evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: Utilization of Electrochemistry for detection of bacteria on a 3D printed flow chip

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Název projektu: Mezinárodní spolupráce v oblasti "in vivo" zobrazovacích technik

Why determine just MRSA?

- Pathogen resistant to β-lactam antibiotics
- Prevalence and spread in hospital environment, food industry (meat)
- Transmission of infection between patients
- Difficult and lengthy treatment

New diagnostic systems

- optical, mechanical, magnetic and electrochemical methods, labeled strategies
- for quickly and simple identification of presence MRSA
- method based on the principle of aggregation AuNPs (Wang, et al., 2011)

Steps of detection

1. Cultivation of bacteria from the sample
2. Isolation of DNA using the AuMNPs
3. Amplification of gene using AuNPs modified complementary primers for specific gene (*mecA*)
4. Spectrophotometric detection of AuNPs using NaCl as an indicator of color changes

Material and methods

- Synthesis of AuNP
  - into $HAuCl_4 \bullet 3H_2O$ solution was added aqueous solution of sodium citrate tribasic dihydrate (0.5 ml, 40 mM). The color of the solution slowly changed from yellow to violet.

- Synthesis od AuMNPs
  - Prepared from 2 solutions: $Fe(NO_3)_3 \bullet 9H_2O$ and 25% NH_3

- Labeling of AuNPs by thiolated primers

Cultivation of bacteria strains

- *S. aureus, E. coli,* MRSA*, S. typhimurium, L. rhamnosus* – model bacteria
- Clinical specimens including wound swabs were obtained from Trauma Hospital of Brno → selective cultivation → identification of bacterial strains using mass spectrometry MALDI-TAF/TOF

Detection of *mecA* gene fragment using AuNPs probe

- The 10 µl of AuNPs probe was mixed with 5 µl of PCR product (26 µM). Afterwards was the mixuture heated 95 °C 5 min and slowly cooled to 25 °C.
- For the *mecA* gene detection was the 10 µl of 5 M NaCl added into the cooled mixture (to final concentration of 2 M).

3D printed chip design and fabrication

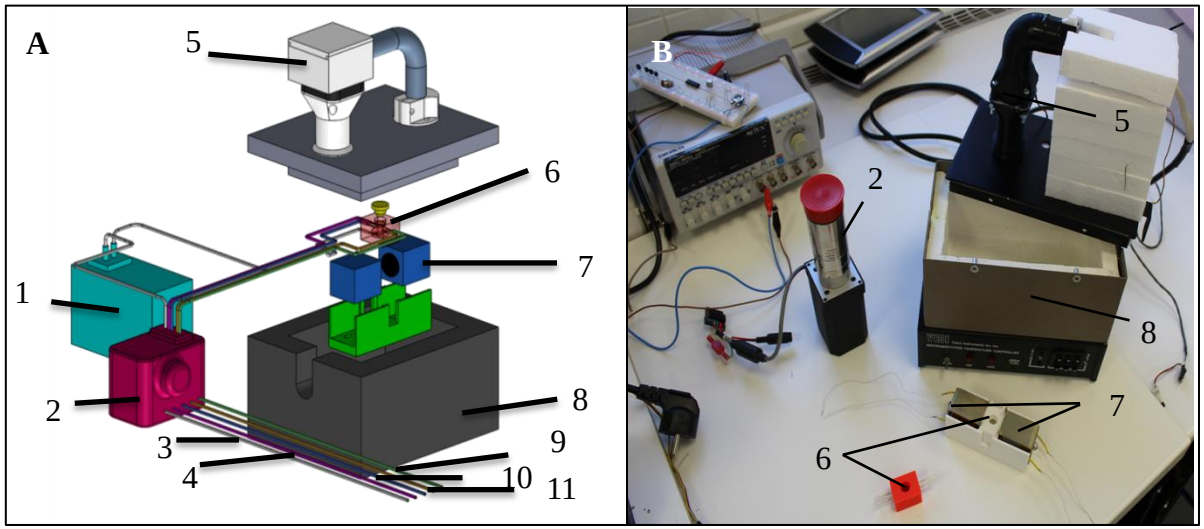
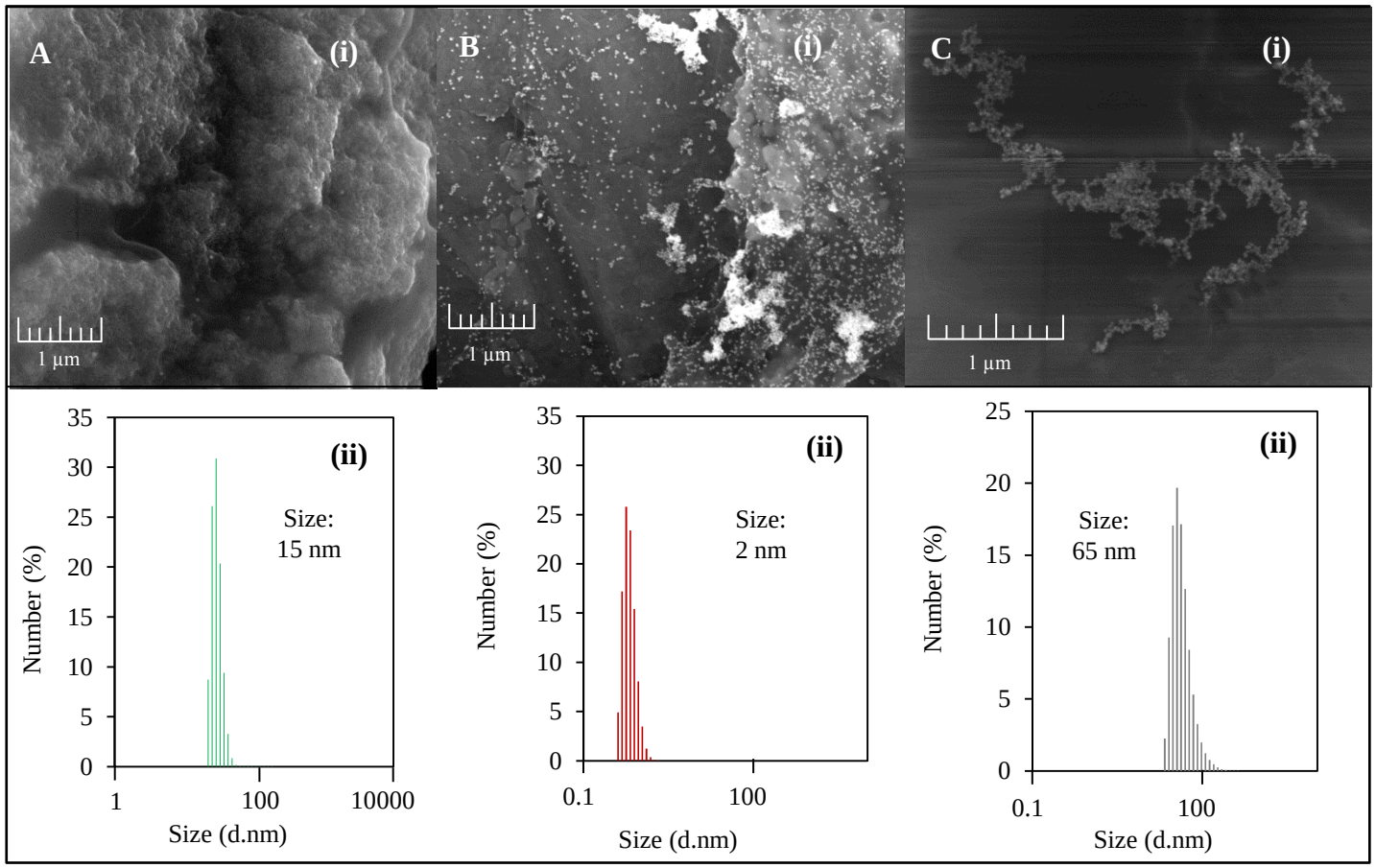


Fig. **A)** Scheme of 3D printed biochip for the detection and confirmation of methicillin-resistant S. aureus presence using binding of MRSA to the gold nanoparticles with specific primers in the chip, **B)** system for the identification of MRSA in the sample and reaction chamber of 3D printed chip: 1 – spectrophotometric detector, 2 – pump with the valves, 3 – outlet, 4 – the first inlet hose, 5 – thermoregulatory system, 6 – cultivation chip, 7 – electromagnet, 8 – thermoisolating box, 9 – the second inlet hose, 10 – the third inlet hose, 11 – the fourth inlet hose.

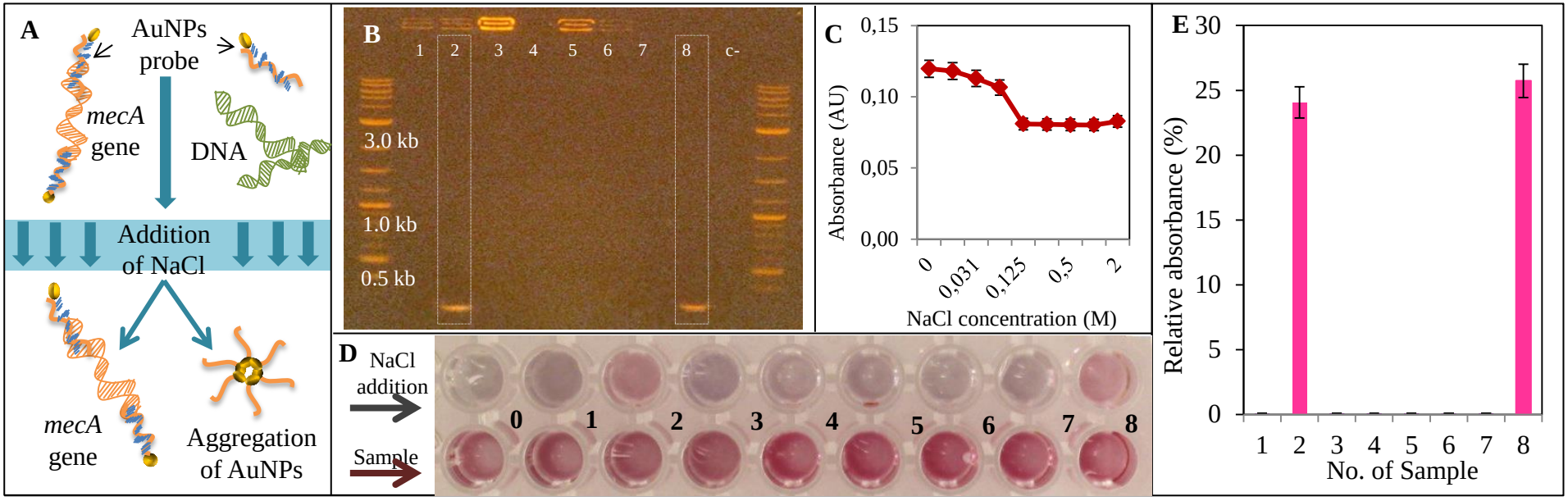
- using 3D printer (Profi3Dmaker, Aroja, Czech Republic) controlled by G3Dmaker v1.0 software (Aroja, Czech Republic). The reaction chamber was made from acrylonitrile butadiene styrene (ABS) (Printplus, Aroja, Czech Republic), which is sufficiently rigid and wiry for the final application of the product

Characterization of AuMNPs and non-aggregated or aggregated AuNPs



Microscopic and electrochemical characterization of **A)** 1 mg/ml AuMNPs, **B)** 1 mM AuNPs and **C)** aggregated 1 mM AuNPs dissolved in water by **(i)** Scanning electron microscope (magnification for AuMNPs 40 kX, AuNPs 40.9 kX and aggregated AuNPs 64.6 kX), and **(ii)** the measurements of AuNPs size distribution by Zetasizer. Measuring conditions were: detector angle 173°, wavelength 633 nm, refractive index 0.30, a real refractive index 1.59, and a temperature 25 °C. Other details are mentioned in materials and methods.

Identification of *mecA* gene fragment to the gold nanoparticles probes



A) Experimental scheme of *mecA* gene detection. **B)** Amplification of and *mecA* genes by PCR with AuNPs probe contained *mecA* primers in various PCR products of bacterial strains: 1 *S. aureus*, 2 MRSA, 3 *E. coli*, 4 *S. typhimurium*, 5 *L. rhamnosus* and three clinical specimens which was confirmed to *S. aureus* presence (sample number 6 – 8). In sample 2 and 8 was confirmed the expression of *mecA* gene. **C)** Titration curve of AuNPs with addition of 0 – 2 M NaCl. The results are expressed as a ± of standart devitation. **D)** The color change of reaction mixtures before 2 M NaCl addition and after NaCl addition into sample: 0 AuNPs, 1 *S. aureus*, 2 MRSA, 3 *E. coli*, 4 *S. typhimurium*, 5 *L. rhamnosus* and three clinical specimens which was confirmed to *S. aureus* presence (sample number 6 – 8). **E)** Comparison of reaction mixtures relative absorbance: various PCR products of bacterial strains 1-8. The results are expressed as percentage of the AuNPs signal (100 %).

Conclusion

- Fast and easy method for detection of pathogen MRSA
- Possibility of modification method for detection of other bacterial species
- Utilization in many different fields

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- NANOLABSYS CZ.1.07/2.3.00/20.0148

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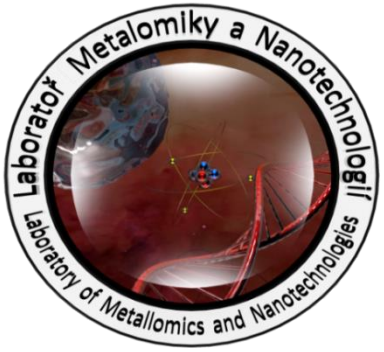

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Thank you for your attention!

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