SUPPLEMENTARY INFORMATION

Ultrasound activated silica particles for efficient eradication of dental biofilms

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Materials, Methods and Instrumentation

Dynamic Light scattering (DLS) and **ζ-potential** data were recorded by a Malvern Panalytical Zetasizer ZS instrument equipped with a H-Ne 633 nm laser at 25 ºC and with a backscattering of 173° using milliQ water as a dispersant. The instrument was controlled with a Malvern DTS 7.03 software. All sizes were determined based on 5 measurements containing 11 runs on each. ζ-potential was determined after measuring 100 runs per sample at 140v, 25 °C in triplicate.

Solid-state UV/Vis spectra were recorded using a Cary 5000 dual beam UV/Vis spectrophotometer in reflectance mode equipped with a solid-state attachment. **Solution UV/Vis spectra** were recorded using a Cary 5000 dual beam UV/Vis spectrophotometer or a Cary 60 UV/Vis spectrophotometer.

Nitrogen porosimetry. The N_2 sorption analysis was performed at 77 K on a Nova porosimeter instrument in the relative pressure range 10−6–0.99 P/P_0 to avoid N_2 condensation on the micrometre scale. The samples (50–70 mg) were dried and degassed at 150°C overnight in the Nova instrument. The samples were analysed using 12 mm wide glass tubes. The pore size distribution was obtained using the 3Flex Software using the heterogeneous surface nonlinear DFT model (HS-NLDFT).

Scanning Electron Microscopy (SEM) images were recorded in a XL30 ESEM-FEG (Phillips) under a high vacuum at 20 kV electron beam and 20,000-25,000x magnification. SEM samples were prepared by mounting onto a carbon coated aluminium stub before splutter coating with gold (Quorum Emitech K550X splutter coater). **Transmission Electron Microscopy (TEM)** images were recorded in a JEOL 1400 electron microscope and FEI TECNAI F20 (Phillips). TEM samples were prepared by placing 0.1 mg/mL of silica particles in 1 mL of MilliQ water, then a drop of the suspension was placed on a TEM grid (carbon coated copper film, 200 mesh) and dried. **Scanning TEM (STEM)** was carried out on a Talos™ F200X G2 TEM (Thermo Scientific™). All images for particle-size were analysed by ImageJ software.

Thermogravimetric analysis was performed in a TGA 8000 PerkinElmer thermogravimetric analyser, controlled by a Pyris Software version 13.3.1.0019. Samples were loaded into ceramic sample crucibles, circa 6.6 mm in diameter and 1.95 mm in height. Samples were dried at 120 ºC for 2 h before the measurement. The temperature is held at 30 ºC for 1 min then increased from 30 to 1000 ºC at 2 ºC min^1. Finally, samples were cooled down from 1000 ºC to 30 ºC at a 30 ºC min^{-1} rate. All thermal decompositions are performed under nitrogen flow.

Mass spectrometry (MS) analyses were performed on a Bruker ultrafleXtreme instrument. A pulsed Nd:YAG laser at a wavelength of 355 nm was operated at a 100 Hz frequency. The course was operated in the positive mode with a delayed extraction time of 30 ns. Data were acquired and processed with the Flex Analysis 3.10 software. Samples (1 mg) prepared for matrix-assisted laser desorption/ionization time-of-flight/time-of-flight (MALDI-TOF/TOF) MS were added to a matrix (100 uL) α-cyano-4-hydroxy-cinnamic acid (CHCA) 10 mg/mL in MeOH:ACN + 1% formic acid + 100 uL MeOH) and 20 µL was spotted on a steel MTP384 anchor chip plate.

Fourier-transform infrared (FT-IR) spectroscopy was performed on samples using the Varian 640-IR spectrometer (Agilent Technologies).

Potassium iodide Oxidation

The chemical effects of cavitation can be monitored by the production of I_3^- by the oxidation of I^- ions from OH radicals formed by cavitation in water.

A 0.2 M solution of KI dissolved in MilliQ water was used and ultrasound was applied at P5, P10, P15 and P20 for 5 mins (n = 3). The absorbance of I_3^- was measured (Cary60) at λ = 355 nm using

a 1 mL quartz cuvette with path length of 1 cm. The rate of reaction was calculated using the following equation:

$$k = \frac{AV}{\varepsilon l t}$$

Where k is the rate of reaction (mol s^{-1}), ε is the molar absorption coefficient of I_3^- (ε = 26,303 $dm^3mol^{-1}cm^{-1}$), A is the absorbance, l is the cuvette length (1 cm), V is the solution volume (dm^3), and t is sonication time (s).

Synthesis of SiO_2

The Stöber method with modifications was used to synthesise the non-porous silica nanoparticles.[1, 2] First a solution containing EtOH (25 mL), NH_4OH (1.9 mL, 0.93 M), H_2O (1.6 mL, 2.9 M) and TEOS (2.4 mL, 12.4 mmol, 0.41 M) were added together. The reaction was left to stir (750 rpm) for 9 h at rt. SiO_2 were isolated as a white solid (0.32 g); diameter 280 ± 60 (DLS by intensity distribution), PDI = 0.31, ζ-potential = - 45 ± 6 mV

Synthesis of m-CPC⊂SiO_2

The Stöber method with modifications was used to synthesise the non-porous silica nanoparticles.[1, 2] First a solution containing EtOH (25 mL), NH_4OH (1.9 mL, 0.93 M), H_2O (1.6 mL, 2.9 M) and TEOS (2.4 mL, 12.4 mmol, 1 equiv, 0.41 M) were added together. The reaction was left to stir (750 rpm) for 3 h at rt. After 3 h solution of CPC (0.465 g, 1.36 mmol, 0.1 equiv) in H_2O (3 mL) was added and stirred at RT for 6 h. SiO_2 were isolated as a white solid (0.54 g); diameter 420 ± 50 nm (DLS intensity distribution), PDI = 0.23, ζ-potential = - 32 ± 6 mV.

Preparation of MCM-41 (mesoporous) silica nanoparticles

Mesoporous silica nanoparticles (MCM-41s) were synthesised as follows. C_{16}TAB (0.25 g, 0.68 mmol) and NaOH (12.5 mM, 1.5 mmol) were dissolved in ultrapure H_2O (120 mL), then TEOS (1.25 mL, 5.6 mmol) was added dropwise over 10 min. The solution was stirred at 750 rpm and heated to 80 °C for 2 h. The white precipitate formed in the reaction was isolated by centrifugation (15 min, 7830 rpm) and washed with water three times. For the removal of the surfactant template (CTAB), the isolated white solid was calcinated at 600°C for 8 h. The nanoparticles are centrifuged and washed with MeOH. The nanoparticles were dried under vacuo to yield a white solid powder (0.56 g); diameter 160 ± 40 nm (DLS intensity distribution), PDI = 0.07, ζ-potential = - 21 ± 5 mV. .

Synthesis of CPC@MCM-41

The previously synthesised MCM-41 were added to a solution of CPC dissolved in H_2O (2 mg/mL). The mixture was stirred at room temperature for 24 h. After **CPC@MCM-41** was isolated by centrifugation (15 min, 7830 rpm) and dried under vacuo to yield a white solid powder (0.73 g); diameter 135 ± 25 nm (DLS intensity distribution), PDI = 0.37, ζ-potential = + 4 ± 5 mV.

Synthesis of templated drug loaded CPC⊂MCM-41

CPC (250mg) was dissolved in ultrapure H_2O (120 mL) with NaOH (12.5 mM, 0.15 mmol), then TEOS (1.25 mL, 5.6 mmol) was added dropwise over 10 min. The solution was stirred at 750 rpm and heated to 80 °C for 2 h. The **CPC⊂MCM-41** particles were washed and isolated by centrifugation (15 min at 7830 rpm) then dried under vacuo to yield a pale-yellow solid powder (0.46 g); diameter 200 ± 53 nm (DLS intensity distribution), PDI = 0.31, ζ-potential = - 36 ± 5 mV.

Drug loading/encapsulation

The drug concentration was measured from absorbance using UV-Vis spectroscopy with a Cary60 UV-Vis spectrophotometer of supernatants and subsequent washings.

Encapsulation efficiency (%) is calculated as the percentage of drug that is successfully entrapped into the nanoparticle eq (1):

$$Encapulation\ efficiency = \left(\frac{total\ drug\ added - free\ non\ entrapped\ drug}{total\ drug\ added}\right) \text{x } 100. \quad eq\ (1)$$

The encapsulation efficiency is converted into weight percent (wt%) eq (2):

$$\frac{\left(\frac{Encapsulation\ efficiency}{100}\right) x\ total\ drug\ added}{mass\ of\ nanoparticles\ synthesised}\ x\ 100 \qquad eq\ (2)$$

***In vitro* release experiments**

For all samples, dry solid particles were immersed in the release media of ultrapure H_2O (10 mg/mL) at pH 7.4, 37°C with and without exposure to US. For a measurement of release, 1 mL of the particle suspension was removed and centrifuged for 5 min at 7830 rpm. The supernatant was collected and analysed by UV/Vis spectrometry by monitoring drug absorbance at the wavelength maximum (λ_{max}). The concentration of drug was calculated by its calibration curve that determined the absorption coefficient (ε, $M^{-1}cm^{-1}$). The amount of drug (μg) per drug loaded SiO_2 (mg) could then be determined. The drug solution was then returned to the stock media and release process was repeated.

Ultrasound-responsive triggered drug release at short intervals

To confirm the drug release from the SiO_2 structure with an external trigger the ultrasonic scaler (P5 Newtron XS, Satelec, Acteon, France) with a US frequency of 28 KHz and tip 10P was used for mechanical cavitation at low, medium, or maximum power (P=10, 15 or 20 respectively). The power settings relate to the dial control on the ultrasonic scaler. The ultrasonic scaler hand-held piece was positioned inside a 50 mL centrifugal tube, immersed in 10mL release media, and fixed at distance of 10 mm above the SiO_2. Subsequently, cavitation was applied in 2 min intervals using the tip of the hand-held piece. After each cycle, 1 mL of the particle suspension was removed and the measurement procedure from *in vial* release experiments was followed.

The power of each setting on the scaler is calculated as follows:

Power (W) = Amps (A) x Volts (V)

Power setting	Handpiece current (mA)	Volts (V)	Power (W)
P10	52	5.2	0.27
P15	76	7.6	0.58
P20	100	10	1

Biological studies

General procedure & chemicals

Brain heart infusion (BHI, CM1135, Oxoid, Dorset, uk) broth and agar (BHI, CM1135, Oxoid, Dorset, UK) were used to culture *Streptococcus sanguinis* (ATCC 10556), from frozen stocks stored at -80°C. Bacteria from frozen stocks were streaked on BHI agar and incubated overnight (37 °C, 5 % CO_2 environment). Liquid cultures were prepared with a single colony in 5 mL BHI broth before incubating for 20 h in an orbital shaker (37 °C, 100 rpm).

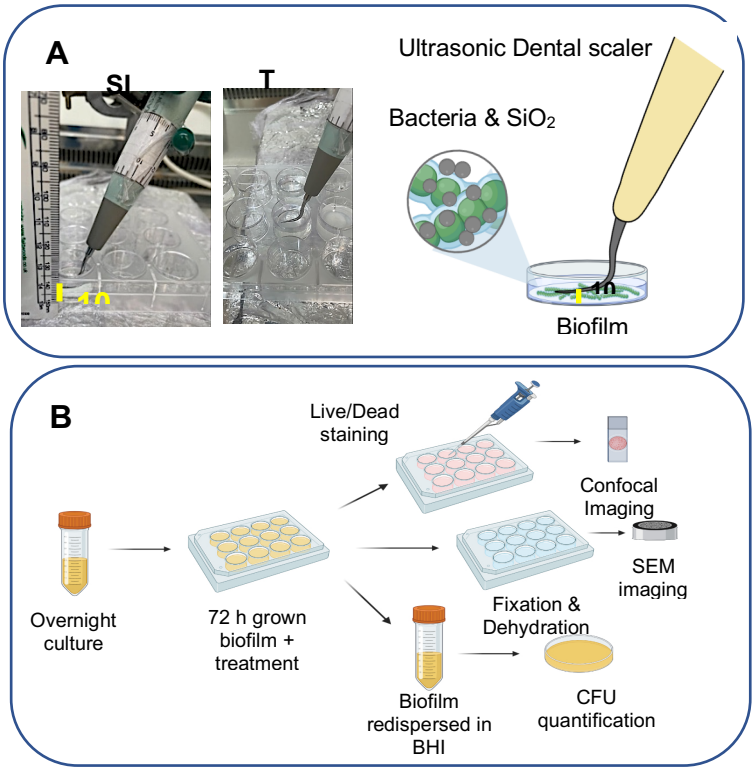
Minimum inhibitory concentration (MIC) assay

The MIC of antimicrobial agents or particles was determined as follows. Overnight cultures were prepared in BHI broth (5 mL) as described above. The OD_{600} of the overnight culture was adjusted to 0.001 by diluting the bacterial inoculum with fresh BHI broth. Sterile sample stock solutions were made and 100 µL of required dilutions were added to 100 µL of diluted culture into a 96-well plate.The plate was then incubated for 24 h at 37 °C, 5 % CO_2. For controls: 1:1 ratio of bacteria to BHI broth as positive control, 200 µL BHI broth was used to show no contamination (negative control) and an empty well to obtain background reading of the well plate. The growth and no growth were determined visually and by measuring the OD_{600} using a microplate reader (ELx800, BIO-TEK instruments Inc.) in triple triplicates (N=9) with the Gen5 data analysis software for all measurements.

Agar Diffusion Assay

Overnight bacterial cultures were prepared. BHI agar plates were inoculated by swabbing with an overnight culture of bacteria to create an evenly distributed lawn of bacteria. In each agar plate, five wells of 6 mm in diameter were cut with the larger end of a sterile pipette tip, approximately equal distances apart. Each well was loaded with 50 µL of sample from stock solutions. Afterwards the plates were incubated at 37 °C in 5 % CO_2 for 20 h in static conditions. At the end of incubation time the bacterial growth was confluent on the agar surface, except at areas of growth inhibition where there was a clear zone surrounding the well. The zones of inhibition were measured using a millimetre ruler. All experiments and measurements were performed in triplicates and the mean and standard deviations were obtained. All samples were dispersed in ultrapure water (negative control).

Biofilm formation model



To prepare artificial saliva a modified method described by Prattern *et al.*[63] was used. To ultrapure water (1 L) the following were added sequentially, accurately to 0.001g: Sodium chloride (NaCl, 0.35 g, VWR 102415K), potassium chloride (KCl, 0.2 g, Sigma 60129), calcium chloride ($CaCl_2$, 0.2 g, WVR 007103020), yeast extract (2 g, Oxoid LP0021), lab lemco powder (1 g, Oxoid L29), hog gastric mucin (type III, partially purified) (2.5 g, Sigma M177) and protease peptone (5 g, Oxoid L85). The mixture was left to stir for 1 h, then autoclaved. After sterilisation 40 % sterile urea (1.25 mL, 0.5 g urea, Oxoid SR20, in 1.25 mL ultrapure H_2O) was added using a 0.22 µm filter. Once the urea was added the media was wrapped in aluminium foil to exclude light and prevent protein degradation and stored at 4 °C. For biofilm BHI medium: 1 % sucrose was added to sterilised BHI broth. First the sucrose (3.5 g, Sigma S0389) was mixed with sterilised filtered ultrapure H_2O (3.5 mL) then added to BHI broth (350 mL) with a sterile syringe.Phosphate buffered saline (PBS, Dulbecco A, Sigma P4417) solution was prepared by dissolving one tablet in ultrapure H_2O (200 mL) and autoclaved for sterilisation. *S. sanguinis* were used to form a single species biofilm model for treatment with SiO_2 and ultrasonic scaler. An overnight culture was prepared. Thermo Scientific™ Nunc™ Thermanox™ Coverslips (13 mm diameter, cell culture-treated one side) were added to a 24-well plate and primed with artificial saliva for 15 min.[63] The artificial saliva was removed and the overnight bacterial suspension was diluted to $1x10^3$ in fresh BHI medium with 1 % sucrose and 1 mL was added to each coverslip. The 24-well plate was incubated at 37°C, 100 rpm for 24 h. Afterwards the medium was removed and replaced with 2 mL fresh medium. This was repeated two more times to obtain biofilms grown for 72 h.

Biofilm viability studies & ultrasonic scaler experimental set-up

The Thermanox coverslips were then moved to a 12-well plate, and either treated with the sample solution and no cavitation or with sample solution and cavitation and then incubated at 37°C, 100 rpm for 30 min. The biofilm treatment process was achieved using a Satelec P5 Newtron XS scaler with a 10P tip in all experiments. The handpiece of the scaler was fixed to a manual clamp stand and adjusted to 10 mm from the biofilm surface and the orientation of the tip was kept in a horizontal position relative to the biofilms. The biofilm coverslips were fixed in place to the surface of 12-well plate using double-sided tape and wells were filled with H_2O (3 mL) to completely immerse the tip surface of the scaler (note the cooling water flow unit part of the scaler was not used in these

experiments). The ultrasonic scaler was operated at low (P10), medium (P15) or high (P20) power for 10 seconds.

Live/dead staining assay

Filmtracer™ LIVE/DEAD® Biofilm Viability Kit (Invitrogen, California, USA) was used to stain the biofilms after treatment and incubation. A stock solution of the stain was prepared with SYTO® 9 stain (3 µL) and propidium iodide (3 µL) in filter-sterilised ultrapure H_2O (1 mL). The biofilms were washed with PBS solution and the staining solution (~200 µL) was added gently to the biofilm. The biofilms were incubated for 20-30 min at room temperature and protected from light exposure with foil. The stain was removed, and biofilms were washed by rinsing gentle once with PBS to remove all excess strain. To fix the biofilms, the coverslips were placed onto microscope slides (biofilm facing up) and a drop of Invitrogen™ ProLong™ Gold Antifade Mountant (ThermoFisher Scientific, Massachusetts, USA) was added before putting a 22 x 26 mm cover glass on top. The slides were left to solidify and dry for 24 h and were stored in the dark to preserve fluorescence.[3]

Confocal laser scanning microscopy imaging

The biofilms' viability was imaged with a confocal laser scanning microscope (LSM 700, Carl Zeiss GmbH, Germany). The images were obtained with the Zeiss Zen lite 2011 software. A x40 oil immersion objective (Zeiss Objective EC Plan-Neofluar 40x/1.30 Oil DIC M27, FWD = 0.21 mm) in combination with immersion oil (Immersol™ 518F) was used. The emission/excitation wavelengths for the stains were 488 nm/<550 nm for SYTO® 9 and 555 nm >550 nm for propidium iodide. Both the green and red channels were imaged together with an image size of 1024 x 1024 pixels. Five random locations were scanned on each biofilm sample and 2-3 Z-stacks of 10-30 µm optical thickness separated by 1.30 µm increments from the surface were obtained for each condition. Z-stacks were examined to calculate the biofilm thickness and for 3D visualisation analysis.

Image analysis of single-species biofilms

The percentage of live and dead bacteria in each image was determined from the confocal fluorescence images. An automated computation method developed and described by S. E. Mountcastle *et al.*[4] was used to evaluate the cell viability. The macro created was a method carried out using the ImageJ software (version 2.1.0). This was achieved by splitting the confocal fluorescence images into two different channels (green and red) and converted into an 8-bit image. A series of erosion, reconstruction and dilation steps had been incorporated into the script to perform on each channel (element size 3). The total bacteria were calculated by number of pixels in the image (green and red), differentiating between background noise depending on intensity values. A Gamma command (set at 1.5) was used to correct for uneven fluorescence intensities and allowed for detection of faint bacteria. Then segmentation and thresholding were performed by using the Otsu's threshold. The output was presented as white pixels, which the number of pixels corresponding to the dead (red) bacteria was calculated. This was used to determine the area of dead bacteria, followed by the total number of white pixels, which were used to calculate the total area of all bacteria. Finally, the percentage of viable cells (green) were calculated from the total area of bacteria and red bacteria. The macro is used based on the assumption that the image contains a single-species biofilm and output is a percentage of live cell area.

Colony counting method - colony forming units (CFUs)

Live bacterial numbers were also obtained by counting the number of colony forming units.[67] After treatment the coverslips containing the biofilms were removed from each well and redispersed by using the vortex for 2 min in fresh BHI broth (3 mL). A serial dilution was performed using the Miles and Misra method[67] to count the number of CFUs. BHI broth (180 µL) was aliquoted into each well of a 96 well plate with 20 µL of samples for the first dilution (1×10^{-1}). Subsequently, 20 µL was taken from the first dilution and added to the next well with BHI broth, repeating the process to 1×10^{-8} dilution. For each dilution, 20 µL aliquots were spotted onto BHI agar plates three times. This was repeated for each sample and all plates were incubated at 37 ºC in a 5 % CO_2 environment overnight. Following this, the number of CFU/mL was determined for each sample prepared in triplicate.

Scanning electron microscopy (SEM) imaging

For further visualisation of bacterial biofilms, samples were prepared for SEM imaging by a fixation and dehydration procedure described by Dysktra *et al*.[5] The biofilms were rinsed three times with PBS to remove the culture medium. For fixation: the samples were immersed in 2.5% EM grade glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 for 10 min. Note the fixative must be prepared fresh but can be kept up to 1 week in the fridge. For a 10 mL stock solution of the fixative, ultrapure H_2O (4 mL), 0.2 M sodium cacodylate buffer pH 7.3 (5 mL, prepare in advance, pH with sodium hydroxide, the pH is important) and 25% EM grade glutaraldehyde (1 mL, CAS 111-30-8, Sigma-Aldrich) were mixed.Dehydration of each specimen was performed by removing water slowly in ethanol solutions of increasing concentration, for at least 10 min in each %: 20, 30, 40, 50, 60, 70, 90, 95 (twice) and 100 % (twice). The ethanol (100 %) was completely removed and hexamethyldisilane (HMDS, CAS 999-97-3, Sigma-Aldrich) was added quickly to cover the surface before the sample could dry out and left to evaporate overnight in a fume cupboard. After the fixation and dehydration process the biofilms were prepared for imaging with the SEM. The biofilm coated coverslips were adhered onto aluminium stubs using 12 mm carbon adhesive tabs (Agar Scientific) and secured with single-sided conductive copper tape. All samples were sputter coated with gold (Quorum Emitech K550X, Kent, UK) before insertion into the SEM. The morphology and changes to the bacteria before and post treatment were examined using a Zeiss Evo MA-10 SEM (Carl Zeiss Jena GmbH, Germany) and SmartSEM software. Imaging was performed at x10-25 k magnifications with a working distance of 8-10.5 mm and accelerating electron beam voltage of 20 kV.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) cell viability assays. Cytotoxicity of studied nanoparticles was assessed by MTT cell viability assays using an epithelial squamous cell carcinoma cell line (H400). Cells were seeded at 10,000 cells per well in 96-well plates for 24 h at 5 % CO_2, 37°C, >90% humidity for attachment to occur. The next day, the media was removed and fresh media (200 μL) containing the nanoparticles was added. Following treatment for 18 h (at 5 % CO_2, 37°C, >90% humidity) the media was removed, and the cells were incubated with 50 μ L of fresh media containing 1 mg/mL MTT solution for 2 hours at 5 % CO_2, 37°C, >90% humidity. The media was removed and reduced MTT was solubilized by adding DMSO (200 μ L). The absorbance at 570 nm was measured in a plate reader (Tecan Infinite 200Pro) as an indicator of mitochondrial reductive activity and cellular viability. All samples were normalised to a (DMSO) blank, and the results represent the mean of three experiments carried out in triplicate (n= 3 ± SD).

Statistical analysis

All statistical analyses were performed in GraphPad Prism (v. 5.03). In biofilm viability studies the percentages of live and dead *S. sanguinis*, were represented by the mean with standard deviation for each group. Normality tests were performed to determine the distribution of data in each group using Kolmogorov-Smirnov, D'Agostino & Pearson and Shapiro-Wilk tests for comparison. Statistical tests performed are described in figure captions.

Table S1. Hydrodynamic diameter and ζ-potential measurements of SiO_2.

	D_H			
	Intensity	**Number**	**PDI**	**ζ-potential / mV**
SiO_2	280 ± 60	270 ± 65	0.31	-45 ± 6
m-CPC⊂SiO_2	420 ±50	420 ± 65	0.23	-32 ± 6

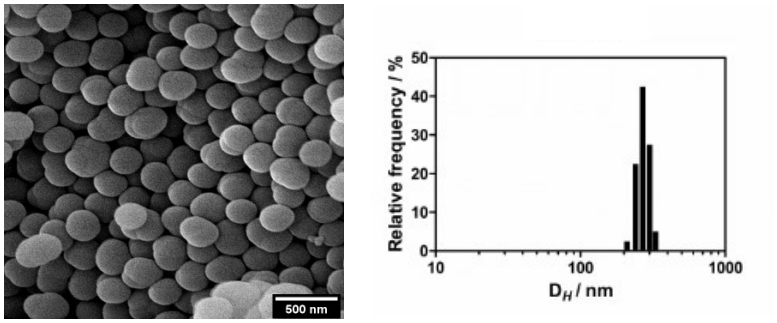


Figure S1 – Scanning electron microscopy (SEM) images and size distribution histograms of measured sizes of **SiO_2**. SEM magnification 35 kx**,** 20.0 kV electron beam. Histogram represents 40 independent measurements.

Table S2. Hydrodynamic diameter and ζ-potential measurements of MCM-41, **CPC@MCM-41** and **CPC⊂MCM-41** in water.

	D_h / nm				
	Intensity	**Volume**	**Number**	**PDI**	**ζ-potential / mV**
MCM-41	160 ± 40	155 ± 46	130 ± 35	0.07	-21 ± 5
CPC@MCM-41	135 ± 25	130 ± 30	120 ± 25	0.37	+4 ± 5
CPC⊂MCM-41	200 ± 53	210 ± 60	190 ± 50	0.31	+36 ± 5

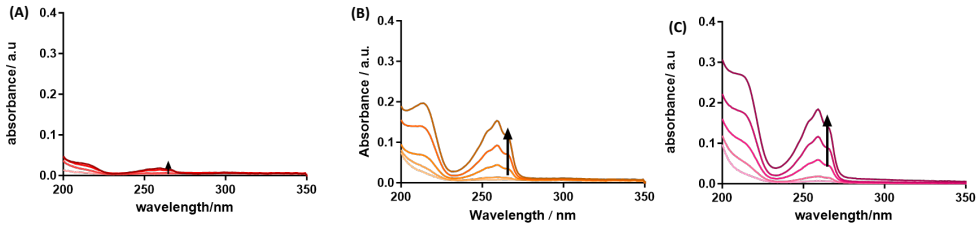


Figure S2. UV-Vis spectra of monitoring CPC release (associated with Figure 3) from **m-CPC⊂SiO$_2$** (2 mg/mL, H_2O, 37 °C) after exposure to (A) no US, (B) US P10, (C), US P15, 2 min pulses for a total of 10 min.

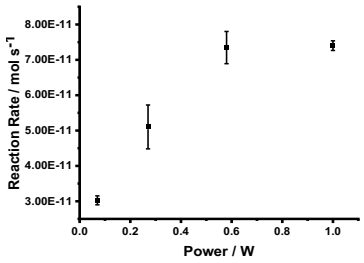


Figure S3. Effect of increasing power on the rate of oxidation of KI to I_3^- showing the chemical effects of cavitation.

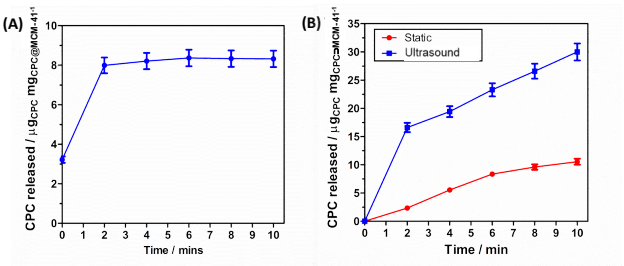


Figure S4. Drug release profile of adsorbed (a) **CPC@MCM-41** (2 mg/mL, H_2O, 37 °C, pH 7.4) and (b) **CPC⊂MCM-41** (2 mg/mL, H_2O, 37 °C, pH 7.4) under stimuli responsiveness from the ultrasonic scaler at P20, total 10 min. Release of CPC monitored by UV/Vis absorbance at λ_{max} 260 nm and CPC released (μg) per drug loaded NP (mg) calculated using TGA drug loading amount.

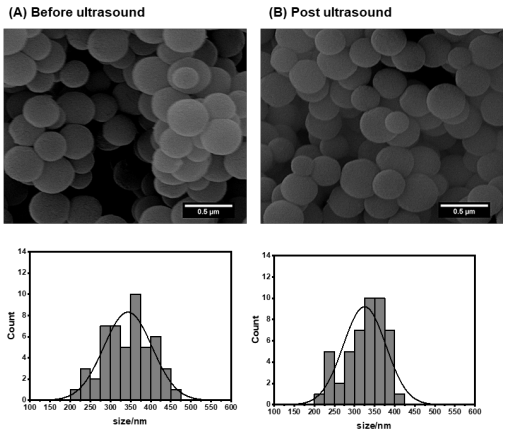


Figure S5. Scanning electron microscopy (SEM) of **m-CPC⊂SiO$_2$** (A) before ultrasound, (B) post ultrasound (P20, 10 min) and corresponding histograms of measured sizes (n=50). SEM magnification 35 kx, 20.0 kV electron beam.

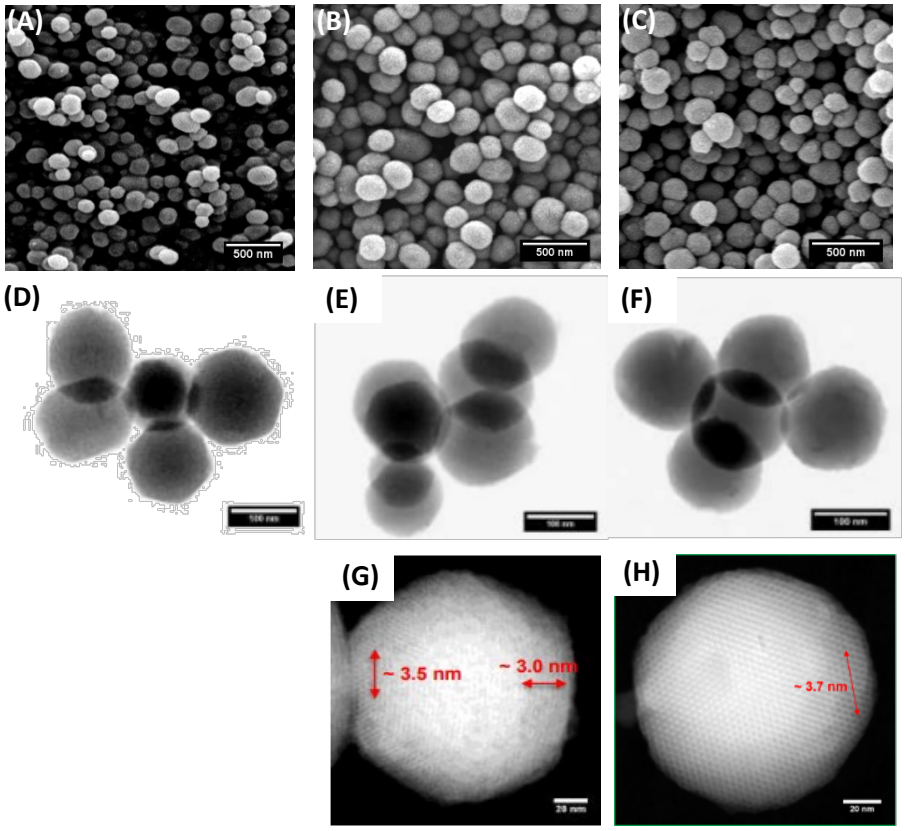


Figure S6. Scanning electron microscopy (SEM) images of (a) **MCM-41**, (b) **CPC@MCM-41** and (c) **CPC⊂MCM-41**. SEM magnification 35 Kx, electron beam 20 kV. Scanning transmission electron microscopy (STEM) micrographs of (d) **MCM-41**, (e) **CPC@MCM-41** and (f) **CPC⊂MCM-41**. HAADF-STEM to show porous network and sizes of pores in (g) **CPC@MCM-41** and (h) **CPC⊂MCM-41**. Images analysed with ImageJ software (version 2.1.0).

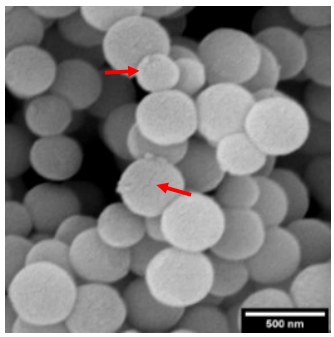


Figure S7. SEM of **m-CPC⊂SiO$_2$** in MeOH (1.5 mg/mL stirred at 400 rpm, 72 h). SEM magnification 35 Kx, 20.0 kV electron beam.

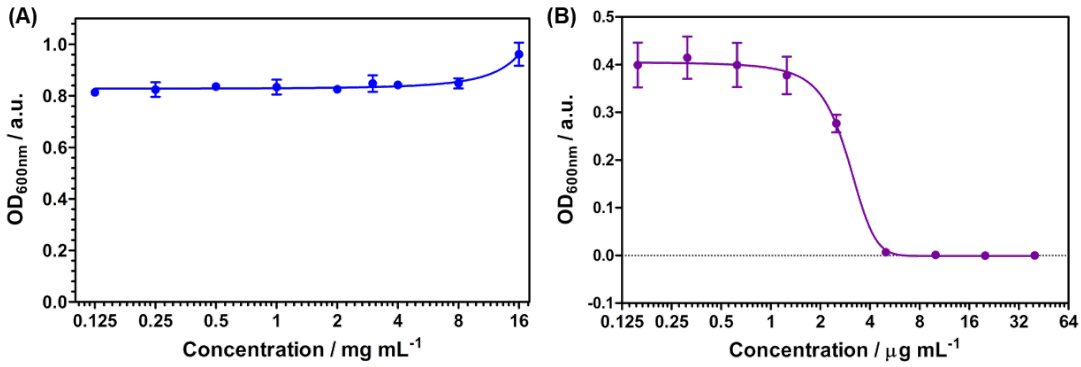


Figure S8. Minimum inhibitory concentration of *S. sanguinis* against a) SiO_2 a b) CPC measured by OD_{600} nm.

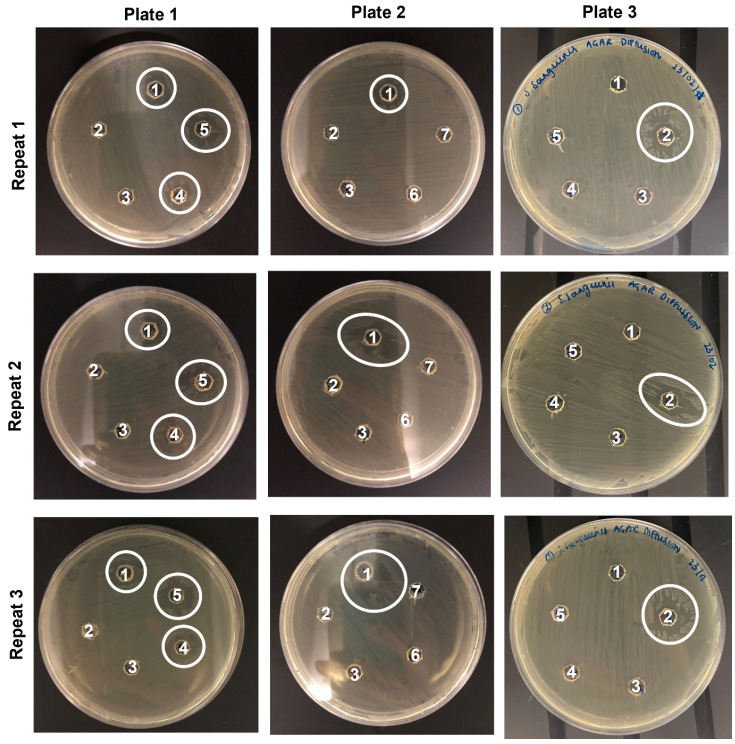


Figure S9. Agar diffusion assay showing zones of growth inhibition around wells with 50 µL of sample. Plate 1 shows: 1: CPC (0.05 % w/w), 2: **MCM-41** (2 mg/mL), 3: ultrapure H2O, 4: **CPC⊂MCM-41** (2 mg/mL), 5: **CPC@MCM-41** (2 mg/mL). Plate 2 shows: 1: CPC (0.05 % w/w), 2: SiO_2 (2 mg/mL), 3: ultrapure H2O 6: **m-CPC⊂SiO₂** (2 mg/mL), and 7: **m-CPC⊂SiO₂** (5 mg/mL). Plate 3: 1: H2O, 2: CPC (0.036 mM, 14 µg/mL), 3: ***m*-CPC⊂SiO₂** (2 mg/mL), 4: **m-CPC⊂SiO₂** (10 mg/mL) and 5: **SiO₂** (10 mg/mL). The diameters of the zones of inhibition were measured and averages calculated (n=3). Plates inoculated with *S. sanguinis*.

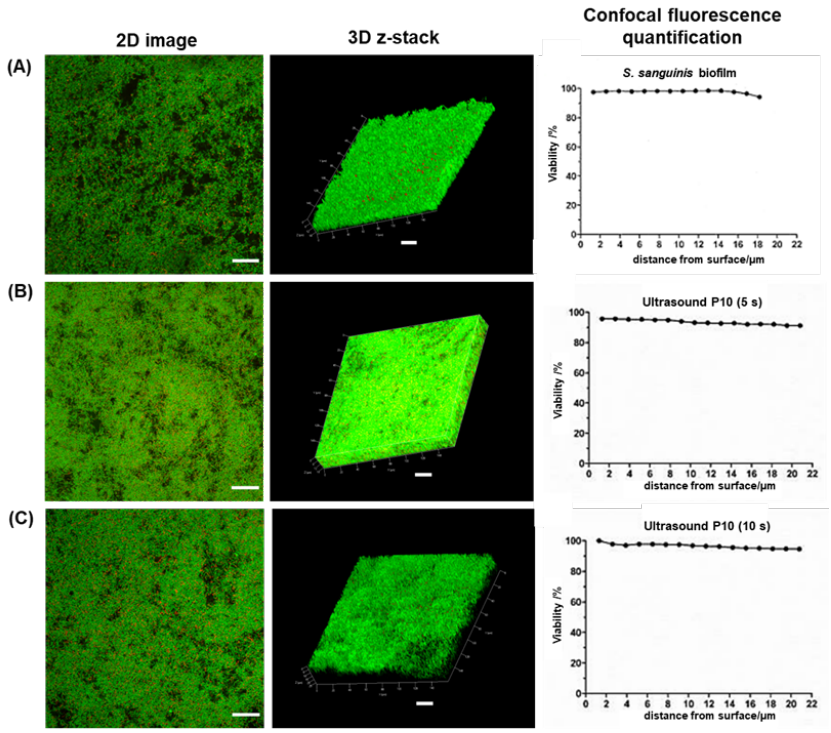


Figure S10. 2D and 3D confocal fluorescence images showing visual representation of biofilms and confocal fluorescence quantification of (a) control, (b) ultrasound P10 5 s, (c) ultrasound P10 10 s. Scale bar represents 2 µM and 20 µM in 2D and 3D images respectively. Viability depth analysis through 3D reconstruction. Image analysis was performed on at least five confocal images of each of three biological replicates (n=3) Average cell viability *S. sanguinis* with no cavitation (negative control) 90.27 ± 5.37 %, 5 s cavitation 92.37 ± 4.44 % ($p = 0.2529$) and 10 s cavitation 93.65 ± 4.11 % ($p= 0.0567$). Statistical analysis obtained using a Mann Whitney U, unpaired t-test (two-tailed, non-parametric).

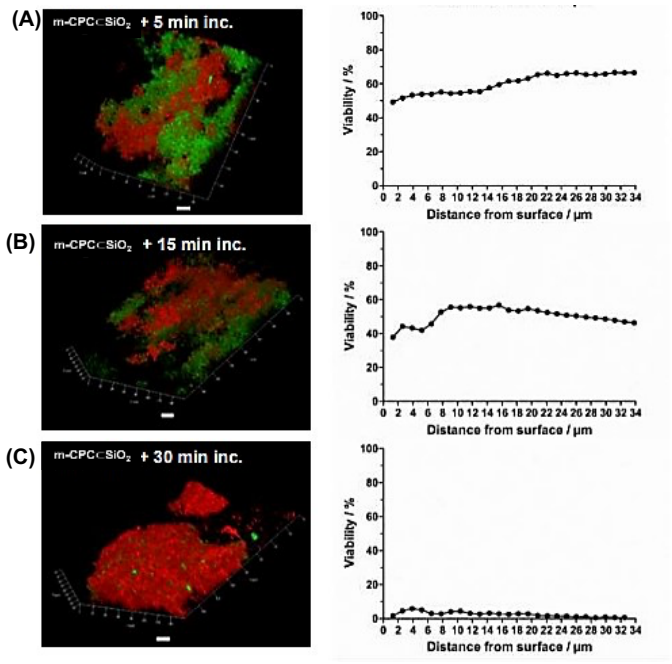


Figure S11. 3D confocal fluorescence images showing visual representation of biofilms after treatment with **m-CPC⊂SiO$_2$** (10 mg/mL) and ultrasound (P10, 10s) and incubation for 5-, 15- or 30 min. Scale bar represents 2 µM in 3D images. The 2D confocal fluorescence images were analysed with ImageJ to quantify the percentage of live bacteria and 3D images obtained by z-stacks.

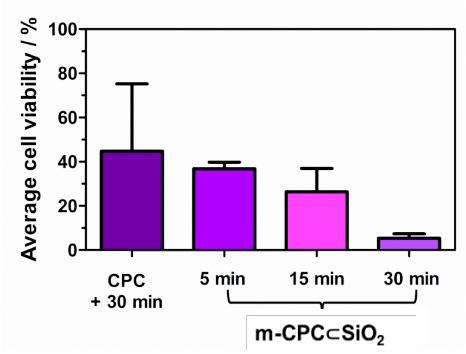
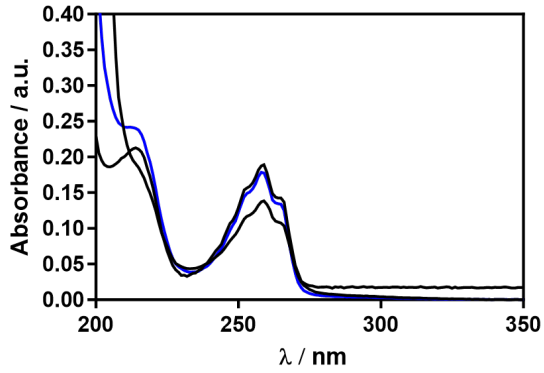


Figure S12. Average cell viability of *S. sanguinis* 72h biofilms after treatment with **m-CPC⊂SiO$_2$** (10 mg/mL) and ultrasound (P10, 10s) and incubation for 5-, 15- or 30 min incubation. Statistical analysis obtained using a one-way ANOVA non-parametric Kruskal-Wallis test, followed by a post-test conducted using Dunn's test multiple comparison. A value of P <0.05 was considered significant.



	CPC released (μg/mL)
Repeat 1	11.8
Repeat 2	15.2
Repeat 3	14.9
Average	14.0 ± 1.5

Figure S13. UV-Vis spectra of CPC (λ_{max} = 259 nm, ε = 4190 $M^{-1}cm^{-1}$) to determine the amount drug released from **mCPC⊂SiO₂** (10 mg/mL) triggered by ultrasound (P10, 10s) with 30 min incubation at 37 °C. The table show the average CPC calculated from each replicate for biofilm treatments.

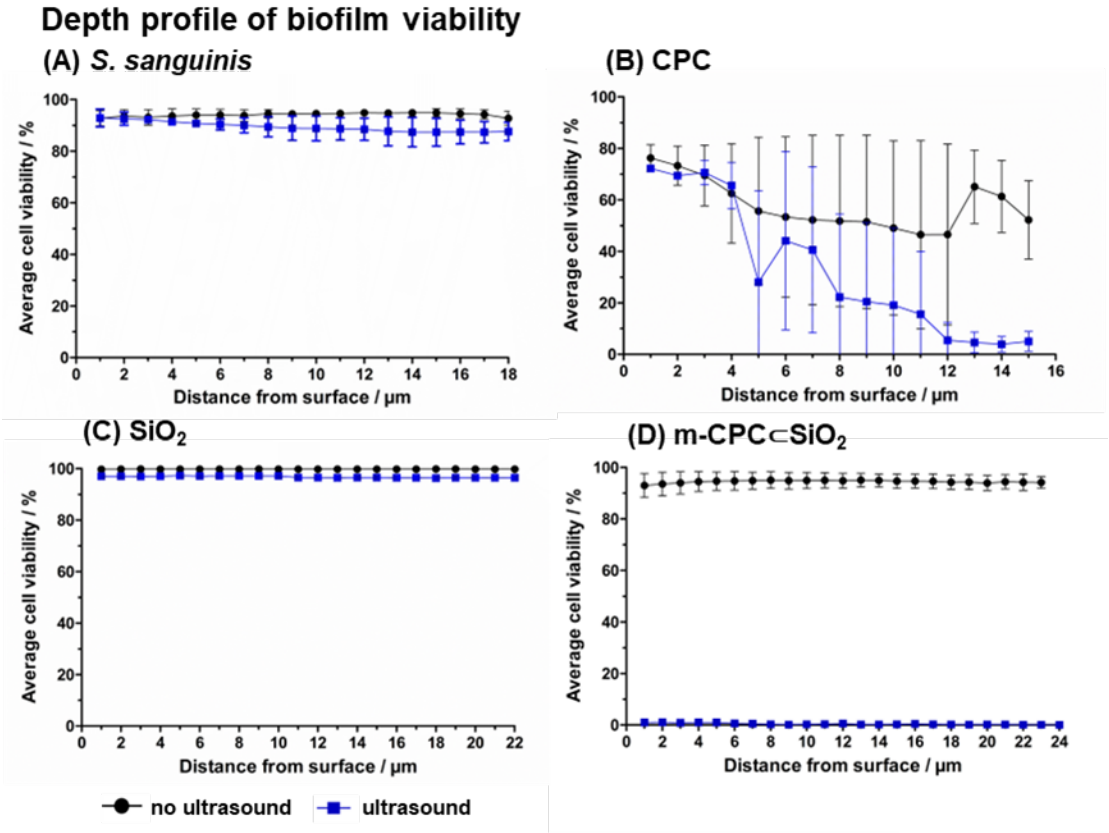


Figure S14. 2D images to form z-stacks were processed with automated image analysis and depth profile plotted to show viability throughout biofilm for (A) *S. sanguinis* biofilm with no treatment, (B) CPC (14 μg/mL), (C) SiO_2 (10 mg/mL), (D), **mCPC⊂SiO₂** (10 mg/mL). All conditions tested with and without application of ultrasound (P10, 10 s) and post-treatment incubation for 30 min. Analysis of statistical significance, non-parametric Mann WhitneyU test (p<0.05). Five random areas of each biofilm were analysed, and experiments repeated in triplicate.

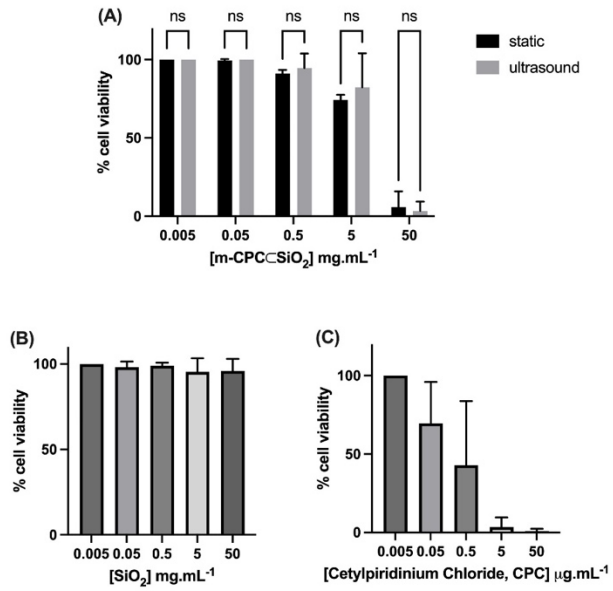


Figure S15. MTT cell viability assay using the H400 cell line incubated with (A) SiO_2, (B) CPC, and (C) **m-CPC⊂SiO_2** for 18 h. Significance was calculated based on 1-way ANOVA followed by Dunnett's t-test corrected for multiple comparisons, ns=no significance, n=3.

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