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Assessment of electrochemical properties of a biogalvanic system for tissue characterisation

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ABSTRACT

Biogalvanic characterisation is a promising method for obtaining health-specific tissue information. However, there is a dearth of understanding in the literature regarding the underlying galvanic cell, electrode reactions and their controlling factors which limits the application of the technique.

This work presents a parametric electrochemical investigation into a zinc–copper galvanic system using salt (NaCl) solution analogues at physiologically-relevant concentrations (1.71, 17.1 & 154 mM). The potential difference at open cell, closed cell maximum current and the internal resistance (based on published characterisation methods) were measured. Additionally, independent and relative polarisation scans of the electrodes were performed to improve understanding of the system.

Our findings suggest that the prominent reaction at the cathode is that of oxygen-reduction, not hydrogen-evolution. Results indicate that cell potentials are influenced by the concentration of dissolved oxygen at low currents and maximum closed cell currents are limited by the rate of oxygen diffusion to the cathode. Characterised internal resistance values for the salt solutions did not correspond to theoretical values at the extremes of concentration (1.71 and 154 mM) due to electrode resistance and current limitation. Existing biogalvanic models do not consider these phenomena and should be improved to advance the technique and its practical application.

1. Introduction

There is a clinical need in many medical interventions to obtain specific information regarding the health of biological tissues. This is particularly pertinent to minimally invasive surgery, where the loss of haptic feedback has limited the information available to the surgeon during a procedure. Research spanning a number of sensing modalities has looked to address this problem. Such proposed techniques include: determination of tissue mechanical properties through force sensing surgical probes [1] or ultrasound based elastic imaging [2]; time-dependent electrical properties in the field of Bioimpedance Spectroscopy (BIS) [3]; and most recently chemical composition analysis through near-real-time spectroscopic analysis of cauterised tissue vapour [4,5]. Although research within these modalities has shown potential, some application-specific issues remain making alternative sensing strategies desirable. In addition, aggregation of multiple sensing modalities can often lead to an improved depiction of the region of interest through exploitation of the individual technique strengths.

A biogalvanic characterisation technique proposed by Golberg et al. [6] combines electrochemical and electrical principles to allow passive determination of a tissue's resistive properties. Dissimilar metal electrodes (copper and zinc) are coupled to the tissue of interest creating a galvanic cell. Subsequent connection of the system through external resistors allows regulation of cell current (I). The voltage measured across the external resistor (R_{EXT}) can be applied to a mathematical model of the system (Eq. (1)) allowing an internal resistance (R_{INT}) to be determined. Extension of this technique proposed by Chandler et al. [7] uses a full set of measured voltages across the range of external resistors to allow more accurate determination of the internal resistance. In addition, this technique allows the Open Circuit Voltage (OCV), which is the potential difference between the galvanic cell electrodes when no current flows, to be determined without direct measurement.

$$\left(\frac{OCV}{I}\right) - R_{EXT} = R_{INT} \tag{1}$$

For characterisation, a zinc and copper galvanic cell is established and used as the current generating power source with the cell current being passively regulated using external resistors. This reduces measurement system complexity as external power supply and current control electronics are not required, in contrast to BIS measurement. The simplicity of the biogalvanic method makes it an attractive sensing modality. However, with the infancy of this technique comes a dearth of scientific understanding. Previous application to porcine tissues

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ex vivo and *in vivo* showed sensitivities to mechanical contact condition (strain levels) and resistor switching direction as well as presenting unexpected transient currents between resistor switching [7]. In addition, and crucially, little is known of the electrochemistry that governs the characterisation process.

1.1. Electrochemical theory

For the copper and zinc galvanic cell proposed, information presented by Golberg et al. [6,8] suggests the primary standard electrode reactions of:

$$Zn^{2+}(aq) + 2e^- \rightarrow Zn(s) \quad E^0 = -0.76\text{V (SHE)} \tag{2}$$

$$2H^+(aq) + 2e^- \rightarrow H_2(g) \quad E^0 = 0.00\text{V (SHE)}. \tag{3}$$

Giving the full cell reaction and galvanic potential difference under standard conditions of:

$$Zn(s) + 2H^+(aq) \rightarrow Zn^{2+}(aq) + H_2(g) \quad \left(\Delta E^0 = 0.76\text{V}\right). \tag{4}$$

The actual half-cell reaction potentials are influenced by the cell conditions in accordance with the Nernst equation, where the actual reduction potential, E_{red} is a function of the standard half-cell reduction potential E^0 and the chemical activity of the reducing agent, a_{red} and the oxidising agent, a_{ox}. For dilute solutions the activity coefficient tends to unity leaving the chemical activity interchangeable with ionic concentration. For the hydrogen evolution reaction (Eq. (3)) to be thermodynamically favourable at pH 7, a potential more negative than −0.41 V (SHE) is required at the cathode. The measured Open Circuit Potential (OCP) of copper under comparable conditions, and measured in this study is +0.1 V (SHE), making hydrogen evolution unfavourable [9]. The oxygen reduction reaction (Eq. (5)) at pH 7 is however thermodynamically feasible at potentials lower than +0.81 V (SHE), suggesting that this is the primary reaction at the copper cathode under open cell conditions. Therefore the full cell reaction within the galvanic cell would be that of Eq. (6).

$$2H_2O + O_2 + 4e^- = 4(OH)^- \quad E^0 = +0.4\text{V (SHE)} \tag{5}$$

$$Zn + H_2O + \frac{1}{2}O_2 \rightarrow Zn^{2+} + 2(OH)^- \quad \left(\Delta E^0 = 1.16\text{V}\right) \tag{6}$$

Biogalvanic characterisation within the range of expected tissue resistivity (0.2–50 Ωm [10]) using the proposed external resistance range will necessitate moving the cell from near open cell conditions towards short circuit. For high current levels the electrode potential must shift away from the equilibrium potential by an amount ΔV, in accordance with the Tafel Eq. (7). The term α represents the charge transfer coefficient and the terms F, R and T represent the Faraday constant, the universal gas constant and absolute temperature, respectively [11]. The sign within Eq. (7) indicates the reaction type with positive representing an anodic process and a negative representing a cathodic process. It is possible that the required potential shift for the cathodic reaction supporting the anodic dissolution of the zinc metal will become sufficient to cause change from solely oxygen-reduction to a mixed system also including hydrogen-evolution.

$$I \propto exp\left[\pm\frac{\alpha F}{RT}\Delta V\right] \tag{7}$$

1.2. Corrosion considerations

The measurement system is fundamentally based on the corrosion of zinc metal. As such, the corrosion mechanisms for zinc dissolution as well as the supporting cathodic reactions should be considered. Electrochemical studies have been conducted looking at zinc and copper in isolation, and as part of a galvanic cell. In neutral and basic solutions the anodic polarisation of zinc produces oxides and hydroxides, although passivation of the electrode is not achieved [12]. García-Antón et al. [13] suggest that these oxide regions may cause reduced reaction kinetics for the zinc oxidation reaction. This could lead to increasing resistance of the zinc electrode with time. However, shorter time scales and surface treatment between tests should mitigate or at least reduce the effect of this potential issue. Cathodic polarisation of copper in neutral aqueous solution will be dominated by the reduction reactions of water and of dissolved oxygen; Eqs. (3) and (5), respectively. In particular, the rate of the oxygen-reduction reaction, Eq. (5), has been shown to be limited at a high overpotential by the mass transport of dissolved oxygen to the electrode surface [14]. As part of a Zn–Cu galvanic couple, the copper electrode has been shown to be highly polarisable with respect to the zinc electrode [15]. Therefore the behaviour of the copper electrode under cathodic polarisation will be likely to dominate the behaviour of the galvanic cell.

1.3. NaCl solution model

In order to characterise the electrochemical properties of the system, tests have been conducted within salt solutions (NaCl (aq)) of varied concentration. This offers improved control over the system parameters in comparison to testing with biological tissue. In particular, a salt solution model allows control over the salt bridge conductivity giving meaningful validation to the biogalvanic characterisation system. The applicability of using an aqueous sodium chloride system is based on a number of assumptions: (1) the primary tissue current pathway is through extracellular fluid, (2) the dominant ionic components of extracellular fluid are Na^+ and Cl^-, and (3) the electrochemistry is dominated by the NaCl medium and the electrode properties. It is common within BIS characterisation to consider biological cells in a capacitive nature, due to their non-conductive lipid bilayer cell membrane [16,17]. At low frequency the current pathway will therefore be predominantly through the extracellular fluid surrounding the cells. The major ionic species within extracellular fluid are Na^+ and Cl^- making the use of NaCl solution an appropriate model [16,18]. Initial comparisons between the NaCl solution and tissue results have been made within this study to understand further the efficacy of this model. The analysis of a salt solution system can thus help to validate the biogalvanic system and demonstrate the influence of electrochemical factors that may need to be addressed for reliable use in tissue characterisation. Specific testing of OCV, closed cell currents and transition currents was undertaken. This paper reports the influence of salt solution conductivity within a physiological range on these independent aspects of the galvanic cell. Additionally, comparisons have been made between the independent electrochemical findings and the applied characterisation process, with reference made to published tissue data.

2. Materials and methods

Tests were conducted in isolation from mechanical considerations through the use of an aqueous sodium chloride electrolyte. Salt solutions of 1.71, 17.1 & 154 mM (0.01, 0.1 & 0.9 wt.%, respectively) NaCl were prepared through volumetric combination of analytical grade NaCl (Fisher Scientific) with distilled water. These concentrations represent a conductivity range spanning across that of soft tissues [10]. Test solutions were maintained at 25 +/− 1 °C for all tests using a temperature-controlled hotplate (MR Hei-Standard, Heidolph). Axially aligned flat faced copper and zinc 12 mm diameter cylindrical

electrodes were set in non-conducting resin and connected to external control and measurement equipment through copper wire. Electrode surfaces were wet ground to 1200 grit and rinsed with distilled water prior to each test. Scheme 1 shows the geometric arrangement and experimental setup used for galvanic testing in salt solutions.

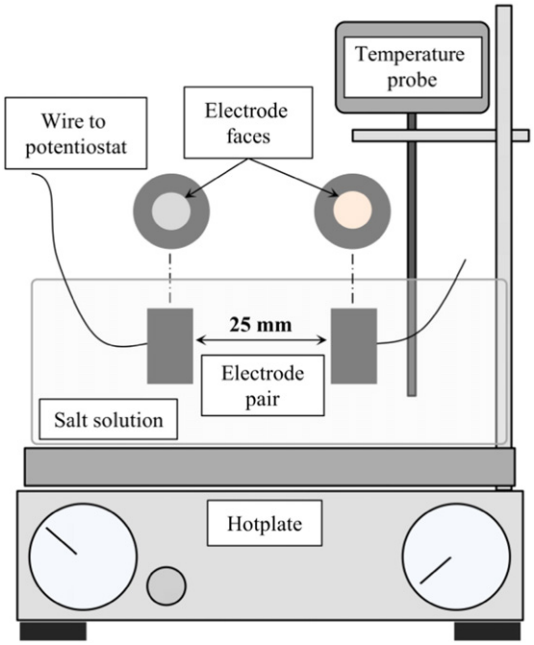
2.1. Measurements

2.1.1. System assessment

Preliminary testing was performed in order to establish typical current behaviour of various types of cells during the biogalvanic characterisation process. The current was measured using a Zero Resistance Ammeter (ZRA) (Compact Stat, Ivium Technologies) during a biogalvanic characterisation of 17.1 mM NaCl solution. The system was tested using 15 fixed external loads switched in descending order at 100 second intervals. In addition, identical characterisation of an electronic model with an OCV of +0.8 V and internal resistance of 10.2 kΩ, was performed and the current monitored as a function of time. The resistance value used in this model is within 1% of the theoretical resistance of 17.1 mM NaCl solution under the test geometry. For comparison a current profile attained during a single biogalvanic characterisation of *ex vivo* human rectum was performed. Freshly excised human rectal tissue was obtained in accordance with NHS and Leeds Teaching Hospital ethics procedures. The biogalvanic characterisation was performed over 20 fixed external load values switched in descending order at 10 second intervals. The current trace during the characterisation was recorded for comparison to the salt solution tests.

2.1.2. Open circuit voltage

The OCV was determined using two separate techniques. Firstly, individual OCP values were measured for each electrode relative to a Ag/AgCl reference electrode (Thermo Scientific). Subsequently the difference between the individual electrode potentials was calculated to give the expected OCV for the galvanic couple. Secondly, the OCV was measured directly from the galvanic couple through external connection of a high resistance voltmeter. To test the OCV, each electrode/electrode-pair was placed into the test solution and allowed to stabilise for 30 min. The OCP/OCV measurements were then conducted using a precision potentiostat (CompactStat, Ivium Technologies). Each measurement recorded the potential for 30 min with the determined OCV being calculated using average potentials over this period. Statistical analysis of the influence of concentration on the galvanic OCV was conducted using a single-factor analysis of variance (ANOVA) test (n = 5).



Scheme 1. Geometric arrangement and test setup for the axially aligned galvanic test cell.

2.1.3. Closed cell current

In order to measure the current levels produced under closed cell conditions a Zero Resistance Ammeter (ZRA) (Compact Stat, Ivium Technologies) was connected in series with the cell. Upon closing the galvanic cell through the ZRA a large initial transient was typically present. To determine the steady state closed current the system was monitored for 1 h with data from the final 30 min used to obtain steady-state average values. Fig. 1 shows the transient behaviour seen when establishing the closed cell current along with the steady-state variation seen in the final 30 min of testing. The variation of closed cell current with concentration was assessed statistically through application of a single-factor ANOVA test (n = 5).

2.1.4. Transition currents

Assessment of the transition currents was performed using three methods: (1) the method of resistor switching employed during biogalvanic tissue characterisation [7], involving sequential external series resistor switching and current monitoring (Fig. 2), (2) polarisation of the individual electrodes against a non-polarisable counter electrode (Pt) using a stable third electrode (Ag/AgCl) as reference, and (3) polarisation of the copper electrode, controlled against the zinc electrode.

2.1.4.1. Galvanic characterisation. For typical galvanic cell characterisation, the electrode pair was submerged in the test solution and connected as an open cell for 30 min prior to resistor switching. External resistor values were then switched every 100 s from high resistance to low resistance over 15 fixed values. An external resistor switching time of 100 s was implemented in order to allow transient voltages caused by discrete switching to settle before being used to determine internal resistance. The system was connected in series with the ZRA to allow measurement of current during this period. The resistor switching pattern and typical resultant current trace can be seen in Fig. 2(A). The internal resistance of the cell was determined using the model and fitting method described by Chandler et al. [7].

2.1.4.2. Polarisation scans. Polarisation scans were undertaken using the individual electrodes of the galvanic cell as the working electrode in a typical three-electrode cell. A combination Ag/AgCl reference and Pt counter electrode (Thermo Scientific) was employed. Polarisation was undertaken after a 30 minute OCP settling period. The zinc and copper electrodes were polarised from OCP in the anodic (increasing) and cathodic (decreasing) potential directions respectively. A scan rate of 0.5 mV/s was employed in all tests. Each polarisation was conducted

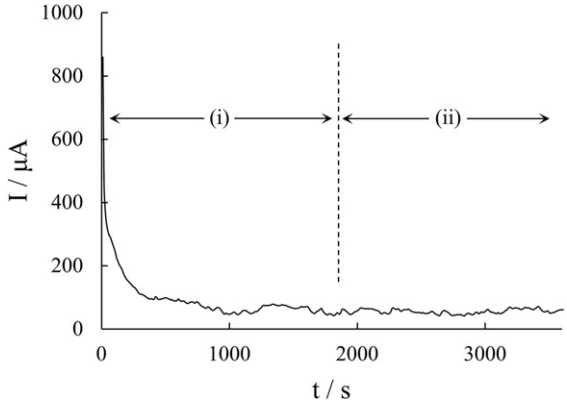


Fig. 1. Typical closed cell current trace showing (i) initial transient settling period and (ii) region used to determine average steady state closed cell current.

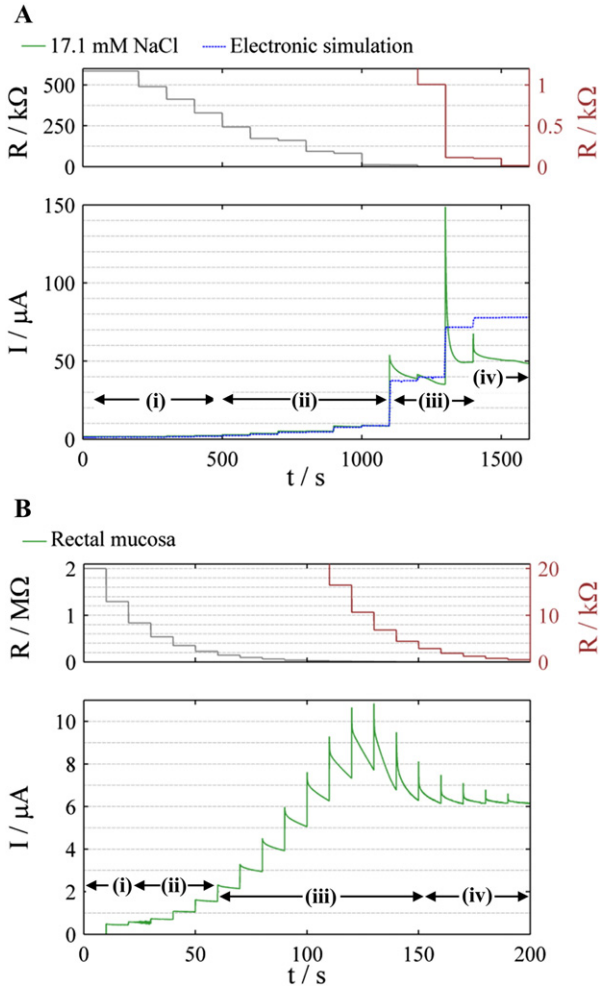


Fig. 2. Current–time profiles during biogalvanic characterisation of: (A) 17.1 mM NaCl at 25 °C (green line); and an equivalent electronic simulation of R_{INT} = 10.2 kΩ and OCV = 0.8 V (blue dashed line), and (B) human rectum tissue *ex vivo*. External resistor values as a function of time are also shown above each current trace. A secondary ordinate axis (right) has been used to show the low external resistance values. The identified features for salt solution and tissue data are: (i) low current towards open cell; (ii) current level step transitions at low current; (iii) transient behaviour occurring at higher currents after switching; and (iv) closed cell maximum current.

to 1 V against OCP in the test direction specified. Five repeat tests were conducted for each electrode at each NaCl concentration.

2.1.4.3. Relative polarisation scans. Relative polarisation of the galvanic cell was conducted through cathodic polarisation of the copper electrode against a zinc counter/reference electrode of the same geometry. Consequently, data more representative of the galvanic cell under internal resistance characterisation could be obtained. The copper potential was controlled from OCV to closed cell value (0 V), with corresponding current response being measured. Five repeat tests were conducted for each of the test solution concentrations. Additionally, a relative polarisation scan was conducted on freshly excised human colon tissue for comparison to the salt solution data. Tissue was obtained in accordance with NHS and Leeds Teaching Hospital ethics procedures.

3. Results

3.1. System assessment

The current–time trace from biogalvanic characterisation of 17.1 mM NaCl solution is shown in Fig. 2(A). It can be seen that numerous standard features are present, these are identified as: (i) the established Open Circuit Voltage (OCV) at open cell conditions (no current flow between electrodes); (ii) transition currents showing no transient behaviour; (iii) transition currents with significant transient behaviour; and (iv) established maximum current under closed cell conditions. Fig. 2(A) also shows the current response for characterisation of an electronic model. The two systems show similar current behaviour for stages (i) and (ii). However, discrepancies are seen at higher current levels where the transient behaviour and limited maximum current are seen to be typical only of the salt solution.

The current profile attained during biogalvanic characterisation of *ex vivo* human rectum tissue is shown in Fig. 2(B). The profile shows that the outlined features seen within the NaCl model are also apparent in the biological tissue test. In particular, similarities in the transient behaviour and limitation at high current are shown. This indicates that the present characterisation model assumption of a pure internal resistance is not appropriate over the full testing range for pure salt solution or biological tissue.

3.2. Open and closed circuit

Fig. 3 shows the averaged OCV values and data range for varied salt solution concentrations; obtained using two different methods. OCV values range from 0.8–0.9 V, with statistically significant differences ($p < 0.05$) being shown between mean values, determined using a galvanic couple. For comparison, Fig. 3 also presents *in vivo* OCV results obtained in a separate study [7]. These values represent the mean and range of five repeats tested on a single porcine specimen at three different tissue locations. The OCV values for a tissue salt bridge are all lower than for aqueous NaCl. Additionally, the values span from 0.3–0.7 V and are specific to the tissue type tested.

Fig. 4 shows the average steady state maximum current obtained for varied solution concentration. No statistical significance ($p < 0.05$) between average closed cell currents at varied test concentrations was found. However, the average current for the 1.71 mM NaCl solution is lower than either of the more concentrated solutions. Variability in results is large for all concentrations, with standard deviations ranging from 4–8 μA.

3.3. Transition currents

3.3.1. Galvanic characterisation

Fig. 5 shows the characterised internal resistance values determined for the varied salt solution concentrations. The internal resistance values measured using the galvanic method follow the trend of the theoretical data, although errors are seen to be large at the extremes of solution concentration. For the lowest NaCl concentration (1.71 mM), the

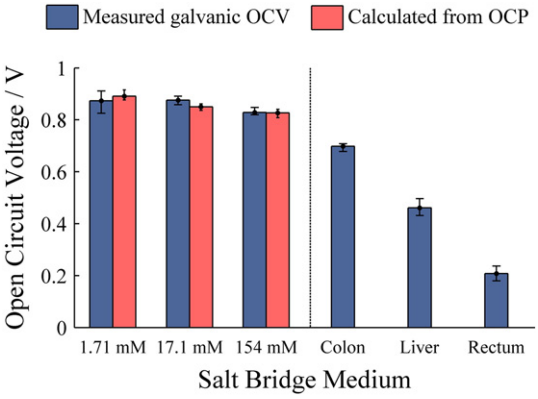


Fig. 3. Averaged OCV determined for solutions of varied [NaCl] using galvanic determination and calculated from independent electrode OCP measurements; full data range indicated (N = 5). OCV values from *in vivo* porcine tissue tests also shown for comparison [7].

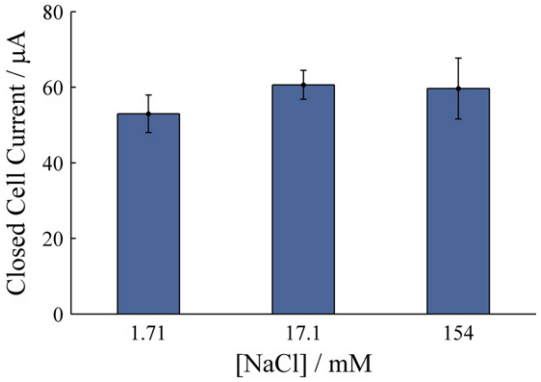


Fig. 4. Average closed cell current for varied [NaCl]; showing +/− 1SD (N = 5).

galvanic method gives resistance values much lower than theory, and shows a high degree of variability for repeat tests. The 17.1 mM concentration gives measured values in line with theory, with the mean internal resistance being 6% larger and with low repeat variation. For the highest concentration (154 mM), measured resistance values were consistently greater than theory with a high degree of variability.

3.3.2. Polarisation

Fig. 6 shows the current profile produced by polarisation of each electrode for the three concentrations tested. The mean polarisation curves are represented by solid lines with the range from five repeats indicated *via* the corresponding shaded boundary. The anodic polarisation of the zinc electrode shows a typical exponential potential-current response for an electrode under charge transfer control; as described by Eq. (7). The range of zinc polarisation profiles is small for all concentrations, indicating that polarisation of this electrode is highly repeatable. The NaCl concentration has the influence of altering the potential-current response. Specifically, for the same overpotential the current is higher for a higher NaCl concentration. This is likely due to a reduction in the losses associated with uncompensated solution resistance as the solution resistance drops. In contrast, the cathodic polarisation of copper breaks from linear behaviour at potentials more negative than 0.1 V from OCP, showing influence from mass transport kinetics. This is exemplified by the large range of polarisation profiles seen from repeat tests; particularly in more concentrated solutions. The OCP values of the copper electrode are more negative for increasing NaCl concentration. The point of equal current for the anodic and cathodic profiles of the galvanic cell has been indicated for each concentration. This is predictive of the maximum current attainable by the cell, although the influence of polarisation scan rate and internal resistance losses are not accounted for.

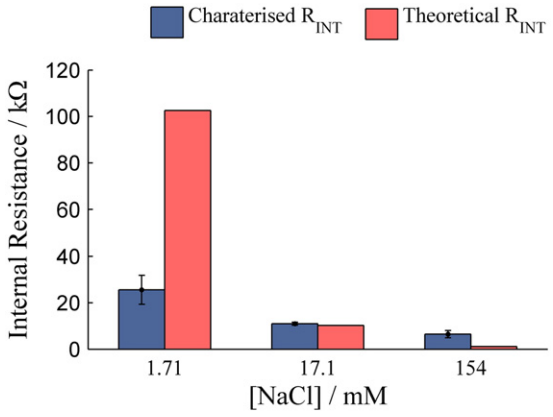


Fig. 5. Averaged internal resistance ± standard deviation, determined using: the galvanic characterisation method, and from theory using conductivity data.

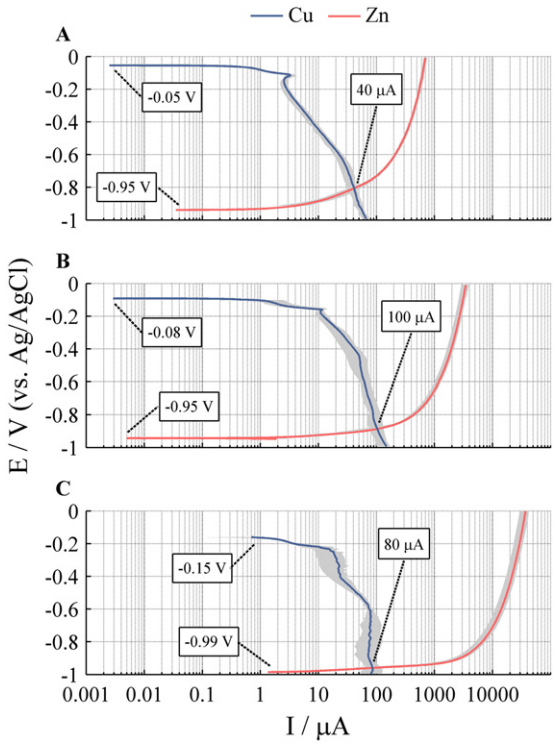


Fig. 6. Average polarisation data (N = 5) for axial electrodes tested in (A) [NaCl] = 1.71 mM, (B) [NaCl] = 17.1 mM, and (C) [NaCl] = 154 mM; zinc and copper polarised in the anodic and cathodic direction by 1 V from OCP, respectively. The range seen within repeats is shown for each test case as the shaded region. Predicted average closed cell current and individual electrode OCP values are indicated.

3.3.3. Relative polarisation

Fig. 7(A–C) shows the polarisation of the copper electrode against a zinc counter for three NaCl concentrations. The current profile produced shows a combination of the features seen within the individual polarisation curves of Fig. 6. All concentrations tested tend towards a similar limiting current, similar to the closed cell current results of Fig. 4. Increased non-linear potential-current behaviour is seen with increased concentration. The profiles demonstrate three distinct regions: (1) activation-controlled response at potentials close to the OCV, where small potential changes cause large changes in current; (2) an approximately linear increase in current with increase in potential; and (3) mass transport limited regime where current becomes independent of potential. The duration of each stage varies for each of the test concentrations. The relative polarisation data for a cell connected through *ex vivo* human colon tissue is shown in Fig. 7(D). The profile produced shows cell features predicted from salt solution tests, with large activation controlled and current limited regions. These features were also predicted from the biogalvanic current trace in Fig. 2(B).

4. Discussion

4.1. OCV

Average OCV values measured from the galvanic couple show statistical significance for varied NaCl concentration. Salt ion concentrations within physiological range alter the standard electrode potentials, generating a range of OCV values spanning 0.1 V from 0.8–0.9 V. Variations in electrode potentials can be accounted for through temperature and local ion concentration fluctuation, in accordance with the Nernst equation. Salt solution results are in contrast with OCV values measured *in vivo* on porcine tissues [7]. Tissue results are presented for comparison in Fig. 3. A much larger range spanning from 0.2–0.8 V is shown, with differences between tissue types being statistically significant.

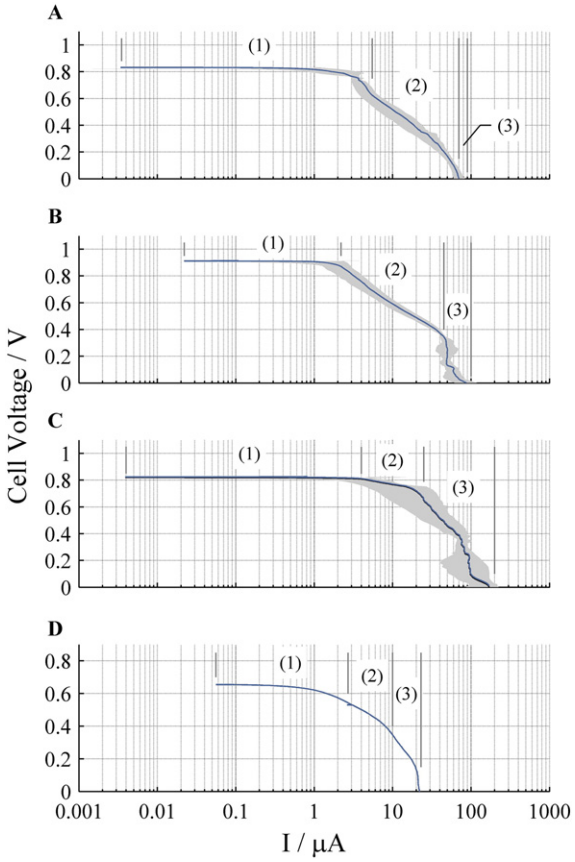


Fig. 7. Average relative polarisation data (n = 5) for axial copper electrode against a zinc counter & reference for salt bridge mediums of (A) [NaCl] = 1.71 mM, (B) [NaCl] = 17.1 mM, (C) [NaCl] = 154 mM, and (D) human rectal mucosa (*ex vivo*). Copper polarised in the cathodic direction from OCV; (A–C) show repeat testing range shown as shaded region (N = 5). Potential current control methods annotated as: (1) activation control, (2) internal resistance control, and (3) mass transport control.

The much lower OCV values seen *in vivo* may be related to altered reaction mechanisms. In particular, the lower OCV may be caused by a lower open circuit potential at the copper electrode. For an aqueous system with a low dissolved oxygen concentration, the electrode potential at the cathode may become more negative to thermodynamically support the hydrogen-evolution reaction of Eq. (3). If tissues have type specific dissolved oxygen concentrations, then specific OCV values for zinc–tissue–copper galvanic cells would be expected. Carreau et al. [19] showed that there is significant variation in the oxygen partial pressure (pO_2) between tissue types, specifically indicating a lower pO_2 for liver tissue compared to intestinal tissue. The influence of oxygen can also be seen within the salt solution system (Fig. 6); where the OCP at the copper electrode becomes more negative as the oxygen solubility is reduced by higher NaCl concentrations. For NaCl concentrations from 0–171 mM the solubility of oxygen reduces from 8.22–7.79 mg/l (~5%) [20].

4.2. Closed cell current

Fig. 4 shows the closed cell current to be insensitive to the concentration of NaCl. No statistically significant differences in steady state current values are shown between concentrations. This suggests that at maximum current the system is not limited by the resistance of the salt bridge. It can be seen directly in Fig. 2(A) that for an electronic model of equivalent internal resistance, the current at low external resistor values is higher than that produced in the galvanic cell. Therefore, at high current levels the characterisation method is no longer influenced by the solution resistance but by a limited reaction rate. Additionally, the fluctuations at maximum current are large indicating instability of the current limiting mechanism. In particular the fluctuations were noted to be sensitive to temperature and agitation which are typically associated with a mass transport limiting, diffusion controlled processes [14,21].

4.3. Galvanic characterisation

The internal resistance values predicted using the biogalvanic characterisation method show discrepancies with theoretical values determined using conductivity data for the corresponding solution concentrations. For the 1.71 mM solution, the measured internal resistance is much lower than theory (25%). This is due to the method of characterisation not being specific to the internal resistance, and thereby measuring the influence of electrode activation. For the 154 mM solution, internal resistance values were measured as being larger than those predicted theoretically. This can be accounted for through the mass transport limited current under closed cell conditions being a dominant factor over the solution resistance. In addition the characterised resistance is highly variable within the same conductivity of solution which corresponds with the fluctuation seen at closed cell current levels. The resistance of the electrodes are also not accounted for within the characterisation model which will inevitably lead to a larger prediction of internal resistance if the system is assessed over the full current range. The internal resistance determined of the 17.1 mM solution shows agreement with theory, and also indicates little variation with repeat testing. Fig. 5 indicates that the galvanic characterisation method is inadequate at determining effective solution resistances for extremes of NaCl concentration. Inaccuracies may be caused by factors influencing the characterisation process such as mass transport limitations at the cathode, large relative resistance of the cell electrodes, and the discrete external resistor range not allowing even characterisation over the full current range. However, the relative pattern follows that of the theoretical resistance values and predicted values at 17.1 mM are within 7% of the theoretical value, indicating that the system may be accurate when sufficiently optimised to the test case.

4.4. Polarisation

Polarisation tests allow the individual electrode current response to be examined over the range of possible potentials experienced during galvanic characterisation. From Fig. 6 it can be seen that the polarisation involved during galvanic characterisation necessitates the anodic and cathodic polarisation of the zinc and copper electrode respectively. For the same electrode areas the zinc electrode requires a much smaller overpotential than the copper electrode to achieve the maximum current of the closed galvanic cell. This indicates that the system is particularly dominated by the cathodic polarisation of the copper electrode. For the 17.1 and 154 mM NaCl solution, a near vertical current response is seen in Fig. 6 at potentials more negative than −0.4 V from the OCP of the copper electrode. For the same concentrations under relative polarisation (Fig. 7), large mass transport limited regions are also shown. This current saturation is associated with the diffusion limited oxygen reduction reaction of Eq. (5), commonly seen in the cathodic polarisation of copper in aqueous solution [14,22].

The value of the maximum current for a system under this type of control is determined by a number of factors, described by Eq. (8) [23]. The current (I) is controlled by the charge transferred per mole (nF), electrode area (A), diffusion coefficient (D), concentration of the diffusing species in the bulk solution (c_b), and the diffusion layer thickness (δ). For the system in solution the values can all be considered constant with the exception of the diffusion layer thickness. The diffusion layer thickness for a static (unstirred) system will be time varying in accordance with an expanding concentration gradient as the reaction proceeds. For a planar electrode, this is typically modelled as Eq. (9) [23],

where t represents time. This model predicts an ever expanding diffusion layer which, in conjunction with Eq. (8), would propose a current tending to zero. It can be seem from Fig. 1 that this is not found experimentally; instead the system appears to fluctuate around a steady state value. The non-zero current results from natural convection resupplying electrolyte of bulk concentration to the depleted diffusion layer. An effective limit is reached on the diffusion layer thickness which is dependent on the natural convection within the system. Tobias et al. [24] advise that the often quoted diffusion layer thickness of 0.5 mm can lead to erroneous predictions of limiting currents under natural convection, and actual thickness values are highly system specific.

$$I = \frac{nFADc_b}{\delta} \tag{8}$$

$$\delta = \sqrt{\pi Dt} \tag{9}$$

This diffusion limiting mechanism is of critical pertinence to the characterisation method. Dropping the external resistance to a level where the current demanded becomes greater than that of the diffusion-limited current will cause the system to operate in a non-linear regime, inconsistent with the proposed characterisation model. Effectively, an additional resistance becomes prominent within the system, thereby restricting determination of the internal salt bridge (tissue) resistance.

4.5. Relative polarisation

Control of the potential across the galvanic cell using polarisation of copper against zinc is most representative of the system during biogalvanic characterisation. Utilising a slow potential transition mitigates the influence of large transient potentials caused by the discrete external resistor switching. Comparison of Fig. 6 with Fig. 7 indicates that the galvanic system behaves as a combination of the two polarised electrodes, with the copper dominating the current response. The diffusion limited oxygen-reduction reaction is again evident in the response for higher concentrations, indicating that it will be present during internal resistance characterisation. An activation controlled region is also present at potentials close to OCV. This indicates that at the extremes of the current range phenomena at the electrode will dominate, thereby reducing the efficacy of internal resistance determination.

The relative influence of the various cell phenomena is proposed as being qualitatively associated with the accuracy of the internal resistance characterisation. The profiles can be divided into three distinct regions: (1) activation controlled potential-current response, at potentials close to the OCV; (2) a steep drop in potential for a small increase in current caused primarily by the internal resistance; and (3) mass transport limited regime where current becomes independent of potential. These regions have been highlighted in Fig. 7 to allow for compassion between concentrations. It is proposed that, when using the current characterisation method, accurate determination of internal resistance can only take place when region (2) is dominant and the external resistor range generates currents primarily spanning this region. It can be seen that for the 17.1 mM NaCl solution the activation control region is small and the onset of mass transport limitation is close to the closed cell condition. Therefore, for the majority of the potential–current profile the system is under internal resistance control, leading to more accurate characterisation. In contrast, galvanic polarisation of 154 mM NaCl shows a very early onset of mass transport limited behaviour at cell voltages of less than 0.7 V, while internal resistance control is only seen at voltages between 0.8–0.7 V. As a result, the internal resistance characterisation will pick up primarily on the effective resistance of the mass transport limiting mechanism. This may be responsible for the over prediction of the salt bridge resistance seen in the 154 mM solution. For the low resistance system of the 1.71 mM NaCl solution, the galvanic polarisation shows little influence of the mass transport limitation (cell voltages <0.2 V). This exposes a large region of internal resistance (0.6–0.2 V) which should allow for accurate characterisation. However, the system also shows a large portion of the profile under activation control (0.8–0.6 V). In conjunction with the fixed range of external resistances, it is postulated that the characterisation of internal resistance in this solution is dominated by this activation region, leading to an under prediction of internal resistance.

Fig. 7(D) shows the response for relative polarisation of the cell connected through tissue. Electrochemical features seen during NaCl tests are again present here with large activation potential losses and mass transport limited current. The tissue system is subject to the same phenomena seen in NaCl, demonstrating that the salt solution model is appropriate for examining the system's electrochemical properties. However, the specific potential–current relationship may not be exactly captured within a particular NaCl system and tissue data should also be examined independently when making biogalvanic characterisations.

4.6. Applicability of findings on biogalvanic characterisation

The primary aim of the biogalvanic characterisation method is to determine an accurate measurement of tissue resistance. This study has shown that the biogalvanic characterisation method is subject to a number of potential errors caused by the electrochemistry of the system. Firstly the dissolved oxygen concentration will change with the variable salt concentration of different tissues. As the oxygen concentration is directly related to the potential of the copper cathode, the cells OCV will be influenced by tissue type. However, the use of appropriate model to fit to the measured data can account for this variation, making it a useful metric in conjunction with the internal resistance. Secondly, and of more significance, is the electrode resistance inherent in the biogalvanic system due to the use of only two electrodes. All measurements will contain contributions from losses at the electrodes along with those within the tissue. This will cause an additional resistance to be present in the system, contributing to the characterised internal resistance. If the electrode resistance is constant and small relative to that of the tissue medium then this systematic error would not preclude the measurement of a specific tissue resistance, although the accuracy will be reduced. Electrode resistance will be influenced by the material type, geometry and current test range making choice of these parameters important. Finally, for high current demands through the cell, it has been shown that the system becomes limited by the rate of diffusion of oxygen to the copper anode. This non-linear behaviour will cause an additional error in the characterisation. This process has been shown to be present during tissue testing (Fig. 2(B)) but may vary due to the range of oxygen concentrations expected. Mitigation of this during biogalvanic characterisations could be achieved by restricting tests to lower cathodic overpotentials (and therefore lower current densities) through the adjustment of the relative electrode areas. Although significant potential errors are present within the biogalvanic characterisation system it is feasible that these can be minimised or mitigated through careful equipment and experimental design, allowing the method to deliver representative tissue resistances. This is supported by the accuracy and repeatability achieved during 17.1 mM NaCl characterisation.

5. Conclusions

Assessment of copper–zinc galvanic cells using typical (OCP and polarisation scans) and atypical (closed cell current, internal resistance characterisation, and galvanic polarisation) electrochemical measurements has improved our understanding of the biogalvanic system. Specifically, it has been shown to be predominantly controlled by processes at the copper electrode. The proposed reaction of hydrogen-evolution is thermodynamically unfavourable relative to the oxygen reduction

reaction at the measured cathode OCP. Under tested conditions the oxygen reduction reaction is occurring and persists as the cathode potential becomes more negative. The OCV of the galvanic cell is proposed to therefore be sensitive to the concentration of dissolved oxygen in the system. This may explain the significant variation in OCV values seen between porcine tissues *in vivo* [7].

Previous work reporting biogalvanic characterisation [6–8] may have underestimated the system complexity. In particular, the assumption of a sole internal resistance is an oversimplification. Galvanic polarisation has shown that electrode activation behaviour at high cell potentials (low current) and transport limitations of oxygen to the cathode at lower cell potentials (high current) may skew the characterisation metric, leading to inaccurate predictions of tissue resistance.

There are potential benefits to biogalvanic characterisation although application of this modality requires repeatable and accurate results across a range of operating conditions. To fully assess the efficacy of this method, tissue assessment incorporating the findings presented in this paper is of primary importance. Mitigation of the issues demonstrated may be achieved through optimisation of the characterisation system, specifically selection of electrode material and geometry, and through appropriate selection of external resistive loads to limit the cathodic overpotential to the activation and internal resistance control regimes, thereby avoiding current limiting oxygen diffusion effects. Additionally, inclusion of the OCV parameter during assessment may yield more reliable metrics pertaining to tissue health, as this parameter is linked to the known variations in tissue oxygen concentrations.

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