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Potential Application of Raman Micro-Spectroscopy as an *In vitro* Drug Screening and Companion Diagnostic Tool for Clinical Application: Chemotherapeutic Drug Mechanism of Action, Cellular Effects and Resistance

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Abstract

To be considered as an *in vitro* companion diagnostics technique to screen for personalised therapies, Raman micro-spectroscopy should be able to monitor sub cellular interaction with chemotherapeutic drugs and to characterise cellular resistance. Investigations demonstrate the ability of Raman micro-spectroscopy not only to track the sub cellular accumulation of the drug as a function of time, but also to identify its mechanism of action, the subsequent cellular response and to identify cellular resistance. Despite the fact that different cell lines show different chemotherapeutic resistance, the chemical binding signature appears to be identical for anti-cancer drugs which belong to the same chemotherapeutic group, with implications of different mechanisms of action as a function of time and dose.

Keywords: Raman micro-spectroscopy; Chemotherapeutic drugs; Mechanism of action; Cellular effects; Resistance; Companion diagnostic

Introduction

Traditional diagnostic methods are largely based on identification of morphological changes in cells or tissues, rather than analysis of the underlying biochemistry, and so are subjective and prone to error. Therefore, there is a need to develop more effective non-invasive diagnostic and prognostic techniques for a single clinical investigation, either in real time diagnosis or an *in vitro* way which could be applied to *in vivo* situations. An increased emphasis on *in vitro* techniques for evaluation of drug mechanisms and efficacies has also emerged from the introduction of European and US legislation which restrict the use of animal models in cosmetic and pharmaceutical development (EU Directive-2010/63/EU and US Public Law 106-545, 2010, 106th Congress), adding further to the demand for novel biological screening methodologies.

As an alternative, Raman micro-spectroscopy, a vibrational spectroscopy, is an optical technique based on inelastic scattering of light by vibrating molecules, demonstrated by C.V. Raman in 1928 [1-4]. It is based on the interaction of photons with the vibrational states of the molecules in the sample, causing them to scatter in elastically, giving rise to the "Raman effect". As the vibrations are molecularly specific, the technique can provide chemical fingerprints of complex biological samples.

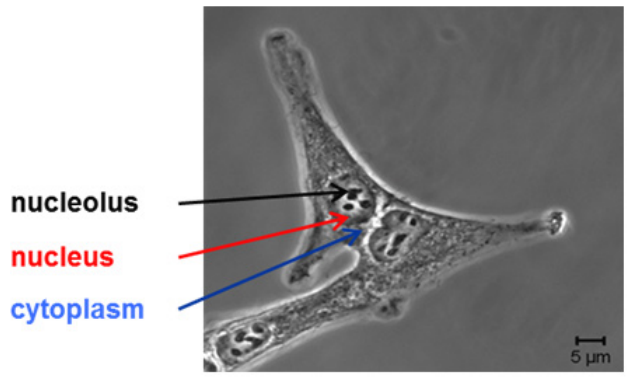

Figure 1: Visible image of human lung adenocarcinoma cell line A549.

As an *in vitro* molecular fingerprinting technique with optical resolution, Raman micro-spectroscopy is able to monitor biochemical processes, drug uptake, efficacy and mode of action and mechanisms of interaction of chemotherapeutic drugs at a sub cellular level and therefore, can help guide drug design

and discovery, and potentially even evaluate signatures of drug resistance, towards potential applications in personalised therapy and as a companion diagnostic tool (Figure 1).

To this end, different lung cell lines were used in order to explore the potential of Raman micro-spectroscopy to elucidate drug pathways, chemical binding signatures, mechanisms of action and efficacy, and physiological cellular responses to the drug exposure. Spectra were taken from the three cellular compartments, namely nucleolus, nucleus and cytoplasm, as shown in Figure 1. As chemotherapeutic agents, Doxorubicin (DOX) and Actinomycin D (ACT), both anthracyclines widely used clinically, especially for lung cancer, were employed as pilot molecules.

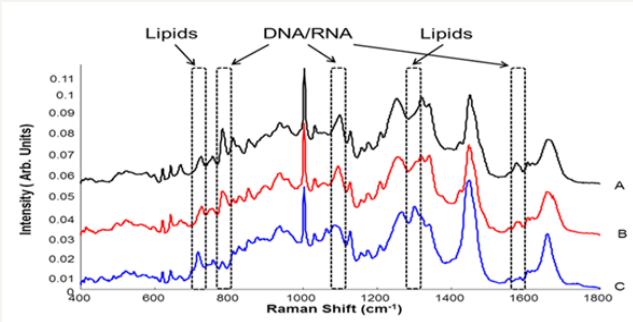

Figure 2: Raman spectra of A. Nucleolus, B. Nucleus and C. Cytoplasm of A549 cell line.
Highlighted regions correspond to DNA/RNA and lipids features.

In Figure 2, typical Raman cellular spectra, with specific features related to cellular components, for example DNA, RNA and lipids, are shown.

Raman Micro-Spectroscopy for Chemotherapeutic Screening and Cellular Resistance

Many studies have reported the use of vibrational spectroscopy to monitor the effects of anticancer agents on cancer cells, including, polyphenols [5] cardiotonic steroids [6], platinum compounds [7], epidermal growth factor inhibitor [8], gold based metallo drugs [9], plant alkaloids [10-13] and anthracyclines [14-18].

Using DOX, Raman micro-spectroscopy clearly demonstrates that it accumulates and saturates first the nucleoli, selectively targeting the RNA, then the nucleus, before it accumulates in the cytoplasm, after nuclear disruption [19,20].

Raman micro-spectroscopy can also differentiate the biochemical responses associated with the sub cellular regions of nucleolus, nucleus and cytoplasm, both in terms of the mechanisms of action (DNA intercalation in the nuclear area and ROS production in cytoplasmic region), and the subsequent cellular metabolic responses for the same cell lines and between different cell lines with difference in resistance, evidenced by an increase in protein features, related to expression of anti-apoptotic proteins and tolerance to DNA damage and implication of DNA repair mechanisms manifest as an increase in DNA signatures [15,20]. A similar response profile was observed for ACT in the same cell lines, both in terms of time evolution and cellular pathways, which suggests that the anthracycline chemotherapeutic group targets the nucleolus first, binding with RNA, and nucleus second, binding with DNA, before accumulating in the cytoplasm, and spectroscopic signatures of the mechanism of action, by DNA intercalation, despite the fact that ACT exhibits higher toxicity than DOX. So, Raman micro-spectroscopy has shed further light on the current understanding of the mode of action of the anthracyclines which is considered to interact only with nuclear DNA in parallel with cytoplasm [21-23].

Moreover, the studies of Cisplatin and Vincristine demonstrate that a drug can have different modes of action, dependent on dose [11,24]. In fact, Cisplatin, an alkylating agent which binds with DNA forming inter and intra strand crosslink's, and Vincristine, an alkaloid which binds to microtubules, appear to intercalate into DNA at high doses.

Conclusion

The ability of Raman micro-spectroscopy not only to track the kinetics and accumulation of chemotherapeutic drugs, *in vitro*, at a sub cellular level, but also to identify their different mechanisms of action according to different time points and doses and to identify cellular resistances, opens up potential clinical applications as a Companion Diagnostics tool, and ultimately personalised medicine approaches as a predictive tool for patient responses in individualised treatment.

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