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A technique for measuring the frictional torque of articular cartilage and replacement biomaterials

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Abbreviations

OA..... Osteoarthritis

ECM.....Extracellular matrix

ABSTRACT

Understanding the tribological behaviour of articular cartilage enables the development of effective replacement biomaterials. This study presents a technique for the investigation of the frictional torque of articular cartilage, for the assessment of replacement biomaterials. A calcium alginate hydrogel was used as the biomaterial for this study. Three different specimen types were examined to include articular cartilage, calcium alginate hydrogel, alone, and in combination with articular cartilage. An axial load, varying from 10 to 100 N, was applied to the specimen and the frictional torque measured whilst an indenter underwent axial rotation from 0° to 2° to 0° for 100 cycles. The resulting frictional torque magnitude was evaluated with a smooth curve fitting function. Linear regression identified a statistically significant relationship between torque magnitude and axial load ($p < 0.05$) for all specimen variations. From 10 to 100 N of applied load, mean torque magnitude ranged from 0.08 ± 0.010 to 0.11 ± 0.013 N m, 0.08 ± 0.012 to 0.09 ± 0.016 N m and 0.07 ± 0.017 to 0.09 ± 0.020 N m (mean \pm standard deviation), for articular cartilage, calcium alginate separately and in combination with articular cartilage, respectively. This study has established a suitable frictional torque testing protocol for potential cartilage replacement biomaterials.

Keywords:

Articular cartilage

Axial-rotation

Calcium alginate

Frictional torque

Osteoarthritis

Replacement biomaterials

1. Introduction

Synovial joints consist of articular cartilage and surrounding synovial fluid, including subchondral bone [1]. The articular cartilage conceals the bone ends in synovial joints [2], contributing to a low coefficient of friction reported in the range of 0.002 to 0.02 [3]–[5], aided by a surface roughness of 80 – 170 nm [6]. The lubrication mechanisms of articular cartilage contribute to its low friction characteristics [7], [8], minimalising wear [8], [9].

Osteoarthritis (OA) is a disease that can affect synovial joints [1]. OA development includes degradation of articular cartilage due to the “imbalance of synthesis and catabolism” [10] as a result of the disruption of chondrocyte cells [10]. OA induces a rough, fibrillated appearance to the surface of cartilage, greatly different to its original smooth surface configuration [11]. OA contributes to the increased friction of articular cartilage whilst the joint undergoes motion. Further, OA is not solely associated with articular cartilage, as the underlying subchondral bone also plays a role. During the earlier stages of OA, increased bone remodelling is observed [12]–[14].

As a result of the incidence of OA, cartilage repair methodologies are required for the treatment of damaged articular cartilage tissue [15], [16], such as hydrogels [17], which are defined as a network of polymers of a hydrophilic gel [18]. Hydrogels are commonly used as tissue replacements [19], due to the biocompatibility [17], biodegradability and absence of toxicity, specifically of those with a polysaccharide origin [18]. Alginate is a linear copolysaccharide, constituted of monomers β -D-mannuronate (M) and α -L-guluronate (G) [20]. For the formation of a biocompatible gel [19], [21], the G monomer components are combined with cations of Ca^{2+} [19], synthesising a calcium alginate hydrogel [21]. The calcium alginate hydrogel is well-known for its use in several biomedical applications [19], [21], [22], particularly for use in cartilage tissue engineering [23], [24].

The successful development of cartilage replacement biomaterials requires an understanding of the frictional characteristics of articular cartilage [25]. Previous studies have explored the effect of sliding speed [26], [27], time [28] as well as cyclic loading [29] on the coefficient of friction of articular cartilage. However, the previously measured coefficient of friction does not consider the

rotational motion of articular cartilage, but is limited to the movement in linear sliding. The frictional torque of articular cartilage as a result of the response to a rotational motion is unknown, yet is important to measure, as the underlying kinetic closely mimics joint movement *in vivo*. Consequently, for the development of a successful cartilage replacement biomaterial, it is essential to compare the frictional torque to that of articular cartilage.

The aim of this study was to develop a technique for the evaluation of the frictional torque of articular cartilage, to which a biomaterial can be compared. Based on the use of calcium alginate in several biomedical engineering applications, it was selected as the biomaterial for this study. For insertion of the hydrogel for investigation, a small cartilage surface defect was induced.

2. Materials and Methods

2.1 Tissue storage and handling

Twelve bovine humeral heads were obtained from a supplier (Dissect Supplies, Kings Heath, Birmingham, UK) extracted from animals between the ages of 18-30 months at slaughter. Bovine articular cartilage has been identified as a suitable model for human cartilage [30]. See our previous work on the protocol for storage and identification of suitable cartilage areas for testing [31], [32]. The underlying subchondral bone was used for the secure fixation of the specimen for friction measurements. Prior to testing, all specimens were immersed in Ringer's solution for 30 minutes [30].

2.2 Preparation of articular cartilage specimens

To evaluate the frictional torque of articular cartilage, rectangular specimens were extracted from the central area of the humeral head. This region was selected as it is typically flat. Also, the central segment acts as a central point of contact within the shoulder joint [33]. All specimens were extracted across six different humeral heads with a 300 mm bi-metal hacksaw blade (RS Components Ltd, Corby, UK). The specimens were approximately 11 mm thick, with a cross-section of 26 mm × 21 mm. In total, eighteen individual cartilage specimens, six per torque test, were prepared. The three torque tests focused on: i. cartilage alone, ii. cartilage with a 2 mm insert

of calcium alginate, iii. cartilage with a 10 mm insert of calcium alginate.

To prepare the 2- and 10 mm inserts for calcium alginate, a 2- and 10 mm diameter drill head (EW Equipment, Stockport, UK) attached to a drill (Makita UK Ltd, Milton Keynes, UK), was respectively used to create a cavity, of an approximate depth of 3 mm, within the surface of the cartilage specimen. The induced cavity allowed for the insertion of the biomaterial. A simple schematic is displayed in Fig. 1 to represent the various cavities for each specimen type.

2.3 Manufacture of calcium alginate biomaterial for specimen preparation

Calcium alginate hydrogel was formed by mixing 7.5 g of sodium alginate (Sigma-Aldrich, Dorset, UK) with 125 ml of deionised water [21]. 12.5 g of calcium chloride dihydrate (EMD Millipore, Darmstadt, Germany) was mixed with an additional 125 ml of deionised water and transferred into the sodium alginate mixture [21]. An image of the formed calcium alginate hydrogel post-48 hours of setting is presented in Fig. 2.

An identical 5 mm diameter region was tested with a single indenter across all specimens; i) 5 mm diameter region of articular cartilage, ii) 2 mm hydrogel with surrounding 3 mm of articular cartilage, iii) 10 mm hydrogel. This allowed the biomaterial alone and in combination with articular cartilage to be assessed.

To prepare the specimen with the 2 mm insertion, a cork-borer with a diameter of 5 mm was used to obtain a core of the biomaterial for insertion within a cavity perforated at the surface of the articular cartilage (Fig. 3a; Fig. 3b). This procedure was repeated for the 10 mm diameter biomaterial insertion, with use of a hand cork-borer with an outer diameter of 11 mm (Fig. 3c; Fig. 3d).

2.4 Frictional torque

To assess the frictional torque a Bose ElectroForce SDWS testing machine operated with the Bose WinTest 4.1 software (Bose Corporation, ElectroForce Systems Group, Minnesota, USA) was used. Testing involved compressing a stainless-steel indenter against the specimen surface and

applying an axial-rotation. The indenter was manufactured from polished stainless-steel to match previous studies which reported the Ra as 0.02 μm [25], [34].

The indenter was designed with a diameter of 5 mm, 7.5 mm in length and with a 0.5 mm chamfered end to prevent damage to the cartilage [31], [35]. The chamfered-polished end ensured a smooth surface for the assessment of the frictional torque. The indenter was attached to a stainless-steel plate with a diameter of 110 mm which was secured to the upper plate of the testing machine. All specimens were secured within a customised circular aluminium test rig via two screws positioned through the subchondral bone for fixation. The rig was designed with a diameter of 63 mm and a thickness of 13.5 mm. Ringer's solution was used as the lubricant similar to previous cartilage friction work [3], prepared as described previously [31]. For the preservation of the hydration of the tissue during testing, the Ringer's solution was inserted within the test rig [35]–[37]. The test rig was secured on a stainless-steel fixture [38], followed by the insertion within a water bath for mounting onto the machine [38]. The overall experimental set-up is shown in Fig. 4a, to illustrate the position of the indenter, test-rig with specimen, water bath as well as rotation stage. Fig. 4b and Fig. 4c display the indenter and test-rig.

A static load of 10 N was applied onto the specimen, followed by a rotation from 0° to 2° to 0°, completed in one second for 100 cycles. This testing method was repeated for a further nine static loads from 20 to 100 N, evaluating the effect of an induced stress range from 0.51 to 5.09 MPa, of a physiological range [39], [40]. All frictional torque tests were completed at 1 Hz. The frictional torque of articular cartilage on-bone was evaluated, to include six repeat tests per load, on each of six cartilage specimens, for 360 individual experiments. A separate six cartilage specimens were assessed to obtain the torque of the 2 and 10 mm calcium alginate inserts, for which the 2 mm insert was located below the centre of the stainless-steel indenter. Based on a 1 minute load removal for friction measurements in a previous study [3], all specimens were left to recover for 5 minutes between each increase in load, ensuring consistent inner fluid recovery.

2.5 Quantification of the frictional torque magnitude

The magnitude of the frictional torque was evaluated for the final 10 cycles from each 100-cycle test per load. The selection of the final 10 cycles for analysis is based on a suitable endpoint that is of the form of a dynamic “steady-state”. Identical ranges in the absolute frictional torque of articular cartilage are identified between the final 10 and 20 cycles, for instance a range of 0.16 to 0.29 N m is shown at 100 N, respectively. Similarly, an identical range was identified as 0.17 to 0.29 N m at 10 N, at both the final 10 and 20 cycles. Yet, due to the nature of biological material where variations in the frictional behaviour are expected, the final 10 cycles accounts for potential instabilities in the friction that may occur earlier on in the test. The maximum frictional stability is therefore predicted at the final 10 cycles, where the extraction at this segment allows repeatable dynamic data to be recorded. MATLAB (Version R2017a, MathWorks, Cambridge, UK) was used to fit a sinusoidal wave to the last 10 cycle plot of the frictional torque generated with respect to time, for a fitted smooth curve fit from the original torque plot. The absolute maximum and minimum curve points were used to obtain two distinct torque values, for which the calculated absolute difference was reported as the torque magnitude. This process was performed for all specimen repeats.

2.6 Data analysis

Regressions analysis was used to assess the relationship between axial load and mean frictional torque magnitude for all specimens (Sigmaplot Version 12.0 (Systat Software Inc., London, UK)). Statistical significance was $p < 0.05$.

3. Results

3.1 Effect of axial load on the frictional torque magnitude

The mean torque magnitude with respect to axial load across all six specimens is presented in Fig. 5a-c, for cartilage on-bone, and the 2 and 10 mm calcium alginate inserts, respectively. The relationship between torque magnitude and axial load is represented by a line of best fit as described by equation (1):

$$T = D + AL \tag{1}$$

where T is the frictional torque magnitude in N m, L is the load (in N), A and D are constants. Across all six cartilage on-bone specimens, the mean frictional torque magnitude with respect to all loads tested, ranged from 0.08 to 0.11 N m (Fig. 5a). The range for the 2 mm calcium alginate insert was of 0.07 to 0.09 N m (Fig. 5b), whilst the range for the 10 mm calcium alginate insert was of 0.08 to 0.09 N m (Fig. 5c), at 10 and 100 N, respectively. The linear regression fits of the mean torque magnitude and load curves indicated statistical significance for all specimen types, $p < 0.05$, (Fig. 5a, b, c, respectively).

4. Discussion

This is the first study to develop a technique to evaluate the frictional torque of articular cartilage, exploring the resulting effect of axial load, incorporating a method for the comparable assessment of replacement biomaterials. The frictional torque magnitude of articular cartilage increased with load, ranging from 0.08 to 0.11 N m at 10 and 100 N, respectively. The frictional torque magnitude of calcium alginate was also observed to increase with load, from 0.08 to 0.09 N m. Greater variability in the obtained data was noticed during the initial few cycles of testing, which is typical of soft connective tissues. Measurements are to within 0.01 N m, based on the assessment of the initial and final datasets obtained per samples tested.

The 0 to 2 to 0° range of rotation reflects the internal rotation of the hip at standing of 1° when putting on lower garments, and of the right hip at 2° when tying shoe laces and picking up objects [41]. Note, the method developed in this study establishes a controlled and repeatable approach, whilst not with the aim to replicate complete joint movement. Limited studies have investigated the rotation of articular cartilage [42], which can investigate the boundary lubrication mode [43], representing the lubrication present at the cartilage-cartilage boundary *in vivo* [42]. Thus, this study is novel in its approach to report the frictional torque magnitude of articular cartilage.

The linear regression increase in the frictional torque with load of articular cartilage indicates the relationship which a suitable biomaterial should adopt, from a tribology perspective. The torque magnitude of a biomaterial should be no greater than the range identified in this study, of 0.08 to 0.11 N m (cylindrical sample 5 mm in diameter within the test parameters used). Note, within the test parameters of 100 N and a rotation of 0 to 2°, this range would act as target values. For future studies, loading may vary beyond 2° and 100 N, and as a result of the evaluation of a wider range, this study provides a broader test protocol which can be used to evaluate 'baseline' data.

Several studies have shown similar trends in the observed increase in the friction of cartilage; commonly with varied load, time and sliding speed. Previous work has reported an increase in the coefficient of friction of bovine articular cartilage against stainless-steel, with time [34]. That study was conducted under a reciprocating motion, with an observed rise in the coefficient of friction from 0.005 to 0.50 when testing with synovial fluid, and up to 0.57 for Ringer's solution [34].

The estimated coefficient of friction which can be approximated from our study is compared to existing literature. For our study, at a normal force of a defined indenter radius, the estimated coefficient of friction is estimated at 0.32 to 0.44, at the maximum applied load of 100 N, for the minimum (0.08 N m) and maximum (0.11 N m) frictional torque, respectively. The minimum load of 10 N is associated with an estimated friction coefficient range of 0.03 to 0.04, at the minimum and maximum obtained torque values, respectively. Thus, the values inferred from this study are close to the range observed in previous work described above [34]. In addition, the estimated coefficient of friction from our study is comparable to previous work that identifies a coefficient of friction range of approximately 0.01 to 0.28, of cartilage against metal, in Ringer's solution [3]. It is also similar to previous work that demonstrates a coefficient of friction of approximately 0.22 of cartilage against stainless-steel, on completion of 20 minutes of testing [44], and a value of 0.25 on completion of 4-hours of testing for cartilage against stainless-steel [45].

Previous work has explored the coefficient of friction of bovine articular cartilage subject to sliding against glass, at varied frequencies from 0.05 to 1 Hz [46] for which the coefficient of friction is reported to increase with time at 1 Hz [46]. Therefore, both the time in that study [46], and the load

in our study, have resulted in the increase in the friction of articular cartilage. With reference to the previous study [46], when the loading frequency is lowered from 1 Hz to 0.05 Hz, the friction coefficient is observed to increase [46]. Therefore, it is anticipated that the frictional torque would also follow this trend on application of a lower frequency (e.g. 0.05 Hz).

Previous studies on articular cartilage have noted an increase in the coefficient of friction [42], linking their results to rotation movement and postulating that interstitial fluid leaves the surfaces in contact, with implications for the extracellular matrix and its loading [42], [44]. However, in our study we have found a linear increase in frictional torque with normal load, which aligns directly to standard equations for calculating friction and torque (regardless of the type of material or whether fluid is present/absent). Yet, previous work suggests testing in multi-directional configurations can lead to the decline in boundary lubricants at the contact surfaces, and therefore, increase the resulting friction [25].

The frictional torque magnitude for the hydrogel is suitably comparable to articular cartilage, with an observed 12.5 % and 28.6 % increase for the 10- and 2 mm inserts, respectively. To mimic the boundary lubrication mode of articular cartilage, the biomaterial was assessed in an identical manner to the cartilage.

A limitation of the study may be that the cartilage was tested as an isolated tissue sample. *In vivo*, the surrounding ligaments and tendons, as well as the attachment to bone, may alter the resulting frictional torque. However, cartilage specimens extracted from a joint allows for direct friction measurements (or indeed frictional torque), with enhanced control of the tribological protocol [3].

Although the metal testing configuration utilised in this study is not a physiological representation, an increase in the start-up coefficient of friction for both cartilage-cartilage and cartilage-metal with stationary loading time was identified [3]. This suggests that no difference in the relationship between coefficient of friction and loading time were observed between metal and cartilage [3]. In addition, the use of an indentation procedure is repeatable, and has previously been used for the evaluation of the mechanical behaviour of articular cartilage [47], including failure [31]. Note,

although the homogeneity in stress cannot be confirmed below the indenter as it is expected for a different spatial stress distribution to exist amongst the specimens, the forces have been applied equally to each specimen. This ensures a comparable assessment of the torque. Thus, despite a limitation, the conclusions presented in this study remain unaltered.

A further potential limitation may be the existence in error of misalignment of the cartilage surface, as a result of the extent of the surface flatness. However, misalignment error has been controlled in this study by the extraction of cartilage specimens from the central, and therefore flattest region of the humeral head. Although an error in misalignment may still have occurred, for which an exact figure would be difficult to estimate, a previous study demonstrates the feasibility in obtaining sheets of cartilage (40 × 20 mm), from which curvature was not observed visually [37].

Although the measurement of frictional torque is not a material property, but rather limited to the specimen size, this technical note describes a novel technique for a consistent comparison of the torque between articular cartilage and a replacement biomaterial. This new method is advantageous in comparison to the pin-on-disc set-up, due to the direct extraction of the unknown frictional torque of articular cartilage with respect to rotation.

5. Conclusions

The technique developed in this study can be implemented in future studies for evaluating potential articular cartilage replacement biomaterials, from a frictional torque perspective. This study has identified a mean frictional torque magnitude of bovine articular cartilage of 0.08 to 0.11 N m at testing 10 through to 100 N, whilst a statistical difference is observed between the torque magnitude and load ($p < 0.05$).

6. Declarations

Ethics approval and consent to participate

Not required.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

None declared.

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Authors' contributions

HM carried out the experimental work, design of the study, data analysis and drafted the manuscript. DETS and DME participated in design of the study, data analysis and critically revising the manuscript. All authors have read and approved the final manuscript.

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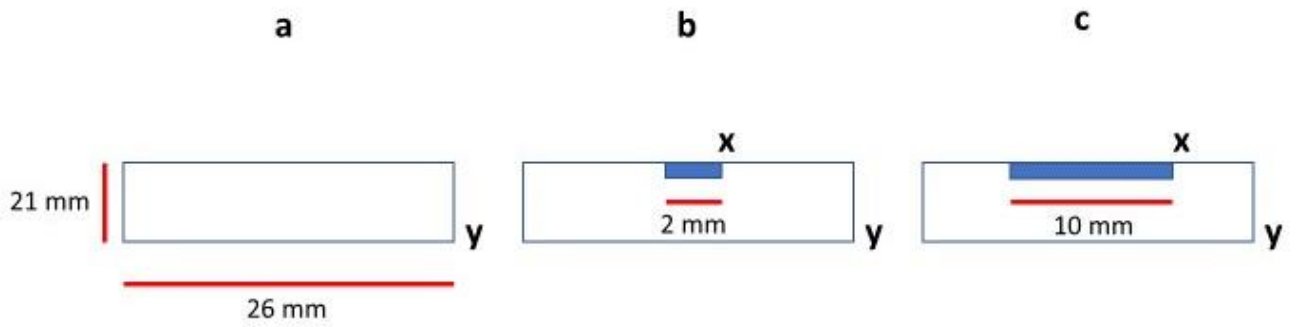


Figure 1: Schematic to represent the articular cartilage specimen for each testing group. a) cartilage on-bone specimen, b) 2 mm calcium alginate insert specimen, c) 10 mm calcium alginate insert specimen. 'y' represents the cartilage specimen for each case, as indicated by the white rectangular space. The insertions for both the 2- and 10-mm calcium alginate hydrogels are shown by the blue shaded area highlighted by 'x'. Key dimensions are shown with a scale bar

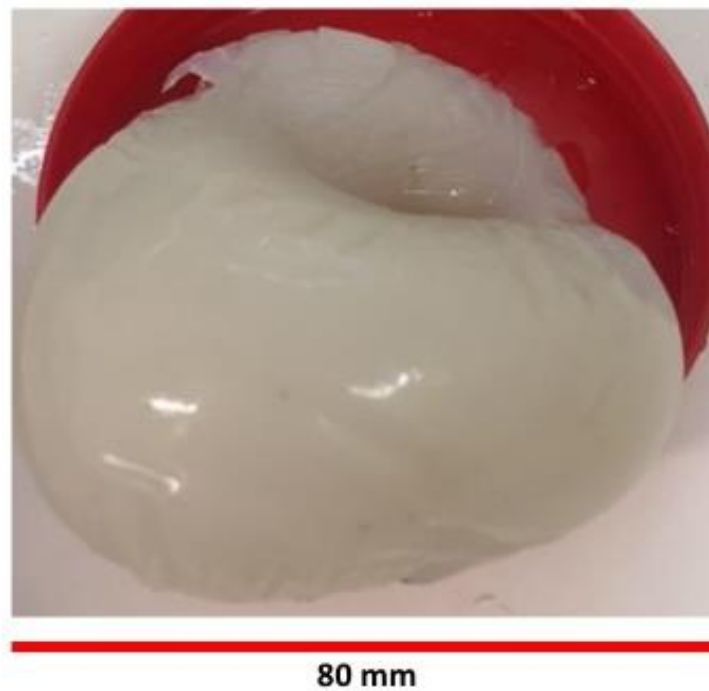


Figure 2: Calcium Alginate Hydrogel: synthesised result post 48-hours of setting. Scale bar is included

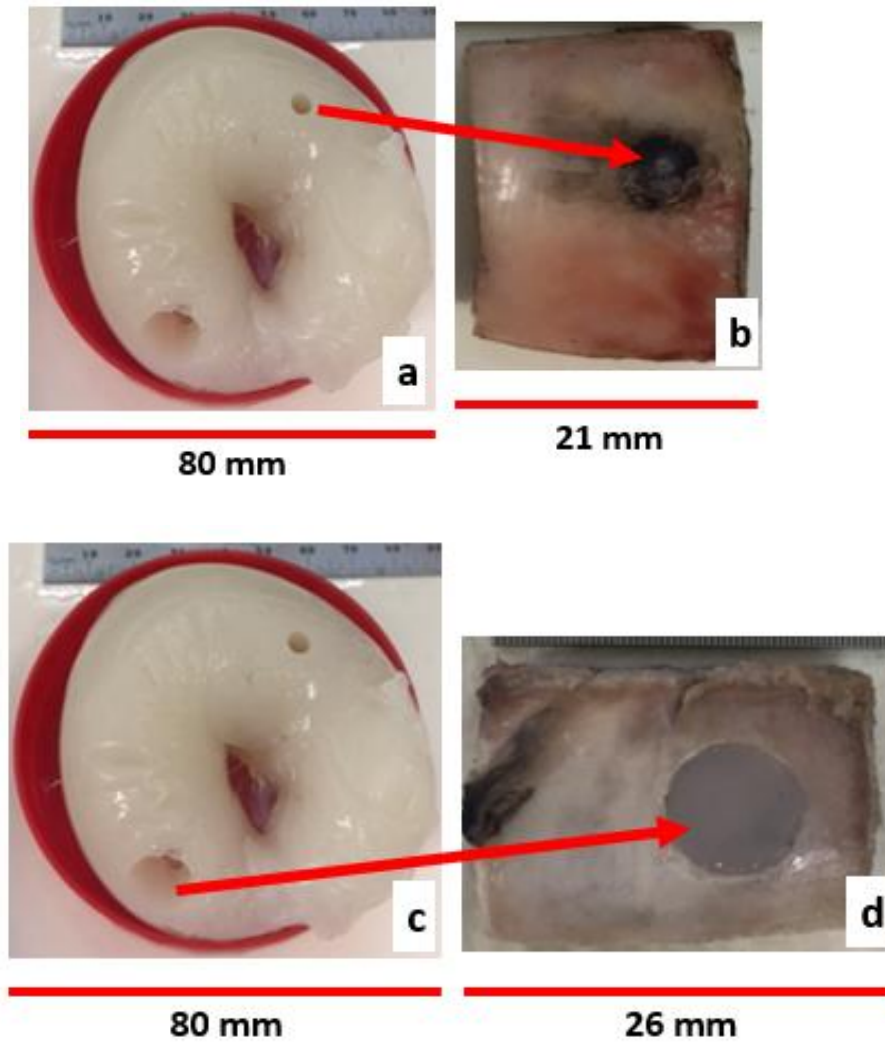


Figure 3: Illustration of the insertion procedure of the calcium alginate hydrogel within the cartilage on-bone specimen, for both 2- and 10-mm hydrogel inserts: a) 2 mm hydrogel extraction. This is inserted into the cartilage on-bone specimen as shown in part b). The black surrounding circular staining of the hydrogel insert in part b) is the use of India ink to locate the insertion location for the biomaterial. The inserted biomaterial is highlighted with a red arrow. c) 10 mm hydrogel extraction. This is inserted into the cartilage on-bone specimen as shown in part d). The inserted biomaterial is highlighted with a red arrow. Scale bar is included

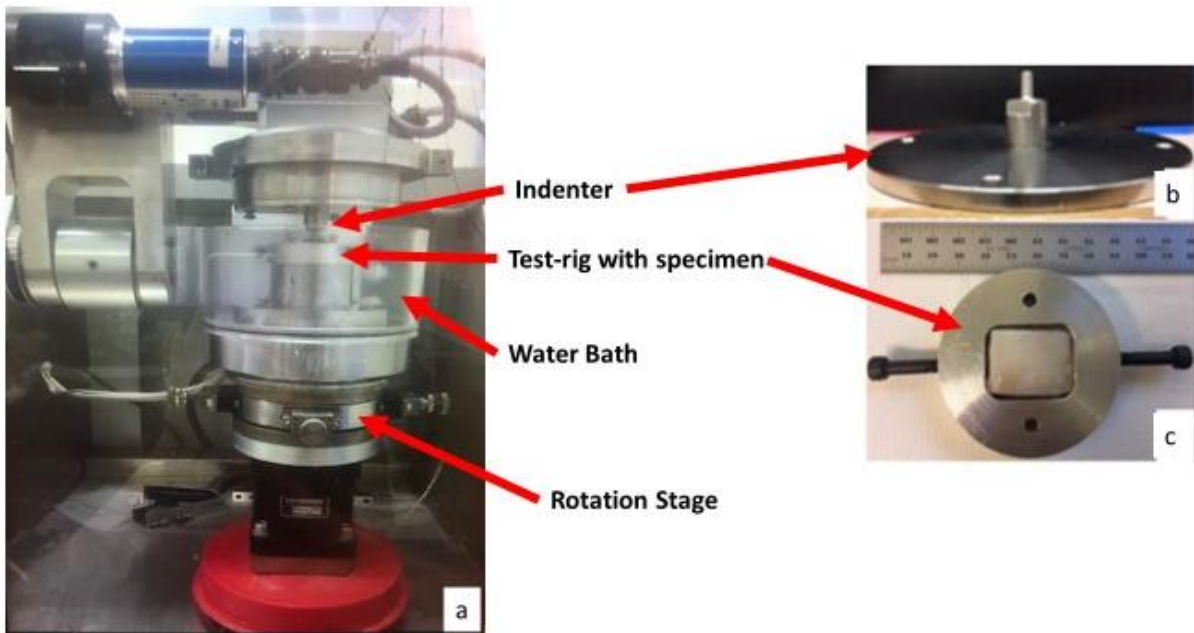
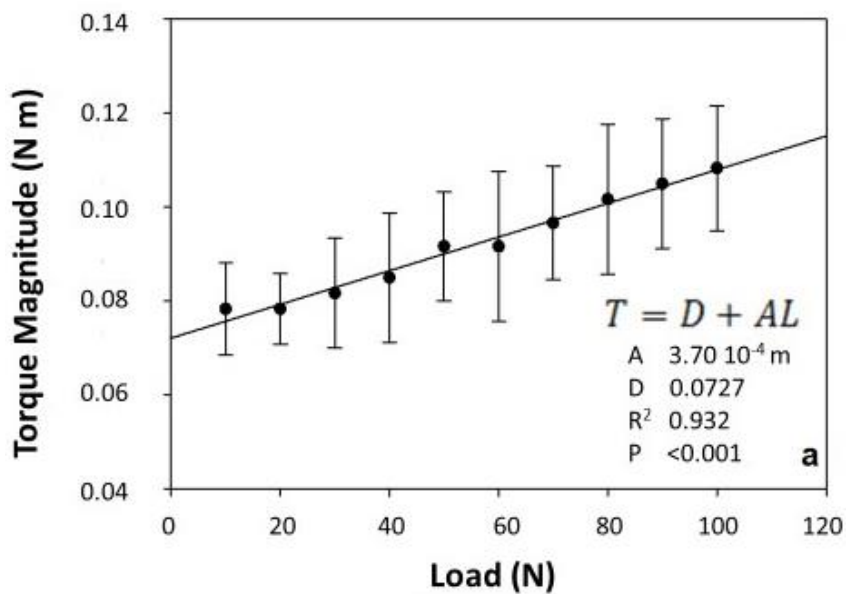


Figure 4: Testing Apparatus: a) Overall schematic of the experimental set-up on the Bose ElectroForce SDWS to display the stainless-steel indenter, test-rig with specimen, water bath and rotation stage. Part b) shows the stainless-steel indenter, part c) displays the test-rig to contain the specimen which is tightened in position with the two black screws either side



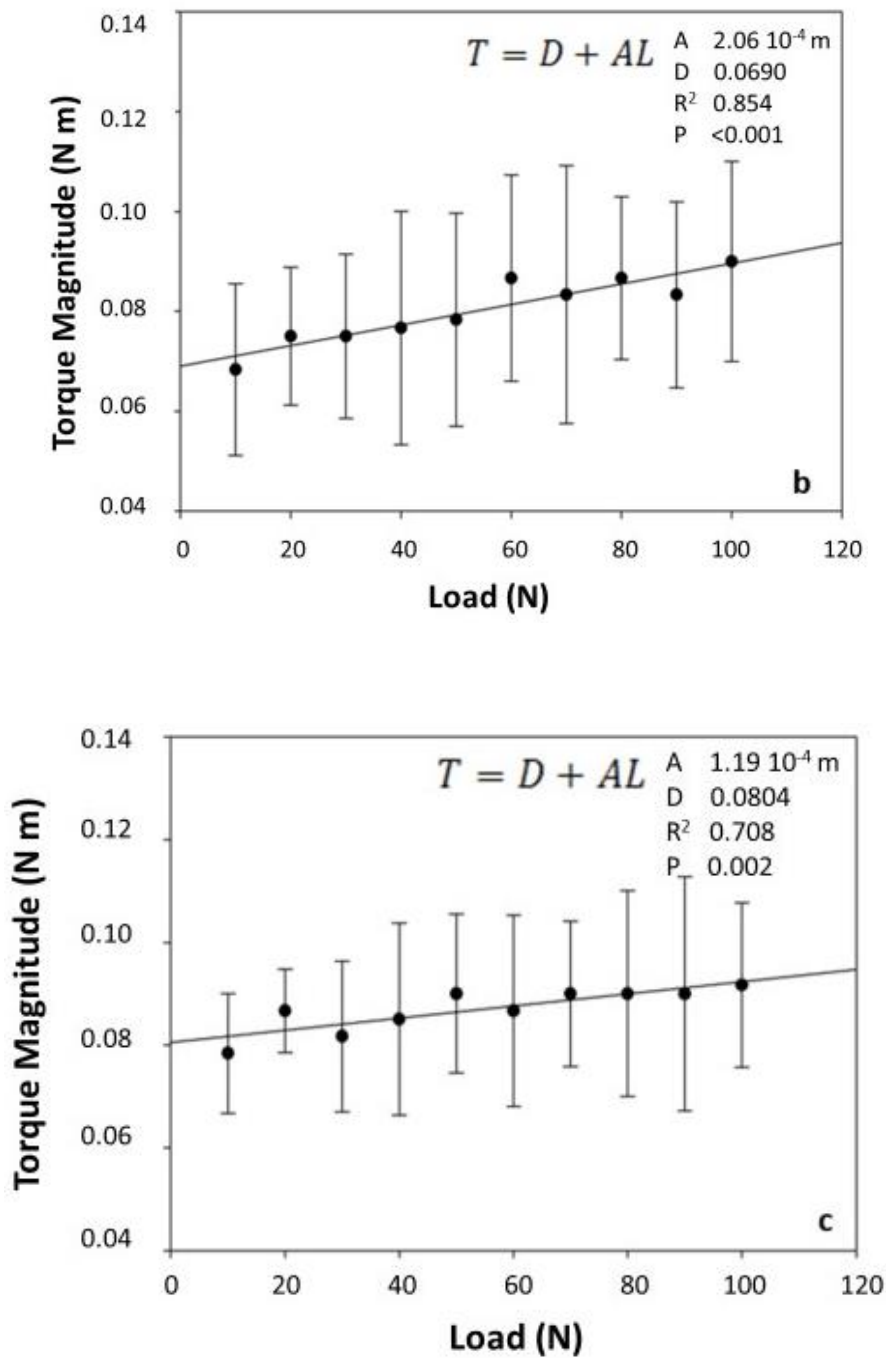


Figure 5: Mean frictional torque with respect to axial load: a) cartilage on-bone; mean of 36 data points calculated across six specimens (n=6). b) 2 mm calcium alginate insert; mean of 6 data points calculated across six specimens (n=6). c) 10 mm calcium alginate insert; mean of 6 data points calculated across 6 specimens (n=6). Error bars represent standard deviation. Linear fit details derived from equation (1) are included within each plot. P-value less than 0.05 indicates statistical significance