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Toxicity of four novel Polyhedral Oligomeric Silsesquioxane (POSS) particles used in anti- cancer drug delivery

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ABSTRACT

The wide use of nanomaterials in medicine and especially in drug delivery had heightened the demand for a safe use of nanoparticles (NPs) before delivered to patients. NPs are used to reduce the toxicity of some drugs; however, the nanocarriers themselves impose risk on the patient health. The creation of NPs safe use guidelines is based on experimental investigation on human cell lines to eliminate the risk of newly synthesized NPs. In this study, toxicity of four novel POSS particles were tested using 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and crystal violet assays and exposed for 1,24 and 48h to human skin keratinocytes (HaCaT). Five concentrations investigated ranging between (0.001-100mg/mL) for each particle (size 100nm in diameter). TrisilanolIsooctyl POSS particles significantly reduced the metabolic activity and relative cell number of HaCaTcells lines after 24h exposure. Trisilanol Phenyl, Cyclopentyl and Cyclohexal POSS particles did not show any sign of toxicity; therefore, toxicity may attributed to the shape of the particle. Trisilanol Phenyl, Cyclopentyl and Cyclohexal POSS particles show a promising application in anti-cancer drug delivery.

INTRODUCTION

Nanotechnology is the science that involve the synthesis, design, characterization and application of devices and materials whose smallest function in at least one dimension is on the nanometre scale up or one billionth of a meter (Silva, 2004). At these scales, it is important to consider the individual molecule and interacting groups of molecules in relation to the properties of bulk macroscopic materials and devices (Silva, 2004). Because it is on a small scale, the properties of materials such as magnetism, color and ability to regulate electricity may be tailored to desired end user applications (Grassian, 2008). This resulted in different characteristics that could produce a wide range of novel products (Grassian, 2008). Nanotechnology applications cover communications, electronics, cosmeceutical, food, energy, and agricultural industries among others (Wilkinson, 2003). Currently, nanomaterials are used in environmental remediation, medical devices and pharmaceutical areas giving scientists a chance to modify matter in varies parts of work and life (Martin, 1994). Some of the important applications in the field of medicine is in drug delivery, fluorescent biological labels, bio-detection of pathogens, detection of proteins, tissue engineering, tumour destruction, separation and purification of cells and molecules (Salata, 2004). Nanoparticles (NPs) are in the same size domain as proteins making it suitable for labelling and bio tagging. This property is underutilised currently for biological tagging (Salata, 2004). NPs could be attached with biological or molecular layer to act as biological interface such as antibodies, collagen or monolayers that are able to make nanoparticles biocompatible. Polyhedral oligomeric silsesquioxane (POSS) nanostructures have potential interest in many biomedical applications such as dental composites, drug delivery, biomedical devices, biosensors and tissue engineering (Ghanbari *et al*., 2011a). Cytocompatibility and non-toxicity are the features for making POSS suitable for biomedical applications (Gao *et al*., 2012; Jain and Bar-Shalom, 2014).

One of the major applications of POSS is its use in the development of cardiovascular implants. The incorporation of POSS with biocompatible polymers leading to the development

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of nanocompsoite materials that enhanced antithrombogeneicity, hemocompatibiltiy; reduce inflammatory response and calcification resistance (Ghanbari *et al*., 2011c). Currently, POSS containing polymers are under intensive investigations to develop a new generation of cardiovascular implants such as bypass grafts, heart valve prosthesis and coronary stents. In the meanwhile, there is a demand for more investigation on POSS NPs cytotoxicity for human cell lines (Singh *et al*., 2010). In this study, we evaluate the potential cytotoxicity of four novel POSS carriers. Toxicity of Trisilanol ethyl, trisilanolisooctyl, trisilanol phenyl, trisilanolcyclopentyl and trisilanolcyclohexyl POSS NPsafter exposure to human skin keratinocytes (HaCaT) was determined by 3- (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and crystal violet assays.

MATERIALS AND METHODS

Reagents and NPs

All reagents were purchased from Sigma Aldrich, Australia unless otherwise stated. These included, [3- (4,5-dimethylthiazol-2yl)-diphenyltetrazolium bromide] MTT, The culture media used was Roswell Park Memorial Institute (RPMI) with 10% heat-inactivated fetal bovine serum (FBS) (HYQ®, Hyclone, Utah, USA), penicillin and streptomycin. Millipore Milli-Q water with resistivity = 18.2 MΩ.cm was used for all the experiments. POSS particles were synthesised by the Department of Chemical and Physical Sciences, Flinders University in South Australia and the physical and chemical properties mentioned in our previously published study (Almutary and Sanderson, 2016).

Cell Culture

HaCaT cell line was obtained from American Type Culture Collection (ATCC). Cells were grown in RPMI medium with 10% fetal bovine serum (FBS) and incubated at 37 °C, 5% CO_2 in a humidified incubator. The cells growth started from $2x10^6$ cells/ml and subcultured when confluence reached 60-70% every 2-3 days.

For Toxicity Assays

HaCaT cells were seeded at 10,000 cells/well in a 96-flat plate then incubated for 18 hours to allow adherence. POSS particles were diluted in fresh medium. After incubation, cells were treated with five POSS particles range between (0.001-100mg/mL) for 1, 24 and 48h. The solution was removed and the cells were washed twice with phosphate buffered saline (PBS) twice to remove excess POSS residue.

3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide Assay Experimental Procedure

The toxicity of POSS particles determined by MTT assay as described (Mosmann, 1983). $1x10^4$ cells were seeded in volume of 100 µl into 96-well flat bottom plate. MTT was added to each well at 0.5 mg/mL, and then plates were incubated at 37 °C for 4h, then 80 µl of 20% SDS in 0.02 M HCl was added to each well. The plates were kept in the dark at room temperature for overnight. OD was read on ELISA reader at 570 nm, with 630 nm as reference wave length. In each experiment a standard curve was run to convert the OD values to cells/well.

Crystal Violet Assay

HaCaT Cells were stained with crystal violet after exposed to particles. Live cells remained adhered to the plate and dead cells were washed away (Smith *et al*., 2016, Chiba *et al*., 1998). Three replicate plates were performed for cell count using crystal violet assay, each plate included 6 technical replicate wells per treatment. After exposure (see above), the plates were washed and 50 µl of crystal violet stain was added and incubated at room temperature for 15 minutes. Then, the Stain was washed off with demineralized water and the plates were left to dry overnight. A 33% (v/v) acetic acid was added to dissolve the stain and the absorption was read using microplate reader at 570 nm. The results were expressed as percentage viability compared to untreated control.

Statistical Analysis

The data were expressed as mean +/- SD of at least three independent experiments using one-way analysis of variance (ANOVA) and Tukey–Kramer multiple comparisons test using SPSS software to compare exposure groups. All comparisons were considered significant level $p< 0.05$.

RESULTS AND DISCUSSION

Toxicity of POSS particles was investigated using two classical colorimetric assays. TrisilanolCyclohexyl, Cyclopentyl and Phenyl POSS particles did not interrupt the metabolic activity of HaCaT cell line. However, TrisilanolIsooctyl POSS particle after 24h exposure significantly reduced the cell growth by 75% compared to untreated control. Interestingly, 4h exposure to TrisilanolIsooctyl POSS increased the cell growth by 50% compared to untreated control Fig.1. Due to POSS particles excellent compatibility with most monomers and polymers, functionalized POSS molecules can be easily incorporated into a variety of polymers (Ghanbari *et al*., 2011c). The main characteristic in POSS particles is the biocompatibility which resulted from the solid nature and low inflammatory reaction in the centre of the silicon reach area (Ghanbari *et al*., 2011a). In addition, non-toxicity and cytocompatibility are other features of POSS particles, therefore it a common used particles in biomedical applications (Kim *et al*., 2007). Several mechanisms are the cause of most of NPs toxicity; however, most intracellular and *in vivo* toxicity is a result of high level of reactive oxygen species (ROS) (Foldbjerg *et al*., 2009; El Badawy *et al*., 2010; Chang *et al*., 2012; Buonocore *et al*., 2010). For example, oxidative stress induced by NPs happen during the dissolution of iron-based NPs, in which stimulate ROS generation and form radicals from H_2O_2 (Auffan *et al*., 2008; Frohlich, 2013). In addition, some NPs do not raise directly ROS production, but are capable under biological

conditions such as targeting the mitochondrial (Xia *et al*., 2006; Sharma *et al*., 2012).

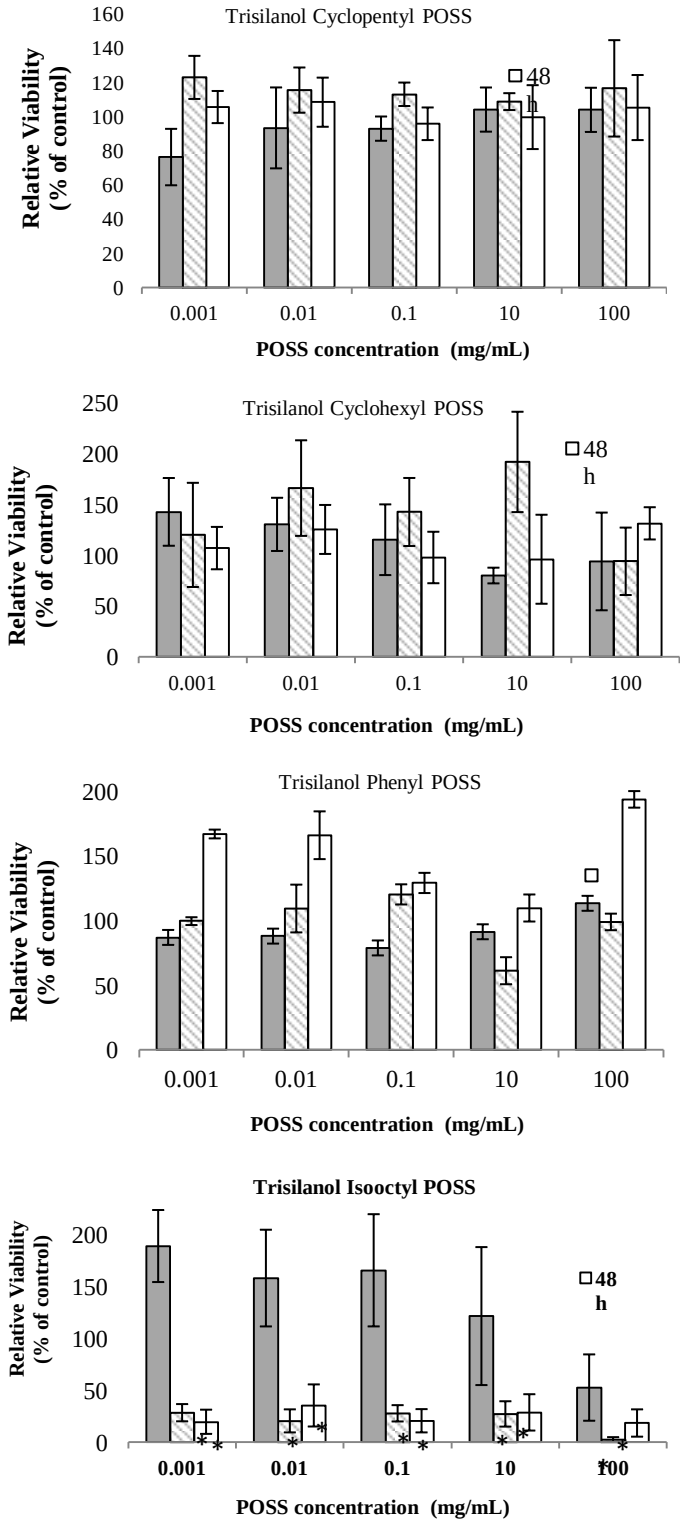

Fig. 1: Metabolic activity of HaCat cell line after exposure to four types of POSS particles. The cells growth significantly decreased after 24 h exposure to TrisilanolIsooctyl POSS; other POSS particles did not show a growth inhibition compared to untreated control. Data are shown as relative survival (%) compared to the untreated control and are presented as mean ± SEM, n = 3.

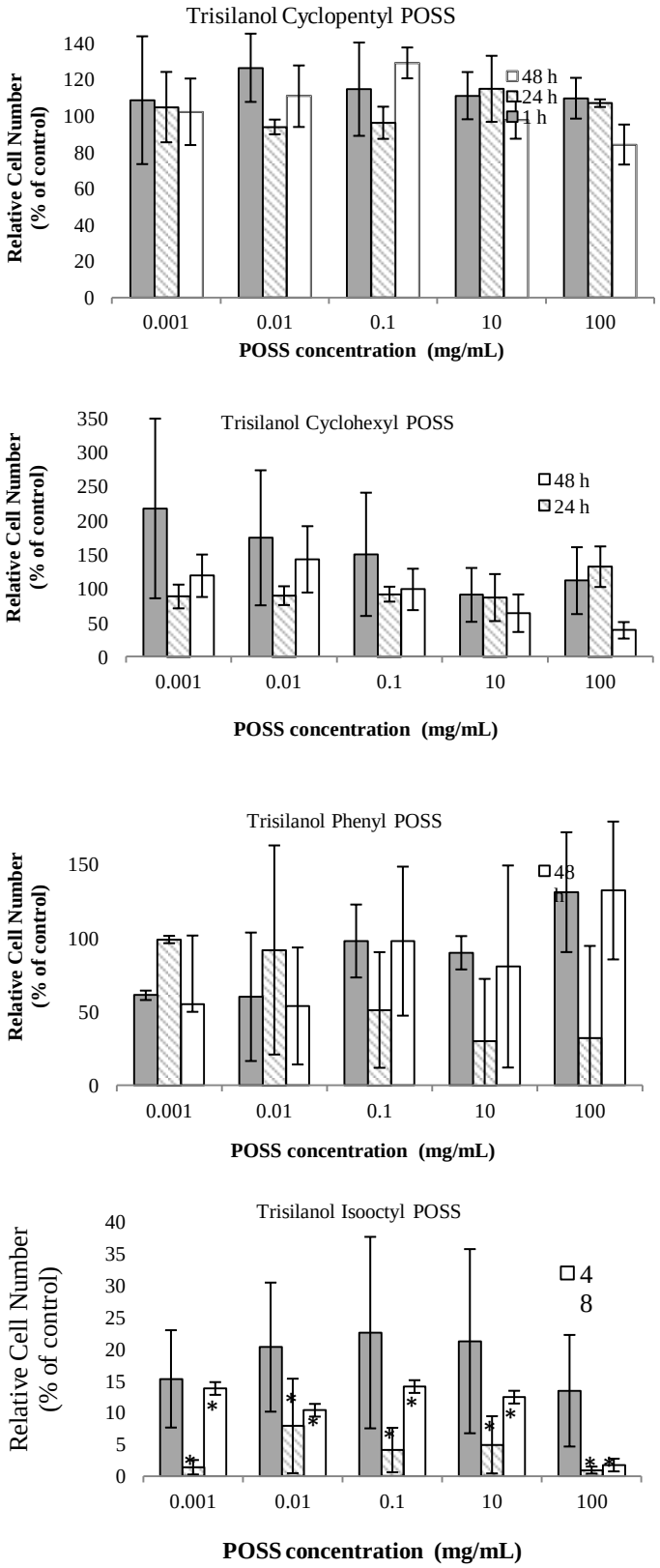

Fig. 2: Treatment of HaCaT cell line with POSS particles assessed using the crystal violet assay. TrisilanolIsooctyl POSS significantly restricted the cell growth after 24 h exposure. Trisilanol Phenyl and Cyclohexal POSS at high and variable doses showed toxicity to HaCaT cells. Data are shown as relative cell number (%) compared to the untreated control and are presented as mean ± SEM, n = 3.

In figure 2, variable impact observed for Trisilanol, Cyclohexyl, Phenyl and Isooctyl using crystal violet assay. TrisilanolIsooctyl POSS significantly reduced the cell number of HaCaT cells after 24 and 48h exposure in all concentrations. The methods that have been used to identify the toxicity of NPs including general toxicity assessment, testing toxicokinetics of NP in humans and animals, in vitro and in vivo assays revealed inconsistent results (Bakand and Hayes, 2016). The source of NPs toxicity can be the particle size, size distribution, shape, surface area and surface chemistry (Kelly *et al*., 2003, Saptarshi *et al*., 2013). Therefore, the only way to avoid testing every single NPs and it variant in toxicological test, is to correlate the physicochemical characteristics with their toxicity in a structure activity relationship model (Saptarshi *et al*., 2013, Balachandran *et al*., 2005).

CONCLUSION

Four novel Polyhedral Oligomeric Silsesquioxane (POSS) particles were tested for biocompatibility for a promising application in anti-cancer drug delivery. We previously investigated the interference ofthe four POSS particles and others particles with MTT and crystal violet assay. The interference of these particles was correlatedto some of the physical and chemical properties. Toxicity of the four POSS particles seems to be a shape or size dependent. At 100 nm size in diameter, only TrisilanolIsooctyl POSS interrupted the metabolic activity also reduce relative cell number of HaCaT cell line. The other POSS particles did not show any sign of toxicity in both assays although particles were at the same concentrations. Trisilanol Phenyl, Cyclopentyl and Cyclohexal POSS particles could be a potential platform for anti-cancer drug delivery.

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