Biomechanical Stimulation for Soft Tissue Simulation in vitro
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Introduction
Physical forces play an essential role in the proliferation and differentiation of many cell types, including fibroblasts [1]. The development of biomaterials and cell-based therapies could be enhanced by using a bioreactor system with perfusion and biomechanical stimulation during cell proliferation and differentiation. Recent investigations of biomaterials comparing static and dynamic cultivation systems clearly demonstrated a significant difference with respect to the expression of extracellular matrix proteins, the stability and the elasticity of the materials. For that purpose pressure and shear forces of wound tissue were applied to develop an implant material for gingival soft tissue augmentation [2].

Materials and Methods
Sponges made of natural collagen were seeded with oral primary fibroblasts (isolated from tissue grafts of the gum) and cultured under static or dynamic (perfusion [P]) or perfusion + pressure and shear forces [PD]) conditions for 14 days. The in-house developed and build bioreactor system consists of 6 chambers, able to apply pressure and shear forces and permanent perfusion on the specimen (Fig. 1). Vitality and cell number determination was investigated by MTT test. Characterization of extracellular matrix proteins was performed by using ELISA and immunohistological methods. Expression patterns of proteins were investigated by rtPCR. Groups of different treated specimen were tested for significance using ANOVA.

Results
Cell proliferation on collagen sponges under PD culture conditions is statistically significant increased compared to static cultured specimen (p<0.05). Volume reduction of 22% of the material’s original volume occurred after 14 days of biomechanical stimulation, but was not cell dependant. CollagenI and fibronectin expression were statistically increased (p<0.001) compared to static cultured specimen. The most striking results were obtained when tenascin-c secretion of different cultivated cell/material constructs were analyzed. A 13fold increase of tenascin-c amount in the medium was detected in PD compared to static or P cultures. This result was confirmed by semiquantitative rt PCR and immunhistological staining.

Discussion and Conclusions
In vitro experiments without biomechanical stimulation have limited value for the investigation and development of biomaterials for soft tissue augmentation. The prototype, which performed best under biomechanical stimulation, subsequently underwent an animal study in the dog. No complications were noted during 84 days of trial and augmentation was comparable to values of the soft tissue graft.

References
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Disclosures
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