	Experiment title: Distribution and fate of CdSe and InP quantum dots in primary human keratinocytes and human skin explants	**Experiment number**: MD902
Beamline:	**Date of experiment**: from: 05/10/2015 to: 09/10/2015	**Date of report**: 01/12/2015
Shifts:	**Local contact(s)**: Rémi Tucoulou	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Marie Carriere (*), Giulia Veronesi (*)		

Report:

Introduction:

Quantum dots (QDs) are semi-conductor nanocrystals with exceptional optical properties; for this reason they are used in a variety of high tech products, as well as for medical imaging and diagnostic. The QDs that are presently used in the highest number of commercial products are CdSe QDs; emerging alternative QDs are based on InP. Their intensive use poses the question of their impact on human health, which is largely dependent on their dissolution. Moreover, since these nanocrystals are used in electronic devices such as TV or mobile phone screens, human exposure would rather occur via skin contact.
The aim of the proposal was to evaluate the intracellular distribution of InP and CdSe quantum dots when accumulated inside skin cells, using µXRF imaging, and to monitor their intracellular dissolution via local µ-X-ray absorption spectrocopy analysis of Cd and In-rich areas.

Materials and methods:

For this experiment, we grew cells on appropriate sample holders and exposed them to CdSe or InP QDs. Our initial objective was to use primary keratinocytes extracted from skins of human donors, as well as human skin explants, but for safety reasons we were not authorized to use such biological models and we rather used the HaCat human skin cell line. The used QDs were commercial CdSe samples (ThermoFisher), provided as a suspension in an organic solvent, that were further coated with penicillamin that permitted their transfer to aqueous solution via ligand exchange. We also used home-made InP quantum dots that consisted of a core composed of InZnP, either coated with a shell of ZnSeS (core-shell QDs) or not (core-only). These InP QDs were then transferred to aqueous solution via ligand exchange using penicillamin, as for CdSe QDs. HaCat cells were exposed to core-shell or core-only InP QDs or CdSe QDs at sublethal concentrations for 24 h, then cryofixed and freeze-dried using the ID16 lyophilizer. They were transferred to the ID16B stage and directly imaged using the pink beam at 29.6 keV.

Results:

Two types of images were acquired, i.e., large low resolution images (250 x 250 nm² images), focusing on one cell, and small high resolution images (50 x 50 nm²) using either 100 ms or 500 ms per point. Images were acquired on control sample (cells not expoxed to QDs), or on cells exposed to CdSe QDs, or to core-only InP Qds or to core-shell QDs.

We successfully imaged the cellular matrix thanks to imaging of P and S distributions, and some In, Zn or Cd-rich regions were observed inside the cells (see, as an example, Figure 1). High magnification images showed that these Cd or In-rich regions showed a punctated morphology, which would suggest that InP and CdSe QDs accumulated as agglomerates inside cells. In InP-exposed cells, when In distribution and Zn distribution were maped in red and blue, respectively, we obtained images showing purple regions, proving the colocalization of both metals that would suggest that the QDs had not dissolved intracellularly. Unfortunately, we could not analyse these regions via µXAS due to lack of time. These In and Cd-rich regions were distributed throughout the cells, without any specific location.

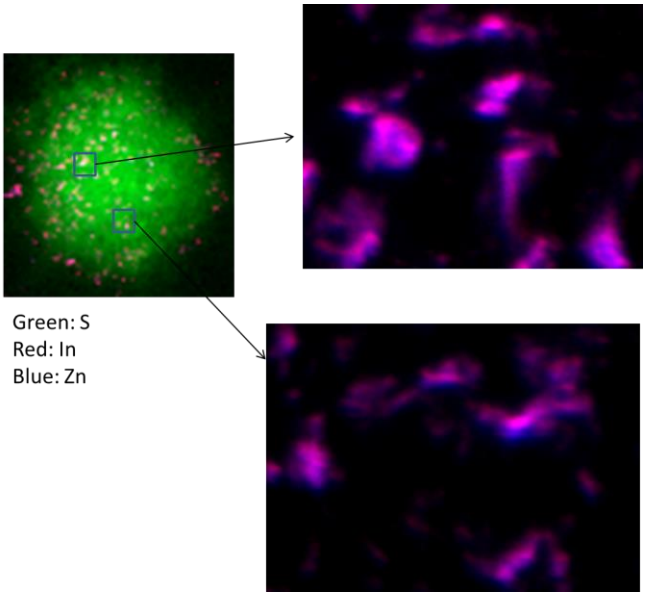

Figure 1. µXRF image of an HaCat cell exposed to core-shell InP QDs for 24 h, showing the cellular matrix (imaged via S elemental map), In accumulated inside cells (represented in red) and Zn accumulated inside cells (represented in blue). Since the In and Zn-rich regions appear pruple, it proves colocalization of In and Zn as intracellular precipitates.

Conclusions:

This experiment allowed successful imaging of CdSe and InP QDs inside a human keratinocyte cell line. These QDs did not show any specific distribution inside cells, an seemed not to have dissolved after their intracellular accumulation.