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Transplantation of mesenchymal stromal cells on mineralized collagen leads to ectopic matrix synthesis *in vivo* independently from prior *in vitro* differentiation

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Background

Tissue engineering using mesenchymal stromal cells (MSC) represents a promising approach for bone regeneration. Nevertheless, the optimal constructs have yet to be determined. It still remains unclear if there is a benefit of in vitro *differentiation of MSC prior to transplantation or if undifferentiated MSC hold the optimal potential concerning new tissue formation.*

Methods

After isolation and in vitro *expansion, MSC were seeded on mineralized collagen sponges and transplanted in a heterotopic SCID mice model (*$n = 12$*). While group A contained undifferentiated MSC, in group B cells were cultivated for 14 days* in vitro *under osteogenic conditions prior to implantation. Results were compared with non-loaded scaffolds (group C). Animals were killed for investigation at 4 and at 8 weeks.*

Results

In situ *hybridization demonstrated integration of MSC for up to 8 weeks in groups A and B. Histology revealed significantly more extracellular matrix synthesis in MSC-seeded scaffolds containing calcium phosphate and collagen type I at 4 and 8 weeks after transplantation compared with unloaded controls. At a biochemical level, higher levels of specific alkaline phosphatase expression were detected in MSC-loaded scaffolds (*$P < 0.05$*). Scaffolds containing undifferentiated and differentiated MSC did not appear to differ in terms of matrix synthesis and protein expression, while the number of avital cells was significant higher in those probes loaded with differentiated MSC (*$P < 0.01$*).*

Discussion

The integration of transplanted cells and MSC-associated matrix synthesis encourages the use of MSC-loaded mineralized collagen for tissue engineering of bone. Furthermore, our data suggest that in vitro *differentiation of MSC does not have a positive influence in terms of improved matrix synthesis.*

Keywords

bone regeneration, in situ *hybridization,* in vitro *differentiation, mesenchymal stromal cells, SCID mouse, tissue engineering.*

Introduction

Replacement of bone defects remains one of the major challenges in current orthopedic research. It is needed for repair and replacement of damaged tissues in cases of trauma, congenital and degenerative diseases and cancer. The market for artificial bone materials has increased tremendously in recent years, reaching an estimated 411.1 million US dollars in the European community and the USA in 2002 [1]. Artificial bone grafts, most of them based on calcium and phosphate, which are components of natural bone, usually only provide osteoconductive properties, which allow adjacent osteoblasts to migrate [2].

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In contrast to these artificial biomaterials, autogenous spongiosa grafts combine osteogenic, osteoinductive and osteogenic properties and must be accepted as the gold standard for bone replacement. However, the amount of autogenous spongiosa available is limited, and graft harvesting, for example from the iliac crest, leads to significant morbidity [3–6].

Recently, approaches based on tissue engineering techniques for bone replacement have been considered the most promising for this major clinical problem [7,8]. According to the tissue engineering concept, isolation and *in vitro* expansion of viable cells followed by three-dimensional (3D) cultivation on an appropriate scaffold is one of the most promising approaches to the construction of adequate bone grafts. With this approach, the osteoconductive properties of the scaffold can be combined with osteoinductive and osteogenic properties. The latter can only be provided by viable cells that themselves contain osteogenic potential.

The existence of multipotential marrow stromal stem cells was known as early as 1968 from the work of Alexander Friedenstein *et al*. [9]. Under appropriate conditions, these give rise to a broad spectrum of differentiated tissues, including cartilage, bone, adipose tissue and fibrous tissue [10,11]. This osteochondral potential has been demonstrated both *in vitro* and *in vivo* [9,12,13]. Because of their multilineage potential and extensive self-renewal, these cells have been given the name mesenchymal stromal cells (MSC) [14]. MSC can easily be isolated from BM aspirates.

The potential of MSC-seeded constructs has already been demonstrated in bone defects of critical size [15–19] but the optimal conditions for cultivation, osteogenic differentiation and 3D cultivation on appropriate scaffolds have not yet been determined. Less is known about the efficacy of 3D cultivation of MSC with osteogenic media supplements *in vitro* prior to *in vivo* implantation. It is not known whether the tissue-specific morphology and composition of constructs seeded with pre-differentiated MSC are superior to those of constructs containing undifferentiated stem cells following implantation *in vivo*. When undifferentiated cells are used, differentiation to an osteogenic lineage is driven solely by factors provided by the local microenvironment of bone. If direct application of cells without prior 3D cultivation proves to be equally effective, this will mean savings in terms of both time and money when they are used in clinical practice.

Because of its excellent seeding properties and adequate 3D cell alignment, mineralized collagen seems to be a promising candidate as a bone tissue engineering scaffold. In an earlier study, our group demonstrated effective 3D culture of hMSC on mineralized collagen, excellent seeding and adherence properties of the mineralized collagen and effective osteogenic differentiation of hMSC cultured on mineralized collagen *in vitro* [20,21]. The scaffold has an overall porosity of more than 95%, the diameter of the pores ranges from 4 μm to 200 μm, and the combination of mineralized collagen sponges with fresh BM aspirates has proved to induce new bone formation, which resulted in higher spinal fusion rates in a rabbit model [22] and therefore has already been approved for clinical use.

The aim of the present study was to examine the integration of MSC on mineralized collagen *in vivo* and to investigate the influence of *in vitro* differentiation prior to transplantation on the ability to produce extracellular matrix in unloaded conditions. The hypothesis that MSC already committed to osteoprogenitors *in vitro* is more responsible for the local environment in bone and produces a better bone matrix than the use of undifferentiated cells was investigated. For this purpose, heterotopic implantation of cell–matrix constructs in a SCID mouse model was established in accordance with earlier reported papers [13,23–25]. This animal model allows studying the influence of *in vitro* manipulation prior to transplantation in the absence of local osteoinductive influences (such as osteoinductive growth factors and mechanical loading) that might mask effects of *in vitro* manipulation. Because this animal model allows sufficient vascularization at the same time and has been used successfully in earlier studies [13,23–25], it was chosen for the present study [23].

Methods

Ethical approval

This work was approved by the Heidelberg University Ethical Board (Heidelberg, Germany; 042/2000 and 251/2002) and animal experiments performed in this study were approved by the local federal government (AZ 35-9185.81/G-69/03).

Human MSC

After informed consent, BM aspirates (10–30 mL) were obtained from six hematologically healthy donors aged