MAGMA (2005) 18: 35–40
DOI 10.1007/s10334-004-0092-2

RESEARCH ARTICLE

K. Wachowicz
R. E. Snyder

A continuous-flow perfusion system for the maintenance and NMR study of small tissue samples in vitro

Received: 26 February 2004
Revised: 29 September 2004
Accepted: 4 October 2004
Published online: 7 February 2005

The authors wish to acknowledge the Canadian Institutes of Health Research (CIHR) for funding and the In Vivo NMR Facility of the University of Alberta for infrastructural support

K. Wachowicz (✉) · R. E. Snyder
Department of Biomedical Engineering,
1098 Research Transition Facility,
University of Alberta,
Edmonton, AB, Canada T6G 2V2
E-mail: wachow@ieee.org
Tel.: +1-780-492-8098
Fax: +1-780-492-8259

Abstract To describe and evaluate a novel perfusion system developed to maintain excised tissue in a flowing, oxygenated bathing solution during acquisition of nuclear magnetic resonance (NMR) data, and in addition allow precise data to be acquired continuously while altering the composition of the bathing solution surrounding the tissue. A chamber to house the tissue sample was constructed of interlocking sections of polyethylene tubing, and had approximate internal dimensions of 4 mm in diameter and 4 mm in height. Temperature-controlled, physiologically appropriate buffer solution was pumped via an infusion pump through the chamber, entering and exiting by way of small openings on either end. Immediately surrounding the polyethylene chamber was a tight-fitting four-loop solenoid RF coil. Measured proton NMR parameters were found to be fairly insensitive to the flow rate of the buffer if this coil was used only for reception and a larger-volume transmit-only coil was used for excitation. Temperature control of the sample was successfully implemented between 25 and 40°C. The perfusion system was found to be resistant to the effects of flow rate, as well as a useful tool for the administration of drugs or agents to the tissue. Changes in buffer composition could be performed on the fly without the need to reposition the sample each time a change was made. This avoidance of repositioning was found to yield a fivefold improvement in the precision of T_2 spectral parameters (using frog sciatic nerve as a sample).

Keywords NMR spectroscopy
In vitro · Tissue · Perfusion · T_2 relaxation

Introduction

Samples of excised tissue are frequently studied in vitro to better understand the nuclear magnetic resonance (NMR) characteristics and properties of the tissue. Non-imaging, in vitro proton studies of relaxation and diffusion are generally performed in one of two conditions: with the tissue placed in an NMR tube and submersed in a static buffer solution, or with the tissue covered by a sealant to prevent dehydration [1–9]. The continuous perfusion of tissue is often avoided because of possible flow-related distortions to measured parameters. This paper describes a system that continuously supplies a fresh, oxygenated buffer solution to an excised tissue sample throughout the course of an NMR study, together with a coil configuration resistant to the detrimental effects of buffer motion. Brief reports of preliminary studies have been published [10, 11].

Materials and methods

Chambers to house the tissue samples to be studied were constructed from two types of material: one was machined from polycarbonate and a second assembled from interlocking

sections of polyethylene tubing. Both types of chamber were cylindrical, having inner dimensions of approximately 4 mm in diameter and 4 mm in height. These dimensions were appropriate for the tissue samples evaluated for test purposes in this study, but could be varied to accommodate samples of different sizes. Polyethylene tubing, having an internal diameter of approximately 0.8 mm, was pressure-fitted to the chambers through holes in either end to allow a physiologically appropriate buffer solution to flow through the chamber and around the tissue (Fig. 1a). These pressure-fits were sufficient to maintain a leak-proof system without any sealing agents. The solution was forced through the chamber against gravity by a dual infusion pump (KD Scientific Inc., Model 210) located approximately 4 m from the magnet. The pump maintained a constant flow rate that was varied between 0 ml/h and 21 ml/h, and could continue uninterrupted for as long as the syringe reservoir (60 ml) lasted. A manual valve, which could select one of two solutions to flow through the chamber, was placed in the inflow tubing approximately 15 cm before the chamber. By adjusting the valve it was possible to change the solution flowing through the chamber within approximately 30 s. For comparison, during a different set of studies designed to assess the precision of measured parameters, samples of tissue were placed in a 5-mm NMR tube containing a buffer solution.

The tissue used to evaluate the system was sciatic nerve taken from *Xenopus laevis*, the African clawed frog, although any excised tissue in general could be studied with this system. Frog sciatic nerve was chosen, as it has a well-characterized multicomponent T_2 spectrum [9,12] that can serve to assess performance of the system. Details regarding sample preparation and buffer solution may be found in the literature [12]. The results from all the frog nerve experiments that are presented in this work were obtained while bathed in a solution at room temperature ($\sim 20^\circ$C). All animal procedures were approved by the Health Sciences Animal Policy and Welfare Committee of our institute.

Frog sciatic nerve has been reported to have an oxygen consumption rate of $6–12 \times 10^{-6}$ mol/h/g (dry weight) at 20°C [13–15], corresponding to an estimated consumption rate of $40–80 \times 10^{-9}$ mol/h for a typical nerve sample (estimated at just under 7 mg dry weight) used in our studies. Allowing a buffer solution whose oxygen content was initially at equilibrium with room air at atmospheric pressure ($pO_2 = 159$ mmHg) to deplete through consumption to 40 mmHg would deliver approximately 1.5×10^{-7} moles of oxygen per millilitre of solution. Thus, an adequate delivery of oxygen would require a flow rate of 0.27–0.54 ml/h. If one were to use such a flow rate, 30–60 min would be required for 99% replacement of the previous buffer when switching from one solution to another (based on the given dimensions of the chamber, and assuming a well-stirred solution). We thus used typical flow rates of 5–8 ml/h that afforded sufficient oxygenation of the tissue, a reasonable switching time (2–3 min), and the avoidance of turbulence that might disturb the tissue with a higher flow rate. Rat sciatic nerve at 35°C has been reported [16] to have an oxygen consumption rate of four to eight times that of frog nerve, thus requiring a flow rate of up to 4 ml/h. Additional oxygen could be supplied, if warranted, by further increasing the flow rate, and/or by saturating the buffer solution with pure oxygen at atmospheric pressure.

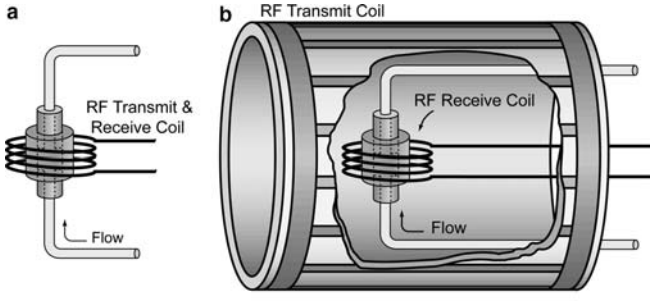

Fig. 1 Schematic of single-coil (**a**) and two-coil (**b**) systems for excitation and reception of RF signals. In both systems a valve (not shown and outside of the RF transmit coil), which allows for the switching between different buffer solutions, is included in the inflow tubing

For all experiments performed, the perfusion chamber was centred in the bore of a 3-T, 80-cm-bore, whole-body NMR system controlled by an SMIS console. In an attempt to allow accurate data acquisition during continuous buffer flow, an RF system was developed in which a larger volume coil surrounded the entire chamber apparatus, and a smaller, four-loop, tight-fitting solenoid coil surrounded the chamber (Fig. 1b). During some studies, the smaller coil both transmitted and received RF signals (single-coil system), while during others, the larger volume coil transmitted and the smaller coil received the signals (two-coil system). The larger was a shielded birdcage coil having 16 elements and active decoupling circuitry to turn it off during RF acquisition by the smaller coil. The dimensions of the larger coil were approximately 7.3 cm in diameter (at the elements), 12.3 cm in length, with a shield diameter of 11 cm. The smaller coil was equipped with a crossed-diode circuit to prevent interaction of the coils during transmission by the larger coil [17]. Both RF coils used in this study were manufactured in house. The exact dimensions of the coils are not important, provided the transmit coil can provide a uniform B_1 over the entire chamber and a sufficient length of the inflow tubing.

When it was desired to increase the temperature of the buffer solution flowing through the chamber above ambient, a section of the polyethylene tubing that supplied solution to the chamber was passed through a circulating water bath located just outside the chamber. Water to the bath was supplied through insulated 1-cm-diameter polyethylene tubing that passed through a variable-temperature heating system located outside of the magnet. To monitor the chamber temperature, a fibre-optic temperature sensor (Model FOT-M, FISO Technologies) was placed in the tubing immediately beyond the outflow opening of the chamber. Using the temperature-control system as described, temperatures of up to 40°C in the chamber could be achieved and maintained with a variation of $< \pm 1^\circ$C. The temperature within the chamber stabilized within approximately 10 min following selection of a new bath temperature.

Transverse-relaxation decay curves were acquired using a phase-cycled Carr–Purcell–Meiboom–Gill (CPMG) sequence that included 4, 8, or 16 averages and transformed into T_2-relaxation spectra using nonnegative least-squares (NNLS) analysis [18], details of which may be found in the literature [12].

Results and discussion

Perfusion chamber material

Transverse-relaxation spectra were obtained for the two types of chamber (polycarbonate and polyethylene) while perfused with water at flow rates of 5–8 ml/h. The single-coil system was used for this experiment, and no tissue was present. Both chambers yielded the expected T_2 spectral line at approximately 1600 ms resulting from the water protons. However, the polycarbonate chamber produced artifactual T_2 lines (over 10% of the total signal), particularly in the region between 10 ms and 500 ms where T_2 components of soft tissue are most often reported. The relatively large amount of unwanted signal in this region made it necessary to rule out the use of machined polycarbonate chambers. The polyethylene chamber had significantly fewer artifactual lines in this region, and was therefore used in all subsequent studies. There was, however, one large spectral line originating from the use of the polyethylene at approximately 1 ms, but as its T_2 relaxation time was considerably lower than that of the tissue components, it did not prove to be a significant problem.

Comparison of spectral-parameter precision between chamber and NMR tube

Transverse-relaxation spectra were obtained from nerve contained in either an NMR tube or the perfusion chamber using the single-coil system. (Buffer flow was always maintained in the perfusion chamber unless otherwise stated.) Both yielded three-component spectra similar to spectra reported in the literature [9, 19], thus demonstrating a comparative performance of the perfusion chamber to an NMR tube, the latter considered the gold standard for in vitro analysis.

In studies in which the buffer solution is altered from one composition to another, the tissue is generally removed from an NMR tube, placed in the altered solution for a period of time, and then reintroduced into the tube together with the altered solution. A study consisting of four experiments was thus performed to determine the level of precision in T_2 spectral component parameters due to repositioning, as well as to inter-preparation variation. In the first experiment, a freshly excised piece of nerve was placed in the two-coil perfusion chamber, and twenty T_2 spectra were acquired. In the second experiment, the same procedure was repeated using a conventional NMR tube and a single transmit/receive RF coil. (A different nerve preparation was used in the second experiment.) In the third experiment, eight T_2 spectra were acquired from the same nerve preparation as in Experiment 2, except that in this case, the nerve was repositioned in the NMR tube before each acquisition. Finally, in a fourth experiment, seven T_2 spectra were acquired from seven different nerve preparations in an attempt to assess inter-preparation variation. The statistical variances of the resulting T_2 spectral parameters were calculated, and are presented in Table 1. To summarize, multiple spectral measures using either the chamber or the NMR tube (columns 1 and 2) yielded similar standard deviations for corresponding parameters; however, repositioning (column 3) increased the standard deviations by a factor of approximately 5. Inter-preparation standard deviations (column 4) were fairly close to those of the repositioning experiment.

Variation with flow rate

Measurements were made to assess the stability of the results with change in flow rate. Freshly excised nerve was placed in the perfusion chamber using the single-coil system, and T_2 spectra were recorded for 15 different flow rates varying from 0 to 14 ml/h. All spectra contained three components attributable to the nerve plus one resulting from the buffer solution. As a function of flow rate, only minor variation was observed in component T_2-relaxation times (data not shown); however, relative component sizes did exhibit variation (Fig. 2a). To account for this variation, it was hypothesized that the decay curve of flowing buffer solution encountered differing levels of distortion, dependent on the flow rate. Two different factors could account for this distortion: signal loss from solution flowing outside the sensitive range of the RF coil, and errant signal from the solution within the inflow and outflow tubes that received imperfect excitation and inversion pulses. These differences in the buffer decay curve could then affect the nerve and buffer component sizes as seen through the analysis routine.

A computer simulation was performed to test the above hypothesis in order to determine whether a newly designed coil system was justified. In the simulation it was assumed that the primary source of variation with flow rate resulted from signal losses derived from excited buffer flowing beyond the range of sensitivity of the RF coil during the acquisition period. The loss of excited buffer was taken to increase linearly with time, generating a signal loss having the form of a linearly increasing plot modulated by an exponentially decaying envelope associated with the transverse signal decay of the buffer solution. The simulation assumed immediate transitions in coil sensitivity, and thus neglected any period of transition, both on the inflow and outflow sides of the chamber. The predicted signal losses were calculated with the following equation:

$$\mathrm{SignalLoss}(n) = S_V \cdot R_F \cdot n\mathrm{TE} \cdot e^{n\mathrm{TE}/T_{2\mathrm{buffer}}}, \quad (1)$$

where n is the echo number, S_V is the estimated signal strength per volume, and R_F is the flow rate. The predicted signal loss versus acquisition (echo) time for a range of

Table 1 Standard deviations for transverse-relaxation component parameters in frog sciatic nerve, as evaluated by an NNLS algorithm

	Chamber ($N=20$)		NMR tube ($N=20$)		Repositioning ($N=8$)		Inter-preparation ($N=7$)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Size A (%)	22.16	0.33	22.69	0.42	20.58	0.76	19.54	1.95
Size B (%)	41.63	0.94	39.69	0.63	38.82	3.26	38.71	4.06
Size C (%)	36.21	0.55	37.62	0.86	40.60	4.66	41.75	3.98
T2 A (ms)	18.09	0.27	17.07	0.54	17.54	1.56	16.97	1.13
T2 B (ms)	82.33	0.58	96.17	0.77	97.60	8.55	82.87	3.96
T2 C (ms)	268.68	3.01	301.25	4.10	287.75	24.71	231.61	15.46

Note In the first of the four columns, one nerve preparation was viewed 20 times using the two-coil perfusion chamber. In the second column, a different nerve preparation was viewed 20 times in a conventional NMR tube. The third column utilized the same nerve preparation as in column 2, but this time the nerve was repositioned in the NMR tube between each spectra acquired. In the last column, seven spectra were acquired using seven different nerve preparations placed in NMR tubes

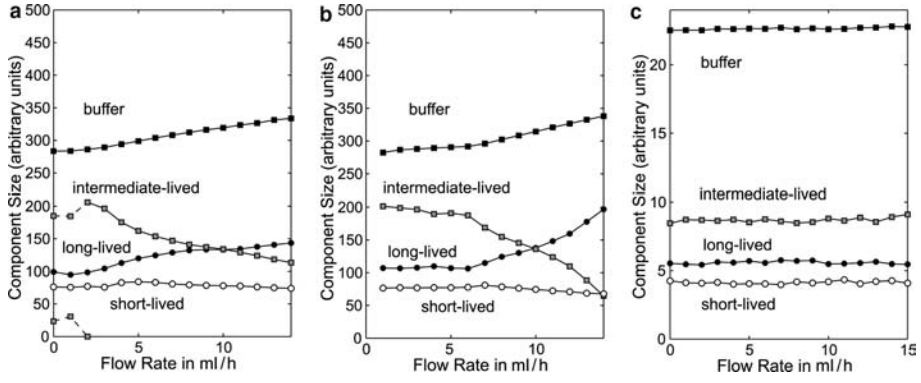

Fig. 2 Variance of nerve and buffer component sizes with changes in flow rate. **a** Experimental results from a nerve sample in the single-coil system. **b** Results generated when theoretical flow loss curves for 1 ml/h to 14 ml/h are subtracted from the zero flow data in **a**. **c** Experimental results from a nerve sample (different from **a**) in the two-coil system. Note the lack of variation of the components with flow rate

flow rates is shown in Fig. 3a (dashed), where it is compared to a measured loss at the same rates (solid). The latter were obtained by measuring the signal difference between nerve experiments with flowing and static buffer solutions. Comparison of these two sets of curves suggests that the outflow of solution excited while in the chamber may account for a substantial portion of the signal loss. To determine whether the predicted signal loss could account for the variation of component size with flow rate (as seen in Fig. 2a), loss predictions for flow rates between 1 ml/h and 14 ml/h were each subtracted from an experimental zero-flow data set. The resulting decay curves were then converted to T_2 spectra with the nonnegative least squares (NNLS) algorithm. The magnitudes of the components from the resulting spectra are shown in Fig. 2b and show qualitative agreement with the trends experimentally determined in Fig. 2a.

Two-coil system

In order to allow for more precise quantitative studies a new experimental setup had to be devised to reduce the dependency of measured parameters on flow rate. Thus, as a means to avoid flow-related signal losses, it was decided that the RF transmission and reception responsibilities should be assumed by two separate coils. In this two-coil system, a larger birdcage coil would transmit RF energy over a region of the apparatus that included the chamber as well as the inflow and outflow perfusate tubing. The smaller single coil that circumscribes the perfusion chamber would then act as a dedicated receive-only coil (Fig. 1b). As a result of the larger coil, all buffer solution from which signal is acquired (whether in the chamber or the tubing) would receive correct excitation and inversion pulses.

To test for signal loss as a function of acquisition time, decay curves were acquired from buffer solution and frog sciatic nerve over a number of different flow rates, varying between 0 ml/h and 15 ml/h (using the two-coil system). All the flow data sets were then subtracted from the static buffer data set to measure experimental loss curves due to flow. A sampling of loss curves measured over a range of

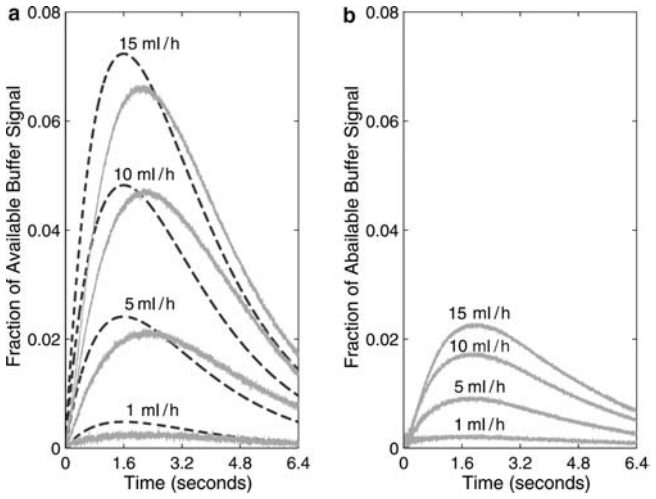

Fig. 3 **a** Experimentally determined flow loss curves for the one-coil system (*solid*) as well as theoretical loss curves (*dashed*) based on the flow model. **b** Experimentally determined flow loss curves for the two-coil system

flow rates is shown in Fig. 3b. As can be seen, the results show a marked reduction in signal losses when the two-coil system is used. The remaining losses probably originate from a decrease in measured buffer T_2 as the flow rate increases. Unlike the losses modeled by Eq. 1, these should not cause distortion in measured tissue parameters, since the exponential nature of the buffer signal would not be compromised; only its decay constant would change. To determine the effect of the two-coil system on variation in component size with flow rate, T_2 spectra were calculated from the decay curves obtained above. The resulting component sizes are displayed in Fig. 2c. Comparing these to results using only the single-coil system, (Fig. 2a) shows a marked improvement in stability.

Conclusions

Experimental data from the use of the perfusion chamber were found to equal the quality of those obtained using standard NMR tubes. This novel perfusion system is able to offer several advantages, however. The two-coil system was successful in minimizing effects of the flowing buffer on the results, allowing quantitative measurements of NMR parameters while the buffer composition is changed on the fly. Fresh buffer solution can be supplied to a tissue sample at flow rates high enough to provide for its oxygen requirements. Agents can also be introduced into the chamber in a longitudinal study without the need to reposition the sample. This is of significant benefit, as a fivefold increase in the precision of measured parameters was found to result when the repositioning of the sample was avoided. The system is also capable of controlling the temperature of the sample between 25°C and 40°C.

References

1. Bertram HC, Rasmussen M, Busk H, Oksbjerg N, Karlsson AH, Andersen HJ (2002) Changes in porcine muscle water characterized during growth—an in vitro low-field NMR relaxation study. J Magn Reson 157:267–276
2. Does MD, Beaulieu C, Allen PS, Snyder RE (1998) Multicomponent T_1 relaxation and magnetization transfer in peripheral nerve. Magn Reson Imag 16:1033–1041
3. English AE, Joy MLG, Henkelman RM (1991) Pulsed NMR relaxometry of striated muscle fibers. Magn Reson Med 21:264–281
4. Cutillo AG, Chan PH, Ailion DC, Watanabe S, Albertine KH, Durney CH, Hansen CB, Laicher G, Scheel RF, Morris AH (1998) Effects of endotoxin lung injury on NMR T_2 relaxation. Magn Reson Med 39:190–197
5. Henkelman RM, Stanisz GJ, Kim JK, Bronskill MJ (1994) Anisotropy of NMR properties of tissue. Magn Reson Med 32:592–601
6. Graham SJ, Ness S, Hamilton BS, Bronskill MJ (1997) Magnetic resonance properties of ex vivo breast tissue at 1.5 T. Magn Reson Med 38:669–677
7. Nightingale T, MacKay A, Pearce RH, Whittall KP, Flak B (2000) A model of unloaded human intervertebral disc based on NMR relaxation. Magn Reson Med 43:34–44
8. Beaulieu C, Fenrich FRE, Allen PS (1998) Multicomponent water proton transverse relaxation and T_2 discriminated water diffusion in myelinated and non-myelinated nerve. Magn Reson Imag 16:1201–1210
9. Peled S, Cory DG, Raymond SA, Kirchner DA, Jolez FA (1999) Water diffusion, T_2, and compartmentation in frog sciatic nerve. Magn Reson Med 42:911–918
10. Wachowicz K, Bonilla I, Snyder RE (2003) The use of a decoupled coil system and relaxation agents to improve perfusion chamber data. In: Proceedings of the 11th Annual Meeting of ISMRM, Toronto, p 2427
11. Wachowicz K, Snyder RE (2000) A perfusion chamber suitable for the measurement of transverse relaxation spectra of tissue maintained in vitro. In: Proceedings of the 8th Annual Meeting of ISMRM, Denver, p 1987
12. Wachowicz K, Snyder RE (2002) Assignment of the T_2 components of amphibian peripheral nerve to their microanatomical compartments. Magn Reson Med 47:239–245
13. Connelly CM (1959) Recovery processes and metabolism of nerve. Rev Mod Phys 31:475–484

14. Hurlbut WP (1958) Effects of azide and chloretone on the sodium and potassium contents and the respiration of frog sciatic nerves. J Gen Physiol 41:959–988
15. Brink F Jr (1975) Components of O_2-uptake by excised frog nerve dependent upon external supplied sodium ions. Proc Nat Acad Sci USA 72:3988–3992
16. Low PA, Schmelzer JD, Ward KK (1986) The effect of age on energy metabolism and resistance to ischaemic conduction failure in rat peripheral nerve. J Physiol (London) 374:263–271
17. Barberi EA, Gati JS, Rutt BK, Menon RS (2000) A transmit-only/receive-only (TORO) RF system for high-field MRI/MRS applications. Magn Reson Med 43:284–289
18. Whittall KP, MacKay AL (1989) Quantitative interpretation of NMR relaxation data. J Magn Reson 84:134–152
19. Does MD, Snyder RE (1995) T2 relaxation of peripheral nerve measured in vivo. Magn Reson Imag 13:575–580