Published in final edited form as:

ACS Appl Mater Interfaces. 2019 March 27; 11(12): 11194–11201. doi:10.1021/acsami.9b00394.

Magnetic Manipulation of Blood Conductivity with Superparamagnetic Iron Oxide-Loaded Erythrocytes

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Abstract

The active and passive electrophysiological properties of blood and tissue have been utilized in a vast array of clinical techniques to noninvasively characterize anatomy and physiology and to diagnose a wide variety of pathologies. However, the accuracy and spatial resolution of such techniques are limited by several factors, including an ill-posed inverse problem, which determines biological parameters and signal sources from surface potentials. Here, we propose a method to noninvasively modulate tissue conductivity by aligning superparamagnetic iron oxide-loaded erythrocytes with an oscillating magnetic field. A prototype device is presented, which incorporates a three-dimensional set of Helmholtz coil pairs and fluid-channel-embedded electrode arrays. Alignment of loaded cells (~11 mM iron) within a field of 12 mT is demonstrated, and this directed reorientation is shown to alter the conductivity of blood by ~5 and ~0.5% for stationary and flowing blood, respectively, within fields as weak as 6–12 mT. Focal

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Author Contributions

G.R.P., B.G., and J.W.M.B. conceived and designed the experiments. B.G. designed and fabricated magnetic coils. G.A.P.-J. designed and performed the alginate alignment experiments. A.A. and M.M. designed the RBC loading procedure. G.R.P. designed and constructed the prototype device, performed cell loading procedures and experiments, analyzed data, and prepared the manuscript. All authors discussed the results and edited the manuscript.

The authors declare the following competing financial interest(s): Bernhard Gleich is an employee of Philips Research Europe.

modulation of conductivity could drastically improve numerous bioimpedance-based detection modalities.

Graphical Abstract

Keywords

blood conductivity; electrophysiology; bioimpedance; iron oxide nanoparticles; red blood cells

INTRODUCTION

Rudolf Höber presented the first evidence that biological cells are composed of conductive electrolytes surrounded by a resistive dielectric membrane in 1910. These findings, and those provided in his two subsequent papers on the topic, elucidated the electrical properties of erythrocytes (red blood cells, RBCs) and frog muscle tissue.^{1–3} Thus began the field of bioimpedance research, which was built upon previous studies of the passive electrical properties of cells and tissue (i.e., their response to externally applied potentials), beginning with the work of Galvani in the 1790s.^{4,5} The active electrical properties of cells and tissue (i.e., internal generation of electrical currents) had been explored by Emil Du Bois-Reymond in the 1850s/60s, leading to Einthoven’s practical electrocardiogram in 1903. These two branches continued to develop in parallel, forming the broad field of electrophysiology.⁶

The varying arrangements of electrically conductive fluids and insulating cell membranes that are comprised in the human body present complex profiles of bioelectrical impedance. These properties vary with structure, tissue composition, and health, along with the frequency of the associated currents. This allows their exploitation for non-invasive characterization of tissue anatomy and physiology, as well as diagnosis and quantification of a wide variety of pathologies.⁷ A vast array of related techniques have been developed and applied clinically, including “passive property”-based methods such as bioelectrical impedance analysis,⁸ electrical impedance tomography/spectroscopy (EIT/EIS),^{9,10} impedance cardiography (ICG),¹¹ and impedance plethysmography (IPG);¹² and “active property”-based methods such as electrocardiography (ECG),¹³ electroencephalography (EEG),¹⁴ electromyography,¹⁵ electroretinography,¹⁶ and many others.

Unfortunately, the basis of these methods also gives rise to the most significant obstacle in their effective implementation. Due to their structure, various types of tissue are electrically anisotropic.¹⁷ Because electrical current “takes the path of least resistance”, it does not propagate through tissue in a linear fashion, preventing the simple application of standard tomographic reconstruction techniques (e.g., the Radon transform). The inverse problem, which calculates the impedance properties or signal sources from the measured surface potentials, is nonlinear and ill-posed in the sense of Hadamard.¹⁸ This imposes limits on the accuracy and spatial resolution of tomographic medical imaging techniques in general but the effect is notably severe in those modalities based on bioimpedance.¹⁹ This issue is compounded by the fact that the current distribution is increasingly “blurred” with distance from the electrode, diminishing high frequency spatial components that are vital to high-resolution image reconstruction. Furthermore, the number, placement, and density of surface electrodes are limited, particularly in comparison to the spatial sampling of methods that employ rotating sources/sensors (e.g., X-ray computed tomography). The aggregate of these issues produces a convoluted reconstruction problem.

Entire fields of study have developed to address this problem in the context of specific methods, including EIT,^{7,20} ECG,²¹ and EEG.^{22,23} Standard approaches to this problem involve various methods of mathematical estimation because no unique solution exists. However, Hebden and Kruger remarked: “Suppose that the resistivity of a small volume of tissue could somehow be modulated, harmlessly and reversibly. A change in the current flowing through the tissue would reflect the change in resistance, and perhaps reflect the inherent (steady state) electrical conductivity of that small volume”.²⁴

Hebden and Kruger went on to propose Acoustically Modulated EIT (a.k.a. acoustoelectric tomography, AET; ultrasound modulated EIT, UMEIT), in which a measurable change in tissue impedance is caused by the propagation of a high intensity acoustic pulse. The velocity of the pulse’s wave front through the tissue under test is utilized to estimate its position, allowing the localization of the impedance modulation. Recent UMEIT research has focused on improved image reconstruction methods but the results are still limited.^{25,26} Furthermore, because high intensity ultrasound pulses can induce large thermal fluctuations or inertial cavitation damage in soft tissues, additional investigation of safety concerns is necessary.²⁷

By revisiting Höber’s examination of RBCs, we find inspiration for a new approach to noninvasive modulation of tissue impedance. RBCs possess not only an electrically insulating membrane but also a biconcave ellipsoid shape.²⁸ As a result, blood (which can be considered a suspension of insulating RBC disks in a conductive fluid) is electrically anisotropic and its conductivity is dependent on the orientation of the RBCs.^{29–31} In fact, this orientation-induced impedance change comprises a significant portion of the signal measured by ICG.^{11,32,33} It follows that the impedance of blood, and thus tissue, could be modulated by aligning RBCs alternately with and against the flow of electrical current.

Here, we propose a method to noninvasively modulate the conductivity of tissue by aligning RBCs with an oscillating magnetic field (Figure 1). Higashi et al. previously showed that RBCs align with their disk planes parallel to an external magnetic field.³⁴ However,

complete alignment occurred within a span of 5 min in a field of 8 T, with decreased effects observed in weaker fields. In vivo application of cell alignment to bioimpedance techniques, particularly at useful frequencies, will require more efficient control of RBC orientation. We accomplish this by loading RBCs with superparamagnetic iron oxide (SPIO) nanoparticles,^{35–37} making them more responsive to magnetic manipulation. We demonstrate that these iron-loaded RBCs align with fields as low as 6 mT and that this alignment produces functional changes in the conductivity of both stationary and flowing blood. This technique could be employed to dramatically improve the spatial resolution and source localization of a vast array of bioimpedance-based modalities.

RESULTS

Human Erythrocyte Loading with SPIO Nanoparticles.

Dialysis in a hypotonic buffer enabled encapsulation of SPIO nanoparticles in human RBCs. After loading, intracellular iron was quantified using Ferene-S spectrophotometric assay. The amount of encapsulated iron increased with increasing amounts of nanoparticles present during dialysis, with 0, 100, 200, 400, and 800 μL Resovist stock producing 0.0 ± 0.0 , 1.8 ± 1.0 , 4.3 ± 0.3 , 6.4 ± 1.2 , and 10.7 ± 2.7 mM iron in 44% hematocrit (hct) RBC suspensions, respectively (Figure 2). All subsequent experiments were performed using RBCs loaded with 800 μL Resovist per mL of 70% hct RBCs.

Transmission electron microscopy (TEM) showed that the encapsulated SPIO was monodisperse (nonaggregated) and distributed throughout the entire cytoplasm (Figure 3). This homogenous distribution of iron within the disk-shaped RBCs effectively creates anisotropic objects that are susceptible to alignment in relatively weak magnetic fields.

Design of Conductivity Manipulation Apparatus.

We applied a three-dimensional set of Helmholtz coil pairs to generate a steerable, uniform magnetic field of up to 12.3 mT (60 A, ~250 W continuous). A clear Lexan enclosure ensures safety, and a removable sample tray allows experimental versatility by accepting various chamber slides and electrode arrays. The prototype apparatus is shown in Figure 4. Custom electrode arrays allow 4-pole measurement of conductivity of flowing or stagnant fluid samples (Figure 5). Flow at variable velocity is driven by a peristaltic pump, enabling continuous examination of sample conductivity within varying magnetic fields. All subsequent experiments were performed using this prototype system.

Loaded Erythrocyte Alignment in a Uniform Magnetic Field.

To demonstrate the alignment of RBCs within a magnetic field, it is necessary to image the cells at a magnification sufficient to visualize their orientation, while keeping electronics and ferrous metals isolated from the field. This was accomplished by suspending loaded and unloaded cells in alginate, subjecting this suspension to a uniform vertical field (normal to the surface of the slide), and then crosslinking the alginate to form a gel. This gel preserved cell alignment while the samples were removed from the field and moved to a digital microscope for imaging. Unloaded cells remain flat, with the disk plane being parallel to the surface of the slide, as expected in stationary fluid.¹¹ Loaded cells appear on the edge, with

the disk plane being normal to the surface of the slide, having rotated to align with the vertically oriented field (Figure 6).

Magnetic Manipulation of Blood Conductivity.

The ability of this RBC alignment to manipulate the conductivity of blood was demonstrated by subjecting loaded and unloaded samples to magnetic fields of alternating direction. The samples consisted of RBCs suspended at 44% hct in *N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid (HEPES) buffer with electrolytic conductivity being consistent with that of human plasma (1.57 S/m at 37 °C).³¹ For nonflowing experiments, the samples were gently agitated, inserted into electrode arrays, and subjected to magnetic fields of increasing strength (3, 6, 9, and 12 mT). The field direction was alternated between 0 and 90° (i.e., parallel and orthogonal to current flow). In this context, the conductivity of loaded samples increases as the cells align with current and decreases as they align against it, changing by more than 5% for field transitions of 6 mT or higher (Figure 7a). The conductivity of unloaded samples did not change significantly (Figure 7c). For flowing blood experiments, the samples were circulated through electrode arrays at a rate comparable to that of capillaries/small venules (0.78 mm/s) and subjected to a 12 mT field of alternating direction (0 and 90°). The conductivity of flowing loaded samples changed predictably, as in nonflowing experiments, but at a lower magnitude of approximately 0.5% (Figure 7b). Again, the conductivity of unloaded samples did not change significantly (Figure 7d).

DISCUSSION

The proposed method enables noninvasive modulation of the electrical impedance of blood. Because blood possesses significantly higher conductivity than solid tissues (particularly at low frequencies),³⁸ the electrical current preferably flows through blood vessels and changes in blood conductivity have relatively strong effects on overall tissue conductivity. For example, at 100 Hz, the conductivity of blood (~0.7 S/m) is approximately 10× higher than that of brain tissue (0.06–0.09 S/m).³⁹ Although this relationship begins to break down at high frequencies (>1 MHz, where membrane impedance is reduced by capacitive effects), relevant electrophysiological signals fall within lower frequency bands.

The clinical application of this technique would involve *ex vivo* loading and transfusion of autologous blood. Such procedures have been applied clinically (loading various therapeutic agents) up to Phase 3 trials⁴⁰ and involved the injection of 50–400 mL of loaded RBCs, representing an *in vivo* dilution of 1:100 to 1:12.5 (v/v; given total human blood volume of ~5 L). The experimental parameters utilized in our experiments can be plausibly extrapolated within these ranges.

While the cell loading here was accomplished using the SPIO formulation Resovist (i.e., 0.5 M ferucarbotran), the RBC encapsulation procedure reduces the heterogeneous size distribution of the nanoparticles,⁴¹ and the overall size and uniform size distribution properties of the nanoparticles are of less importance in this application than in MRI or magnetic particle imaging (MPI).^{42–44} The purpose of the encapsulated iron is to increase the overall magnetic susceptibility of cells, so that they may be more quickly and easily aligned with external magnetic fields. Various other SPIO nanoparticles have been

successfully loaded into RBCs³⁶ and could be viable alternatives for this application. An additional benefit of this SPIO encapsulation is that enclosing this exogenous material within RBCs serves to conceal it from the reticuloendothelial system (RES), preventing phagocytosis and prolonging its utility in in vivo applications.³⁵ No evidence of cytotoxic effects has been shown with RBC-encapsulated SPIO.⁴⁵

Spatially specific application of this method requires focal manipulation of magnetic field gradients, which has been accomplished previously in the development of MPI.⁴² In MPI, a pair of opposing magnets in a Maxwell configuration generate a selection field, which is uniform at all points except for the center, which exhibits a zero field and is known as the field free point (FFP).⁴⁶ SPIO nanoparticles outside of this FFP are saturated, unable to move, whereas those within the FFP are free to be manipulated by a weaker excitation field or by the gradients at the edges of the FFP as it is moved through the volume under test. In the same manner, it is possible to focally control the orientation of SPIO-loaded RBCs, thus modulating the conductivity of a chosen voxel. This is accomplished with the device constructed for our experiments by simply inverting the connections of one coil in each Helmholtz pair (Figure 8). The voxel can be steered in space by physically moving the subject/coils or by modulating the current driven through each coil.

Blood and tissue conductivity are fundamental elements in a plethora of electrophysiological tools and techniques, and the accuracy and spatial resolution of these methods are reduced significantly due to several factors, including the ill-posed inverse problem. The proposed method of noninvasively modulating the conductivity of a controllable voxel could be employed to drastically improve upon these shortcomings. Application to passive property-based imaging techniques such as EIT is fairly straightforward, as described by Hebden and Kruger.²⁴ Similarly, IPG could be refined from simply detecting the presence of venous thrombosis to isolating thrombus location. ICG calculates several cardiodynamic parameters from the change in impedance between randomly oriented RBCs and those aligned by shear forces in higher velocity flow.¹¹ By orienting RBCs in the “random” state orthogonally to the direction of flow, one might increase the impedance differential and thus the SNR of ICG measurements. Further ICG improvement might be achieved by fixing the orientation of RBCs through the cardiac cycle, reducing the influence of pulsatile flow on impedance measurement.³²

Electrophysiological techniques that examine endogenously generated signals would also benefit greatly from controlled, noninvasive modulation of tissue conductivity. ECG researchers continue to grapple with the inverse problem as they work to generate personalized cardiac electrophysiology models from surface potentials.⁴⁷ Furthermore, an exceptionally promising potential application for the proposed method is found in the EEG field. Among the various noninvasive modalities that examine neural activity, EEG boasts superior temporal resolution and is a direct measure of neuroelectrical activity. However, its spatial resolution is comparatively poor due to diminished high frequency spatial components and limited sampling, and signal source localization is once again impeded by an ill-posed inverse problem. The proposed method could be employed to generate a carrier wave at a chosen location, effectively amplitude modulating neural signals generated within

the FFP. These signals could then be demodulated from the EEG data, isolating information that originates within this voxel and directly resolving the source localization problem.

This approach to improving EEG provides a pertinent example for extrapolation of the presented results to eventual in vivo application. First, the effectiveness of the proposed method is expected to vary in particular applications depending on the context of the surrounding tissue. In the worst cases, the liver and spleen tissue of certain patients (i.e., those with thalassemia or sickle cell disease) can reach iron concentrations of 18.4 ± 3.8 and 19.3 ± 3.7 mg/g dry weight, respectively.⁴⁸ In contrast, brain tissue contains only 0.06–0.33 mg/g,⁴⁹ and thus the tissue iron contribution should be significantly less in EEG applications. Second, in the hypothetical case that the only conductivity change was that of the blood (i.e., $\pm 0.5\% \times 10\%$), the tissue would experience a 0.1% full swing conductivity change with flowing blood. EEG signals are on the order of 100 μV , and thus a conductivity change of 0.1% should translate to a 0.1% signal change of approximately 100 nV. The total contact resistance with 100 high-quality EEG electrodes at ~ 1 k Ω impedance (for example) is less than 40 Ω , which translates to an electrical noise of approximately 0.9 nV/ Hz. A 100 nV signal can easily be measured in comparison to this noise.

It should also be noted that stronger magnetic field gradients would produce larger changes in conductivity and would be easily produced using an eventual production-grade instrument (while the included experiments were limited to 60 Amps per coil pair, producing 12 mT). Further increases in conductivity change could be achieved through increased iron loading of RBCs, which is possible via dialysis with increased volumes of nanoparticles.⁴³

Beyond sensing and/or imaging applications, the proposed approach also shows potential for numerous therapeutic applications. Magnetic orientation of RBCs has been utilized to reduce blood viscosity, which is a key factor in vascular disease.⁵⁰ Improving the efficiency of RBC alignment via iron loading of cells could produce a clinically relevant, non-pharmaceutical method of manipulating viscosity. Various drugs could also be encapsulated with SPIO in autologous RBCs and injected, preventing rapid RES clearance and allowing targeted delivery by focally rupturing the cells with shear stress in high frequency fields.

METHODS

Cellular Encapsulation of SPIO Nanoparticles.

Human RBCs were loaded with commercially available iron nanoparticles (Resovist/ferucarbotran, 28 mg/mL Fe or 0.5 M, Meito Sangyo Co. Ltd., Nagoya, Japan), which have a mean diameter of 60 nm and are coated with carboxydextran. Cell loading was accomplished using a method previously described by Antonelli et al.³⁵ In brief, fresh blood was collected from healthy volunteers into heparinized tubes, and RBCs were isolated by centrifugation (1400g, 4 °C, 10 min), then washed twice with HEPES buffer (10 mM HEPES, 154 mM NaCl, 5 mM glucose, pH = 7.4) and resuspended at 70% hct. Samples of 1 mL each were mixed with varying amounts of Resovist: 0, 100, 200, 400, and 800 μL (0, 2.8, 5.6, 11.2, and 22.4 mg Fe) and then dialyzed for 75 min. in membranes with a cutoff of 12–14 kDa in 100 $\times$ volume of dialysis buffer (10 mM NaHCO_3 , 10 mM NaH_2PO_4 , 20 mM glucose, 4 mM MgCl_2 , pH 7.4, 64 mOsm) containing 2 mM adenosine triphosphate (ATP)

and 3 mM reduced glutathione. The cells were then collected and incubated (37 °C, 45 min) with 0.1% v/v of PIGPA (5 mM adenine, 100 mM inosine, 2 mM ATP, 100 mM glucose, 100 mM sodium pyruvate, 4 mM MgCl₂, 194 mM NaCl, 1.61 M KCl, 35 mM NaH₂PO₄, pH = 7.4). RBCs were isolated by centrifugation and washed four times (400g, 4 °C, 10 min) with HEPES buffer to remove unencapsulated particles.

Intracellular Iron Quantification.

Encapsulated iron was quantified using a Ferene-S (3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-5',5''-disulfonic acid disodium salt; Sigma-Aldrich, St. Louis, MO)-based spectrophotometric assay developed by Hedayati et al.⁵¹ Samples of RBCs loaded with varying amounts of Resovist (0, 100, 200, 400, and 800 mM) were diluted and digested with 70% nitric acid at 90 °C for 2 h, then cooled to room temperature and neutralized with 10 N NaOH. They were then combined with a working solution (5 mM Ferene-S, 0.2 M L-ascorbic acid in 0.4 M ammonium acetate buffer, pH ~ 4.3) at a concentration of 1:10 and incubated at room temperature in the dark for 30 min. The absorbance was measured with a Wallac Victor 3 plate reader (PerkinElmer Life and Analytical Sciences, Boston, MA) at 570 nm in a standard 96-well plate (150 μ L per well in triplicate). Iron standards of 0.078–10 μ g/mL with eight concentration levels were prepared from a stock certified standard reference material (FeCl₃, Iron Standard for ICP, 1000 $\pm$ 2 mg/L Fe in 2% nitric acid; Sigma-Aldrich, St. Louis, MO).

Transmission Electron Microscopy.

RBC pellets were fixed in 2% glutaraldehyde and 4% formaldehyde in 0.1 M sodium cacodylate buffer (pH = 7.4) with 3% sucrose and 3 mM CaCl₂. Postfixation was done with 2% osmium for 2 h. RBCs were stained en bloc with 2% uranyl acetate in distilled water for 30 min and subsequently dehydrated in a graded ethanol series. Embed 812 (EMS) was used as the embedding medium. Thin sections (70–80 nm) were cut on a Reichert Jung Ultracut E microtome and placed on formvar-coated 100 mesh copper grids. Grids were stained with uranyl acetate followed by lead citrate. TEM was performed using a Zeiss Libra 120 instrument equipped with a Veleta camera (Olympus Soft Imaging Solutions GmbH, Munster, Germany).

Magnetic Field Generation and Conductivity Measurement Apparatus.

To generate a steerable, uniform magnetic field, a three-dimensional set of square Helmholtz coils was constructed (102 mm across, equivalent radius 57 mm, separation 136 mm, 35 windings). Each coil pair was determined to have an inductivity of 380 μ H and a resistance of 69 m Ω at direct current (DC), allowing the generation of a continuous 12.3 mT field per axis when driven at 60 A (dissipating ~250 W). A model 1693 switching DC power supply (B&K Precision, Yorba Linda, CA) was connected to each coil pair with heavy gauge (4 AWG) drive cables, allowing variable current/flux density. The coils were encased in a clear Lexan enclosure, which includes a removable sample tray, ports for instrument/drive cables and flow tubing, and two cooling fans (120 mm, 70 CFM) in a push/pull configuration.

The sample tray utilizes a set of spring-loaded pins to connect and secure interchangeable electrode arrays, which were custom etched by Applied Biophysics (Troy, NY). Each array

includes eight electrodes (24K gold, 3 mm × 4 mm) in a linear configuration, embedded in a 90 μL flow channel (0.36 mm × 5 mm × 50 mm) with a Luer connection at each end. The cell constant of the electrode array was determined to be $K = 88.24 \text{ cm}^{-1}$ using a standard reference solution (H7030L, 12 880 $\mu\text{S}/\text{cm}$; Hanna Instruments, Woonsocket, RI). A Model 880 LCR meter (B&K Precision, Yorba Linda, CA) was connected to the system, allowing measurement of conductance (G) in a four-pole configuration at 100 Hz, from which conductivity was calculated as $C = K \times G$. All conductivity values were converted from the recorded temperature t to a standard temperature of 25 °C using an estimated temperature coefficient of 2% ($\alpha = 0.02$) and the following equation

$$C_{25} = \frac{C_t}{1 + \alpha(t - 25)}$$

To simulate small venule/capillary blood flow, the cell samples were circulated through the electrode array at 0.78 mm/s⁵² using a BT101S peristaltic pump with a DG6-1 head (Golander, Norcross, GA).

Imaging of Cell Alignment.

Loaded (10.7 mM Fe, 44% hct) and unloaded RBCs were washed four times with HEPES buffer. After this step, RBCs were 10× diluted in a Krebs–Ringer–Hepes (KRH) solution without calcium ions and suspended in 3.4% w/v ultrapure low viscosity guluronic alginate (NovaMatrix, Sandvika, Norway) to a final 100× dilution. Then, 100 μL of each alginate-suspended RBC sample was plated in an 8-well chamber slide system (Thermo Scientific). The slide was placed in the Helmholtz coil apparatus (in place of the electrode array) and subjected to a vertically oriented magnetic field (12 mT) for 5 min. A 100 mM CaCl_2 solution was then added to crosslink the alginate, and the samples were left in the field for 5 min to allow complete gelation. The alginate gel with embedded RBCs was washed four times with KRH solution to remove excess CaCl_2 and imaged using phase contrast with a bright-field microscope (Zeiss) at 20× magnification. A Z-stack image of 40 slices with a thickness of 2.30 μm was acquired and processed using ImageJ software (NIH, Bethesda, MD).

ACKNOWLEDGMENTS

We thank Carol Cooke for assistance with TEM, Jay Burns for mechanical consultation and machining, and Anna Jablonska for assistance with multiple laboratory techniques. This work was supported by the National Institutes of Health (R24 MH109085) and unrestricted University of Urbino funds.

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Figure 1. Alignment of RBCs with the direction of magnetic field B . Cells orient with their disk planes parallel to the field lines.

Figure 2. Encapsulation efficiency of RBCs for varying amounts of Resovist, as quantified by Ferene-S-based spectrophotometric assay.

Figure 3.

TEM image of (a) unloaded and (b, c) SPIO-loaded RBCs at (a, b) 20 000 $\times$ and (c) 63 000 $\times$ magnification. RBC pellets were fixed and stained with 2% uranyl acetate, cut into thin sections (70–80 nm), and placed on uranyl acetate and lead citrate stained 100 mesh copper grids.

Figure 4. Prototype apparatus. (a) Top view, showing the custom flow chamber on the sample tray, within three-dimensional Helmholtz coils. The entire apparatus is placed within a Lexan enclosure for safety and is cooled by dual 120 mm fans. (b) Side view, showing a removable sample tray and instrumentation cable that connects to the LCR meter. (c) Front view, including 60 A power supplies that drive the coils.

Figure 5. Custom electrode arrays for 4-pole conductivity measurements of flowing samples. (a) Circuit etching proof. (b) 24K gold electrodes in a linear configuration. (c) Blood sample in the flow channel.

Figure 6. Reorientation of RBCs in a vertical magnetic field of 12 mT. (Left: unloaded cells appear flat, oriented with their disk planes parallel to the slide surface, unaffected by the field. Right: SPIO-loaded cells appear on the edge, oriented with their disk planes being perpendicular to the slide surface, aligned with the field).

Figure 7.

Conductivity changes of (a, b) SPIO-loaded and (c, d) unloaded RBCs at orthogonal field transitions in (a, c) stagnant and (b, d) flowing (0.78 mm/s) blood. As the field is switched between parallel and orthogonal (with respect to the direction of electrical test current), the conductivity of loaded cell samples changes. This effect increases with flux density but plateaus beyond 9 mT. No significant change is observed for unloaded cells. Conductivity values at each time point were mathematically converted from recorded temperatures to a standard of 25 °C to correct for the influence of transient coil temperatures on conductivity.

Figure 8.

Experimental setup of Helmholtz coil pairs in opposing configuration, generating a uniform selection field with a symmetric field free point (FFP), in which SPIO-loaded RBCs can be selectively manipulated to modulate conductivity.