Stiffness of callus tissue during distraction osteogenesis

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Accepted: 10 November 2009

Summary
Introduction: The treatment of limb length discrepancies by distraction osteogenesis represents a significant challenge of predicting the load-bearing capacity. Today, in vivo stiffness measurements by applying compressive, bending or torsional stress on the callus tissue are quantitative methods. Therefore, it is relevant to know how regenerating bone tissue regains its various stiffness characteristics. Knowledge of the development of each type of stiffness is important in order to prevent an over- or underestimation of the actual patients’ load-bearing capacity.

Hypothesis: Various types of stiffness are supposed to evolve similar during consolidation.

Materials and methods: In this ex vivo study, an analysis of torsional, compressive and bending stiffness of callus tissue during consolidation was performed on 26 sheep tibiae after distraction osteogenesis.

Results: This study indicates differences within the quantity of stiffnesses during consolidation.

Discussion: Thus, in vivo stiffness measurements have to be interpreted carefully in order to prevent false estimation of the load-bearing capacity of new bone.

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Introduction

Distraction osteogenesis is a successful treatment for correcting limb length discrepancies and for reconstruction of bone defects. During this treatment, the surgeon faces the challenge of predicting the load-bearing capacity of the callus tissue. Information about the healing process is necessary to adjust patients load-bearing capacity as well as the time for removal of the external fixator. Currently, X-ray follow-
ups and manual clinical examination are commonly used to estimate the status of regenerative callus tissue in clinical daily routine. However, both methods have been proven to be inappropriate [1–3]. To address this shortcoming, the measurement of bone stiffness was proposed to support the orthopaedic surgeon with this task of predicting the load-bearing capacity. With stiffness measurements authors try to use a proven correlation between stiffness and strength. For in vivo stiffness measurements only minimal, reversible deformation of the callus tissue in either torsion, bending or axial compression occurs. In previous ex vivo studies, a significant correlation between compressive, torsional and bending stiffness in anteroposterior and mediolateral orientation and the load-bearing capacity was proven [4]. Thus, all mentioned types of stiffness seem to be good predicting parameters of the load-bearing capacity. Various principles of in vivo stiffness measurements were published in earlier studies: torsional stiffness [5–6], bending stiffness [7–11]) and compressive stiffness [8]. Richardson and Dwyer [9,11] revealed by empirical studies a safe point for removal of the external fixator as soon as the regenerating bone has a certain value of bending stiffness. Cunningham et al. [8] determined a mechanical end-point as well for compressive stiffness. These results showed a safe removal of the external fixator as soon as the regenerated bone recovers a certain fraction of the stiffness-characteristics of the contralateral, intact tibiae [12]. However, up to today no study exists investigating possible difference in types of stiffness during consolidation. During motion bony substance is loaded by a combination of torsional, compressive and bending stress. It is not distinctive whether all types of stiffness represent the true stiffness appropriately. It is important to know whether bone tissue regains similar amounts of the various types of stiffness during consolidation or whether these amounts differ. According to this, in vivo measurements have to be judged appropriately to predict the load-bearing capacity and to avoid a possible over- or underestimation of the actual strength of new callus tissue.

In this study, a comparison of the regained amount of torsional, compressive and bending stiffness of the callus tissue during consolidation was conducted. Furthermore, an approximation of the development of torsional, compressive and bending stiffness in relation to the load-bearing capacity during consolidation was determined. The setup of this study does not admit information about the evolution of stiffness over time. However, the samples represent different levels of the load-bearing capacities and thus characterize different states of bone healing. Thus, this study presents information about the interpretation of in vivo stiffness measurements for predicting the load-bearing capacity of regenerated bone. By now no data were previously available comparing various kinds of stiffness during callus distraction and bone healing.

**Hypothesis**

During consolidation of callus tissue after fracture or callus distraction, the callus tissue regenerates continuously. Thus, it can be hypothesized that different type of stiffness regenerate similarly during consolidation.

**Methods**

Diaphyseal osteotomies were performed on 26 right tibiae of mature, female domestic sheep ranging between 2 and 4 years of age. The experiments were conducted in accordance with the approval of the German federal animal welfare legislation.

As the first step of operative procedure, the tibiae were stabilized using an Ilizarov external half-ring fixator mounted by six bicortical half-pins to the tibia. This frame consisted of three rings and one custom-made device for torsional in vivo stiffness measurement connected by three rods as described previously [5,6]. As the second part of the surgery, a mid-diaphyseal osteotomy was performed using an oscillating saw, followed by the control of complete transection. After surgery, the sheep were allowed unrestricted motion and loading. Following a 4-day latency period, the tibiae were lengthened at a rate of 1.25 mm per day in two increments (0.75 mm and 0.5 mm) for 20 days. On the 74th day, the sheep were sacrificed and both tibiae were dissected at the knee, harvested and labelled. This timing was evaluated before in a pilot study. After 74 days of consolidation, 50–70% of the characteristics of intact tibiae were regained by the distracted limb. After the external fixator, half-pins and soft tissue were removed. The ends of the tibiae were embedded in PMMA (Technovit® 4004, Heraeus Kulzer, Wehrheim, Germany) and frozen at −20 °C until tested. Before embedding, the distraction gap was marked precisely using X-ray examination. The callus tissue bridging the distraction gap was located by its higher X-ray transparency and inhomogeneous bony structure. Furthermore, specimens were reproducibly embedded over a standardized length close to the distraction gap in an alignment jig in order to assure proper alignment and to avoid artifacts due to gripping or grasping of specimens. The contralateral, non-distracted tibiae served as reference for biomechanical testing and represented intact physiologic tibiae. As a result of a parallel study, callus tissue was treated with different combinations of growth factors and carrier materials, resulting in four treatment groups plus the additional contralateral control group.

Before biomechanical testing, the frozen tibiae were thawed at room temperature and kept moist using gauze soaked in 0.9% NaCl solution during the entire test period. The embedded specimens were mounted in specially constructed jigs for compressive, 4-point-bending, or torsional testing in a Material Testing System (MTS [Model 858, MTS Corp., Minneapolis, USA]). The order of stiffness testing was randomised and measurements were performed on each specimen by using compressive, bending (anteroposterior and mediolateral) and torsional load. The resulting deformation was detected by custom-made compression, torsion (Fig. 1) and deflection (Fig. 2) sensors (LVTD and precision potentiometer). For each stiffness testing-procedure, a pre-conditioning of 10 cycles was conducted before the actual testing in order to assure repeatability. The callus tissue within the specimens was loaded during the different types of testing up to 15 Nm for torsional, to 750 N for compressive and to 6.5 Nm for bending load. During testing, load and deformation were simultaneously recorded in order to determine stiffness, which is defined as the slope of the
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Figure 1 Photograph of the special custom-made jig for torsional testing including the specially constructed rotation sensor.

Figure 2 Illustration of the custom-made jig for bending testing including the specially constructed deflection sensor.

Figure 3 Diagram for determination of stiffness (sheep no. 820 — bending stiffness ap).

Figure 4 Comparison of regained stiffness characteristics of distracted tibiae at the 74th postoperative day in relation to intact tibiae.

Statistics

Stiffness was calculated by regression of linear (nondestructive) part of the load—displacement curves using Origin 4.0 (Microcal Software Inc., MA, USA) (Fig. 3).

The mean values of each kind of stiffness for all distracted tibia specimens in percentage of the mean value of the intact tibiae were assessed. Thus, it is possible to compare the percentage of each kind of stiffness, which all specimens in mean regained during the consolidation period at the 74th postoperative day. An analysis using the paired student’s t-test was conducted.

Furthermore, the load-bearing capacity (abscissa) and the different types of stiffness measurements (ordinate) were plotted in a diagram. A linear function describing the relation between these parameters was computed using a linear regression analysis. For each measured parameter an analysis of variance (ANOVA) was performed with SPSS-Software (SPSS, SPSS Inc., Chicago, USA). The regression line describes an approximation of the relation between each type of stiffness and the load-bearing capacity. In order to compare these approximations of the evolution of each type of stiffness in relation to the load-bearing capacity, the characteristics of each specimen were indicated as a percentage of the mean value of the intact contralateral tibiae.

Results

The distracted tibiae regained on average 36.37% of the compressive stiffness, 39.72% of the torsional stiffness, 56.15% of the bending stiffness in anteroposterior orien-
Table 1 Description of the function of linear regression ($f = b \cdot x + a$) for each type of stiffness including coefficient of determination ($r^2$) and $p$-value for each linear regression.

<table>
<thead>
<tr>
<th>Stiffness</th>
<th>Regression coefficient $b$</th>
<th>Point of intersection with the ordinate $a$</th>
<th>$r^2$</th>
<th>$p$-value (by ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torsional stiffness</td>
<td>1.06</td>
<td>$-16.9$</td>
<td>0.77</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Bending stiffness ap</td>
<td>1.10</td>
<td>$-2.75$</td>
<td>0.70</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Bending stiffness ml</td>
<td>1.19</td>
<td>$-4.28$</td>
<td>0.66</td>
<td>$&lt;0.0001$</td>
</tr>
<tr>
<td>Compressive stiffness</td>
<td>0.96</td>
<td>$-15.0$</td>
<td>0.60</td>
<td>$&lt;0.0001$</td>
</tr>
</tbody>
</table>

Figure 5 a–d: evaluation of torsional, bending stiffness in anteroposterior and mediolateral orientation and compressive stiffness.

The regression coefficient ‘‘$a$’’ representing the slope of the linear regression was greatest for bending stiffness in mediolateral orientation compared to the ones for the other types of stiffness (Table 1, Fig. 5 a–d and Fig. 6). The point of intersection with the ordinate ‘‘$b$’’ also varies for the various linear regressions. The point of intersection was lower for torsional and compressive stiffness than for bending stiffness.

Furthermore, there is a great variance in maximum torsional moment achieved by the specimens. Thus, variable stages of bone healing had been achieved at the time of harvesting of the specimens (i.e., after 74 days). Samples which failed at a low amount of stress compared to the one of intact bones were postulated to be at an early stage of the healing process. Samples that failed at a greater amount of stress similar to the intact samples were postulated to be at the end of the healing process. In order to compare the approximation of the evolution of each kind of stiffness, the function of linear regression for each type of stiffness was analysed. Thereby, the approximation of the evolution of each type of stiffness shall be illustrated in relation to the load-bearing capacity assessed by the maximum torsional moment. The function of linear regression for each type of stiffness is described as $f = a \cdot x + b$, whereas ‘‘$a$’’ is the regression coefficient expressing the slope of the function and ‘‘$b$’’ is a constant expressing the intersection with the ordinate (y-axis).
Discussion

In this study, a comparison of the regained amount of various types of stiffness after callus distraction was conducted. Furthermore, approximations of the developments of torsional, compressive and bending stiffness of callus tissue during consolidation according to the load-bearing capacity were determined. To our knowledge, the results facilitate novel insights into the utility of stiffness measurements since no data about stiffness comparison during bone healing were previously available.

The data of the present study shows that 74 days postoperative, the callus tissue regained significantly more bending than torsional or compressive stiffness (Fig. 4). In relation to the characteristics of intact tibiae, there is a difference of up to 23% within the regained amount of stiffness after 74 days of distraction depending of the type of stiffness. There seems to be a strong rationale that an estimation of the load-bearing from in vivo bending stiffness measurement might lead to relative overestimation, while torsional and compressive stiffness tend to an underestimation of load-bearing capability during early phase of healing.

The evolution of stiffness over time is described in several earlier studies. These studies showed during time progression an increase of stiffness and thus of stability of regenerated bone after fracture healing [13]. However, the way stiffness increases is considered controversial. Some authors revealed that stiffness rises exponentially up to healing [11—14], while others found a significant logarithmic increase between week 3 and week 7 after fracture healing [10]. Both studies analysed bending stiffness development. Furthermore, previous studies disclosed that fracture strength recovers more slowly than stiffness [15—17]. An experimental study analyzing biomechanical behavior of bone showed that radii regained bending stiffness faster than bending strength [15]. Hiltunen et al. analysed the evolution of strength and stiffness over time by determining strength and bending stiffness of fractured mouse tibiae harvested at different times after surgery. In this study, stiffness values increased more rapidly than ultimate failure loads [17]. Another study using tibial osteotomy on rabbits also indicated that bone stiffness recovers faster than bone strength [16]. Whereas, a study on fractures of canine tibiae showed that maximum torque and torsional stiffness increased between 2 and 8 weeks, before leveling off at values of 44 and 29% of values for intact tibiae after 12 weeks [18].

However, to our knowledge, no study exists comparing the evolution of various types of stiffness. Although studies exist determining different types of stiffness, no study compares the evolution of stiffness or the regained amount of stiffness except for one study [19]. Wade et al. stated that stiffness in two orthogonal planes may differ widely with a maximum difference in two orthogonal planes of 9.0 Nm/◦ and a mean difference of 4.1 Nm/◦ [19]. Thus, this study supports the results of the current study showing a difference in quantity of various stiffness characteristics of bone callus harvested after 74 postoperative days in relation to the mean value of intact tibiae.

The strength of this study is that, to our knowledge, it is the first study comparing the regained characteristics of callus tissue in the form of torsional, bending (anteroposterior and mediolateral) and compressive stiffness. Thus, in order to adjust the information regained by in vivo stiffness measurement for the determination of the time for the removal of the external fixator appropriately, it is necessary to know how various kinds of stiffness develop. This study was performed ex vivo in order to avoid influence by soft-tissue.

As a limitation of this study, all specimens were gained at the same point of time, so that no analysis of the evolution of various kinds of stiffness over time is possible. For the current study, it was assumed that the load-bearing capacity is a parameter characterising the status of healing. Thus, only an approximation in relation to the load-bearing capacity was determined. In the future, an in vivo analysis of the development of the various kinds of stiffness would be helpful to support the results of the current study. Furthermore, the load-bearing capacity was determined only by torsion. However, it was necessary to choose one type of load out of the three possibilities because of the amount of specimens.

This study demonstrates that different stiffness properties develop differently during healing after distraction osteogenesis. It provides information about the evolution of different types of stiffness in relation to the maximum torsional moment as an indicator for healing at various stages of healing. Regenerated bone regained a significant greater amount of anteroposterior and mediolateral bending compared to compressive and torsional stiffness. As a consequence, stiffness measurements for the prediction of the load-bearing capacity have to be judged accordingly: bending stiffness tends to overestimates the loading capacity, while compressive and torsional stiffness tends to underestimate the loading capacity.

Financial disclosure
Fixator parts, used in this study, were a generous gift of Smith + Nephew, Hamburg, Germany.
Parts of this study were supported by Wyeth, Cambridge, MA.

Conflict of interest
The authors declare that they have no conflict of interest.

Acknowledgement
The animal experiments followed the principles of laboratory animal care and were conducted in accordance with the approval of the German federal animal welfare legislation.

References


