Monitoring Cellular Stress: A New Strategy for Rapid Susceptibility Testing

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Abstract

Background. Methods currently used for determining the susceptibility of bacteria and other cells to therapeutic agents are based on assessing growth-related processes. This reliance on proliferation leads to prolonged incubation periods before test results can be made available, forcing clinicians to prescribe therapies empirically. This, in turn, can lead to inappropriate treatments and poor patient outcomes. A new approach based on monitoring the stress developed by cells in the presence of therapeutic compounds is described for determining drug susceptibility. The development of cellular stress is immediate, enabling susceptibility results to be obtained rapidly irrespective of the growth rate.

Methods. The stress response and associated ancillary processes developed by bacteria, yeast, and mammalian cells during exposure to therapeutic agents were monitored by recording changes in the dielectric properties of the suspension using differential impedance sensing methods. Stress in bacterial and yeast cells was induced by antimicrobial and antifungal compounds while the human cells were treated with the anti-cancer drug mitoxantrone.

Results. Data are presented for strains of *Escherichia coli*, *Candida albicans*, and the human lymphoma cell line HL-60 all known to be resistant and susceptible to particular therapeutic agents. Qualitatively similar responses were measured for susceptible cells irrespective of cell type while resistant mutants were consistent with the absence of a measurable stress response. In all cases, the response from drug-resistant cells could be distinguished from drug-susceptible cells in less than one hour. All measurements were correlated with known susceptibilities (MIC) determined by conventional methods.

Conclusions. Monitoring the development of stress in prokaryotic and eukaryotic cells treated with therapeutic agents can be used to distinguish between susceptible and resistant populations in near real-time.

Introduction

Laboratory methods currently used to determine the susceptibility profiles of bacteria and other cells to therapeutic agents are based on assessing growth-related processes. This reliance on cell proliferation leads to prolonged incubation times delaying the availibility of test results and forcing clinicians to prescribe therapies empirically. This, in turn, can lead to inappropriate treatments, poor patient outcomes, and the development of drug resistance. Monitoring the stress developed by cells in the presence of therapeutic compounds has now been described as a new approach to determine the effects of a drug on a cell.[1, 2] Because the development of physiological stress is immediate, drug susceptibility results can be obtained rapidly irrespective of the growth rate of the cell including unculturable cells.

We have found that monitoring the dielectric permittivity of a cell suspension, a readily measurable electronic property, is a simple, non-invasive, and practical method for sensing the physiological changes associated with stressed cells. The inclusion of differential impedance sensing methods in our system enables the signal from stressed cells to be isolated within complex biological samples.

Data are presented comparing the dielectric responses of bacteria, yeast, and mammalian cells exposed to therapeutic agents.

Methods

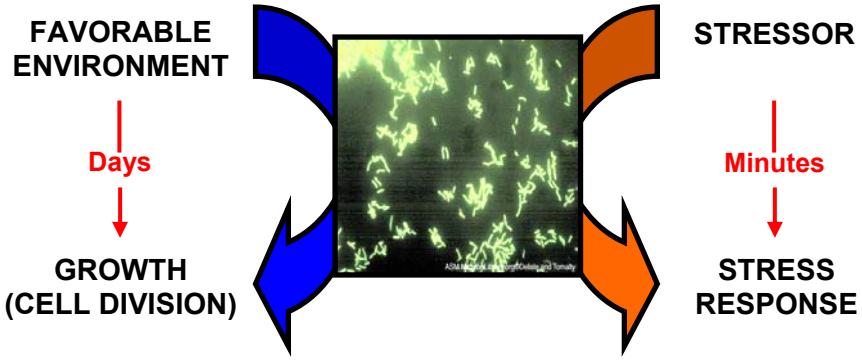
The impedance responses from drug-treated and untreated *Escherichia coli*, *Candida albicans*, and the human lymphoma cell line HL-60 were measured using the BioSense Z-Sense™ differential impedance sensing platform. The stress responses and associated ancillary processes were induced in bacterial and yeast cells by gentamicin (GEN) and fluconazole (FLU), respectively, while the human cells were treated with mitoxantrone (MIT). The impedance signals from two identical 100 µL test volume chambers embedded within a credit-card sized cassette held at 37ºC were continuously recorded by a PC-based Z-Sense data acquisition system. The capacitance components of the respective impedance signals were analyzed together to minimize interfering background signals and produce the corresponding Normalized Impedance Response (NIR) Profiles.

Z-Sense™ Differential Impedance Sensing Platform.

Rapid Susceptibility Testing: Stress versus Growth

Assessing the inhibition of cellular growth in the presence of therapeutic agents is the conventionally accepted method for determining drug susceptibility. For example, a bacterial strain is judged to be susceptible or resistant to an antibiotic based on the value of the minimum inhibitory concentration (MIC), the amount of drug needed to prevent the replication of the bacteria *in vitro*.

An alternative way to characterize the effect of a drug on a cell is to obtain complementary information that assesses the metabolic status of treated cells directly. Our approach accomplishes this by quantifying the physiological stress generated by a drug-treated cell. For example, an untreated cell maintained under optimal growth conditions will develop no stress. Similarly, the same cell when exposed to the drug to which it is fully resistant will also develop no stress. However when exposed to a lethal concentration of a therapeutic compound to which it is susceptible, the cell will be highly stressed in its quest to survive.



Two Possible Metabolic Pathways for Measuring the Susceptibility of Cells to Therapeutic Agents.

Results

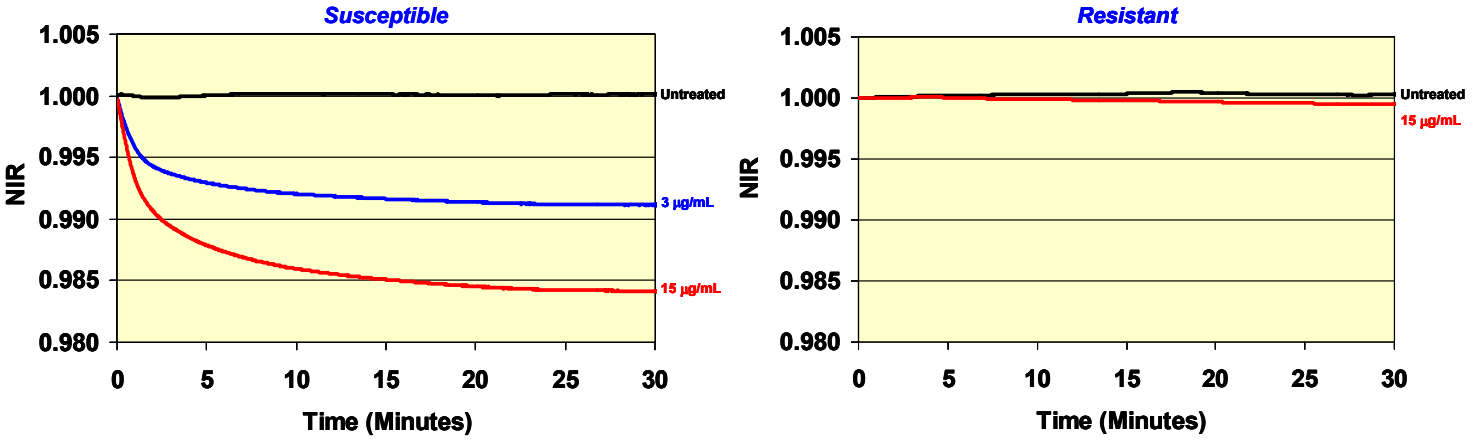
Data are presented for bacterial, yeast, and mammalian cells each known to be resistant or susceptible to the aforementioned therapeutic agents. The Normalized Impedance Response profiles obtained for all susceptible cell-drug combinations are seen to be qualitatively similar. These profiles are characterized by an immediate and continuous decrease in the NIR values with an intensity that is proportional to the drug concentration used as well as the inherent susceptibility of the cell.

NIR profiles were also obtained for isolates having known resistance to these same drugs. In these measurements, no statistically significant differences between the profiles for the drug treated and untreated cells were found, implying the absence of any measurable metabolic response to the drug in resistant cells.

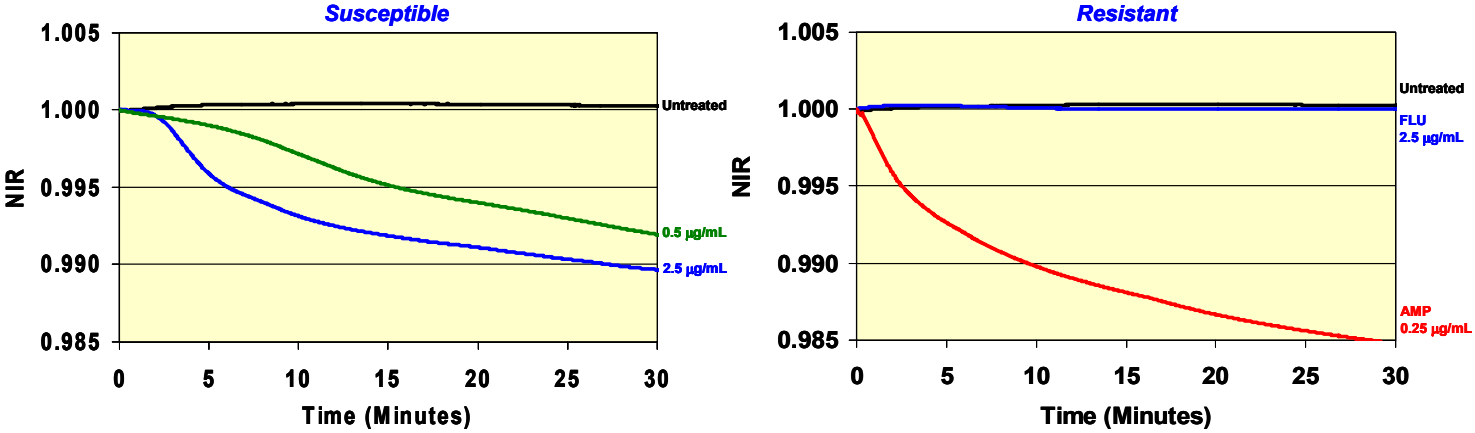
In all cases, significant differences in the NIR profiles for the susceptible cells and drug-resistant cells could be distinguished in less than one hour irrespective of cell type.

All data obtained with Z-Sense technology were in good agreement with known susceptibilities determined by conventional methods.

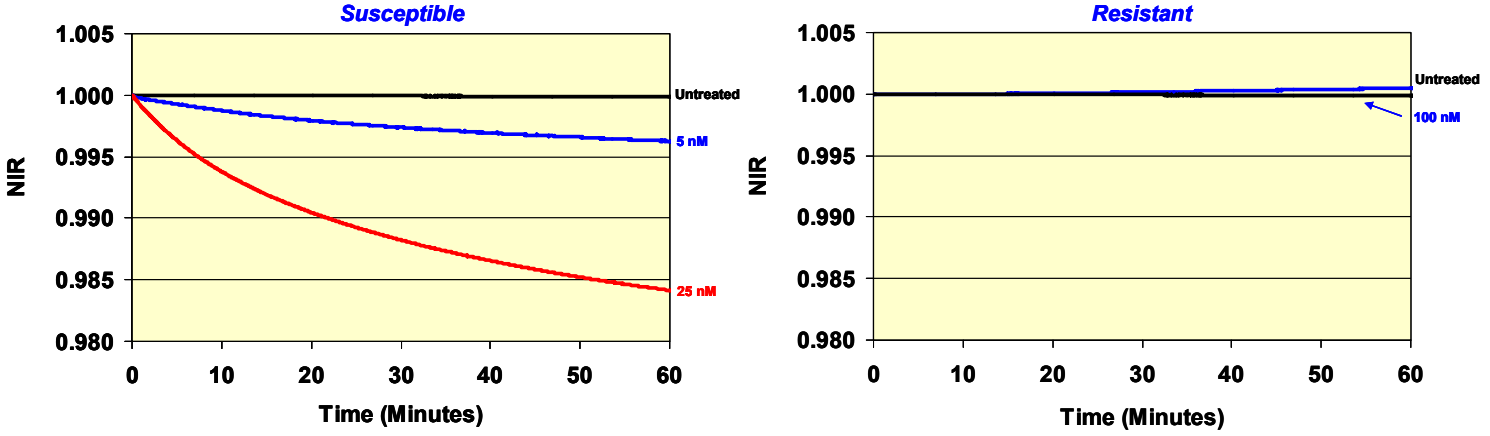
Impedance Response from *E. coli* Treated with Gentamicin



Impedance Response from *C. albicans* Treated with Fluconazole



Impedance Response from HL-60 Cells Treated with Mitoxantrone



Summary and Conclusions

Drug susceptibility measurements currently require days to weeks to obtain results because of the reliance on the growth rate of the cells. This presentation describes an alternative approach that provides commensurate information in near real-time by assessing the degree of physiological stress developed by cells in response to a therapeutic agent.

In our studies, we have discovered that a fast, easy, and sensitive way to detect the development of this stress is to measure changes in the dielectric permittivity of a cellular suspension using differential impedance sensing methods.

The data presented here show that therapeutic agents having different mechanisms of action produce a characteristic and concentration-dependent response irrespective of the cell type. This provides a straightforward means to characterize the susceptibility profiles and distinguishes between susceptible and resistant cells in near real-time.

Taken collectively, these data support our proposition that effective monitoring of the cellular stress developed in response to a therapeutic compound is a powerful tool for identifying drug resistant cells and determining the most suitable therapy rapidly.

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References

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[2] Tiligada E. (2006). Chemotherapy: induction of stress responses. Endoc Relat Cancer;13:S115-S124.