Biocompatibility of nanoclays for future applications in biomedicine

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Abstract Nano clays as sepiolite and clinoptilolite, have attracted increasing interest in biomedicine for their potential applications as nanovehicles. However, no attempts have been made to evaluate the potential undesirable effects of sepiolite and clinoptilolite nanoparticles. The cytotoxicity of this two nano clays with different chemical structures was systematically evaluated in various macrophages types, such as human peripheral blood, mice bone marrow and cell line 264,7, by measuring cell viability and apoptotic pathway of death. In vivo evaluation was performed exposing CD1mice to suspensions of nano sepiolite and clinoptilolite at different concentrations (1, 2.5 and 5 mg/Kg of weight) by different ways (cutaneous, subcutaneous, intramuscular and intraperitoneal). No significant cytotoxic or toxic effects could be seen in both cases (in vitro and in vivo), but nano sepiolite was determined to be slightly more toxic than clinoptilolite in terms of cell viability and inflammation, this may be due to the differences in the structure. It is, therefore, expected that nano clays could be promising candidates for novel inorganic drug delivery carriers.

Introduction The origin of nanoscience and nanotechnologies is often attributed to a concept advanced by Nobel Laureate Richard P. Feynman, who in a 1959 lecture at the California Institute ofTechnology, stated: “There is plenty ofroom at the bottom. Many of the cells are very tiny, but they are active: they manufacture substance; they walk around; they wiggle: and they do all kinds ofmarvelous thingsall on a very small scale. Also they store information. Consider the possibility that we too can make things very small which does what we want when we want-and that we can manufacture an object that maneuvers at that level”.[1] Nanoparticles are used in various fields such as photonics, catalysis, magnetics, and biotechnology including cosmetics, pharmaceutics, and medicines. In particular, research interest has focused on their potential as drug delivery nano vehicles and nano medicines[2,3] It is, therefore, needed to answer the questions on their safety issue by performing vigorous toxicological evaluation based on various models[4-7], and to understand their interaction mechanism as well. Such a toxicological study can provide not only the critical information on the biological applications of nanoparticles, but also help to avoid any undesirable effects[8]. However, only a few studies have evaluated the safety of nano-sized materials and their potential adverse effects on biological systems[9].

Materials and methods. Parasite culture *Entamoeba histolytica* HM1-IMSS trophozoites were axenically grown in TYI-S33 medium supplemented with each nano clay so that the final concentrations were as follows: 1, 10, 50, and 100 mg/ml. Amoebic trophozoites viability was assessed employing two different methods, (1) vital marker trypan blue and (2) carboxyfluorescein diacetate (CFDA Vibrant kit) and propidium iodide done at 24, 28 and 72 hours under a microscope using a haemocytometer. **Cellular assays** Macrophages from human peripheral blood, for each experiment $1x10^5$ cells per well were placed in 96 well-plate with 100µl of RPMI 1640 supplemented and enough nanoclay to reach 0.1, 1, 10 and 100mg/ml of medium in each well. Apoptosis and necrosis were analysed each 12 hours for 48 hours by flow cytometry. In vivo tests CD1 mice were treated orally, cutaneously, subcutaneously, intramuscular and intraperitoneal with 1, 2.5 and 5mg of nano clay/Kg of weight and analytes were measured for kidney and kiver function.

Results

There is no significant effect in the cultures of Entamoeba histolytica treated with clinopiloite but the one that can be seen is dose and time dependent like for sepiolite but for this one the effect is significant for the higher concentrations (50 and 100mg/ml) it can be associated with the structure of the clays cause it is known that sepiolite is a fibrous clay until clinoptilolite have octahedric structure (Figure1). In the case of HPB (human peripheral blood) macrophages the effect is significant for the two clays at the higher concentrations (10, 100µg/ml) but is largest the percentage of cells affected with nano sepiolite (25%) than with nano clinoptilolite (20%) (Figure2). For in vivo tests we observed the accumulation of nano clays without inflammation (Figure3), but orally the mice stomachs treated with nano sepiolite have a size reduction and black color in some areas (Figure4) however kidney and liver function are normal (Figure 5).

References

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Figures

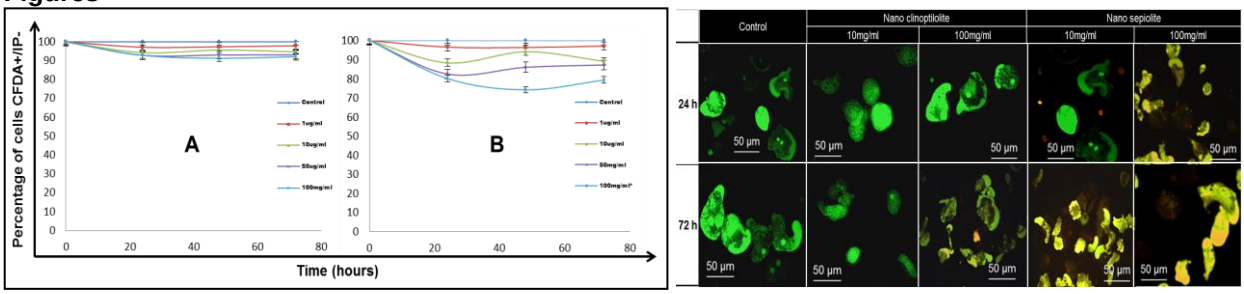

Figure 1. *Left* **A** Cultures of Entamoeba histolytica treated with nano clinoptilolite. **B** Cultures of Entamoeba histolytica treated with nano sepiolite. Right Analyze with CFDA and PI that shows in green the live cells and in red the nucleus of dead cells.

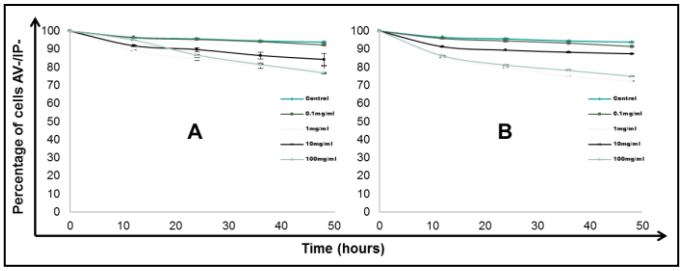

Figure 2. A Macrophages of HPB treated with nano clinoptilolite. **B** Cultures of macrophages of HPB treated with nano sepiolite. Cells were analyzed by flow cytometry in a FacsCanto cytometer.

Figure 3. Accumulation of nano clays in the administration areas in CD1 mice.

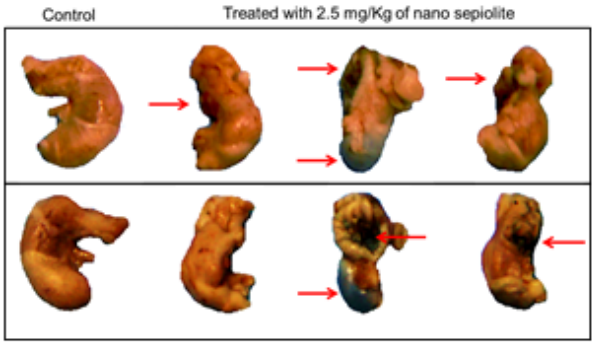

Figure 4. Effect on the stomach morphology and colouration of CD1 mice treated with nano sepiolite.

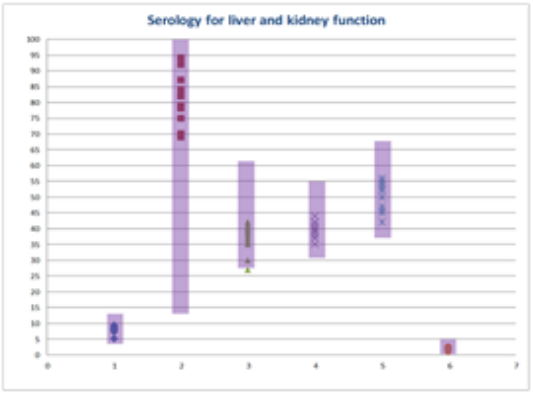

Figure 5. Analytes for liver and kidney function that are in the normal range.

Conclusions Nano clinoptilolite seems to be a biocompatible nano clay that in the case of nano sepiolite more studies are necessary but the effect is higher in cultures and animals.