Isolation of osteogenic cell populations from bone fragments harvested during lumbar spine fusion procedures

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Introduction
When performing posterior lumbar fusions, a number of bone graft options are available including autograft, allograft, and various bone graft substitutes. Autograft iliac crest bone is still considered surgical standard due to its osteogenicity and osteoconductivity (1). However, harvesting such graft carries risk of postoperative pain and infection (2,3). Utilizing local lamina bone is an attractive alternative, as it typically must be removed to accomplish neural decompression. With the availability of bone graft extenders and other materials that provide osteoconductive and osteoinductive potential, a local autograft may be more effectively relied upon to replace autogenous bone autografts (ICBG) in posterior lumbar fusions. It would be important, therefore, to better characterize the osteogenic potential of local autograft bone. The objective of this study was to determine the presence of viable osteogenic cells in local autograft bone fragments. The secondary objective was to compare the osteogenic potential compared to mesenchymal stem cells.

Materials and Methods

Donor information and harvesting procedures: Twenty patients (8 females and 12 males; mean age 64.7 ± 12.9; range 33–86) undergoing laminotomy or laminectomy alone were included in the study after approval by the Scripps Institutional Review Board. The bone samples were collected in one of two ways. i) Rongeurs were used to remove laminar bone or ii) a Lukens trap was used to collect high-speed burr shavings. The bone samples were then placed in a tube containing 4% calcium citrate solution and stored at 4°C. All samples were processed for cell isolation 2–6 hours of the procedure. Isolation of cells from bone burr fragments: Harvested bone fragments were washed in Hank’s buffered salt solution and then plated into 6-well plates with MSC medium (Lonza). Expansion of cells: Upon detection of a cell colony from the bone fragments, the cells were expanded until confluent or a high cell density was observed in a colony (passage zero). These were replated in a T75cm² flask and further expanded (passage 1) until a sufficient number of cells were available for an osteogenic assay. Human bone marrow derived mesenchymal stem (MSC) cells: were harvested from one 24-year-old female donor (From Texas A&M). Osteogenic assay: Cells were seeded at a density of 1x10⁵ into at least 6 wells of a 12-well plate and cultured in MSC medium until confluence. At least three wells were cultured in osteogenic medium (Lonza) and three wells were maintained in MSC medium (controls) for three weeks. MSC were also cultured in identical conditions (positive control). After three weeks, one well from each treatment was either stained in monolayer for von Kossa (calcium deposition), alkaline phosphatase (AP) activity or harvested for RNA extraction for RT-PCR to examine osteogenesis-related gene expression (Col1a1, Col10a1, Runx2, BGLAP/osteocalcin, and MMP13).

Results

Of a total of 35 samples from 20 donors, 28.6% (10 samples) produced cell colonies that emigrated from the bone fragments over a 2–3 week period. Cell colonies were only observed from bone fragments removed using rongeurs (10 from 19 samples: 53%) and never from bone burr samples (zero from 15 samples). Cell morphology changes during culture in osteogenic medium indicated a treatment effect with control cells remaining fibroblast-like and treated cells with a more rounded and larger morphology, which stained positive for AP and von Kossa (Figure 1). Gene expression profiles indicate osteogenic differentiation with a 3- to 4-fold higher expression of Col10a1 and RUNX2 gene expression compared to control cultured cells (Figure 2). A large variation in osteocalcin/BGLAP expression was observed between donors (range: minus 28-fold to plus 4.3-fold) indicating different stages of osteogenic differentiation by 3-weeks. MSC showed very high levels mRNA levels of Col10a1 (>60-fold), RUNX2 (8.7-fold) and MMP13 (49-fold) at the same time point compared to controls. MSC were also strongly AP and Von Kossa positive (data not shown).

Discussion

The study indicates that rongeured laminar bone fragments have osteogenic potential, while we were unable to culture cells from burr shavings. This finding is in general agreement with a recent study that reported lower osteogenic capacity in drill shavings compared to bone fragments (4). In a clinical study (5), approximately 80% fusion was reported in one-level fusions using either ICBG or laminar bone. This rate fell to around 20% in two or more level fusions when using laminar bone to about 65% using ICBG. The variance in fusion rates with different sources is likely reflected in our observation that there may be less osteogenic potential in local autograft bone compared to iliac crest bone. The lack of cells from our burr samples may be related to instrument settings, which may lead to cell death. However, previous work (6) indicates that the cell and bone fraction of burr samples show no obvious microscopic damage. Further studies on cell viability following burring are warranted. The osteogenic potential of bone marrow derived cells (MSC) was greater compared to cells derived from the laminar bone fragments in this study. More research is needed to translate in vitro osteogenic potential to clinical success in fusion rates. Since laminar bone fragments do harbor osteogenic cells, these fragments may reduce the need to utilize ICBG, and allow in combination with extenders or other biologic alternatives, may represent a reasonable replacement of ICBG. While burr shavings did not generate viable cells in our culture system, they might have value as graft expanders and used as osteoconductive scaffolds.

Significance

Autograft bone fragments for posterior lumbar fusions should reduce complications arising from harvesting iliac crest bone grafts.

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References