Characterization of a P407 hydrogel dispersing liposomes for sustained release of dexamethasone for deposition on an *in vitro* RWM model

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1. Introduction

Sensorineural hearing loss is a prevalent and often irreversible condition resulting from damage to the inner ear structures or auditory nerve. Current treatments are limited, highlighting a need for innovative therapeutic approaches that can deliver drugs directly to the inner ear. Dexamethasone (Dexa) has shown efficacy in protecting hair cells in the cochlea, but challenges in sustained delivery limit its therapeutic use. The aim of this work is therefore to explore a novel drug delivery system that encapsulates Dexa within liposomes produced via an innovative PGSS (Particles from Gas Saturated Solutions) method based on supercritical CO_2 ($scCO_2$) previously developed in our lab [1]. Liposomes will be dispersed in a Poloxamer 407 (P407) hydrogel that, thanks to its thermosensitive properties, forms a gel at body temperature to allow prolonged and localized drug release directly at the cochlear site. An *in vitro* Round Window Membrane (RWM) model will be used to assess the release and predict the *in vivo* diffusion of Dexa in the inner ear.

2. Materials & Methods

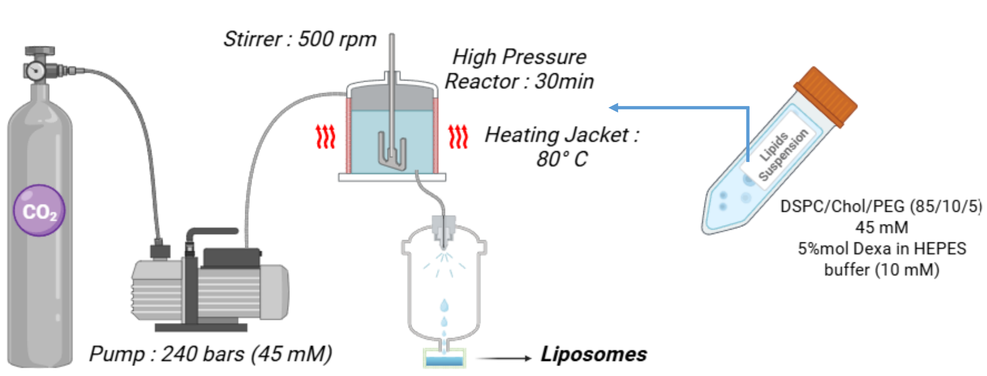


Figure 1. Liposome production method [1,2]

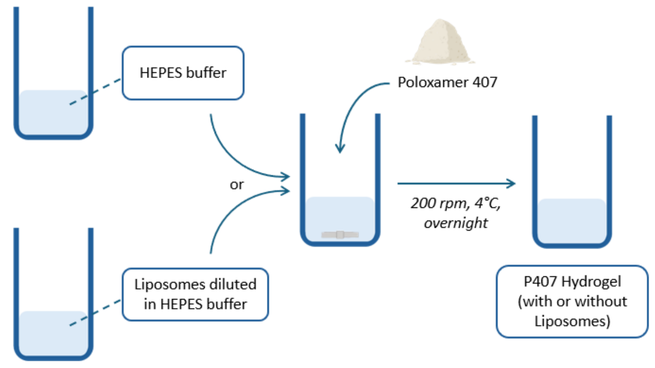


Figure 2. Hydrogel production method [2]

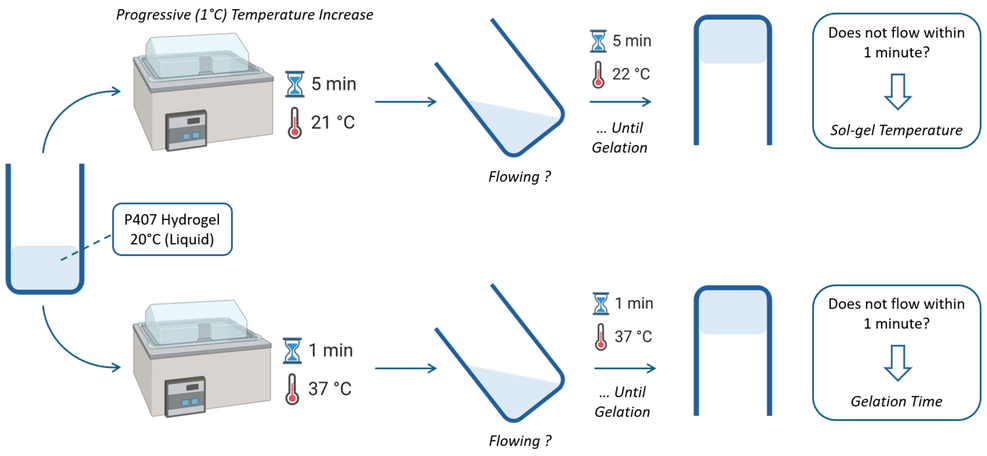


Figure 3. Sol-gel Temperature & Gelation Time determination [2]

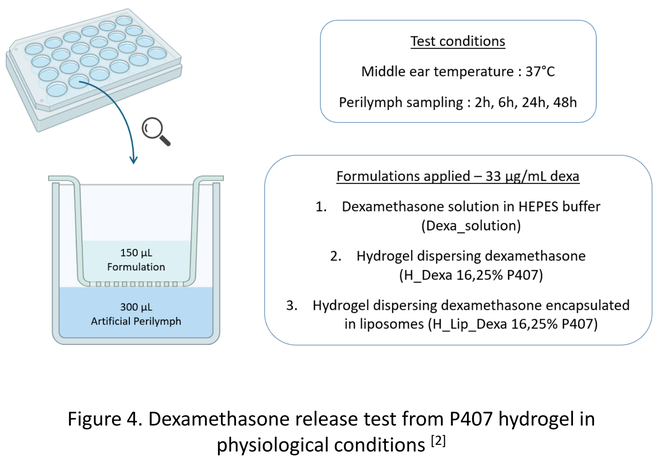
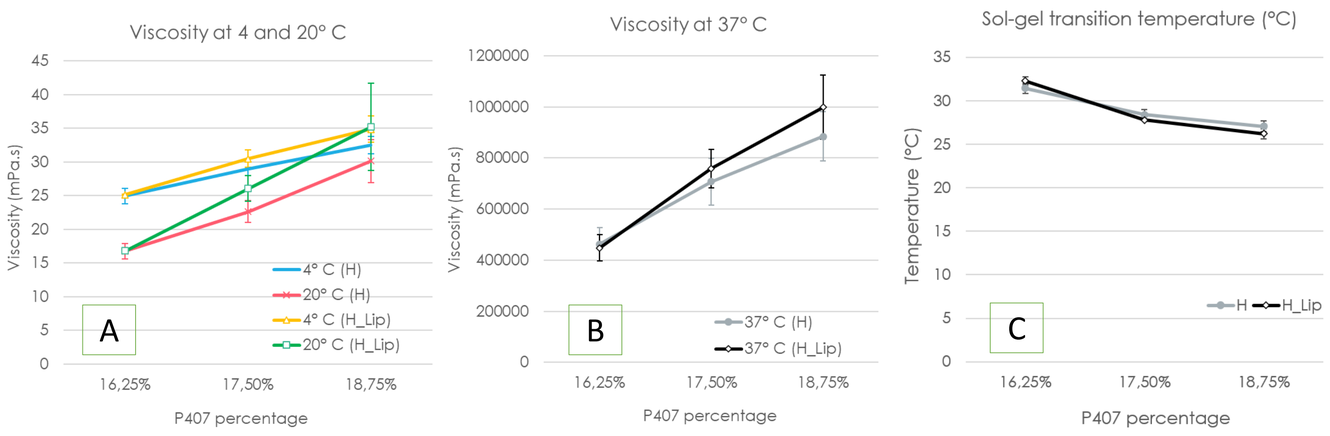


Figure 4. Dexamethasone release test from P407 hydrogel in physiological conditions [2]

3. Results & Discussion



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P407	With Liposomes	Without Liposomes
16,25%	2 ± 0 min	1 ± 0 min
17,50%	1 ± 0 min	1 ± 0 min
18,75%	1 ± 0 min	1,3 ± 0,6 min

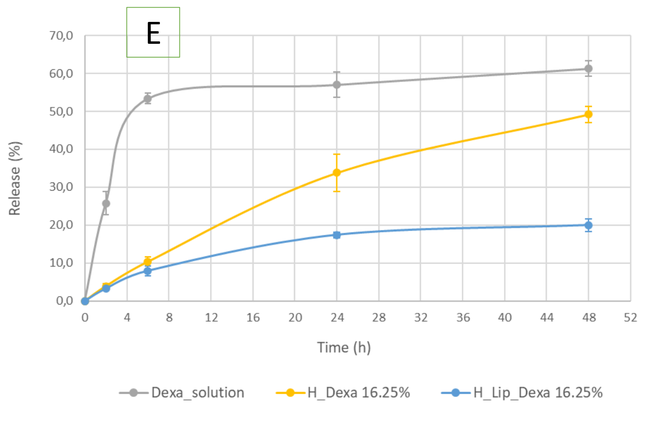
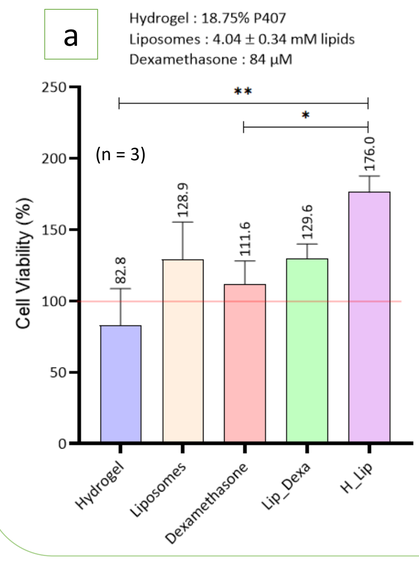


Figure 5. Viscosity (mPa.s) as a function of P407 percentage at 4 and 20°C for liquid formulations (A) and at 37°C for gelled formulations (B) on hydrogels dispersing liposomes (H_Lip) or not (H). Sol-gel transition temperature (C) and gelation time (D) determined by visual tests. Release profiles (E) of dexamethasone over time when in aqueous solution (Dexa_solution), in a hydrogel with 16.25% P407 (H_Dexa 16.25%), or encapsulated in liposomes dispersed in a hydrogel with 16.25% P407 (H_Lip_Dexa 16.25%).



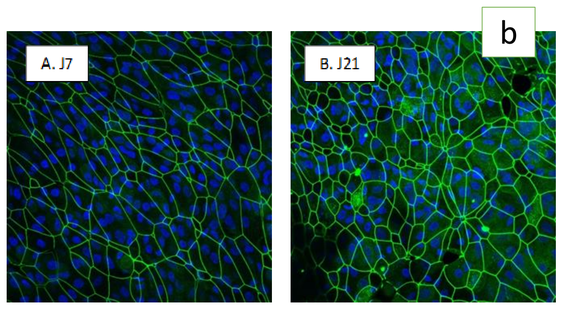


Figure 6. (a) Comparison of the viability of human small airway epithelial (hSAE) cells exposed for 24h to different formulations: a P407 hydrogel, liposomes, dexamethasone, liposomes encapsulating dexamethasone (Lip_Dexa) and a hydrogel dispersing liposomes (H_Lip). (b) Evolution of tight junction formation (anti-ZO1 targeting) in an epithelium composed of hSAE cells after 7 (A) and 21 (B) days of cellular differentiation at the air–liquid interface.

All P407 hydrogels were liquid at 20°C but gelled at 37°C (Figure 5). Higher P407 concentrations increased viscosity and decreased sol-gel transition temperature from 31.4°C to 27.1°C. Gelation time at 37°C was short as expected (± 1.0 min). Adding liposomes did not significantly affect hydrogels properties. The 16.25% P407 hydrogel provides sustained release of Dexa (33.8 ± 5.0 % after 24h), compared to a solution (53.4 ± 1.4 % after only 6h) and this effect is even more pronounced when Dexa is encapsulated in liposomes within the hydrogel (17.4 ± 0.8 % after 24h). The P407 hydrogel, liposomes and Dexa are biocompatible with hSAE cells (Figure 6). The biocompatibility of these formulations will soon be assessed *ex vivo* using cochlear explants. An *in vitro* RWM model in an air-liquid interface was also developed with hSAE cells. As a first step, the integrity of the epithelium was assessed through tight junction staining (ZO-1) following 21 days of differentiation. TEER measurements and a paracellular flux assay with FITC-Dextran will also be conducted to complete the characterization of the membrane.

4. Conclusion & Perspectives

P407 hydrogels offer injectability at 20°C and gelation at 37°C, aiming to prolong Dexa release. Indeed, 16.25% P407 hydrogels demonstrated a prolonged release effect compared to a solution and incorporating it into liposomes further extended this release. The identified gelation time is very short, allowing the patient to remain in a lateral decubitus position for only a few minutes post-administration. All components of the formulation are biocompatible. After completing the characterization of the RWM model, penetration tests will be conducted (Figure 7).

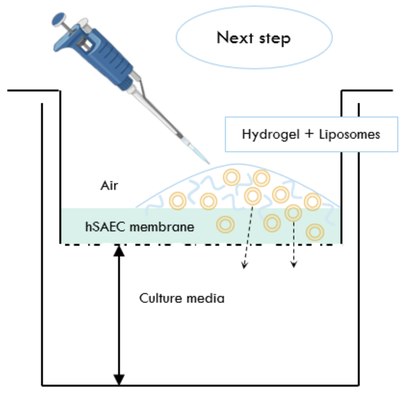


Figure 7. Penetration test of a P407 hydrogel containing liposomes through an *in vitro* RWM model.

5. References

[1] Penoy, N.; Delma, K.L.; Homkar, N.; Karim Sakira, A.; Egrek,S.; Sacheli, R.; Sacré, P.-Y.; Grignard, B.; Hayette, M.-P.; Somé, T.I.; Semdé, R.; Evrard, B. and Piel, G. Development and optimization of a one step process for the production and sterilization of liposomes using supercritical CO_2, Int. J. Pharm. 651, 123769 (2024).

[2] Created with BioRender.

6. Acknowledgements

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