Osteogenic Differentiation of Human Amniotic Membrane in toto
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Introduction
For tissue engineering applications, cells are usually combined with a suitable carrier substrate. Alternatively, the so called cell sheet technology enables transplantation without the use of carrier materials. Concept of our study is to differentiate amniotic membrane, which constitutes a pre-formed sheet of stem cells, in toto without any cell isolation. Our aim was to study a new approach for bone regeneration via osteogenic differentiation of amnion sheet.

Materials and Methods
Osteogenic differentiation of human amnion was induced by osteogenic stimulatory kit (OKit, Stem Cells) or osteogenic medium (OM) as described by Pittenger.1 After two weeks of pre-differentiation some biopsies cultured in in OM or control medium (DMEM, 10% FCS) were transferred into a rotary cell culture system (Synthecon Inc.) and cultured under dynamic conditions for 14 days. To determine osteogenic differentiation, bone-specific mineralization was demonstrated by von Kossa staining of paraffin slides. Differentiation efficiency was further evaluated by quantitative real-time polymerase chain reaction (RT-PCR) for specific osteogenic markers RUNX2 (core binding factor alpha), ALPL (alkaline phosphatase), SPP1 (osteopontin), BGLAP (osteocalcin).

Results
OKit and OM induced osteogenic differentiation at least in three out of four preparations shown by von Kossa stainings. When comparing the expression levels, OKit revealed a greater efficiency to induce osteogenic differentiation of amniotic membrane regarding the selected markers on d14 (Fig. 1). The early marker RUNX2 was not upregulated. In contrast, ALPL showed an upregulation on d14 which was further increased on d28. The mRNA level of osteopontin (SPP1) was upregulated on d14 but may be already decreasing on d28, especially under dynamic conditions. Similarly, under the dynamic conditions the expression of osteocalcin (BGLAP) was also downregulated (Fig. 1).

Discussion and Conclusions
Osteogenic differentiation of amniotic membrane in toto could be achieved by culture in osteogenic media. The additional rotary, dynamic conditions used in our experiments turned out to be not suitable for osteogenic differentiation of amnion. Thus, these results are a first promising step for tissue engineering with preformed sheet of stem cells.

References
1. Pittenger et al. (1999) Science, 284, 143

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Disclosures
We certify that there is no actual or potential conflict of interest in relation to this article.