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EdU-labelling: Raman-based click-free detection of endothelial cell proliferation

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Endothelial dysfunction (ED) has been linked to the development of many lifestyle diseases such as atherosclerosis, diabetes or hypertension 1 and the phenotype of endothelial dysfunction is often linked to altered capacity of endothelial regeneration unable to repair vascular dysfunction. Therefore, new methods to study endothelial cell proliferation are desirable.
Raman imaging of endothelial cells provides valuable information on the chemical changes associated with disease development 2. Although Raman spectroscopy allows label-free detection of key biological compounds i.e. proteins, lipids and nucleic acids, the use of Raman reporters has been shown to improve sensitivity and selectivity of subcellular organelles imaging and tracking specific molecules 3. One example of this is the detection of alkyne-tagged 5-ethynyl-2′-deoxyuridine (EdU).
EdU is a thymidine analogue that incorporates into newly synthesized DNA during replication. EdU subsequent detection, after its cupper-catalysed cycloaddition reaction "Click Chemistry" with a fluorescent azide, has been used in fluorescence imaging to follow DNA synthesis of proliferating cells 4,5. Due to the alkyne tag, EdU could be easily detected in the Raman spectra of cells as it gives a Raman band at ca. 2122 cm-1 in the "silent region" where there are no interferences in the signal from other biological compounds 6.
This study aims to assess the Raman imaging of endothelial cells (ECs) DNA by following the characteristic alkyne band at ca. 2122 cm-1 in EdU-labelled ECs and to investigate the changes in ECs proliferation using EdU as an indicator of DNA replication in in vitro and ex vivo conditions. We studied the effects of cycloheximide (CHX), a protein synthesis inhibitor that inhibits DNA replication, 7 on EdU-tagged ECs. EdU-labelling has been shown to improve Raman imaging of the nuclei of ECs from different origins. Furthermore, CHX pretreated cells showed a significantly lower EdU signal from their nuclei. It was clear that cell proliferation was inhibited after CHX treatment and this effect could be detected using Raman EdU-labelling approach, which was not detectable using label-free Raman imaging. When fluorescent detection of Alexa Fluor® 488-tagged EdU was used as a reference method, the intensity of the signal from Alexa Fluor as well as the percentage of EdU positive cells were decreased in CHX pre-treated cells compared to the control. The Raman imaging results are in agreement with the fluorescence method, moreover, the Raman-based approach omits cell permeabilization step and allows EdU detection in live cells and without the need of additional dyes.
In conclusion, the results of this study show the feasibility of click-free detection of EdU-labelled DNA in endothelial cells using Raman spectroscopy, and this method is being further used and optimized to detect endothelial proliferation in the isolated vessel ex vivo.

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