Programmable Nucleic Acid Polymerization for Nanoparticle-Mediated Imaging of the ECM

Niancao Chen, Shihui Li, Mark R. Battig, Yong Wang.
Department of Biomedical Engineering, Pennsylvania State University, 16802

Statement of Purpose: The ability to characterize the procedure of tissue remodeling *in vivo* is important to the understanding of disease development or therapeutic efficacy. Thus, it is very critical to develop tissue-imaging agents and methods to acquire images with high sensitivity and high contrast.[1] A variety of nanoparticle-based imaging agents have been developed in an attempt to obtain high intensity of imaging signals in the target tissue rather than in the non-target one.[2] However, normal tissues in the human body, e.g., liver and spleen, can nonspecifically entrap a significant amount of nanoparticles; therefore it's still challenging to achieve a high ratio of target to non-target signal. To approach this problem, we herein demonstrated a new concept, in which nanoparticles function as molecular anchors to accumulate small DNA-based molecular beacons locally in the target tissue and program DNA-based molecular amplification on their surfaces. When the DNA molecules carry imaging agents, the imaging signal in the target tissue could be greatly amplified.

Methods: The programmed DNA polymerization was examined in both aqueous solution and on the surface of substrates using gel electrophoresis, surface plasma resonance (SPR), flow cytometry and fluorescence imaging. The bifunctional nanoparticles were functionalized with anti-collagen I antibody and DNA initiator (DI) via biotin-streptavidin interaction. The antibody can mediate the recognition between the nanoparticles and the target ECM; the DI can initiate structural changes and sequential hybridizing of two metastable DNA molecular beacons (DM_1 and DM_2) into a DNA polymer (**Figure 1a**). To study the programmed DNA polymerization for nanoparticle-mediated ECM imaging, the cultured monolayer of human dermal fibroblast cells was treated with bifunctional nanoparticles and FAM-labeled DM_1 and DM_2 mixture sequentially. The images were obtained using a confocal microscope. Moreover, FAM-labeled DM_1 and TAMRA-labeled DM_2 were used to examine DNA polymerization on nanoparticles for potential multiple-modality imaging.

Results: The results of gel electrophoresis and SPR showed a significant increase of the molecular weights of DNA molecules after DNA polymerization. We also observed a strong increase of fluorescence of nanoparticles with the aids of flow cytometry and fluorescence imaging. All these results demonstrated that the DNA polymerization was successful both in aqueous solution and on the surface of nanoparticles. In addition, the images of green fluorescence demonstrated that the programmed DNA polymerization for nanoparticle-mediated ECM imaging (*i.e.* group 1 in **Figure 1b**) was successful. DNA molecular beacons were also labeled with two different fluorophores. The results showed that the nanoparticles acquired two distinct fluorescence signals after the DNA polymerization.

Conclusions: The bifunctional nanoparticles can play dual roles of recognizing target ECM components and inducing signal amplification in the ECM via DNA polymerization. Therefore, nanoparticle-programmed DNA polymerization holds great potential for *in vivo* tissue imaging. Moreover, it is promising to achieve single- or multiple-modality imaging with signal amplification by using two DNA monomers with identical or different imaging agents. This concept is currently under investigation with animal models.

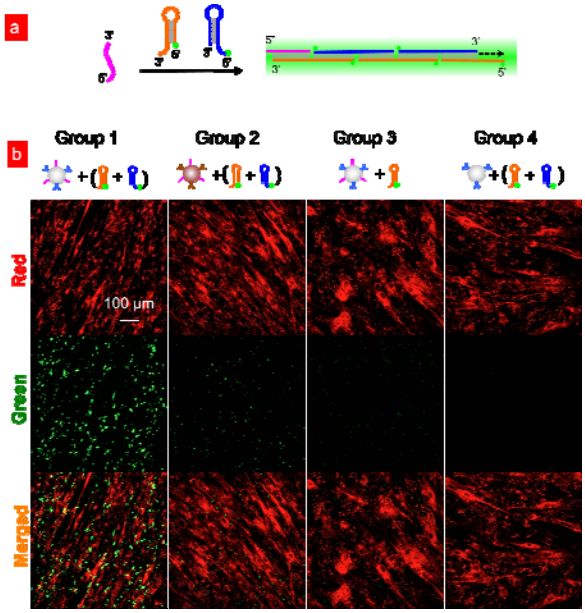

Figure 1. (a) Scheme of the programmed DNA polymerization (the purple strand indicates DI, the brown and blue strands indicate DM_1 and DM_2, the green dot indicates imaging agent). (b) Confocal microscopy images of the ECM. The ECMs were sequentially treated with nanoparticles and the solution of DM_1 or the mixture of DM_1 and DM_2. Both DM_1 and DM_2 were labeled with FAM (green). For clear legibility, the living cells were stained with a Vybrant-cell staining solution (red). The polymerization lasted one hour at 37 °C.

References:
1. Shin, SJ. *Integr. Biol.* 2013; 5: 29-42.
2. Chan, WC. *Curr. Opin. Biotechnol.* 2002; 13: 40-46.

Acknowledgement: This work was supported in part by the US National Science Foundation (DMR-1241461/1322332) and the UConn Research Foundation. The authors declare no competing financial interests.