An acoustic microscopy technique to assess particle size and distribution following needle-free injection

Jamie Condliffea) and Heiko A. Schiffter
Institute of Biomedical Engineering, University of Oxford, Oxford OX3 7DQ, United Kingdom

Robin O. Cleveland
Department of Mechanical Engineering, Boston University, Boston, Massachusetts 02215

Constantin-C. Coussios
Institute of Biomedical Engineering, University of Oxford, Oxford OX3 7DQ, United Kingdom

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Needle-free injection is a novel technique for transdermal drug and vaccine delivery, the efficacy of which depends on the number density and mean penetration depth of particles beneath the skin. To date, these parameters have been assessed optically, which is time-consuming and unsuitable for use in vivo. The present work describes the development of a scanning acoustic microscopy technique to map and size particle distributions following injection. Drug particles were modeled using a polydisperse distribution of polystyrene spheres, mean diameter 30.0 μm, and standard deviation 16.7 μm, injected into agar-based tissue-mimicking material, and later, as polydisperse stainless steel spheres, mean diameter 46.0 μm, and standard deviation 13.0 μm, injected both into agar and into porcine skin. A focused broadband immersion transducer (10–75 MHz), driven in pulse-echo mode, was scanned over the surface of the injected samples. Recorded echo signals were post-processed to deduce particle penetration depth (30–300 μm). Furthermore, post-injection size distribution of the spheres was calculated using a novel, automated spectral analysis technique. Experimental results were validated optically and found to predict penetration depth and particle size accurately. The availability of simultaneous particle penetration depth and particle size information makes it possible for the first time to optimize particle design for specific drug delivery applications. © 2010 Acoustical Society of America. [DOI: 10.1121/1.3314252]

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I. INTRODUCTION

Needle-free particle injection is a technique, which allows pain-free, transdermal delivery of drugs and vaccines. Typically, devices employ a high-speed gas flow, caused by rupturing a pressurized gas canister held at 60 bars, in which particles are entrained. The gas flow, with an exit velocity in the region of Mach 0.8–1.0, is aimed at the skin, and the particles penetrate to depths of hundreds of microns.

Previous work has suggested that, based on calculations using momentum and size, particles penetrate to depths proportional to the product of their density, radius, and velocity. Though no experimental evidence is available, which confirms this hypothesis, it is believed that larger particles penetrate deeper into a target than smaller ones. Work has previously been conducted using monodisperse polystyrene particles, of diameter 48.0 ± 3.8 μm and density of 1050 kg/m³ (Thermo Fisher Scientific), which were fired into agar targets. The penetration depth of these particles was subsequently measured by slicing the sample and analyzing sections using optical microscopy (see Fig. 1). It has been shown that 48.0 μm polystyrene particles penetrate 3% agar to an average depth of around 300 μm over a surface, or injection “footprint,” which is approximately circular with a diameter of 10 mm.

As optical microscopy is time-consuming and inherently invasive, it would be desirable to develop an automated, non-invasive method of measuring particle penetration depth and size in order to assess the efficacy of the injection device, or to assess other forms of subcutaneous dosage administration. Previous work by the authors has shown that ultrasonic imaging techniques show great promise in identifying particles beneath a surface, with work at 15 MHz providing accurate estimations of relative particle number density within a tissue-mimicking material. Furthermore, it has been suggested that frequency domain analysis could provide a means of measuring particle size, though such a technique has never been applied to the case of polydisperse size distributions, nor automated and compared directly to optical measurements in order to assess its accuracy. For these reasons, this study considers the development of an acoustic microscopy technique—over technologies such as optical coherence tomography—which can be used to assess the size distribution and penetration depth of particles injected using needle-free injection. Unlike previous works, this study considers polydisperse particle distributions, injected into both agar and skin targets.

Though the acoustic scattering from particles in agar has been considered previously, to the best knowledge of the
authors, the present work represents the first attempt to develop an acoustic microscopy technique to image particles embedded within skin, following transdermal delivery. Furthermore, this work develops a novel means of non-invasively measuring the size of polydisperse particles surrounded by both inviscid and visco-elastic media. The overall objective is to develop a microscopy technique to measure particle size and penetration depth in agar and skin simultaneously, that can be exploited to optimize particles and devices for a broad range of drug and vaccine delivery applications.

II. THEORY

A. Scattering from an elastic sphere

The acoustic microscopy technique presented here relies on the ability to relate the acoustic signal backscattered by particles embedded in agar or skin samples to the electrical signal received by the interrogating transducer. The scattered wave varies depending on the acoustic properties of the surrounding medium and the scatterer, the frequency of the incident sound, and the size and shape of the scatterer. In general, the surrounding medium and scatterer will support both compressional and shear waves. To simplify the theoretical discussion, the case of a particle surrounded by agar is first considered. Agar has a shear modulus \( G \) of 100 kPa, assumed to be negligible compared to the bulk modulus \( K \) of 2.25 GPa.\(^8\) In what follows, therefore, theoretical modeling is shown, which assumes that a particle is surrounded by agar, treated as an inviscid compressible fluid. This theory is developed into a means of assessing particle size in later sections.

The particles considered here are made of polystyrene and stainless steel. Polystyrene spheres have a density of 1050 kg/m\(^3\), bulk modulus of 5.8 GPa, shear modulus of 1.27 GPa, and Poisson’s ratio \( \nu \) of 0.34; stainless steel particles have a density of 8000 kg/m\(^3\), bulk modulus of 193 GPa, shear modulus of 82 GPa, and Poisson’s ratio of 0.30. The surrounding medium is assumed to be a 3\% agar gel, with density of 1030 kg/m\(^3\), bulk modulus of 2.25 GPa, and shear modulus of 100 kPa.

An exact solution for scattering of sound by an elastic sphere in an inviscid fluid was developed by Faran.\(^9\) This solution is implemented here to model the response of the particles in agar to an incident ultrasound pulse. Figure 2 shows the theoretical variation in differential backscattering cross-section—backscattered power per unit incident intensity— with frequency for a 48.0 \( \mu \)m polystyrene sphere in agar. Also plotted in Fig. 2 is the experimentally acquired frequency response of a single, isolated particle known to be of diameter 48.0 \( \mu \)m, measured using the 10–75 MHz transducer described in Sec. III B, which has been normalized to match the peak amplitude at 20 MHz. It can be seen that the two results are in close agreement, with the theoretical model correctly predicting the sharp resonances of the particle. Similar agreement (data not shown) was obtained for the case of a stainless steel sphere surrounded by agar—a second particle type that will be used in subsequent studies.

B. Spectral method for particle sizing

In Fig. 2, it can be seen that the 48 \( \mu \)m sphere has five distinct resonance peaks below 50 MHz, and the spacing between these peaks is 6.2 MHz. For small values of the non-dimensional size parameter \( ka \), where \( k \) is the wavenumber and \( a \) is the particle radius, it is found that the spacing between the resonance peaks is inversely proportional to the particle diameter. For the particular case of a 48.0 \( \mu \)m polystyrene sphere in agar, this is no longer true above 60 MHz, where the simple, low-order elastic resonances of the particle become confounded with higher-order modes of oscillation. However, over the frequency range containing the strong resonances, the spectral peak spacing can be directly correlated with particle diameter, as illustrated in Fig. 3. The values for inter-peak spacing in Fig. 3 were determined from the Faran solution, which was evaluated for polystyrene and stainless steel spheres.
stainless steel particles of size 1–100 μm. The resonances were detected using a peak finding algorithm, and their spacing was recorded. The relationship shown in Fig. 3 provides a means of non-invasive, real-time particle sizing, under certain assumptions, which are discussed in Sec. III E 1.

C. Multiple scattering

The particle sizing concept described in Sec. II B can only be applied to an experimental situation involving multiple scatterers if it can be assumed that individual scatterers do not interact. The multiple scattering criterion developed by Waterman and Truell\(^{11}\) states that the single scattering approximation holds when

\[
\frac{n \sigma_s}{k_0} \ll 1,
\]

where \(\sigma_s\) is the total scattering cross-section of the object, \(n\) is the number of scatterers per unit volume, and \(k_0\) is the wave number in the surrounding medium. In the current work, this parameter is of the order of 0.2, even for the highest payload conditions of both polystyrene and stainless steel particles, assuming frequencies in the range 10–75 MHz. It may therefore be assumed that the single scattering approximation holds.

III. MATERIALS AND METHODS

A. Needle-free injection apparatus

The experimental needle-free particle injection system shown in Fig. 4 was used. Particles were weighed using a microbalance (MT 5, Mettler-Toledo, U.K.) and inserted into a disposable cartridge, which fits into the main body of the needle-free device. The number density of particles was chosen to reflect that typically injected in a clinical setting. The particles were fired at the sample from a height of 10 mm above the surface with the “gun” mounted on a specially designed delivery console to ensure that the direction of injection was orthogonal to the target surface.

Two targets were used during this work. The first was a 3% agar gel, which has been previously shown to provide a good experimental model for needle-free injection.\(^4\) In terms of acoustic properties, a 3% agar gel has a density of 1030 kg/m\(^3\) and sound speed of 1540 m/s.\(^8\) These values compare favorably with the acoustic properties of human skin, which has a density of approximately 1000 kg/m\(^3\) and a sound speed of 1480 m/s.\(^12\)\(^{–}\)\(^14\) The tissue-mimicking agar material was manufactured in the laboratory by combining agar powder with water, which was heated (\(\sim 80 ^\circ\)C) while stirring, and then degassed under a vacuum (\(\sim 50 \) kPa). The resulting molten agar was injected into specially designed molds, and refrigerated for at least 3 h to ensure complete solidification prior to injection of particles.

The second target was excised porcine tissue, which is similar histomechanically and biomechanically to that of humans,\(^15\) and is also of a similar thickness.\(^16\) Pig skin exhibits a sound speed that is slightly higher than that of human skin, at around 1720 m/s.\(^13\) Samples were taken from the inguinal region (inner thigh) and dorsal pinna (back of the ear) of an English white pig. The skin was marked before removal from the body, and its dimensions were noted so that the skin could be tensioned, to restore its dimensions before retrieval during experimentation, providing continuity between the \(\text{in vivo}\) and \(\text{ex vivo}\) cases. Experiments were conducted in the shortest time possible, following harvest from the pig, and in all cases, experiments were performed within 3 days. The skin was at all times stored at a constant temperature of 4 °C.

Monodisperse polystyrene particles of diameter 48.0 μm and density 1050 kg/m\(^3\) have been used in previous works to model drug and vaccine particles.\(^3\) However, the use of monodisperse particle distributions undoubtedly constitutes an over-simplification of the polydisperse particles, which are injected in a clinical context. The work presented here therefore uses polydisperse polystyrene particles, mean diameter of 30.0 μm, and standard deviation of 16.7 μm, and stainless steel particles, mean diameter of 46.0 μm, and standard deviation of 13.0 μm (Thermo Fisher Scientific). The polystyrene spheres are able to penetrate human skin, but we found that these were not able to penetrate deep enough into porcine skin. Therefore, stainless steel particles were chosen for the experiments in the porcine skin because their higher density allowed them to penetrate...
to depths more relevant to the clinical application. We note that this does not affect signal to noise ratio, with the scattering cross-section of 46 \( \mu \text{m} \) stainless steel spheres being of the same order as that of 30 \( \mu \text{m} \) polystyrene spheres over the frequency range investigated here. It should be noted that all references to size distributions hereafter refer to number distributions and not to volume distributions.

B. Scanning acoustic microscopy apparatus

Following injection, targets were exposed to ultrasound using the scanning acoustic microscopy (SAM) set-up pictured in Fig. 5. The basic premise of the experimental layout was that the sample, with embedded particles, was held in a fixed position inside the ultrasound tank while planar scans, parallel to its surface, were performed using an ultrasound transducer.

The sample was mounted on a goniometer stage (GN1/M, Thorlabs) with its free surface facing upwards inside a custom-built water tank, which contained filtered, de-gassed, and deionized water maintained at a temperature of 20 °C. A 10–75 MHz, spherically focused PVDF transducer (PI175–1-R0.50, S/N: 200411, Olympus NDT) was mounted above the sample holder on a software-controlled, three-dimensional motorized positioning system of sub-micron precision (Z825 and TST001, Thorlabs). The transducer was mounted with its acoustic axis normal to the sample surface and driven in pulse-echo mode using a high frequency pulser-receiver (DPR500, JSR Ultrasonics). The waveform received by the pulser-receiver was recorded by a digital oscilloscope (1 GHz bandwidth, 8-bit, Wavesurfer 104MXi, LeCroy), and transferred to a PC (XPS, Dell) via TCP/IP for storage and further processing.

C. Transducer characterization

According to O’Neil’s theoretical model for focused radiators, the focal point of the transducer used in the present study is 13.2 mm from the face of the transducer when calculated at a frequency of 75 MHz. The focal zone depth, defined as the distance between the −6 dB points for pulse-echo response, is 6 mm, and the beam diameter is 120 \( \mu \text{m} \) at the −6 dB points.

The implication of these calculations is twofold. Axially, the focal zone depth of the transducer is such that the pressure amplitude of the ultrasound wave received by the embedded particles can effectively be assumed to be constant over penetration depths of hundreds of microns. The beam diameter further determines the scanning step size required to achieve complete characterization of the sample.

D. Sample alignment and data acquisition

Following mounting of the sample in the water tank, the transducer was moved axially until its focus coincided with the water-sample interface. The goniometer, on which the sample holder was mounted, was then adjusted to ensure that the surface of the sample was perpendicular to the transducer axis. Performing this alignment at several points across the sample surface ensured that the water-sample interface was normal to the transducer axis, and was always coincident with the transducer focus.

Motion control, pulser-receiver settings, and data acquisition from the oscilloscope were controlled using a custom-built graphical user interface developed in MATLAB (Mathworks). The excitation pulse settings were set so as to keep the pulse length short, to improve axial resolution, while also maintaining high signal-to-noise ratio (SNR). Parameters of 330 V driving voltage, 25 \( \Omega \) damping, and “high” energy produced an ultrasound pulse duration of 20 ns, equivalent to a spatial pulse length of 30 \( \mu \text{m} \), which was generated with a pulse repetition frequency of 20 kHz.

Once alignment was achieved, the transducer was moved transversely so that its focus approximately coincided with the center of the needle-free injection site. A scan was launched in a plane parallel to the sample surface, henceforth referred to as the XY plane. Based on the transducer characterization, a transverse step size of 100 \( \mu \text{m} \) was chosen. At each XY position, 50 consecutive traces were averaged on the oscilloscope to enhance SNR before transferring the data to the PC.

E. Post-processing

A typical raw trace, taken from an experiment using polystyrene particles in agar, is shown at the top of Fig. 6. Two features are readily discerned. At 17.6 \( \mu \text{s} \), the discontinuity in acoustic impedance between the water and the agar surface causes a reflection of the incident ultrasound pulse. The second feature, starting at 18 \( \mu \text{s} \), consists of a multiple set of pulses, which corresponds to scattering from a single particle in the agar. Traces taken from experiments performed in skin are extremely similar, though there is an appreciable increase in signal noise present. Post-processing of these raw time traces is required to quantify particle properties and particle position beneath the target surface.

The distinctive features of these waveforms can be used to extract parameters of interest: Signal amplitude and time of flight can yield an estimate of penetration depth, while frequency domain analysis can be used to predict particle...
size. The following two post-processing algorithms were developed to analyze the raw data; the first uses a novel means of frequency domain analysis to measure post-injection particle size non-invasively, while the second uses thresholding and analysis of B-mode images to measure particle penetration depth.

1. Measuring particle size

As illustrated in Fig. 3, the inter-peak spacing of the sharp resonances in the frequency response are unique for a given particle size. To measure the size of the particles from the echo data, an algorithm has been developed, which determines the periodicity of resonances in the frequency domain and matches this to the most probable particle size. First, the reflection from the water-sample interface is gated out, leaving just the signal present as a result of scattering from the particles. This is shown in the top plot in Fig. 6, which shows an echo signal from a 48.0 ± 3.8 μm particle. The dashed box represents the gated signal that is used. The Fourier transform of this part of the signal is taken, providing the frequency spectrum of the signal due to scattering, as is shown in the middle plot of Fig. 6. Finally, the periodicity of the resonance peaks is obtained by taking a Fourier transform of the amplitude spectrum (bottom).

A sharp peak is apparent in this plot, whose position corresponds to the periodicity of the peaks due to scattering in the frequency domain. The peak periodicity, of units MHz⁻¹, is the inverse of the peak spacing. Here, the peak occurs at 0.163 MHz⁻¹, corresponding to a peak spacing of 6.16 MHz. This value can be used to assess particle size using the curve presented in Fig. 3. In the illustrated case, the algorithm would predict that the particle has diameter 49 μm. This procedure is undertaken across the footprint to identify a particle size for each XY point. This algorithm is reliable for the cases where (i) there is one particle of any size in the focal zone of the transducer, and (ii) where there are multiple particles in the focal zone of the same size. For cases where this is not true, it is assumed that multiple particles in the focal region are of the same size. However, optical examination of the injection footprints in several samples reveals that over 90% of the data points will contain only one particle, which implies that sizing errors introduced by signals attributable to multiple particles in a single time trace should be relatively small over the entire injection footprint.

2. Measuring particle penetration depth

The magnitude of the analytic signal (described interchangeably from here on as the “envelope”), generated using the Hilbert transform, is commonly used in medical ultrasound imaging systems to create B-mode images. The B-mode image shown at the top of Fig. 7 was created using this technique. The magnitudes of the analytic signals from each RF line, across a particular line of constant x or y, were calculated, and “stacked” next to each other in a two-dimensional matrix. The magnitudes of each element of the matrix were then converted to a log-compressed greyscale. The resulting images, shown for the case of skin in Fig. 7 (top), clearly show the surface of the sample and the particles lying beneath.

To measure penetration depth, successive B-mode images taken from the footprint were considered separately. Thresholding and image analysis were used to correctly identify particles below the surface, and filtering of object properties allowed the effects of artifacts from skin to be ignored. In each case, the surface of the sample was identified using thresholding. The first return from the agar interface was roughly located along each A-line in the B-mode image using thresholding, whereby the first occurrence of the signal, being greater than five times the amplitude of the noise floor, was sought. The noise floor was calculated as the mean value of the first 1 μs of the envelope signal, which is a part of the signal originating from before the surface of the sample. Upon locating the first occurrence of the signal being above this threshold, the local maximum along the A-line, within 20 ns of the first occurrence, was sought, and the interface between water and sample was defined to be half of an acoustic pulse length prior to this maximum. This was used to define the sample surface across each B-mode image.

In the current case, the scattered power from the particle was greater in magnitude than that reflected by the surface. In fact, this was true over the range of size and particle types used throughout this work. By assessing the return from the agar surface, at a position away from the injection footprint, it was possible to calculate an average return from the surface. A threshold was then set, at 1.5 times the average surface reflection amplitude, above which a peak was assumed to occur due to the possible presence of a particle. This threshold was used to create a binary image. Pixels with a value lower than the threshold were assigned a value of zero, while those greater than the threshold were assigned a value.
of unity. The resulting black and white image is shown in the second sub-plot of Fig. 7, which includes the superposition of the skin surface.

At this point, the spatial characteristics of the objects identifiable on the binary image were analyzed to extract the area, minimum axis length $l_{\text{min}}$, major axis length $l_{\text{maj}}$, and perimeter, where all values were measured in pixels. We observed that particles appeared circular in the B-mode images. As a result, in order for a shape to be identified as a particle, it had to be sufficiently “round” [i.e., have a ratio of $l_{\text{maj}}/l_{\text{min}}$ close to unity and a perimeter approximately given by $\pi (l_{\text{maj}}+l_{\text{min}})$] and have a pixel area within a certain range. Objects, which were sufficiently small, large, or strangely shaped, were excluded, and those of the correct size and shape were included. This process allowed artifacts due to hair follicles, sweat glands, and other skin sub-structures to be ignored in the analysis. Those objects, which were determined to be particles are plotted at the bottom of Fig. 7 as circles, and beneath the skin surface, plotted as a line. Each value of penetration depth was recorded and tagged with the $XY$ position at which it was measured.

F. Validation

Penetration depth measurements were validated using optical microscopy. Following completion of the acoustic scan, the sample was removed from the tank and was sectioned. Previous work has introduced a variety of optical imaging techniques to evaluate particle penetration depths. Agar was sectioned in the laboratory perpendicular to its surface, in the $XZ$ plane, using a specially produced “guillotine” that resulted in slices of around 200 $\mu$m in thickness. The sections were then photographed using a microscope with a CCD-camera (ECLIPSE Ti, Nikon), providing images such as that in Fig. 1. An algorithm developed in MATLAB allowed the upper surface of the gel to be detected and the distance from this surface to the centroid of each particle to be measured. A calibration image from the microscope was then used to convert measurements in pixels to an actual penetration depth that can be directly compared with acoustic measurements.

For the case of tissue, the sample was fixed in a solution of saline buffered 10% formalin, and histology subsequently performed at the Northwick Park Institute for Medical Research (U.K.). The samples were cut to provide transverse slices, sectioned perpendicular to their surface in the $XZ$ plane, and stained with hematoxylin and eosin. Images of the slides were captured using the CCD-camera equipped microscope, and depth measurements were performed using image analysis software (NIS-Elements, Nikon).

All particle size measurements were validated using laser-light diffraction. Measurements were performed using a Mastersizer S (Malvern Instruments), fitted with a 300RF lens and backscatter detector, within a small volume sample dispersion unit. This provided accurate distributions of par-
articles size. Optical microscopy and laser-light diffraction were considered to be the “gold standard” for the purposes of validation of the acoustic technique.

IV. RESULTS AND DISCUSSION

Samples of 3% agar were prepared, and differing payloads of polydisperse polystyrene and stainless steel particles were embedded into each using the experimental needle-free injection device. Porcine tissue samples were also prepared and injected with polydisperse stainless steel particles. Each sample was individually analyzed using the SAM apparatus, in conjunction with the two aforementioned algorithms for measuring penetration depth and estimating particle size, before being sliced and analyzed optically.

A. Penetration depth

Use of the post-processing algorithm described in Section III E 2 can be used to evaluate the distribution of penetration depth in the sample. Figure 8 shows a plot of the distribution of penetration depths for the case of a 350 μg payload of polydisperse polystyrene particles, mean diameter of 30 μm, injected into agar. Alongside values computed from the acoustic data, the reader may observe results acquired from the optical validation, normalized in the same way. It can be seen that the modal penetration depth, represented graphically as the peak of the curve, is accurately measured by the acoustic technique. Furthermore, computation of the mean penetration depth, measured to be 173 μm acoustically and 184 μm optically, and standard deviation, measured to be 105 μm acoustically and 103 μm optically, are in good agreement.

For the sake of brevity, the mean and standard deviation of penetration depth was calculated and recorded for each experiment, which was performed, providing values for ten data sets in total: three corresponding to polydisperse polystyrene particles in agar, three to polydisperse stainless steel particles in agar, and four to polydisperse stainless steel particles in skin. Similar values were calculated from the optical analysis for comparison. Figure 9 (top) shows a scatter plot of mean penetration depth of particles, measured acoustically and optically. It can be seen that there exists strong correlation between the two, with correlation coefficient of $R = 0.9866$. Alongside this strong value of correlation coefficient, the data exhibits an extremely small $p$-value (calculated by transforming the $R$ value into a paired, two-tailed t-statistic) at $p=1.3944 \times 10^{-7}$. Therefore, the results exhibit strong correlation, which is also statistically significant, satisfying a 99.99% confidence interval. The mean penetration is therefore accurately represented by the acoustic technique.

Figure 9 (bottom) shows a similar scatter plot for the standard deviation of penetration, measured both acoustically and optically. Relatively strong correlation is once again observed, with a correlation coefficient of $R=0.9335$, which is again shown to be statistically significant, with a $p$-value of $p=7.8756 \times 10^{-5}$. The standard deviations seem to vary rather more than the mean penetration depth, and it is hypothesized that this is due to slight disruption of the particles during optical validation. As the cutting blade is passed through a sample, particles, especially in the case of those made of stainless steel, are dislodged, and as a result, there is a tendency for increased variation between the two measurements. Regardless, these results suggest that the acoustic technique is capable of accurately representing the mean penetration depth, and provides a good indication of the standard deviation.

B. Particle size

Post-processing of the acoustic microscopy data using the novel algorithm described in Section III E 1 provides estimates of particle size at every point across the acoustic scan. The same algorithm developed in agar was employed to estimate particle size in skin. Shown in Fig. 10 (top) is a

FIG. 8. Comparison between acoustical and optical estimates of particle penetration depth of polydisperse polystyrene particles, mean diameter 30 μm, in agar.
histogram representing particle size distribution measured acoustically for the case of a 350 \mu g payload of polydisperse polystyrene particles injected into agar, and for stainless steel particles injected in skin, as measured acoustically and optically. Values of correlation coefficient \( R \) and significance \( p \)-value are included.

Figure 9. Scatter plots for mean (top) and standard deviation (bottom) of penetration depth data, for polystyrene and stainless steel particles injected into agar, and for stainless steel particles injected in skin, as measured acoustically. The overlaid black curve represents the particle size distribution measured, pre-injection, using laser-light diffraction techniques, and is normalized such that the integral under the curve is equal to that of the histogram. It can be seen that the mean particle size of 30.0 \mu m is accurately measured by the acoustic technique, though the standard deviation of the distribution, at 13.4 \mu m, is smaller than that measured using light diffraction, at 16.7 \mu m. The lack of smaller particles in the distribution may be due to the inability of such particles to penetrate the agar surface; previous experiments have tended to suggest that smaller particles penetrate less effectively, so that very small particles may not penetrate the sample at all. Prior evidence also suggests that larger particles may deform on impact with the target, meaning that they are effectively converted into smaller particles post-injection. This was confirmed by melting an agar sample post-injection and performing laser-light diffraction measurements using the remaining particles, showing a similar decrease in the number of large particles present. This could, therefore, account for the acoustically measured decrease in the number of particles in the larger size range.

Figure 10 (bottom) shows a typical acoustically measured size distribution of polydisperse stainless steel particles in skin (histogram), alongside the size distribution of the same particles measured pre-injection using laser-light diffraction (solid line). It can be observed that, though the modal size (i.e., the size exhibited by most particles; the peak of the distribution) of the acoustically measured distribution is different to that measured pre-injection, the mean at 43 \mu m, and standard deviation at 16 \mu m, are extremely similar to those measured using laser-light diffraction. These observations suggest that the particle sizing technique is, to some extent, affected by the change in surrounding medium from agar to skin. It is worth noting that the sizing technique assumed that the particle, which was being sized, was surrounded by an inviscid fluid. The algorithm could be adapted to compensate for the visco-elastic effects of the surrounding medium.
medium, but as the properties of skin vary so much with depth, site, and age, assigning correct elastic parameters to the model will likely be extremely difficult. The results achieved with the inviscid model were deemed accurate enough, and the algorithm was used throughout this work to measure particle size.

The mean particle size measured acoustically was recorded for each experiment performed, providing data across all ten experiments. The acoustically measured penetration depth is plotted against the mean particle size measured using laser-light diffraction, and this is shown in Fig. 11. It can be seen that there is strong correlation between the acoustic and optic measurements, with a value of correlation coefficient \( R = 0.9556 \). This may also be shown to be statistically significant, with a \( p \)-value of \( 1.611 \times 10^{-5} \), which satisfies a 99.99% confidence interval. The acoustic technique therefore provides a way of measuring particle size, following needle-free injection, non-invasively, in both agar and skin. This has never been achieved in the past, and provides a useful new tool for the developers of needle-free injection. The ability to size and localize particles using a single modality, scanning acoustic microscopy, offers a unique opportunity for the first time to correlate particle size with penetration depth. This is explored in Sec. IV C.

**C. Correlating penetration depth and particle size**

It has been hypothesized in previous works that larger particles will penetrate samples to greater depths due to their greater momentum. The data presented above makes it possible to correlate penetration depth and particle size, as for every position, where a penetration depth was measured, a particle size has been calculated. The use of a bubble plot—a two-dimensional scatter plot where a third variable is represented by the size of the points—allows analysis of correlation between particle size and penetration depth, while also providing an insight into where small and large numbers of points lie. Shown in Fig. 12 are bubble plots from typical experiments performed using polydisperse particles in both agar and skin during this research: polydisperse polystyrene in agar (top) and stainless steel particles in agar (middle) and skin (bottom). The circle diameter on the bubble plot is directly proportional to number of particles, and each circle is centered at the mean particle size and penetration depth of its constituent points. Data was binned along the particle size axis.

![FIG. 11. Scatter plot of mean particle diameter, for polystyrene and stainless steel particles injected in agar, and for stainless steel particles injected in skin, as measured acoustically and optically. Values of correlation coefficient \( R \) and significance \( p \)-value are included.](image1)

![FIG. 12. Variation in penetration depth with particle size, both measured acoustically, for polydisperse polystyrene particles in agar (top), and stainless steel particles in agar (middle) and skin (bottom). Circle diameter is directly proportional to number of particles, and each circle is centered at the mean particle size and penetration depth of its constituent points. Data was binned along the particle size axis.](image2)
of the acoustic technique in establishing the relationship between particle size and penetration depths for particular particle types in different media. The data in Fig. 12 can be acquired non-invasively and in a relatively short time using the novel acoustic technique presented here, and such results have not been acquired in the past. The value of this data for the development of transdermal drug and vaccine delivery modalities should not be underestimated: From a single experiment using a range of particle sizes, it becomes possible to choose the right particle size range for a particular application.

V. CONCLUSIONS AND FUTURE WORK

An innovative acoustic microscopy technique, which is capable of measuring penetration depth and particle size, simultaneously following needle-free injection, has been developed. Acoustic measurements of particle penetration depth are in strong agreement with those measured using optical microscopy, in both agar and porcine skin. In fact, there exists a strong, statistically significant, correlation between mean and standard deviation of particle depth measured acoustically and optically. The novel, automated particle sizing algorithm presented here has also been shown to provide an accurate measurement of post-injection particle diameter. Once more, strong correlation exists between particle diameter measurements performed acoustically and using laser-light diffraction. The unique opportunity to compare size and penetration depth showed that the two are strongly correlated, with larger particles penetrating the sample to greater depths. Scanning acoustic microscopy could provide a viable means of non-invasively optimizing particle size and properties to achieve specific penetration depths for particular transdermal drug and vaccine delivery applications.

The sizing techniques presented here offer the most interesting opportunities for future development. The obvious extension is to develop the technique to assess changing particle size, as real drug-loaded particles tend to dissolve over time. Furthermore, though the technique has been shown to be of use in assessing the efficacy of needle-free injection, it is suggested that it could also be utilized in analyzing many other solid dosage forms administered to the skin, such as implants. It is hoped that the acoustic technique developed as part of the present work will act as a springboard, and a common denominator, in several future studies of particle behavior in skin.

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