Fluid Pressure Induced Osteoclast Activation and Bone Loss is Not Dependent on Fibrous Tissue in the Peri-Implant Zone

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PURPOSE:
Insufficient initial fixation and early implant subsidence are associated with an increased risk of aseptic loosening in total hip arthroplasty. In the setting of instability, a fibrous tissue layer is formed at the bone-implant interface, leading to implant migration and periprosthetic osteolysis. This fibrous layer has been hypothesized to serve two specific roles: 1) a mechanical role leading to component subsidence, and 2) a biologic role in which cytokines are expressed to induce osteoclast differentiation. Osteoclast recruitment is largely controlled by the RANKL/RANK system, and interface tissue from loose total hip replacement prostheses is known to produce RANKL.

In the rat model we have developed, a fibrous tissue membrane is cyclically compressed by a piston creating fluid pressure and flow leading to osteoclast differentiation. In this model, osteoclast activity and bone resorption are inhibited by OPG-Fc, demonstrating RANKL plays an active role in instability-induced osteolysis. The objective of this study was to determine the role of the fibrous tissue layer in osteoclast activity and periprosthetic bone loss. The presence and absence of fibrous tissue in our established rat model for instability induced osteolysis was used to determine whether fibrous tissue is required for both osteoclast activity and peri-implant bone loss.

MATERIALS & METHODS:
Animal Model: 46 rats (Sprague Dawley, 404 ± 24g), were used in a previously validated animal model for instability-induced prosthesis loosening. Briefly, a titanium plate with a central screw was inserted on the proximal tibia into a milled depression on the surface of the cortex. The screw and plate were allowed to osseointegrate for 5 weeks.

Thereafter, the central screw was replaced by a piston, which moved perpendicular to the bone surface and Ti plate (Figure 1). In half of the animals, a five day latency period was allowed prior to initiating loading, during which a fibrous tissue layer formed in the space between the piston and the bone surface.

In the remaining animals, loading was initiated the day after the central screw was replaced by the piston. Each respective group was subdivided into pressurized and non-pressurized controls. Once loading was initiated, an 8N transcutaneous force was applied by pushing the piston towards the bone surface. Twice daily force was applied for 20 cycles, at a frequency of 0.17Hz. The day after the last loading episode each rat was euthanized and the tibiae were harvested for analysis.

MicroCT: Samples were scanned by microCT and reconstructed at 15 μm voxel size. The volume of bone resorption was measured in two regions for each specimen: 1) a central zone, comprised of the 3mm area directly beneath the piston and 1.2 mm deep and 2) a peripheral zone, 0.75mm from the periphery of the central zone (Figure 1). The bone volume (BV) was measured in each zone, so loss of bone is reflected by smaller BV.

Immunohistochemistry: After decalcification, the proximal tibia was processed for immunohistochemical evaluation. Mouse anti-rat Cathepsin K antibodies were used to visualize bone resorbing osteoclasts. Osteoclasts were defined as Cathepsin K positive multinuclear cells located within 0.05 mm from a bone surface counted in the same locations as for microCT.

Statistical Analysis: All analyses were performed blinded for treatment to examine two primary hypotheses statistically by a two-way ANOVA (SPSS, version 19): 1) The fluid pressure induces osteoclast activation and 2) the fibrous tissue has a vital role in the amount of osteoclast activity and bone loss. For both hypotheses osteoclast number was the primary outcome. p<0.05 was considered significant.

RESULTS:
Fluid pressure induced dramatic bone resorption both beneath the central zone below the piston and at the periphery. The presence of fibrous tissue had no effect on the amount of osteoclasts, neither was a combined effect seen by pressure and fibrous tissue.

The amount of bone loss was increased at the peripheral location, but not at the location under the piston (central) (p=0.14). The presence of fibrous tissue had no effect of the amount of bone loss. There was no enhanced effect of bone loss, when pressure and presence of fibrous tissue was combined.

Table 1. Bone Parameters investigated. Values given as mean and SD.

<table>
<thead>
<tr>
<th></th>
<th>Fibrous Tissue</th>
<th></th>
<th>No Fibrous Tissue</th>
<th></th>
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<tbody>
<tr>
<td></td>
<td>Pressure</td>
<td>Control</td>
<td>Pressure</td>
<td>Control</td>
</tr>
<tr>
<td>Central Zone</td>
<td>OcN (a)</td>
<td>37 (10)</td>
<td>14 (7)</td>
<td>31 (17)</td>
</tr>
<tr>
<td></td>
<td>BV (mm³)</td>
<td>1.8 (0.3)</td>
<td>2.0 (0.3)</td>
<td>1.9 (0.3)</td>
</tr>
<tr>
<td>Peripheral Zone</td>
<td>OcN (a)</td>
<td>66 (31)</td>
<td>16 (11)</td>
<td>78 (30)</td>
</tr>
<tr>
<td></td>
<td>BV (mm³)</td>
<td>4.1 (0.8)</td>
<td>5.0 (0.6)</td>
<td>4.5 (0.8)</td>
</tr>
</tbody>
</table>
|               | p<0.05 compared to controls. OcN (Osteoclast Number), BV (Bone Volume)

DISCUSSION:
The fibrous tissue membrane surrounding loose prostheses has been suggested to play an important role in instability induced osteolysis. The current animal model mimicked this scenario through a compressed fibrous tissue membrane. However, the fibrous membrane had no effect on osteoclast activity and bone loss. In the absence of fibrous tissue, the pressurized specimens demonstrated similar osteoclast activity and peri-implant bone loss when compared to the presence of fibrous tissue. Therefore, we suggest that cytokine signals are released directly from the surrounding bone, rather than from the fibrous tissue layer. These findings are in line with previous in vivo studies where mechanical regulation and bone trauma led to osteocyte apoptosis, in which cleaved caspase-3 expression preceded osteoclast activation. Specifically, osteocytes close to apoptotic osteocytes expressed RANKL, demonstrating a signaling pathway leading to osteoclast activation. In a previous study using intermittent pressure, empty osteocyte lacunae were present in pressurized areas. Therefore, the signaling pathway within the bone adjacent to loose implants may be more important for the progression of osteolysis rather than the activity of cytokines in the fibrous tissue itself.

SIGNIFICANCE:
Our findings suggest that the bone tissue surrounding an implant induces osteoclast differentiation and bone loss due to mechanical factors such as fluid flow and pressure, even in the absence of a fibrous tissue membrane.

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REFERENCES: