THE ROLE OF PERIOSTEUM AND BONE MARROW DURING RABBIT BONE REGENERATION

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In vitro, the mesenchymal stem cells obtained from the bone marrow can give rise to myoblasts, chondroblasts, adipocytes, tendon cells, fibroblasts or osteoblasts. Some of the informative factors or culture conditions able to induce the differentiation in one way or another have been described. Periosteal stem cells can form either bone or cartilage depending on the culture conditions. In the same way, in vitro, muscle satellite cells, adipocytes or pericytes were able to be oriented toward the osteoblast phenotype. Thus, the pathways leading to osteoblast differentiation seem to be numerous in vitro.

In vivo, it is known that osteoblasts can form from fibroblast-like cells from the mesenchyme or from the hypertrophic chondroblasts during enchondral ossification or from chondroid cells during chondroid ossification. The aim of this study was to evaluate the role of the different periosteous tissues in the supply of bone forming cells during bone regeneration. The model chosen was bone lengthening as it is known to stimulate bone formation. We have histologically studied bone formation in the regenerate during lengthening when bone marrow, periosteum, both or none of them were made unavailable.

Materials and methods

Surgical procedure
19 pure-bred immature 2.4-3.0 kg New Zealand white rabbits were fitted on one randomly chosen femur with a lateral external fixator. They were randomly assigned to 4 surgical groups. Group 1. Periosteum and bone marrow preservation (5 animals). The periosteum was elevated up to its proximal and distal insertions. After positioning of the external fixator, a corticotomy was performed using a high speed steel cutter preserving the bone marrow. Group 2. Periosteum preservation and bone marrow destruction (5 animals). The surgical procedure used in Group 1 was performed, but the bone marrow was removed and scrapped up to the metaphyseal cancellous bone using a curette. The cavity was filled with a radiotransparent surgical polymethylmetacrylate. Group 3. Periosteum destruction and bone marrow preservation (5 animals). Using the same lateral approach, the muscle fibers were elevated from the periosteum, then the well individualized periosteum stripped from the bone. A corticotomy was performed as in Group 1. Group 4. Periosteum and bone marrow destruction (4 animals). It combined procedures used in Group 2 and 3.

Histology study
Qualitative analysis was performed on transverse and longitudinal sections while histomorphometry was done on longitudinal sections only. For the histomorphometric study, the reference points for the measurement through the work station were (1) the osteotomy site and adjacent zones; and (2) lines along the inner and outer cortical lines. Several interest areas were defined: the total intercortical area (ICA) between the proximal and distal cortices, the periosteal (PA) and medullary (MA) reaction area, fixed as the measurement window of the computer screen at a 10 times magnification. The thickness (in mm) of the periosteal (PT) and medullary (MA) reaction were also assessed.

The parameters measured were the relative surface area of bone (in ICA, PA and MA), cartilage and the dense healing fibrous tissue (in ICA), and thickness of reaction (in mm) on the periosteal side (PT) and on the medullary side (MT).

Statistical analysis
At the cortical site of the regenerate, the effect of periosteum/bone marrow/interaction on bone, cartilage, fibrous tissue formation was analyzed with a two-way ANOVA. At the periosteal and bone marrow sites, the effect of periosteum/bone marrow interaction on bone formation activity was studied with a two-way ANOVA. The activity of bone formation was separately recorded as the area percent of calcified tissue in the juxta-cortical window, and the reaction thickness (in mm).

For dependent parameters with significant effect, a multiple comparison (Kruskal-Wallis test with pair-wise comparisons) was performed to determine where the difference lies between each group.

Results and discussion:
This study showed that:
• Both bone marrow and periosteum could heal the bone tissue during distraction.
• No other tissues than bone marrow or periosteum in the periosteous tissues can give osteogenic stem cells in vivo. The removal of bone marrow periosteum suppressed the bone healing ability.
• There was no difference in the process of bone formation during healing by bone marrow or periosteum. Three ossification modes were detected: direct, enchondral and chondroid.
• Both the presence of bone marrow and periosteum had a positive effect on the bone density in the distraction zone. There was an interaction between bone marrow and periosteum for periosteal density and thickness (table 1)
• It indicated that there were some informations whose nature is not known exchanged between the periosteum and the bone marrow.
• Migration of periosteal or bone marrow cells was limited in the period of time studied

Table II. Brown-Forsythe Test for the Effect of 1) Periosteum and Bone Marrow Effect on Cortical Bone Formation, at the Distraction Gap ; 2) of Periosteum, Bone Marrow/Endosteum, and Their Interaction on Bone Formation Density at the Periosteal and Endosteal Windows ; 3) of Periosteum, Bone Marrow/Endosteum and Their Interaction on Bone Formation Thickness at the Periosteal and Endosteal Sites.

<table>
<thead>
<tr>
<th>Effect/area</th>
<th>Periosteal and Endosteal Thickness (mm)</th>
<th>Periosteal</th>
<th>Endosteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periosteum +</td>
<td>0.26 0.36 9.35E-08</td>
<td>0.0258 0.0594</td>
<td></td>
</tr>
<tr>
<td>Bone Marrow -</td>
<td>0.0372 0.1319</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction +</td>
<td>0.0013 0.1241</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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