Age-Related Optimization of Screw Placement for Reduced Loosening Risk in Locked Plating

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Received 8 September 2015; accepted 28 January 2016

Published online 14 October 2016 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/jor.23193

ABSTRACT: When using locked plating for bone fracture fixation, screw loosening is reported as one of the most frequent complications and is commonly attributed to an incorrect choice of screw configuration. Choosing a patient-optimized screw configuration is not straightforward as there are many interdependent variables that affect device performance. The aim of the study was to evaluate the influence that locking screw configuration has on loosening risk and how this is influenced by bone quality. This study uses finite element models that incorporate cortical bone heterogeneity, orthotropy, and geometrical nonlinearity to examine the effect of screw configuration on variables associated with loosening and interfragmentary motion. Strain levels within the bone were used as indicators of regions that may undergo loosening. The study found that, in healthy bone under axial loading, the most important variables influencing strain levels within the bone were the size of the bridging span (working length) and the plate rigidity. Unlike healthy bone, osteoporotic bone was found to be particularly sensitive to the spacing of the screws within the plate. Using two empty screw holes between the screws closest to the fracture was found to reduce the strain levels at the first screw by 49% in osteoporotic bone (compared to only 2.4% in healthy bone). The study also found that under torsional loading the total number of screws used was the most important variable with a 59% reduction in the strain around the screws closest to the fracture when using six rather than four screws in osteoporotic bone. © 2016 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 34:1856–1864, 2016.

Keywords: bone strains; interfragmentary motion; bone quality; osteoporosis

The mechanical behavior of plates used for internal fixation can be substantially altered depending upon the type of screws used and the placement of those screws.^{1,2} Locking plates have advantages over conventional plates; the plate does not need to be fastened tightly against the bone^{3,4} which can improve vascularity around the fracture.⁵ When a plate (locking or conventional) is in contact with the bone, construct rigidity can be relatively insensitive to screw placement;^{6,7} however, when using locking plates in a "bridging mode," spanning the fracture, the configuration of the screws considerably alters the stiffness and strength of the construct.^{2,8} This can affect the course of fracture healing which is sensitive to the mechanical stimulus at the fracture site or interfragmentary motion (IFM).⁹ Biomechanical studies have shown that the location of screws can also influence device strength¹⁰ and the likelihood of periprosthetic refracture.¹¹ Additionally, when screw loosening occurs, it is generally attributed to incorrect choice of screw placement.^{12–14}Therefore, the placement of screws is of critical importance when selecting a device configuration for a patient. Choosing a screw configuration that is optimized for a patient's bone quality or age is not straightforward as there are many interdependent variables relating to device configuration.

The distance between the two screws on either side of the fracture (working length) has been reported to be the single most important configuration parameter

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influencing IFM in locked plating;² despite this, current biomechanical guidance relating to working length is somewhat unclear. In the case of narrow fracture gaps, where interfragmentary contact can occur, some studies recommend increasing working length in order to reduce stress concentrations within plates;^{15,16} however, in wider gap situations, excessive working length can make the plate overly flexible¹⁴ and can result in plate breakage.¹³ The influence of working length on screw loosening has not been previously discussed. Screw positioning variables such as the number and spacing of screws have also been held responsible for cases of plate breakage, screw loosening, and periprosthetic re-fracture.^{12,17–19}

The fixed angles of the locking screws are also thought to improve the strength of fixation in poorer quality bone,^{3,11,20} although loosening or cut-out is still reported as the most frequent complication,²¹ particularly among the elderly.²² It is accepted that different fixation techniques are often required in osteoporotic bone; complications are more likely to be related to bone failure rather than implant failure.²² It is likely, therefore, that screw positioning guidance that applies to healthy bone may not be appropriate for osteoporotic bone. While there has been some consideration of different screw types in osteoporotic bone,^{11,23,24} the influence of the position of the screws in varying bone quality has not been investigated.

It is known that aging causes the cortex to thin and the cross-section of the bone to become wider.²⁵ It is also recognized that cortical bone is not isotropic but is well represented by orthotropy.²⁶ Osteoporotic bone is known to deteriorate more transversely and radially than axially, meaning its resistance to transverse forces is compromised.²⁷ Despite this, isotropic assumptions are generally used in simulation.^{2,28–30}

Conflicts of interest: There is no conflicts of interest to declare. Grant sponsor: Osteosynthesis and Trauma Care Foundation; Grant number: 2011-PPHS.

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Computer simulation allows the prediction of local mechanical environment in the bone around screws which is difficult or impossible to measure experimentally. Nonlinear contact mechanics has been previously shown to greatly influence the prediction of stress at the screw-bone interface.³¹⁻³³ Fully-bonded representations mean that tensile strains can develop where in reality separation would occur, substantially altering the stress-strain environment.³⁴ It is also important to include screw threads to capture stress concentrations at the first few threads.³⁵ These factors are likely to influence the predictions of strain within the bone and so should be included in any computational models evaluating screw loosening risk.³⁴ Additionally, geometrical nonlinearity has been previously shown to be important for prediction of IFM in organscale models.^{31,34}

The aim of this study was to examine the effect of screw configuration on variables associated with screw loosening and interfragmentary motion (IFM), using finite element models that incorporate cortical bone heterogeneity, orthotropy, contact mechanics, and geometrical nonlinearity.

METHODS

A Stryker AxSOS 5.0 mm narrow locking plate implant used was scanned using a 3D laser scanner (NextEngine, Inc., Santa Monica, CA). An idealized geometry of the tibial diaphysis was created using an extruded cross-section (dimensions and material properties described later). These geometries were used to create three-dimensional finite element models in ABAQUS (6.10/CAE, Simulia, Providence, RI). Symmetry was assumed at the centre of the plate; other than this no restraint was applied to the model (Fig. 1). The total effective length of bone-plate construct was 445 mm (including symmetry). A bone-plate off-set of 2 mm was used. Locking screws were modeled with an outer diameter of 4.5 mm and a thread depth of 0.5 mm. The screw threads were explicitly modeled with a triangular profile in idealized rings. The plate and screws were considered to be stainless steel and were modeled as a homogeneous isotropic material with a Young's modulus and Poisson's ratio equal to 205 GPa and 0.3, respectively. The influence of different plate material properties was considered and is described later.

The material and geometric characteristics for the bone were varied to represent healthy and osteoporotic bone qualities. The properties included: material orientations for orthotropy; heterogeneous variation in the radial direction; and geometrical changes associated with osteoporosis (cortical thinning and periosteal apposition;^{25,36}) (Fig. 2). The



Figure 1. Idealized model of the bone-plate system showing loading and boundary conditions.

material orientations were specified using cylindrical orientations (Fig. 2a). Previous studies have shown increased porosity and bone loss at the endosteal aspect and a clear pattern of increasing porosity from the periosteal, to the middle, to the endosteal aspect in all age groups.^{25,37} In this study heterogeneous variation was incorporated using an orthotropic elasticity tensor for points near the periosteum and endosteum³⁸ and interpolated for intermediate locations across the cortical thickness (Fig. 2b).³⁹ These properties are summarized in Table 1.^{27,36} Clinically, locking screw loosening generally occurs toward the diaphyseal end of the plate;¹²⁻¹⁴ therefore, only cortical bone was included in the models, similar to previous experimental studies⁴⁰⁻⁴² and numerical studies.^{32,43} The geometric characteristics of the bone were selected to match reported values of cortical thickness and cross-sectional areas for an average female at 40 and 80 years old.^{25,36} The cortical thickness and crosssectional area was 5.1 mm and 319.2 mm² for healthy bone and 3.64 mm and 265.3 mm² for osteoporotic bone (Fig. 2c).

At the near cortex, screw-bone contact interfaces were modeled as sliding interactions using Coulomb friction coefficient of 0.3.^{31,44} Similar to previous studies, the peak strains were located at the near cortex;^{31,43} therefore, to simplify the analysis, interactions at the far cortex were modeled as tie constraints. Recent studies^{45,46} that have compared experimental results with numerical simulations have shown that the assumption of a tied screw-plate interface overestimates the stiffness of the screw-plate system. Consequently, the screw-plate connection was modeled using linear springs with a spring stiffness derived from experimental data.⁴⁵

Locking plates are often used for comminuted fracture patterns where individual fragments and the fracture site are bridged.¹⁵ A 10 mm osteotomy gap was used to represent this situation; the fracture pattern would be included in AO/OTA fracture classification 42C1-3.⁴⁷ This fracture pattern is often associated with high energy fracture such as car accidents where the fibula is also commonly fractured.³⁰

The bone was loaded axially up to 250 N which is similar in magnitude to the values used in previous studies^{2,40,48} and represents partial weight-bearing (approximately 14% of peak physiological knee joint loads during level walking).⁴⁹ Load was evenly distributed over the end of the bone and was selected to represent the shafts of long bones such as the tibia and femur. A similar model was used in a recent study by Bottlang et al.¹¹ to examine metaphyseal and diaphyseal plating. Screw configurations were also examined under torsional loading of 2 Nm representing internal/external moments experienced during level walking (approximately 25% of peak physiological knee joint loads).⁴⁹ Quasi-static (implicit) analyses were conducted using geometric nonlinearity (ABAQUS/Standard).

The influence of the following screw positioning variables was investigated (Fig. 3):

- The total number of screws used (on one side of the fracture);
- The working length—the distance between the screws closest to the fracture on either side of the fracture (i.e., bridging length);
- Screw spacing—the proximity of the first and second screws closest to the fracture site on the same side of the fracture.

In each case the influence of bone quality and plate rigidity were examined. The influence of the plate rigidity was evaluated by varying its Young's modulus, E; in these models,



Figure 2. The material properties incorporated showing (a) orthotropic material orientations; (b) heterogeneity—gradient from endosteal to periosteal surfaces; and (c) the geometrical changes—periosteal apposition and endocortical resorption associated with osteoporosis.³⁸

the material properties of the screws were not changed. In all cases, symmetrical screw configurations were used. The influence of screw positioning was assessed for three variables: (i) interfragmentary motion (IFM); (ii) maximum von Mises stress within the plate; and (iii) localized strain levels around screws. To quantify the risk of loosening, the volume of bone above 0.02% equivalent strain around each screw hole location at the near and far cortices was quantified and designated as EqEV (equivalent strain volume); an example of such regions is marked in Figure 3. Although this value of 0.02% strain is low, it is only intended to be an indication of regions of relative high strain and consequent loosening.^{22,50} This measure is also indicative of the risk of micro-motion induced loosening as strain concentrations are associated with gap opening on the opposite side of the screw or screw thread.³² As the majority of EqEV was found to occur at the first two screws, the use of a larger value would have obscured any comparisons with subsequent screws. Thus the choice of this threshold was based on its ability to highlight the variation of the strain environment around different screws; it is recog-

Table 1. Material Properties for Different Directions Used in the $Study^{38}$

| | Young/Healthy | | Old/Osteoporotic | |
|----------|---------------|-----------|------------------|-----------|
| (GPa) | Periosteum | Endosteum | Periosteum | Endosteum |
| E11 | 18.5 | 16.6 | 12.9 | 3.2 |
| E22 | 18.8 | 17.1 | 14.6 | 6.0 |
| E33 | 22.4 | 21.4 | 19.3 | 11.2 |
| G12 | 7.2 | 6.6 | 5.4 | 1.8 |
| G13 | 6.9 | 6.4 | 5.4 | 2.2 |
| G23 | 7.0 | 6.5 | 5.7 | 3.0 |
| $\nu 12$ | 0.28 | 0.27 | 0.24 | 0.16 |
| $\nu 13$ | 0.26 | 0.24 | 0.20 | 0.07 |
| $\nu 23$ | 0.26 | 0.24 | 0.22 | 0.14 |

Directions 1–3 refer to radial, circumferential, and axial directions, respectively.

nized that some of these small interfacial strains may aid osseointegration in the long term.

A mesh convergence study was conducted and appropriate mesh resolutions for different parts of the model were determined based on their influence on the equivalent strain volume (EqEV) predictions. Linear tetrahedral elements used for the bone and screws while quadratic tetrahedral elements were used for the plate. The approximate number of elements used in the bone, each of the screws and the plate was: 200,000; 13,000; and 57,500, respectively. The average element edge length around screw holes was 0.3 mm. Doubling the number of elements in the bone, plate, and screws changed the predictions of EqEV (equivalent strain volume) by 2.36%, 2.72%, and 3.14%, respectively. Doubling the number of elements within the plate changed interfragmentary motion (IFM) predictions by 0.21%. As a consequence, the FE model with the above stated number of elements was considered to be appropriate for analysis.

RESULTS

The maximum interfragmentary movement (IFM) was found to occur at the cortex furthest from the plate (or the far cortex). Predictions of IFM at this location for selected screw configurations and varying bone quality are shown in Figure 4. For each configuration, the positions of the screws is denoted using the numbers of the plate holes and their proximity to the fracture; that is, if screws were used in the first three screw holes closest to the fracture, the configuration would be labeled "C123."

The maximum von Mises stress within the plate for selected configurations in shown in Figure 5.

The equivalent strain volume (EqEV) predictions were recorded under axial loading for different total numbers of screws (Fig. 6), working lengths (Fig. 7), screw spacing (Fig. 8), and varying plate rigidity (Fig. 9). Finally, the influence of selected configurations on EqEV levels under torsion is presented in Figure 10.



Figure 3. Cross-section through the centre of the plate showing the regions with elements above 0.02% equivalent strain (EqEV) and various screw positioning variables examined using the model.

Overall, the two most influential variables affecting EqEV were found to be the working length and plate rigidity. Larger working lengths were found to not only increase IFM (Fig. 4) and plate stress (Fig. 5), but also increase EqEV within the bone (Fig. 7). In healthy bone, doubling the size of the working length increased EqEV levels by 68% at the screw closest to the fracture site; tripling the working length caused a 99% increase in EqEV (Fig. 7). As expected, reduced plate rigidity increased IFM, however, EqEV levels were also increased (Fig. 9). A plate with a Young's Modulus equal to that of titanium (105 N/mm^2) produced EqEV levels at the first screw 80% greater than stainless steel (205 N/mm^2) .

Increasing the number of screws beyond three on either side of the fracture was found to have minimal influence on EqEV predictions (Fig. 6) regardless of the position of the screws. This was because the first two-screws closest to the fracture, on either side of the fracture, were found to have the largest EqEV values in all cases (Fig. 8).

Reduced bone quality had minimal influence on IFM and plate stress (Figs. 4 and 5) but substantially altered EqEV levels under axial loading (Figs. 6–9). Increasing the number of screws used did not benefit osteoporotic bone any more than healthy bone (the percentage reduction in EqEV was similar), however, the influence of screw spacing was substantial (Fig. 8).



Figure 4. Interfragmentary movement (IFM) predictions for selected screw configurations demonstrating the influence of working length (WL) and bone quality.



Figure 5. Maximum von Mises stress predictions in the plate for selected screw configurations.

EqEV levels in osteoporotic bone were found to be lowest when using a two-hole spacing between screws on either side of the fracture (Fig. 8). In this case, EqEV at the first screw was reduced by 49% compared to a configuration with no spacing. In healthy bone, the influence was much smaller, reducing the EqEV levels by 2.6% and 3.4% for one-screw, and two-screw spacing, respectively (Fig. 8). Additionally, the proportion of EqEV in the near cortex was measured for various screw configurations. In osteoporotic bone, the EqEV at the near cortex was, on average, 53% of the total compared to around 77% in healthy bone (Table 2).

Under torsion, the total number of screws and the proximity of the screws to the fracture were found to be the most influential variables (Fig. 10). Increasing the number of screws from two to three reduced the EqEV at the first screw by 59% and 52% in healthy and



Figure 6. Predicted volumes of bone above 0.02% equivalent strain (EqEV) for different numbers of screws. (a) Screw arrangements: C123456; C1234; C123; and C12. EqEV values at different screw locations for (b) healthy bone and (c) osteoporotic bone. Load of 250 N is applied from above and the fracture is located below.



Figure 7. Predicted volumes of bone above 0.02% equivalent strain (EqEV) for different working lengths. (a) Screw arrangements C123; C234; and C345. EqEV values at different screw locations for (b) healthy bone and (c) osteoporotic bone. Load of 250 N is applied from above and the fracture is located below.



Figure 8. Predicted volumes of bone above 0.02% equivalent strain (EqEV) depending upon the proximity of the second screw from the first. (a) Screw arrangements: Cl26; Cl36; Cl46; and Cl56. EqEV values at different screw locations for (b) healthy bone and (c) osteoporotic bone. Load of 250 N is applied from above and the fracture is located below.

osteoporotic bone, respectively. Under axial loading, the reduction was 25% and 26%, respectively. Under torsional loading, however, both bone qualities produced relatively similar levels of EqEV compared to axial loading.

DISCUSSION

The study found that screw configuration and plate properties substantially affect regions of high strain around the screw-bone interface in locked plating. Locking plates are commonly used to stabilize tibial plateau and pilon fractures, the findings of this study can be applied to the shaft fixation in these clinical situations. In many aspects, osteoporotic bone was found to behave similarly to healthy bone; however, it was found to be much more sensitive to screw spacing (the distance between first two screws closest to the fracture site, on either side of the fracture) than healthy bone.

The importance of allowing sufficient screw spacing (between screws on the same side of the fracture) has been voiced previously; Gautier and Sommer⁵¹ recommended that fewer than half of the plate holes should be filled. This study found that allowing a screw spacing of one or two empty screw holes produced the greatest reduction in EqEV (equivalent strain volume)



Figure 9. Predicted volumes of bone above 0.02% equivalent strain (EqEV) for differing Young's moduli of plate: 305; 205; and 105 N/mm^2 . EqEV at different screw locations (configuration C126 is used in all cases) is shown for (a) healthy bone and (b) osteoporotic bone.



Figure 10. Predicted volumes of bone above 0.02% equivalent strain (EqEV) under torsional loading. (a) Screw arrangements: C123456; C1234; C123; C126; C136; and C12. EqEV values at different screw locations for (b) healthy bone and (c) osteoporotic bone. Torque of 2 Nm is applied from above and the fracture is located below.

146

156

Average

| First Screw for Selected Screw Configurations | | | | |
|---|-------------|------------------|--|--|
| Configuration | Healthy (%) | Osteoporotic (%) | | |
| 123456 | 73.0 | 45.2 | | |
| 1234 | 75.7 | 46.1 | | |
| 123 | 78.4 | 48.5 | | |
| 12 | 72.7 | 48.2 | | |
| 234 | 63.1 | 44.6 | | |
| 345 | 63.1 | 38.3 | | |
| 126 | 71.1 | 42.6 | | |
| 136 | 89.7 | 63.9 | | |

90.5

91.1

76.8

74.5

78.4

53.0

Table 2. Proportion of EqEV at the Near Cortex in theFirst Screw for Selected Screw Configurations

levels. The percentage reduction of EqEV was larger in osteoporotic bone and was attributed to the smaller cortical thickness, total cross-sectional area and lower Young's moduli. Additionally, our osteoporotic bone model captured the effects of highly directional deteriorations in stiffness, and the influence this would have on the strain response under the different loading scenarios considered; this effect is likely to have been less pronounced if transversely isotropic or isotropic assumptions were made.

It is known that reducing the stiffness of external fixation devices, by using titanium screws or a more flexible screw arrangement, causes high strains around screws, which can lead to loosening.^{33,43} The present study confirmed that this also applies to locked plating; increasing working length; and reducing the stiffness of the plate both increased EqEV levels. This was attributed to changes in the angle of screws during plate deformation and thus strains at the screw-bone interface. Doubling the size of the working length increased EqEV levels by 68% at the screw closest to the fracture site; tripling the working length caused a 99% increase in EqEV. Working length, however, is known to be the most important determinant of IFM.² Therefore, this study has demonstrated that there is a compromise between producing greater IFM, advocated by several studies,^{29,40,42} and reducing local strain levels around screws. It is important to recognize that while EqEV illustrates the variation of strain environment for different configurations, it is only the relatively large local strains that will lead to loosening; some of the small interfacial strains may aid osseointegration.

This study found that no significant reduction in EqEV was obtained by using more than three screws on either side of the fracture in either healthy bone or osteoporotic bone (less than 8% reduction even when using six screws on either side of the fracture). It has been argued, however, that additional screws can add redundancy, thereby protecting against sequential failure.¹ There has also been some discussion as to whether two locking screws on either side of the fracture may be enough in selected scenarios such as humeral fractures.^{23,52} This study found that there

was a considerable reduction of EqEV under both axial loading and torsion at the screw closest to the fracture site when using three screws compared with two.

Compared to healthy bone, osteoporotic bone had a more even distribution of EqEV at the near and far cortices. This indicates that in healthy bone the entrant cortex carries the majority of the load, whereas in bone of poorer quality the far cortex plays a more important role. This provides a biomechanical explanation as to why bi-cortical fixation is important in poorer bone quality and supports clinical recommendations that bicortical screws should be used in osteoporotic bone.¹

Obese patients are known to present a high risk when using locked plating.^{53,54} Patients of different weights, however, are currently treated similarly^{53,54} despite manufacturers warning against the use of plating in obese individuals.⁵⁵ This study found that EqEV, plate stress, and IFM all increase nonlinearly with load, indicating that patient weight should be taken into account when selecting a plate type and screw configuration.

In simple fractures, fracture reduction is recognized as being more important than screw placement.^{1,20} In some situations, such as comminuted fractures, the fracture zone may be "bridged" and the locking plate must support the full weight-bearing loads. This study agreed with the findings of Stoffel et al.² that screw placement can greatly influence IFM in this situation. Additionally, the regions of high strain induced in the bone around the screw-bone interface, not previously investigated, are also influenced by device configuration. These high strains are thought to be responsible for screw loosening.²²

This study found that bone quality did not significantly influence interfragmentary motion (IFM) (<8% difference). Much of this difference can be attributed to the larger cross-section of osteoporotic bone (6.8% larger than healthy bone) resulting in an increased eccentricity of the plate from the loading axis. This means that, for the prediction of IFM, the geometry of a fractured bone is more critical than its material properties. Uhl et al.⁴⁰ found similar results where changes in bone density influenced IFM considerably less than overall construct stiffness. Unfortunately, the ideal combination of these factors to support healing for a given fracture is not vet known.¹⁴ This study found, however, that additional flexibility of locking plates increased the levels of EqEV indicating that excess flexibility should be avoided, particularly in osteoporotic bone which has larger EqEV levels than in healthy bone.

Finally, the risk of screw loosening can also be mitigated by the placement of remaining screws beyond the working length. This study found that osteoporotic bone was much more sensitive to screw spacing than healthy bone. Gautier⁵¹ previously noted that this variable is clinically important, however, this study is the first to emphasize the particular importance of the proximity of two screws on either side of the fracture (four screws closest to the fracture). We also found that, regardless of bone quality, the use of more than three screws was only beneficial under torsional loading. Additionally, in osteoporotic bone, the far cortex plays a significant role in load sharing and thus bi-cortical screws should be used.

The majority of previous studies evaluating the mechanical behavior of locking plates have used specimens with cylindrical cross-sections to simulate long bone fractures.^{2,4,11,29,41} Unlike these previous studies, the current study predicted strain levels within the bone requiring more complex material and geometrical properties. We used a standardized tibial cross-section which was then modified to match previously reported age-dependent geometric characteristics.²⁵ The specimen length was selected by taking the approximate length of a human tibia (405 mm) plus 20 mm at either end to approximate the distance to the centers of rotation at the knee and ankle joints.⁵⁶

One of the benefits of locked plating is the ability to off-set the plate from the bone, however, off-sets larger than 2 mm have been shown to compromise construct strength and stiffness.⁵⁷ If an off-set is not used, then the spacing of the screws becomes less important; for example, a previous study found that working length had no effect on axial stiffness when the plate was in contact with the bone.⁶ An off-set of 2 mm was used in the current study, consistent with some previous studies.^{2,4,29}

If a fracture union is not achieved, the implant-bone construct will eventually fail, with screw loosening being a typical failure mode.²¹ The total magnitude of load transmitted by the device has been shown to reduce as healing progresses.⁵⁸ The presence of callus formation in the fracture region was therefore not included in the analyses in order to provide a worstcase scenario where the plate is transmitting the entire load via the screws that traverse the bone. This study used symmetrical screw configurations in order to reduce the size of the models, however, non-symmetrical screw configurations, which may not be in the same plane, may be used clinically and would be an interesting aspect for future studies to consider. Previous studies using nonlinear contacts have found that the strains at the near cortex are much larger than those at the far cortex.^{31,43} Tie constraints were used at the far cortex in the present study order to simplify the analysis. It is possible that even larger differences between the two bone qualities could be seen had nonlinear contacts also been used at the far cortex. The models included geometric and contact nonlinearities but did not incorporate material nonlinearity. This was because none of the screw configurations tested in healthy or osteoporotic bone produced maximum or minimum principal strains greater than the tensile or compressive yield strains of cortical bone (0.5% or 0.7%), respectively).^{59,60} While this study was limited to two bone qualities, it would be possible to incorporate patient-specific bone properties in the models. It is likely, however, that the majority of patients would fall within the extreme cases considered here.

AUTHORS' CONTRIBUTIONS

All authors have given approval of the final submitted manuscript and contributed with the following roles: A. MacLeod: Study design, data acquisition, analysis, interpretation, drafting, and critically revising paper. H. Simpson: Study design, data interpretation, and critically revising the paper. P. Pankaj: Study design, data interpretation, and critically revising paper.

ACKNOWLEDGMENT

We gratefully acknowledge the support of The Osteosynthesis and Trauma Care Foundation.

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