Fluorescent PLGA nanoparticles: *in vitro* toxicity study using a co-culture of human keratinocytes and dermal fibroblasts

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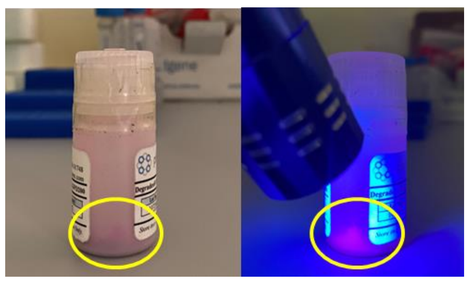
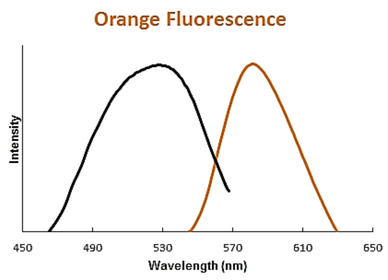
AIM. Fluorescent poly(lactic-co-glycolic acid (PLGA) nanoparticles (NPs) possess unique properties, including biodegradability, biocompatibility, tunable degradation rate, prolonged drug release, stability, formulation flexibility, and Food and Drug Administration (FDA) approval, that make them promising candidates for various nanomedicine applications. This study aimed to investigate the effects of different concentrations of these nanoparticles on a **co-culture of human keratinocytes (HaCaT cell line) and dermal fibroblasts (CCD-1070Sk cell line)**.

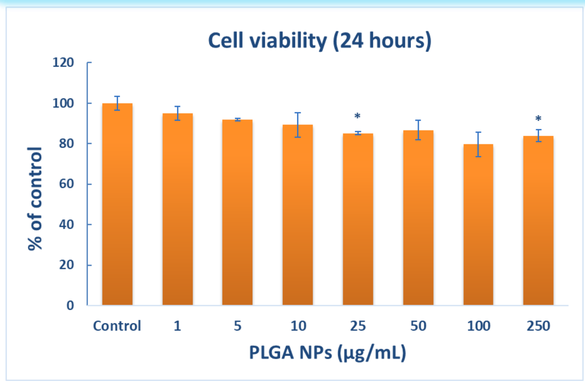
NANOPARTICLES. Degradex ® PLGA NPs are made of poly(D,L-lactide-co-glycolide) with an L/G ratio of 50/50 and a MW of 30,000. They contain 8.5% mannitol. PLGA has the density of 1.3 g/cm^3.

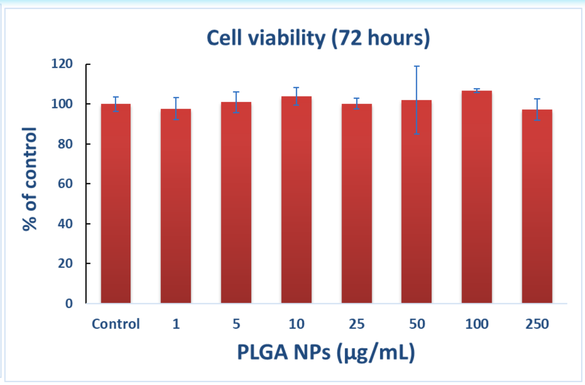


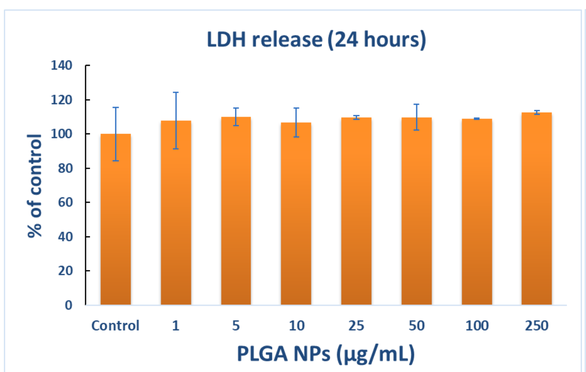


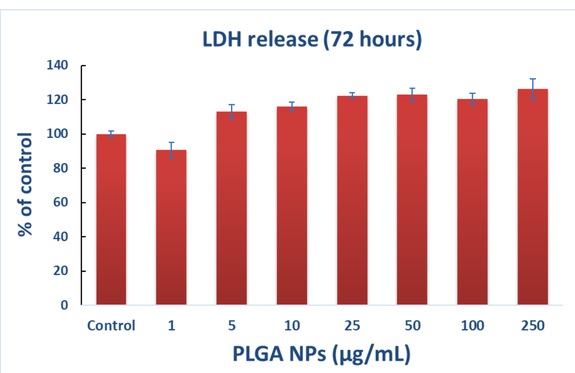
Parameter	Hydrodynamic size (d.nm)	Polydispersity index (PdI)	Zeta potential (mV)	Excitation/emission (nm)
PLGA NPs	171.30 ± 2.26	0.09 ± 0.01	-7.3 ± 0.7	530/582

RESULTS. Concentrations higher than 25 µg/mL caused a slight reduction in cell viability after 24 hours, but viability recovered to control level by 72 hours of exposure, indicating **minimal cytotoxicity**.

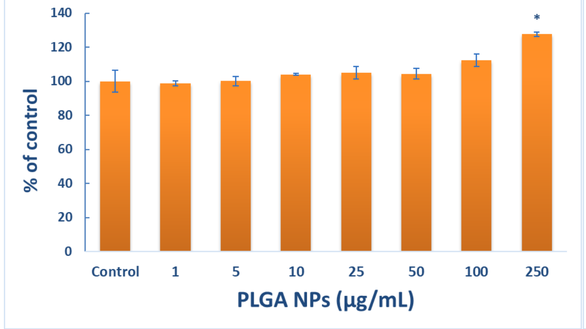


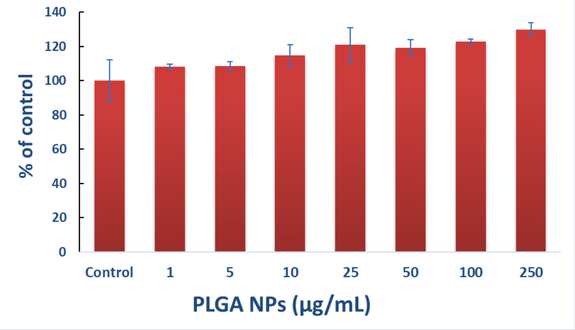




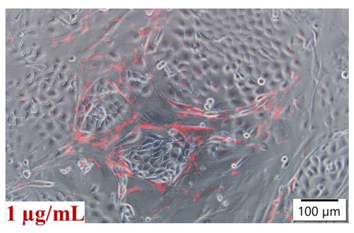


RESULTS. The highest concentration tested (250 µg/mL) induced elevated nitric oxide levels and lactate dehydrogenase (LDH) release after 72 hours, suggesting potential inflammatory response and membrane permeability, respectively, after incubation with PLGA NPs.

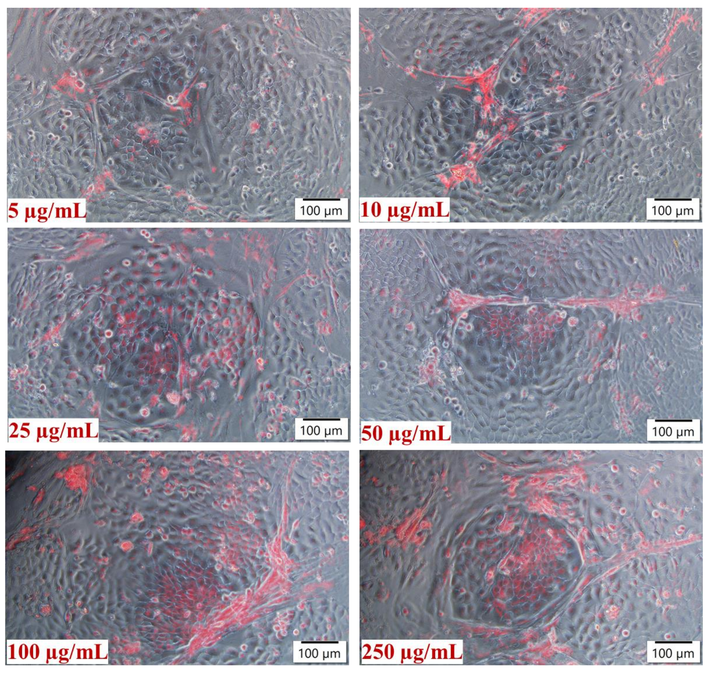




➢ Phase-contrast microscopy images of co-culture morphology overlayed with PLGA NPs fluorescence after 72 hours of incubation

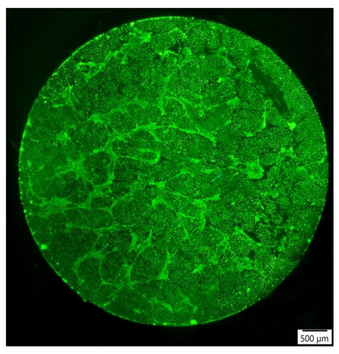


⇒ High fluorescence intensity and accumulation of NPs inside cells



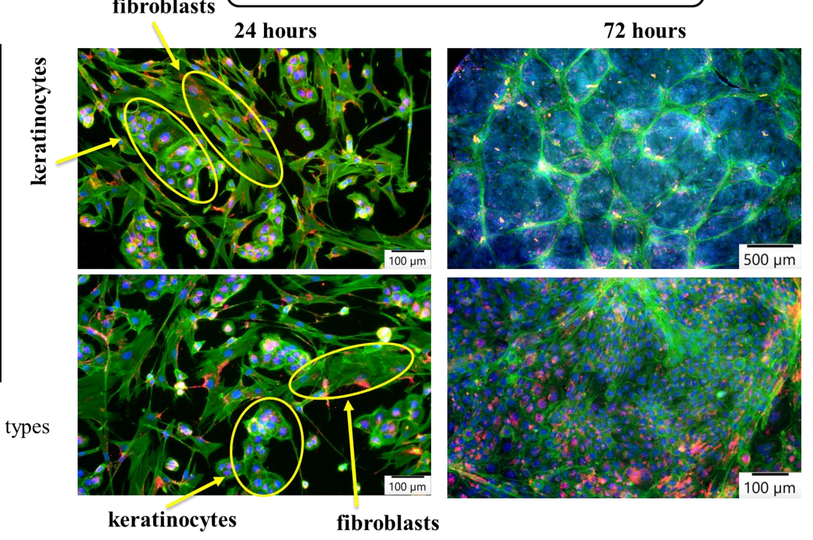
RESULTS. Efficient cellular **internalization** and intracellular dispersion of nanoparticles were observed after exposure to 25 µg/mL PLGA NPs. Fluorescent labeling of the actin cytoskeleton enabled visualization of co-culture morphology and cell organization, providing information on cell-to-cell interactions.

➢ After 72 h of incubation

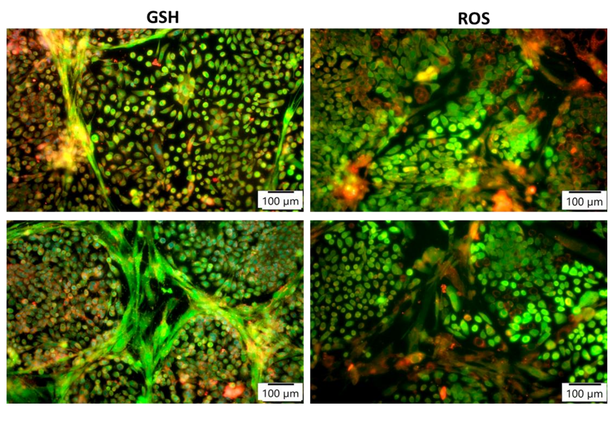


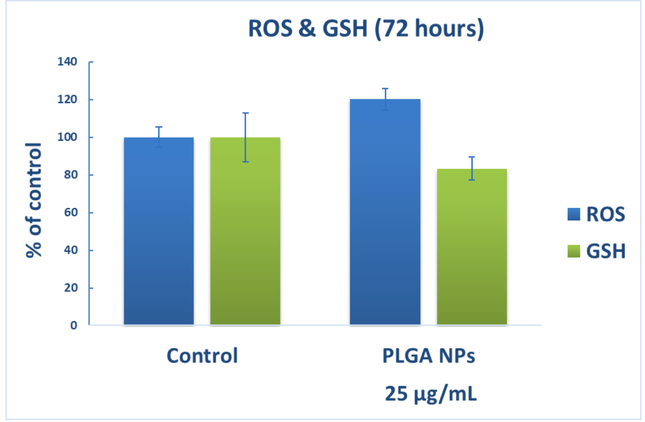
Co-culture stained for GSH
→ shows the arrangement of the two cell types

Actin cytoskeleton organization after 24 and 72 h of incubation with PLGA NPs (25 µg/ml).



RESULTS. A correlation was established between increased level of reactive oxygen species (**ROS**) and decreased glutathione (**GSH**) concentration, as confirmed by fluorescence imaging after 72 hours of exposure to 25 µg/mL PLGA NPs.





CONCLUSIONS.

- ✓ Through this **co-culture model of skin**, a deeper understanding of the dynamic behavior and interaction between the different cell types (keratinocytes and fibroblasts) was gained.
- ✓ Our results advance the **understanding of cellular responses to PLGA NPs** (in terms of viability, inflammatory response, actin cytoskeleton organization, and redox dynamics) and offer perspectives for safer and more efficient nanomedicine strategies.

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