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MORE BULLETS FOR PISTOL: LINEAR AND CYCLIC SILOXANE REPORTER PROBES FOR QUANTITATIVE 1H MR OXIMETRY

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Tissue oximetry can assist in diagnosis and prognosis of many diseases and enable personalized medicine. Previously, we reported the ability of hexamethyldisiloxane (HUDSON) for

accurate measurements of tissue oxygen tension (PO_2) using Proton Imaging of Siloxanes to map Tissue Oxygenation Levels (PISTOL) magnetic resonance imaging. Here we report the feasibility of several commercially available linear and cyclic siloxanes (molecular weight 162–410 g/mol) as PISTOL-based oxygen reporters by characterizing their calibration constants. Further, field and temperature dependence of PO_2 calibration curves of HMDSO, octamethyltrisiloxane (OMTSO) and polydimethylsiloxane (PDMSO) were also studied. The spin-lattice relaxation rate R_1 of all siloxanes studied here exhibited a linear relationship with oxygenation ($R_1 = A' + B' \cdot PO_2$) at all temperatures and field strengths evaluated here. The sensitivity index $\eta (= B'/A')$ decreased with increasing molecular weight with values ranged from 4.7×10^{-3} – $11.6 \times 10^{-3} \text{ torr}^{-1}$ at 4.7 T. No substantial change in the anoxic relaxation rate and a slight decrease in PO_2 sensitivity was observed at higher magnetic fields of 7 T and 9.4 T for HMDSO and OMTSO. Temperature dependence of calibration curves for HMDSO, OMTSO and PDMSO was small and simulated errors in pO_2 measurement were 1–2 torr/ $^\circ\text{C}$. In summary, we have demonstrated the feasibility of various linear and cyclic siloxanes as PO_2 -reporters for PISTOL-based oximetry.

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regarding oxygenation. The current magnetic resonance imaging (MRI) based oximetry techniques can be further sub-divided into a) qualitative techniques: Blood Oxygen Level Dependent (BOLD), oxygen-enhanced MRI, Tissue Oxygen Level Dependent (TOLD) and b) quantitative oximetry techniques: hypoxia targeted MRI (F NMR), Electron Paramagnetic Resonance (EPR) perfluorocarbon emulsions, Fluorocarbon Relaxometry using F¹⁹ Echoplanar imaging for Dynamic Oxygen Mapping (FREDOM), and Proton Imaging of Siloxanes for MRI of hexafluorobenzene). mapping Tissue Oxygenation Levels (PISTOL)

H) techniques are minimally ¹F and ¹⁹EPR and MR oximetry (invasive and provide quantitative oxygenation information via measuring the change in linewidth or spin lattice relaxation time, respectively, of an exogenously administered paramagnetic spin-probe as it interacts with the molecular oxygen. They allow for non-invasive and repeated measurement of oxygenation at multiple locations. Some of the EPR probes are lithium phthalocyanine (LiPc), lithium naphthalocyanine (LiNc), Fusicone, Gloxy, India Ink and triarylmethyl (TAM), of which India Ink is approved for clinical F MR oximetry uses exogenous perfluorocarbon. The use of ¹⁹F or ⁴¹reporters such as perfluoro-15-crown-5-ether (15C5) as oxygen reporters and uses the linear relationship of fluorocarbon spin-lattice relaxation rate R₁ with oxygenation. Exploiting the same concept, our group has previously shown the feasibility of accurate and repeated measurements of oxygen using hexamethyldisiloxane (HMDSO) in thigh muscle and in tumor regions. We have also shown the ability of siloxane based nanoemulsions for tissue oximetry. Siloxanes can be synthesized in a variety of forms (linear or cyclic, increasing chain length, with or without functional groups) and have been used in various applications. While such as biomedicine, cosmetics, and food processing reporter and has a large HMDSO has been shown to be a reliable PO_2 reporter, the values of spin lattice sensitivity, dynamic range and high pO_2 under hypoxic conditions can be as long as 11 s, leading to long measurement times. This raises the question whether any of the other siloxanes could be used as PO_2 reporter molecules and how chain length and structure (linear versus cyclic) exhibited sensitivity and dynamic range of T_2 influence the PO_2 under different oxygenation conditions. In this study, we have characterized the calibration curves of various low molecular weight linear and cyclic siloxanes and assessed their utility as pO_2 H MR oximetry. The siloxanes reporter molecules for use with investigated here are: linear siloxanes HMDSO, octamethyltrisiloxane

Adequate availability of oxygen is critical to the efficient functioning of many vital organs and tissues indicative of a disruption in homeostatic conditions which are ischemic heart, wounds prevalent in pathologies such as tumors, and traumatic brain injury, metabolic disorders, disease oxygen requirement changes between cells, tissues and organs and thus each tissue type exhibits a distinct normal range of oxygenation. For example, the normal tissue oxygen level in the brain is ~34 torr. The lack of adequate oxygen (mmHg) while that in the muscle is ~29 torr oxygen in cells and tissues is termed as hypoxia and could result from diminished blood flow, low blood oxygen saturation, elevated oxygen metabolism and increased cellular proliferation. Oxygen homeostasis and hypoxic stress are being recognized as important factors for development and physiology of cells and tissues. These factors also influence the pathophysiology of diseases as they regulate various intracellular signaling pathways for processes such as angiogenesis, cell proliferation and protein synthesis. Malignant cell proliferation and protein synthesis tumors are known to have regions with low oxygen tension known as hypoxia which is a major driving force behind tumor progression and Hypoxia presents itself as an ideal target resistance to therapies for the development of anti-cancer therapies due to the role that it plays in the progression of cancer. Thus, measurement of oxygen is essential for monitoring the function of organs as well as for diagnosis, treatment planning and studying treatment response of pathologies. Consequently, there is an increased need for an oximetry technique that can facilitate repeated, non-invasive and accurate assessment of oxygen and can be translated to the clinic.

Many qualitative and quantitative oximetry techniques have been developed for oximetry such as polarographic needle electrode, Near Infrared (NIR) spectroscopy, fiber optic probes, positron emission, immunohistochemical probes, fluorescence and single photon emission computed tomography (PET). Polarographic needle electrode and fiber tomography (SPECT) optical probe techniques are invasive, susceptible to pressure artifacts and cannot facilitate simultaneous measurement of multiple locations and repeated measurements, while immunohistochemical hypoxia analysis. NIR spectroscopy is a non-invasive technique but *ex-vivo* can only detect the changes in vascular oxygen saturation and cannot distinguish between signals from oxyhemoglobin, deoxyhemoglobin, PET and SPECT based techniques lack oxidase and cytochrome spatial resolution and cannot provide quantitative information

k is a constant that determines the solubility of oxygen in the agent and is different for different agents. Thus, net relaxation rate becomes

(OMTSO), decamethyltetrasiloxane (DMTSO), dodecamethylpentasiloxane (DDMPSO), trimethylsiloxy-terminated polydimethylsiloxane (PDMSO, M.W. 410) and cyclic siloxanes octamethylcyclotetrasiloxane (OMCTSO) and decamethylcyclopentasiloxane (DMCPSO). These siloxanes are ¹H resonance commercially available, inexpensive and have a single . Further, field and temperature dependence of the ⁴⁸around 0.1 ppm calibration curves of HMDSO, OMTSO and PDMSO were also ²PO studied.

$$1=A'+B'*pO_2 \quad R_1=A'+B'*pO_2$$

(3)

$$1/k_1p \text{ and } B' = R_{1d} \text{ where } A' = R$$

Since longitudinal relaxation rate is a function of temperature we assume a linear dependence of constants A' and B' on temperature (for relevant physiological range) which empirically can be defined as

$$A'=A+C*T \quad A'=A+C*T$$

(4)

$$B'=B+D*T \quad B'=B+D*T$$

(5)

-Substituting value of A' and B' in Eq. [3] results in a temperature dependent model for net relaxation rate

Theory

Quantitative MR oximetry exploits the Fermi contact interactions that leads to a ⁴⁹between paramagnetic oxygen and reporter molecules) dependent relaxation of the nuclear₂ concentration (and hence pO₂O MR oximetry must preferably possess *in vivo* spins. Probes used for the following characteristics: high oxygen solubility, hydrophobicity (so that diffusion of aqueous ions is restricted), should have a single resonance so that there are no chemical shift artifacts in the MR on temperatures. Due to ₁images and minimal dependence of R paramagnetic nature of molecular oxygen, it tends to shorten the nuclear relaxation times and relaxes the nuclear spins faster thereby increasing the spin-lattice (longitudinal) and spin-spin (transverse) respectively of the reporter molecule. The ₂ and R₁relaxation rate R on the spin-lattice ₂principle is based on the linear dependence of pO relaxation rate of the probe.

If x is the molar fraction of oxygen the net spin lattice relaxation rate ⁴⁹ is given by ₁R

$$1=(1-x)*R_{1d}+x*(R_{1d}+R_{1p})=R_{1d}+x*R_{1p}$$

$$R_1=(1-x)*R_{1d}+x*(R_{1d}+R_{1p})=R_{1d}+x*R_{1p}$$

$$R \quad (1)$$

= diamagnetic or anoxic component of the relaxation rate _{1d}Where R and

= paramagnetic component of the relaxation rate due to the _{1p}R contribution of oxygen.

is directly related to xAs per Henry's law, the dissolved mole fraction the partial pressure of oxygen.

$$pO_2=k*x \quad pO_2=k*x$$

p

(2)

levels can be ₂Inverting the above equation, quantitative pO determined more accurately and reliably if temperature is also monitored.

Relative Signal siloxane (α)	η (X10 = B'/A')	Slope B' $^{-1}(s \cdot \text{torr})$	Intercept $^{-1}A'$ (s)	Molecular wt. (g/mol)	Siloxane
1.00	11.6	$0.0013 \pm 2.09 \times 10^{-5}$	$0.1125 \pm 1.38 \times 10^{-3}$	162.4	HMDSO
0.98	11.4	$0.0012 \pm 8.38 \times 10^{-5}$	$0.1597 \pm 7.50 \times 10^{-3}$	236.5	OMTSO
0.97	8.4	$0.0015 \pm 3.60 \times 10^{-5}$	$0.1780 \pm 3.30 \times 10^{-3}$	310.7	DMTSO
0.97	8.2	$0.0017 \pm 7.44 \times 10^{-5}$	$0.2062 \pm 6.70 \times 10^{-3}$	384.8	DDMPSO
0.91	5.6	$0.0016 \pm 3.87 \times 10^{-5}$	$0.2827 \pm 3.50 \times 10^{-3}$	296.6	OMCTSO
0.92	4.7	$0.0015 \pm 3.47 \times 10^{-5}$	$0.3169 \pm 3.10 \times 10^{-3}$	370.8	DMCPSO

$$C * TB + D * TpO2 = R1 - A - C * TB + D * T \tag{7}$$

Therefore, estimation of these parameters (A, B, C and D) is crucial measurement. Equation [7] also allows us to relate ρ_2 for accurate pO estimation at a particular oxygenation level and ρ_2 the errors in pO temperature to the potential errors or uncertainty in temperature applications. Also, for a given *in vivo* measurement, particularly for determination per 1 °C ρ_2 measurement, the relative error in pO₁R error in temperature estimation at a particular temperature T and can be derived as: oxygenation level pO

of the siloxane Table 1 Summary of calibration constants and α various linear and cyclic siloxanes at 4.7 T (37 °C).

$$pO2\Delta T = |C + D * pO2| / |B + D * T|$$

$$\Delta pO2\Delta T = |C + D * pO2| / |B + D * T|$$

Results

Calibration curves of linear and cyclic siloxanes at 4.7 T

of the linear and cyclic siloxanes (Fig. 1) OMTSO, DMTSO, ρ_1 R DDMPPO, OMCTSO and DMCPSO were measured as a function of at 4.7 T and 37 °C and fit to the Eq. [3] to yield the calibration ρ_2 pO of all the ρ_1 constants A' and B'. At a fixed temperature (37 °C), the R > 0.99) (Fig. 2). In ρ_2 (R₂ siloxanes showed a linear dependence on pO the linear siloxanes, it was observed that with increasing molecular weight, the intercepts of the linear fits increased (ranging from) but the slopes were almost similar (ranging from 1.3×10^{-1} –0.32 s). The cyclic compounds had higher $^{-1} \cdot \text{torr}^{-1} \cdot \text{s}^{-3}$ -1.7×10^{-3} 10 than the linear compounds at each oxygen concentration and ρ_1 R showed a similar trend with increasing molecular weight as the linear siloxanes. The recovery curves for all siloxanes showed a monoexponential behavior (Supplementary Fig. S1). Table 1 lists the values of the calibration constants A' and B' for all the linear and values. ρ_2 cyclic siloxanes at 4.7 T and 37 °C along with the α HMDSO calibration constants at 4.7 ρ_2 were included from our for comparison. ρ_3 previously published work

While comparing signals from same volumes of various siloxanes (as), the *in vivo* typically a fixed volume of reporter probe would be used , of each (compared to HMDSO) ρ_2 relative theoretical signals, α can be computed by accounting for the density differences and the mole fraction of protons in a mole of the siloxane.

$$\text{siloxane} = \rho_{\text{siloxane}} * N_{\text{siloxane}} * H * M_{\text{WH}}$$

$$M_{\text{DSO}} \rho_{\text{HMDSO}} * N_{\text{HMDSO}} * H * M_{\text{Wsilox}}$$

$$\alpha_{\text{siloxane}} = \rho_{\text{siloxane}} * N_{\text{Hsiloxane}} * M_{\text{WHM}}$$

$$D_{\text{SO}} \rho_{\text{HMDSO}} * N_{\text{HHMDSO}} * M_{\text{Wsiloxane}}$$

Figure 1

ρ_{siloxane} is the density of the siloxane under ρ_2 is the molecular weight of $M_{\text{Wsiloxane}}$ $M_{\text{Wsiloxane}}$ and the siloxane (162 g/mol for HMDSO). The theoretical values of α are listed in Table 1.

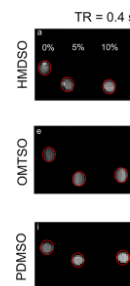
at 7 T was characterized as a function of temperature.

At a given temperature and magnetic field the linear relationship and temperature (defined by Eq. 3) determines the ρ_2 between PO. The intercept A' represents the ρ_2 to changes in PO, sensitivity of R relaxation rate observed under anoxic condition (the diamagnetic, ρ_1) and its inverse represents ρ_1 oxygen independent contribution R displayed by the probe. The slope B' represents the ρ_1 maximum T sensitivity of the probe's relaxation rate to the changes in oxygenation (ρ_2) of oxygen to the ρ_1 and is a ratio of the paramagnetic contribution (R relaxation rate of the probe and the solubility of oxygen in the probe). The ratio $\eta = \text{slope/intercept} (B'/A')$ is a parameter that helps in determining and comparing the sensitivity index of different MRI reporter molecules. A larger slope B' and smaller intercept A' ρ_2 PO but also indicates ρ_2 represent greater sensitivity to changes in PO longer imaging times. Since a smaller A' implies a larger maximum (observed under anoxic or hypoxic conditions which are usually of ρ_1 T interest in studying pathologies), an adequate sampling of the recovery curves would require the use of longer recovery time TR for imaging. We characterized the relaxation behavior of the linear and cyclic siloxanes by comparing the magnetization recovery curves (Supplementary Fig. S1). ρ_2 vs 21% O₂ (0% O₂ after bubbling with N₂) behavior was not observed in any of the ρ_1 Bi-exponential T magnetization recovery curves of the evaluated linear and cyclic siloxanes suggesting that the availability of oxygen to all the protons (e.g end chain vs backbone for linear siloxanes) was unhindered. We observed a decrease in η with respect to an increase in chain length of the linear siloxanes and the η values ranged from 8.2–11.6 \times sensitivity. Another ρ_2 with only small changes in PO⁻¹ torr⁻³10 important observation was that anoxic relaxation rate (A') increased with increasing chain length of the siloxanes and was higher for cyclic siloxanes than the linear siloxanes. This is again consistent with and is a ⁵¹ and alkanes⁵⁰ the observations for perfluoroalkanes consequence of the reduction of molecular tumbling rate for larger chain lengths leading to an increase in relaxation rate. The higher and hence ρ_1 anoxic relaxation rate indicates a shorter maximum T mapping for the larger siloxanes ρ_1 potentially less time needed for T which can be further exploited to map tissue oxygenation faster than HMDSO. The B' value remained similar between the linear and cyclic siloxanes indicating that the solubility of oxygen and its proximity to the methyl protons remains similar between the siloxanes of different chain lengths and structures.

Performing MR oximetry at higher magnetic field strengths has the following advantages: 1) resonances have larger chemical shift separation between them which aids in selective excitation of the siloxane resonance as well as the suppression of the water and fat resonances, 2) increase in net magnetization, leading to improved over the ρ_1 signal-to-noise, 3) improved dynamic range in T range resulting in more accurate ρ_2 physiological PO measurements. On the other hand, the relaxation times tend to ρ_2 PO which might result in an ^{52,53} increase at higher magnetic fields mapping. Our goal was to ρ_2 increase in the total imaging time for PO s of HMDSO and OMTSO at 7 T and ρ_1 evaluate the relationship of T 9.4 T and also help in determining the choice of siloxane for applications requiring higher temporal resolution. Our results suggest = 0 torr and ρ_2 was constant at 9 s at PO₁ that at 37 °C for HMDSO, T = 160 torr on changing the field ρ_2 ranged from to 3.5–3.2 s at PO obtained ρ_1 strength from 7 T to 9.4 T, which differed by ~ 2% from T determination as ρ_2 . Also, the calculated relative error in PO³⁸ at 4.7 T given by Eq. [8] at 37 °C was ~ 0.7 torr/°C at 7 T and ~ 1 torr/°C at value was 5 torr. Since the maximum ρ_2 9.4 T when the actual PO (and hence potentially imaging time) at 7 T and 9.4 T was same as ρ_1 T for 4.7 T with no significant increase of temperature-fluctuation determination, PISTOL oximetry using ρ_2 induced error in PO HMDSO would be improved at higher magnetic field strengths. = 0 torr ρ_2 ranged from 6.3–6.8 s at PO₁ Similarly, for OMTSO the T

) A of PDMSO on (ρ_1 Dependence of spin lattice relaxation rate R) 7 T. B and temperature at (ρ_2 PO

Figure 6



calibration ρ_2 maps from the pO₁ Representative MR images and T) h–e), OMTSO (d–a maps for HMDSO (ρ_2 along with predicted pO) at 37 °C and 7 T. In each figure the tubes from left l–i and PDMSO (), ρ_2 (balance N₂ to right) were bubbled with 0%, 5%, 10% and 21% O respectively. All the images were analyzed using MATLAB R2018b (MathWorks, <https://www.mathworks.com/products/matlab.html>).

Discussion

reporter ρ_2 HMDSO has been previously characterized as a pO H MR ¹ molecule for quantitative oximetry using the PISTOL and HMDSO based nanoemulsions have also ^{38,39} oximetry technique . ^{44,45} at 4.7 T *in vitro* and *in vivo* been used to report oxygenation Furthermore, PDMSO nanoemulsions have been used for cell at 7 T. In this ⁴⁶ labelling and oximetry of neural stem/progenitor cells study we had several goals. Firstly, we aimed to expand the utility of the PISTOL technique for MR oximetry by identifying other reporter molecules. Temperature-dependent ρ_2 siloxanes for use as PO calibration of HMDSO was conducted at 7 T and 9.4 T to study the effect of magnetic field on calibration of HMDSO and to extend its sensor (at 4.7 T) to higher ρ_2 previously demonstrated utility as PO fields (7 T and 9.4 T). Based on initial observations at 4.7 T, the reporter OMTSO was further evaluated at higher ρ_2 promising PO fields (7 T and 9.4 T) and calibration curves were characterized as a function of temperature. Finally, given the prior application of , its calibration curve ⁴⁶ PDMSO at 7 T for cell labelling and oximetry

siloxanes.

In summary, we have demonstrated for the first time the feasibility of ^1H -sensing probes for ^2O of various linear and cyclic siloxanes as pO₂ MR oximetry. Of these OMTSO can be identified as a promising probe which could enable faster mapping of tissue oxygenation ^2O than HMDSO without a significant drop in sensitivity. Alternatively, for applications requiring better temporal resolution or for cell labelling applications, one can use cyclic or long chain linear siloxanes, such as PDMSO, along with a recently developed pulse sequence for faster ^1H MR oximetry. In general, all the siloxanes ^1H MR oximetry sequence for faster studied here, with a broad range of boiling points and dynamic range of ^1H MR ^1s , can be used for diversifying the applications of ^1H MR oximetry.

Materials and Methods

The linear siloxanes HMDSO, OMTSO, DMTSO, DDMP SO, and cyclic siloxanes OMCTSO and DMCP SO were purchased from Sigma-Aldrich (St Louis, MO). PDMSO (MW = 410, viscosity = 2 cSt) was purchased from Alfa Aesar (Tewksbury, MA). All the materials were used as received and all the experiments were conducted without any dilutions i.e. used 'neat'.

For the sample preparation, each siloxane (1 ml) was placed in 4 gas tight NMR glass tubes (Wilmad Taperlok, Buena, NJ) and saturated by bubbling for 15 minutes with varying standard of gases including N_2 , O_2 , respectively. Gases with O_2 (balance N_2) 0%, 5%, 10%, and 21% O_2 varying oxygen concentrations were made by mixing nitrogen and air in varying proportions in a HypoxyDial (STARR Life Sciences meter was connected in line with the O_2 Corp.; Oakmont, PA). A PO₂ output of the HypoxyDial in order to verify the accuracy of the HypoxyDial. The tubes were then sealed. For measurement of the ^1H MR, the temperature of the water pad was ^1H temperature dependence of T varied between 17 to 52 °C. A fiber optic probe (FISO Technologies Inc., Quebec, Canada) was used to measure the temperature of the tubes.

MR experiments were performed on a Varian Inova 4.7 T, Bruker BioSpec 7 T and Varian Inova 9.4 T. The tubes were placed together measurements were performed ^1H on a pad with circulating water and T after the tube temperature ^1H using previously described methods was allowed to equilibrate at the desired value for 10–20 mins using a measurement was conducted by ^1H surface or volume coil. Briefly, T using pulse-burst saturation recovery with a variable TR ranging from data were fit to a single exponential, 3-parameter ^1H 0.1–55 ms. T magnetization recovery equation using the Levenberg-Marquardt algorithm. The data at each temperature was then fit into the Eqs. [4–7] described earlier to obtain the corresponding calibration values. MATLAB R2018b (MathWorks, Natick, ^1H constants and T maps and ^1H MA) was used to analyze the images and compute T maps. Using equation [8], the dependence of errors in $^2\text{pO}_2$ determination per 1 °C change due to temperature fluctuations $^2\text{pO}_2$ was simulated for oxygenation levels in relevant hypoxic range (0 torr to 50 torr) at 37 °C.

= 160 torr over the range of magnetic fields 2 and around 2.8 s at pO₂ strengths studied. Thus, the changes in the fields strength will not result in a substantial increase in the imaging time for OMTSO and 2 are similar to that of HMDSO siliconethe η and relative signal (α) (Table 1). Further, the calibration of OMTSO and PDMSO demonstrated that longitudinal relaxation rate of both the siloxanes at temperatures in the 2 varied linearly with respect to changes in PO₂ physiological range, demonstrating the potential of OMTSO and 2 . At a temperature 2 PDMSO to measure dynamic changes in tissue PO₂ of 37 °C OMTSO and PDMSO had an oxygen sensitivity similar to 2 at all ^1H torr 1 HMDSO (B' values ranging from 0.0011 to 0.0013 s three fields. It should be also noted that boiling point of OMTSO (153 °C) is higher than the boiling point of HMDSO (101 °C). This suggests that it maybe be more advantageous to use OMTSO for generating nanoemulsions for cell labelling applications than 2 as it would be less volatile during the ^45H HMDSO (used previously determination 2 . The simulated errors in pO₂ emulsification process due to temperature fluctuations for OMTSO as well as PDMSO were found to be in the same range as HMDSO at 4.7 T, 7 T and 9.4 T.

in vivo We have previously demonstrated the feasibility of mapping following intra-tissue injection of 'neat' HMDSO 2 pO₂ ^1H . Dilution of the 39,44,55 as well as HMDSO based nanoemulsions calibration curve 2 siloxane in a solvent can potentially affect the PO₂ ^1H in a "concentration-dependent" manner by 1 or R_1 (and hence T changing the intercept due to changes in the dipole-dipole interactions of the siloxane protons with the solvent protons, although ^1H studied the dependence *et al* this was not tested here. Jamrogiewicz ^1H relaxation times of linear HMDSO on dilution using a mixture ^1H of carbon tetrachloride with deuterated benzene and found that did not significantly depend on the analyte concentration in the ^1H T. Dilution of 48 sample or the mutual ratio of the solvents used siloxanes in solvents can also affect the slope of the calibration curve as the oxygen solubility may change based on solvent used and the siloxane concentration. Dilution in tissue by use of less siloxane administered per tissue volume or by diluting a siloxane emulsion is unlikely to affect the calibration as the siloxane is restricted to a local partitioned environment consisting solely of other siloxane molecules and dissolved gasses in either case. We recommend using undiluted mapping applications in order to maintain the highest 2 probes for PO₂ signal to noise ratio.

In general, siloxanes are considered non-toxic or minimally toxic and ^1H . However, 47 toxicity decreases with increasing molecular weight use. *in vivo* individual siloxanes should be evaluated for safety before Previous studies have shown that HMDSO is quite inert, and exhibited minimal toxicity in rats tested for subchronic inhalation (> 5 ml/kg) was found in rats and 50 . No oral toxicity (LD₅₀ 56,57 toxicity no irritation and acute toxicity was reported in Draize tests of skin or ^1H . In our previous studies, we saw 58 eye irritancy in a study in rabbits no overt signs of toxicity, inflammation or discomfort after injection into muscle, although no 44 or HMDSO nanoemulsions 39 of HMDSO microscopic analyses were performed. Cytotoxicity analysis of HMDSO nanoemulsions showed that the half maximal inhibitory in 3T3 45) at a concentration of 0.4–1% (v/v) 50 concentration (IC₅₀ > 2% (v/v) was reported for PDMSO 50 fibroblast cells while a IC₅₀ ^1H . These 46 nanoemulsions in mouse neural progenitor/stem cells findings indicate that the use of siloxanes, especially longer chain siloxanes may be feasible for human applications. In particular, the use of siloxane emulsions for labelling transplanted cells and monitoring cell health is promising due to the trace amounts used. ^1H MR oximetry in ^1H Further, the PISTOL technique used for conjunction with siloxanes utilizes pulse sequence components that are readily available on clinical MRI scanners such as selective RF pulses (for excitation of the siloxane resonance and suppression of fat mapping). This ^1H and water signals) and echoplanar readout (for fast T₂ ^1H MR oximetry using ^1H adds to the promise of clinical translation of

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