Anionic Ultrasmall Quantum Dots for Long-term Intravital Vascular Imaging

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Optical-based intravital imaging of vasculature is an emerging modality for studying vascular structure, function and angiogenesis. Although many probes and contrast agents have been developed for imaging of microvasculature, issues related to stability and bioavailability have yet to be overcome. A multivalent and biologically compatible platform for the development of fluorescent imaging agents is still needed for long-term *in vivo* imaging. As a biological imaging agent, quantum dots (QDs) possess a number of distinct advantages including high quantum yield, enhanced photostability, narrow emission band, and long fluorescence lifetime. However, until now, all previous studies [1-2] only reported *in vivo* QDs distribution in tumors at the organ-level or by *ex vivo* sections. The limited resolution and penetration depth of conventional *in vivo* imaging techniques make it difficult to obtain a clear real-time dynamic of QDs at the cellular level *in vivo*.

In this study, we developed mercaptosuccinic acid (MSA) capped cadmium telluride/cadmium sulfide (CdTe/CdS) ultrasmall QDs (3.5 nm in diameter). Then we evaluated the QD concentrations in mouse plasma and these QDs displayed a bi-exponential decay in systemic blood circulation with long half-lives of 7.67 h ($t_{1/2\Phi\#177;}$) and 2363.19 h ($t_{1/2\beta}$). Short and long-term intravital images of mouse livers were collected from 30 seconds after injection using **multiphoton microscopy** (MPM) coupled with **fluorescence lifetime imaging** (FLIM) [3]. As shown in **Figure 1B, F** and **J**, vasculature of normal and fibrotic liver tissue and hepatocellular carcinoma are clearly visualised by highly intense fluorescence signals of QDs after 30 min of intravenous injection. Unlike highly regulated and controlled sinusoids in normal liver tissues, tumor tissues exhibited disordered and tortuous vasculature, mainly due to tumor aniogenesis and lack of blood perfusion. Neovessels were found to be connected with larger vessels. These images further confirmed that QDs were retained in the vasculature and were not taken up by hepatocytes or tumor cells (**Figure 1A, E** and **I**). As shown in **Figure 2**, the fluorescence of QDs reached maximum at 2 min post-injection, and was evenly distributed in blood vessels. The QDs labelled vascular images were obvious up to 36 hours after injection, but QD signals gradually disappeared at 48 hours post-injection. In addition to fluorescent signals, FLIM images further clearly confirmed the QDs labelled vasculature of normal and fibrotic liver tissue and hepatocellular carcinoma, where orange color represents longer lifetime of QDs (≥ 10 ns, **Figure 1D, H** and **L**) and green color (< 2.5 ns, **Figure 1C, G** and **K**) represents shorter lifetimes of autofluorescence from hepatocytes or tumor cells.

In summary, we show that anionic ultrasmall QDs can be used to *in vivo* visualise the vasculature in normal liver, fibrotic liver, and liver tumor in mice with cellular resolution up to 36 hours after intravenous injection. MPM coupled with FLIM has great potential as emerging tools in diagnosis and monitoring of treatment response by intravital vascular imaging.

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References

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Figures

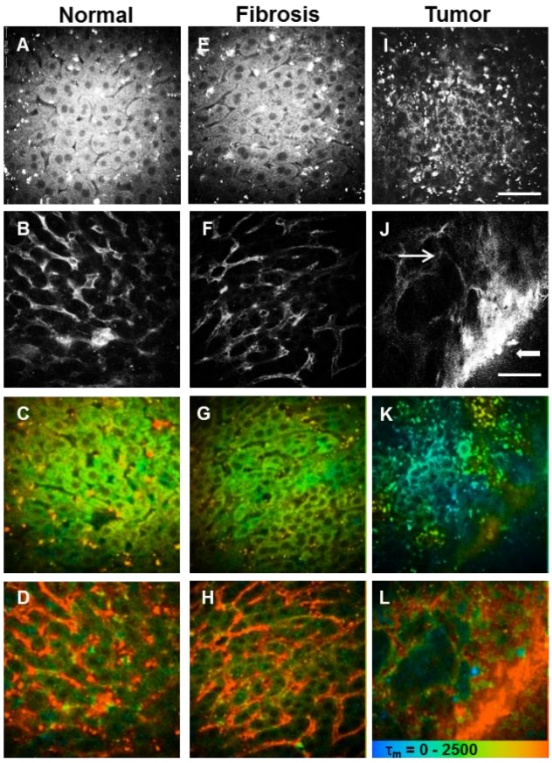

Figure 1 MPM-FLIM images of normal, fibrosis liver tissues and hepatocellular carcinoma. Narrow arrow indicates disordered and tortuous vasculature of hepatocellular carcinoma with inefficient blood perfusion and filled arrow indicates larger vessels connected to the tumor vasculature. (**A, E** and **I**) Fluorescence intensity image recorded at $\lambda_{Exc}/\lambda_{Em}$: 740/350 to 450 nm; (**B, F** and **J**) Fluorescence intensity image recorded at $\lambda_{Exc}/\lambda_{Em}$: 900/515 to 620 nm; (**C, G** and **K**) Pseudocolored FLIM image (τ_m: 0-2500 ps; blue-green-red) recorded at $\lambda_{Exc}/\lambda_{Em}$: 740/350 to 450 nm or (**D, H** and **L**) recorded at $\lambda_{Exc}/\lambda_{Em}$: 900/515 to 620 nm. (Scale bar: 20 μm).

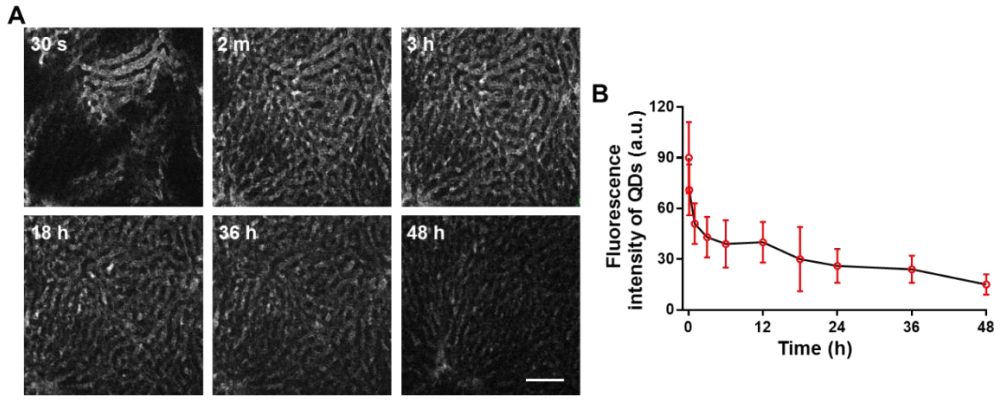

Figure 2 Time profile of QDs intensity in vasculature of normal liver after bolus injection. (**A**) Real-time hepatic disposition of QDs, (Scale bar: 40 μm); (**B**) Time profile of QDs intensity per pixel. The symbols represent measured data and the line represents the connecting curve. (n = 3).