Peer Review File

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Reviewer A

Very well constructed study and statically well organized

Reply: The authors would like to thank Reviewer A for their time reviewing this study.

Reviewer B

The study that you have reported in this paper is important and clinically relevant. Therefore, I would like to congratulate you on a wise selection of the study question. Please do not be discouraged by my comments, I believe it is an important study and I hope you will be able to improve it with the help of my comments.

Reply: The authors would like to thank Reviewer B for their time reviewing this study and will make every effort to provide complete responses and edits accordingly.

Study Hypothesis:

In the introduction you did not mention any reference about the fact that nailing can result in lower variance of mechanical properties across specimens. Therefore, it may indicate that this hypothesis was formulated after you have seen the results. Please defend.

Reply: Thank you for the thorough examination of the study design. It has not been previously reported specifically that nails would cause lower variance, however we did hypothesize that the nail would be comparable or superior to that of a lateral locking plate and due to the axial location of the implant, we suspected that it would be a superior construct for a preclinical model. The observation that a lower variance occurred in the IMN group was not specifically hypothesized, but rather a reported finding, and therefore the clause 'with less variance of mechanical properties across specimens.' has been removed.

Changes in text: Line 52-53 ~~'with less variance of mechanical properties across specimens.~~

Line 57: You wrote that the sheep were without history of any orthopedic diseases, but actually do you have any prove of that? If you took the hindlimbs from slaughterhouse, just report it.

Reply: The sheep limbs that were used were taken from sheep that had been euthanized for a separate orthopedic implant study involving the axial skeleton, and therefore their histories were known to the researchers.

Changes in text: Line 56-57 Sixteen ovine hindlimbs were acquired from skeletally mature Columbia x Rambuoillet research sheep (body weight average 70kg) with no known history of orthopedic disease, which had previously been enrolled in a separate study of the spine. The metatarsi were visually inspected prior to implant placement to verify that no macroscopic abnormalities were observed prior to inclusion.

Line 57: standard deviation must be reported with the average bodyweight.

Reply: Approximate standard deviation is provided. The study sheep were required to be as uniform as possible for in vivo testing, however exact weights of the sheep used are unknown for this study purpose. The implication for relevance in this mechanical study is considered to be minimal due to the known history and selection of the research herd.

Changes in text: Line 57 estimated average 70±3 kg

Line 68: Please make it clear. Did you start with the osteotomy and then nailing?

Reply: The procedure is first described in brief and then detailed method is provided. Clarification has been added to the text. We did do the osteotomy first in the IMN group and then placed the IMN using the custom cylindrical guide. This enabled consistency in the osteotomy gap creation without risking damage to the IMN

Changes in text: Line 68 Detailed implantation methods are provided as follows:

The hindlimb was stabilized to simulate a dorsally recumbent animal. The limb was clipped free of wool. A 5cm incision was made through the skin over the lateral aspect of the estimated midpoint of the metatarsus. The periosteum was elevated circumferentially and a 3cm osteotomy was made first using an oscillating saw in the mid-diaphysis of the metatarsus. A custom cylindrical guide was then placed enabling consistency in the osteotomy gap creation without risking damage to the IMN after placement.

Line 93: The IFU acronym was not explained before.

Reply: An acronym has been added to the initial usage in line 78.

Changes in text: Line 78 The IMN was then applied using the I-Loc® IM Fixator (BioMedtrix) system according to the Manufacturer's Instructions for Use (IFU)

Line 100: No information about the LCP implants? Provider?

Reply: Provider has been added.

Changes in text: Line 100 A 3.5mm, 9-hole LCP (Depuy Sythes) was applied to the lateral aspect of the metatarsus

Lines 100 – 106. I am not sure if this is an appropriate way of placing an implant. I am afraid that putting a screw twice in the same hole would degenerate bone-screw interface thus degenerate the mechanical properties of the construct. Please convince me if I am wrong, ideally with references.

Reply: Thank you for this observation. Routinely, in clinical settings, screws will be removed and replaced through the same hole for either optimization of implant placement or for changing the length of the screw to avoid surrounding anatomical structures. This practice has not been shown to weaken the construct, so long as care is taken to replace the screw without

stripping the threads of the bone or the implant. Once a thread hole is drilled and tapped, no further tapping or drilling takes place. Careful removal and replacement does not alter the bone-screw interface. In the use of locking compression plates, the strength of the construct is largely dependent on the screw plate interface, and far less reliant on the bone-screw interface. (Alisdair R. MacLeod, A. Hamish R.W. Simpson & Pankaj Pankaj (2015) Reasons why dynamic compression plates are inferior to locking plates in osteoporotic bone: a finite element explanation, Computer Methods in Biomechanics and Biomedical Engineering, 18:16, 1818-1825, DOI: 10.1080/10255842.2014.974580)

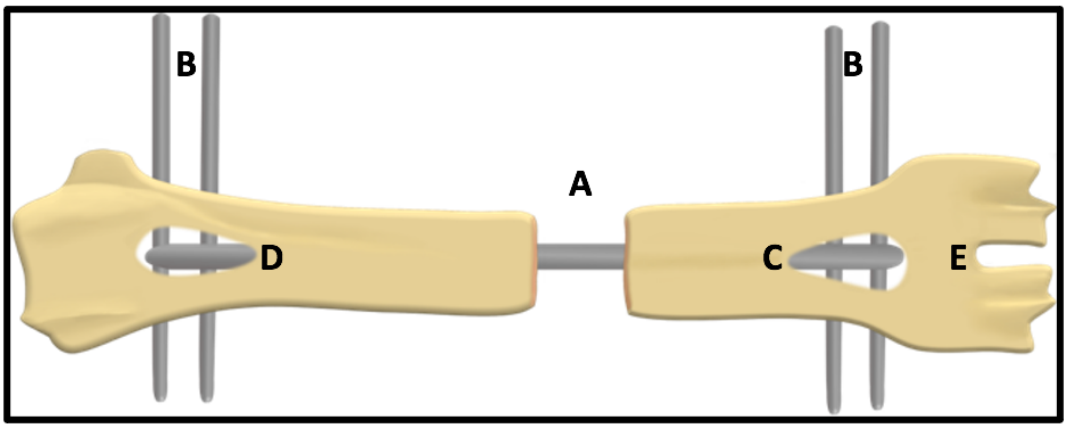
For these reasons, we chose to place the locking plate on an intact metatarsus for ideal, consistent and anatomically correct implant placement. Then we removed the plate in order to make an osteotomy without inadvertently damaging the implant with the bone saw. Then we replaced the implant on the ostectomized metatarsus using the previously drilled holes. This is the same process used in the Yang et al. reference included in the study.

General questions to biomechanical testing: Please provide some reasoning why have you decided to work with strain gauges? In my opinion it would be easier to reach similar conclusions just by evaluating the machine data (calculate stiffness of the construct?) Why was this not done?

Reply: Thank you for your question on this topic. We had hoped to provide detailed comparison of strains specific to the materials of both the bone and the implant in presenting this study. We agree that the machine data for overall construct stiffness would have been a helpful addition, however this data was not captured during the study. We feel that the strain gauge data is still pertinent and provides adequate comparison for the conclusions made in this manuscript.

Please provide pictures from the setup of the biomechanical tests. Also, how and where the strain gauges were placed.

Reply: No pictures were available of the biomechanical tests, however the description of the setup is consistent with previously described methods of testing for similar constructs. The strain gauge placements are indicated in Figure 3 included in the manuscript.



Generally working with strain gauges (SG) is quite challenging, therefore you should provide a protocol how you have placed the SG on the bone it? How/if did you prepare the surface of the bone? Which glue did you used? This in my opinion should be detailed to an extend that would allow for repeating the study.

Reply: Thank you for this attention to detail. The exact method used for fixation of SG has been added and is consistent with previous studies cited within the manuscript. (Gadomski, B. C., McGilvray, K. C., Easley, J. T., Palmer, R. H., Ehrhart, E. J., Haussler, K. K., … Puttlitz, C. M. (2014). *An In Vivo Ovine Model of Bone Tissue Alterations in Simulated Microgravity Conditions. Journal of Biomechanical Engineering, 136(2), 021020.* doi:10.1115/1.4025854)

Changes to text: Lines 109- 117- The metatarsal constructs were harvested and fitted directly to the bone with three multidirectional strain gauge rosettes (Micro Measurements, C2A-XX-062WW-350) on both the proximomedial and distomedial metatarsus approximately 3cm from the proximal and distal most ends of the metatarsus stabilized with either the IMN or LCP. A third gauge was placed on the lateral aspect of the IMN or medial aspect of the LCP at the center of the defect site. The strain gauge rosettes were applied directly to the bone or metal of the construct, depending on location. Bone was prepared by removing all periosteal tissue then sanding and dehydrating the application area with ethanol. For application to the hardware, the metal surface was sanded with fine grit sandpaper and cleaned with a strain gauge manufacturer supplied solution. For the LCP, the screw hole at the center of the defect was filled with an elastomer, then sanded flush to the surface to ensure the strain gauge was uniformly attached to the surface. The stiffness of the elastomer was negligible compared to that of the plate contributing negligible resistance to the applied loads while experiencing similar strains. The rosettes were bonded to all surfaces using ethyl cyanoacrylate (Loctite Super Glue Precision Pen (Product# 2066118)).

The bones were fixed at the proximal and distal ends in custom-built potting boxes using epoxy (M325, Smooth-On, Inc., Macungie, PA). The constructs were tested in a servo-hydraulic material testing frame. (MTS Landmark, MTS Systems Co., Eden Prairie, MN).

You wrote that the loads apply in your study corresponds with the physiological loadings. Please provide a reference that it is the case.

Reply: The loads applied were calculated based on the average weight of the specific breed of sheep used in this study as well as the understanding that the hindlimb would have less associated force than a forelimb in a quadruped. The explanation of calculations is included in lines 128-131. The authors are unaware of an exact reference for the in vivo biomechanics of the normal physiologic loading of an ovine metatarsus. The biomechanical testing specifications were duplicated from previous studies referenced in the manuscript (Gadomski, B. C., McGilvray, K. C., Easley, J. T., Palmer, R. H., Ehrhart, E. J., Haussler, K. K., … Puttlitz, C. M. (2014). *An In Vivo Ovine Model of Bone Tissue Alterations in Simulated Microgravity Conditions. Journal of Biomechanical Engineering, 136(2), 021020.* doi:10.1115/1.4025854; Yang YP, Labus KM, Gadomski BC, Bruyas A, Easley J, Nelson B, et al. Osteoinductive 3D printed scaffold healed 5 cm segmental bone defects in the ovine metatarsus. Sci Rep [Internet]. 2021;11(1):1–12. Available from: https://doi.org/10.1038/s41598-021-86210-5

Changes to text: Line128-129- Due to lack of reported in vitro physiologic loading of the ovine metatarsus, compression values were estimated to approximate a 70kg sheep bearing roughly 70-75% body weight on the limb.

Line 142: It is a good practice to report the software that you have used for statistics .

Reply: GraphPad Prism was used. This has been added to the text.

Changes to text: 144-145- A Wilcoxon rank-sum test was used for statistical comparisons when data were not normally distributed (GraphPad Prism).

Line 176 -178: Here you provide information about overall construct stiffness. Term "stiffness" was used once in the abstract and once in discussion. Not in M&M nor results sections. very confusing. You should clearly define what you evaluate in the materials and methods, then report this in results section. All the time the vocabulary should be consistent, otherwise the content is difficult to understand.

Reply: In this study, the strain measured in the material components of the construct was used to define stiffness by displaying relative deformation of the constructs under loading conditions. In an effort to bridge this vernacular, additional explanation has been added into the discussion.

Changes to text: Line 175-177- The stiffness of constructs in this study was defined as the measured strain experienced by the material components of the constructs, including the bone and implant. Greater strain implies greater deformation and therefore reduced stiffness of the construct. It is therefore possible to conclude that an IMN is an acceptable alternative in a metatarsal osteotomy model when compared to the laterally placed LCP.

Lines 188 to 197: I believe this information belong rather to the introduction, as they do not discuss the results of your study in relation to the studies that you are mentioning.

Reply: This information is introduced in the introduction (Lines 29-38) and was revisited in the discussion for ease of reference when evaluating our study findings. This section can be deleted if there is no perceived value to the repetition.

Line 198: It is very important that to report well your protocol of strain gauge placement. My concern would be: did the person who was placing the strain gauge started from the LCP group, hence this high variability among specimens due to a learning curve in how to place the strain gauges? Please defend.

Reply: The protocol for strain gauge placement has been added to the text as per responses above. The protocol used is consistent with previously utilized protocols in citations provided . The placement was performed by an individual familiar and practiced with said protocol., and therefore we do not believe that the variability is subject to learning curve differences. All placements throughout the LCP group were uniform between constructs. (Gadomski, B. C., McGilvray, K. C., Easley, J. T., Palmer, R. H., Ehrhart, E. J., Haussler, K. K., … Puttlitz, C. M.

(2014). *An In Vivo Ovine Model of Bone Tissue Alterations in Simulated Microgravity Conditions. Journal of Biomechanical Engineering, 136(2), 021020.* doi:10.1115/1.4025854));

Reviewer C

Introduction:

Overall the introduction is well written and informative. I just have a few small comments as below:

Line 34- extra space after recently

Line 37 - "has" should be "have"

Reply: these edits have been executed as described.

Materials and methods:

The authors do a fantastic job of describing their methodologies. They go into great detail describing the operative procedures for the intramedullary nail as well as for the LCP. Additionally, measurement methodologies are described in excellent detail. Statistical analysis seems sound, they tested first for normality and then used T test for normally distributed data and Wilcoxon rank sum for non-normally distributed data. All this seems fine to me and indeed more detailed than in other manuscripts I have reviewed.

Reply: Thank you for your complimentary and detailed review.

Results:

Results are clearly presented. The authors find that the magnitude of strain differences amongst the specimens is lower in the IMN group. They record higher stiffness values for the IMN. As an orthopedic surgeon, I find these results to be of interest. The authors report that their methodology for placement of LCP involved bicortical, locking head srews. Traditionally our orthopedic teaching would suggest that bicortically placed locking screws would be an extremely stiff construct, likely more stiff than a IMN construct.

Reply: Thank you for this commentary. We believe this to be due to the difference in axial implant location of IMN vs abaxial placement of a lateral LCP under direct compression.

This is an important finding if the ovine osteotomy model is to be used as a fracture model, especially with regards to lower variability in strain experienced at the fracture site. If there is significant variability in strain across specimens, obviously this would distort results.

Discussion:

Discussion is well laid out and I have no grammatical edits. The authors do a good job of describing previous work. The authors appropriately described their limitations, such as the fact that the ex vivo nature of the study limits the ability to conclude definitively the reduction

in variability of results in a fracture healing model. I do take issue with their statement that different size/thickness of hardware would almost surely change the measured strains, but not necessarily the differences between an LCP and IMN. While differing size of hardware may not change the measured differences between an LCP and IMN, the size of the osteotomy gap and the specific fracture principles may. For example, a comminuted fracture will exhibit lower strain when fixed with a bridge plate construct. In this instance, the authors chose to leave the middle three holes of the LCP unfilled. Obviously, this will greatly decrease the stiffness of the construct. I'm not necessarily saying this negates the results, just saying that the generalizability may be affected by the specific fracture model that they created.

The study by Yang et al which the authors mention, for example, utilized a Bony defect measuring 5 centimeters as well as the IMN which they then applied rh BMP 2 to evaluate for its efficacy in aiding fracture healing utilizing a 3D printed scaffold. So obviously there is lack of standardization across methodologies in these ovine studies, and this is probably worth mentioning in the discussion.

Reply: Thank you for this commentary. There is indeed lack of standardization of methodology in this area and we have added commentary to reflect the points that you have raised.

Changes to text: Lines 236-238- Only a single size of LCP and IMN were analyzed for this study, different size/thickness of hardware would almost surely change the measured strains. Due to a lack of standardization of fracture healing models in the literature, further examination of these differences may provide a more complete understanding of the ideal methodology for repeatability and consistency between constructs.

My conclusions:

overall, I feel that this is a very well done study. It adds to the literature in a necessary way if the IMN metatarsal model is to take the place of the LCP model (or at least is to be used more commonly in lieu of the LCP model). The study is well written and the methodologies are extremely sound. The study is novel to my literature review. I recommend this study for publication with minimal revisions.

Reply: Thank you for your detailed review and contribution to this manuscript.