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# CANPAC AND ALLOPAC® ALLOGRAFTS CONTAIN BIOLOGICAL FACTORS KNOWN TO SUPPORT BONE HEALING

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# CanPac and AlloPac® Allografts Contain Biological Factors Known to Support Bone Healing

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## INTRODUCTION

It is widely accepted that bone graft material of adequate quality and quantity is fundamental to achieve bone healing and a solid spine fusion. To aid the fusion process, specifically for scoliosis correction surgeries, autologous rib or iliac crest autograft has primarily been utilized.[1] However, use of autograft tissue can increase the operative duration, increase blood loss, cause donor site morbidities and may not provide an adequate amount of tissue that is required for fusion.[1] Due to these challenges, alternatives such as allograft bone have gained widespread use as a graft extender or used alone to improve fusion rates.[1,2] The purpose of this study is to review the composition and presence of innate, retained biological factors of two allografts and determine if they contain the necessary elements known to support bone repair. These elements include growth factors and lipids, which have been shown to influence bone fusion by the biological process of differentiating patient bone marrow derived mesenchymal stem cells (BM-MSCs) into bone forming osteoblasts.[3,4] Cancellous bone is inherently osteoconductive and its large trabecular surface area encourages revascularization and incorporation. It serves as an osteoconductive scaffold and supports ingrowth of new bone.[3]

CanPac (AlloSource Inc, Centennial, CO, US) is an allograft that consists of minimally manipulated ground cancellous bone (particle size range of 1.0 – 9.5mm), that is produced to retain native lipids and marrow. In contrast, AlloPac (AlloSource, Inc) consists of minimally manipulated ground cancellous bone (particle size range of 1.0 – 9.5mm) and cortical bone (particle size range of 1.0 – 4.0mm) and is also produced to retain native lipids and marrow. CanPac and AlloPac are available in aseptic and electron beam irradiated configurations. This study analyzed CanPac and AlloPac to assess the presence of biological factors that have been shown to aid bone healing.

CanPac

AlloPac®

## MATERIALS AND METHODS

### Cell Differentiation

Five aseptic CanPac samples and five irradiated AlloPac samples were collected and tested from a total of nine different research consented donors. Bone marrow derived mesenchymal stem cells (BM-MSCs) (AlloSource, Centennial, CO, USA) were placed in culture until confluent. Cells were then counted and placed into a 6-well Transwell plate at 100k cells per well. The bottom of an 8.0-µm Transwell® insert (Thermo Fischer Scientific, Waltham, MA, USA) was seeded with 100,000 BM-MSCs. The cells were then incubated for 24 hours, under standard conditions, to allow for cell attachment. To examine cell differentiation, CanPac and AlloPac samples were weighed and placed into the top of the Transwell plate containing the BM-MSC's, to avoid direct contact with the cells, and then covered with 4mL media to co-culture for 7 days under standard conditions. Media was made with Corning® Minimum Essential Medium (MEM) (Corning, Corning, NY, USA), 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), and 1% Gibco Antibiotic-Antimycotic (Anti-Anti) (GibCO, Gaithersberg, MD, USA). Media from the well plate was removed and stained with alizarin red (iXCells Biotechnologies LLC, USA). Images were taken using a Nikon Eclipse Ci (Nikon, Tokyo, Japan) microscope and imaging software.

### Growth Factor Analysis

Five aseptic CanPac samples and five irradiated AlloPac samples were weighed and then placed into a 6-well plate, covered with 6mL media (MEM +10%FBS +1%Anti-Anti). Samples were cultured under standard conditions for 7 days. At the end of 7 days, the samples were removed from the incubator, the media was collected and strained through a 100-micron cell strainer, followed by centrifugation at 250G for 30 minutes to remove debris. The supernatant was collected and stored at -80°C until testing was conducted. The thawed samples were then tested using bone morphogenic protein 2 (BMP-2), insulin like growth factor 1 (IGF-1), and platelet derived growth factor (PDGF) enzyme-linked immunosorbent assays (ELISA). A Quantikine ELISA protocol was followed to conduct BMP-2, IGF-1 and PDGF sandwich ELISA testing (R&D Systems, Minneapolis, MN, USA). Colorimetric readings were gathered at 450nm with a Biotek Synergy H1 Hybrid Reader (Biotek, Winooski, VT, USA) and normalized curve fitting was performed with built-in software. Data was then normalized to the weight of the sample.

### Lipid and Metabolite Analysis

CanPac and AlloPac samples were weighed and analyzed by Omix Technologies (Aurora, Colorado, USA) using targeted mass spectrometry-based quantitative proteomics and metabolomics analyses.

## RESULTS

### Cell differentiation

Alizarin red staining is a commonly used stain to identify calcium containing osteocytes in differentiated osteoblasts. As shown in Figure 1, the results of Alizarin red staining demonstrated that the control group of BM-MSCs alone did not indicate deposition of calcium. However, the BM-MSCs co-cultured with CanPac or AlloPac showed presence of calcium deposits. This demonstrated that the CanPac and AlloPac samples may be involved in inducing the osteogenesis of the BM-MSCs in this controlled environment (Figure 1, Images A, B and C).



**A:** BM-MSCs Alone **B:** BM-MSCs with CanPac **C:** BM-MSCs with AlloPac

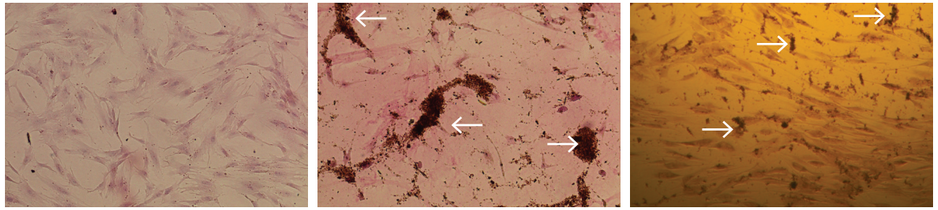

**Figure 1.** Image A shows the control sample of BM-MSCs alone, which is absent of calcium deposition, however the cells are healthy and confluent. Images B and C, CanPac and AlloPac, respectively, (both tested with BM-MSCs) showed many pockets of calcium deposits dispersed throughout the culture plate (identified by the arrows), as well as healthy BM-MSCs that may display characteristics of osteogenesis.

### Growth factors

All CanPac and AlloPac samples tested positive for the following growth factors: bone morphogenic protein 2 (BMP-2), insulin like growth factor 1 (IGF-1) and platelet derived growth factor (PDGF). The biological functions of these growth factors in the bone healing process are described in published literature and are summarized in Table 1.

| GROWTH FACTOR | IMPACT ON BONE HEALING |
|---|---|
| PDGF | • Stimulates cell proliferation and differentiation to promote tissue regeneration and revascularization.[4]<br>• May increase the replication and synthesis of matrix proteins, playing an important role in the remodeling and construction of new bone.[5]<br>• Increases bone formation and fracture healing.[6] |
| IGF-1 | • Stimulates bone formation and has been reported to increase osteoblast recruitment and differentiation, leading to enhanced trabecular bone formation and decreased bone loss.[7,8]<br>• Impacts bone modeling and periosteal growth.[9]<br>• Stimulates MSC differentiation to osteoblasts.[10] |
| BMP-2 | • Induces bone and cartilage formation. Promotes differentiation of MSCs into preosteoblasts, fibroblasts and chondroblasts.[11]<br>• May act as a mediator in osteoblast-osteoclast coupling and may critically affect the rate of bone remodeling.[12] |

**Table 1**

### Metabolome and Lipidomes Evaluation

All CanPac and AlloPac samples tested positive for lipids and metabolites (Omix Technologies, Aurora, CO, USA), indicating that these biologic components have been retained in the products. The biological functions of lipids and metabolites in the bone healing process, as described in published literature, are outlined in Table 2.

| COMPOUND | IMPACT ON BONE HEALING |
|---|---|
| **Metabolites: Animo Acids, Glucose** | • Deliver essential energy to bone resident cells during remodeling.[13]<br>• Intake of protein and amino acids may be beneficial for bone health.[14] |
| **Lipids: Fatty Acids, Triglycerides, Phospholipids** | • Aid bone homeostasis, skeletal health and metabolism.[13]<br>• Provide energy during bone remodeling and cell differentiation.[15] |

**Table 2**

## DISCUSSION

CanPac and AlloPac, in the described cell differentiation test, appeared to show in vitro osteogenic cell differentiation of autologous bone marrow derived MSCs. This may be the result of growth factors present in CanPac and AlloPac, which published literature describes as playing a key role in fracture repair due to the growth factors' fundamental biological function in the differentiation of MSCs becoming bone forming osteoblasts.

The described growth factor test showed the presence of growth factors PDGF, IGF and BMP-2. PDGF is known to regulate cell proliferation and differentiation, to promote bone regeneration and revascularization. IGF-1 stimulates bone formation by increasing osteoblast recruitment and differentiation. Published studies have shown that the interaction between these two growth factors cause an increase in bone formation and a decrease in bone loss. BMP-2 supports osteogenesis of MSCs and stimulates the production of bone. BMP-2 can also upregulate other growth factors such as PDGF and IGF-1, further supporting bone healing.

Lipids and metabolites were found to be present in the CanPac and AlloPac products. Published literature shows these biologic components are involved in the bone remodeling process by providing the necessary fuel required to support cell differentiation.

The data obtained in these tests demonstrate that CanPac and AlloPac (minimally processed ground bone allograft products) retain inherent growth factors that may contribute to in vitro demonstration of calcified deposits, that may be the result of osteogenic differentiation of MSCs. The data also demonstrates the retention of lipids and metabolites. Finally, published literature also demonstrates that growth factors such as PDGF, IGF and BMP, lipids and metabolites are integral in bone healing processes and support skeletal health.

## CONCLUSION

CanPac and AlloPac as minimally processed ground bone allografts, retain biological factors and the inherent bone micro-structure of cancellous bone that published literature indicates are required for bone healing.

Allograft bone can be used as an autograft extender or can be used alone to fill boney voids.



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