Toward precise arterial input functions derived from DCE-MRI through a novel extracorporeal circulation approach in mice

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Purpose: Dynamic contrast-enhanced MRI can be used in pharmacokinetic models to quantify functional parameters such as perfusion and permeability. However, precise quantification in preclinical models is challenged by the difficulties to dynamically measure the true arterial blood contrast agent concentration. We propose a novel approach toward a precise and experimentally feasible method to derive the arterial input function from DCE-MRI in mice.

Methods: Arterial blood was surgically shunted from the femoral artery to the tail vein and led through an extracorporeal circulation that resided on the head of brain tumor–bearing mice inside the FOV of a 9.4T MRI scanner. Dynamic 3D-FLASH scanning was performed after injection of gadobutrol with an effective resolution of 0.175 × 0.175 × 1 mm and a temporal resolution of 4 seconds. Pharmacokinetic modeling was performed using the extended Tofts and two-compartment exchange model.

Results: Arterial input functions measured inside the extracorporeal circulation showed little noise, small interindividual variance, and typical curve shapes. Ex vivo and mass spectrometry validation measurements documented the influence of shunt flow velocity and hematocrit on estimation of contrast agent concentrations. Modeling of tumors and muscles allowed fitting of the recorded dynamic concentrations, resulting in quantitative plausible parameters.

Conclusion: The extracorporeal circulation allows deriving the contrast agent dynamics in arterial blood with high robustness and at acceptable experimental effort from DCE-MRI, previously not achievable in mice. It sets the basis for quantitative...
1 | INTRODUCTION

Dynamic contrast-enhanced (DCE) MRI is a common and promising clinical technique to study benign and malignant pathologies in various body regions. The procedure consists of injection of a gadolinium-based contrast agent (CA) and repeated acquisition of $T_1$-weighted images. Analysis of the derived dynamic data sets can be performed at different levels of complexity: (1) solely based on visual analysis of dynamic images, (2) based on semi-quantitative descriptive parameters (eg, area under the curve, time to peak) or (3) based on pharmacokinetic modeling. Only the latter approach allows absolute quantification of perfusion and permeability, but also demands the most elaborate image acquisition and data processing. Two-compartment models can be fitted to the acquired time course of CA concentration characterizing the dynamic transfer of CA between plasma and tissue. Accuracy of the calculated parameters is heavily dependent of the quality of the measured dynamic tissue and arterial blood concentrations, the so-called arterial input function (AIF). Acquisition of the AIF is typically the most difficult part and largest source of error, as it must be measured in voxels of “pure” arterial blood. Depending on the tissue of interest, the AIF measurement is carried out in a heart cavity, within the aorta, or smaller arterial vessels. It is therefore challenged by motion, rapid and dynamically changing blood flow, and partial-volume effects. These effects are complex and difficult to compensate for, as recording of AIFs demands a high temporal resolution.

Significant challenges in AIF measurements are already present in human DCE-MRI; however, these are heavily aggravated in small animals, especially in mice. A variety of approaches have been proposed to measure individual AIFs in different body regions, but none have been established as a standard. Moreover, validation of quantitative precision of these methods was not done. Particularly problematic are the high level of noise and the high degree of variance of AIF magnitude and curve shapes, both in and between individual studies, even with comparable injection protocols. Accordingly, many small animal DCE studies relinquish to measure individual AIFs. The alternative approaches used include adaptation of population-based AIFs and deducing the AIF from a reference region. Population-based AIFs neglect the interindividual variance of AIFs. Moreover, the appropriateness of adaptation of population-based AIFs or reference regions from mice of different strains, age, weight, disease model, and therapeutic regimen is questionable. More bias derives from the measurement-related imperfections of provided population-based AIFs and characterized reference regions. These approaches might be suitable for study concepts in which quantitative precision is not pursued. However, accurate and precise quantification would significantly support the translational potential of, for example, preclinical studies that aim to establish DCE-derived parameters as biomarkers for therapy monitoring. Thus, accurate and precise AIF recordings in mice are highly desirable.

In this study, we introduce a novel approach for AIF measurements in mice. Instead of measuring the arterial blood CA concentration within the mouse, the arterial blood is shunted through an extracorporeal circulation that is included inside the MRI FOV. This way, the blood signal intensity is measured outside the body, largely circumventing motion and partial-volume effects.

2 | METHODS

2.1 | Experimental setup

All experiments were conducted in accordance with the German Law on the Care and Use of Laboratory Animals and approved by the Landesamt für Natur, Umwelt und Verbraucherschutz of North Rhine-Westphalia, Germany. Female NMR nu/nu mice (Janvier, France), 8 to 12 weeks old, were housed at constant temperature (23°C) and relative humidity (45%-60%) under a regular light/dark schedule. Food and water were available ad libitum.

Eleven mice were orthotopically (intrastriatal injection, coordinates in relation to bregma: lateral $-2.0$ mm, anterior–posterior $+0.5$ mm, dorsal–ventral $-3.0$ mm), implanted with $2 \times 10^5$ human U87 dEGFR-LITG cells in $2 \mu$L normal saline under combined anesthesia (isoflurane, fentanyl). Imaging was performed 12-15 days after implantation. Imaging experiments, including interventional and surgical vessel preparation, were performed as terminal experiments under combined anesthesia: Mice were prepared with two intravenous tail vein catheters (one in each lateral tail vein). Surgical contralateral femoral vein cannulation was used when double tail vein cannulation was not successful. A total of 20 $\mu$L blood was withdrawn for hematocrit precise pharmacokinetic modeling in small animals to enhance the translatability of preclinical DCE-MRI measurements to patients.

**KEYWORDS**

AIF, arterial input function, DCE-MRI, pharmacokinetic modeling, preclinical MRI
(HCT) measurement. Body mass equal to 4 µL/g of a 100 international units/mL heparin solution was injected subcutaneously. Subsequently, the right femoral artery was surgically catheterized with an intravascular polyurethane tubing (0.007 × 0.014 in/1Fr; Instech, Plymouth Meeting, PA). The extracorporeal circulation consisted of (1) femoral artery, (2) intravascular polyurethane tube, (3) transparent silicon tube (200 mm/0.3 mm), (4) glass capillary (10 mm/0.94 mm, proximal reservoir Rprox), (5) transparent silicon tube (200 mm/0.3 mm), (6) glass capillary (10 mm/0.94 mm, distal reservoir Rdist), (7) black silicon tube (200 mm/0.3 mm), (8) measuring chamber, (9) black silicon tube (100 mm/0.3 mm), and (10) tail vein catheter (Figure 1A, length and inner diameter in brackets). The total extracorporeal blood volume was approximately 65 µL.

After positioning of the mouse on the MR scanner bed, the two reservoirs (glass capillaries, Rprox and Rdist) were fixated in a custom-made polymethylmethacrylate holder to assure parallel angulation and reproducible spacing. A probe with a fixed gadobutrol (Gadovist/Gadavist) concentration (1 mM) in a tube of 0.58-mm inner diameter was placed in the middle of the two reservoirs Rfix. Dynamic scanning was performed after T2-weighted imaging and T1 mapping (Figure 1B-E). After 1 minute of baseline scanning, contrast injection was initiated consisting of a 80 µL saline bolus + 100 µL 35 mM gadobutrol in saline followed by a 45 µL saline bolus with a power injector at 1 mL/min (Harvard Apparatus, Holliston, Massachusetts). Mouse temperature was maintained at 37° ± 1°C during the whole experiment. Isoflurane concentration was adapted to keep breathing frequencies at 50-65 min⁻¹ and typically amounted to 2.0-2.5% in 100% oxygen. Overall MR measuring time for one mouse was approximately 1 hour. After the measurement, again 20 µL of blood was withdrawn from the circulation for HCT estimation (this HCT value was used for conversion into plasma cGd), and in four mice an additional immediate withdrawal of 60 µL blood for mass spectrometry was performed.

Human whole blood with defined amounts of gadobutrol (0.0, 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mM) was used for ex vivo validation experiments. The blood was circulated with a power injector at defined flow velocities of 0, 30, 50, and 70 µL/min through Rprox and Rdist in a setup otherwise unchanged to the in vivo experiments. In an additional experiment, the effect of varying HCTs (47%, 40%, 34%, and <20%) was assessed. A dead mouse was positioned in

![Figure 1](image-url)
the animal bed to ensure similar coil loading. Proper temperature adjustment to 37°C was approximated by placing a temperature sensor near the reservoirs. Human blood was withdrawn from healthy donors, and CA was added by a pipette. Seven (Figure 2A,C) or 12 DCE (Figure 2B) time frames were performed for each combination of concentration and flow velocities.

### 2.2 MRI scanning

MRI was performed with a 9.4T small-animal MR scanner (Bios-Spec 94/20; Bruker BioSpin MRI, Ettlingen, Germany) and a helium-cooled cryoprobe (Bruker BioSpin MRI). The system was operated using the software ParaVision 5.1. (Bruker BioSpin MRI). Two-dimensional $T_2$-weighted rapid imaging with refocused images (RARE) images were obtained with a matrix $= 192 \times 192$ pixels, 28 contiguous axial slices, slice thickness $= 0.5$ mm, FOV $= 16 \times 16$ mm$^2$, spatial resolution $= 0.083$ mm, flip angle $= 180°$, TE $= 10$ ms, TR $= 6200$ ms, RARE factor $= 20$, and number of averages $= 6$. $T_1$ mapping was performed in a single axial slice with equivalent geometry to a central $z$-plane of the DCE FOV containing the brain tumor. Mapping was performed based on fitting measurements with varying TR ($5500$ ms, $3000$ ms, $1500$ ms, $800$ ms, $400$ ms, $200$ ms, and TE $10$ ms) using ROCKETSHIP.\(^{20}\) For modeling of temporal muscles, the mean $T_1$ of the measured mice was used, as not all muscles were equally well covered by $T_1$ mapping. A 3D-FLASH sequence with matrix $80 \times 80 \times 8$ pixels was used for DCE. The FOV ($14 \times 14 \times 8$ mm$^3$) was positioned to completely cover the blood-filled reservoirs and the tumor. Spatial resolution was $0.175 \times 0.175 \times 1$ mm$^3$, temporal resolution of $4015$ ms, 225 repetitions, with a total length of 15 minutes. Anti-aliasing in Z was 1.25, flip angle $= 15°$, TR $= 5.019$ ms, and TE $= 1.961$ ms.

### 2.3 Inductively coupled plasma mass spectrometry

Whole blood samples of 60 µL were withdrawn from the extracorporeal circulation. A total of 50 µL of whole blood was weighed and 500 µL of concentrated HNO$_3$ was added to determine the total gadolinium (Gd) concentration. The samples were heated to $80°C$ for approximately 60 minutes until no solid components were visible for complete digestion. Subsequently, the samples were diluted with water and Eu as internal standard was added at a final concentration of 1 ng/L. For quantification, an external ex vivo validation was performed with 11 standard solutions ranging from 0.1 to 40 µg/L diluted from a Gd inductively coupled plasma mass spectrometry stock solution (1000 mg/L) and a blank. All standard solutions also contained 1 ng/L Eu and 1% HNO$_3$ (vol/vol). The isotopes $^{153}$Eu, $^{156}$Gd, and $^{159}$Gd were recorded in triple-quantum mode with oxygen as reaction gas on the corresponding $^{16}$O mass shift and a dwell time of 100 ms. Evaluation
was performed based on the \textsuperscript{155}Gd isotope. Linear regression showed good correlation ($R^2 = 0.9993$) and recovery rates for the internal standard ranged between 95% and 107%. The limit of detection was 7.3 ng/L and the limit of quantification was 24 ng/L, calculated with the 3σ criteria and 10σ criteria.

### 2.4 Data processing

Volume-of-interest (VOI) based image analysis was performed using an in-house-developed image processing software. A VOI containing $8 \times 8 \times 2.3$ voxels ($1.4 \times 1.4 \times 2.3$ mm$^3$) was placed to cover and exceed each reservoir ($R_{\text{prox}}$ and $R_{\text{dist}}$) and the fix probe in x and y for the extra-corporeal blood measurements. Glass and polymethylmethacrylate structures inside the FOV were neither discernible to air by signal nor by noise levels. We therefore assumed homogeneous noise in the surroundings and subtracted its contribution based on another VOI placed in air. The discrepancy between VOI volume and true volume of the reservoirs was corrected by multiplying the signal with the respective proportion of volumes. This VOI geometry was chosen because it showed best correspondence of area under the curve (AUC) between the two reservoirs, especially compared with smaller VOIs that covered only part of the reservoirs. The VOIs were placed with a minimal distance of 2 mm in z to the blood inflow into the FOV. Input functions from venous sinuses were based on a set of 3-6 voxels (typically in different Z-slices), providing the maximum dynamics of signal intensity after injection. A population AIF was generated as the mean signal of providing the maximum dynamics of signal intensity after injection and delay to finally extract the femoral artery AIF from $R_{\text{prox}}$. For dispersion correction, the $c_{\text{Gd}}$ curve of $R_{\text{prox}}$ was convolved using the following mono-exponential kernel:

$$k(t) = 0 \quad \text{for} \ t < \Delta t$$

$$k(t) = \frac{e^{-(t-\Delta t)/\tau}}{\tau} \quad \text{for} \ t \geq \Delta t$$

with dispersion constant $\tau$ and delay $\Delta t$ determined to match the $R_{\text{dist}}$ curve (i.e., $c_{\text{Gd,Rdist}} = c_{\text{Gd,Rprox}} \times k$). For deconvolution, the $R_{\text{prox}}$ curve was fitted to

$$c_{\text{Gd,Rprox}}(t) = 0 \quad \text{for} \ t < 0$$

$$c_{\text{Gd,Rprox}}(t) = (a_1 e^{-t/\tau_1} + a_2 e^{-t/\tau_2} + a_3) e^{-t/\tau_3} \quad \text{for} \ t \geq 0$$

with fitting parameters $n, a_1, a_2, a_3, \tau_1, \tau_2, \tau_3$. The fit was then numerically deconvolved with the determined kernel $k(t)$. Precise delay correction for the resulting AIF was performed manually, based on the onset of the Gd signal in the tumor tissue in the dynamic images. For estimation of the potential error of dispersion underestimation, a different set of further deconvolved AIFs with $\tau = 8$ seconds for every mouse was used based on the dispersion-corrected individual shunt-derived AIFs.

Voxel-wise pharmacokinetic modeling was performed with the software \textit{ROCKETSHIP} version 1.2,\textsuperscript{20} using the extended Tofts model (ETM)\textsuperscript{23} and two-compartment exchange model (2XCM)\textsuperscript{24} with the shunt-derived preprocessed AIFs. Pharmacokinetic modeling of VOIs in the bilateral temporal muscles (mean of both temporal muscle VOIs, 12-27 voxels) was performed using \textit{PMOD} version 3.703 (PMOD Technologies, Zürich, Switzerland).

The VOIs were positioned equally to animal experiments for the phantom experiments; however, the longitudinal VOIs were split in three VOIs of 1.4 × 1.4 × 1 mm$^3$ for each reservoir. Additional VOIs were positioned at the blood entry into the FOV (Figure 2C). Baseline was adapted from the median of frames 2-7/2-12 from the measurements with native blood. Assumptions were otherwise equal to animal experiments, and $c_{\text{Gd}}$ calculations were performed for frames 2-7/2-12 for every VOI. No significant drift was observed; therefore, no correction was performed.
2.5 | Statistics

Pearson correlation coefficients and respective $P$ values were calculated with MATLAB; $P < .05$ was considered significant.

3 | RESULTS

3.1 | Experimental procedure

An overview of setup and representative recording slices is given in Figure 1. A time-lapse video of the 3D-FLASH dynamic series is available online (Supporting Information Video S1). The mouse blood was shunted from the surgically cannulated femoral artery to one of the two conventionally cannulated tail veins. The shunt contained two reservoirs (R$_{prox}$ and R$_{dist}$) that resided inside the FOV on the head of the brain tumor–bearing mice. Shunted arterial blood signal intensity was measured inside the extracorporeal reservoirs, and tumor signal was simultaneously measured in the mouse head. Overall, the procedure was generally well-tolerated by the animals. Preparation of the venous catheters and surgical femoral artery cannulation took approximately 45 minutes; MRI measuring procedure including mouse positioning took 60 minutes. In addition to the 11 animals shown, two mice died of unknown reasons during scanning and one was excluded due to a missing shunt flow.

3.2 | Ex vivo validation experiments

In the first session of the ex vivo validation experiments, different gadobutrol concentrations (HCT: 51.6%) were measured at different flow velocities (Figure 2). An approximate 1.5-fold overestimation was noted and a relationship between flow velocity and calculated $c_{Gd}$ was observed, indicated by a more pronounced overestimation of high $c_{Gd}$ at high shunt flow velocities and small $c_{Gd}$ at lower flow velocities (Figure 2A). In the second measurement session, the influence of varying HCT values was investigated at a fixed whole-blood $c_{Gd}$ of 0.4 mM (Figure 2B). The results show an overestimation of $c_{Gd}$ at high HCT values and an underestimation at HCT values typically observed in the mouse experiments. In both measurement sessions, only central to distal z-planes with respect to the blood flow direction were analyzed as indicated in the inset of Figure 2A. Positioning the VOIs at the entry z-plane with respect to blood flow (Figure 2C) in the first measurement session led to a considerable increase of flow dependency on $c_{Gd}$ calculation. A similar, strong flow dependency was observed when performing DCE with a 2D FLASH (data not shown).

3.3 | AIF measurements in mice

Figure 3 displays the dynamic CA blood concentrations of 11 mice derived from the extracorporeal shunt and the dispersion-corrected AIF. Animal 4 demonstrated an exceptional low shunt flow velocity of $<10 \mu$L/min and was excluded for further analysis. The remaining 10 animals (average weight of 28.7 ± 2.1 g) displayed a mean shunt blood flow of 45.5 ± 4 µL/min and a mean peak of the AIF of 0.81 ± 0.12 mM. The mean ratio of $c_{Gd}$ peak to $c_{Gd}$ after 15 minutes was 9.8 ± 3.4. The relative net difference of $c_{Gd}$ between R$_{prox}$ and (delay-adapted) R$_{dist}$ at the end of the measurement was only 4% ± 3%. The average HCT as directly measured after DCE-MRI was 38% ± 4%. Noise level was overall low. A significant negative correlation of calculated shunt flow and dispersion constant $r$ for deconvolution to the AIF was noted ($R = -0.77$, $P = .0095$), in line with a lower dispersion effect at rapid passing of the bolus through the tubing. Despite a positive correlation in the ex vivo validation experiments, in vivo HCT was neither correlated with AIF peak height ($R = -0.48$, $P = .16$) nor with $c_{Gd}$ after 15 minutes ($R = 0.3$, $P = .4$, delay-adapted). No significant correlation of mouse body mass with AIF peak height was found ($R = -0.06$, $P = .86$).

3.4 | Evaluation with mass spectrometry

To evaluate the quantitative precision and accuracy of $c_{Gd}$ calculation in the mouse experiments, 60 µL blood of each of four mice (mice 3-6) was withdrawn immediately after the DCE measurements. The value of $c_{Gd}$ of the blood samples was determined with inductively coupled plasma mass spectrometry, and results were correlated with the concentration in R$_{prox}$ corrected to the respective time point based on shunt flow, the exact time of blood withdrawal, and the volumetric distance between R$_{prox}$ and the blood withdrawal line. An underestimation of the true CA concentrations by DCE-MRI was noted for every mouse with little variation of the ratios of true to calculated concentration among the four animals (Figure 4; mean ratio mass spectrometry to DCE-MRI-derived CA concentration: 1.43 ± 0.07).

3.5 | Pharmacokinetic modeling

The method presented here was compared with four alternative AIF approaches derived from the measurements (Figure 5, top row) and tested for their modeling performance using the ETM in healthy muscles. The only anatomical structure that featured a typical AIF-like signal intensity slope was the venous sagittal sinus. The variance of the sinus-derived AIFs as well as the resulting modeling
parameters, the volume transfer constant $K_{\text{trans}}$ and the extravascular extracellular space fractional volume $v_e$, were considerably higher compared with the shunt-derived AIFs (Figure 5, middle row). Furthermore, a median shift to higher values for $K_{\text{trans}}$ and $v_e$ and a shift to likely unphysiological values for the fractional plasma volume $v_p$, was observed. Additionally, a lower goodness of fit was noted (Figure 5, middle row). The shape of the shunt-derived and sinus-derived curves appeared markedly different, with a shorter peak and steeper increase and decrease with the sinus-derived AIFs. This observation raised the question of whether the differences in modeling parameters were driven primarily by the differences of AIF-AUCs or by a potentially insufficient dispersion correction of our method. Further deconvolution of the shunt-derived AIFs with an additional dispersion constant of $\tau = 8$ seconds led to AIFs most closely resembling the shape of the sinus-derived curves. Using these further deconvolved AIFs resulted in a minor increase of $K_{\text{trans}}$ and $v_e$ with 10% and 7%, and a moderate decrease of $v_p$ of 22%. Use of a population AIF led to an increased parameter variance and to a lower goodness of fit.

Three mice featured comparable growth of U87 glioblastoma and were selected for voxel-wise pharmacokinetic modeling using the ETM and 2XCM using the individual shunt-derived AIFs (Figure 5, bottom row, and Figure 6). The resulting parameter maps indicate tumor heterogeneity not visible in the $T_1$-weighted and $T_2$-weighted morphological images (Figure 6). Further deconvolving AIFs by 8 seconds
as well as the choice of models had only a minor effect on the resulting parameters \(K^{\text{trans}}\) and \(v_e\). However, only in the 2XCM were major differences demonstrated between the different degrees of AIF deconvolution for \(v_p\). Additionally, a strong variation of plasma flow \(F_p\) was noted. The residuals were slightly lower for the additionally deconvolved AIFs, indicating best goodness of fits (Figure 5).

4 | DISCUSSION

Measuring AIFs in mice in vivo is a challenging endeavor. To the best of our knowledge, we are first to present a method for AIF measurement for DCE-MRI in mice using an extracorporeal circulation.

4.1 | Advantages of extracorporeal AIF measurements

Major challenges and sources of error in conventional AIF measurements are small vessel diameter and the resulting partial-volume effects and respiratory and cardiac motion. Measuring the arterial blood concentration in an extracorporeal circulation and not inside the mouse allows us to overcome these sources of error and to perform robust measurements independent of specific constraints of the examined body region. The large inner diameter of the reservoirs surrounded by glass/poly(methylmethacrylate), not contributing to MR signals, makes partial-volume effects negligible or at least correctable. Moreover, measuring from a large and standardized volume leads to a superior low level of noise. The extracorporeal circulation allows convenient and fast arterial blood withdrawal to cross-validate the MRI concentration estimation, such as by mass spectrometry (Figure 4). The setup also facilitates multimodal imaging approaches, such as for parallel dynamic PET imaging. As indicated in Figure 1A, the extracorporeal circulation features a MR-compatible measuring chamber that can be equipped with radiation detectors for parallel dynamic measurements of radiotracer blood concentrations.

The in vivo results displayed in this publication have been acquired consecutively, and only unforeseen incidents such as unintended deaths (but not poor measurement quality) were reasons for exclusion. The displayed data thus allow an authentic valuation of the advantages and feasibility of the experimental setup. Influence of the shunt on circulatory properties or tracer excretion was not specifically tested, but a strong effect does not appear likely as the shunted blood volume represents less than 5% of the mouse blood volume. It should especially be of minor relevance compared with the effect of intravenous tracer injections, which in mouse DCE-MRI studies often exceeds 10% of the mouse blood volume (as in our study). Femoral artery cannulation impedes longitudinal measurements due to induced hind leg ischemia. This constraint however could be circumvented by using alternative access ways such as the (internal) carotid artery.

4.2 | Ex vivo and mass spectrometry validation experiments

The extracorporeal circulation uniquely enables using a nearly identical experimental setup for in vivo animal and ex vivo validation/calibration experiments. The ex vivo validation measurements demonstrated an almost linear relationship between calculated and true \(c_{\text{Gd}}\) with a HCT-dependent offset and a flow-modulated slope (Figure 2A). The relationship between calculated concentration and HCT (Figure 2B) matches well with the coherence of HCT and whole-blood \(T_1\). Validation of the Gd content of whole-blood samples by ICP-MS showed a consistent relative underestimation of the true concentration of a factor of 1.4, reflecting a systematic bias. In agreement with the ex vivo validation experiments, the low HCT of the analyzed mice (mean HCT: 37%) should lead to an underestimation of the true concentration. However, the degree of underestimation is higher than expected from the ex vivo validation measurements.
Temperature influences both blood $T_{10}$ and relaxivity.\textsuperscript{21,22,25} The temperature of $37^\circ$C was adopted as the basis for both values. The effect of temperature changes was obvious in one animal (animal 2, Figure 3), in which a defect of the warming system led to heavily fluctuating temperature of the mouse and, ultimately, the calculated $c_{\text{Gd}}$. As the reservoirs lie outside the body, an offset of the reservoir temperature to the mouse core temperature of $37^\circ$C must be considered. Although the experimental setup is designed to provide close accordance, a temperature offset cannot fully be excluded to contribute to the observed underestimation of the true CA concentration. Especially in the ex vivo validation experiments prewarming of the blood is difficult to control.

Different blood flows have a minor influence on $c_{\text{Gd}}$ estimation, as observed in the ex vivo validation measurements (Figure 2A). Deviation from the baseline flow during a measurement, however, could have a higher influence on the calculated contrast agent concentration. In 4 of 11 (mice 8-11) mice, an injection-induced minor change (e.g., mouse 10, Figure 3) of the baseline signal intensity inside the reservoirs before arrival of the contrast bolus was observed, putatively reflecting a change of shunt blood-flow velocity. Such flow changes are likely not a unique feature of the shunt setup, but can be assumed to occur also in conventional measurements. The large diameter of the reservoirs drastically reduces linear flow rates by two orders of magnitude (i.e., 0.0012 m/s for 50-µL/min volumetric flow rate in comparison to up to 0.15 m/s in the mouse carotid artery).\textsuperscript{26} Besides the potential miscalculation of $c_{\text{Gd}}$ due to varying flow, however, a second potential reason for bias depicts a unique feature of the extracorporeal circulation: The assumption of mirroring the intravascular blood curve shape and sole modulation by dispersion relies on a constant inflow of blood into the extracorporeal circulation; dynamic changes of flow interfere with this assumption and could principally lead to distortion of the AIF curve shape and miscalculation of the dispersion effect. Overall, a major bias from these dynamic flow changes is not expected, considering the minor amplitude (estimated with a maximum of 19 µL/min in the most affected mouse [mouse 10]) and length of the changes with typical adaption to baseline before arrival of the bolus in the first reservoir and the excellent conservation of $c_{\text{Gd}}$ integrals between the two reservoirs. Mice displaying flow changes also demonstrated

**FIGURE 5** Top row: Different AIFs: 0-240 seconds, excluding mouse 4. Individual shunt AIFs (ind): corresponding to Figure 3. Population AIF (pop): representing the mean of the individual shunt AIFs. $8\ s$ deconv shunt AIF ($8s$): individual shunt AIFs further deconvolved with $r = 8$ seconds. Venous sinus (ven): volume of interest in the superior sagittal sinus. #9 and #11: the four different AIF types in two individual mice. Middle row: VOI-wise modeling using the ETM of the bilateral temporal muscles. The plotted parameters represent the mean of the left and right temporal muscle. $\chi^2$ indicate goodness of fitting to the model. AUC represents area under the curve of the input functions of the first row relative to the individual shunt AIFs. Bottom row: Whole tumor VOI covering voxel-wise fitted tumors of mice #9-11 using the extended Tofts model (ETM) and two-compartment exchange model (2XCM). $Fp$ is only included in the 2XCM, but not in the ETM. Residuals indicate the goodness of fitting to the model.
an increase of respiratory frequency after injection (from 75 min$^{-1}$ to 110 min$^{-1}$ in mouse 10). Both can effectively be avoided by a well-balanced anesthesia with a respiratory frequency of 50-65 min$^{-1}$ or a reduction of the injection volume.

### 4.4 Pharmacokinetic modeling

Pharmacokinetic modeling of vasculature-related parameters in the temporal muscles of 10 mice was performed using the ETM with different alternative AIFs, the individual shunt-derived AIF, a population-based AIF, sagittal sinus-derived AIFs, and—to match the shape of the sagittal sinus AIFs—a further deconvolved individual shunt AIF. Only the shunt-derived AIFs, but not the sinus-derived AIFs, led to parameters in very good agreement with published literature values ($K_{\text{trans}}$ range ~ 0.02-0.13 min$^{-1}$ in mouse paravertebral muscle,$^{18}$ 0.034 ± 0.006 min$^{-1}$ in mouse epicranial muscle,$^{8}$ 0.13 min$^{-1}$ ± 0.06 in rat skeletal muscle,$^{27}$ $v_p$ ~ 0.08 in rat back muscle,$^{18,28}$ 0.19 ± 0.05 in rat skeletal muscle,$^{27}$ $v_p$ ~ 0.012-0.04 in skeletal muscles in various species,$^{29}$ compared with Figure 5, middle row). Together with the observed higher AUC and parameter variance, these results are highly indicative of a lower precision and accuracy of the sagittal sinus AIFs, despite low levels of noise and characteristic AIF curve shapes. The minor changes in $K_{\text{trans}}$ and $v_p$, by further deconvolving the shunt-based AIFs, suggest that imperfections in the AIF AUC due to partial-volume effects but not curve shapes primarily drive these errors. These results underline the need for cautious evaluation of AIF measurements in small vessels in small animals.

Population-based approaches are a frequently used alternative to individual AIF measurements. The observed smaller variance and better goodness of fits in the individual AIFs emphasize the gain in precision of individual AIF measurements compared with population AIFs.

In three mice, the ETM and 2XCM were used to perform voxel-wise modeling of brain tumors. Although the permeability-related parameters $K_{\text{trans}}$ and $v_p$ were grossly unaffected by the choice of model and by additional AIF deconvolutions, the perfusion-related parameters $v_p$ and $F_p$ were significantly affected by additional AIF deconvolution only in the 2XCM. Strikingly, additional deconvolution shifted $v_p$ from implausible to plausible values for the 2XCM in two out of three mice. Considering the modeling results from tumors and temporal muscles, we conclude that the correct
AIF AUC is most critical for the permeability-related parameters $K_{\text{trans}}$ and $v_e$. In contrast, and especially when blood flow is incorporated into the model, the correct AIF curve shape appears to be crucial to precisely estimate perfusion-related parameters.

Comparing the sinus-derived AIFs and modeling with the deconvolved shunt AIFs indicate that our method might feature an underestimation of dispersion. We can only speculate whether this reflects true differences in dispersion between the intracranial vessels and the femoral artery or imperfections in describing the dispersion in the silicon tubings and glass capillaries as a mono-exponential convolution with equal dispersion between the femoral artery to the first reservoir and between the two reservoirs. However, we could demonstrate that this potential error has little effect on the permeability-related parameters $K_{\text{trans}}$ and $v_e$.

4.5 | Outlook

The novel shunt method bears the potential of setting a new standard of precision in DCE-MRI AIFs as a basis for quantitative precise pharmacokinetic modeling–derived parameter maps in mice. We propose consideration of it being a significant extension to alternative DCE-MRI approaches. Although the extracorporeal circulation features additional experimental effort, we currently see at least three major potential application scenarios: (1) to appear in preclinical studies that emphasize the quantitative aspects of DCE-MRI-derived parameters, (2) to serve as a validation standard of comparison for conventional experiments, and (3) to establish population-based AIFs in a subset of animals for individual studies. In future experiments, we will further refine our method by addressing remaining limitations such as the inability to perform longitudinal experiments and the inaccuracies in concentration and dispersion estimation. Thus, we will compare our results to recordings from central blood vessels and aim to test alternative arterial access ways that can be maintained over time. Furthermore, we aim to use a positron detector within the measuring chamber to simultaneously record dynamic radiotracer blood concentrations, setting the basis for multimodal cross validation using radiotracer tracers and even radio-labeled MR CAs.

5 | CONCLUSIONS

We introduce a novel method to measure AIFs in DCE-MRI in mice. An extracorporeal circulation now allows us to simultaneously record CA dynamics in arterial blood and tissues with high robustness, low noise, and at acceptable experimental effort in mice. It features several advantages compared with conventional measurements of AIFs and sets the basis toward quantitatively precise pharmacokinetic modeling in mice. Accordingly, this method bears the potential to enhance the clinical translatability of preclinical DCE-MRI measurements.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found online in the Supporting Information section.

**VIDEO S1** Time lapse of a 15-minute DCE scan (mouse 11). The left panel corresponds to the slice chosen in Figure 1. The right panel displays the propagation of signal intensity in $R_{\text{prox}}$, $R_{\text{dist}}$, and a circular tumor volume of interest.