Nanoimprint lithography based bioelectronics

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Potentiometric measurement of bacterial activity on imprinted electrodes

Background

Bacterial infections and biofilm formation on surfaces represent the major issue for implantable devices, independently of their function. The irreversibility of the biofilm formation process make this type of infections arduous to treat and predisposed to relapse. Traditional methods to solve implant-associated infections involve the use of antimicrobial or antibiotic agents released by the implant itself or administered as a drug, however, this approach often results in overuse, side-effects and on a larger scale, causes antimicrobial resistance (AMR).

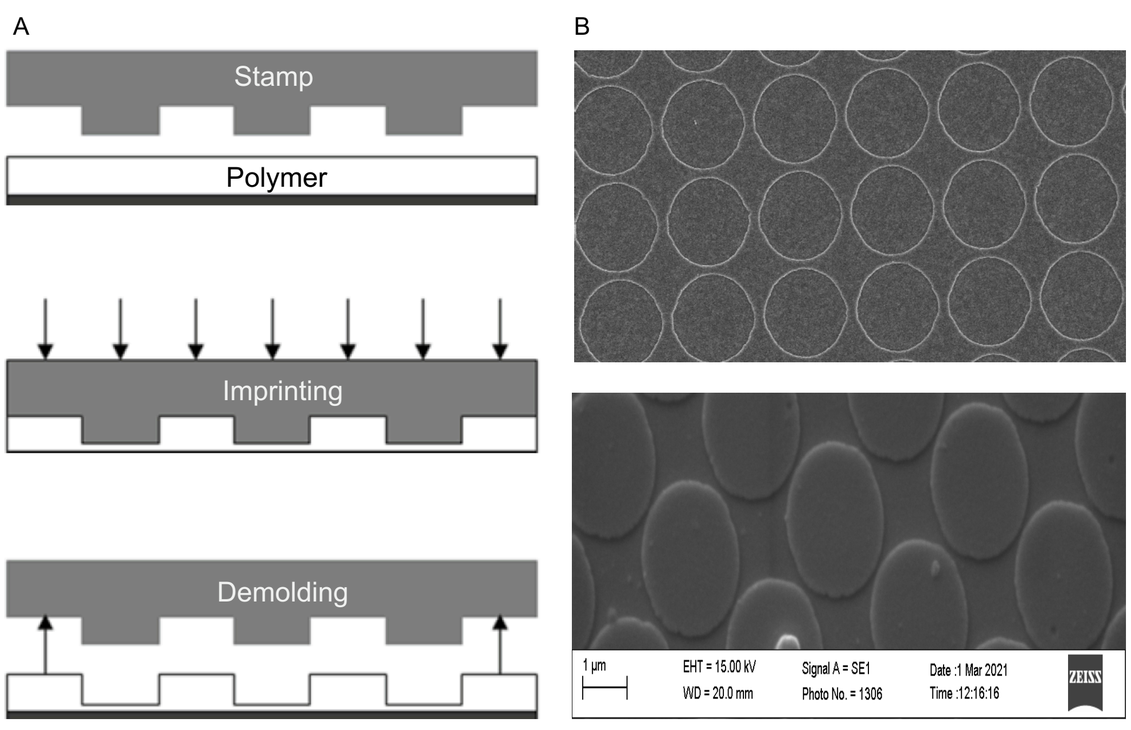
An alternative solution, is the fabrication and design of surface topographies. The surface pattern at the micro and nano scale can affect the bacterial adhesion and biofilm formation by reducing the bacterial attachment but at the same time promote cellular proliferation. Additionally, the tuning of surface features can provide well controlled responses by bacteria or cells, either promoting or inhibiting their interactions with the surface enabling antimicrobial or sensing properties, respectively.

In this research a cost-effective and large scale imprinting techniques (NIL) is employed for the fabrication of patterned electrodes while a non-destructive electrochemical method (open circuit potential, OCP) is used to monitor bacterial and cellular activity on the electrodes

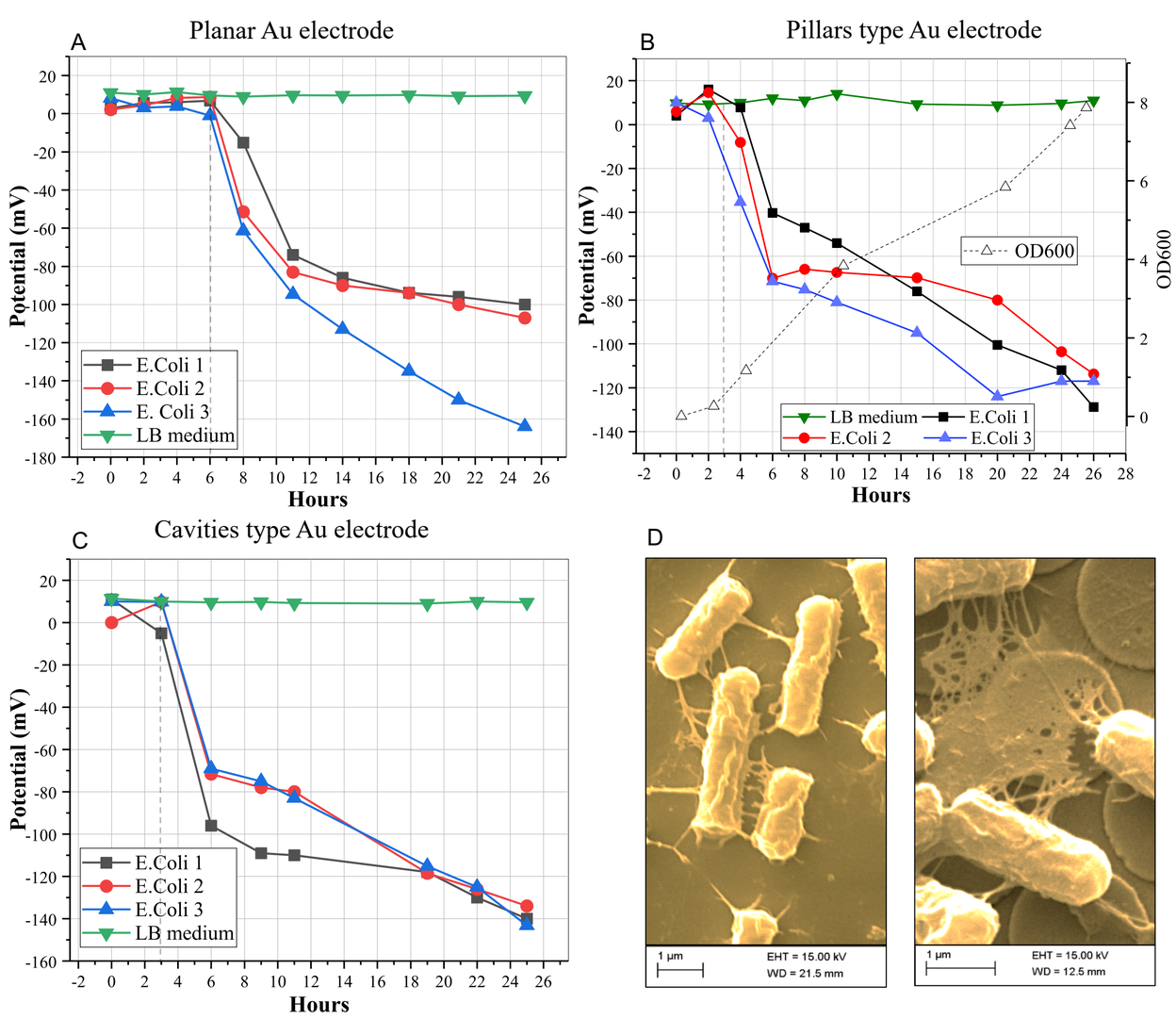
Methods

Nanoimprint lithography (NIL) was used for the fabrication of the patterned electrodes. Unlike conventional lithographic methods, such as photolithography or electron beam lithography, NIL relies on mechanical modification allowing high-throughput combined with high resolution. The process begins with a hard stamp being pressed into a polymeric material under controlled temperature and pressure conditions, transferring thereby the pattern in the polymeric material. Then the pressure is released and the stamp is demolded with a following cooling of the polymer. To perform electrochemical measurements, 100 nm gold layer was deposited on the imprinted polymer via electron-beam vapor deposition.

E.coli strains were grown in LB medium for 18 hrs at 37 °C with shaking. The solution of 0.1 OD_{600} was then diluted by a factor of 30 and incubated in contact with the electrodes. The development of the potential was measured for 25 hrs at regular intervals against an Ag/AgCl reference electrode. The bacterial attachment was observed in terms of potential decrease, owed to the presence of negatively charged biomolecules and extracellular electron transfer (EET) process by which microorganism exchange intracellular electrons with extracellular electron acceptors.



A) Schematic steps of nanoimprint lithography. B) Upper picture shows the negative pattern with cavities. The lower picture displays the complementary positive pattern with pillars. The diameter of the structures is 2 µm with a depth/high of 60 nm.



A, B, C) OCP measurements of planar and imprinted electrodes incubated with *E.coli* for 25 hours. D) SEM images display biofilm formation on planar (left) and pillar (right) type electrodes after 25 hours incubation. Electrodes with cavities displayed similar features.

Results

- The OCP measurements were performed in triplicates with one additional electrode incubated in sterile LB medium as a control.
- The absence of microorganisms in LB medium resulted in a steady and positive OCP value throughout the whole experiment which demonstrated its correlation to the processes occurring during bacterial growth.
- For the electrodes placed in the chambers containing *E.coli* cultures, the OCP value decreased with the increase of bacterial concentration.
- SEM images of the incubated electrodes showed the presence of bacterial cells on all the electrodes. The positive pattern promoted the highest level of bacterial adhesion, with a visible expression of *fimbria/pili-like appendages* extending towards the substrate and neighbouring cells and the presence of the biofilm matrix. Smaller colonies were visible on the planar and the negative pattern with a prevalence of isolated bacterial cells.

Conclusions

- This study described the application of a versatile and high performing micro/nano imprinting technique allowing the fabrication of orderly patterned electrodes.
- The OCP transduction demonstrated the feasibility of detecting and monitoring in real time bacterial activity on the surface, exhibiting an inverse correlation with the bacterial growth. It was however, impossible to assess whether the transduction signal was generated by biofilm formation or general bacterial metabolic activity.
- The extremely low aspect-ratio of imprinted structures, yielding to a generally flat surface, did not allow to make a correlation between the surface pattern and the detected signal.

The ongoing study is focused on the NIL fabrication of patterns with higher aspect-ratio aiming to provide a more distinctive transduction signal compared to the flat control surface.

Future plans are:

- Investigate possible applicability of OCP transduction for monitoring cell adhesion (e.g. Fibroblasts)
- Introduce biocompatible materials for NIL processing.

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