Size exclusion chromatography of radioactive polymers (SERP) for tracking in vivo biodegradation of nanoparticles

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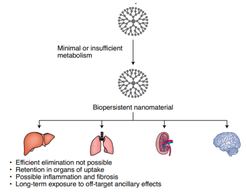
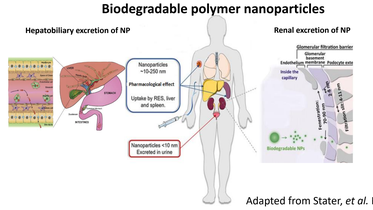
INTRODUCTION

- Clinical translation of new excipients used in nanomedicines requires characterizing their *in vivo* pharmacology (absorption, distribution, metabolism and excretion)
- *In vivo* biodegradation of polymer can inform on their safety
- New, simple, and broadly applicable methods are needed to track polymeric molecules and their degradation products after *in vivo* administration

Biochemically stable polymer nanoparticles



Biodegradable polymer nanoparticles



Adapted from Stater, *et al.* Nat. Nanotech., 2021

HYPOTHESIS

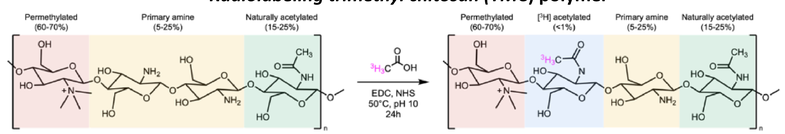
Radiolabeling and chromatography can be combined to inform on the degradation and excretion of pharmaceutically-relevant biopolymers

OBJECTIVE

To develop a method for tracking the biodisposition and evaluating biodegradation of model polymeric nanoparticles in complex biological media

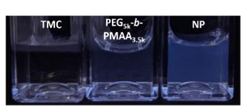
METHODS

Radiolabeling trimethyl chitosan (TMC) polymer

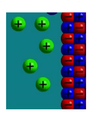


Scheme for acetylation of TMC with [^{3}H]-acetic acid forming radiolabeled TMC

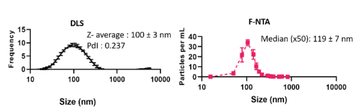
TMC nanoparticle synthesis, purification and characterization



Nanoparticles prepared by ionic gelation of TMC and PEG-PMAA resulted in opalescent formulations

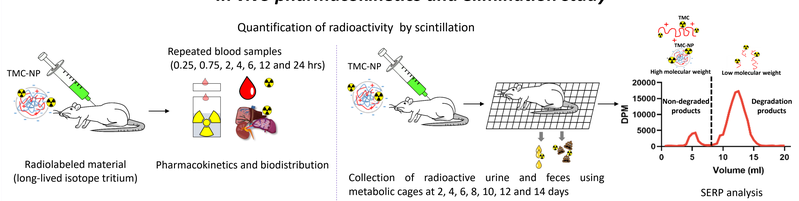


Negatively charged nanoparticles were separated from free TMC via Ion exchange chromatography



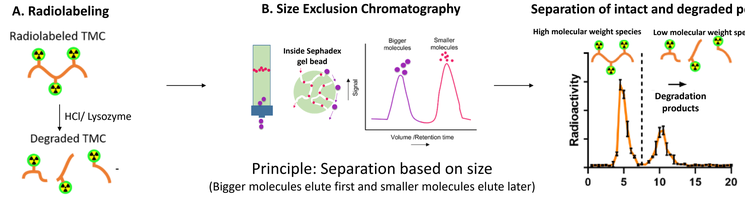
TMC nanoparticles prepared using a fluorescent PEG-PMAA-Cy3 polymer, observed by fluorescence nanoparticle tracking analysis (F-NTA), had sizes similar to those measured by dynamic light scattering (DLS)

In vivo pharmacokinetics and elimination study



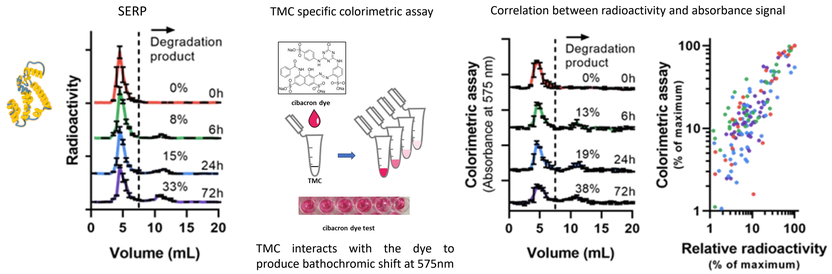
RESULTS

What is SERP?



Principle: Separation based on size
(Bigger molecules elute first and smaller molecules elute later)

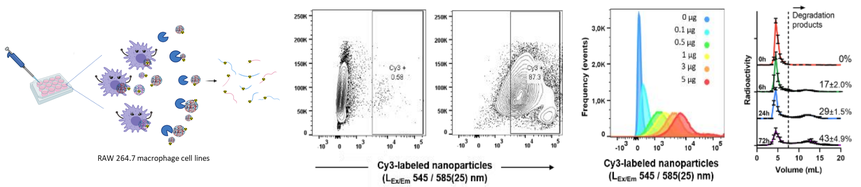
1 *The radiolabel remains attached to TMC and its degradation products upon hydrolysis with lysozyme*



TMC interacts with the dye to produce bathochromic shift at 575nm

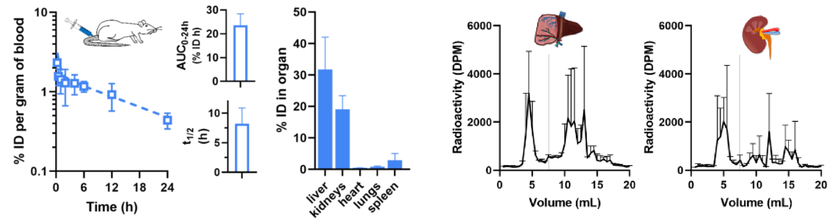
SERP quantified the proportion of degradation products vs. the larger molecules eluting in the void volume. Good correlation is obtained between the radioactivity signal and the TMC-specific colorimetric assay. This support that the radiolabel remains attached to the TMC fragment after hydrolysis. Panels represent means +/- SD (n = 3)

2 *Flow cytometry, followed by SERP analysis shows that RAW macrophages can degrade TMC nanoparticles*



RAW 264.7 macrophages internalize Cy3-labelled TMC nanoparticles in a concentration dependent manner. Interactions of TMC nanoparticles with macrophages result in the gradual degradation of the polymer over time. Data represent means ± SD (n = 3)

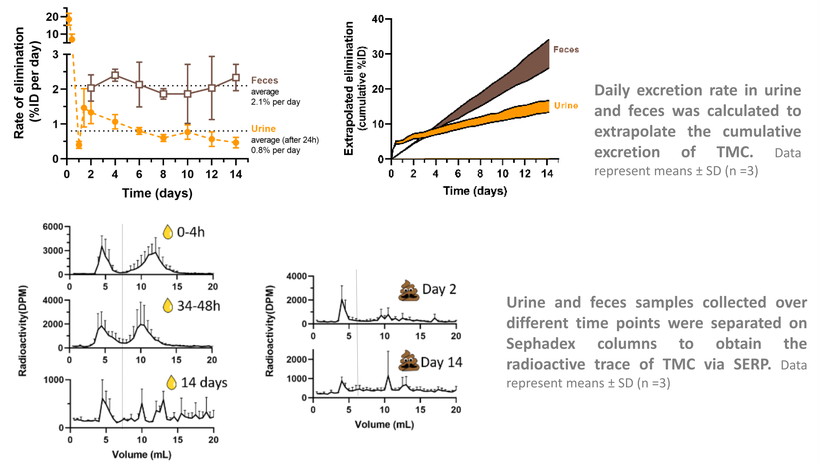
3 *Biodistribution of TMC nanoparticles, followed by SERP revealed the production of degradation products within organs*



Pharmacokinetic parameters and biodistribution of radiolabeled TMC nanoparticles in rats showed rapid clearance from the blood and distribution in organs of mononuclear phagocyte system. Data represent means ± SD (n = 3)

SERP confirmed the presence of large molecular weight species in the organs. Degradation products were observed mostly in the liver. Data represent means ± SD (n = 3)

4 *SERP analysis on urine and feces samples showed the presence of excreted native and degraded TMC*



Daily excretion rate in urine and feces was calculated to extrapolate the cumulative excretion of TMC. Data represent means ± SD (n =3)

Urine and feces samples collected over different time points were separated on Sephadex columns to obtain the radioactive trace of TMC via SERP. Data represent means ± SD (n =3)

CONCLUSIONS and PERSPECTIVES

- Size Exclusion of Radioactive Polymers (SERP) allows to monitor biodegradation
- SERP can be used in cell culture, *in vivo* and in excreta
- Trimethyl chitosan was degraded *in vivo* and excreted in urine and feces of rats
- SERP could be applied to determine the biological fate of other polymers used for drug delivery such as poly(lactic acid), poly ε(caprolactone), etc

REFERENCES

- Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Guidance for Industry Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients 2005 FDA-2002-D-0188.
- A. Dikpati, et al., Size Exclusion of Radioactive Polymers (SERP) informs on the biodegradation of trimethyl chitosan and biodegradable polymer nanoparticles *in vitro* and *in vivo*. *J Control Release*. 2022, 346:20-31.

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