Making the Hospital a Safer Place by the Sonochemical Coating of the Fabrics with Antibacterial and Antibiofilm Nanoparticles.

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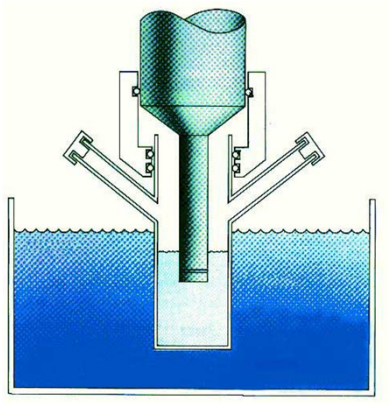
LECTURE at the NANOTEK 2014 Conference
on Decmber 3 , 2014
San Francisco, CA
USA

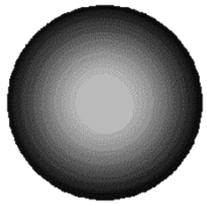
What is Sonochemistry?

The use of sound energy to produce chemical or physical changes in a medium through

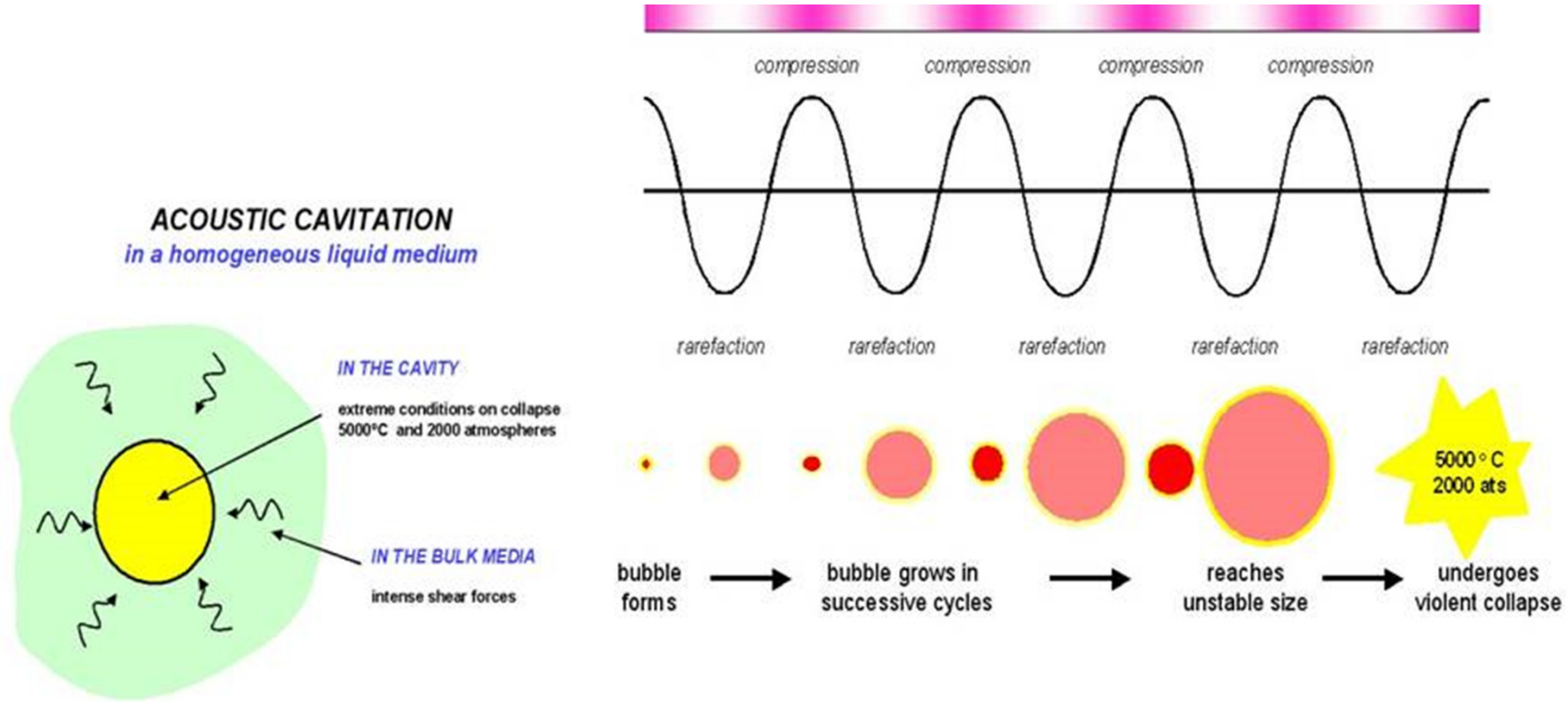
ACOUSTIC CAVITATION



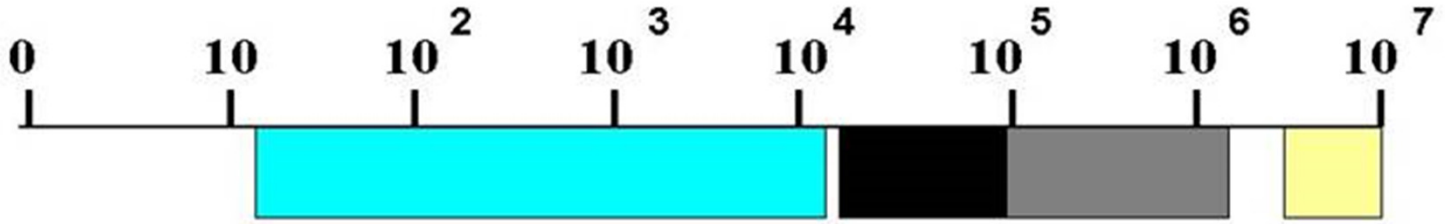
Typical sonochemical apparatus



ACOUSTIC CAVITATION
generation of the bubble:



Frequency ranges of sound



Human hearing		16Hz - 18kHz
Conventional power ultrasound		20kHz - 100kHz
Extended range for sonochemistry		20kHz - 2MHz
Diagnostic ultrasound		5MHz - 10MHz

“Anything you can do I can do better”

What can be done better with Sonochemistry?

1. Prepare Amorphous Nanoparticles.

2. Deposit Nanoparticles on Flat and Curved Surfaces

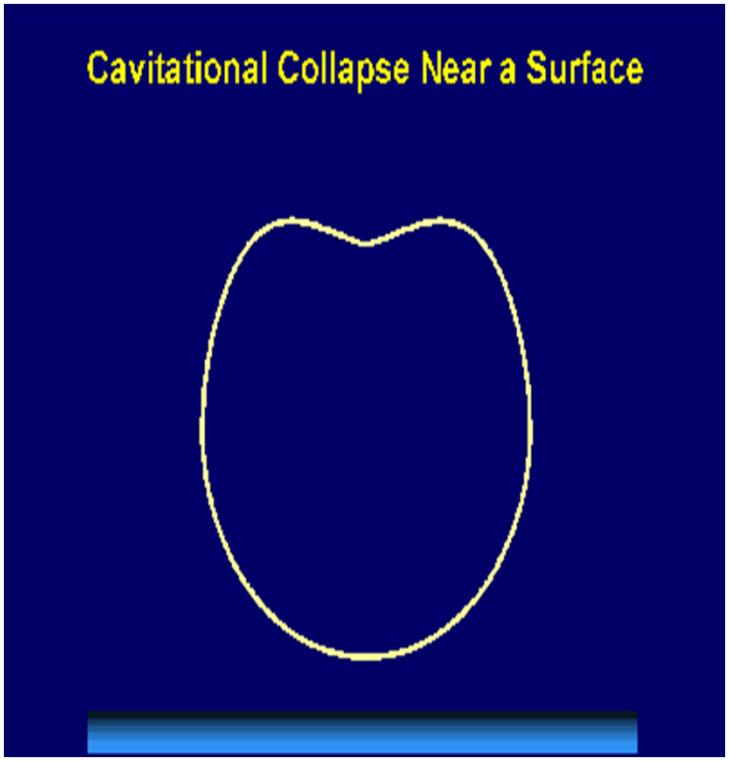
    Ceramic, Polymeric, Metallic, Glass, Textile Substrates.

3. Insert Nanoparticles into Small Size Pores

(Mesoporous Materials, CNT)

4. Make Proteinaceous Micro and Nano Spheres.

Why Sonochemistry is an effective method for coating?



What surfaces were coated?

- Ceramics
- Polymers (Polyethylene, Polycarbonate, Polyester)
- Metals (Stainless steel)
- Glass
- Paper
- Protein microspheres

What kind of materials were sonochemically coated on these substrates?

Successfully coated both inorganic and organic materials on surfaces providing various functionalities.

- **Inorganic**

✓**Antibacterial** - particles such as silver, ZnO, CuO, and MgO

✓**Magnetic** - iron oxide

✓**Conductive** - silver and gold

✓**Catalytic** - Pt, Ru

- **Organic**

✓**Antibacterial** – thymol, tannic acid, chitosan

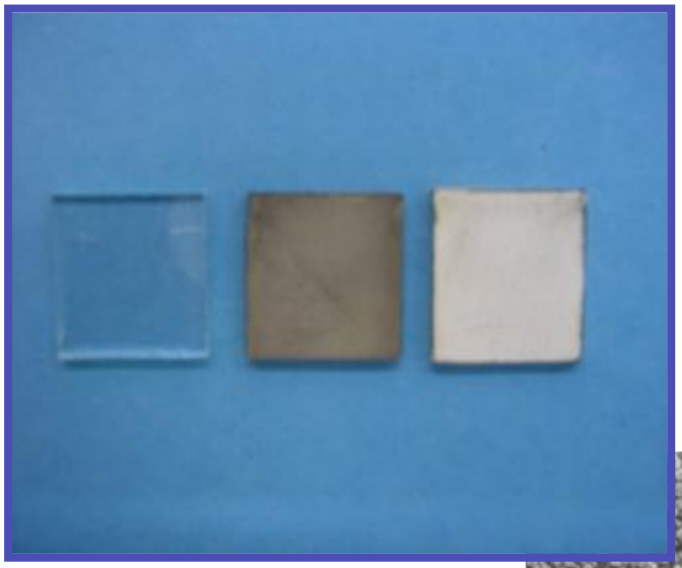
✓**Catalytic** - amylase

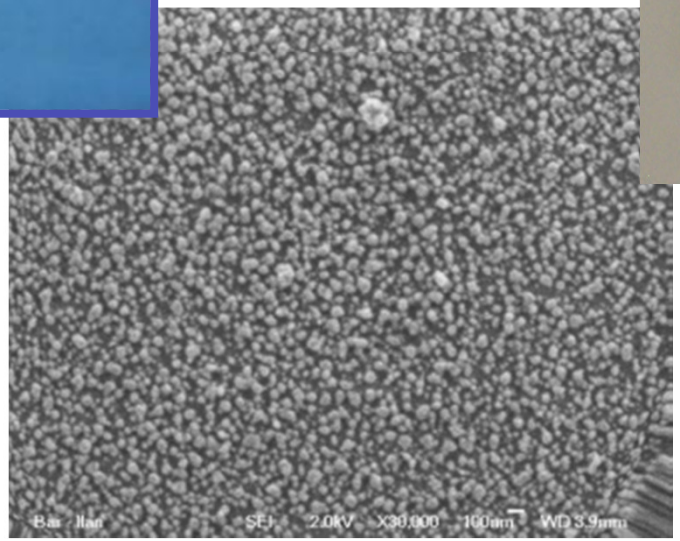
✓**Antiviral** - mercaptoethyl solfonate bonded to silver

✓**Optical** - Rohdamine 6G

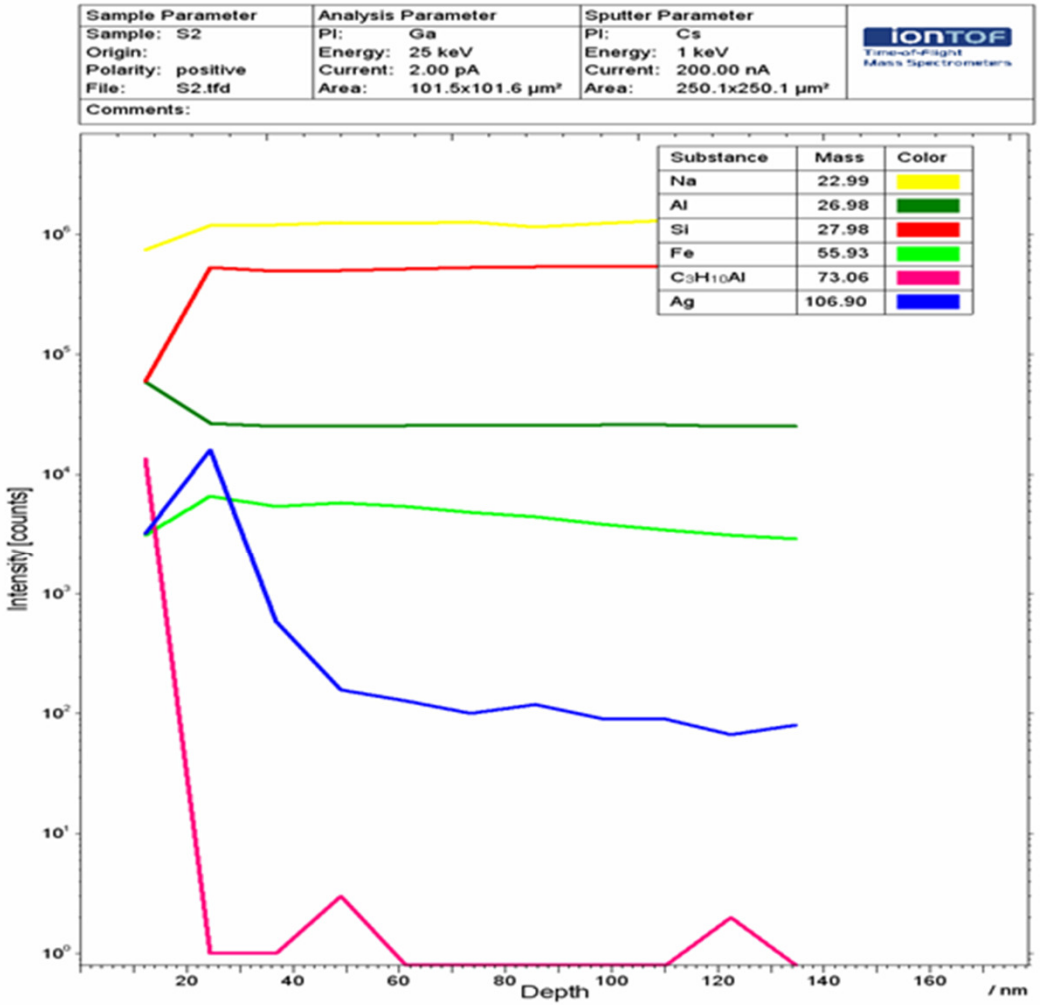
✓**Cosmetic** - hyaluronic acid

Glass coated with silver (Ag) nanoparticles by sonochemical method





TOF SIMS results on penetration of silver into glass



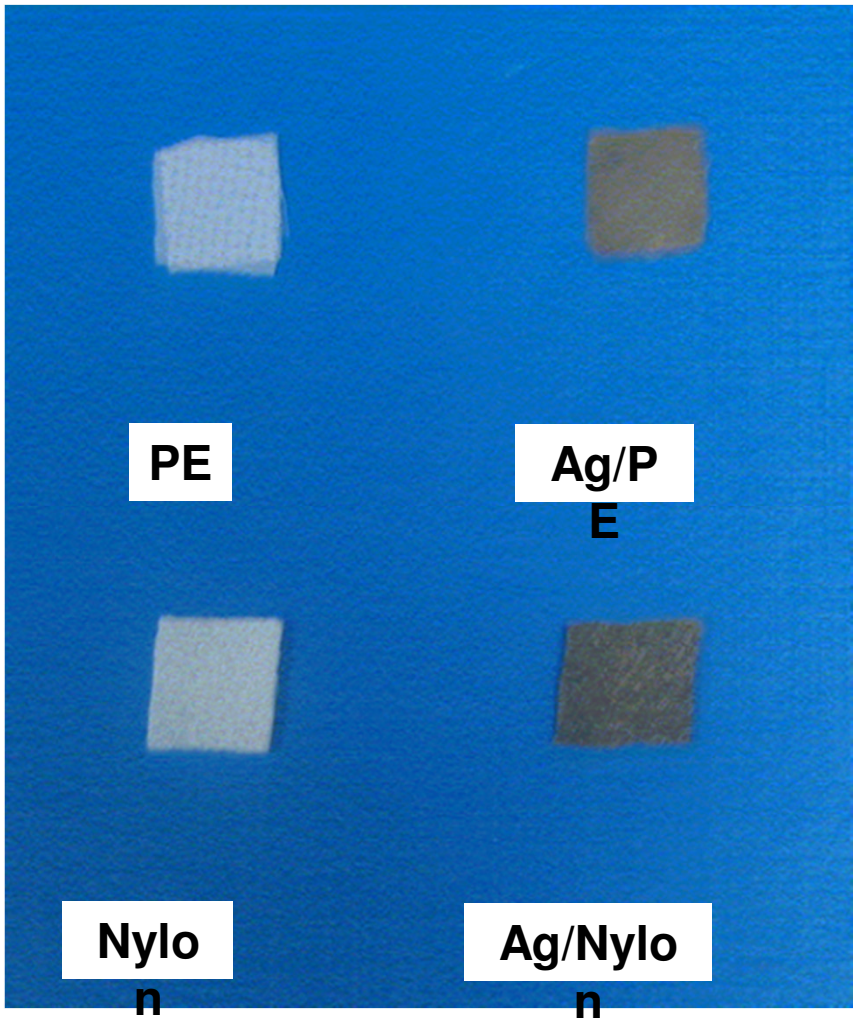
State of The Art

Why? Because 1 in 10 patients are affected by hospital-acquired infections .90,000 deaths are caused by hospital-acquired infections in Europe

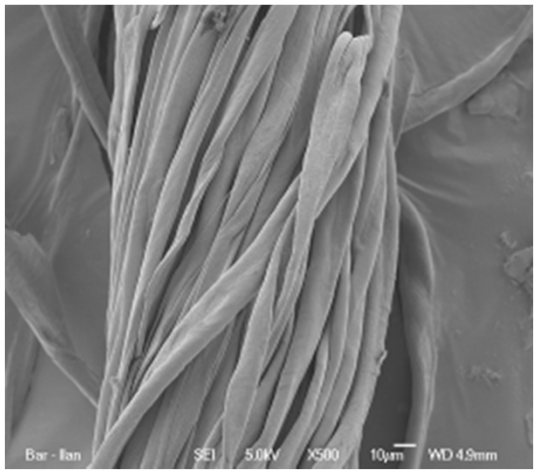
A **hospital-acquired infection**, also known as a **HAI** or in medical literature as a **nosocomial infection**, is an infection whose development is favored by a hospital environment, such as one acquired by a patient during a hospital visit or one developing among hospital staff.

Ag/Nylon composite was used as master-batch for synthesis of Nylon fibers and yarns

Samples of coated fabrics

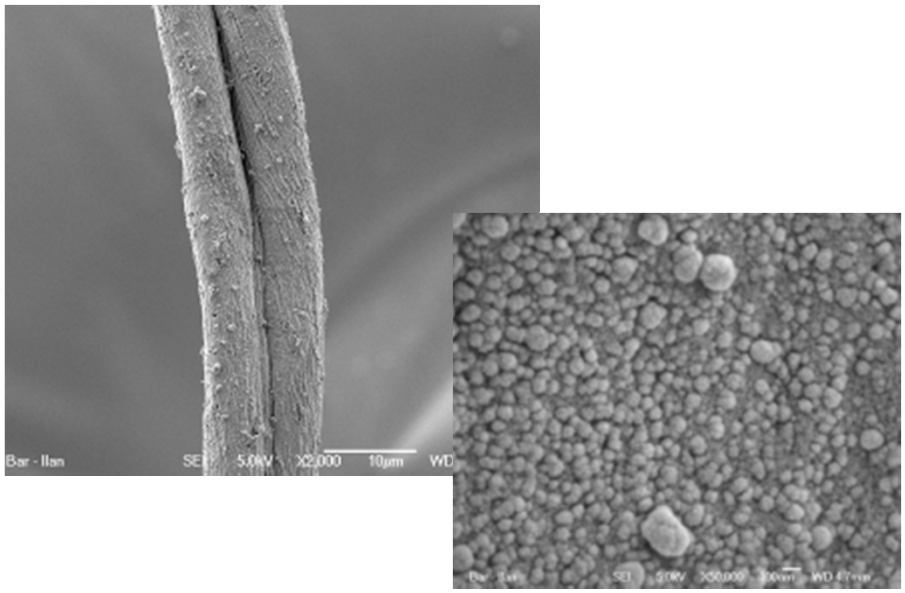


Morphology of Ag-fabric nanocomposite



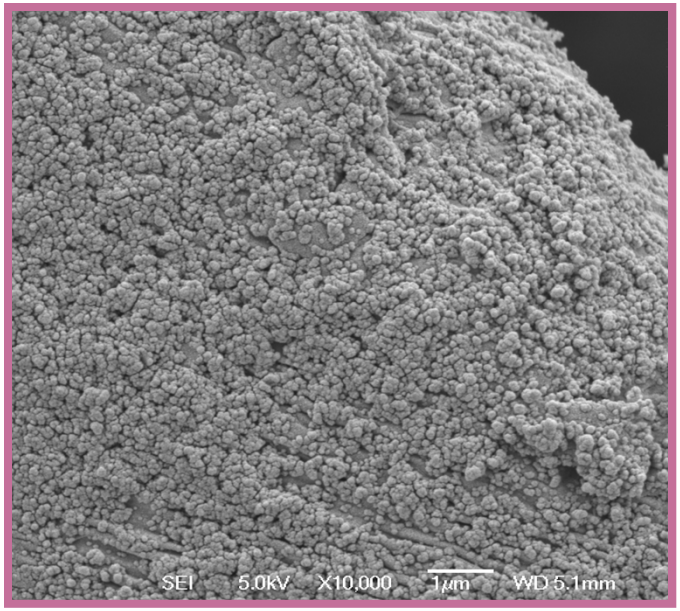
Before coating

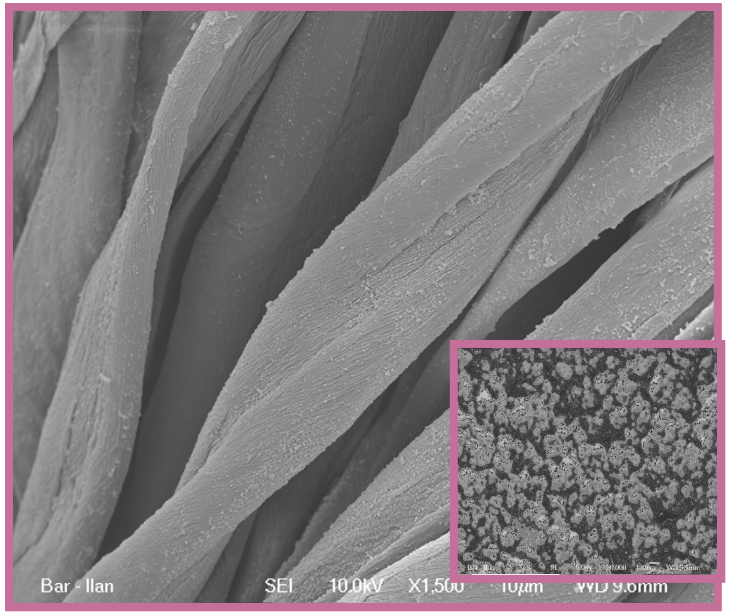
After coating with Ag nanoparticles



The calculated average size of the silver nanoparticles deposited on the surface of the cotton fibers is 80 nm

HRSEM of ZnO coated fabric





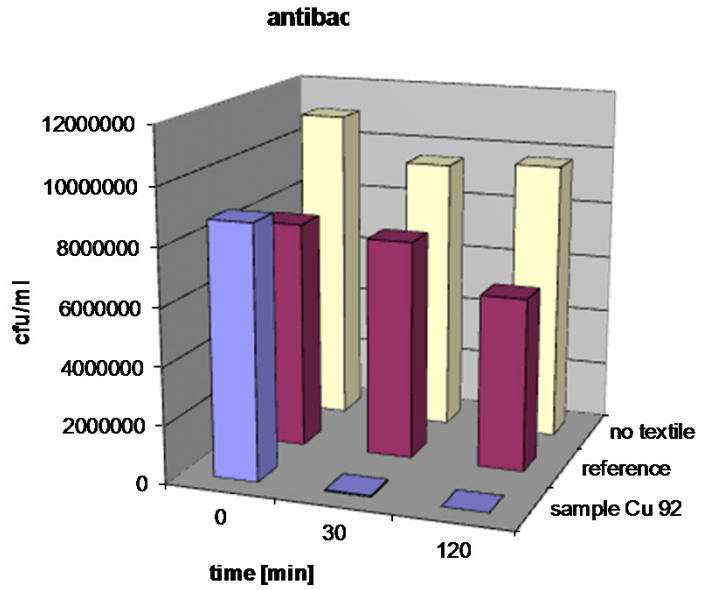
Fibers after sonication are homogeneously coated with nanoparticles.

The distribution of the particles is quite narrow, primary particles are in a very low nanometric range (~ 30 nm)

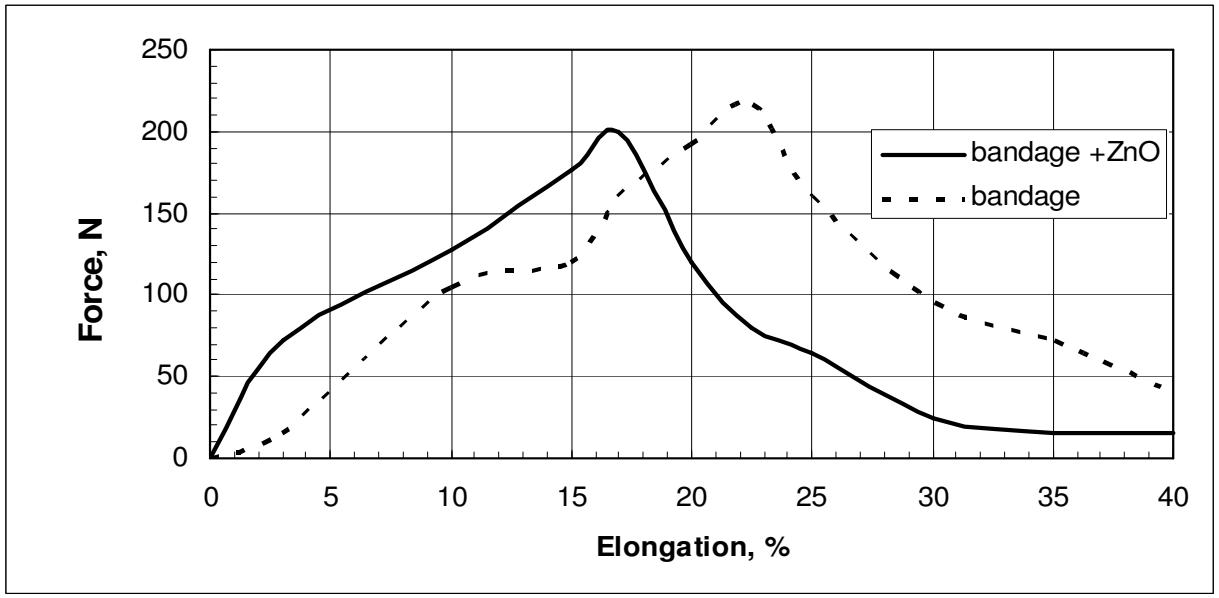
Antibacterial properties of CuO coated bandage

Antibacterial tests: Antibacterial effect could be revealed with the coated bandage even at the concentration of 0.6 wt%.

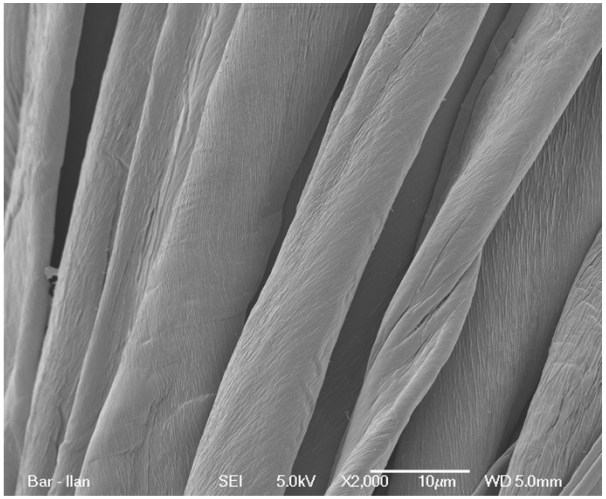
We observed 100% reduction of the bacteria after 1h for both strains of bacteria: *E. coli* and *Staph. aureus*

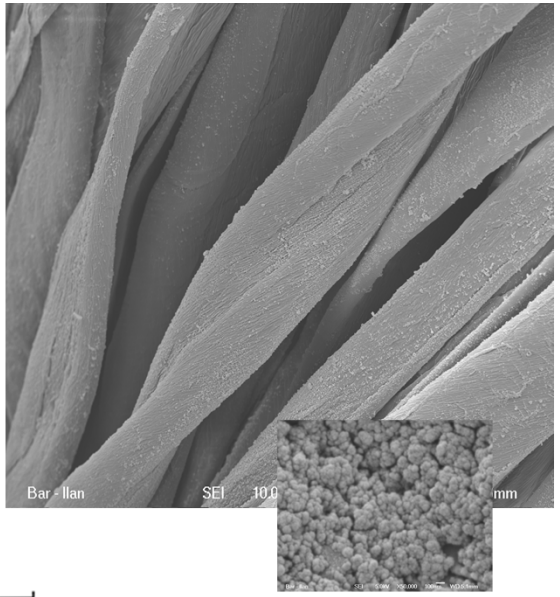


Mechanical properties of the non-coated and ZnO coated bandages

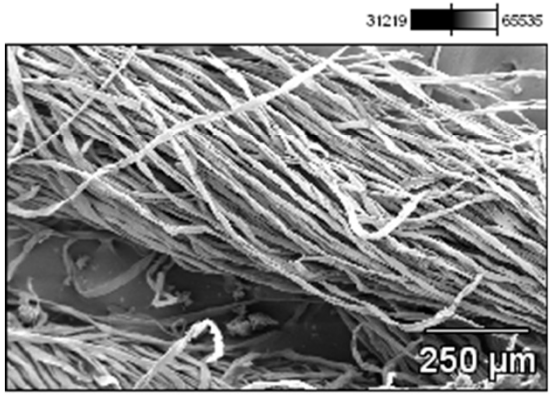


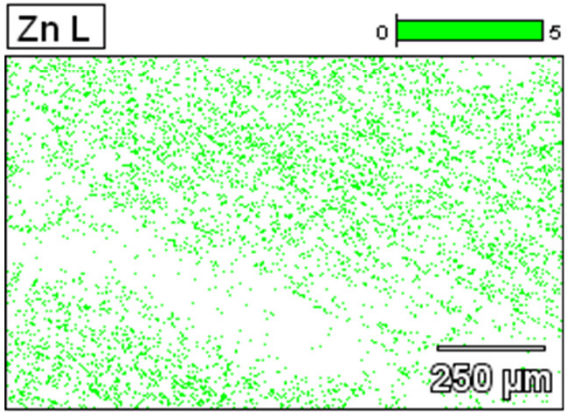
- The mechanical properties of the fibers after sonochemical radiation are similar to the pristine material

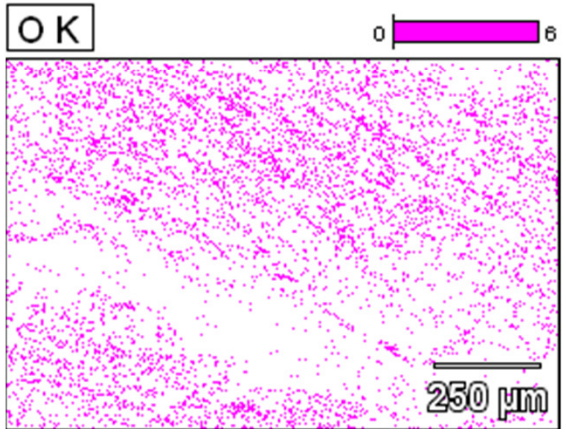

Bar - Ilan
SEI 5.0kV X2,000 10µm WD 5.0mm


Bar - Ilan
SEI

Base(4)


31219
65535
250 µm

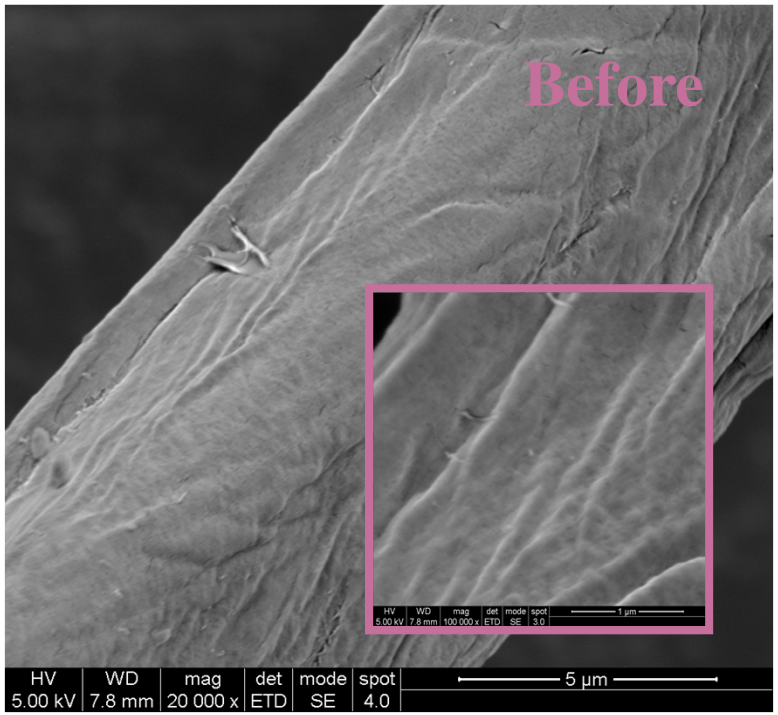

Zn L
0
5
250 µm


O K
0
6
250 µm

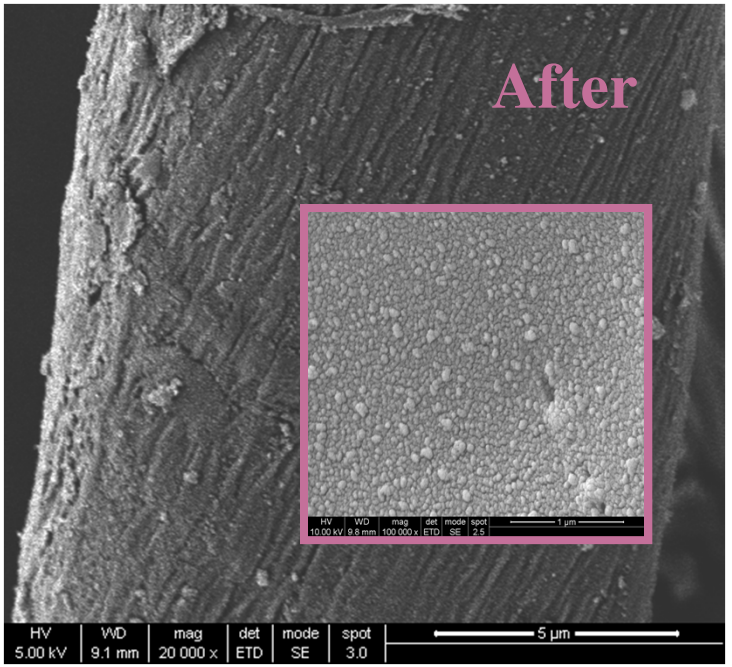
Sonicator.wmv

Roll to Roll Coating Machine

Morphology of CuO coated fabric (HRSEM)



A narrow size distribution is obtained and the primary particles are in a very low nanometric range (~ 10-15 nm).



2 Pilots have been built in the frame of the SONO project

Piezoelectric system at Italy

Magnetostrictive system at Romania

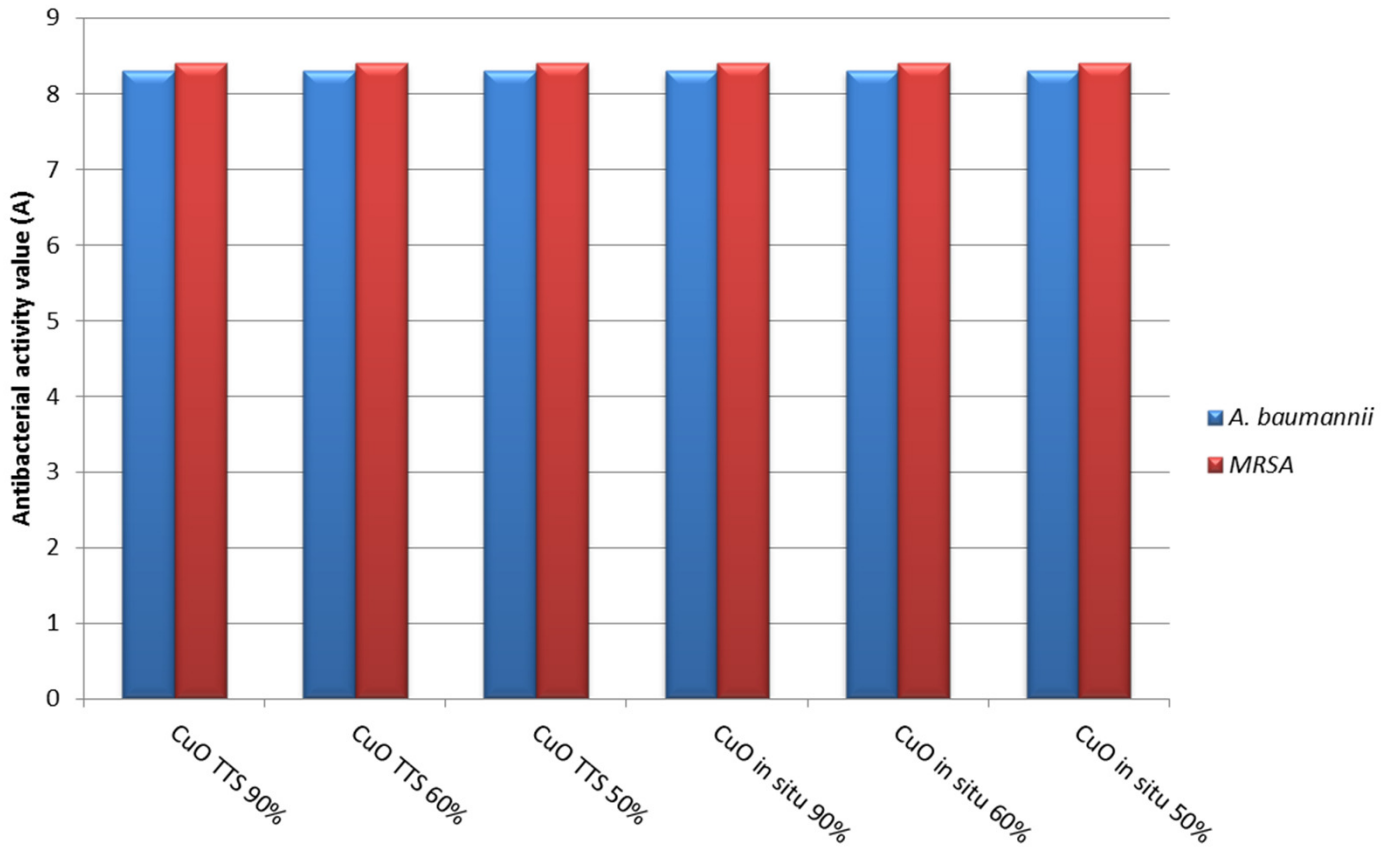
In the deposition reactions, the ultrasound can be applied in 2 modes of operation:

1. *"In-situ" reaction* - the formation of the particles and their following deposition on the fabrics in one step reaction.

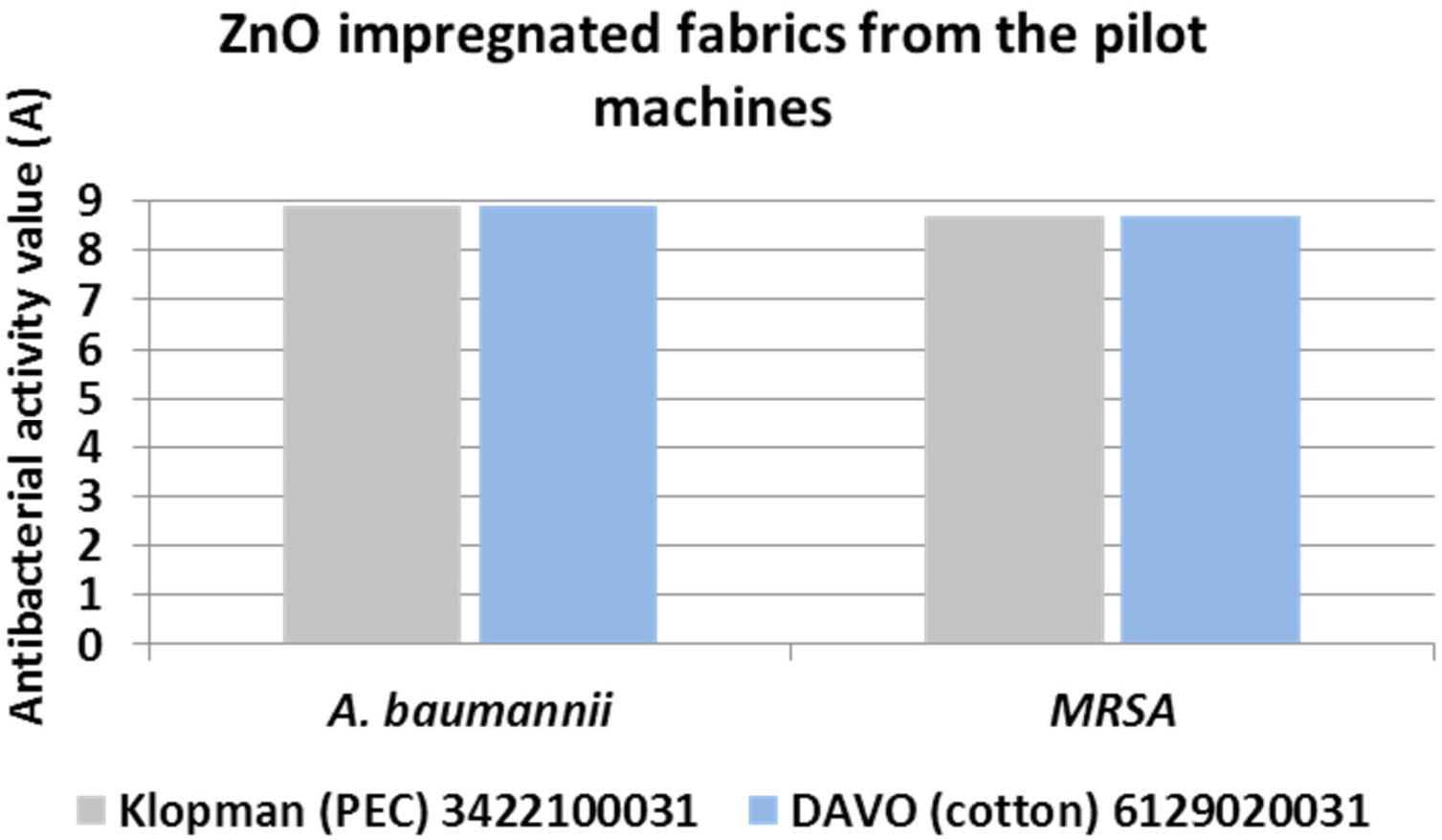
   In the current project the MeO NPs are formed by the basic hydrolysis of $Me(Ac)_2$

2. *"Throwing stones"* technique for deposition of the previously synthesized or commercially available materials. The ultrasonic waves and the microjets throw the commercial NPs at the textile and form a coated layer.

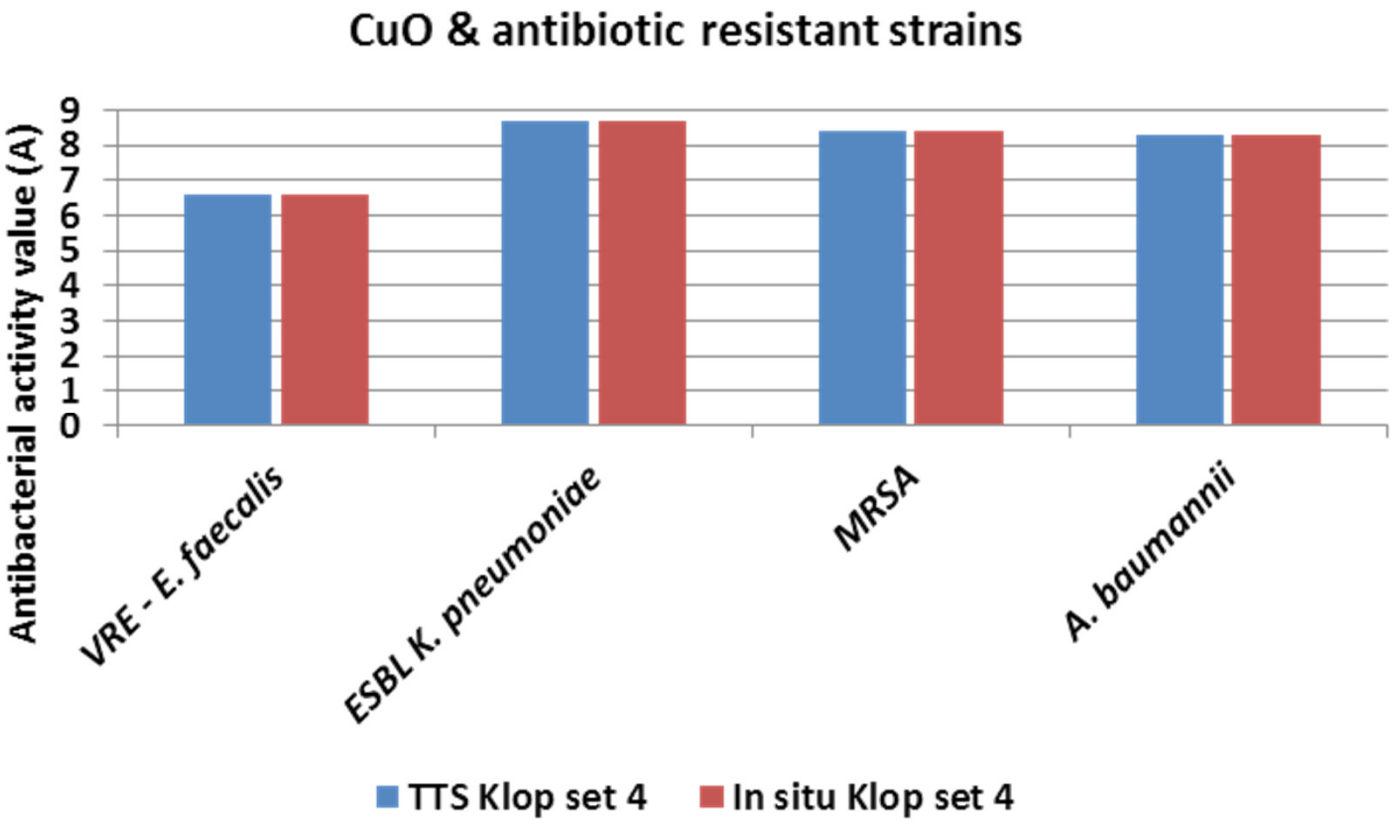
Antibacterial activity of CuO coated textiles



Antibacterial activity of ZnO coated textiles



Killing of resistant bacteria



Washing of textiles coated with MeO NPs

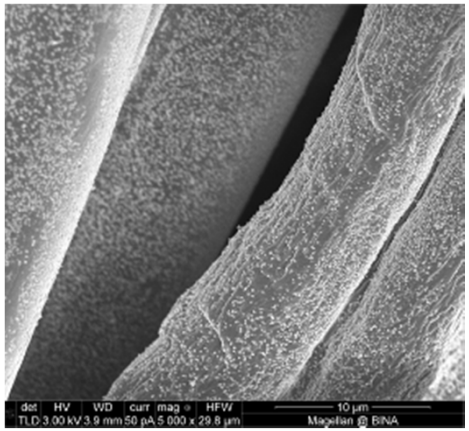
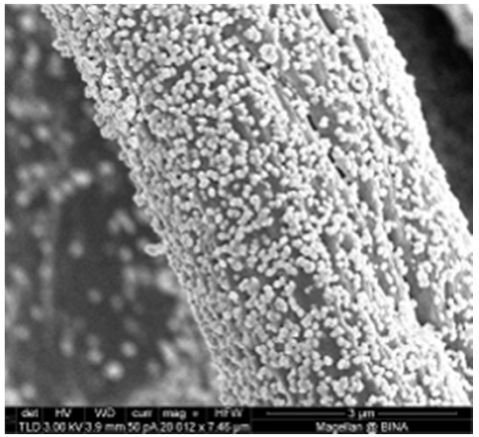
Washing requirement - Type of Washing Cycles

Type	Washing Cycles
I	Antibacterial activity after 50 washing
II	Antibacterial activity after 20 washing
III	Antibacterial activity without washing (for disposable products)

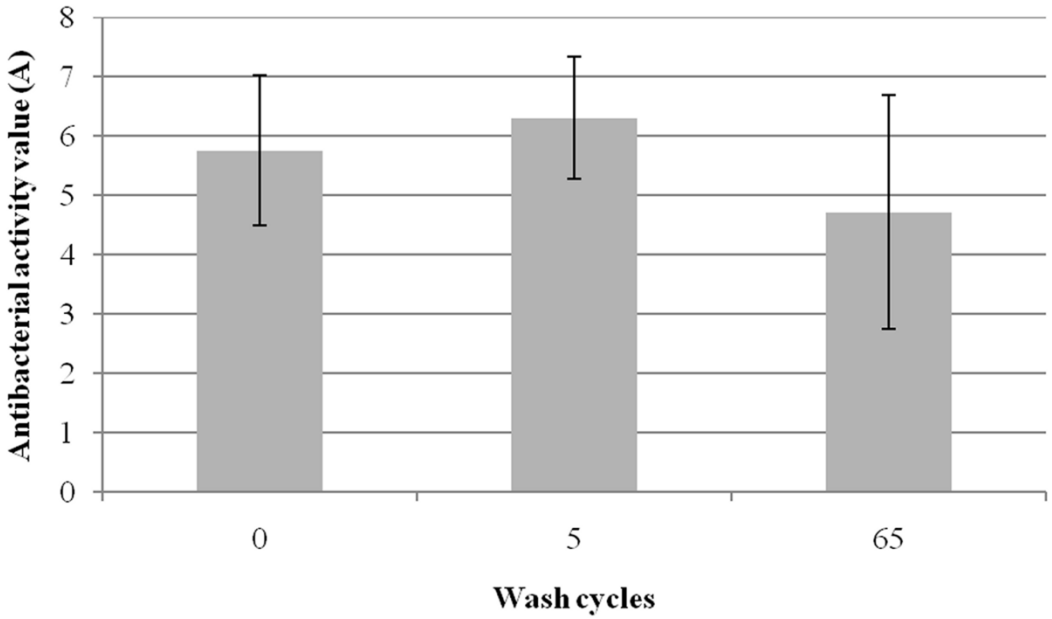
- The SONO project has demonstrated that textiles coated with MeO NPs underwent 65 washing cycles at 75^0C, and were found highly antibacterial after this long process.

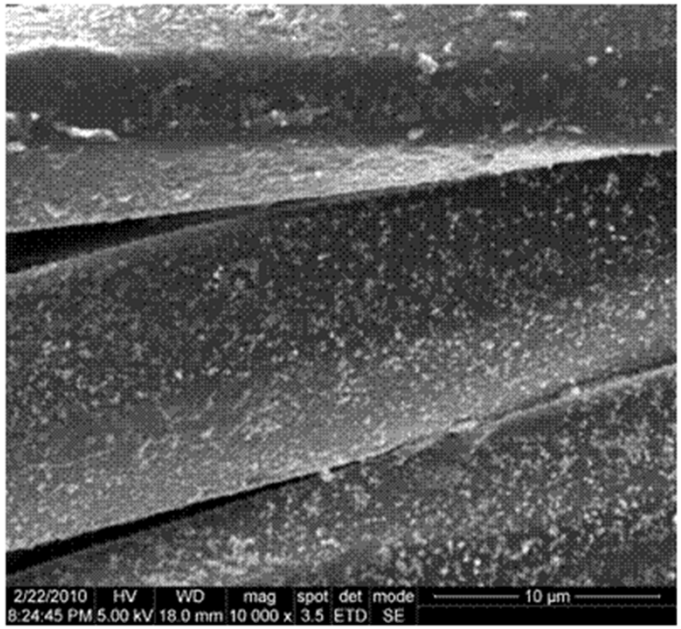
- The samples were washed with a market detergent (ECOS) **with neutral pH**, *SR EN ISO 6330/A1/2010 ".*

Main Results

CuO% before washing	CuO% after 65 washings	SEM before washing	SEM after washing (Neutral detergent ECOS)
0.9 → 0.74 17%			

The Sonochemical Coating of Cotton Withstands 65 Washing Cycles at Hospital Washing Standards and Retains its Antibacterial Properties





- Antibacterial activity values of washed CuO coated fabrics against *Staphylococcus aureus* (ATCC 6538). Testing was carried out according to the absorption method from BS EN ISO 20743:2007.

ROS Production On ZnO Surface

Suggested mechanism:

$$H_2O + Os^- \longleftrightarrow \cdot OH + OHs^-$$

$$2OHs^- \longleftrightarrow H_2O + \square + Os^{2-}$$

$$O_2 \longleftrightarrow 2Os$$

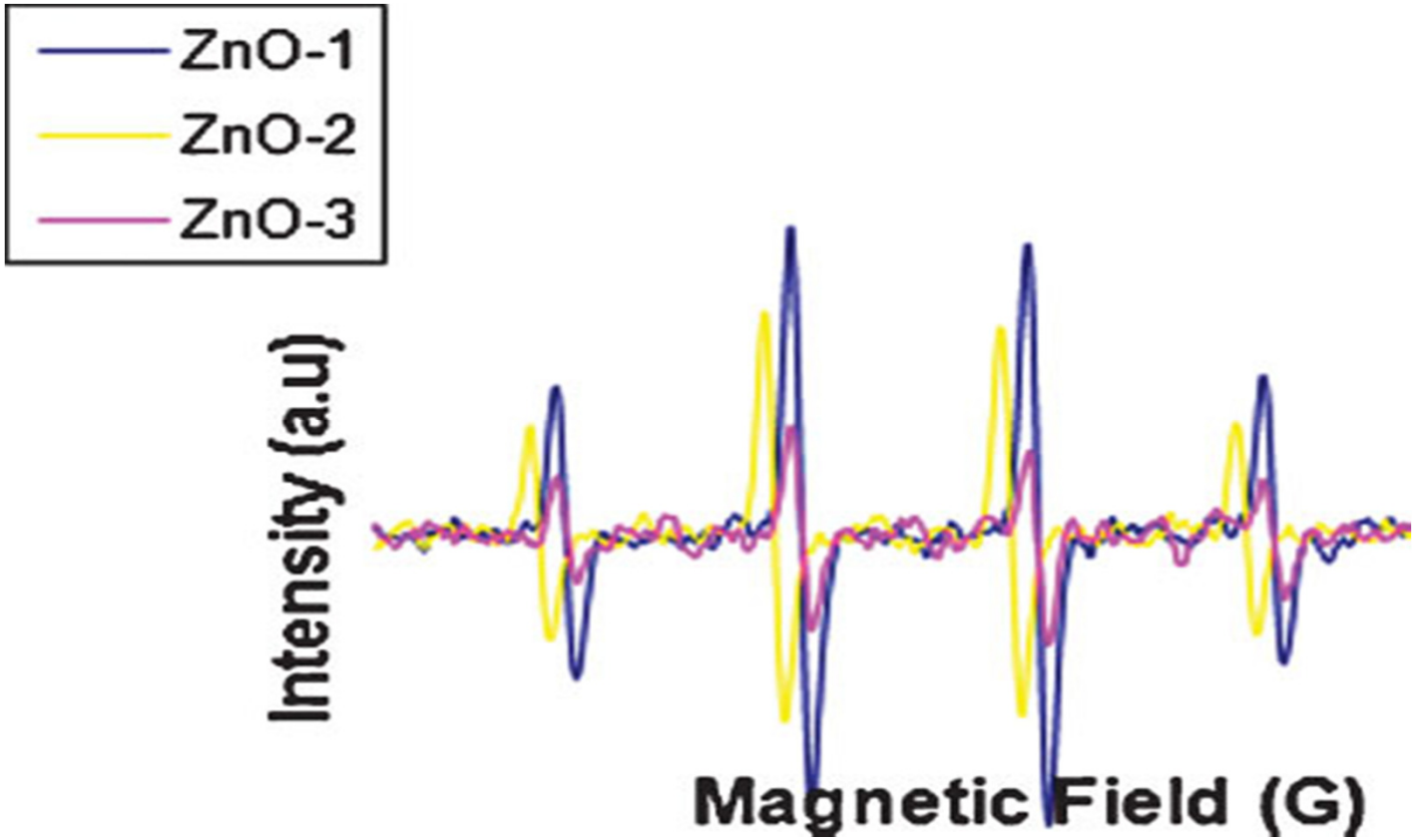
$$Os + \square + Os^{2-} \longleftrightarrow 2Os^-$$

$\square$ refers to an oxygen vacancy and the subscript "s" refers to surface species.

The net reaction can be written as follows:

$$O_2 + 2H_2O \longrightarrow 4OH\cdot$$

ESR-Spin Trapping Technique



Smaller sizes of ZnO produce greater amount of hydroxyl radicals

37 subjects enrolled in total:
- 21 on SONO textiles (patients)
- 16 on standard type of hospital textiles (controls)

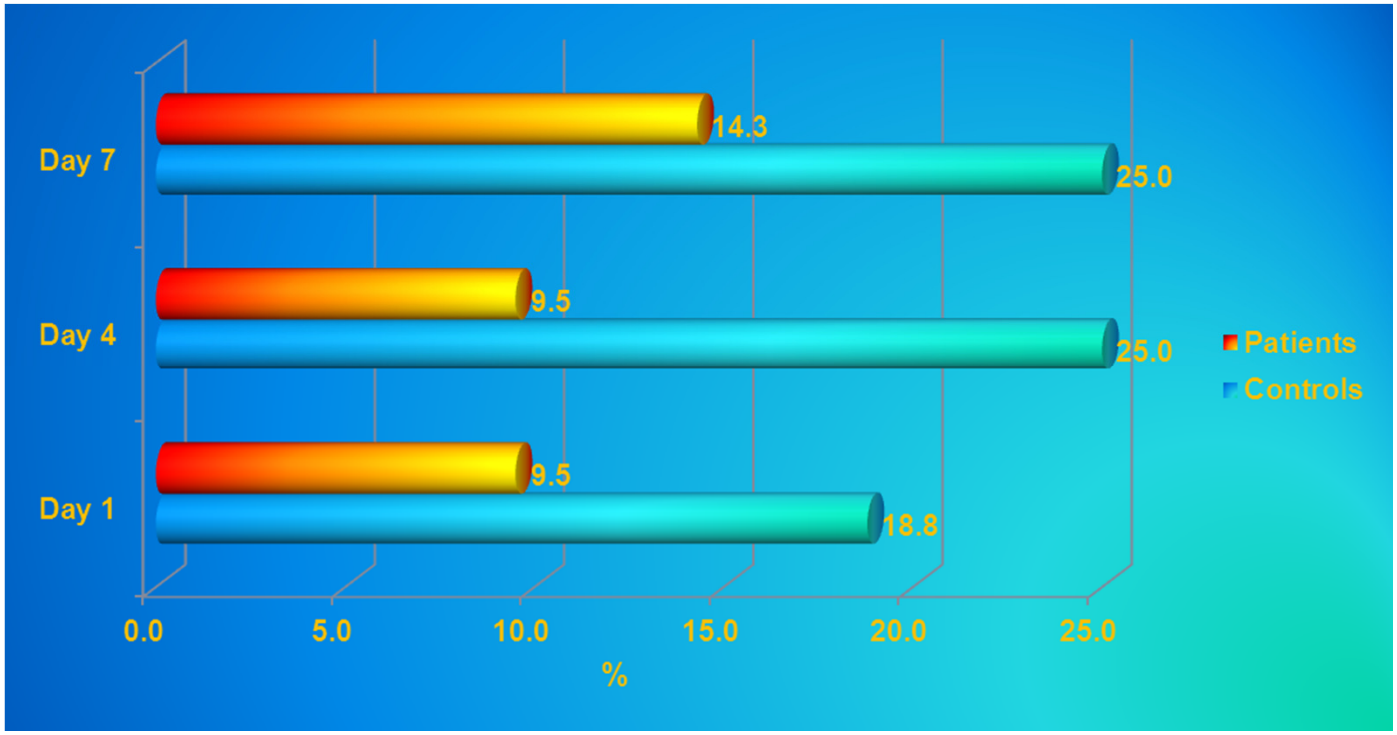
each subject – sets of textiles changed every 12 hours (7 am & pm)

Characteristics of the washing:

duration of washing
60 min
temperature 60°C; detergent with neutral pH

Clinical Results

Microbiology assessments – Colonization of throat

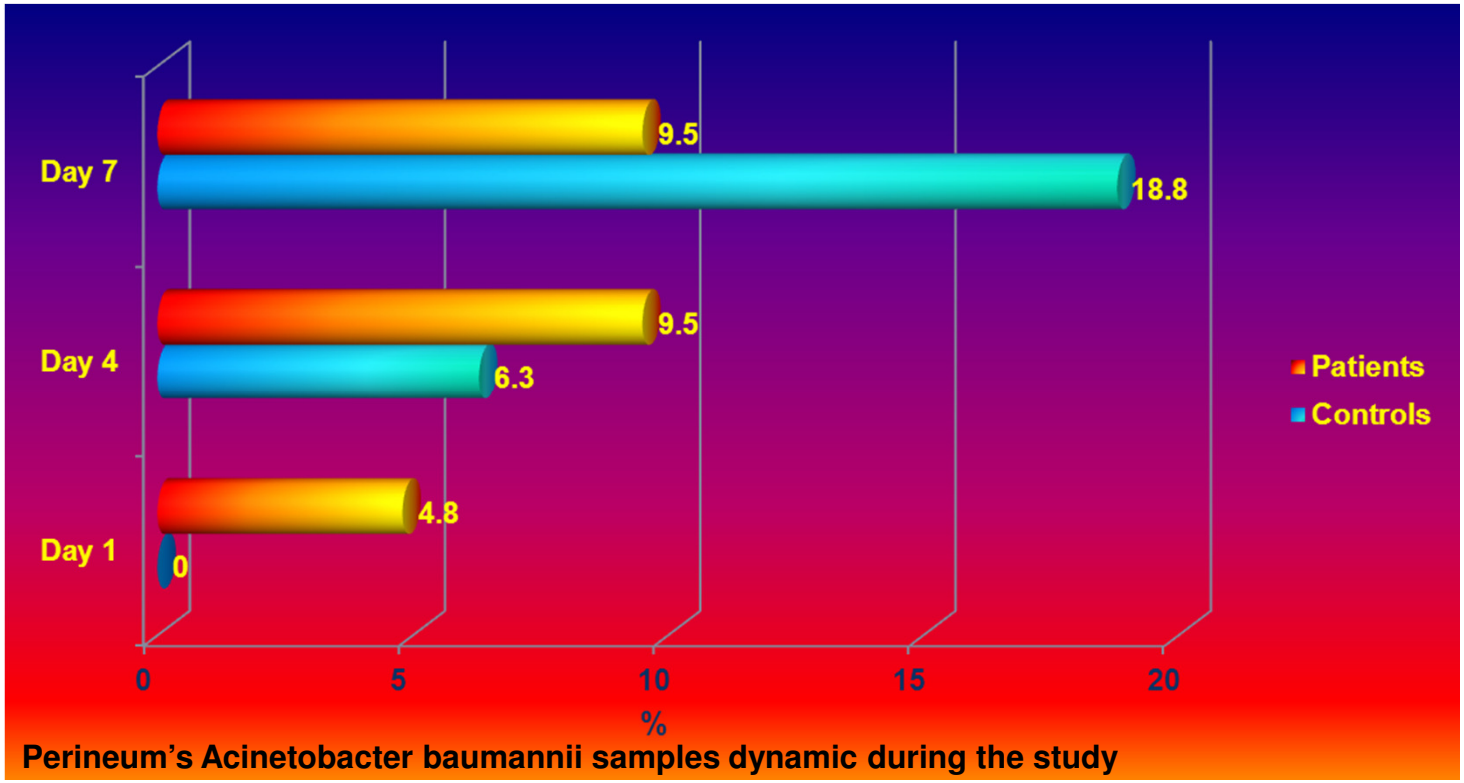


Throat S. aureus MSSA samples dynamic during the study

Throat contamination in patients remains at stable levels, while in controls increases from Day 0 to Day 4

Clinical Results

Microbiology assessments – Colonization of perineum

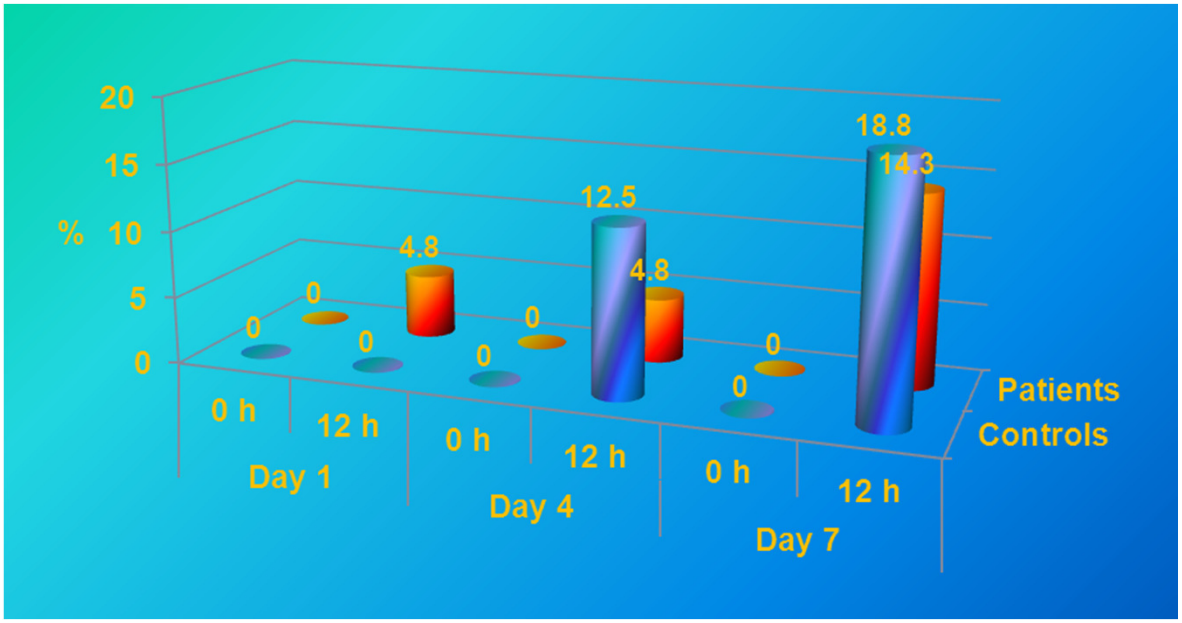


Perineum's Acinetobacter baumannii samples dynamic during the study

Perineum colonization of controls with Acinetobacter baumannii (representative of nasocomial infections) increases from Day 0 to Day 7 from 0% to 18%, while in patients remains comparatively stable

Clinical Results

Microbiology assessments – Colonization of textiles



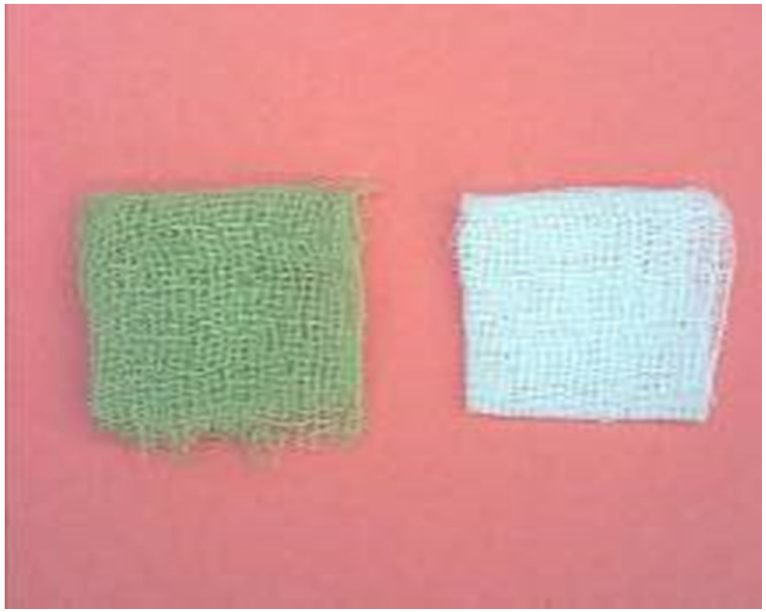
Colonization of bed linen of controls with Acinetobacter baumannii increases significantly from Day 0 to Day 7 (from 0% to 18%) , while in patients remains comparatively stable with small increase in the end of the studied period.

Note: One of the most difficult for managing nasocomial agents not affected by the known antiseptics and lots of antibiotics) .

Zn-doped CuO Nanocomposite

- ✓ ZnO is known for its bacterial growth inhibition initiated by the catalysis of reactive oxygen species (ROS) produced in water
- ✓ The creation of ROS depends on the presence of defect sites in the structure of the metal oxide NPs
- ✓ The doped metal oxides are characterized by increased defects that can make them more effective antibacterial agents due to higher ROS production
- ✓ ZnO- CuO NPs were synthesized and simultaneously deposited on cotton fabric using ultrasound irradiation

Deposition procedure



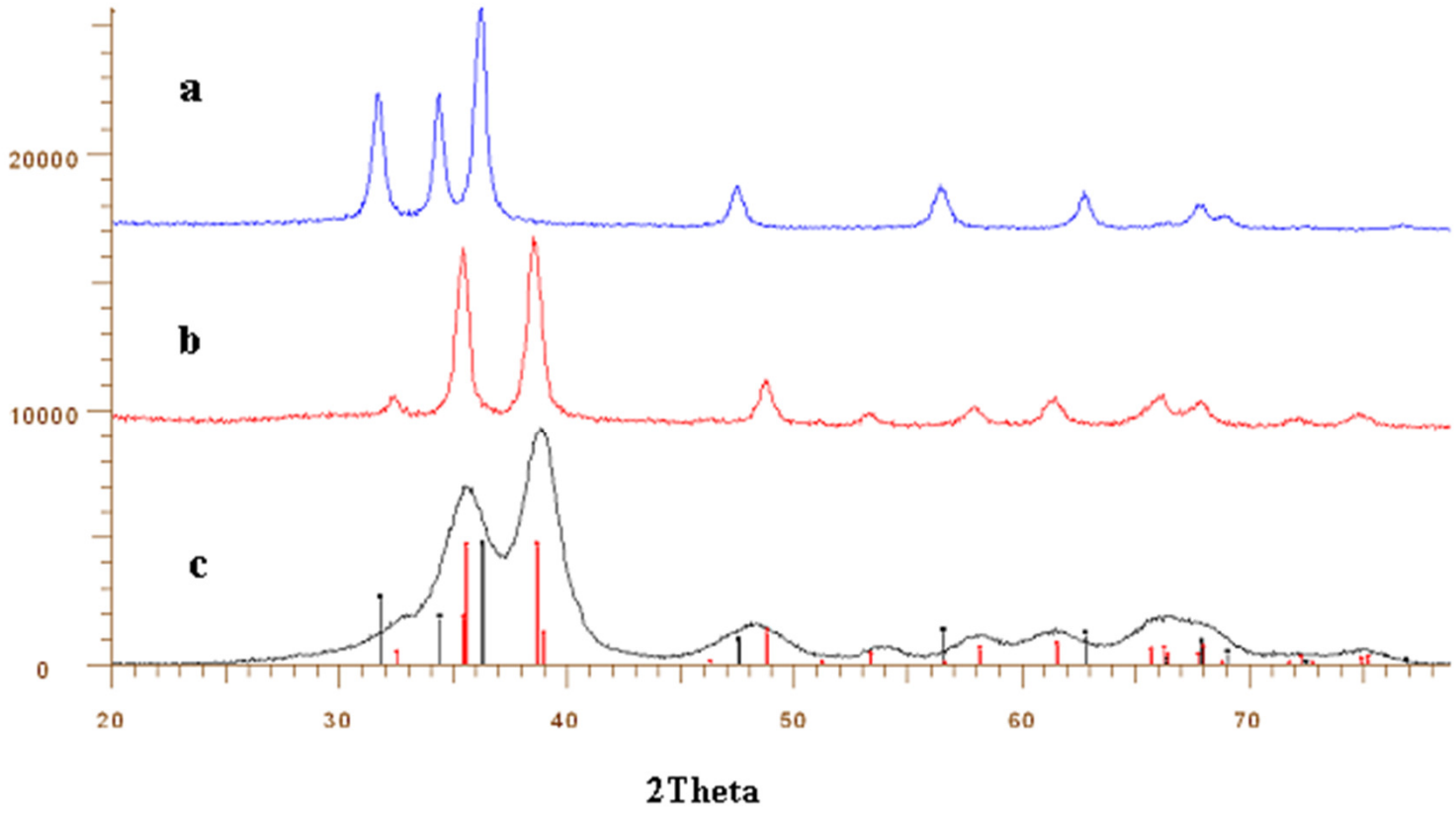
Content of metal ions on the coated cotton fabrics (by ICP method)

Sample name	Concentration in working solution (mol/L)		Deposition (wt %)	
	$[Cu^{2+}]$	$[Zn^{2+}]$	$[Cu^{2+}]$	$[Zn^{2+}]$
1 - low	0.015	0.005	0.41	0.05
2 - medium	0.0075	0.0025	0.95	0.12
3 - high	0.00375	0.00125	2.15	0.27

Antibacterial activity table results using *E. coli and S. aureus*

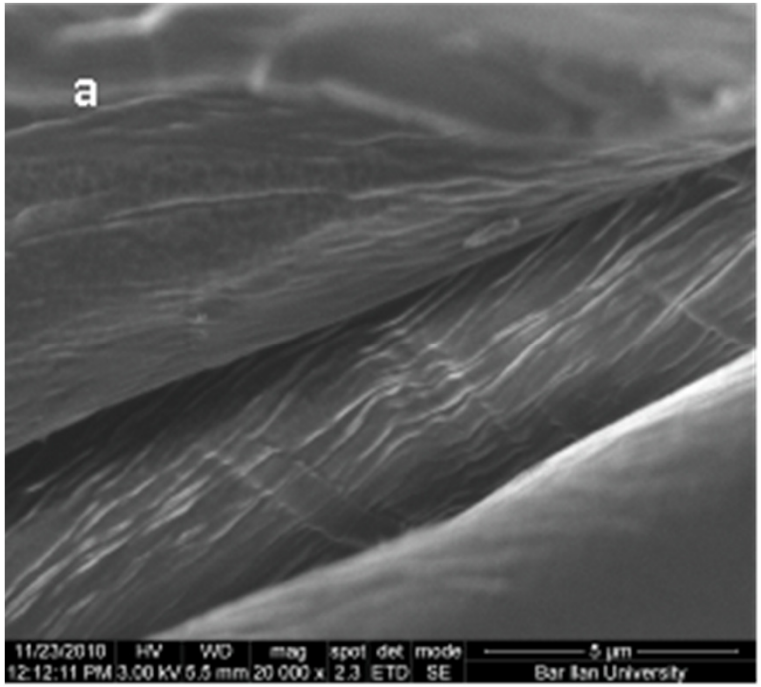
Treatment	Duration of treatment			
	10min		30min	
	$CFUml^{-1}$	Difference in viability (log)	$CFUml^{-1}$	Difference in viability (log)
E. coli T_0 $2.5x10^8$				
Clean fabric	$2.47\ x10^8$	-0.005 ± 0.049	$2.49\ x10^8$	-0.005 ± 0.077
Zn-CuO	$1.07\ x10^3$	-5.37 ± 0.009	$1.9\ x10^1$	-7.14 ± 0.23
S. aureus. T_0 $1.47x10^6$				
Clean fabric	$1.62\ x10^6$	-0.02 ± 0.08	$1.06\ x10^6$	-0.14 ± 0.09
Zn-CuO	$2.5\ x10^1$	-4.78 ± 0.27	$4\ x10^0$	-5.62 ± 0.41

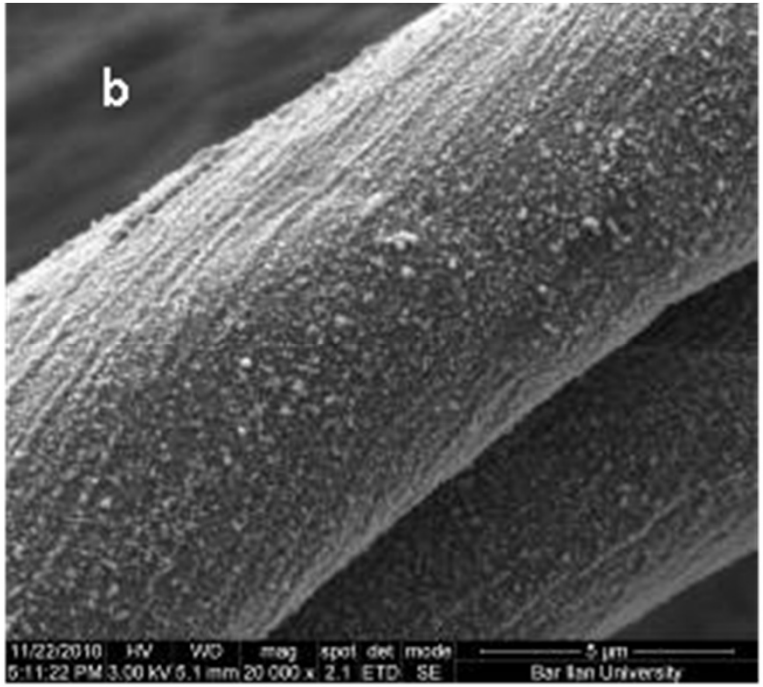
XRD diffraction patterns of: (a) ZnO (b) CuO (c) Zn-CuO



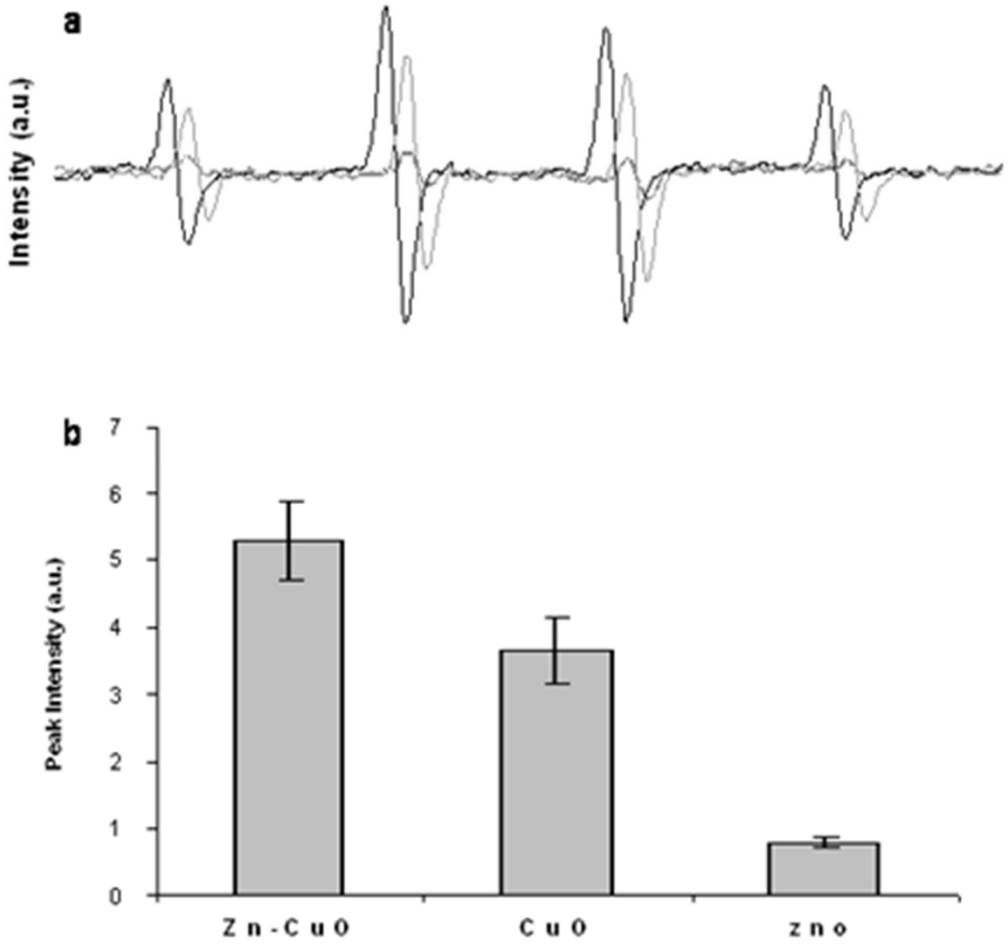
The cell parameters of the CuO lattice changed from: a = 4.6890; b=3.4200; c=5.1300 for CuO to a = 4.6829; b=3.4201; c=5.1429 for the Zn-CuO

SEM images of the cotton fabric:
(a) pristine cotton fabric ,
(b) cotton fabric coated with Zn-doped CuO NPs



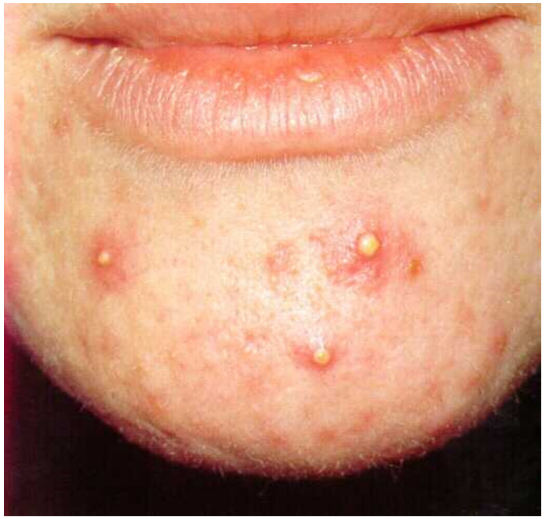


ESR studies



(a) ROS generation by ZnO (bold gray line), CuO (gray line) and Zn-CuO (black line) NPs'; **(b)** The relative ESR signal intensity of the DMPO-OH spin adduct generated from Zn-CuO, CuO and ZnO NPs'

(a) Zn-CuO (b) Zn-CuO after 300°C under air (c) Zn-CuO after 550°C under air (d) Zn-CuO after 550°C under N_2 (e) DMPO control.



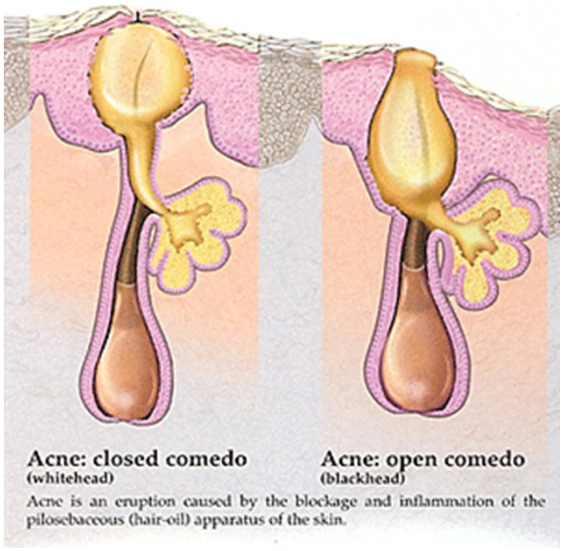
Acne : overview

Definition:

Acne Vulgaris is a Multi factorial disease

characterized by

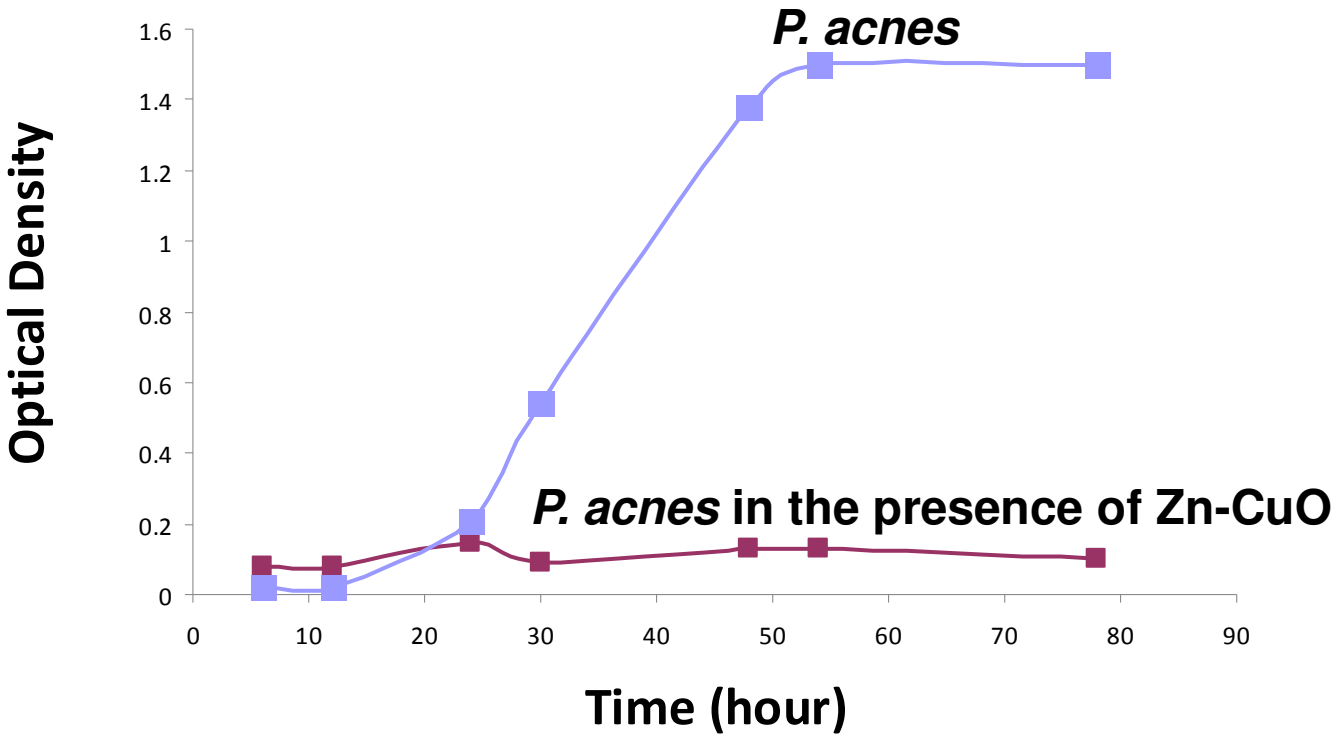
- abnormalities in sebum production,
- follicular desquamation,
- **bacterial Proliferation**
- inflammation.



Propionibacterium acnes
is the bacterium
responsible for
inflamed acne breakouts.

Controlling *Propionibacterium acnes* population is a crucial step in reducing acne

Zn-CuO NP's sonochemically coated on bandages cause immediate inhibition on *P. acnes* growth



What is a Biofilm?

A biofilm is a complex aggregation of microorganisms marked by the excretion of a protective and adhesive matrix to form a protected mode of growth that allows cells to survive in hostile environments

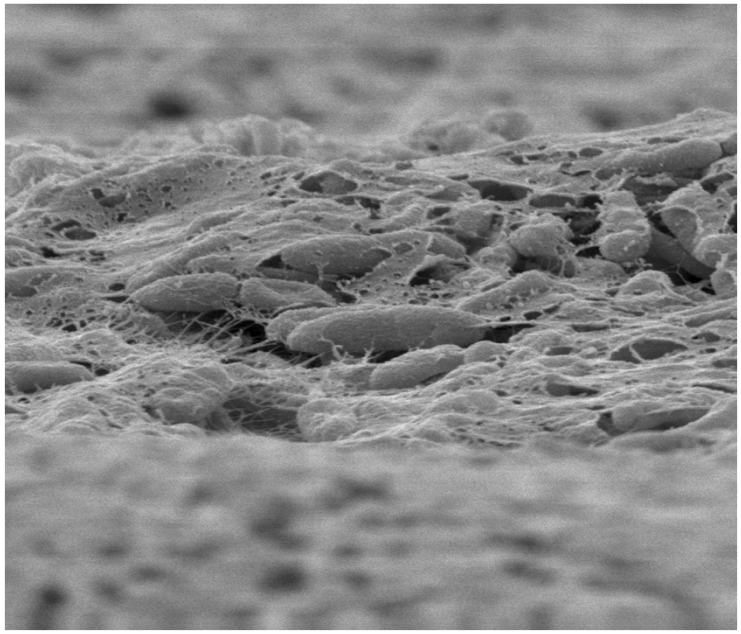
We have found that Nanoparticles of MgF_2 prevent the formation of Biofilms.
The nanoparticles were deposited on glass slides and the results below will demnostrate their activities.
The modes of deposition of the nanoparticles on the glass were sonochemistry and Microwave

Biofilm and biofouling

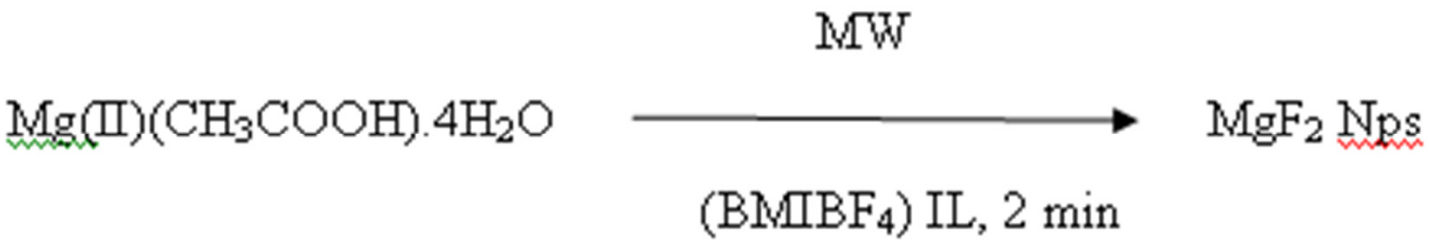
Biofilm - a structured community of bacterial cells enclosed in a self-produced polymeric matrix

Biofilms are a protective mode of growth that allow survival in hostile environments.

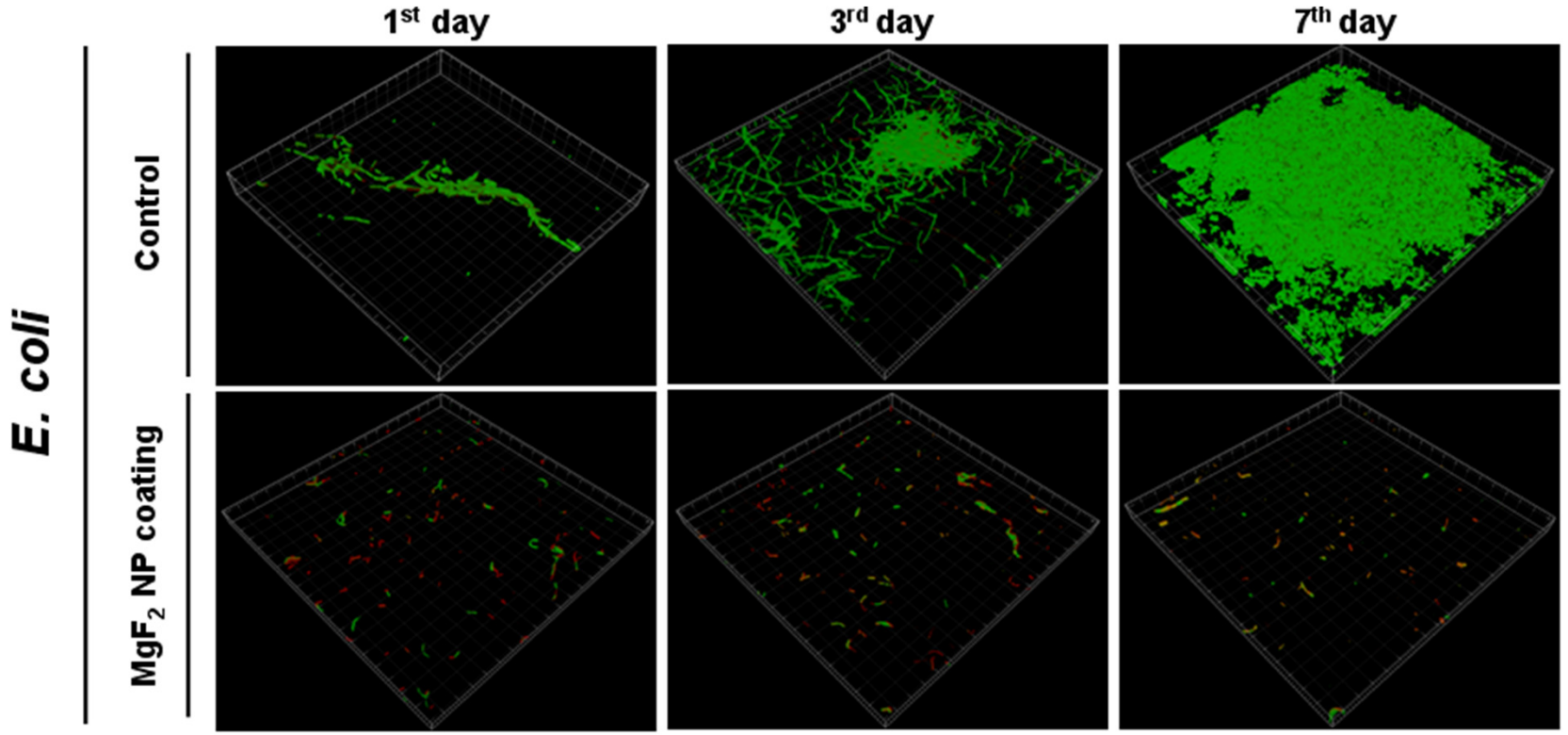
Increased resistance to disinfection – major problem



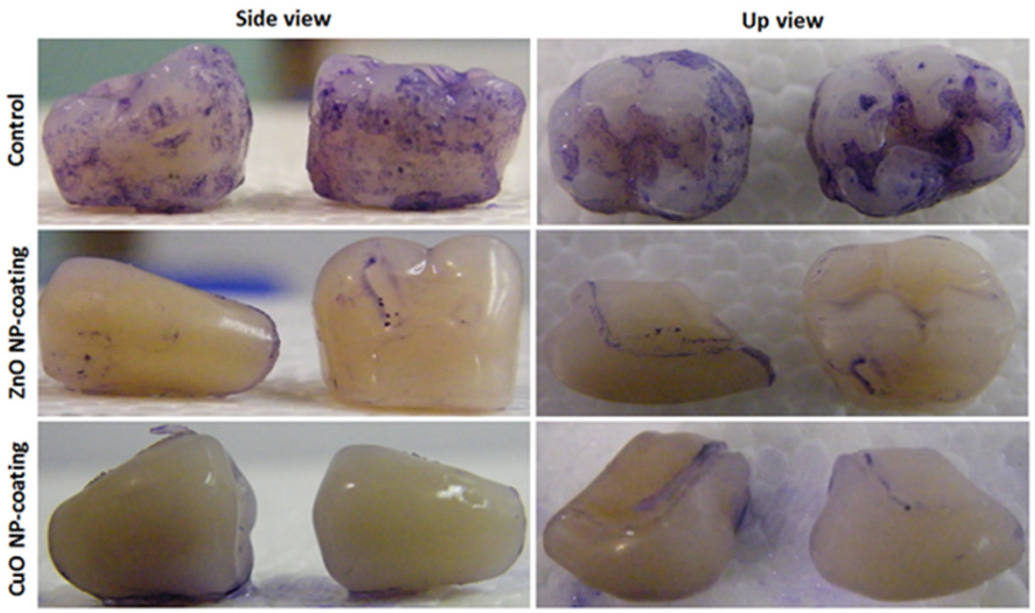
Synthesis of magnesium fluoride nanoparticles from 1-Butyl-3-methylimidazolium tetrafluoroborate Ionic Liquid (BMIBF4) and magnesium acetate salt under microwave irradiation.



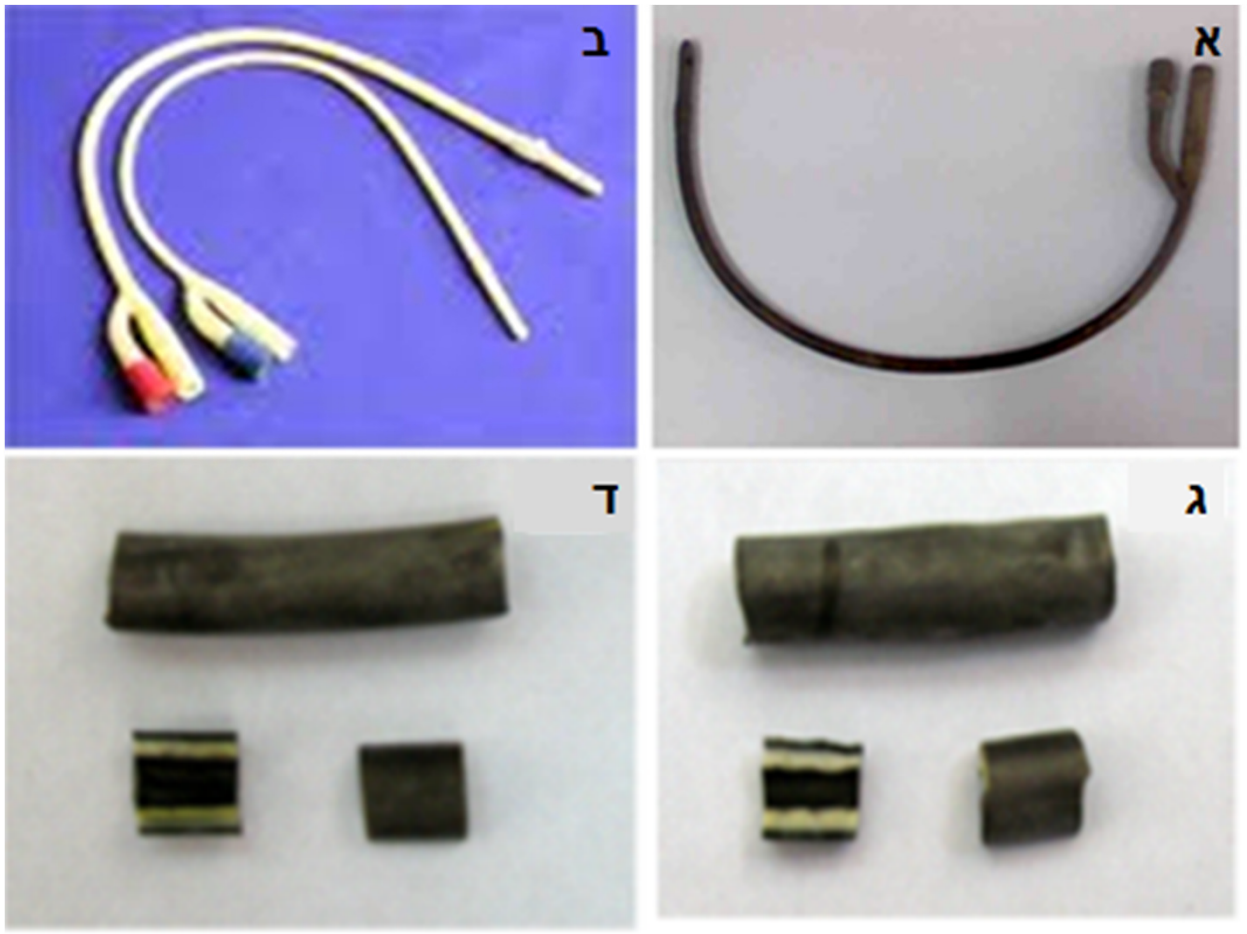
MgF_2 coated surfaces inhibit bacterial biofilm formation



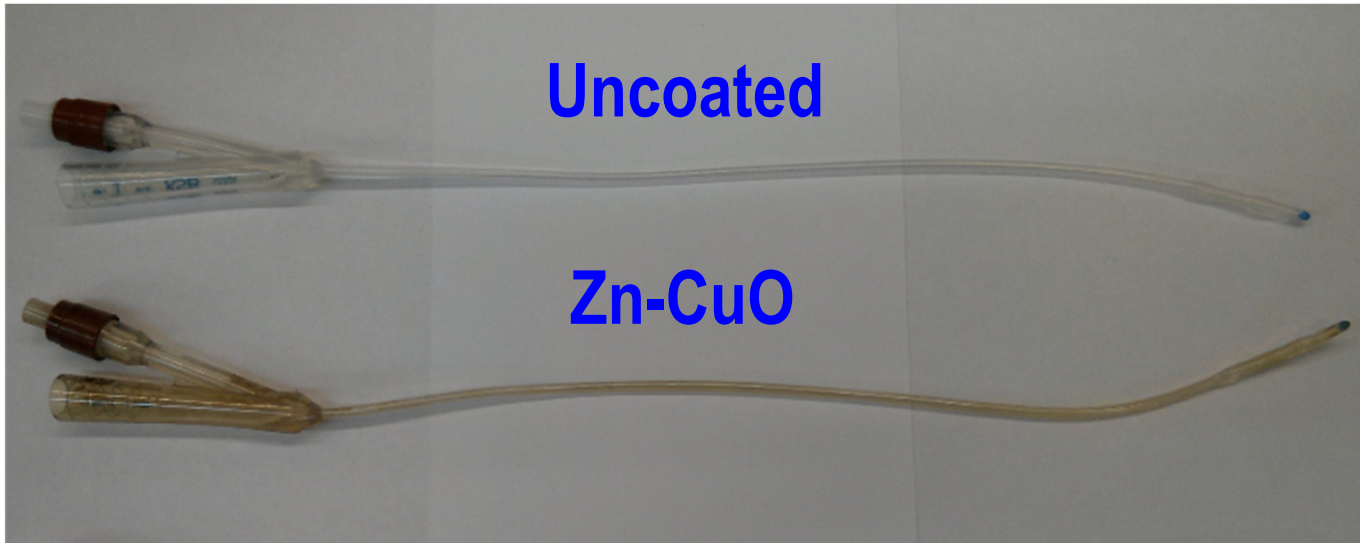
A



Ag coated catheters



Catheters coated with Zn-CuO



In vivo rabbit catheter model

In-Vivo Test With Indwelling Urinary Catheters

In vivo test with coated indwelling urinary catheters using coating type I to prevent the potential catheter-associated Urinary Tract Infection (CAUTI) and biofilm formation in the New Zealand White male rabbit, the exposure period was for 7 successive days.

Control Group – Urinary catheter without antibacterial protection	
Rabbit #	**Indication Day of Urinary Tract Infection (UTI)**
1	4
2	4
3	4
4	4
5	4

Test Group – Urinary catheter with antibacterial protection type II	
Rabbit #	**Indication Day of Urinary Tract Infection (UTI)**
1	7
2	7
3	2
4	**No indication** of UTI on day 7
5	**No indication** of UTI on day 7

Rabbit experiment results

- **General** - no mortality, no apparent clinical signs, no change in body weight.

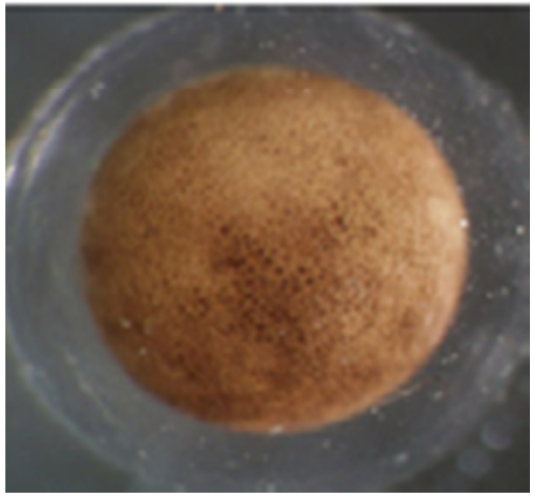
- **Histopathological** - no apparent differences in the range of lesions and/or severity of epithelia response. No signs of irritation.

- **Hematology & biochemistry (blood tests)** – no change between the rabbits with the *coated catheter* versus uncoated *catheter*.

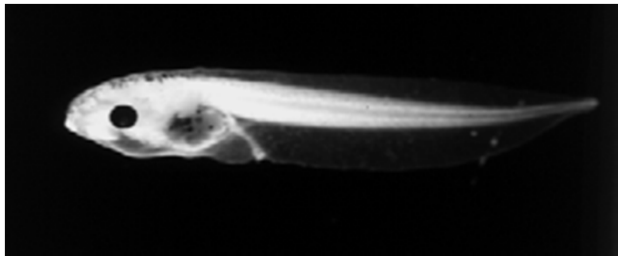
- **No irritating effect**

IS THE ENVIRONMENT AT RISK FROM LEACHING OF NANOPARTICLES FROM COATED FABRICS?

The biological effects on *Xenopus* embryos was performed, focusing on the possible interactions of particles with the biological structures at the nanoscale and on the role of the metal ions dissolved from NPs in inducing the toxic effects.



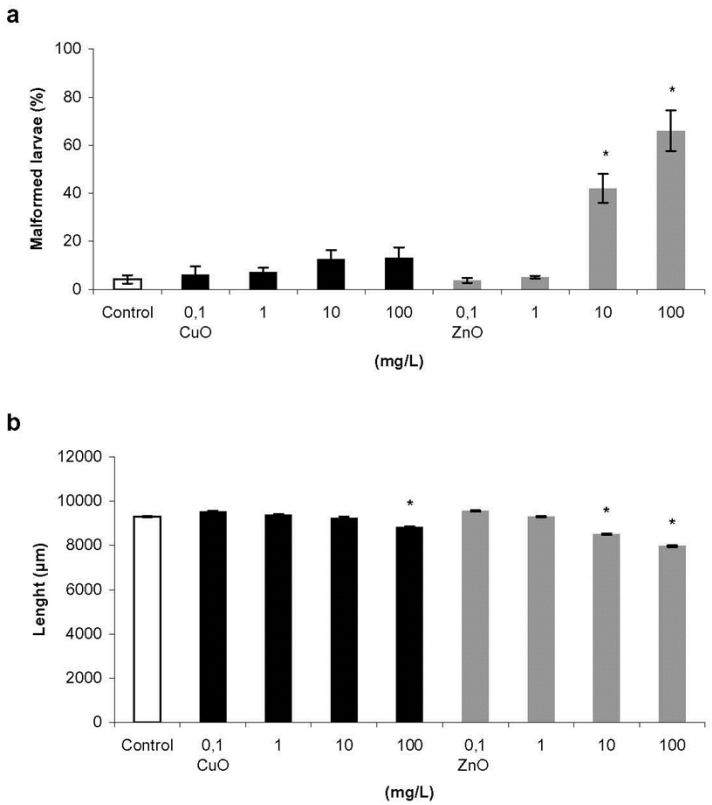
96h, NP exposure nCuO; nZhO at 0.1 – 500 mg/L



At the end of the test, the different endpoints (mortality, malformations, growth retardation) were registered and embryos preserved for further microscopical and biochemical analyses.

Nano Sono Cooperation

Embryotoxicity of sonochemically produced CuO and ZnO NPs (0.1-100mg/L), as evaluated by FETAX test



a) percentages of malformed larvae at the end of the test;
b) growth retardation measured in larvae at the end of the test. Black bars= CuO NPs; grey bars= ZnO NPs.

*significantly different from control at $p<0.05$.

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No lethal effects were registered in embryos exposed to 0.1-500 mg/L nCuO and nZnO.

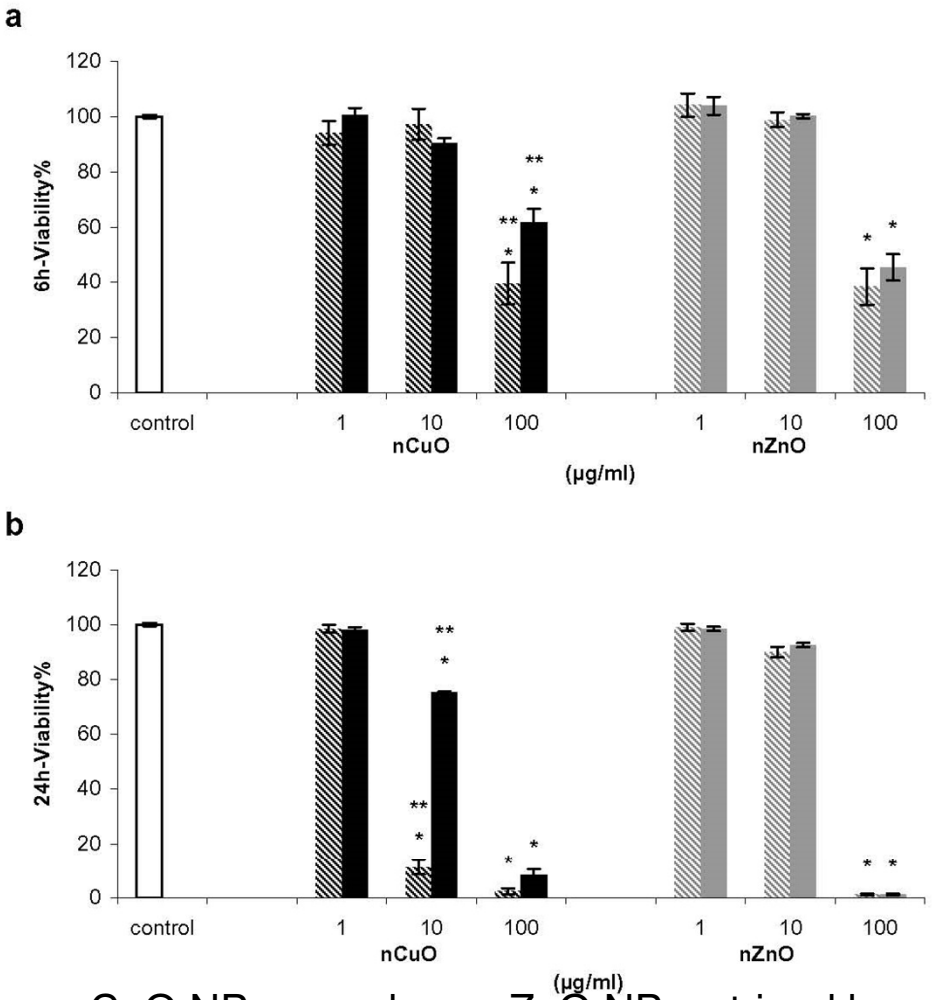
Malformation and growth retardation effects were observed starting from 10 mg/L

Control

nZno

Nano Sono Cooperation

Comparative cytotoxicity of commercial and sono CuO and ZnO NPs on human lung cells, A549, as evaluated by MTT assay at 6h (panel a) and 24h (panel b) exposure.



Black bars= CuO NPs; grey bars= ZnO NPs; striped bars= commercial NPs; solid bars=sono NPs.
* significantly different from control, ** significantly different from correspondent exposure group (sono *vs* commercial) at $p<0.05$.

Nano Sono Cooperation

Acknowledgements

Grants

EC Grants: 6th Program NAPOLYDE, SELECTNANO, LIDWINE, MULTIPOL consortia GM grant
7th program: SONO, Parylens, NOVO, NANOCI, INFACT

People: Dr. Ilana Perelshtein, Dr. Nina Perkas, Dr. Anat Lipovsky, Dr. Guy Applerot, Dr. Ulyana Shimanovich, Partners of LIDWINE and SONO

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