INTRODUCTION:

Development of improved technologies for bone regeneration is important in numerous clinical applications including craniofacial and dental reconstruction, and fracture repair. Many current approaches to bone repair rely on use of bone graft materials such as autologous iliac crest bone, and allograft and xenograft-derived materials. Significant drawbacks to use of these materials include limited amount and availability of donor material, donor site morbidity, possible disease transmission, and the potential for immune reaction and delayed healing. Development of an artificial bone graft material is desirable and should include the three critical components contained in iliac crest bone: 1) osteogenic cells, 2) osteoinductive proteins, and 3) an osteoconductive matrix.

1) As an alternative to cells from iliac crest bone, gingival fibroblasts, periosteal-derived mesenchymal cells, and fat-derived cells reportedly possess varying degrees of osteogenic potential and could serve as more plentiful and easily harvested cell sources for bone graft material.

2) Sonic hedgehog (SHH), a key secreted, regulatory protein involved in craniofacial morphogenesis, has the potential for increasing the commitment of various cell types into an osteogenic phenotype thereby serving as an osteoinductive component of the graft.

3) A novel osteoconductive matrix consists of a mixture of alginate and collagen I.

We report on development of a gene enhanced tissue engineering approach using SHH-expressing cells in a novel osteoconductive matrix to stimulate repair of calvarial bone defects in rabbits.

Our goal is to develop a cell-based artificial bone graft material from easily harvested and plentiful cell sources that have bone regenerative properties similar to those of autologous cancellous bone. Toward that end, gingival fibroblasts, periosteal-derived mesenchymal cells, and fat-derived cells were assessed for ability to regenerate bone. In addition, the impact of SHH genetic enhancement on the bone regenerative capacity of cells was determined.

METHODS:
The complete SHH cDNA was cloned from human fetal lung tissue by RT-PCR into a retroviral vector under the control of the β-actin promoter which drives low-level constitutive expression of SHH. Rabbit primary gingival fibroblasts, periosteal-derived mesenchymal cells, and fat-derived cells were stably transduced with SHH expressing or control retrovectors. Prior to implantation of transduced cells, SHH expression was confirmed at the RNA level by RT-PCR and SHH secretion was quantitated by ELISA. Cells (2 x 10^5) were combined with an absorbable alginate/collagen I matrix and inserted into 8 mm full-thickness calvarial bone defects in skeletally mature (>7 months) New Zealand White rabbits. Additional controls included matrix alone and empty defect (N=6 for each group). Bone regeneration (% bone in the full-thickness defect) was assessed at 6 weeks by digital analysis of histological sections.

Retroviral Vectors

Retroviral vectors used in this study. LTR: long terminal repeat; Neo+: neomycin resistance gene, fact : rat β-actin promoter; Shh: sonic hedgehog gene. Constructs are based on the LN series containing the selectable neomycin resistance gene driven by the 5’ LTR. Retroviral vectors were generated as amphotrophic retroviral vector particles from PA317 cells.

RESULTS: In Vitro

Steady state levels of free SHH present in 72 h. conditioned medium was assessed by ELISA from transduced cells grown in vitro prior to implant.

Table 1: SHH Expression In Vitro

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>SHH (ng/10⁶ cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GF-LNB-SHH</td>
<td>1.0</td>
</tr>
<tr>
<td>FDSC-LNB-SHH</td>
<td>4.1</td>
</tr>
<tr>
<td>MSC-LNB-SHH</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Control transduced cells do not produce significant amounts of SHH.

RESULTS: In Vivo

Experimental preparations containing cells transduced with SHH resulted in significantly higher quantities of regenerated bone tissue. Control defects did not demonstrate any substantial bone repair.

DISCUSSION:

Gingival fibroblasts, periosteal-derived mesenchymal cells, and fat-derived cells can be easily harvested, genetically enhanced with SHH expressing retroviral vectors, and rapidly expanded in culture. Transduced cells expressed SHH at the RNA and protein level. Composite grafts of alginate, collagen I and cells are easily manufactured and implanted into bone defects. Empty defects, matrix alone, and grafts with control transduced cells do not regenerate substantial amounts of bone in 6 weeks. FDSC often form undesirable cyst-like structures in the regenerating defects. Autopsy findings at 6 weeks show no abnormalities in other tissues (brain, lung, kidney, liver, and eye). Bone regeneration is not complete in 6 weeks. SHH genetic enhancement of all three cell types significantly improves bone regenerative capacity of grafts at 6 weeks.

This is the first report demonstrating that an artificial bone graft material containing tissue engineered SHH gene enhanced cells results in significant bone regeneration in vivo.

AFFILIATED INSTITUTIONS FOR CO-AUTHORS:

**Long Island Jewish Medical Center, New Hyde Park, NY