CONFORM™ Cube:  
An Evaluation of a Fully Demineralized  
Cancellous Scaffold Seeded with  
Human Mesenchymal Stem Cells  

SUMMARY  
CONFORM Cube is a fully demineralized cancellous scaffold designed to have compressible and wickable properties, as well as the ability to be rehydrated with BMA, blood, or any other hydrating agent. Due to the inherent properties of the natural scaffold in combination with the proprietary demineralization and pH restoration processes that are used, CONFORM Cube aids in bone formation by exposing bone morphogenetic proteins and providing a cell-friendly environment. Bone marrow aspirate (BMA) can be easily retrieved from multiple anatomic sites, including the iliac crest, vertebral bodies, tibial metaphyses, malleolus, calcaneus and others. In posterolateral lumbar spine procedures, the surgically-exposed local vertebral body is also a suitable alternative site for bone marrow aspiration. Clinically, bone marrow aspiration is used to recover bone marrow cells to add to bone grafts. This technique provides cells and additional biological growth factors that assist in the healing and regeneration of bone. Cells can populate CONFORM Cube through the use of bone marrow aspirate or migration of host cells into the scaffold following implantation. To evaluate the cellular attachment and distribution characteristics, an in vitro study was conducted in which CONFORM Cubes were seeded with human mesenchymal stem cells (hMSCs). Following specified incubation periods, the tissue was assayed to determine cell counts, and confocal microscopy was employed to evaluate cell distribution. The results demonstrate that CONFORM Cube provides a biocompatible environment where cells can attach and produce extracellular matrix.
MATERIALS AND METHODS

Scaffold Preparation: CONFORM Cubes were prepared from two donors using a proprietary process that fully demineralizes the tissue, as well as restores the pH to physiological conditions. The tissue was subsequently cut into cylinders with a diameter of 3.5mm ± 0.5mm and a height of 2.5mm ± 0.5mm.

Cell Seeding: The tissue was rehydrated and placed inside an agarose gel cast. Each sample was then seeded with 500,000 hMSCs cultured from commercially available hMSCs (Lonza). The tissue was incubated at 37°C for specified time periods and assayed to determine cell counts and analyze cell distribution.

Cell Count: Cell counts were obtained using the CellTiter-Glo® Luminescent Cell Viability Assay at three time points: 10 minutes, 30 minutes, and 3 hours. The assay was performed in triplicate for each donor.

Cell Distribution: Prior to seeding the cells onto the tissue, cells were fluorescently stained with CellTracker™ Green CMFDA (5-chloromethylfluorescein diacetate), and the tissue was fluorescently stained with Alexa Fluor® 633. Once stained, the cells were seeded onto the tissue and incubated. At incubation periods of 30 minutes, 3 hours, and 24 hours, samples were fixed in 4% formalin and imaged using confocal microscopy.

Cellular Differentiation: Cellular differentiation was observed using histological analysis. At 7 days, samples were fixed in 10% formalin, embedded per standard procedures, sectioned and stained with hematoxylin and eosin (H&E).

RESULTS

Cell Count: The CellTiter-Glo® Luminescent Cell Viability Assay quantifies the amount of adenosine-5’-triphosphate (ATP) by lysing the hMSCs and producing a luminescent signal proportional to the amount of ATP. From the luminescent signal, the cell number can then be calculated, as shown in Figure 1.

Upon addition of the 500,000 hMSCs to the scaffold, ~46% of the hMSCs adhered to the scaffold. Cell retention is based on the method of cell application as well as the porosity of the scaffold. The result showed that once seeded onto the tissue, the cells remained viable and metabolically active, as the cell counts for each incubation period remained similar (p=0.2185).

![Cell Number for Seeded Samples](image)

**Figure 1:** The average cell number for CONFORM Cube seeded with hMSCs. The numbers are an average of two donors ± the standard error.

Cell Distribution: Confocal microscopy was used to obtain images of the hMSCs seeded onto CONFORM Cube. Over an incubation period of 30 minutes and 3 hours, the cells attached to the surface of the tissue. By 24 hours, the cells have begun to produce extracellular matrix, demonstrating biocompatibility of the scaffold, and further that the tissue provides a cell-friendly environment conducive to cell growth, as shown in Figure 2.
Cellular Differentiation: Histological images were used to analyze the cellular activity of the hMSCs seeded onto CONFORM Cube. Over an incubation period of 7 days, the cells continue to produce extracellular matrix and qualitative histological assessment shows that the cells are beginning to exhibit a cuboidal shape consistent with the osteoblast phenotype, as shown in Figure 3.

CONCLUSION

These study results demonstrate that CONFORM Cube provides a biocompatible environment for human mesenchymal stem cells to attach and produce an extracellular matrix. After the addition of hMSCs to CONFORM Cube, the cell viability remains consistent for 3 hours, and by 24 hours the cells have begun to produce an extensive extracellular matrix. Cells continue to produce extracellular matrix after 7 days and show osteogenic differentiation via qualitative histological analysis.
All in vitro data on file at MTF.

In vitro test results may not necessarily be indicative of clinical performance.

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