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4.3b *In vitro* analysis of nanoparticles and nanodust released, from polymer/nanostructured-material composite, during drilling process.

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Background and objectives

Nanomaterials provide a new avenue of progress into technological development. By manipulating materials on the very basic atomic and molecular levels the property of a given material can be specifically altered to suit the purpose of intended applications. However, nanomaterials (nanoparticles and nanolayers) have a more complex nature in physiochemical properties and surface reactivity than their larger counterparts. Therefore, the release of these nanomaterials as dust during crushing or drilling may lead to serious health hazards for humans and the surrounding environment.

This study, supported by the NEPHH (Nanomaterial-related Environmental Pollution Health Hazards), addresses two important questions about nanomaterials:

- Whether nanomaterials can be released from physical process of nanoproducts.
- Toxicity potential of nanodusts generated from nanoproducts in comparison with reference products.

Both questions address a massive gap in knowledge for toxicity and more specifically nanomaterial toxicity.

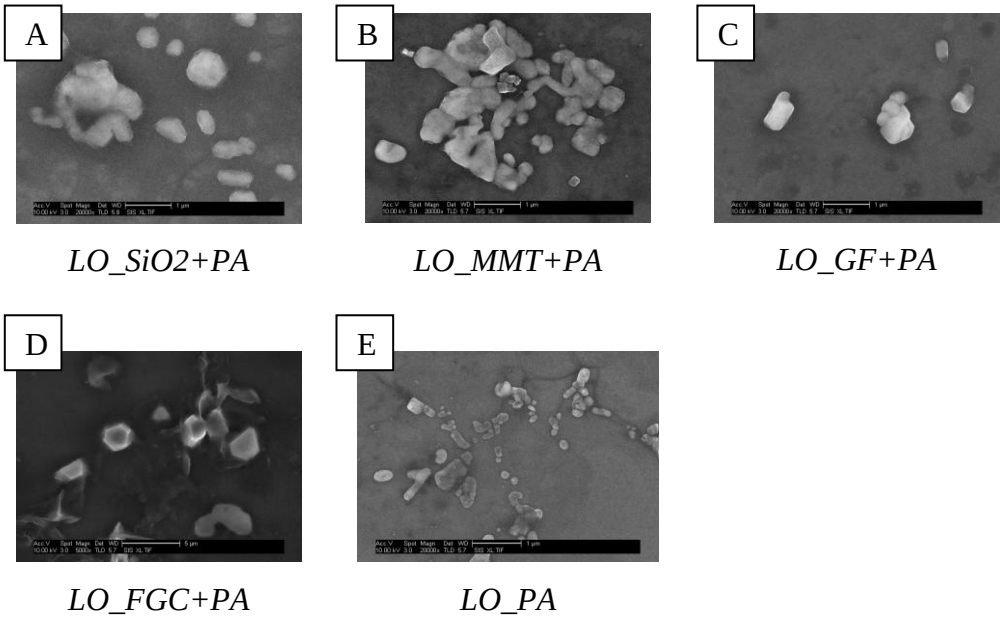
Study description

Polyamide (PA) nanocomposites panels utilising nanosilica and organically modified montmorillonite as nanofillers were manufactured using compression moulding technique and further utilised for toxicology investigations. To complement the study, PA composites that contained microscale size filler materials (glass fibre and foam glass crystal) were also manufactured and studied in addition to pristine PA polymer panels that contained no other reinforcement materials. Drilling was used to generate nanodust from these composite materials, this drilling process aimed to mimic a real situation which may lead to nanodust release. This nanodust was later characterised and tested *in vitro* to assess the toxicity potential.

Results & Discussion

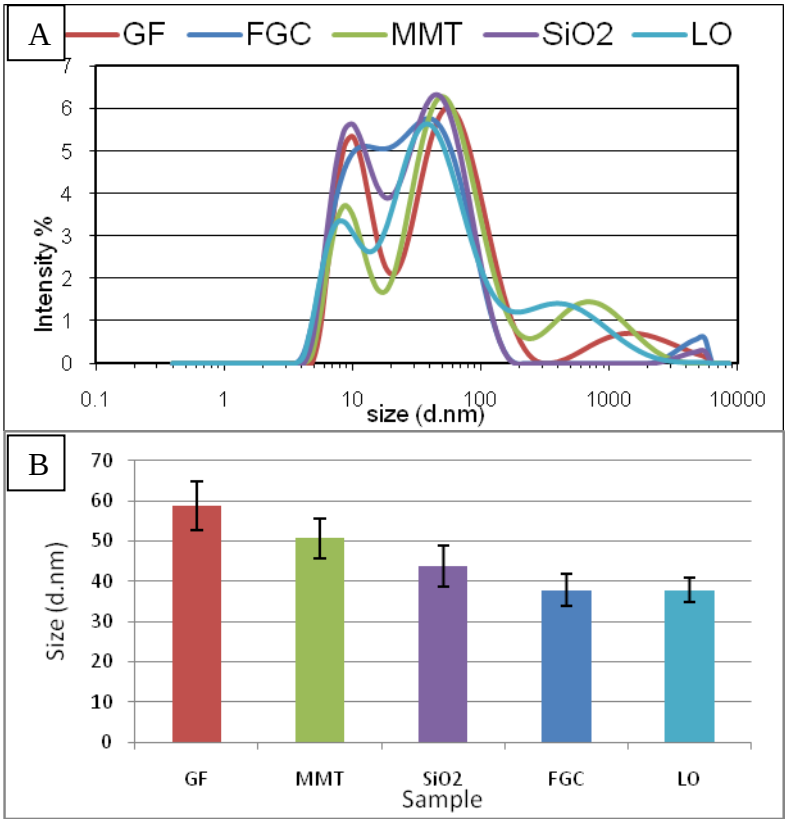
The results showed that the nanodust samples generated were of nanoscale, <100 nm and also showed a reduction in viability over a 72 hour period when treated to A549 cells in culture media.

Figure 1: SEM images of PA nanodust produced by drilling and filtration to achieve nanosized. A) PA and SiO_2 composite; B) PA and MMT composite; C) PA and GF composite; D) PA and FGC composite; E) PA reference



Scanning electronic microscopy examination, as shown in figure 1, revealed that the nanodusts contained predominantly nanoparticles of polymer matrix or reinforcement materials embedded within the polymer matrix.

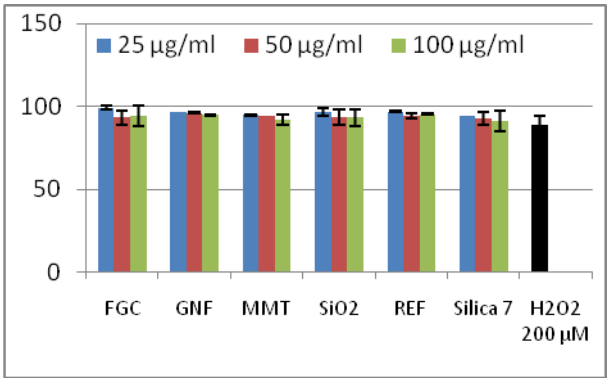
Figure 2: A) DLS size distribution of nanodust in culture medium of each sample; B) average size intensity of each nanodust sample. Nanodusts were dispersed in culture media at 50 µg/ml.



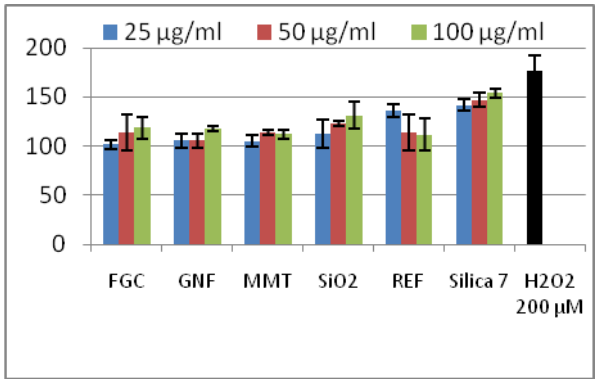
The average diameter of the nanodusts in cell culture medium was less than 100 nm, sown in figure 2 the DLS size distributions of each nanodust sample. These collected nanodusts were used to treat cells at doses of 25-100 µg/ml, nanodusts from all the PA polymers induced little generation of ROS and reduction of cell viability over time, which were not associated with the damage in membrane integrity.

Figure 3: MTT, ROS generation and LDH leakage of treated A549 cells with the corresponding nanodusts; MMT and LDH measured at 24, 48 and 72 hours; ROS generation measured at 12, 24, 48 and 72 hours. Silica 7 and H_2O_2 were used as positive controls in each of the assays.

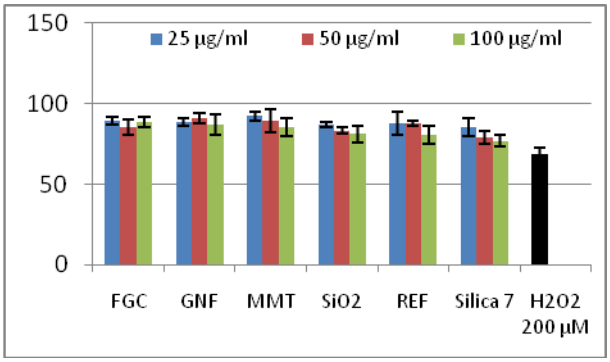
24 hour MTT



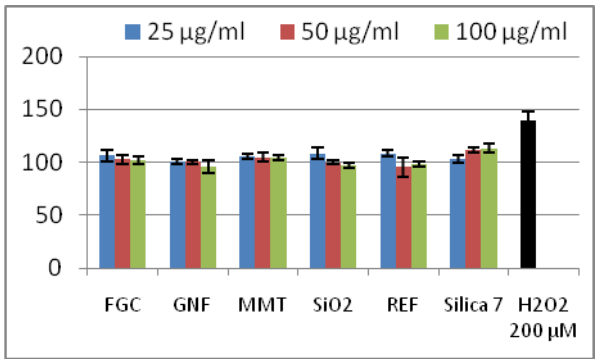
12 Hour ROS



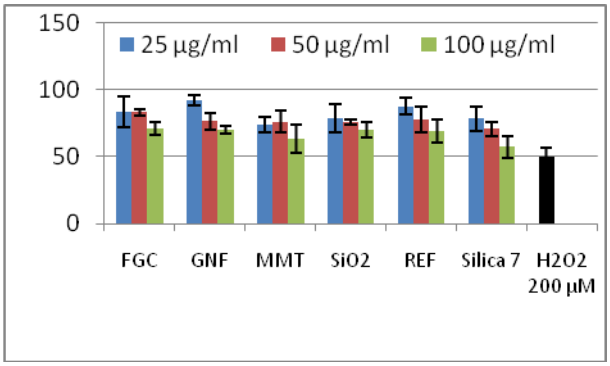
48 hour MTT



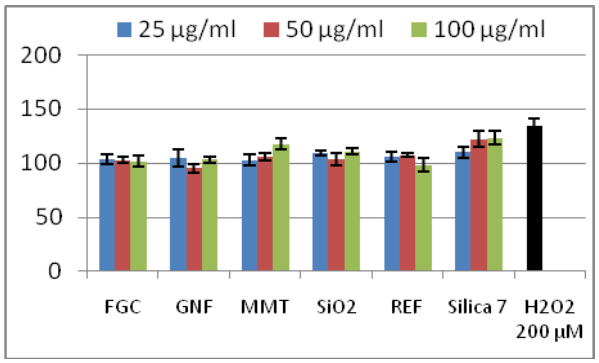
24 hour ROS



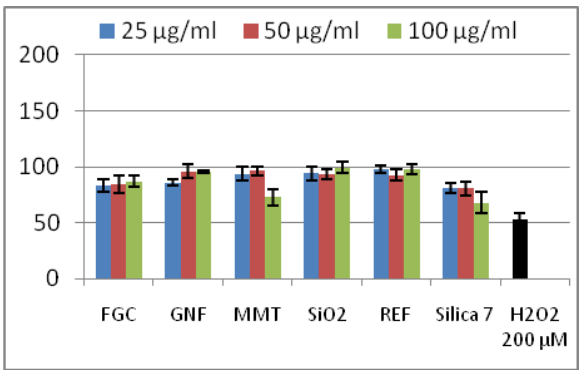
72 hour MTT



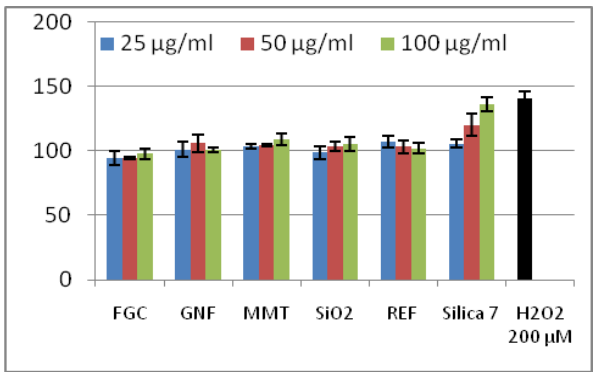
48 hour ROS



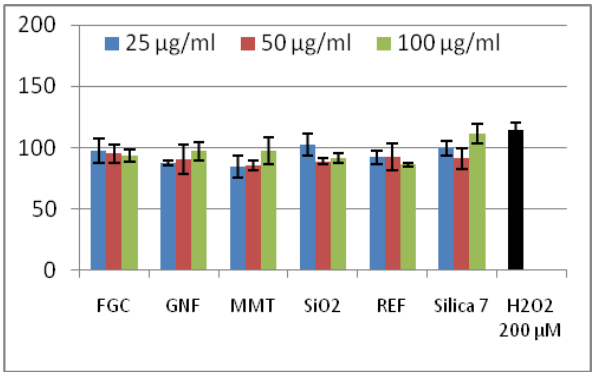
72 hour ROS



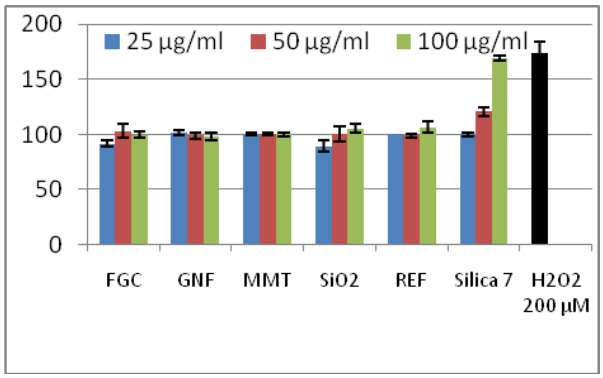
48 hour LDH



24 hour LDH



72 hour LDH



No significant difference was detected in toxicity potential among the different nanodusts generated from the PA reinforced composites with and without nanofiller materials. However, it can be detected from the graphs above in figure 3 that the cell viability reduces slightly with time. This response is seen to be more time dependant rather than a dose dependant reduction of cell viability. The generation of ROS however shows that the nanodust do not necessarily produce an elevated level of ROS.

Conclusions

The toxicity results showed that there is very little if any variation which occurs between the different nanodust samples. This lack of variation requires further analysis to relate which nanodust characteristic impacts on the toxicity potential of the nanodust. The model developed in this project for assessment of nanoproducts safety in relation to a specific scenario during product life cycle is of potential value in supporting safe development of novel materials.

Acknowledgements

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