Investigation of bacterial attachment patterns on micro- and nano-restricted surface topographies
Outline of presentation

• Overview
• Aims
• Outcomes
  Bacterial attachment behaviours on ultrafine grained titanium
  Bacterial attachment behaviours on subnano- and nano-scale topography
  Bacterial attachment behaviour on self-cleaning surfaces
  Improvement of anti-biofouling properties
• Conclusion
• Practical outcomes
Utilisation of titanium in biomedical industry

• Benefits:
  Biocompatible – non-toxic
  Light
  Strong
  Corrosion resistant

• Medical applications:
  Dental implants
  Orthopaedic implants
  Surgical instruments
Infections associated with implants

**Staphylococcus aureus (34%)**

1 μm diameter; non-motile; cell surface adhesin proteins, other EPS

**Pseudomonas aeruginosa (8%)**

0.5-0.8 μm x 1.5-3.0 μm; flagellum; fimbriae, polysaccharide capsule, EPS

Frequency of main pathogenic species among orthopaedic clinical isolates of implant-associated infections (Campocia, 2006)
Theoretical model of biofilm formation

Factors influencing biofilm formation on non-biological surfaces

- Surface charges
- Hydrophobicity: thermodynamic approach, DLVO or extended DLVO theories.
- Surface topography
Aims of the research

• Fabricate and investigate surfaces with topographic features ranging from micro-scale to sub-nanometric-scale

• Conduct systematic and comprehensive studies to investigate the impact of micro-/nano-topography on bacterial attachment

• Explicate bacterial attachment response to changes of the surface topography

• Development of anti-biofouling coatings for titanium surfaces
Production of ultrafine-grained titanium

As-received

ECAP

Pressure

Punch

Sleeve

Heater

Shaping insert

Preform

Stopper plate

Following with mechanical polishing and the combination of chemical and mechanical polishing
Bacterial attachment on ultrafine-grained titanium

S: S. aureus  
P: P. aeruginosa

How does surface topography modulate bacterial attachment?

Chemico-mechanical polishing  
Mechanical polishing

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Fabrication of Ti thin films

To control Ti thin film thicknesses at atomic scale, with average roughness ranging from 1.2 nm—0.2 nm
Surface topography

\[ R_a = 1.22 \pm 0.27 \text{ nm} \]
\[ R_q = 1.61 \pm 0.34 \text{ nm} \]
\[ R_a = 0.92 \pm 0.06 \text{ nm} \]
\[ R_q = 1.16 \pm 0.06 \text{ nm} \]
\[ R_a = 0.58 \pm 0.08 \text{ nm} \]
\[ R_q = 0.73 \pm 0.14 \text{ nm} \]
Attachment of bacteria on Ti thin films

**P. aeruginosa**

**S. aureus**

Red: bacterial cells; Green: exopolysaccharide produced by bacteria

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*P. aeruginosa* and *S. aureus* on the surfaces with nano-topographic features
Fabrication of hierarchical structures on titanium surfaces

Schematic of femtosecond laser ablation setup (Courtesy of Elena Fadeeva, Hannover Laser Zentrum)

10 pulses  
20 pulses

Ripple effect

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Surface topography and self-cleaning effect

As-received vs Structured
Attachment of bacteria on lotus-like Ti surfaces

**P. aeruginosa**
- As-received Ti
- Structured Ti

**S. aureus**
- As-received Ti
- Structured Ti

Low attachment of *P. aeruginosa* on structured Ti

Induced attachment of *S. aureus* on structured Ti

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Attachment behaviour of *S. aureus* and *P. aeruginosa* on micro-/nano-topography

Localisation of spherical *S. aureus* on nano-restricted surface topography

Increased surface area favourable for the attachment of spherical *S. aureus*, not of rod-shaped *P. aeruginosa*
Improving anti-biofouling properties for titanium surfaces

Plasma-enhanced chemical vapour deposition system

Terpinen-4-ol
Performance of polyterpenol coatings on titanium surfaces

Green: viable
Red: dead
Conclusion

Surface topography with dimensions ranging from micro- to subnano-features may control the extent of bacterial attachment

The extent of bacterial attachment on hierarchic structures of superhydrophobic surfaces is variable and dependant on the bacterial morphology

Bacterial attachment on the subnano-/nano-smooth surfaces cannot be explained by previously known mechanisms, e.g. flagella, fimbriae, production of EPS, which are believed facilitating the bacterial attachment
Practical outcomes

Improvement of anti-biofouling properties achieved using polymerised terpinen-4-ol, main constituent of antibacterial tea tree oil

Understanding the relationship between bacterial attachment and surface roughness assisting in the design of biomaterials with minimum risk of bacterial infections

To optimize the process of osseointegration on modified titanium surfaces, investigation of competitive colonization between eukaryotic and prokaryotic cells required in the future direction
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References