Cell Viability Comparison of Trinity ELITE® and Osteocel® Plus Bone Grafts

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INTRODUCTION:

Trinity ELITE allograft (TE) is a cryopreserved cellular bone graft that contains living mesenchymal stem cells (MSCs) and osteoprogenitor cells (OPCs). These cells are endogenous to the cancellous bone particles and are maintained within the bony matrix during processing. In addition to the viable cancellous component, TE also contains elongated particles of demineralized cortical bone. These particles entangle with each other allowing the graft material to be cohesive and moldable. As a result, TE possesses putty-like handling characteristics without the addition of any synthetic carriers and the formulation contains 100% allograft bone. Once thawed, TE can be sculpted into various geometries as preferred by the user and does not require physical containment to prevent loss of graft material. In addition, TE does not leave any noticeable tissue on surgical instruments or gloves during manual manipulation thus minimizing any loss of graft material prior to implantation.

This combination of bone components yields a graft that provides the essential osteoconductive, osteoinductive, and osteogenic elements that are needed for new bone formation as well as providing the user with superior handling properties and ease of use (Figure 1). By possessing these characteristics, TE may be considered to be an effective substitute for autograft bone without the added risks of donor site morbidity, insufficient quantity, or uncertain tissue quality.

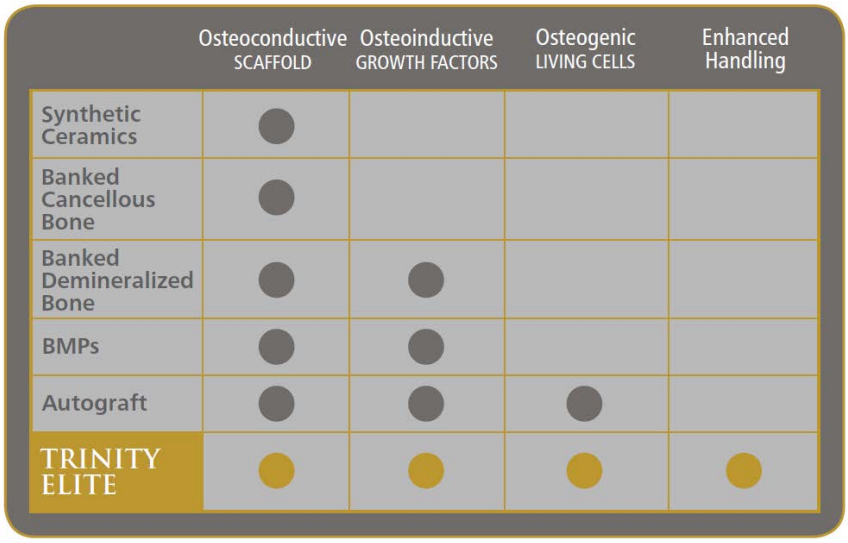

Figure 1: *In contrast to synthetics, devitalized bone, demineralized bone, and BMPs, TE supplies all three components necessary for bone growth with the added benefit of enhanced handling properties.*

The MSCs and OPCs in the viable cancellous component of TE have previously been shown to be capable of proliferating and differentiating into bone-forming cells after thawing and culturing the cryopreserved tissue.[1] These types of cells have also been widely noted to secrete trophic factors that may promote bone healing, and thus play an important role in facilitating the process of bone regeneration.[2,3] To maximize and preserve cell health for TE, donors are strictly screened to provide the highest quality tissue. MTF's screening criteria exceed the requirements of the American Association of Tissue Banks (AATB), as well as the guidelines for screening and testing of tissue donors set forth by the US Food and Drug Administration (FDA). Screening begins with a comprehensive medical and social history that

includes the cause of death. Tissue and blood samples are tested for infectious diseases, including hepatitis, HIV and syphilis. A team of medical/technical specialists from the infectious disease and tissue banking fields evaluates all information including test results before the donor is released for processing. As a result of the extensive donor screening process and strict donor criteria, only 3% of all donors screened are accepted.

Donor bone processing is initiated within 72 hours of death in order to minimize loss in viability (Figure 2). Subsequently the tissue is cryopreserved through a controlled-rate freezing method and stored at -185°C in vapor phase liquid nitrogen, which inhibits any enzymatic and chemical activities that may cause cell damage.[4, 5]

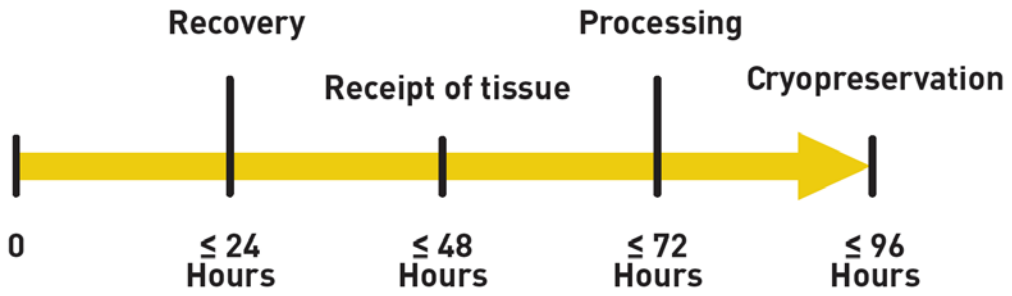

Figure 2: *Processing timeline for TE.*

As part of the quality control procedure, each lot of TE is evaluated for cell viability following processing and frozen storage to ensure successful cryopreservation and the maintenance of cell health. The viability of the cells in TE is determined by a quantitative metabolic assay that provides a measure of cellular activity. A higher cellular activity level has been shown to directly correlate with increasing numbers of healthy, viable cells that retain their functionality and proliferative capacity.[6] By using this method, the viability of TE was compared to Osteocel Plus, another commercially available cryopreserved bone allograft containing living cells. In this study, the viability levels of TE and Osteocel Plus were evaluated immediately post-thaw for multiple donor samples, and then a subset from each group that were similarly aged were also compared for both immediate post-thaw and 2-hour post-thaw conditions.

METHODS:

Prior to evaluating the viability of TE and Osteocel Plus, both tissue forms were stored at their recommended storage temperature until testing. Following their respective product insert instructions, units of both TE and Osteocel Plus were thawed in a 37°C bath until the contents were free-flowing. The cryoprotectant solution was then decanted and rinsed from each tissue sample. TE was rinsed and kept hydrated in sterile 5% dextrose in lactated Ringer's (D5LR) solution; whereas Osteocel Plus was rinsed and hydrated in a warm (37°C) sterile normal saline solution according to its package insert recommendation.

Cell viability was determined by measuring cell activity levels for both tissue forms using a quantitative metabolic assay. To determine the immediate post-thaw viability of TE and Osteocel Plus, data was

collected from the quality control record of 200 consecutive TE tissue lots from a 7-month period of processing. The average viability of these lots was compared to the average viability of 8 lots of Osteocel Plus. In order to demonstrate that possible differences in tissue aging between TE and Osteocel Plus samples were not a potential cause of differences in viability levels, a subset of 5 lots of Osteocel Plus was selected to match the average age (9 months from date of production) of 5 lots of TE. Subsequently, these samples were comparatively evaluated at both immediate post-thaw and 2 hours post-thaw conditions.

Cellular activity levels reported from every donor sample included in a single group were averaged and standard error (SEM indicated by error bars) was calculated for each condition. Statistical analysis was performed via a one-factor ANOVA with a p-value ≤ 0.05 representing statistical significance.

RESULTS:

In comparison to the 8 lots of Osteocel Plus, the viability of TE taken from production lots was determined to be significantly greater for the immediate post-thaw condition. Here, the average cellular activity from 200 production lots of TE was approximately 4 times greater than Osteocel Plus cell activity (Figure 3).

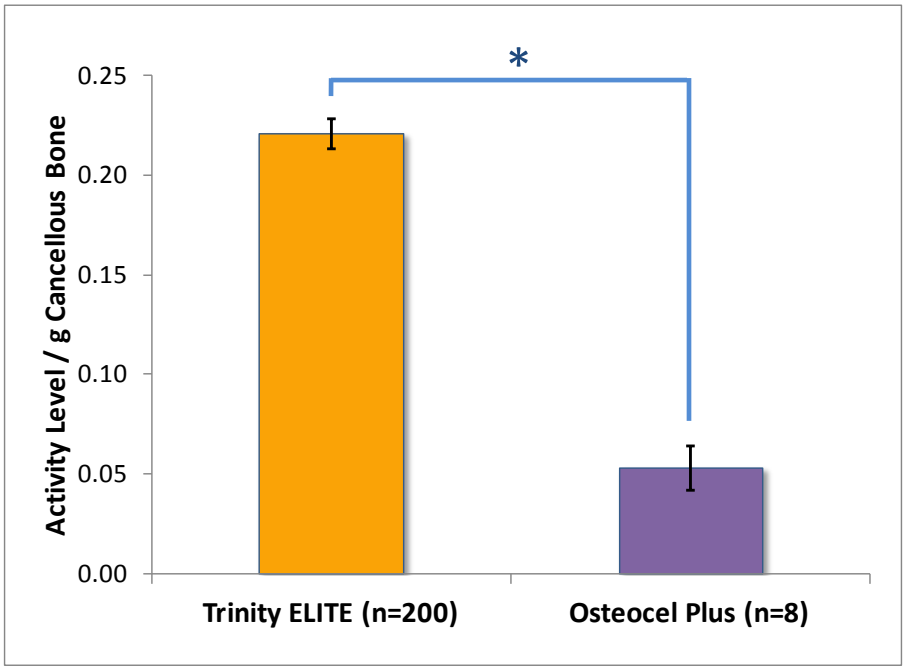

Figure 3: *Releasable TE tissue has significantly higher cellular activity levels immediately after thawing compared to Osteocel Plus (*p≤0.0001).*

To simulate operating room conditions where the tissue may not be immediately implanted, TE (n=5) and Osteocel Plus (n=5) samples at the 9 month time point were tested at 2 hours after thawing in addition to the immediate post-thaw condition. The immediate post-thaw results of the five donors of TE used in this part of the study were comparable to the results from the 200 production lots suggesting that this sample was a reasonable representation of the population. In comparison to Osteocel Plus, TE had a nearly 4-fold higher level of cellular activity immediately after thawing (Figure 4). In addition, TE demonstrated better

stability when compared to Osteocel Plus, with only an 18.4% decrease in cellular activity at the 2 hour post thaw time point. In the same time frame, Osteocel Plus had a 41.3% decrease in cellular activity at the 2 hour time point. It is also important to note that the cellular activity of TE at 2 hours after thawing was measured to be approximately 3.2 times greater than that of the immediately post-thawed Osteocel Plus tissue (Figure 4).

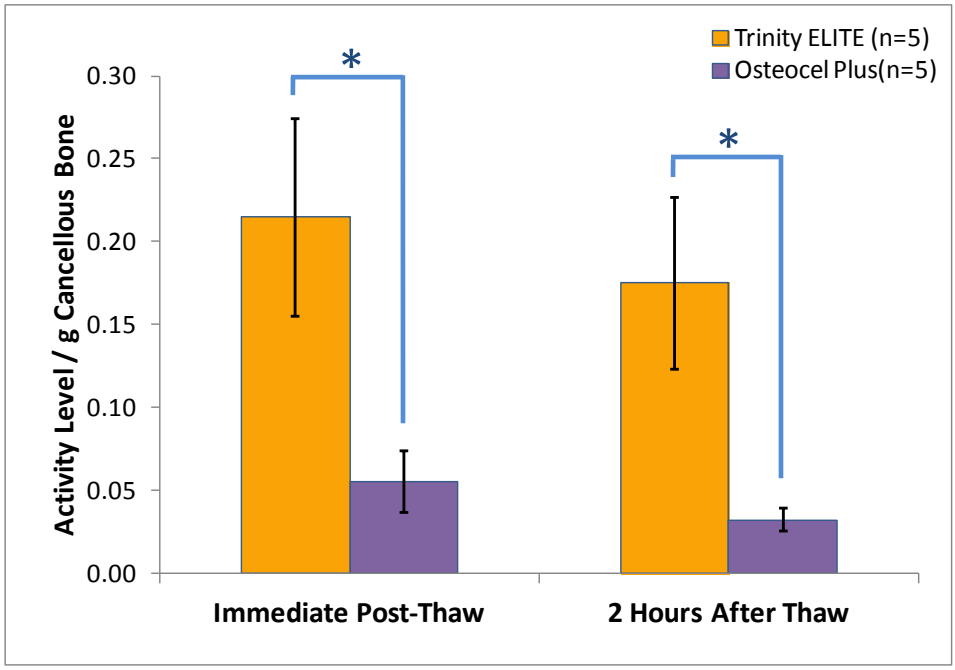

Figure 4: *TE maintained higher time-elapsed cellular activity levels than Osteocel Plus immediately after thawing, and 2 hours post thaw(*p≤0.05).*

CONCLUSION:

In this study, Trinity ELITE allografts consistently demonstrated significantly greater cellular activity levels than those measured for Osteocel Plus when evaluated acutely after thawing. Additionally, these differences were maintained over time as higher activity levels were measured for TE compared to Osteocel Plus when the grafts were tested at two hours post-thaw. Since metabolic activity levels are proportional to the number of living, functional cells present in the tissue, these findings are indicative of cell viability. While it may be difficult to attribute such differences in viability between TE and Osteocel Plus to any single factor, there may be variations in donor selection, tissue formulation, processing methods, and cryopreserved storage conditions that could account for the higher levels of cellular activity observed in TE. All of these parameters were important considerations in the design of Trinity ELITE where the goal is to provide a bone graft that supplies an optimal combination of osteoconductive, osteoinductive and osteogenic elements, while also possessing desirable handling properties for the end user.

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