Electrical impedance dermography differentiates squamous cell carcinoma in situ from inflamed seborrheic keratoses

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Running title: EID differentiates SCC and SK

Abbreviations: EID, electrical impedance dermography, ICC, intra-class correlation; kHz, kilohertz; SCC, squamous cell carcinoma; SCC in situ, squamous cell carcinoma in situ, SK, seborrheic keratosis

ABSTRACT

There are no currently available low cost, non-invasive methods for discerning depth of squamous cell carcinoma (SCC) invasion or distinguishing SCC from its benign mimics such as inflamed seborrheic keratosis (SK). We studied 35 subjects with subsequently confirmed SCC or SK. Subjects underwent electrical impedance dermography (EID) measurements, at six frequencies, to assess the electrical properties of the lesion. Averaged greatest intra-session reproducibility values were 0.630 for invasive SCC at 128 kHz, 0.444 for SCC in situ at 16 kHz, and 0.460 for SK at 128 kHz. EID modeling revealed significant differences between SCC and inflamed SK with normal skin ($p<0.001$), and also between invasive SCC and SCC in situ ($p<0.001$), invasive SCC and inflamed SK ($p<0.001$), and SCC in situ and inflamed SK ($p<0.001$). A diagnostic algorithm classified SCC in situ from inflamed SK with an accuracy of 0.958, sensitivity of 94.6%, and specificity of 96.9%; also SCC in situ against normal skin with an accuracy of 0.796, sensitivity of 90.2%, and specificity of 51.2%. This study provides preliminary data and a methodology that can be utilized in future studies to further advance the value of EID and inform biopsy decision-making in patients with lesions suspicious for SCC.

INTRODUCTION

Squamous cell carcinoma (SCC) is the second most common form of skin cancer in the U.S. (Madan et al., 2010). SCC may present as invasive disease involving the dermis (Figure 1a) or in superficial form (termed SCC in situ, Figure 1b) in which keratinocyte atypia is confined to the epidermis (Kivisaari, 2013). While SCC is often diagnosed clinically, the tumor subtype and extent of invasion and cellular differentiation often cannot be appreciated by visual examination alone (Glazer et al., 2017). SCC in situ is best biopsied by shave technique and can usually be treated without surgery (by destruction or topical therapy), while invasive SCC is best biopsied by punch technique to assess depth and histologic differentiation of invasive tumor and usually requires surgical excision that may be associated with substantial morbidity (Motley et al., 2002). Seborrheic keratosis (SK) is a benign neoplasm characterized by epidermal hyperplasia that commonly arise in older individuals (Sun and Halpern, 2022). SK occasionally become irritated and inflamed (Figure 1c) (Mansur and Yildiz, 2019), which may signal the development of SCC in situ within the lesion (Chen et al., 2017). Distinguishing SCC in situ from invasive SCC and inflamed SK represent clinical conundrums that have important implications for determining optimal biopsy approach and urgency of evaluation. Thus, there is a clinical need to develop new technologies to augment visual skin examination to guide biopsy-decision-making and improve management of lesions suspicious for SCC.

Electrical impedance dermography (EID) is a new technology that can be used to detect electrical conduction differences between benign and malignant skin tissues (Luo et al., 2022). These electrical properties indicate how strongly tissue resists or conducts alternating electrical current and they can be used as a diagnostic biomarker (Nagy et al., 2019). Similar to other existing electrical impedance-based techniques (Martinsen et al., 1999), EID measures the skin

impedance applying a painless, low-intensity electrical alternating current to the tissue in a given area using two electrodes in contact with the skin and then uses a different pair of electrodes to measure the resulting voltages. The underlying electrical properties of the skin can then be calculated from the impedance recorded, with less conductive skin corresponding to higher impedances and vice versa. It would be predicted that differences in tissue composition and structure between SCC and SK (e.g., resulting from epidermal hyperplasia, tumor cells in epidermis and dermis, keratin pearls in SCC, and UV-induced elastosis and infiltration of inflammatory cells) would change the ionic content of the skin and thus affecting its ability to conduct electrical current also compared to normal skin.

We recently reported a pilot study testing the first version of a new EID device (termed URSKIN) for measuring skin and found significant skin electrical differences between basal cell carcinoma (BCC) and adjacent normal skin (Luo et al., 2022). Here, we report an investigation using URSKIN to evaluate whether EID can differentiate invasive SCC, SCC in situ, and inflamed SK.

RESULTS

Subject recruitment and lesion characteristics

A total of 40 subjects with skin lesions clinically suspicious for SCC or inflamed SK were recruited for the study. Subject demographics and clinicopathologic characteristics of the lesions are detailed in Table 1. Given the intended use of the device for distinguishing SCC subtypes and inflamed SK, we targeted enrollment to palpable scaly lesions. All lesions were assessed by an experienced dermatologist (D.G.) and histopathologic diagnoses reviewed by an experienced dermatopathologist (S.R.F.). Following EID measurements using URKSIN, all lesions were biopsied and a total of 8, 14, and 13 lesions were confirmed to be invasive SCC, SCC in situ and inflamed SK, respectively. Two subjects were technical failures due to loss of Bluetooth connectivity between URSKIN and the smartphone app, biopsies of two lesions showed actinic keratosis, and one lesion showed scar; these five subjects/lesions were excluded from the analyses.

Intra-session reproducibility

We performed at least three repeated measurements on each lesion and adjacent normal skin. The test-retest reproducibility results of the 35 included cases are summarized in Table 2, which provides data for the overall values for each of the measurements and their variability expressed as 95% confidence intervals. Due to the unique electrical signature across the range of frequencies measured of the different conditions studied, intra-session intra-class correlation (ICC) values varied with frequency as well as the dataset measured. In general, the data were found more reproducible at the mid-low frequency range probably due to the technical difficulty of measuring accurately at high frequencies, with highest individual ICC values for conductivity

and relative permittivity of 0.701 at 32 kHz and 0.937 at 64 kHz for invasive SCC, 0.689 and 0.808 both at 16 kHz for SCC in situ, 0.584 at 8 kHz and 0.625 at 16 kHz for SK, and 0.557 at 8 kHz and 0.884 at 128 kHz for normal skin.

Electrical impedance dermography of SCC, inflamed SK, and normal skin

We used a random mixed effect linear model to describe the multi-frequency skin electrical properties in both longitudinal and transverse directions between inflamed SK and SCC in situ (Figure 2) and SCC in situ and invasive SCC (Figure 3). Additional multi-frequency curve plots between inflamed SK, invasive SCC, SCC in situ with normal skin are shown in Supplementary Figures 1-3, respectively. The values of conductivity and relative permittivity show an increasing and decreasing dependence with the frequency, respectively, a result that is consistent with in a biological tissue in its response to the application of alternating electric field across the tissue sample. Further, transverse conductivity values were lower than longitudinal conductivity values, indicating that the electrical properties of skin have a directional dependence in these lesions. Consequently, EID devices that obtain only skin impedance measurements in one direction are prone to obtaining values that will change depending on the relative position of the electrodes with respect the skin lesion. We avoided this electrode-position dependence with the URSKIN device that could potentially cause confounding measurement artifacts, and limit diagnostic accuracy, by automatically measuring in four different directions. The statistical results of all these comparisons between multi-frequency modeling analyses are summarized in Table 3. Our linear modeling approach adopted allowed us to "collapse" the frequency dependence of the skin electrical properties in a single parameter of interest, namely the intercept, which has the most direct relevance to skin physiology. The intercept parameter represents the ability of the skin to

conduct low frequency electrical current, which due to the capacitive nature of the cells' membranes, is mainly determined by the extracellular medium. In our regression models, the intercept represents the mean value of the response variable (e.g., electrical values) when all of the predictor variables (i.e., frequencies) in the model are equal to zero. In short, all the above comparisons resulted in significant differences ($p<0.001$) in the intercept parameter, indicating distinct electrical properties in the extracellular composition associated with each lesion type.

Machine learning classification of SCC in situ from inflamed SK and normal skin

Finally, we developed a machine learning (ML) algorithm for classifying SCC in situ from SK lesions and normal skin. Invasive SCC lesions were not included given the small number of cases. Figure 4A shows the receiver operator characteristic (ROC) curve from the nested loop random forest approach developed comparing SCC in situ versus inflamed SK. The averaged area under the curve (AUC) was 0.968, accuracy was 0.958 (95% confidence intervals 0.941 and 0.971), sensitivity 94.6%, specificity 96.9%, positive predictive value (PPV) 96.5%, and negative predictive value (NPV) 95.2%. Figure 4B shows the confusion matrix to compare the diagnosis with those predicted by the ML model. The overall performance of the ML algorithm was 94.6% and 96.9% for correct predictions of SK and SCC in situ lesions, respectively. We also developed a ML model to classify SCC in situ from normal skin, the resultant ROC curve is shown in Figure 4C. The AUC was 0.825, accuracy was 0.796 (95% confidence intervals 0.720 and 0.859), sensitivity 90.3%, specificity 51.3%, PPV 83%, NPV 66.7%. Finally, Figure 4D shows the confusion matrix comparing the diagnosis of SCC in situ versus healthy skin with those predicted by the ML model.

DISCUSSION

Here we demonstrated the potential clinical utility of EID by detecting differences between SCC subtypes, inflamed SK, and normal skin. These results build on our prior study of BCC (Luo et al., 2022), and support the future use of EID as a bedside technology serving as a simple, inexpensive, easily-applied, and rapid diagnostic biomarker for non-melanoma skin cancers. The basis for the electrical differences observed is likely due to the distinct aspects of skin pathology associated with these conditions. For invasive SCC and SCC in situ, the presence of enlarged tumor cells, keratinization, and changes in the extracellular medium of the dermis likely creates heterogeneities in the tissue that will alter EID values from those in normal skin. For inflamed SK, impedance values are likely affected by the presence of epidermal acanthosis and dermal inflammatory infiltrate. Our method of acquiring this information in real time in the clinic could help the provider determine whether a lesion should be biopsied (e.g., if more likely to be SCC in situ than inflamed SK) and whether a punch or shave technique would be optimal (e.g., for invasive SCC vs. SCC in situ). It could also prove useful when deciding on whether to perform concurrent destruction (e.g., electrodessication and curettage) of suspected malignancies at the time of biopsy, which may be particularly helpful for patients traveling long distances to clinic.

In this study, we aimed to assess performance of the URSKIN device under real-world clinical conditions. Lesions were measured immediately prior to biopsy, and the process usually took less than five minutes to complete. We found the degree of reproducibility measuring invasive SCC, SCC in situ and inflamed SK across the range of frequencies measured to be comparable to that in our previous BCC study (Luo et al., 2022). To compare between SCC in situ, inflamed SK, and normal skin, we pooled the skin multi-frequency data from all subjects for each group (SCC in situ, invasive SCC, inflamed SK, and normal skin) and analyzed these using

a mixed-effects linear model with random intercept and slope. Our analyses revealed significance of intercept parameter of skin electrical properties ($p<0.001$) associated with invasive SCC, SCC in situ and inflamed SK. Compared to non-linear models, modeling multi-frequency skin EID data using a mixed-effect linear model is appealing in its relative simplicity to "collapse" the multi-frequency dependency into a reduced number of model parameters, however, these are not the only approaches to analyze the multi-frequency dependency of skin EID data. Another potentially more powerful method for skin lesion classification is to combine all the multi-frequency skin electrical data with machine learning techniques, as we did here and discussed below.

While accurate diagnosis of SCC currently requires biopsy and histologic confirmation, there are several established non-invasive modalities that can facilitate visual examination of deeper structural components of the skin. Dermoscopy is primarily used for pigmented lesions to assist in the identification of melanoma, but also can be used to identify features associated with SCC that include dotted vessels, yellow opaque scales, and microerosions (Zalaudek et al., 2012). However, there is significant overlap of dermoscopic features between SCC and inflamed SK (Papageorgiou et al., 2021). Confocal microscopy allows direct visualization of histologic structures beneath the skin surface and can enable identification of SCC (Shahriari et al., 2021). Nonetheless, this technology has significant limitations, including visualization only to the depth of the superficial dermis, high cost, and difficulty in image interpretation that requires extensive training (Jain et al., 2018). Although machine learning has primarily been applied to diagnosis of melanoma, these approaches are also being developed for non-melanoma skin cancers. However, a recent meta-review concluded that published machine learning algorithms trained on 2-dimensional BCC and SCC images have not performed well in real-world clinical settings and

were inferior to trained dermatologists utilizing dermoscopy (Sharma et al., 2022). Perhaps not surprisingly, these algorithms can only be as effective as the information available in the images used for training. Lesional features that are not readily visible in such images would limit the diagnostic potential of these image-based algorithms, for example, to differentiate subtypes of BCC and SCC.

Prior impedance studies using an earlier version of the Nevisense system found no difference in SCC subtypes (Malvehy et al., 2014; Sarac et al., 2020), although these were not initially designed for this purpose. It is worth highlighting that their approach was limited by the measurement approach used and number of frequencies measured. In addition, the depth of measurement with the Nevisense may be insufficient to include the lower dermis. In our previous study (Luo et al., 2022), we included computer simulations results that allowed us to quantify the depth of measurement with URSKIN that was estimated to be 7 mm. This depth of measurement is sufficient to interrogate most skin lesions including those with a deeply invasive or infiltrative component. More recent versions of Nevisense have been designed to measure skin-electrode impedance contact changes in pigmented lesions to distinguish melanoma from nevi. In addition to melanocytic lesions, 48 BCCs and 7 SCCs were evaluated in a multi-center trial that demonstrated 100% sensitivity but a specificity of only 34% (Malvehy et al., 2014). Although a follow-up study confirmed the ability of Nevisense to accurately distinguish skin cancers from benign lesions, it did not attempt to discriminate between invasive and in situ SCC and the specificity was only 41% (Sarac et al., 2020). Although a few inflamed SK were also evaluated in the trial (Malvehy et al., 2014), it was not clear whether Nevisense could distinguish between inflamed SK and SCC in situ. The low specificity and impracticality of Nevisense (high cost, large instrument size, measuring times of up to 20 minutes) likely explain its limited clinical use

in the U.S. Despite the limited number of lesions measured here, we obtained an averaged AUC of 0.968, accuracy of 0.9581, sensitivity of 94.6%, specificity of 96.9%, PPV of 96.5%, and NPV 95.2% for diagnosis of inflamed SK and SCC in situ. Slightly worse performance was obtained when discerning normal skin from SCC in situ, with an average AUC of 0.825, accuracy of 0.796, sensitivity of 90.3%, specificity of 51.3%, PPV 83%, and NPV 66.7%. These results show promise of our approach, which is based on physiologically meaningful EID data and can be quickly obtained at the bedside without using expensive instrumentation or requiring highly trained examiner skills.

There were several limitations of our study. First, there were a small number of cases from a single practitioner's clinic and dermatopathologist at a single institution and lack of validation on a separate external cohort. In addition, we did not have sufficient case numbers to determine potential differences between SCC in situ and invasive SCC or actinic keratosis, SCC in situ with and without follicular involvement, SK with and without inflammation, and SK with and without atypical features. Still, we found significant intercept difference between invasive SCC, SCC in situ and normal skin, thus warranting further study. Indeed, these represent important clinical distinctions that we may be able to resolve in future studies with additional cases. Future work is warranted to establish the relationships between EID values and quantitative biopsy data in order to obtain deeper insights into electro-histological status of SCC subtypes and inflamed SK. We have not yet incorporated more sophisticated machine learning approaches that may help to improve the overall diagnostic performance over analysis of EID data alone. Another limitation is the relationship between the histological characteristics of SCC lesions and their EID signatures still remains unknown. Combining EID data with patient information (e.g., gender, age, lesion location), recent history of SCC, and histologic

characteristics (e.g., epidermal thickness, degree of dermal involvement, and dermal changes such as elastosis and collagen loss associated with chronic sun damage). Finally, 4 of the SCC lesions were transected such that the dermis could not be visualized by the dermatopathologist. This may result from the biopsy being too superficial, in which case only epidermis was sampled, or from mal-orientation of the tissue during processing such that only epidermis makes it onto the slide for microscopic examination. Because the underlying dermis is not visualized, an invasive SCC extending beyond the epidermis cannot be ruled out. In these cases, when the lesion is clinically superficial (i.e. no palpable nodule under the skin), it is assumed to be SCC-in-situ and usually treated as such. These were included in the analyses with the other SCC-in-situ lesions.

EID remains very much in development, and it is likely that further advances in the technique will lead to improved diagnostic accuracy. We anticipate that future data collection and technical advances, mainly involving the use of newer electronic circuits, electrodes, and controlling the pressure applied against the skin, will make it possible to obtain even more reproducible data across the range of frequencies measured for these types of lesions. Clearly, the greater the reproducibility and diagnostic performance, the greater will be the clinical value and potential utility of this technique as a diagnostic biomarker. Ultimately, our study shows the potential utility of EID for primary care physicians and dermatologists by improving (pre-biopsy) accuracy of diagnosis and guiding biopsy-decision-making, thereby improving management of lesions suspicious for SCC.

MATERIALS & METHODS

Study subjects

This study was approved by the Institutional Review Board (#141288) at University of Utah and all participants gave prior written, informed consent. The URSKIN device received IRB approval for human testing as a non-significant risk investigational device. A total of 40 patients were enrolled in this study. The inclusion criteria were age 18 or older with a lesion clinically suspicious for SCC at least 5 mm in diameter that was to be biopsied. Subjects with lesions on the face, haired scalp, or genital area were excluded. Only one lesion was measured on each subject.

Experimental protocol

Our protocol for obtaining EID measurements is described previously (Luo et al., 2022). Briefly, after cleaning both the electrodes and the skin site, at least three measurements were performed of each lesion and the adjacent normal skin. Subjects then underwent biopsy of the suspicious lesion, which was sent for routine processing and histologic analysis.

EID data collection

URSKIN (Figure 5 A) performs an automated sequence of 4-electrodes skin electrical impedance measurements in 4 different directions, namely 0 degrees (Figure 5 B), 45 degrees (Figure 5 C), 90 degrees (Figure 5 D), and 150 degrees (Figure 5 E) , resulting in a total of 16 electrodes arranged along two concentric circles. For each direction, the device applies alternating electrical current from 8, 16, 32, 64, 128, and 256 kHz between a pair of electrodes in the outer circumference and then measures the resultant voltage the electrodes located in the inner

circumference. Once the measurement is completed, the device switches the electrodes for current injection and voltage sensing and repeats the measurement sequence in another direction until all 4 directions have been measured. A skin impedance measurement takes just a few seconds to complete and the user is notified through a custom smartphone app developed for this purpose. Skin impedance data measured includes the resistance and the reactance at six frequencies and four directions. These data are then processed by the URSKIN device using a mathematical algorithm to estimate the underlying electrical properties of the skin, that is the conductivity and the relative permittivity in both longitudinal and transverse directions, perpendicular to each other, and along the surface plane of the skin. Longitudinal and transverse directions are determined by the conductivity in these directions, with larger conductivity values in the longitudinal directional than in the transverse direction. The results of these calculations are 24 different datasets: 2 values (conductivity and relative permittivity) × 2 directions (longitudinal and transverse directions) × 6 frequencies (8, 16, 32, 64, 128, and 256 kHz). These data are pre-filtered by the mathematical algorithm to remove non-physiological data (artefacts), with conductivity cut-off values in the measured frequency range of 0 and 2 S/m. Finally, in the current prototype, raw measured and filtered data automatically transferred from the device to the app via Bluetooth. These data were then analyzed as described below.

Data analysis

Skin EID data were analyzed using R software (R Foundation for Statistical Computing, Viena, Austria). Intraclass correlation coefficients were calculated to determine the technique's intrasession reproducibility as well as its 95% confidence intervals. Spuriously negative ICC values were set to 0. Multi-frequency paired analysis was performed using a random mixed

linear effects model test for each dataset with random intercept and slope terms to account for within-subject correlations and between-subject variability. P values <0.05 were considered significant.

Finally, we trained and tested a machine learning random forest disease classification algorithm (in R) using all multi-frequency skin conductivity and relative permittivity data in both longitudinal and transverse directions for SCC in situ, inflamed SK, and normal skin. In order to accommodate for the stochastic nature of random forest model, we trained and evaluated 1000 random forest models. We split our data into two parts for the learning algorithm: 80% of it was assigned to the training data, and 20% was designated to be the "out of bag" test set that were exclusive of training samples. We then performed a nested 10-fold cross-validation approach for prediction with random forest in two loops. An inner loop was allocated to determine the individual training data estimates and their performance, whereas the outer loop was used for checking the ability of these estimates in making predictions (classifications) on the test set. Averaging the performance of each test set from all of the outer loops provided the final evaluation output of the learning algorithm to ensure that the predictions were robust and all plausible variations in the data were taken in consideration. Finally, we created receiver operator characteristics (ROC) curves from all the variables used in our model to check our machine's performance, with the area under the curve providing the probability of the given learning model's ability to correctly classify SCC in situ from inflamed SK. We then extracted the accuracy, sensitivity, specificity, positive predictive value, and negative predictive value for each of the two-category measurements. Finally, we created a confusion matrix to evaluate the overall diagnostic performance.

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CONFLICTS OF INTEREST

Dr. Sanchez holds equity in Haystack Dx, a company that develops clinical needle impedance technology for neuromuscular evaluation. The company has an option to license patented needle impedance technology where the author is named an inventor. He also holds equity and serves as Scientific Advisory Committee Member of Ioniq Sciences, a company that develops clinical impedance technology for early lung and breast cancer detection. Dr. Sanchez serves as Scientific Advisory Board Member of B-Secur, a company that develops wearable ECG and impedance technology. He consults for Myolex, Inc., a company that develops surface impedance technology. The company has an option to license patented surface impedance technology where the author is named an inventor. Dr. Sanchez also serves as a consultant to Impedimed, a company that develops clinical impedance technology for early detection of secondary lymphedema. The company has an option to license patented impedance technology where the author is named an inventor. He also serves as a consultant to Texas Instruments, Happy Health, and Maxim Integrated, companies that develop impedance related technology for consumer use. Dr. Grossman is an investigator for Skin Analytics, Inc, Dermtech, Inc. and Orlucent, Inc., companies developing non-invasive technologies for skin cancer/melanoma

diagnosis, and also serves on the Advisory Board of Orlucent. The other authors have no conflicts to declare.

AUTHOR CONTRIBUTION STATEMENT

Developed the methods: B.S. Conceived and designed the experiments: D.G., B.S. Performed the experiments: T.S., M.D. Analyzed the data: E.W-Y.W, S.P., H.C., K.M.B., S.R.F. Wrote the paper: D.G., B.S. All authors reviewed and approved the final draft of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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FIGURE LEGENDS

Figure 1. Histologic and clinical presentations of lesions. (A) Invasive SCC, tumor islands fill dermis. (B) SCC in situ, epidermis filled with atypical cells, not invading into dermis. (C) Inflamed SK, epidermal hyperplasia with perivascular and interstitial inflammatory cell infiltrate in superficial dermis. Measurements of normal skin were made outside the dotted lines around each lesion.

Figure 2. Conductivity (A) and relative permittivity (B) of SCC in situ and inflamed SK. SCC in situ (blue) and inflamed SK (red) circles represent individual values obtained in longitudinal and transverse directions including repeated measurements for all subjects with confirmed SCC in situ and inflamed SK diagnosis. The solid lines are the modeled trajectories of the random mixed effect linear model test ($p<0.05$) and shaded areas represent respective 95% predicted confidence intervals. Sample size SCC in situ of 14 patients and 197 data points, inflamed SK of 13 patients and 177 data points.

Figure 3. Conductivity (A) and relative permittivity (B) of SCC in situ and invasive SCC. SCC in situ (blue) and invasive SCC (red) circles represent individual values obtained in longitudinal and transverse directions including repeated measurements for all subjects with confirmed SCC in situ and invasive SCC diagnosis. The solid lines are the modeled trajectories if the random mixed effect linear model test ($p<0.05$) and shaded areas represent respective 95% predicted confidence intervals. Sample size SCC in situ of 14 patients and 197 data points, invasive SCC of 6 patients and 93 data points.

Figure 4. Machine learning results. Receiver operator characteristic curve of SCC in situ versus SK (A) and representation of **a** machine learning algorithm performance classifying SCC in situ and inflamed SK (B)**.** Receiver operator characteristic curve of SCC in situ versus normal skin (C) and representation of a second manchine learning algorithm performance classifying SCC in situ and normal skin (D).

Figure 5. Electrical impedance dermography. (A) View of URSKIN with dimensions. (B) Detail showing the URSKIN circuit board and electrodes used for measuring skin in (B) 0, (C) 45, (D) 90, and (E) 150 degrees.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Conductivity (A) and relative permittivity (B) of seborrheic keratosis (SK) and adjacent normal skin. SK (blue) and normal (red) circles represent individual values obtained in longitudinal and transverse directions including repeated measurements for all subjects with confirmed SK diagnosis. The solid lines are the modeled trajectories of the random mixed effect linear model test ($p<0.05$) including 95% predicted confidence intervals. Sample size SK of 13 patients and 177 data points, normal skin of 37 patients and 518 data points.

Supplementary Figure 2. Conductivity (A) and relative permittivity (B) of invasive squamous cell carcinoma (invasive SCC) and adjacent normal skin. Invasive SCC (blue) and normal (red) circles represent individual values obtained in longitudinal and transverse directions including repeated measurements for all subjects with confirmed invasive SCC diagnosis. The solid lines are the modeled trajectories of the random mixed effect linear model test ($p<0.05$) including 95% predicted confidence intervals. Sample size invasive SCC of 6 patients and 93 data points, normal skin of 37 patients and 518 data points.

Supplementary Figure 3. Conductivity (A) and relative permittivity (B) of squamous cell carcinoma in situ (SCC in situ) and adjacent normal skin. SCC in situ (blue) and normal (red) circles represent individual values obtained in longitudinal and transverse directions

including repeated measurements for all subjects with confirmed SCC in situ diagnosis. The solid lines are the modeled trajectories of the random mixed effect linear model test ($p<0.05$) including 95% predicted confidence intervals. Sample size SCC in situ of 14 patients and 197 data points, normal skin of 37 patients and 518 data points.

TABLES

Table 1. Subject demographics and clinicopathologic features of lesions.

Subject #	Age	Sex (M,F)	Body site	Lesion size (mm)	Diagnosis
1	78	M	Upper Back	5	SK, with brisk inflammation*
2	79	F	Left Shin	7	SCC, superficially invasive, well-differentiated
3	79	M	Left Dorsal Hand	5	SCC in situ
4	54	F	Right Shoulder	6	SK, with brisk inflammation
5	67	F	Left Dorsal Hand	5	SK, with brisk inflammation
6	46	M	Upper Forehead	5	SCC in situ, plus suppurative folliculitis
7	75	M	Left Dorsal Hand	5	SCC in situ, with follicular involvement
8	89	M	Left Upper Chest	5	SCC in situ
9	62	F	Upper Chest	5	SK, with non-brisk inflammation
10	54	F	Left Dorsal Hand	5	SCC, superficially invasive
11	76	M	Right Lower Leg	5	SCC in situ
12	66	M	Left Scalp Vertex	5	SCC in situ, with follicular involvement
13	57	M	Right Dorsal Hand	5	SCC in situ, with follicular involvement
14	66	M	Left Forearm	5	SCC in situ
15	68	M	Right Posterior Shoulder	5	SK, with non-brisk inflammation
16	70	M	Left Clavicle	5	SCC in situ
17	77	M	Mid Scalp	5	SCC in situ, with follicular involvement
18					No measurement--device complications
19	55	F	Right Upper Chest	7	SK, with brisk inflammation
20					No measurement--device complications
21	63	M	Right Forearm	5	SCC in situ
22	71	M	Left Upper Chest	5	Actinic Keratosis, plus suppurative folliculitis
23	77	M	Left Elbow	5	SCC in situ, with actinic keratosis
24	68	M	Left Forearm	5	Inflamed scar
25	72	M	Base of Right Neck	6	SK, with brisk inflammation
26	73	F	Right Upper Chest	5	SK, with non-brisk inflammation

27	70	M	Left Hand	5	SCC in situ
28	86	M	Right Hand	5	Actinic Keratosis
29	81	M	Left Upper Forehead	10	SCC (transected)**
30	81	M	Left Inner Thigh	5	SCC (transected)
31	83	M	Left Temple	5	SK, with non-brisk inflammation
32	63	F	Left Mid Back	5	SK, with brisk inflammation
33	63	F	Left Clavicle	6	SK, with non-brisk inflammation
34	78	M	Posterior Scalp Vertex	7	SCC (transected)
35	50	F	Left Posterior Shoulder	5	SK, with brisk inflammation
36	65	M	Right Dorsal Hand	5	SCC (transected)
37	68	M	Left Upper Chest	6	SK, with non-brisk inflammation
38	76	M	Right Shoulder	10	SCC, invasive, well-differentiated
39	64	M	Left Shin	7	SCC, invasive, well-differentiated
40	49	M	Left Forearm	5	SK, with brisk inflammation

*Inflammation graded as brisk (present along advancing edge) or non-brisk (patchy and perivascular).
**Transected lesions were likely SCC in situ but an invasive SCC component could not be ruled out.
SCC, squamous cell carcinoma; SK, seborrheic keratosis
Subjects 18, 20, 22, 24, and 28 were excluded from the analyses.
Sex: M, male; F, female.
SCC, squamous cell carcinoma; SK, seborrheic keratosis.

Table 2. Summary of test versus retest reproducibility for multi-frequency conductivity and relative permittivity values. Estimates of intra-class correlation coefficients (ICC) and 95% confidence intervals at the measurement frequencies.

		Longitudinal Conductivity		***Transverse Conductivity***		***Longitudinal Relative Permittivity***		***Transverse Relative Permittivity***	
Frequency (kHz)		*Estimates*	*Conf. Int. (95%)*	*Estimates*	*Conf. Int. (95%)*	*Estimates*	*Conf. Int. (95%)*	*Estimates*	*Conf. Int. (95%)*
8	*Invasive SCC*	0.557	0.2 - 0.836	0.247	-0.243 - 0.808	0	-0.496 - 0.195	0.360	-0.115 -0.846
	SCC in Situ	0.359	0.012 - 0.705	0.288	-0.047 - 0.655	0.389	0.073 - 0.714	0.409	0.061 - 0.735
	Inflamed Keratosis	0.140	-0.188 - 0.58	0.584	0.238 - 0.847	0.557	0.194 - 0.837	0.557	0.2 - 0.836
	Normal	0.482	0.25 - 0.695	0.557	0.335 - 0.747	0.000	-0.335 - -0.01	0.374	0.142 - 0.61
16	*Invasive SCC*	0.625	0.183 - 0.912	0	-0.418 - 0.23	0.584	0.064 - 0.937	0.824	0.428 - 0.978
	SCC in Situ	0.689	0.359 - 0.901	0	-0.282 - 0.38	0.278	-0.116 - 0.705	0.808	0.548 - 0.943
	Inflamed Keratosis	0.000	-0.373 - 0.542	0.315	-0.085 - 0.787	0.029	-0.317 - 0.622	0.625	0.183 - 0.912
	Normal	0.192	-0.03 - 0.463	0.118	-0.111 - 0.405	0.287	0.052 - 0.549	0.299	0.053 - 0.564
32	*Invasive SCC*	0.114	-0.323 - 0.794	0.701	0.261 - 0.945	0.011	-0.334 - 0.659	0.399	-0.129 - 0.865
	SCC in Situ	0.034	-0.271 - 0.513	0.123	-0.199 - 0.583	0.068	-0.278 - 0.558	0.111	-0.207 - 0.574
	Inflamed Keratosis	0.290	-0.292 - 0.871	0.408	-0.026 - 0.886	0.000	-0.625 - 0.428	0.114	-0.323 - 0.794
	Normal	0.261	0.015 - 0.537	0.501	0.25 - 0.723	0.104	-0.095 - 0.374	0.509	0.266 - 0.725
64	*Invasive SCC*	0.033	-0.274 - 0.538	0.577	0.032 - 0.937	0.937	0.753 - 0.993	0.247	-0.201 - 0.842
	SCC in Situ	0.000	-0.38 - 0.361	0.202	-0.125 - 0.636	0.656	0.311 - 0.888	0	-0.344 - 0.452
	Inflamed Keratosis	0.208	-0.175 - 0.681	0	-0.433 - 0.272	0.414	0.033 - 0.79	0.033	-0.274 - 0.538
	Normal	0.552	0.296 - 0.767	0.352	0.076 - 0.633	0.62	0.38 - 0.809	0.341	0.063 - 0.625
128	*Invasive SCC*	0.381	-0.013 - 0.762	0.109	-0.274 - 0.723	0.861	0.574 - 0.977	0.649	0.18 - 0.933
	SCC in Situ	0.221	-0.109 - 0.65	0.345	-0.039 - 0.74	0.391	0.017 - 0.762	0.144	-0.192 - 0.604
	Inflamed Keratosis	0.000	-0.336 - 0.419	0.054	-0.216 - 0.505	0	-0.252 - 0.451	0.381	-0.013 - 0.762
	Normal	0.314	0.067 - 0.576	0.240	-0.003 - 0.515	0.884	0.788 - 0.943	0.201	-0.026 - 0.474
256	*Invasive SCC*	0.795	-0.248 - 1						
	SCC in Situ								

	Inflamed Keratosis	0	-0.942 - 0.993	0.678	-0.713 - 1	0	0 - -1.035	0.795	-0.248 - 1
	Normal	0.296	-0.508 - 0.999	0.198	-0.277 - 0.998	0	0 - -0.187	0	0 - -1.369

Units: Conductivity, Siemens per meter; Relative permittivity, dimensionless; Frequency, kilohertz.

Table 3. Summary of modeled conductivity and relative permittivity fixed effect parameters. Estimates of intercept, 95% confidence intervals and statistical significance *p*-values.

		Longitudinal Conductivity (S/m)			Transverse Conductivity (S/m)			Longitudinal Relative Permittivity (dimensionless)			Transverse Relative Permittivity (dimensionless)		
	Predictors	*Estimates*	*Conf. Int. (95%)*	*P-Value*	*Estimates*	*Conf. Int. (95%)*	*P-Value*	*Estimates*	*Conf. Int. (95%)*	*P-Value*	*Estimates*	*Conf. Int. (95%)*	*P-Value*
SK vs Normal	Intercept	0.10307	0.08391 - 0.12222	**<0.001**	0.06478	0.05491 - 0.07015	**<0.001**	163179.97	149243.39 - 177116.55	**<0.001**	84683.44	82121.29 - 87245.59	**<0.001**
	Frequency	0.00114	0.00094 - 0.00134	**<0.001**	0.00026	0.00020 - 0.00031	**<0.001**	-863.59	-1010.29 - -716.89	**<0.001**	-448.93	-476.58 - -421.28	**<0.001**
SK vs SCC in situ	Intercept	0.11766	0.08662 - 0.14870	**<0.001**	0.61860	0.55524 - 0.06848	**<0.001**	167414.01	143150.42 - 191677.60	**<0.001**	83976.49	80347.76 - 87605.23	**<0.001**
	Frequency	0.00114	0.00083 - 0.00146	**<0.001**	0.00026	0.00019 - 0.00032	**<0.001**	-856.37	-1057.90 - -654.84	**<0.001**	-438.32	-474.99 - -401.66	**<0.001**
Invasive SCC vs Normal	Intercept	0.09115	0.07212 - 0.11017	**<0.001**	0.06661	0.06074 - 0.07248	**<0.001**	141350.45	119568.92 - 163131.97	**<0.001**	84300.99	81721.15 - 86880.85	**<0.001**
	Frequency	0.00117	0.00096 - 0.00138	**<0.001**	0.00024	0.00018 - 0.00031	**<0.001**	-765.08	-929.42 - -600.74	**<0.001**	-460.49	-488.91 - -432.07	**<0.001**
Invasive SCC vs SCC in situ	Intercept	0.09729	0.06307 - 0.13150	**<0.001**	0.06579	0.05651 - 0.07507	**<0.001**	133283.92	103262.64 - 163275.21	**<0.001**	82939.4	79209.37 - 86668.71	**<0.001**
	Frequency	0.00123	0.00085 - 0.00160	**<0.001**	0.00022	0.00011 - 0.00032	**<0.001**	-699.44	-892.12- -506.76	**<0.001**	-453.75	-494.6746 - -412.8238 4	**<0.001**
SCC in situ vs Normal	Intercept	0.09738	0.07755 - 0.11720	**<0.001**	0.06398	0.05893 - 0.06902	**<0.001**	151354.97	141621.50 - 161088.43	**<0.001**	84764.01	82423.99- 87104.03	**<0.001**
	Frequency	0.00120	0.00098 - 0.00142	**<0.001**	0.00026	0.00021 - 0.00032	**<0.001**	-817.82	-924.25 - -711.40	**<0.001**	-460.86	-486.44 - -435.27	**<0.001**

Abbreviations: SK, seborrheic keratosis; SCC, squamous cell carcinoma. Conductivity units: S/m, Siemens per meter.