Assessment of the initial viscoelastic properties of a critical segmental long bone defect
reconstructed with impaction bone grafting and intramedullary nailing.

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Abstract

Introduction: This study compared the initial viscoelastic properties of a segmental tibial defect stabilized with intramedullary nailing and impaction bone grafting to that of a transverse fracture stabilized with intramedullary nailing.

Materials and Methods: Seven sheep tibiae were tested in compression (1000 N), bending and torsion (6 Nm) in a six degree-of-freedom hexapod robot. Tests were repeated across three groups: Intact tibia (Intact), transverse fracture stabilized by intramedullary nailing (Fracture), and segmental defect stabilized with a nail and impaction bone grafting (Defect). Repeated measures ANOVA on the effect of group on stiffness/phase angle were conducted for each loading direction.

Results: The Intact group was significantly stiffer than the Fracture and Defect groups in bending and torsion ($p<0.022$ for both loading directions), and was marginal for the Defect group in compression ($p=0.052$). No significant differences were found between the Fracture and Defect groups ($p>0.246$ for all loading directions) for stiffness/phase angle. In compression and bending, phase angles were significantly greater for the Fracture and Defect groups compared to Intact ($p<0.025$), with no significant differences between groups in torsion ($p=0.13$). Sensitivity analyses conducted between the Fracture and Defect group differences found that they were not of clinical significance.

Conclusion: The initial properties of a segmental defect stabilized with intramedullary nailing and impaction bone grafting was not clinically significantly different to that of a transverse fracture stabilized with intramedullary nailing.

Keywords: Bone allograft; Segmental Defect; Impaction bone grafting; Biomechanical testing, Compression stiffness; Bending stiffness; Torsion stiffness; Fixation stability; Fracture stability; Tibia; Sheep; Phase Angle
1. Introduction

Adequate fracture stability is paramount not only for healing but also for minimizing complications, such as malunion and failure of fixation. Equally important, the stability of a fracture-internal fixation construct has a great influence on patient rehabilitation. These injuries can take between 3-12 months or more to heal [1], and even longer periods for complex and complicated cases, and are recognized to have a high cost burden [2]. Therefore, regaining early unrestricted loading through the affected limb is of major importance, not only for the patient, but also for reducing the cost burden on society and the health system.

The association of a segmental bone defect compounds the healing of a long bone such that they require a longer time to heal [3, 4], and pose increased difficulty in providing the stability that allows safe mobilization with functional loading, which we defined as weight bearing as tolerated by pain, as well as healing.

The most common treatment for femoral and tibial shaft fractures is intramedullary nailing [5-7]. Stabilizing a simple mid-diaphyseal fracture by an intramedullary nail allows inter-fragmentary load sharing and immediate weight bearing [8]. Although loading and dynamisation is thought to stimulate bone healing across a fracture/nonunion site [9-11], this process was shown to be detrimental to healing in situations with bone defects and/or unstable fixations [7]. Cancellous bone allograft has been successfully used as an adjuvant bone filler in fracture nonunion and to reconstruct segmental bone defects [4]; however, without compaction the construct has to be protected from physiological loading until advanced stages of healing and bone graft integration are achieved [12]. By contrast, immediate stability and weight bearing (as tolerated) has been achieved when bone defects were reconstructed using compacted cancellous bone allograft with the technique of impaction bone grafting [13-16]. However, the role of impaction bone grafting in orthopaedic
trauma is yet to be established. Such a technique could be very beneficial to the treatment of some complex human posttraumatic pathology, which would otherwise involve lengthy periods of restricted weight bearing [4]. A recently published study investigated the concept of impaction bone grafting to treat critical segmental bone defects as a salvage procedure in patients in which none of the established techniques were thought to be applicable [16]. The technique proved to be successful in all three cases in which it was applied and, importantly, it also provided a strong enough construct to allow the patients to recommence immediate postoperative weight bearing for the first time in 8-36 months [16].

We hypothesized that the initial viscoelastic properties (stiffness and phase angle - a measure of energy absorption) of a critical segmental diaphyseal defect treated with intramedullary nailing and impaction bone grafting is not clinically significantly different to a transverse fracture treated with intramedullary nailing. To test this hypothesis we measured the initial stiffness and phase angle of a critical segmental diaphyseal defect [17] stabilized by a combination of intramedullary nailing with impaction bone grafting contained with compliant mesh wire in a sheep model. This was compared with the stability of a transverse diaphyseal fracture stabilized with an intramedullary nail, as well as with the mechanical properties of the intact bone as a control.

2. Methods

2.1. Specimen preparation

Seven fresh sheep tibiae were obtained from a local abattoir (age: three years, weight: 50-55 kg), sealed in plastic bags and frozen at -30C. Prior to the day of testing, all soft tissue was removed leaving the periosteum intact, after which the bone was wrapped in saline soaked gauze, re-sealed in plastic bags and frozen. The tibiae were then thawed at 4C overnight and
stood at room temperature for a minimum of three hours prior to testing. The proximal and
distal ends of each tibia were resected to the same length and cut parallel to each other using
a bandsaw.

Cylindrical fixation cups were used to secure each end of the tibia. Additional fixation was
achieved using three 6 mm stainless screws with pointed tips, which were inserted into the
cortices of each end and tightened using a wrench. A custom-made alignment device was
used to ensure that the superior and inferior cups were parallel to each other and the tibia was
aligned such that its longitudinal axis was perpendicular to each cup surface. The alignment
device consisted of a vertically orientated precision linear rail and bearing block mounted to
two fixation plates. The base of the linear rail was mounted perpendicular to a fixed lower
plate and the bearing block was mounted perpendicular to an identical mobile plate above.
The cylindrical fixation cups were bolted to each plate such that their centers were aligned,
therefore the potted ends of the tibia were always parallel to each other (Figure 1).

2.2. Biomechanical testing
All testing was conducted in a custom-developed six degree of freedom hexapod robot [18].
Briefly, the hexapod robot was based on the concept of the Stewart Platform and employs six
servo-controlled ball screw driven actuators that precisely position a mobile upper plate with
respect to a fixed base plate (Figure 2a). Specimens were bolted between the fixed base and
the mobile upper plate. Displacements and rotations of the specimen were directly measured
by six linear optical encoders with an incremental resolution of 0.5 μm (B366784180185;
LDM54, MicroE Systems, Inc., MA, USA) that were positioned independently to the loading
frame (i.e. actuators) and load cell. Therefore, system compliance was eliminated from the
measurement of specimen behavior. Forces and moments were measured by a six axis load
cell (MC3A-6-1000, AMTI, Watertown, MA, USA), having a maximum axial compressive force capacity of 4,400 N and 110 Nm of axial torque. The displacement measurements were independently validated prior to this study to NATA standards based on ISO 10360: “Geometrical product specifications - Acceptance and reverification tests for coordinate measuring machines (Part 2)”. The load accuracies were based on NATA calibrations provided by the manufacturer (AMTI, Watertown, MA, USA). The accuracy of the measured compressive force and axial torque was ±9 N and ±0.2 Nm respectively, with displacement and rotation angle accuracy being ±0.01 mm and ±0.006° respectively. At the time of this study the control system required to perform tests under load control (forces/moments) was still under development, therefore all tests were conducted in position control (translations/rotations).

For the axial compression and axial rotation tests, the specimen was bolted onto the fixed base of the hexapod. The load cell was zeroed prior to connecting the specimen to the mobile upper plate. After the specimen was bolted to the mobile plate, all forces and moments about each of the three axes that arose during mounting were removed by repositioning the mobile plate. Each specimen was considered to be in its neutral position when all three forces were less than 5 N and all three moments less than 0.5 Nm.

Loads applied to the tibia were adopted from a study that applied 750 N of axial compression and 5 Nm of axial torsion [19]. An axial compressive load of 1000 N and axial torsion moment of 6 Nm was chosen for this study. Four-point bending was not conducted in the study of Brown, et al., 2007, however, a 6 Nm bending moment was chosen to match the axial torsion moment. These values were chosen to ensure that the tibia sustained loads that were representative of physiological loading, while ensuring that the bone or implant did not
fail. Importantly, the applied loads were chosen to ensure that the response was beyond the toe region of the non-linear viscoelastic curve and into the linear region of loading, which was used for calculation of stiffness.

2.2.1. Axial compression

A compressive preload of 50 N was first applied using the hexapod robot at a slow displacement rate (0.01 mm/s), and the compressive displacement recorded. The tibia was then further loaded until approximately 1000 N of compression was achieved, after which it was unloaded to 50 N preload as the starting load for the test. Stress relaxation occurred immediately after applying the displacement, requiring additional displacement to be applied until the load relaxed to a steady-state level above 1000 N. The change in displacement between 50 N and 1000 N was calculated and used as the input amplitude for the test. A haversine displacement waveform was then applied to load the tibia for 5 cycles at 0.1 Hz to approximately 1000 N. Forces, moments, translations and rotations about all three axes for all test directions were recorded at 10 Hz.

2.2.2. Axial rotation

After loading in axial compression, any residual forces and moments were removed by returning the specimen to its neutral, unloaded position. A 50 N axial compression preload was applied and the specimen was subjected to 6 Nm of axial torque using a sinusoidal waveform at 0.1 Hz for 5 cycles. Sinusoidal loading resulted in twisting the tibia in both directions (internal and external rotation).

2.2.3. Four-point bending
After completion of axial compression and axial rotation testing, each tibia was removed from the fixation cups and placed in between the rollers of a four-point bending device in the hexapod robot for loading in the medio-lateral direction (Figure 2b). Separation between each roller was set to 60 mm, creating a distance of 180 mm between the outer two lower support rollers and 60 mm between the inner two loading rollers. A compressive load of 200 N was applied, resulting in a constant bending moment of 6 Nm between the inner two rollers. The tibia was preloaded to 50 N and loaded to 200 N using a haversine waveform for 5 cycles at 0.1 Hz.

2.3. Surgical technique/methods

The testing described above was first conducted on all intact tibiae (Intact group), after which the bones were cut in half through a transverse osteotomy. All procedures were performed by an orthopaedic surgeon. Following this the osteotomised tibias were reduced anatomically [20] and stabilized with a locked, reamed Austofix intramedullary nail (Fracture group) (diameter 8 mm, length 220 mm, Austofix Pty Ltd, SA, Australia) as previously described [21], and the bones were retested in axial compression, four-point bending and axial rotation.

After completion of the second round of biomechanical testing the nails were removed and a diaphyseal segment with a length of 20 mm was removed from the distal segment. Following that, the internal fixation was reapplied using the same locking holes, thus in effect creating a 20 mm segmental diaphyseal defect in each tibia. The segmental defects created were then contained with a rectangular piece of compliant mesh wire (Stryker, Mahway, NJ, USA) that was rolled around the tibial shaft and secured to each fragment with cerclage wire, as illustrated in Figure 3a. After the defects were first stabilized with the nail and then contained with the mesh wire, they were filled with coarsely milled human bone allograft (Defect
that had been irradiated at 25kGy, prepared as previously described [14, 21], and impacted with a set of flat headed packers and a hammer as per the techniques described and used in revision hip surgery (Figure 3: b and c) until no more bone could be inserted. The bones were then retested in axial compression, four-point bending and axial rotation.

2.4. Statistical and data analysis

All data were analyzed using a custom software program written in Matlab R2011b (The Mathworks Inc., Natick, MA, USA). Recordings were first partitioned into cycles representing the applied displacements/rotations and data from the final cycle was analyzed. Stiffnesses were calculated using linear regression from the linear loading region between 600 N and 900 N for axial compression, 3 Nm and 5 Nm for four-point bending, and a variable range for axial torsion (typically between 4-6 Nm in each direction of rotation). The variable stiffness range for axial torsion arose because of the stress relaxation reported earlier. In some cases the torque did not reach 6 Nm, or there were asymmetries between each direction of rotation. In all cases, the stiffness range was chosen to lie in the linear loading region. Phase angles for each direction of loading were calculated between the input displacements/rotations and measured forces/moments using the Cross Spectral Density Estimate function (Matlab: CSD.m).

Separate repeated measures ANOVA having a within-subjects factor of Group (Intact, Fracture, Defect) on the dependent measure of stiffness/phase angle were performed for each direction of loading (axial compression, axial rotation and four-point bending). Statistically significant differences were accepted when p<0.05 (two-tailed), and a Bonferroni adjustment was used for all statistical post-hoc pairwise multiple comparisons. All statistical analyses were conducted using SPSS Version 18 (PASW 18, IBM, NY, USA).
Where no statistically significant differences were found, analyses were conducted by comparing the pairwise mean difference, and the 95% confidence interval (CI) range of this difference, to the reported clinically significant differences between the Fracture and Defect groups for each direction of loading. This provided important information to further support the interpretability of clinical significance.

All stiffnesses and phase angles were then normalized as a fraction of the Intact tibia group and presented graphically to allow the reader to compare the magnitude of group differences between loading directions.

3. Results

Example loading curves in each direction of loading for one specimen are shown in Figures 4–6. Mean (95% CI range) stiffnesses and phase angles for each direction of loading and group are presented in Tables 1 and 2. Mean (95% CI range) normalized stiffnesses and phase angles for all specimens in each group and for each direction of loading are shown in Figures 7 and 8.

Statistical analyses for stiffness revealed that the within-subjects effects was significant between groups across all directions of loading (p=0.031 for axial compression, p<0.002 for four-point bending and axial rotations). For axial compression, pairwise comparisons revealed that stiffness difference between the Intact tibiae and the Defect group was marginal (p=0.052), no significant differences were present between Intact and Fracture (p=0.207), or between Fracture and Defect (p=0.246). Pairwise comparisons between groups for four-point bending and axial rotation (both internal and external) revealed that the Intact group was
significantly stiffer than both Fracture and Defect (p<0.022 for each direction), with no significant differences existing between the Fracture and Defect (p=1).

Statistical analyses for phase angle between each group revealed that the overall effect of group was significant for axial compression (p<0.001) and four-point bending (p=0.007), with no significant effect of group overall for axial rotation (p=0.13). Pairwise comparisons revealed that no significant differences were present between the Fracture and Defect groups (p=0.451 for compression; p = 0.353 for four-point bending), however both groups absorbed significantly more energy than the Intact tibia (p<0.006 compression, p<0.025 four-point bending).

4. Discussion

In this study we investigated whether the initial stiffness and phase angle (energy absorption) achieved in a critical segmental diaphyseal defect managed with locked intramedullary nailing and impaction bone grafting in a compliant wire mesh was comparable with that of a transverse fracture of the same bone stabilized by locked intramedullary nailing. The results of this study demonstrated that the stiffness and phase angle of a critical defect with impaction bone grafting in addition to a locked intramedullary nail (Defect) were not statistically different from those of a simple transverse fracture stabilized with a locked intramedullary nail (Fracture).

These findings are encouraging for the translation and possible establishment of impaction bone grafting as an alternative technique for the management of segmental long bone defects that could allow immediate and unrestricted postoperative weight bearing, similarly to current postoperative recommendations after simple (transverse) femoral and tibial fractures treated
with intramedullary nailing [8]. This could have a significant clinical impact into the
management of these difficult cases as the current established techniques to treat critical
segmental long bone defects, distraction osteogenesis and vascularized fibula grafts, are
known to require many months of non weight bearing and partial weight bearing [4, 25].

Since all tests were conducted in position control, the maximum recorded loads varied
between each specimen and group in axial compression, four-point bending and axial
rotation. These variations were due to stress relaxation of the specimen between the initial
application of load and performing the test (in the order of a few minutes). This study
employed a repeated measures design, where each intact tibia served as its own control prior
to the creation, repair and testing of a transverse fracture and segmental defect. A state of the
art, six degree of freedom hexapod robot was used to conduct the testing where the tibia was
first subjected to axial compression, followed by axial rotation loading, without having to be
removed from the robot until the application of four-point bending.

Determining a clinically relevant effect size was not straightforward since we were seeking
information that connected initial stability/interfragmentary motion (axial compression,
bending and axial rotation) to evidence of fracture healing from in-vivo studies. We
considered the in-vivo experimental data from Klein et al., 2004 and Claes et al., 1997 to
calculate clinically significant effect sizes [22, 23]. Claes et al., 1997 used an in-vivo sheep
model to investigate the progressive weekly change in axial compressive tibial
interfragmentary motion repaired with an external ring fixator over a period of 9 weeks for
three fracture gap sizes (1, 2 and 6 mm) based on an initial interfragmentary motion of
approximately 7% or 31% strain. One of their conclusions was that initial interfragmentary
motions of less than 0.5 mm were correlated with union and healing of the fracture. However,
no data on interfragmentary motions during bending and axial rotation were provided. Klein et al., 2004 compared an unreamed tibial nail to an external fixator in an in-vivo sheep tibial fracture model. They measured the interfragmentary motions in axial compression, bending and axial rotation two days postoperatively and then conducted in-vitro tests to determine the compressive, bending and torsional stiffness of each repair after 9 weeks. They found that healing occurred in all tibiae treated with external fixation compared to the unreamed nail, where healing was delayed. The increase in initial interfragmentary motion between the two treatments were 0.8 mm for axial compression, 5.4° for axial rotation, and 2° for mediolateral bending. We chose to use this data as reasonable evidence of clinically significant initial interfragmentary motions that differentiate between successful and unsuccessful healing.

We used the largest mean (SD) stiffness data from Klein et al., 2004 for the external fixator for axial compression (1959 (634) N/mm), axial rotation (3.4 (0.3) Nm/°) and bending (32.4 (9.6) Nm/°) and assumed that a clinically significant increase in interfragmentary motions (and a corresponding decrease in stiffness) by the above-described magnitudes was required to delay healing. Based on this information, and assuming that the load-displacement behaviour in each loading direction was linear, we calculated the reduced stiffnesses to be 763 N/mm (axial compression), 0.63 Nm/° (axial rotation) and 4.74 Nm/mm (four-point bending). From these stiffness differences and assuming that the standard deviations were the same as calculated by Klein et al., 2004, we calculated effect sizes of 1.9, 9.2 and 2.6 for axial compression, axial rotation and four-point bending respectively. Since the worst-case sample size is defined by the smallest effect size, we used an effect size of 1.9. The comparison between the fracture and defect repairs is equivalent to a paired t-test and after conducting an a-priori power analysis using GPOWER, a power analysis software program [24], with a type II error of 0.2, and a Bonferroni adjusted type I error of 0.0167, we
calculated a total required sample size of 6 tibiae. Therefore, our chosen sample size of 7 tibiae would have allowed us to detect an effect size of at least 1.62 (GPOWER, paired t-test sensitivity power analysis) [14].

Sensitivity analyses revealed that the mean difference (and 95% CI range of this difference), between the fracture and defect groups for axial compression was 287 (-165, 739) N/mm and this difference was not found to be statistically significant (p=0.246). Based on the clinically relevant difference of 1196 N/mm (i.e. 1959 – 763 as per effect size calculations detailed above) determined from the data of Klein et al., 2004, we are confident that the difference between these two groups has no clinical significance. Similar sensitivity analyses were performed for four-point bending and axial rotation. The mean pairwise difference between the fracture and defect groups in four-point bending was 1.0 (-5.3, 3.2) Nm/mm, which was not statistically significant (p=1). A clinically significant difference of 26 Nm/mm was calculated for four-point bending based on the data of Klein et al., 2004, indicating that the small difference between the two groups was not of clinical relevance. For axial rotation, the calculated clinically significant difference was 2.8 Nm/°, which was orders of magnitude larger than the difference between the fracture and defect groups (0.11 (-0.60, 0.37) Nm/° for internal rotation (p=1), and 0.08 (-0.29, 0.46) Nm/° for external rotation (p=1)). Therefore, these non-significant statistical differences were also found not to be clinically significant.

We chose not to include additional groups such as defect + nail alone and defect + nail + mesh (no graft) to demonstrate the contribution to stiffness of the impacted graft, since these groups are clearly not clinically relevant. The present study design compared the nail in a fracture to a nail + impacted graft + mesh in a defect, from which we can conclude that the total nail + impacted graft + mesh construct is as stable as the nail + fracture treatment. It is
possible that the majority of the stability was provided by the nail and that the mesh and impacted graft contributed little to this stability. Although we did not test the defect + nail alone construct, an experimental study found that the torsional stability of a nail in a cadaver femur decreased with increasing length of mid-shaft defect [26]. This suggests that the impacted graft + mesh construct in our study served to increase the stability of the defect in torsion beyond that of the nail alone, since the stiffness did not decrease to clinically significant levels with the introduction of the defect and repair.

A limitation of this study was the use of sheep tibiae instead of human cadaver tibiae. Sheep tibiae were chosen for this research as their long bone size is comparable to those of humans, and this enables the use of metal implants designed for human application [27]. Also, the loads and moments acting through the sheep hind limbs were shown to be about half of those determined for humans during normal walking [28, 29]. Aside from the usual challenges with obtaining human specimens for study, along with large variations in bone quality, age, and disease state of donors, we believe that using sheep bone was justified in the context of our question, where we compared initial construct repair stiffness and were seeking an understanding of the relative magnitude of stiffness differences compared to the intact control.

We calculated the axial compressive force, bending moment and axial torque based on reported in-vivo strain measurements of sheep tibiae during gait (Gautier et al., 2000 [30]). From this study, the axial, bending and torsional tibial strains for slow walking were 140 με, 500 με, and 1,200 με respectively. We used the mean (SD) outer bone radius of 10.1 (0.8) mm and inner radius of 5.75 (1.0) mm taken from measurements of the sheep tibiae in this study to calculate average cross-sectional area, second moment of area (I), and polar moment...
of area (J). The Young’s modulus (E) and shear modulus (G) of the cortical bone were taken to be 30.9 GPa and 5 GPa respectively, based on experimental ultrasonic velocity measurements [31]. Axial compressive load, bending moment and axial torsion were then calculated using elementary formulae for stress in prismatic bars of linearly elastic materials. Table 3 compares the values used in the present study to the calculated loads and in-vivo measurements of knee loads during gait from the studies of Kutzner et al., 2010, 2013 [32, 33] based on a person having a mass of 70 kg. We note that our chosen axial load of 1,000 N is a close match to those calculated and measured in-vivo, with our applied bending moment and axial torque of 6 Nm being lower than calculated. While there are differences between bending moment and axial torque, we note that these differences range between a factor of 2-3. Given the challenges with undertaking in-vivo strain and load measurements, these differences are within a reasonable range to those moments applied and were not expected to result in significant changes in stiffness as calculated from the present study.

In a study by Cristofolini and Viceconti, 2000, the mean (95% CI) torsional stiffness of a cohort of eight intact cadaver tibiae was found to be approximately 2.6 (2.4) Nm/° [34] compared to 2.6 (0.4) Nm/° for our sheep tibiae (average of internal and external rotation stiffnesses). Similarly, Cristofolini and Viceconti, 2000 measured a four-point bending stiffness of approximately 1,300 (600) N/mm for lateral-medial bending, compared to an equivalent of 983 (160) N/mm for our sheep tibiae. The bending stiffness from our study is lower; however they applied a considerably larger bending moment of 31 Nm to their specimens, which may have resulted in a larger stiffness. Reasonable agreement with the stiffnesses measured in our study was evident, considering that their human tibiae resulted in larger 95% confidence intervals. Klein et al., 2004 reported on the stiffnesses of repaired mid-shaft sheep tibia implanted with a locked unreamed tibial nail [22]. They measured the
following mean (95% CI) stiffnesses: axial compression 1776 (1052) N/mm and axial rotation 1.4 (0.7) Nm/°. Our fracture repair group stiffnesses (Table 1) are larger for axial compression, although the confidence intervals of the means do overlap, and are comparable for axial rotation.

The reporting of energy absorption in the assessment of fracture repair biomechanics is scarce. Energy absorbed at failure of fracture fixation constructs has been reported [35-37], however this does not provide a measure of the intrinsic viscoelastic response of the construct during sub-failure loading. We included phase angle in this study to provide an improved understanding of how the viscoelastic response is affected by these treatments. While bone stores energy more like an elastic material, our measured phase angles (Table 2) are consistent with those reported for soft tissues such as the medial collateral ligament (approximately 4° [38], 6° - 14° [39]), and the human intervertebral disc (3° - 11° [40]). The results for phase angle revealed that the Fracture and Defect groups absorbed more energy (i.e. larger phase angle) compared to the Intact groups, with the exception of axial rotation where no significant differences were found. The largest changes in phase angle were found in four-point bending, where almost a doubling in energy absorption occurred in the Fracture repair group compared to the Intact tibia. After repairing the Defect, a trend of decreased energy absorption by approximately 35% occurred compared to the Fracture group, although this was not significant. The phase angle of the hexapod itself was not evaluated prior to this study. Since we have decoupled the loading frame (i.e. actuators) and load cell from the sensing frame (i.e. linear encoders and specimen testing region), we do not expect any system compliance that would contribute to the measured phase angle. In fact, a systemic phase lag exists in conventional materials testing systems due to the compliance/hysteresis of the load.
cell, which is included in the measurement of specimen deformation. This phenomenon does not occur in our hexapod robot.

Impaction bone grafting is an established technique used to reconstruct bone stock while providing immediate stability in revision hip replacement surgery [13-15]. Recently, a few studies have investigated the feasibility of this technique to reconstruct posttraumatic segmental long bone defects. Bullens et al., 2009 investigated the stability of impacted morsellized bone graft in isolation as an in vitro reconstruction model of segmental diaphyseal defects [41]. They showed that the stability of the impacted bone graft was not influenced by the type of cage that the graft was contained in, but rather that the quality of the fit between the cage used and the bone reconstructed was important. As opposed to that study, the present study investigated the stability of the entire composite of long bone with segmental defect reconstructed with intramedullary nailing and impaction bone grafting. In addition, the present study investigated the stability of the reconstruction using human bone graft and used an impaction grafting technique that is applicable to defects of various shapes, as opposed to the study of Bullens et al., 2009 [41], which used bovine bone graft and an impaction grafting technique that is applicable only for defects that are perfect cylinders. Bullens et al., 2009 have also demonstrated in an animal model that segmental bone defects can be treated successfully with impaction bone grafting of the defect contained in a titanium cage with intramedullary nailing [42]. When the animals were sacrificed at 26 weeks the defects had healed and the bones had regained 67% of the mechanical torsion strength of the intact bone. In a further study, the same group investigated the role of static or dynamic mode of nail fixation on the healing of such defects and found no statistically significant difference [43]. The authors concluded that the impaction bone grafting is a viable option to reconstruct segmental bone defects in an animal model. Solomon et al., 2013, in a case report on three
patients, showed that impaction bone grafting was a viable option to reconstruct segmental bone defects in human femoral non-unions, where the defects were contained in compliant wire mesh [16]. One advantage of the compliant wire mesh is that, as opposed to the titanium cages that can only be used to reconstruct cylindrical defects, the compliant wire mesh can also be used to contain the irregular metaphyseal and epiphyseal defects [16]. Besides being successful in reconstructing post-traumatic segmental long bone defects in humans, this study also showed that immediate weight bearing as tolerated after impaction bone grafting was possible, and did not compromise the results [16]. Therefore, by demonstrating that the initial mechanical stability of a segmental diaphyseal defect repaired with an intramedullary nail and impaction bone grafting is similar to the one of a simple transverse diaphyseal fracture repaired with an intramedullary nail, the present study represents an important step in the successful translation of this technique for routine use in human trauma. However, before a possible routine use of such a technique to reconstruct posttraumatic long bone segmental defects in humans, many other factors need to be given consideration. Such an important factor is the presence of an intact healthy periosteum which was shown to be essential for the healing of large long bone segmental defects in an animal model [44]. Since the presence of a healthy periosteum will influence the vascularization and remodelling of the impacted allograph, questions like the timing of the application of such a reconstructive technique in the acute or subacute phase of the injury causing the defect, need to be answered. If proven, this technique could have multiple benefits for both the patient and the health care system, having the potential to reduce the high complication rates associated with the current established technique to treat critical segmental long bone defects [4, 25], and the advantage of allowing for immediate weight bearing as tolerated.

5. Conclusions
This study demonstrated that the initial viscoelastic properties (mechanical stiffness and energy absorption), in axial compression, four-point bending and axial rotation, of a critical tibial defect stabilized with impaction bone grafting, in addition to a locked intramedullary nail, was not clinically significantly different to that of a simple transverse fracture stabilized with a locked intramedullary nail.
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presence of periosteum is essential for the healing of large diaphyseal segmental bone defects
Figure Legends

Figure 1. a) and b) Front and oblique photographs of the alignment device used to ensure that the potted ends of the tibia were parallel to each other and aligned with the vertical axis of the hexapod robot. The alignment device consisted of a vertically orientated precision linear rail and bearing block to which each fixation cup was mounted.

Figure 2. a) Intact tibia in the hexapod robot ready for axial compression (1000 N) and axial torsion (6 Nm) testing. The specimen was inserted between the rigid base and mobile upper plate (arrow). A six axis load cell (indicated in photo behind actuator frame) is connected between the mobile upper plate and actuator frame. Six ball screw actuators produce the required displacement or rotation. Displacements and rotations of the specimen were directly measured by six linear optical encoders (arrow) that were positioned independently to the loading frame. Therefore, system compliance was eliminated from the measurement of specimen behavior. b) Intact tibia placed in a four-point bending device in the hexapod robot. The distance between the lower support rollers was 180 mm, and 60 mm between the upper two loading rollers. A bending moment of 6 Nm was applied to each specimen.

Figure 3. a) 20 mm segmental defect of a sheep tibia contained with a compliant wire mesh and cerclage wires. b) The same bone after the defect was filled with impacted bone allograft. c) Close up view of the reconstruction. d) and e) The reconstructed bone defect after removal of the nail and sheep tibia at the end of biomechanical testing.

Figure 4. Example plot of axial compression loading behavior between each group (Intact, Fracture, Defect).
Figure 5. Example plot of four-point bending loading behavior between each group (Intact, Fracture, Defect)

Figure 6. Example plot of axial rotation loading behavior between each group (Intact, Fracture, Defect).

Figure 7. Mean (95% CI) normalised stiffnesses expressed as a fraction of the intact tibia stiffness for each direction of loading. Significant differences relative to the Intact group are denoted by asterisks.

Figure 8. Mean (95% CI) normalised phase angles expressed as a fraction of the intact tibia phase angle for each direction of loading. Significant differences relative to the Intact group are denoted by asterisks.
Figure 1b
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Figure 4

Comparison of Treatment Groups for Specimen 5 in Axial Compression

Axial Displacement (mm)

Compressive Force (N)

C5I, Intact: Stiffness = 3597 N/mm
C5FN, Fracture: Stiffness = 2276 N/mm
C5DG, Defect: Stiffness = 1741 N/mm

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Figure 5
Comparison of Treatment Groups for Specimen 5 in Four-Point Bending

Symbols:
- BSi: Intact
- BSFN: Fracture
- BSDD: Defect

Stiffness:
- BSi: 30 Nm/mm
- BSFN: 8 Nm/mm
- BSDD: 12 Nm/mm
Figure 6
Comparison of Treatment Groups for Specimen 5 in Axial Rotation

Axial Rotation (°)
Axial Torque (Nm)

Comparison of Treatment Groups:
- T5I: Intact
- TSFN: Fracture
- TDGG: Defect

Stiffness values:
- T5I: 2.41 Nm/° and 3.16 Nm/°
- TSFN: 0.859 Nm/° and 1.27 Nm/°
- TDGG: 1.28 Nm/° and 0.729 Nm/°
Mean (95% CI) Normalized Stiffnesses for Each Direction of Loading

Axial Compression

Normalized Stiffness

Intact | Fracture Treatment Group | Defect

Four-Point Bending

Normalized Stiffness

Intact | Fracture Treatment Group | Defect

Axial Rotation - Internal

Normalized Stiffness

Intact | Fracture Treatment Group | Defect

Axial Rotation - External

Normalized Stiffness

Intact | Fracture Treatment Group | Defect
Mean (95% CI) Normalized Phase Angles for Each Direction of Loading

Axial Compression

Four-Point Bending

Axial Rotation
Table 1. Mean (95% confidence interval range) stiffness for each direction of loading and treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Axial Compression (N/mm)</th>
<th>Four-point Bending (Nm/mm)</th>
<th>Axial Rotation (Internal) (Nm/°)</th>
<th>Axial Rotation (External) (Nm/°)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact</strong></td>
<td>3766 (3010, 4522)</td>
<td>29.5 (23.5, 35.5)</td>
<td>2.60 (1.88, 3.33)</td>
<td>2.69 (1.92, 3.47)</td>
</tr>
<tr>
<td><strong>Fracture</strong></td>
<td>2710 (1828, 3593)</td>
<td>10.3 (7.0, 13.6)</td>
<td>1.07 (0.73, 1.42)</td>
<td>1.13 (0.91, 1.35)</td>
</tr>
<tr>
<td><strong>Defect</strong></td>
<td>2423 (1585, 3261)</td>
<td>11.3 (9.8, 12.9)</td>
<td>1.18 (0.96, 1.41)</td>
<td>1.05 (0.86, 1.23)</td>
</tr>
</tbody>
</table>

Notes: Sample size: N = 7 tibiae

Intact represents the intact tibia

Fracture represents a mid-diaphyseal transverse fracture stabilised by intramedullary nailing

Defect represents repair of a 20 mm segmental defect stabilised with a nail and impacted bone graft in a compliant mesh
Table 2. Mean (95% confidence interval range) phase angles for each direction of loading and treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Axial Compression (°)</th>
<th>Four-point Bending (°)</th>
<th>Axial Rotation (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact</strong></td>
<td>7.6 (6.9, 8.3)</td>
<td>5.1 (4.4, 5.8)</td>
<td>9.6 (7.1, 12.1)</td>
</tr>
<tr>
<td><strong>Fracture</strong></td>
<td>10.5 (9.1, 11.9)</td>
<td>10.1 (7.1, 13.2)</td>
<td>8.7 (6.9, 10.5)</td>
</tr>
<tr>
<td><strong>Defect</strong></td>
<td>11.6 (9.3, 13.9)</td>
<td>8.2 (7.0, 9.4)</td>
<td>11.6 (9.4, 13.8)</td>
</tr>
</tbody>
</table>

Notes: Sample size: N = 7 tibiae

Intact represents the intact tibia
Fracture represents a mid-diaphyseal transverse fracture stabilised by intramedullary nailing
Defect represents repair of a 20 mm segmental defect stabilised with a nail and impacted bone graft in a compliant mesh
Table 3. Summary of loading magnitudes used in the present study for each loading direction compared to calculated loads based on in-vivo tibial strain measurements in sheep during gait [30] and from telemeterised in-vivo measurements of human knee joint loads during gait [32, 33].

<table>
<thead>
<tr>
<th>Loading direction</th>
<th>Present study</th>
<th>Calculated loads</th>
<th>In-vivo loads</th>
</tr>
</thead>
<tbody>
<tr>
<td>Axial compression (N)</td>
<td>1,000</td>
<td>939</td>
<td>1,031-1,373</td>
</tr>
<tr>
<td>Four-point bending (Nm)</td>
<td>6</td>
<td>11</td>
<td>17-19</td>
</tr>
<tr>
<td>Axial torsion (Nm)</td>
<td>6</td>
<td>17</td>
<td>7-9</td>
</tr>
</tbody>
</table>