**Intra-callus pressure and oxygen tension during normal bone healing in the sheep tibia**

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**Introduction:** The healing of bone following a fracture requires mechanical stability and an adequate blood supply. Stability influences the magnitude of mechanical stimuli in the callus, which has been shown to be important for the proliferation and differentiation of callus tissue. The blood transports oxygen and thereby maintains tissue oxygen tensions. Oxygen tension is also believed to regulate cell differentiation, with mesenchymal cells thought to differentiate preferentially to form chondrocytes at low oxygen tensions. However, limited information exists as to the actual values of pressure and oxygen tension occurring in vivo during bone healing. In this study, novel methods are applied to investigate in vivo the pressure and oxygen tension in the external callus of a healing tibial osteotomy.

**Materials and Methods:** A mid-shaft tibial osteotomy, distracted to 3mm, was created in six skeletally-mature female sheep. The osteotomy was stabilized with a standard mono-lateral external fixator that has been previously characterized. Prior to closure of the skin incision, a multi-parameter catheter (Neurovent PTO, Raumedic AG, Germany) for measurement of pressure and oxygen tension was inserted. The catheter was inserted through a second skin incision proximal to the osteotomy and then guided using a puncture cannula until the sensor was positioned adjacent to the osteotomy gap on the medial periosteal side. Prior to insertion and for the remainder of the operation the catheter was connected to a portable data logger (MPR2-logO, Raumedic AG, Germany). Postoperative measurement of pressure, oxygen tension and temperature was performed daily with the animals standing up until the 10th day postop. The catheter was connected to the data logger and data was collected continuously for 1 min. Dynamic measurements during gait were performed on day 3 and 7. For this period, the portable data logger was affixed to the sheep’s back and data was recorded continuously. Video capture using a conventional video camera enabled identification of gait events (HS: heel strike, TO: toe off). The maximum pressure and amplitude were calculated for a complete gait cycle. The ground reaction force and interfragmentary movement were measured simultaneously according to a previously described protocol. Statistical comparison of gait parameters at 3 and at 7 days was performed using the Wilcoxon’s signed-rank test for paired samples (SPSS 14, SPSS Inc., USA). A P-value of less than 0.05 was taken as a significant difference.

**Results:** During the stance phase of gait, the intra-callus pressure varied accordingly with the ground reaction force and the interfragmentary movement (Figure 1). Two distinct peaks were observed, one shortly after heel strike and one prior to toe-off. The maximum pressure was determined shortly before heel strike. The maximum pressure (Median [Min-Max]) during the gait cycle increased significantly (p = 0.028) from 3 days (39.3 [19.1–63.9] mmHg) to 7 days (69.0 [60.2–95.4] mmHg). There was no significant change (p = 0.138) in weight bearing of the operated limb between day 3 (40 [39–41] % body weight) and day 7 (37 [36–38] % body weight). The amplitude of interfragmentary movement was determined to be approximately 1.2 mm axial compression with a rotation of 2.5°. The median [Min-Max] oxygen tension on conclusion of the operation was 53.4 mmHg [52.9 – 54.0]. A steady decrease in oxygen tension occurred over the first 5 days postoperative (Figure 2). Between day 6 and day 10, the oxygen tension varied between 12 and 20 mmHg.

**Discussion:** In this study, pressure and oxygen tension were measured in the external callus during early phase of bone healing. The external callus was chosen since it is here, that tissue regeneration is most active in the early phase of healing. The intra-callus pressure measured during the stance phase of gait showed a similar profile to the ground reaction force and the interfragmentary movement (Figure 1), suggesting a causal relationship. However, the observation that the peak pressure during gait occurred during the swing phase implies that muscle contraction may be more significant than weight bearing for the local mechanical conditions in the early callus. This component of loading is typically neglected in the analysis of the mechanical conditions during bone healing. In regards to the actual magnitude of the measured pressure, it was below that thought necessary to promote cartilage differentiation. However, pressure in the external callus may only increase at later stages of healing when mineral callus formation is more advanced. The oxygen tension in the callus was found to be relatively high initially, taking five days to reach a minimum. These results contradict the perception, that a low oxygen environment develops immediately post-operative and suggests that oxygen in the blood is trapped through formation of the haematoma. The steady decline in oxygen tension over the first few days of healing may be explained by the gradual consumption by cells invading the haematoma. The present study provides valuable new insights into the actual environmental conditions experienced by regenerating tissues in vivo.


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