Label-free microscopy for biophysical and biochemical profiling of live organoids

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Abstract: Organoids are a simplified version of an organ produced *in vitro* in three dimensions. CLASS microscopy and Raman spectroscopy, two complementary label-free techniques, can be used for comprehensive non-destructive profiling of live organoids.

1. Introduction

Translating findings from traditional cell lines and animal models to humans poses a significant challenge in the drug discovery pipeline. Two-dimensional cell cultures are not representative of real cell environments, and numerous studies have identified biological processes that are unique to the human body and cannot be accurately modeled in animals [1]. Organoids, which are miniature self-organized three-dimensional tissue cultures derived from stem cells, offer a promising solution to overcome these challenges [2]. Organoids can be created to replicate much of the complexity of an organ, and their potential to extend basic biological research, medical research, and drug discovery into a more physiologically relevant human setting is becoming widely appreciated. However, a major challenge when working with organoids is effectively monitoring their maturation and response to different drug candidates. Often, multiple markers at the gene, protein, and functional level need to be screened simultaneously, but existing methods are mostly destructive and lack the ability to measure several markers at once. As a result, large sample volumes are required, which can be problematic given the known heterogeneity of organoids. An alternative approach is to use a label-free method that allows non-destructive interrogation of numerous markers [3]. We recently showed that confocal light absorption and scattering spectroscopic (CLASS) microscopy and Raman spectroscopy, two complementary label-free spectroscopic techniques, can be used for profiling various biophysical and biochemical changes in live organoids [4].

2. Results

An important biophysical property for both stem cell maturation and epigenetic drug discovery is chromatin organization. To pack a 2-m-long DNA strand into a nucleus that has a diameter of only a few microns, chromatin must be organized into distinct domains. Some of these domains display a more open form of chromatin, with high gene density and high gene expression, while others are more densely packed, exhibiting a closed chromatin state. Unfortunately, techniques for probing chromatin organization are mostly destructive and involve extensive manipulations of the sample. An alternative is label-free CLASS microscopy, which extends the principles of light scattering spectroscopy [5, 6] to subcellular imaging and can also sense the state of the chromatin organization in live cells [4]. CLASS microscopy employs a broadband light source and confocal microscope, with the light scattered from within the confocal volume (see Fig. 1A) delivered to a built-in spectrometer. The light that is scattered by the chromatin can be easily extracted from the organoid nuclei using white light reflectance images (Fig. 1B) and processed using techniques described in detail elsewhere [4]. We monitored live human induced pluripotent stem cell (hiPSC) organoids undergoing differentiation over a period of 16 days. This 16-day period begins with human embryoid bodies (hEBs), followed by a four-stage protocol that forms definitive endoderm, foregut endoderm, hepatoblasts, and mature hepatocytes, respectively. Over the sixteen-day period, we observed a steady decrease in open chromatin, which was in excellent agreement with transmission electron microscopy measurements carried out on the same organoids.

Another important consideration when monitoring organoid maturation and drug response is changes to various biochemical concentrations [7]. However, most of the traditional techniques that are used require sacrificing of the sample and cannot be used to monitor biochemical changes in live organoids. An alternative is label-free Raman spectroscopy, a technique that is sensitive to the vibrational modes of molecules, and as a result, can detect a wide range of molecules (including carbohydrates, lipids, proteins, nucleic acids, specific metabolites, drugs, and minerals) at the single organoid level. We collected Raman spectra from live differentiating hiPSC organoids over a period of 16 days, with this 16-day period corresponding to the four stages of the protocol described above. The

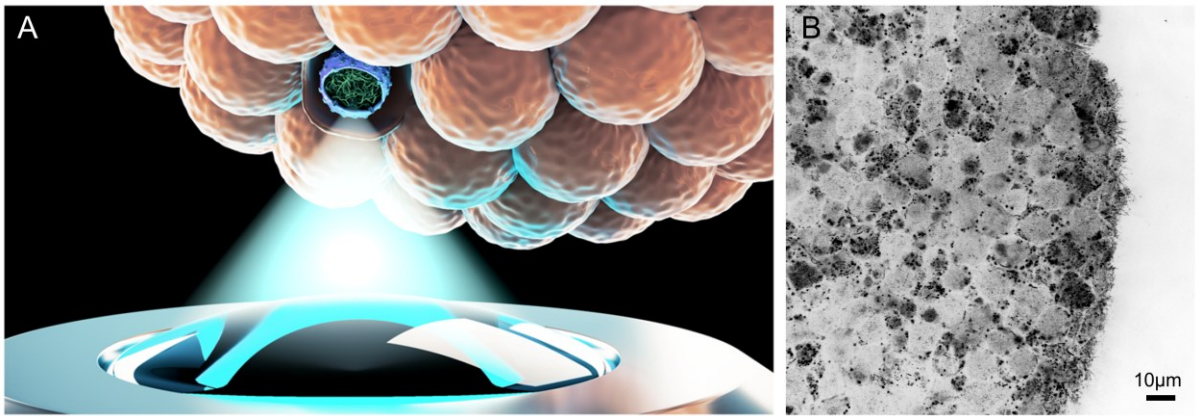

Fig. 1. (A) Rendering of a chromatin measurement in the nucleus of an organoid. Light scattered from within the confocal volume is delivered to a spectrometer. (B) White light reflectance image (presented with inverted colors) provides a high contrast image of the organoid, with both the edge of the organoid and nuclei boundary easily identifiable. Panel A adapted from [4].

Raman spectroscopy system used a low-magnification Raman microscopy setup with a 785-nm excitation wavelength laser delivering excitation light to the organoids. To analyze the spectra, we employed a spectral unmixing approach. This allowed us to quantitatively evaluate the concentrations of various biochemicals by reconstructing the Raman spectra of organoids from the base spectra of biochemicals whose concentrations are known to be changing during differentiation. With this approach, we monitored changes in albumin, α-fetoprotein (AFP), glycogen, and hepatic lipids. The observed changes were in good agreement with published studies carried out using traditional techniques.

3. Conclusion

Organoids can replicate much of the complexity of organs, providing a more physiologically relevant platform for advancing biological research and drug discovery programs. However, monitoring the maturation of organoids and their response to various drug candidates is difficult due to their inherent heterogeneity. A promising approach to help overcome these difficulties is the combination of chromatin-sensitive CLASS microscopy and biochemical–sensitive Raman spectroscopy. These complementary techniques can be used to profile live organoids in an efficient, non-destructive, and straightforward manner.

4. References

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