Dexamethasone loaded PLGA-microparticles intracellularly induce an anti-fibrotic, anti-inflammatory macrophage phenotype for cell therapy applications

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Statement of Purpose: Macrophages are key players of the innate immune system and the primary initiators of inflammatory responses. They differentiate from monocytes infiltrating the wound site and are involved in different stages of wound healing, tissue repair, angiogenesis and fibrosis. Macrophages are commonly described as either pro-inflammatory M1 or anti-inflammatory, pro-healing M2 phenotype. However, as highly plastic cells, they exist on a spectrum of diverse phenotypes that dynamically respond to changing environmental stimuli and interact via different functionalities specific to their environment. While M2 macrophages are necessary for wound healing and tissue regeneration by the release of anti-inflammatory molecules and growth factors, an overproductive and dysregulated response can lead to fibrosis, impairing a proper regenerative process. Dexamethason (Dex), a small molecular drug used for the inhibition of inflammation, was shown to have anti-fibrotic properties in the treatment of lung fibrosis. However, the application of free Dex, which acts on numerous cell types, can lead to unwanted side effects. Utilizing monocytes for cell therapy which migrate to and differentiate into macrophages at the wound site, has not only the advantage that Dex will induce the anticipated macrophage phenotype but also increase macrophage accumulation, which will be helpful for diseases characterized by impaired immune cell recruitment.

Methods. We used PLGA-microparticles as the drug vehicle, as these particles can be easily adjusted in size and release kinetics. Dex-loaded microparticles of around 1 µm were added to murine or human monocytes to allow phagocytosis. After two hours, unphagocytosed particles were removed and cells cultivated for up to seven days, partly with pro-inflammatory stimuli. The phenotype was analyzed by qPCR, ELISA and flow cytometry.

Results. In this study, we could show that dex-loaded monocytes (murine and human) differentiate into an anti-inflammatory, anti-fibrotic macrophage phenotype. This phenotype was characterized by the downregulation of inflammatory markers and cytokines (e.g., IL1b, TNFa) in M0 and M1 macrophages. Moreover, macrophages showed an expression pattern of MMPs and TIMPs (markers involved in inhibiting/promoting fibrosis) as well as of growth factors that was previously shown to be effective in downregulating fibrosis in lung or kidney.

Thus, our approach of an anti-fibrotic and anti-inflammatory cell therapy strategy is promising for the treatment of various diseases associated with increased inflammation and fibrosis, while limiting the direct effects of Dex only on monocytes/macrophages.

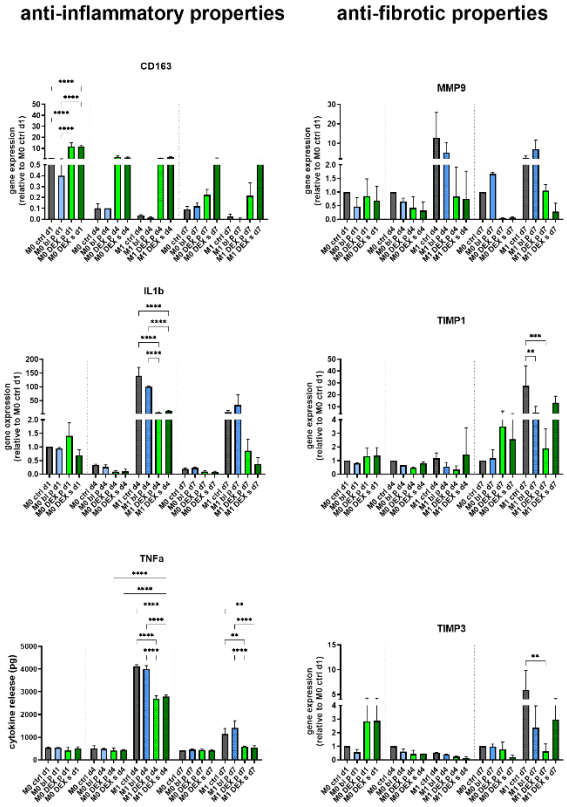

Figure 1 Macrophages treated with Dex-loaded PLGA particles show an anti-inflammatory phenotype (downregulation of IL1b and TNFa and upregulation of CD163) as well as characteristics of an anti-fibrotic phenotype (downregulation of MMP9, especially on later time points and TIMP1 and TIMP3 in inflammatory (M1) macrophages).