Assessment of the advanced material molybdenum disulfide (MoS_2) and layered double hydroxides (LDHs) nanosheets effects *in vitro* using zebrafish liver cells

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INTRODUCTION

Molybdenum disulfide (MoS_2) and layered double hydroxide (LDHs) nanosheets are very promising novel materials for use in environmental remediation among other applications. During production, use and disposal, these materials will reach the environment. However, the environmental hazard they pose is poorly understood. The use of *in vitro* models can contribute to increased understanding of molecular and cellular mechanisms in a high-throughput and cost-effective manner and can address the challenge of the plethora of nanomaterials (NM) continuously produced and released in the environment.

Aim: to elucidate the behavior, biocompatibility and effects of the advanced NM MoS_2 and Mg-Al-LDH *in vitro* using the zebrafish liver cell line (ZFL) and to evaluate its suitability in nanotoxicology studies.

APPROACH

Nanomaterial synthesis → Dispersion stability and characterisation → Effects on ZFL cell line

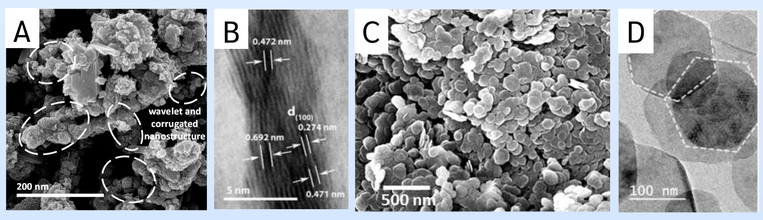
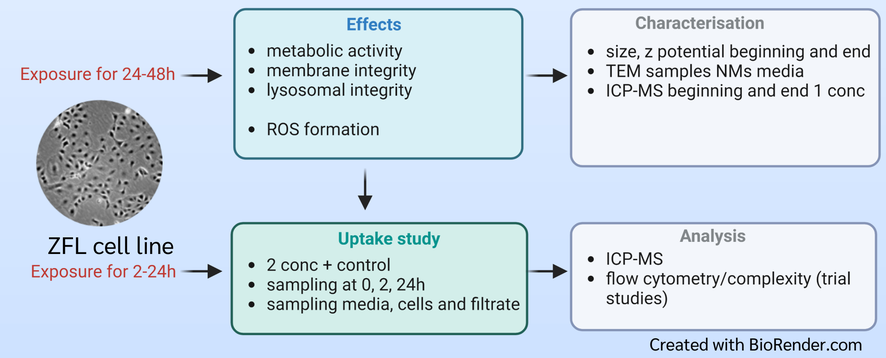


Figure 1. Nanomaterials used in the study. Scanning (A, C) and transmission (B, C) electron microscopy images of (A, B) **MoS_2 nanosheets** and (C, D) **Mg-Al-LDH layered nanosheets**. *Panchal et al. 2021. Appl. Surf. Sci. 553; Singh et al. 2024. Appl. Mater. today 36.*

Probe sonication

- MilliQ water (NanoReg SOP)
- Bovine serum albumin in PBS (NIST SOP)
- Humic acid/MQ SOP (Enhanced NanoReg)
- Exposure media over time
- ICP-MS, electron microscopy



RESULTS

Characterisation

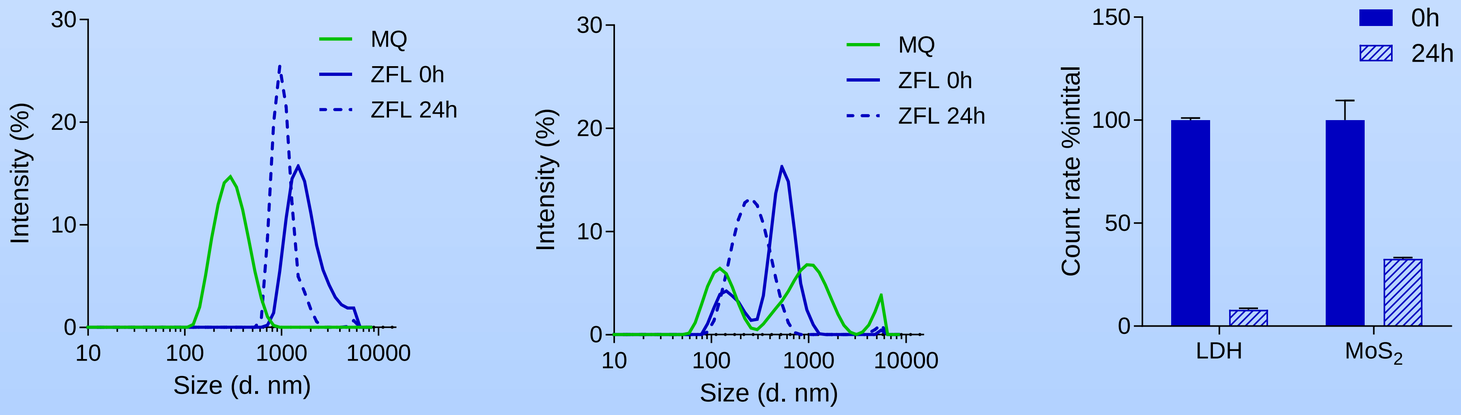
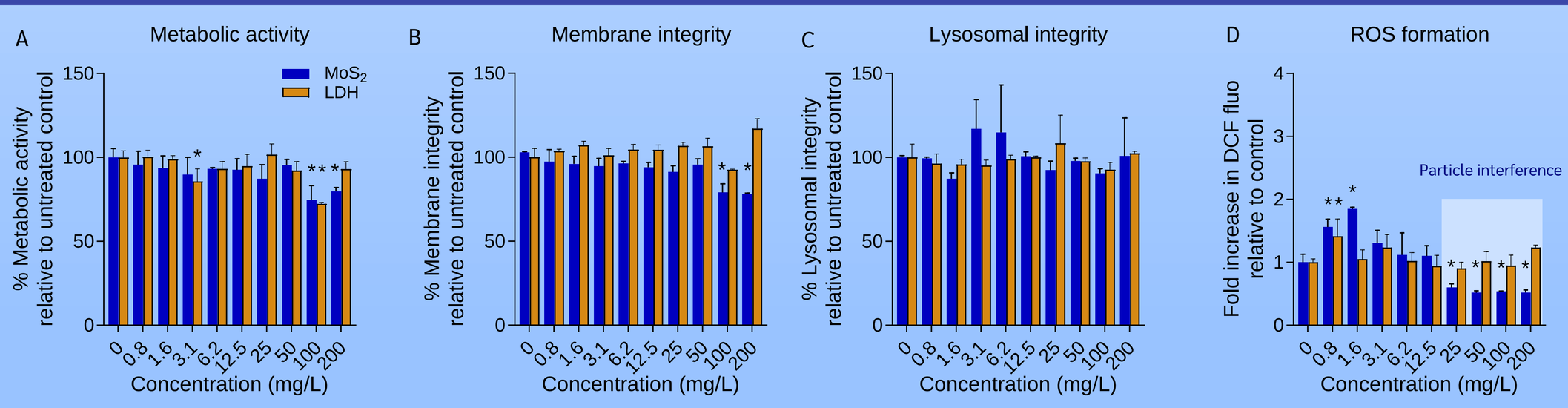


Table 1. Characterisation (polydispersity index and surface charge) of Mg-Al-LDH and MoS_2 nanosheets in MilliQ water and ZFL cell culture media (without FBS) over time.

Nanomaterial	Media	Polydispersity index	Zeta potential (mV)
Mg-Al-LDH	MQ	0.14 ± 0.005	42.5 ± 0.4
	ZFL media 0h	0.22 ± 0.03	- 7.6 ± 1.2
	ZFL media 24h	0.35 ± 0.05	- 12.4 ± 0.4
MoS_2	MQ	0.9 ± 0.09	- 49.7 ± 4.2
	ZFL media 0h	0.61 ± 0.04	- 20.4 ± 0.4
	ZFL media 24h	0.24 ± 0.01	- 19.1 ± 0.5

Figure 2. Characterisation of (A) Mg-Al-LDH , (B) MoS_2 nanosheets and (C) their stability over time in the ZFL exposure media by dynamic light scattering.

Effects of MoS_2 and Mg-Al-LDH nanosheets



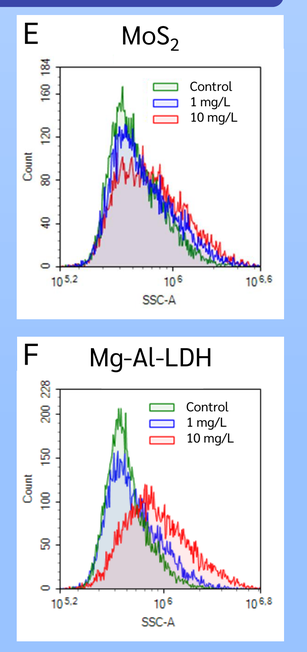


Figure 3. Effects of MoS_2 and Mg-Al-LDH nanomaterials on the (A) metabolic activity, (B) membrane integrity, (C) lysosomal integrity and (D) reactive oxygen species formation on the ZFL cell line. The cells were exposed for 24h to increasing concentration of the nanomaterials. Cell interaction assessment of (E) MoS_2 and (F) Mg-Al-LDH nanomaterials by flow cytometry (forward and side scatter analysis) after exposure to 1 and 10 mg/L for 24 hours. Asterisks denote significant difference from the untreated controls.

HIGHLIGHTS

- **Mg-Al-LDH NM** did not induce adverse effects
- **MoS_2 NM** led to decreased metabolic activity and membrane integrity at highest conc. and induced ROS at the lowest conc.
- ROS formation was the most sensitive endpoint (effects at the lowest conc.)
- **MoS_2 particle interference** observed with the ROS assay (at conc. > 12.5 mg/L)
- Trial studies show concentration-dependent NM uptake by FCM

ONGOING WORK

- Cellular uptake studies (ICP-MS and flow cytometry)
- Genotoxicity and gene expression studies (immune response genes, metabolism, xenobiotic defense)
- Comparison of human vs zebrafish liver cell responses

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