ViviGen® Cellular Bone Matrix

The right cells. The right time.
Cell Types Involved in Bone Healing

Bone is a highly dynamic tissue and undergoes remodeling, which is a balance between the removal of old mineralized bone and the formation of new bone. As a result, bone is in a constant state of renewal. During the bone remodeling and repair processes, osteogenic cells are required to reach the defect site to directly participate in these processes.² Bone cells that are responsible for this remodeling process have very specific roles and functions (Figure 1).

These include:

**Osteoblasts** are bone building cells. They secrete the collagen matrix and synthesize proteins such as osteopontin and osteocalcin that are involved in the mineralization of the matrix²,³. Osteoblasts can differentiate into osteocytes.

**Osteoclasts** are bone removing cells. They are responsible for the absorption and removal of old bone.

**Osteocytes** are terminally differentiated osteoblasts that are entrapped in the mineralized matrix. They are able to send signals based on stress and strain felt within the bone to begin the remodeling process.

**Bone lining cells** are cells that line the surface of bone. They direct mineral uptake and release in bone.

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*Figure 1. Cell types involved in bone healing.*
The Bone Cell Advantage

ViviGen® Cellular Bone Matrix represents a paradigm shift in the field of bone repair. A body of evidence has emerged supporting the fact that bone cells are the preferred cells for bone formation compared to undifferentiated Mesenchymal Stem Cells (MSCs), because they are fully differentiated and are committed solely to laying down bone matrix.

Pre-Clinical Studies Suggest Bone Cells

- Remain at the defect site longer\(^5\), directly participate in the bone formation process and deposit a higher quality of bone than Mesenchymal Stem Cells\(^5\)

- Secrete chemotactic factors such as IGF-1, which may play a major role in recruitment of osteoblasts during bone formation\(^6,7\)

- Secrete cytokines to stimulate angiogenesis\(^6\) and work in concert to facilitate further MSC differentiation\(^8\)

- Maintain viability, proliferation potential and osteoblastic function later in life compared to an MSC\(^9,10,11,12\)
3 Key Components of ViviGen

ViviGen contains the elements necessary for bone formation\(^1\)

1. **Viable Cells (Osteogenic)**
   The processing of ViviGen removes bone marrow components including Mesenchymal Stem Cells while retaining the desirable bone cells (osteoblasts, osteocytes and bone lining cells)\(^1\)

2. **Corticocancellous Chips (Osteoconductive)**
   Provide a natural scaffold for cell attachment, migration and proliferation

3. **Demineralized Bone (Osteoinductive)**
   A patented demineralization process exposes natural growth factors within the bone matrix that recruit host cells and stimulate bone forming activity

ViviGen is a Human Cells, Tissues, and Cellular and Tissue-based Product (HCT/P) comprised of cryopreserved viable, lineage committed bone cells within a corticocancellous bone matrix and demineralized bone delivering all of the properties necessary for bone formation. It is the first cellular allograft focused on protecting viable, lineage committed bone cells from recovery to implantation\(^1\).
In Vitro Assays

ViviGen Bone Cells are Viable

In order to determine the presence of viable cells, ViviGen bone chips were thawed after processing and cryopreservation and were plated in culture wells. The cells were able to migrate from the bone chips and attach to the culture plate demonstrating that they survived the freezing and thawing process¹ (Figure 2).

ViviGen Bone Cells are able to Proliferate

The proliferation profile of the viable ViviGen bone chips were further evaluated using an Alamar blue assay. Assays that can demonstrate the proliferation potential of cells are the ultimate test of viability since these assays test cell function and not just membrane integrity of cells. Results show that ViviGen cells can not only survive cryopreservation and processing but can also proliferate over time¹ (Figure 3).

ViviGen is Osteogenic¹

ViviGen derived cells were analyzed to assess their capacity to generate bone when stimulated with a standard osteogenic differentiation media. A substantial deposition of calcium was seen in the ViviGen derived cells as early as day 7. When allowed to differentiate to 14 - 21 days, extensive matrix deposits were evident by their positive red staining for calcium in the entire well¹ (Figure 4).
Safety

LifeNet Health prides themselves on their safety record over the last 30+ years. They hold the longest continuous accreditation from the American Association of Tissue Banks, and have a comprehensive range of measures in place to validate the safety of their allograft bio-implants; this includes stringent donor screening methods and release criteria. To obtain suitable donors, LifeNet Health maintains an extensive network of recovery partners. Additionally, LifeNet Health is a leading, federally designated Organ Procurement Organization. LifeNet Health only accepts donors from federally designated Organ Procurement Organizations and qualified tissue recovery partners. These partners are regularly audited to document that their recovery process meets current FDA regulations, AATB standards and LifeNet Health’s own stringent guidelines.

Donor Criteria

Every donor for ViviGen must meet LifeNet Health’s strict medical and behavioral risks assessment in addition to microbial and serological testing.

Aseptic Processing

LifeNet Health utilizes aseptic techniques in ISO certified clean rooms. Each lot of final product is tested for sterility.

ViviGen Cells are Non-Immunogenic¹

Staining for CD45, a type I transmembrane protein present on all hematopoietic cells, confirmed the presence of hematopoietic cells in the bone matrix prior to processing. Post processing, cryopreservation and thawing, marrow components as well as CD45 positive cells were absent, which confirmed that the marrow components were negligible. This demonstrates that the processing reduces the number of potentially immunogenic cells from the bone marrow, also reducing the risk of eliciting an immune response.¹

A Mixed Lymphocyte Reaction (MLR) Assay was conducted to ensure the bone cells derived from ViviGen are non-immunogenic.

Lymphocytes from ViviGen donors were combined with peripheral blood mononuclear cells (PBMC) to illicit an immune reaction. As expected, lymphocytes stimulated a statistically significant proliferative response from the non-matched PBMCs. ViviGen derived bone cells from the same donors were also mixed with PBMC resulting in no proliferation. This illustrates the absence of an immune response from the ViviGen derived bone cells¹.

Figure 5. Immunohistochemistry staining of CD45 positive cells (brown color) preprocessing and post cryopreservation and thawing.

Figure 6. Mixed lymphocyte reaction. Lymphocytes from ViviGen donors were combined with PBMC to elicit an immune reaction (grey bars). ViviGen-derived bone cells from the same donors were mixed with PBMC (blue bars). PBMC alone was used as a negative control (green bar).
Processing Time is Important

ViviGen is cryopreserved 24 hours faster than competitive cellular allografts.¹⁴

Rapid Thawing is Crucial for Cell Viability¹³
The thin walls of the ViviGen pouch allow for an efficient energy transfer, which facilitates a controlled freezing and a rapid thaw. The rapid heat transfer of the ViviGen pouch not only allows for all sizes to thaw in less than 5 minutes but is also vital for cell viability. The rapid thaw prevents ice crystals from forming intracellularly, ultimately maintaining viability. In addition, the port at the end of the ViviGen pouch allows for the quick and efficient removal of the cryopreservation media and rinsing solutions¹.

Features and Benefits

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<tr>
<th>FEATURES</th>
<th>BENEFITS</th>
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<tr>
<td>Osteogenic</td>
<td>Contains viable, lineage committed bone cells that are able to proliferate in vitro post cryopreservation and thaw</td>
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<tr>
<td>Osteoconductive</td>
<td>Contains corticocancellous chips that provide a natural scaffold for cell attachment, cell migration and cell proliferation</td>
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<td>Osteoinductive</td>
<td>Demineralization of the cortical bone exposes the natural growth factors within the matrix</td>
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<tr>
<td>Safety</td>
<td>Every lot is aseptically processed and all final product is tested for sterility using USP &lt;71&gt; standards</td>
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| Packaging        | The rapid heat transfer not only allows for all sizes to thaw in less than 5 minutes but is also vital for cell viability  
                   | The ported pouch allows for the quick and efficient removal of the cryopreservation media and rinsing solutions |
| Processing Time  | ViviGen reaches cryopreservation within 72 hours maximizing cell viability 24 hours sooner than competitive products |
| Maximized Cell Viability | The processing of ViviGen is focused on protecting viable, lineage committed bone cells from recovery to implantation¹ |
Ordering

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All ViviGen orders are placed with LifeNet Health directly by phone: 1-888-847-7831, by fax: 1-888-847-7832 or by email: orders@lifenethealth.org. Business hours are Monday through Friday, from 7am to 7pm EST.

Shipping

Free Next-Day Delivery

ViviGen is shipped for next day delivery by 10:30am free of charge. Next day early AM and FedEx are also available upon request.

1. Data on file LifeNet Health DHF 12-008
14. Trinity Evolution® Product Brochure

Findings from an in vitro assay are not always predictive of human clinical results.