Characterizing the biological behavior of Fe_3O_4 nanoparticles conjugated with acridine orange using *in vitro* co-culture systems relevant to skin, lung and gut barrier models

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AIM. Fe_3O_4 nanoparticles (NPs) can be conjugated with acridine orange to create a hybrid nanomaterial with unique properties, such as the magnetic characteristics of magnetite and the fluorescence of acridine orange, making them useful for a variety of applications, including cell imaging, drug delivery, and magnetic separation. In this context, we aimed to provide a **biological evaluation of this type of NPs using in vitro co-culture models of human skin, lungs, and intestine.**

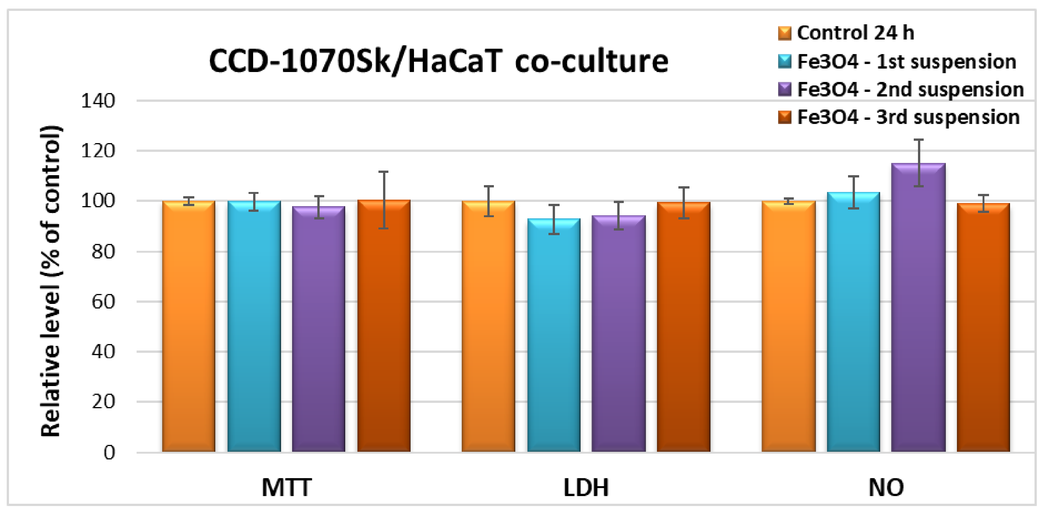
NANOPARTICLES. Fe_3O_4 NPs were obtained by the co-precipitation method from Fe^{2+} and Fe^{3+} (1:2 molar ratio). The concentration of acridine orange in an aqueous NH_4OH solution was 0.00025%. The product was washed several times with ultrapure water, redispersed, and centrifuged thrice at 6000 × *g* for 10 min. Each supernatant was collected, obtaining 3 different NPs suspensions.

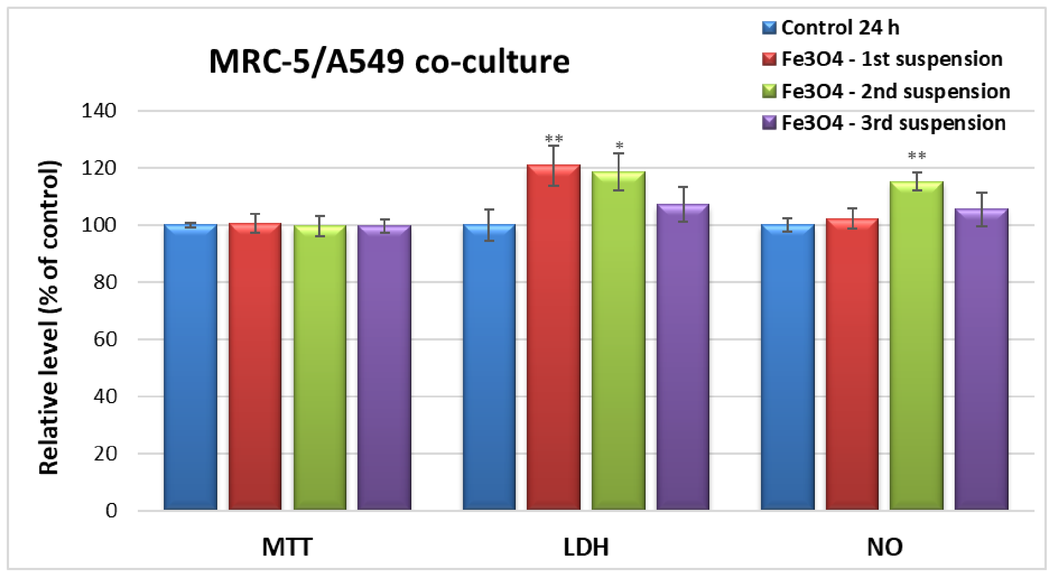
Nanoparticles (dispersed in culture medium)	Hydrodynamic size Z-average (d.nm)	Polydispersity index (PdI)	Zeta potential (mV)
Fe_3O_4 – 1st suspension	98 ± 0,5	0,621 ± 0,006	-10,3 ± 0,51
Fe_3O_4 – 2nd suspension	351 ± 5,6	0,965 ± 0,009	-11,2 ± 0,70
Fe_3O_4 – 3rd suspension	38 ± 2,7	0,827 ± 0,110	-8,6 ± 0,11

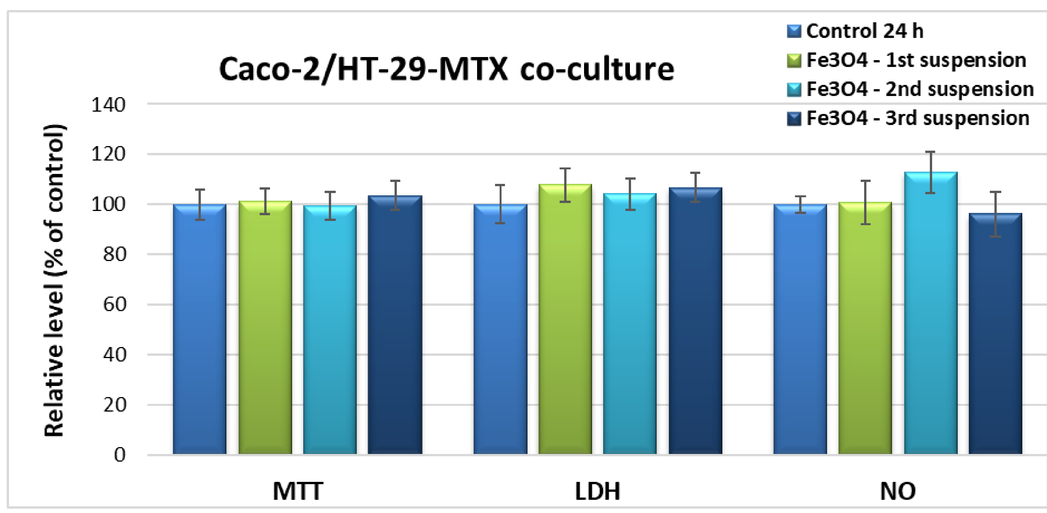
RESULTS. We developed one model of **skin barrier** using a co-culture of **human keratinocytes** (**HaCaT** cell line) and **dermal fibroblasts** (**CCD-1070Sk** cell line), one model of **intestinal** barrier composed of **human Caco-2 enterocytes** and **HT-29-MTX mucus-producing intestinal cells**, and one model of **pulmonary** barrier made of **A549 epithelial cells** and **MRC-5 fibroblasts**.

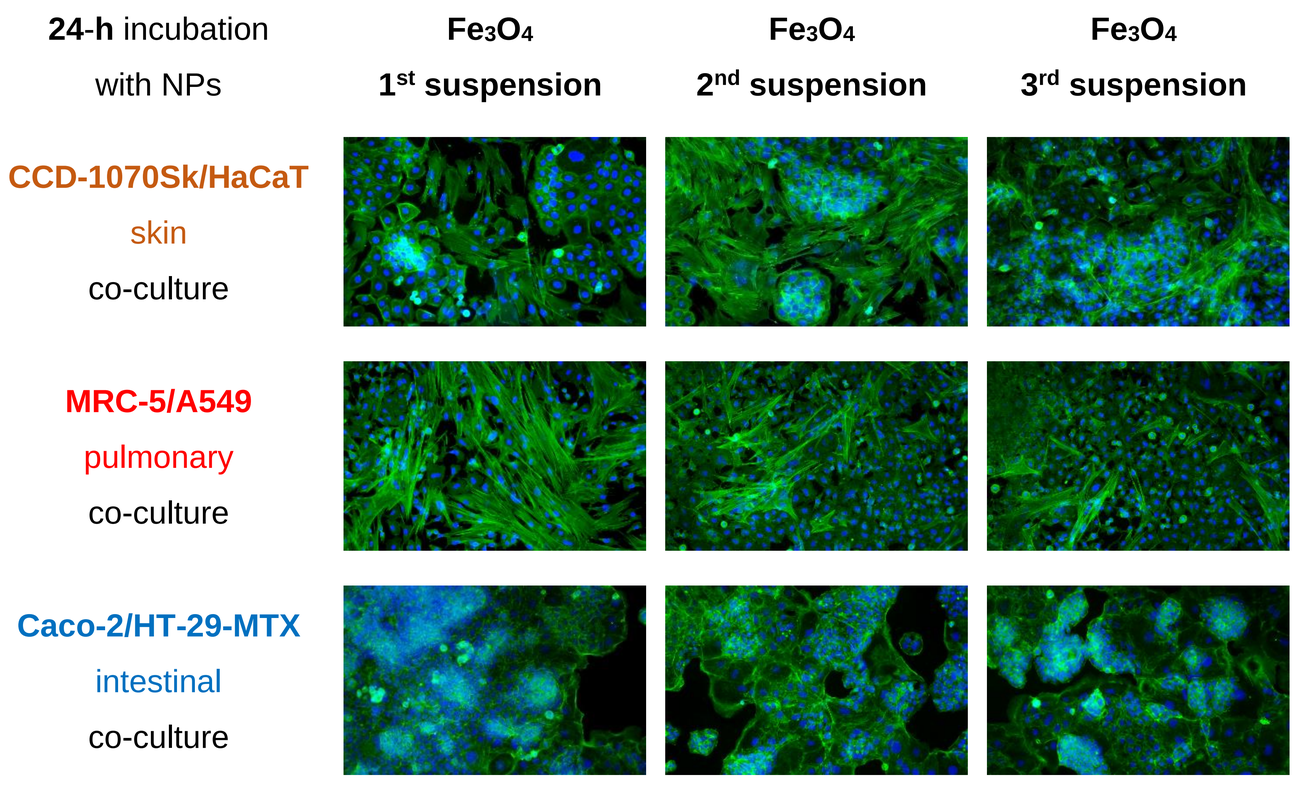
Our results showed that none of the NP suspensions influenced the cell viability of the co-culture systems, suggesting their ***good biocompatibility*** on short-term exposure (24 hours) according to the cytotoxicity assays performed.

In addition, we observed a specific cell orientation in the co-culture systems, being maintained after one-day exposure to the three suspensions of NPs.









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CONCLUSION. Fe_3O_4 NPs conjugated with acridine orange could be promising hybrid nanomaterial with good biocompatibility and special properties for future applications in biomedicine.