# Characterization of a novel gadolinium-based high molecular weight polymer as an intravascular MR contrast agent

**Anne Kirchherr[1], Danielle Franke[1], Thomas Kaulisch[2], Detlef Stiller[2], and Andreas Briel[1]**

*[1]nanoPET Pharma GmbH, 10115 Berlin, Germany*
*[2]In-Vivo Imaging, Target Discovery Research,*
*Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach, Germany*
*anne.kirchherr@nanopet.de*

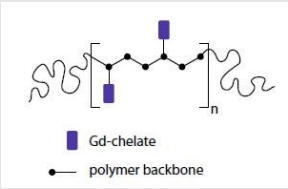
## Introduction & Objective

Blood pool contrast agents used in magnetic resonance imaging (MRI) are characterized by intravascular distribution and prolonged persistence in the blood compared with conventional contrast agents in clinical use such as Gd-DTPA (Magnevist®, Bayer Pharma AG, Germany). Since the size of the contrast agent molecule is a main factor determining the distribution and elimination of the agent *in vivo*, macromolecules are excellent candidates as blood pool contrast agents. Due to their larger size, macromolecular agents can potentially overcome the limitations of small molecules such as the rapid elimination from the blood pool, nonspecific extravasation into surrounding tissue, and poor relaxation enhancement efficiency [1]. In addition, due to the enhanced permeation and retention (EPR) effect, macromolecular contrast agents can extravasate from fenestrated blood vessels into surrounding tissue of inflamed or tumorous areas. Thus, besides assisting contrast-enhanced magnetic resonance angiography (MRA), blood pool agents can also be used to target e.g. necrotic myocardium [2, 3], or to detect various tumors [4, 5]. In the present work, a novel blood pool contrast agent composed of Gd-DTPA conjugated to a biodegradable polymer of high molecular weight (GadoSpin™ P, Miltenyi Biotec, Germany) was characterized *in vitro* as well as *in vivo*.

## Materials & Methods

### Synthesis of the gadolinium-based polymer

Diethylene triamine pentaacetic acid (DTPA) was dissolved in dimethyl sulfoxide (DMSO) under heating, sonication and addition of 4-dimethylaminopyridine (DMAP). Dicyclohexylcarbodiimide (DCC) and hydroxysuccinimide (NHS) were dissolved in DMSO and added to the DTPA solution.
The biodegradable polymer of high molecular weight ($M_r$ ~ 200,000) was dissolved in DMSO, added to the activated DTPA solution and left to react overnight at room temperature under stirring. The reaction mixture was filtered, dialyzed over 5 days against water and then lyophilized. For chelation of the gadolinium, the polymer-DTPA conjugate and $GdCl_3$ were dissolved in distilled water under stirring at room temperature for 1 h. The mixture was dialyzed against distilled water for 5 days and was then lyophilized.




*Schematic diagram of the gadolinium-based polymer*

To obtain a well-tolerated, ready-to-use product, the gadolinium-based polymer was dissolved in water for injection (at a concentration of 25 mM Gd) and the osmolality and pH were adjusted to physiological conditions. Finally, the polymer solution was sterilized.

### Relaxivity measurements

The $T_1$- and $T_2$-relaxation times of water containing increasing concentrations of Gd-DTPA or polymeric Gd-DTPA (2.5 - 15.0 mM, n = 3) were determined at 37 °C using a nuclear magnetic resonance pulse spectrometer (MiniSpec, Bruker BioSpin, Ettlingen, Germany) running at 1.41 T. Relaxivity values ($r_1$ and $r_2$) were calculated by estimating the slope of the relaxation rate (1/T) as a function of concentration.

### *In vivo* characterization

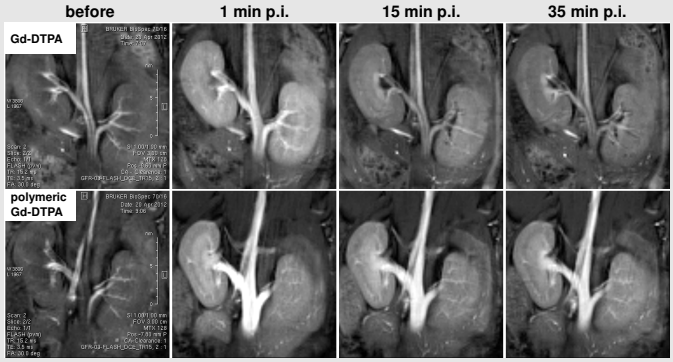
*In vivo* experiments were performed in C57BL/6 mice (n = 6 per group) after intravenous injection (lateral tail vein) of 100 µL of the polymeric Gd-DTPA or Gd-DTPA solution per 25 g mouse, corresponding to a dose of 100 µmol Gd/kg body weight. $T_1$-weighted images were acquired on a 7 T Bruker BioSpec 70/16 scanner (Bruker BioSpin, Ettlingen, Germany) using a FLASH sequence. The following parameters were used: TR 15 ms, TE 3.5 ms, FA 30 deg, FOV 30 mm, slice thickness 1 mm, 90 repetitions, 1 image per min.

## Results & Discussion

The longitudinal and transverse relaxivities, $r_1$ and $r_2$, of the gadolinium-based polymer in water at 37 °C and 1.41 T were found to be 10.2 $mM^{-1}s^{-1}$ and 12.2 $mM^{-1}s^{-1}$, respectively (see table). These values are more than 3 times higher compared to Gd-DTPA, the conventional contrast agent in clinical use, indicating a high contrast enhancement.

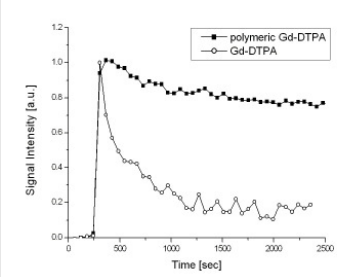
| | Relaxivity (37 °C, 1.41 T, in water) | |
|---|---|---|
| | $r_1$ [$mM^{-1}s^{-1}$] | $r_2$ [$mM^{-1}s^{-1}$] |
| polymeric Gd-DTPA | 10.2 | 12.2 |
| Gd-DTPA | 3.1 | 3.7 |

After intravenous administration of Gd-DTPA the signal intensity rapidly disappeared from the blood as expected due to strong extravasation and fast renal elimination. The blood half-life was approx. 6 min determined by mono-exponential fit of the time curve.




*Coronal MR images ($T_1$-weighted) of the mouse kidney and adjacent blood vessels before and after intravenous injection of 100 µL per 25 g mouse (100 µmol Gd/kg bw) of the novel polymeric Gd-DTPA (lower row) compared to the conventional Gd-DTPA (upper row).*

In comparison to Gd-DTPA, the polymeric Gd-DTPA remained within the vascular system for a prolonged period of time. Due to the long blood circulation and complex pharmakokinetics of the molecule, a standard mono- or bi-exponential fit could not be applied. The blood half-life was therefore graphically estimated and found to be approx. 2 h.
No adverse effects were observed after injection of the novel polymeric Gd-DTPA. The blood pool agent was mainly excreted via glomerular filtration and was completely eliminated after 24 h.




*Representative time course of the signal intensity in $T_1$-weighted images of the vena cava after intravenous injection of 100 µL per 25 g mouse (100 µmol Gd/kg bw) of the novel polymeric Gd-DTPA compared to the conventional Gd-DTPA.*

## References

[1] A.M. Mohs *et al.* Expert Opin Drug Deliv. 2007; 4 (2): 149–64.
[2] L.J. Kroft *et al.* J Magn Reson Imaging. 1999; 10 (3): 395–403.
[3] A. Muhler *et al.* Magma. 1995; 3 (1): 21–33.
[4] F. Kiessling *et al.* Curr Med Chem. 2007; 14 (1): 77–91.
[5] H.E. Daldrup *et al.* Radiologe.1997; 37 (9): 733–40.

## Conclusion

In this study we report on a novel gadolinium-based polymer that acts as an intravascular MR contrast agent (blood pool contrast agent) having a high contrast efficiency as well as optimal biocompatibility and clearance properties. The agent enables higher resolution through a higher efficacy and a pronounced steady state period for a wide acquisition timeframe in magnetic resonance angiography (MRA). Additionally, the novel agent can be of use in studies of renal structure and function.

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