Sonochemical production of nanoparticle metal oxides for potential use in dentistry

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Abstract

Two types of ultrasonic instruments used in dentistry have been compared with a sonochemical horn for the production of copper oxide and zinc oxide nanoparticles. The nanoparticles can be produced from benign reagents using dental instruments as the source of sonochemical enhancement. The process has been operated in resin models of teeth in a preliminary exploration of the potential of the method for enhancing procedures such as root canal surgery. The techniques is potentially useful but further work is needed for a full assessment of using in-situ generated nanoparticles as an aid in disinfection during some types of dental surgery.

Highlights

- Ultrasonic dental instruments produce sufficiently intense sound fields to perform sonochemistry
- Zinc and copper oxides have been produced sonochemically using benign reagents
- Reactions have been performed under conditions simulating dental surgery

Keywords: cavitation; dental instrument; nanoparticle; metal oxide; sonochemistry

1. Introduction

This paper describes work that combines two areas in which ultrasound has been extensively applied – preparation of nanoparticulate materials and dentistry – in a preliminary attempt to demonstrate a potentially new method for enhancing enhance the effectiveness of some dental treatments.

Ultrasound technology has found a number of uses in dentistry over many years[1]. At a straightforward level, ultrasonic cleaning is used for the preparation and sterilization of instruments[2]. More relevant to this work, a metal tip oscillating at ultrasonic frequencies – often 25 – 35 kHz – is used in *descaling*, the mechanical removal of plaque, biofilm and other deposits from teeth[3]. Other instruments are used for cutting and shaping teeth and bone during extraction or dental surgery[4] Ultrasonically vibrating needles and files are employed in ultrasonically activated irrigation (UAI) during root canal treatments[5, 6]. A dentist will gain access by drilling through the enamel and dentine to remove diseased internal tooth pulp using UAI.

A common application of sonochemistry has been the preparation of nanomaterials[7, 8]. All of the various effects of cavitation – rapid heating and cooling within collapsing bubbles, high shear forces and the generation of reactive intermediates such as organic or hydroxyl radicals have been exploited. Nanoparticulate metals including iron[9], gold[10], silver[11] and platinum[12] have been produced as have a range of mixed metal nanoparticles (NPs). Oxide[13] and sulfide[14] materials have also been produced. In many cases[15], the use of ultrasound gave control over particle size or morphology and allowed the preparation of structures not possible by more conventional techniques. Several of the materials that can be produced sonochemically such as silver, copper oxide and zinc oxide have been shown to be toxic towards bacteria and algae[16] including those implicated in causing dental disease. Gedanken and co-workers[17, 18] have exploited these antibacterial properties to produce textiles impregnated with silver or zinc oxide NPs which are stable and retain their antiseptic properties for extended periods of time. Research into the use of

nanoparticles in dentistry has also demonstrated that they may be useful for a range of applications[19].

So how could these seemingly unrelated applications of ultrasonics be combined? Recent work, including by the present authors, has shown unambiguously that significant levels of cavitation[20, 21, 22] can be generated around ultrasonic dental instruments. Both qualitative studies using *e.g.* luminol mapping to visualize cavitation and quantitative measures such as Fricke or terephthalate dosimetry have been used. It was also demonstrated that sonochemical reactions[23] such as the bleaching of a dye due to reaction with hydroxyl radicals can be promoted by dental ultrasound sources. The hypothesis for the current work was that employing sonochemically promoted synthesis of nanoparticles as a final stage during endodontic treatment could deposit antibacterial NPs into the fine root canals and/or embedding onto the inner dentine surfaces leading to longer lasting root canal fillings by preventing reocurrence of infection. The first stage was to investigate whether nanoparticle synthesis could be achieved using dental sources. This was followed by attempts to conduct the synthesis in situations simulating a clinical environment.

2. Experimental

2.1 Materials

All reagents were analytical grade or better, obtained from Sigma-Aldrich Co., UK, unless otherwise stated, and were used as received. The water used to prepare solutions was deionised water from a MilliQ system and had a resistance > 10 MΩ.

2.2 Ultrasound Sources

The ultrasound horn used was a Sonic Systems L500-20 Sonic Processor operating at a nominal frequency of 20 kHz fitted with a ~ 1 cm diameter tip. The intensities used were measured using calorimetry in the usual manner[24] while the quoted displacements are taken from the generator setting. The dental equipment was powered by an Electro Medical Systems (EMS) miniMaster

Piezon Scaler (Nyon, Switzerland) into a piezoelectric transducer mounted in a handpiece at a nominal frequency of 30 kHz (Figure 1). Tips of varying shape and size to accommodate different dental procedures can be fitted to the handpiece; two types were used in this work. The first was a tip (EMS 'Tip – A') usually used for enlarging and preparing root canals in teeth while the second type was a file normally used for fine endodontic work (EMS 0.35 mm). For this preliminary exploration, the former was operated at half-power, corresponding to an intensity of 2.5 ± 0.6 W cm^{-2}, while the endodontic tip was operated at the manufacturers recommended maximum setting, corresponding to an intensity of 0.56 ± 0.14 W cm^{-2} both measured calorimetrically as reported previously[25]. Quantification of sound intensities is more complex here than with a horn since emission does not occur consistently along the length of the tip. However, the values quoted are sufficient for comparative purposes. Likewise, the vibration amplitude varies along the length. For comparison purposes the maximum displacement values are quoted below, the detailed values having been published previously[22].

Figure 1. Ultrasound sources used in this work. (left to right) 20 kHz sonochemical horn, EMS Tip A, EMS0.35

2.3 Cavitation Analysis

The cavitation activity of the sonochemical sources were assessed by monitoring the emission of (sono)chemiluminescence from luminol solution[26]. Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) solution was prepared by dissolving in deionised water 1×10^{-3} mol dm^{-3} luminol (Aldrich, 97%), 0.1 mol dm^{-3} sodium carbonate (Fisons, ≥ 99.9%), 1×10^{-4} mol dm^{-3} hydrogen peroxide and 1×10^{-4} mol dm^{-3} ethylenediamine tetraacetic acid (EDTA; Acros Organics, 99%), and adjusting to pH 12 by addition of sodium hydroxide (ACS reagent ≥ 97.0%)[27]. The solution was stored in the dark and kept for no more than two weeks. A Canon EOS 500D digital single-lens-reflex camera with a Canon EF-S 60 mm f/2.8 USM Macro lens set

at ISO 3200 was used to record the emission from ~ 40 cm^3 of solutions with exposures between 30 s and 180 s. The resolution was 15 Megapixels. The apparatus and camera were placed in a light-proof box. The intensity of the luminol emission was calculated using[28] ImageJ with the background image for the silent reaction subtracted from the image. All further image post-processing was performed with the aid of ImageJ. For the work reported here, no quantitative analysis was performed so the editing process was not considered to influence any conclusions drawn.

For Fricke dosimetry, 20 cm^3 of a solution of 0.001 mol dm^{-3} $FeSO_4.7H_2O$ (BDH, ≥ 99.5%) and 0.005 mol dm^{-3} H_2SO_4) contained in a jacketed vessel was sonicated with the sonochemical horn at 20 ± 2 ˚C. 3 cm^3 of solution was removed for analysis at 30 s or 1 min intervals and returned to the vessel after measuring the absorbance at 304 nm due to Fe^{3+} using an Agilent 8453 UV-visible spectrometer. Using the endodontic tips, the reaction was carried out directly in a 4 mL cuvette, with care taken to keep the metal component away from the walls. The low intensities and short duration of sonication meant that no temperature control was necessary; the temperature rose by < 1 ˚C. To eliminate any thermal effects, aliquots of the FeSO4 solution were heated to a range of temperatures between 20 and 40 ˚C; no changes in absorbance were observed.

Hydroxyl radical production was quantified using terephthalic acid dosimetry[29] by recording fluorescence spectra with a Perkin Elmer 4300 fluorimeter using excitation and emission wavelengths of 340 nm and 425 nm respectively. A 0.002 mol dm^{-3} solution of terephthalic acid (Aldrich, 98%) in 0.005 mol dm^{-3} sodium hydroxide buffered to pH 6-11 with non-fluorescent phosphate buffer solutions (Fisons) was prepared and 50 cm^3 (sonochemical horn) or 20 cm^3 (dental source) of the solution sonicated at 20 ± 2 ˚C. Aliquots of 3 cm^3 were taken, their fluorescence emission measured and returned to the vessel.

2.4 Tooth models

Bovine incisor teeth, obtained from a local abattoir after sterilization, were used to construct a realistic dental model. Moulds of the teeth were made in Siligum silicone compound (Pébéo) following the manufacturers' instructions. When set, the tooth was removed and the cavity – duplicating the shape of the tooth - filled with an acrylic clear resin (East Coast Fibreglass Supplies). A short piece of plastic tube was inserted to provide a cavity in the tooth mould to simulate a root canal access and the resin allowed to set hard.

2.5 Nanoparticle Production

The preparation of copper oxide nanoparticles, CuO-NPs, was based on the method[30] of Angi *et al.* and involved a solution of 0.9990 g of copper acetate, $Cu(O_2CCH_3)_2$ (Sigma-Aldrich, ≥ 99.0%) in 50 cm^3 of distilled water being held under nitrogen gas for at least 30 minutes in a vessel maintained at 20 ± 4 ˚C. 10 wt% hydrogen peroxide was dropwise over the first 3 min of sonication and 1 cm^3 aliquots removed periodically for analysis by UV-Visible spectrophotometry at 750 nm. On completion of the experiment, the mixture was centrifuged at 4500 rpm for 45 min and the resultant solid oven dried at 80 ˚C overnight. Analysis was performed using a BRUKER AXS D8 Advance, equipped with a Vantec-1 detector, using CuKα radiation for powder X-Ray Diffraction (PXRD) and a JEOL JSM6480LV scanning electron microscope (SEM) fitted with an Oxford INCA X-ray analyser for elemental analysis and mapping.

The method[31] of Askarinajad *et al.* was adapted for the synthesis of nanoparticulate zinc oxide (ZnO NP) work. Solutions of 0.20 mol dm^{-3} zinc acetate, $Zn(O_2CCH_3)_2$ (Sigma-Aldrich, ≥ 99.0%) and 0.01 mol dm^{-3} NaOH were prepared and mixed in a 1:4 ratio. A 3 mL sample was removed and the UV-Visible spectrum recorded. The sample was returned to the reaction vessel, maintained at 20 ± 4 ˚C and sonication commenced. For the ultrasound horn, experiments were run for 60 min with aliquots taken every 10 min; for the descaler tip, samples were taken each 1 min for 10 min. Blank experiments were performed for each source by using the same setup in the absence of ultrasound. The solid ZnO was recovered by centrifuging the product for 1 h at

4500 rpm and oven drying at 80 ˚C overnight. Analysis was performed using PXRD and SEM as above.

3 Results and Discussion

3.1 Characterization of sonochemical activity

The first stage in the work was to confirm that cavitation occurred around the dental ultrasound sources and that it was possible to use them to initiate suitable chemical reactions. Figure 2 shows sonochemiluminescence emission when the instruments were operated in luminol solution. Emission from a sonochemical horn system has been described previously[32] and consisted of the usual cone of high luminescent activity directly under the horn tip. The cavitation field around Tip A was clear from the characteristic blue emission concentrated around the middle of the tip. Previous work [20] on similar systems has shown that this corresponds with the vibration antinodes along the length of the tip. The image for the EMS0.35 file suggested only a very small level of cavitation that was hardly distinguishable from the background even at the maximum exposure available (Figure 2(b)).

Figure 2. Images for emission from luminol solution for (a) Tip A (Exposure 90 s at 2.5 ± 0.6 W cm^{-2} and (b) the EMS0.35 tip (Exposure 180 s at 0.56 ± 0.14 W cm^{-2})

In an attempt to quantify the varying levels of cavitation, two dosimetry methods were used. Figure 3 shows the results from the terephthalate dosimeter, which is specific for hydroxyl radical production, and the Fricke dosimeter which indicates the total level of oxidation reactions occurring in the system. As expected the results show that oxidation reactions can be initiated by each of the sources since each was active within the Fricke system. Interestingly, the dental sources showed similar levels of activity to the sonochemical horn when operated at low intensity. As noted above, the quoted intensity values for these sources is difficult to quantify as ultrasound

is not emitted from the whole area; local intensities may be much higher. The thinner endodontic source did not produce observable amounts of hydroxyl radicals in agreement with the emission from luminol described above.

While these results indicate that sonochemical reactions can be promoted by the two dental sources, the low levels indicated for the endodontic EMS0.35 file led us to concentrate the remainder of this preliminary study on the other Tip A.

Figure 3. Dosimetry results for sonochemical systems (a) Fricke dosimeter (b) terephthalate dosimeter. ○ Sonochemical horn, 21.8 ± 2.8 W cm^{-2} ; displacement = 8 μm ● Tip A 2.5 ± 0.6 W cm^{-2} ; maximum displacement = 45 μm □ Sonochemical horn, 5.3 ± 0.7 W cm^{-2}; displacement = 2 μm ◆ EMS0.35 0.56 ± 0.14 W cm^{-2}

3.2 Production of nanoparticles

The reaction to form CuO was selected since it involves an oxidation reaction using hydrogen peroxide and so is likely to benefit from sonication of the type produced by sonochemical systems. It uses benign reagents and so could potentially be used in a dental situation. Angi and co-workers[28] reported a rapid reaction using a sonochemical horn, albeit in relatively low yield, to form particles with diameters of 100 – 200 nm. In our hands, a black precipitate of CuO formed rapidly using both the horn and the dental Tip A. However, the particle size, as shown in Figure 4 was significantly higher than previously reported. This suggests that the reaction is occurring very quickly on a local scale so that particle growth is rapid. XRD analysis showed that, with both sources, CuO was formed although a large amount of amorphous material also resulted.

Differences in the morphology of the particles produced in the two systems were notable; those from the dental source had a clear layered structure within each particle rather than the less well defined particles produced on the horn. A reason for this loss of structure could be that with the more intense source, the particles were formed more quickly and so the large particles are formed of many individual small layers. Alternatively the higher intensity may have caused many

interparticle collisions due to increased mixing which would break up the structured particles and cause agglomeration to give the particles shown.

Figure 4. Comparison of CuO NPs produced using the dental Tip A and the sonochemical horn at an intensity of 15.7 ± 2.0 W cm^{-2}. Upper images: magnification × 500; Lower images: magnification ×5000

Attention was then turned to zinc oxide particles. Again the procedure was chosen to avoid the use of toxic or corrosive reagents, unlike the more common preparative methods[33] using ethylene or propylene glycols. Again, ZnO particles were produced with both ultrasound sources while blank experiments conducted in the absence of ultrasound produced solid products only at extended reaction times. Powder X-ray diffraction analysis confirmed by comparison with literature sources[34] that ZnO in the Wurtzite structure was the only observable product (Figure 5). The mechanism by which ZnO is formed is not entirely clear but probably involved the formation of $Zn(OH)_2$ as an intermediate which then transforms via elimination of water to ZnO. A range of particle shapes and sizes were observed with agglomerates of small particles. Similar effects have been observed in the sonochemical synthesis of e.g. calcium carbonate[35].

Figure 5. PXRD patterns of sonochemically produced ZnO

Scanning electron micrographs of the products revealed a large range of particle sizes but a significant proportion of the samples were in the nanoparticle range. The sample produced on the sonochemical horn had a different appearance in the laboratory. Elemental mapping of the sample indicated why, as shown in Figure 6. The particles are well under 1 μm in diameter and the larger particles look to be agglomerates of smaller versions. More significantly, the element maps all showed high amounts of zinc and oxygen, as expected, but some of the particles indicate the presence of large amounts of titanium. The source of the titanium was suspected to be from wear

of the horn tip. To confirm this, the preparation was repeated in an ultrasound bath, where no titanium contamination could occur. In none of these products, or those arising from the dental tip, was titanium detected (a typical example is shown Figure 7). As a caution to other workers in the area, we conducted several preparations of silver nanoparticles on the sonochemical horn and a significant number of titanium particles were detected.

Figure 6. Elemental analysis of ZnO produced on the sonochemical horn. Spectra (a) and (b) indicate only ZnO; spectra (c) and (d) indicate the presence of titanium.

Figure 7. Elemental analysis of ZnO produced on the dental Tip A.

Figure 8. SEM micrographs of ZnO. (a) ultrasound bath (× 500); (b) Dental Tip A (× 500); (c) no ultrasound (× 500); (d) Dental Tip A (× 4500); (c) no ultrasound (× 5000)

Figure 8 shows SEM micrographs of the ZnO particles. The sizes of the particles are not dramatically different between the different experiments although there is a higher density of smaller particles in the sonicated samples. The surface of the sonicated particles appears smoother at higher magnification, presumable due to interparticle collisions and/or shock waves from collapsing cavitation bubbles[36].

Having shown that the dental source could promote the formation of ZnO, attempts to simulate operation during a dental procedure were attempted. Model teeth were prepared from an acrylic resin and holes drilled to simulate root canals, as shown in Figure 9. Note that the teeth used are rather larger than human teeth, to facilitate these preliminary experiments. The cavity was filled with approx. 0.1 cm^3 of the zinc acetate / sodium hydroxide solutions described above. Some of the liquid was expelled due to the ultrasonic action and was replaced during the experiment. Sonication was maintained for 5 - 10 min in order to reproduce the timescale of a

dental treatment. Blank experiments were conducted in the same manner without switching on the ultrasound.

Figure 9. Bovine teeth and resin models. The arrows indicate the drilled channels (3 mm diameter)

Figure 10 shows the SEM micrographs of a resin model. After treatment, the resin cast was dried and broken open to image the inside of the cavities. To obtain the micrographs, the resin had to be coated with gold to prevent charging which may influence the resolution achieved in the images. It was important to note that the lower magnification images show only slight differences suggesting that no damage was done to the surface of the resin as a result of potential cavitation effects. There is some suggestion of particulate matter in the larger magnification image of the resin that had been subjected to ultrasound but these are rather smaller than expected for ZnO NPs. It may be that the majority of particles, once formed, do not embed within the walls of the cavity and so are washed away during cleaning.

Notwithstanding the absence of large numbers of individual particles, elemental mapping of the inside of the cavities was carried out with typical results shown in Figure 11. No signal for zinc was found in cavities that had not been exposed to ultrasound. In contrast, it was clear that exposure to ultrasound resulted in some zinc being strongly attached to the cavity walls. The signal is weak due to partial obscuring by the gold coating. The washing and cleaning process was the same for each resin model so that the presence of the zinc is correlated with the ultrasound treatment.

Figure 10. SEM micrographs of resin tooth models treated with ZnO.

Figure 11. Elemental mapping of resin cavities treated with ZnO. (a) blank experiments (no ultrasound) (b) irradiated with endodontic Tip A for 10 min.

3.3 Discussion

Our results show a very preliminary attempt to demonstrate a new approach to using ultrasound to enhance the effectiveness of dental surgery. Using instruments typically used in dental procedures, metal oxide nanoparticles can be prepared from readily available, benign compounds over a timescale compatible with, for example, the cleaning and rinsing phases of root canal surgery Optimisation of concentrations, instrument design and treatment regimes should allow better control over particle size. The resin models employed here involved a single large cavity. Teeth have a series of microscopic tubules running through the dentine and soft tissue that can be around 1 μm in diameter so that nanoparticles could be deposited within the tubules without blocking them. The approach has been suggested previously for introducing agents to assist tooth repair[37, 38, 39] although these have all had to be prepared in advance. Synthesis during treatment would offer advantages and flexibility in use. Recent work by Walmsley and co-workers [40] has shown that it is possible to deposit silica particles coated with a surfactant into teeth, offering a vehicle for introducing therapeutic agents. Using our approach would deposit material that could remain active for some time releasing ions that act as a disinfectant to kill remaining bacteria and to prevent reinfection. The dental ultrasonic instrument used here has a broader shape compared to the 'needles' typically used to clean root canals. The latter have been shown to produce cavitation, albeit in smaller amounts so that there is a need to increase the cavitation and focus it in the smaller dimensions of the tooth. The scaler is used on the outside of the tooth and there may be further benefit to this aspect of the tooth structure from the *in-situ* production of nanoparticles. Considerable work also needs to be done in determining the long term efficacy of such a nanoparticle approach as well as to optimise the treatment but our initial results suggest an interesting avenue of approach.

4 Conclusion

Our work has shown that ultrasonic instruments used in dentistry produce sufficient sound intensities to initiate sonochemical reactions similar to those using a sonochemical horn. Copper oxide and zinc oxide nanoparticles, known to be toxic to a range of bacteria have been produced under conditions that simulate root canal treatments. The method shows promise as a preliminary exploration of the possibility of using in-situ generated nanoparticles as a disinfection aid during some types of dental surgery although a good deal of further work is needed for a full assessment of its potential.

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