Metabolic reprogramming of classically activated microglia by surface-functionalized enzymatic nanoreactors

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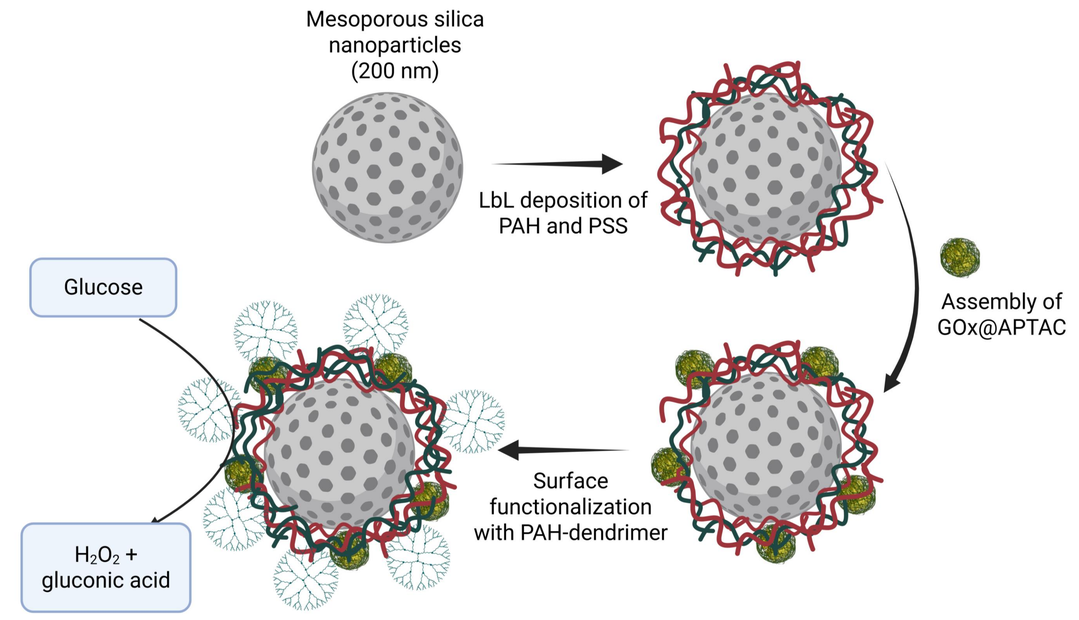
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ABSTRACT

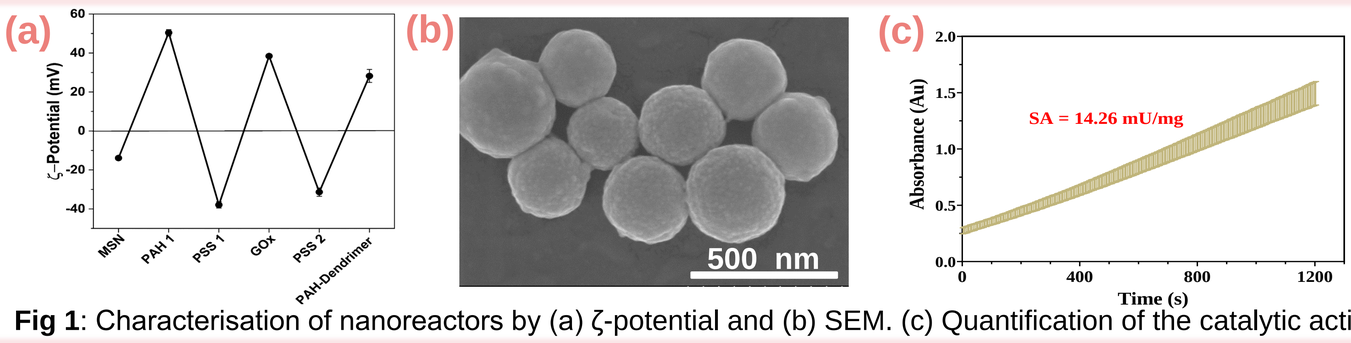
Microglia, the resident immune cells of the brain, maintain cerebral homeostasis by exhibiting metabolic plasticity. The rapid switch between the pro-inflammatory and anti-inflammatory state is governed by distinct cytokine secretions making **metabolic reprogramming** a potent therapeutic approach. By assembling **glucose oxidase (GOx)** encapsulated in (3-Acrylamidopropyl)trimethylammonium chloride (GOx@APTAC), a **single enzyme nanogel (SENs),** in nanoreactors and functionalizing the surface of the nanoreactors with a **G4-dendrimer**, we successfully alleviated **inflammation** in LPS-activated microglia.

METHODS

Layer-by-layer (LbL) approach



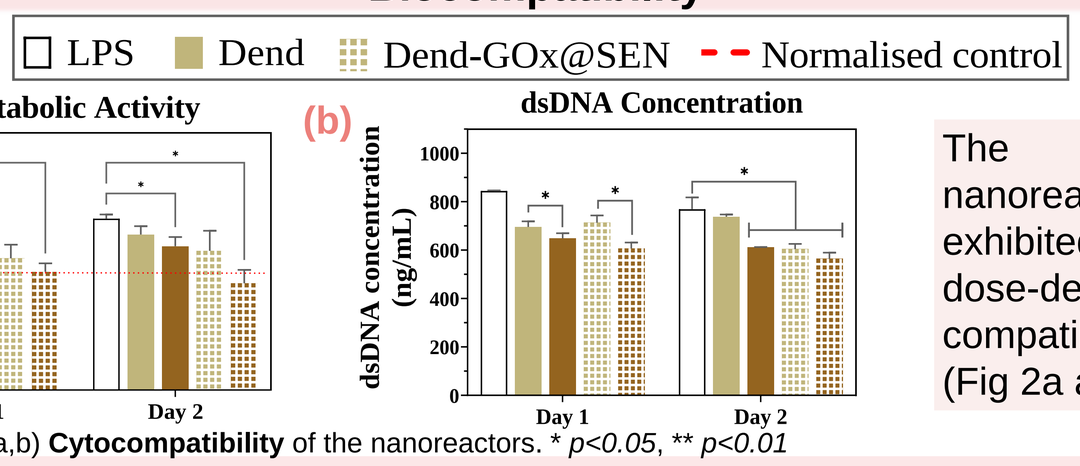
Characterisation of Nanoreactors



The correct assembly of the building blocks (PAH, PSS, GOx@APTAC) and the dendrimer was demonstrated by ζ-potential (Fig 1a). The nanoreactors preserved their catalytic activity after the synthesis procedure (Fig 1c).

Fig 1: Characterisation of nanoreactors by (a) ζ-potential and (b) SEM. (c) Quantification of the catalytic activity following the Michaelis-Menten equation.

Biocompatibility



The nanoreactors exhibited dose-dependent compatibility (Fig 2a and b).

Fig 2: (a,b) **Cytocompatibility** of the nanoreactors. * *p<0.05*, ** *p<0.01*

Internalisation

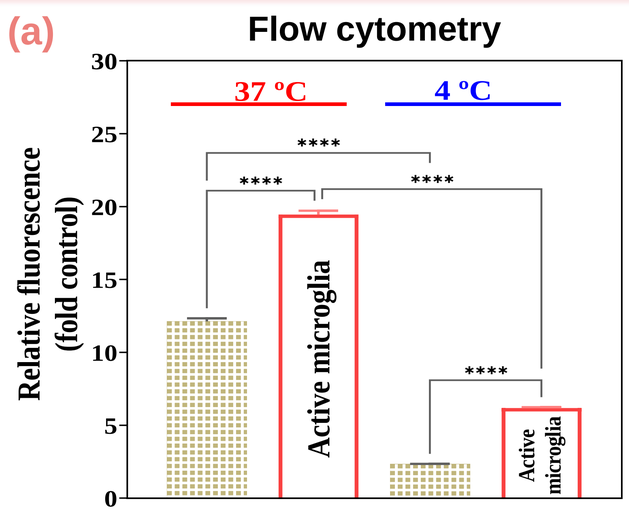


Fig 4. (a) Quantification of fluorescent signal in cells. **** *p<0.05.* (b) Confocal images of **internalised nanoreactors**.

RESULTS

Genetic Profiling

Pro-Inflammatory / Anti-Inflammatory

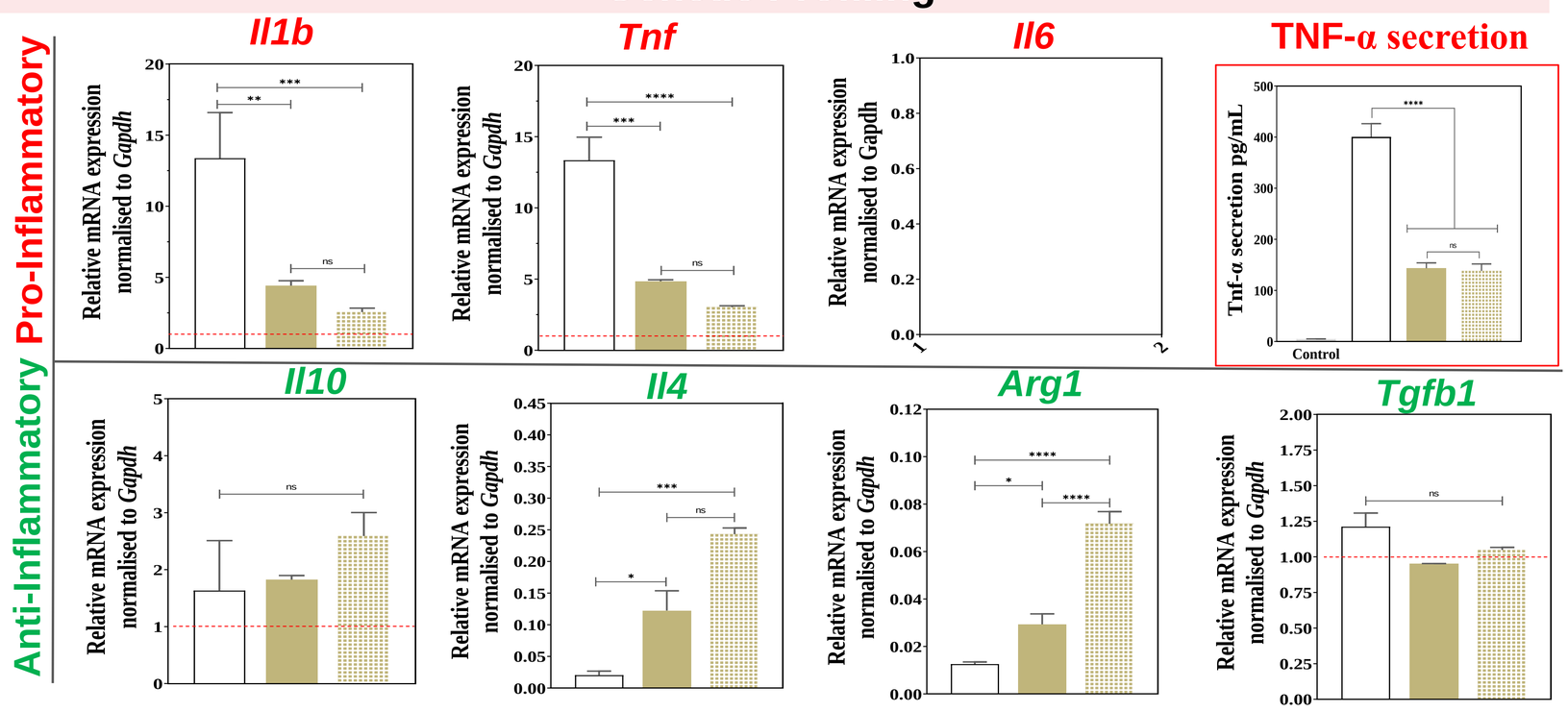


Fig 3: The **gene expression** of microglia quantified by **RT-qPCR**. Cells were activated with **100 ng/mL of LPS** for 14 h, prior to the 24 h incubation with nanoreactors. (ns *p>0.05*, * *p<0.05*, ** *p<0.01*, *** *p<0.001, **** p<0.0001).*

>98% of the nanoreactors were **internalised** after 4 h via energetic and non-energetic pathways (Fig 4a and b). They induced a **~70% downregulation** in pro-inflammatory cytokines and **upregulated** the expression of anti-inflammatory cytokines by **~20 folds** (Fig 3).

CONCLUSIONS

- ✓ **Metabolic Reprogramming:** GOx@APTAC nanoreactors successfully shift inflamed microglia toward a protective state.
- ✓ **Internalisation Mechanism**: Dendrimer-functionalisation facilitates nanoreactor uptake via dual energetic and non-energetic mechanisms.
- ✓ **Platform Versatility**: The LbL-SEN approach provides an effective vehicle for delivering bioactive enzymes into the intracellular environment.

REFERENCES

[1] J. Cheng et al., 2021, J. Neuroinflammation,18, 2021.

[2] A. Mirarchi et al., 2024, Int. J. Mol. Sci., 25.

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