

## RADIOLABELING OF MAGNETIC TARGETED CARRIERS WITH SEVERAL THERAPEUTIC AND IMAGING RADIOISOTOPES

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**INTRODUCTION:** Magnetic Targeted Carriers (MTCs) are magnetic microparticles made from metallic iron and activated carbon [1]. The average microparticle diameter is approximately 1  $\mu\text{m}$  (0.5-5  $\mu\text{m}$ ). The activated carbon component of the MTCs is capable of adsorbing a wide variety of pharmaceutical agents including chemotherapeutic drugs such as doxorubicin. This technology is under clinical investigation in a Phase I/II clinical trial in patients with hepatocellular carcinoma investigating the safety and tolerability profile of MTC-Doxorubicin in human patients [2].

The efficacy of a chemotherapeutic treatment depends on the tumor sensitivity to the drug as well as the effective intra-tumoral drug concentration. Radiation therapy is a good alternative for some chemoresistant tumors. Furthermore, local or intra-tumoral radiotherapy could be a better therapeutic approach as it would minimize side effects typically associated with external beam radiation therapy. We recently prepared MTC microparticles labeled with the therapeutic radioisotope <sup>188</sup>Re [3]. The labeling efficiency was higher than 95% and the *in vitro* characteristics satisfactory. However, due to the limited availability of Re generators, we chose to study other commercially available radionuclides. We investigated the radiolabeling of MTCs with <sup>90</sup>Y, <sup>111</sup>In, <sup>125</sup>I and <sup>131</sup>I. The physical properties of these radioisotopes are summarized in Table 1. These radioisotopes have been used for many years in diagnostic and therapeutic radiopharmaceuticals.

The aim of this paper is to report the optimal conditions for the radiolabeling of MTCs with these radioisotopes and testing their stabilities *in vitro*.

Table 1. Physical properties of radioisotopes used for MTC radiolabeling.

Isotope	Decay mode	Half-life	Max. Particle Energy (%)	Max. Range	$\gamma$ Energy (%)
<sup>90</sup> Y	$\beta$	2.67 d	2.29 MeV (100)	11.9 mm	-
<sup>111</sup> In	$\gamma$	2.83 d	-	-	171 keV (90) 245 keV (94)
<sup>131</sup> I	$\beta, \gamma$	8.0 d	807 keV(1) 606 keV(86) 336 keV(13)	2.4 mm	364 keV (81)
<sup>125</sup> I	EC	60 d	-	0.02 mm	35 keV (7)

**METHODS:** Several methods to bind <sup>90</sup>Y, <sup>111</sup>In, <sup>125</sup>I and <sup>131</sup>I to MTCs were investigated. Direct incubation of MTCs with the radioisotopes were studied as well as adsorption using different radiolabeled chelators or molecules (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid = DOTA; 2-p-aminobenzyl-1,4,7,10-

tetraazacyclo-dodecane-1,4,7,10-tetraacetic acid = ABz-DOTA), oxine for <sup>90</sup>Y and <sup>111</sup>In; iodogen, MIBG, and Iodohippurate. The parameters investigated were concentration, mole ratios, temperature, buffers and incubation time, were investigated. Thin Layer Chromatography (TLC) was used to analyze the chelation efficiency. The binding stability of the radiolabeled MTCs was determined in human plasma at 37 °C for 7 days for <sup>90</sup>Y or <sup>111</sup>In and for 28 weeks for <sup>131</sup>I by measuring the activity released in human plasma and comparing it to the activity still bound on the MTCs

**RESULTS:** The chelation and binding efficiencies of MTCs labeled with different radioisotopes after optimization of the reaction parameters are summarized in Table 2.

Table 2. Optimized chelation and binding efficiencies.

Radiolabeled MTC	Chelation efficiency	Binding efficiency
<sup>90</sup> Y-MTC		98.5% $\pm$ 1.2% (n=3)
<sup>90</sup> Y-DOTA-MTC	97.0%	100% (n=3)
<sup>90</sup> Y-ABz-DOTA-MTC	99.7% $\pm$ 0.4% (n=4)	100% (n=12)
<sup>90</sup> Y-oxine -MTC	97.6% $\pm$ 0.1% (n=10)	100% (n=5)
<sup>111</sup> In-MTC		83.7 $\pm$ 1.2% (n=3)
<sup>111</sup> In-DOTA-MTC	95.1 $\pm$ 2.5% (n= 5)	84.9 $\pm$ 2.6% (n=3)
<sup>111</sup> In-ABz-DOTA-MTC	96.9% $\pm$ 2.2% (n= 5)	97.7 $\pm$ 0.9% (n=3)
<sup>111</sup> In-oxine -MTC	100%	100% (n=3)
<sup>125</sup> I-MTC	-	10.8% $\pm$ 5.2% (n=3)
<sup>125</sup> I-Iodogen-MTC		88.1% $\pm$ 2.0% (n=3)
<sup>131</sup> I-iodohippurate-MTC		76.1% $\pm$ 0.8% (n=3)
<sup>131</sup> I-MIBG-MTC		97.9% $\pm$ 0.1% (n=3)

Binding stability profiles of these different radiolabeled MTC products in human plasma at 37°C are shown in Figures 1 through 3.

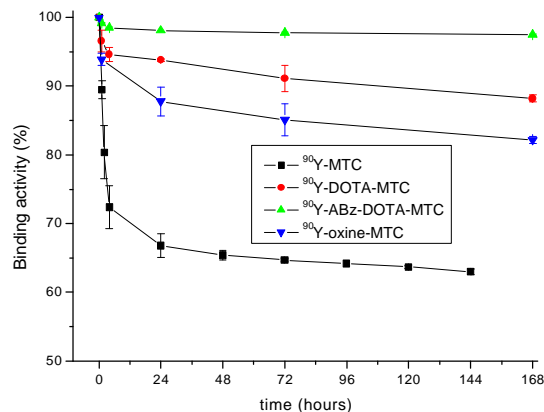


Fig. 1: Plasma stability of  $^{90}\text{Y}$ -MTC,  $^{90}\text{Y}$ -DOTA-MTC,  $^{90}\text{Y}$ -ABz-DOTA-MTC, and  $^{90}\text{Y}$ -oxine-MTC.

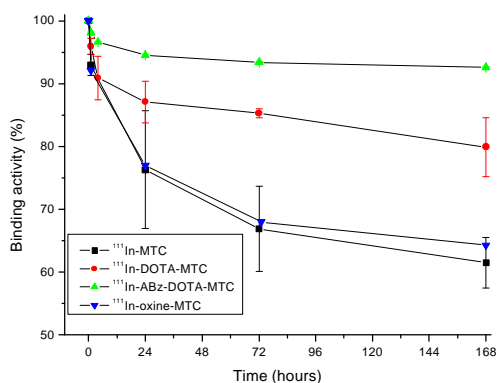


Fig. 2: Plasma stability of  $^{111}\text{In}$ -MTC,  $^{111}\text{In}$ -DOTA-MTC,  $^{111}\text{In}$ -ABz-DOTA-MTC and  $^{111}\text{In}$ -oxine-MTC.

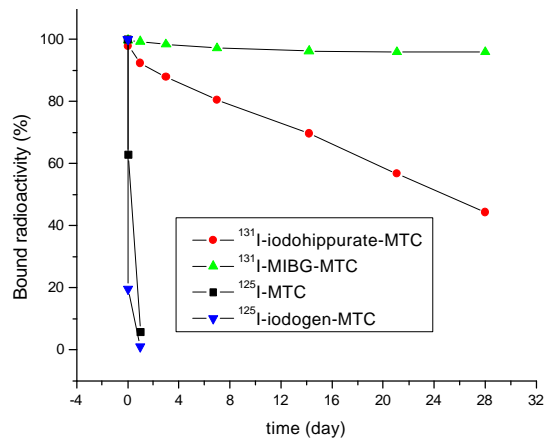


Fig. 3: Plasma stability of  $^{125}\text{I}$ -MTC,  $^{125}\text{I}$ -iodogen-MTC,  $^{131}\text{I}$ -iodohippurate-MTC and  $^{131}\text{I}$ -MIBG-MTC.

The TLC system consisting of Sil G/UV<sub>254</sub> as stationary phase and 8:2 of 10% NH<sub>4</sub>Ac (pH 4): methanol as the mobile phase is appropriate for the analysis of  $^{90}\text{Y}$ -labeled compounds (Figure 4). In this system, free  $^{90}\text{Y}^{3+}$  migrates with the solvent front ( $R_f$ : 0.8-1.0) while  $^{90}\text{Y}$ -MTC (or  $^{90}\text{Y}$  colloid) stays at the origin ( $R_f$ : 0). The  $R_f$  of  $^{90}\text{Y}$ -DOTA is 0.2-0.4 and  $^{90}\text{Y}$ -ABz-DOTA 0.3-0.5. This system is also suitable for the characterization of the  $^{111}\text{In}$ -compounds.

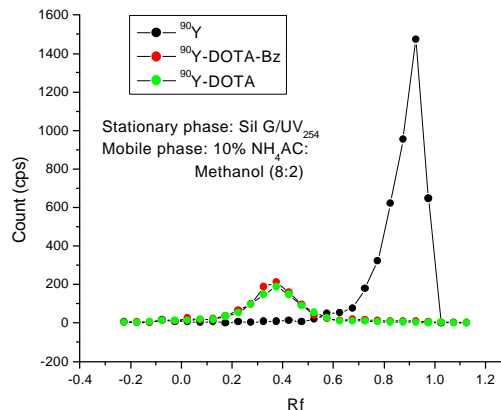


Fig. 4: TLC system for analysis of  $^{90}\text{Y}$ -DOTA,  $^{90}\text{Y}$ -ABz-DOTA and  $^{90}\text{Y}$ -oxine-MTC.

However, not every radiopharmacy uses a radiation scanner, and analysis should also be made possible by cutting the TLC in two pieces and counting them with a  $\gamma$ - or  $\beta$ -counter. We therefore evaluated additional TLC systems. The Tec-strip and ITLC systems with methanol as solvent, or Whatman 17 paper with water as solvent also separated free and chelated  $^{111}\text{In}$  ( $^{90}\text{Y}$ ) (Figure 5). The strips can both be cut into two pieces at 1 cm from origin and counted in a  $\gamma$ -counter. As In colloids could be counted together with free  $^{111}\text{In}$ , a control should be run to determine the eventual concentration of colloids vs free when using these three TLC systems.

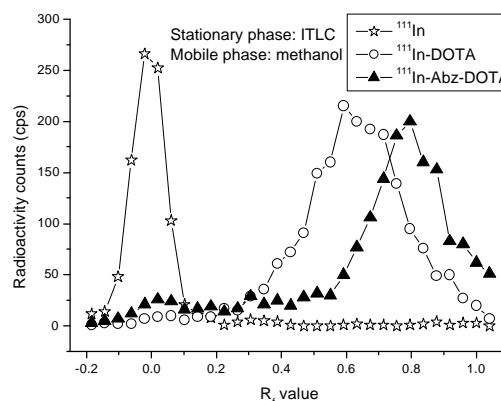


Fig. 5: TLC system for analysis of free  $^{111}\text{In}$ ,  $^{111}\text{In}$ -DOTA and  $^{111}\text{In}$ -ABz-DOTA.

**DISCUSSION & CONCLUSIONS:** *In vitro* results described here indicate the potential for the MTC technology to be used for the site-specific targeting of therapeutic and imaging radioisotopes. The chelation and binding efficiencies, and stability profiles in human plasma for  $^{90}\text{Y}$ -ABz-DOTA-MTC and  $^{131}\text{I}$ -MIBG-MTC are particularly encouraging.

MTCs can be targeted to specific sites in the human body using a small, externally positioned permanent magnet which creates a localized magnetic field within the body at the desired site. MTCs are administered intra-arterially with the catheter positioned proximal to the targeted area. The physical force created by the magnetic field pulls the MTCs out of circulation through the endothelial wall (extravasation) into the interstitial space, resulting in

localization and retention of the microparticles at the targeted site. The magnet is removed approximately 15 minutes after infusion of MTCs [2]. In the Phase I/II clinical trial investigating safety and tolerability profile in patients with primary liver cancer, MTC-Doxorubicin microparticles have been shown to be efficiently targeted to liver tumors. In addition, it had been previously demonstrated in healthy swine that MTCs were efficiently and selectively targeted to different organs using  $^{99m}\text{Tc}$  labeled MTCs [5].

Therefore, the development of MTCs labeled with therapeutic radioisotopes could lead to radiopharmaceuticals suited for the intra-tumoral radiotherapy of solid tumors.

Preliminary *in vivo* investigation of the binding stability and localization was performed in normal swine. Eleven mCi of  $^{90}\text{Y}$ -ABz-DOTA-MTC was administered intra-arterially to a swine liver via catheterization of the hepatic artery. Blood samples were taken following the administration, which indicated that less than 3% of the total injected activity was circulating 30 minutes following the administration and decreased over time. In addition, a  $\gamma$ -camera image taken 24 hours after the injection using the Bremsstrahlung emission associated with  $^{90}\text{Y}$ . While purely qualitative, the picture shows a single source of emission in the region of the liver where the  $^{90}\text{Y}$ -ABz-DOTA-MTCs were targeted (Fig. 6).

In conclusion, *in vitro* results suggest that the MTC technology may be used for the site-specific delivery of radioisotopes.  $^{90}\text{Y}$ -ABz-DOTA-MTCs is potentially a good candidate for the intra-tumoral radiotherapy of solid tumors.

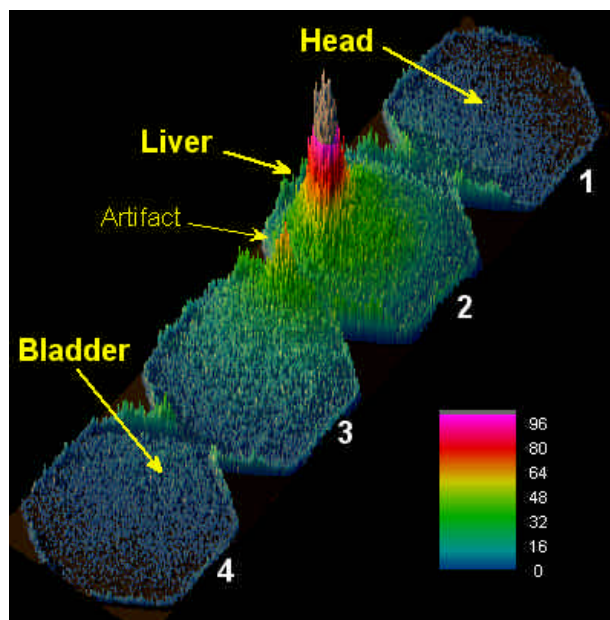


Fig. 6: Bremsstrahlung image taken 24 hours after intra-arterial delivery of  $^{90}\text{Y}$ -ABz-DOTA-MTCs to the right liver lobe of a swine. The animal was lying on its back. Four consecutive scans were taken and combined in this figure. The artifact is due to imperfect alignment of the scans 2 and 3 (they were taken too close together).

## REFERENCES:

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