OCT guided microinjections for mouse embryonic research

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ABSTRACT

Optical coherence tomography (OCT) is gaining popularity as live imaging tool for embryonic research in animal models. Recently we have demonstrated that OCT can be used for live imaging of cultured early mouse embryos (E7.5-E10) as well as later stage mouse embryos in utero (E12.5 to the end of gestation). Targeted delivery of signaling molecules, drugs, and cells is a powerful approach to study normal and abnormal development, and image guidance is highly important for such manipulations. Here we demonstrate that OCT can be used to guide microinjections of gold nanoshell suspensions in live mouse embryos. This approach can potentially be used for variety of applications such as guided injections of contrast agents, signaling molecules, pharmacological agents, cell transplantation and extraction, as well as other image-guided micromanipulations. Our studies also reveal novel potential for gold nanoshells in embryonic research.

Keywords: Optical Coherence Tomography (OCT), embryonic development, mouse, microinjection, gold nanoshells.

1. INTRODUCTION

Embryonic studies in mouse models are highly valuable for understanding the mechanisms of normal development and congenital defects. A variety of mouse mutants associated with human defects are available already, and a number of large-scale, international, genome-wide screens for new or advanced mouse models of human disease will yield hundreds of lines over the next few years, raising an urgent need for better research tools (references). Optical coherence tomography (OCT) is increasingly gaining popularity as a live embryo imaging tool in mice [1, 2] and other animal models [3-5]. Previously we have shown that OCT can be used for live visualization of the entire externalized cultured mouse embryos until E10.5 [1, 2]. The resolution of this imaging modality is sufficient to image individual circulating blood cells and small groups of cells [6]. At later stages (E12.5 through the remainder of embryogenesis), live mouse embryos can be imaged with OCT through the uterine wall following externalization of the uterine horn, allowing repeated imaging of the same living embryos from E12.5 to birth to characterize temporal changes in organ development [7].

While live OCT imaging of mouse embryos has been demonstrated and is currently used to study developmental mechanisms, an ability to perform precise image guided micromanipulations at the same spatial resolution as the imaging would be highly beneficial. This would provide a possibility for targeted delivery of signaling molecules, drugs, cell transplantation and extraction and to combine delivery with imaging to analyse the effects. Here we report OCT guided microinjection of the gold nanoshell suspension into the vasculature of the E9.5 live cultured mouse embryo. Gold nanoshells are considered to be nontoxic [8, 9] and their light scattering capability can be tuned [10], which makes them an attractive contrast agent for biomedical

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applications. This work demonstrates the possibility of OCT image guided micromanipulations and reveals the potential of gold nanoshells in embryonic research.

2. MATERIALS AND METHODS

Imaging system. The imaging was performed with a home-built Spectral Domain OCT (Figure 1). The laser source of the SDOCT was a MICRA 5 Titanium:Sapphire laser (Coherent Inc.) with a central wavelength of 800 nm and a bandwidth of ~ 100 nm. A-scan acquisition rate of the system was set at 50 kH for the experiments. The resolution of the system was measured as 5 um (in air) in axial and 6 um in lateral directions.

Animal Manipulations. CD-1 mouse timed matings were set overnight and checked for vaginal plugs daily. The day of the vaginal plug was counted as E0.5. The embryos were dissected out of the uterus with the yolk sac intact at E8.5 or E9.5 in culture medium containing 89% DMEM/F12, 1% Pen-strep solution, and 10% FBS (Invitrogen). The dissection station was maintained at 37 ^{o}C. After the dissection, embryos were transferred to a humidified incubator maintained at 37 ^{o}C and 5% CO_2. To immobilize the embryos for the injection, a 2% agarose gel was prepared with the same culture media. The gel was made approximately 1 to 2 mm thick in a small culture dish. Prior to transferring the embryo into the gel, a small well of about the same size as an embryo was made in the gel and the dish was filled with the culture medium.

Gold-Silica Nanoshells. Gold-silica shell-core nanoshells with near-infrared (NIR) extinction were synthesized via a four-step process as described previously [11, 12]. The diameter of the silica cores was approximately 120 nm, and the total diameter was approximately 150 nm. Nanoshell size was verified with transmission electron microscopy (JEOL 1230), and optical properties were evaluated using UV-Vis spectroscopy (Varian Cary 50 Bio). To prevent particle flocculation upon injection in the mouse embryo, nanoshell surfaces were passivated with thiol-terminated poly(ethylene glycol) (PEG-SH, Laysan Bio, MW = 5000 kDa).

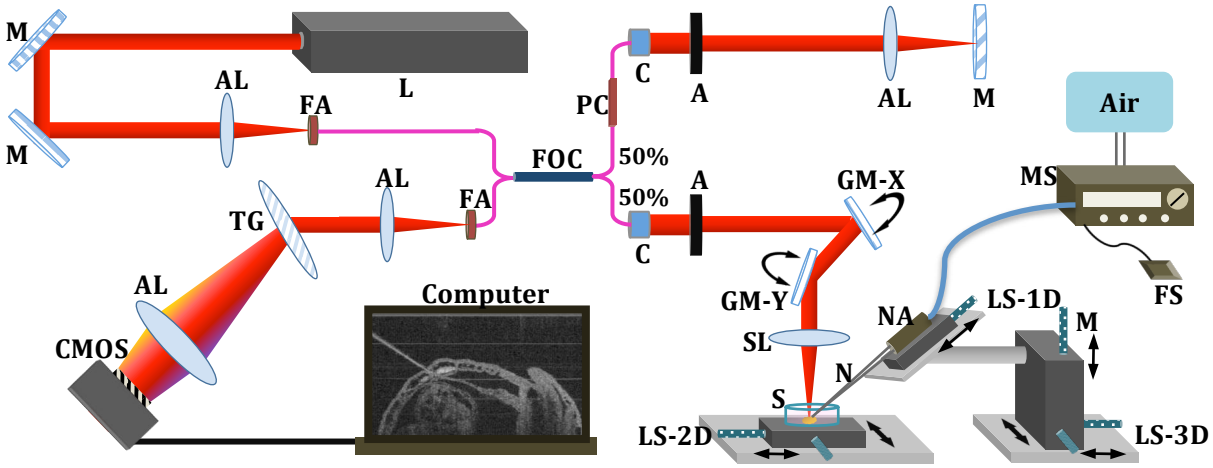

Figure 1. Experimental setup for OCT guided injections. L – laser, M – mirror, AL – achromatic lens, FA – fiber adaptor, TG – transmission grating, FOC – fiber optics coupler, PC – polarization controller, C – collimator, A – aperture, GM-X – galvo mirror-X, GM-Y – galvo mirror-Y, SL – scan lens, S – sample, N – needle, NA – needle adaptor, M – Micrometer, LS-1D – 1-D linear stage, LS-2D – 2-D linear stage, LS-3D – 3-D linear stage, MS – microinjection system, FS – foot switch.

3. RESULTS AND DISCUSSION

For image-guided injections, the OCT scanning head, a dish with an embryo, and the microinjection needle were placed on three independent translational stages (Figure 1). The imaging plane was aligned along the injection needle prior to the experiment. Such configuration allowed us to manipulate the embryo position, choose the injection site, guide needle to the desired location, and visualize the injection.

Figure 2 shows an example of the OCT guided microinjection in the vascular system of the cultured mouse embryo. The representative frames were taken from the time lapse acquired at 50 fps. The tip of the microinjection needle was inserted in the lumen of the blood vessel (Figure 2A). When the needle was positioned properly, the suspension of the gold nanoshells was microinjected in the circulation (Figure 2B). We used gold-silica nanoshells approximately 150 nm in diameter with a peak extinction at 800 nm. The OCT signal enhancement due to the nanoshells was clearly visible in the lumen of the vessel during the injection. After the injection, the needle was removed from the vessel, while the gold nanoshells were taken into the circulation (Figure 2C). The needle can be retracted from the vessel without producing significant damage to the vessel or leakage of the material.

These results demonstrate that the presented approach can be successfully used to perform guided injections in the vasculature of the cultured embryos, providing a useful tool for manipulation of the live mouse embryo. This tool can potentially be used for variety of applications such as guided injections of contrast agents, signaling molecules, pharmacological agents, cell transplantation and extraction, as well as other image-guided micromanipulations. Our results also reveal novel the potential for gold nanoshells in embryonic research. Gold nanoshells provide significant OCT signal enhancement and can be used as a contrast agent. Potentially they can be targeted to specific tissues by antibody conjugation.

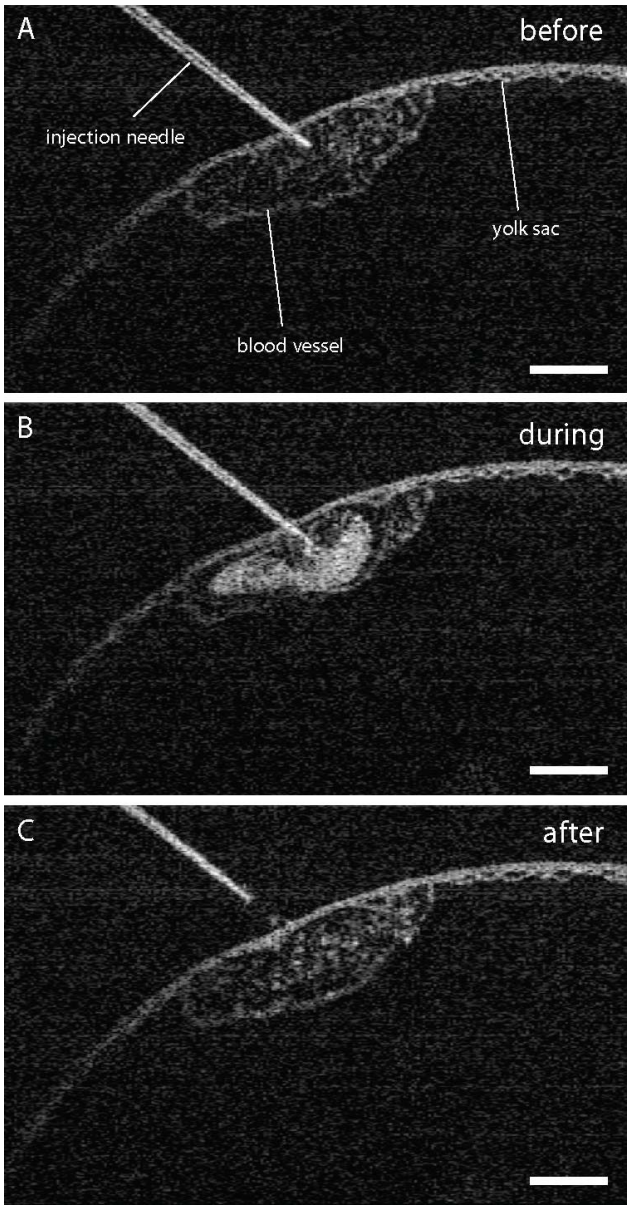

Figure 2. OCT guided microinjection of gold nanoshells into the embryonic blood circulation system. A series of frames from a time lapse of an OCT guided injection into the blood vessel of the E9.5 cultured mouse embryo. The scale bar corresponds to 200 μm.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

[1] I. V. Larina, N. Sudheendran, M. Ghosn, J. Jiang, A. Cable, K. V. Larin, and M. E. Dickinson, "Live imaging of blood flow in mammalian embryos using Doppler swept source optical coherence tomography," Journal of Biomedical Optics 13 (6), 0605061-3, (2008).

[2] K. V. Larin, I. V. Larina, M. Liebling, and M. E. Dickinson, "Live Imaging of Early Developmental Processes in Mammalian Embryos with Optical Coherence Tomography," Journal of Innovative Optical Health Sciences 2 (3), 253-9, (2009).

[3] M. W. Jenkins, L. Peterson, S. Gu, M. Gargesha, D. L. Wilson, M. Watanabe, and A. M. Rollins, "Measuring hemodynamics in the developing heart tube with four-dimensional gated Doppler optical coherence tomography," J Biomed Opt 15 (6), 066022, (2010).

[4] S. A. Boppart, G. J. Tearney, B. E. Bouma, J. F. Southern, M. E. Brezinski, and J. G. Fujimoto, "Noninvasive assessment of the developing Xenopus cardiovascular system using optical coherence tomography," Proc Natl Acad Sci U S A 94 (9), 4256-61, (1997).

[5] I. V. Larina, K. Furushima, M. E. Dickinson, R. R. Behringer, and K. V. Larin, "Live imaging of rat embryos with Doppler swept-source optical coherence tomography," J Biomed Opt 14 (5), 050506, (2009).

[6] I. V. Larina, S. Ivers, S. Syed, M. E. Dickinson, and K. V. Larin, "Hemodynamic measurements from individual blood cells in early mammalian embryos with Doppler swept source OCT," Optics Letters 34 (7), 986-988, (2009).

[7] S. H. Syed, K. V. Larin, M. E. Dickinson, and I. V. Larina, "Optical coherence tomography for high-resolution imaging of mouse development in utero," J Biomed Opt 16 (4), 046004, (2011).

[8] M. J. Connor EE, Gole A, Murphy CJ, Wyatt MD., "Gold nanoparticles are taken up by human cells but do not cause acute cytotoxicity," Small 1 (3), 325-327, (2005).

[9] S. C. Gad, K. L. Sharp, C. Montgomery, J. D. Payne, and G. P. Goodrich, "Evaluation of the toxicity of intravenous delivery of auroshell particles (gold-silica nanoshells)," Int J Toxicol 31 (6), 584-94, (2012).

[10] R. Weissleder and V. Ntziachristos, "Shedding light onto live molecular targets," Nat Med 9 (1), 123-8, (2003).

[11] S.J. Oldenburg, R.D. Averitt, S.L. Westcott, and N. J. Halas, "Nanoengineering of optical resonances," Chemical Physics Letters 288, 243–247, (1998).

[12] A. M. Gobin, M. H. Lee, N. J. Halas, W. D. James, R. A. Drezek, and J. L. West, "Near-infrared resonant nanoshells for combined optical imaging and photothermal cancer therapy," Nano Lett 7 (7), 1929-34, (2007).