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

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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 allogeneic 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, such as white blood 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).¹

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

¹ Data on file at LifeNet Health, 65-0347
ViviGen bone cells do not elicit in vitro immune cell proliferation.

In design validation, a mixed lymphocyte reaction (MLR) assay, additionally demonstrates that the lineage committed bone cells comprising ViviGen do not elicit immune cell proliferation (Figure 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. 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.

Mixed Lymphocyte Reaction Study (FDA Guidance)

![Graph showing mixed lymphocyte reaction results](image)

**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.

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6 Data on file at LifeNet Health, 65-0347
**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. In order to test this, ViviGen processed bone chips were stained for the presence of the MHC II antigen presentation receptors. As see in Figure 4, 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 graft. This finding is consistent with the lack of immune cell proliferation response noted in the MLR assay.

**Summary**

Taken altogether, the process to cleanse the bone of its immunogenic marrow components (like CD45 expressing cells) in addition to the negative MLR 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.

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10 Data on file at LifeNet Health, 65-0347