Temporal *in vivo* Monitoring of Fracture Healing in Sheep Tibiae


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**Introduction**

Not only size and shape of a healing bone, but also stiffness and strength undergo a certain change due to callus formation. Different treatment regimes can lead to different healing progression, which in turn lead to different patterns in the temporal history of physical parameters. Structural stiffness of healing bone is a parameter, which increases with time, and clinically this change is apparent to both the patient and the surgeon1. Measurement of the stiffness of the healing bone *in vivo* delivers quantifiable, reproducible, technical values, which serve as a tool for the evaluation of the way in which different treatments may modulate bone healing. We wish to optimize this experimental method by use of a modified fixation device for accuracy and ease of use and by minimizing the number of animals necessary. What makes our method special is the ability to apply a known force directly to the bone fragments in order to measure stiffness. This reduces inaccuracies due to force dissipation into the surrounding soft tissues or to the use of ground reaction forces as input. In contrast to other methods, this method allows measurement of stiffness between short intervals, which leads to a temporal overview of bone healing in each animal. Furthermore, if the healing of an ovine tibial osteotomy, as assessed by callus stiffness, is not affected by similar healing of the contralateral bone, a model could be established, where the opposite limb might also be used, as an experimental sample. This approach could minimize the number of animals needed. The rationale of this project was, to determine whether or not an animal model could be established, where both limbs could be used as separate experimental samples.

**Method**

For increased reproducibility, instead of a fracture, a 3mm wide osteotomy was created on the midshaft portion of the sheep tibia by means of a custom made saw- guide. For practical stiffness measurements, a known force $F$ is applied and the resultant displacement $x$ is measured. The term $dF/dx$ is then termed stiffness $k$ of the bone. For comparisons however, normalized values are easier to use. Therefore, the stiffness values obtained were normalized to the initial stiffness of the intact bone resulting in a dimensionless factor $f_S$ given by $f_S = k_{pre-op} / k_{post-op}$ (stiffness factor = stiffness pre-op / stiffness post-op). The temporal assessment of stiffness *in vivo* is dependent upon a reproducible, rigid connection to the bone segments on either side of the osteotomy. For this purpose a stiff external fixator was constructed and mounted using 2 bicortical Schanz’ screws 5x100mm (#294.782, Synthes® AG, Switzerland) in each bone fragment. The fixator was constructed with a removable central section between the two inner Schanz’ screws. When the section is removed, this allows a clear view of the osteotomy gap for X-rays and CT-scans. Additionally, it allows free movement of the fracture- ends during stiffness measurements while the two other plates remain connected to the Schanz’ screws and thus to the bone. The force needs to be applied such that the bone fragments receive the full amount of force applied and measured and bend consistently in the same plane. In contrast to other authors2, we applied the force to the bone using a removable loading frame with a hinge, which converted a linear force into a pure moment around the neutral axis of the osteotomy. The device was displacement controlled (opening of the fracture gap $\leq 1.5^\circ$) with force cutoff at 100 N. Force $F$ was measured with a piezoresistive force transducer (Type 9021, KISTLER Instrumente AG, Winterthur, Switzerland) and the displacement $x$ with a resistive displacement transducer (LP-100FE, Pewatron AG, Wallisellen, Switzerland). Both values were then fed into a data acquisition system (Measurements Group, Messtechnik GmBH, Graefelfing, Germany) displayed and processed in real time and collected in a PC- based system. The stiffness was calculated from the force versus displacement curve stored on the PC using a spreadsheet program (Microsoft® Excel 95). We used 2 study groups of 5 Swiss mountain sheep each. In group A, only the right tibia was
treated. These animals survived for 10 weeks. In group B, both hind limbs were treated, and the animals survived for 12 weeks. The results of group B were put in 2 subgroups Br (right leg) and Bl (left leg). Weekly measurements of the bending stiffness of the healing bone were performed and the stiffness factor calculated. Clinical radiographs were taken every week to observe any unusual conditions. Those animals would have been eliminated from evaluation. For statistical comparisons, we used repeated measures as well as multivariate analysis of variance. All weekly values of the subgroups Br and Bl were compared up to the 12th week and a comparison of Br vs. A and Bl vs. A was made up to the 10th week.

Results

During the observation period, we did not face any unusual conditions and no animal had to be excluded from the study. In all animals, an increase in stiffness occurred not earlier then two weeks after treatment. Between week 2 and 4, an increase could clearly be measured (fS: 0.06±0.03 at 3 wks). This was followed by a more distinct increase in stiffness during week 4 and 6. The stiffness factor fS was 0.66±0.16 at six weeks. After six weeks, the increase in stiffness of Group A slowed down and reached an fS of 0.79±0.15 at 8 weeks where the stiffness begun to level up on that value. After a comparable pattern in the first six weeks, the stiffness factor of group B reached a value of 0.74±0.15 (right) and 0.7±0.22 (left) after the 7th week. The stiffness was, in the latter time coarse, not as consistent as in group A. It fluctuated at 0.74±0.04 (right) and 0.72±0.04 (left).

The multivariate tests as well as the repeated measures ANOVA revealed no statistical difference in temporal stiffness between Br and Bl as well as between Br and A and Bl and A.

Discussion

The temporal stiffness of sheep tibiae with a 3mm-osteotomy gap was measured by applying a known displacement force directly to the bone over a geometrically defined framework. This increases the reproducibility of the measurements, due to constant lever arms of the forces applied. Additionally this avoids errors due to forces distributed into the surrounding soft tissues of the bone or due to the use of ground reaction forces as input. The pattern of stiffness of a healing, osteotomized sheep tibia, expressed by a stiffness factor, shows an s-shaped curve where an up-bend could be detected at around 2 weeks. A steady increase was observed between the 4th and 6th weeks. Leveling of the stiffness could be detected after 7 weeks. No statistical difference in temporal stiffness could be observed in either experimental group. Therefore, the measurement method presented allows right-left comparisons in the same animal to lower the number of animals used, as well as the costs involved.


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