Trinity Evolution

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Technology

Trinity Evolution

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Abstract: *Trinity Evolution Cryopreserved Cell Viable Bone Matrix is a minimally manipulated, human cellular, and tissue-based allograft containing adult mesenchymal stem cells, osteoprogenitor cells, and a demineralized cortical component. The cancellous bone used to produce Trinity Evolution is derived from freshly recovered donor tissue by Food and Drug Administration–registered facilities and processed under aseptic conditions. Preclinical in vivo and in vitro testing as well as strict donor screening has demonstrated the safety of Trinity Evolution as well as its osteoinductive and osteogenic potential contained within a natural osteoconductive matrix.*

Keywords: mesenchymal stem cell allograft; bone healing; foot and ankle surgery

Autograft is considered the gold-standard source for bone graft because it contains the 3 structural and physiologic components necessary to achieve bone growth: an osteoconductive matrix, osteoinductive growth factors, and mesenchymal stem cells.

Donor site complications such as pain, hematoma, seroma, and inadequate quality and/or quantity of donor bone have led to the development of a virtual plethora of bone graft substitutes, expanders, and extenders.[1] Although synthetics, demineralized bone matrices, bone morphogenic proteins, and others may possess osteoconductivity and/or osteoinductivity, none contain an osteogenic cellular component. In order for bone healing to occur, surgeons therefore rely on cells from the local environment or the addition of bone marrow aspirate or autograft from a second site. However, patient age and cell health make these unreliable sources. Allogeneic stem cells provide the osteogenic component of bone healing in a known quantity for the surgeon, providing more confidence that bony healing can and will occur in the most challenging of patients.

Mesenchymal Stem Cell Science

Since the identification of the mesenchymal stem cells (MSCs) in 1966,[2] their properties have been well elucidated. The MSC is able to self-renew and is multipotential, capable of delineating into bone, cartilage, or fat depending on cues received from the local environment.[3,4]

Although autograft does provide MSCs, which naturally occur in a patient's bone marrow, the cell number and quality can vary depending on a patient's age and medical history (see Figure 1). Medical history factors such as diabetes as well as dietary and smoking habits may affect the number and quality of stem cells present in patients and therefore affect their bone healing. In addition to changes in quantity of MSCs,[5] there is also evidence that aging diminishes quality, or the ability to differentiate or regenerate, as well as mobilization capacity.[6,7]

Human MSCs Decline With Age

The most essential and unique property of MSCs that allow surgeons the ability to employ their implantation as a safe and effective allograft is that they are immune privileged as well as immune modulatory.[8,9]

> "Trinity Evolution obviates the unpredictability of the quality and quantity of the cellular content inherent in autograft, bone marrow aspirate, and the local environment being grafted."

Normally, allogeneic cells are eliminated by the host immune response. However, MSCs do not express major histocompatibility complex (MHC)/human leukocyte antigen (HLA) class II antigens and co-stimulatory molecules required to cause T cell proliferation.[10] In addition, MSCs secrete cytokines that modulate immune reactions. These characteristics

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Figure 1.

Mixed lymphocyte reaction assays. Caplan A. Clinics in Plastic Surgery 1994. Estimates obtained by colony-forming unit–fibroblast (CFU-F) assay.

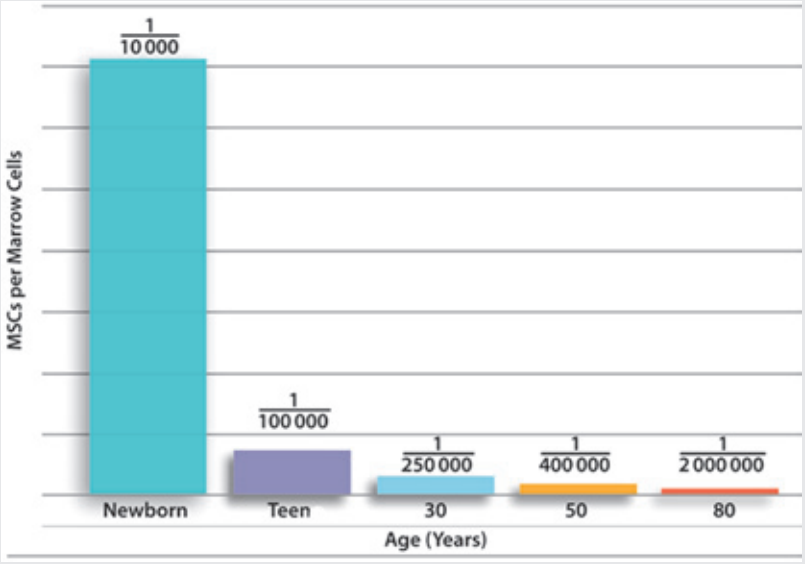
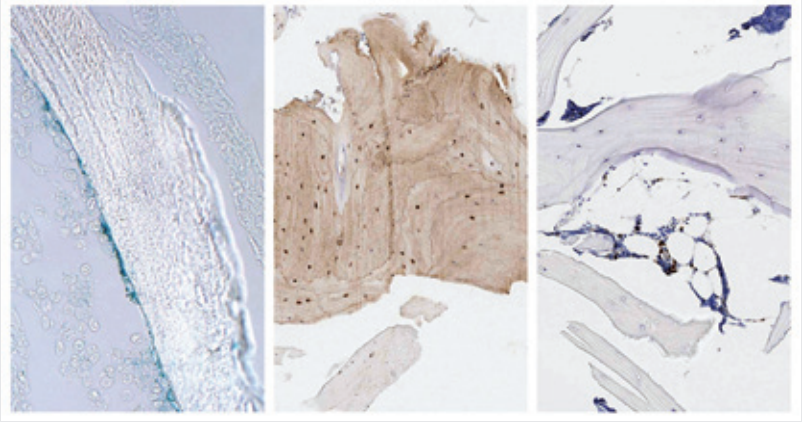


Figure 2.

Trinity Evolution demonstrates the presence of mesenchymal stem cells and osteoprogenitor cells and the absence of hematopoietic cells.



of MSCs persist following osteoblastic differentiation to a much greater extent than after chondrogenic and adipogenic differentiation.[11] MSCs therefore can be transplanted between unmatched individuals without risk of provoking an immune reaction. Preclinical tests such as the mixed lymphocyte reaction (MLR) and complement activation tests allow for direct testing of stem cell–based allografts to provide confirmation of their safety.

Trinity Evolution

Trinity Evolution is an allograft processed from human tissue donors by the Musculoskeletal Transplant Foundation (MTF) according to stringent quality control to ensure proper concentrations of osteoconductive matrix, osteoinductive factors, and osteogenic cells, as well as controlled particle size for optimum handling. Trinity Evolution obviates the unpredictability of the quality and quantity of the cellular content inherent in autograft, bone marrow aspirate, and the local environment being grafted.

Value of Cold Storage

Storage at extremely low temperatures stabilizes cells and maximizes their viability because cell metabolism effectively ceases at –130°C. The lower the temperature, the longer the viable storage period.[12,13] Trinity Evolution undergoes controlled rate freezing to minimize crystallization, followed by storage at –185°C in vapor-phase liquid nitrogen to maximize the cell health. Every lot of Trinity Evolution is tested to confirm the presence of a reliable number of viable MSCs and osteoprogenitor cells (OPCs).

Cell Identification

Clusters of differentiation (CDs) are cell surface markers used to identify and characterize cells. Although specific cell surface markers unique to MSCs have not been identified, there is a consensus on a combination of markers that can be used to identify MSCs.[10] Preclinical studies have demonstrated that cells contained within the matrix of Trinity Evolution stain positive for CD 166, a marker for immune-privileged MSCs and OPCs. OPCs are those MSCs that have begun to differentiate along an osteogenic lineage and are demonstrated by a positive stain for osteocalcin. The absence of hematopoietic stem cells was confirmed through the use of CD34 and CD45 stains, showing that Trinity Evolution has an insignificant number of immune-competent hematopoietic cells (HSCs) that have been confirmed through immunogenic testing (Figure 2).

Immunogenicity Testing

The MLR is an in vitro test used to assess compatibility or, conversely, the

Figure 3.

Based on the mixed lymphocyte reaction assay, Trinity Evolution does not stimulate an immune response.

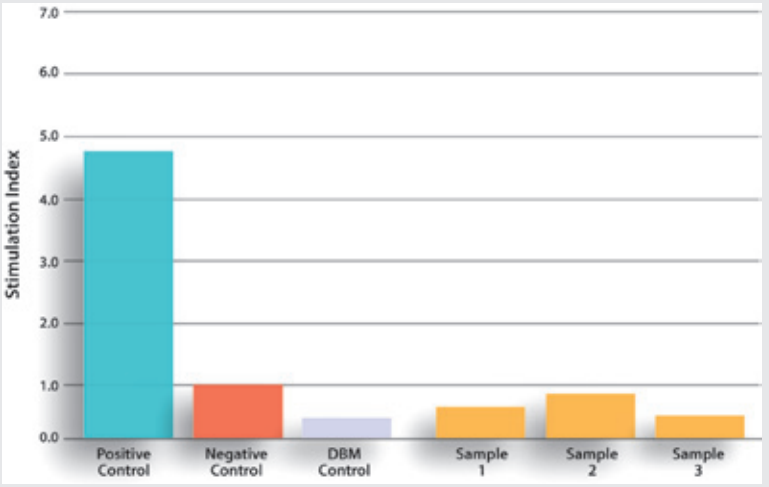


Figure 4.

Athymic rat muscle pouch. New bone formation is evident in athymic rat intramuscular pouch model.

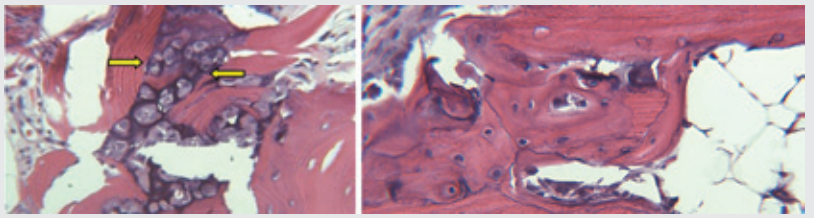
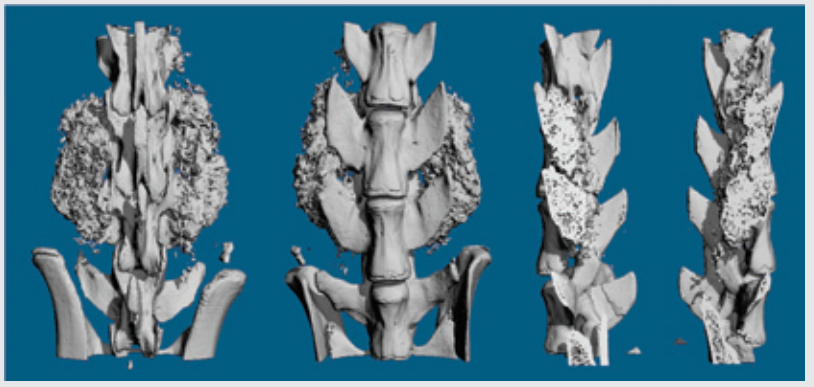


Figure 5.

Athymic rat posterolateral lumbar fusion in 8 weeks. Micro-CT scan of rat spinal posterolateral fusion model.



capacity of cells from 2 different individuals to stimulate an immune response, when cultured together. Cells from Trinity Evolution were cultured with immune cells from another individual and demonstrated a negative MLR (ie, they did not stimulate cell proliferation in the MLR). The cells from Trinity Evolution are thus demonstrated to be immune privileged (nonimmunogenic) and can be safely transplanted into individuals without HLA matching (Figure 3).

In Vivo Osteoinduction and Osteogenesis

In vivo testing of Trinity Evolution was performed in an immune-compromised animal to avoid xenographic rejection. The osteoinductive and osteogenic qualities of Trinity Evolution were verified in an intramuscular pouch as well as in a posterolateral lumbar fusion (Figures 4 and 5).

How Trinity Evolution Is Supplied

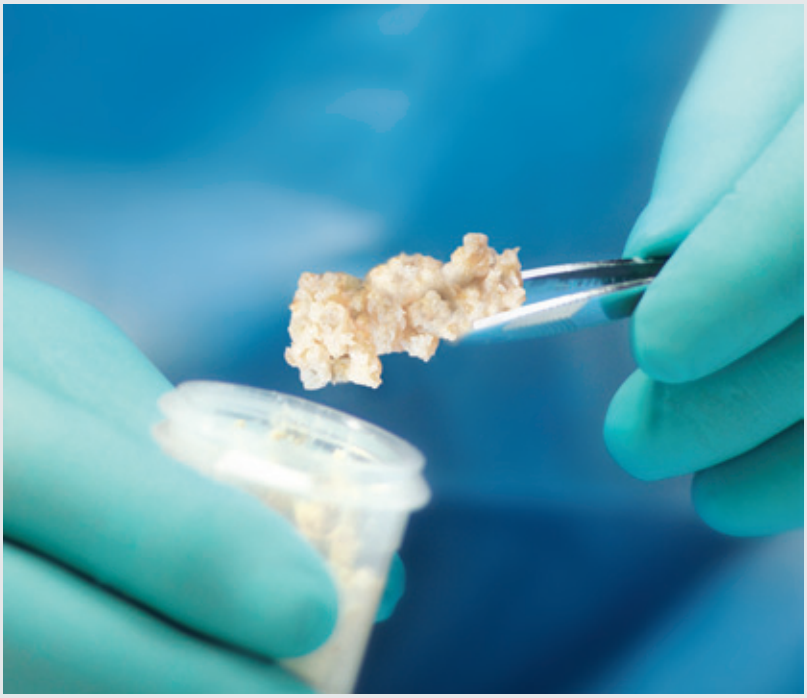
Trinity Evolution is supplied to the surgeon at –80°C in 1-, 5-, 10-, and 15-cc vials. Once removed from a –80°C freezer or dry ice, it is thawed in a saline bath, and the cryopreservative and basal medium are decanted and replaced with 5% dextrose in lactated ringer's solution (D5RL). The D5RL is decanted just prior to implantation at the operative site, which, for maximum cell viability, should be done within 2 hours after thawing (Figure 6).

Summary

Trinity Evolution is an allogeneic cancellous bone matrix containing viable OPCs and MSCs as well as a demineralized component prepared from tissue donors by the Musculoskeletal Tissue Foundation. A proprietary process depletes immunogenic HSCs and preserves immune-privileged MSCs and OPCs in their native matrix. Extensive preclinical and in vivo testing has validated its safety profile, confirming its immune privilege nature, biocompatibility, and its osteoinductive and osteogenic potential. FAS

Figure 6.

Postthaw Trinity Evolution handles much like autograft.



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