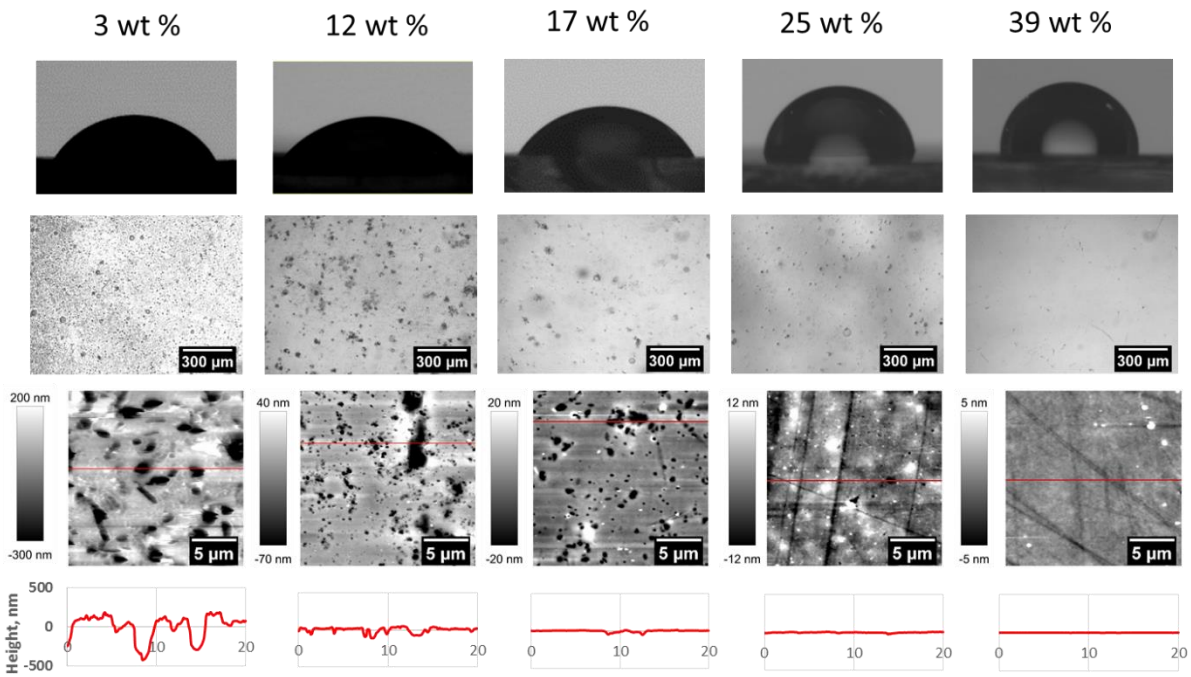


Figure 4. First row: Contact angle measurements; Second row: Optical Microscope images of surface features of the hybrid; Third row: AFM images (20 µm x 20 µm) showing that the surface features to be small pits on hybrid surface; Bottom row: height profile across cross-section of AFM images, showing typical depth of pits and general surface roughness.

3.2 Short term testing results

3.2.1 Friction results

Figure 5A shows the friction coefficient for cartilage against the hybrid materials at 5 seconds and 3500 seconds and against negative and positive controls. Full friction traces for all tests are included in Supplementary Material S2. In these tests, the cartilage pin experienced a non-migrating contact, meaning there was no opportunity for fluid replenishment into the cartilage during the test. Thus, the interstitial fluid in the cartilage flowed out of the contact area, resulting in less fluid load support and increased solid-solid contact. This in turn led to a rise in the friction coefficient with time [16].

After 5 seconds, there was no statistically significant difference in the measured friction coefficient between the positive cartilage-cartilage control and any of the hybrid materials against the cartilage, or the negative CoCrMo control against the cartilage ($p=0.178$). At t=5 seconds, the cartilage was still hydrated and the friction coefficient was at its lowest. This is representative of the conditions in a healthy joint.

After 1 hour, the cartilage in contact lost its interstitial fluid resulting in a worst-case friction coefficient. Therefore, after 1 hour, the coefficient of friction for all hybrid materials and the positive control increased, and the friction coefficient increased as SiO_2 content in the composition decreased. This increase was lowest for 39 wt% SiO_2 sample, which reached an average friction coefficient of 0.17. This increase was less than that observed for the CoCrMo control, but greater than the cartilage/cartilage control at 1 hour.

3.2.2 Wear

For all hybrid formulations, typical surface maps and the average surface roughness, measured by WLI, as received and after all tests, are shown in Figure 5 B,C. The surface maps show that the surface roughness was highest for hybrids with low silica content and decreased as silica content increased, which was consistent with the AFM scans (Figure 4).

For each hybrid, the average surface roughness after testing was compared to the surface roughness in the as received condition using a one-way ANOVA with significance of 0.05. This showed there was no statistically significant change in surface roughness. This is visually demonstrated in the surface maps which show no discernible change in the morphology after testing.

Although no wear was observed on the hybrid samples, the cartilage pins had surface damage that was observable under the optical microscope. This damage was limited in area so that a boundary between damaged and undamaged cartilage was visible in most cases. Optical images of the surface

damage at higher magnification showed the formation of fibrous structures or rolls as well as exposed cartilage lacunae (Figure 5D). This indicates that there was disruption to the surface amorphous layer which is the uppermost layer of cartilage formed of a mixture of proteins, lipids and glycosaminoglycans [24, 25]. Surface area measurements of the damaged region, taken using Image J, showed the extent of this damaged region decreased as silica content increased from 12.8 mm^2 for 2.5 wt% SiO_2 to 5.2 mm^2 for 39 wt% SiO_2. This is despite the lower contact pressure for the softer hybrids with a lower wt% SiO_2 content.

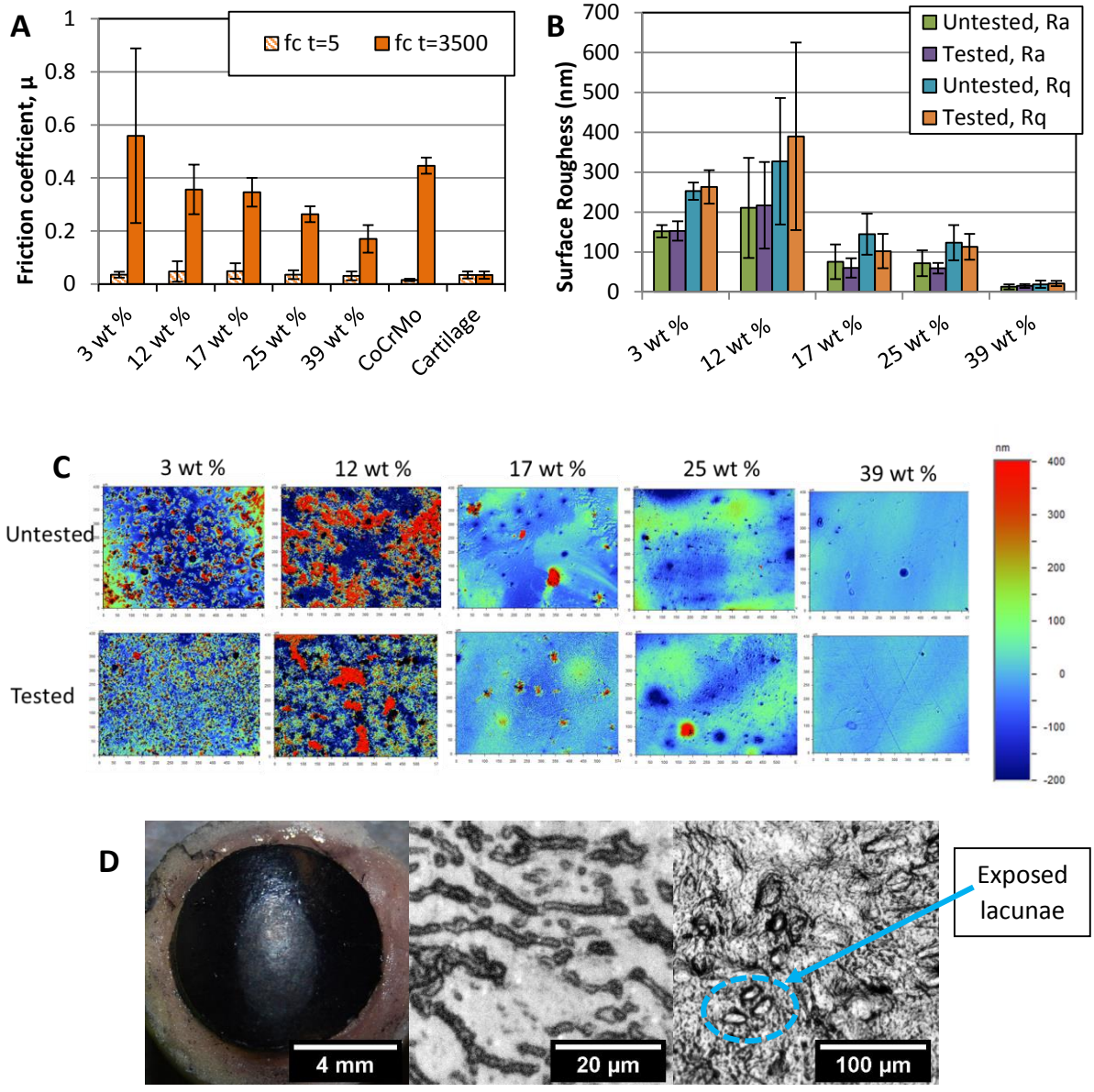


Figure 5. Friction and wear results for short term tests for cartilage pins against the hybrid materials of different wt% of inorganic component: (A) friction coefficient at t = 5 s and t = 3500 s

for cartilage pins lubricated by bovine serum against hybrids with different wt% of inorganic components, a CoCrMo disk (negative control) and a cartilage flat (positive control); (B) average surface roughness (R_a) and root mean square roughness (R_q), measured by WLI, showing no change before and after friction tests; (C) typical WLI height maps of hybrid materials, showing variation with wt% of inorganic components; (D) optical microscope images of a cartilage plug showing a worn area; increasing magnification showed line or rolled features (middle) and exposed lacunae where chondrocytes would be situated (right).

3.3 Bioreactor test results

3.3.1 Friction measurements

For each testing pair (cartilage against cartilage or 25 wt% SiO_2 hybrid against cartilage), the friction coefficient at the end of 1 hour of testing was calculated as the average of the last 5 cycles (Figure 6A). The measured friction for each pair stayed consistent over five days (with the exception of the third hybrid-cartilage pair on day 4 which was due to a set-up error where the ball came loose on the supporting shaft and so no friction data is shown for this day), even though there was some variation between testing pairs. This variation is typical of tissue tests where the exact geometry and tissue properties change from sample to sample. The average friction coefficient for the four cartilage-cartilage pairs was 0.013 ± 0.008, which was statistically significantly different to the average friction for the hybrid-cartilage pairs of 0.005 ± 0.002 ($p < 0.001$). The friction trace for the 8 sample pairs over the whole 1 hour duration is included in Supplementary Material S3.

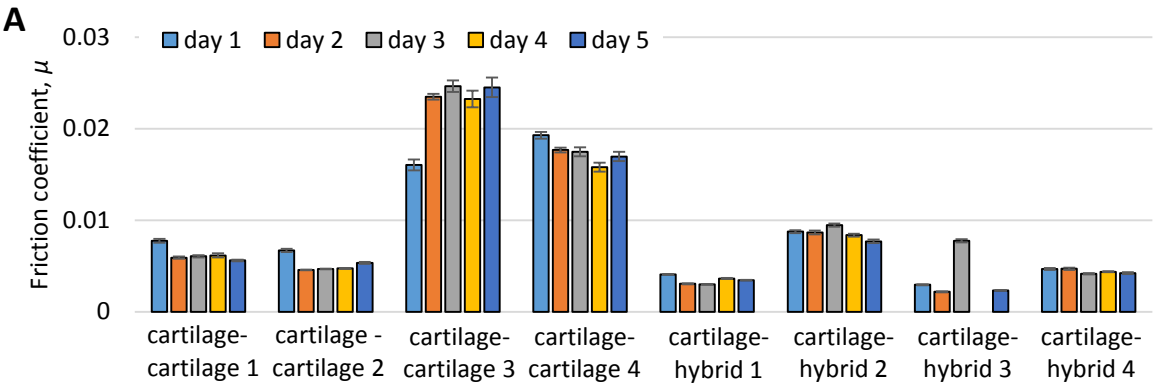
3.3.2 Cell viability

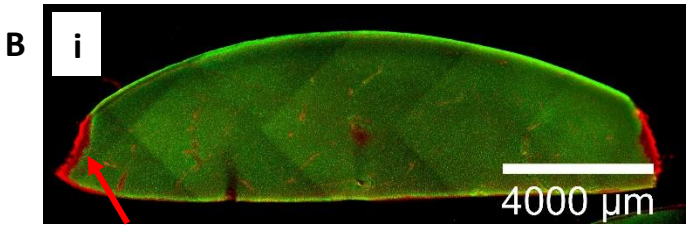
For cartilage-cartilage pairs, an image of the entire cross section of the flat side was captured by combining several images at 5x magnification. This whole cross section image demonstrated that there was good cell viability through the depth of the cartilage seven days after harvest (Figure 6B). Regions of dead cells were observed at the edges of the cross section at the interface with the PE wafer. There was no sign of increased cell death in the area of articulation.

On the cartilage balls, a clear area of dead cells was seen in the loaded surface region. This region varied in thickness but extended to an average depth of 0.4 mm below the surface. Cell viability was measured for a sample area of 0.25 mm^2 in the loaded region. The average cell viability in this area was 29 ± 9 % and 34 ± 13 % for cartilage-cartilage and hybrid-cartilage pairs respectively showing no statistically significant difference (*p=0.527*), Figure 6C.

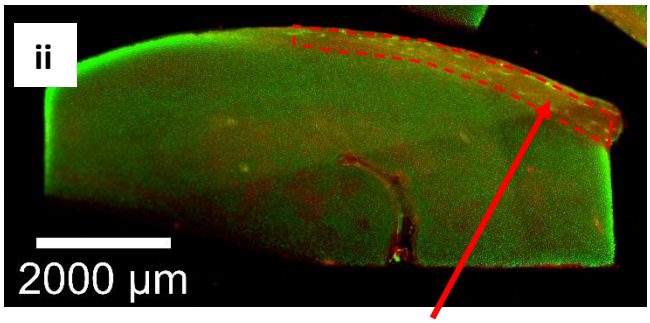
3.3.3 GAG release into media

For cartilage-cartilage testing pairs there are two possible sources of GAG release (the cartilage ball and cartilage flat) whilst the hybrid system only has one cartilage surface, the cartilage flat. To compare GAG release between the systems, it was assumed that the cartilage ball and flat would equally contribute to GAG release and so this measurement was divided by 2. The average GAG release into the test media over five days was between 7-18 µg mL^{-1} per day (Figure 6D), with larger variations in GAG release for cartilage-cartilage pairs. The average release over all five days for the cartilage-cartilage group was 13.6 ± 4.84 µg mL^{-1} which was slightly higher than the 8.0 ± 2.8 µg mL^{-1} measured for the cartilage-hybrid testing groups. A one-way ANOVA shows the reduction to be statistically significant with $p < 0.001$.

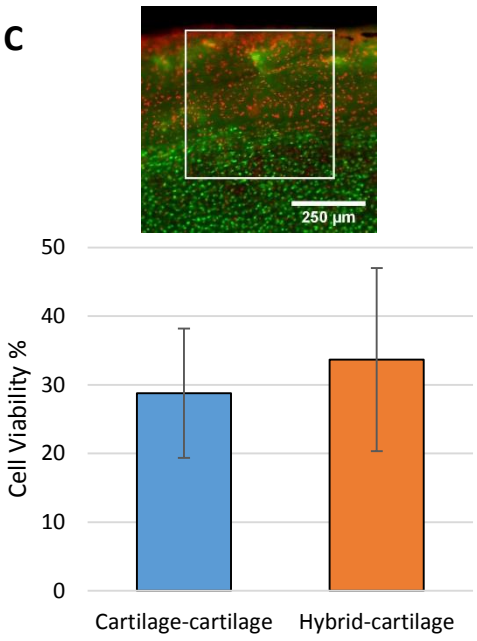




Dead cells at interface with PE wafer



Dead cells in articulating region of cartilage ball



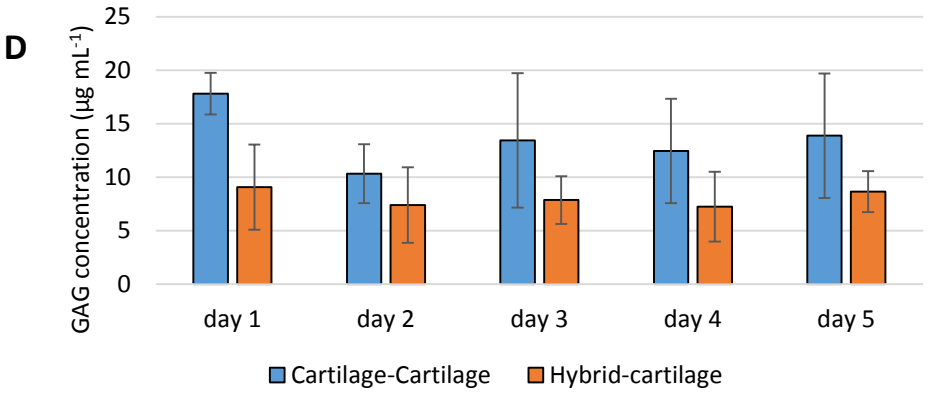


Figure 6 Friction and wear results for bioreactor tests of cartilage against cartilage and 25 wt% SiO_2 hybrid against cartilage: (A) friction coefficient for each testing pair at end of 1 hour of testing, daily; (B) stitched fluorescence images showing LIVE/DEAD stain across the cross section of (i) a cartilage flat (ii) a cartilage ball; (C) typical sampling region to assess cell viability and average

results for testing combinations; (D) average GAG release into test media over 5 testing days as measured by DMMB assay, normalised to number of cartilage surfaces.

3.3.4 Hybrid wear measurements

Following testing, the hybrid samples were examined under an optical microscope and no signs of wear were observed. WLI measurements found that the average surface roughness (R_a) and root mean square roughness (R_q) of the tested hybrid varied between 52-76 nm and 68-98 nm but were not significantly different to the untested control (as prepared unloaded hybrid, Figure 7). The values were also similar for the hybrid that was soaked in the media but not friction tested (*p=0.311* and *p=0.298* respectively).

From TGA measurements, the initial organic content of the hybrid of 82.9 wt% was reduced by soaking (with no loading) by $\delta_{soaking}$ = -1.2 wt% compared to the loaded samples which show an average additional loss of polymer due to wear of δ_{wear} = -0.2 wt%. ICP-OES measurements show a small peak of Si release of 2.3±0.4 µg mL^{-1} at day 1, reducing to below 1 µg mL^{-1} after day 4.

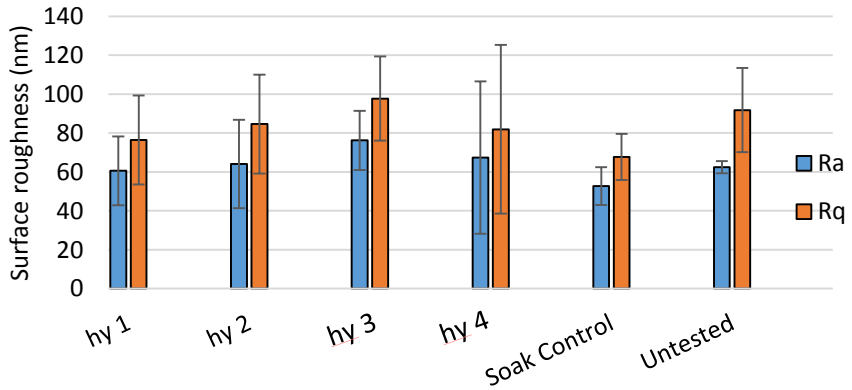


Figure 7. Average Surface Roughness (R_a) and root mean square roughness (R_q) of the hybrid materials, measured by WLIF, showing no change before (Untested) and after friction tests (hy1-4). Hybrid materials that were soaked in the media but not loaded (Soak control) were also tested.

4. Discussion

This is the first study to investigate the tribology of silica/polymer hybrid materials designed for chondral repair applications. Increasing the silica content of the hybrid material improved both the friction and wear performance (Figure 5) and when tested against cartilage in a bioreactor, the hybrid formulation with 25 wt% SiO_2 had similar performance to a cartilage-cartilage pair in terms of friction forces, cell viability and cartilage wear (Figure 6). There were no surface changes in the hybrid material, indicating little wear (Figure 7). The amount of polymer released from the hybrid material was not increased due to friction testing. This evidence is supportive of further development of the hybrid material for chondral repair applications.

The five formulations of hybrid materials tested had different surface properties in terms of hydrophobicity (Figure 4) and surface roughness (Figure 5), both of which can impact the friction performance of a material. Some proteins when adsorbed on hydrophobic surfaces can increase friction forces [26, 27], whilst for the nanoscale roughness seen here other polymers have shown an increase in friction with higher roughness [28, 29]. As an increase in SiO_2 content increased hydrophobicity (Figure 4) but reduced surface roughness (Figure 5). These changes oppose each other and it is only possible to determine the relative effects through friction testing, which showed increasing SiO_2 content improved the overall results (Figure 6).

AFM showed the presence of holes in the hybrid surface (Figure 5), causing roughness, which were more frequent at higher solvent evaporation rates, i.e. at lower silica contents [25]. By controlling the solvent evaporation process more closely, formation of the holes could be reduced, potentially improving the friction performance of materials with a lower SiO_2 content. Removal of these holes which act as stress concentrations would also reduce the likelihood of crack formation in the material. However, at our chosen composition of 25 wt% silica, the holes were low in number.

For materials that articulate against cartilage, low friction prevents high shear strains and stresses in the cartilage which can lead to chondrocyte death and collagen cleavage [30]. In our test that was

most representative of the *in vivo* situation (moving surfaces in media), at t = 5 s there was no statistical difference between the friction coefficient for cartilage-cartilage contact or cartilage-hybrid contact for any formulation (Figure 5A). However, as fluid was lost from the cartilage, the friction force increased as significant wear of the cartilage surface was generated.

For the testing configuration that was a worst-case scenario [17] (cartilage pins), wear was observed on the cartilage pin when it had made contact with the hybrid material (Figure 5). The wear observed was more severe than would be expected in practice, but the test allowed discrimination between test variables and allowed us to choose the hybrid formulation to test in the bioreactor (25 wt% SiO_2). Optical images of the worn area showed a disruption of the continuous surface amorphous layer of the cartilage and exposure of lacunae. The size of the worn area decreased as SiO_2 content in the hybrid increased, which may be explained by an increase in compressive stiffness of the hybrid. Whilst this agrees with the friction results indicating increasing SiO_2 content is beneficial this must be balanced against the risk of increasing contact pressures which can also be detrimental to tissue health.

For bioreactor testing, the 25 wt% formulation was chosen as this had a high SiO_2 content, giving good friction and wear properties but also had a compressive modulus closest to cartilage. In agreement with the short-term tests, the coefficient of friction of the hybrid-cartilage couple was on average lower than a cartilage-cartilage pair when tissue fluid levels were maintained (Figure 6). This is most likely due to the more regular geometry of the hybrid samples rather than an improvement on the frictional performance of cartilage. The hybrid samples are manufactured so have a repeatable, flat geometry. The cartilage samples are retrieved and as mentioned in section 2.1 cartilage flats had a convex curvature with a typical radius of 20mm. This curvature will have some effect on the measured tangential forces during sliding, and so will cause greater variation between test pairs in the calculated friction coefficient. Healthy cartilage lubricated with synovial fluid is expected to see a shear strain of approximately 0.02-0.05 based on a coefficient of friction of 0.01

[31]. In the bioreactor, friction coefficients between 0.005 and 0.025 were measured for testing pairs. This gives a maximum lateral force of 0.25 N, which if we assume to act over the cross-sectional area of the sample (5 mm x 14 mm) the maximum shear stress was approximately 3.6 kPa. Assuming a cartilage shear modulus, *G,* of 0.26 MPa [31], maximum shear strains can be estimated to be 0.014, which is in the range that healthy tissue is exposed to and so not expected to cause tissue damage. This is consistent with our LIVE/DEAD assay of the "flat" cartilage specimens, but not consistent with the observed cell death seen in the articulating region for the cartilage balls. The difference in cell viability may be due to the source location of the cartilage: for the cartilage balls, the cartilage was taken from the rim of the trochlear grove, which is not usually subjected to high loads during motion, whereas the cartilage flats were taken from the femoral condyles which do experience loading during gait. This is a factor to consider in further experiments of this type. Although cell viability was reduced on the articulating surface of the ball, subsurface viability did not change, nor did the viability of the surface within 500 µm of the articulating surface for the cartilage-cartilage or the hybrid-cartilage pair, indicating that it was not caused by the properties of the hybrid material.

The release of GAG from cartilage tissue is associated with cartilage degeneration and may indicate early wear of samples. The concentrations of GAG measured here were around 10 µg mL^{-1} for a one hour period, which is similar in magnitude to the 60 µg mL^{-1} for three hours reported by Trevino *et al.* [19]. The amount of GAG released was actually lower for the cartilage-hybrid tests than it was for the cartilage-cartilage pairs, even when adjusted for the number of cartilage surfaces. This may be due to lower contact pressures in the hybrid-cartilage pairing either due to the reduced compressive modulus of the hybrid compared with cartilage or the more conformal geometry of the hybrid-cartilage system.

The hybrid material tested here is designed to biodegrade with time and be replaced by repaired cartilage tissue. Dissolution studies of the material at 37°C in PBS have previously shown that over 7

496 days the material will release PCL, causing an increase in the inorganic/organic ratio [8], but it was
497 not known whether the rate of material dissolution would increase due to loading, or if changes in
498 the composition would impact the friction and wear performance of the material. Here TGA
499 measurements after 5 days in media at 37°C found the relative organic content of the hybrid
500 decreased by 1.4 wt% but the decrease due to friction testing was not significant (δ_{wear} = -0.2 wt%)
501 when compared to the control that was soaked in the same media without friction testing ($\delta_{soaking}$ = -
502 1.2 wt%). ICP-OES measurements showing a peak in Si release of 2.3±0.4 µg mL^{-1} at day 1 are
503 consistent with unloaded, soaked samples tested previously [8]. The combination of the TGA and
504 ICP-OES results suggest that over the 5 days of testing, as expected, both inorganic and organic
505 phases degraded at very slow rate, with a predominant loss of polymer. No changes in friction forces
506 or increases in GAG release were observed for the hybrid-cartilage testing pairs over the five day
507 testing period, suggesting that the release of small amounts of PCL does not affect the tribological
508 performance. There was also no difference in cell viability observed at the end of 5 days testing
509 which suggests any products released from the hybrid are non-toxic.

510 Although this is the first SiO_2/PTHF/PCL-diCOOH hybrid material to be tested for cartilage repair
511 applications, some composite materials have previously been examined. Composite scaffolds
512 containing Bioglass particles have been suggested for osteochondral regeneration [5, 32], but with
513 no tribology or wear studies. A concern is that Bioglass® particles would be released which would
514 lead to mechanical damage of the cartilage surface through abrasive wear. Implants used to repair
515 focal defects that have metal bearing surfaces, that articulate against cartilage, can cause damage to
516 the opposing cartilage, and that the damage can be as severe as leaving the defect untreated [11,
517 12, 33]. Although often overlooked, tribological testing of proposed biomaterials for osteochondral
518 repair is a crucial part of the design process that will act as a safeguard against damaging devices and
519 will increase the chances of a successful translation into the clinic.

This study was designed to compare 5 different formulations of a new hybrid material and so a simplified geometry and motion relative to that found in the human knee joint were used. Further tests are needed to capture the effects of speed, cross-shear and variable loading on the tribological properties or surface chemistry of the material. Although this study shows no increase in friction or wear related to PCL degradation over a much longer time scale than other studies, it is still short of the time needed for full breakdown of the material and growth of replacement cartilage tissue. Longer term testing will build on these results and form part of the ongoing material development process.

5. Conclusions

Here a SiO_2/PTHF/PCL-diCOOH hybrid material has been tested against a cartilage pin, to discriminate between hybrid compositions and then tested in a more physiological conditions, using a new bioreactor testing rig designed specifically to look at the friction and wear properties under extreme conditions, to give an indication of *in vivo* performance. The silica content of the hybrid material strongly affected the friction coefficient and hence shear force applied to the opposing articular cartilage. The best frictional and wear properties were achieved through increasing the silica content of the hybrid, although this must be balanced against introducing high contact pressures which can be detrimental to cartilage tissue. After five days of testing against "living" cartilage, no difference in friction or wear was seen for the hybrid rotating against cartilage pair compared with the control cartilage-cartilage pairs. Wear of the hybrid was not observed despite a reduction in the material PCL content over this period. This information will inform the development or chondral and osteochondral scaffolds.

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