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Experimental and Computational Micromechanics at the Tibial Cement-Trabeculae Interface

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Abstract

Aseptic loosening of the tibial component in cemented total knee arthroplasty remains a major concern. We hypothesize that micromotion between the cement and trabeculae leads to increased circulation of interstitial fluid which in turn causes fluid-induced resorption of the trabeculae. Another mechanism for implant loosening is trabecular strain shielding. Using a newly developed experimental setup and digital image correlation (DIC) methods we were able to measure micromotion and strains in lab-prepared cement-trabeculae interface specimens (n=4). Finite element (FE) models of these specimens were developed to determine whether differences in micromotion and strain in morphologically varying specimens could be simulated accurately. Results showed that the measured micromotion and strains correlated well with FE model predictions ($r^2 = 0.59-0.85$; $r^2 = 0.66-0.90$). Global specimen strains measured axially matched well with the FE model strains ($r^2 = 0.87$). FE model cement strains showed an increasing trend with distance from the cement border. The influence of loss of trabecular connectivity at the specimen edges was studied using our FE model results. Micromotion values at the outer edge of the specimens were higher than the specimen interior when considering a very thin outer edge (0.1 mm). When the outer edge thickness was increased to about one trabecular length (0.8 mm), there was a drop in the median and peak values. Using the experimental and modelling approach outlined in this study, we can further study the mechanisms that lead to loss of interlock between cement and trabeculae at the tibial interface.

1. Introduction

In cemented knee arthroplasty, aseptic loosening is a major cause of (early) revision surgery (Furnes, Espehaug et al. 2007). Although aseptic loosening has been studied extensively in terms of arthroplasty survival rates, the underlying mechanisms behind this commonly occurring complication are still incompletely understood. In particular, early aseptic loosening of tibial components remains an unexplained phenomenon, as PE particle wear

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debris leading to osteolysis is more of a long-term effect (Fisher, Jennings et al. 2010). Some studies have reported tibial component debonding contributing to early aseptic loosening (Cheng, Pruitt et al. 2006; Arsoy, Pagnano et al. 2013). There has also been considerable research into osteolysis induced by fluid flow, fluid pressure and fluid shear stress as an additional stimulus for bone resorption (Johansson, Edlund et al. 2009; Fahlgren, Bostrom et al. 2010; Mann and Miller 2014). These two factors may also be affected by implant design (Bertin 2007; Foran, Whited et al. 2011). Given the sharp rise in the projected number of knee arthroplasties in the near future (Kurtz, Lau et al. 2009), it is crucial to better understand the mechanisms leading to aseptic loosening as this would provide valuable insights into measures to improve the initial fixation of knee arthroplasties.

One hypothesis for early aseptic loosening is that micromotion between bone cement and trabeculae leads to increased circulation of interstitial fluid which causes fluid-induced resorption of the trabeculae. Moreover, strain shielding of the trabecular bone below the tibial plateau could also cause bone resorption. The complex micromechanics occurring in the tibial cement-bone interface have only been minimally assessed. Interactions in bone-cement composite specimens have been studied using micro-finite element (FE) models in the case of vertebroplasty (Kinzl, Boger et al. 2011; Helgason, Stirnimann et al. 2013). FE models of the cement-bone interface have also been used to study the stress-displacement behaviour of the interface and also crack formation in the cement layer (Waanders, Janssen et al. 2009; Waanders, Janssen et al. 2010). From a computational modelling perspective, it is very challenging to create a model of the interlocked bone-cement interface that is both geometrically accurate as well as capable of correctly predicting the contact mechanism. In this study, we present an experimental setup to measure micromotion and strain at the tibial cement-bone interface using Digital Image Correlation (DIC). Using FE models of the same specimens we compared DIC measurements at the specimen surface with corresponding simulated values. In clinical practice, cement penetration depth can vary depending on the cementation technique used, as well as on the bone quality (Cawley, Kelly et al. 2013). Here, we selected bone-cement interface specimens that were representative of these variations and aimed to develop finite element models that were able to accurately simulate interface behaviour irrespective of these specimen differences. We further studied whether surface micromotion is representative of the internal behaviour of the entire cement-bone interface. Our main research questions were therefore: (1) Can finite element models of lab prepared cement-bone specimens accurately predict micromotion and strain at the cement-trabeculae interface as measured experimentally? (2) Are differences in global experimental results obtained from morphologically varying specimens also reflected in the finite element model results?

2. Materials and Methods

Micromotion and strain along the cement-trabeculae interface were measured using DIC in lab-prepared specimens of interdigitated tibial bone and cement. Finite element (FE) models of these specimens were subsequently validated using the DIC measurements of micromotion and strain. A total of 4 specimens with varying cement interdigitation depths and bone volume fraction were obtained from 2 donor tibiae.

2.1 Specimen Preparation

Two fresh-frozen tibias were obtained from the SUNY Upstate Anatomical Gift Program (1 male, age 86 years; 1 female, age 43 years). Two donors were selected to account for differences in the bone quality. Soft tissue was removed from both donor bones. The tibial plateaus of both bones were resected and cleaned with pulse lavage. Tibias were warmed to 37°C in calcium-buffered saline with a bacteriostatic agent (Race, 2005) prior to the application of cement. Radiolucent surgical bone cement (Radiolucent Simplex P, Stryker Orthopedics, Mawah, NJ) was vacuum mixed. After the cement reached a state of “does not stick to glove”, cement was applied to the proximal tibia and pressurized with a cement mixing spatula. To account for varying amounts of cement interdigitation depth on the results, one half of the each tibia received approximately double the amount of cement than the other half of the tibia. Cemented tibias were then soaked in the calcium buffered saline solution with bacteriostatic agent for 3 days at room temperature. A previously described method (Mann 2008) was used to section the tibias into cement-bone interface specimens that were nominally 4×4 mm in cross-section and about 15 mm in axial length. From each donor, two specimens were created and the cement interdigitation depth varied between 1.1 – 5.2 mm.

2.2 Experimental Apparatus and Mechanical Testing

Each specimen was positioned between a rigid, vertical fixture and a load cell mounted on a screw-driven loading stage. The long ends of the specimens were bonded to the test fixture using dental cement. The specimens were non-destructively loaded in compression to the equivalent of approximately 1 body weight (BW) which equated to 1 MPa (Figure 1a). Axial load was applied to the specimens by a screw-driven motor with a displacement rate of 1.5 mm/min.

During testing, images of the top face of the cement-bone interface were captured at 0 and 1 MPa for image analysis. The images were captured with a digital camera (Diagnostic Instruments, Sterling Heights, MI) attached to a microscope (Nikon, Tokyo, Japan). The image resolution was 0.5 µm/pixel, with a field of view equal to 0.8 × 0.6 mm (Figure 1b). Since the field of view was too small to capture the whole specimen, the loading apparatus was mounted on a 3-dimensional CNC vertical mill stage (Intelitek, Manchester, NH). After each loading and unloading cycle, the loading apparatus was moved via the CNC stage to a new region of the cement-trabeculae interface. This protocol was repeated until the entire interface was imaged.

Each specimen was also imaged at a lower resolution of 3 µm/pixel in order to obtain global displacements and strains in different regions of the specimen. Displacements at different locations within the cement layer and supporting bone were determined and used to obtain global strains in the loading direction (Figure 2).

2.3 Digital Image Correlation Analysis

Micromotion between cement and trabeculae was quantified using digital image correlation (DIC) analysis on the high resolution images (0.5 µm/pixel). Graphite was lightly distributed on the top surface of the specimen to provide suitable texture for the DIC software.

Measurements in the interdigitated region were spaced at 250 μm increments along the cement-trabeculae interfaces (Figure 3). To measure micromotion, DIC software (MatchID 2D, Catholic University College Ghent, Ghent, Belgium) was used to quantify the relative displacement between pairs of discrete DIC sample boxes (30×30 pixels, 0.0002mm^2) across the cement-bone interface (Figure 1b). Based on preliminary error analysis of the DIC system, Root Mean Square Error (RMSE) in displacement measurements at 0.5 $\mu\text{m}/\text{pixel}$ and 3 $\mu\text{m}/\text{pixel}$ resolution were 0.06 μm and 0.08 μm , respectively.

2.4 Finite element model generation and set-up

The four cement-bone interface specimens were scanned at 12 μm isotropic resolution using a micro-CT scanner (Scanco Inc. Media, PA, USA) prior to loading. The images were segmented based on an image greyscale ranging from -1024 to -769 using Mimics 14.0 (Materialise, Leuven, Belgium). The same threshold ranges for bone and cement were used to create each of the four models. Each model consisted of three parts – cement, interdigitated bone and supporting bone. Interdigitated bone was the bone enclosed within the cement layer and supporting bone was defined as that which lies distal to (below) the cement layer (Figure 1a). The segmentation mask of the cement part was used to identify interdigitated bone using the procedure described by Mann et al. (Mann, Miller et al. 2012). Using this procedure, the interdigitated bone was isolated, enabling easier post-processing of this region of interest. A non-manifold 3D surface mesh was then created using the three segmentation masks where $2 \times 2 \times 2$ matrix reduction was used. In order to limit the number of very small solid elements, smoothing, mesh reduction and remeshing was applied in 3-matic 5.1 (Materialise, Leuven, Belgium). This non-manifold mesh consisted of five surfaces; cement, interdigitated bone and supporting bone formed the three main parts. The other two surfaces were the non-manifold interfaces cement-interdigitated bone and interdigitated bone-supporting bone. The complete surface mesh of each of the three parts was obtained by duplicating the two interfaces and then merging with the appropriate surfaces. The surface meshes were then automatically corrected for any errors (3-matic 5.1). The non-manifold approach ensured that there were minimal initial penetrations between the cement and the bone mesh, which has the advantage of significant reduction in simulation time required for the contact analysis. Solid, 4-node tetrahedral meshes were created using the three surface meshes (Patran Mesher in Mentat 2012, MSC Software Corporation, Santa Ana, CA, USA). Due to the variation in cement interdigitation depth, the total number of elements ranged from 4 to 8 million with 1 to 2 million nodes (Figure 2).

Material properties for both bone and cement were assumed to be linearly elastic and isotropic. Young's modulus for the cement and bone was set to 3,000 MPa (Lewis 1997) and 14,000 MPa, respectively. A Poisson's ratio of 0.3 was applied for both materials. The cement-bone contact interface was modelled as unbonded (Waanders, Janssen et al. 2010) and a double-sided segment-to-segment contact algorithm was used (MSC Marc 2012).

The four models were loaded in compression at 1 MPa axially and constrained at both long ends, allowing only vertical movement (y-direction), consistent with the physical experiments. Micromotion between the interlocked trabeculae and cement was calculated on a nodal basis using pairs of contact nodes. The contact node pairs were determined using a

minimum distance calculation between each surface node in the interdigitated bone node set and all surface nodes in the cement node set. This operation was performed once at the beginning of the simulation, before application of the load. Each node pair was then followed throughout the simulation, during which the total and incremental micromotion was calculated.

2.5 Studied results

Global differences between the four specimens were studied by measuring the bone volume fraction, cement interdigitation depth and strains within cement and bone. Total specimen strains were determined in the direction of loading. Corresponding strain values from the FE models were compared to the experimental measurements.

To compare the experimental surface DIC micromotion values with those from the simulation, micromotion was calculated in two dimensions (x–y) as the difference in displacements of 2 nodes across the interface at each corresponding DIC measurement location (Figure 3). Experimental interdigitated bone strains were measured using a virtual extensometer method. From the high resolution DIC images, interdigitated bone strains were determined by first centering a 250 μm window on the trabeculae at each location at which micromotion was measured. The difference between the displacements at the top and bottom of the window was divided by the window length (250 μm) to obtain the bone strain at that location. A similar approach was used to determine displacements from the FE models at nodes corresponding to the top and bottom of the 250 μm window. The cement strains were also determined using a virtual extensometer method with a gauge length of 300 μm using the lower resolution (3 $\mu\text{m}/\text{pixel}$) DIC images. Average axial displacement over the width of the specimen was measured at each 300 μm interval using DIC. Corresponding displacement values were obtained from the FE models. Median FE cement strains were also determined at each 300 μm interval. Linear regression was used to study the correlation between the experimental and simulated micromotion and strain; the distribution of errors between the experimental and simulated micromotion was also determined.

An unavoidable aspect of experimental analyses on cut surfaces of specimens is that the results may be confounded by loss of continuity of the specimen structure. To determine if the micromotion measured on the surface was representative of micromotion measured away from the cut surface, we used the finite element models in this study to compare the outer micromotion (near the cut surface) with the remaining inner values (away from cut surface). The thickness of the outer segment was varied from 0.1 mm to 0.8 mm to determine whether any perceivable difference was found between the outer and inner values.

3. Results

3.1 Micromotion analysis

DIC measurements of the four specimens showed that there was limited micromotion in the region where the bone was completely encapsulated by cement. Median DIC micromotions for the four specimens were 0.78, 0.42, 0.56 and 0.09 μm and those for the FE models were 0.61, 0.43, 0.11 and 0.06 μm . Median errors in the four models were 0.25, 0.04, 0.39 and

0.04 μm respectively. Micromotion increased when moving distally through the cement layer (Figure 3). FE models of the specimens were able to capture this trend of increasing micromotion considerably well. The r^2 coefficient for the four models was between 0.59 and 0.85 (Figure 4a). Higher values of micromotion ($\sim 2\text{--}6\ \mu\text{m}$) were measured experimentally at the distal interface. Due to the under-prediction by the FE models, the absolute error values (DIC – FE) between DIC and FE prediction in the distal region was higher than the proximal region (Figure 4b). However, the relative error (%) was found to be generally the same throughout the interdigitated region. The error distribution shows that the majority of errors were centred between 0 and 1 μm (Figure 4b). Specimen 3 showed the highest median error; this specimen also had the highest difference (28%) between the experimental and FE global strain of the four specimens.

3.2 Strain analysis

The interdigitated bone strains calculated using FE were in general good agreement with the experimental measurements (Figure 5). Some locations at which micromotion was measured did not have axially oriented trabeculae with a sufficient strain window length of 250 μm . These locations were omitted from the strain analysis to avoid erroneous results. Our results show a good correspondence between experimental and FE values for bone strain as a function of interdigitation depth (Figure 6). Higher bone strains occurred at the distal interface and a decreasing trend was observed with increasing interdigitation depth. DIC measurements of Specimen 3 showed some high strain values deeper within the cement; these were not predicted by the FE model.

3.3 Global specimen behaviour

The cement interdigitation depth varied between specimens (1.1, 2.2, 4.1 and 5.2 mm) and the bone volume fraction for the four specimens was 0.12, 0.17, 0.18 and 0.24 (Figure 2). Global specimen strain in the loading direction was measured experimentally for each specimen and corresponding FE model global strains matched well, $r^2 = 0.87$ (Figure 7). This result shows that we are able to simulate the mechanics of morphologically different specimens under compression loading on a global level. Experimental and FE strains in the supporting bone also correlated well, $r^2 = 0.89$. The FE model of specimen 1 showed a higher supporting bone strain than the experimental measurement (Figure 7). Further analysis of specimen behaviour showed that strains in the cement layer were considerably lower for the FE models compared with the experimental results. The expected bulk cement strain is about 1 MPa/3000 MPa or 333 $\mu\epsilon$, which is in the range of the error in strain measurement from DIC (RMSE 0.08 $\mu\text{m}/300\ \mu\text{m}$). FE median cement strains were determined for the DIC measured surface change when moving through the interdigitation depth (Figure 8). As one might expect, strains are lower near the cement border and increase deeper in the interdigitated region. FE cement strains were in the range of the bulk cement strain.

3.4 Surface micromotion vs. internal micromotion

Comparing micromotion in the outer segment of the FE model with the remaining inner segment, we found that the outer segment resulted in higher values. Figure 9 shows the results for specimen 1. When the thickness of the outer segment was increased to 0.8 mm,

we noticed a reduction in the peak and median values of micromotion in the outer segment. The same general effect in outer values was observed for all other specimens (Table 1). In the case of specimen 2 however, median micromotion values for inner and outer segments remained constant with both segment definitions. Median micromotion in the inner segments also changed depending on the segment definition chosen. Comparing the inner values for the 0.8 mm segment definition, we see that the median micromotion is equal to the outer values in specimens 1 and 2. Specimens 3 and 4 show higher inner values compared to the outer segments. These observations indicate that the results obtained depend on the morphology of the interdigitated bone, which is defined based on the extent of cement interdigitation.

4. Discussion

The purpose of this study was to experimentally measure micromotion along the tibial cement-trabeculae interface and subsequently compare those with the same quantities obtained from FE models of the same specimens. The results showed that we could obtain reasonable agreement between the interface specimens and the FE models. Correct simulation of interface micromechanics using FE modelling depends on how accurately the model is able to capture the complexities of the bone-cement interface morphology. This depends, in large part, on the resolution of the μ CT data used to create the models. Surface measurements on the interface specimens showed that the cement and trabeculae are not in direct apposition everywhere along the interface, forming minute gaps in the range of 4 μ m wide. The maximum amplitude of micromotion measured experimentally was 10 μ m which is lower than the 12 μ m spatial resolution of the μ CT data used here. Further loss of geometric accuracy could also occur due to the matrix and surface mesh reduction techniques used to limit the total number of elements. Other studies involving micro-FE models of trabecular bone specimens have used voxel resolutions anywhere between 10 – 80 μ m (Bevill and Keaveny 2009; Bauer, Sidorenko et al. 2014; Dall'Ara, Barber et al. 2014) which is comparable to this study (24 μ m, after the 2 voxel reduction). Given the reasonably good comparison between our FE models and the experimental data (Figures 4–6) and to save computational costs, we did not study the influence of further refining our FE models and believe that the present mesh density is adequate for our purposes.

The robustness of our model development methods and FE simulations was also tested by using specimens that showed substantial morphological variation of cement interdigitation depth and trabecular bone volume fraction. By comparing global specimen deformations obtained experimentally and with those from finite element models, we were able to show that total specimen strains and supporting bone strains corresponded well. As expected, we also found that supporting bone strains from DIC and FE showed an inverse relationship with bone volume fraction. The cement strain results from DIC and FE models did not compare well in all four specimens. The reason for this is the experimental limitation in measuring displacements and strains over a small gauge length (300 μ m). The resolution of the DIC imaging camera (3 μ m/pixel) results in an RMSE error of 0.08 μ m based on a known 5 μ m excursion. In terms of strain measures, the error estimate is then 267 μ e (0.08/300). The interdigitated bone strains measured experimentally and from the FE models are however of a larger order than the cement strains, particularly around the distal interface.

Other sources of error could be differences in the boundary conditions between the actual specimens and the models. The specimens were fixed in cement at the supporting bone end whereas the models were created by cropping the segmentation mask at the appropriate level.

Micro-FE modelling has been used in other studies to investigate bone-cement interface strength and damage mechanics (Waanders, Janssen et al. 2009; Waanders, Janssen et al. 2010; Tozzi, Zhang et al. 2014; Zhang, Tozzi et al. 2014). Attempts to validate trabecular bone-cement interface FE models have also been made by comparing the deformation observed under 3D real time compressive loading in specific specimen sub-volumes with those of FE models (Tozzi, Zhang et al. 2012). This is the first study where micromotion and strain in trabecular bone-cement FE models have been directly compared with experimental data. We observed some inter-specimen variation in the correlation between the experimental data and the FE model predictions ($r^2 = 0.59 - 0.85$). FE models of specimens with lower cement interdigitation depth (1 and 2) seem to show better correlation with the experimental micromotion results. Accurate modelling of a cut, specimen surface presents certain difficulties due to the mesh smoothing operations necessary to avoid convergence issues. Some details of the cut faces are inevitably lost, making direct comparison at certain locations on the specimen face difficult and subject to artefacts. The overall r^2 coefficient for all four specimens (all values) grouped together was 0.70.

Another point of discussion is that surface measurements of micromotion may not accurately represent micromotion throughout the specimen interface. Other authors have previously acknowledged the fact that loss of continuity at the specimen boundaries leads to a higher compliance of the trabecular network at the cut faces (Harrison and McHugh 2010; Lievers, Petryshyn et al. 2010). Minimizing these side-artefacts would require larger specimen size such that the ratio between the volumes of the side-artefact and the remaining specimen is relatively small. Due to the computational challenges involved with increasing specimen/model dimensions, we studied the extent to which the side-artefact actually occurs in our models. We compared the micromotion in the outer segment of the model with that of the remaining interior segment for different segment ratios (Figure 9b). Changes in micromotion in the outer segment of the specimen, when comparing the two different segment ratios, provides an indication of the extent of the side-artifact due to cutting specimens from a continuum. It should however be noted that the specimens differ in morphology and cement interdigitation depth in the defined outer segment. In other words, the interdigitated bone is not homogeneous throughout the specimen. These factors could also play a role in the extent to which side artifacts are measurable/observable. From our results we conclude that side-artifacts are measurable with the FE models, but in general the higher specimen outer micromotions are comparable with the interior specimen values. When the outer segment thickness is chosen to equal approximately one trabecular length (0.8 mm case), median micromotion values were found to match better with interior values.

Our results showed that micromotion and bone strain obtained from micro-FE models of tibial cement-bone interface specimens do compare well with experimental DIC measurements. Global deformations of the morphologically different specimens tested were also predicted accurately by the FE models. However, the resolution of the μ CT data used

can be a limiting factor. This study lays the groundwork to investigate the micromechanics of the tibial cement-bone interface incorporating the use of FE models. Using the experimental and computational methods adopted here, we will be able to further our understanding of the mechanisms that lead to bone resorption in cemented tibial knee components. In future studies, we will focus on possible mechanisms for the bone resorption observed in post-mortem retrievals (Miller, Goodheart et al. 2014) such as strain shielding.

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Figure 1.

a) Schematic of experimental set-up showing specimen under compression loading in a CNC loading stage; a DIC camera captures motion at the region of interest (black square). b) High resolution image (field of view 0.8×0.6 mm) captured by DIC camera showing detailed bone-cement interface and sample boxes used to measure displacement under load.

Figure 2.

Finite element models of the four specimens showing different levels of cement penetration depth; from left to right, cement interdigitation depths (ID) are 1.1, 2.2, 4.1 and 5.2 mm. The specimens also varied in bone volume fraction (BV/TV) ranging from 0.12 to 0.24. Element edges are not shown for image clarity. Experimental and FE strains were measured in all specimens for the cement mantle, interdigitated bone, supporting bone and total specimen over the lengths shown.

Figure 3.

Specimen 3 face showing experimental micromotion in different parts of the cement-trabeculae interface. Values shown are averaged over all DIC measurements taken within the boxed areas. Example sample pairs of DIC boxes have been shown along the cement-trabeculae interface at 250 μm spacing. Higher micromotion was measured around the cement border (distal to the implant) and minimal micromotion deeper within the cement layer (proximal to the implant).

Figure 4.

a) Comparison between DIC and simulated trabeculae-cement micromotion for the four specimens. Measurement locations were spaced $250\ \mu\text{m}$ apart along the trabeculae. Specimens with larger interdigitation have more points of comparison. b) Distribution of errors between experimental and simulated micromotion for the four specimens.

Figure 5.

Interdigitated bone strains show good comparison between DIC and FE. Strains were measured using axial displacements over a 250 μm gauge length (using 0.5 μm/pixel resolution images). Strain measurements were taken at the same locations as micromotion measurements.

Figure 6.

Interdigitated bone strains measured using DIC and those predicted by the FE models show an increase towards the distal cement-bone interface (interdigitation depth = 0).

Figure 7.

Global specimen strain in the direction of loading from the experimental measurements and FE models showed good agreement. Global specimen strain from the FE model of specimen 3 was under-predicted (S3 ●). Supporting bone strain from experimental measurements and from the FE models correlated well, with an over-prediction for the FE model of specimen 1 (S1 ○).

Figure 8.

a) Cement strains were evaluated by determining axial strains predicted by the FE models on the specimen face used for DIC (b). Each marker represents the median strain over a 0.3 mm interval of the cement interdigitation depth (ID). A trend of increasing cement strains with distance from the cement border (ID=0) was found for all specimens.

Figure 9.

a) Two distinctions were made for the inner versus outer segment of the interdigitated bone; thickness of the outer segment was either 0.1 mm or 0.8 mm. The second thickness was chosen to represent one trabecular length b) Micromotion in the inner and outer segments were compared to highlight the influence of loss of connectivity of trabeculae at the sides of the specimen. The thinner outer segment showed higher median micromotion compared to the thicker segment.

Table 1

Median micromotion for inner and outer segments of the cement-bone interface. When the outer segment was increased from 0.1 to 0.8 mm there was a reduction in the median value of micromotion for most specimens.

Model	Median micromotion for 0.1 mm outer segment (μm)		Median micromotion with 0.8 mm outer segment (μm)	
	Inner	Outer	Inner	Outer
Specimen 1	0.28	0.43	0.29	0.29
Specimen 2	0.23	0.22	0.23	0.23
Specimen 3	0.14	0.16	0.18	0.13
Specimen 4	0.05	0.07	0.06	0.05