The Influence of Engineered Nanomaterials on Keratocyte-Fibroblast-Myofibroblast Transformation in the Corneal Stroma

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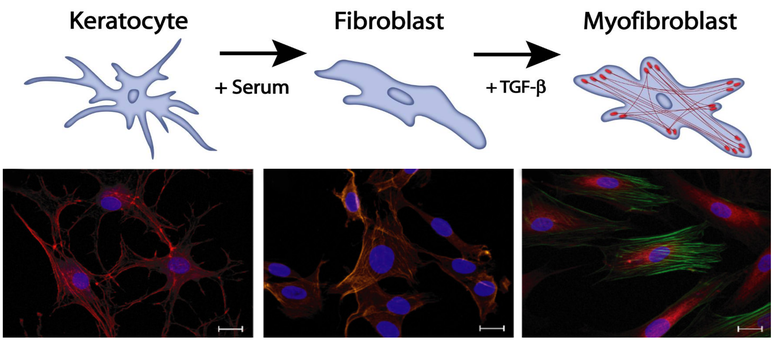
Introduction

- Demand for engineered nanomaterials (ENMs) embedded in consumer products has dramatically increased in the current years resulting in deposition of ENMs in the air, water, and soil during their manufacturing, use and disposal[1].
- The eye is primarily exposed to substances in air, a major route of exposure to ENMs. In addition, the eye is a current target of ENM based therapeutic delivery[2-4].
- Upon corneal stromal wounding, changes in the microenvironment of the wound promote transformation of the quiescent keratocyte to the activated fibroblast and subsequently the differentiated myofibroblast (KFM transformation, **Figure 1**).
- The most important cytoactive factor to induce this pathway is TGF-β1
- A better understanding of the effects of ENMs on the genesis and persistence of the myofibroblast within the corneal wound space is critical to understanding their impact on corneal stromal wound healing.
- The purpose of this study is to determine the effects of ENMs on KFM transformation in the presence and absence of TGF-β1.

Figure 1: The K-F-M Differentiation Pathway



Keratocytes: DAPI (blue) + TRITC-phalloidin (red)

Fibroblasts: DAPI + TRITC-phalloidin

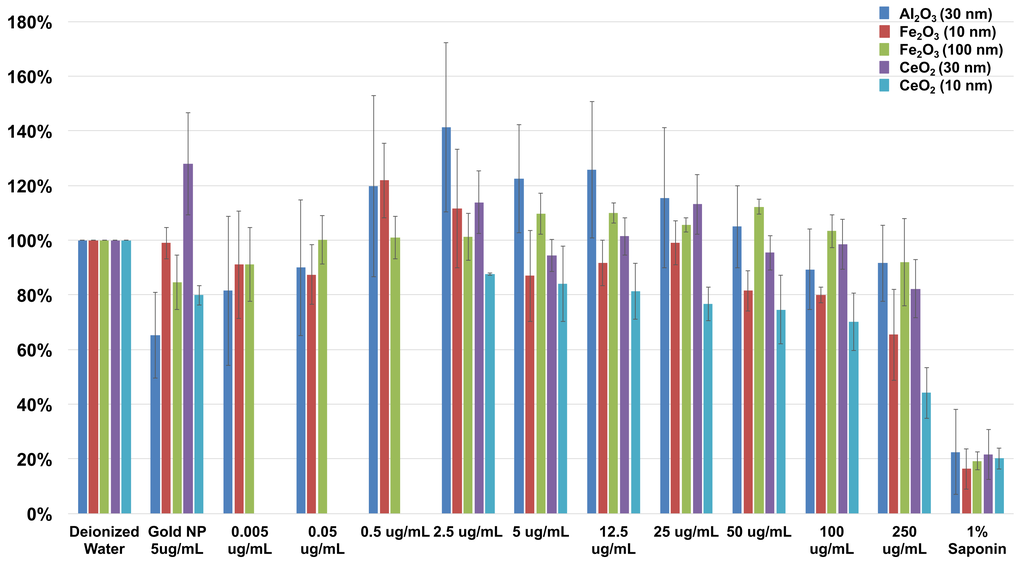
Myofibroblasts: DAPI + anti-αSMA-FITC (green) + TRITC-phalloidin

Methods

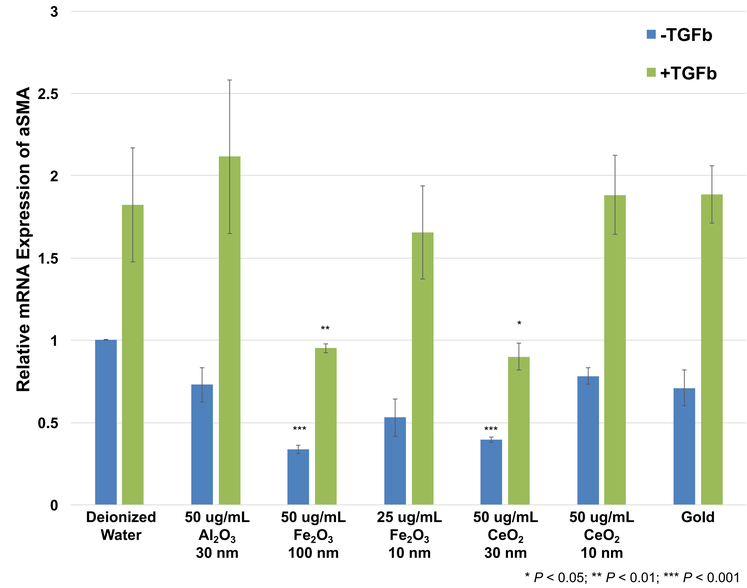
- Primary rabbit corneal fibroblasts (RCFs) were seeded in 96-well plates and cultured with media for 24 h. Cells were treated for 24 h with varying concentrations of ENMs. Deionized water, gold nanoparticle, and 1% saponin were used as controls.
- MTT assay and Calcein AM assay were conducted to determine cytotoxicity.
- RCFs seeded in 6-well plates and cultured with media for 24 h. Cells were treated for 24 h with ENMs +/- TGF-β1.
- RNA was harvested and quantitative PCR was performed to determine expression of myofibroblast phenotypic markers α-smooth muscle actin (α-SMA).
- One and two way ANOVA was used to determine statistical differences in mRNA expression.

Results

MTT Assay on RCF treated with ENMs for 24 hours; n=3



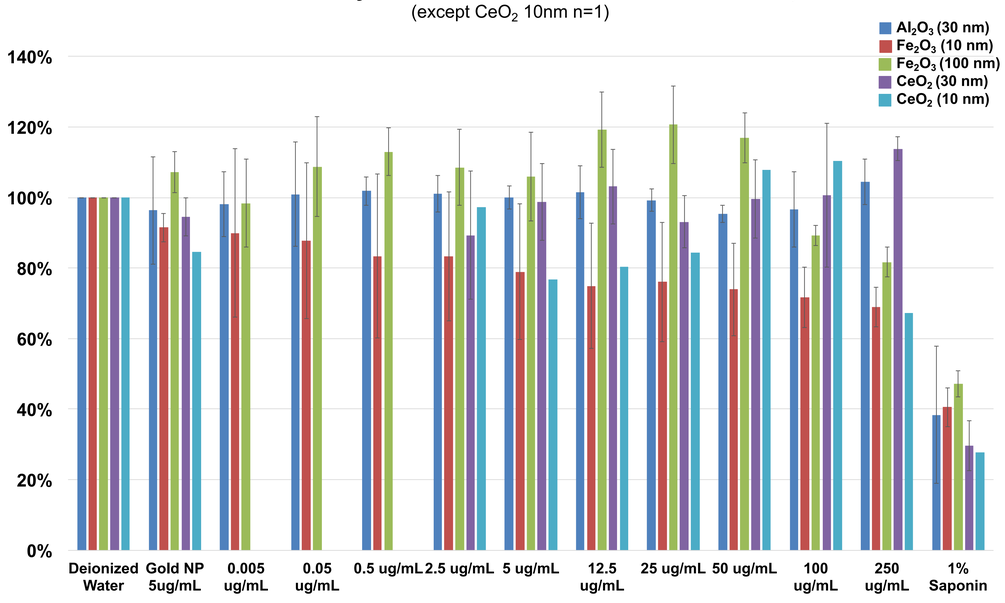
qPCR of RCFs treated with ENMs for 24 h; n=3



* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

None of the ENMs tested had to have a significant effect on cell viability using the two different assays. However there was a trend towards decreased cell viability with the 10 nm iron oxide and 10 nm cerium oxide ENMs at the 50, 100, and 250 μg/ml concentrations.

Calcein AM Assay on RCF treated with ENMs for 24 hours; n=3
(except CeO_2 10nm n=1)



Treatment of RCFs with iron oxide (100 nm) and cerium oxide (30 nm) significantly decreased expression of αSMA in the absence and presence of TGF-β1.

Conclusions

- The ENMs tested did not markedly alter viability of the RCFs.
- Iron oxide (100 nm) and cerium oxide (30 nm) inhibited KFM transformation.
- Future studies will entail assessing ENMs on corneal wound healing *in vivo*.

Acknowledgements

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