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Iron-oxide nanoparticles powered micro optical coherence tomography for *in situ* imaging the penetration and swelling of polymeric microneedles in skin

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Abstract

In recent years, polymeric microneedles (MNs) have attracted keen interests among researchers for their applicability in transdermal drug delivery and interstitial skin fluid (ISF) extraction. When design and characterize such devices, it is critical to monitor their real-time *in vitro* and *in vivo* performances to optimize the desired effects, yet most of the existing methods are incapable of such functions. To address this unmet need, we develop a real-time non-invasive imaging methodology by integrating iron oxide (Fe_3O_4) nanoparticles into polymeric MNs to enhance image contrast for micro-optical coherence tomography (μOCT) imaging. Using the Fe_3O_4 integrated polystyrene-block-poly (acrylic acid) (PS-b-PAA) MNs as an example, we evaluate the influences of Fe_3O_4 concentrations on contrast enhancement in μOCT imaging and visualize the real-time swelling process of polymeric MNs in biological samples for the first time. Our results show that a concentration of ~4-5 wt% Fe_3O_4 nanoparticles not only helps achieve the best contrast to noise ratio (CNR) in μOCT imaging, which is 10 times higher than that without Fe_3O_4 nanoparticles in air and hydrogel, but also enables the MNs' real-time profile changes to be observed clearly in their swelling process in skin tissues. Based on such findings, we utilize the optimized concentration of Fe_3O_4 nanoparticles to further quantitatively study the swelling kinetics of PS-b-PAA MNs in agarose hydrogel and fresh skin tissues, which lasts ~20s and ~30-35s, respectively. The suitability of such a methodology for enhancing μOCT imaging would greatly facilitate the development and clinical translation of MN-based medical technologies.

Introduction

Microneedles (MNs) are arrays of needle-like protrusions with micrometre (μm) dimensions. They can penetrate through the skin's protective barrier (i.e., stratum corneum) without contacting the underlying nerve endings in the dermis, resulting in minimal pain and inflammation. Since their introduction by Prausnitz *et al.*, MNs have been attracting extensive interests and been widely utilized for transdermal drug and vaccine delivery.¹⁻³ In recent years, with reports dictating that dermal interstitial fluid (ISF) contains biomarkers of interests for systemic and dermatological analysis, MNs have been further explored as potential tools for extraction of ISF in the development of diagnostic sensors.⁴⁻⁷ To meet the needs of various applications, MNs have been fabricated using a wide range of materials, including silicon, ceramics, metals such as titanium and stainless steel, non-degradable polymers such as a copolymer of methylvinylether and maleic anhydride (PMVE/MA), biodegradable polymers such as poly-lactic-co-glycolic acid (PLGA) and natural biopolymers such as hyaluronic acid.^{1, 8}

When design and develop the MNs, it is often necessary to visualize and characterize the MNs' penetration depth, swellability, and/or dissolution in skin for determining the key parameters such as drug release profiles and efficacy of ISF extraction. Currently, researchers rely mainly on *ex-vivo* techniques such as biopsy and histological staining.⁹ However, these traditional methods are cumbersome and time-consuming requiring the excision and processing of tissue specimens. In addition, the results derived with those destructive methods are often inaccurate as skin tissue is elastic and usually do not retain the microstructure generated during MN intrusion. Therefore, imaging technologies that provide real-time non-invasive, *in-situ* or *in vivo* images of the MNs' penetration and swelling in skin are highly desired.

Optical coherence tomography (OCT) is a well-established powerful non-invasive imaging technique that uses reflections of light waves to generate cross-sectional images with axial resolutions of 1-15 μm . It typically employs near-infrared light, which allows the imaging of tissue as deep as several millimetres.⁹ The development of micro-optical coherence tomography (μOCT), a new form of OCT, further pushes the resolution down to 1–2 μm allowing the visualization of a single cell.¹⁰ Currently, OCT is not only a routine imaging tool for eye disease diagnoses,¹¹ but has also shown promise in other clinical research areas such as skin,¹² airway function evaluation,¹³ cardiology and gastroenterology.^{10, 14-16}

In light of these applications, there have been growing interests in applying OCT to image MNs behaviours in transdermal drug delivery.¹⁷⁻²⁰ Donnelly *et al.* adopted a swept-source OCT (SS-OCT) to study the influences of MNs' geometry and application force on their penetration characteristics and dissolution effects in porcine skin *in vitro*.¹⁷ Through utilizing a classical OCT system, Enfield *et al.* reported the first real-time OCT imaging of MN's skin penetration *in vivo*, meanwhile, the MNs geometry, penetration depth, together with the skin healing characteristics and micropore closure rate were also successfully measured.¹⁸ Similarly, with lab-built OCT systems, Liu *et al.* assessed the

dissolving process of silk MNs in different carriers for the first time,¹⁹ while Tsai *et al.* investigated the temporal effects of both morphological and vascular changes in mouse skin induced by MNs' insertion.²⁰ However, it is noteworthy that the images obtained in all these studies have only limited contrast. Specifically, although false colours can be applied onto the obtained OCT images to differentiate between the MNs and the skin layers with post-processing software,^{17, 19-20} the real-time visualization of MNs' dissolution effects and their real-time temporal behaviours in their swelling process still lacked clarity and resolution. Such results are due to the weak light backscattering abilities of the transparent MN materials, which result in poorly-defined structures and image artefacts in the above studies.

In a previous work, we reported the use of iron oxide (Fe_3O_4) nanoparticles to significantly enhance μOCT signal in the imaging of cellular-uptake of $\text{Au-Fe}_3\text{O}_4$ nanoparticles.²¹ Inspired by such results, we integrate Fe_3O_4 nanoparticles into polymeric MNs to improve the contrast in μOCT imaging of MNs' real-time swelling behaviours in transdermal application. In addition, as Fe_3O_4 nanoparticles are biodegradable and have been widely used in preclinical/clinical studies as magnetic resonance imaging agents and magnetic hyperthermia agents,²²⁻²³ their presence in MNs would bring more potentials in multimodal imaging and therapy without the concern about safety issues. Using the polymeric MN platform as an example, we determine that the presence of Fe_3O_4 nanoparticles in polymeric MNs is capable of enhancing contrast by more than 10 times for μOCT imaging in both air and hydrogel. The enhanced contrast enables *in situ* monitoring and quantitative characterization of the MNs' swelling properties in different biological samples (hydrogel, mouse and human skin). For the first time, this methodology realizes the real-time non-invasive visualization and quantitative analysis of polymeric MNs' transdermal swellability performance.

Results and Discussion

Fabrication and Characterization of the MN patch

The swellable Fe_3O_4 MN arrays were prepared with polystyrene-block-poly (acrylic acid) (PS-b-PAA) and polystyrene (PS) via a solvent casting method as shown in Figure 1. PS-b-PAA polymer can absorb fluid when being placed within tissue, resulting in a swollen morphology.²⁴ PS polymer was used as the base material of the MN structure to provide mechanical strength and structural integrity. Specifically, we firstly fabricated a negative polydimethylsiloxane (PDMS) mold from a stainless-steel MN template (Figures 1a-1b). Fe_3O_4 nanoparticles with a diameter of 40 nm and zeta potential of +18.2 mV were mixed with 100 mg/ml of PS-b-PAA in different weight percentages (wt%) using dimethylformamide (DMF) as the solvent. The nanoparticle-polymer mixture was stirred for 24 hours vigorously before it was placed in a vacuum oven to degas for 2 hours and casted onto the PDMS negative mold. After being dried for 48 hours in a fume hood, a thick film was formed at the tip region of the mold due to capillary forces (Figure 1c). The PS pellet was then melted on the top of the thin PS-b-PAA film remaining in the mold at 180 °C under vacuum for 4 hours to produce the

double layered needle structure (Figures 1d-1e). The designed MNs have a height of approximately 700 μm with a base width of about 300 μm and inter-needle spacing of 400 μm .

Figure 1: Schematic illustration of the fabrication process of the double-layered Fe₃O₄ PS-b-PAA/PS MN patches. Fe₃O₄ nanoparticles were localized on the tips of the needles. (a) Stainless steel MN mold (b) PDMS mold (c) PS-b-PAA polymer solution with Fe₃O₄ nanoparticles solvent casted onto PDMS mold (d) PS layer is melted to form double-layer structure (e) Top view of a MN array after peeling off from PDMS mold.

Without the presence of Fe_3O_4 nanoparticles, PS-b-PAA/PS MN array is translucent with the MNs displaying a distinct double layered structure (Figures 2a-2b). The incorporation of Fe_3O_4 nanoparticles resulted in a brown color with a clear distinction of Fe_3O_4 nanoparticles encapsulated at the MN tips (Figures 2d-2e). Regardless of the presence of Fe_3O_4 nanoparticles, the MNs swelled after immersion into 1.4 wt% agarose hydrogel for 1 minute (min), which was accompanied with a slight color fading. These images shown in Figure 2 acquired with conventional microscopy allows the visualization of the morphology changes after fluid absorption, e.g., the MN tips swell and become round-shaped (Figures 2c and 2f) as the carboxylic acid groups present becomes ionized in the presence of water. However, such images do not provide any accurate quantifications of the fluid volumes extracted. to the real-time monitoring of the *in-situ* MNs' swelling process could not be achieved either.

Figure 2: PS-b-PAA/PS MN patches without and with Fe_3O_4 nanoparticles. (a, b) PS-b-PAA/PS MN patch; (c) PS-b-PAA/PS MN patch after immersion in the agarose hydrogel for 1 min; (d, e) Fe_3O_4 -PS-b-PAA MN patch; (f) Fe_3O_4 -PS-b-PAA MN patch after immersion in the agarose hydrogel for 1 min. (Scale bar: 1mm)

Therefore, to monitor the MNs' real-time swelling characteristics and quantitatively analyze their swellability in biological tissues non-invasively, we adopt a lab-built μOCT system for MNs' penetration imaging. The Fe_3O_4 nanoparticles are integrated into the polymeric MNs to enhance μOCT imaging contrast. However, although Fe_3O_4 nanoparticles may provide a significant contrast enhancement for real-time monitoring of polymeric MNs' temporal behaviors, the addition of Fe_3O_4 nanoparticles should not significantly alter the properties of the PS-b-PAA/PS MNs such as mechanical strength and swellability. Hence, we should optimize the concentration of Fe_3O_4 nanoparticles in the MN patch for OCT imaging.

The concentration of Fe_3O_4 nanoparticles was tuned between 2 wt% and 10 wt% in the PS-b-PAA/PS MNs. The mechanical strength of the MNs was studied with an axial compression test, in which the MNs were compressed and the deformation at various loads was recorded. Compressive stress and strain were calculated and plotted as a stress-strain diagram (Figure 3a). As can be seen, when Fe_3O_4 nanoparticle concentration was 6 wt% or less, there was no significant change in the mechanical strength of the MNs. However, exceeding this concentration, it is observed that the MN tips bend easily when being peeled off from the mold. This phenomenon is mostly because the entire tip structure of the MN is filled with the inorganic nanoparticles, causing it to become very soft. Furthermore, the mechanical strength and structural integrity of the MNs is imparted by the PS inner core. The increasing Fe_3O_4 concentration in the PS-b-PAA polymer could possibly alter the extent of cross-linking between the PS block in PS-b-PAA layer and the PS homopolymer layer at the molecular level. It is also observed that the double-layered structure is less prevalent with increasing nanoparticles as the PS base layer occupies lesser cavity space in the MN mold. Therefore, the loading should be controlled between 2.0 wt% and 6.0 wt% to retain the mechanical strength of MNs.

The swelling ability of the MNs was evaluated by inserting MNs with different concentrations of Fe_3O_4 nanoparticles into agarose hydrogel at room temperature for 1, 3, and 5 mins. A thin strip of Parafilm M® was used to cover the surface of the agarose hydrogel. The film mimics the stratum corneum barrier and prevents the contact of the MN base with the hydrogel. We quantified the swellability of the MNs by weighing the MN patches before and after their immersion in the hydrogel. The mass increase was computed as a percentage swelling ratio and compared against blank PS-b-PAA/PS MNs. As shown in Figure 3b, a longer immersion time in hydrogel resulted in a higher swelling ratio, and such a trend was observed consistently in different Fe_3O_4 concentration MNs. However, the encapsulation of Fe_3O_4 nanoparticles did not significantly influence the swelling ratio, regardless of their concentration. Such an observation should be due to the small percentage of Fe_3O_4 nanoparticles in the polymer matrix and the relatively smaller size of the nanoparticles in comparison to the micrometre-sized MN tips.

We further examined the influences of Fe_3O_4 concentration on the contrast to noise ratios (CNRs) of MNs' OCT images in Figure 3c. MN patches with different concentrations of Fe_3O_4 nanoparticles were prepared and utilized for μOCT imaging. Cross-sectional images of a row of MNs were acquired and averaged (8 images were utilized), while the contrast of such obtained images was adjusted to their best values for CNR comparison. The CNR improved with an increasing Fe_3O_4 concentration until it reached a plateau at 4 wt%. This should be due to the saturated light-backscattering effects with a Fe_3O_4 nanoparticles concentration higher than 4 wt%.

It is critical that the presence of nanoparticles should minimally or even not affect the mechanical properties and swellability of PS-b-PAA MNs. To make a compromise between the CNR enhancement and MNs' mechanical properties, we adopted the MNs with 5 wt% Fe_3O_4 concentration for the following experiments.

Figure 3: Characterization of Fe₃O₄-PS-b-PAA/PS MNs. (a) Mechanical strength (b) Swellability and (c) CNR of Fe₃O₄-PS-b-PAA/PS MNs with different percentages of Fe₃O₄ nanoparticles (5wt%).

Contrast enhancement of Fe₃O₄ nanoparticles in μ OCT imaging of MNs

The presence of Fe₃O₄ nanoparticles in the MNs allowed the capture of the tomography of PS-b-PAA MNs with μ OCT imaging. Figure 4a is a cross-sectional image of the PS-b-PAA/PS MNs without Fe₃O₄ nanoparticles in the air and Figure 4b is a 3D reconstruction image of the same patch. As shown in Figures 4a and 4b, the MNs are hard to be identified from the background. When the MNs were inserted into the hydrogel (Figures 4c-4d), the backscattered light signal from MNs is even lower than the background (i.e. hydrogel), which makes visualization of MNs' insertion and swelling in hydrogel impossible.

In contrast, the presence of Fe₃O₄ nanoparticles within Fe₃O₄-PS-b-PAA/PS MNs significantly improved the MNs' OCT signals by increasing the backscattered light signals from the polymeric MNs. As shown in Figures 4e-4f, the morphology of MNs was clearly seen in both 2D and 3D images. When the MNs were inserted into the hydrogel (Figures 4g-4h), they still presented identifiable OCT signals. To have a better understanding about the difference, the CNR in these images was also measured (Figures 4a-4h). Both area of interests (AOIs, i.e., the MNs) and background noise regions (BNRs, i.e., the background) were marked with yellow dashed boxes as shown in Figures 4a and 4e. In both air and hydrogel environments, the CNR of the OCT images for Fe₃O₄-PS-b-PAA/PS MNs is more than 20dB (i.e., at least 10 times) higher than that of PS-b-PAA/PS MNs (Figure 4i).

Figure 4: Contrast enhancement of Fe₃O₄ nanoparticles in MNs' μOCT imaging: (a-d) 2D and 3D images of PS-b-PAA MNs (e-h) 2D and 3D images of Fe₃O₄-PS-b-PAA MNs in air and agarose hydrogel respectively; (i) Calculated CNRs for a-h by using the OCT signals in AOIs and BNRs in the corresponding images. (Scale bars: vertical-0.1 mm; horizontal-1 mm. yellow arrows: MN base; green arrows: MNs.)

μ OCT assessment of MNs penetration into the agarose hydrogel

The contrast enhancement of μ OCT imaging of MNs in the static condition motivates us to further explore whether this would allow the real-time imaging of MNs' swelling in moisture environment. We inserted Fe_3O_4 -PS-b-PAA/PS MNs into the agarose hydrogel and focused the scanning beam onto a row of MNs and acquired a time-series B-mode frames for 45 seconds (Figure 5a). To quantitatively evaluate the morphology change of MNs in hydrogel, we modelled each MN to be a circular cone, and calculated the average volume of those MNs within the hydrogel at different time. The swelling behaviour of the MNs was measured by the average MN volume changing ratio, which is defined to be the ratio of average MN volume in the hydrogel at different time to that at 1s. As shown in Figure 5b, the swelling was minimal from 1s to 3s, when MNs started to get into the hydrogel. After the MNs were completely inserted into the hydrogel (3s to 10s), the swelling was fast. In 7 seconds, volume of MNs increased by $\sim 200\%$. 10 seconds later, the polymer matrix was saturated with water and thus there was only $\sim 28\%$ volume increase in the next 30 seconds. For the real-time swelling behaviour of MNs in hydrogel, readers are referred to the supplementary video (Supporting Video 1).

Figure 5: Real-time μ OCT imaging of MNs' insertion into the agarose hydrogel. (a) Time-lapsed cross-sectional view of MNs during the insertion process; (b) Quantification of swelling kinetics of MNs at different time points. (Scale bars: vertical-0.1 mm; horizontal-0.5 mm.).

μOCT imaging of MN penetration and swelling in mouse skin tissue

We further examined the swelling behaviour of the MNs in fresh mouse ear skin tissues. When imaging, the skin tissue was placed onto the glass window on the portable OCT scanning head, while the MN patch was placed on a motion stage and moved to penetrate the skin tissue. The dynamic insertion and swelling process of the MNs was recorded with the constructed μOCT system (Figure 6a). The first 15 seconds were the insertion process. While after 50 seconds in skin, the MNs were pulled out from the skin. Similar to the analysis of MNs' insertion and swelling in hydrogel, the swelling process of the MNs in mouse skin was also quantified by their volume changing ratio over time (Figure 6b).

During the first 10s' insertion process (Figure 6a), the MNs' profile change was not obvious since the skin was not punctured due to skin elastics and the existence of the stratum corneum layer. However, once the stratum corneum layer was punctured, and the MNs were inside the skin (10 seconds onwards), the MNs swelled, which lasted for about 30 seconds until the volume stabilized. The swelling process (30 seconds) of MNs in skin is much slower as compared with that in hydrogel (~18 seconds), which is believed to be due to the lower water content in the skin (~60%) than that in hydrogel (>99%). When being pulled out from the tissue (65 seconds onwards), the MNs shrank due to the evaporation of water in air. It is worth mentioning that within the whole process of MNs' insertion into and pulling out from the skin, although the distinction between the MN profiles and the skin tissue was not as clear as those without Fe_3O_4 ,¹⁷ the real-time MN profile changes could be observed clearly. Such is because addition of Fe_3O_4 nanoparticles helps improve the light backscattering effect from the MNs, which thus improves the contrast between the MNs and the image background. For the real-time swelling process, readers are referred to the supplementary video (Supporting Video 2).

Figure 6: Real-time μ OCT imaging of MNs' swelling kinetics in mouse ear skin tissues. (a) Time-lapsed cross-sectional view of MNs during the swelling process (b) Averaged volume changing ratio per MN measured at different time points. (Scale bars: vertical-0.1 mm, horizontal-0.5 mm. Yellow arrows: skin stratum corneum; green arrows: MNs.).

μ OCT imaging of MN penetration in human skin tissue

This methodology was finally extended to the human skin tissues. Before MNs insertion and imaging, the fat tissue was removed and then the processed skin tissue was placed on top of the glass window of the portable OCT scanning head. The MN patch was placed on a motion stage and moved to penetrate the skin tissue, and the penetration process was recorded for ~5 mins. The penetration took about 15 seconds and MNs were left in the human skin tissue for 251 seconds in total (Figure 7a). After the penetration application, the MN patch was removed from the skin tissue.

Similar to the above MN swelling quantification, the MNs' swelling in human skin was also characterized by their volume changing ratio, which was defined as the ratio of averaged overall MN volume to that of an MN at 5s (Figure 7b). The swelling process in human skin lasted for around 35s, which was slightly longer than that in mouse ear skin (30s). While during the time from 40s to 75s, the average volume of those MNs kept within a certain range as those needles reached their best swelling abilities.

The skin morphology before and after MNs' penetration was also recorded with the μ OCT system. Figures 7c-7f depict the 3D and *en-face* images at an imaging depth of 120 μ m before and

after MNs' insertion into skin tissue. The black holes as shown in Figure 7f illustrate the areas left by those MNs. Such imaging results demonstrate that the MNs can achieve significant penetration in biological tissues, and therefore, could act as viable tools for chemical diffusion across the tissue. Readers are referred to the supplementary video for MNs' real-time swelling process in human skin (Supporting Video 3).

Figure 7: Real-time μ OCT imaging of MNs' swelling kinetics in human skin. (a) Time-lapsed cross-sectional view of MNs during the swelling process (b) Averaged volume changing ratio per MN measured at different time points. (c-d) 3D and *en-face* images of the skin tissue before MNs' insertion (e-f) 3D and *en-face* images of the skin tissue after the MNs' insertion (Scale bars: vertical-0.1 mm, horizontal-0.5mm. Yellow arrows: skin tissue; green arrows: MNs, black arrows: glass surface.).

Conclusion

This study demonstrates the use of Fe_3O_4 nanoparticles as contrast agents for real-time high resolution OCT imaging of polymeric MNs in transdermal applications. With finely tuned concentrations, the presence of Fe_3O_4 nanoparticles in the MNs not only improves contrast in μOCT imaging by more than 10 times in both air and hydrogel, but also enables the *in-situ* MN profile changes to be observed clearly without significant alteration to the mechanical properties of MNs. As a proof-of-concept, we examined the penetration and swelling behaviours of Fe_3O_4 -PS-b-PAA MNs in agarose, mice skin and human skin tissues for the first time non-invasively and quantitatively evaluated the swelling process using μOCT . Fe_3O_4 -PS-b-PAA MNs swelled rapidly once they were inserted into the hydrogel/tissues, and the swelling process lasted for ~20s in agarose hydrogel and ~30-35s in the skin tissues. This technology would allow researchers to monitor the *in-vivo* and *in-situ* performances of MNs with greater ease and accuracy, and thus analyse key parameters such as MNs penetration depth, drug release profile, and skin recovery rate more easily. Moreover, it can greatly facilitate and drive the development of MN-based therapy and diagnosis.

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Author contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Specifically, C.J.X., L.L conceived the project and wrote the paper. R.Z. performed the MN synthesis. X.Y. performed the OCT imaging. C.H. performed mechanical test. R.Z., X.Y. analysed the data. C.P. provided valuable input for the potential roles of Fe_3O_4 nanoparticles in the microneedle field.

Conflict of Interest

The authors declare no conflict of interest.

Supporting Information

The following files are included as supporting information:

- Supporting information document showing the detailed experimental methods and MNs' fabrication process (PDF);

- Supporting video 1 showing the MNs' real-time penetration into hydrogel and the swelling process (AVI);
- Supporting video 2 showing the MNs' real-time penetration into mice skin and the swelling process (AVI);
- Supporting video 3 showing the MNs' real-time penetration into human skin and the swelling process (AVI);

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