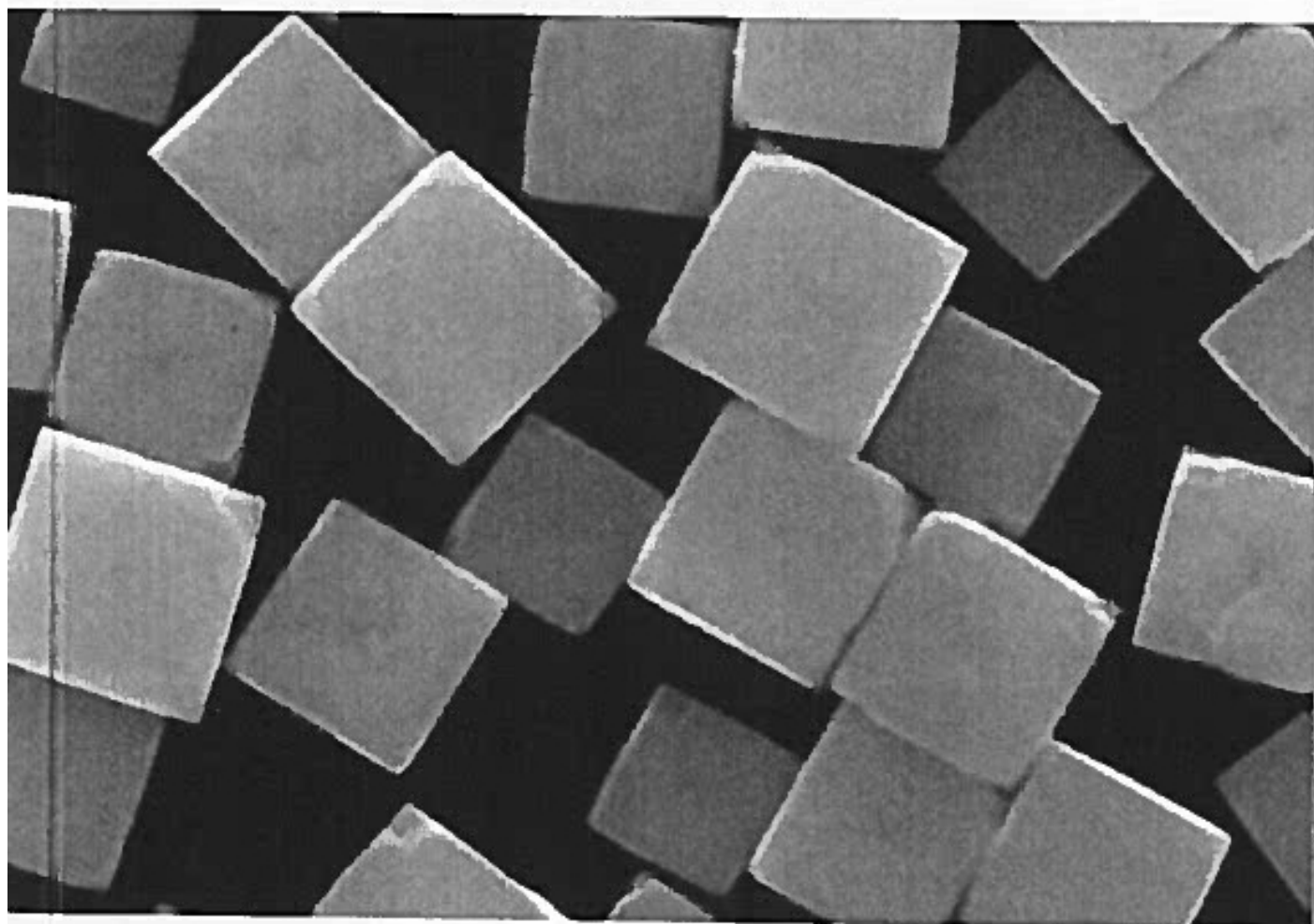


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## Does surface charge play a role in nanoparticulate- systems toxicity?

When evaluating the toxicological effects of nano- and microparticulate-systems, it is of utmost importance to characterise the physicochemical properties that are likely to influence cell and tissue processes. In fact, specific physicochemical properties of materials at the nano- and microscale, such as size, charge or hydrophobicity can greatly differ from the ones of the bulk material and, thereby, can also drive unpredictable biological interactions and effects. In particular, charge is one of the determinant properties of biological interaction and cationic particles have been shown to produce more effects on various cells than neutral or anionic particles [1].

The aim of the present work was to compare the toxicity of relevant biomedical acrylic based particulate systems (polymethylmethacrylate - PMMA), with different charges, within the same size range ( $\approx 500\text{nm}$ ). Specifically, PMMA (negatively charged) and PMMA-Eudragit (positively charged) formulations were considered. Both particles, hereinafter represented by PMMAp and PMMA-EUDp, were obtained by single-emulsion solvent-evaporation methodology [2]. The surface charge of particles was evaluated through zeta-potential measurement (Malvern Zetasizer Nano Z). Surface charge was measured in water dispersions, as well as in different media aiming to identify cell culture conditions, specifically ionic strength and FBS (fetal bovine serum) concentration, that would have a direct impact on particles charge.

Toxicological effects of both particulate systems were evaluated by cytotoxicity (MTT assay), stress response ( $\text{H}_2\text{DCFDA}$  fluorescence test) and

genotoxicity (Comet assay) in fibroblast L929 cells (as recommended by [3]). To confirm cellular effects, uptake studies were also undertaken by confocal microscopy analysis.

Results showed a significant reduction in the absolute charge values of the particles with the increase in the ionic strength of the media. It should be pointed that at 0.12 M, which is the reported salt concentration in physiological solution, both particles showed a surface charge close to zero. Also, results indicated that particles did not retain their original charge once they were put in contact with FBS. A complete inversion of PMMA-EUDp surface charge was observed when exposed to FBS, even at low concentrations (as low as 0.01%), while PMMAp surface charge tends to neutrality.

Concerning the evaluation of particles toxicological effects assessed by *in vitro* cellular assays, it was concluded that both particles were internalized in L929, after only 1h of exposure. Particles cytotoxicity, evaluated by the MTT, did not show any evidence of toxicity. Also, genotoxicity testing showed that PMMAp and PMMA-EUDp were not genotoxic *in vitro*, given that no significant induction in DNA damage was found through the comet assay for either particle type, as compared to negative controls. Furthermore, using FPG-modified comet assay, no significant oxidative DNA lesions occurred. This absence of oxidative damage was confirmed with the  $\text{H}_2\text{DCFDA}$  oxidative stress assay, since no significant rise in ROS was detected.

Overall, both PMMAp and PMMA-EUDp proved to be safe on the tested cell line and within the conditions employed on the various assays, showing promising biological properties for

potential use as carriers in drug-delivery applications. Considering that in the biological tested conditions none of the evaluated particles had positive charge, no conclusion could be made in what concerns comparing the toxicity of particles with opposite charge. Our study clearly shows that careful standardization of procedures must be undertaken, before evaluating potential biomedical application of particulate systems.

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#### References

- [1] Louro L, Bettencourt A, Gonçalves LM, Almeida AJ, Silva MJ. Role of nanogenotoxicology studies in safety evaluation of nanomaterials. In: S. Thomas, Y. Grohens, N. Ninan, ed. *Nanotechnology Applications for Tissue Engineering*. Amsterdam: Elsevier. 2014 (In Press)
- [2] Bettencourt, A, Almeida, AJ. *J Microencapsul.* 29(4) (2012) 353-67.
- [3] ISO 10993-5:2009: Biological evaluation of medical devices – Part 5: Tests for *in vitro* cytotoxicity.