Optimized preservation of biological properties of ViBone

ViBone™

Summary

ViBone was designed to advance viable bone grafts beyond the standard story of osteogenesis, osteoinduction, and osteoconduction. A deep scientific understanding of tissue quality was the basis for optimized processing, resulting in a novel bone graft closer to the gold standard of autograft. The success of human tissue for transplant depends on how well the processing protects the tissue. An excellent marker of overall tissue quality is the health of the endogenous cells. Here data is presented from sophisticated in vitro assays of apoptosis, as well as measures of functionality like cell proliferation and increased production of osteogenic factors. Ultimately, these results translate to better osteogenic potential and a graft of superior quality for use in bone regeneration.

The balance between life and death…of bone cells

Bone is a dynamic organ that constantly undergoes remodeling to maintain normal healthy bone or to repair damaged bone due to age or trauma. Bone remodeling involves coupling of bone formation and bone resorption processes and requires the coordinated activity of different bone cells from two lineages. Cells of the osteoblast lineage (osteoprogenitor cells, osteoblasts, osteocytes, and bone-lining cells) and bone-resorbing cells (osteoclasts) work together to facilitate normal bone remodeling processes. Osteoblasts originate from differentiated mesenchymal stem cells and are responsible for bone matrix synthesis and its subsequent mineralization. In contrast, osteoclasts are large, multinucleated cells derived from hematopoietic marrow cells and are responsible for resorbing bone tissue.[1]

Apoptosis, programmed cell death, controls bone formation

During bone formation, osteoprogenitor cells and osteoblasts undergo a sequence of events including proliferation, maturation, mineralization and ultimately termination.[1] At termination, these bone cells can follow three fates: (1) osteocytes that are embedded within the lacunae of newly formed bone tissue; (2) quiescent bone lining cells; or (3) undergo cell death through apoptosis.[2] (Fig 1). Apoptosis is a programmed cell death mediated through caspase activation that leads to cell death within a few hours to a few days after initiation.[3] Toxin and external factors trigger apoptosis through the intrinsic pathway, which begins in the mitrochondria. A well studied pro-apoptotic protein is cytochrome c, which along with other stimuli activates a cascade of caspases. Activation of caspase-9 leads to cleavage of the executioner caspases-3 and -7 and cell death. Interestingly, cells undergoing apoptosis will continue to exhibit markers of living cells (such as trypan blue exclusion) until final cell death.[1] Since it is a delayed effect, more sophisticated assays are needed to detect apoptosis. It is the balance between the birth of osteoblasts from mesenchymal predecessors and controlled apoptotic death that dictates the number of cells critical in achieving normal bone homeostasis.[2,4] The second pathway for cell death is necrosis. Necrosis results in immediate cell death and is an acute, uncontrolled process characterized by cell swelling and rupture, compared to the controlled shrinkage process of apoptosis.[7] Historically, viability of our allograft has been characterized by counting live and dead cells. This counting is a snapshot of cell viability including cell death due to either pathway. This assessment cannot be used to gain insight into the proportion of cells triggered to die due to apoptosis. This requires a more sophisticated, detailed measure of cell, and therefore allograft, condition.

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Premature apoptosis leads to poor bone regeneration

Although apoptosis is integral for normal bone remodeling, improper stimulation of apoptosis can lead to pathologic bone conditions, such as osteoporosis.[5] Specifically, apoptosis can be prematurely triggered by various stress stimuli including exposure to non-physiological environments, such as excessive heat, oxidative stress and chemical exposure,[6-11] all of which are common in traditionally processed human tissue allografts. For example, removal of bone tissue from its physiologic environment leads to the production of reactive oxygen species which is well established to induce apoptosis of osteoblasts.[6] Furthermore, studies have demonstrated that apoptosis prevents osteogenic differentiation of adult human mesenchymal stem cells and impedes the activity of osteoblasts.[12] Essentially, premature apoptosis results in lower number of metabolically active bone forming cells, leading to decreased osteogenic potential (Fig. 2) and ultimately poor bone formation. Therefore, minimizing cell exposure to stress that stimulates apoptosis in bone cells is critical for efficient bone regeneration.

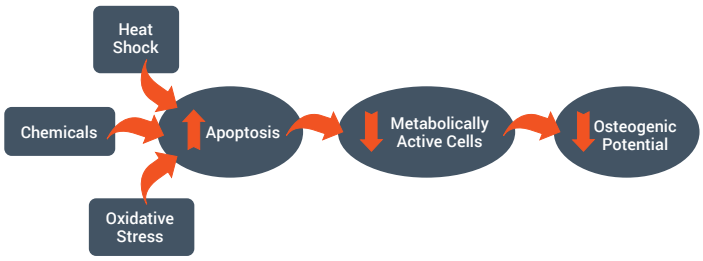

Figure 1. Non-physiologic triggers of Apoptosis can lead to impaired osteogenic potential. Non-physiologic stimuli such as excessive heat, chemical exposure, and oxidative stress can trigger apoptosis of bone cells that result in lower number of metabolically active bone cells resulting to lower osteogenic potential.

ViBone is optimized to preserve the health of native bone cells

ViBone is a viable bone matrix that provides all three components critical for bone regeneration: osteoconductivity, osteoinductivity, and osteogenicity. It is a next generation cellular bone matrix that has been optimized to preserve the health of native bone cells and yield more readily available bioactive agents to enhance new bone formation while ensuring a safe allograft tissue for transplantation.

Aziyo has developed a proprietary process for ViBone that reduces tissue exposure to non-physiological conditions and potential triggers of apoptosis to preserve and optimize the native biological and structural elements beneficial to bone repair. These methods include minimizing tissue contact and the time outside of a physiologic environment. Furthermore, exposure to potentially harmful chemicals frequently used during allograft manufacturing is minimized. In the following sections, the cells within ViBone are characterized and compared against cells from allografts produced using traditional processing methods (control allograft) to demonstrate better cell health, which ultimately may support better bone regeneration.

ViBone has fewer apoptotic cells

As previously described, apoptosis inhibits osteogenic differentiation and osteoblast function and can be prematurely triggered by non-physiological stimuli. To assess the impact of ViBone's proprietary processing on apoptosis, activated caspase levels (indicator for apoptotic cells) were measured from ViBone versus allografts produced using traditional control methods. As indicated by the higher apoptotic index (ratio of caspase levels normalized against ViBone caspase levels), allografts produced from control processing methods resulted in a significantly greater population of apoptotic cells compared to ViBone. Specifically, ViBone exhibited more than 50% reduction of apoptotic population compared to control tissue (Fig. 2).

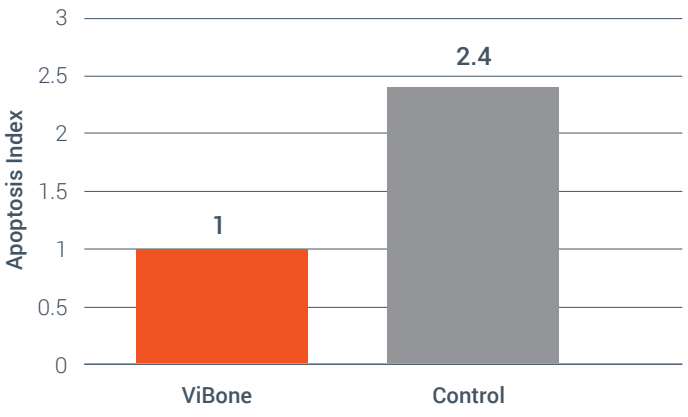

Figure 2. Apoptosis index is the ratio of caspases produced by apoptotic cells normalized against ViBone levels. Control allograft processed using traditional methods yielded a 2.4-fold greater apoptotic population compared to ViBone.

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Greater Cell Proliferative Capacity

To demonstrate the significance of less apoptotic cells within ViBone, proliferation of cells isolated from ViBone versus cells from control allograft (tissue processed using traditional methods) was investigated. Cell proliferation is a good indicator of cell health in that only metabolically active cells will proliferate efficiently. In this study, cells were isolated from both ViBone and control allografts and cultured over seven days. Cell proliferation rates were determined by quantifying cell number at timepoints during the seven day culture and calculating the fold increase compared to day 1 levels. As Figure three demonstrates, cells from ViBone proliferated at a faster rate than cells from control allografts. These data suggest that cells from ViBone are metabolically more active and proliferate faster, thus are healthier than cells from traditionally processed allografts.

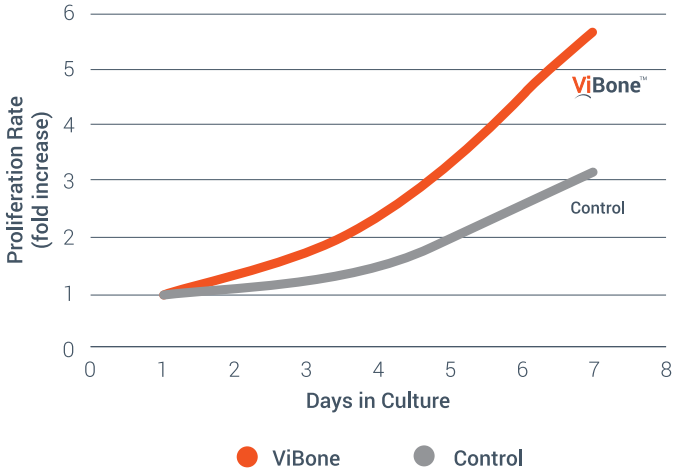

Figure 3. Cell Proliferation Rates. Cells isolated from ViBone and control tissue prepared using traditional processing methods were cultured and quantified over seven days. Cells isolated from ViBone proliferated at a much faster rate (as indicated by steeper slope) than cells isolated from control tissue.

Greater osteogenic potential

To further confirm the health of cells within ViBone, osteogenic potential was examined by quantifying production of osteoblast markers (osteocalcin, osteopontin, collagen type I) during osteogenic differentiation.

Osteocalcin is produced by osteoblasts and is the most abundant non-collagenous protein found in bone. Osteopontin is another non-collageneous protein produced by osteoblasts and is involved in bone mineralization. Collagen type I is the major extracellular matrix component of bone and is primarily produced by osteoblasts. Briefly, cells within ViBone or control allografts were stimulated with osteogenic media for 28 days. Media was collected and measured for production and release of osteoblast markers using enzyme-linked immunosorbent assays (ELISAs). Osteogenic index is defined as the amount of osteoblast markers produced from cells within ViBone normalized against levels from control allografts. Index values over 1 signifies greater production of the specific marker versus control levels.

Figure 4 demonstrates that the osteogenic index of ViBone is greater across all three markers tested. Specifically, cells within ViBone produced increased levels of osteocalcin (20%), osteopontin (50%) and collagen type I (40%) compared to control allografts.

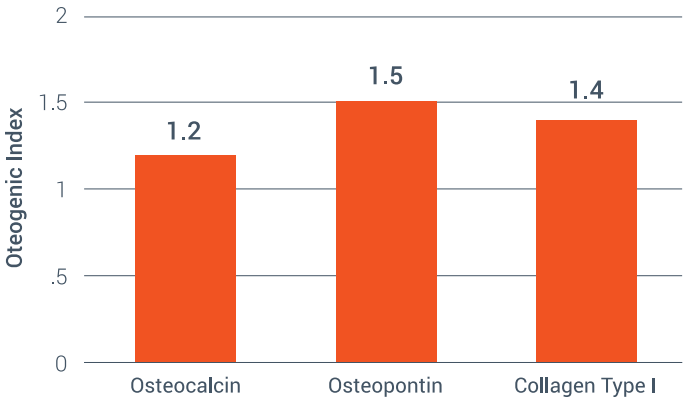

Figure 4. Osteogenic index of ViBone relative to control allografts. Allografts were cultured in osteogenic media for 28 days. Media was collected and measured for osteoblast markers by ezyme-linked immunosorbent assay (ELISA). Osteogenic index is defined as the amount of osteoblast markers produced from cells within ViBone normalized against levels from control allografts.

These data confirm that the fewer apoptotic cells and more healthy cells in ViBone result in greater production of bone forming components, which ultimately contributes to better bone regeneration.

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Greater Host Cell Recruitment Potential

ViBone's role as a chemoattractant for host cells was assessed using an in vitro cell migration assay. Briefly, human mesenchymal stem cells (MSCs) were plated onto the surface of transwell filters and stimulated for 24 hours to migrate across the filter towards conditioned media from ViBone or control allografts. Media supplemented with 10% FBS and media alone served as positive and negative controls, respectively. Cells that migrated onto the underside of the filter were visualized by staining with gentian violet, a cellular dye. As Figure 5 depicts, conditioned media from ViBone demonstrated more cell migration (Fig. 5C) than conditioned media from control allografts (Fig. 5D) suggesting that ViBone is more potent as a chemoattractant for MSCs than control allografts.

ViBone Summary:

Optimized to preserve the health of native bone cell population leading to less apoptosis and ultimately resulting in healthier and more metabolically active cells.

- Higher cell proliferation potential
- Healthier cells produce more bone forming components
- Greater recruitment of host cells to enhance bone regeneration

Ultimately, ViBone is closer to autograft, and therefore has better potential for robust bone formation.

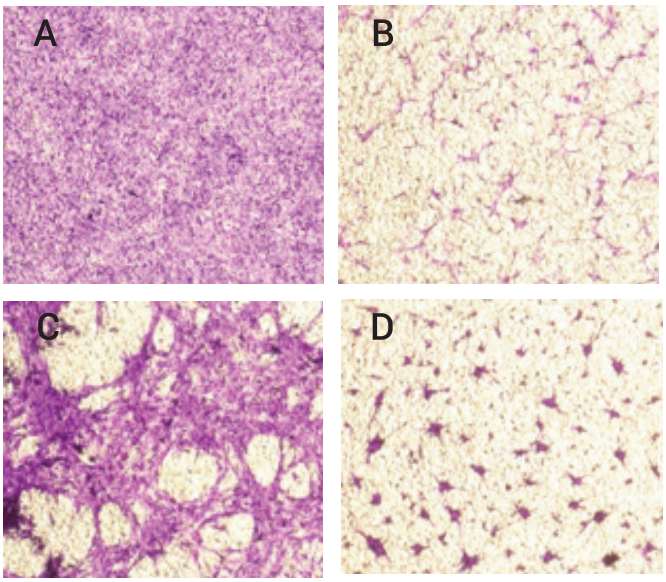

Figure 5. ViBone induced migration of MSCs. Representative images of MSC migration studies demonstrating the chemoattractive effects of ViBone or control allografts on MSC migration. (A) Media + 10% FBS as positive control (B) Media alone as negative control illustrating no migration. (C) Conditioned media from ViBone (D) Conditioned media from control allograft.

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