Local Soft Tissue Compression Enhances Fracture Healing in a Rabbit Fibula

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Abstract Local soft tissue compression of fractures enhances fracture healing. The mechanism remains uncertain. Past studies have focused on intermittent soft tissue compression. We report a preliminary study assessing the relationship between constant soft tissue compression and enhanced fracture healing in an osteotomy model designed to minimize confounding variables. Fibulae of nine New Zealand white rabbits were bilaterally osteotomized, openly stabilized, and fitted with spandex stockinets. Soft tissue at the osteotomy site was unilaterally compressed using a deforming element (load=26 mmHg). The contralateral side was saved as the control and was not compressed. Osteotomies were monitored with weekly radiographs. All fibulae in both groups were healed 6 weeks postoperatively. Micro-CT analysis of bone mineral density (BMD) and bone volume (BV) was then performed on both the experimental and control sides. Radiographic measurement

of transverse callus-to-shaft ratios (TCSR) was compared. BMD of the experimental callus was greater than the noncompressed controls. BV and TCSR were not different between controls and experimental osteotomies. Constant local soft tissue compression produced significant increases in BMD, but not in BV or transverse callus size, indicating significant measurable increases in callus composition without significant change in gross dimensions. Our experimental design minimizes confounding factors, such as micromotion, immobilization, and altered venous flow, suggesting that these are not the primary mechanisms for fracture healing enhancement. Further studies with more animals and study groups are necessary to confirm efficacy and identify optimal compression pressures and schedules.

Keywords soft tissue compression · fracture healing · osteotomy · bone mineral density (BMD) · bone volume (BV) · micro-CT

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Each author certifies that his or her institution has approved the animal protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

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Introduction

Alterations in local pressure and mechanical environment have been shown to enhance connective tissue proliferation in various laboratory, animal, and human models. In vitro studies of fibroblasts have demonstrated increased rates of procollagen synthesis and processing, as well as enhanced stimulatory effects of growth factors with mechanical loading [3, 28]. Pneumatic pressure applied intermittently to soft tissue at a fracture site has been reported to enhance bone and callus formation in rabbits [4–6, 11, 13, 26, 27]. An intermittently pressurized brace for fracture immobilization in a canine model has been shown to produce biomechanically sturdier healing relative to a traditional cast when tested for energy absorption, torque, and angle of rotation [26]. Low-intensity pulsed ultrasound which produces an analogous pressure wave within fracture callus was also found to enhance bone volume (BV) and osteoid thickness in human patients with a delayed union of osteotomized fibulae [29].

Though it is agreed that mechanical conditions in the fracture area influence bone remodeling and fracture healing

[8, 22, 26], there is still much uncertainty regarding the precise biological mechanism of the enhanced fracture healing associated with increased soft tissue pressure [26]. Several possible mechanisms have been considered in the literature [26]. One common suggestion is that pressure placed on the soft tissue initiates electrochemical potentials due to the electrolytes passing in close proximity to charged surfaces, such as capillary pores. This electrochemical phenomenon, commonly referred to as “streaming,” may, in times of mechanical changes, serve as a signal for osteoblasts to form new bone. Cell stimulation due to the electrochemical streaming has been suggested as a possible mechanism for the enhancement of bone formation in both loading and pressure models [2, 23]. Streaming potentials were detected upon application of a pressurized inflatable brace in a canine osteotomy model [23]. Another suggested mechanism of enhancing bone formation is increased fluid flow. Compressing muscle tissue and blood vessels may increase venous blood peak velocity and flow, resulting in an increased arterial, periosteal, and medullary blood flow and subsequent enhancement of bone formation [7, 8, 26, 27]. Possible mechanisms by which an increase in fluid flow facilitates bone formation include stimulation of cells by increased surrounding fluid pressure, delivery of nutrients to cells via the fluid, and possible flow-induced streaming potentials [2]. Others have maintained that a rapid increase in blood velocity, as occurs in soft tissue compression, may stimulate release of nitric oxide, a known mediator in bone formation [7, 26]. A combination of these possibilities may all contribute to the effects of altered fluid flow [2]. Increased interstitial fluid return to circulation has been suggested as a mechanism for enhancing bone healing by intermittent pneumatic compression [7]. The increase in capillary pressure and blood flow was attributed to an increase in venous pressure, as postulated by Starling’s Law [2, 22, 23]. It is possible that increasing the soft tissue pressure decreases blood flow to the tissues surrounding the fracture, thereby diminishing tissue pO_2 . pO_2 was found to be diminished in a rib fracture model [10]. A low-oxygen environment has been suggested to enhance angiogenesis and fracture healing. Increased angiogenic factor output was observed in macrophages exposed to a low-oxygen environment [14].

Some have been reluctant to embrace any of the above hypotheses, since it is possible that the pressurized brace systems provide more efficient immobilization, thereby enhancing bone formation [8, 23]. Microfragmentary or interfragmentary motion at the fracture site has also been reported to be associated with enhanced fracture healing [10] and, as such, was posited as a possible mechanism in both pressurized brace [26] and weight-bearing models [22]. Confounding factors in past studies of soft tissue compression also contribute to uncertainty concerning the mechanism. One study demonstrated that raising venous pressure stimulated periosteal bone formation by using a canine tourniquet model [2]. In a study of intermittent pneumatic soft tissue compression in a rabbit model, the femoral vein was ligated [26]. In both instances, it is difficult to ascertain whether it was the alterations in venous pressure or soft tissue compression that stimulated bone growth. Furthermore, the pressure in the lapine intermittent

pneumatic compression model [26] was raised to 95 mmHg for 2 s separated by 20-s intervals. Past literature concerning compartment syndrome has posited that the critical soft tissue pressure at which ischemia begins ranges from approximately 30 to 45 mmHg [1, 5, 9–14, 19, 22]. Quick alterations in blood flow, inducing cycles of local ischemia followed by longer intervals of reperfusion, may have been a further confounding factor in this study. In our rabbit fibula osteotomy model, we have attempted to minimize the confounding factors present in other studies, while also minimizing the contribution of immobilization and micro-motion. We believe that the design of the compressive device in this study as well as our experimental protocol are better suited to assessing whether soft tissue pressure changes at the osteotomy site are associated with increased fracture healing.

We hypothesize that elevation of the soft tissue pressures at the fracture site results in enhanced callus formation. The primary question of the study is whether soft tissue compression at the osteotomy site is associated with an increase in bone mineral density (BMD) when compared to a control where no pressure is applied. A second question concerns whether soft tissue compression is associated with increased BV at the fracture site in the experimental side when compared to a control. A third question is whether soft tissue compression is associated with an increase in the transverse callus-to-shaft length ratio (TCSR) in the experimental side when compared to a control.

Materials and methods

The soft tissue compression over the osteotomy was produced by placing a cylindrical deforming element of dense foam 1 in. in height and diameter beneath layers of stockinet. Prior to application within an animal model, the number of stockinet plies that would produce a pressure of 30 mmHg or 4,000 Pa on the deforming element was determined. Using $F=PA$ and $A=\pi R^2$ (where F , P , A , and R represent force, pressure, area, and radius, respectively), a weight of 2.06 g would be necessary to produce 30 mmHg pressure over a surface area of 1 in. (Fig. 1). The number of stockinet plies required to produce the equivalent pressure was then empirically determined. The displacement of a spring upon loading with three different known weights was measured, and the spring constant was determined. Displacement is linearly related to the load, as expressed by Hooke’s law, which states that the force exerted upon a spring is in linear relationship to the displacement of the spring ($F=kx$, where F is the force applied, k is the spring constant, and x is displacement). The spring constant was averaged to be 264 N/m. Subsequently, the same spring was used to measure the displacement of the spring with each of three stockinet plies. The relationship of the number of stockinet plies to the force and pressure produced was determined. Three stockinet plies produce a pressure of 3,460 Pa or approximately 26 mmHg. Three stockinet plies were used when applying the stockinet in the animal model.

Nine, 3.5 to 4.0 kg, mature male New Zealand white rabbits underwent osteotomy of both fibulae. Under aseptic

Fig. 1. The deforming element calibration apparatus is shown

conditions, anesthesia was induced with 35 mg/kg ketamine and 5 mg/kg of xylazine intravenously and was maintained using 1.5% isoflurane. The hind limbs were shaved from mid thigh to the ankle, and the fibula was exposed using a lateral approach. The fibula was osteotomized with a 1-mm rotating burr aligned perpendicular to the fibula's longitudinal axis. In a rabbit, the fibulae are fixed to the tibia proximally and distally, obviating the need for fixation at the osteotomy site. Postoperatively, each rabbit was kept in individual stainless steel rabbit housing (Allentown Inc.[®], Allentown, NJ, USA). The rabbits were fed ad libitum with a high-fiber Laboratory Rabbit Diet HF #5326 (Purina Mills

Fig. 2. A bilateral radiograph of specimens harvested 6 weeks postoperatively shows a healed treated specimen (*left*) and an unhealed contralateral control (*right*)

Fig. 3. A micro-CT slice from a typical treated specimen is shown

Inc.[®], Gray Summit, MO, USA). Under manual restraint and without anesthesia, three layers of spandex stockinet were applied to both limbs of all animals between the tenth and 14th day postoperatively, depending on wound healing. The deforming element was placed between the stockinet and the osteotomy site on one randomly selected limb of each animal. The rabbits were allowed unrestricted activity with the stockinet in place for six continuous days. The device was then removed. After 24 h, a new stockinet was reapplied with the deforming element for another 6 days. The animals were seen to bear weight on both limbs without any apparent limp. Under manual restraint and without anesthesia, X-rays were taken at the time of application of the deforming element and weekly from then on to ensure that the deforming element overlay the osteotomy site and check for the progression of bone healing.

Six weeks postoperatively, the animals were sacrificed using pentobarbital 250 mg/kg intravenously, and the lower limbs were harvested. Each specimen was randomly assigned a number from 1 to 18. Radiographs and micro-CT scans (GE Healthcare[®], UK) with 50- μ m cuts were obtained for both limbs on eight animals by a member of the research team (SM) who remained blinded to the identity of control and experimental groups throughout the radiographic and micro-CT scanning and analysis (Figs. 2 and 3).

Table 1 Bone mineral density (mg (HyAp)/cm³)

	BMD-treated	BMD-untreated	Difference
Avg.	288.68	243.92	44.76
SD $p=0.0098$	74.89	78.27	36.01

Table 2 BV at 1,000 Hounsfield units (mm³)

	BV-treated	BV-untreated	Difference
Avg.	0.15	0.17	-0.01
SD $p=0.1497$	0.04	0.03	0.04

A three-dimensional distribution of callus around the osteotomy site was generated from the micro-CT scan using Microview Software (version 2.1.2, GE Healthcare®, UK). BMD and corresponding BV measurements were obtained at threshold values of 1,000, 3,000, and 4,000 Hounsfield units (HU). A Student's paired t test was utilized to compare BMD and BV in the control and experimental sides.

The ratio of the callus span to the fibular shaft in the transverse plane was measured on radiographs. Using a centimeter ruler, the distance from the external edge of the fibular shaft to the external edge of the callus was measured once for each side of each limb by a blinded member of the research team. The two callus measurements (one for each side) for each limb were then averaged. The transverse width of the shaft was measured above and below the osteotomy site for each limb. These two measurements were averaged. All measurements were taken as close to the osteotomy as possible. The callus-to-shaft ratio was subsequently calculated for each of the specimens individually. Because a Student's t test may only be used for interval data, the callus-to-shaft ratios were analyzed using a Wilcoxon signed rank test. The Wilcoxon signed rank test, like the Student's t test, may be used to analyze paired data but unlike the t test does not assume parametric distribution and may be used for analysis of paired ordinal data, such as rank.

Results

The BMD was found to be significantly greater in the experimental side compared to the control ($p=0.01$; Table 1).

No significant difference in BV was demonstrated between the experimental and control limbs at threshold values of 1,000, 3,000, and 4,000 HU (Tables 2, 3, and 4).

No significant difference in TCSR was demonstrated between the experimental and control limbs ($W=0$, $n=8$; Table 5).

One animal was excluded from the quantitative micro-CT and radiographic analyses due to nonunion of the control side.

Table 3 BV at 3,000 Hounsfield units (mm³)

	BV-treated	BV-untreated	Difference
Avg.	0.07	0.08	-0.01
SD $p=0.3506$	0.02	0.02	0.02

Table 4 BV at 4,000 Hounsfield units (mm³)

	BV-treated	BV-untreated	Difference
Avg.	0.03	0.04	-0.01
SD $p=0.3290$	0.01	0.01	0.01

Discussion

Mechanical pressure at a fracture site has been shown to improve fracture healing. Various mechanisms have been suggested for this phenomenon, including local streaming potentials, altered fluid flow, and altered venous blood peak velocity. Some have been skeptical of all of these explanations, preferring to attribute the improved fracture healing to micromotion or superior immobilization. Additionally, past research studying soft tissue compression is characterized by various confounding factors. These include femoral vein ligation and the use of cuffs that alter venous pressure, as well as cyclic intervals of likely ischemia and reperfusion [2, 26, 27]. These confounding factors make it difficult to assert that the local effects of soft tissue compression, and not changes in venous pressure or arterial perfusion, explain observations of improved fracture healing. In our rabbit model, we attempted to minimize differences in micromotion and immobilization between experimental and control limbs. Furthermore, we did not ligate the femoral vein. No cuffs were used that would cause gross venous pressure changes. The pressure applied at the site did not exceed 30 mmHg so as to avoid local ischemia. The compression was maintained throughout the experiment to avoid cycles of diminished perfusion and reperfusion. The primary research question, using this model, was whether soft tissue compression results in significant increase in BMD in the experimental side versus the control. Additionally, we also evaluated differences in BV and TCSR in experimental versus control sides.

Shortcomings of this study include its lack of biomechanical testing. While BMD has been demonstrated to be an excellent predictor of bone strength and Young's modulus in cancellous and cortical bone using a variety of measurement techniques [12, 21, 24, 32], biomechanical testing is a more direct and clinically relevant method of testing bone strength. Furthermore, all animals were sacrificed concurrently, precluding any observations or data concerning possible differences in speed of healing. Future study designs should allow for serial sacrifice of study groups at set time intervals, so as to assess not only changes in healing time but also to identify optimal compression

Table 5 Callus-to-shaft ratio on radiograph

	Ratio-treated	Ratio-untreated	Signed rank
Avg.	0.90	0.83	$W=0$
SD $p=1.0$	0.25	0.30	

schedules. Constant compression of soft tissues produce constantly elevated pressure that in this case fluctuates around 30 mmHg. The pressure, however, cannot be assumed to be a constant number since the animals are allowed unrestricted activity, and the exact pressure may change with limb motion. Future experiments ought to include continuous pressure measurements below the deforming element [15–18, 20, 25, 31]. Future studies should also incorporate instruments, such as laser Doppler probes, to monitor perfusion and flow as reported in past research [27]. Additionally, while this study demonstrates that 30 mmHg is associated with increased BMD, this pressure may not be optimal. Future studies should compare the effects of compression at various pressures. Additionally, our study included no systematic way of measuring adverse affects of compression on the skin. Such measures should be included in future studies. This study did not include an a priori power analysis. Future studies should perform power analyses and use the appropriate number of animals necessary for demonstrating a significant difference if present, while also allowing for justified conclusions concerning what is not affected by increasing soft tissue compression, namely BV and TCSR. Future studies should also take measures to ensure intraobserver reliability during the radiographic data collection and analysis.

Our results support the hypothesis that constant local compression of the soft tissues over the fracture site increases density of callus. Constant soft tissue compression produced a significant increase in BMD without significantly affecting the callus volume or the transverse CTSR in our study. An a priori power analysis is required to meaningfully state that soft tissue compression results in no significant BV or TCSR change. Nevertheless, it is possible that the main effect of soft tissue compression is on the nature of the callus and not gross volume or size. Studies are ongoing to confirm this. The BMD findings in our study correlate with past rabbit osteotomy research showing increased BMD in an intermittent soft tissue compression model [26]. While they also found increases in “mineralized callus at the osteotomy site,” our study fails to find significant differences in markers for callus size rather than content, namely BV and TCSR. Notably, this study differed from our study in that they compressed at pressures much higher than needed to induce ischemia, cycled the pressure from 0 to 95 mmHg, ligated the femoral vein, and compressed proximally and distally to the osteotomy site instead of at the osteotomy site. It is possible that soft tissue compression alone enhances BMD. Yet when compression is combined with various other interventions utilized in past research [26], bone size is also affected. Further research is required to assess this. Past researchers have suggested that micromotion and immobilization contribute to the enhanced fracture healing and have posited these as explanations for the enhanced fracture healing in soft tissue compression models [8, 10, 22, 23, 26]. In our study design, the experimental and control limbs did not differ in regards to these parameters. Our results provide evidence that make interfragmentary micromotion and immobilization unlikely to be key contributors. We

suggest that the Sarmiento bracing technique be revisited so as to assess structural and conceptual differences between his technique and the present concept. According to Sarmiento, motion at the fracture site, not increased soft tissue pressure, enhances osteogenesis [30]. Nevertheless, the increased pressure in the soft tissues produced with the Sarmiento-type cuff may contribute to its success. Our study, provides no new evidence to resolve uncertainties regarding the mechanism of action for soft tissue compression. Increased fluid flow, electrochemical streaming, or altered soft tissue pO_2 may all explain the findings in our study [2, 7, 8, 10, 14, 23, 26, 27].

In this pilot study, soft tissue compression at the site of a fibula osteotomy produced callus with a significantly greater BMD compared to the contralateral control of the same animal. Further studies are in progress to confirm this result. Although this result is compatible with previous reported studies reporting an association between increased soft tissue pressure and increased bone density [26], no similar correlation was found in callus size as measured by BV and TCSR. The present experimental design uses constant, rather than intermittent, soft tissue compression while minimizing confounding factors, such as micromotion, immobilization, ischemia, sudden perfusion shifts, and altered venous flow. Further studies designed in light of an a priori power analysis are needed to confirm our findings, as well as determine the ideal compression pressure and schedule for commencements and cessation of compression. Efforts should also be taken to observe possible adverse effects of local pressure on the skin, and what protocol, especially pressure and schedule, is optimal for avoiding skin degradation.

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