Journal of Mammary Gland Biology and Neoplasia (2019) 24:177–184
https://doi.org/10.1007/s10911-019-09426-2

Detection of Milk Ejection Using Bioimpedance Spectroscopy in Lactating Women during Milk Expression Using an Electric Breast Pump

Hazel Gardner[1] · Ching Tat Lai[1] · Leigh Ward[2] · Donna Geddes[1]

Received: 2 August 2018 / Accepted: 17 January 2019 / Published online: 13 February 2019

Abstract

Milk ejection is essential for effective milk removal during breastfeeding and pumping, and for continued milk synthesis. Many women are unable to accurately sense milk ejection to determine whether their infant is receiving milk or, when pumping, to switch the pump to a more effective expression pattern. To determine if changes in bioimpedance parameters are associated with milk ejection in the lactating breast during pumping. 30 lactating women participated in 2 pumping sessions within 2 weeks of each other. During pumping the breasts were monitored with bioimpedance spectroscopy (on either the pumped or the non-pumped breast), and milk flow rate and volume were measured simultaneously. All mothers completed 24-h milk productions. Linear mixed effects models were used to determine associations between milk flow rate and bioimpedance changes. Changes in bioimpedance parameters were greater at the first milk ejection when measured on the pumped breast (median (IQR): R zero: −7 (−17, −4,) % ($n = 30$); R infinity: −8 (−20, −2) % ($n = 29$); membrane capacitance: −24 (−59, −7) % ($n = 27$). Changes in bioimpedance detected in the non-pumped breast were lower at the first milk ejection, R zero: −3 (−8, −2) % ($n = 25$); R infinity: −5 (−8, −2) % ($n = 23$); membrane capacitance: −9 (−17, 15) % ($n = 24$). Smaller less consistent decreases in the bioimpedance characteristics were detected at the second milk ejection in both breasts. Bioimpedance parameters showed a consistent decrease associated with the first milk ejection when electrodes were placed on the pumped breast. Smaller decreases were observed when the non-pumped breast was monitored for the first and second milk ejection. There was wide variation in the magnitude of changes observed, and hence further development of the methodology is needed to ensure reliability.

Keywords Lactation · Breast · Bioimpedance · Milk ejection

Introduction

Human milk provides optimal nutrition to facilitate growth and development and afford immunological protection to the newborn. Essential physiological processes which enable milk production, milk synthesis and milk ejection are dependent on the developmental changes which occur during pregnancy and the early postpartum period [1]. Milk components are drawn from the maternal bloodstream or produced within the lactocytes. Milk is then secreted into the alveolar lumen, where it is stored for removal via milk ejection [1]. Without milk ejection, little of the synthesised milk can be removed, resulting in inadequate intake by the infant and potential down regulation of milk production [2, 3].

Milk ejection is stimulated by the infant sucking the nipple or by other sensory stimulation, resulting in nervous impulses being transmitted through the spinal cord to the hypothalamus. This results in the posterior pituitary releasing oxytocin, which is transported through the bloodstream to bind with G protein-coupled oxytocin receptors on the mammary myoepithelial cells [1]. These cells contract, expelling milk from the alveolar lumen into the shortened and widened ducts, where increased intra-ductal pressure propels the milk towards the nipple for removal by the infant or breast pump [4].

Most women have multiple milk ejections and the sensations accompanying milk ejection are variable. Around 10–25% of women do not feel the first milk ejection and perception of subsequent milk ejections is rare [2, 3]. Previously, milk ejections have been detected using intra-mammary

✉ Hazel Gardner
Hazel.Gardner@uwa.edu.au

[1] School of Molecular Sciences, Faculty of Science, University of Western Australia, Perth, Australia

[2] School of Chemistry and Molecular Sciences, University of Queensland, Brisbane, Queensland, Australia

pressure, ultrasound imaging and through continuous measurement of milk flow [4–6], all of which require specialised equipment and expertise.

Bioimpedance technology offers a potential alternative method for the detection of milk ejection. Bioimpedance measures the opposition (impedance) to the passage of an alternating electrical current through body tissues. The current is conducted by the electrolytes in the extracellular and intracellular fluids, which are highly conductive. The cell membrane functions as an imperfect capacitor, and is therefore only penetrable by the current in a frequency-dependent manner; at low frequencies current cannot pass across membranes and passes only through the extracellular fluid [7–9]. Breast milk is an extracellular fluid and thus bioimpedance measurements at low frequencies will reflect the resistance of the extracellular space, including milk in the milk ducts. The relationship of resistance to fluid volume is inverse, i.e. resistance is low when extracellular fluid volume is high.

Bioimpedance is most commonly used to predict body composition, i.e. fat mass, fat-free mass and body water [7, 10], although it is also used in specific circumstances to assess localised fluid accumulation, such as in lymphoedema [9]. In relation to the breast, bioimpedance has been explored for the detection of breast pathologies including mastitis and cancer [11–13]. Thus the application of bioimpedance to the lactating breast offers a possibility to detect milk ejection without reliance on maternal perception of milk ejection.

The theoretical basis for using bioimpedance to detect milk ejection is based on the assumption that fluid levels in the breast will alter during milk ejection, resulting in changes in resistance at zero and infinite frequencies (R0 and R∞), which are indicative of the levels of extracellular and total fluid respectively. In addition, membrane capacitance (C_m) reflects the surface area of cell membranes and may change during milk ejection due to the movement of milk from the alveolar lumen through the ducts, as well as the contractile effect of oxytocin on the myoepithelial cells.

The aim of this study was to investigate whether bioimpedance is a viable method to detect changes in both the pumped and the non-pumped breast associated with milk expression.

Methods

Thirty lactating women were recruited through the Australian Breastfeeding Association or by social media. All participants were healthy breastfeeding mothers and infants with no milk production or infant growth concerns. The mothers provided informed consent to take part in the study, which was approved by The University of Western Australia Human Research Ethics Committee (RA/4/1/7897). All participants completed a background questionnaire to collect demographic information. Participants were all accustomed to expressing milk using a breast pump. The majority of the research sessions were undertaken in participants' homes, with only one participant completing sessions in the laboratory at The University of Western Australia.

24- H Milk Profile

Mothers were issued with a baby weigh scale (Medela AG, Switzerland). Each participant completed a 24-h milk profile using the test weighing method [14]. Samples of 1–2 ml were collected in 5 ml polypropylene tubes (P5016SL, Techno Plas Pty Ltd., SA, Australia) before and after each feed or pumping session. These samples were frozen until collection and transportation to the lab, where they were analysed for fat content using the creamatocrit method [15]. Participants entered time, breast (left or right) and milk volume from each feed or expression electronically, and these data were then used to calculate breast fullness and storage capacity using the method described by Kent et al., [2].

Milk Flow and Cumulative Volume Measurements from Experimental Sessions

Two research sessions were conducted one week apart where one breast was pumped each time with either the pumped or non-pumped breast being monitored simultaneously using bioimpedance (Fig.1).

Milk flow rate was measured using the method described by Prime et al. [16]; a balance was used to continuously measure cumulative milk and flow rate (Showmilk, Medela AG, Baar, Switzerland). The data were then analysed to determine the individual milk ejection patterns of each mother [16, 17]. Parameters measured for each milk ejection included duration, peak flow rate and the time taken for milk flow to peak. The volume and cream content of milk removed during the experimental sessions were added to the 24-h milk profile dataset to facilitate calculation of available milk, breast fullness and percent available milk removed (PAMR) during each session. These characteristics were calculated from the flow rate data.

Bioimpedance Measures

Bioimpedance data were collected using an Impedimed SFB7 bioimpedance spectroscopy device (Impedimed, Pinkenba, Queensland 4008, Australia) which measures bioimpedance parameters (impedance (Z), resistance (R), reactance (Xc) and phase (Ph)) at 256 frequencies over a range of 3–1000 kHz. A single frequency scan takes approximately 800 ms and scan data were collected continuously throughout each session. The device uses a tetra – polar electrode arrangement; a pair of voltage sense electrodes spanning the area of interest, with the current being supplied by distally-placed current injection

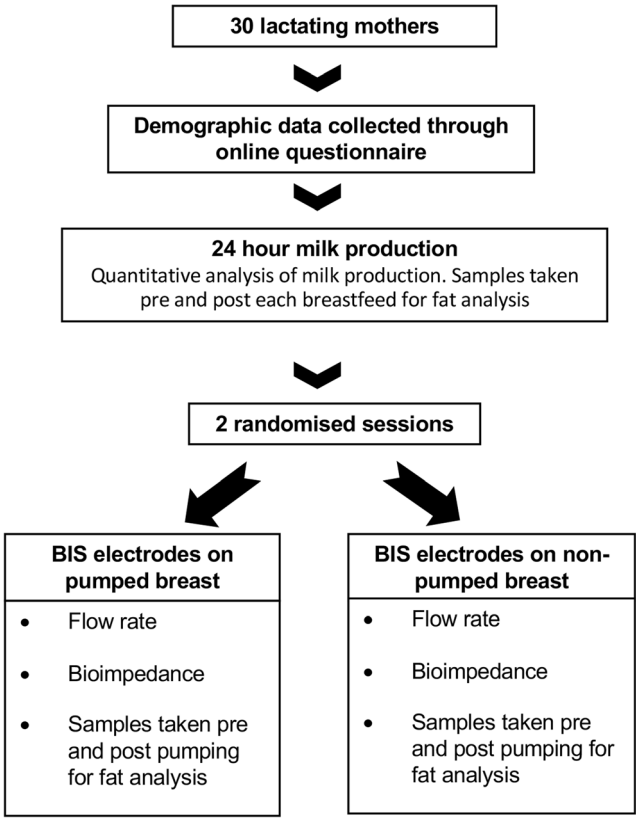

Fig. 1 Flow chart showing study sessions

and return electrodes. Drive current is 200 μA and is totally harmless. The breast was swabbed with an alcohol wipe prior to attachment of electrodes; gel pad electrodes supplied for use with the SFB7 machine were placed on the inferior medial and inferior lateral quadrants of the breast (Fig. 2).

The authors spent a great deal of time developing the optimal methodology outlined above. Some variations in experimental protocol are listed below:

- Electrodes were tested using various arrangements on the breast to determine the optimal position to detect changes.
- Electrodes were tested on both the breast shields and the breasts.
- Silver plated and gel pad electrodes were tested.
- Controls and lactating women participated in initial stages.
- Breast size was recorded but was not found to be related to changes in bioimpedance.

The experimental breast (one breast was pumped during each session) was randomly selected during the first research session and the electrodes were placed in the same positions on the same breast for each of the two monitored sessions, which involved monitoring either the pumped or non-pumped breast. A two-minute calibration period was conducted prior to pumping to collect baseline data before the session began. Duration of pumping with the electric pump (Symphony, Medela AG, Baar, Switerland) was until 10 min after milk ejection (Fig. 2). Milk flow data was used to determine milk ejection characteristics and was synchronised with the bioimpedance data which was then analysed to detect associated changes (Fig. 3).

Data Analysis

Bioimpedance data were analysed according to the conventional Cole model to provide resistance at zero and infinite

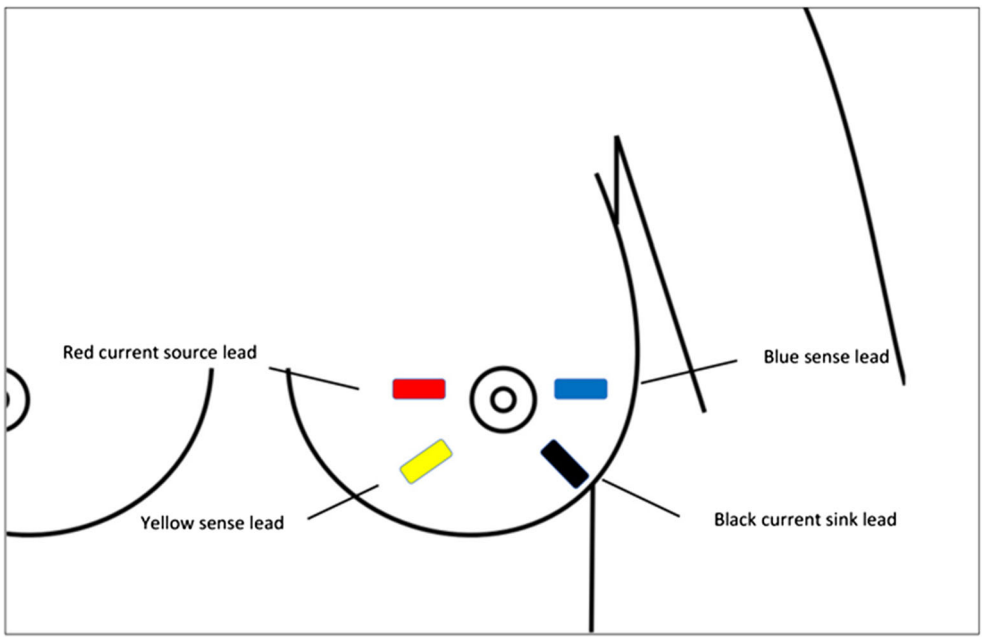

Fig. 2 Bioimpedance electrode placements on the breast for monitoring of milk ejection during milk expression with an electric pump

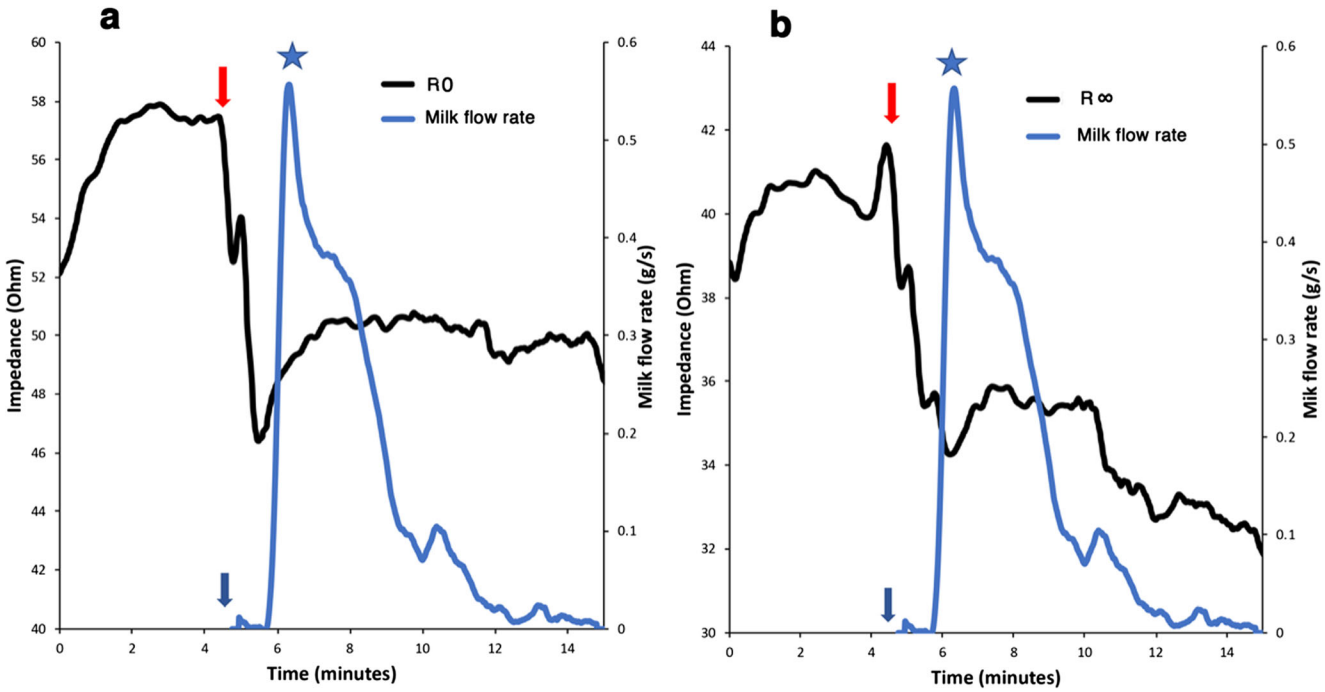

Fig. 3 Bioimpedance measurements with milk flow rate. a. R0. b. R∞. Pump starts Bioimpedance response Milk ejection peak

frequencies (R0 and R∞ respectively). Average tissue membrane capacitance (C_m) was also calculated. Cole modelling was performed using Bioimp (version 5.4.03) software provided with the SFB7 machine. Milk ejection parameters were measured using the methods described above. Statistical analyses were carried out with R Studio Version 1.0.136 [18] using package nlme [19] for linear mixed effect modelling and lsmean [20] for Tukey *post-hoc* comparison. Probability values for the change in the bioimpedance characteristics were determined using the Wilcoxon matched pairs test as the data were not normally distributed.

Results

The participant characteristics are detailed in Table 1. Parity was significantly related to the volume of milk pumped ($p < 0.001$). Most of the infants were exclusively breastfeeding, but some of the older infants were consuming supplementary foods.

There was a mean of 3 milk ejections for participants over the two sessions. There were no significant differences in milk volume pumped, PAMR, time to reach milk ejection or the number of milk ejections between sessions. In addition, there were no significant differences in milk ejection characteristics between the two sessions (Table 2).

Bioimpedance data were plotted together with cumulative milk and flow rate data, and typical example data are presented in Fig. 3. Changes in R0, R∞ and membrane capacitance (C_m) from the 30 s period immediately prior to the initial and second milk ejection to the period immediately following milk ejections were determined and presented in Tables 3 & 4. The magnitude and significance of changes recorded were smaller for milk ejection 2 (Table 4).

Although there were wide variations between individuals and between sessions (pumped breast monitored vs non-pumped breast monitored), most sessions showed a significant decrease in R0 and R∞ indicative of increased milk flow (Tables 3 & 4). In a few sessions, an increase in bioimpedance parameters occurred at the first milk ejection for both the session where the pumped breast was monitored (R0: $n = 2$, R∞: $n = 3$,) and for the non-pumped breast (R0: $n = 4$, R∞: $n = 3$). However, the magnitude of change for R0 and R∞ on the non-pumped breast was considerably less than that of the pumped breast (Table 3). Changes in C_m were more variable, and did not show a consistent relationship with milk ejection.

No significant relationships were demonstrated between the changes in bioimpedance parameters at milk ejection, breast fullness (R0, $p = 0.87$, R∞, $p = 0.63$), milk volume pumped (R0 $p = 0.57$, R∞, $p = 0.57$, C_m, $p = 0.66$), PAMR (R0, $p = 0.93$, R∞, $p = 0.63$, C_m, $p = 0.15$) or milk ejection characteristics (time to first milk ejection, R0, $p = 0.71$, R∞, $p = 0.12$, C_m, $p = 0.37$. Similarly for duration of milk ejection 1, R0, $p = 0.29$, R∞, $p = 0.57$, C_m, $p = 0.09$ and time to peak flow rate for milk ejection 1, R0, $p = 0.35$, R∞, $p = 0.41$, C_m, $p = 0.09$). There was a significant relationship between C_m and breast fullness, but further investigation is required to confirm this, given the variability of changes in C_m in the research sessions.

Table 1 Maternal and infant characteristics

		Mean (SD)	Median	Range
Mother	Age (years)	31(3)	32	24–38
	Parity	2(1)	1	1–5
	Body Mass Index (kgm^{-2})	28(6)	26	19–54
Infant	Gestational age at birth (weeks)	39 (1)	39	37–41
	Birth weight (g)	3653(435)	3642	2660–4645
	Age at monitoring (weeks)	18 (7)	17	5–33
Milk	Storage capacity (left breast; ml)	182 (78)	168	84–455
Production	Storage capacity (right breast; ml)	187 (62)	184	88–286
	Milk removed by baby/pump: left breast (ml)	383 (126)	363	228–741
	Milk removed by baby/pump right breast (ml)	395 (117)	380	221–723
	24 h milk profile (Milk removed by baby/pump) (ml)	778 (169)	773	484–1155

Discussion

This study has shown that bioimpedance parameters change significantly at milk ejection; in particular a reduction in R0 and R∞ at the first milk ejection during the expression of milk with an electric breast pump.

Bioimpedance successfully detected the first milk ejection during a pumping session as a significant drop in R0 (93% of sessions) and R∞ (90% of sessions). This consistent decrease in R0 and R∞ at the first milk ejection may be due to the substantial increase in extracellular fluid i.e. milk, passing through the measurement area during milk ejection. This is akin to a demonstrated decrease in R0 and R∞ when fluid is injected subcutaneously into the arm in humans [21] or the decrease in R0 with lymph accumulation in lymphoedema [9]. The bioimpedance electrodes were placed on the lower half of the breast, where all women were likely to have a substantial amount of secretory tissue, thus increasing the likelihood of detection of milk flow through the ducts at milk ejection. The bioimpedance changes at the subsequent milk ejection were typically smaller [4, 6] in magnitude (Table 4). These decrements are consistent with the reduced responsiveness of myoepithelial cell oxytocin receptors with breast emptying, decreased intra-ductal pressure and reduced volume of milk flowing through the ducts [22].

Reductions in R0 and R∞ were observed when electrodes were placed on both the pumped breast and the non-pumped breast, although considerably greater magnitudes of change

Table 2 Milk ejection characteristics for pumping sessions

Measure (mean ± SD)	BIS electrodes on pumped breast	BIS electrodes on non-pumped breast	*P* Value
Milk volume (ml)	81 ± 55	70 ± 51	0.17
Breast fullness	0.7 ± 0.2	0.7 ± 0.3	0.57
Available milk (ml)	144 ± 64	136 ± 65	0.55
% Available milk removed	58 ± 26	55 ± 27	0.61
Time to 1st milk ejection (s)	105 ± 77	136 ± 65	0.39
Number of milk ejections	3 ± 1	3 ± 1	0.42
First Milk Ejection			
Duration (s)	200 ± 113	182 ± 102	0.37
Time to peak flow rate (s)	69 ± 59	69 ± 39	0.99
Peak flow rate (g/s)	0.7 ± 0.6	0.5 ± 0.3	0.18
Milk volume (ml)	48 ± 48	39 ± 28	0.25
% milk volume removed	57 ± 27	56 ± 26	0.89
Second Milk Ejection			
Duration (s)	126 ± 39	134 ± 49	0.41
Time to peak flow rate (s)	40 ± 16	45 ± 21	0.38
Peak flow rate (g/s)	0.6 ± 0.8	0.4 ± 0.4	0.17
Milk volume (ml)	22 ± 13	25 ± 36	0.67
% milk volume removed	29 ± 13	35 ± 23	0.08

Table 3 Bioimpedance measurements for the first milk ejection during expression of milk with an electric breast pump (*P* values from Wilcoxon matched pairs test)

Measure	Breast	Milk Ejection 1							
		n	Baseline (mean 30s before change in milk flow)		Change associated with milk flow		Percent change		*P* value
			Median (IQR)	Range	Median (IQR)	Range	% Change median (IQR)	Range	
R 0 (Ohm)	Pumped	30	36 (3,43)	16–122	-2 (−5,-1)	−31 - 12	−7 (−17,-4)	−46 - 31	<0.001
	Non-pumped	25	35 (28,53)	17–108	−1 (−2,-1)	−17 - 3	−3 (−8,-2)	−30 - 9	0.007
R∞ (Ohm)	Pumped	29	24 (21,29)	13–66	−2 (−5,-1)	−15 - 12	−8 (−20,-2)	−39 - 43	<0.001
	Non-pumped	23	25 (18,38)	12–68	−1 (−3,0)	−10 - 2	−5 (−8,-2)	−25 - 4	<0.001
C_m (nF)	Pumped	27	52 (34,79)	11–176	−10 (−32,-2)	−81 - 22	−24 (−59,-7)	−92 - 48	<0.001
	Non-pumped	24	42 (25,63)	8–123	−3 (−6,5)	−16 - 63	−9 (−17,15)	−46 - 99	0.48

Significant changes in: R0, R∞ and C_m were found less frequently for the second milk ejection for both sessions (Table 4)

were observed when electrodes were applied on the same breast that was expressed. Although milk ejection occurs simultaneously in both breasts [23], there are differences in the physiological changes when there is no breast stimulation or milk removal. Ramsay et al. [4], reported ductal dilation on the opposite breast during breastfeeding, and it was observed that the milk was redistributed within the breast after the initial milk ejection [4]. This may provide an explanation for the smaller changes in R0 and R∞ observed on the opposite breast. In addition, the non-pumped breast may have contained less milk, as participants would generally select the fuller breast for the study.

Membrane capacitance also decreased when the electrodes were placed on the pumped breast for the first milk ejection, but these measures exhibited wider variability compared to R0, R∞ (Table 3). This suggests that membrane capacitance decreases as extracellular fluid levels increase due to changes between the fluid compartments influencing the membranes role as a capacitor. Alternatively, the capacitive characteristics of the membranes may change with conformational change in the membranes due to the presence of larger volumes of extracellular fluid.

At the second milk ejection, measurable decreases in R0 & R∞ were detected for 26% of sessions where the electrodes were placed on the expressed breast. The smaller change was anticipated as the magnitude of the first milk ejection tends to be greater, resulting in higher milk flow rates, and therefore a greater increase in the extracellular fluid volume [3, 6]. Significant changes in membrane capacitance at the second milk ejection were also observed, which suggest continued physiological changes.

Despite the occurrence of changes in impedance parameters being associated with milk ejection, the magnitude of these changes were not related to breast fullness, milk ejection characteristics or percent available milk removed. This may not be unexpected as the volume effectively measured by impedance constituted a relatively small proportion of the whole breast, whilst these parameters relate to the entire breast.

Table 4 Bioimpedance measurements for the second milk ejection (*P* values from Wilcoxon matched pairs test)

Measure	Breast	Milk Ejection 2							
		n	Baseline (mean 30s before change in milk flow)		Change associated with milk flow		Percent change		*P* value for change
			Median (IQR)	Range	Median (IQR)	Range	% Change median (IQR)	Range	
R 0 (Ohm)	Pumped	8	33 (27,37)	16–65	−2 (−2,-1)	−5 - -1	−4 (−7,-4)	−11 - -3	0.012
	Non-pumped	6	30 (27,41)	20–52	−1 (−1,−1)	-1 - -1	−3 (−4,-2)	−7 - −1	0.028
R∞ (Ohm)	Pumped	8	21 (14,26)	10–38	-1 (−1,0)	−4 - 0	−3 (−7,-3)	−17 - −1	0.012
	Non-pumped	5	21 (18,25)	18–34	0 (−1,0)	-1 - 0	−2 (−4,-1)	−7 - -1	0.043
C_m (nF)	Pumped	9	52 (29,78)	17–85	−13 (−17–8)	−24 - -2	−26 (−37,17)	−57 - -8	0.008
	Non-pumped	12	44 (39,96)	27–133	−6 (−10,-3)	−31 - 1	−10 (−14,-6)	−33 - -3	0.002

A number of limitations to this study must be acknowledged. The study was designed as a proof of concept to determine whether impedance monitoring of the lactating breast may be a fruitful line of research and should not be considered as an established method for monitoring milk production. The complexities of the lactating breast provide a challenge to researchers due to the large individual variability in anatomy and physiology. The variability in R0, R∞ and membrane capacitance reported in this study are likely to be due to a range of physiological changes occurring during milk ejection. The lactating breast has twice as much glandular as adipose tissue, but the distribution of these tissues varies within individuals [4]. The ductal system is also variable, with differences in the width and length of ducts and the number of viable orifices on the nipple for milk removal [24]. Milk ejection results in changes in duct morphology and intramammary pressure as the various lobes are asynchronously emptied [4, 17]. Another factor not measured in this study that might influence the detection of milk ejection is mammary blood flow, which is variable between women but has been shown not to relate to milk production [25]. In animal models, however, blood flow has been reported to increase before milk ejection followed by a decrease during milk ejection [26]. It is not, therefore possible to ascribe with certainty the observed impedance changes specifically to milk ejection per se. Nevertheless, impedance appears to be measuring changes strongly associated with milk ejection.

Impedance measurements are affected by the geometry of the volume being measured. Impedance measurements are best suited to cylindrical-shaped objects not highly variable in shape and volume the human breast. We attempted to mitigate this confounder by locating electrodes in similar relative positions irrespective of overall breast shape and size such that a similar effective volume was measured between the sense electrodes. A disadvantage of this approach is that this volume is small relative to total breast volume and may not be representative of the breast overall. Further work is required to optimise electrode locations on the breast, particularly given the highly variable shape and size of the human lactating breast. Despite these challenges, we have shown that bioimpedance can consistently detect changes occurring at milk ejection, and further modification of the equipment and methods should result in improved performance, particularly if the whole breast is monitored.

Conclusion

This study has demonstrated the potential for impedance monitoring as a feasible method for the detection of the initial milk ejection during expression of milk with an electrical breast pump. Changes in impedance parameters were consistently observed with milk production through pumping, although there was wide variation in the magnitude of changes observed. It is anticipated that optimisation of the impedance method will reduce this variation and these encouraging preliminary observations will stimulate further research. Potentially, inclusion of sensors such as bioimpedance to detect milk ejection in a breast pump may provide biofeedback to enable automatic adjustment of pump settings and improve the efficacy of milk removal.

Acknowledgements This research was funded by an unrestricted research grant from Medela, Ag, Baar Switzerland .

The authors would like to thank the participants, and the Australian Breastfeeding Association (WA Branch) for their support (Approval 2016-1).

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards HG, CTL & DG are supported by an unrestricted research grant from Medela AG.

The study was approved by The University of Western Australia Human Research Ethics Committee (RA/4/1/7897).

All participants provided written informed consent.

Conflicts of Interest Author Ward provides consultancy services to ImpediMed Ltd. ImpediMed had no input into the conception or conduct of the study or the writing of the manuscript.

References

1. Truchet S, Honvo-Houéto E. Physiology of milk secretion. Best Pract Res Clin Endocrinol Metab. 2017;31:367–84.
2. Kent JC, Ramsay DT, Doherty D, Larsson M, Hartmann PE. Response of breasts to different stimulation patterns of an electric breast pump. J Hum Lact. 2003;19(2):179–86.
3. Ramsay DT, Mitoulas LR, Kent JC, Cregan MD, Doherty DA, Larsson M, et al. Milk flow rates can be used to identify and investigate milk ejection in women expressing breast milk using an electric breast pump. Breastfeed Med. 2006;1(1):14–23.
4. Ramsay DT, Kent JC, Owens RA, Hartmann PE. Ultrasound imaging of milk ejection in the breast of lactating women. Pediatrics. 2004;113(2):361–7.
5. Luther E, Arballo J, Sala N, Cordero Funes J. Suckling pressure in humans: relationship to oxytocin-reproducing reflex milk ejection. J Appl Physiol. 1974;36(3):350–3.
6. Prime DK, Geddes DT, Hepworth AR, Trengove NJ, Hartmann PE. Comparison of the patterns of milk ejection during repeated breast expression sessions in women. Breastfeed Med. 2011;6(4):183–90.
7. Kyle UG, Bosaeus I, De Lorenzo AD, Deurenberg P, Elia M, Gómez JM, et al. Bioelectrical impedance analysis—part I: review of principles and methods. Clin Nutr. 2004;23(5):1226–43.
8. Cornish B, Chapman M, Hirst C, Mirolo B, Bunce I, Ward L, et al. Early diagnosis of lymphedema using multiple frequency bioimpedance. Lymphology. 2001;34(1):2–11.
9. Ward LC. Bioelectrical impedance spectrometry for the assessment of lymphoedema: principles and practice. Lymphedema: Springer; 2015. p. 123–32.

10. Bera TK. Bioelectrical impedance methods for noninvasive health monitoring: a review. J Med Eng. 2014;2014:1–28.
11. Gowry B, Shahriman A, Paulraj M, editors. Electrical bio-impedance as a promising prognostic alternative in detecting breast cancer: a review. Biomedical Engineering (ICoBE), 2015 2nd International Conference on; 2015: IEEE.
12. Malecka-Massalska T, Chara K, Golebiowski P, Wladysiuk M, Smolen A, Kurylcio A, et al. Altered tissue electrical properties in women with breast cancer–preliminary observations. Ann Agric Environ Med. 2013;20(3).
13. Tornuev YV, Koldysheva E, Lapiy G, Molodykh O, Balakhnin S, Bushmanova G, et al. Bioimpedancemetry in the diagnostics of inflammatory process in the mammary gland. Bull Exp Biol Med. 2014;156(3):381–3.
14. Arthur P, Hartmann P, Smith M. Measurement of the milk intake of breast-fed infants. J Pediatr Gastroenterol Nutr. 1987;6(5):758–63.
15. Fleet I, Linzell J. Rapid method of estimating fat in very small quantities of milk. J Physiol Lond. 1964;175(1):P15–7.
16. Prime DK, Geddes DT, Spatz DL, Robert M, Trengove NJ, Hartmann PE. Using milk flow rate to investigate milk ejection in the left and right breasts during simultaneous breast expression in women. Int Breastfeed J. 2009;4(1):10.
17. Gardner H, Kent JC, Lai CT, Mitoulas LR, Cregan MD, Hartmann PE, et al. Milk ejection patterns: an intra-individual comparison of breastfeeding and pumping. BMC Pregnancy Childbirth. 2015;15(1):156.
18. Studio Team R, Studio R. Integrated development for R. Boston, MA: R Studio Inc.; 2015.
19. Pinheiro J, Bates D, DebRoy S, Sarkar D. Linear and nonlinear mixed effects models. R package version. 2007;3:57.
20. Lenth RV. Least-squares means: the R package lsmeans. J Stat Softw. 2016;69(1):1–33.
21. Ward L, Sharpe K, Edgar D, Finlay V, Wood F, editors. Measurement of localized tissue water–clinical application of bioimpedance spectroscopy in wound management. J Phys Conf Ser; 2013: IOP Publishing.
22. DeNuccio D, Grosvenor C. Effects of volume and distribution of milk on the oxytocin-induced contraction of the lactating rat mammary gland in vivo. J Endocrinol. 1971;51(3):437–46.
23. Prime DK, Kent JC, Hepworth AR, Trengove NJ, Hartmann PE. Dynamics of milk removal during simultaneous breast expression in women. Breastfeed Med. 2012;7(2):100–6. https://doi.org/10.1089/bfm.2011.0013.
24. Ramsay D, Kent J, Hartmann R, Hartmann P. Anatomy of the lactating human breast redefined with ultrasound imaging. J Anat. 2005;206(6):525–34.
25. Geddes DT, Aljazaf KM, Kent JC, Prime DK, Spatz DL, Garbin CP, et al. Blood flow characteristics of the human lactating breast. J Hum Lact. 2012;28(2):145–52.
26. Eriksson M, Lundeberg T, Uvnäs-Moberg K. Studies on cutaneous blood flow in the mammary gland of lactating rats. Acta Physiol Scand. 1996;158(1):1–6.