BACKGROUND

Advances in RF coil design have recently permitted investigations into the MR relaxation properties of tissue microstructures [1-4]; however, microperfusion apparatus are typically developed for use with light-microscopy staging and are not manufactured with NMR compatibility as a design criterion thus resulting in a recent, unmet need for specialized microperfusion equipment. Here, we describe a home-built microperfusion rig based on previous iterations of external oxygenators built for use in MRI [5] which interfaces with a modified Bruker micro surface-coil. NMR and biological compatibility as well as the ability to operate within the spectrometer bore were key design features.

METHODS

Tissue retention was accomplished as previously described (Fig. 3).6 Perfusion and tissue wells were joined using a silicon gasket and secured by a nylon zip-tie. Gas inlet and exchange chambers were constructed using 5mm and 10mm economy NMR tubes respectively. Gas exchange took place through highly permeable silicone tubing. After testing pH and dissolved oxygen levels (data not shown), timecourse experiments (21h) were conducted on fixed cortical slices (300μm, n = 3) to eliminate the possibility of signal instability resulting from dynamic changes in tissue structure. Continuous perfusion (2ml/min) and no perfusion (static control) groups were compared to determine perfusion’s contribution—if any—to signal variability over time. Signal was recorded in 14 separate but identical DW scans (TR/TE = 2000/11.6ms, b = 1200s/mm², Δ = 1ms, Δ = 6ms, 1.5h). Equivalence tests were conducted assuming a range equal to the total signal change over time (21h) observed in the static perfusion (stable control) group.

RESULTS

Figure 1 shows the modified 500μm diameter micro surface-coil (Bruker, B6370). Figure 2 shows the completed oxygenator, coil assembly, and bubble trap. Figure 3 is an exploded schematic of our tissue retention components and well interface. Figure 4 summarizes stability data allowing us to conclude with 95% confidence that signal from the continuous perfusion group was statistically equivalent to that of the static perfusion group in thirteen out of fourteen separate trials (n = 3). One of the scans which made up the constant perfusion group’s third—and only non-equivalent—time trial exhibited a dramatic and unexplained drop in raw signal which carried over—albeit to a lesser extent—into the fourth trial of the same group.

CONCLUSIONS

Owing to its proximity to the sample, our device solves problems associated with dissolved gas loss from perfusates (mainly low oxygen levels or pH increases due to low CO₂ leading to precipitate formation in bicarbonate buffer systems) which become increasingly problematic as the buffer volume decreases or the perfusion line’s length increases. This allows for precise control of dissolved gas content due to both the rig’s 100% gas saturation ability and subsequent loss prevention achieved by minimizing the distance of travel between the oxygenator and sample. While our stability assessment appears promising, the spike observed in one of three trials for the continuous perfusion group demands caution in interpretation pending further testing.

REFERENCES AND ACKNOWLEDGEMENTS