Quantum Dots in Transmission Electron Microscopy

Valuable Tools to Study Biomedical Processes

Quantum dots (Q-dots) label structures that can be observed in live cell mode by fluorescence microscopy. As light microscopy images alone cannot decipher the final localization of the signal unambiguously, ultrastructural resolution is needed. Transmission Electron Microscopy (TEM) elucidates the target cell and cellular organelles reached by Q-dots.

Introduction

Q-dots are small inorganic nanocrystals emitting fluorescence at a specific wavelength depending on their size. Because of their small size and robustness they are considered as therapeutical vehicle for biomedical treatment [1]. Beside their fluorescence properties Q-dots are also electron-dense and so detectable by TEM. Thus Q-dots became a favorable tool for Correlative Light Electron Microscopy (CLEM) [2]. We will exemplify here both applications and discuss imaging and interpretation. As for any EM-sample preparation the workflow needs to be adapted and modified to the specific requirement of the individual sample.

As a model system for biomedical application and potential therapy we studied vascular injury and inflammation in the mouse cremaster muscle upon application of Q-dots into the blood circulation or the interstitial space.

As an example for CLEM we performed post-embedding immuno-labeling of a cellular structure by applying Q-dot conjugated secondary antibodies. The labeling pattern was recorded by fluorescence and light microscopy and the area of interest carefully prepared by consecutive sectioning and ultrastructural analysis.

Instrumentation and Experimental Set-Up
In several studies we used the inflamed cremaster tissue as in vivo model to investigate the potential of Q-dots to interfere with the inflammatory cascade. The microvascular distribution, as well as cellular interaction of Q-dots were investigated using intravital microscopy (fig. 1a).

The CLEM approach was achieved in cells, cultivated on a gridded cover slip for orientation. By using Q-dots for pre-embedding, labeled areas of interest can be selected by their fluorescence signal at the light microscope and the correlative area is subsequently processed for ultrathin sectioning after epon-embedding (fig. 3).

TEM-imaging of nanoparticles challenge the investigator who needs to discriminate a real signal from background noise. It is therefore recommendable to standardize the imaging procedure first by analyzing pure particle solution dispersed on a formvar coated grid (fig. 1b). The contrast of the nanoparticle itself is not always sufficient to identify Q-dots on the phosphor-screen; therefore a light sensitive CCD-camera is mandatory. While studying the real sample it helps to examine un-stained and stained, consecutive sections in parallel, this is of particular importance, since the counterstain reagents (uranyl-acetate, lead) resemble the size and density of Q-dots (fig. 2a, b, fig. 2d, e). It is also crucial to inspect non-treated samples to envision the endogenous background, which needs to be subtracted while interpreting the signal (fig. 2c, e).

**Results on Real and False Signals (Signal-to-Noise Ratio)**

Q-dots are valuable tools to resolve the ultrastructural environment in the cellular context recorded by light microscopy as fluorescence spots (fig. 1a, fig. 3e).

For example, intravenous injected carboxyl Q-dots in the cremaster are effectively transported via the endothelial layer and become subsequently enriched in
perivascular cells in the interstitium. Whereas direct application into the muscle (intra-scrotal) leads to an enormous accumulation of Q-dots in the endocytic/phagocytic system of cells in close vicinity to the vasculature (fig. 1c). With support of the electron microscope the uptake mechanism of carboxy Q-dots could be elucidated as caveolae-mediated [3]. Macrophages were identified as the main cell type reached by this type of Q-dots applied intravenous or intra-scrotal (fig. 1c). This reflects of course the physiological task of macrophages, clearance of small sized particles, which is an important point and should be taken into account if biomedical therapy is desired. While inspecting macrophages in non-injected animals one can also observe that non-treated animals are exposed to nanoparticles of unclear origin (fig 2c, f).

Interestingly the surface charge of the Q-dots strongly determines their fate in the organism. This became particularly evident when investigating negatively charged amino Q-dots in post ischemic muscle [4]. Amino Q-dots were found associated to microparticles, which themselves are bio-functional vehicles in the circulating blood [5].

Applied in vitro, as CLEM approach, Q-dots are suitable for bridging the light microscopy imaging to ultrastructural resolution. The overlay of light and electron microscopy signal clearly resolves the labeled organelle (fig. 3).

**Conclusions**

The pre-embedding approach with Q-dot- conjugated antibodies has simplified the CLEM approach as the light microscopy signal and electron microscopy signal results from one labeling component.

The systematic application of Q-dots in vivo allows time-lapse imaging of their distribution and cellular interactions. Remarkably when Q-dots are applied as chemically modified reagents they interfere with different pathways of inflammatory processes. Beside these exciting possibilities for in vivo recording one should also reflect the risk of nanoparticles applied to the organism. They might not be cytotoxic at first application but become hepatotoxic, because they cannot be cleared from the body easily [6]. Further encapsulation and modification of nanoparticles are needed to minimize their health risk [7]. As a consequence extensive studies, including TEM analysis should be applied before biomedical application.

**References**

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