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 Abstract: The occurrence of edge loading in hip joint replacement has been associated with many factors such as prosthetic design, component malposition and activities of daily living. The present study aimed to quantify the occurrence of edge loading/contact at the articulating surface and to evaluate the effect of cup angles and edge loading on the contact mechanics of a modular metal-on-polyethylene (MoP) total hip replacement (THR) during different daily activities. A three-dimensional finite element model was developed based on a modular MoP bearing system. Different cup inclination and anteversion angles were modelled and six daily activities were considered. The results showed that edge loading was predicted during normal walking, ascending and descending stairs activities under steep cup inclination conditions 34 (\geq 55°) while no edge loading was observed during standing up, sitting down and knee bending activities. The duration of edge loading increased with increased cup inclination angles and was affected by the cup anteversion angles. Edge loading caused elevated contact pressure at the articulating surface and substantially increased equivalent plastic strain of the polyethylene liner. The present study suggested that correct positioning the component to avoid edge loading that may occur during daily activities is important for MoP THR in clinical practice.

Keywords: edge loading, activities, metal-on-polyethylene, contact mechanics, cup angles

1 Introduction

 Despite the successful outcomes and encouraging long-term clinical performance of hip joint replacement, the clinical complications and unexpected failure of the prostheses linked to edge loading are causing concerns [1-5]. The edge loading, usually described as the contact of the femoral head on the edge of the acetabular component, was observed in many retrieval components and usually identified as the condition under which the maximum depth of penetration of the wear scar occurs at the rim of the cup or the wear scar has a distinct boundary in retrieval studies [6-8]. In numerical studies, true edge loading was specified and defined as the condition where the contact patch between the acetabular and femoral components extends over the rim of the cup [9, 10].

 Edge loading can reduce the tribological performance and may cause unexpected clinical problems [3,6,11-14]. In metal-on-metal (MoM) hip replacement, edge loading can produce accelerated local and overall articulation wear [15, 16] and lead to metallosis, adverse peri prosthetic tissue reactions such as pseudotumours [2,6,17]. In ceramic-on-ceramic (CoC) articulations, edge loading has been associated with accelerated articulation wear, stripe wear on either the femoral or acetabular component, and in some situation, squeaking and fracture of components [11,18-20]. For metal-on-polyethylene (MoP) and ceramic-on-polyethylene (CoP) combinations, although in vitro experimental studies indicated that edge loading induced by steep cup inclination and lateral microseparation did not increase the wear of prostheses compared to that without edge loading [21,22], finite element (FE) studies have shown that substantial increase in the stresses and plastic strain of polyethylene component were predicted for the hip prosthesis under edge loading conditions [13], which may contribute to subsequent fatigue and fracture. Therefore, persistent and sustained efforts to reduce or prevent edge loading should be still made for hard-on-soft articulations.

 It has been recognized that the occurrence of edge loading on the hip joint replacement is related to many factors such as prosthetic design [10,23], malposition of components [9,14,16], impingement and dislocation [24,25], and patient activities [17,26]. Particularly, the malposition of the components has been recognized as an important factor causing the poor outcome of hip joint replacement. Although a golden "safe zone" with cup inclination of $40^{\circ} \pm 10^{\circ}$ and anteversion of $15^{\circ} \pm 10^{\circ}$ was recommended and accepted by most surgeons [27], a large variation in the cup orientation was observed in clinical practice [28, 29]. The adverse effect of malposition of acetabular component on the performance and outcome of the hip joint replacement was also reported [29,30]. Schmalzried et al. conducted a study to investigate the relationship between the design, position and wear of acetabular component and the development of pelvic osteolysis [30]. They demonstrated that the osteolysis of the ilium was associated with a lateral opening of the acetabular component of more than 50 degrees. Kennedy et al. reviewed two groups of total hip arthroplasties with mean inclination 81 angles of 61.9° and 49.7° and concluded that although the postoperative Mayo clinical hip 82 score was similar for the two groups, the group with a mean inclination of 61.9° had higher rate of recurrent dislocation, osteolysis, wear asymmetry and acetabular component migration, compared to the group with a mean inclination of 49.7º [29]. Therefore, the malposition of components on edge loading and performance of hip joint replacement should be examined.

 The important contribution of daily activity patterns on the occurrence of edge loading has been demonstrated in a number of previous studies [17,26,31]. Mellon et al. investigated the effect of function activities (i.e. level walking and stair descent) and cup orientation on the

 edge loading and contact stress of MoM hip resurfacing using FE method and a combination of the computed tomography (CT) and three-dimensional lower limb motion capture data [26]. They suggested that steep cup inclination can cause edge loading and that individual's activity patter can compensate or even override the influence of steep cup inclination and prevent edge loading. Using the same method, Kwon et al. quantified the duration and magnitude of in vivo edge loading during functional activities (i.e. level walking, stair climbing and rising from a chair) in MoM hip resurfacing arthroplasty with and without pseudotumours [17]. They indicated that edge loading in MoM hip resurfacing with pseudotumours (which was associated with higher inclination and anteversion angles) occurred with significantly longer duration and greater magnitude of force compared to that without pseudotumours during daily activities. A study conducted by von Arkel et al. showed that the prevalence of posterior edge loading can be reduced by introducing abduction to activities that require deep flexion such as rising from a chair and stooping [31]. These studies have demonstrated the important contribution of patient's daily activities on the edge loading in total hip replacement (THR). However, these studies were based on in vivo evaluation and therefore the edge loading was roughly evaluated by using either the distance or angle between the hip contact force vector and acetabular cup edge vector. In this case, the magnitude of loading and deformation of the component were not considered in these studies.

 The aims of the present study were, firstly, to determine whether edge loading occurred, the duration of edge loading occurrence and the specific instances over which edge loading occurred during different daily activities under different cup orientation conditions, and secondly, to investigate the effect of cup orientations and edge loading on the contact mechanics of a modular MoP THR during different daily activities using FE method.

2 Materials and methods

 A typical modular MoP total hip system, consisting of metallic acetabular shell, polyethylene liner and metallic femoral head, was analysed. The inside of the acetabular shell is comprised two distinct regions: the central dome region and the locking mechanism. The central dome region covers approximately 140 degrees of the interior of the shell, providing backside support to the liner. Peripheral to the dome is the locking mechanism, which extends to the face of the acetabular shell. The polyethylene liner is mechanically locked with the acetabular shell via the locking mechanism, forming two areas between the acetabular shell and polyethylene liner: the dome spherical region and equatorial region, as shown in Fig. 1.

 The nominal diameters of the femoral head and inner surface of polyethylene liner were 36 mm and 36.6 mm respectively, giving a radial clearance of 0.3 mm between the femoral head and polyethylene liner. The radii of the central dome region of the acetabular shell and outer surface of the polyethylene liner were 24.14 mm and 24 mm respectively, giving a gap of 0.14 mm between the acetabular shell and polyethylene liner at the central dome region (dome spherical region). The outer diameter of the acetabular shell was 56 mm. A polar fenestration with radius of 10 mm was considered in the central dome region of the acetabular shell.

 A three-dimensional FE model was developed to simulate the implantation of the modular MoP total hip system into a hemi-pelvic bone model (Fig. 1). The hemi-pelvic bone model consisted of a cancellous bone region surrounded by a uniform cortical shell with thickness of 1.5 mm [32]. The acetabular subchondral bone was assumed to have been reamed completely prior to implantation.

 All the materials in the FE model were modelled as homogenous, isotropic and linear elastic except the polyethylene liner which was modelled as non-linear elastic-plastic behaviour with the plastic stress-stain constitutive relationship showing in Fig. 2 [33,34]. The femoral head was modelled as a rigid body as the elastic modulus of the metallic femoral head is about 200 140 times that for polyethylene liner. The mechanical properties for the materials are presented in Table 1. The FE model comprised approximately 92,000 elements, including triangular shell elements for the cortical bone with element sizes less than 3 mm, tetrahedral elements for the cancellous bone with element sizes less than 3 mm, hexahedral and wedge elements for the prosthetic components with element sizes less than 0.8 mm and 0.3 mm respectively. Mesh converge studies were conducted for the FE model under normal walking activity under cup inclination angle of 75º and anteversion angle of 0º, an assumed extreme condition under which the polyethylene liner was assumed to have the worst mechanical behaviour with respect to the contact pressures, von Mises stresses and plastic strain. The results showed that when the element size was reduced by half, the change in any of the parameters of interest was within 5%.

 A sliding contact formulation was applied both on the articulating surface between the femoral head and polyethylene liner and at the interface between the acetabular shell and polyethylene liner, with friction coefficients of 0.083 and 0.15 respectively [35,36]. The nodes situated at the sacroiliac joint and about the pubic symphysis were fully constrained. All relative movements were prevented between the pelvic bone and the acetabular shell, simulating a situation where the porous sintered coating and in-grown bone were well bonded. The centre of the femoral head was constrained in rotational degrees of freedom and allowed to move freely along the translational free degrees of freedom to allow self- alignment. The validation of the FE model was presented in a previous study, which demonstrated that good agreements of contact areas at the articulating surface were obtained between the FE predictions and experimental measurements using Leeds Prosim hip joint simulator [34].

 The physiological loadings of six different human activities, which were measured in vivo previously using an instrumented total hip prosthesis [37], were applied to the FE model. These activities were as follows: normal walking (NW), ascending stairs (AS), descending stairs (DS), standing up (SU), sitting down (SD) and knee bending (KB). In order to consider 167 the specific direction and orientation of the forces, the three components of the resultant hip joint forces relative to the pelvis coordinate system in the in vivo study [37] were exported and discretized into 22 or 23 steps, which were then applied directly to the centre of the femoral head in the FE model in a quasi-static manner, as shown in Fig 3. At this case, the global coordinate system in the FE model was assumed to be aligned with the pelvis coordinate system in the in vivo study [37]. A total of 20 orientations of cup angles were considered, with inclination angles varying between 35º and 75º and anteversion angles varying between 0º and 30º, both in 10º increments. The FE analysis was performed using ABAQUS software package (Version 6.9; Dassault Syste`mes Simulia Corp., Providence, RI, United States). Edge loading at the articulating surface was detected and evaluated at each instance during the whole cycle of these activities. In the present study, edge loading was defined to occur when the contact patch between the femoral head and polyethylene liner extends over the rim of the liner, as shown in Fig. 4.

3 Results

Contact pressures distribution during gait

 Fig. 5 shows the distribution and peak value of contact pressures on the articulating surface of the polyethylene liner with different cup inclination and anteversion angles at instance of 17% gait of normal walking activity.

 Generally, the areas of the contact patch were located about the superior region of the liner and shifted toward the superior edge as inclination angle increased. The peak contact pressure was located at the dome spherical region at low cup inclination conditions (i.e. 35° and 45°) and moved to the equatorial region when the inclination angle was increased to 75°. Edge 190 loading started to occur when the cup inclination angle increased to 65°.

Edge loading

 The duration of edge loading and specific instances of cycle at which edge loading occurred during different activities as a function of cup angles are shown in Fig. 6.

 Edge loading was predicted at some instances of cycle during normal walking, ascending and 195 descending stairs activities under steep cup inclination angle conditions (\geq 55°). No edge loading was predicted for standing up, sitting down and knee bending cases for all cup angles considered. For normal walking and ascending stair cases, the combination of steep cup inclination and low anteversion was more likely to cause edge loading. For example, for normal walking activity, the proportion of gait cycle when edge loading occurred increased from 5% (at specific instances of 50-55% of gait cycle) to 50% (at specific instances of 10- 201 60% of gait cycle) as cup inclination angles increased from 55° to 75° with anteversion of 0°. With cup inclination of 65°, the proportion of gait cycle when edge loading occurred 203 decreased from 40% to 13% when the cup anteversion angles increased from 0° to 30°. In contrast, for descending stair activity, the combination of steep cup inclination and high anteversion tended to induce edge loading.

Effect of activities, cup angles and edge loading on contact mechanics

 The activities and cup angles were found to have a synergistic effect on the peak contact pressure at the articulating surface and equivalent plastic strain of the liner (Fig. 7 and 8). Edge loading caused elevated peak contact pressure at the articulating surface and marked increase of peak equivalent plastic strain of the polyethylene liner (Fig. 7, 8 and 9). For normal walking, ascending and descending stairs activities, the cup inclination angles had marked effect on the peak contact pressure and equivalent plastic strain while the cup anteversion angles had minor effect. Considering the cup anteversion, the peak contact pressure over the whole cycle firstly decreased by approximately 7%-12%, 5%-9% and 7%-

 14% for normal walking, ascending stair and descending stair activities respectively when the cup inclination angle increased from 35° to 55°, and then increased by about 18%-26%, 22%- 28% and 27%-33% respectively for the three activities when the cup inclination angle increased to 75°, where edge loading occurred (Fig. 7). Correspondingly, the peak equivalent plastic strain over the whole cycle firstly decreased by approximately 31%-53%, 13%-21% 220 and 15%-28% when the cup inclination increased from 35° to 45° and then increased by 221 about 234%-306%, 179%-231% and 178%-213% when the cup inclination increased to 75° for the three activities respectively.

 In contrast, for standing up, sitting down and knee bending activities, the cup anteversion angles were found to have dominated effect on the peak contact pressure and equivalent plastic strain. Considering the cup inclination, the peak contact pressure and equivalent plastic strain over the whole cycle increased by approximately 14%-24% and 88%-164%, 2%-21% and 57%-148%, 4%-12% and 56%-138% for standing up, sitting down and knee 228 bending activities respectively when the cup anteversion increased from 0° to 30° .

4 Discussion

 Edge loading as an adverse condition that could cause unexpected clinical problems has attracted more and more attentions in biomechanics fields [38,39]. The factors that may lead to edge loading have been recognized and were generally associated with the component positions (i.e. cup angles, head offset/microlateralisation), prosthetic design (i.e. radial clearance, cup coverage), impingement and activities. The contribution and effect of component malposition, prosthetic design, impingement and dislocation on the edge loading of hip replacement have been investigated in a number of previous studies [9,10,23-25,40- 238 42]. The primary purposes of the present study were therefore to investigate the effect of cup orientations and daily activities on the contact mechanics and occurrence of edge loading for a modular MoP THR. The duration of edge loading and instances of cycle at which edge loading occurred during six daily activities were evaluated. To the authors' acknowledge, this was the first to quantify the duration and period of time of true edge loading in THRs during different daily activities, by considering the deformation of pelvic bone and components.

 The FE simulations showed that an individual's activity patterns played an important role on the occurrence of edge loading in MoP THR. For the THR considered in the present study, edge loading occurred at some instances during normal walking, ascending and descending

 stairs activities under steep cup inclination conditions. With increased cup inclination angles, the duration and period of time over which the hip experienced edge loading increased. These were supported by an in vivo study to evaluate edge loading in MoM hip resurfacing patients with and without pseudotumours which showed that edge loading in patients with well- functioning MoM hip resurfacing arthroplasty was observed during functional activities and that edge loading in the hips with pseudotumours (which was associated with higher cup inclination) occurred for a significantly longer period of time compared to that without pseudotumours [17]. The present study also showed that the duration and period of time of edge loading was activity-dependent, with the longest duration of edge loading being observed for normal walking activity. No edge loading was predicted for standing up, sitting down and knee bending activities. These observations, however, were found to be different from the previous in vivo study which indicated that edge loading also occurred for rising from or sitting down to chair activity [17]. A retrieval study conducted by Esposito et al also demonstrated both anterior and posterior edge loading in retrieval ceramic components and they assumed that posterior edge loading may occur during activities such as climbing stairs or rising from a chair [43]. The different conclusions between the present study and the in vivo and retrieval studies may be due to several reasons. Firstly, in vivo study, edge loading was defined to occur when the locus of the force vector intersection with the acetabular component was located within the areas where the distance to the edge of the component was no larger than 10% of the component radius, while in the present study, edge loading was defined as the case when the contact patch extends over the rim of the component. The limitation of the in vivo study was that although the force vector for the rising up/sitting down activities was located in the edge loading zone defined in the in vivo study for a longer period of time, the force magnitude was smaller compared to that in normal walking, ascending and descending stairs activities, leading to a smaller contact patch at the bearing surface of the component. Therefore, if the radius of the contact patch was smaller than 10% of the component radius, edge loading would not occur. However, at this case, edge loading was assumed to still occur in the in vivo study. Secondly, the different design of prosthesis considered in the present study (MoP) and the in vivo (MoM) and retrieval (CoC) studies may be an important factor causing the different conclusions. In the present study, the radial clearance between femoral head and polyethylene liner was 0.3 mm. If a smaller clearance is considered, the contact stresses will be decreased and the contact areas will be increased. At this case, the contact patch will potentially extend over the rim of the polyethylene liner, causing posterior edge loading for rising up/sitting down activities. In fact, in the present simulation, for most instances of rising up/sitting down activities, the contact patch was prone to locating at the posterior area of the bearing surface, having the potential to cause posterior edge loading. Therefore, the effect of prosthetic design such as radial clearances and cup coverages on the occurrence of edge loading will be examined in future studies. Thirdly, the posterior edge loading observed in the retrieval study may be caused by some adverse conditions such as impingement of the components, which has been reported to be common for MoP THR in retrieval studies [28,44]. However, the adverse condition of impingement was not considered in the present study.

 Previous studies have shown that the cup inclination of no larger than 45° is best for achieving stability and preventing wear [45,46]. The present study supported this conclusion 291 that no edge loading occurred when the cup inclination angle was no larger than 45° for all the activities and cup anteversion angles considered. In addition, the cup anteversion was found to have a crucial effect on the duration and occurrence of edge loading as well. For example, under a steep cup inclination angle of 65°, the duration of occurrence of edge 295 loading during normal walking was over 40% gait cycle under anteversion angle of 0° , which 296 reduced to less than 15% gait cycle under anteversion angle of 30° . Edge loading was most likely to occur at the instances between 45-55%, 15-20% and 90-95% cycle time for normal walking, ascending and descending stairs activities respectively. This was a result of the synergistic effect between the force vector and magnitude. Indeed, in a paper to investigate the effect of motion patterns on edge-loading of MoM hip resurfacing, Mellon et al. suggested that the force vector at the instance of 60% gait cycle was closer to the edge of component than any other time during the stance phase of gait [26].

 The analysis of the effect of cup angles on the contact pressures at the articulating surface showed that mild increase of the cup inclination angle resulted in decreased peak contact pressure at the articulating surface of the modular MoP THR for normal walking, ascending and descending stairs activities, which was found to be different from the non-modular THR [33,45]. This was probably due to the factor that at lower cup inclination condition (i.e. 35º), the contact area was mainly located in the dome spherical region of the polyethylene liner in modular MoP THR. When the cup inclination angles increased (i.e. 45º, 55º), the contact area moved to the transition area between the dome spherical region and equatorial region. The different deformation of the polyethylene liner due to the different stiffness of support behind the liner would cause enlarged contact areas at this transition region, leading to decreased contact pressures [47,48]. When the cup inclination angle increased further (i.e. 75º), edge

 loading would occur and the contact pressures increased. For all cup angles conditions and activities considered, plastic deformation of the polyethylene liner was predicted. Similarly, the equivalent plastic strain of the polyethylene liner was first increased and then decreased with increased cup inclination angles.

 It is well known that the cup inclination angles had a marked effect on the contact mechanics and stability of hip joint replacement under both normal and adverse conditions [13,33,45,47]. The present study demonstrated that for normal walking, ascending and descending stairs activities, the cup inclination angles had a leading effect on the contact pressures at the articulating surface and equivalent plastic strain of the polyethylene liner, while for standing up, sitting down and knee bending activities, the cup anteversion had dominated impact. Therefore, it is suggested that the importance of cup anteversion should be considered and recognized during the positioning of cup component in clinical practice.

 The FE analysis also showed that edge loading caused elevated contact pressures at the articulating surface and equivalent plastic strain in the components, which was consistent with previous studies [13,14]. In particular, there was a substantial increase in the equivalent 329 plastic strain when the cup inclination increased from 55[°] to 65[°] and from 65[°] to 75[°] for normal walking, ascending and descending stairs activities, where edge loading occurred. This indicated that obvious plastic deformation would occur under these conditions, as observed in previous in vitro study [21]. The amplified plastic deformation could potentially induce creep and fatigue of the liner [49,50], and also pitting and delamination of the surface at this area, leading to fatigue damage and fracture of the component [51]. Therefore, it is indicated that the positioning of the component is important clinically to avoid severe plastic deformation of the component and that lower cup inclination angle remains a recommendation for implant positioning of the modular THRs.

 There are several limitations to the present study. First, the muscle and ligament surrounding the hip were not considered in the present study, which was proved to play an important role in the stability of hip replacements [52]. Previous study has shown that the muscles inserted into the distal femur, patella or tibia can contribute to edge loading of well-positional cup [31]. Therefore, a large-scale computational model that integrate the FE model and musculoskeletal dynamic model could be developed for getting a better understanding of edge loading during different daily activities in future studies. Second, only six activities were considered in the present study whereas a broad variety of challenge maneuver ensue in activities of daily living which would cause adverse complications such as impingement and

 dislocation [53]. However, the activities considered in the present study did represent the most frequent activities for human daily living [37]. Third, homogeneous, isotropic and linear material properties for the bone and uniform thickness of cortical bone were assumed in the present study. However, a real bone should have a non-homogenous, anisotropic property [54], and previous studies have shown that the thickness of the cortical bone layer and the material properties of the bone were site-dependent and bone density-dependent [55,56]. The effect of bone properties on the results should be evaluated and addressed in the future studies. Moreover, lubrication may play an important role in the occurrence of edge loading which was not considered in the present study. However, a recent study to investigate the contact mechanics and lubrication of ceramic-on-metal total hip replacements demonstrated that the profiles and magnitude of the film pressures calculated using elastohydrodynamic lubrication (EHL) theory was closely similar to those of the dry contact pressures calculated using FE modelling [57]. Finally, the femoral head was assumed to be located perfectly within the liner during all activities in the FE simulation. However, in deep flexion activities such as standing up or sitting down activities, there is possibilities that impingement of the components occurs, causing a posterior subluxation of the femoral head and posterior edge loading in the acetabular liner. These were not simulated in the present study.

 Despite these limitation listed above, the present study suggested that edge loading would occur during some of the functional daily activities such as normal walking, ascending/descending stairs under steep cup inclination conditions. Edge loading induced by these daily activities and steep cup inclination can result in elevated contact pressures at the articulating surface and equivalent plastic strain in the component for the modular MoP THR. Therefore, it is suggested that clinically it is important to optimise the orientation of the components in hip joint replacements to avoid edge loading that may occur during activities of daily living.

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Conflict of interest

John Fisher is a consultant to DePuy Synthes Joint Reconstruction.

Ethical approval

Not required.

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List of figure captions:

- **Fig. 1** The FE modelling and boundary conditions, and cross-section of the modular MoP THR showing the detailed structure and features.
- **Fig. 2** The plastic stress-strain relation for the polyethylene liner [33,34].
- **Fig. 3** Resultant hip joint forces during normal walking. The resultant force was converted to 539 three components (F_X, F_Y, F_Z) and computed as $F = \sqrt{F_X^2 + F_Y^2 + F_Z^2}$. During the simulation process, the resultant hip joint force was discretized into 23 steps.

 Fig. 4 The definition of edge loading in MoP THR in the present study. Left: edge loading did not occur as the contact patch was within the inner surface of the liner; right: edge loading occurred as the contact patch extended over the rim of the liner.

- **Fig. 5** The distribution and peak value of the contact pressures (MPa) on the articulating surface of the polyethylene liner as a function of cup inclination and anteversion angles at 17% gait cycle during normal walking activity.
- **Fig. 6** The duration of edge loading and specific instances at which edge loading occurred on the articulating surface of the liner as a function of cup inclination and anteversion angles during different activities (NW: normal walking, AS: ascending stairs, DS: descending stairs). No edge loading was predicted for standing up, sitting down and knee bending activities.
- **Fig. 7** The peak contact pressure (MPa) at the articulating surface over the whole cycle as a function of cup inclination and anteversion angles during different activities ((NW: normal walking, AS: ascending stairs, DS: descending stairs, SU: standing up, SD: sitting down, KB: knee bending).
- **Fig. 8** The peak equivalent plastic strain in the polyethylene liner over the whole cycle as a function of cup inclination and anteversion angles during different activities ((NW: normal walking, AS: ascending stairs, DS: descending stairs, SU: standing up, SD: sitting down, KB: knee bending).
- **Fig. 9** The maximum contact pressure at the articulating surface of liner during normal walking for different cup inclination angles with cup anteversion angle of 10°. The bold red lines represent the instances when edge loading occurred.

Components	Materials	Young's modulus (GPa)	Poisson's ratio
Polyethylene liner	UHMWPE	L	0.4
Metal shell	Titanium	116	0.25
Cortical shell	Cortical bone	17	0.3
Cancellous bone	Cancellous bone	0.8	0.2

640 **Table 1** Material properties for the components used in the present study [32,33,34].