Reduction of *Pseudomonas aeruginosa* biofilm formation through the application of nanoscale vibration

Shaun N Robertson^{1,2,3}, Peter G Childs^{2,4}, Ayorinde Akinbobola¹, Fiona L Henriquez⁵,

Gordon Ramage⁶, Stuart Reid^{2,3}, William G Mackay^{1*}, Craig Williams¹

¹Institute of Healthcare, Policy and Practice, School of Health & Life Sciences, University of the West of Scotland, High Street, Paisley PA1 2BE, Scotland, UK

²SUPA, Institute of Thin Films, Sensors and Imaging, School of Engineering and Computing, University of the West of Scotland, High Street, Paisley PA1 2BE, Scotland, UK

³SUPA, Department of Biomedical Engineering, University of Strathclyde, 40 George Street, Glasgow G1 1QE, Scotland, UK

⁴Centre for the Cellular Microenvironments (CeMi), School of Engineering, University of Glasgow, G12 8LT, Scotland, UK

⁵Institute of Biomedical and Environmental Health Research, School of Health & Life Sciences, University of the West of Scotland, High Street, Paisley PA1 2BE, Scotland, UK

⁶School of Medicine, Dentistry and Nursing, MVLS, University of Glasgow, 378 Sauchiehall St, Glasgow G2 3JZ, Scotland, UK

***Corresponding author**: William Gordon Mackay, Institute of Healthcare, Policy and Practice, School of Health & Life Sciences, University of the West of Scotland, High Street, Paisley PA1 2BE, Scotland, UK. Phone. +44(0) 1418483000 Email: <u>w.mackay@uws.ac.uk.</u>

	Mean fluorescent intensity	
	Live (SYTO9)	Dead (PI)
Control 1	18.98	13.21
Control 2	14.4	6.88
Control 3	11.72	5.39
Control average	15.03	8.5
Nanovibrational 1	14.07	8.36
Nanovibrational 2	14.31	11.18
Nanovibrational 3	11.05	12.59
Nanovibrational average	13.14	10.71

Table S1 – Mean fluorescent intensity of Live and Dead biofilm images.

Randomly selected microscopic images of each biofilm were used to calculate the mean fluorescent intensity of the green channel (live – SYTO9) and red channel (dead - PI). Statistical analysis was performed using a student t-test between the control and nanovibrational average, there was no significant difference between the mean fluorescent intensity levels of live stained control vs. nanovibrationally stimulated (p = 0.3479) or dead stained control vs. nanovibrationally stimulated (p = 0.4579) staining.



Fig. S1 – Dehydrated ECM of control *P. aeruginosa* 10332 biofilm. Representative SEM images of *P. aeruginosa* 10332 control biofilms at 30 degree tilt. Dehydrated extracellular matrix visbile, a by-product of the dehydration steps used to prepare the samples for SEM analysis. Scale bar 10 μ m.



Fig. S2 - Generation of heat by the reverse piezo effect. (A) An IR laser thermometer was used to measure the apparent temperature of the piezo; this was performed with 3 separate measurements on 3 piezos for each state (on/off). The true temperature was calculated from the apparent temperature. (B) The temperature of the media was measured by use of a thermocouple; 3 separate measurements were taken of the media in each Petri dish at each time point in 3 Petri dishes for each state (piezo on/off). Data are mean \pm SD.



Fig. S3- Dye dispersal with and without nanovibrational stimulation at high frequencies. (A) No significant difference was noted between dye dispersal with and without nanovibrational stimulation at 1 kHz. (B) No significant difference was noted between dye dispersal with and without nanovibrational stimulation at 2 kHz. (A) A difference in dispersal was observed with and without nanovibrational stimulation at 4 kHz, likely due to non-uniform movement of the Petri dish (A) No significant difference was noted between dye dispersal with and without nanovibrational stimulation at 6 kHz. Data are mean \pm SD. n = 3.