ESRF	**Experiment title:** Towards a better understanding of peri-implant inflammation: Intra-cellularisation and chemical modification of titanium nano-particles by human neutrophils.	**Experiment number**: LS-2312
Beamline: ID-21	**Date of experiment**: from: 28/2/2014 to: 3/3/2014	**Date of report**:
Shifts: 12	**Local contact(s)**: Hiram Castillo-Michel	*Received at ESRF:*

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Scientific background:

We aimed to study the intra-cellularisation and potential chemical modification of titanium (Ti) nanoparticles (NP) by human neutrophils using X-ray absorption spectroscopy. In previous SR experiments and with complementary techniques probing human tissues from around Ti implants we have demonstrated distributions of Ti particles (including on the nanoscale) which are present in several species (metallic, oxide, peroxy-complex). In the absence of significant wear processes, mechanically assisted crevice corrosion is implicated and through which the generation of Ti-oxide NPs can be explained. What is less obvious is why Ti-oxide as Rutile is predominant over Ti-oxide as Anatase in situations where implants have been present for longer periods of time and why we observe Ti-peroxo-complexes in these tissues. Neutrophils are the most abundant professional phagocyte and are found in large numbers in chronically inflamed Ti implant sites. We have conducted extensive *in-vitro* studies to demonstrate that Ti-NPs are potent stimulators of extracellular and intracellular neutrophil Reactive Oxygen Species (ROS) generation (superoxide, H_2O_2 etc). However the magnitude of both intra- or extra-cellular ROS production is sensitive to Ti speciation and to particle size which both seem to influence the phagocytic capacity of the cell. Most interestingly preliminary XANES measurements suggest modification of NP speciation inside the neutrophil themselves (**Fig 1**). The findings of this study will add significantly to a broad multidisciplinary research programme targeted at understanding peri-implant inflammatory mediators.

Aims:

The specific aims were to:

1. identify species/size specificity in the cellular internalisation of Ti NPs
2. to identify chemical modification of the internalised Ti NPs using nano/micro-XANES in human neutrophils.

Experiment:

Human neutophils were isolated from heparinised blood of healthy volunteers using a Percoll gradient and erthrocyte lysis. Cells were exposed to size graded high purity Ti NPs (as anatase and rutile at concentrations of 2 or 10 ppm and between 30-60 or 100- 200 nm in size) dispersed in physiological media for fixed time points prior to retrieval, washing and preservation for X-ray measurements. Cells were mounted on SiN windows, freeze dried and XRF mapping was performed using the maximum spatial resolution of ID21 with an incident energy of 5.1 KeV. XANES measurements were acquired and spectra correlated with the control stimuli.

Results:

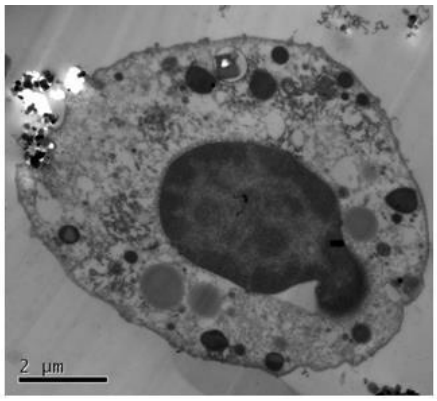

Figure 1: TEM of membrane association of neutrophils with Ti NPs and internalisation into membrane bound phagosomes (exposure 10 ppm Ti as Anatase).

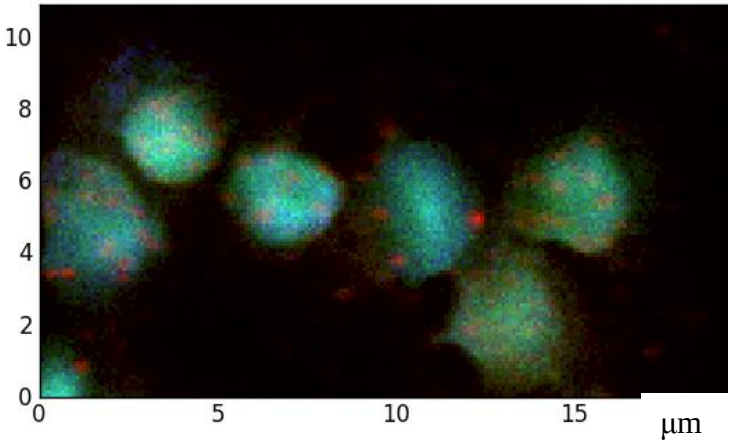

Figure 2: XRF map (P-green, S-blue, Ti –red) showing isolated neutrophils with associated Ti. There is some evidence of degranulation with the morphology of cells towards the lower right of the image. Ti appears discrete and membrane bound, but certainly cellularly associated.

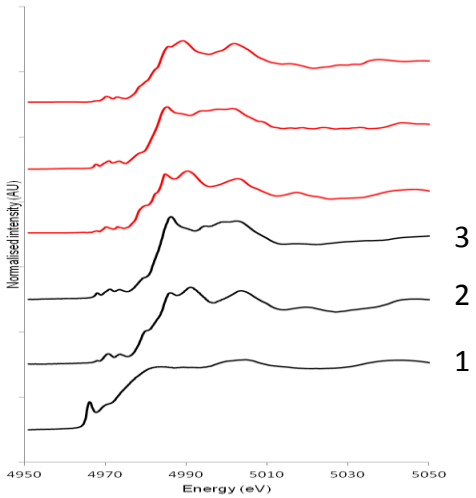

Figure 3: Micro-XANES of experimental spectra taken from identified Ti 'hotspots' from XRF mapping of human neutrophils (red) compared with spectra of the control stimuli anatase (3), rutile (2) and Ti foil (1) as a metallic reference. In general spectra from cellular measurements were noisy and it is suggested that this was due to instability of the beam position moving through the energy range or indeed beam damage of the biological tissue. Analysis of the spectra demonstrated no obvious chemical modification in the oxide species of Ti associated with neutrophils for any of the experimental stimuli used. However, it must be stated that the quality of the XANES spectra were largely poor and this is thought to be largely due to the small size of the NP agglomerations being probed.

Further work:

We are currently working to identify smaple preparation and measurements strategies to improve the XAS measurements on these biological samples. We have generated a large quantity of data that has allowed us to statistically report the percentage of isolated cells interacting with the Ti NP stimulus and this will be used to support forthcoming outputs relating biological assays and in particular stimulation of ROS release to NP interactions.