Supporting Information:

Antimicrobial activity of Piezoelectric Polymer: Piezoelectricity as a reason for damaging bacterial membrane

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S1. PDLLA nanotexture morphology.

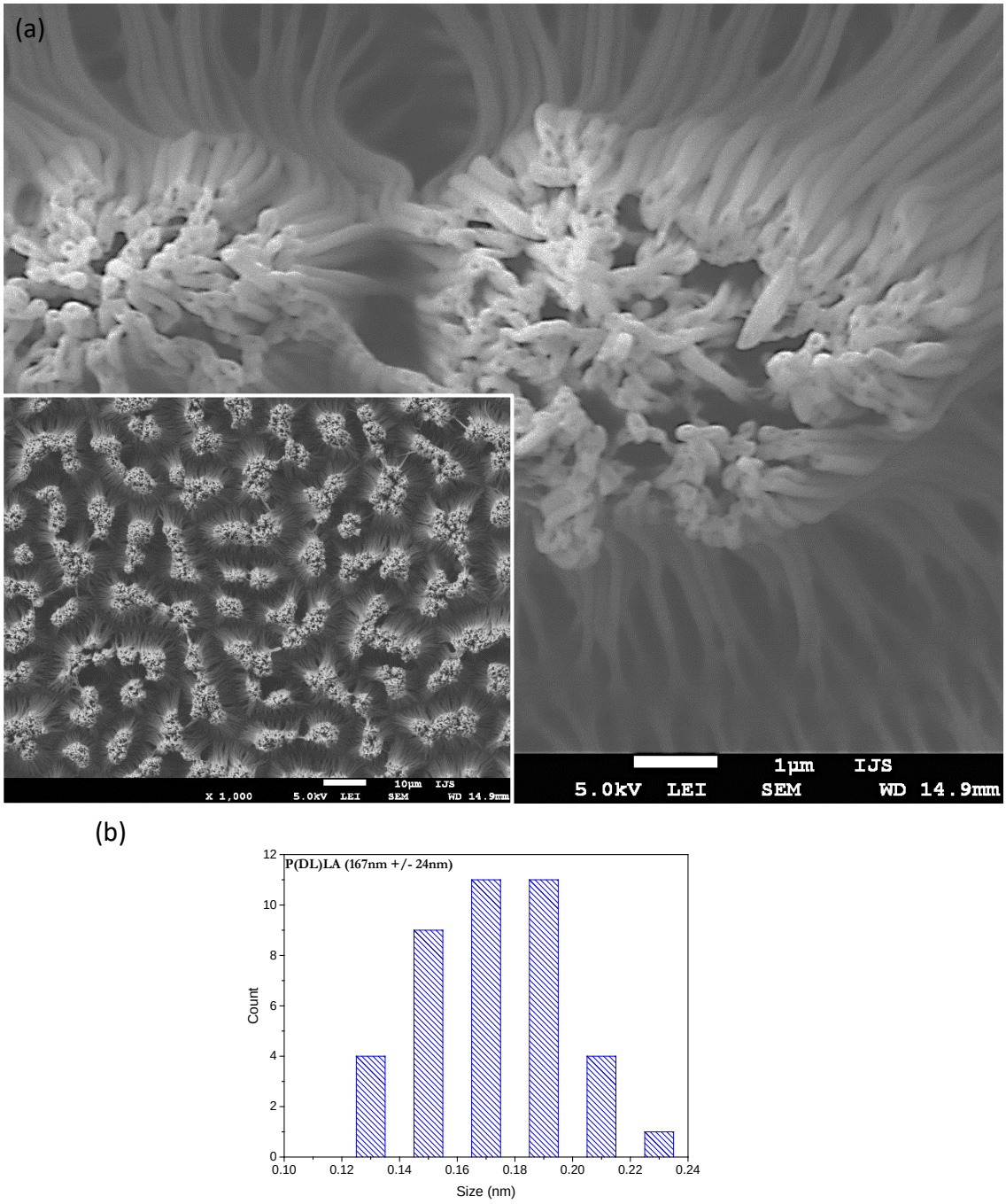

Figure S1. (a) SEM image of P(DL)LA nanotextured film and closer look confirming tube formation and (b) average diameter count from analysing SEM images.

Using the same template assisted method as we used to prepare piezoelectric nanotextured PLLA film we prepared reference sample as non-piezoelectric biodegradable nanotextured P(DL)LA film. Film was used for observing morphological effect on bacteria cells. After analysing some of the SEM images above 10,000x – 15,000x magnification, average pillar estimated diameter was 167 nm ± 24 nm, suggesting the full filling of the template pores (Fig. S1 (a,b)).

S2. Frequency signal comparison between piezoelectric and non-piezoelectric films under ultrasound (US) stimulation.

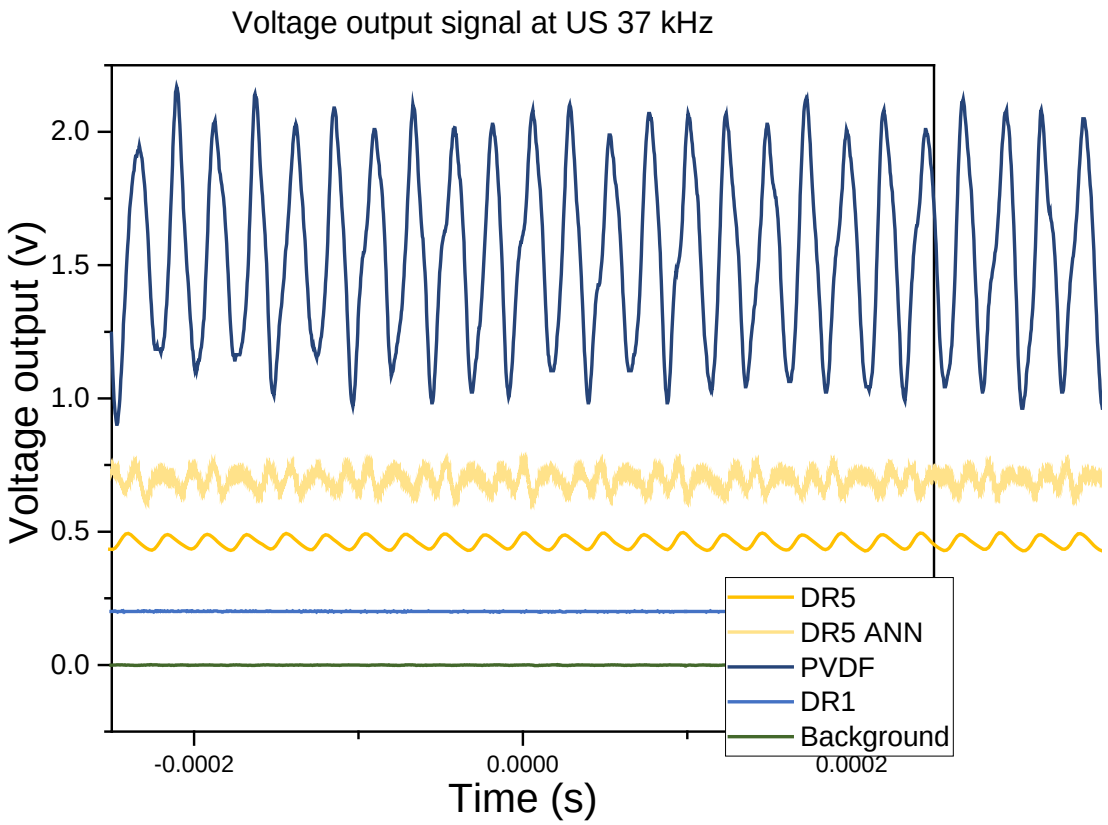

Figure S2. Voltage output as a function of time as frequency signal observed for different films (offset was used). Comparison of Voltage output for drawn film (DR5), non-drawn film (DR1), drawn and annealed (DR5 ANN), PVDF reference sample and background noise.

Clear difference is observed in voltage output frequency for non-drawn therefore non-piezoelectric film (DR1) or background noise compared to all other films, which shows some piezoelectricity (Fig. S2). Therefore, even the measured small signal from DR5 film is confirmed as piezoelectric property of the film.

S3. Methylene blue (MB) degradation in presence of hydrogen peroxide without ultrasound (US) stimulation.

For thin films and films with complex morphology as nanotexture on the surface, global piezoelectric coefficient is hard to be measured, therefore we confirmed it through the piezo potential for piezo-catalyzed organic dye degradation. Methylene blue (MB) acting as organic pollutant can be destroyed by reactive oxygen species (ROS), generated from H_2O_2 catalytic degradation [1]. Our polymer can be used as a piezo catalytic redox reagent to produce ROS (hydroxyl radicals (•OH), superoxide (•O2−), and singlet oxygen) from hydrogen peroxide medium. To activate piezoelectric properties ultrasound stimulation is one and very useful possibility to achieve mechanical deformation which result in polarization effect and charge generation on the film [2].

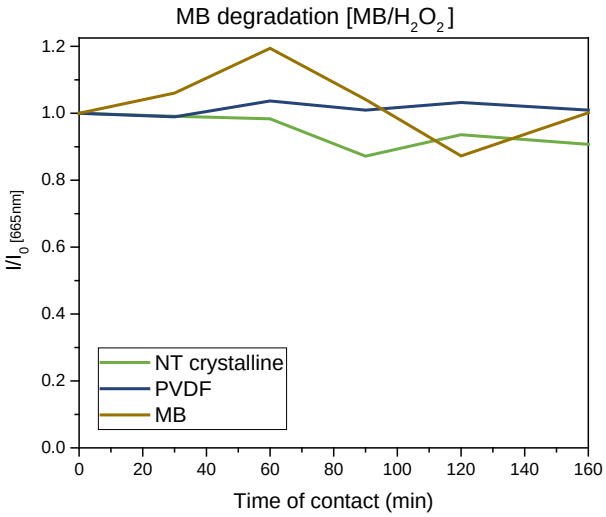

Figure S3. Methylene blue (MB) degradation curve in presence of active component (peroxide) and piezoelectric films without ultrasound (US) stimulation.

For MB degradation test, with 0.5 M H_2O_2 present, no degradation was observed for annealed NT film and PVDF reference, if no US stimulation was used after 2h of mixing, presented in the graph (Fig. S3). Those samples showed highest response with US stimulation under same addition of peroxide.

S4. Methylene blue (MB) degradation in presence of peroxide with US and added 0.5 M hydrogen peroxide as active component.

Methylene blue (MB) dye can be degraded with reactive oxygen species (ROS) over time. In our experiment, hydrogen peroxide is added as a source of formed ROS and piezoelectric films act as piezo-catalysers for peroxide reduction. Proposed mechanism of ROS production and MB degradation is:

$$ultrasound + piezo\ PLA \rightarrow e_{piezo} + h^+_{piezo}$$

$$H_2O_2 + e_{piezo} \rightarrow \boldsymbol{OH^{\cdot}} + OH^- + O_2$$

$$OH^- + h^+_{piezo} \rightarrow \boldsymbol{OH^{\cdot}}$$

$$H_2O_2 + h^+_{piezo} \rightarrow H^+ + HOO^{\cdot}$$

$$\boldsymbol{OH^{\cdot} + MB \rightarrow CO_2 + H_2O}$$

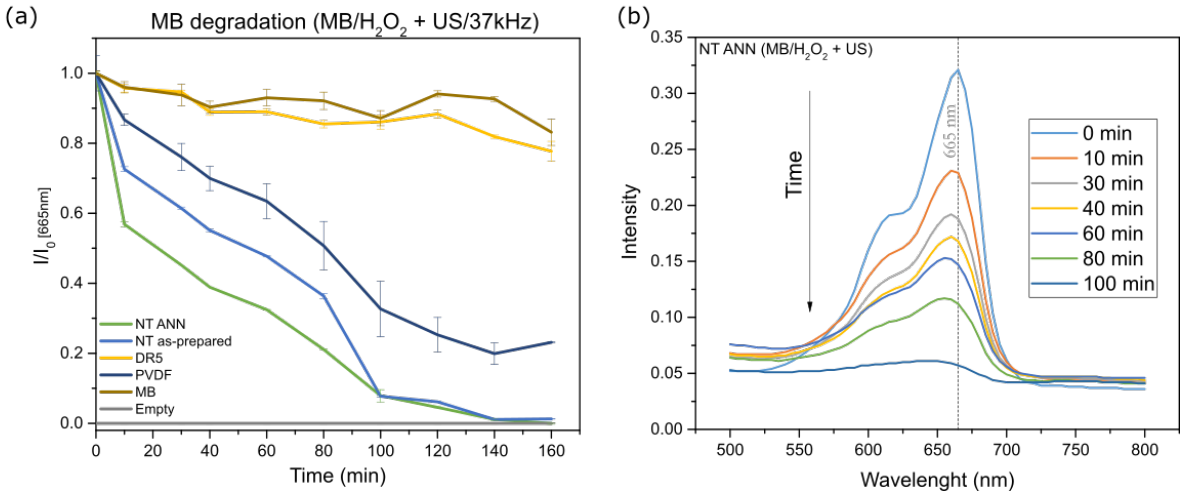

Figure S4. (a) Methylene (MB) degradation with added active component (peroxide) in presence of piezoelectric films (NT as prepared, NT annealed (NT ANN), drawn film (DR5), reference sample (PVDF)) under ultrasound (US) stimulation. (b) Associated absorbance spectra for NT ANN sample after different time of US stimulation.

By adding 0.5 M hydrogen peroxide (Fig. S4 (a,b)), the degradation process is slower and almost reaches 100 % degradation in 2 hours for nanotextured samples (NT ANN and NT as-prepared), compared to the previously added 1 M peroxide process completed before 1 hour of stimulation in the US for nanotextured samples and PVDF. Highest degradation rate is observed for nanotexture annealed sample, with similar end degradation after 2 h of US stimulation as NT as-prepared film. PVDF showed slower degradation rate, indicating lower piezoelectric potential compared to nanotextured films. Drawn piezoelectric film showed no degradation of MB dye.

Piezoelectric potential of prepared films is expected to be at least 2x higher at 37 kHz compared to higher frequency, since effect of mechanical stretching is higher. For piezoelectric properties films were measured at 37 kHz frequencies. Therefore, to see what is happening at conditions used for bacteria testing, which does not disturb bacteria growth, 80 kHz was used and observed the MB/H_2O_2 degradation (Fig. S5). Results again shows similar behaviour of the curves for NT ANN film and PVDF reference with slower degradation rate. Also confirming that piezoelectric effect is responsible for change in degradation rate, since at 37 kHz degradation is faster compared to 80 kHz.

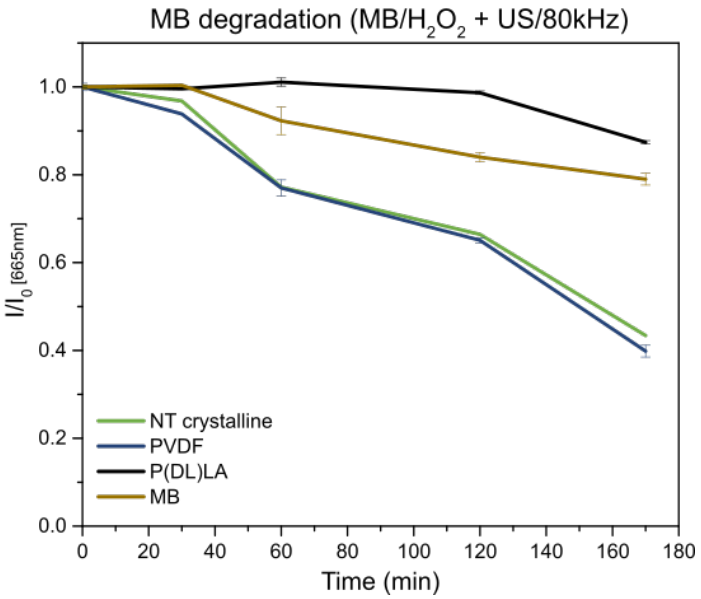

Figure S5. Methylene (MB) degradation with added active component (peroxide) in presence of piezoelectric films under ultrasound (US) stimulation at 80 kHz frequency.

Kinetics of MB degradation in presence of peroxide (0.5 and 1 M) and films under US stimulation (37 kHz and 80 kHz), and comparison with voltage output measurements for smooth films ($V_{pk.-pk.}$) is presented in Table S1.

Table S1. Comparison of calculated degradation rate for different piezoelectric and non-piezoelectric films, with 0.5 or 1M hydrogen peroxide addition, stronger (37 kHz) or milder (80 kHz) ultrasound (US) stimulation, and measured direct voltage output under US stimulation.

Sample	H_2O_2 [**0.5M**] + US [**37kHz**]		H_2O_2 [**1M**] + US [**37kHz**]		H_2O_2 [**0.5M**] + US [**80kHz**]		Piezo with US mechanic stimulation (V_{p-p})	
	k (min^{-1})	Degradation at 1h of US	k (min^{-1})	Degradation at 1h of US	k (min^{-1})	Degradation at 1h of US	**37kHz**	**80kHz**
NT crystalline	0.0146	68 %	3.6154	97 %	0.0035	23 %	Not applicable	
NT amorphous	0.0136	52 %	1.0893	66 %	Not measured		Not applicable	
DR5	0.0013	11 %	Not measured		Not measured		59 mV	24 mV
PVDF	0.0098	34 %	3.1149	95 %	0.0037	23 %	1.2 V	450 mV
NT P(DL)LA film	0.0009	0 %	Not measured		0.0004	1 %	Not applicable	
MB	0.0008	7 %	0.14121	13 %	0,001	8 %	/	/

Values show faster kinetic for higher addition of peroxide (1M), since more ROS can be formed. However, it is also observed that with stronger US (37 kHz) compared to milder (80 kHz) for the same piezoelectric film (NT ANN for instance) degradation rate is 5 times higher (0.015 compared to 0.003

min^{-1}), indicating that piezoelectric properties effect degradation rate and ROS production from peroxide.

S5. Contact angle measurements of representative piezoelectric samples.

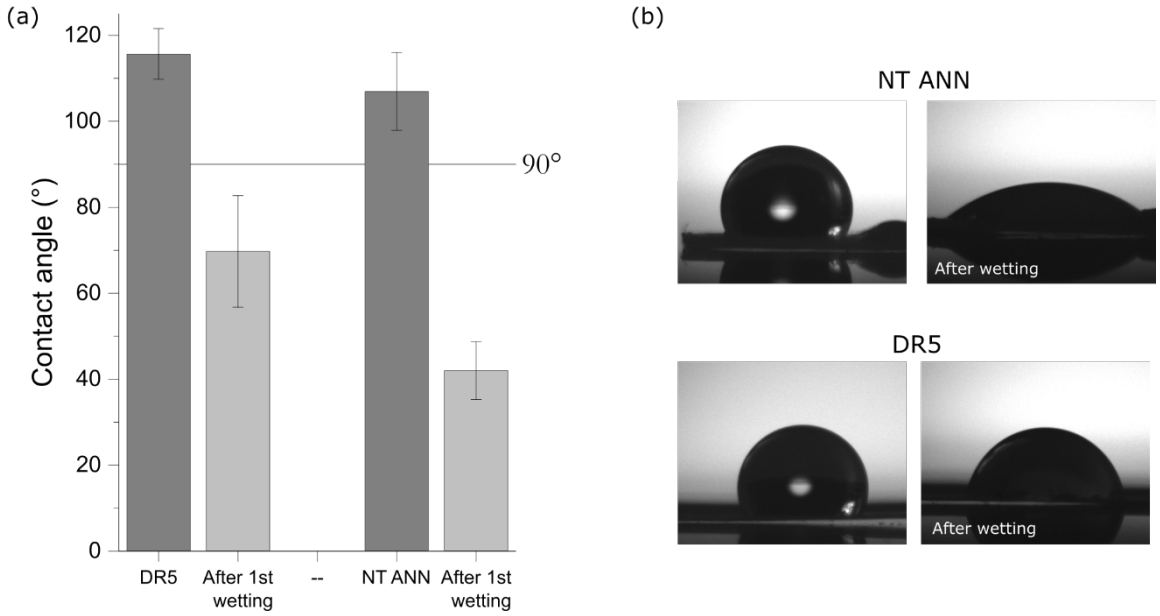

Figure S6. (a) Contact angle measurements of dry piezoelectric drawn (DR5) and nanotextured annealed film (NT ANN) and after wetted and wiped surface; (b) pictures of contact angle measurement for beforementioned samples.

For piezoelectric stretched (DR5) and nanotextured samples (NT ANN), contact angles were observed for 5 µl water dropped on the dry film surface (Fig. S6 (a,b)). We observe high angles of our samples, indicating more hydrophobic nature of the films. However, after the surface was initially wetted and wiped (not dried completely), observed angles were much lower (70° and 42° for DR5 and NT ANN films respectively), even more for NT sample. We believe in our assays after short time film is wetted and good contact is achieved between bacteria and suspension, however hydrophobicity can inhibit the contact of bacteria with film surface, suspecting mostly for the drawn DR5 films, where no effect of films was observed on bacteria viability and membrane integrity, even when ultrasound stimulation was used. Using US may improve needed bacteria-material contact and help to overcome hydrophobic properties.

S6. Antibacterial testing for *E. coli* and *S. epidermidis* in saline solution.

After contact test, where bacteria suspension was added on films, sonicated (80 kHz, 100% power, 30 min) and incubated in saline solution for 24 h, washed film solution was mixed with growth medium (LB) and viability of bacteria was estimated through fluorescence measurement of Presto blue indicator changing colour into more fluorescence after metabolic degradation of initial dye. It is clearly observed that only for non-piezoelectric film (DR1) and E. coli, bacteria freely grew and change colour into more fluorescent (Fig. S7). For nanotextured films no survival of bacteria was observed. Similar was confirmed when films were put on solid agar plate to really observe any remained bacteria on the

film (Fig. S7 right). Again, for nanotextured piezoelectric films no growth was observed. Since all bacteria on the films were dead, bacteriocidic effect of piezoelectric nanotexture film was confirmed.

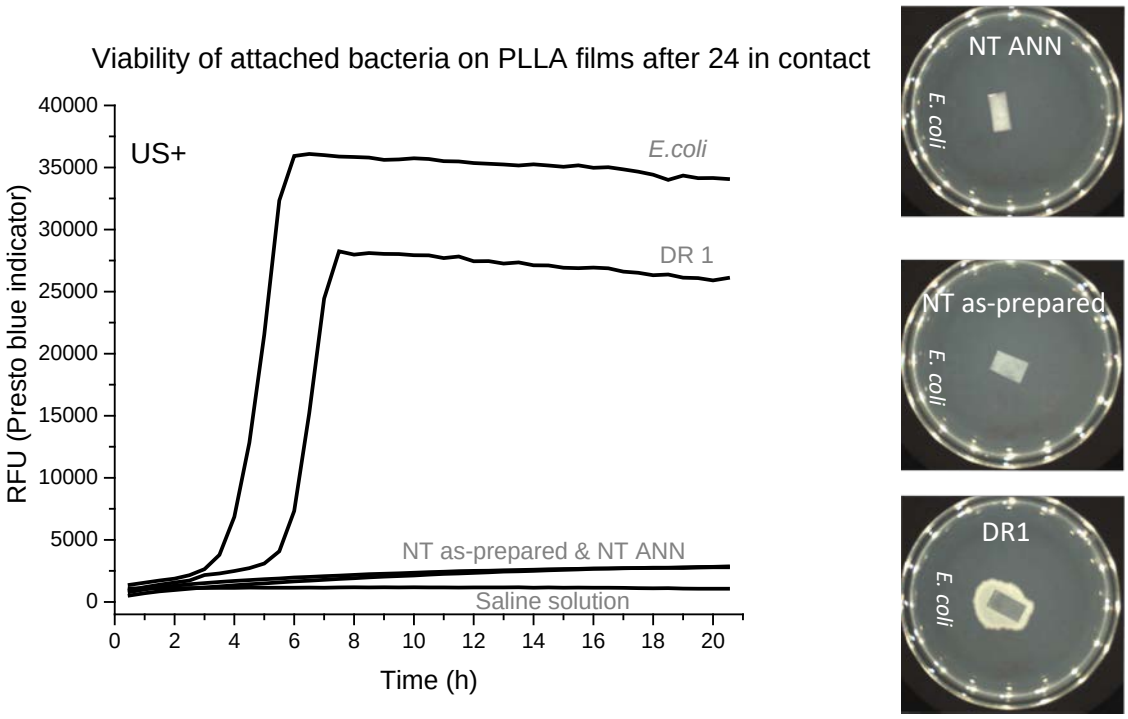

Figure S7. Viability of attached E. coli bacteria on tested films (DR1, NT as-prepared and NT ANN) after incubation at 30 min of US and in saline solution for 21 h, with pictures of petri dishes with films put on solid agar.

Similar was observed when contact test with *S. epidermidis* bacteria was performed. Observing the growth of only attached bacteria, for nanotextured films none colony was formed, when around DR1 film the growth is obvious (Fig S8).

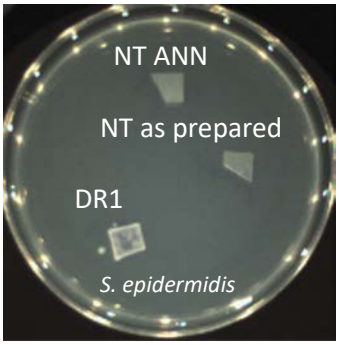

Figure S8. Agar plate presenting survival of attached S. epidermidis bacteria on tested films (DR1, NT as-prepared and NT ANN) after incubation at 30 min of US and in saline solution for 24h.

We also observed viability of washed bacteria (Fig. S9 (a,b)) after contact test to clearly confirm dead bacteria. For NT films with or without ultrasound stimulation, in close contact test, both bacteria (*E. coli* and *S. epidermidis*) was killed. No viability through presto blue fluorescence was observed, compared to smooth piezoelectric films or reference bacteria. Small effect of piezoelectricity for drawn film was observed (suspected less bacteria at start due to the shift of curve increase to later time), if US was used, compared to the curve without US stimulation. Since for the bacteria with or without stimulation, curve is the same.

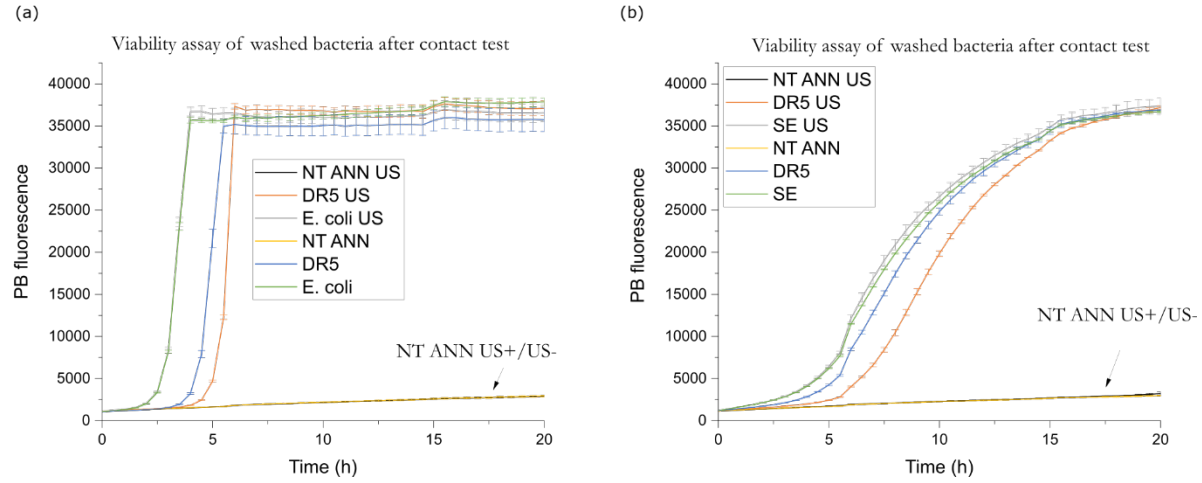

Figure S9. Viability assay through Presto blue fluorescence measurement. Washed bacteria after close contact with piezoelectric nanotextured (NT ANN), piezoelectric drawn (DR5) films and reference (a) E. coli and (b) S. epidermidis bacteria with or without US stimulation.

After contact testing, piezo-degradation potential for methylene blue dye was examined for NT ANN samples after the contact test preformed twice with *E. coli* bacteria and washed with 70% ethanol. If we observe washed sample under SEM without Glutaraldehyde fixation, most bacteria or bacteria leftovers are washed away, therefore active piezo-potential was expected (Fig S10 (a)). Conditions for piezo-potential testing were the same (0.5 mg/ml MB, 0.5 M peroxide), however samples were smaller, therefore reference samples with the same size were compared to assess the piezo-potential loss. Result show some decrease in piezo-potential (60 %), however still present (Fig. S10 (b)). Some degradation due to US or bacteria, ethanol spraying can be the reason for some loss in piezoelectric signal.

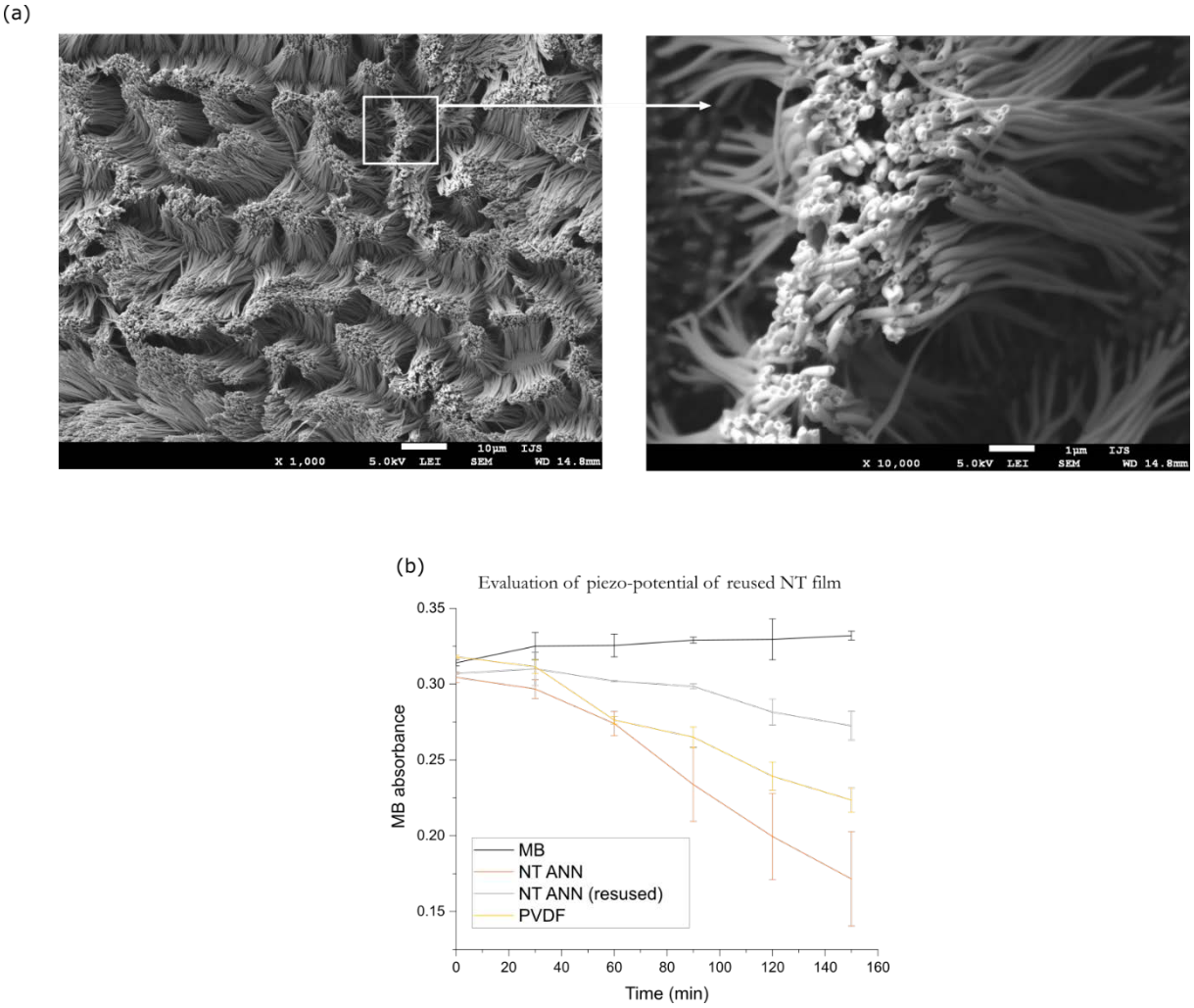

Figure S10 (a) SEM images of nanotextured annealed sample (NT ANN) after close contact test with E.coli and washing with 70% ethanol and closer look on the right; (b) methylene blue (MB) degradation potential assessment for NT ANN samples compared to non-used NT ANN sample, PVDF reference and MB dye.

S7. SEM observation of annealed and as prepared nanotubes after 30 min of ultrasound (US) sonication.

SEM analysis showed difference before and after sonication in ultrasonic bath at frequency 80 kHz for 30 minutes, as were the typical process parameter for bacteria testing. For as-prepared films (Fig. S11 (a)) fibers are all over the place after sonication for 30 minutes, when for annealed samples (Fig. S11 (b)), they stay in island formation, as was the initial construction for both samples. After 30 minutes of sonication, almost all fibers stay attached on the film substrate.

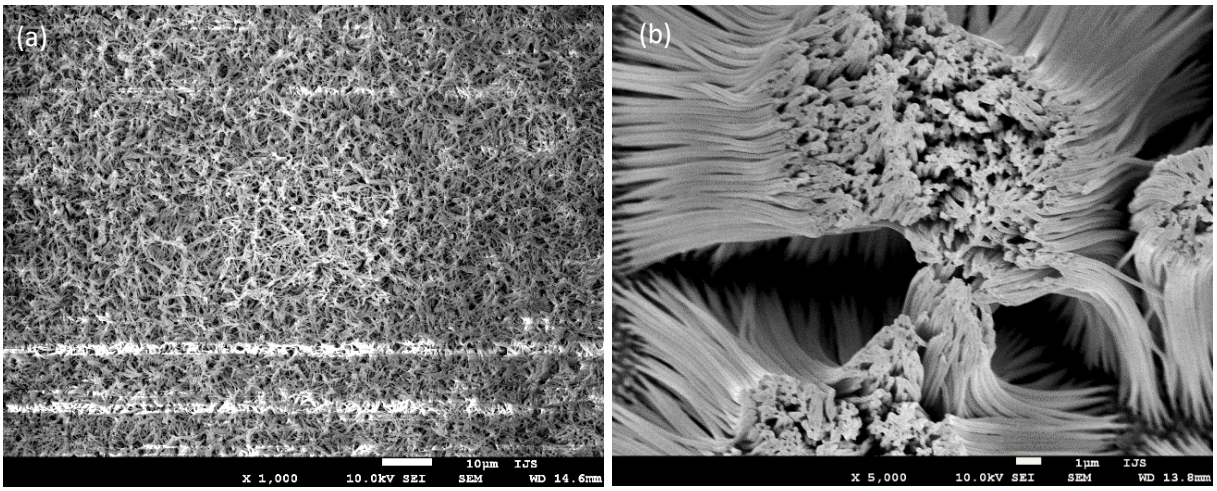

Figure S11. SEM images of nanotextured films after 30 min of US sonication (80 kHz) for (a) NT as-prepared sample and (b) NT ANN sample.

S8. Haemolysis test for piezoelectric films with references without US or US at 80 kHz with 100 % power.

Any damage of red blood cells (RBC) were excluded when nanotextured or smooth, piezoelectric or non-piezoelectric films were in contact with RBCs, if US was not implemented (Fig. S12, left part of the picture), observed through released haemoglobin detection (absorbance measurement) from damaged cells. Similar was observed (less than 2 % damage of RBC) for US stimulated under 80 kHz for 30 min and only 30 % power. Undamaged RBCs with films still inside were also visually confirmed with observing Eppendorf tubes right after US (Fig. S13), where turbid liquid for NT ANN, NT as-prepared and DR5 confirm undamaged RBC and clear red liquid (RBC in water) means dead RBCs. However, difference occur if full power US was used at 80 kHz, where 40 % (NT ANN and NT as-prepared) or 90 % (NT P(DL)LA) damaged cells were observed for nanotextured films, regardless of their piezoelectric properties. This observation clearly indicates negative morphological effect on RBC only after more powerful US stimulation. When comparing piezoelectric with non-piezoelectric films with same morphology, piezoelectricity help survive more RBC compared to more damaged cells in contact with non-piezoelectric samples (NT P(DL)LA and DR1).

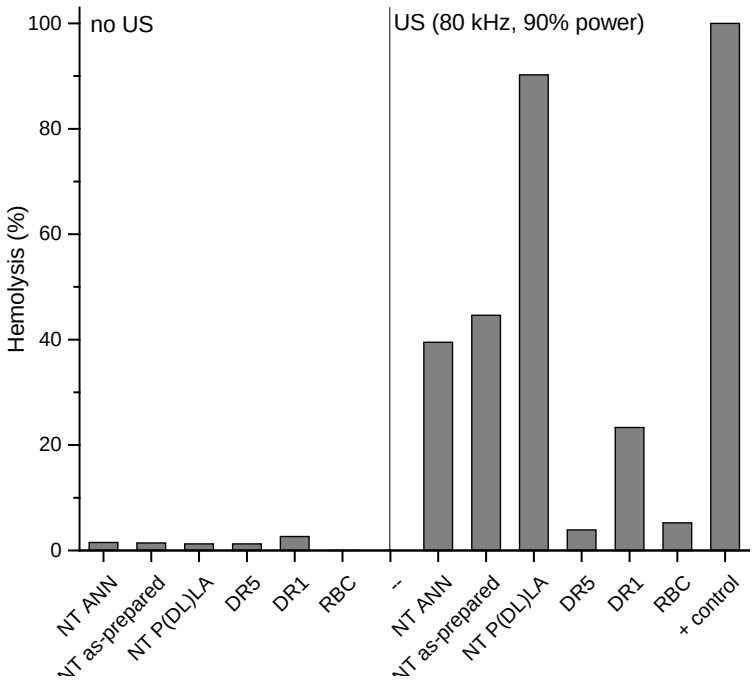

Figure S12. Hemolysis test for detecting damaged red blood cells (RBC) in contact with films (nanotextured piezoelectric- NT ANN, NT as- prepared and non-piezoelectric- NT P(DL)LA; or smooth piezoelectric- DR5 and non-piezoelectric- DR1) without US (left part) or with 80 kHz US for 30 min and 100 % power (right part).

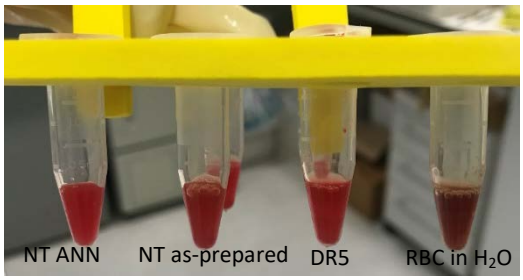

Figure S13. Visual observation of tested films in RBC solution (NT ANN, NT as-prepared, DR5 and fully damaged RBC in water) after US stimulation in Eppendorf tubes.

Literature:

[1] L. I. Jinga *et al.*, "Chemical Degradation of Methylene Blue Dye Using TiO2/Au Nanoparticles," *Nanomaterials*, vol. 11, no. 1605, pp. 1–10, 2021.

[2] G. G. Genchi *et al.*, "P (VDF-TrFE)/ BaTiO 3 Nanoparticle Composite Films Mediate Piezoelectric Stimulation and Promote Differentiation of SH-SY5Y Neuroblastoma Cells," 2016.