Dielectric Spectroscopy of MDCK Cell Activity in Microfabricated Three-Dimensional Scaffolds

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Statement of Purpose: Traditional methods of evaulating cell proliferation involve biochemical assays which require distruption of cell activity. Moreover, cells seeded within scaffolds require indirect chemical assays or scaffold sacrificing in order to further study the cultured cells [1]. Therefore, non-invasive methods are needed to provide accurate analysis of cells *in vitro*, which allows for continuous, unperturbed *in vitro* cell culture. This research project introduces dielectric spectroscopy as a non-invasive tool in monitoring cellular behaviour within three-dimensional scaffolds. Marbine-Derby Kanine Cells (MDCK) are cultured within microfabricated, geometrically-controlled scaffolds and allowed to differentiate to hollow cyst-like structures [2]. This transformation within the three-dimensional environment is monitorined through dielectric spectroscopy while maintaining cell culture *in vitro*.

Methods: Poly (DL-lactide-co-glycolide) acid (PLGA) with 15% NaCl particles (53-63 μm) dissolved in Methyl Ethyl Ketone at a 1:1 ratio were overlayed layer-by-layer using a 3D bioplotter (Envisiontech, Germany) to produce scaffolds with a controlled pore geometry of ~350 μm. The microfabricated scaffolds were cut into 3.6 mm thick and 5 mm diameter discs, post-processed by immersion in water for 6 hours at 50°C, and vacuum dried overnight. Following UV and ethanol series sterilization, scaffolds were immersed in DMEM media overnight, blot dried and prepared for cell seeding. MDCK cells, obtained from ATCC, were embedded in collagen I gel, neutralized with 0.34 N NaOH/10X Waymouth solution (2:3 ratio), and seeded onto the scaffolds where gelation ensured homogeneous cell distribution. The cell-gel seeded scaffolds were cultured with DMEM media containing 10% FBS for 7 days, allowing MDCK cells to differentiation into epithelial, hollow cyst-like structures. Through the cell culture period, cell-seeded scaffold measurements were performed using a measurement cell containing a cylindrical chamber measuring 10 mm in diameter and 5 mm in height. Dielectric measurements for capacitance and conductance were performed using an Agilent 4294a impedance analyzer (Palo Alto, CA) and collected data was converted to permittivity and conductivity values. Throughout the cellular transformation, the measured values were analyzed and fitted to analytical effective media approximations (EMAs), including Hanai-Asami-Kozumi (HAK) and Pauly Schwann (PS) relations, as well mathematical cellular models [3] depicting cell morphology in order to arrive at relations accurately describing cellular activity within the measured scaffolds. The measurement results were confirmed through morphometry using a Zeiss LSM 510 confocal microscope (Jena, Germany).

Results: Dielectric measurements of bare scaffolds immersed in water provided distinct dielectric relaxation spectrums for scaffolds possessing varying pore sizes, which shows that dielectric spectroscopy may also be utilized to differentiate between scaffolds of varying pore geometries. Prior to seeding cells onto scaffolds, mathematical simulation of MDCK cell dispersion in an electric field, performed in COMSOL Multiphysics, provided an approximate value for the appropriate volume fraction of cells to seed in order to accurately be described by EMAs, which was in the range of 0.05-0.2. Therefore, using a seeding volume fraction in the appropriate range, cell-gel seeded scaffolds were measured at day 1,3,5, and 7 (Fig. 1). The obtained dielectric spectra showed a distinct variation in the relaxation curves and inherent dielectric constants. Non-linear curve fitting was performed using mathematical cell models for single and double shell structures within a heterogeneous solution described by the Pauly Schwann EMA. The measured spectra was accurately fitted and described by the aforementioned models, yielding an accuracy of >95%.

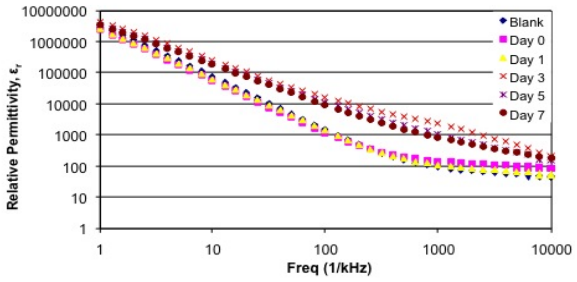

Figure 1 - Relative permittivity of the MDCK cell-gel seeded scaffolds.

Conclusions: A non-invasive cell monitoring technique based on dielectric spectroscopy is a powerful tool that can be applied for cellular monitoring of biological processes *in vitro*, within a three-dimensional scaffold. In this study, we presented a system that is capable of measuring MDCK cells, embedded within a collagen I gel that is seeded onto a microfabricated three-dimensional scaffold. Dielectric measurements, coupled with analytical mathematical models, showed >95% accuracy in monitoring MDCK cellular activity, volume fraction, morphology, proliferation, and differentiation. These results provide a valuable foundation stone for the development of non-invasive monitoring systems for a myriad of cells with complex geometries.

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References:

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