Investigation of Carbon Nanotube Biodegradation by Human Neutrophils using Raman Spectroscopy

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The main routes of exposure of living cells to nanomaterials are environmental, industrial, laboratory or clinical. Traditionally the distribution, degradation and excretion of drugs or other substances in organisms are studied using blood or urine samples. One of the key expectations in nanomedicine is that in the near future it will produce new drugs incorporating nanocarriers for use in the human body. It is therefore essential not only to understand the destiny of these nanocarriers in the organism but also to develop a reliable method for evaluating the uptake and degradation of these nanomaterials, many of which undergo poor or no biodegradation.

Raman spectroscopy was employed to assess the biodegradation of single walled carbon nanotubes (SWCNT) by myloperoxidase (MPO) in human neutrophils. Using SWCNT as a model of such poorly-degraded nanoparticles and Raman spectroscopy, it is demonstrated here that this non-invasive, non-destructive, spectroscopic method enables clear discrimination between intact and degraded SWCNT in biological systems. The Raman spectra of SWCNT have a number of distinct bands making it possible to measure their integrity with particular emphasis on any change in the relative intensities of the D to G band. Since neutrophils (or polymorphonuclear leucocytes) represent the first line of innate immune defence, we exposed them to SWCNT at different time points (0 hours, 2 hours, 4 hours and 8 hours). The key metabolic component of these cells, utilised in neutralising pathogens, is the enzyme called myloperoxidase. Subsequently the structural integrity of these SWCNT was characterised after the various time-points using Raman.

At 0 hours the Raman spectra of the SWCNT indicated the presence of intact, pristine nanotubes whereas from 2 hours on the typical characteristic Raman spectra of the SWCNT showed signs of degradation. The intensity of the disorder-induced D-band increased whereas the tangential mode G-band decreased thus suggesting oxidation of the graphene side-wall. This degradation increased with incubation time and the Raman spectra taken at the 8 hour time-point indicated the highest level of degradation. All of the spectra measured were then analysed using principle component analysis to objectively separate, on a score plot, the pristine and biodegraded SWCNT.

In summary we have developed a new technological approach to measure the integrity of SWCNT in human neutrophils. This non-invasive method can be applied to studies on a range of nanomaterials interacting with human cells.