Cellular and proteomic characterization of Cellular Bone Matrix Allografts

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INTRODUCTION: Autografts remain the clinical gold standard for bone grafting due to their inherent osteogenic, osteoinductive, and osteoconductive properties; however, limitations such as donor site morbidity and restricted availability have accelerated the use of allografts, which now account for over half of the bone graft market. Cellular Bone Matrices (CBMs) represent a next-generation allograft, distinguished by the presence of viable osteogenic cells. While the presence of these cells may not be the primary driving force for the product, they might be capable of contributing to bone regeneration. Beyond their cellular component, CBMs also retain extracellular matrix–associated growth factors that may further support osteogenic activity. In this study, we aimed to investigate the cellular properties of CBMs and assess the extracellular matrix–associated growth factors that may modulate cell behavior and enhance their bone-forming potential.

METHODS: Frozen CBM samples were obtained from 12 male and female cadaveric donors, aged 15 to 55 years. To investigate cellular viability and migration capacity, cancellous bone component was isolated from the CBMs and cultured in Minimum Essential Medium alpha (MEM-α) supplemented with 20% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cultures were maintained at 37 °C with 5% CO_2 and 5% O_2 for 28 days. Following incubation, cell presence on bone surfaces was evaluated using lactate dehydrogenase (LDH) staining and live/dead fluorescence assays, while microscopy imaging was employed to document cell migration. Quantitative PCR (qPCR) was performed to assess the expression levels of osteogenic markers including Osteopontin (SPP1), Osteocalcin (OC), and Alkaline phosphatase (ALP) in migrated cells and compared to human osteoblasts (hOB) as the reference control. Additionally, the expressions of CD90, CD73, CD105 and CD166, markers associated with progenitor cells, were quantified via flow cytometry. A quantitative proteomic array was conducted on whole CBM units to characterize their angiogenic and bone-modulating protein profile. Potential immunogenic responses of the intact grafts were further assessed using a Mixed Lymphocyte Reaction (MLR) assay, measuring interferon-gamma (IFN-γ) expression following co-culture with peripheral blood mononuclear cells (PBMCs).

RESULTS SECTION: After 28 days, LDH staining confirmed the presence of proliferating cells on the cancellous component of the CBMs (Figure 1), supported by microscopy images showing cell migration from the bone to the culture flask (Figure 2). qPCR analysis demonstrated that CBM-derived cells expressed osteogenic genes at levels comparable to hOB, with increased expression of key markers such as SPP1, osteocalcin, and ALP (Figure 3). Flow cytometry data demonstrated homogeneity of the migrated cells with high expression of CD90, CD73. CD105, CD166 markers, suggesting an osteoprogenitor phenotype. Quantitative proteomic analysis of whole CBM units revealed a diverse range of proteins involved in bone modulation and angiogenesis, including Angiostatin, IGF-1R, BMP-2, BMP-5, BMP-7, and Galectin-1, underscoring the complex regenerative environment provided by CBMs. MLR assay further showed no increase in IFN-γ levels in PBMC cultures exposed to CBM units, indicating a lack of immunogenic response.

DISCUSSION: These findings demonstrate that CBMs retain viable, functional cells capable of migrating from the graft matrix, confirming their osteogenic and osteoconductive property by providing a scaffold that supports cell movement and new tissue formation. The elevated expression of key osteogenic genes underscores the strong osteogenic potential of CBM-derived cells in promoting bone matrix synthesis and mineralization. Moreover, the proteomic array revealed a diverse profile of proteins involved in bone modulation and angiogenesis such as BMP-2, BMP-5, BMP-7, IGF-1R, Angiostatin, and Galectin-1, highlighting the osteoinductive capacity of CBMs to stimulate bone formation and vascularization.

SIGNIFICANCE/CLINICAL RELEVANCE: The findings of this study demonstrate the osteogenic, osteoinductive, and osteoconductive properties of CBM bone allografts, positioning them as high-potential substitutes for bone autografts.

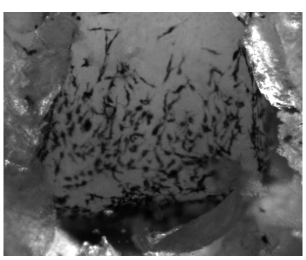

Figure 1. LDH staining after 28 days showing cells (black) present on the surface of the cancellous component of the CBMs.

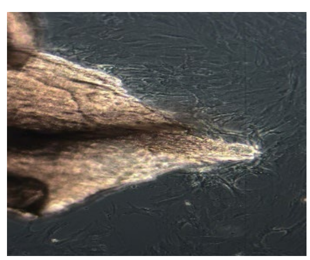

Figure 2. Microscopy images showing cell migration from the bone surface into the culture flask after 28 days.

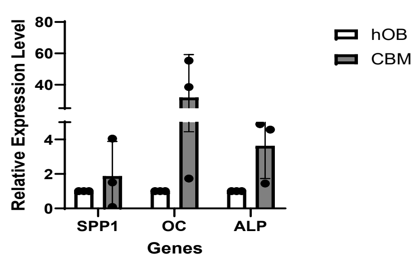

Figure 3. qPCR analysis showing CBM-derived cells expressing osteogenic genes comparable to hOB, including SPP1, Osteocalcin, and ALP.