Concentration-Dependent Effects of Graphene Oxide Engineered Nanomaterials on Corneal Epithelial and Fibroblast Cell Viability and Proliferation

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Introduction

- As engineered nanomaterials (ENMs) become increasingly prevalent in consumer products, human exposure is dramatically increasing.[1]
- A major interaction site of ENMs is the eye, particularly the cornea.
- The deposition and retention of ENMs within the eye may induce adverse molecular and pathophysiological changes.
- Relevant exposure scenarios of diverse ENMs with different physicochemical properties on immortalized human corneal epithelial (hTCEpi) and rabbit corneal fibroblast (RCF) cells were created to determine the concentration-dependent effects of ENMs on cell viability and proliferation *in vitro*.
- We hypothesize that carbon-based ENMs will impair hCTEpi and RCF cell viability and proliferation *in vitro*.

Methods

- Two different sizes of graphene oxide (GO) (110nmx110nm) and GO (250nmx250nm) ENMs were studied.
- The hTCEpi cells and RCF cells were incubated for 24 h with ENMs and cell viability was assessed with MTT assays - 0.1% Saponin, gold (5 µg/ml) and distilled water (DI) were used as positive, negative, and vehicle controls, respectively.
- Using a 96-well plate, 7,000-8000 cells/well of hTCEpi cells and 1,000-2,000 cells/well of RCF cells were plated 24 hours prior to ENM treatment and incubated at 37º C with 5% CO2.
- Cytotoxicity assays (MTT and Calcein AM) were used to evaluate cell viability.
- All data sets were compared with one-way ANOVA followed by a Holm-Sidak pairwise comparison test to compare significant data sets.

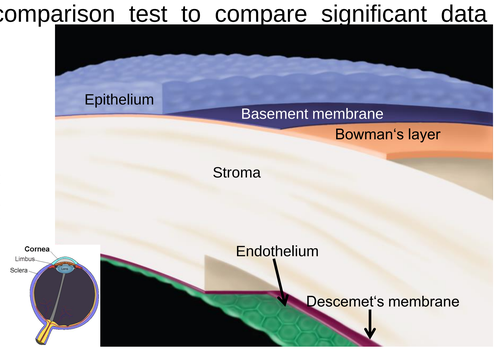
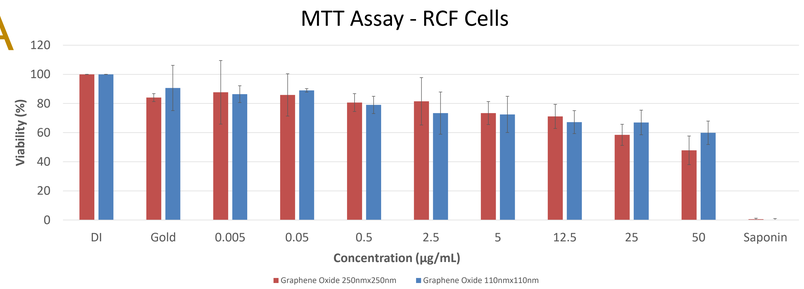


Figure 1. The anatomy of the human cornea comprised of 6 main layers. The epithelium is the outermost layer of cells that is several layers thick. The thickest layer of the cornea is the stroma which is comprised of regular arrangements of collagen fibrils and sparsely distributed cells called keratocytes. These cells can differentiate into fibroblasts and play a major role in wound healing.

A



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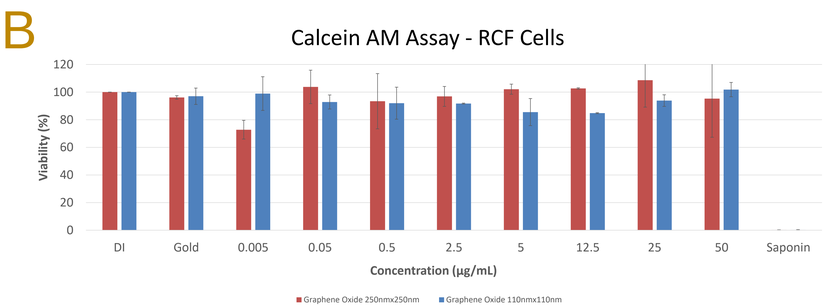
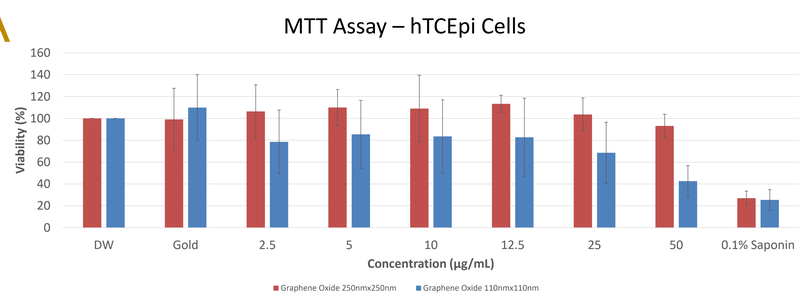


Fig. 2: RCF cell exposure to various concentrations of GO (110nmx110nm) and GO (250nmx250nm) resulted in significantly decreased viability at concentrations ≥12.5 µg/mL with GO (110nmx110nm) and ≥25 µg/mL with GO (250nmx250nm) compared to vehicle control (DI) (n=3). Data are mean ± SD (P<0.05).

A



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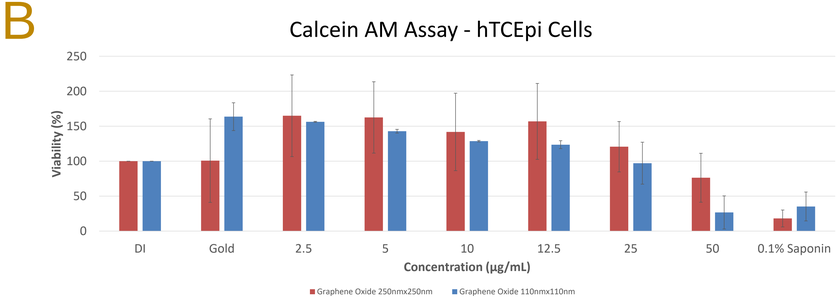

Fig. 3: Exposure of hTCEpi cells to various concentrations of GO (110nmx110nm) and GO (250nmx250nm) did not significantly impact hTCEpi cell viability versus vehicle control (DI) (3A, 3B).

Results

- MTT assays (2A) demonstrate significantly decrease RCF cell viability at concentrations ≥12.5 µg/mL with GO (110nmx110nm) and ≥25 µg/mL with GO (250nmx250nm) compared to vehicle control (DI).
- No significant decrease in hTCEpi cell viability (3A) was observed with GO (250nmx250nm) and GO (110nmx110nm) exposure (n=3).
- Calcein AM assays show no significant decrease in RCF (2B) and hTCEpi (3B) cell viability compared to controls.
- Graphene oxide ENMs have a minimal effect on RCF and htCEpi cell viability.

Conclusion

- Carbon-based nanoparticles are capable of decreasing corneal epithelial and fibroblast cell viability in a concentration-dependent manner *in vitro*, however, no significant decreases in RCF and htCEpi cell viability can be concluded from this study.
- Additional studies needed to evaluate effects of ENMs on wound healing *in vitro* and to determine the mechanism of action of graphene nanoparticles.
- Ultimately, results from the *in vitro* screening of ENMs will be used to determine which ENMs have the most potential to induce corneal toxicity prior to pursuing *in vivo* experiments.

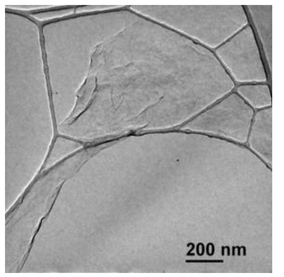

Fig. 4: Transmission Electron Microscopy (TEM) image of GO (250nmx250nm).

Reference:
[1]Arun Kumar, Prashant Kumar, Ananthitha Anandan, Teresa F. Fernandes, Godwin A. Ayoko, and George Biskos, Engineered Nanomaterials: Knowledge Gaps in Fate, Exposure, Toxicity, and Future Directions. Journal of Nanomaterials. 2014; 130198[2]Reinero CR, Cohn LA. Interstitial Lung Diseases. Veterinary Clinics of North America: Small Animal Practice 2007;37:10.

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