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# Penetration of Sub-micron Particles into Dentinal Tubules using Ultrasonic Cavitation

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**Abstract**

**Objectives**: Functionalised silica sub-micron particles are being investigated as a method of delivering antimicrobials and remineralisation agents into dentinal tubules. However, their methods of application are not optimised, resulting in shallow penetration and aggregation. The aim of this study is to investigate the impact of cavitation occurring around ultrasonic scalers for enhancing particle penetration into dentinal tubules.

**Methods**: Dentine slices were prepared from premolar teeth. Silica sub-micron particles were prepared in water or acetone. Cavitation from an ultrasonic scaler (Satelec P5 Newtron, Acteon, France) was applied to dentine slices immersed inside the sub-micron particle solutions. Samples were imaged with scanning electron microscopy (SEM) to assess tubule occlusion and particle penetration.

**Results**: Qualitative observations of SEM images showed some tubule occlusion. The particles could penetrate inside the tubules up to 60µm when there was no cavitation and up to ~180 µm when there was cavitation.

**Conclusions**: The cavitation bubbles produced from an ultrasonic scaler may be used to deliver sub-micron particles into dentine. This method has the potential to deliver such particles deeper into the dentinal tubules.

**Clinical Significance:** Cavitation from a clinical ultrasonic scaler may enhance penetration of sub-micron particles into dentinal tubules. This can aid in the development of novel methods for delivering therapeutic clinical materials for hypersensitivity relief and treatment of dentinal caries.

**Introduction**

Exposure of dentinal tubules can lead to dentinal caries or dentinal hypersensitivity [1, 2]. The causative agent of caries is bacteria which invade the dentinal tubules with a direct path to the pulp of the tooth. The elimination of the bacteria is fundamental in the treatment of caries [2] and this may be achieved by the use of antimicrobials. For treatment of dentinal hypersensitivity, research is directed at reducing the permeability of the dentine, achieved through partially or fully blocking the tubules [3], which may lead to reduced sensitivity and discomfort [4]. The use of tubule blocking agents is a major area of interest within the oral healthcare industry for treating dentinal hypersensitivity [4].

Nanoscale materials have attracted interest as a more efficient method of delivering antimicrobials and remineralisation agents into dentinal tubules [5, 6]. Sub-micron particles (SMPs) with diameters between 100-1000 nm are considered to be safe and have been shown to cause less inflammation than nanoparticles (NPs) [7]. Both NPs and SMPs have a large surface area to volume ratio, which increases solubility and reactivity. If they are non-agglomerated and dispersed, they can easily enter dentinal tubules as their diameter is smaller than the tubule diameter, which ranges from 2-4 µm. The size and reactivity of NPs and SMPs may allow them to be delivered further into dentinal tubules, with an enhanced potential for decontamination, remineralisation and reduced sensitisation compared with contemporary treatment regimens [5]. This may offer advantages such as allowing antimicrobials to reach bacteria deep in the tubules, and also for increasing the retention time of remineralising and tubule occluding materials.

An effective penetration depth into dentinal tubules remains a key issue for the development of NP and SMP technology. Besinis et al. showed how acetone could be incorporated into nanoparticle solution to act as a carrier to deliver NPs further into dentinal tubules [8]. The potential of the solvent to displace water in the tubules may allow deeper particle

penetration. Furthermore, reduced particulate aggregation using surfactants has been reported to improve delivery within tubules, reduce the surface interactions with dentine and increase occlusion of tubules [9].

Cavitation is the generation and collapse of gas or vapour bubbles in a liquid, occurring when the local pressure falls below the saturated vapour pressure, which can occur when an ultrasound field is applied in a liquid [10]. These bubbles can grow to many times their original size and rapidly collapse, releasing high amplitude shock waves and high velocity micro jets [11]. Previous researchers have demonstrated successful penetration of chitosan NPs into dentinal tubules using cavitation [12, 13]. However, in these studies the ultrasound was delivered by a large-scale transducer that may not be suitable for a clinical application. An ultrasonic scaler produces cavitation and may provide a more practical method of directing SMPs deeper into dentinal tubules [14, 15].

Consequently, the aims of this research are to investigate the impact of cavitation from ultrasonic scalers, combined with acetone, in enhancing SMP penetration into dentinal tubules.

**Materials and Methods**

*Dentine specimen preparation & particle application*

Intact, caries free, single-rooted human molars and premolars were used in this study. The use of teeth was authorised by a licence from the United Kingdom Human Tissue Act (HTA) (Licencing number: 12313, Clinical Research Network Consortium Reference: BCHCDent355.1548.TB). Teeth were decoronated at the cemento-enamel junction and 2 mm thick longitudinal slices were cut from the external surfaces of the roots using a low speed water cooled saw (Buehler, Isomet, UK). The area of each section was 8 ±1 mm length, 5 ±1 mm width. The outer surfaces of the slices were ground using silicon carbide (SiC) paper with grit size P500 until nominally flat, and then polished using P1200 and P4000 grade SiC paper to remove microscale grooves. The tooth slices were then immersed in 10% citric acid (BDH, Poole, England) for 2 minutes to remove the smear layer and expose the dentinal tubules as described previously [9]. Specimens were subsequently ultrasonicated in an ultrasonic cleaner (In-ceram, Vitasonic) in 200 ml reverse osmosis (RO) water for 10 minutes prior to imaging using a stereomicroscope (Zeiss PrimoTech, Oberkochen, Germany) (resolution 17 pixels/µm), to determine the direction of the dentinal tubules. Only specimens with dentinal tubules orientated perpendicular to the surface were used in this study to mimic the clinical situation where the tubules on the outer portion of the cervical third of the root become exposed. The specimens were stored in a humidified container prior to testing.

In this study we used silica ($SiO_2$) sub-micron particles prepared in the same manner as those used by Claire et al. to compare the effect of the cavitation and acetone addition on the particle distribution on the dentine surface. The silica particles were prepared according to a published method [16] containing a luminescent ruthenium complex (tris-(2,2'-bipyridyl)ruthenium(II) dichloride) for introducing a luminescent core for potential

luminescence imaging. Dynamic Light Scattering (DLS) was used to estimate the particle size distribution (Zetasizer Nano ZSP, Malvern Instruments, UK). The zeta potential of the particles was also measured (Delsa Nano particle analyser, Beckman Coulter, USA). 1% w/v solutions of sub-micron particles were prepared by adding 0.1 g particles (dry weight) to 10 ml RO water and 10 µl Tween 20 surfactant (Sigma-Aldrich) [9]. The solution was dispersed via ultrasonication for 10 min and then centrifuged at 8000 rpm for 5 minutes. The supernatant was discarded and the particles were re-suspended in either 10 ml RO water or 5 ml RO water mixed with 5 ml acetone. Solutions were ultrasonicated for 10 min before application to the tooth slices to disperse the particles.

Each particle solution (5 ml) was pipetted into a compartment into 12-well culture plate, and a tooth slice was placed inside (Figure 1). An ultrasonic scaler (P5 Newtron, Satelec, Acteon, France) with tip 10P was fixed 0.5 mm away from the tooth slice using a translation stage accurate to 10 µm (PT3, Thorlabs, USA) (Figure 1). The scaler was operated for 20 s at either power 1 (lowest power setting, no cavitation) or power 10 (medium power setting, with cavitation) (n=6). The same procedure was used for both particle solutions.

*Scanning Electron Microscopy*

Samples were then dried for 1h in a $60^0$C oven and gold-coated (K550X, Quorum Technologies, UK) for SEM (EVO MA10, Carl Zeiss, Germany). After imaging the surface of the samples, they were cryogenically frozen in liquid nitrogen and fractured manually. The fractured specimens were mounted onto SEM stubs using conductive paste (Leit C Plast, Agar Scientific, UK), sputtered with gold and imaged using SEM to determine the depth the particles had travelled into the dentinal tubules. Acceleration voltages of 5-20 kV were used in secondary electron mode for SEM imaging.

A representative dentine slice from SEM measurements was also analysed using focussed ion beam (FIB) SEM (Quanta 3D FEG, FEI, Netherlands). The electron beam was operated at 20 kV with spot size 6-8 and the ion beam was operated at 30 kV. The initial trench was cut using milling parameters of 7 nA at 52$^{\circ}$, and subsequent milling was performed at 2 nA, and then, 1 nA at 54$^{\circ}$.

*Image Analysis*

Image analysis was performed using the Fiji distribution of the ImageJ software [17] (U. S. National Institutes of Health, Bethesda, Maryland, USA). The coverage of the dentine surface by the sub-micron particles was calculated for the different settings (with/without cavitation/acetone). The brightness was reduced and contrast was increased manually to prevent the algorithm from falsely segmenting the open dentinal tubules. The same brightness and contrast adjustments were applied to all the images being tested. The images were then segmented automatically using the 'minimum' threshold. This was chosen after trying all of Fiji's auto thresholding methods as it segmented the tubules with the least amount of noise. The percentage of surface covered by the particles was determined from the histogram of the image, by calculating the ratio between the number of pixels with the value 255 and the total number of pixels in the image. SigmaPlot (Systat Software, USA) was used for analysis and data plotting. The One Way ANOVA test was used to test for statistical significance with significance defined as $\alpha=0.05$.

SEM images were also used to confirm the size of the particles together with DLS studies. The size of the sub-micron silica particles was calculated by thresholding the images using the Huang auto-thresholding method, and retaining objects with size 30-300 and circularity >0.95 to remove noise and aggregated particles. The area of each particle was calculated using Fiji's particle analysis plugin. The particle diameter was calculated from the area, assuming circularity.

## Results

Dynamic light scattering provided the particle size distribution in solution to be $960 \pm 210$ nm (intensity distribution) and $910 \pm 210$ nm (number distribution). The poly-dispersion index was 0.2 (number distribution) and 0.3 (intensity distribution). The mean particle diameter as calculated from the area of particles in the SEM images was $960 \pm 70$ nm. The zeta potential of the particles was -48 mV.

The ability of the SMPs to occlude and penetrate inside dentinal tubules was first investigated. Dentine tubule occlusion was found using SEM (Figure 2). Full and partial occlusion of some tubules at the surface was observed for all settings (with and without cavitation, with and without acetone) and there was no visible difference in the amount of tubules obstructed. Some dentinal tubules remained open with no occlusion at the surface. For the settings where cavitation was applied, micro indentations were seen on the dentine surface.

The particles covered approximately 40% of the dentine surface in all of the cases (Figure 3). There was no statistical difference in the area covered by the particles between the different cases (*p=0.7*). There were larger agglomerations of particles on the dentine surface when cavitation was not applied (Figure 3 c & d) compared to when cavitation was applied (Figure 3 e & f).

Penetration of particles into the dentinal tubules was observed for all settings (Figures 4-6). For the particles in acetone, this was seen up to a distance of approximately 80 µm when cavitation was not applied (Figure 4c) and up to ~120 µm when cavitation was applied (Figure 5). Particles that had been dispersed in only water were observed to enter into the tubules up to a distance of approximately 60 µm (Figure 4 a,b) when there was no cavitation and up to ~180 µm when there was cavitation (Figure 6).

Fracture face SEM images showed that in some cases the particles had obstructed the tubules near to the surface of the dentine (Figures 5e and 6c).

Tubules could be milled to view particle penetration near to the surface using FIB SEM (Figure 7). One particle was found inside the tubule near the surface in one of the cases (Figure 7 e) but for other cases there were no particles inside the tubules near to the surface.

**Discussion**

There is no optimal method for rapidly delivering particles into dentinal tubules for occlusion and antimicrobial purposes. In this study we investigated how cavitation from ultrasonic scalers and acetone enhance SMP application to dentine.

SMPs occluded and penetrated the tubules for all ultrasonic scaler settings, showing that infiltration also occurs without cavitation. Acoustic streaming in the fluid around the scaler [18] could be the mechanism that is assisting in the infiltration, as it may also occur at low power without cavitation. The depth that the particles had travelled increased by at least two-fold when cavitation was applied (Figures 4-6), suggesting that cavitation is able to push the SMPs further into the tubules. In this study the maximum penetration depth observed was 185 μm, whereas Shrestha et al. found particles at up to 1 mm inside the dentinal tubules [13]. However they used an 8 mm diameter disc shaped ultrasound transducer which would have a larger area of cavitation compared to the ultrasonic scaler tip used in this study, where the cavitation occurs in an area of approximately 2 mm x 1mm [14].

Besinis et al. have noted that as acetone is used clinically in adhesive systems for dental resin composite restorative materials, it could aid in particle infiltration into dentinal tubules [8]. In the current study, there was no observable difference in the penetration depth when acetone was included in the particle solution, but there were a larger number of tubules occupied by particles. We speculate that acetone is able to initially aid in the particle delivery and then cavitation is able to increase penetration. Further work is needed to confirm these observations.

The majority of the SMPs had deposited on the surface of the dentine and many tubules were partially or completely occluded. Although complete occlusion would be ideal, partial occlusion is also likely to reduce pain because the fluid flow inside the tubules is

proportional to the tubule radius to the power 4 ($r^4$) [19]. Doubling the tubule radius therefore causes a 16-fold increase in the fluid flow rate in the tubule, so partial occlusion of the tubule may increase the potential to significantly reduce the pain experience of the patient. Some tubules remained completely exposed and SEM cross-sections and FIB SEM demonstrated that particles often remained on the surface and failed to penetrate inside the tubules (Figures, 5, 6, 7). The particles may be collecting around the entrance to the tubules due to the presence of electrostatic charges on the surface. The zeta potential of the particles was -48 mV indicating good stability, although inter-particulate attractive forces could still be contributing to cause agglomeration and retention of the particles on the dentine surface (Figures 2 & 3). The different mineralisation levels between peritubular dentine and intertubular dentine are also likely to affect the particle infiltration. Citric acid removes the smear layer and consequently, ions on the dentine surface (e.g. $Ca^{2+}$, $Na^+$, $K^+$, $Mg^{2+}$), however, it may not enter into all of the dentinal tubules. By removing ions, citric acid may expose more biological material such as collagen, which may influence the movement of the particles. Therefore, the ion concentration could be different on the surface dentine compared to the dentine inside the tubules, and the particles may be repelled by the charge inside the tubules. Future work can include determining the ions present by SEM and on investigating the effect of different demineralising acids on the penetration of particles.

In the cases where cavitation was applied from the ultrasonic scaler, pits were observed in the dentine surface, which had a similar diameter to the silica particles (Figure 2f). This may be a result of the particles being accelerated towards the surface due to both the cavitation and acoustic microstreaming forces occurring in the liquid, and their high velocity impact caused an indentation in the dentine. Such pitting may offer an advantage in that the slurry of liquid and particles will impact on the surface removing debris.  The presence of these indentations also confirms that the cavitation is able to accelerate the particles toward the

dentine surface, so it is likely they are also accelerated into the dentinal tubules. This correlates with the finding that there was deeper particle infiltration when cavitation was applied (Figures 4-6).

A limitation of the work is that as the samples were only fractured in one location on the sample, only that area was observed. It is possible and likely that particles infiltrated other tubules that could not be imaged. In addition, the sample preparation techniques such as fracturing and drying could have displaced the particles from their original deposition location inside the tubules. The penetration of particles was not quantitatively analysed in this study therefore future work can focus on developing an imaging protocol which enables the penetration distance tubule occluding materials to be quantified. A non-destructive imaging technique such as nanotomography can be investigated for such work. Another use of the cavitation from the ultrasonic scaler may be in removing bacteria, which have entered into the dentinal tubules. The scaler could then be used in a 2-step method where the tubules are first cleaned and then the particles are delivered. Optimal settings for cleaning and delivery can be determined using further work, in addition to developing antimicrobial and remineralising sub-micron particles.

These findings open up different avenues of research investigations in the use of sub-micron sized particles on the dentine surface.  In order to increase the particle infiltration frequency and depth, parameters such as the duration of the cavitation, the frequency and power of the ultrasonic scaler and the distance it is held from the dentine surface can be altered.

**Conclusion**

This preliminary study has confirmed that the cavitation from ultrasonic scalers may be used to direct sub-micron Silica particles which have entered into dentinal tubules. The work

shows considerable promise although further research is necessary before such a technique may be advocated as a clinical application.

## Acknowledgements

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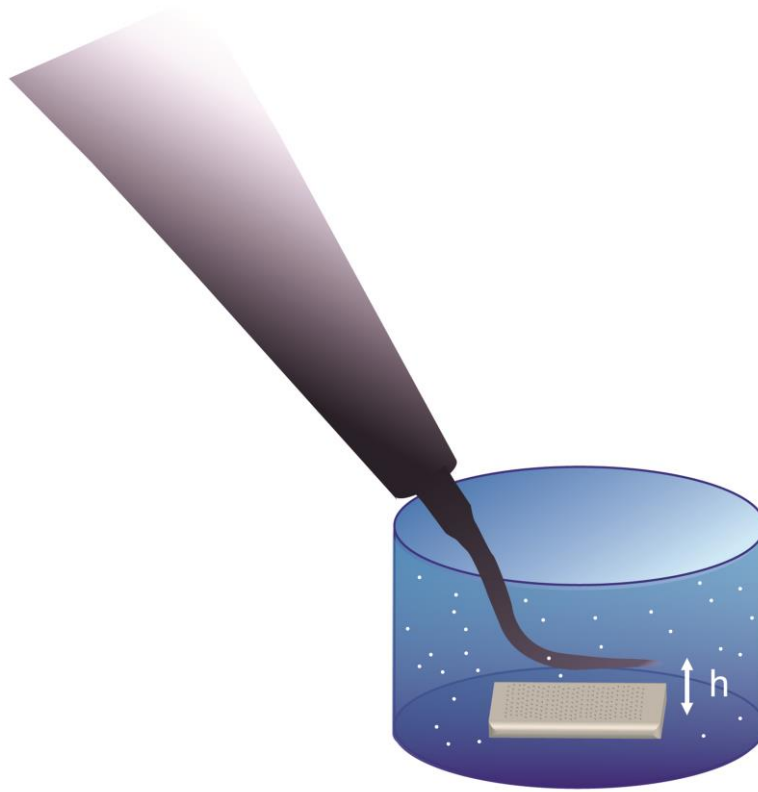
**Figures**



Figure 1 Schematic of the experimental setup showing the orientation of the ultrasonic scaler. The tip was placed inside a well of a 12 well plate filled with 5 ml particle solution. The dentine slice was placed at the bottom of the well and the scaler was positioned at a fixed height h above the slice (h = 0.5 mm)
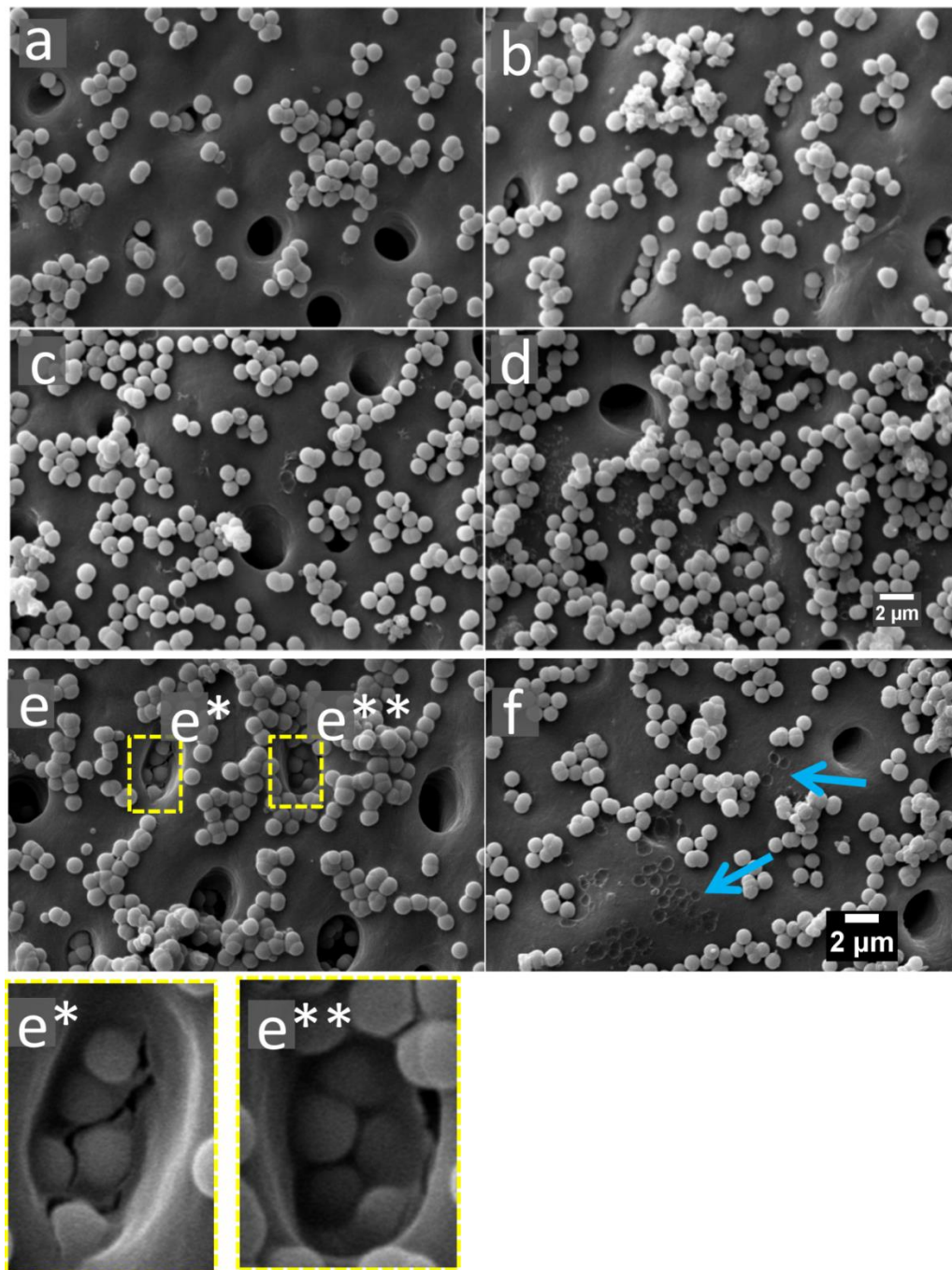
Figure 2 Electron micrographs of the silica particles on the dentine surface at x11,000 magnification (a) particles in 50% acetone, no cavitation applied (b) particles in water, no cavitation applied (c) particles in 50% acetone, cavitation applied (d) particles in water, cavitation applied.(e) SEM images showing the silica particles sealed inside some dentinal tubules, outlined in yellow. Particles in water, no cavitation applied. (f) SEM images showing indentations in the dentine when cavitation was applied (particles in water)
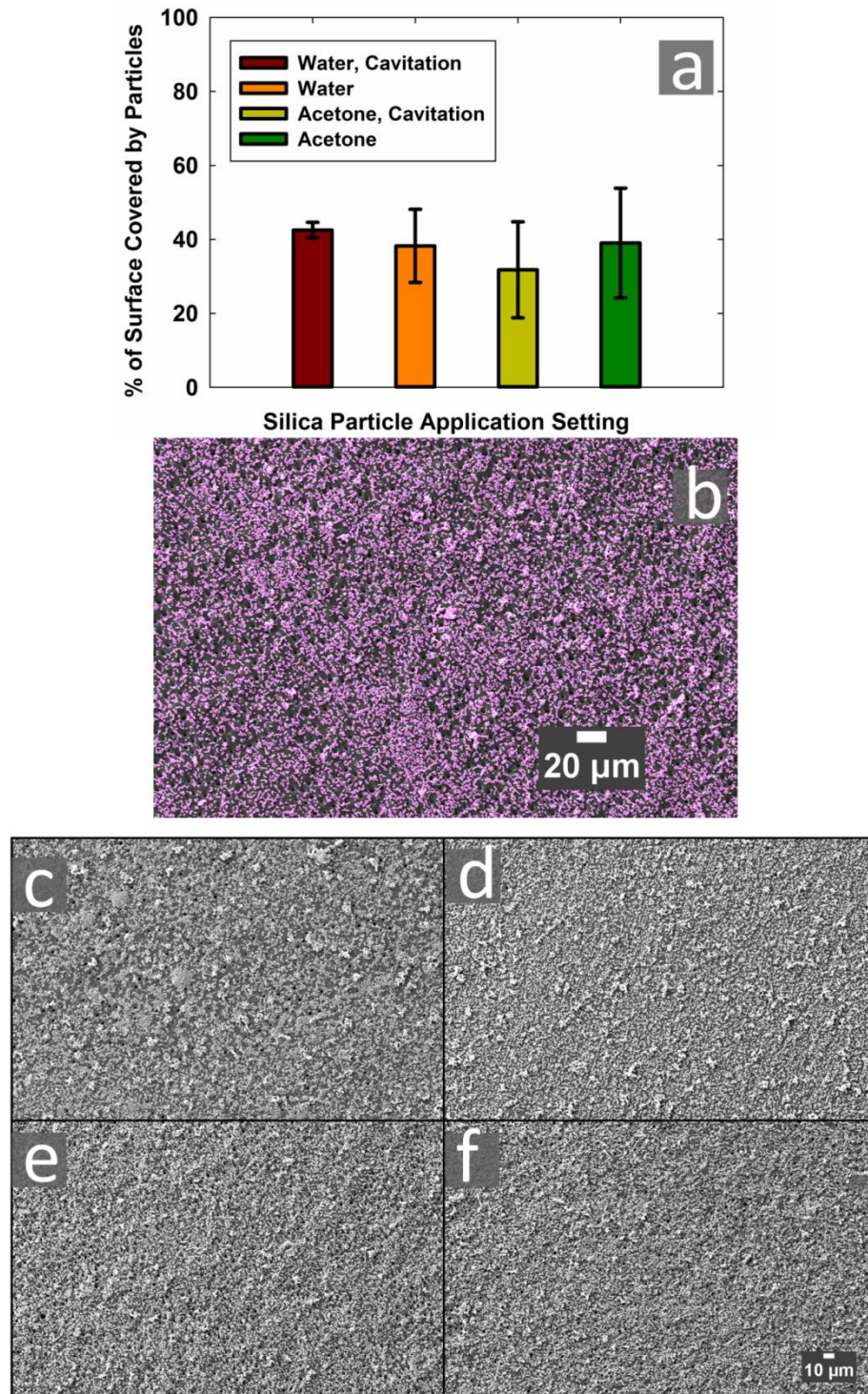
Figure 3 (a) Percentage of the dentine surface covered by the silica particles calculated using image analysis (b) Example showing of one of the thresholded images used for calculating the area covered by particles, merged with the original SEM image. (c-f) Low magnification SEM image of the particles on the dentinal surface showing larger agglomerations of particles when cavitation was not applied (c) particles in 50% acetone, no cavitation applied (d) particles in water, no cavitation applied (e) particles in 50% acetone, cavitation applied (f) particles in water, cavitation applied.
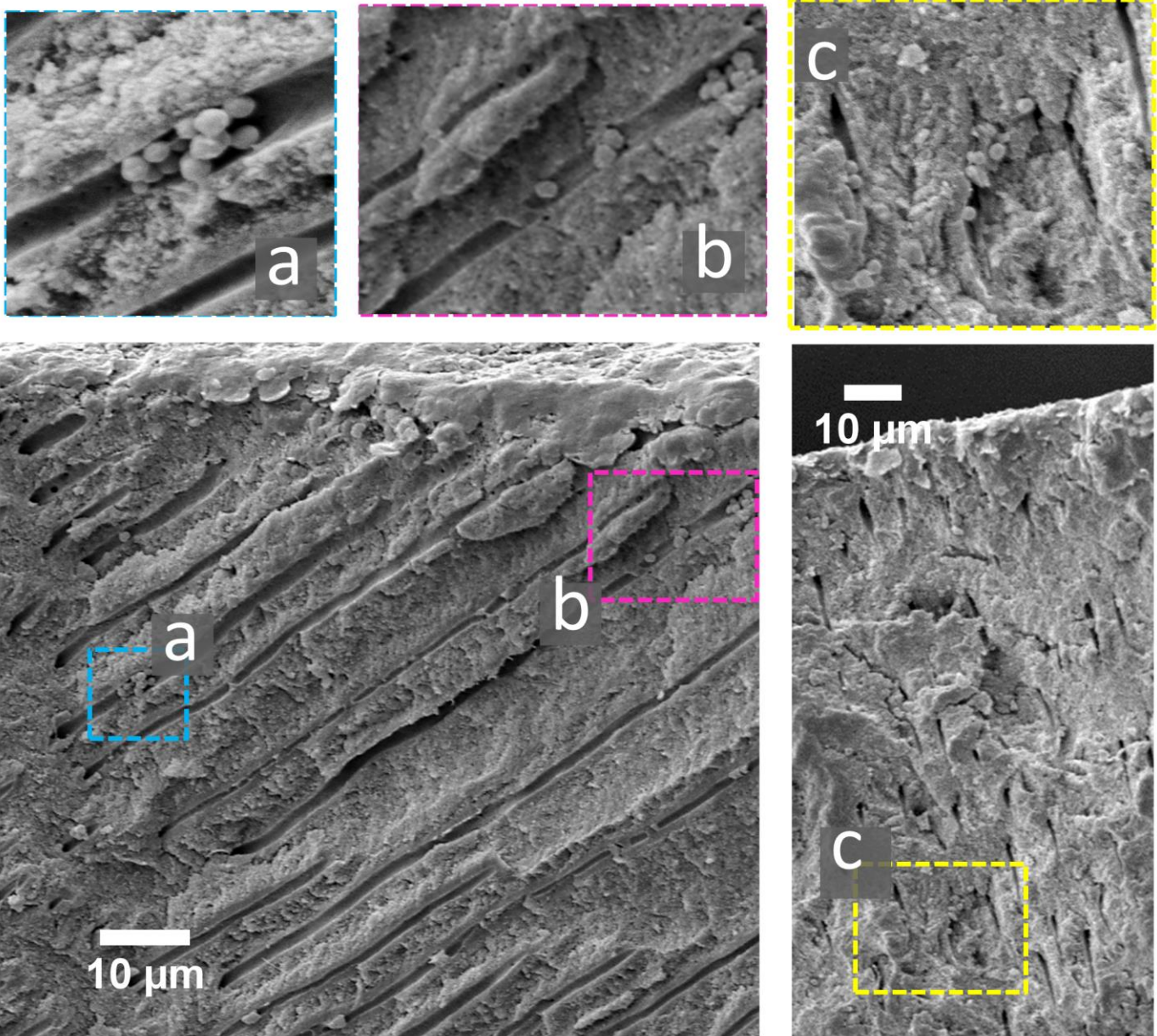
Figure 4 (a,b) Cross section of a dentine specimen with particles in water, without cavitation, showing penetration of the particles into the dentinal tubules. (c) Cross section of a dentine specimen after application with particles in acetone, without cavitation, showing penetration of the particles into the dentinal tubules.
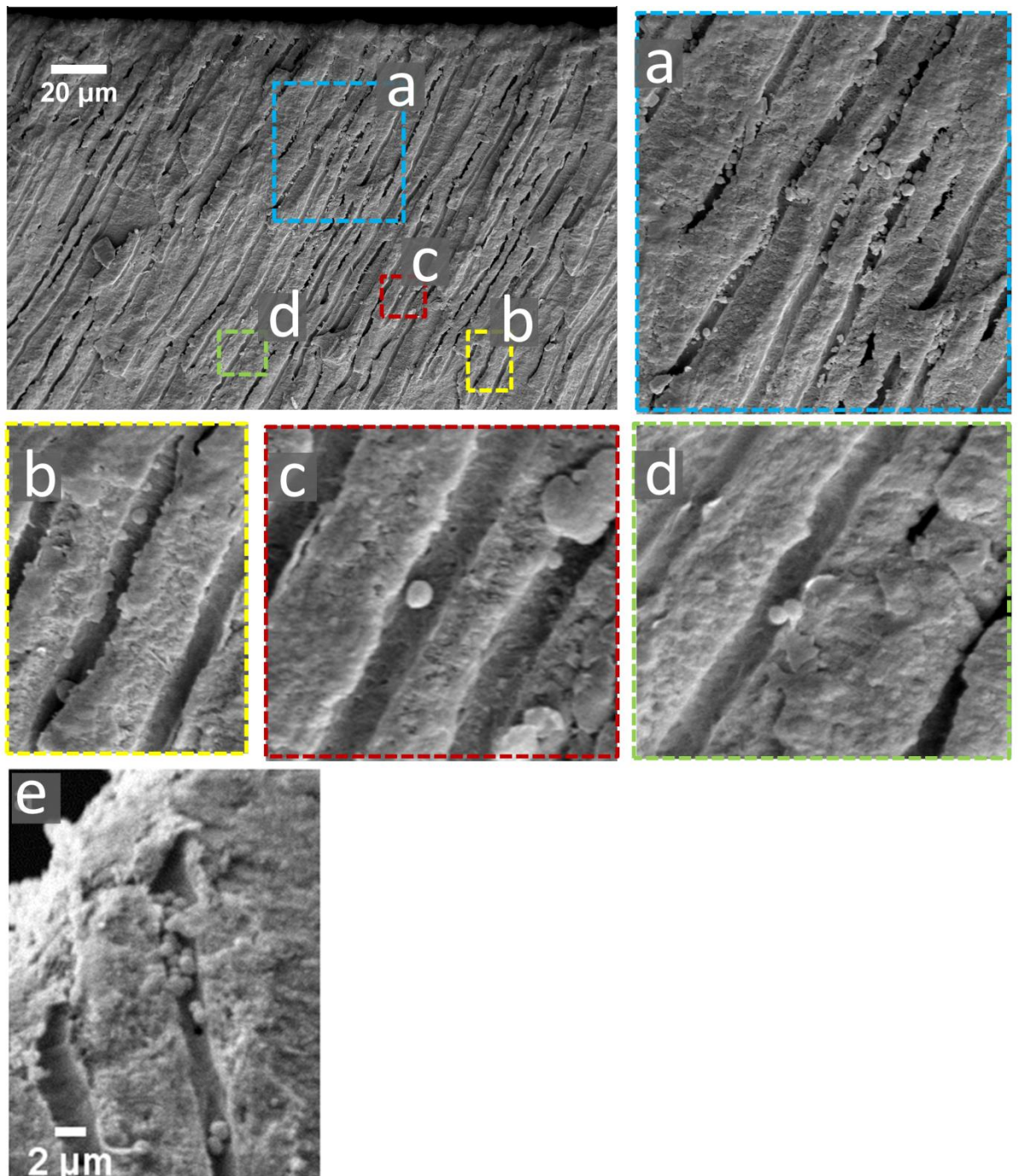
Figure 5 (a-e) Cross section of dentine specimens with particles in acetone with cavitation applied, and corresponding magnifications showing penetration of the particles into the dentinal tubules and occlusion near the surface (e).
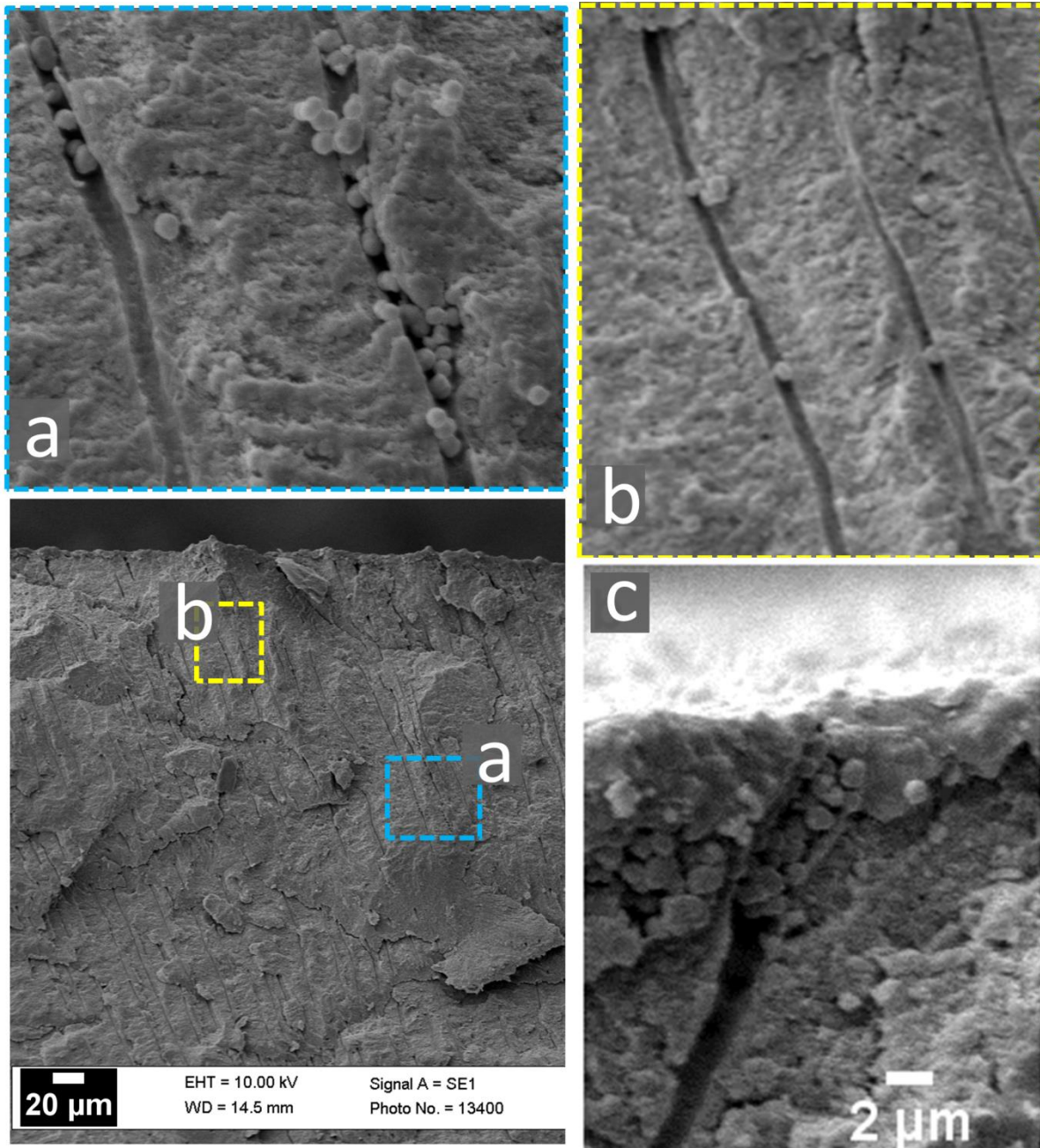
Figure 6 (a-c) Cross section of dentine specimens with particles in water with cavitation applied, and corresponding magnifications showing penetration of the particles into the dentinal tubules and occlusion near the surface (c).
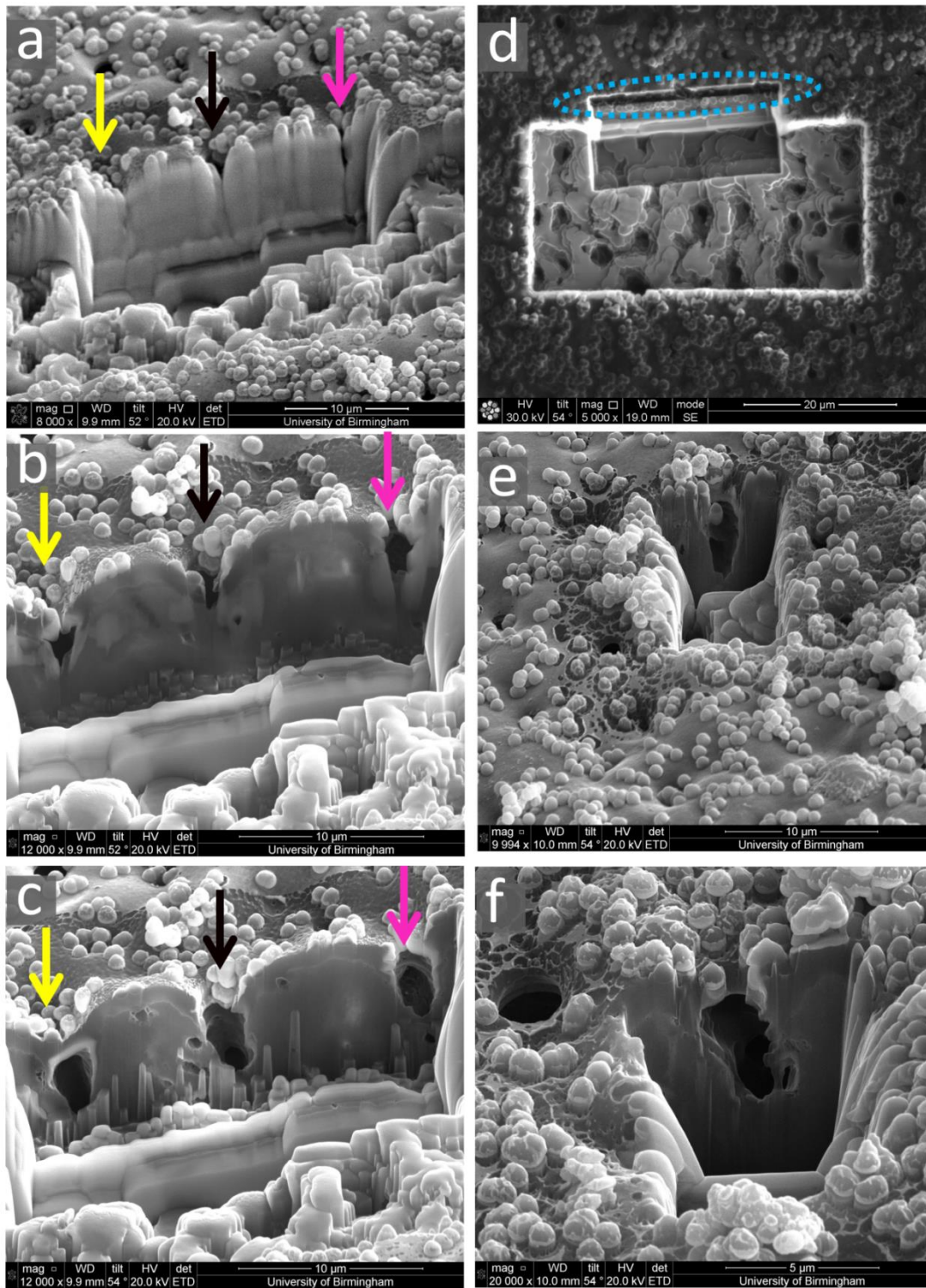
Figure 7 (a) (b) and (c): successive FIB-SEM images showing 3 tubules being milled by the FIB beam. The tubules are not occluded under the surface. Viewing angle: 52/54⁰ (d) FIB secondary electron image showing the trenches milled to acquire images a,b and c. The area shown in a, b and c is circled in blue. (e) and (f): SEM images of single tubules after being milled by the focussed ion beam. Viewing angle: 54⁰