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Establishing principles of macromolecular crowding for *in vitro* fibrosis research

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Introduction: An *in vitro* model that mimics vocal fold (VF) fibrogenesis is highly desirable but is not realized so far. Such a model would be of great value for rapid screening of antifibrotic compounds before going into larger expensive clinical trials and would create a real alternative to animal studies. The development of a fibrogenesis model has been hampered by several biological and technical obstacles. The biggest challenge is to guarantee a sufficient *in vitro* production of collagen and its incorporation into a pericellular matrix. Fibrogenic cells in monolayer culture do not lay down significant amounts of stable collagen in a useful time for testing antifibrotic compounds. Many of these obstacles might be overcome by implicating the principles of the excluded volume effect (EVE) and macromolecular crowding (MMC).

Methods: VF fibroblasts from Sprague-Dawley rats were cultured either in standard medium, as well as in "crowded medium" by adding inert macromolecules (Ficoll®). Differentiation into myofibroblasts was achieved by administration of TGFβ-1 (transforming growth factor beta-1). Cells were cultured in standard medium/crowded medium, with or without administration of TGFβ-1. After five days culture medium and cell layer were collected separately and analyzed.

Results: Collagen deposition increased significantly under crowded conditions in both culture medium and cell layer. Administration of TGFβ-1 resulted in a significant increase of collagen deposition compared to zero-control. Amounts of collagen in the cell layer were significantly higher under crowding conditions compared to administration of TGFβ-1 alone.

Discussion: Crowding of the culture medium resulted in a significant increase of collagen deposition compared to standard medium in both culture medium and cell layer. In this specific environment collagen processing was enhanced, resulting in more favorable conditions for studying fibrogenesis compared to conventional cell culture medium. This can be the first step not only towards developing a rapid and robust *in vitro* model for testing of antifibrotic compounds, but also in the construction of ECM components.

Biography

Matthias Graupp is a resident for otorhinolaryngology at the Medical University of Graz since 2011. He has completed his study of medicine at the age of 25 years at the Medical University of Graz. In 2012 he started his PhD studies with the project "Establishing a novel *in-vitro* model for vocal fold scarring". He has published eight papers in reputed journals.

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