CELL GROWTH IN BONE BIOPSIES FROM FROZEN HUMAN FEMORAL HEAD ALLOGRAFTS.

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Introduction
Impacted morsellized bone grafts are successfully used to treat bone loss in revision total hip arthroplasties. For this technique we use femoral heads from our own bone bank, processed according to the Musculo-skeletal Council of the American Associates of Tissue Banks (AATB) (1) and the European Association of Musculo-skeletal Transplantation (EAMST) (2). The femoral heads are stored at –80°C for at least six months before they are implanted, because of a window period. After implantation new bone is formed in the graft. It is generally thought that the method of processing of the fresh frozen femoral heads kills all cells. On the other hand there is general concern about introducing viral/bacterial contamination and/or oncogenesis with allograft bone (3,4). Therefore, in the present study we wished to determine whether the frozen bones contain any viable bone cells, as assessed by their capacity to give rise to proliferating cells in tissue culture.

Materials and Methods
Human femoral heads, obtained from 5 donors who got a primary total hip arthroplasty (THA), were stored at –80°C for 3 to 31 months (mean ± SEM, 17 ± 6 months) in our bone bank. Under sterile conditions, bone biopsies were taken from the center of the femoral heads, and processed as described in Figure 1. Collagenase was used to remove the soft tissue from the bone fragments.

Cell cultures were established according to earlier described methods for human bone cell cultures (5) (Figure 1). Microscopic evaluation of cell growth, number, and aspect was performed during culture. To test their osteoblastic phenotype, 6 weeks old cultures were incubated for 3 days with or without 10⁻⁸ M 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), and monolayers were assayed for alkaline phosphatase activity.

Discussion
The present study shows for the first time that femoral heads from a bone bank processed according to the AATB and the EAMST contain living bone cells with growth capacity. The cells showed aspects of mature osteoblastic cells (5). The osteoblastic phenotype of the cells was also suggested by their responsiveness to 1,25(OH)₂D₃. The outgrowth of bone cells from femoral head bone which was stored frozen at ~80°C suggests that the bone matrix prevents bone cell damage as a result of freezing. Another indication for a protective role of the bone matrix for bone cells during frozen storage is the somewhat faster outgrowth of bone cells from collagenase-treated bone fragments (where all dead bone is probably removed, allowing the living cells to grow out faster) compared to untreated fragments (where dead bone slows the cells down in their outgrowth).

In conclusion, bone biopsies from human femoral heads from a bone bank according to the AATB and the EAMST contain living bone cells with growth capacity. These observations warrant further studies on the use of synthetic bone materials in the treatment of bone loss in revision arthroplasty.

Table 1. Bone cell outgrowth from frozen human femoral head biopsies.

<table>
<thead>
<tr>
<th>donor age</th>
<th>sex</th>
<th>storage time</th>
<th>cell outgrowth (A)</th>
<th>cell outgrowth (B)</th>
<th># cells after 6 wk culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>76 y ♀</td>
<td></td>
<td>3 m</td>
<td>11 d</td>
<td>11 d</td>
<td>+</td>
</tr>
<tr>
<td>70 y ♀</td>
<td></td>
<td>3 m</td>
<td>11 d</td>
<td>15 d</td>
<td>++</td>
</tr>
<tr>
<td>77 y ♀</td>
<td></td>
<td>24 m</td>
<td>6 d</td>
<td>11 d</td>
<td>+++</td>
</tr>
<tr>
<td>83 y ♀</td>
<td></td>
<td>24 m</td>
<td>inf</td>
<td>35 d</td>
<td>+</td>
</tr>
<tr>
<td>63 y ♀</td>
<td></td>
<td>31 m</td>
<td>6 d</td>
<td>15 d</td>
<td>++</td>
</tr>
</tbody>
</table>

After the bone fragments were put into culture (day 0), cell outgrowth was monitored from collagenase-treated (A) and untreated (B) fragments. m, months; wk, weeks; d, days; inf, infected; +, 0-10 cells; ++, 10-100 cells; ++++, 100-1000 cells.

References

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