Selenium nanoparticles and their utilization in scaffolds

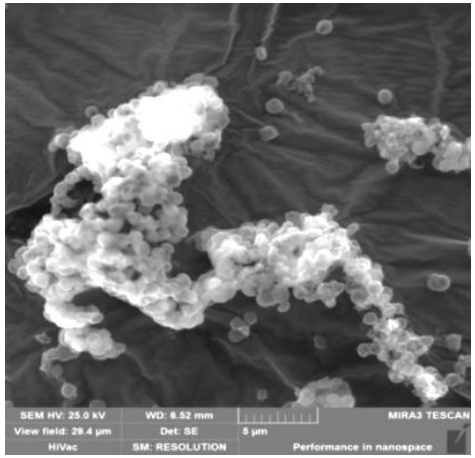
Prof. RNDr. Vojtech Adam, Ph.D.
Mgr. Dagmar Hegerova, Ph.D.
Mendel University in Brno

Content

1. Nanoparticles – silver, selenium
    - Staphylococcus aureus; Bacterial infections
    - Antibiotic resistance
2. Product SelenBact
3. Nanoparticles in scaffolds

Staphylococcus aureus

- G^+, facultative anaerobic coccal bacterium of the genus *Staphylococcus* (Ø 1 µm)
- In 1/3 of the population naturally presented in the human respiratory tract, on the skin and mucous membranes



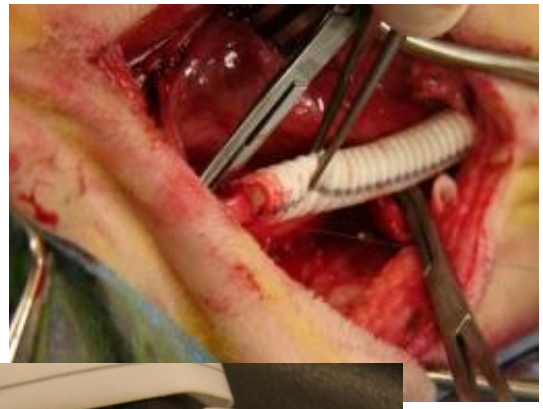
S. aureus under electron microscopy

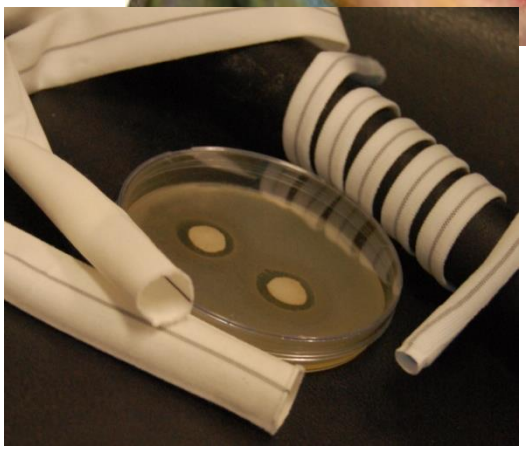
Colonies of *S. aureus*

Colonies of *S. aureus* on Petri dish

Staphylococcus aureus

- Not always pathogenic, but often a serious agent of the bacterial infections, emerging after transplantation of implants (e.g. vascular grafts etc.)
- Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies



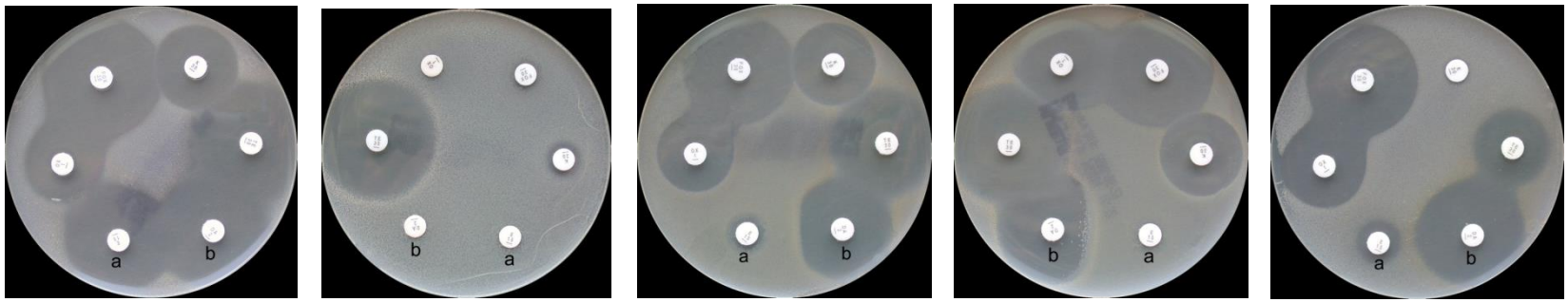


Bacterial infections: treatment and prevention

- Localized infections – surgically
- 90 % of staphylococci are resistant to penicillin → use of penicillin, which inhibits beta-lactamase
- Macrolides, cephalosporins, aminoglycosides, tetracyclines etc.
- Prevention – vaccines to stimulate specific immunity
- Antibiotic-resistant strains (e.g. MRSA) = worldwide problem in clinical medicine

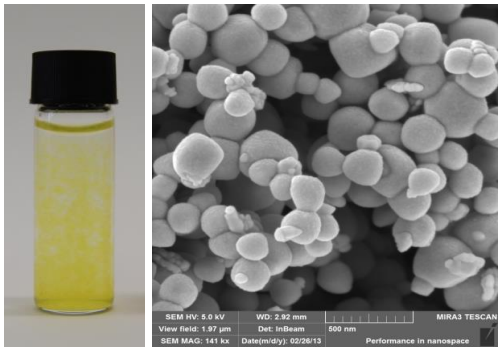
Resistance of microorganisms

- Biological resistance = resistance x environment effects,
- Resistance genes code genetic information, which can be changed by environmental factors.



Characterization of nanoparticles

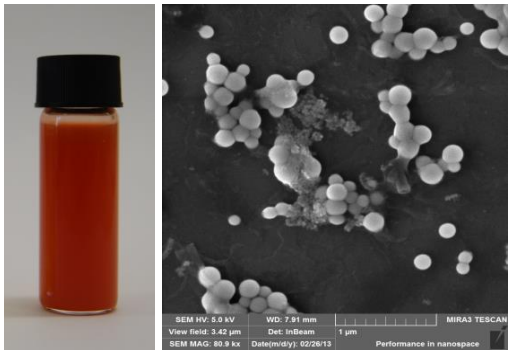
- Silver phosphate nanoparticles (SPNPs)



$Na_2HPO_4 \cdot 7H_2O$ + solution of SPNPs nitrate + ACS → yellow colloidal SPNPs

- (200-300 nm) square or spherical character

- Selenium nanoparticles (SeNPs)



$Na_2SeO_3 \cdot 5H_2O$ + $HSCH_2CH_2CO_2H$ + ACS → SeNPs

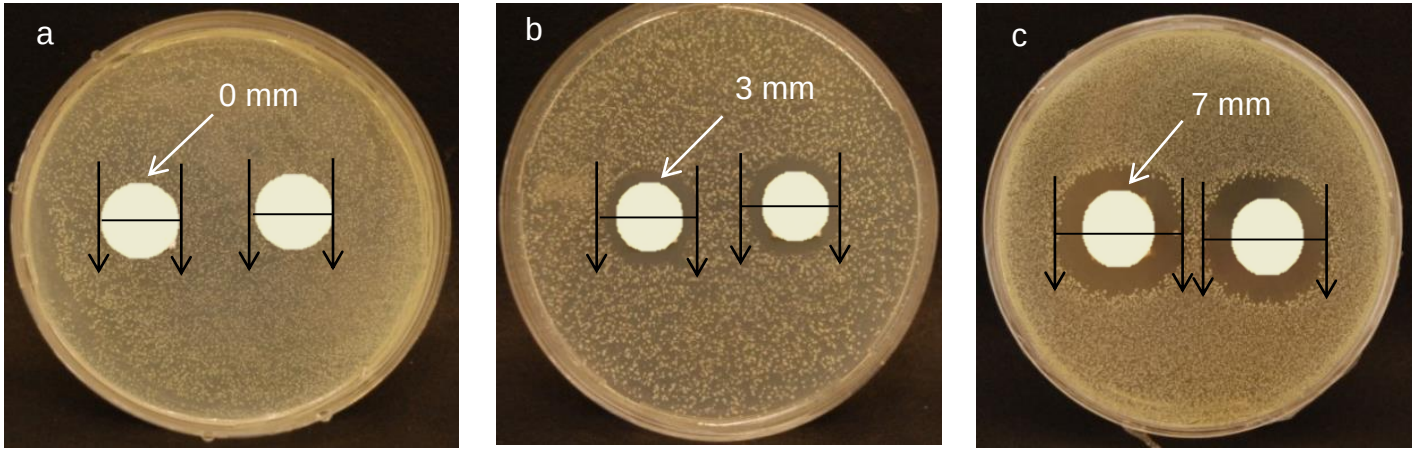
- (50-100 nm) small spherical particles

Chudobova et al., Comparison of the effects of silver phosphate and selenium nanoparticles on Staphylococcus aureus growth reveals potential for selenium particles to prevent infection FEMS Microl. Letters, 351(2), 195-201

Antimicrobial effect of nanoparticles

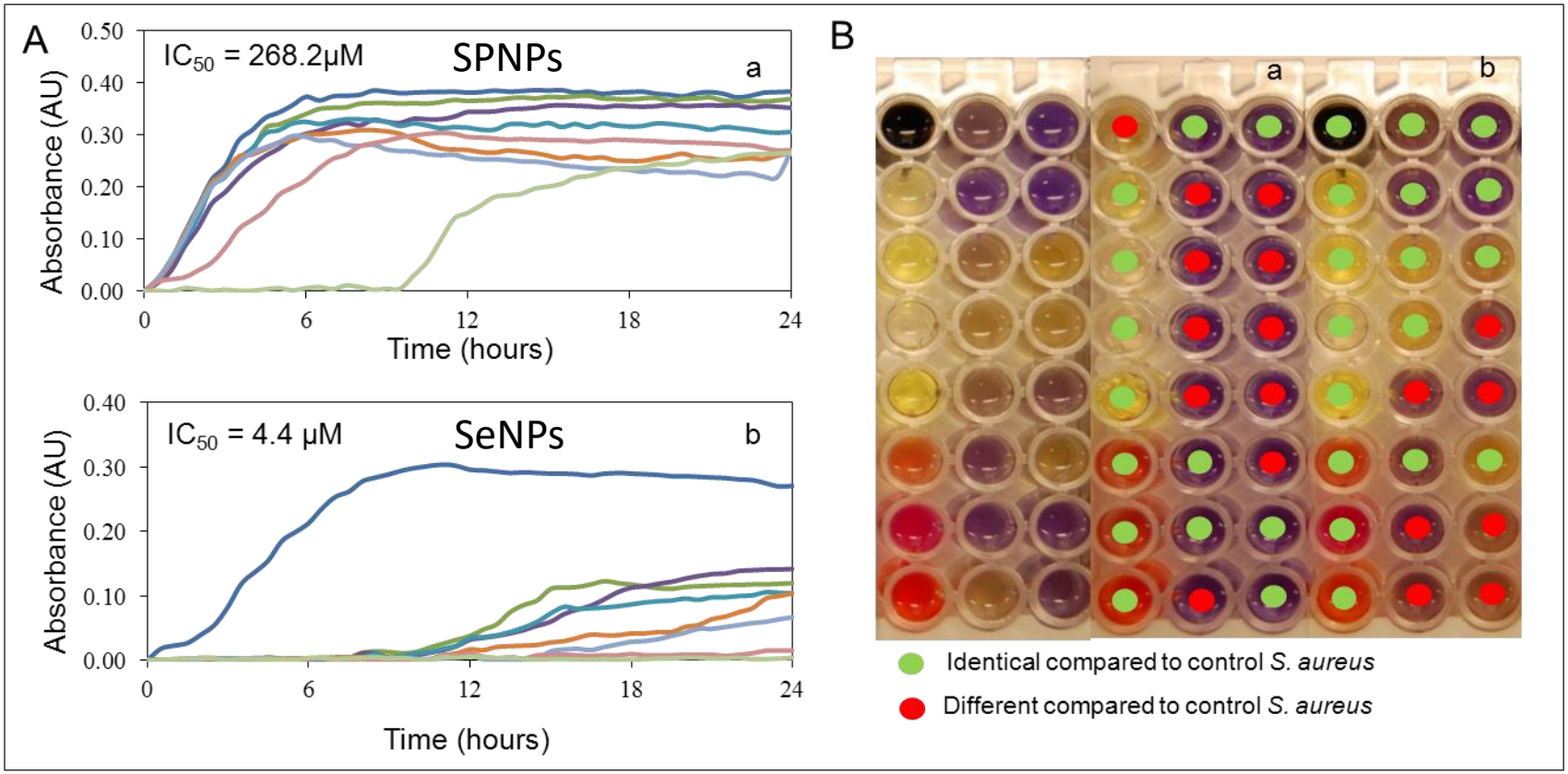
- Growth inhibition zones in bacterial culture after application of 300 μM concentration of nanoparticles
- Significant growth inhibition after application of smaller-sized SeNPs (even twice larger zones - 7 mm) were observed in comparison with larger SPNPs (3 mm zones)

S. aureus | Silver nanoparticles | Selenium nanoparticles



Antimicrobial effect of nanoparticles

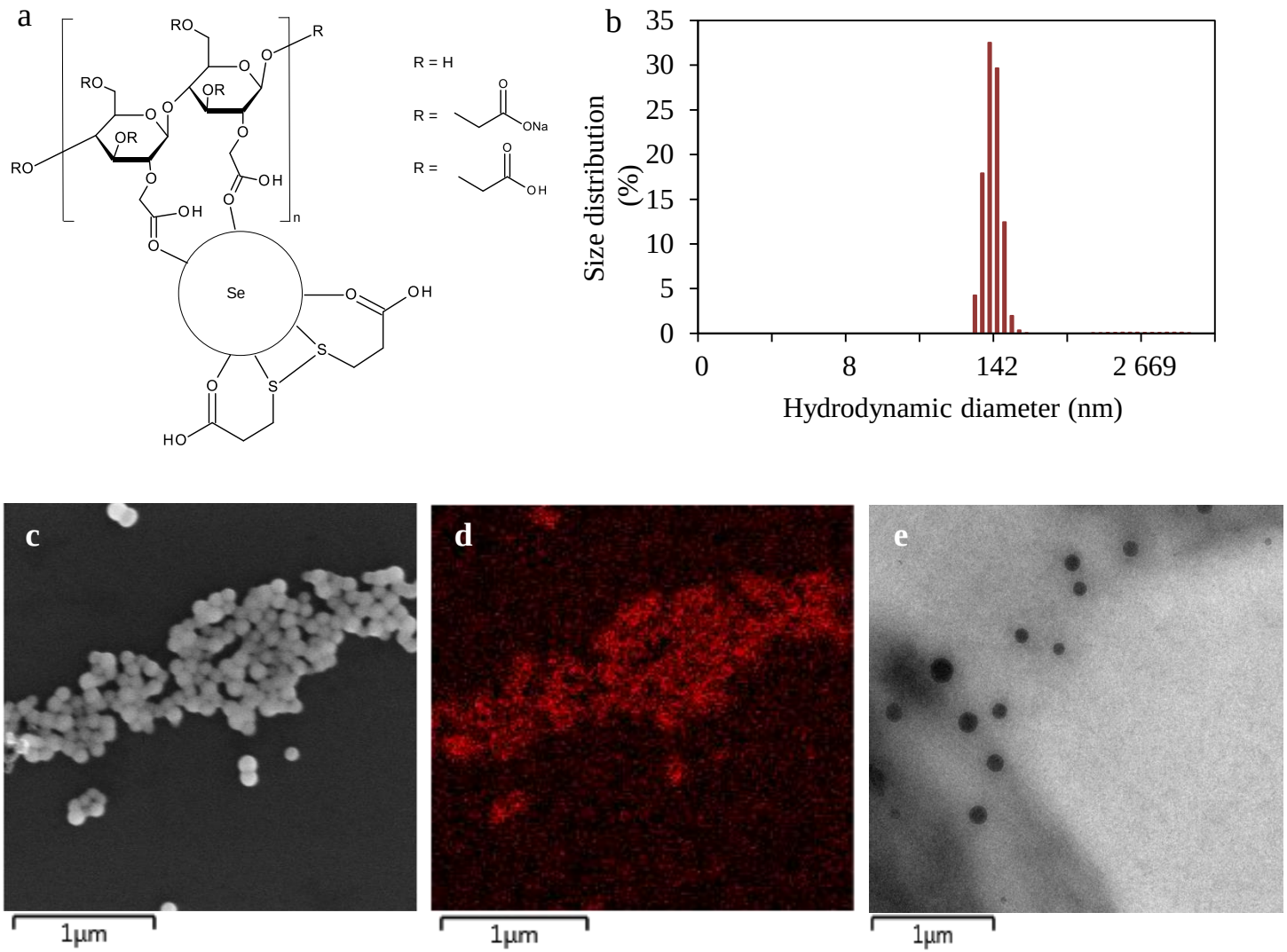
- (A) Spectrophotometric analysis of the growth of *S. aureus* bacterial culture with SPNPs (a) or SeNPs (b) in concentrations of ● 0, ● 10, ● 25, ● 50, ● 75, ● 150, ● 225 and ● 300 μM and (B) colorimetric study of changes in biochemical parameters after application of 300 μM concentration of nanoparticles (B).



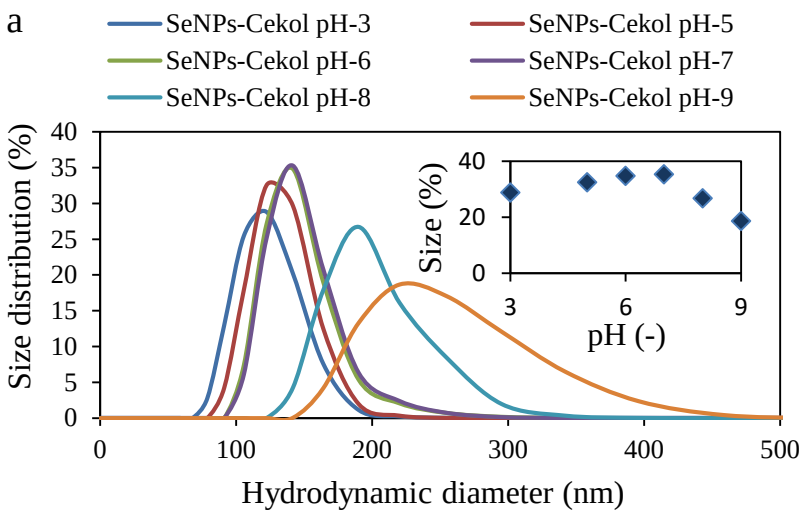
Product SelenBact

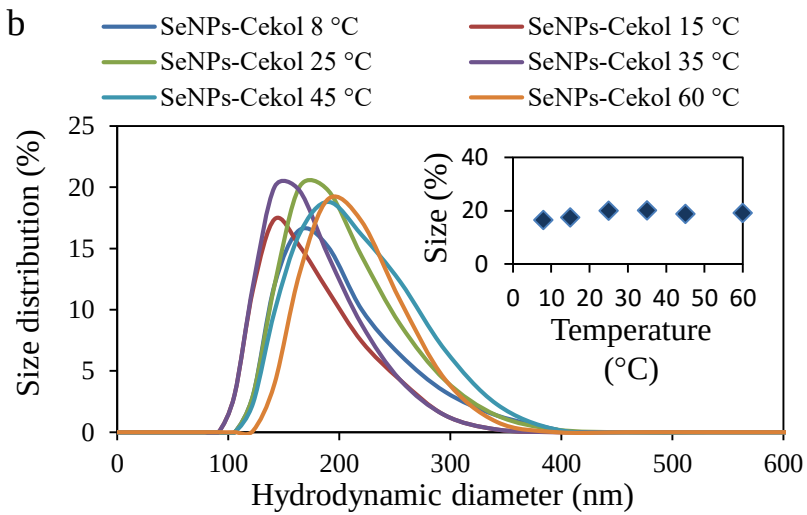
- **MedicProgress Ltd.**
- Characteristics:
  - pH 6.8 – 7.2
  - Content of Se 158 ppm ± 10 % (selenium content in solution was characterized by AAS)
  - Size 50 – 100 nm
  - Viscosity 1500 – 4500 mPa.s
  - Color – orange

Chemical structure of SelenBact

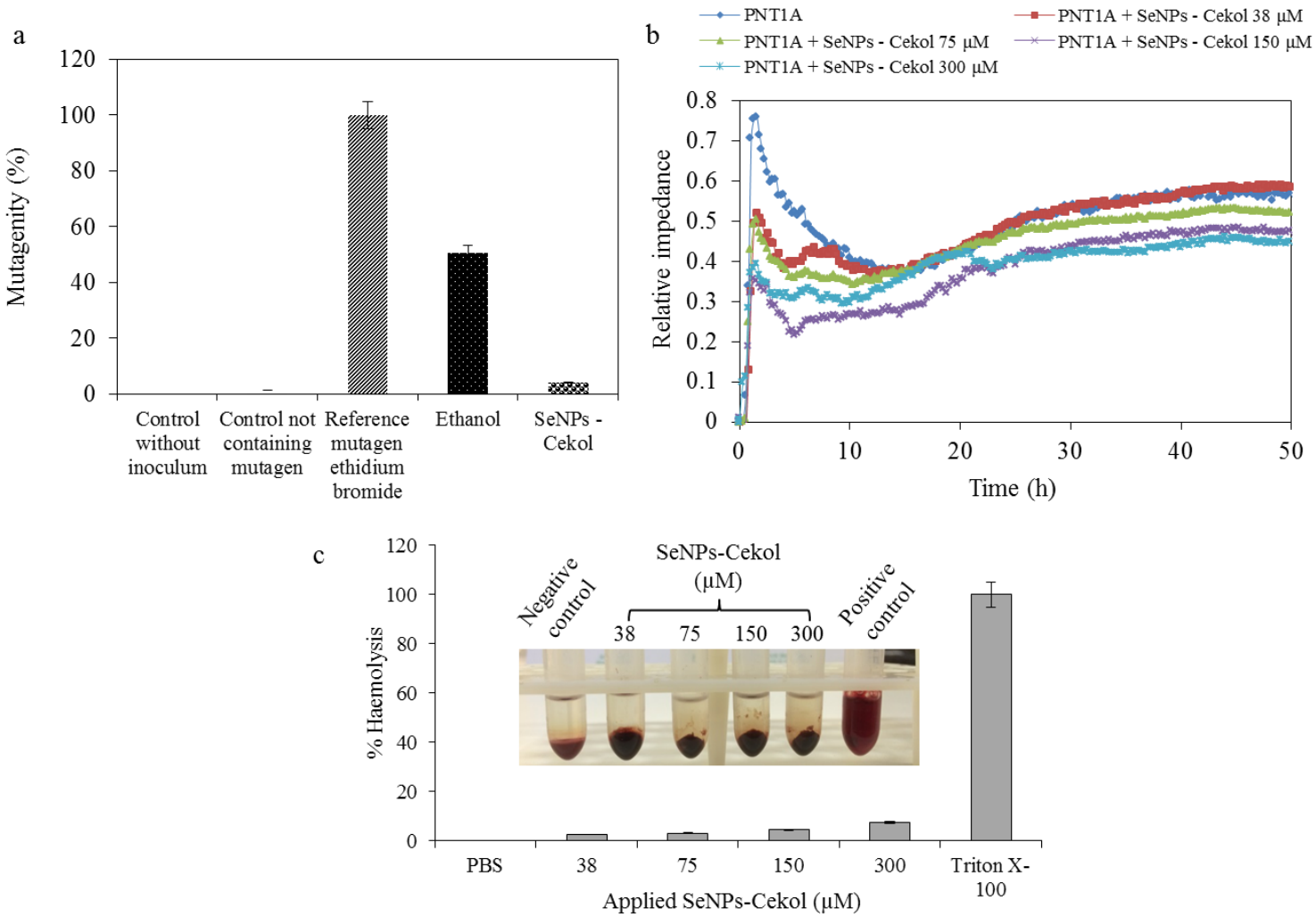


Determination of SelenBact stability

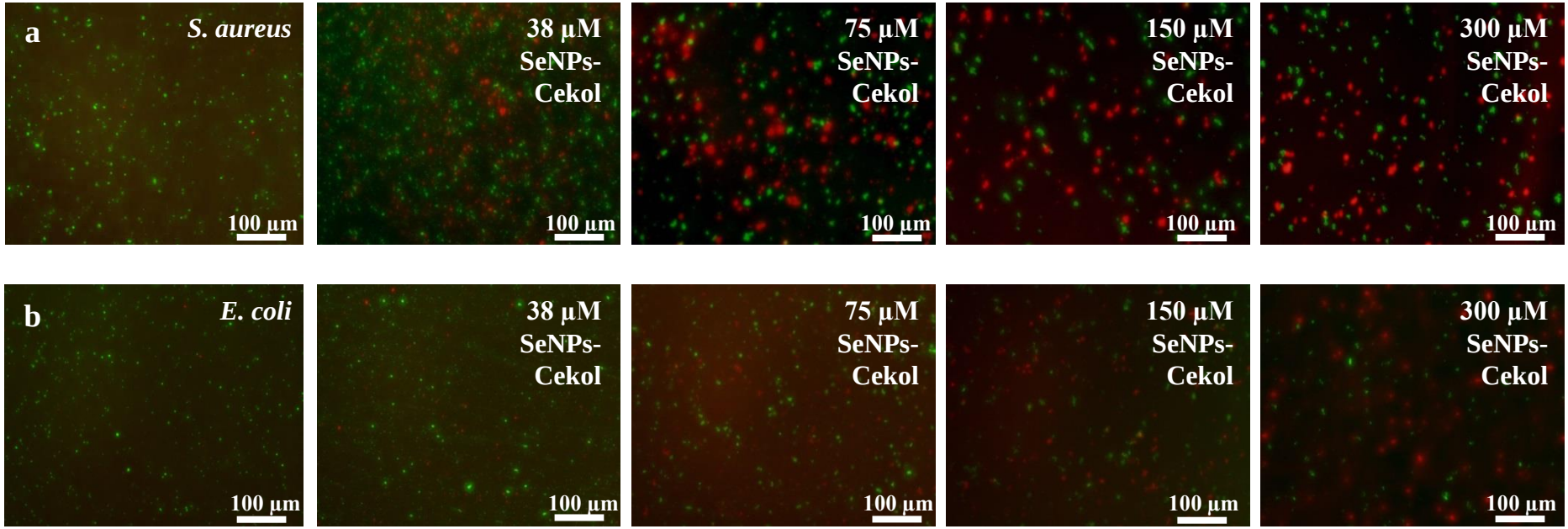




Testing of toxicity and mutagenity of SelenBact on eukaryotic cells

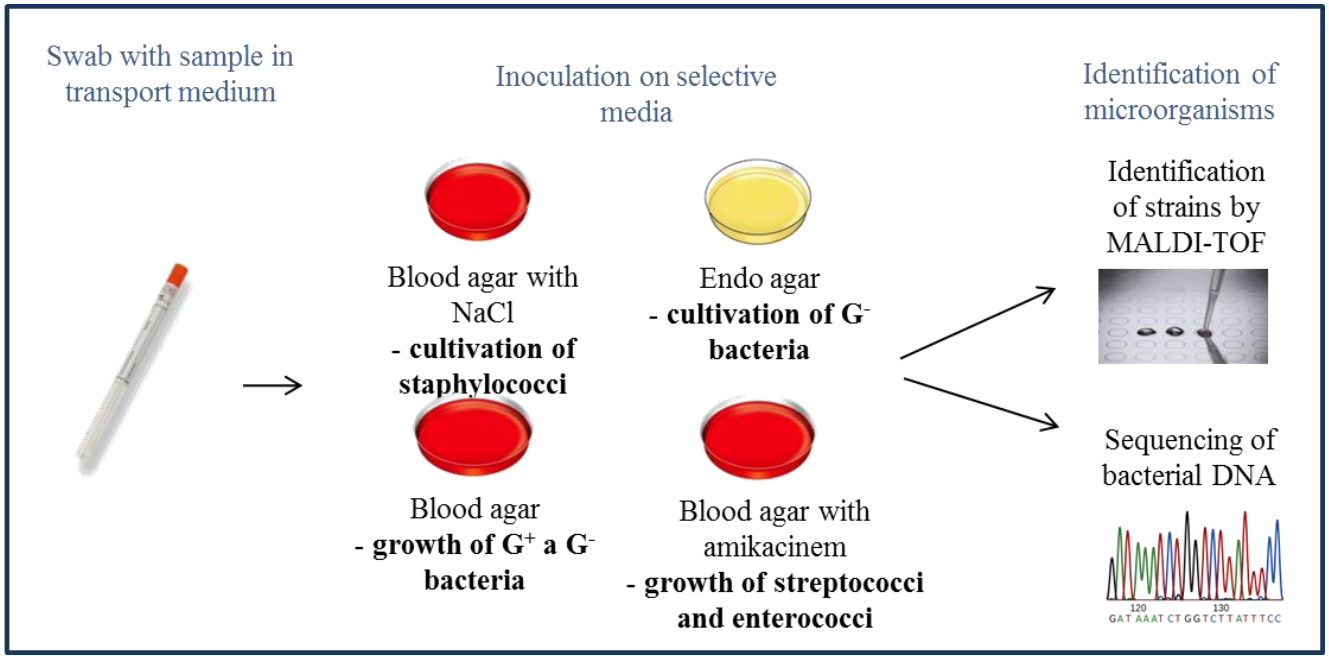


Fluorescence microscopy of bacteria treated with SelenBact



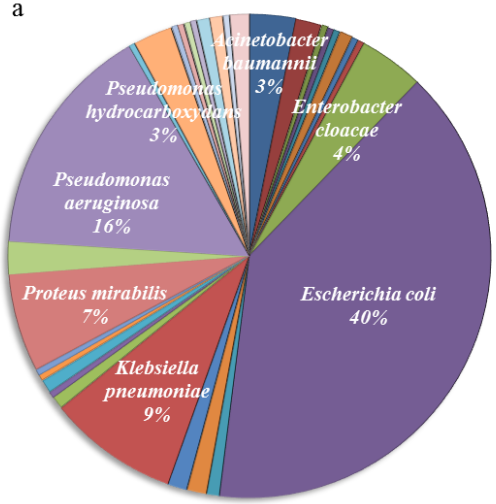
Bacterial isolates identification by MALDI

- Cooperation with Trauma Hospital in Brno
- Collection of swabs (300 patients) from surface wounds infected by bacterial specimens
- Isolation of these strains
- Identification by different methods (microbiology, PCR, sequencing, MS)



Bacterial isolates identification by MALDI

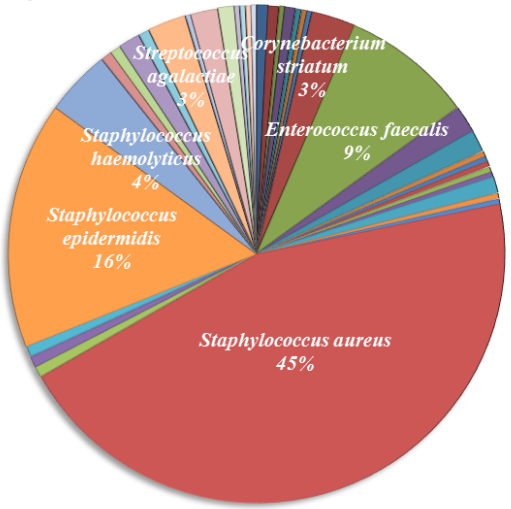
a



b

Specie of gram-negative bacteria	Occurence (%)	Specie of gram-negative bacteria	Occurence (%)
Acinetobacter baumannii	3.1	*Neisseria perflava*	0.9
Acinetobacter genomospecies	1.7	*Neisseria weaveri*	0.4
Acinetobacter lwoffii	0.4	*Pantoea agglomerans*	0.4
Aeromonas caviae	0.4	*Proteus mirabilis*	6.6
Aeromonas media	0.4	*Proteus vulgaris*	2.2
Alcaligenes faecalis	0.9	*Pseudomonas aeruginosa*	15.7
Arthrobacter parietis	0.4	*Pseudomonas alcaligenes*	0.4
Citrobacter freundii	0.4	*Pseudomonas hydrocarboxydans*	2.6
Enterobacter cloacae	4.4	*Pseudomonas mendocina*	0.4
Escherichia coli	39.7	*Pseudomonas nitroreducens*	0.4
Escherichia ferqusonii	0.9	*Pseudomonas oryzihabitans*	0.4
Hafnia alvei	1.3	*Pseudomonas syringae*	0.4
Klebsiella oxytoca	1.3	*Raoultella ornithinolytica*	0.9
Klebsiella pneumoniae	8.7	*Serratia marcescens*	0.9
Morganella morganii	0.9	*Serratia ureitica*	0.4
Neisseria gonorrhoeae	0.4	*Stenotrophomonas maltophilia*	1.3

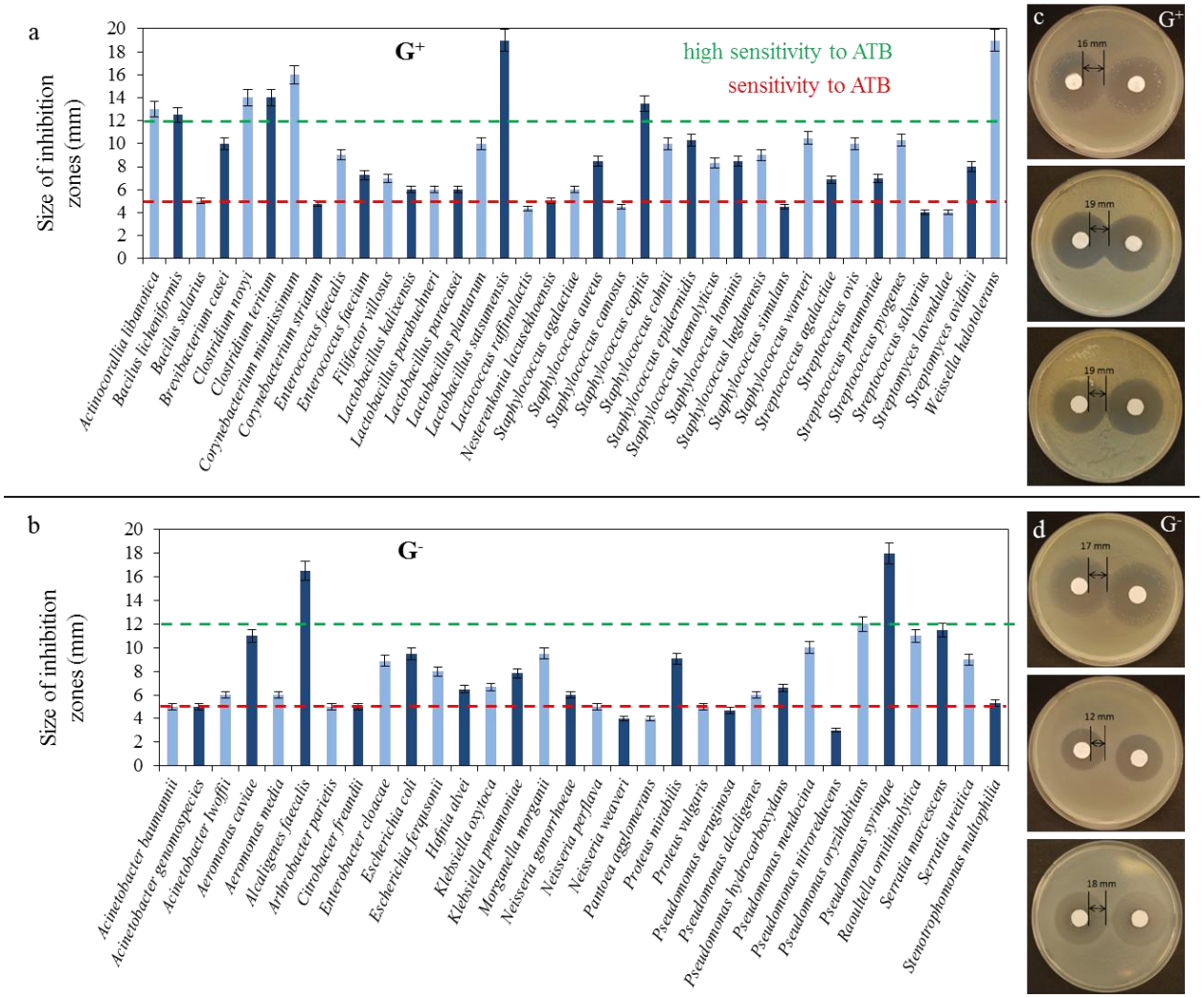
c



d

Specie of gram-positive bacteria	Occurence (%)	Specie of gram-positive bacteria	Occurence (%)
Actinocorallia libanotica	0.7	*Staphylococcus aureus*	45.0
Bacilus licheniformis	0.7	*Staphylococcus camosus*	0.7
Bacilus salarius	0.4	*Staphylococcus capitis*	0.7
Brevibacterium casei	0.7	*Staphylococcus cohnii*	0.7
Clostridum novyi	0.4	*Staphylococcus epidermidis*	16.1
Clostridum teritum	0.4	*Staphylococcus haemolyticus*	4.3
Corynebacterium minutissimum	0.4	*Staphylococcus hominis*	0.7
Corynebacterium striatum	2.9	*Staphylococcus lugdunensis*	0.7
Enterococcus faecalis	8.6	*Staphylococcus simulans*	1.4
Enterococcus faecium	1.8	*Staphylococcus warneri*	0.7
Filifactor villosus	1.4	*Streptococcus agalactiae*	2.5
Lactobacillus kalixensis	0.4	*Streptococcus ovis*	0.4
Lactobacillus parabuchneri	0.4	*Streptococcus pneumoniae*	1.8
Lactobacillus paracasei	0.4	*Streptococcus pyogenes*	1.1
Lactobacillus plantarum	0.4	*Streptococcus salvarius*	0.4
Lactobacillus satsumensis	0.4	*Streptomyces lavendulae*	0.4
Lactococcus raffinolactis	1.1	*Streptomyces avidinii*	0.4
Nesterenkonia lacusekhoensis	0.4	*Weissella halotolerans*	0.4
Staphylococcus agalactiae	0.4		

Effects of SelenBact on bacterial isolates

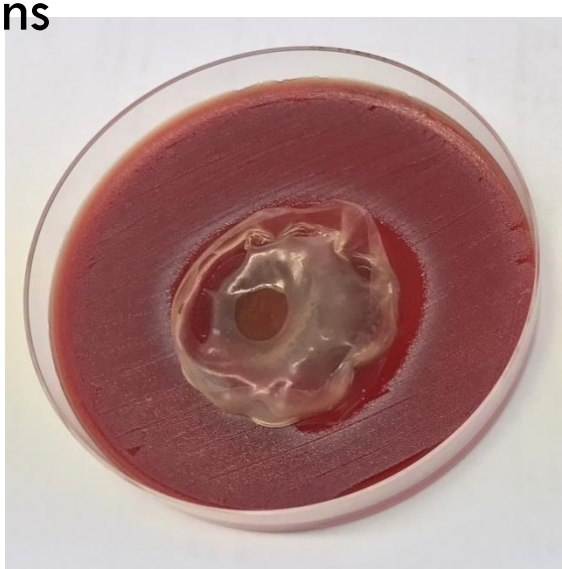


Selenium nanoparticles in scaffolds

- Advanced nanomaterials – potential in treatment of surface bacterial infections
- Attachment to the surface of cover material
    - Long-term sterility
    - Elimination and reduction the risk of spreading bacterial infection
    - Acceleration of tissue regeneration
    - Reduction the cost of treating infections

Selenium nanoparticles in scaffolds

- Physical and chemical fixation by cold plasma
- Promising results in terms of excellent transparency
- Non-adherent specimens – newly created tissue
- Ensuring the inhibition of the pathogens
    - In the contact area
    - Across the spectrum of bacterial strains



Antimicrobiality of SeNPs on scaffolds

- Surface treated with plasma

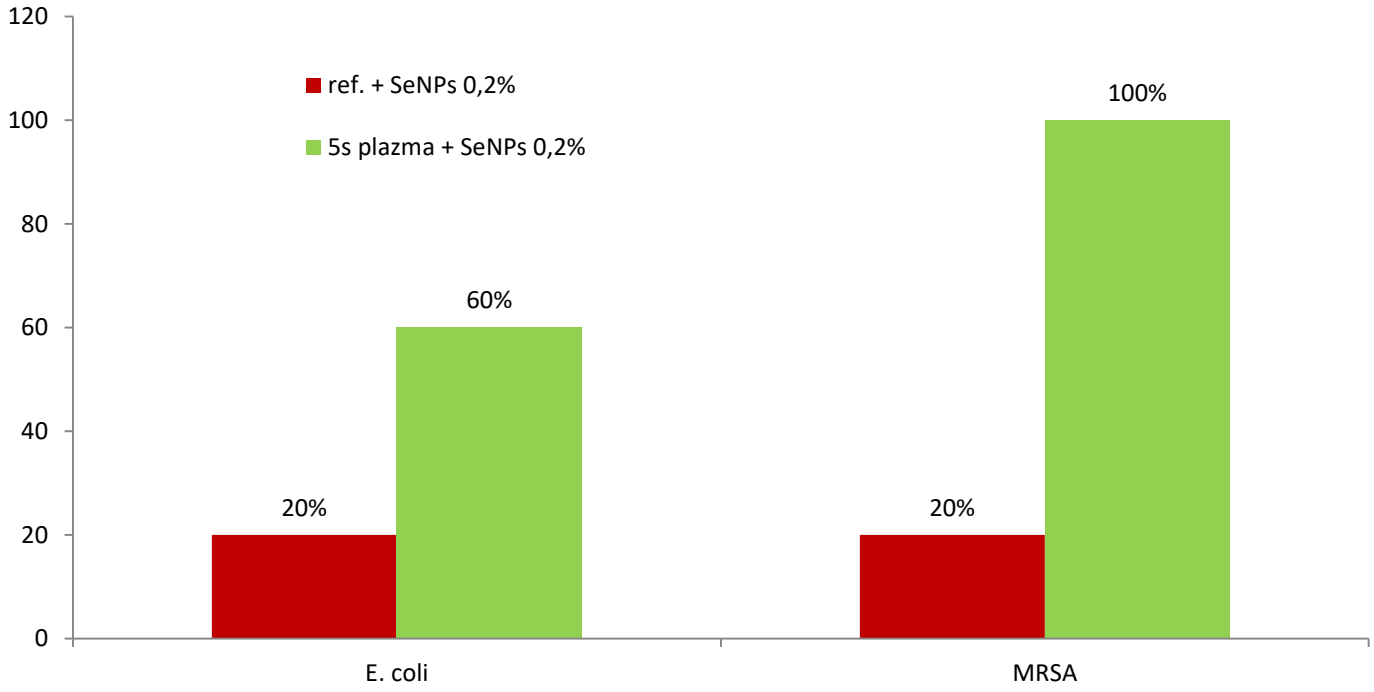
- Samples
  - ref. + SeNPs 0.2%
  - ref + SeNPs 0.2% + 1s plasma
  - 1s plasma + SeNPs 0.2%
  - 5s plasma + SeNPs 0.2%
  - 1s plasma + SeNPs 0.2% + 1s plasma
  - 1s plasma
  - 5s plasma
  - Control (pure fabric)

Size of inhibition zones

Fabrics	*E. coli*	MRSA
1. ref. + SeNPs 0.2%	1	1
2. ref + SeNPs 0.2% + 1s plasma	0	1
3. 1s plasma + SeNPs 0.2%	2	2
4. 5s plasma + SeNPs 0.2%	3	5
5. 1s plasma + SeNPs 0.2% + 1s plazma	2	4
6. 1s plasma	1	0
7. 5s plasma	1	0
8. Control (pure fabric)	0	0

*** Most suitable to reduce bacterial growth is fabric 5s plasma + SeNPs 0.2%**

Comparison of effects of SeNPs and SeNPs with plasma



Acknowledgment

Thank you for your attention