Effects of Extensive Periosteal Stripping on the Microstructure and Mechanical Properties of Cortical Bone

A Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Andrew Mercurio, DVM

Graduate Program in Comparative and Veterinary Medicine

The Ohio State University

2011

Thesis Examination Committee:

Dr. Matthew Allen, Advisor

Dr. Eric Green

Dr. Joel Mayerson
Abstract

The periosteum is a thin connective tissue layer that covers the external surfaces of most bones and is essential to normal cortical bone biology. Periosteal stripping (PS) is a significant risk factor for post-radiation pathologic fracture following surgery for extremity soft tissue sarcomas. The purpose of this study was to determine the effects of PS on cortical bone structure and mechanical properties. 62 adult female mice underwent PS (N=32) or sham surgery (N=30) on the left femur. For PS, the periosteum was circumferentially removed from an 8-mm segment of the mid-diaphysis. For sham surgery, the bone was isolated without manipulation. At 2, 6, 12, or 26 weeks following surgery, the left femora were examined by micro-CT. Cortical thickness (CtTh), cross-sectional area (CSA), bone volume (BV) and polar moment of inertia (PMI) were measured from the mid-diaphysis. 3-point mechanical bend testing was performed and peak load (PL), stiffness and energy to failure (EF) were determined. Data from the groups were compared at each time point using a Student’s T-test (p<0.05), and two-way ANOVA was used to examine the parameters. PS resulted in significantly decreased CtTh, CSA, BV and PMI at all time points. PS resulted in significantly decreased PL, stiffness, and EF at 2 weeks, with further decreases at 6 and 12 weeks. There were no significant differences in mechanical properties between the two groups at 26 weeks. Correlation analysis revealed strong and significant relationships between microstructural...
and mechanical parameters. In conclusion, extensive PS causes early and significant decreases in cortical bone structural and mechanical properties, with eventual recovery of strength.
I would like to acknowledge The Ohio State University Canine Intramural Fund for the financial support of this project. I would also like to sincerely thank Dr. Matthew Allen for all of his guidance and patience, without whom this project would not have been possible. Finally, and most importantly, I would like to thank my wife, Hannah, for her unconditional love and support throughout the entire residency.
Vita

2003 ............................................................... B.A. Biology, Assumption College

2007 ............................................................... D.V.M., Tufts University Cummings School of Veterinary Medicine

2007 - 2008 ....................................................... Internship, Small Animal Medicine and Surgery, College of Veterinary Medicine, University of Georgia

2008 to present .............................................. Residency, Small Animal Surgery, Department of Veterinary Clinical Sciences, The Ohio State University

Publications

Fields of Study

Major Field: Comparative and Veterinary Medicine

Specialty: Small Animal Surgery
# Table of Contents

Abstract .......................................................................................................................... ii  
Acknowledgements ....................................................................................................... iv  
Vita ................................................................................................................................... v  
List of Tables ................................................................................................................... viii  
List of Figures ................................................................................................................. ix  
Chapter 1: Introduction .................................................................................................. 1  
Chapter 2: Methods ....................................................................................................... 7  
Chapter 3: Results ......................................................................................................... 12  
Chapter 4: Discussion .................................................................................................... 19  
References ..................................................................................................................... 24
List of Tables

Table 1: Summary of cortical morphometry results.......................................................... 13

Table 2: Summary of mechanical test results from 3-point bend testing.............................. 14

Table 3: Correlation matrix summarizing the relationship between mechanical and microstructural parameters.............................................................. 15
List of Figures

Figure 1. Surgical procedure for circumferential periosteal stripping .......................... 8
Figure 2. Set-up for 3-point bend testing of the murine femur ...................................... 9
Figure 3. Micrographs demonstrating the presence (A) and absence (B) of the periosteum
in sham femora and stripped femora respectively ......................................................... 16
Figure 4. Representative 3-dimensional micro-CT reconstructions of femora from mice
that underwent periosteal stripping or sham surgery ............................................... 17
Figure 5. Graphs depicting changes in peak load (A) and bone volume (B) over time in
PS and sham femora ..................................................................................................... 18
The periosteum is a highly vascular, osteogenic connective tissue layer that covers the external surfaces of most bones. Histologically, periosteum is composed of two distinct layers. The outer, “fibrous” layer consists primarily of fibroblasts, collagen, and elastin fibers within a well-developed nerve and microvascular network. This layer provides elasticity and flexibility. The inner “cambium” layer is in direct contact with the bone surface. It is highly cellular, containing mesenchymal progenitor cells, osteoblasts, and fibroblasts. The osteoblasts of the cambium layer unique; they have a greater mechanosensitivity to strain and a lower threshold of responsiveness to various osteogenic compounds, such as parathyroid hormone. The periosteum has a well-developed blood supply. An intrinsic periosteal vascular system consists of a net of longitudinal blood and lymphatic vessels that run parallel to the long axis of the bone, and circular vessels that encircle the bone. The main periosteal vessels give off perpendicular arterioles that anastamose with the nutrient arteries and medullary blood supply. Associated with its relatively high vascularity, the periosteum contains many endothelial pericytes. These pericytes are in physical contact with capillary endothelial cells, and they have the ability to differentiate into many cell types, including osteoblasts. Cultured pericytes mineralize in vitro, and synthesize the osteoblast marker alkaline phosphatase as well as bone matrix proteins including osteocalcin, osteonectin, osteopontin, and bone sialoprotein. The pericytes form an osteogenic tissue that mimics
bone-derived tissue and responds to osteogenic stimuli such as PTH and BMP\textsuperscript{1}. Normal periosteum and cortical bone biology plays a major role in fracture prevention, and periosteal expansion of the cortical shell significantly increases bone strength. This important tissue layer is often disturbed in the setting of musculoskeletal oncology and orthopedic surgery, but the effects of periosteal trauma on underlying bone structure and strength are poorly understood.

Disruption of the periosteum, whether traumatic or elective, has been known to accelerate growth in the developing skeleton. Periosteal transection and elevation has been used in the treatment of angular limb deformity in veterinary medicine, and in the treatment of leg-length discrepancies in children\textsuperscript{3, 4}. More specifically, hemi-circumferential periosteal stripping has been used in the treatment of carpal valgus deformity in foals, where it can be performed as an alternative to or in combination with transphyseal bridging\textsuperscript{5, 6}. The technique is based on the results of research in chickens, rabbits and rats, which suggests that the periosteum acts a fibroelastic tube, exerting tension on the proximal and distal physes of long bones\textsuperscript{3, 7, 8}. Incising and elevating the periosteum in an immature animal may provide tension relief, ultimately resulting in accelerated growth along the cis-cortex. Though its use is widely accepted in equine practice, its efficacy has been questioned, and the effects of periosteal stripping on immature long bones is an area of ongoing research\textsuperscript{4}.

Recent work evaluating the effects of periosteal resection on tibial growth in weanling lambs seems to validate the results of previous studies and offers insight into the mechanism by which periosteal disruption alters growth\textsuperscript{9}. A 1cm wide circumferential periosteal resection distal to the proximal tibial physis resulted in
increased growth velocity in every lamb beginning as early as 48 hours after surgery and persisting throughout the 4 week study period. Histomorphometric and stereological assessments of chondrocyte kinetic parameters were performed on the growth plates, demonstrating an increase in axial elongation of the hypertrophic chondrocytes in treated limbs compared to controls\textsuperscript{9}. These studies offer insight into the normal function of the periosteum as a mechanical tissue layer, but do not investigate the relationship of the periosteum to cortical bone biology.

A unique clinical oncologic problem has stimulated interest in the normal relationship between the periosteum and the underlying bone in adults and has led to research into the effects of peristeal trauma on intact bone. The standard treatment of malignant extremity soft tissue tumors in veterinary and human medicine includes wide surgical resection followed by adjuvant radiation therapy\textsuperscript{10, 11}. The combination of surgery and radiation therapy greatly improves local control of these tumors, allowing the limb to be spared in the cases of extremity tumors. When the tumor lies adjacent to bone, some of the periosteum is removed to achieve adequate surgical margins. This is especially true of tumors affecting the cranial or medial compartment of the thigh, where resection including the periosteum combined with radiation therapy results in a high probability of long term disease control\textsuperscript{12-14}. One of the potential complications following surgery and radiation therapy for extremity soft tissue sarcomas is pathologic long bone fracture. This is a devastating complication that may lead to multiple revision surgeries or even amputation. The reported incidence of post-radiation fracture after excision of extremity soft tissue tumors in humans ranges from 1.2\% to 7\%\textsuperscript{12, 13, 15}. 

3
Several clinical studies have identified periosteal stripping as a significant risk factor for post-radiation pathologic fracture following surgery for extremity soft tissue sarcomas\textsuperscript{12, 13, 16}. In patients treated for soft tissue sarcomas of the thigh, moderate to extensive periosteal stripping is associated with an 18- to 20-fold increase in the risk of pathologic femoral fracture, with the incidence of fracture at 5 years ranging from 29\% to 32\%.\textsuperscript{12, 13} For the femur, Helmstedter defines moderate periosteal stripping as involving 10-20 cm of the bone, and extensive periosteal stripping as involving greater than 20 cm of the length. In the case series reported by Lin\textsuperscript{13}, most of the patients with fractures had massive, often near-circumferential stripping of the periosteum. The development of pathologic fracture after combination therapy is devastating, with very high patient morbidity. Treatment of these fractures is fraught with complications including a high rate of fracture nonunion and deep infection requiring multiple revision surgeries or even amputation\textsuperscript{12, 13}.

The negative effects of therapeutic radiation on bone are well documented and include radiation osteitis, avascular osteonecrosis, stress microfractures, pathologic fracture and delayed fracture healing\textsuperscript{17-19}. The risk of fracture following radiation therapy may be related to vascular fibrosis and impairment of osteoblastic function or increased osteoclast-mediated bone resorption leading to net bone loss and subsequent bone weakening. Identifying the exact mechanisms behind the effects of radiotherapy on bone is an area of continued investigation. In a murine model, profound declines in trabecular bone volume and reduced bone mineral density occurred following exposure to clinically relevant sources of whole body radiation\textsuperscript{17}. Wernle et al\textsuperscript{20} demonstrated that local hind limb irradiation causes dose dependent decreases in femoral trabecular bone with
increased fragility at 12 weeks following treatment. Interestingly, bone mineral density (BMD) and other total bone parameters were retained over time in irradiated femora, highlighting the challenge in predicting fracture risk using traditional methods.

The periosteum is an important tissue layer, and it is not surprising that injury has deleterious effects on the bone. We know that the periosteum plays a major role in fracture repair. It provides a rich blood supply necessary to support fracture healing. The cambium layer is responsible for producing the initial mass of cartilaginous callus that subsequently undergoes endochondral ossification. Using a well-standardized rib fracture model in the mouse, Li\textsuperscript{21} demonstrated considerable proliferation of cambial periosteal cells as early as two days after the fracture event. Following cell replication, differentiation into osteoblastic and chondrogenic lineages occurred with subsequent regeneration of periosteal cartilage and bone directly from the region of the fracture site\textsuperscript{21}. Periosteal stripping removes osteoprogenitor cells within the cambium layer and may reduce the healing potential and regenerative capacity of bone. It also disrupts the nutrient vessels to bone, thereby compromising blood supply to the outer cortex. In long bone models, periosteal stripping has been shown to decrease cortical perfusion and impair fracture healing\textsuperscript{22, 23}.

It is important to recognize that neither radiation therapy alone nor periosteal stripping alone results in an appreciable rate of fracture in patients treated for extremity soft tissue sarcomas. It is also interesting to note that total radiation dose not seem to be associated with the development of fractures after combination therapy\textsuperscript{12, 13, 24}. It is logical to consider the possibility of an additive effect of periosteal stripping and radiation therapy leading to the significantly increased fracture risk in this patient.
population. Because of the substantial risk of fracture in patients treated with combination therapy that includes periosteal stripping and the significant morbidity associated with pathologic fracture, this is a clinical problem that warrants additional investigation.

The effects of periosteal stripping on intact bone strength have not been studied using a controlled experimental model. The ability to define and quantify the effects of periosteal stripping on bone strength and structure will improve our understanding of bone biology with potential applications in the fields of orthopedic surgery and traumatology. Studying the effect of periosteal stripping alone and in combination with radiation therapy will also contribute to our knowledge of bone biology and pathophysiology in the context of musculoskeletal oncology. Controlled, experimental studies using an animal model are indicated as a foundation for this research. After establishing a model to study the effects of periosteal stripping on bone, the effect of periosteal stripping combined with radiation therapy and the effect of the varying degrees/locations of periosteal stripping can be studied. The specific aims of this study were to develop an experimental model of extensive periosteal stripping in a skeletally mature animal, and to determine the effects of periosteal stripping on bone structure, mass and mechanical properties.
Chapter 2: Methods

The following study was reviewed and approved by the local Institutional Animal Care and Use Committee. Sixty-two skeletally mature (12-14 week old) female Balb/c mice were housed in standard micro-isolator cages (4 mice per cage) and fed a standard laboratory mouse chow with ad libitum access to water. The mice were randomly allocated to undergo periosteal stripping (N=32 mice) or sham surgery (N=30 mice) on the left femur. General anesthesia was induced and maintained with inhaled isoflurane (1-5% in oxygen) delivered via facemask. Standard aseptic technique was used for all surgeries. For periosteal stripping (PS) surgery, a lateral approach to the left femoral diaphysis was performed, and the bone was isolated by bluntly elevating the surrounding musculature from the bone surface. A micro-periosteal elevator (Figure 1A) was used to elevate and remove the periosteum circumferentially from an 8 mm length of the mid-diaphysis (Figure 1B). For sham surgery, a lateral approach was used to expose and isolate the left femoral diaphysis, but the periosteum was left intact. For all surgeries, lavage with 0.9% saline solution preceded routine closure of the muscle fascia and skin using non-absorbable suture.

Buprenorphine (0.01 mg/kg) was administered subcutaneously immediately following surgery and every twelve hours for three days. Mice were allowed unrestricted activity and access to food ad libitum. They were monitored daily for clinical signs of
Figure 1. Surgical procedure for circumferential periosteal stripping. A micro-elevator was used to strip the periosteum circumferentially from the mid-diaphysis (A), resulting in an 8-mm long segment of denuded femur (B).
pain or distress, and general ill health. 7-9 mice per group were euthanatized at two, six, twelve or twenty-six weeks. Euthanasia was performed by cervical dislocation under isoflurane anesthesia.

The left femora were harvested and examined by micro-computed tomography using a Skyscan 1172 scanner operating at a nominal resolution of 13.9 µm. The images were reconstructed using proprietary software (NRecon; Aartselaar, Belgium) and visualized using medical imaging software (Mimics v. 13.0; Materialize, Ann Arbor, MI). Morphometric analysis was performed on a 1-mm thick slab of cortical bone located at a consistent location within the femoral diaphysis using the trochanteric fossa as a reference point. Cortical thickness (mm), cortical area (mm²), bone volume (mm³) and polar moment of inertia (mm⁴) were determined using standard morphometry software (CTAn; Skyscan, Aartselaar, Belgium). Three-point mechanical bend testing was performed using a custom test fixture with the outside loading points placed eight millimeters apart and the femur placed with the cranial surface facing upwards (Figure 2). Testing was performed on a screw-driven materials testing machine (Insight 1; MTS Corporation, Eden Prairie, MN) fitted with a 50N load cell. Tests were conducted with no preload and at a displacement rate of 0.155 mm/second to failure.²⁵, ²⁶ Load and displacement were recorded continuously, and the resulting load-displacement curve was used to calculate peak load (N), stiffness (N/mm) and energy to failure (N*mm) for each sample.

Within each time point (2, 6, 12 and 26 weeks), data from the sham and periosteal strip groups were compared using an unpaired Student’s t-test with significance set at p<0.05. Correlation analysis was used to examine the relationship between
Figure 2. Set-up for 3-point bend testing of the murine femur. The femur was placed with the caudal surface in contact with two supports separated by a distance of 8 mm.
microstructural estimates and mechanical parameters. Temporal patterns in both microstructural and mechanical properties in the two groups were compared using two-way analysis of variance (ANOVA) with Tukey post-hoc testing as appropriate. A significance level of p<0.05 was used throughout.
Chapter 3: Results

All of the mice survived the study and there were no significant peri- or post-operative complications. Specifically, there were no episodes of persistent lameness and there were no incidences of femoral fracture. Periosteal stripping resulted in complete, circumferential removal of the periosteum from the cortex, as demonstrated histologically in samples taken immediately after surgery within a pilot group of animals (Figure 3). Three-dimensional micro-CT reconstructions demonstrated relatively thinned diaphyses in femora that underwent periosteal stripping (Figure 4). On analysis of the CT-derived cortical morphometry data, periosteal stripping resulted in significantly decreased cortical thickness, cortical cross-sectional area, bone volume and polar moment of inertia at all time points (Table 1). Mechanical testing data demonstrated that periosteal stripping resulted in significantly decreased peak load, stiffness, and energy to failure at 2, 6 and 12 weeks (Table 2). There were no significant differences between the sham and periosteal strip groups for any of the mechanical testing parameters at 26 weeks.

The relationship between bone microstructure and mechanical properties was examined using correlation analysis. There were strong and statistically significant correlations between microstructural parameters (BV, CSA, CtTh and PMI) and peak load ($r=0.695$ to $0.729$, $p<0.001$), stiffness ($r=0.661$ to $0.679$, $p<0.001$) and energy to failure ($r=0.653$ to $0.720$, $p<0.001$) (Table 3).
**Table 1.** Summary of cortical morphometry results derived by micro-CT analysis. Data are presented as mean ± standard deviation for 6 to 8 mice per group at each time point. PS = periosteal strip. Superscripts indicate statistically significant differences between sham and PS treatments (paired t-test) at p< 0.05 (a), p< 0.01 (b) or p<0.001 (c).

<table>
<thead>
<tr>
<th>Group (time)</th>
<th>Cortical Thickness (mm)</th>
<th>Cortical Cross-Sectional Area (mm²)</th>
<th>Bone Volume (mm³)</th>
<th>Polar Moment of Inertia (mm⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (2 wks)</td>
<td>0.27 ± 0.02</td>
<td>1.08 ± 0.05</td>
<td>1.09±0.05</td>
<td>0.43 ± 0.03</td>
</tr>
<tr>
<td>PS (2 wks)</td>
<td>0.24 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.82 ± 0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.83±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.28 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sham (6 wks)</td>
<td>0.30 ± 0.02</td>
<td>1.08 ± 0.10</td>
<td>1.09±0.10</td>
<td>0.42 ± 0.06</td>
</tr>
<tr>
<td>PS (6 wks)</td>
<td>0.25 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.97±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.36 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sham (12 wks)</td>
<td>0.32 ± 0.03</td>
<td>1.15 ± 0.11</td>
<td>1.16±0.11</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>PS (12 wks)</td>
<td>0.26 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.85 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.85±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.27 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Sham (26 wks)</td>
<td>0.34±0.01</td>
<td>1.19±0.07</td>
<td>1.20±0.07</td>
<td>0.48±0.06</td>
</tr>
<tr>
<td>PS (26 wks)</td>
<td>0.29±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.05±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.06±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.38±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 2. Summary of mechanical test results from 3-point bend testing. Data are presented as mean ± standard deviation for 6 to 8 mice per group at each time point. PS = periosteal strip. Superscripts indicate statistically significant differences between sham and PS treatments (paired t-test) at p< 0.05 (a), p< 0.01 (b) or p<0.001 (c).

<table>
<thead>
<tr>
<th>Group (time)</th>
<th>Peak Load (N)</th>
<th>Stiffness (N/mm)</th>
<th>Energy to Failure (N*mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham (2 wks)</td>
<td>21.21 ± 1.74</td>
<td>82.53 ± 3.85</td>
<td>4.60 ± 0.65</td>
</tr>
<tr>
<td>PS (2 wks)</td>
<td>16.00 ± 2.2^c</td>
<td>70.53 ± 14.12^a</td>
<td>3.00 ± 0.43^c</td>
</tr>
<tr>
<td>Sham (6 wks)</td>
<td>23.79 ± 3.20</td>
<td>91.72 ± 8.65</td>
<td>5.08 ± 0.99</td>
</tr>
<tr>
<td>PS (6 wks)</td>
<td>16.47 ± 0.84^c</td>
<td>75.44 ± 4.06^c</td>
<td>3.16 ± 0.71^b</td>
</tr>
<tr>
<td>Sham (12 wks)</td>
<td>26.65 ± 3.72</td>
<td>95.70 ± 6.92</td>
<td>6.00 ± 1.10</td>
</tr>
<tr>
<td>PS (12 wks)</td>
<td>16.43 ± 3.40^c</td>
<td>77.14 ± 11.76^c</td>
<td>2.76 ± 1.02^c</td>
</tr>
<tr>
<td>Sham (26 wks)</td>
<td>26.11±3.34</td>
<td>100.27±12.0</td>
<td>5.07±0.74</td>
</tr>
<tr>
<td>PS (26 wks)</td>
<td>23.44±6.81</td>
<td>97.55±9.33</td>
<td>4.49±1.87</td>
</tr>
</tbody>
</table>
Table 3. Correlation matrix summarizing the relationship between peak load and microstructural parameters derived from micro-CT analysis. Results are presented as Spearman product-moment (r) values for data from all 62 animals. Asterisks denote p<0.01.

<table>
<thead>
<tr>
<th></th>
<th>Bone Volume (mm³)</th>
<th>Cross-Sectional Area (mm²)</th>
<th>Cortical Thickness (mm)</th>
<th>Polar Moment of Inertia (mm⁴)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Load (N)</td>
<td>0.729**</td>
<td>0.728**</td>
<td>0.695**</td>
<td>0.715**</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>0.679**</td>
<td>0.678**</td>
<td>0.669**</td>
<td>0.661**</td>
</tr>
<tr>
<td>Energy to Failure (N*mm)</td>
<td>0.713**</td>
<td>0.713</td>
<td>0.653**</td>
<td>0.720**</td>
</tr>
</tbody>
</table>
Figure 3. Micrographs demonstrating the presence (A) and absence (B) of the periosteum (arrows) in sham femora and stripped femora respectively. Stripping resulted in complete removal of the periosteum from the middle 8 millimeters of the femoral diaphysis.
Figure 4. Representative 3-dimensional micro-CT reconstructions of femora from mice that underwent periosteal stripping (PS) or sham surgery (sham). Images reflect observations at 12 weeks but similar findings were seen at all four time points.
The temporal patterns of changes in mechanical and structural properties were analyzed using 2-way ANOVA. Representative plots illustrating these patterns are presented in Figure 5. Statistically significant differences were identified for treatment, time point and for the treatment *time interaction.

Figure 5. Graphs depicting changes in peak load (A) and bone volume (B) over time in PS and sham femora. Two-way ANOVA confirmed that there were significant differences between treatments and time points. Additionally, there was a statistically significant interaction between time and treatment. Similar results were seen with the other mechanical and microstructural measures.
Chapter 4: Discussion

The results from this study demonstrate the potential for early and significant decreases in bone structural and mechanical properties following extensive periosteal stripping. While fractures were not seen in these animals, the mechanical test data clearly demonstrate the increase in bone fragility following periosteal stripping. On comparison of the sham and strip groups, there was a significant decrease in femoral strength and stiffness at 2 weeks, with further decreases seen at 6 and 12 weeks. At 12 weeks, stripped femurs were only 61% as strong as control femurs. Interestingly, the adverse effects of extensive periosteal stripping on bone strength appear to be recoverable, with stripped femurs demonstrating similar mechanical properties to sham controls at 26 weeks post-surgery.

The relationship between structural and mechanical properties was strong and significant, with decreases seen in bone volume, cross-sectional area, cortical thickness and polar moment of inertia for stripped femurs. Despite the recovery of mechanical properties in our model, however, a significant improvement in microstructural properties over time was not observed. It is important to recognize that whole bone mechanics depend not only on bone volume and architecture, but also on the intrinsic material properties of bone, and we suspect that the improvements in mechanical properties seen between 12 and 26 weeks reflect changes in the degree of bone organization and/or
mineralization. Additional research is indicated to evaluate the material properties of cortical bone in light of the observed changes in skeletal phenotype.

The negative effects of periosteal stripping on bone may be related to cortical vascular disruption and increased bone resorption. One of the principal functions of the periosteum lies in its contribution to cortical blood supply. It has been erroneously suggested that the periosteum contributes little to cortical blood supply in the uninjured long bone. Using gamma spectrometry to quantify vascularization of the guinea pig femur, Chanavaz and others demonstrated that the periosteal circulation is responsible for 70-80% of the arterial supply to the bone cortex and 90-100% of the venous return when compared to centromedullary blood supply. Using microangiography of the dog femur, Silberman demonstrated that circumferential periosteal stripping and removal resulted in almost complete absence of vascular paths in the diaphysis when examined at 7 days. In adults, persistence of diaphyseal vascularization was seen at 15 days, and the vascular patterns approached normal at 1 month. Kowalski and others studied the effect of periosteal stripping on cortical bone perfusion using a juvenile sheep model. In this experiment, extensive periosteal stripping along the medial aspect of the tibia acutely resulted in an approximately 25% decrease in cortical bone perfusion as measured by laser Doppler flow measurements. Periosteal microvascular disturbances including decreased nutritive perfusion and increased vascular permeability can also be seen following closed soft tissue injury. In our model, loss of periosteal contribution to cortical blood supply is likely contributing to the pathologic changes we observed. While the exact mechanism remains unclear, it may be related to increased cortical bone resorption leading to decreased bone volume.
The adverse effects of periosteal stripping may also be due to the removal of cambial osteoprogenitor cells and impaired bone turnover. In a rat femur model of periosteal stripping, histological evidence of periosteal regeneration was seen as early as 2 weeks following stripping. The regenerative tissue was initially seen as a thick layer of osteoblasts and undifferentiated cells. By eight weeks, regenerated periosteum in the stripped diaphyses had histomorphometric characteristics that were not significantly different than those of control periosteum. The gain in mechanical strength in our model may be related to regeneration of the periosteum and increased bone formation. Even if osteoblast depletion does not significantly alter bone structure and the primary insult is vascular in nature, periosteal regeneration and revascularization may play an important role in the recovery observed between 12 and 26 weeks.

When used as an adjunct therapy in the surgical management of patients with extremity soft tissue tumors, periosteal stripping is typically combined with radiation therapy, which is itself known to induce bone loss and bone fragility in vivo. As previously suggested, the periosteum should not be excised unless it is necessary to obtain an adequate surgical margin, and patients undergoing periosteal stripping and radiation therapy should be monitored closely following surgery. Management of pathologic long bone fractures secondary to periosteal excision and radiation therapy is fraught with complications, including fracture nonunion and deep infection. In particularly high-risk patients, prophylactic internal fixation should be considered.

There are several limitations recognized with this model and study design. The degree of periosteal stripping performed was very extensive and, at least in terms of the relative area of bone involved, does not directly correlate with periosteal stripping
typically performed in the context of soft tissue oncologic surgery. The model was designed to be relatively aggressive in order to identify and quantify the effect of periosteal stripping on bone microstructure and strength. Given the magnitude of the effect demonstrated with this study, it would be appropriate to use a similar model to study the effects of less extensive periosteal stripping. Using a model to investigate long bone growth after femoral periosteal stripping in rats, Garcés et al. observed significantly decreased diaphyseal diameter following 4 mm circumferential stripping as early as 2 weeks after surgery. While mechanical and structural evaluation of the femurs was not performed, these results at least suggest a measurable effect with less extensive stripping. Another limitation recognized with this study design is that only one loading condition (3 point mechanical bend testing) was used to evaluate bone strength. While axial or torsional loading would be more appropriate in evaluating fracture risk from a clinical perspective, 3-point bend testing was chosen because this is the most appropriate test for looking at cortical bone strength, which was the focus of this experiment.

This experimental model will serve as a foundation for future research. Ongoing studies include a histomorphometric assessment of the relative contributions of changes in bone formation and resorption rates to the reduction in bone volume identified in the current study. In addition, the role of vascular injury in the pathogenesis of bone loss following periosteal stripping will be examined using a combination of microspheres (to quantify blood flow) and immunohistochemistry (to identify and quantify vascular structures in/around bone). Ultimately, the periosteal strip model presented in this paper will be combined with the previously established local radiation therapy model to provide a clinically relevant test system for exploring interventional strategies for preventing
bone loss and/or restoring bone volume and bone strength in patients that undergo periosteal stripping and adjunct radiation therapy.
References


