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Monitoring the topical delivery of ultrasmall gold nanoparticles using optical coherence tomography

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Abstract

Background: Optical coherence tomography (OCT) is a promising imaging modality for skin cancer diagnosis. However, this capability has been hindered by the low contrast between normal and neoplastic tissue. To overcome this limitation, gold nanoparticles have been used to enhance the contrast in OCT images and are topically administered to reduce the risk of systematic side effects associated with intravenous injection. To ensure efficient penetration and distribution of the nanoparticles, an enhanced delivery strategy is required. In this porcine study, we assessed two delivery methods: (a) using dimethyl sulfoxide (DMSO) and (b) via sonophoresis.

Materials and Methods: The gold nanoparticles were topically applied on pig skin before evaluating DMSO and sonophoresis as penetration enhancers in topical administration. The OCT images were taken from the same locations to monitor signal change.

Conclusion: The combination of DMSO and sonophoresis is an effective method to enhance the penetration and diffusion rate of nanoparticles during topical administration.

Significance: Topical administration of nanoparticles is advantageous in dermatological applications. Nevertheless, efficient topical delivery remains a challenge. DMSO and sonophoresis can be used as two effective approaches to enhance topical delivery of nanoparticles.

Keywords

gold nanoparticles; optical coherence tomography; sonophoresis; topical delivery

1 | INTRODUCTION

In dermatology, the early detection of pre-malignant and malignant lesions is crucial for disease detection and treatment.^{1–3} Currently, the excisional biopsy is the gold standard for

skin cancer diagnosis.⁴ However, since excisional biopsy is invasive, susceptible to sampling errors, and can cause patient discomfort, the development of imaging-based non-invasive diagnostic techniques with high a sensitivity and specificity is gaining attention.^{5,6}

Among imaging modalities, optical coherence tomography (OCT) has shown a great promise for skin cancer detection.^{7,8} OCT is a non-invasive optical imaging technique that enables micrometer-scale imaging⁹ and is particularly promising for dermatological applications because it provides real-time 2-D and 3-D cross-sectional images of skin subsurface structures.^{10,11} OCT is based on the principle of Michelson interferometry and uses low coherent near-infrared light at the wavelength of about 1300 nm.¹² However, the primary limitation for using OCT in early cancer detection is the low contrast between physiologic and neoplastic tissue. In response, OCT contrast agents including nanoparticles have been developed and explored.¹³ Various nanoparticles were designed and invented to possess many beneficial characteristics, such as transport vehicle for drug delivery,^{14,15} contrast agent for imaging,^{16,17} and photosensitizer for photodynamic therapy.^{18,19} Specifically, gold nanoparticles (Au NPs) have been used as contrast agents for a variety of applications.^{20,21} Au NPs have numerous advantages as in vivo OCT contrast agents including biocompatibility and surface functionalization with additional biomolecules.^{22–24} Au NPs are effective OCT contrast agent because of their flexible optical properties which be tailored by modifying the size and shape of nanoparticles.^{25–27} To ensure sufficient OCT signal from Au NPs and to improve sensitivity, it is crucial to deliver the Au NPs efficiently, evenly, and specifically to the targeted area.²⁸ For dermatological applications, therefore, a topical administration is preferable.

In our study, a commercialized OCT was used to monitor the diffusion of sub-resolution ultrasmall 10 nm gold nanoparticles by topically applying the particles on pig ear skin. We hypothesized that DMSO and ultrasonic force would improve the penetration and distribution of Au NPs and thus enhance topical delivery. The intensity of OCT images over 40 minutes at different depths of skin was analyzed and compared.

2 | METHODS

2.1 | OCT system setup

A commercialized high-resolution swept-source OCT (SS-OCT) scanner with handheld probe from Michelson Diagnostic™ was used (Figure 1A). The imaging system is FDA-approved for dermatological study, has a central wavelength of 1305 nm, and a bandwidth of 30 nm. The A-line rate is 10 kHz, and the frame rate is 6 fps. The B-scan images obtained with this OCT system have a size of 6 × 2 mm with lateral and axial resolutions of 7.5 and 10 μm, respectively. The penetration depth of the system was measured as 1.5 mm in healthy human skin. The capabilities of this system for skin cancer detection have been described previously.^{29,30}

2.2 | Dimethyl sulfoxide (DMSO)

Dimethyl sulphoxides (DMSO) is one of the most prevalent penetration enhancers. It is a powerful aprotic solvent which hydrogen bonds with itself rather than water. DMSO helps

facilitate diffusion through the stratum corneum, activates the formation of deposits in the dermis, and facilitates transport into the local blood vessels. The stratum corneum acts as the major barrier to any active substance delivered from the surface designated for deeper layers of the skin. The four factors that influence penetration of any given material through any membrane are as follows: (a) membrane's diffusion coefficient,³¹ (b) the agent's concentration in the vehicle,³² (c) the partition coefficient between the membrane and the vehicle,³³ and (d) the membrane thickness.³⁴ Penetration enhancers are intended to influence all or some of these factors without causing permanent structural or chemical modification of the membrane. Since modifying the thickness of the membrane is not practical, most penetration agents, including DMSO, are used to reversibly modify principals 1–3. DMSO increases diffusion through the stratum corneum by disruption of the barrier function in the stratum corneum layer, aprotic interactions with intercellular lipids, and reversible distortion of lipid head groups which generate a more permeable packing arrangement. DMSO also contributes to partitioning by forming solvent microenvironments within the tissue. Lastly, DMSO has a solubilizing effect on less soluble agents, improving penetration by delivering a higher concentration to the membrane barrier.^{35,36}

2.3 | Sonophoresis

Sonophoresis is a method that disrupts the stratum corneum lipid bilayer by facilitating the delivery of molecules with low molecular weight through the skin. It increases the fluidity of lipids and thus intracellular drug permeation. Sonophoresis is typically used in the form of low-frequency pressure waves < ~100 kHz. Ultrasound creates microbubbles which collapse at the surface of stratum corneum and produces many shock waves or acoustic microjets rendering the skin permeable.^{37,38} Other mechanisms have been reported, including thermal effects³⁹ and radiation pressures⁴⁰ that further add to the sonophoretic permeation enhancement.

2.4 | Nanoparticles delivery on ex vivo pig skin

Pig ear skin was used due to its similarity to the human skin with particular emphasis on the epidermal structure, thickness, and the epidermal/dermal junction.⁴¹ The pig ear was purchased from a local slaughter house, the skin was removed, and the hair was carefully shaved prior to the experiments. 2 μ L of 10 nm Au NPs (NanoHybrids, Inc) was topically administered on top of the pig skin. DMSO was used as a chemical penetration enhancer for transdermal drug delivery, and the pig skin was treated with it (#472301, Sigma Aldrich Inc) prior to Au NP application. 200 μ L DMSO was applied and rubbed on pig skin for 3–5 minutes before adding the nanoparticles on top of that. Next, the sonophoretic method was used. After the Au NPs topically dropped on the skin, 1 MHz sonicator (Sonifier 250, Branson) applied ultrasonic force by placing the sonicator tip about 2 mm and 45 degree away from the Au NPs drop surface (Figure 1A, iii and iv).

2.5 | Image acquisition and processing

The OCT probe was stably fixed using a clamp to acquire images from the same area of pig skin, and diffusion of the nanoparticles was monitored using OCT. The first set of OCT images were taken when the nanoparticles disappeared on the skin surface and were completely diffused inside the skin. The second set of OCT images were taken 40 minutes

later. The intensity of these two sets of OCT images was analyzed over 13 different depths and analyzed using Matlab.

The OCT images were analyzed over 13 different depths and at two different time points. In terms of depth, the images started from the top surface of the skin to 0.565 mm (130 pixels) downwards with 0.044 mm (10 pixels) steps. The two time points were the disappearance of the Au NPs on skin surface, and 40 minutes after that.

To compensate for the effect of laser energy drop over time, the OCT signal was calibrated at 40 minutes to the time point which we collected the first set of images. Six areas of pig skin without DMSO treatment and Au NPs administration were used for compensation. The mean OCT signal intensity over 13 different depths was calculated at those two different time points. The calibration factor for each depth was calculated as the intensity at 40 minutes divided by the intensity at initial time point. This calibration factor was used for all the data at 40 minutes to correct the laser energy drop. For each depth, a region of interest (ROI) was chosen with the size 400 by 10 pixels. The mean intensity of the ROI was calculated and normalized as OCT signal intensity. The standard deviation was calculated and normalized as the homogeneity of the ROI.

3 | RESULTS

The OCT monitored the Au NPs topical diffusion in real-time. The results in Figure 1B clearly show the Au NP drop on the pig skin surface. The first set of OCT images ($t = 0$ minute) were acquired when the Au NPs were completely diffused inside the skin surface. The OCT probe was fixed during the entire imaging period to image the same location after 40 minutes ($t = 40$ minutes). The skin structure can be clearly seen up to 0.565 mm (130 pixels) below the top surface. Therefore, we analyzed the OCT signal intensity of pig skin at 13 different depths with the steps of 0.044 mm (10 pixels). Each depth is considered as one ROI with a width of 400 pixels. The intensity of each ROI was expressed as the mean intensity value of all the pixels. The mean intensity values were plotted over 13 different depths for two different time points $t = 0$ minute (blue line) and $t = 40$ minutes (red line) (Figure 2A,B).

The OCT signal decreased over depth for both two time points (Figure 2A,B). In Figure 2A, i, without adding any Au NPs, the OCT signal dropped over time for the same location, especially at depths 0.05 mm to 0.25 mm. Thus, the signal at 40 minutes for each depth was calibrated to reach the OCT signal at initial time point ($t = 0$ minute) (Figure 2A, ii) and the calibration factors were calculated and later applied to the corresponding depth.

First, the spontaneous diffusion of Au NPs on skin surface was tested without any enhancers. 2 μ L Au NP was added on skin surface. After the calibration, the OCT signal at 40 minutes was still lower than the signal at $t = 0$ minute (Figure 2B, i). On the OCT images, it is important to note that the intensity dropped at 40 minutes (Figure 1C, ii) compared to the OCT image at 0 minute (Figure 1C, i). Next, two non-invasive approaches, namely DMSO and sonophoresis, were tested. DMSO was applied and rubbed on skin surface before adding the Au NPs. After the calibration, the OCT signal at 40 minutes did not

decrease significantly (Figure 2B, ii). The OCT signal at depth 0.044 mm and 0.088 mm dropped at 40 minutes compared to the signal at $t = 0$ minute. For the following depth below 0.088 mm, however, the OCT signal at 40 minutes is higher than the signal at $t = 0$ minute. Then, the two methods were combined. DMSO was rubbed on the skin surface first, the Au NPs were dropped on top of skin, and then sonophoresis was administered until the Au NPs were completely diffused inside the skin. Following calibration, the OCT signal at depth 0.044 mm and at 40 minutes is slightly lower than the signal at $t = 0$ minute. For the depths below 0.044 mm, the OCT signal at 40 minutes is much higher than the signal at $t = 0$ minute. On the OCT images, the intensity of the skin increased at 40 minutes (Figure 1C, vi) compared to the OCT image at 0 minute (Figure 1C, v). The use of sonophoresis on the DMSO-treated skin greatly enhanced the results (Table 1).

Additionally, the homogeneity of skin with and without enhancing methods was compared. In Figure 2C, i, the homogeneity of skin when using Au NPs alone decreased over time. However, the homogeneity of skin was almost unchanged over time (Figure 2C, ii, iii) when the DMSO and sonophoresis were applied as enhancers.

4 | DISCUSSION

Despite a growing number of current studies involving nanomedicine in dermatology and the cosmetic industry, the penetration and distribution of nanoparticles inside the skin requires further investigation. Additionally, efficient delivery of Au NPs is crucial to obtain a sufficient signal in a variety of applications. Many imaging-based techniques including light microscopy, confocal microscopy, and electron transmission microscopy have been used to evaluate the penetration and distribution of nanoparticles. However, these techniques are limited in penetration depth and field of view.

Optical coherence tomography can be used to visualize nanoparticle diffusion real-time inside the skin. The OCT signal for the same location was observed to drop over approximately 40 minutes because of reduced OCT laser energy and dehydration of the skin. Since skin is excised and exposed in the air during surgery, and the imaging period lasts 40 minutes, this dehydration can result in an OCT signal drop. Six different areas from the skin without DMSO and Au NPs were used in order to compensate for this effect. The calibration factors were calculated for each depth and were applied to the corresponding depth in our analyses.

It was found that the addition of DMSO and sonophoresis increased the diffusion rate and penetration of Au NPs in skin. Over time, the OCT signal showed an increase after using DMSO and sonophoresis, whereas the Au NPs alone showed an OCT signal decrease. These findings indicate that the combination of DMSO and sonophoresis may be an effective method for nanoparticle delivery. Additionally, the topical administration can minimize the concerns of toxicity, drug overdose, and frequent drug administration. Advances in nanotechnology such as these have consistently expanded the field of nanomedicine in various applications.

5 | CONCLUSION

The present study demonstrates an effective approach to improve the topical delivery of gold nanoparticles and enhance OCT image contrast. The combination of DMSO and sonophoresis was demonstrated to be an effective method to improve the penetration and diffusion rate of gold nanoparticles in skin. These findings may provide a new paradigm for enhancing in vivo OCT images and, ultimately, improve the capability of OCT in the early diagnosis of cancer.

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REFERENCES

1. Jerant AF, Johnson JT, Sheridan CD, Caffrey TJ. Early detection and treatment of skin cancer. *Am Fam Physician*. 2000;62(2):357–368. [PubMed: 10929700]
2. Friedman RJ, Rigel DS, Kopf AW. Early detection of malignant melanoma: the role of physician examination and self-examination of the skin. *CA Cancer J Clin*. 1985;35(3):130–151. [PubMed: 3921200]
3. Friedman LC, Bruce S, Weinberg AD, Cooper HP, Yen AH, Hill M. Early detection of skin cancer: racial/ethnic differences in behaviors and attitudes. *J Cancer Educ*. 1994;9(2):105–110. [PubMed: 7917894]
4. Zuber TJ. Punch biopsy of the skin. *Am Fam Physician*. 2002;65(6):1155–1158, 1161–2, 1164. [PubMed: 11925094]
5. Pickett H. Shave and punch biopsy for skin lesions. *Am Fam Physician*. 2011;84(9):995. [PubMed: 22046939]
6. Fujimoto JG, Pitris C, Boppart SA, Brezinski ME. Optical coherence tomography: an emerging technology for biomedical imaging and optical biopsy. *Neoplasia*. 2000;2(1–2):9–25. [PubMed: 10933065]
7. Welzel JJSR. Optical coherence tomography in dermatology: a review. *Skin Res Technol*. 2001;7(1):1–9. [PubMed: 11301634]
8. Pierce MC, Strasswimmer J, Park BH, Cense B, de Boer JF. Advances in optical coherence tomography imaging for dermatology. *J Invest Dermatol*. 2004;123(3):458–463. [PubMed: 15304083]
9. Gambichler T, Moussa G, Sand M, Sand D, Altmeyer P, Hoffmann K. Applications of optical coherence tomography in dermatology. *J Dermatol Sci*. 2005;40(2):85–94. [PubMed: 16139481]
10. Huang D, Swanson EA, Lin CP, et al. Optical coherence tomography. *Science*. 1991;254(5035):1178–1181. [PubMed: 1957169]
11. Fercher AF, Drexler W, Hitzinger CK, et al. Optical coherence tomography-principles and applications. *Opt Lett*. 2003;28(2):239.
12. Leitgeb R, Hitzinger C, Fercher A. Performance of fourier domain vs. time domain optical coherence tomography. *Opt Express*. 2003;11(8):889–894. [PubMed: 19461802]
13. Lee TM, Oldenburg AL, Sitafulwalla S, et al. Engineered microsphere contrast agents for optical coherence tomography. *Opt Lett*. 2003;28(17):1546–1548. [PubMed: 12956374]
14. Cho K, Wang X, Nie S, Chen ZG, Shin DM. Therapeutic nanoparticles for drug delivery in cancer. *Clin Cancer Res*. 2008;14(5):1310–1316. [PubMed: 18316549]

15. De Jong WH, Borm PJ. Drug delivery and nanoparticles: applications and hazards. *Int J Nanomedicine*. 2008;3(2):133. [PubMed: 18686775]
16. Hainfeld J, Slatkin DN, Focella TM, Smilowitz HM. Gold nanoparticles: a new X-ray contrast agent. *Br J Radiol*. 2006;79(939):248–253. [PubMed: 16498039]
17. Kim EH, Lee HS, Kwak BK, Kim B-K. Synthesis of ferrofluid with magnetic nanoparticles by sonochemical method for MRI contrast agent. *J Magn Magn Mater*. 2005;289:328–330.
18. Roy I, Ohulchanskyy TY, Pudavar HE, et al. Ceramic-based nanoparticles entrapping water-insoluble photosensitizing anticancer drugs: a novel drug-carrier system for photodynamic therapy. *J Am Chem Soc*. 2003;125(26):7860–7865. [PubMed: 12823004]
19. Chatterjee DK, Fong LS, Zhang Y. Nanoparticles in photodynamic therapy: an emerging paradigm. *Adv Drug Deliv Rev*. 2008;60(15):1627–1637. [PubMed: 18930086]
20. Daniel M-C, Astruc D. Gold nanoparticles: assembly, supramolecular chemistry, quantum-size-related properties, and applications toward biology, catalysis, and nanotechnology. *Chem Rev*. 2004;104(1):293–346. [PubMed: 14719978]
21. Sperling RA, Rivera Gil P, Zhang F, Zanella M, Parak WJ. Biological applications of gold nanoparticles. *Chem Soc Rev*. 2008;37(9):1896–1908. [PubMed: 18762838]
22. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. *Adv Drug Deliv Rev*. 2008;60(11):1307–1315. [PubMed: 18555555]
23. Ghosh SK, Pal T. Interparticle coupling effect on the surface plasmon resonance of gold nanoparticles: from theory to applications. *Chem Rev*. 2007;107(11):4797–4862. [PubMed: 17999554]
24. Hemmatian Z, Jalilian E, Lee S, et al. Delivery of cargo with a bioelectronic trigger. *ACS Appl Mater Interfaces*. 2018;10(26):21782–21787. [PubMed: 29905062]
25. Chen J, Saeki F, Wiley BJ, et al. Gold nanocages: bioconjugation and their potential use as optical imaging contrast agents. *Nano Lett*. 2005;5(3):473–477. [PubMed: 15755097]
26. Zhang Q, Iwakuma N, Sharma P, et al. Gold nanoparticles as a contrast agent for in vivo tumor imaging with photoacoustic tomography. *Nanotechnology*. 2009;20(39):395102.
27. Jain S, Hirst DG, O'sullivan JM. Gold nanoparticles as novel agents for cancer therapy. *Br J Radiol*. 2012;85(1010):101–113. [PubMed: 22010024]
28. Sokolov K, Follen M, Aaron J, et al. Real-time vital optical imaging of precancer using anti-epidermal growth factor receptor antibodies conjugated to gold nanoparticles. *Cancer Res*. 2003;63(9):1999–2004. [PubMed: 12727808]
29. Turani Z, Fatemizadeh E, Blumetti T, et al. Optical radiomic signatures derived from optical coherence tomography images improve identification of melanoma. *Can Res*. 2019;79(8):2021–2030.
30. Xu Q, Adabi S, Clayton A, Daveluy S, Mehregan D, Nasirivanaki M. Swept-source optical coherence tomography-supervised biopsy. *Dermatol Surg*. 2018;44(6):768–775. [PubMed: 29381540]
31. Leekumjorn S, Sum AK. Molecular study of the diffusional process of DMSO in double lipid bilayers. *Biochimica et Biophysica*. 2006;1758(11):1751–1758.
32. Castro CA, Hogan JB, Benson KA, Shehata CW, Landauer MR. Behavioral effects of vehicles: DMSO, ethanol, Tween-20, Tween-80, and emulphor-620. *Pharmacol Biochem Behav*. 1995;50(4):521–526. [PubMed: 7617697]
33. Pelletier JS, Stewart KP, Capriotti K, Capriotti JA. Rosacea blepharoconjunctivitis treated with a novel preparation of dilute povidone iodine and dimethylsulfoxide: a case report and review of the literature. *Ophthalmol Ther*. 2015;4(2):143–150. [PubMed: 26525679]
34. Shinoda W. Permeability across lipid membranes. *Biochimica et Biophysica*. 2016;1858(10):2254–2265.
35. Stoughton R, Fritsch W. Influence of dimethylsulfoxide (DMSO): on human percutaneous absorption. *Arch Dermatol*. 1964;90(5):512–517. [PubMed: 14200645]
36. Capriotti K, Capriotti JA. Dimethyl sulfoxide: history, chemistry, and clinical utility in dermatology. *J Clin Aesthet Dermatol*. 2012;5(9):24–26.

37. Smith NB. Perspectives on transdermal ultrasound mediated drug delivery. *Int J Nanomed.* 2007;2(4):585.
38. Tezel A, Mitragotri S. Interactions of inertial cavitation bubbles with stratum corneum lipid bilayers during low-frequency sonophoresis. *Biophys J.* 2003;85(6):3502–3512. [PubMed: 14645045]
39. Ng K-Y. Enhancing transdermal drug transport with low-frequency ultrasound. *Drug Discovery Today.* 2004;9(21):913.
40. Prausnitz MR. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev.* 2004;56(5):581–587. [PubMed: 15019747]
41. Kratkiewicz K, Manwar R, Rajabi-Estarabadi A, et al. Photoacoustic/ultrasound/optical coherence tomography evaluation of melanoma lesion and healthy skin in a Swine model. *Sensors.* 2019;19(12):2815.

FIGURE 1.

Optical coherence tomography (OCT) imaging setup and OCT images of pig ear skin. A, The OCT machine from Michelson Diagnostic TM (i) and imaging setup for Au NPs topical delivery with (iii, iv) and without (ii) sonophoresis; B, the OCT images of skin with a drop of Au NPs on top; C, the OCT images of skin after applying Au NPs at 0 min (i, iii, v) and 40 min (ii, iv, vi) for no DMSO treatment and sonophoresis (i, ii), using DMSO treatment only (iii, iv), and using DMSO treatment and sonophoresis (v, vi). Au NPs, gold nanoparticles; HSL, high speed scanning laser

FIGURE 2.

The optical coherence tomography (OCT) signal change over depth at different time points. A, the OCT signal at two different time points before (i) and after (ii) calibration; B, the OCT signal change over depth for no DMSO treatment and sonophoresis (i), using DMSO treatment only (ii), and using DMSO treatment and sonophoresis (iii); C, the homogeneity of pig skin at two different time points for no DMSO treatment and sonophoresis (i), using DMSO treatment only (ii), and using DMSO treatment and sonophoresis (iii)

TABLE 1

The nanoparticle diffusion rate over time. NP alone diffused at 0.1144, while adding DMSO increased the relative rate to 0.5612 and adding both DMSO and sonophoresis (US) increased the diffusion rate to 1.0628

NP alone	NP + DMSO	NP + DMSO + US
0.1144	0.5612	1.0628
NP = nanoparticles	DMSO = dimethyl sulfoxide	US = ultrasound