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***In vitro* method for assessment of the biomechanics of the patellofemoral joint following total knee arthroplasty**

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Keywords

Total knee arthroplasty, patellofemoral joint, biomechanics, Oxford knee rig, preclinical testing

Abstract

The patellofemoral joint is a common site of pain and failure following total knee arthroplasty. A contributory factor may be adverse patellofemoral biomechanics. Cadaveric investigations are commonly used to assess the biomechanics of the joint, but are associated with high inter-specimen variability and often cannot be carried out at physiological levels of loading. The present study aimed to evaluate the suitability of a novel knee simulator for investigating patellofemoral joint biomechanics. This simulator specifically facilitated the extended assessment of patellofemoral joint biomechanics under physiological levels of loading.

The simulator allowed the knee to move in six degrees of freedom under quadriceps actuation and included a simulation of the action of the hamstrings. Prostheses were implanted on synthetic bones and key soft tissues were modelled with a synthetic analogue. In order to evaluate the physiological relevance and repeatability of the simulator, measurements were made of the quadriceps force and the force, contact area and pressure within the patellofemoral joint using load cells, pressure sensitive film, and a flexible pressure sensor.

The results were in agreement with those previously reported in the literature, confirming that the simulator is able to provide a realistic physiological loading situation. Under physiological loading, average standard deviations of force and area measurements were substantially lower and comparable to those reported in previous cadaveric studies respectively. The simulator replicates the physiological environment and has been demonstrated to allow the initial investigation of factors affecting patellofemoral biomechanics following total knee arthroplasty.

Introduction

Increasing life expectancy, coupled with growing rates of obesity, is fuelling an unprecedented growth in total knee arthroplasty (TKA) procedures across the developed world.¹ Procedures in the USA alone are predicted to increase sevenfold, from around 400,000 per year in 2003 to 3.48 million in 2030.¹ While survival rates at 15 years are frequently reported to be in excess of 80%,²⁻⁴ patient expectations are increasing, and 20% are not satisfied with their new joint.⁵ Up to a third of patients report that their joint does not feel normal following TKA.⁶

The patellofemoral joint (PFJ) is implicated as a factor in 20% of revision cases,³ and up to 25% of unrevised patients report difficulties with everyday extension activities or anterior knee pain (AKP).⁷ The exact causes of AKP are not fully understood. It is thought that changes in PFJ loading magnitudes and patterns may be contributing factors, causing changes to tissue homeostasis, initiating bone remodelling, and stimulating intraosseous nerve cells.⁸⁻¹¹

In vivo studies provide the most physiologically relevant assessment of the mechanics of the human body. However, they are often purely qualitative, time consuming, and limited in scope. *In vitro* studies, although not without their own limitations, allow for more invasive investigations into parameters that influence the biomechanics of the PFJ.¹²⁻¹⁹ *In vitro* studies are most commonly carried out using cadaveric knees, which are characterised by high inter-specimen variability and are susceptible to damage under physiological levels of loading.¹² This limits the number of variables that can be assessed on a single specimen or the level of load under which tests can be carried out.¹²⁻¹⁹

Simulators using non-cadaveric knee models are commonly used for wear testing and have previously been employed to assess the PFJ following TKA.^{20, 21} Any synthetic joint model will be simpler than the

human knee and may be limited by the absence, or simplification, of some soft tissue structures.^{21, 22} However, use of non-cadaveric models reduces inter-specimen variability, allows systematic assessment of the influence of a single parameter on the PFJ, and the completion of extended testing under physiological loads.^{21, 22}

Previous non-cadaveric knee models have replaced the action of the collateral ligaments, two of the primary stabilising structures within the knee, with a purely compressive force.^{21, 22} This maintains joint integrity but does not replicate the natural guiding role of the ligaments.²² Non-cadaveric knee models also commonly do not include the flexor mechanism,^{20, 21} despite the biomechanical significance of hamstring co-contractions.²³⁻²⁷

A novel non-cadaveric knee simulator, which includes synthetic models for the collateral ligaments and the hamstring complex, has been developed. The present study aimed to evaluate the physiological relevance of the new simulator through comparisons with previous cadaveric studies. The study also aimed to assess the importance of testing under physiological loading conditions when assessing PFJ biomechanics.

Methods & Materials

The simulator is a derivative of the Oxford Knee Rig and designed to allow the knee joint to move in all six degrees of freedom (Figure 1). The simulator is intended to model the squatting motion of an average UK female, and is the development of a previously reported study.²⁸

[Insert Figure 1 Revised.png]

Figure 1: Kinematic test rig, with pertinent features highlighted

Motion at the knee joint is induced by a single quadriceps actuator (SKF Care 33A, SKF, Luton, UK) mounted with a Q angle of 16°. ²⁹ For the purposes of this study, the knee was actuated against two different simulated body weights; the first replicated a physiological peak flexion moment of 43 Nm, ³⁰⁻³² and the second a reduced level of loading (approximately 19Nm peak moment). This reduced level of loading accommodated the load rating of one of the sensors (Pliance, Novel, Munich, Germany) and replicated the loading often used in cadaveric testing. ^{12-19, 33} The level of simulated body weight was modified by altering the counterweight on the knee simulator (**Figure 1**). It was also possible to position the knee at fixed flexion angles to allow static testing.

A 3 mm steel cable was used to model the patella and quadriceps tendons. ²⁸ Cotton webbing was glued to the cable above and below the patella construct to reduce friction and distribute the load when the tendon contacted the femoral component in deep flexion (Figure 1). ^{34, 35}

Collateral and popliteofibular ligaments are modelled using a synthetic analogue, which a previous study had demonstrated to have appropriate tensile properties. ³⁶ The proximal attachments points of all three ligaments were aligned with the cylindrical flexion axis of the knee. ³⁷ The distal insertion points of

the ligaments were based on locations reported in the literature.³⁸⁻⁴¹ The hamstring mechanism of the knee joint is also simulated using two constant force springs with a stiffness of 50 N each.⁴² The two springs were attached to the simulator to represent the actions of the biceps femoris, and the combined action of the semitendinosus and semimembranosus muscles.⁴³⁻⁴⁶ Preliminary tests during the development of the simulator indicated that addition of the hamstring model significantly increased the required quadriceps force during extension of the knee.

For the purposes of this study all tests were carried out using Scorpio NRG PS size 7 (Stryker, NJ, USA) femoral and tibial components, which were cemented on to medium size synthetic bones (Sawbones, Pacific Laboratories, WA, USA) by an orthopaedic surgeon using the recommended surgical protocol. A concentric dome patella button was used for all tests (Scorpio, Stryker). Mediolaterally, the dome was mounted centrally with regards to the quadriceps tendon to represent an optimal *in vivo* placement. The patella was located using the three polyethylene pegs on the back of the patella button. In order to allow changes of position, the patella button was not cemented in place. In order to allow changes of position, the patella button was not cemented in place. The proximodistal position of the patella button was based on a normal modified Insall-Salvati index of 2.⁴⁷ In order to achieve this, the patella construct was positioned so that the distance from the distal pole of the patella button to the tibial attachment of the patella tendon was twice the proximodistal length of the patella implant. An alignment jig was developed to allow the femur and tibia to be repeatably secured in mounting pots using a low melting point alloy. The femoral and tibial components were aligned so as to maintain the geometric proportions of the average UK female and a neutral mechanical axis.³⁰ The same components were used for all tests. Since the number of cycles the implants were subjected to was negligible compared to the expected life of a knee implant, factors such as wear should not have had an impact on the results. For

dynamic testing both the tibiofemoral joint (TFJ) and the PFJ were lubricated using industrial grease to minimise friction.⁴⁸

An experimental study was carried out using the simulator to assess the effect of the magnitude of loading on the measured PFJ biomechanics and the physiological relevance and repeatability of the simulator. The test protocol comprised four stages. Firstly, the rig was extended from 90° to 20° of flexion under the reduced level of loading (19 Nm peak moment). During this stage of testing the capacitive Pliance X patella sensor array (Novel GMBH, Munich, Germany) was placed within the PFJ to assess the joint centre of pressure (COP). The COP measurements were referenced to the centre of the patella component. The Novel system sensor was supplied as calibrated by the manufacturer. The tibiofemoral (TF) flexion angle, quadriceps force, patella compressive force and PTMA were also recorded during this dynamic extension.

The TF flexion angle was monitored using the known implant dimensions and the measurement of the hip joint height relative to the ankle, which was assessed using a displacement transducer (VPA-40, UniMeasure, Oregon, USA).²⁸ The quadriceps load and the compressive force on the patella component were measured using single axis load cells (Quadriceps: bespoke sensor; Patella: LC8125-312-500, Omega, Manchester, UK). In order to measure the force on the patella implant relative to the quadriceps tendon, the inner race of the patella loadcell was rigidly attached to the patella implant and the outer race rigidly attached to the frame securing the steel cable modelling the patella and quadriceps tendons.

The patella tendon moment arm (PTMA) was assessed using 2D images. Regular dot markers were placed on the simulator to locate the knee joint centre of rotation, the patella tendon and the femoral and tibial anatomical axes. A camera (AVT Marlin, AVT, Germany) was used to take sagittal images of the

joint at 15 fps during the extension movement. Matlab (Mathworks, MA, USA) scripts were utilised,⁴⁹ to assess each image, automatically locate the markers, and calculate the patella moment arm. Preliminary work indicated that this method was accurate to ± 1 mm.

For the second stage of the testing, Prescale LLW film (Fujifilm Europe GmbH, Düsseldorf, Germany) was used to measure the PFJ contact area statically whilst the joint was subjected to the reduced level of loading.⁵⁰ The Pliance sensor was removed and the knee joint positioned at 10° intervals from 20° to 90° of TF flexion. At each TF flexion angle the film was placed within the PFJ and load applied for 5 seconds. The developed films were scanned using a standard flatbed scanner and a Matlab script was utilised to compute the joint overall, lateral, and medial contact areas. This process was calibrated according to the manufacturer's guidelines.

The third element of the testing involved repeating the dynamic tests at the physiological level of loading (43 Nm peak moment), without the Pliance sensor in place. The knee was cycled from 20° to 90° of TF flexion for two complete flexion-extension cycles to allow the knee to settle. Measurements of the TF flexion angle, quadriceps force, patella compressive force, and PTMA were recorded for the third extension cycle. Lastly, the static tests were repeated at the higher level of loading. The full protocol was repeated six times. Between each repeat, the femoral and tibial components were repositioned and remounted.

Statistical Methods

The sample size was six. Data has been reported using mean \pm standard deviation to give an indication of the variability. Where appropriate, the data were normalised relative to the results at 20° of TF flexion. Differences between the normalised results at the two levels of loading (43 Nm and 19 Nm peak

moments) were assessed using Wilcoxon signed rank tests ($\alpha = 0.05$). Non-parametric tests were used to assess differences between the loading conditions to reduce the threat of outliers within the relatively small sample.

Results

The quadriceps force and patellofemoral compressive force measured under both loading conditions decreased non-linearly with extension of the TFJ. The decrease in measured forces from 90° to 20° of flexion was not consistent between the two loading conditions. Under physiological loading the average quadriceps force decreased during extension on average by a factor of 7.9 ± 0.8 (mean \pm standard deviation) from a peak of 1555 ± 53 N, and the average patellofemoral force by a factor of 13.6 ± 1.2 from a peak of 1220 ± 16 N (Figure 2). Conversely, under the lower level of loading, the average quadriceps force only decreased by a factor of 5.7 ± 0.7 from a peak of 392 ± 29 N, and the average patellofemoral force by a factor of 7.2 ± 0.6 from a peak of 315 ± 14 N (Figure 2). The normalised quadriceps force and the normalised patella compressive force were statistically higher under the physiological loading from 90° to 30° of TF extension (Figure 2).

The average recorded standard deviations were 24.2 N (6% of measured value) for the quadriceps force and 10.5 N (4% of measured value) for the patella compressive force. The calculated PFJ pressures followed similar patterns to the measured forces (Figure 3).

Under both loading conditions, the PTMA increased non-linearly with extension by 5 ± 2 mm over the tested flexion range. Throughout the TF extension motion, the PTMA under physiological loading was 3 ± 1 mm smaller than that recorded under reduced loading. The normalised PTMA values for the two conditions showed no statistical differences (Figure 2).

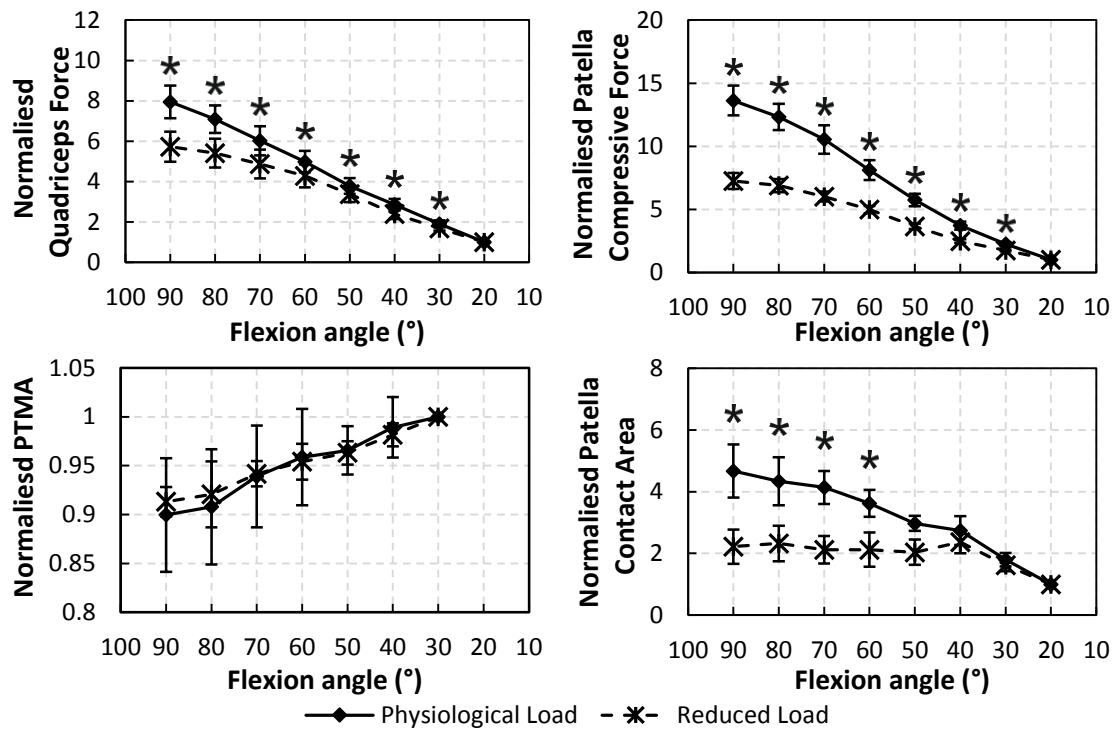


Figure 2: Normalised results. * p < 0.05 Reduced Load vs Physiological Load.

The patterns of measured contact area were also different under the two loading conditions. The average standard deviation of the measured contact areas was 0.03 cm² (12% of measured value). Under physiological loading the contact area decreased on average by 5.2 ± 1.2 from a peak of 0.48 ± 0.05 cm² during the tested range of extension. The decrease was only a factor 2.7 ± 1.0 from a peak of 0.24 ± 0.04 cm² under the reduced level of loading. The normalised contact area was significantly greater under the physiological loading at flexion angles greater than 50° (Figure 2).

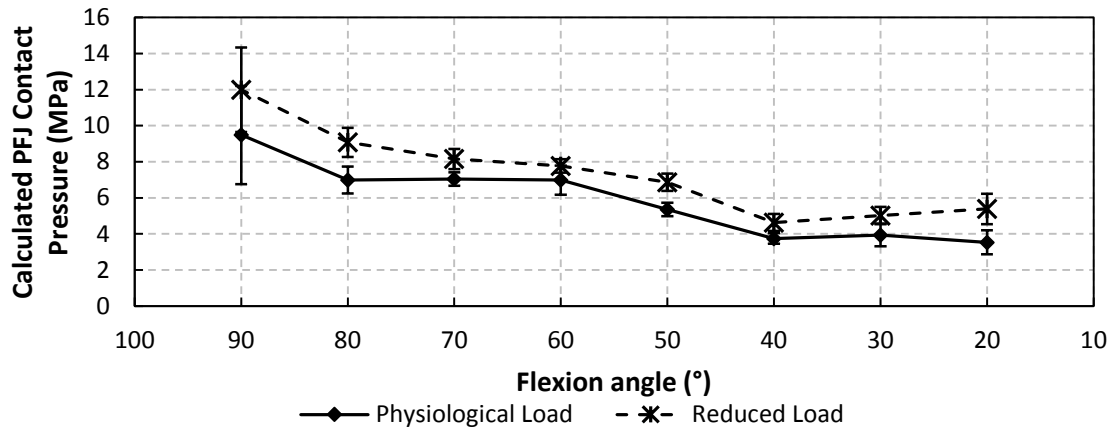


Figure 3: Variation in patellofemoral contact pressure with flexion angle for each loading condition (mean \pm standard deviation).

Under both loading conditions, at least 65% of the contact area was on the lateral aspect of the patella button throughout the tested flexion range. The COP, which could only be measured under the reduced load, was also consistently on the lateral aspect of the patella button (Figure 4). The COP tracked inferiorly with extension (Figure 4). The COP measurements were subject to relatively high standard deviations, averaging 4.7 mm.

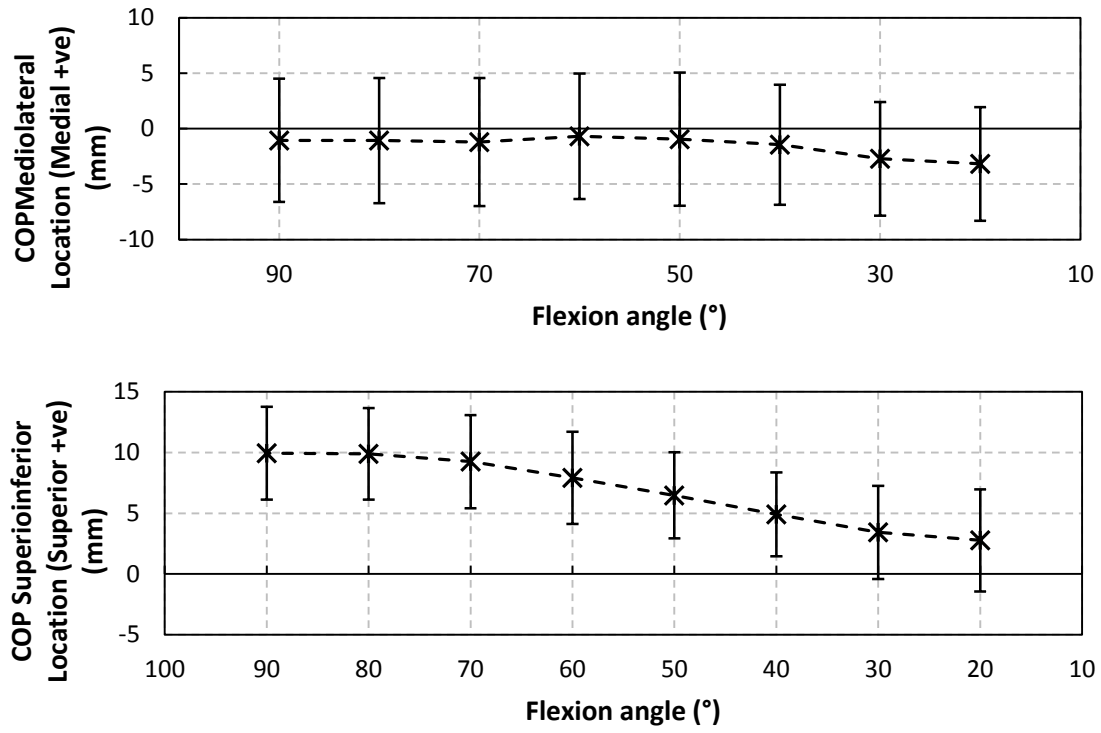


Figure 4: Variation in the COP location, relative to the centre of the patella button, with TF flexion angle under reduced loading (mean \pm standard deviation).

Discussion

The present study aimed to assess the repeatability and physiological relevance of a novel knee joint simulator which was developed to facilitate extended non-cadaveric *in vitro* investigations, under physiological loads, of the effect of specific parameters on PFJ biomechanics following TKA. This was achieved through comparisons with *in vitro* data in the literature (Table 1). The effect of the level of applied load on PFJ biomechanics was also assessed.

The quadriceps force required to complete a task after TKA is a good indicator of the ability of a patient to carry out activities of daily living.^{51, 52} The PFJ compressive force and the magnitude of joint contact area following TKA are also important to assess as they indicate the potential risk of long term pain and failure.⁸⁻¹¹ The simulator facilitated measuring these variables with good repeatability. The recorded standard deviations of quadriceps and patella compressive force were substantially lower than those reported using cadaveric models (St.Dev. of peak quadriceps force 53 N vs 156 N; St.Dev. of peak patella compressive force 17 N vs 278 N).⁵³ The reported contact area measurements also demonstrated comparable variability to those previously reported using the same measurement method and cadaveric specimens (St.Dev. of peak contact area 0.06 cm² vs 0.02 - 0.03 cm²).^{15, 54} The demonstrated low variability supports the assertion that the presented simulator would allow differences, in terms of joint forces and contact areas, between test conditions to be demonstrated using as many or fewer specimens than comparable cadaveric systems. The COP measurements were subject to high measurement variability. This was likely due to the low sensor density of the Pliance system and would limit the power of future studies assessing COP differences between test conditions.

Table 1: Pertinent details of literature studies to which the results achieved using the developed simulator were compared

Reference	Experimental Methods	Implant System	Outputs
Anglin, Brimacombe (2010) ¹²	Oxford knee rig Sub-physiological loading (peak quadriceps force of 450N) Cadaveric	Nex Gen Legacy PS (Zimmer, Warsaw, IN, USA) with a central dome patella	Contact force and COP measured using I-Scan 5051 pressure sensor (Tekscan, Boston, Mass) during <i>TF flexion</i>
Browne, Hermida (2005) ⁵³	Oxford knee rig 40 Nm peak flexion moment Cadaveric knees	Scorpio CR (Stryker) with concentric dome patella component	Quadriceps force and Patella compressive force monitored during <i>TF flexion</i>
Fornalski, McGarry (2012) ⁵⁴	Oxford knee rig Sub-physiological loading Cadaveric	Encore Total Knee Arthroplasty System CS (Encore Medical Corporation, Austin, TX) with resurfaced patella	Contact area measured using Fujifilm
Heegaard, Leyvraz (2001) ³³	3D finite element PFJ model	IB2 Total Condylar Prosthesis (Zimmer) with concentric dome patella	Patella contact pressure and COP calculated during <i>TF flexion</i>
Lee, Gerken (1997) ¹⁵	Oxford knee rig Sub-physiological loading (peak quadriceps force of 200N) Cadaveric	Kirshener Performance System (Kirshner Medical corporation, MD, USA)	Contact area measured using Fujifilm
Müller, Lo, Wünschel et al (2009) ⁵⁵	Oxford knee rig Ankle reaction force varied from 25 to 250 N Cadaveric	n/a	Quadriceps force measured during <i>TF flexion</i>
Ostermeier and Stukenborg-Colsman (2011) ⁵⁶	Fixed femur rig Isometric extension against a constant 31Nm extension moment Cadaveric Knees Hamstrings loaded	Triathlon PS (Stryker) with no patella resurfacing	Quadriceps force monitored during <i>TF extension</i>
Present Study	Oxford knee rig Physiological quadriceps actuation against a peak flexion moment of	Scorpio PS (Stryker) with dome patella	Quadriceps force, patella compressive force, patella contact pressure and COP

43Nm Hamstrings modelled	measured during TF <i>extension</i>
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The simulator was able to replicate physiological knee joint loading situations which were comparable to more commonly used cadaveric systems. In agreement with a previous *in vitro* cadaveric study on the same implant,⁵³ the required quadriceps force decreased with extension under physiological levels of loading. The recorded quadriceps force fell with increasing extension as a result of the body weight moment arm decreasing, and a reduction in the effective patella moment arm. The recorded peak quadriceps force achieved using this simulator and a physiological peak flexion moment (1555 N ± 53 N at 90° flexion), was higher than previously reported because measurements were made during TF extension, rather than flexion.⁵³ The magnitude of quadriceps force measured using the developed simulator was consistent with work carried out using an alternative simulator and a similar single radius implant design.⁵⁶

The patellofemoral compressive force also decreased with extension but appeared to level out slightly in deeper flexion. This has also been shown in *in vitro* cadaveric studies,^{12, 53} and highlights that the simulator was able to replicate the physiological contact of the quadriceps tendon with the distal femur in deeper flexion. This reduced the load carried by the PFJ.^{34, 35} The patellofemoral compressive force decreased by a greater amount than the quadriceps force during extension (13.5 vs 7.9 under physiological motion) because of the superiorinferior motion of the joint contact point (Figure 4), which altered the effective patella moment arm.³² Similar to the peak quadriceps force, the recorded peak patellofemoral compressive force under physiological loading was higher than that previously reported using the same implant, as a result of taking measurements in extension rather than flexion. The peak force was consistent with *in vivo* estimates.³²

Throughout the extension movement there was greater contact on the lateral aspect of the patella and the PFJ COP was lateral (Figure 4). This is in distinct contrast to the more even loading generally seen in the native knee, but has been previously reported in TKA studies with alternative implants.³³ The PFJ COP was also consistently superior, becoming more inferior with increasing extension. This is again consistent with previously reported cadaveric studies.¹² These results suggest that the simulator has modelled a sufficient number of soft tissue structures to facilitate the replication of physiological patellofemoral biomechanics.

The pressure within the PFJ following TKA is a strong indicator of the risk of wear of the implanted patella button and pain within the joint.^{9, 57, 58} Previous studies, have used the Pliance system^{14, 18} or the K-Scan and I-Scan systems (Tekscan, Boston, USA)^{17, 19, 59} to measure contact area and pressure dynamically within the PFJ. However, these were not suitable for use in this study due to the low sensor density and relatively large thickness (2 mm) of the Pliance sensor, and the limited pliability and high expense of the Tekscan systems.^{60, 61}

Preliminary testing indicated that dynamic and static contact area measurements showed no notable differences. This is consistent with literature reports that the presence, or lack thereof, of motion does not affect PFJ kinematics.⁶² The resultant force vector on the patella button was unlikely to have corresponded directly with the measurement axis of the single-axis load cell positioned behind the button. However, compression was assumed to be the largest component of force in the joint. Dynamic PFJ contact pressures were therefore calculated at 10° intervals of extension, for both loading situations, using the recorded compressive force and contact area values.

The contact area and pressure within the PFJ, following TKA with the Scorpio NRG system, or its predecessor the Scorpio system, have not previously been assessed. The geometry of the femoral and

patella components has a significant effect on the contact within the PFJ comparisons with measurements made using different implant systems are therefore not valid.^{63, 64} The pattern of recorded contact area under physiological loading was however consistent with modern kinematic understanding showing a consistent reduction with TF extension as the patella component traversed through the intercondylar notch, into the trochlear groove and, closer to full extension, superior to the groove.^{35, 65} The estimated pressure values were therefore considered to give a fair indication of the loading magnitude on the patella component during testing and the potential risk of long term *in vivo* component failure and pain.^{9, 57, 58}

The results associated with the two loading conditions, i.e. the reduced and physiological loading levels, suggest that they are not comparable. The measurements of quadriceps forces, patellofemoral compressive forces and patellofemoral contact areas all demonstrated distinctly different patterns between the two loading conditions. The normalised values for the quadriceps forces, patellofemoral compressive forces and patellofemoral contact areas were statistically significantly different under the two levels of loading in deeper TF flexion (60 - 90°) (Figure 2). Similarly, the measured forces and areas increased with extension by approximately a factor of 2 more under physiological loading rather than under reduced loading. This has been previously reported for quadriceps forces using a cadaveric model, but has not been demonstrated with regards to the patella compressive force or contact area.⁵⁵

Increased levels of hip loading resulted in increased levels of friction within the PFJ, which increased the required quadriceps force. In deeper flexion, the required quadriceps force also increased due to the increased body weight moment, which exacerbated the differences. This pattern was reflected in the PFJ compressive force as the two are biomechanically linked. The significantly higher patellofemoral compressive force under physiological loading was observed to cause elastic deformation of the

relatively soft UHMWPE button which resulted in the higher reported contact areas. Using simple linear-elastic theory at the peak measured load (1220 N) this deformation can be approximately calculated to be in the order of 0.3 mm at the location of contact.

In contrast, the PTMA results demonstrated similar patterns under both levels of loading. The normalised results were not statistically different (Figure 2). There was however a consistent reduction in the PTMA, of 3 mm, under the physiological loading. The Pliance sensor array was 2 mm thick, which accounted for a proportion of this reduction under the physiological loading when the sensor was not used. The remainder of the reduction in PTMA may be attributed to compression of the UHMWPE button under the higher loading. The reduced PTMA reported under the physiological loading condition will also have contributed to the increased quadriceps forces.

Many studies carry out testing under less than physiological levels of loading, to protect cadaveric soft tissues and sensor systems.^{12-19, 33} The results of this study however demonstrated that it is important to carry out tests under physiological levels of loading, and that this can be achieved using the present simulator.

This study was limited by not including cadaveric testing to provide a direct assessment of the physiological relevance of the simulator. However, comparisons to a published cadaveric study using the same implant were possible.⁵³ The elimination of cadaveric tissue from the model resulted in improved reliability. However, this was at the cost of some physiological relevance. Specifically, the simulator only modelled a limited number of the soft tissue structures found in the knee. Specifically, the patella retinaculum was not modelled. Such soft tissue structures are only a primary constraint to patella motion during early flexion,⁶⁶ which is not facilitated by the present rig. It would only be feasible to account for more soft tissue structures using cadaveric specimens or by increasing complexity in the

present simulator and adding further soft tissue analogues. Given the relatively low forces within the PFJ during early flexion, and hence the low risk of pain and failure during such movements, the lack of patella retinacula is not considered to be a significant limitation for initial biomechanical investigations designed to assess the factors which influence the long term risk of pain and component failure following TKA. However, it may be prevent the measurement of physiological kinematics.

The use of a single set-up as opposed to the more common use of multiple cadaveric specimens also limits the relevance of results produced using the simulator to the population as a whole. This is an inherent limitation of the method but does not prevent its use for initial comparative tests. It may be advisable however, to follow up on any hypothesis developed as a result of such initial tests with cadaveric investigations.

Conclusions

Cadaveric specimens provide the best *in vitro* model of the natural knee, but are limited with respect to inter-specimen variability and cannot be tested for extended periods of time under physiological loads. This study has highlighted the effect of simulated body weight on PFJ biomechanics. The patterns of loads and contact areas reported at a substantially reduced level of loading did not correspond to those reported under physiological loads. It is therefore important to carry out biomechanical evaluations under physiological levels of loading and understand the limitations of testing carried out under reduced loading

The developed simulator allowed the extended assessment of the PFJ after TKA under physiological loading. This study also showed, through comparisons with published studies, that the simulator is able to model physiological knee motions and loading conditions. The simulator facilitated the assessment of forces, areas and pressures within the PFJ, in many cases with a higher degree of repeatability than more commonly used cadaveric systems. This study indicated that the simulator would be appropriate for use in initial comparative studies to systematically assess, under physiological loads, the effect of specific parameters on PFJ biomechanics.

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Declaration of conflicting interests

The authors have no conflicts of interest relating to this work.

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