Rapid Detection of Oral Cancer: Electrophysiological Characterization by Dielectrophoresis technology

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ABSTRACT

Microengineered medical devices offer many potential benefits for point-of-care healthcare and rapid diagnosis, particularly in the field of rapid cancer detection. We have developed a microengineered system using the electrostatic phenomenon dielectrophoresis (DEP) to non-invasively determine the electrophysiological parameters of normal and cancerous oral brush biopsies, looking at both samples consisting primarily of keratinocytes and those consisting primarily of fibroblasts. The cells were isolated from patients after informed consent and the normal, dysplastic/ cancerous state of oral biopsies were confirmed histopathologycally. Cytoplasmic conductivity and specific membrane capacitance were determined using DEP. Cancerous brush biopsies exhibited significantly different electrophysiological fingerprints to normal oral mucosa. KCND2, a gene encoding a member of voltage-activated potassium ion channels was found to be differentially expressed between CAFs and NOFs.

Keywords: oral cancer, fibroblasts, detection, electrophysiology, dielectrophoresis

1 INTRODUCTION

Oral cancer accounts for 3% of all diagnosed cancers and 2% of all cancer deaths worldwide and the incidence rate is increasing [1]. In countries such as Pakistan, Bangladesh, India and Sri Lanka oral cancer is the most common form of cancer, accounting for up to 30% of all diagnosed cancers. Moreover, the incidence of the disease in young people is growing [2-4] Ninety percent of oral cancers are oral squamous cell carcinoma (OSCC). A greater understanding of the mechanisms underpinning the formation of oral cancer would offer benefits in diagnosis and treatment. To this end, we have developed a microengineered system using the electrostatic phenomenon dielectrophoresis (DEP) to non-invasively determine the electrophysiological parameters of normal and cancerous oral brush biopsies, as well as matched pairs of normal oral (NOF) and squamous oral cell carcinoma-derived (CAF) fibroblasts.

Microengineered medical devices offer many potential benefits for point-of-care healthcare and rapid diagnosis. DEP in particlar has a number of benefits for cellular medicine; it is rapid, non-invasive and label-free, providing insight into the electrophosiological properties of the cell suspensions. Once a frequency-based "DEP fingerprint" has been established for a cell line, markers such as the cytoplasmic conductivity (cytoplasm ionic strength), membrane conductance (how well ions conduct across membrane), and specific membrane capacitance (relating to membrane morphology) can be determined by mathematical determination of key frequencies an polarisations of the cells.

In the studies presented here, cells were isolated from patients after consent. The fibroblast ability to stimulate invasion of oral cancer cells was investigated in 3D organotypic models. Furthermore, normal, dysplastic/ cancerous states of oral biopsies were determined using immunohistopathology. DEP results showed CAF cells exhibited lower membrane capacitance and conductance than NOFs. Cancerous brush biopsies exhibited significantly different electrophysiological fingerprints to normal oral mucosa. KCND2, a gene encoding member of voltage-activated potassium ion channels was found to be differentially expressed between CAFs and NOFs. Our results showed the potential of this technology for rapidly detecting oral cancer. Furthermore, CAFs and NOFs electrophysiological differences may warrant further modulation studies of KCND2 expression for impairing oral cancer invasion.

2 MATERIALS AND METHODS

2.1 Cell Collection

i. Fibroblast collection

Primary oral fibroblasts were isolated from normal oral mucosa of healthy individuals (n=7) and from lesions of oral squamous cell carcinoma (n=6) after informed consent. For some patients with oral cancer (n=5), pairs of oral carcinoma-associated fibroblasts and normal oral fibroblasts were isolated from the cancer lesion and the contra lateral healthy site (confirmed histologically), respectively. The cells were grown from explants in

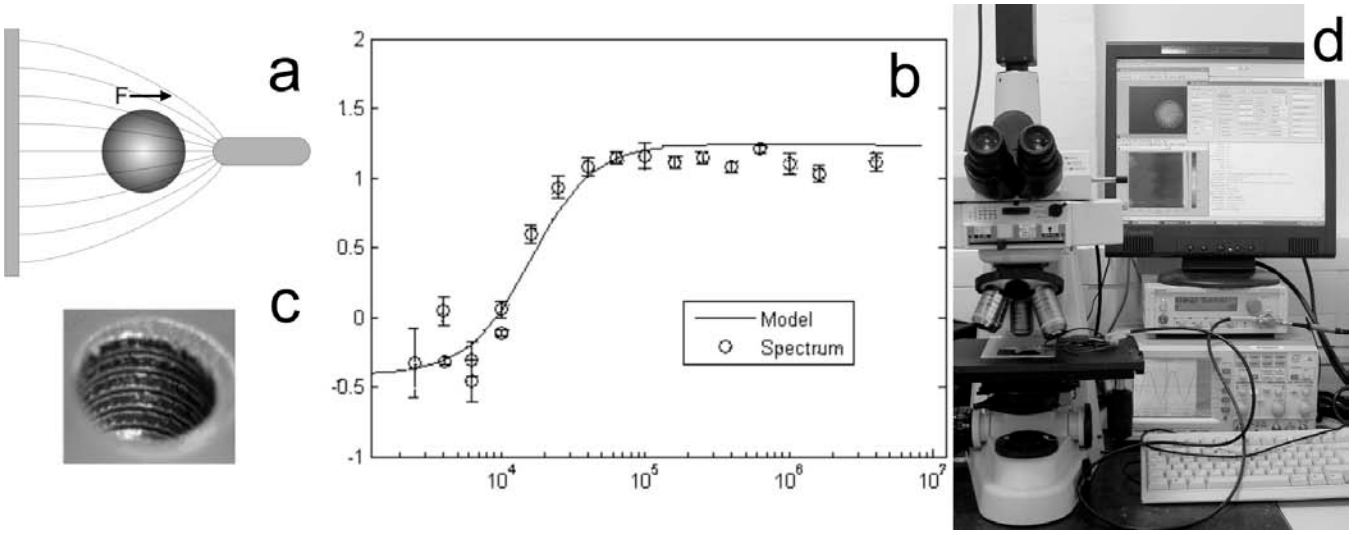

Figure 1. The experimental setup used in these experiments. (a) Dielectrophoresis occurs due to the interaction between a non-uniform field and suspended cell, inducing movement towards or away from the electrodes (b) Plotting the movement of the cell (motion towards the electrodes being positive) as a function of frequency yields a spectrum such as this. The data show the experimental values, the line is a best-fit (based on maximizing correlation coefficient) to a mathematical model that yields electrical data for the cells. (c) The DEP-Well chip used in this experiment to determine these properties, consisting of a well 2mm deep and 1mm in diameter, with 12 electrodes striped down the well wall. (d) A photograph of the data acquisition system; the chip is observed from a microscope camera which records the net motion of particles when an electric field is imposed by the signal generator, with all processing and control coming from a PC running MatLab.

fibroblast-specific medium (DMEM, 10% FBS, all from Sigma, St. Louis, MO, US) and propagated in culture for 1 to 5 passages for further experiments. The cells were analyzed by flow cytometry and immunohistochemistry for the expression of lineage-specific markers, and their gene expression profile (CAFs vs. NOFs) was assessed genome-wide on Affymetrix U133 plus 2 arrays.

ii. Epithelial cell collection

Subjects were recruited at UCL Eastman Dental Institute. Oral cells were harvested by a dental clinician by using a Rovers® Orcellex® Brush. The brush based cell collector was rotated around over the point of interest for at least ten rotations in order to collect cells, as advised by the manufacturer. The brush heads were then suspended in sample collection media containing high glucose (4.5mg/ml) DMEM and 1% penicillin streptomycin. Samples were collected from normal healthy volunteers and patients referred to the UCL Eastman Dental institute with suspected or confirmed OSCC. Ten samples were collected from normal, healthy volunteers without OSCC and 15 normal samples with OSCC and 4 samples were collected from OSCC tissue. The patient sample experiments were carried out as a blind survey. The harvested cells were maintained at 2-8ºC for a maximum of 48 hours. To process samples for DEP experiments the brush head was agitated in the medium to release the cells and then discarded. The cell sample was centrifuged at 260xg. The resulting pellet was resuspended in DEP medium and washed twice, as described in 2.2. The solution was resuspended in 200µl of DEP medium.

2.2 Sample Preparation

Iso-osmotic DEP medium was prepared containing 0.3% glucose and 8.5% sucrose in deionised water, adapted from [5,6]. The medium was adjusted to a conductivity of 5mS m-1 by addition of PBS and verified with a Jenway 470 conductivity meter. Cells were harvested from culture vessels by a brief incubation period with trypsin-EDTA (Sigma Aldrich, Poole, U.K.), washed twice by centrifugation at 150xg in DEP medium and resuspended at a cell concentration of around $2x10^6$ cells ml^{-1}.

2.3 Dielectrophoresis Experiments

A DEP-well electrode chip was used in the experiments. The fabrication of the chip is described elsewhere [7,8]. For each experiment, the cell motion in response to a non-uniform AC electric field was measured for five frequencies per decade from 4kHz to 20MHz. To carry out each frequency reading, approximately 1µl of cell solution was injected into a well in the DEP-well electrode chip. The sample was viewed under a Nikon Eclipse 50i microscope with a 4x magnification. A camera connected to a PC captured images of the well using Photolite software. The

DEP well chip was energised by a sinusoidal signal produced by a Thurlby/Thandar signal generator. The signal was monitored by an ISO-Tech IDS710 digital oscilloscope. Images of the well were captured at time 0 before the electric current was applied and every 3 seconds thereafter, for a period of 60 seconds. Each experiment was repeated five times. A MatLab (The Mathworks Inc, Nantick USA) script was used to assess the change in light intensity in the well over the duration of time that the electric current was applied. The change in light intensity was normalised to the image captured at time 0. Following each frequency run, the sample was extracted from the DEP well and a new sample from the cell solution was injected to prevent any possible harmful effects the electric current may have on the cells. The dielectric properties were inferred by fitting a 'single-shell' model to the line of best fit through the dielectric spectra, as outlined by Broche et al [9]. The line of best fit had a correlation co-efficient of 0.9 or above for all experiments. Radii data was obtained by measuring at least 50 cells per experiment through a microscope and using image analysis software.

3 RESULTS AND DISCUSSION

The preliminary data derived from dielectrophoretic modeling of the cells are shown in table 1. Considering the fibroblast samples first, it can be seen that the mean membrane conductance of the two samples within 2% of one another, indicating the conductance of the two cell types are closely matched and that any variation in ion transport across the membrane has not significantly varied as the cell phenotype has changed. However, the membrane capacitance has varied significantly, from 20.1 (normal) to 15.1 (cancerous), indicating a morphological smoothing of the membrane and a reduction of surface features. When the cytoplasmic conductivity was measured – a parameter closely related to the membrane potential [10] – the parameter was higher in the cancerous cells than the normal cells, indicating elevated membrane ion transporter activity.

Similar trends were revealed when the keratinocytes were examined; the values of the parameters were substantially different when compared to fibroblast cells, indicating a substantial difference in underlying morphology and function, highly similar trends are seen in the transition from normal to cancerous. For example, the cytoplasmic conductivity is elevated by approximate y 25% in both instances, possibly indicating common processes in ion channel overexpression. Similar trends were observed in membrane capacitance, where the cancerous cells exhibited a reduced capacitance – indicative of a less complicated, smoother structure – when compared to healthy cells. Note that the dielectric properties of the keratinocytes were significantly different to those of the fibroblasts when compared against each other; this is in keeping with the radically different morphology and function of the two cell types.

	Fibroblasts		Keratinocytes	
	Normal	Cancerous	Normal	Cancerous
C_{spec}	20.1	15.1	3.3	1.0
σ_{cyto}	0.25	0.32	.05	.06

Table 1. A summary of the DEP data derived from the samples; C_{spec} (units, Fm^{-2}) and σ_{cyto} (units Sm^{-1}) are the specific membrane capacitance and cytoplasmic conductivity respectively

Since the interaction of ion channels and ion pumps embedded in the membrane can contribute to the electrophysiological properties of the cells, we have further investigated the ion channel expression in the fibroblasts. This was checked on a microarray database generated from analyzing and comparing the gene expression of 7 strains of CAFs and 5 strains of NOFs on Affymetrix U133 plus 2 arrays. A couple of proteins members of the voltage-activated potassium ion channels were found to be differentially expressed in CAFs vs. NOFs. CAFs expressed 4.46 fold more KCND2 and 2.86 fold more KCNE4 than NOFs ($p<0.05$). Ongoing studies are validating this results on patient biopsies and tests the possibility of modifying the electrophysiological and biological, cancer-stimulative properties of CAFs by targeting these molecules.

4 CONCLUSIONS

This study brings evidence that when CAFs are different from NOF in terms of their electrophysiological properties. Ongoing studies are investigating if the modulation of their electrophysiological parameters through alterations of KCND2 expression can be used for impairing oral cancer invasion.

5 ACKNOWLEDGEMENTS

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6 REFERENCES

1. Cancer Research UK. [cited; Available from: http://info.cancerresearchuk.org/cancerstats/types/oral/incidence/.

2. Warnakulasuriya, S., *Global epidemiology of oral and oropharyngeal cancer.* Oral Oncology. **45**(4-5): p. 309-316.
3. Annertz, K., Anderson, H., Biörklund, A., Möller, T., Kantola, S., Mork, J., Olsen, J.H., and Wennerberg, J., *Incidence and survival of squamous cell carcinoma of the tongue in Scandinavia, with special reference to young adults.* International Journal of Cancer, 2002. **101**(1): p. 95-99.
4. Shiboski, C.H., Schmidt, B.L., and Jordan, R.C.K., *Tongue and tonsil carcinoma.* Cancer, 2005. **103**(9): p. 1843-1849.
5. Stephens, M., Talary, M.S., Pethig, R., Burnett, A.K., and Mills, K.I., *The dielectrophoresis enrichment of CD34(+) cells from peripheral blood stem cell harvests.* Bone Marrow Transplantation, 1996. **18**(4): p. 777-782.
6. Pethig, R., Bressler, V., Carswell-Crumpton, C., Chen, Y., Foster-Haje, L., García-Ojeda, M.E., Lee, R.S., Lock, G.M., Talary, M.S., and Tate, K.M., *Dielectrophoretic studies of the activation of human T lymphocytes using a newly developed cell profiling system.* Electrophoresis, 2002. **23**(13): p. 2057-2063.
7. Hoettges, K.F., Hubner, Y., Broche, L.M., Ogin, S.L., Kass, G.E.N., and Hughes, M.P., *Dielectrophoresis-Activated Multiwell Plate for Label-Free High-Throughput Drug Assessment.* American Chemical Society, 2008. **80**(6): p. 2063-2068.
8. Hubner, Y., Hoettges, K.F., Kass, G.E.N., Ogin, S.L., and Hughes, M.P., *Parallel measurements of drug actions on erythrocytes by dielectrophoresis, using a three-dimensional electrode design.* Nanobiotechnology, IEE Proceedings-, 2005. **152**(4): p. 150-154.
9. Broche, L.M., Labeed, F.H., and Hughes, M.P., *Extraction of dielectric properties of multiple populations from dielectrophoretic collection spectrum data.* Physics in Medicine and Biology, 2005. **50**(10): p. 2267-2274.
10. Coley, H.M., Labeed, F.H., Thomas, H. and Hughes, M.P. *Biophysical characterization of MDR cell lines reveals the cytoplasm is critical in determining drug sensitivity.* Biochimica et Biophysica Acta 2007. **1770** p: 601-608