Non-invasive Measurement of Fibrin Concentration by Fast Field-Cycling NMR Technique

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Introduction and Aim

Fast Field-Cycling (FFC) is a technique used in NMR since the 1970s [1], and more recently in MRI [2]. It involves changing the strength of the main magnetic field during the pulse sequence, typically during polarisation, evolution and detection. FFC NMR offers many possibilities since it allows using low evolution fields to investigate the dispersion of the relaxation rate (R_1), and switching back to higher field during the detection phase to provide a high signal-to-noise ratio in the data. FFC NMR relaxometry has been used since the 1980s for the detection of the ^{14}N-^1H quadrupolar relaxation effect [3], which can be seen in various biological samples as characteristic peaks in the R_1 dispersion curve of water ^1H, called the 'quadrupolar peaks'. These peaks occur at well-defined magnetic fields (16 mT, 49 mT and 65 mT) at which the ^{14}N nuclear quadrupole resonance frequency and the ^1H NMR frequency coincide; the height of the peaks has previously been shown to be proportional to the concentration of immobilised protein [4].

The present work uses FFC NMR relaxometry to investigate the linear relationship suggested by the theory [5] between the amplitude of the quadrupolar peaks generated from cross-linked human fibrin (the main constituent of thrombus), with its concentration. The long-term aim of this study is to test the feasibility of thrombus detection and characterisation by FFC-MRI.

Methods and Materials

The samples were prepared using different concentrations of human fibrinogen (Hyphen BioMed, France, cat. PP001C, lots 090703g and 0310270) from 0.1 to 10 mg.mL^{-1}. The preparations were performed from frozen samples of fibrinogen, defrosted gradually and diluted with fibrin plate buffer (75 mM Tris, 22 mM NaCl, 50 mM CaCl$_2$). Agarose (Sigma Aldrich) was added at 44°C to maintain a good homogeneity for all the samples. The clotting was produced using thrombin at 0.4 U.mL^{-1} (Sigma Aldrich). Then the samples were incubated at 37°C for 180 min and were left at room temperature overnight. The experiment was repeated three times on three different days.

The measurements of relaxation rate were carried out using a commercial bench-top FFC NMR relaxometer (SMARtracer, Stelar s.r.l., Italy). The samples were kept at 20°C +/- 0.1°C during data acquisition by the relaxometer's temperature control system. An inversion-recovery sequence was used employing 30 different evolution fields, linearly selected between 1.5 and 3.5 MHz (equivalent proton Larmor frequency). No averaging was used in order to keep the experimental time short and make sure there was no sample degradation during the measurement. The polarisation time and field used were 5 s and 5 MHz respectively and the field ramp time was set to 2.5 ms. The acquisition field was fixed at 7.2 MHz and the acquisition parameters were carefully selected to avoid out-of-scale ADC conversions.

Figure 1 presents a data set obtained from the 10 mg.mL^{-1} fibrin sample (dots) and the curve obtained by the fitting algorithm (solid line). The curve fitting used for the evaluation of R_1 and the quadrupolar signal was performed with Matlab (Mathworks) using its curve fitting toolbox. The values of T_1 were calculated using a monoexponential model, checking that the R^2 values of the fits were greater than 0.999. The model used to fit the quadrupolar peaks in R_1 used Lorentzian-shaped bells with a power-law background curve [4].

The amplitude of the dips, denoted ΔR_1, was given by the fit and correspond to the difference between the highest point of the peak and the baseline, as indicated in Fig. 1 for the 65 mT peak.

Results

The samples prepared had typical values of T_1 of 1.5 s and could be analysed in 45 min each. Fig. 2 presents the amplitude of the quadrupolar peaks measured for different fibrin concentrations from all three experiments performed. The linear fits obtained have p-values of 0.87 and 0.96 for the 49 and 65 mT peaks respectively. The average slope of the 65-mT peak is 1.18 times lower than that of the 49-mT peak (5.94 +/- 0.69 x10^{-3} mg.s^{-1}.mL^{-1} and 4.80 +/- 0.65 x10^{-3} mg.s^{-1}.mL^{-1} respectively), which is attributed to the effect of the broadening of the ^{14}N energy levels with increasing magnetic fields.

The signal-to-noise ratio measured from the averaged results indicates a detection threshold of 1.04 mg.mL^{-1} fibrin concentration. This can be lowered by using repeated data points for averaging during the data acquisition, but is still significantly lower than the typical physiological concentrations of fibrinogen in plasma (2.35 mg.mL^{-1} [6]).

Conclusion

The presence of a quadrupolar peak signal in the R_1 dispersion curve of human fibrin has been demonstrated and its linearity has been established over a range of concentrations that covers physiological conditions. Further work is planned to determine if this signal can be used to determine the content and rigidity of model thrombi. This could potentially lead to a novel method using FFC MRI to estimate the rigidity of *in vivo* thrombi, which is an important clinical problem.

Fig. 1: R_1 measured in a sample of 10mg.mL^{-1} fibrin in the region of the quadrupolar dips. The baseline is a power law provided by the model used for the curve fitting; the quadrupolar peaks appear clearly above it. The F-test gives a p-value of 0.945 here.

Fig. 2: Variations of amplitude of the quadrupolar dips with different concentrations of fibrin. The error bars stand for the 95% confidence bounds from the fit of the quadrupole dips.

References

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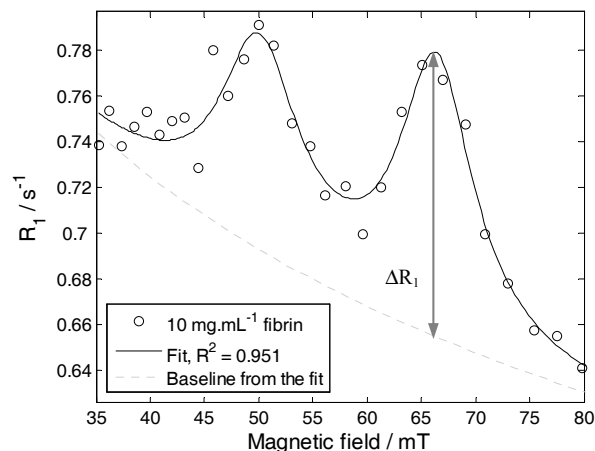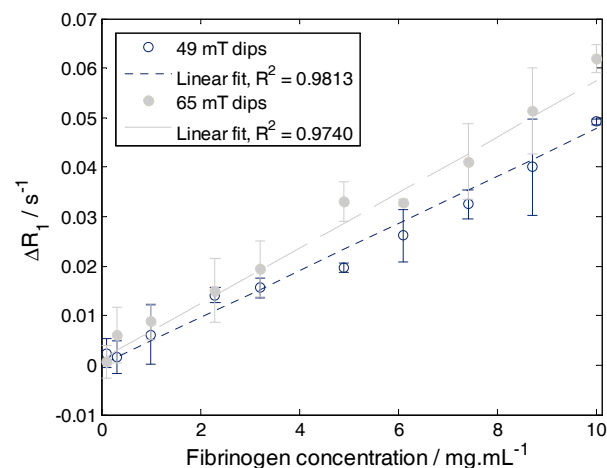[6] Halvorsen S., Skjonsberg O.H. and Godal H.C., *Thrombosis Research* **72**, *1993*, 305-313