V92™

CELLULAR BONE MATRIX

A PARAGON 28®
PRESERVE™ PRODUCT

V92™ CELLULAR BONE MATRIX

TECHNICAL MONOGRAPH

Exclusively from Earth's Paragon 28®

V92™ Cellular Bone Matrix Safety Profile

V92™ Cellular Bone Matrix is an allogeneic bone matrix product containing viable osteoprogenitor cells. It contains the key components needed for bone formation: an osteoconductive matrix, a demineralized matrix component with osteoinductive potential and osteogenic cells. It is safe and non-immunogenic. It is an ideal alternative to autograft in a variety of orthopaedic and spine applications.

Bone Grafting

Bone grafting is a common surgical procedure for treating fractures and securing a bony arthrodesis in a range of indications. Autograft has been the gold standard graft material for these procedures because it possesses all three key properties needed for new bone growth: osteoconductivity, osteoinductivity and osteogenicity. Additionally, it is by definition histocompatible and non-immunogenic.

While autograft use typically results in high fusion rates, autograft is of variable quality and quantity depending on the donor and site of harvest. Additionally, there are concerns associated with autograft harvest which include increased surgical time, limited volume availability, surgical site morbidity, potential for blood loss and infection.[1] There are now a wide range of alternative allogeneic and synthetic graft materials available to the surgeon. One grafting material option of particular interest is an allogeneic bone graft that contains mesenchymal stem cells.

Mesenchymal Stem Cells are Multipotent and Non-Immunogenic

Mesenchymal stem cells (MSCs) are a type of adult stem cell that have the ability to self-renew and differentiate. They are 'multipotent', meaning they can differentiate into more than one type of specialized cell of the body, but not all types. These cells can produce all tissues derived from the mesoderm, which includes bone, cartilage, fat, nerve, muscle, and tendon. In the skeletal system, MSCs are the osteogenic cells that are required for bone repair, remodeling and maturation. Under the appropriate conditions, MSCs differentiate into osteoblasts that subsequently make new bone.

A sub-population of the MSCs present in bone marrow are a developmentally immature and highly homogeneous cell type called marrow-isolated adult multi-lineage inducible cells (or MIAMI cells) that share a similar phenotype with human embryonic stem cells.[2] These MIAMI cells have a high expansion capacity, a unique molecular profile and retain the ability to differentiate into multiple lineages.[3]

MSCs also have special immunological properties. They do not stimulate allogeneic rejection and are not eliminated by the host immune response.[4] This lack of allogeneic reaction has been supported by in vitro studies as well as current clinical data for cellular bone matrices.[1]

Cellular Bone Matrices: Viable Stem Cell-Containing Bone Graft Substitute

The V92 cellular bone matrix consists of bone marrow-derived cells and a bone scaffold component. The two components are combined at the time of surgery prior to graft placement. Bone marrow contains two types of stem cells: mesenchymal stem cells and hematopoietic stem cells. The bone marrow component in V92 cellular bone matrix is harvested from the vertebral body region of the donor, an area known to be rich in mesenchymal stem cells. Prior studies have reported that the highest concentration of mesenchymal stem cells is found in the pelvic girdle and vertebral body regions.[5]

In addition, it has been reported that aging decreases the quantity of osteogenic MSCs.[6] There is also evidence that aging may decrease the ability of the cells to differentiate or regenerate.[7] The donor criteria for the V92 cellular bone matrix requires donors between the ages of 15 and 55, younger than typical for allograft bone products.

Cell Viability

Viable osteogenic cells are a key component of the V92 cellular bone matrix. V92 is extensively tested to assure consistently high concentrations of mesenchymal stem cells. Following processing of the bone marrow using conventional cell isolation methods, the cell component contains an enriched population of osteoprogenitor cells.

Characterization of each lot assures that cell viability during processing exceeds 80% in all donors and cell number is a minimum of 150,000 viable cells/cc of allograft. The average viability of cells from thawed V92 cell component vials is 92%.

Optimized Bone Scaffold Component

The bone scaffold component of V92 is a mixture of demineralized cortical bone and mineralized cortical and cancellous bone. The demineralized bone component (40%) contains growth factors and has osteoinductive potential. The mineralized scaffold component (60%) is a balanced combination of cortical and cancellous bone for cell attachment and bone formation. The scaffold components are combined and freeze dried.

Novel Cryoprotectant to Maximize Cell Viability

V92 utilizes a novel, next- generation cryo-protectant for storage and preservation of the cellular component. It is a non-toxic alternative to a traditional DMSO- based cryo-preservation media. It provides improved cell viability and functionality when used to preserve biological cells and tissues. This novel cryo-protectant is used in the UTMB process of viable bone allograft preparation; retaining cell function, sustaining cell viability, and providing dependable cell identity that supports therapeutic intervention in orthopaedic and other regenerative medicine applications.

Dimethyl sulfoxide (DMSO) is the most widely accepted cryoprotectant for cell preservation in spite of concerns around its cytotoxicity and negative effect on cell differentiation.[8] Avoiding or reducing the DMSO concentration has been a key research focus in stem cell preservation.[9] In addition, the use of a DMSO cryoprotectant requires extra steps in the operating room. The cellular bone matrix must be washed after thawing and the DMSO solution must be decanted and replaced with sterile saline or 5% dextrose prior to implantation due to the potential cytotoxicity of the DMSO.

The preservation of cells is critical to successful bone grafting. The cell component of V92 cellular bone matrix is stored at very low temperatures. The novel cryoprotectant stabilizes the cells and maximizes cell viability during the storage period. Cells are typically frozen within 48 hours post-mortem. A controlled rate of cooling is implemented to minimize crystallization and any negative effects on the cells. The cell component of V92 is stored at -80° C until release. The product is shipped on dry ice (-65°C to -80° C). With V92, the ability to re-suspend the cells in saline and utilize them directly ensures no loss of cells and maximal therapeutic dose without cell-damaging, DMSO-based cryopreservants.

Safety Profile – Tissue Processing

V92 cellular bone matrix is processed in an aseptic manner in a Class 100 clean room using procedures and screening criteria that exceed those required by the American Association of Tissue Banks (AATB). It is a minimally-manipulated, human cellular and allograft tissue product regulated by the FDA Center for Biologics Evaluation and Research as human cells, tissues, and cellular- and tissue-based products (HCT/Ps). Such HCT/Ps are regulated under FDA CFR 21 Part 1271.

Microbiological testing is performed throughout the process and samples are taken for histological and pathological analysis. All graft material (scaffold and cells) is processed from the same donor per AATB and FDA guidelines. The donor criteria for the V92 cellular bone matrix require donors between the ages of 15 and 55.

Tissue is recovered and processed according to a strict timeline. Tissue must be recovered and processed within 24 hours post-mortem. The bone marrow component is processed, frozen and packaged within 120 hours post-mortem. Proprietary processes preserve the condition of the stem cells and reduces the components that could stimulate an immune response. The bone scaffold component (DBM, cortical/cancellous bone) is processed within the following 2 weeks.

Donor Criteria and Screening for Tissue Safety

Donor Testing:

- HIV-1 and 2 Antibody
- HIV-1 PCR Nucleic Acid Test
- Hepatitis B Surface Antigen
- Hepatitis B Core Antibody (IgG & IgM)
- Hepatitis B Virus Nucleic Acid Test (if applicable)
- Hepatitis C Virus Antibody
- Hepatitis C Nucleic Acid Test
- Human T-Lymphocytic Virus Antibody I/II (if applicable)
- Syphilis Rapid Plasma Reagin Screen or T.pallidum IgG Screen

Donor Screening:

- Medical and social history review
- Physical examination
- Medical record evaluation, including autopsy report (if performed)
- Licensed physician review of donor record

Safety Profile – Cell Characterization

V92 cellular bone matrix has been subjected to a number of tests in order to ensure that the allograft does not stimulate an immune response. Mesenchymal stem cells are known to be immune privileged cells and the manufacturing process also acts to minimize the potential presence of other cells likely to elicit an immune response.

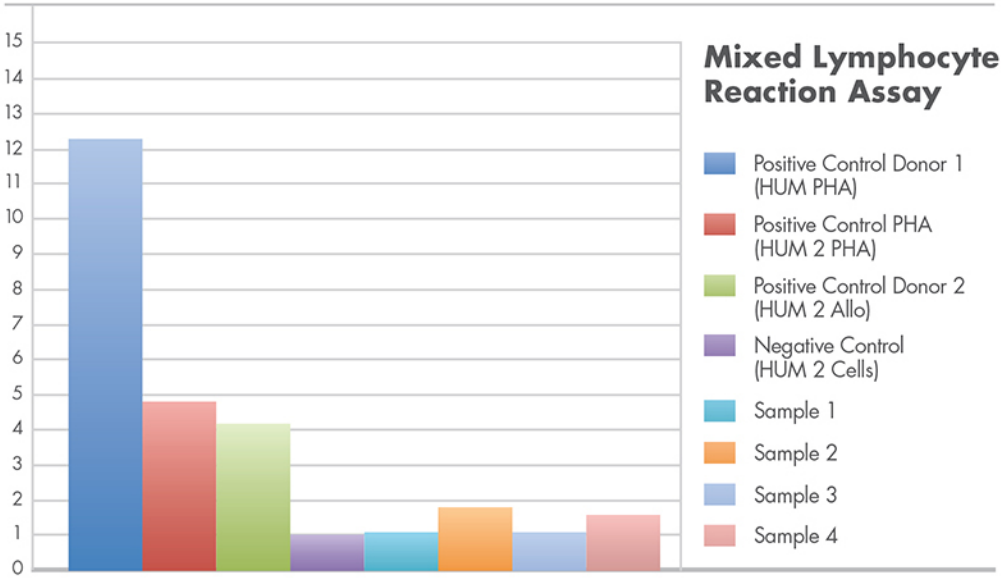
Mixed Lymphocyte Reaction Assay:

A mixed lymphocyte reaction (MLR) assay has been performed to assess the potential for activation of T-cell proliferation. Peripheral blood mononuclear cells (PBMCs) are isolated from consented donors. Freshly isolated PBMCs from one donor are used as the primary responder cells. Cryopreserved PBMCs from a separate donor are used as control cells to emulate the cryopreserved V92 bone matrix product.

Stimulator cells are treated with mitomycin C which binds to DNA rendering the cells non-proliferative. Responder cells left untreated are therefore able to proliferate when stimulated. Phytohema- glutinin (PHA) acts as a mitogen to stimulate cell division for the positive control. Fresh PBMCs are labeled as HUM and mitomycin C treated cells labeled as HUMX. The cryopreserved human PBMCs (control) are labeled as HUM 2 and the mitomycin C treated cells as HUM 2X. Product from four representative donors are depicted as SAMPLE 1-4.

The methodology followed standard protocols. Once the cells are treated and added to the 96-well culture plate, the plate is incubated for six days at 37° C with 5% CO2 and >95% humidity. On day six, BrdU is added to the plate wells and the plate returned to the incubator overnight. On day seven, the plate is removed, the wells visually observed and the BrdU ELISA proliferation test performed. Comparisons of the stimulated test cells with control, non-stimulated responder cells yields a stimulation index which can then be used to compare proliferation in the various cell combinations.

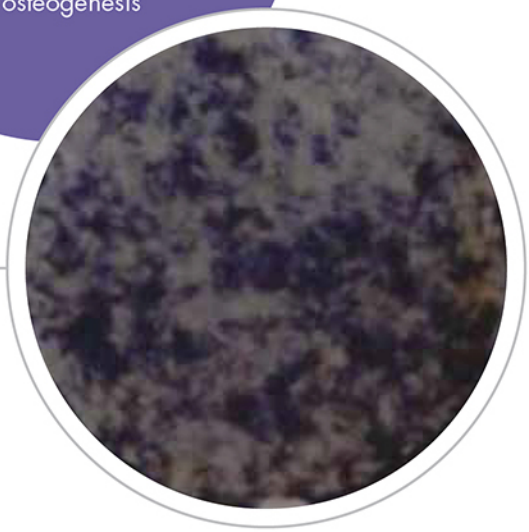
Because the test samples (Samples 1-4) did not stimulate a significant response, compared to the negative control, the MLR assays demonstrated that the V92 cellular bone matrix is not immunogenic.



Stimulation indices for the test samples were near or below that for the negative control, while positive controls performed as expected and demonstrated robust response. V92, therefore, does not stimulate an immune response.

Osteogenesis: Alkaline Phosphatase Staining

Robust staining for Alkaline Phosphatase indicates capacity for osteogenesis



Osteogenic Capacity: Alkaline Phosphatase Staining

The cells isolated for the V92 cell component were directly cultured to determine osteogenic capacity utilizing alkaline phosphatase as an indicator of osteogenic differentiation. Cells isolated for the V92 cell component colonize and express alkaline phosphatase within 14 days (as shown right).

Flow Cytometry Analysis:

The cells were evaluated for their mesenchymal stem cell content from frozen vials as obtained/utilized by the clinician. Dual-color flow cytometry analysis was done with a LSR II system (Beckman-Dickson). Utilizing fluorescent antibodies, the cells were stained with a mesenchymal stem cell marker CD105 and hematopoietic marker CD45. The percentages of cells that were negative for CD45 and were positive for CD105 were recorded. These limits are important because CD105 can also be expressed on some hempatopoietic cells which can result in inflated results.

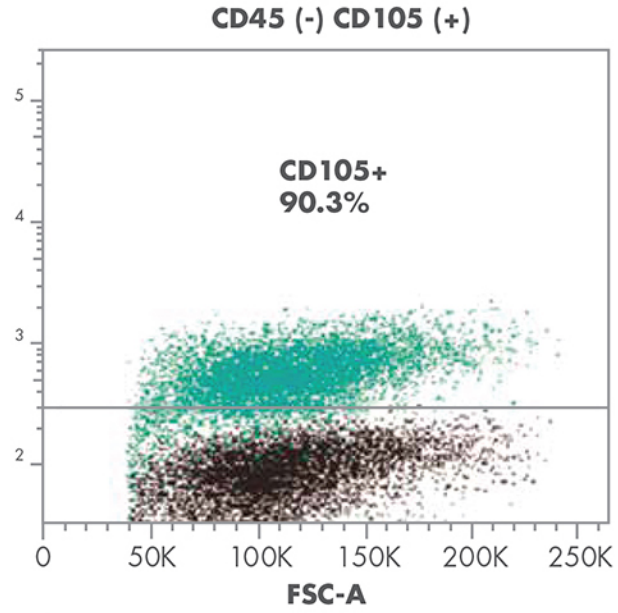
For further verification, cells were labeled with T-cell marker CD3 and B-cell marker CD37. The percentage of cells positive for CD3 or CD37 were equal to isotype controls. Hence, B- and T-cells are not present in the V92 cell component vials.

The flow cytometry analysis of cell surface markers on V92 cell component reveal >50% mesenchymal stem cells because they express CD105 and not CD45. Additionally, the V92 cell component is highly osteogenic because >70% of cells express STRO-1 on their surface and are negative for GlycoA. The presence of such a large percentage of mesenchymal stem cells in the V92 cell component vials, as well as the lack of B-cells and T-cells, further ameliorates potential for any immune response.

Safety Profile – Preliminary Clinical Experience

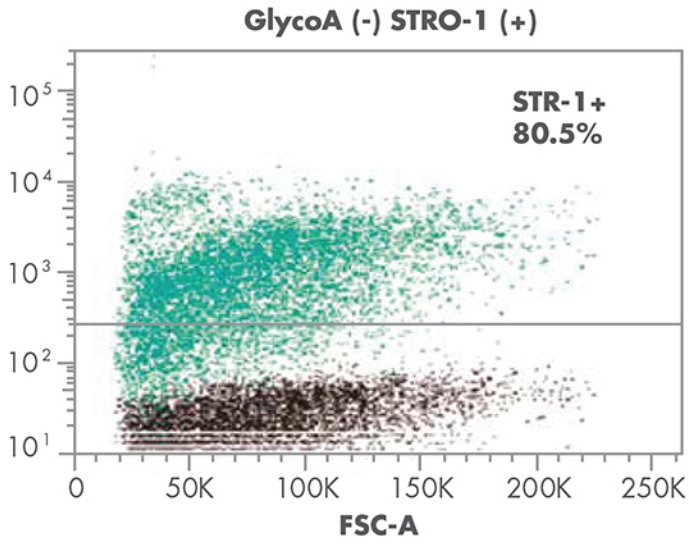
Over 2000 units of this graft have been distributed since August 2014. Implantations have occurred in a range of clinical applications including spinal fusion, extremity fracture and oncological reconstruction. These initial implantations have been followed and the users have actively assessed any potential graft-related complications. Specific inquiries regarding signs of fever, infection, seroma, wound drainage and neuritis have been made to the clinicians. To-date none have reported any potentially graft-related adverse events. A longitudinal cohort study of the material is underway.

Flow Cytometry Analysis



Freq. of intact cells: 80.4%

Dot plot showing positive expression of CD105



Freq. of intact cells: 74.9%

Dot plot showing positive expression of STRO-1 which further ensures amelioration of an immune response.

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For more information on
V92™ please contact:

Paragon 28, Inc.
4B Inverness Ct. E, Ste. 280
Englewood, CO 80112, U.S.A.
888.728.1888
www.paragon28.com

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