Regeneration of Periosteum in Denuded Bone

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INTRODUCTION
The periosteum is a protective layer around bone which actively participates in bone remodeling and turn-over. A recent one-stage bone-transport procedure has shown that mesenchymal stem cells from vascularized periosteum, even in the absence of the bone marrow, is capable of generating bone de novo to repair critical sized defects in long bone [1,2].

Fig. 1. Schematic of one-stage bone-transport procedure (yellow – bone, red – periosteum). A portion of diaphyseal bone is denuded of periosteum, osteotomized, and transported over an intramedullary nail to the site of the 2.54-cm critical-sized defect, where it is docked to the distal femur. The periosteal sheath is left in situ to surround the new defect site, leading to full infilling of the newly created defect with woven bone within 2 weeks after surgery and healing fully within 16 weeks post-surgery.

METHODS
Undecalcified Bone Samples: Bone segments previously preserved in polymethylmethacrylate (PMMA) were cut into 250 µm thick sections using a diamond wire saw (Well Diamond Wire Saw Model 4240, Norcross, GA) and polished to a final thickness of 100 µm (Buehler Automat Polisher, Lake Bluff, IL). The sections were stained using Giema and Eosin and mounted using 100% Eukitt.

Giemsia and Eosin Staining: The surfaces of the sections were etched using a 1% formic acid solution. The sections were then stained using a 15% Giemsa solution for 30 minutes at 55°C and then with a 1% Eosin solution for 1 minute at room temperature, rinsing with deionized water in between. The sections were then dehydrated using a series of ethanol washes at 70%, 96%, and 100% concentration.

Calculation of Major and Minor Axes, Histomorphometry: Digital images of the sections were obtained using a high resolution scanner and the major and minor centroidal axes (CA) were calculated using a macro in ImageJ [3]. Brightfield images of the periosteum at the major and minor CA were taken at 20x magnification (Leica Research Grade microscope).

RESULTS
The histology of the perfect control sections (from the bone segments that were removed to create the original defect) showed a very clear separation between the bone, periosteum, and muscle (Fig. 2). The inner and outer borders of the periosteum were very smooth and the dark blue nuclei were a clear indication of the difference between the cellular layer and the fibrous layer of the periosteum. A similar protective layer was observed in the denuded bone (Fig. 3); however, the separation between the muscle and the newly-formed layer is much less defined. The difference between the two layers of the periosteum is also more difficult to distinguish.

Fig. 2. Periosteum on perfect control bone. The periosteum on the perfect control bone can be clearly distinguished between the bone and the muscle around it. The cell nuclei (stained dark blue) delineate the cambium and fibrous layer of the periosteum.

Fig. 3. Newly-formed layer surrounding denuded bone. Histological images show the newly-formed layer around denuded bone surface that is A) smooth and B) uneven. Cells are larger in number and in size (as indicated by the dark blue cell nuclei), indicative of active osteoclastic remodeling in these areas.

DISCUSSION
Based on histological evaluation of denuded bone in comparison with healthy control periosteum, it is clear that a protective layer of some form is regenerated after denuding of the transport segment. Areas of active osteoclastic remodeling are also apparent in a large portion of the denuded bone at 16 weeks after the one-stage bone transport procedure. Quantitative analyses of bone resorption, bone apposition, and periosteal regeneration along denuded surfaces are underway to better elucidate periosteal physiology in health and disease. We are particularly keen to understand the capacity of the periosteum to regenerate itself, given previous studies demonstrating the remarkable capacity of the periosteum to rapidly generate bone de novo.


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