Development of Bone Constructs Taking into Account Physiological Aspects

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Introduction

One main objective in bone tissue engineering is the construction of precise scaffolds in order to support and guide cell growth and differentiation. These scaffolds should substitute the extracellular matrix (ECM) characteristics and should form the desired structure with similar physical properties to bone tissue. Moreover, to ensure proper function of the tissue mechanical loading has to be applied. Mechanical strain mimics physiological environment and thus supports the differentiation process.

In this work, osteoblast like SAOS-2 cells and bone marrow stromal cells (BMSCs) were cultured onto ceramic scaffolds. The viability on the scaffolds was investigated using MTT-Test and the differentiation was analyzed by alkaline phosphate assay. Additionally, a cyclic mechanical stress was applied to maintain and enhance the differentiation and physiological properties of the cells. Extracellular matrix protein levels were detected and semi-quantified using RT-PCR. Activation of signal transduction proteins was measured using the western blot technique.

Mechanical strain experiments

Human bone marrow aspirates were obtained during routine orthopaedic surgical procedures involving exposure of the iliac crest. BMSCs were isolated using a density gradient. Human bone marrow aspirates were obtained during routine orthopaedic surgical procedures. In this work, osteoblast like SAOS-2 cells and bone marrow stromal cells (BMSCs) were cultured onto ceramic scaffolds. The viability on the scaffolds was investigated using MTT-Test and the differentiation was analyzed by alkaline phosphate assay.

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Experiments with Sponceram®

Scaffolds: macroscopic ceramic Sponceram®

Cell seeding: scaffolds were incubated for 24 h in DMEM 10% FCS, antibiotics, +/- differentiating conditions (1 μM demecolcine, 10 mM β-glycerolphosphate, 50 μg/ml ascorbic acid at 37°C, 5% CO2). An excess of osteoblast-like SAOS-2 cells or BMSCs was seeded on each scaffold in 96-well plates for 30 min at gentle stirring. Before each of the following tests the scaffolds were placed into a new 96-well plate. Since it was not possible to estimate the attached number of cells on the scaffolds, the first measurements were performed directly after cell seeding. Cell metabolism was assessed using MTT-Test. Total protein content was measured using BCA assay. Alkaline phosphatase (AP) was determined by an assay based on the hydrolysis of p-nitrophenylphosphate to p-nitrophenol.

Thereafter, experiments were performed on the first 8 days. Due to high confluence on the scaffolds, the BMSCs viability decrease rapidly after 10 days. In comparison, the viability of SAOS-2 cells remains more stable.

Conclusion

The results obtained in this work showed that Sponceram® is an appropriate scaffold for bone tissue engineering. Moreover, the application of mechanical strain accelerates the differentiation of BMSCs. Fors further experiments a combination of the used scaffolds and mechanical strain is planned to induce specific osteogenic differentiation.

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