Magnetic tissue engineering of the vocal fold: generation of 3D cell constructs using superparamagnetic iron oxide nanoparticles

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Introduction

The principles of biology and engineering are combined in tissue engineering to generate functional replacement for damaged tissue. Growing interest on new approaches, including nanotechnology, are promising solutions for overcoming the rejection of transplanted organs. Tissue engineering comprises the isolation of autologous cells from healthy tissue or stem cells, cultivation and proliferation of these cells and finally generation of 3D-structures resembling functional tissue structures. Even though expertise of these methods in tissue engineering has been established, there is still scope for enhancement. *In vivo* tissue has a complex cellular organization and defined arrangements of cells need to be established. Therefore, techniques to manipulate and remotely control cellular behaviour can deliver a powerful tool for tissue engineering. Such a tool bargains Magnetic Tissue Engineering (MTE) [3,4]. The underlying concept includes cellular uptake of magnetic nanoparticles and the usage of external magnetic fields to manipulate and remotely control magnetized cells and their behaviour. As the voice is the basic instrument of oral communication [1], tissue defects in this region lead to serious aggravation in quality of life. Briefly, voice is produced by vibrations of the vocal fold via an air flow from the lungs. Until now, no satisfactory possibilities for vocal fold replacement exist [2].
We aim for an establishment of a functional vocal fold transplant in a rabbit model by MTE using superparamagnetic iron oxide nanoparticles (SPION).

Methods

Rabbit vocal fold fibroblasts (VFF) were incubated for 24 h with different concentrations of SPIONs (20, 40 or 80 µg/cm^2). Vocal fold cell behaviour under SPION treatment was tested extensively for adhesion, spreading and migration, which are important for formation of 3D-structures. The possibility of magnetic guidance of SPION-loaded cells was tested in 2D. 1 x 10^5 SPION loaded VFFs were seeded in 6-well plates were a 24-well magnet-plate (Fig. 1, B) was positioned below. Cells were allowed to adhere for 24 h. To generate 3D VFF cell-constructs, 1 x 10^6 cells loaded with 20 µg/cm^2 SPIONs were placed in a 24- well plate with a magnet above to induce MTE (Fig. 2).

Results

The effects of SPIONs on cell behaviour were dose-dependent for adhesion, with good tolerability observed up to the nanoparticle concentration of 20 µg/cm^2, migration and spreading were not significantly influenced by SPION uptake up to 80 µg/cm^2 [6]. Magnetically guidance of cells loaded with SPIONs (20 and 40 µg/cm^2) was demonstrated in 2D with cells only growing in areas where a magnet is present [5,6] (Fig.1). To establish a 3D structure of VFFs, a magnet (0.7 T) was placed above the cell culture-plate and cells loaded with 20 µg/cm^2 were able to generate a 3D cell-construct after 24h. (Fig. 2)

Conclusion and Discussion

Here, we present first results of Magnetic Tissue Engineering (MTE) for voice rehabilitation. To develop 3D cell-structures, cell behaviour, which affects cell-cell interactions, must not be affected by SPION uptake. Therefore, cell features including adhesion, spreading and migration were proven to be intact after SPION treatment. As a proof of principle for magnetic cell guidance, SPION loaded vocal fold fibroblasts were allowed to “choose” either to grow on the side were a magnet or none was placed. Interestingly, 5 and 20 µg/cm^2 are sufficient to induce cell growth solitary at site of the magnet in 2D. Furthermore, magnetic cell guidance as only initiator for 3D cell-construct formation was proven to work with very low amounts of SPIONs 5 µg/cm^2). Next steps include the isolation of epithelial cells and establishment of functional multi-layered 3D co-cultures, as well as the proof of functionality in a flow channel model of the rabbit larynx. Our results will constitute a solid basis for a successful transfer of this technique into humans, in order to provide a functional and personalized vocal fold transplant. This is particularly important for patients, who suffer from dysphonia as a consequence of a vocal fold tissue defect, and will help to improve their quality of life.

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References

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Figures

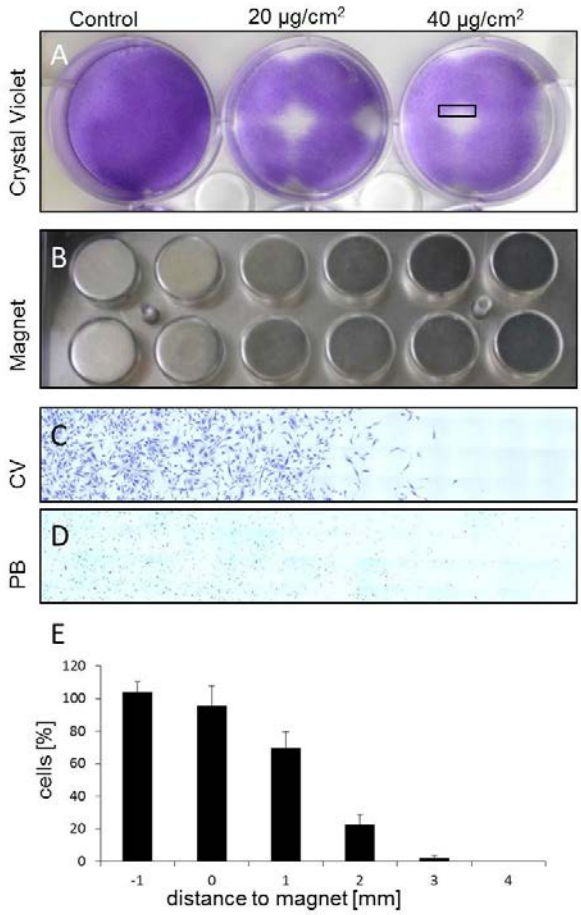

Figure 1. 2D cell-control: A: Magnetic field induced VFF cell-growth, control cells are not loaded with SPIONs, VFFs (SPION concentrations as indicated) only grow in magnetic zones. B: 24- well magnet-plate. C and D: magnification of A (rectangle) with cells stained with Crystal Violet (C) and Prussian Blue (iron) (D). F: Cell concentrations of magnetized VFFs in distance to magnet.

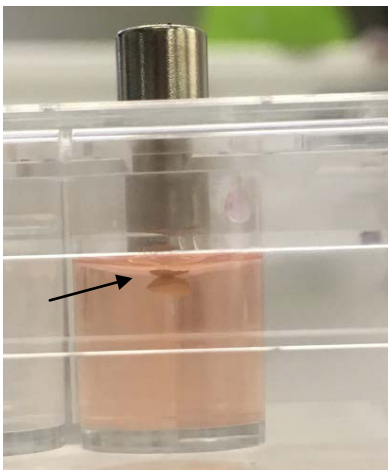

Figure 2, 3D VFF cell construct (arrow) in 48 well-plate after 24h. Magnet on top (outside of lid) retains magnet in well. Cells were incubated for 24 h with 20 µg/cm2 SPIONs. 1 x 10^6 cells were used for construct formation.