Human femoral neck has less cellular periosteum, and more mineralized periosteum, than femoral diaphyseal bone

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ABSTRACT

Periosteal expansion enhances bone strength and is controlled by osteogenic cells of the periosteum. The extent of cellular periosteum at the human femoral neck, a clinically relevant site, is unclear. This study was designed to histologically evaluate the human femoral neck periosteal surface. Femoral neck samples from eleven male and female cadavers (ages 34-88) were histologically assessed and four periosteal surface classifications (cellular periosteum, mineralizing periosteum, cartilage, and mineralizing cartilage) were quantified. Femoral mid-diaphysis samples from the same cadavers were used as within-specimen controls. The femoral neck surface had significantly less ($p < 0.05$) cellular periosteum (18.4 ± 9.7 %) compared to the femoral diaphysis (59.2 ± 13.8%). A significant amount of the femoral neck surface was covered by mineralizing periosteal tissue (20-70%). These data may provide an alternate explanation for the apparent femoral neck periosteal expansion with age and suggest the efficiency of interventions that stimulate periosteal expansion may be reduced, albeit still possible, at the femoral neck of humans.

47 **Key words: histomorphometry-human, calcification, periosteal expansion**

48

INTRODUCTION

The risk of hip fracture increases exponentially with age (10). Predictions for future trends in hip fractures are staggering, estimated at more than 6 million by 2050 (11), compared to 1.5 million in 1990 (12). Hip fractures localized to the femoral neck present unique difficulties for treatment (6) and carry with them the highest rate of fracture-related morbidity (10). Although the factors that lead to a femoral neck fracture are numerous, it is well-accepted that the structural geometry of the neck is significantly related to fracture risk.

Periosteal expansion occurs throughout life. The rate of expansion is high during the pubertal years (9), slower during the adult years (23, 25) and, in women, accelerated again after menopause (1). This apposition is manifested through the cells of the periosteal cambium layer, which is in direct contact with the periosteal bone surface and contains a rich supply of osteogenic cells. Independent of other changes, expansion of the periosteal surface increases the strength of long bones and decreases the risk of fracture (19).

The existence of periosteum at the femoral neck is commonly debated. Early observational (20, 21) and histological (4) studies suggest the human femoral neck lacks a periosteum. The absence of callus formation following femoral neck fractures in adults supports these observations (20, 26). Despite recent studies that suggest the absence of periosteum at the femoral neck is not absolute (3, 13, 22, 24), publications continue to state that the femoral neck lacks a periosteal covering (14-16). If periosteum is to be a future therapeutic target to enhance bone strength (2) the extent of periosteum at this clinically relevant site must be clarified. This study was designed to examine the periosteal surface

of the human femoral neck, through quantification of cellular periosteum and characterization of other types of surface coverings.

METHODS

Samples of femoral neck and femoral mid-diaphyseal tissue were obtained from eleven cadavers at the Indiana University School of Medicine (Table 1). Cadavers were embalmed within 24-48 hours of death, thus preserving structural and cellular detail at that point. Mid-diaphyseal tissue was used for an internal control for any possible changes in cell detail lost during the processing of tissue, as cellular periosteum is known to cover a substantial percentage of this surface (28). Entire cross-sections of both femoral neck and mid-diaphyseal samples were divided into quadrants (four and two, respectively) to ease demineralization and sectioning processes. Samples were demineralized in 5% formic acid buffered formalin, dehydrated in sequential ethanols, cleared with xylene, and embedded in paraffin for histological sectioning. Serial transverse cross-sections (4 μm) were cut using a Reichert-Jung 2050 microtome (Magee Scientific, Inc., Dexter, MI) and stained with Massons trichrome.

For all surfaces, one of four classifications was noted (Figure 1). *Cellular periosteum* contained multiple cells (independent of cell morphology) within ~50 μm of the periosteal surface. Cells did not have to be continuous along the surface yet multiple cells had to be present within a focal area (~50 μm of surface) to be counted. *Mineralized periosteum* was in a similar spatial location to cellular periosteum (within ~50 μm of periosteal surface) although no cells were observed. Rather mineralizing nodules/tissue lacked any lamellar pattern and were clearly distinct from the periosteal bone surface. *Cartilage (hyaline)* consisted of a blue stained matrix with abundant chondrocyte lacunae;

(D) *Mineralized cartilage (hyaline)* consisted of red stained matrix with islands of blue unmineralized cartilage surrounding chondrocyte lacunae. Variable surface percentages in each specimen did not conform to any of the four criteria yet displayed no other discernable tissue patterns. For each specimen, one entire cross section was analyzed from each location using a semiautomatic digitizing system (Bioquant System 4, R&M Biometrics, Inc., Nashville, TN) attached to a microscope with a bright-field light source (Nikon Optihot 2 microscope, Nikon, Tokyo, Japan). The length of surface covered by each tissue classification is expressed as a percentage of total surface length. Differences in periosteal surface tissue composition between femoral neck and diaphysis were compared using a Wilcoxon signed ranks test for matched samples; correlations between femoral neck surface tissues and age were assessed using Spearman rank order analysis. Data are presented as percentage or mean ± SD. For all tests, a p value of < 0.05 was deemed statistically significant.

RESULTS

Femoral mid-diaphyseal bone served as an adequate control tissue for periosteal assessment, having a cellular cambium layer on greater than 40% of the surface in all specimens (Figure 2 and 4A). In sharp contrast, the femoral neck had significantly less cellular periosteum (18.4 ± 9.7 %) compared to the diaphysis, with individual subjects ranging from 2-33% (Figure 3A and 4A). In all subjects the amount of cellular periosteum on the diaphysis was > 2 fold higher compared to the femoral neck. The lower cellular periosteum surface percentage at the femoral neck compared to mid-diaphyseal bone are inversely associated with increased amounts of mineralized tissue (Figure 3B and 4B). Notably absent on diaphyseal bone, this mineralized tissue, which is distinctly discernable

116 histologically from lamellar bone, covers between 20-70% of the femoral neck surface
117 (Figure 4B). There was no cartilage covering any portion of the diaphyseal bone, while
118 variable amounts of both mineralized and non-mineralized cartilage were quantified at the
119 femoral neck (Table 2). There was no significant correlation between subject age and the
120 percent of cellular ($R^2 = 0.18$) or mineralized ($R^2 = 0.07$;) periosteum at the femoral neck
121 (Figure 5). Similarly, there was no correlation between age and cellular periosteum at the
122 femoral diaphysis ($R^2 = 0.05$).
123

124 **DISCUSSION**

125 The results of this study document that the human femoral neck has significantly less
126 cellular periosteum than diaphyseal bone. Unlike the diaphysis, the majority of the femoral
127 neck periosteal surface is covered by mineralizing tissue located spatially where cellular
128 periosteum would be expected. The majority of studies that have addressed the issue of
129 periosteum at the femoral neck have been qualitative (3, 4, 20, 21, 24, 27) and rarely define
130 whether cellular periosteum exists at this location. To our knowledge only one study (22)
131 has quantitatively evaluated the human femoral neck; their measurement of cellular
132 periosteum (16%) is comparable to the results of the current study (18.4%). All subjects in
133 this previous study (22) were over 75 years of age. In the current study, which includes
134 younger individuals, the 34, 42 and 49 year old subjects had < 32% of cellular periosteum
135 covering the femoral neck surface. This, along with the lack of correlation between age
136 and cellular periosteum (Figure 5) suggests that periosteal cellularity at the femoral neck is
137 significantly lower than in diaphyseal bone even in young adults.

Perhaps more striking than the lack of a cellular periosteum at the femoral neck was the large extent of mineralizing tissue (20-70% of surface). There is precedent for such mineralizing tissue on the periosteal surface of other human bones (28), yet this is the first study to quantitate such tissue at the femoral neck. Zagba-Mongalima observed that after the age of 48, "periosteal calcifications" on diaphyseal bone were evident in over 75% of the subjects and were characterized by dense calcified aggregates throughout the inner layer of the periosteum which are devoid of osteocyte lacunae (28). The histological observations of the current study conform to this description (Figure 3B). This tissue appears to be one of two types of mineralizing tissues that have been described near the periosteal surface (7, 8, 24, 27). Calcified fibrocartilage exists at the femoral neck as early as age 20 and is observable using backscatter electron microscopy (7, 8, 24, 27), synchotron imaging (7, 8, 24, 27), and standard histology (7, 8, 24, 27). Although we did not observe any calcified fibrocartilage (we noted mineralized hyaline cartilage) in the current study, others have documented both types of calcified tissue in a given specimen (27). As only one cross-section from each location was assessed, we cannot discount the possibility that femoral neck surfaces may vary along the length of the femoral neck. However, previous studies provide no indication of such spatial differences with respect to various tissue types (24).

These results have two main implications. From the perspective of reducing femoral neck fractures, the fact that 20% of the femoral neck surface has cellular periosteum suggests that anabolic osteogenic therapies may be effective in strengthening this clinically relevant site. As periosteal cells have greater sensitivity to mechanical (17) and pharmacological (18) stimuli compared to marrow cells, even limited cellular

161 periosteum may be sufficient for enhancing periosteal apposition. These cells likely do
162 serve to expand the periosteal diameter, as the femoral neck experiences age-associated
163 radial expansion (5, 23, 25). It may be, however, that the limited quantity of cells limits the
164 rate of expansion, resulting in less than optimal bone geometry and therefore elevated
165 fracture risk.

166 Alternatively, these data may present supporting evidence that the femoral neck
167 exhibits an alternative means of periosteal apposition. Previous studies have documented
168 that both periosteal calcification and calcified fibrocartilage undergo osteonal remodeling
169 (27, 28). Although this study did not document any calcified fibrocartilage, the abundant
170 periosteal mineralized tissue did contain individual osteons, clearly separate from the
171 periosteal bone surface, in some regions. Such a mechanism could be an alternative
172 explanation for femoral neck periosteal expansion with age. Thus, rather than
173 circumferential lamellae being laid down on the periosteal surface and subsequently
174 remodeled into osteons, as occurs in diaphyseal bone, mineral accumulates separate from
175 the periosteal surface with subsequent osteonal remodeling necessary for incorporation into
176 the existing bone. The highly irregular surface of the femoral neck, as compared to the
177 relatively smooth periosteal surface of diaphyseal bone, certainly supports this hypothesis
178 although further study is necessary.

179 Our data document that the human femoral neck has significantly less surface
180 covered by cellular periosteum than the femoral diaphysis. Such differences appear to
181 manifest during the early adult years and exist in both genders. It remains unclear whether
182 periosteal apposition at the femoral neck is mediated through the limited number of
183 periosteal cells or if alternate means of expansion (soft tissue mineralization followed by

184 remodeling) exist. From a clinical perspective, the relatively sparse cellular periosteum on
185 the femoral neck may reduce the efficacy of interventions (both pharmacological and
186 mechanical) that stimulate periosteal apposition at this site.
187

Figure Legends

Figure 1: Periosteal surface tissue classifications. In each image, the periosteal surface (P) is noted. (A) *Cellular periosteum* contained multiple cells (independent of cell morphology) within ~50 μm of the periosteal surface. Cells did not have to be continuous along the surface yet multiple cells had to be present within a focal area (~50 μm of surface) to be counted. Original magnification x 200, bar = 50μm; (B) *Mineralized periosteum* was in a similar spatial location to cellular periosteum (within ~50 μm of periosteal surface) although no cells were observed. Rather mineralizing nodules/tissue lacked any lamellar pattern and were clearly distinct from the periosteal bone surface. Original magnification x 100, bar = 100μm; (C) *Cartilage (hyaline)* consisted of a blue stained matrix with abundant chondrocyte lacunae. Original magnification x 100; bar = 100μm; (D) *Mineralized cartilage (hyaline)* consisted of red stained matrix with islands of blue unmineralized cartilage surrounding chondrocyte lacunae. Original magnification x 200, bar = 50μm. All sections stained with Massons trichrome.

Figure 2: Photomicrographs of femoral diaphysis periosteal surface. Cellular periosteum (arrowheads) is clearly observed near the periosteal surface. Section is from an 81 year-old female cadaver stained with Massons trichrome. Original magnification x 200, bar = 50μm.

Figure 3: Photomicrographs of femoral neck periosteal surface. Cellular periosteum (A) and mineralizing periosteum (B) are clearly observed near the periosteal surface. Mineralizing periosteum is noted directly near (arrows) the bone surface. Sections are

from an 81 year-old female cadaver stained with Massons trichrome. A: Original magnification x 200, bar = 50μm. B: Original magnification x 40, bar = 100μm.

Figure 4: Quantification of cellular (A) and mineralizing (B) periosteum at the femoral neck and mid-diaphysis. Data presented as the percent of total periosteal surface covered by each tissue type. ***** significantly different ($p < 0.01$) compared to alternate site.

Figure 5: Correlation between age and periosteal tissue type at the femoral neck. There was no significant relationship between age and either cellular or mineralizing periosteum.

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222 **Table 1: Specimen characteristics**

Gender	Age	Cause of death
Female	34	Renal carcinoma
Female	42	Breast carcinoma
Male	49	Lung carcinoma
Female	66	Metastatic carcinoma
Male	68	Aortic aneurysm
Male	70	Cardiac arrhythmia
Female	75	Lung carcinoma
Female	76	Congestive heart failure
Male	77	Renal disease
Female	81	Lung carcinoma
Female	88	Lung carcinoma

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Table 2: Cartilage surface on femoral neck

Subject	Age	Cartilage surface, %	Mineralized cartilage surface, %
Female	34	1.63	0.55
Female	42	9.89	6.91
Male	49	0.00	15.78
Female	66	4.13	1.12
Male	68	0.00	0.00
Male	70	3.33	0.52
Male	77	17.95	4.80
Female	81	1.29	0.00
Male	81	12.50	15.71
Female	88	1.62	0.00

Data are expressed as the percentage of the femoral neck periosteal surface covered by each tissue type. There was no cartilage tissue on the periosteal surface of mid-diaphyseal bone.

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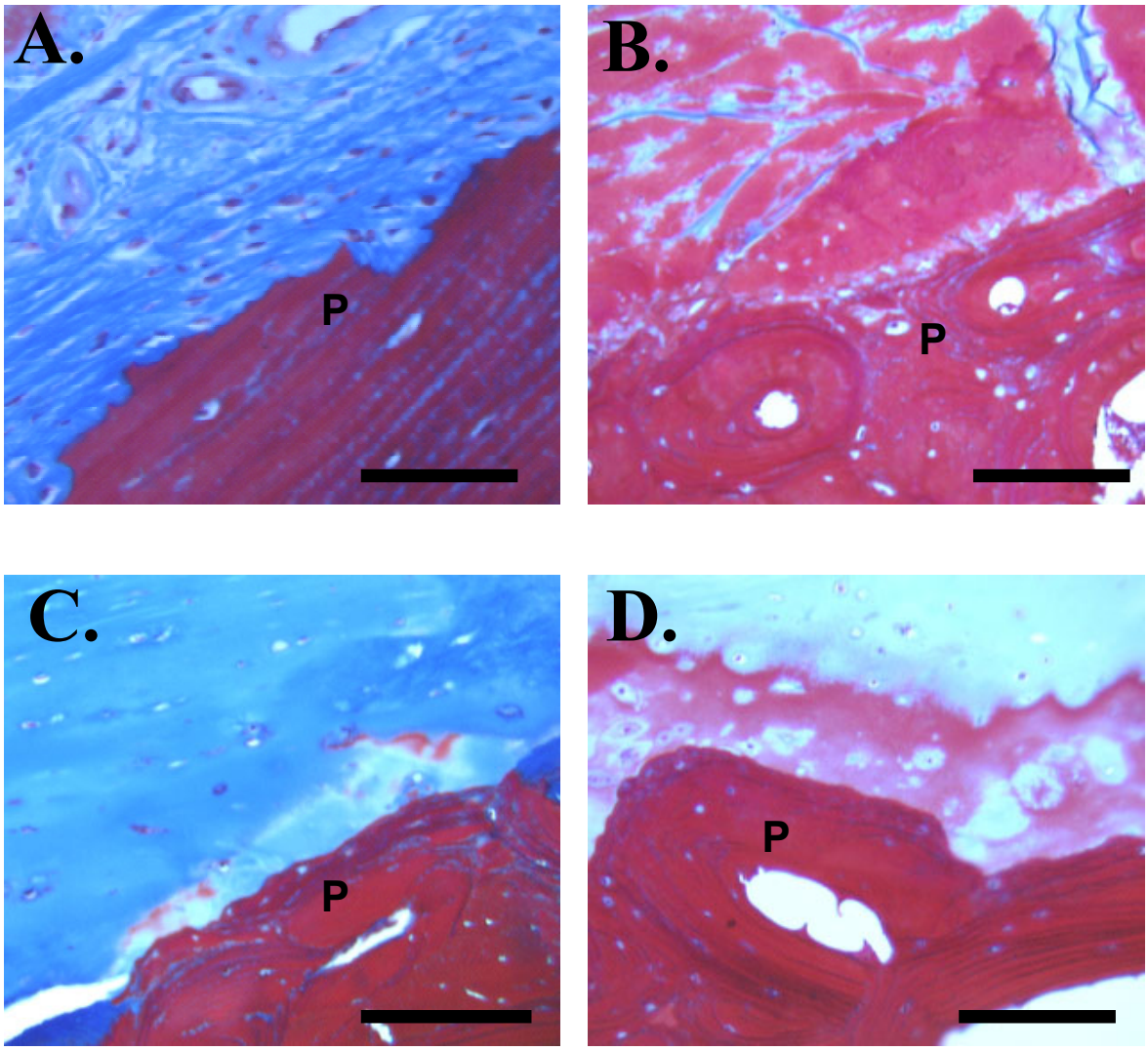
Figure 1



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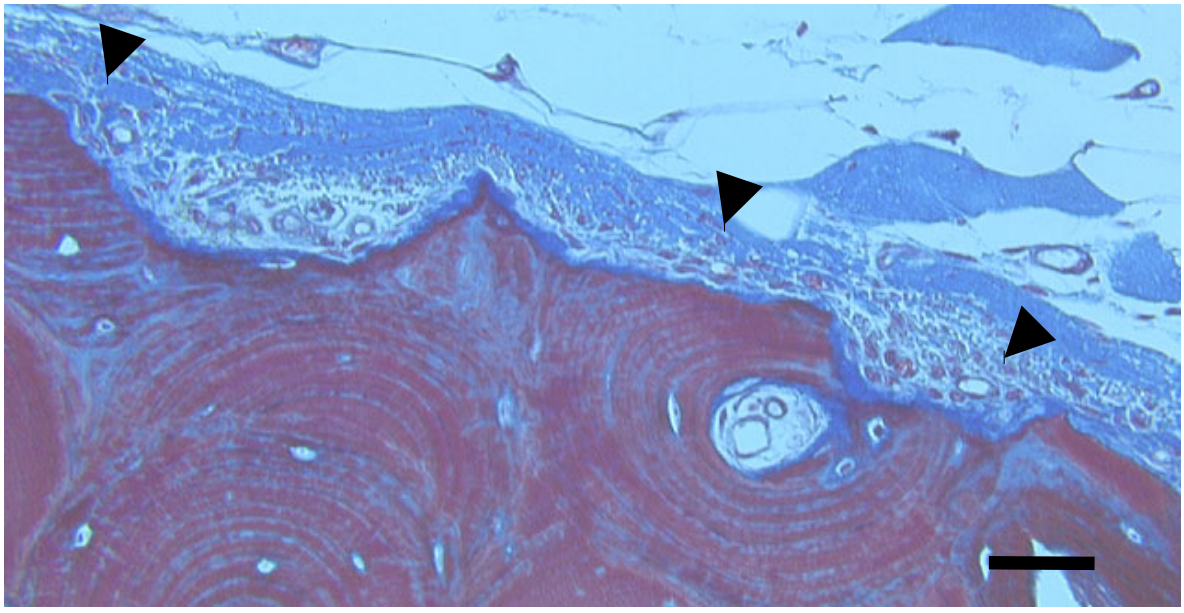

373 Figure 3
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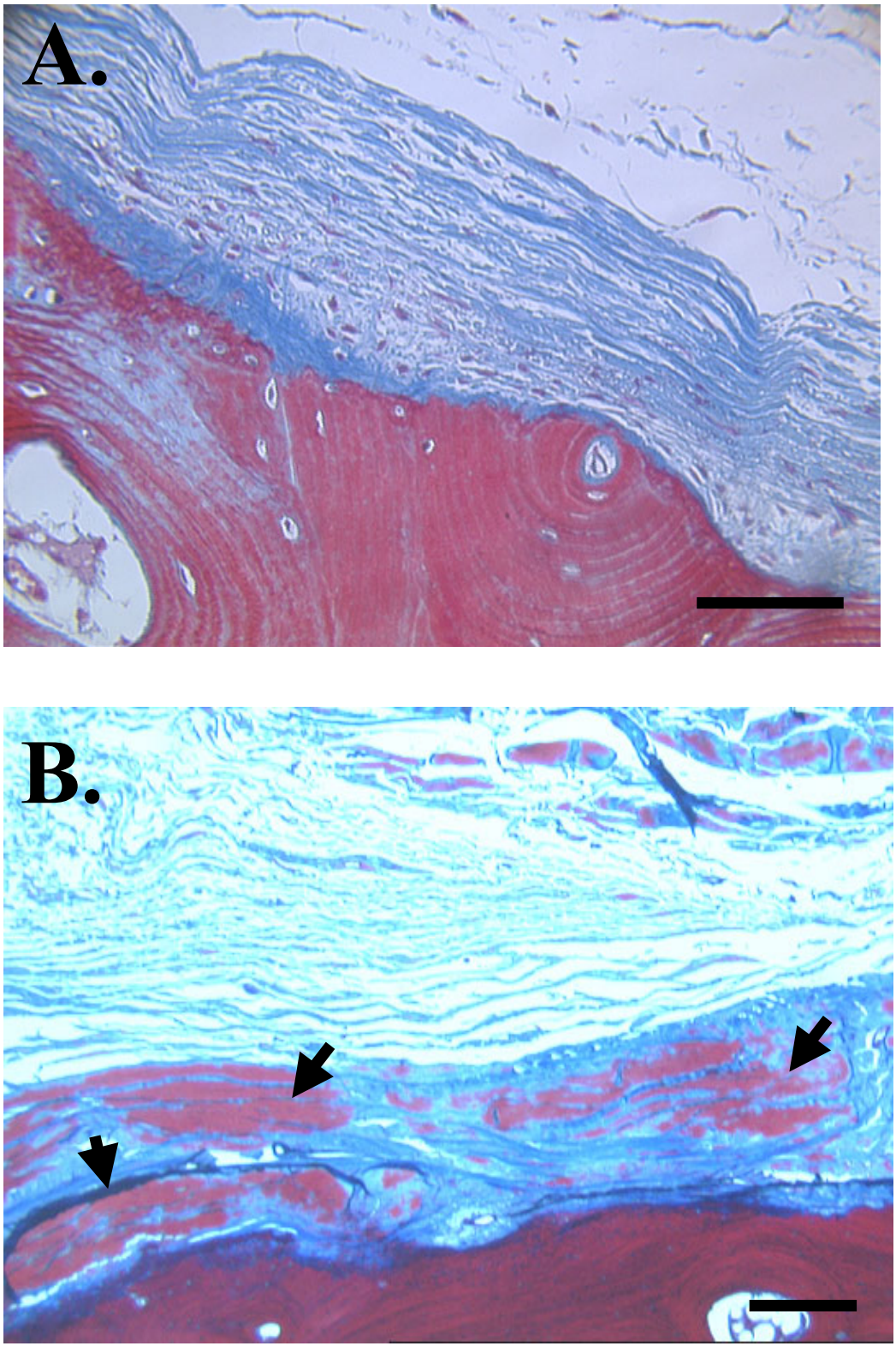

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376 Figure 4

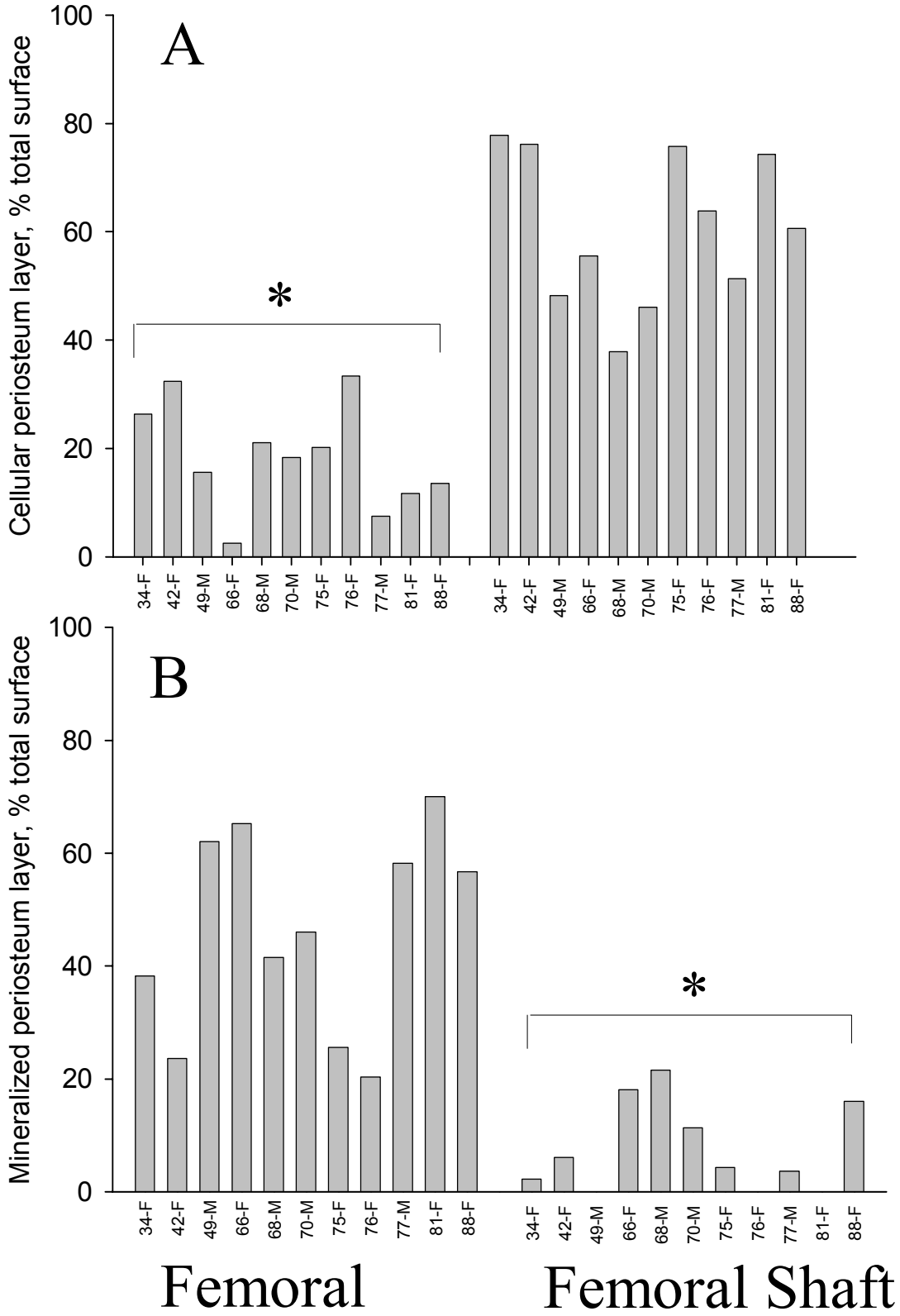

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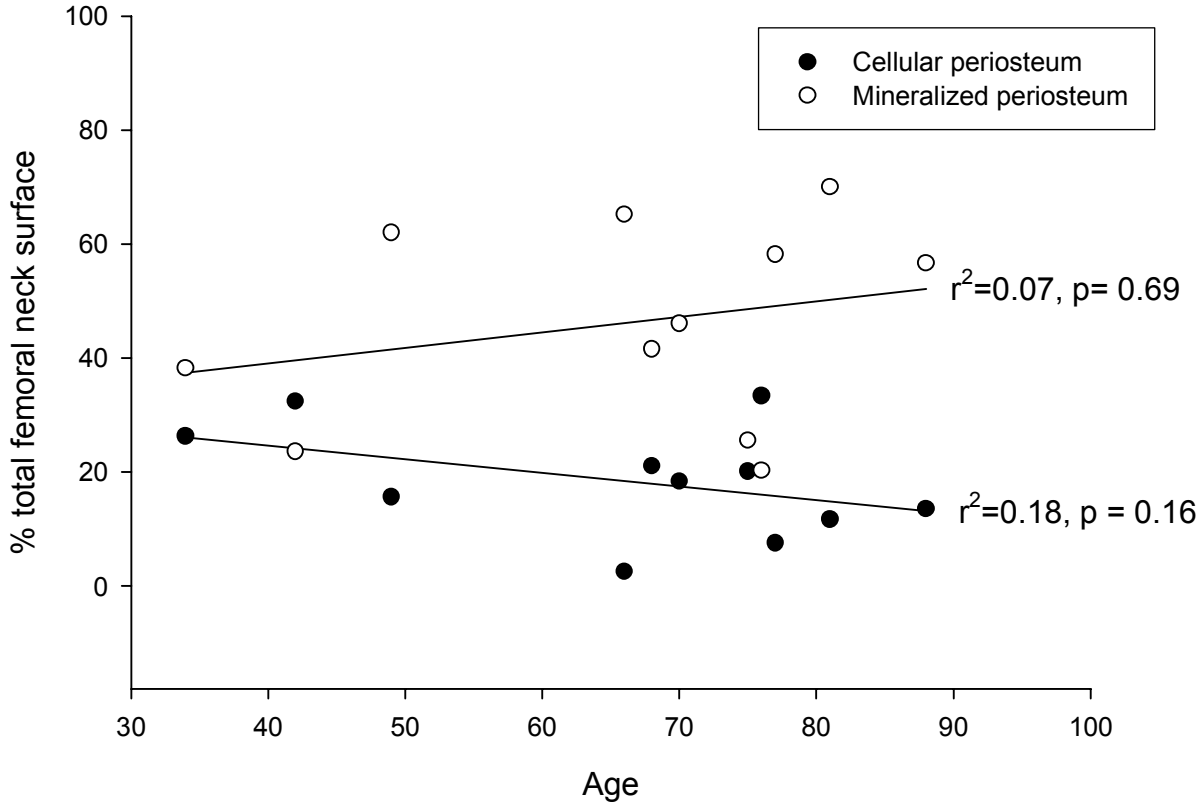
Figure 5


Cellular periosteum
Mineralized periosteum
r2=0.07, p= 0.69
r2=0.18, p = 0.16
% total femoral neck surface
Age
100
80
60
40
20
0
30
40
50
60
70
80
90
100