WHITE PAPER

Characterization of Growth Factors in the Demineralized Bone Fiber Component of Trinity ELITE™ Allograft

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INTRODUCTION

Trinity ELITE™ allograft, processed by the Musculoskeletal Transplant Foundation (MTF Biologics), is a cryopreserved viable bone graft composed of demineralized cortical bone fibers (DBF) and fresh milled cancellous bone. It is intended for the treatment of musculoskeletal defects in a variety of surgical applications where the availability of autogenous bone is limited or the risk of an additional surgical site for harvesting autogenous tissue precludes autograft as an option. As a biologic alternative to autograft, Trinity ELITE allograft contains all three physiologic components necessary for the bone healing process: an osteoconductive allogeneic bone matrix, osteoinductive growth factors in the DBF component, and viable osteogenic cells resident in the cancellous bone.

The DBF component of Trinity ELITE allograft serves as a source of both osteoconductive and osteoinductive elements of the graft. It is composed of a proprietary blend of cortical bone fibers that function as a scaffold for cell attachment and remodeling. The size and geometry of the fibers are engineered to promote self-entanglement, resulting in a cohesive, putty-like graft material which can be readily molded by the end user. A key characteristic of the DBF component is that endogenous osteoinductive growth factors in the bone matrix are exposed through a demineralization process, whereby the tissue is stripped of inorganic minerals while retaining the organic collagen matrix. Thus, cells which attach to the DBF scaffold not only have an osteoconductive material on which to expand and remodel, but are also able to more effectively interface with local growth factors on the surface, resulting in a positive synergistic effect on bone formation.

These intrinsic properties of Trinity ELITE allograft DBF act in concert to support the bone healing process and are only maintained in demineralized bone that has been carefully processed in a way that preserves the biological integrity of the tissue. Traditional bone processing and sterilization techniques are harsh and result in irreparable damage to growth factors; this subsequently diminishes osteoinductivity in the demineralized bone[1,2]. MTF Biologics is able to avoid this issue through its proprietary, validated processing method which limits exposure of the bone to harsh processing chemicals, thereby retaining native growth factor activity in Trinity tissue. Furthermore, MTF Biologics follows stringent procedures for aseptic processing, eliminating the need for growth factor-damaging terminal sterilization.

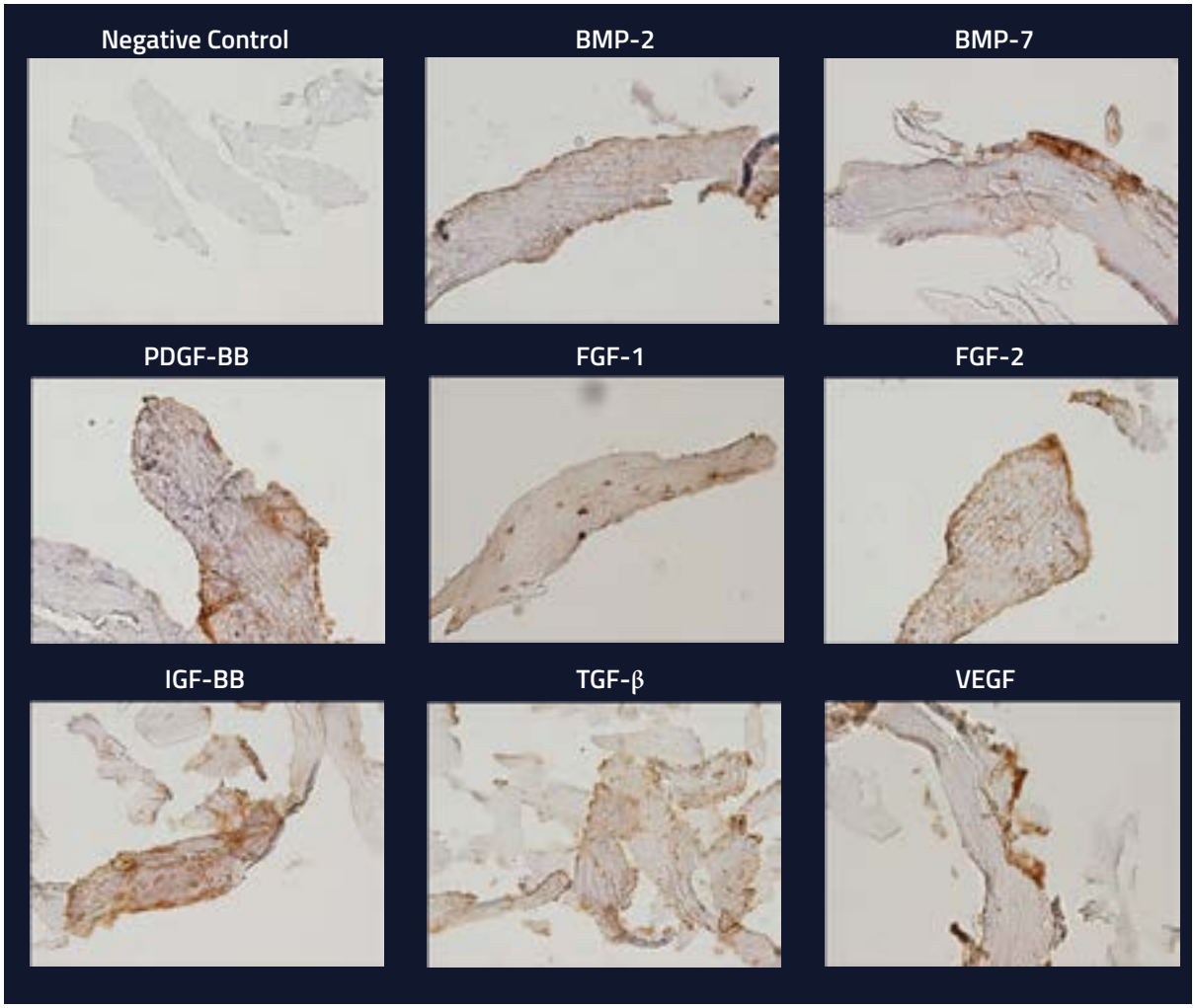
The study detailed below presents evidence of growth factor presence in Trinity ELITE allograft tissue, obtained from immunohistochemical (IHC) staining of the DBF component. As a whole, these results suggest that MTF Biologics' processing methods successfully preserve the osteoinductive elements of the allograft. This represents a potential clinical advantage in the use of Trinity ELITE allograft over competitive bone grafting materials that are exposed to harsh chemical processing and sterilization methods that may impact their inherent properties.

HISTOLOGICAL EVALUATION OF ENDOGENOUS GROWTH FACTORS

In this study, the DBF component of Trinity ELITE allograft was aseptically processed from the cortical bone of three distinct donors using proprietary methods. A cleaning procedure removed residual lipids, blood, and other potentially immunogenic factors from the bone. The cortical bone was then milled into fibers and minerals were removed from the tissue via an acid extraction procedure. Rinsing and buffering steps followed to ensure neutral pH of the demineralized fiber mixture. Processed DBF was stored frozen until sent for histological analysis. At the time of sampling, the tissue was thawed, placed in a fixative solution, and shipped to an external lab to be embedded, sectioned, and stained for a panel of growth factors known to be relevant to bone healing (BMP-2, BMP-7, PDGF-BB, FGF-1, FGF-2, IGF-1, TGF-**β**, VEGF).

Results of this IHC staining are visualized in Figure 1 using conventional microscopy imaging. In these images, growth factor presence in Trinity ELITE allograft DBF is represented by brown coloration, which can be seen throughout the fiber particles. A negative control, which was not exposed to primary antibody during the staining process, was also prepared from the DBF samples for comparison.

Figure 1: Immunohistochemical staining (DAB-Brown) of growth factors present in Trinity ELITE DBF



All growth factors expressed positive staining, indicating they were present in the DBF tissue after processing. Table 1 summarizes these results and presents brief descriptions of the role played by each growth factor in bone healing. All growth factors assessed in this study are known to contribute to the bone repair process and the expression of these growth factors varies during the different phases of the bone healing cascade.

Table 1: Growth factors found in Trinity ELITE DBF and their respective roles in the bone healing cascade [3,4]

Growth Factor	Role in Bone Healing Cascade	Presence
BMP-2	Differentiation of MSCs into osteoprogenitor cells, chondrocytes and osteoblasts	✓
BMP-7	Differentiation of osteoprogenitor cells into osteoblasts	✓
PDGF-BB	Mitogenic for MSCs and osteoblasts and responsible for macrophage chemotaxis	✓
FGF-1	Mitogenic for MSCs, chondrocytes, and osteoblasts. Promotes vascularization	✓
FGF-2	Mitogenic for MSCs, chondrocytes, and osteoblasts. Promotes vascularization	✓
IGF-1	Promotes proliferation and differentiation of osteoprogenitor cells	✓
TGF-β	Pleiotropic growth factor responsible for stimulation of undifferentiated MSCs	✓
VEGF	Promotes migration and proliferation of osteoblasts. Promotes angiogenesis	✓

RECOMBINANT GROWTH FACTOR BIOLOGICS & DEMINERALIZED BONE

Within the broad range of options for bone grafting materials currently available, two major categories of products containing osteoinductive growth factors exist: (1) biologics dosed with recombinant growth factors and (2) demineralized allograft bone such as Trinity ELITE allograft which have been minimally processed to expose endogenous factors.

Biologics that incorporate recombinant growth factors have been shown to aid in the bone healing process[5]. However, there have also been well-documented complications associated with their use, such as ectopic bone growth, inordinate retropharyngeal swelling and osteolysis[6]. While the exact causes of these side effects are difficult to determine, the use of exogenous growth factors at concentrations significantly greater than physiological levels may play a role in eliciting undesired responses from host cells and tissue.

Trinity ELITE allograft DBF contains a broad spectrum of endogenous growth factors identified as necessary in the bone healing cascade (BMP-2, BMP-7, PDGF-BB, TGF-β, IGF-1, FGF-1, FGF-2, and VEGF) and is minimally processed to expose these osteoinductive factors at a normal, physiological level. Moreover, the endogenous growth factors in demineralized bone are slowly released over time as the demineralized matrix is remodeled[7].

THE MTF ADVANTAGE

Amongst bone allografts, the demineralized component of each can differ considerably with regard to its osteoinductive potential. Several factors may account for variability of osteoinductive potential of demineralized bone grafts. These factors include the quality of donor tissue, the processing techniques, sterilization methods and storage conditions[8]. Industry standards exist for donor selection criteria that address both the safety and efficacy of bone grafts. However participation in these industry organizations are voluntary. In addition, regulations governing processing practices can be met through a variety of methods, some of which may have an impact on the quality of these grafts[9,10].

With respect to these considerations, MTF Biologics aims to provide the highest quality tissue by following the most stringent standards in the industry. MTF Biologics' quality and safety criteria consistently meet or exceed the best practice standards of the American Association of Tissue Banks (AATB), as well as the guidelines for screening and testing of tissue donors set forth by the Food and Drug Administration (FDA). With regards to processing, certain techniques used to clean, process, and sterilize demineralized bone have been shown to be detrimental to the quality of the tissue. In contrast, MTF Biologics' proprietary processing techniques are specifically designed to expose the endogenous growth factors in Trinity ELITE allograft DBF while preserving the osteoinductive potential of the tissue.

CONCLUSION

Current understanding of bone healing indicates a critical role for key growth factors in effecting osteogenic cell activity and regulating the overall process of new bone formation[3,11,12]. While some biologics have attempted to artificially augment these processes through concentrated recombinant growth factors, there have been risks of complications and adverse events documented clinically[6]. Meanwhile, bone allografts which achieve bioactivity via demineralization, have a strong history clinically in the bone healing process. However, their potency may be impacted depending on how the bone allograft is prepared[8].

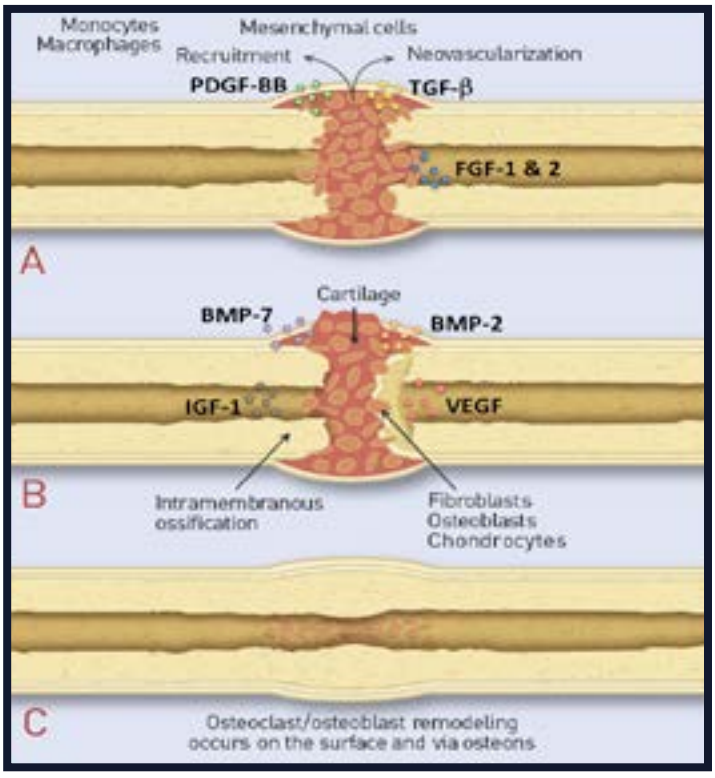
Trinity ELITE allograft remains distinct within both categories of bone-healing products by virtue of the non-traditional, aseptic techniques with which it is processed and MTF Biologics' commitment to consistently screen for the highest quality donors. The results of these efforts are empirically demonstrated in this study, which establishes that the DBF component of Trinity ELITE allograft naturally contains a broad spectrum of endogenous growth factors known to be essential during the bone healing cascade. The presence of these growth factors suggests that the osteoinductivity of the allograft is preserved during processing – a factor which may contribute to successful clinical outcomes for a variety of orthopedic applications.

APPENDIX: BONE HEALING CASCADE

Three components are necessary for bone healing and/or bone graft incorporation: the presence of bone-forming cells, a signal to trigger differentiation of the host cells to bone-forming cells, and a scaffold or matrix on which the new bone can form. When bone fracture or injury occurs, there is a loss of mechanical integrity of the bone and a disruption of the blood supply. The healing cascade begins immediately and occurs in three distinct but overlapping phases: inflammation, repair, and remodeling[11].

Inflammation is the process by which host cells remove debris from the injured site, prepare the local matrix to support cell growth, and enable new bone to be formed; during this phase, revascularization required for new bone growth also begins. The repair phase includes the recruitment and differentiation of mesenchymal stem cells (MSCs) into osteoblasts which produce new bone at the injured site. Lastly, remodeling is the resorption of immature or extraneous bone coupled with reorientation of bone along the direction of mechanical loading to provide adequate structure support. These phases are regulated by the local release of growth factors which include BMP-2, BMP-7, PDGF-BB, TGF-β, IGF-1, FGF-1, FGF-2, and VEGF. An overview of growth factor involvement in the bone healing cascade is shown in Figure 2.

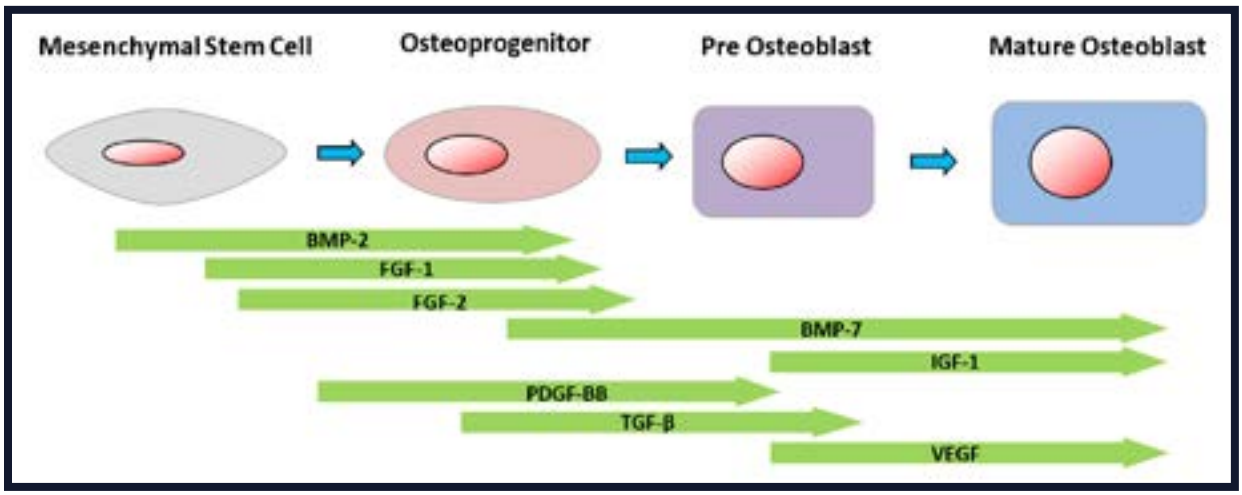
Figure 2: Growth factors involved in the Inflammation (A), Repair (B), & Remodeling (C) phases of the bone healing cascade*



*This graphic is a simplified illustration which shows cytokines only where they are most highly expressed during the bone healing process. The majority of the cytokines shown are continually expressed at varying levels throughout the three major phases of bone healing.

In addition to regulating the overall process of bone healing, osteoinductive growth factors are also essential for the differentiation of MSCs into mature bone-forming osteoblasts. A simplified diagram depicting the expression profiles of various growth factors during osteoblastic differentiation is shown in Figure 3.

Figure 3: Cascade of various growth factors in the differentiation of osteoblasts for bone repair and remodeling[3,9*]



*Adapted from "Effects of growth factors and cytokines on osteoblast differentiation" by Hughes F J, et al. Periodontology 2000. 41:48-71.

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