In-vivo study of self-assembled glycol chitosan nano-radiopharmaceutical for liver imaging

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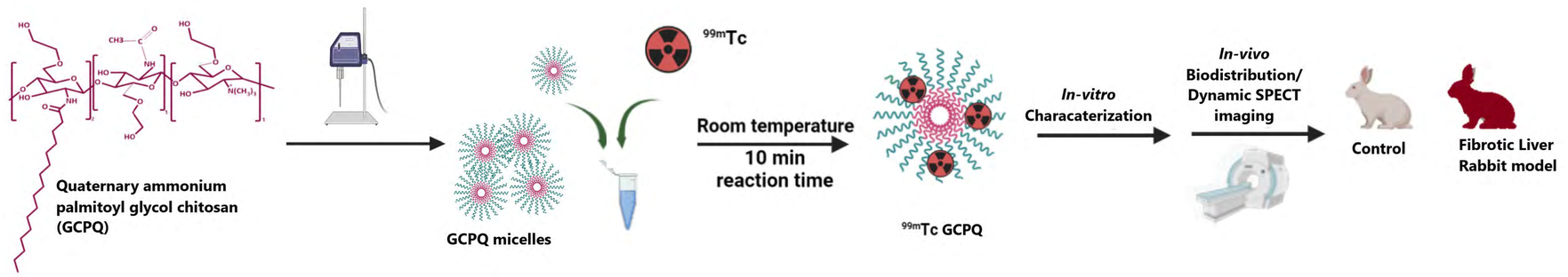
1. Introduction

Hepatocellular carcinoma (HCC) constitutes about 90 % of primary liver cancer cases but its prognosis is very poor (median survival of < 1 year, after diagnosis). The poor prognosis is associated with delayed diagnosis that comes with advanced stage (highly dispersed cancer) and limited treatment options. Ability to non-invasively image hepatocyte's functional capacity would be helpful in early diagnosis of HCC and other hepatocyte-specific diseases. We propose to develop GCPQ (quaternary ammonium palmitoyl glycol chitosan) polymeric micelles, loaded with radiotracer (Tc-99m), with preferential localization to hepatocytes for non-invasive imaging of hepatocyte's functional capacity.

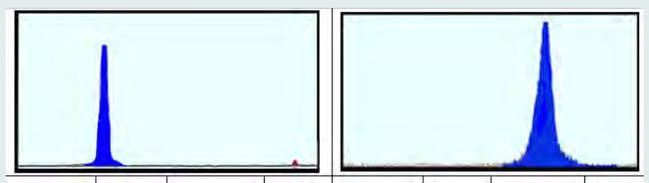
2. Methodology



Schematic illustration of the radiolabelling of GCPQ polymeric micelles with Technitium-99m (^{99m}Tc) and subsequent *In-vitro* and *In-vivo* studies.

3. Characterization

Percentage Radiolabeling

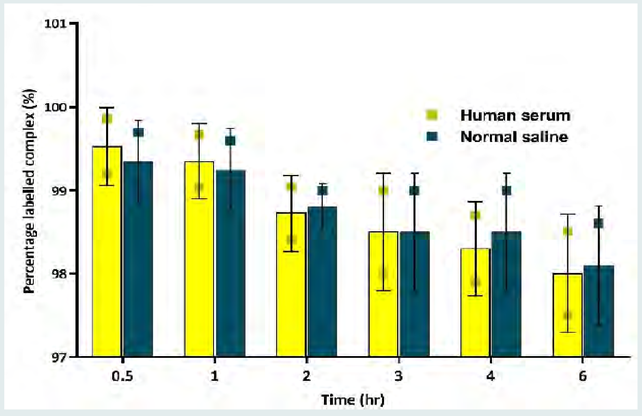


Tc-99m	Color	Retention (Rf)	ROI (%)	Tc-99m	Color	Retention (Rf)	ROI (%)
Complex + HR		0.056	99.75	TcO_2		0.011	0.03
TcO_4^{-1}		0.996	0.25	Complex + TcO_4^{-1}		0.672	99.87

Normal saline- Strip 1 Acetone- Strip 2

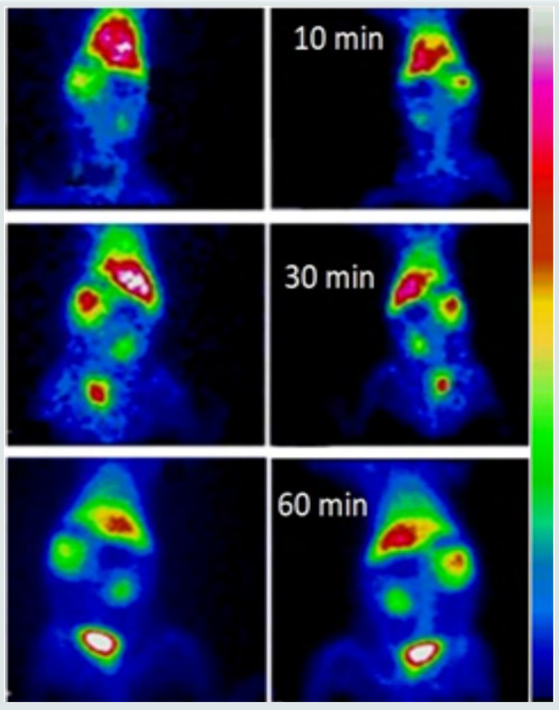
Chromatogram of radiochemical analysis showing labelling efficiency of ^{99m}Tc GCPQ micelles using double-strip method. Strip one, ITLC-SG Chromatogram of ^{99m}Tc GCPQ micelles and hydrocolloids at RF = 0 and Free TcO_4^{-1} at Rf = 1. Strip two, ITLC-SG Chromatogram of hydrocolloids at RF = 0 and Free TcO_4^{-1} and ^{99m}Tc GCPQ micelles at Rf = 0.672.

Serum radiolabeling stability

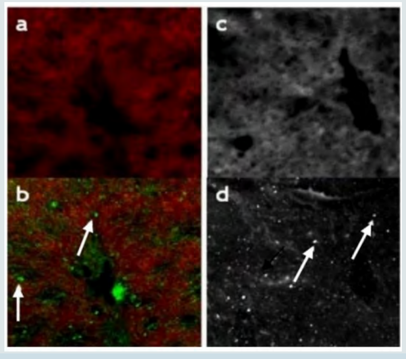


***In vitro* radiolabeling stability of ^{99m}Tc GCPQ micelles at 37 °C in normal saline and human serum over a period of 6 h, with the standard error of the mean (SEM), n=3. Paired t-test.**

4. Biodistribution study

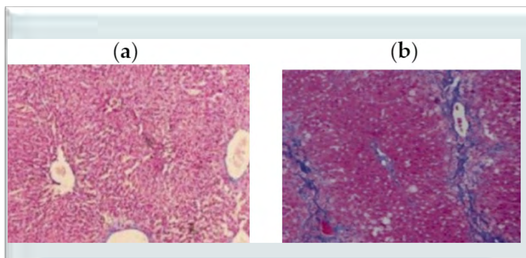


Representative dynamic SPECT images were taken by Dual-Head Gamma Camera (Anterior: right and Posterior: left) after IV administration of ^{99m}Tc GCPQ micelles at 10, 30, and 60 min.

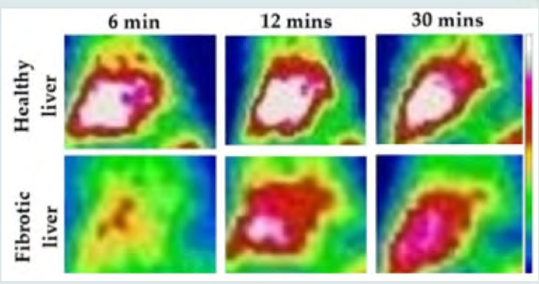


Excised liver tissue images captured after 1 h after FITC GCPQ/India ink administration; (a,b) confocal images, green spots of FITC GCPQ in hepatocytes and black spot of India ink in Kupffer cells; (c,d) light microscope images, white spots of FITC GCPQ in hepatocytes and black spotS of India ink in Kupffer cells, *n* = 3

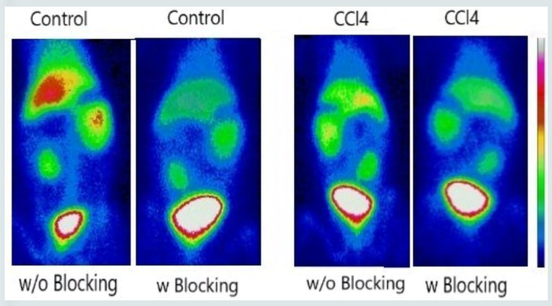
5. Fibrotic liver model study



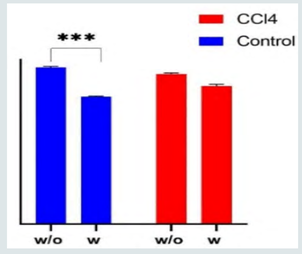
Masson's-stained liver tissue of; (a) Healthy rabbit liver; (b) fibrotic rabbit liver after CCl_4 administration for 9 weeks, fibrotic liver tissue is showing increased collagen (purple)



Representative ^{99m}Tc GCPQ dynamic SPECT images at different time points for fibrotic and healthy rabbit liver.



Blocking studies of ^{99m}Tc GCPQ in fibrotic mice. Representative SPECT image of healthy and fibrotic-liver rabbit at 60 min pi ^{99m}Tc GCPQ (80 MBq) with or without GCPQ for blocking. Images were adjusted using the same scale for all animals.



Hepatic uptake of ^{99m}Tc GCPQ derived from SPECT images by drawing the ROI of the whole liver, uptake is normalized for each group, $p < 0.001$.

Acknowledgements

Reference

Nashmia Zia, Zafar Iqbal, Abida Raza, Aadarash Zia, Rabia Shafique, Saiqa Andleeb, and Gilbert C. Walker. 2022. "Glycol-Chitosan-Based Technetium-99m-Loaded Multifunctional Nanomicelles: Synthesis, Evaluation, and In Vivo Biodistribution" *Nanomaterials* 12, no. 13: 2198. https://doi.org/10.3390/nano12132198