ESRF	Experiment title: Investigating distribution and speciation of micro- and nanoparticles originating from tattoo colorants in human skin and lymph node tissues	Experiment number : MD974
Beamline :	Date of experiment:	Date of report:
ID21/ ID16	8-12/Apr 2016 and 01-05/July 2016	1. Sep. 2016
Shifts: 6 (ID21), 12 (ID16B)	Local contact(s): Bernhard Hesse (ID21), Julie Villanova (ID16B)	
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Background:

The ever-increasing prevalence of tattoos gives rise to safety concerns regarding the distribution of pigment particles inside the human body. In this study, biopsies of human skin and regional lymph nodes were used to address biokinetics, in terms of general tissue load with tattoo-related elements, by means of synchrotron X-ray fluorescence techniques at the micro (ID21) and nano (ID16B) scales. Together with additional mass spectrometric analyses, we provide evidence for the simultaneous transport of organic pigments and inorganic titanium dioxide from skin to lymph nodes. Mean particle sizes of primarily rutile titanium dioxide were ~200 nm. The organic pigments displayed a polydisperse size distribution with the smallest potentially being below ~ 100 nm and an absence of the micro-meter ranged particles in the lymph nodes.

<u>ID21</u>:

Beam size was about 0.7(vertical) x 0.4 μ m (horizontal). We have scanned a total of 9 skin and 5 lymph node tissue sections on ID21. We have collected fast maps at 3-10 μ m resolution to identify ROI's for subsiquent mapping at 1 μ m resolution. From all sections we have collected Ti and Cr K-edge XANES spectra. The prelimanry data suggest:

- Particle agglomeration up to two-digit micrometer sizes, primary size below the beamlines resolution
- Ti in rutile form in most cases, in few cases presence of both rutile and anatase (Fig. 1 C)
- Cr mixture of metal and Cr III in all samples

<u>ID16B:</u>

Beam size was about 0.1(vertical) x 0.1 μ m (horizontal). We have scanned a total of 8 skin and 4 lymph node tissue sections on ID16B which were previously characterized on ID21. We have collected maps at 1 μ m resolution to identify ROI's for subsiquent mapping at 50 nm steps. In 9 out of 12 samples we identified the

presence of Ni which was subsiquently specified by means of Ni K-edge spectroscopy in 7 samples were the intensity of Ni was high enough. The prelimanry data suggest:

- Particle sizes (preliminary data based on only one samlple so far (Dubelloy- skin)):
 - \circ Cr: 100 300 nm
 - Cu: 100 200 nm
 - Fe: 150 300 nm
 - $\circ \quad Mn{:}\; 100-300\;nm$
 - o Ni: 150 300 nm
 - Ti:150 250 nm
- Ni spectroscopy:

No metallic Ni was found in neither of the samples. The obtained spectra suggest the presence of soluble Ni which is therefore able to trigger contact allergies in sensitized individuals.

Please note that the beamsize was about 100 nm, therefore particles smaller than 100 nm will not be assessed by their real size.

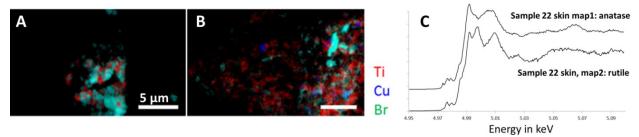


Figure 1: Distribution of TiO_2 and brominated copper phthalocyanine green in human skin (A) and lymph node (B). C shows XANES spectra originating of two different regions of the same sample. The shown maps in A and B have been collected at 17.5 keV with pink beam setup, all other maps have been collected at 9.5 keV to allow for subsequent spectroscopy of the Ni edge.

Summary:

The beamtime on D21 and ID16B went technically extremely well and the results are very promissing. Parts of the data are already part of a ongoing publication *. We expect to publish the final outcome of our analysis of the experiment MD974 during the first half of 2017 in an high impact journal.

*Schreiver Ines et al., Localization, characterization and local effects of tattoo pigment particles in human skin and lymph nodes; ongoing submission