Analysis of Osteoblastic and Stem Cells from a Bone Allograft[1]

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INTRODUCTION

Bone grafting materials are often used to facilitate new bone formation. Successful bone fusion is reliant on three key components: an osteoconductive matrix, an osteoinductive signal, and osteogenic cells[2-4]. Autograft bone has been widely used and is the traditional gold standard for bone grafting due to it containing all three components needed for bone healing.

Trinity Evolution® and Trinity ELITE® allografts are cryopreserved, human donor bone grafts consisting of a viable cancellous and a demineralized bone component. The Trinity allografts are reported to contain viable adult human mesenchymal stem cells (MSCs) and osteoprogenitor cells (OPCs) that are retained within the cancellous bone matrix. Similar to autograft, the Trinity allografts have also been reported to possess osteogenic, osteoinductive, and osteoconductive elements needed for successful bone grafting. In the present study, we investigated the marker proteins and gene expression in bone chips to validate their potential osteogenic and therapeutic abilities.

METHODS

A total of 4 different donor batches of 5cc units of Trinity Evolution and Trinity ELITE samples (n=2 each) were examined. The samples were thawed per the package insert of the tissue processor. Once thawed each sample was minced/homogenized using a Hard Tissue Omni Tip probe (USA Scientific, Inc., Orlando, FL) and then briefly centrifuged before starting RNA isolation. RNA was isolated with TRIzol® Reagent (Invitrogen Life Technologies, Grand Island, NY) and purified with RNeasy Plus Mini Kit (Qiagen, Germantown, MD).

TaqMan® Reverse Transcription Reagents (Invitrogen Life Technologies) were used for making cDNA, and subsequent qPCR was done using Power SYBR® Green PCR Master Mix (Invitrogen Life Technologies) and appropriately designed and synthesized primers (Primer-Blast, NIH; Integrated DNA Technologies).

Cultures of human bone marrow stromal cells (BMSCs) derived from either fresh human bone marrow (Lonza Group Ltd, Basel, Switzerland) or from other Trinity Evolution samples samples were used for comparison to the homogenized Evolution and Elite samples.

Relative levels of genes associated with stem cells (Oct-4), osteocytes (SOST), osteoblasts (bone sialoprotein (BSP), collagen type I (Col-I), osteocalcin (OC), Runx2, osterix (OSX), osteoclasts (Cathepsin K, TRAP), and monocytes (CD-11b, CD-14, monocyte chemoattractant protein-1 (MCP-1), and NFAT-2) were compared with ribosomal protein, RPL13A.

RESULTS

RNA isolation techniques yielded sufficient amounts of RNA for all of the intended analysis. Subsequently, findings indicated that RNAs associated with MSCs (Oct-4) and bone-forming cells (OC, Runx2, OSX, BSP, and Col-I) were expressed at high or intermediate levels in both Trinity Evolution and Trinity ELITE samples (Figure 1). For nearly all of these genes, levels of expression were either similar to or greater than cultures of BMSCs. Monocyte/macrophage (MCP-1, CD-11b) and osteoclast (Cathepsin K, TRAP) markers were either absent or expressed at very low levels.

Figure 1: Gene expression comparison of BMSCs and cells in Trinity allografts

Gene	BMSCs Sample A	BMSCs Sample B	Evolution Sample A	Evolution Sample B	ELITE Sample A	ELITE Sample B
Stem Cell Marker						
Oct-4	+	+	+++	+++	+++	+++
Osteocyte Markers						
SOST	-	-	++	++	++	++
Osteoprogenitor/Pre-Osteoblast Markers						
RUNX2	++	++	++	++	+++	+++
OSX	-	-	++	++	++	++
Osteoblast Markers						
BSP	-	-	+	++	++	++
Col-I	+++	+++	++	++	++	++
OC	+	+	++	++	++	++
Osteoclast Markers						
Cath K	++	+	-	-	-	-
TRAP	-	-	+	-	+	+
Monocyte/Macrophage Markers						
CD-11b	-	-	-	-	-	-
CD-14	+	-	+	+	+	+
MCP-1	+++	++	-	+	-	-
NFAT-2	+	-	-	-	-	-

+++	High expression or extremely high expression (X ≥ 0.1000)	+	Low expression (0.0100 > X ≥ 0.0010)
++	Intermediate expression (0.1000 > X ≥ 0.01000)	-	No expression or extremely low expression (0.0010 > X)

CONCLUSION

In this study, Trinity Evolution and Trinity ELITE were demonstrated to have a cell population that exhibits high gene expression levels of markers associated with MSCs, osteoprogenitors, and bone-forming cells.

In contrast, either no expression or very low levels of genes associated with immunoreactive cells were measured indicating that these types of cells are present in an insignificant amount within the tissue suggesting that this allogeneic graft material is unlikely to elicit an immune response.

Overall, these observations suggest that Trinity allograft materials possess an active osteogenic component that may be capable of contributing to bone healing in a clinical setting.

REFERENCES

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