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Human Spinal Bone Dust as a Potential Local Autograft: In vitro Potent Anabolic Effect on Human Osteoblasts

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The manuscript submitted does not contain information about medical device(s)/drug(s).

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Abstract

Study Design: In Vitro Study

Objective: To evaluate the effect that factors released from human posterior spinal bone dust have on primary human osteoblast growth and maturation.

Summary of Background Data: Bone dust, created during spinal fusion surgeries has the potential to be used as an autologous bone graft by providing a source of viable autologous osteoblasts and mesenchymal stem cells with osteogenic potential. To date, no information is available on whether bone dust also provides a source of anabolic factors with the potential to enhance osteoblast proliferation and maturation, which would enhance its therapeutic potential.

Methods: Bone dust was collected from consenting patients undergoing elective posterior spinal fusion surgeries, andprimary human osteoblasts were cultured from patients undergoing elective hip or knee arthroplasty. Growth factors and cytokines released by bone dust were quantified using enzyme-linked immunosorbent assay (ELISA). Primary human osteoblast proliferation and gene expression in response to bone dust were assessed using ^{3}H-thymidine incorporation and real-time polymerase chain reaction (qPCR), respectively.

Results: Human bone dust released anabolic cytokines (IL-1β and IL-6) **andgrowth factors** (TGF-β, VEGF,**FGF-Basic and**PDGF-BB)**in increasing concentrations over a 7-day period. In vitro, the anabolic factors released by bone dust increased osteoblast proliferation by 7-fold, compared with osteoblasts cultured alone. In addition, the factors released from bone dust up-regulated a number of osteoblastic genes integral to osteoblast differentiation, maturation and angiogenesis.**

Conclusion: This study is the first to demonstrate that human posterior spinal bone dust released anabolic factors that potently enhance osteoblast proliferation and the expression of genes that favorbone healing and bone union. Given that bone dust is anabolic and its harvest is fast, simple, and safe to perform, spinal surgeons should be encouraged to ‘recycle’ bone dust and harness the regenerative potential of this free autologous bone graft.

Figure 2.Bone dust ‘pate’.

Image of bone dust collected from one patient during posterior spinal fusion surgery.

Figure 3.In vitro methodology.

Schematic diagram demonstrating the experimental set up. Primary human osteoblasts are seeded in 24-well plates, above which bone dust is placed in tissue culture inserts with a 1μm pore size. The pores in the tissue culture inserts allow factors released from the bone dust to act on primary human osteoblasts.

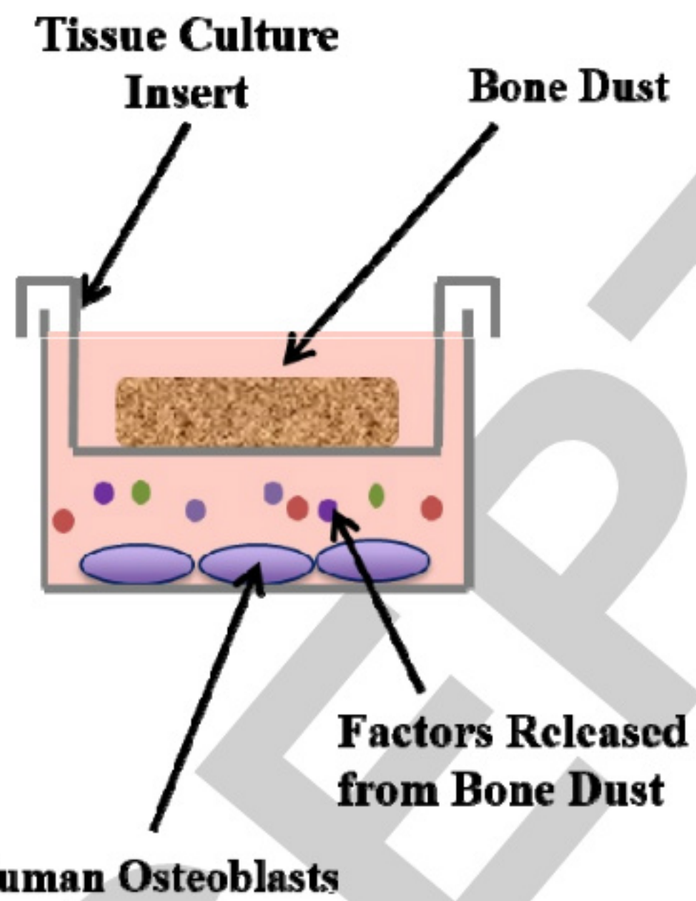


Figure 4.Bone dust acts as a source of growth factors and cytokines.

Enzyme-linked immunosorbent assay (ELISA) results demonstrating the presence of a number of anabolic growth factors (TGF-β,VEGF, FGF-Basic and PDGF-BB) and cytokines (IL-1β and IL-6) released from human posterior spinal bone dust over a 7-day period. Data presented are mean of four patient samples ± SEM. Statistical analysis was performed using one-way ANOVA and post-hoc Dunnett's test *p<0.05 (n=4)

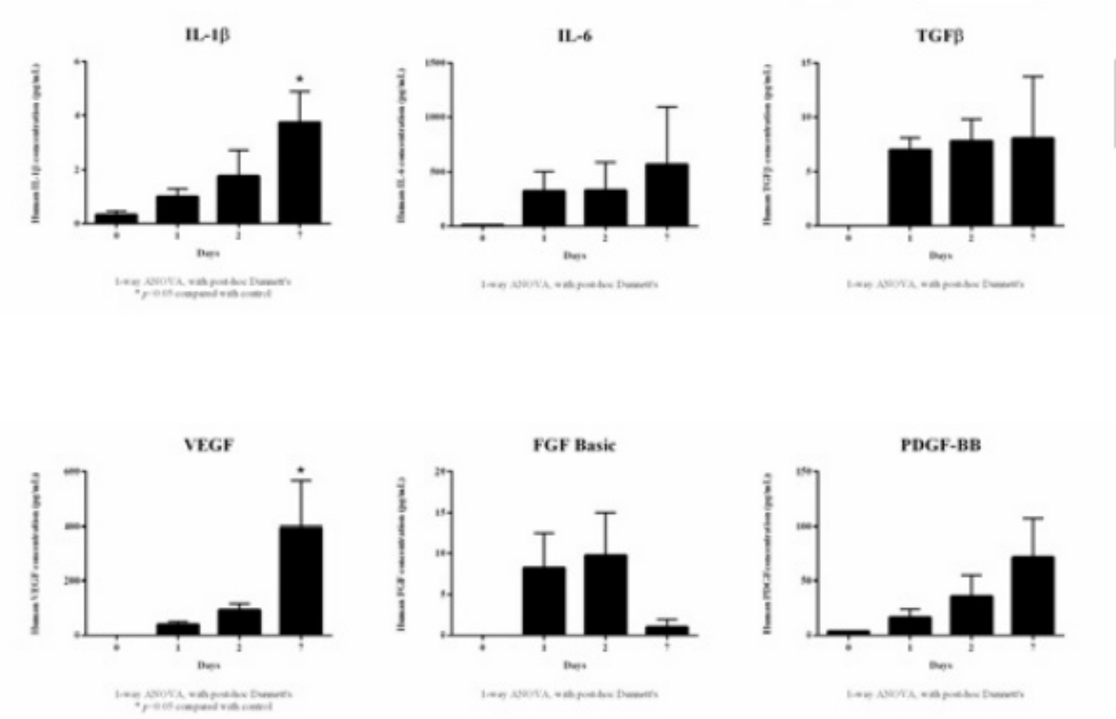
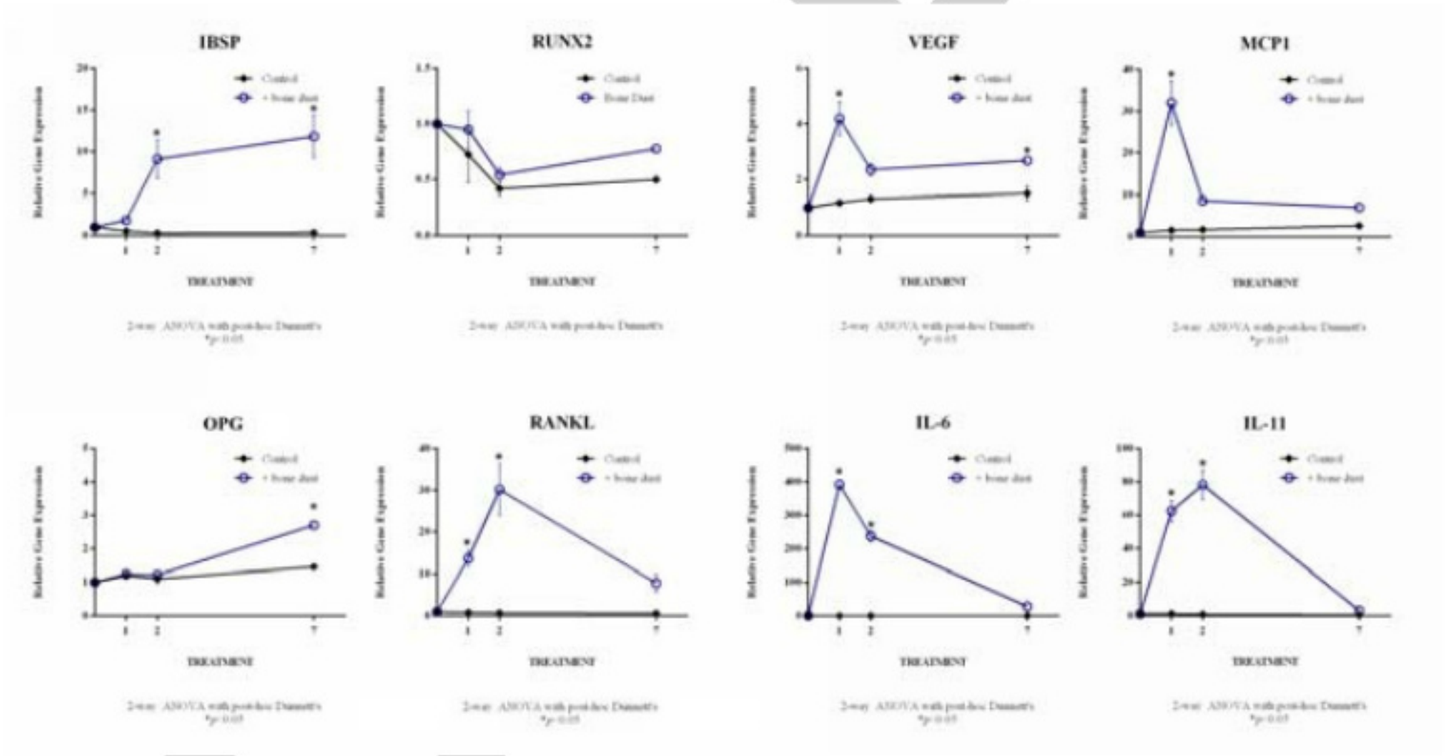


Figure 6.Bone dust increases osteoblast gene expression.

Expression levels of osteoblastic marker genes, and genes important in inflammation and angiogenesis, in primary human osteoblasts exposed to bone dust for up to 7 days, as determined by real-time PCR. Results are presented relative to the expression of each gene on day 0 in the control group. The osteoblasts cultured without bone dust (control) are represented by black diamonds; open circles represent osteoblasts treated with bone dust. Data presented are mean ± SEM. Statistical analysis was performed using two-way ANOVA and post-hocDunnett'stest *p<0.05. Representative data from one patient shown.



Preliminary findings. Pre-white paper submission results.
October 2016

Title: Evaluation of Autograft Bone Tissue Recovered with the Hensler Bone Press™

Sponsor: Hensler Surgical Products

Hospital for Clinical Study: University of Alabama at Birmingham.

Medical Director: Dr. Steven Theiss

Type of Study: Qualitative Lab Study, Autograft Analysis from Hensler Bone Press.

Primary Aim

Hypothesis

i. Intra-operative collection of autologous bone graft using the Hensler Bone Press contains osteogenic and osteoconductive factors for the promotion of spinal fusion.
ii. These osteogenic and osteoconductive factors can be used to improve the success rate of a complete fusion and reduce pseudoarthrosis from primary spinal fusion surgeries.

Secondary Aim

Hypothesis

iii. To test the viability of cells and tissue collected using the Hensler Bone Press and to quantify the presence of growth factors.

Study design with description

While performing cervical, thoracic, and lumbar spine surgery, a burr is used to remove the lamina and facets. This morselized bone material mixed in native body fluids will be suctioned to a collection container called the Hensler Bone Press (HBP). The HBP is a sterile 'inline' collection device between the surgical field and the waste container. The HBP is a 510-K exempt, Class II disposable device. The FDA Establishment number for the HBP is: 3009657922 and Regulation number 880.6740. The HBP was designed for the purpose of autologous bone graft collection and separation, and we are using it for this same reason. The method for bone sample collection with the HBP is the same for every surgery, although the procedures themselves may vary. The standard operational procedures will be performed according to normal standards – the only additional step that will be included during surgery is collection of the morselized bone with the HBP rather than a waste container.

For our purposes, bone from only the lamina and facets, minus the articular cartilage, will be aspirated and collected in the HBP. Once collected this autologous bone graft will then be passed off the sterile field in the HBP, placed in a biohazard bag and taken to a laboratory on UAB campus. Once at the lab, the bone press can be used to separate the solid bone matter from its native fluids and blood.

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Background Information and Scientific Rationale:

Spinal arthrodesis (fusion) is indicated for several pathologies in the cervical, thoracic and lumbar spine. Briefly, during spine surgery the vertebrae are realigned and neural elements are decompressed. In performing these tasks, the spine is destabilized necessitating internal fixation with various options (pedicles screws, hooks, rods, interbody devices). These devices provide 'temporary' stabilization, however, the ultimate the goal is for bony union (fusion) to occur across the operative segments. If complete fusion between the bones does not occur, a pseudoarthrosis will develop leading to fatigue/failure of the hardware, spinal instability, and potential neurologic dysfunction. Spinal fusion is performed by placing bone graft across the motion segments. Two forms of bone graft can be used: 1) Autograft (from the patient) or 2) Allograft (cadaveric or synthetic). Autograft is the gold standard as it contains all the necessary osteogenic factors for bone union to occur. However it does carry the down side of donor site morbidity. Allografts do not have this risk of complications from harvesting autograft bone, but there are concerns about its efficacy in promoting fusion. The idea is to collect and condense morselized bone material that is created during spine surgery and reapply it later in the surgery as an autologous graft source. Normally this bone and tissue gets thrown away, but it could potentially be a valuable source of osteogenic cells and growth factors.

Potential Risks and Benefits:

Risks: None to the patient – there is no change in any component of the procedure that the patient will be receiving. The only difference is that bone/tissue/body fluids that are normally thrown away will be collected and tested at a lab. Even the method of collection is extremely similar to methods that do not involve the HBP.

Benefits: If this study shows that there is osteogenic capability of the autologous bone collected using the HBP, it will be beneficial to orthopedic surgery in many ways:

1. Effective use autograft material (gold standard material) without the morbidity associated with grafting from another location, since it is collected from the same site that it will be applied. Harvesting a graft from another location will cause two points of trauma for the patient and can cause chronic pain or other problems.
2. Faster surgery times since there will no longer be separate graft harvesting when using an autograft.
3. No need for purchasing allograft materials or synthetic growth factors, effectively decreasing the cost of surgery.
4. Promote osteogenesis and complete fusion more effectively than allograft materials can.