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Advancements in mucosal delivery of *Porphyromonas gingivalis* antigens using chitosan-coated PLGA nanoparticles

NANOTECHNOLOGIES

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INTRODUCTION

Emerging evidence is pointing towards a potential etiological link between *Porphyromonas gingivalis* (*Pg*) and Alzheimer's disease (AD) — *Pg* might infiltrate systemic circulation via weakened oral/intestinal barriers, subsequently breaching the blood-brain barrier (BBB) and precipitating AD pathology within the brain. **APPROACH:** An oral nanovaccine based on total *Pg* antigens targeting the gut-associated lymphoid tissue (GALT) may elicit both mucosal and systemic immunity, thereby hampering *Pg* ability to breach the oral/intestinal barriers and the BBB. **AIM OF THE STUDY:** Optimization and *in vitro* evaluation of a candidate chitosan-coated poly(lactic-co-glycolic acid) (PLGA-CS) nanovaccine with suitable characteristics for oral delivery.

METHODS

1. Preparation and characterization of *Pg* antigens;
2. Preparation of PLGA-CS nanocarrier by double emulsion solvent evaporation method [2] — optimization of process and formula parameters to target the desired nanocarrier features: mean particle size 200-350 nm, polydispersity index (PdI) < 0.3, and positive ζ-potential;
3. Preparation and characterization of the nanovaccine — particle size, PdI, ζ-potential, encapsulation efficiency (EE), drug loading (DL), morphology;
4. Integrity assessment of encapsulated *Pg* extract antigens — SDS-PAGE and Western blotting;
5. Cellular assays of nanovaccine using THP-1 macrophages — cell uptake and viability studies.

RESULTS/DISCUSSION

— The candidate PLGA-CS nanocarrier was successfully produced and optimized;

— Upon antigen encapsulation, the resulting nanovaccine presented suitable characteristics for oral delivery;

— Encapsulation process proved effective at preserving antigen integrity;

— The nanovaccine was taken up by macrophages, while showing low cytotoxicity.

Process/Formula Optimization

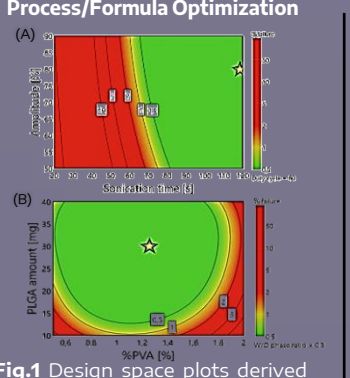

Fig.1 Design space plots derived from the models for process (A) and formula (B) optimization of the nanocarrier. Optimal setpoint represented by '☆'.

Nanovaccine characterization

Tab.1 Characterization of *Pg* extract-loaded PLGA-CS NPs (mean ± SD, n = 3).

NPs Form.	Extract (µg)	Size (nm)	PdI	ζ (mV)	EE (%)	DL (%)
Empty	0	305.0 ± 4.3	0.213 ± 0.013	+18.4 ± 1.1	–	–
Extract-loaded	250	309.8 ± 4.6	0.214 ± 0.012	+18.4 ± 0.4	39.4 ± 5.7	0.33 ± 0.05
	500	314.8 ± 15.7	0.226 ± 0.002	+19.4 ± 0.8	63.9 ± 5.1	1.07 ± 0.09
	750	334.6 ± 8.4**	0.230 ± 0.008	+22.8 ± 4.6	56.1 ± 2.1	1.40 ± 0.05
	1000	324.1 ± 7.2	0.233 ± 0.020	+18.5 ± 1.2	55.3 ± 3.5	1.84 ± 0.12

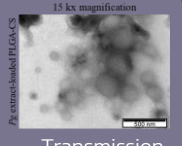

Fig.2 Transmission electron micrographs of *Pg* extract-loaded PLGA-CS NPs at 15 kx magnification.

Integrity of antigens

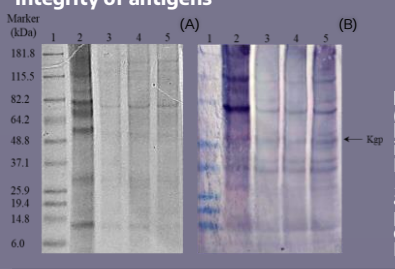

Fig.3 SDS-PAGE (A) and WB (B) of *Pg* extract antigens before and after encapsulation into the PLGA-CS nanocarrier. Lanes: (1) MW marker (2) native *Pg* extract antigens; *Pg* extract antigens from PLGA-CS NPs prepared with antigen amounts of (3) 500, (4) 750, (5) 1000 µg. Kgp gingipain: 56 kDa.

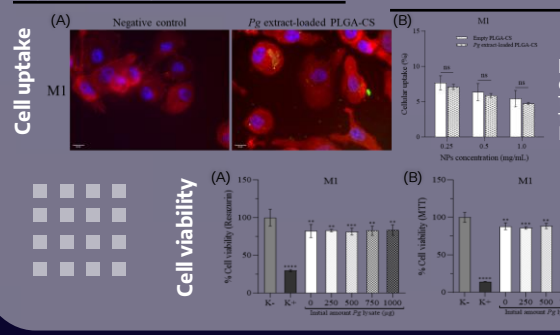

Fig.4 Qualitative (A) and quantitative (B) cell uptake evaluation by fluorescence microscopy and fluorometry in THP-1 M1 macrophages exposed for 2 h to empty and *Pg* extract-loaded coumarin-6-labelled NPs (green).

Fig.5 Cell viability by resazurin (A) and MTT (B) assays of THP-1 M1 macrophages exposed for 24 h to *Pg* extract-loaded PLGA-CS NPs with increasing amounts of *Pg* extract antigens (mean ± SD, n = 6). (K-) negative control (medium); (K+) positive control (SDS). Relative to K-: ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$.

CONCLUSION

These findings underscore the potential of PLGA-CS NPs as carriers for antigen mucosal delivery, paving the way for further investigations into their applicability as vaccine candidates against *Pg*.

INSTITUTION AND CONTACTS

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Acknowledgements: financial support from FCT (UIDP/04138/2020, CEECIND/03143/2017, 2021.07660.BD)

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TEXAS The University of Texas at Austin