ViviGen® Cellular Bone Matrix is designed to minimize acute immune potential

Michael Romanko PhD, Mark Moore PhD, MBA
LifeNet Health
Virginia Beach, VA

The proprietary processing of ViviGen Cellular Bone Matrix removes the potentially immunogenic marrow components from the product.

In order to minimize host response to ViviGen, LifeNet Health's proprietary process removes potentially immunogenic marrow components from sourced donor bone while retaining native bone cells. This is demonstrated through immunohistochemical analysis of ViviGen derived bone chips prior to processing, as well as after cryopreservation and thawing.[1] Prior to processing, large amounts of marrow components are present in the bone matrix (Figure 1A) as evidenced by staining for CD45, a type I transmembrane protein present on all hematopoietic cells. In contrast, those cells were absent (Figure 1B) following processing, cryopreservation, and thawing thus confirming removal of marrow components, which lowers the risk of immune response. In addition, the retention of native bone lineage cells is indicated by positive osteocalcin staining within the bone matrix following processing (Figure 2).[2]

These data indicate the process to prepare ViviGen both removes marrow and white blood cells while retaining a marker for active osteoblasts. Together ViviGen is intended to yield an osteogenic and biocompatible material that also does not elicit an acute immune response.

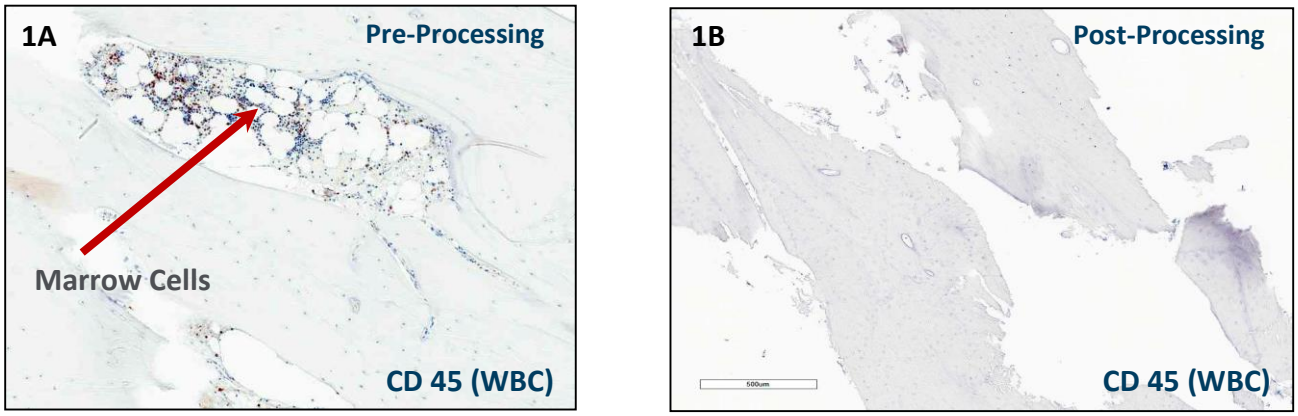

Figure 1. LifeNet Health's proprietary processing removes marrow components from sourced donor bone.
1A) Immunohistochemistry for white blood cell (WBC) marker CD 45 (stained red and identified with a closed arrow), 1B) demonstrating an absence of WBC staining following processing, cryopreservation and thawing of ViviGen.

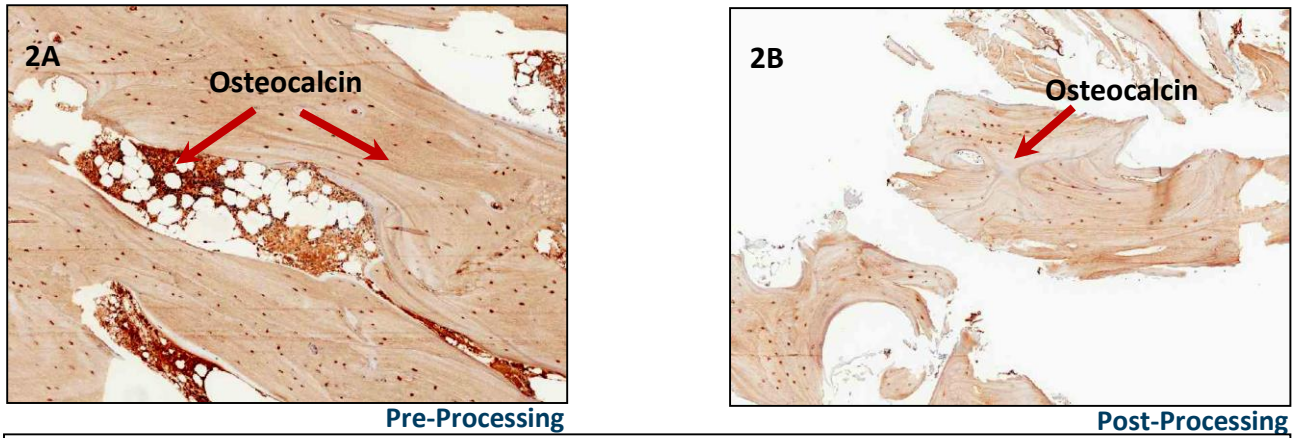

Figure 2. LifeNet Health's proprietary washing and processing affords retention of osteoblasts in sourced donor bone.
2A) Immunohistochemistry for osteoblast cell marker osteocalcin (stained brick-red and identified with a closed arrow), 2B) demonstrating the retention of putative osteoblast marker following processing, cryopreservation and thawing of ViviGen.

[1] Data on file at LifeNet Health DHF 12-008, DHF 15-001
[2] Data on file at LifeNet Health DHF 12-008, DHF 15-001

ViviGen bone cells do not elicit *in vitro* immune cell proliferation.

In validation of this design, a mixed lymphocyte reaction (MLR) assay, additionally demonstrates that the lineage committed bone cells comprising ViviGen do not elicit immune cell proliferation (Figure 3).[3] The MLR assay has been traditionally used to assess the histocompatibility of cell antigens between recipients and donor and is a test recommended by the FDA to measure the functional immune response mediated by T-cells against foreign antigens.[4, 5, 6] In this assay, a target population of responsive HLA-mismatched peripheral blood mononuclear cells (PBMC) were separately subjected to lymphocytes sourced from ViviGen donors and ViviGen-derived bone cells recovered from the same respective donors. Not surprisingly, the results show that *lymphocytes* sourced from ViviGen donors induce a statistically significant proliferation in the "target PBMC" population, thus indicating immune cell activation. Conversely, ViviGen-derived bone cells from those same respective donors did not induce additional proliferation in the "target PBMC" population, as they were comparable to the baseline proliferation of untreated control target PBMCs. This lack of proliferation indicates that the ViviGen-derived bone cells did not induce an immune cell activation response *in vitro*. While the experiment utilized cells from a thawed cryogenically preserved end product, it is important to note that samples tested prior to cryopreservation also showed no significant immune cell proliferation. This also confirms that following the processing, the product "even prior to cryopreservation" does not elicit an immune cell proliferation response *in vitro*.[7]

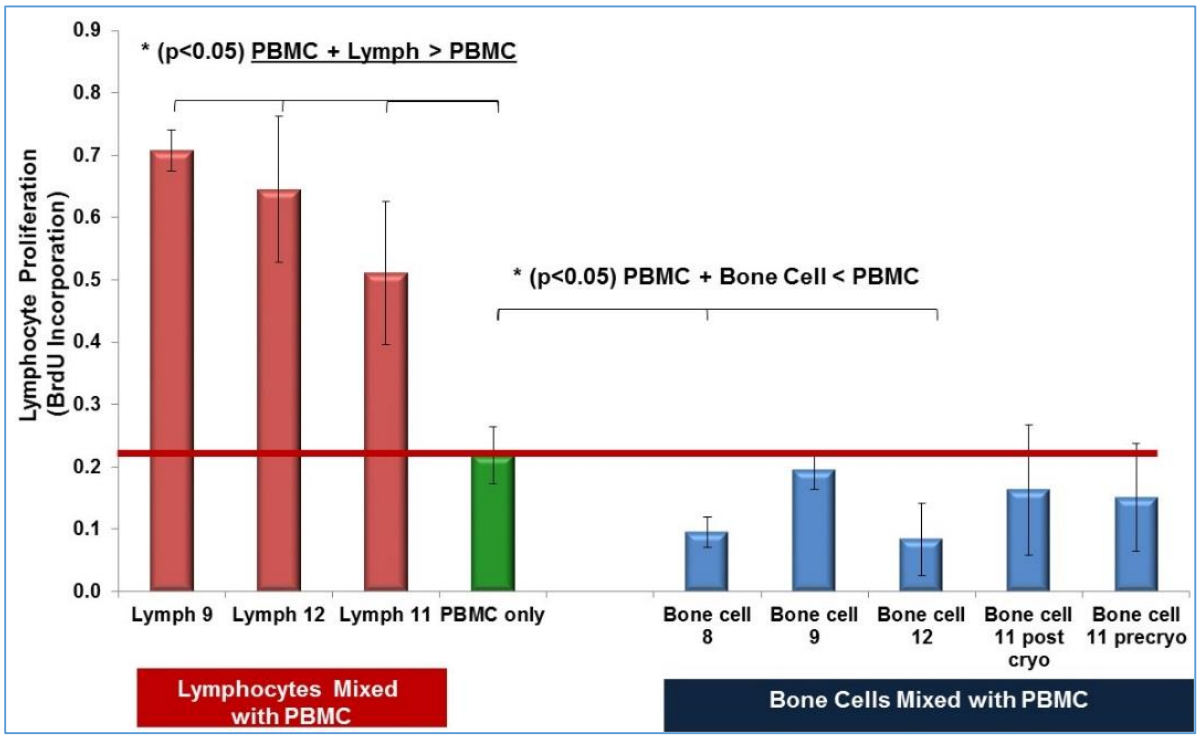

Figure 3. ViviGen-derived Bone cells do not induce immune cell proliferation. MLR assay indicating that cells derived from several donor lots of ViviGen did not induce "target" PBMCs to proliferate (blue bars) when compared to baseline "target" PBMC proliferation levels (green) indicating no immune cell activation. Conversely, lymphocytes recovered from the same respective donors serves as a positive control and induces "target" PBMCs to proliferate (red) indicating immune cell activation.

[3] Dirckx N, Van Hul M, Maes C. Osteoblast recruitment to sites of bone formation in skeletal development, homeostasis, and regeneration. Birth Defects Res C Embryo Today. 2013; 99(3):170-91
[4] Schwarz M. The mixed lymphocyte reaction: an in vitro test for tolerance. The Journal of Experimental Medicine. 1967; 127: 879-893.
[5] Mangi R, Kantor F. The multiple mixed lymphocyte reaction: variables important in the test as a measure of lymphocyte competence in man. The Yale Journal of Biology and Medicine. 1975; 48: 217-228.
[6] FDA, Guidance for Industry and FDA Reviewers (May 6, 1999) Immunotoxicity Testing Guidance.
[7] Data on file at LifeNet Health DHF 12-008, DHF 15-001

ViviGen derived bone cells are not antigen presenting cells.

To help explain the lack of immune cell proliferation response seen in the MLR assay, it is important to understand the surface antigen characteristics of bone cells. It is noted that all nucleated human cells possess major histocompatibility I (MHC I) class surface receptors which present *intracellular* proteins to immune cells and also identify the cells as "self". In addition, some cells also possess major histocompatibility class II (MHC II) surface receptors that present *extracellular* antigens to immune cells and are thus referred to as antigen presenting cells. However, some cells may either not express MHC II, express low levels of MHC II, or express altered conformations of MHC II receptors, and as a result may avoid detection by immune cells.[8, 9, 10] In order to test this, ViviGen processed bone chips were stained for the presence of the MHC II antigen presentation receptors. As see in Figure4, immunohistochemistry for presence of MHC II receptors show that very few cells stain for MHC II receptors in the Haversian canals and no cells within the bone matrix stain positively for MHC II receptors in the final post-cryopreserved ViviGen product.[9] This finding is consistent with the lack of immune cell proliferation response noted in the MLR assay.

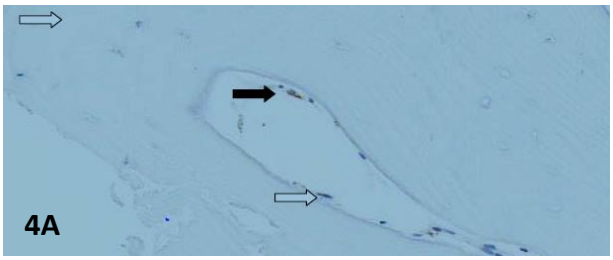

Figure 4. Immunohistochemistry for MHC II in Post-Cryopreserved ViviGen.

4A) Few cells in the Haversian canal stained positively for MHC II (closed arrow). Cells inside the bone matrix and lining the Haversian canal were not stained positively for MHC II (open arrow).

4B) Cells within the bone matrix tissue do not stain positive for MHC II (open arrow).

Summary

Taken altogether, the process to cleanse the bone of its immunogenic marrow components (like CD45 expressing cells) in addition to the negative Mixed Lymphocyte Reaction Assay and lack of MHC II expression, data suggest that ViviGen cellular bone matrix does not contain the necessary immunogenic proteins to elicit immune cell proliferation.

About ViviGen Cellular Bone Matrix

ViviGen comprises cryopreserved viable bone cells within a corticocancellous bone matrix and demineralized bone. ViviGen is processed from donated human tissue and is intended for repair, replacement, or reconstruction of musculoskeletal defects. ViviGen contains viable cells that are committed to produce bone in concert with the osteoconductive scaffold and osteoinductive signals naturally found within the demineralized bone.[1]

[8] Kevin McIntosh, et al., The Immunogenicity of Human Adipose-Derived Cells: Temporary Changes In Vitro. Stem Cells 24:1246-1253, 2006.
[9] Elena Klyushnekova, et al., T cell responses to allogenic human mesenchymal cells: Immunogenicity, tolerance, and suppression. Journal of Biomedical Science 12:47-57, 2005.
[10] Baskar, Sivasubramanian, et al., Constitutive expression of B7 restores immunogenicity of tumor cells expressing truncated major histocompatibility complex class II molecules. Proceedings of the National Academy of Sciences. 90.12, 5687-5690, 1993.
[9] Data on file at LifeNet Health DHF 12-008, DHF 15-001

DSUS/MOC/0416/0483 10/16
68-20-162.00