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A Biphasic Apatite/Sulphate Bone Substitute, Cerament™ Induces Bone In A Skeletal Muscle Cell Line

General Topics / Implants & Biomaterials

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Introduction

Bone defects are either non-critical and can heal on their own, or require bone grafting. Autografts and allografts serve as conventional replacement options in such cases. However, they have limitations mainly due to supply. We have used a biphasic composite of hydroxyapatite and calcium sulphate, Cerament™ with and without gentamycin in several clinical cases. It resembles inorganic components of bone and its microporous architecture makes the material highly osteoconductive. Moreover, the material is radiopaque and injectable and allows surgeons to deliver the material mini invasive with high precision. The material sets in 10- 15 min in situ making it a suitable choice for filling of critical bone voids.

Objectives

During clinical trials, we have observed in some cases, the muscle in direct contact with Cerament™ undergoes bone formation 4-6 weeks post-implantation. This motivated us to see whether the material itself can induce differentiation of muscle cells into bone cells in an in vitro model.

Methods

Skeletal muscle cells C2C12 were seeded on Cerament™ and Cerament™| G discs and the cell material interactions were assessed using various methods over a period of several weeks. We have looked at the proliferation profile (MTT), alkaline phosphatase activity (ALP), immunocytochemistry (ICC) of osteogenic markers like Collagen type I, Osteocalcin, Osteopontin and Runt related transcription factor (RUNX-2) and polymerase chain reaction (PCR). Cell- material interactions were also visualized using scanning electron microscopy (SEM).

Results

1. Cell Proliferation: Proliferation of C2C12 cells on Cerament™ and Cerament™| G did not show any significant difference as observed from MTT analysis.
2. Scanning Electron Microscopy: The cells were homogeneously distributed across the scaffolds with no adverse reaction from the material as observed from SEM images.
3. Alkaline Phosphatase activity: C2C12 cells seeded on Cerament™ and Cerament™| G expressed significantly higher amount of ALP ($p < 0.001$) 3 days post seeding while the cells on plastic controls had 3-4 fold less activity indicating onset of differentiation.
4. Immunocytochemistry: ICC analysis on 21st day post seeding revealed that cells seeded on

the biomaterials had undergone differentiation leading to expression of osteogenic proteins like RUNX2, OCN, COLI and OPN. Cells in the control groups did not express such proteins.

5. Polymerase chain reaction: PCR results confirmed expression of osteoblastic genes like RUNX2, COLI, OCN and BSP 21- days post seeding in C2C12 cells seeded on Cerament™. Proliferation of muscle cells on Cerament™ and Cerament™| G compared to polystyrene plates with a 3-4 fold increased ALP activity indicates early onset of mineralization. No differences were observed between materials after adding gentamycin. SEM images clearly show morphology of rounded cells seeded on Cerament™, which other wise form myotubes. Expression of osteoblastic markers like RUNX2, COLI, OCN and OPN clearly indicated osteogenic differentiation of muscle cells into bone cells. PCR results confirm that the cells seeded on the biomaterial underwent osteogenic differentiation. C2C12 cells are multipotent muscle cells capable of differentiating into bone cells when stimulated either with bone morphogenic protein-2 or 7 or if they are in contact with bone. Our results suggest that the niche provided by Cerament™ is sufficient for their differentiation.

Conclusions

The above results clearly indicate that the biphasic ceramic material, Cerament™ is osteoinductive and provides an appropriate niche for muscle cells to differentiate into osteogenic lineages.