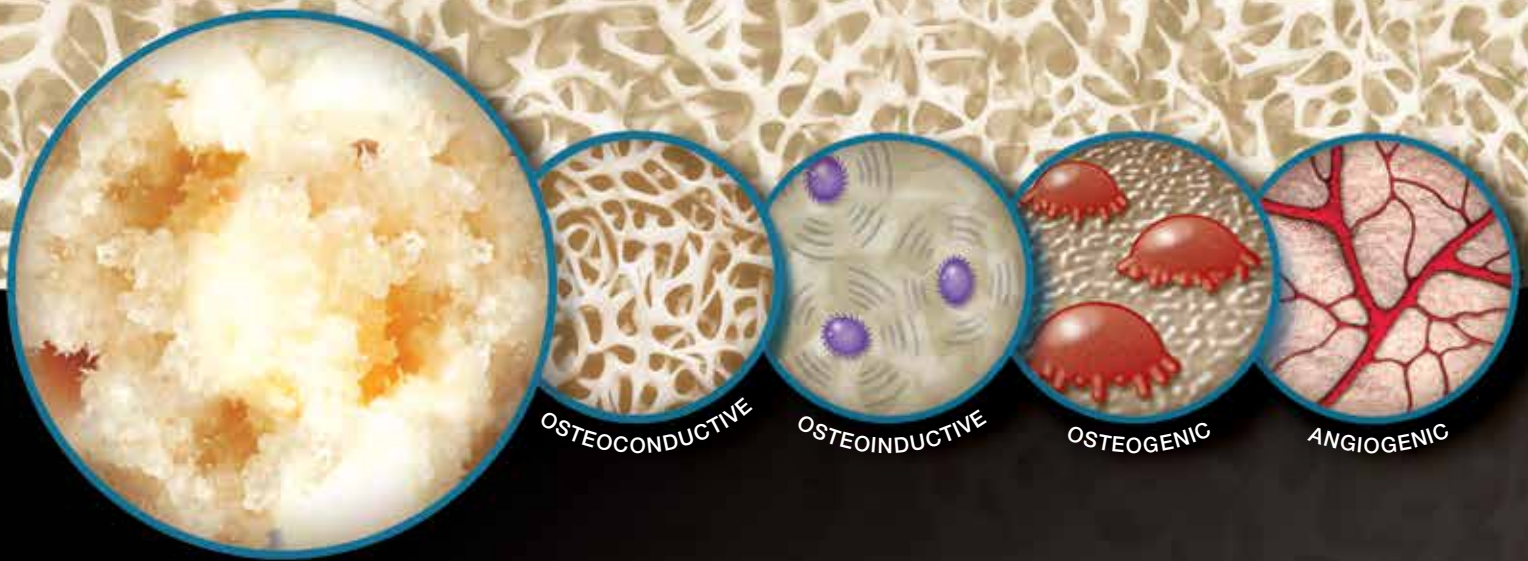


BIO4™

Viable Bone Matrix



BIO⁴™ Viable Bone Matrix

































BIO⁴ – The Next Generation Viable Bone Matrix

BIO⁴ is the next generation viable bone matrix as it offers four components for bone formation – scaffold for osteoconduction, signals (growth factors) for osteoinduction, viable cells for osteogenesis and in addition, retains signals (growth factors) that support angiogenesis.

As illustrated in Table 1, to date, no other non-cultured cellular bone allografts on the market are promoted as supporting angiogenesis. Further, no other bone graft materials on the market have been marketed as supporting all four components for bone formation

(Figure 1).

Figure 1. In comparison to other bone graft materials, BIO⁴ and autograft are the only ones to contain all four characteristics supporting bone formation.^[1,2,3,4]

	Osteoconductive (Scaffold)	Osteoinductive (Signals / Growth Factors)	Osteogenic (Cells)	Angiogenic (Signals / Growth Factors)
Autograft¹				
BIO⁴™ - Viable Bone Matrix²				
Vitoss® - Synthetic Ceramics (+BMA)³				
Trinity / Osteocel - Viable Bone Matrix (Allografts) ^{4, 5, 6}				
Augment - rhPDGF-BB (+TCP) ⁸				
Infuse - Bone Morphogenetic Proteins (+Collagen) ⁷				
Deminerallized Bone Matrix (Allograft)				
Cancellous Bone Chips (Allograft)				

BIO⁴ Overview

Stryker Corporation entered into an exclusive, worldwide partnership with Osiris Therapeutics to expand our biologics portfolio and bring you the next generation viable bone matrix, BIO⁴. The association of a global leader in Orthopaedics, Stryker, and the creators of the original allograft cellular matrix, pioneers and leaders in regenerative medicine, Osiris Therapeutics, brings physicians and patients an innovative bone allograft that is one step ahead of the other commercially available cellular bone allografts.

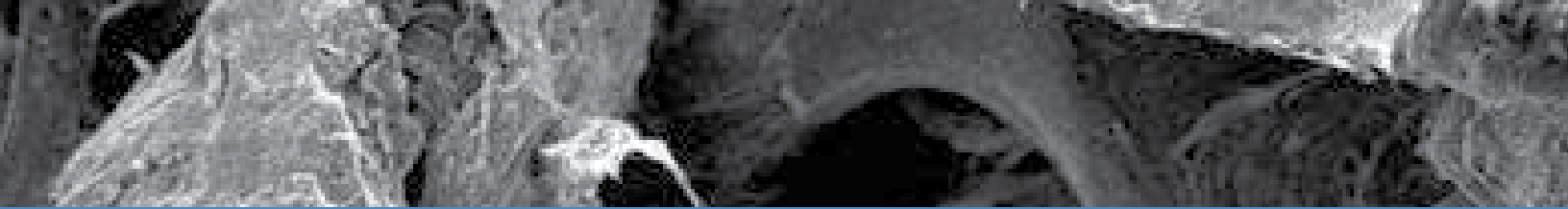
The innovative principle behind BIO⁴ is to offer the next generation viable bone matrix that retains not only osteoconductive, osteoinductive and osteogenic properties of bone, which are provided by bone allograft products, but also preserves the endogenous signals (growth factors) for supporting angiogenesis.

Definition: BIO⁴ is a viable bone matrix containing endogenous bone forming cells including mesenchymal stem cells, osteoprogenitor cells and osteoblasts as well as osteoinductive and angiogenic growth factors. BIO⁴ possesses all four characteristics involved in bone repair and regeneration: osteoconductive; osteoinductive; osteogenic; and angiogenic.^[2,10,11]

Osiris Therapeutics, the creators of the original allograft cellular bone matrix, developed BIO⁴ as an alternative to autograft to minimize the potential for harvest site co-morbidities. Features for BIO⁴ include:

- Next generation viable bone matrix
- Lot tested for the presence of VEGF (vascular endothelial growth factors)^[10]
- Ready to use out of the package; no decanting is required and thaws in 15 minutes
- Differentiated handling compared to the competition
- Contains on average at least 600,000 cells (endogenous bone forming cells including mesenchymal stem cells, osteoprogenitor and osteoblasts) per cc^[10]
- Lot tested for 70% cell viability post-thaw^[10]
- Non-immunogenic^[10]

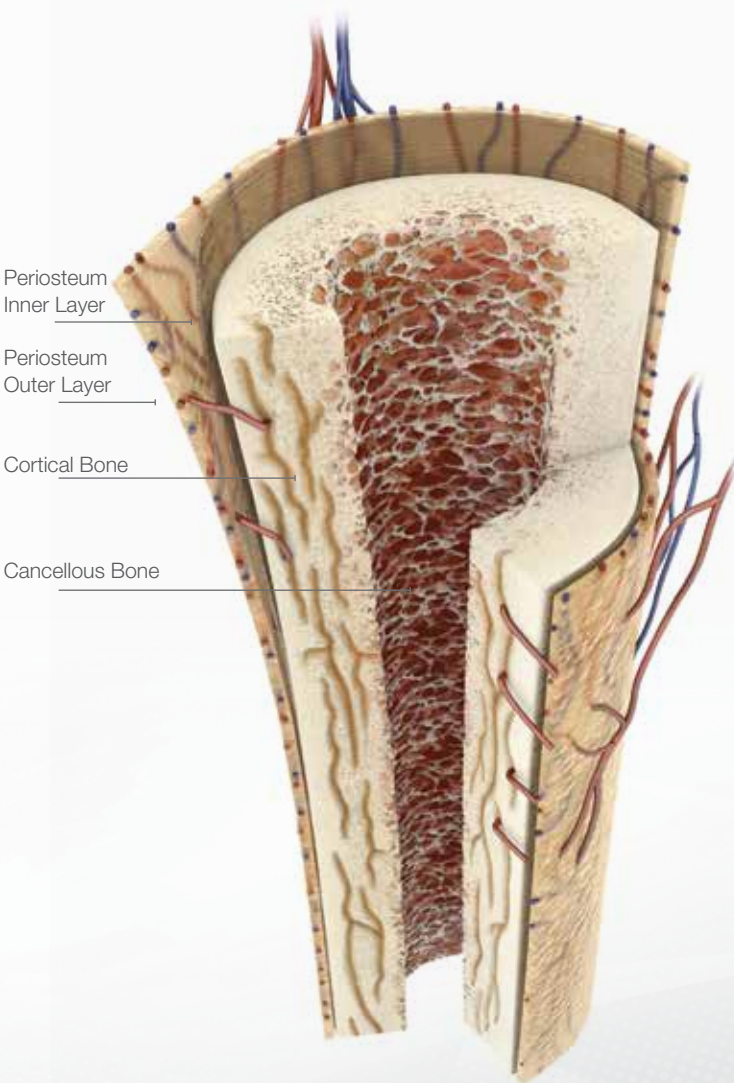
BIO⁴ is processed utilizing proprietary methods. Each BIO⁴ lot is processed from a qualified single donor that undergoes extensive serology, sterility and bioburden testing and a full review of medical records by the tissue bank Medical Director.



BIO⁴ Composition

BIO⁴'s proprietary formulation includes all components of bone – cancellous and cortical bone and periosteum (**Figure 2**). Careful processing preserves these components needed for bone formation while removing the elements that might elicit an immunogenic response. The scaffold, cells and signals are derived from cancellous bone, bone chips and demineralized cortical bone. In addition, signals that support angiogenesis are derived from bone periosteum, which is carefully processed to preserve angiogenic growth factors. The presence of periosteum further improves handling properties.^[10]

Figure 2. Components of the Bone



BIO⁴ Processing

All components of BIO⁴ are from allograft tissue: cancellous bone, cortical bone and periosteum. Processing of the bone periosteum retains growth factors that support angiogenesis, such as the vascular endothelial growth factor VEGF.^[11] Each BIO⁴ lot is processed from a qualified single donor.

Processing of BIO⁴ is performed using aseptic techniques. In addition, all tissues are soaked in an anti-microbial solution for an amount of time required to minimize bioburden level.

Osiris has implemented criteria for tissue lot and donor release for BIO⁴. All assays have been qualified through extensive qualification studies.^[10] Prior to release, each lot is tested to ensure a minimum of 70% cell viability, minimum of 600,000 viable cells per cc, and the presence of VEGF.^[10] The tissue testing and release criteria are summarized in **Figure 3**.

Figure 3. Lot Release Criteria for BIO⁴.^[10]

Assay	Source	Criteria
Viral Serology Panel	Donor Serum	Pass
Total Bioburden	Pre-antibiotic Treatment Sample	Pass
Sterility per USP <71>	Final Tissue	Pass
Cell Viability	Final Tissue	≥70%
Cell Count	Final Tissue	≥600,00 cells/cc
VEGF	Final Tissue	Present

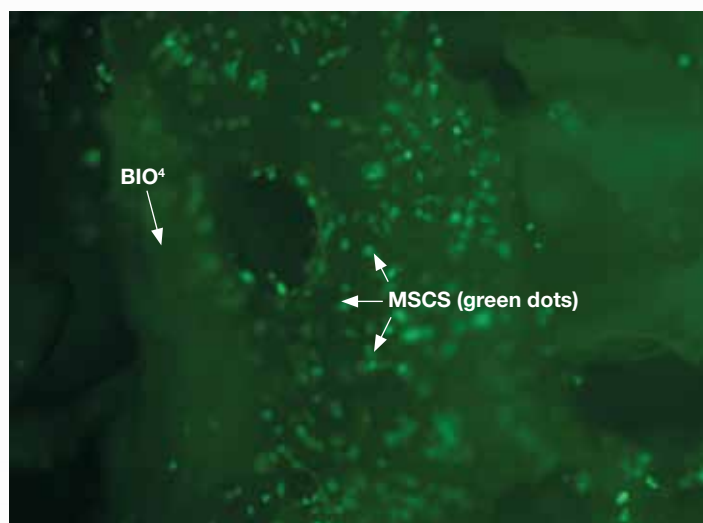
BIO⁴™ Viable Bone Matrix

BIO⁴ Scaffold

The osteoconductive bone chips comprising BIO⁴ offer a natural scaffold for host cell attachment.

The bone chips comprising BIO⁴ have been shown to provide excellent natural scaffold for bone formation.^[10] The osteoconductivity of the BIO⁴ matrix was confirmed by assessing hMSC (human mesenchymal stem cells) attachment *in vitro*. For this assay, fluorescently labeled hMSCs were seeded onto culture wells containing BIO⁴. The cells were allowed to attach, and then the wells were irrigated to remove non-adherent cells. The well was evaluated using fluorescence microscopy for the presence of attached cells. The retention of hMSCs on the matrix after irrigation demonstrates that BIO⁴ is an osteoconductive matrix (**Figure 4**).

Figure 4. Human MSCs are shown to attach to the BIO⁴ matrix (in bright green via fluorescence microscopy after 1 hour), illustrating that BIO⁴ is an osteoconductive matrix that supports cell attachment.



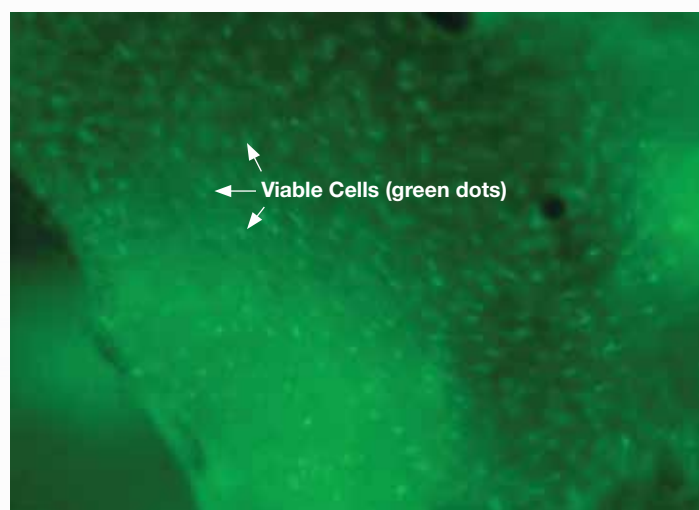
BIO⁴ Cells

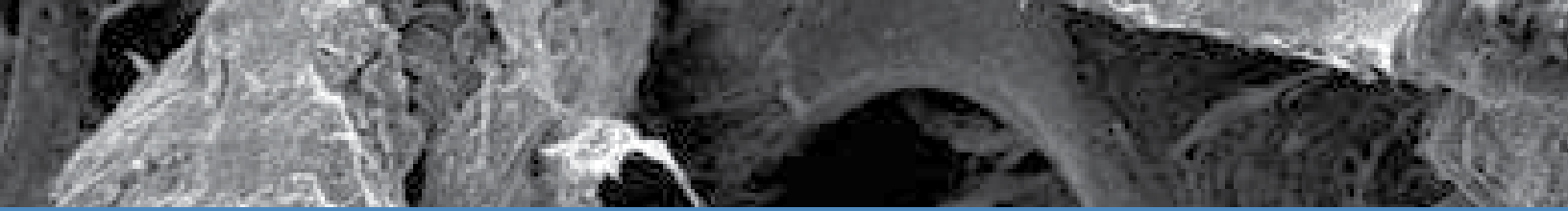
BIO⁴ contains endogenous viable cells, including bone-forming osteoblasts, osteoprogenitor cells and mesenchymal stem cells.

Osteogenesis is the ability of the graft to produce new bone and requires viable cells in the allograft; specialized processing and cryopreservation techniques have been developed to preserve the native bone cells in BIO⁴. Each lot of BIO⁴ is tested and contains a minimum of 600,000 viable cells per cc.^[10]

To visualize the viable cells that are retained in BIO⁴ after cryopreservation and thawing, samples of BIO⁴ were stained with a live/dead stain. Only live, viable cells are capable of metabolically converting the non-fluorescent calcein-AM dye into calcein, which emits a green fluorescent signal. Viable cells are visualized as green fluorescent dots under fluorescence microscopy (**Figure 5**). Quantification of the number of viable cells in cryopreserved and thawed BIO⁴ showed that greater than 70% of cells remain viable, using trypan blue staining.^[10] Cell viability testing is performed on every BIO⁴ lot that is released.

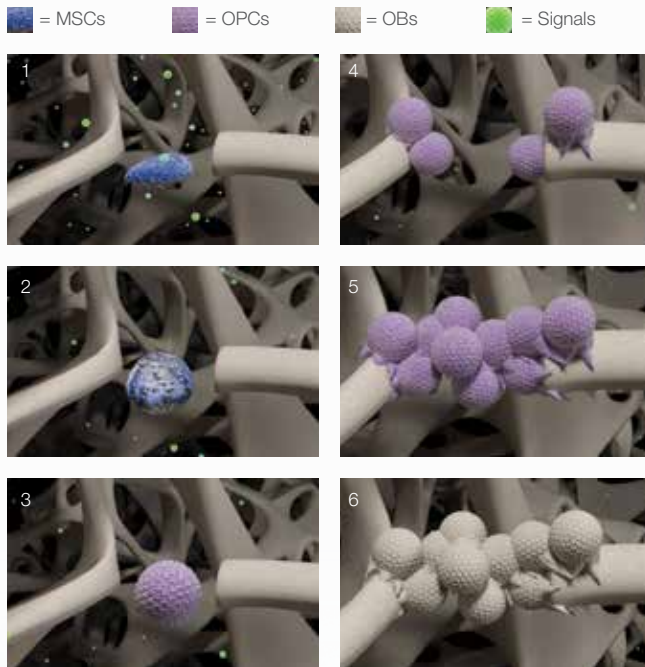
Figure 5. BIO⁴ contains viable cells. BIO⁴ was incubated with live/dead stain and viable cells were visualized as bright green dots under fluorescence microscopy.





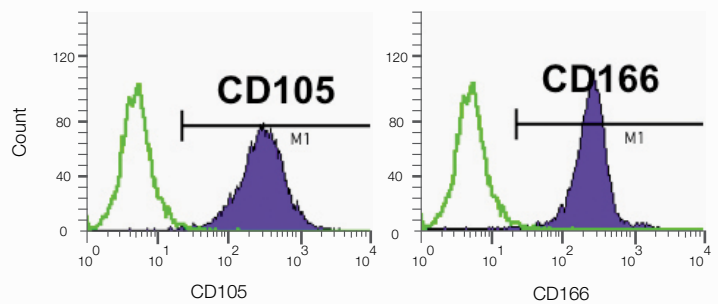
Repairing and regrowing damaged bone requires not only viable cells, but specifically osteoprogenitor cells that have the potential to form new bone (osteogenic potential). These osteoprogenitor cells originate from adult mesenchymal stem cells (MSCs) (**Figure 6**). MSCs reside in bone and other tissues, and represent a subset of stem cells that respond to cues in the cellular environment to differentiate into a variety of cell types, including osteoprogenitor cells.^[12] MSCs committed to the osteogenic lineage will give rise to osteoblasts, the cells that form the bone matrix.^[13]

Figure 6. Animation images of mesenchymal stem cell (MSCs) differentiation into osteoprogenitor cells (OPCs), which are precursors to bone-forming osteoblasts (OBs).



The cell types retained in BIO⁴ were identified using fluorescence activated cell sorting (FACS) analysis of specific cell surface markers that are characteristic of MSCs.^[12] The cancellous chips present in BIO⁴ were placed in culture medium for 3 weeks to allow for cells to migrate out of the bone chips. These cells present in BIO⁴ were stained and characterized by FACS. **Figure 7** shows that viable cells in BIO⁴ express the known MSC markers CD105 and CD166.

Figure 7. Representative FACS histograms showing cell specific markers of MSCs present in BIO⁴. The green histograms in both graphs represent the isotype control (non-specific staining) and the violet histograms represent staining for MSC markers CD105 (left graph) and CD166 (right graph).



In addition to MSCs, BIO⁴ contains osteogenic cells as detected through staining for alkaline phosphatase, a marker of osteogenesis.^[12] **Figure 8** shows that cells on BIO⁴ are alkaline phosphatase positive, supporting that BIO⁴ contains viable osteogenic cells. Taken together, BIO⁴ retains at least 70% viable cells in the allograft and contains a verified subpopulation of MSCs and osteoprogenitor cells.^[10]

Figure 8. Cells on cultured BIO⁴ bone chips were labeled with BCIP/NBT Alkaline Phosphatase Substrate for detecting alkaline phosphatase (ALP) activity, a known osteogenic marker. Osteogenic cells stain positive (dark dots) for ALP, as visualized under light microscopy.

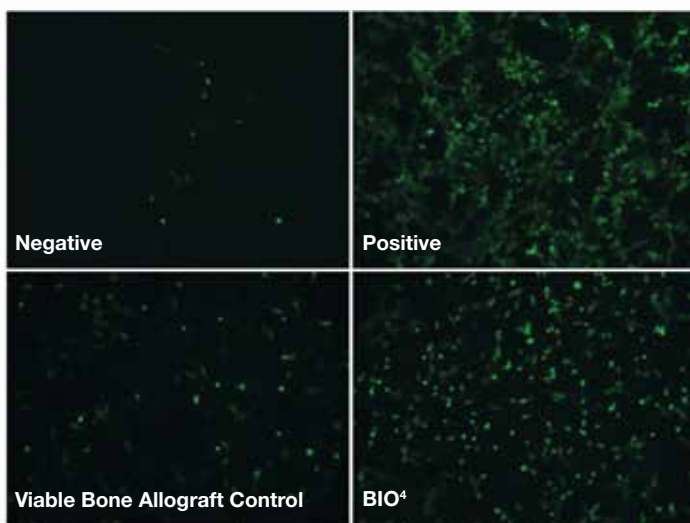


BIO⁴ – Signals (Osteoinductive)

BIO⁴ exhibits osteoinductive potential characterized by its ability to stimulate MSCs to migrate and differentiate into bone-forming osteoblasts.

Osteoinductive grafts have the ability to stimulate cell migration to sites of damaged bone where mesenchymal stem cells and osteoprogenitor cells can subsequently differentiate into mature bone-forming cells (osteoblasts).^[14] To demonstrate the capacity of BIO⁴ to recruit MSCs, an *in vitro* MSC migration assay was used. In contrast to the negative control, MSCs (fluorescently labeled green) were shown to migrate in the presence of soluble extracts of BIO⁴, as when in the presence of an osteoinductive positive control (**Figure 9**). These *in vitro* data support that BIO⁴ exhibits the potential to recruit MSCs, which fulfills one criterion defining osteoinduction.

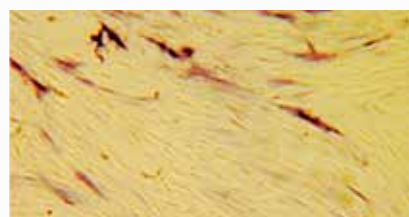
Figure 9. BIO⁴ induces mesenchymal stem cell migration. Fluorescently labeled MSCs were seeded onto a transwell filter. Cell medium supplemented with soluble extracts from BIO⁴ were added to the tissue culture wells. Medium without growth factors served as the negative control, while medium supplemented with 10% FBS was the positive control known to induce cell migration. The effect of the medium supplemented with these factors was examined and compared to the effects of a viable bone matrix control that contains viable cancellous bone and DBM, but lacks the periosteum. Cell migration to the underside of the transwell insert was evaluated under fluorescence microscopy.



Once localized to sites of damaged bone, key osteoinductive signals, such as bone morphogenetic proteins (BMPs), trigger the differentiation of mesenchymal stem cells and osteoprogenitor cells into osteoblasts that form bone.^[15]

To demonstrate the ability of BIO⁴ to induce MSC differentiation, undifferentiated human MSCs were cultured with and without osteogenic growth factors (positive and negative controls, respectively) and in the presence of BIO⁴ extracts. In contrast to the negative control, cells exposed to the BIO⁴ extracts and to the positive control showed alkaline phosphatase staining, a known marker of osteogenic bone-forming cells (**Figure 10**). This data supports the ability of BIO⁴ to induce MSC differentiation into bone-forming cells.

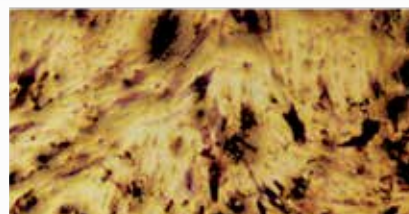
Figure 10. BIO⁴ tissue extracts induce osteogenic differentiation. hMSCs were incubated for seven days with the soluble factors extracted from BIO⁴ into cell culture media. Cells were then fixed in 10% formalin and stained with BCIP/NBT Alkaline Phosphatase Substrate to detect ALP activity, as shown by dark staining.



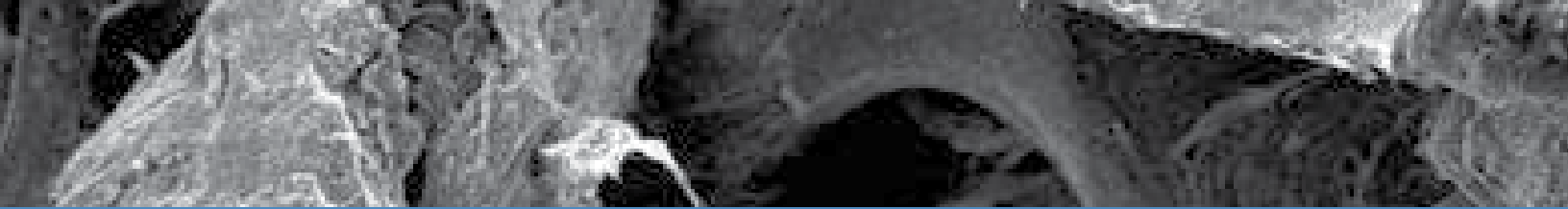
Negative



Positive



BIO⁴



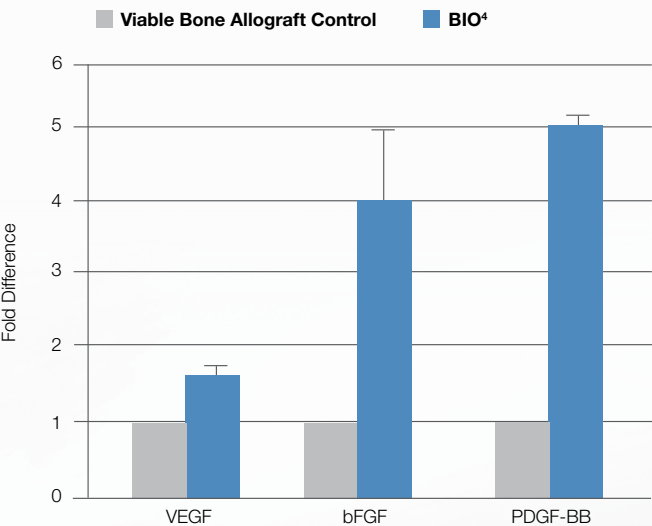
BIO⁴ – Signals (Angiogenic)

BIO⁴ contains naturally occurring angiogenic growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF), which have been reported to be important for bone repair at sites of damaged bone.^[16]

Angiogenesis refers to the ability to grow new blood vessels from pre-existing ones, which is a natural step in the process of bone healing.^[14] Key signaling factors involved in angiogenesis include growth factors such as the VEGF, PDGF and bFGF.^[15] All three factors have been shown to recruit and/or promote the proliferation of MSCs and endothelial cells to sites of revascularization and play a role in new blood vessel formation.^[17,18,19,20,21]

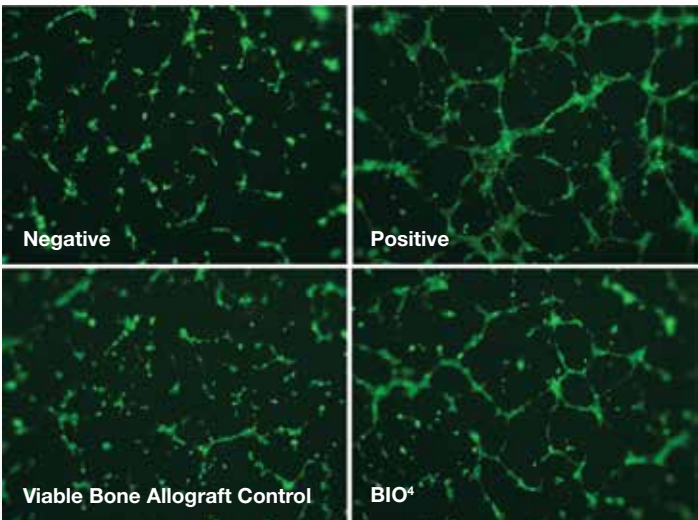
As shown in **Figure 11**, the presence of angiogenic signals in BIO⁴ was confirmed using ELISA. BIO⁴ showed higher amounts of endogenous angiogenic growth factors compared to a similar bone allograft formulation lacking the periosteum (viable bone allograft control). VEGF, bFGF and PDGF-BB are detectable to a higher degree in BIO⁴ due to the presence of periosteum.

Figure 11. BIO⁴ contains the angiogenic signals VEGF, bFGF, and PDGF-BB in greater concentrations than matrix lacking periosteum.



The angiogenic potential of BIO⁴ was established in an *in vitro* assay evaluating the formation of tubes, which is an initial step in the formation of blood vessels.^[21] In this assay, the formation of 3D tube structures from endothelial cells is visualized as interconnected green lines under fluorescence microscopy.^[22] Human umbilical vein endothelial cells (HUVEC), cells are reactive to angiogenic signals, were exposed to cell medium supplemented with BIO⁴ extracts, serum and VEGF media (positive control) or serum- and VEGF-free media (negative control). **Figure 12** shows that BIO⁴ supports the formation of tube-like structures from viable endothelial cells to a similar degree as that observed in the positive control. In contrast, minimal tube formation is detected when HUVEC cells are exposed to the BIO⁴ formulation that lacks the periosteum. The tube formation shown in this *in vitro* assay supports the angiogenic potential of BIO⁴.

Figure 12. BIO⁴ extracts induce *in vitro* tube formation characteristic of angiogenesis. HUVECs were suspended in EBM medium then seeded onto wells coated with Reduced Growth Factor Matrigel. Wells containing either Endothelial Basal Medium (EBM, negative control), Endothelial Growth Medium (EBM + EBM2 Bullet Kit with serum and VEGF – Lonza, positive control), BIO⁴ tissue lysate supplemented EBM, or an EBM-supplemented viable bone matrix lacking periosteum. HUVEC cells were cultured with the described media for 5 hours and viable cells were stained with Calcein-AM and imaged under fluorescence microscopy.



Bone Grafting Overview

Bone grafting has become a widely used, safe and efficient surgical procedure to facilitate bone repair and new bone formation in a variety of orthopaedic indications, such as spine fusion, repair of traumatic defects, non-unions and arthrodesis.^[2,23] The success of bone grafting relies on factors including the selection of the appropriate bone graft and bone substitute biomaterials. The biology of bone grafts and their substitutes requires an understanding of bone formation, which involves osteoconduction, osteoinduction, osteogenesis and angiogenesis.^[3,4,14,23,24,25]

Osteoconduction - The process by which an implanted scaffold passively allows ingrowth of host vasculature, cells and tissue. Osteoconduction occurs when the bone graft material serves as a scaffold for host cells, which is required for new bone growth that is perpetuated by the native bone.

Osteoinduction - Involves the stimulation of osteoprogenitor cells to migrate, proliferate and differentiate into osteoblasts, which then begin new bone formation. New bone formation requires the active recruitment of host mesenchymal stem cells from the surrounding tissue, which differentiate into bone-forming osteoblasts. Osteoinduction occurs due to the presence of growth factors within the graft, principally bone morphogenetic proteins (BMPs).

Osteogenesis - The ability of the graft to produce new bone, relying on cellular elements within the donor graft, which synthesize new bone at the recipient site. Osteogenesis occurs when viable cells originating from the bone graft material contribute to new bone formation.

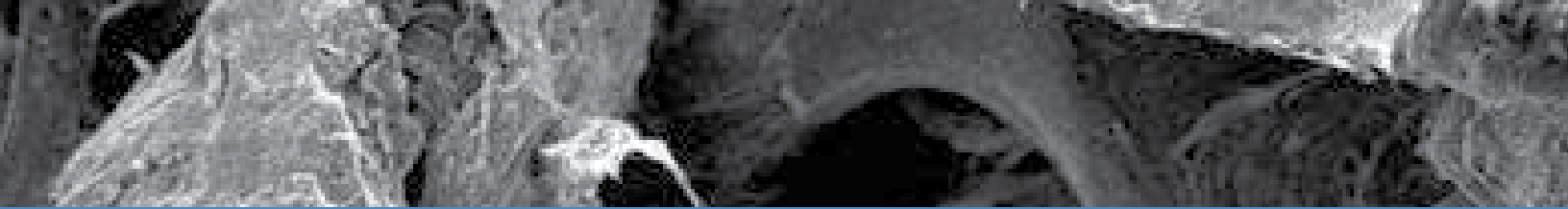
Angiogenesis - Refers to the formation of new blood vessels, which is a natural part of the bone repair process. New blood vessels restore blood flow and delivery of oxygen and nutrients at the site after the injury, which is essential for bone repair. Angiogenesis is mediated by the presence of growth factors within the graft, principally vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF) and basic fibroblast growth factor (bFGF).^[26]

Successful new bone formation involves these four physiological properties of osteoconduction, osteoinduction, osteogenesis and angiogenesis.

Autograft Bone

Autograft bone comprises all four properties that are involved in new bone formation: osteoconductive, osteoinductive, osteogenic and angiogenic. For this reason, autograft has remained the “gold standard” for bone grafting.^[14] Recovery sites for autograft bone include iliac crest, vertebral body and extremities (epiphysis) of long bones, such as distal femur or proximal tibia. Autograft bone may then be further morselized for better handling before placement at the site of bone injury. Autograft bone contains cortical and cancellous bone and periosteum, which together provide all the principal elements to support bone formation processes.

The outside covering of the bone is the periosteum, a complex membrane tissue composed of an outer fibrous layer that lends structural integrity and an inner cambium layer that possesses osteogenic potential. During growth and development, it contributes to bone elongation and modeling, and when the bone is injured, it participates in its recovery.^[27] The periosteum contains angiogenic growth factors such as VEGF, which has been documented to be important for bone repair.^[28]



Bone Formation and Bone Repair Overview

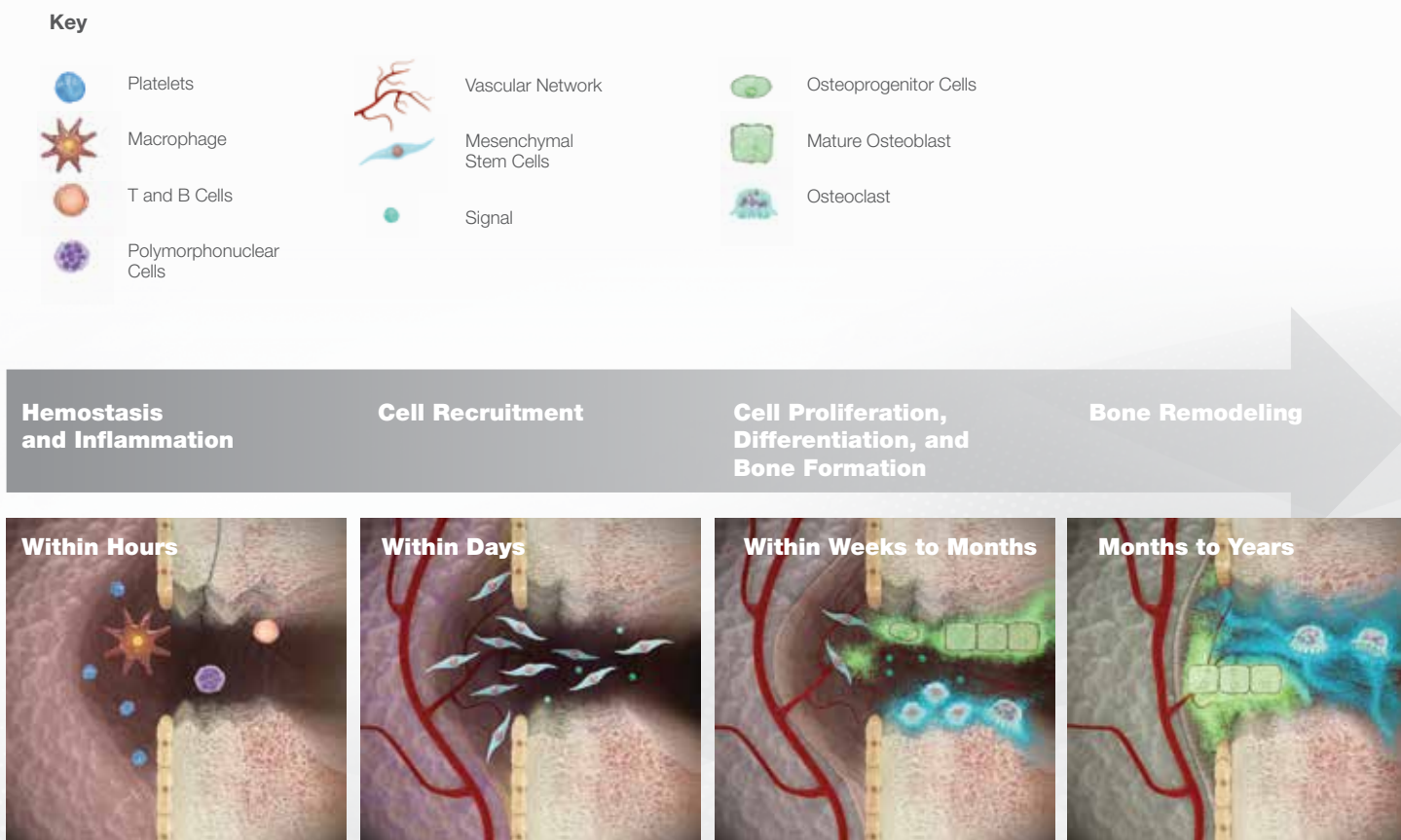
Bone repair occurs in a sequential progression of overlapping processes – hemostasis, inflammation, angiogenesis, repair and finally, remodeling of the damaged bone (**Figure 13**).^[29] For bone to repair, scaffold, cells and signals must collectively ensure the restoration of tissue structure and function.^[2] Once hemostasis is achieved following the initial injury, an inflammatory phase commences a cascade of molecular events. Signals in the form of growth factors and cytokines are released by cells involved in the inflammatory response, which then recruit host cells, including mesenchymal stem cells (MSCs), to the injury site to support bone formation. Host cells, such as MSCs, may originate from the surrounding host tissue, migrate to the injury site and attach to the implanted scaffold.

The formation of new vasculature from existing blood vessels is termed angiogenesis and is supported by angiogenic signals. Nutrients and oxygen, which are delivered through blood vessels, are required for tissue repair. Angiogenesis is a component of bone development and repair and also for bone graft integration.^[30] Studies have shown that the more vascularized the bone graft, the faster the graft integration.^[16]

Once MSCs have attached to the scaffold and host bone and encounter the appropriate signals, they begin to differentiate into osteoprogenitor cells (OPCs). MSCs and OPCs subsequently release additional factors that recruit more bone-forming cells to the injury site to further assist in bone repair and remodeling. OPCs, in turn, become mature osteoblasts, capable of forming new bone. Osteoblasts ultimately facilitate bone formation by depositing the mineralized matrix, a characteristic of healthy bone. Bone remodeling is a balance between osteoclasts (bone resorbing cells) and osteoblasts (bone-forming cells) through a continuing activation-resorption-formation sequence.

Bone formation and bone repair need a trio of components: a scaffold for osteoconduction, cells for osteogenesis and signals for osteoinduction. Another noteworthy component – signals for angiogenesis – is also a beneficial element of a bone graft to repair and form bone.

Figure 13. Bone Repair Schematic.



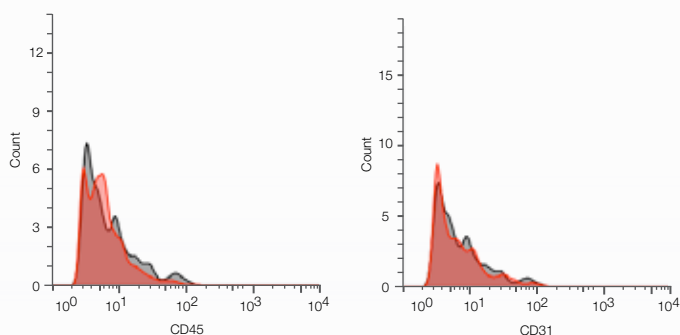
BIO⁴ Lack of Immunogenicity

The cellular components in BIO⁴ have been shown not to elicit an immune response.

BIO⁴ is formulated using a process that depletes immunogenic cells while retaining osteogenic cells. The removal of immunogenic cells was examined via fluorescence activated cell sorting (FACS) analysis of cell surface markers characteristic of immune cells. The immunogenic cell surface markers CD45 (hematopoietic, leukocyte marker) and CD31 (endothelial cell marker) were undetectable in BIO⁴ (**Figure 14**).

The periosteum is known to contain immunogenic hematopoietic and endothelial cells.^[27] A live/dead cell assay and fluorescent microscopy confirmed that no viable cells were present in the periosteum component of BIO⁴. This data demonstrates the lack of immune response associated with BIO⁴.^[10]

Figure 14. Representative FACS histograms show the lack of detection of immunogenic cell surface markers CD45 (hematopoietic, leukocyte cell marker) and CD31 (endothelial cell marker) in BIO⁴. Grey histogram represents the isotype control (non-specific staining) and the red histogram represents specific staining for CD45 (left graph) and CD31 (right graph). The overlap of the two histograms indicates that there is no specific staining detected for the given immunogenic markers.



The lack of BIO⁴ immunogenicity was confirmed in a modified version of the Mixed Lymphocyte Reaction (MLR), a test routinely performed as surrogate predictor of donor graft rejection.^[31] MLR is a functional assay which measures activation of T lymphocyte (T-cells) expected after exposure to unmatched immunogenic cells derived from another individual. The readout of T-cell activation may include measurement of expression of activation markers on the surface of T-cells, or T-cell proliferation, or secretion of inflammatory cytokines. The modified version of the MLR assay takes advantage of the fact that T-cells (Peripheral blood mononuclear cells - PBMC) secrete the interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α). Therefore, the level of the immune response correlates with the levels of IFN- γ and TNF- α secreted by activated T-cells (detected by ELISA). Three different lots of BIO⁴ were tested in this modified MLR assay, and all resulted in non-detectable IFN- γ levels, similar to the negative controls (**Figure 15**). In addition, two different lots of BIO⁴ were tested for secretion of TNF- α in MLR, and both resulted in non-detectable TNF- α levels, similar to the negative controls (**Figure 16**).

Figure 15. MLR assay applied to BIO⁴ shows non-detectable IFN- γ levels, similar to the negative controls, demonstrating a lack of immune response. Peripheral blood mononuclear cells (PBMC 1 or 2) derived from two different unmatched donors were used alone as negative controls, the mixture of the two was the positive MLR control. IFN- γ secretion by PBMCs was measured and indicates PBMC activation. Relative PBMC activation assessed by a Stimulation Index (SI) represents the lymphocyte activation in the positive control or test samples versus the negative control. Adding PBMC 1 or 2 to BIO⁴ samples (unmatched donors) derived from three different donors showed no detectable levels of IFN- γ ,

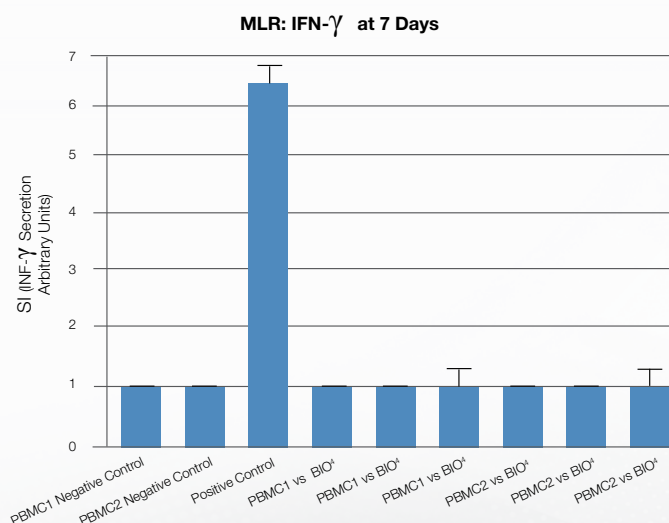
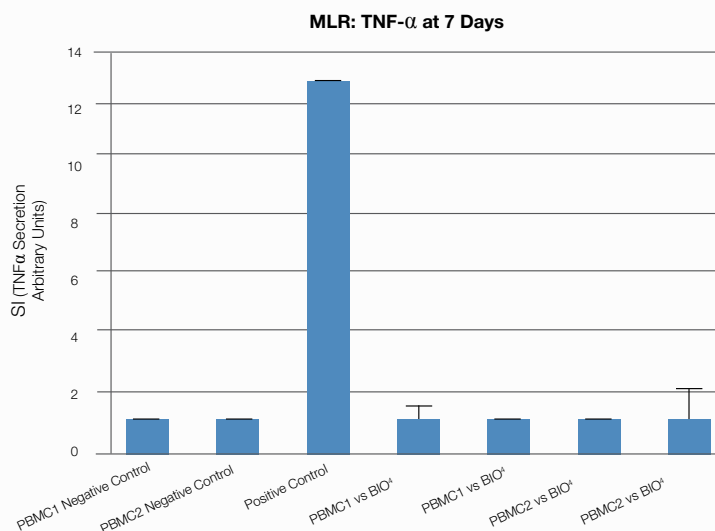
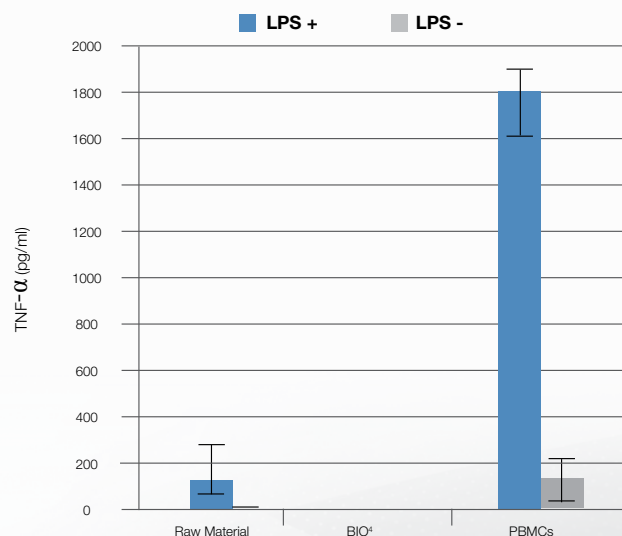


Figure 16. MLR assay applied to BIO⁴ show non-detectable TNF- α levels, similar to the negative controls, demonstrating a lack of immune response.



Furthermore, immunogenicity was examined in a lipopolysaccharide (LPS) challenge assay. In this assay, LPS interacts with membrane receptors on immune and endothelial cells and stimulates secretion of inflammatory cytokines such as TNF- α .^[31] A high secretion of these cytokines, including TNF- α , indicates the presence of immunogenic cells in a test sample. Two different lots of BIO⁴ were tested in the LPS assay, and both resulted in non-detectable TNF- α levels indicating a lack of immunogenic cells in BIO⁴. The corresponding starting Raw Material and PBMC positive control exhibited increased TNF- α in response to LPS stimulation. **(Figure 17).**^[10] All this data combined demonstrate the lack of immunogenicity of BIO⁴.

Figure 17. LPS assay applied to BIO⁴ shows non-detectable TNF- α levels demonstrating a lack of immunogenic cells present in BIO⁴. LPS was added to PBMCs (positive control) or BIO⁴ and the secretion of TNF- α was measured. When LPS was added to BIO⁴, no detectable TNF- α was detected, indicating a lack of immunogenic cells in BIO⁴.



BIO⁴™ Viable Bone Matrix

BIO⁴ — Quality in Tissue Banking

Osiris Therapeutics' commitment to quality and safety is evident in all aspects of tissue recovery, processing and characterization. Osiris ensures that BIO⁴ is processed consistently and preserves the inherent functionality of the tissue matrix.

Each BIO⁴ lot undergoes sterility and bioburden testing. BIO⁴ is processed from a qualified single donor that has undergone extensive serology testing and full review of medical records by the tissue bank Medical Director. Donor screening starts with a thorough medical and social history evaluation, physical examination and medical record evaluation. Each donor that may be selected by Osiris must then pass high quality and safety standards that meet the requirements of the American Association of Tissue Banks (AATB) as well as the guidelines established by the U.S. Food and Drug Administration (FDA). **Figure 18** lists these extensive and thorough tests to ensure safety and quality of the donors selected for BIO⁴.

BIO⁴ is processed by Osiris Therapeutics, which is accredited by the AATB and registered with the FDA as a established tissue bank. Osiris further complies with the licensures in the States of California, Florida, Maryland and New York.

All tissue services are committed to the principles of safety, quality and consistency. Osiris maintains rigorous requirements, standards and qualification methods. All tissue preparation takes place in a clean room environment. All tissue undergoes rigorous testing before being released.

Osiris's highly qualified and innovative team continually develops new products applying new designs, methods and assays. Founded in 1992, Osiris developed a technology that led from commercializing its first generation bone reparative product, Osteocel,^[33] to approval of the world's first stem cell drug, remestemcel-L for graft versus host disease. Osiris's innovative tissue products include allograft tissues for bone repair, acute and chronic wounds, and cartilage repair. Osiris is a fully integrated company with capabilities in research, development, manufacturing and distribution. Osiris has developed an intellectual property portfolio to protect the company's technology and commercial interests.^[32]

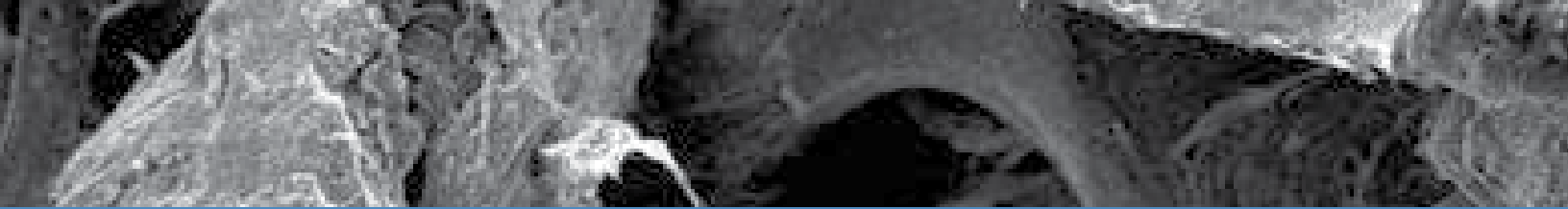


Figure 18. List of tests conducted for donor screening and testing.

Institution	Screening Criteria
Osiris	<p>Hepatitis B virus, Hepatitis C virus, HIV 1&2, Malaria, Sepsis, Syphilis, Transmissible Spongiform Encephalopathy (TSE), Vaccinia, West Nile Virus (WNV), Clinically significant metabolic bone disease, Leprosy, Polyarteritis nodosa, Rabies, Rheumatoid arthritis, Sarcoidosis, HIV/HCV RNA test, HIV I/II Antibody, Human T-Cell Lymphotropic Virus Type I & II Antibody, Group A Streptococcus, Mycobacterium, Encephalitis, Epstein Barr Virus (Mononucleosis), Herpes Virus, Hepatitis A, Meningitis, Poliomyelitis, Varicella zoster, Bacteremia, Chagas Disease, Condyloma, Clostridium difficile infection, Creutzfeld Jakob Disease (CJD), Endocarditis, Gonorrhea, Histoplasmosis, Meningitis, Methicillin Resistant Staphylococcus Aureus (MRSA), Osteomyelitis, Peritonitis, Pneumonia, Pyelonephritis, Rheumatic fever, Sepsis, Syphilis, Systemic Mycoses, Tuberculosis, Urinary tract infection, Vancomycin Resistant Enterococcus (VRE), Ankylosing Spondylitis, Antiphospholipid Syndrome, Autoimmune Hemolytic Anemia, Autoimmune Lymphoproliferative Syndrome, Autoimmune Thrombocytopenic Purpura, Autoimmune Vasculitis, Celiac Disease, Cold Agglutinin Disease, Crohn's disease, Dermatomyositis, Goodpasture's Syndrome, Grave's Disease, Guillain-Barre Syndrome, Hashimoto's Thyroiditis, Lupus Erythematosus, Meniere's Disease, Mixed Connective Tissue Disease, Multiple Sclerosis, Myasthenia Gravis, Pemphigus, Polyarteritis Nodosa, Reactive Arthritis (Reiter's Syndrome), Sarcoidosis, Scleroderma, Sjogren's Syndrome, Vitiligo, Wegener's Granulomatosis, Adult respiratory distress syndrome (ARDS), Alzheimer's Disease, Amyotrophic lateral sclerosis (ALS), Basal Cell Carcinoma, Burns, Cancer, Carbon monoxide poisoning, Chronic Obstructive Pulmonary Disease (COPD), Corneal scarring consistent with vaccinia keratitis, Corticosteroid therapy, Cystic Fibrosis, Diabetes Mellitus, Disseminated lymphadenopathy, Emphysema, Eczema vaccinatum (lesion or scab), Genetic disease, Hemophilia, Huntington's Disease (or Chorea), Idiopathic Thrombocytopenic Purpura, Kaposi's Sarcoma, Leukemia, Marfan's Syndrome, Multiple Myeloma (Plasmacytoma), Muscular Dystrophy, Myelodysplastic Syndrome, Parkinson's Disease, Polycythemia, Rash, generalized vesicular/vaccinia, Sick Cell Anemia, Vaccination, Vaccinia</p>



BIO⁴™ Viable Bone Matrix

BIO⁴ Preparation

Post-processing, BIO⁴ is cryopreserved with a minimal amount of cryopreservative medium using a controlled cooling rate, which is required for preservation of endogenous bone cells in the graft material. The minimal amount of cryopreservation medium eliminates the need to decant the medium (**Figure 19**) before usage.

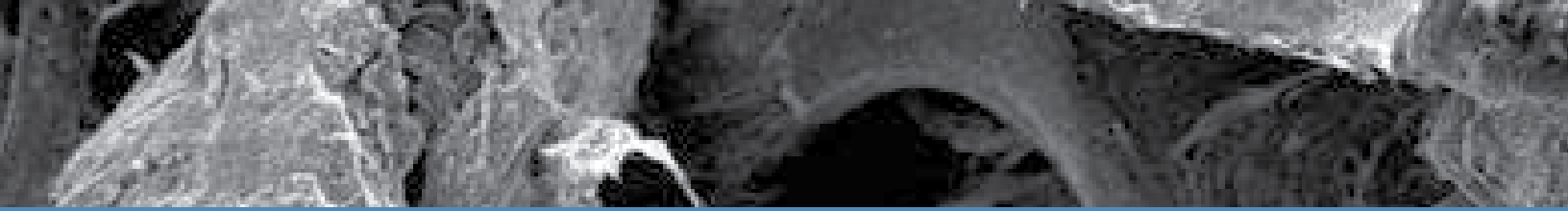
Figure 19. BIO⁴ preparation does not require decanting after thawing. BIO⁴ should be used within 1 hour of thawing.



Using aseptic technique, add sterile saline until volume is below the lid. Incubate for 15 minutes. **Temperature of thawing solution should not exceed 39 °C (102 °F).**



After 15 minutes, transfer product to lid or your hand and test for malleability; product should be soft and easy to shape.



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