Coating of Sub-Micrometric Keratin Fibers on Titanium Substrates: A Successful Strategy for Stimulating Adhesion and Alignment of Fibroblasts and Reducing Bacterial Contamination

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Background and rationale – 1: Titanium can come in contact with different biological entities...

**Soft tissue** contact at gum level

- **Name:** Fibroblast
  - **Dimension:** 15-20 μm
  - **Particular signs:**
    - Preferentially adhere to smooth surfaces, sensitive to contact guidance
    - Some proteins (e.g. keratin) can improve their adhesion and activity

**Hard tissue** contact at bone level

- **Name:** Osteoblast
  - **Dimension:** 20-25 μm
  - **Particular signs:**
    - Preferentially adhere to rough surfaces
    - Adhesive proteins/moieties (e.g. fibronectin, RGD) can favor their adhesion

**Risk of bacterial penetration**

- **Name:** Bacteria
  - **Dimension:** 1-2 μm
  - **Particular signs:**
    - Preferentially adhere to rough surfaces (Ra>0.2 μm)
    - Don’t have specific adhesion proteins but can recognize adhesive moieties

**Example:**
- Transmucosal dental implants
- Similar conditions for percutaneous devices
**The idea:** design of the surface in order to favor fibroblast adhesion-orientation and to obstacle bacteria penetration

- Currently used
  - Smooth surfaces

- Suitable for fibroblast adhesion and limited bacterial attachment
  - Possibility to align fibroblasts
  - Avoid the increase of bacterial adhesion
  - Improvement of fibroblasts adhesion, proliferation and activity. Eventual doping by antibacterial metal ions

- Oriented nanogrooves
  - Final Ra<0,2µm
  - Keratin nanofibers

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S. Ferraris, S. Spriano, A. Varesano, C. Vineis, V. Guarino, L. Ambrosio, L. Rimondini, A. Cochis, *Superficie di titanio modificata, impianto medicalet dotato di una o più di tali superfici e procedimento di realizzazione di una tale superficie, TO2015000070808, patentpending*

S. Ferraris et al. *Materials Science and Engineering C* 76 (2017) 1–12
Research outline and final aim: surface able to favor fibroblast adhesion-orientation and obstacle bacteria penetration

- Electrospinning of keratin extracted from wool
  - Stationary collector: randomly oriented fibres
    - Fibroblasts adhesion and proliferation
  - Rotating collector: aligned fibres
    - Fibroblasts adhesion, proliferation and alignment

- Silver doping of keratin fibres
  - Fibroblasts adhesion, proliferation (and alignment, for aligned fibres)
  - Antibacterial activity

Possible application of keratin fibres coatings: dental implant collars
- Soft tissue contact
- High risk of bacterial contamination
Randomly oriented keratin nanofibres: deposition results

Electrospinning of keratin extracted from wool

Stationary collector: randomly oriented fibres
- Fibroblasts adhesion and proliferation

- Low density deposition, not complete surface coverage, possible cell stimulation by substrate topography

- High density deposition, almost complete surface coverage, cell stimulation mainly driven by keratin fibres (not directional)
**Aligned keratin nanofibres:** deposition results

Electrospinning of keratin extracted from wool

Rotating collector: aligned fibres
- Fibroblasts adhesion, proliferation and alignment

**Low density deposition,** not complete surface coverage, possible cell stimulation by substrate topography combined with keratin fibres (both oriented)

**High density deposition,** almost complete surface coverage, cell stimulation mainly driven by keratin fibres (directional)
Randomly oriented vs Aligned keratin nanofibres: fibroblasts response

Stationary collector: randomly oriented fibres

Rotating collector: aligned fibres

Fibroblast growth with random orientation

Fibroblast alignment in the fibres direction
Randomly oriented keratin nanofibres: silver doping

Low density deposition of fibers, silver loading (ionic form) in keratin fibres and silver precipitation (metallic form) on the substrate.

High density deposition of fibers, mainly silver loading (ionic form) within keratin fibres.
Randomly oriented keratin nanofibres: silver doping & antibacterial activity

Silver loading confers significant antibacterial activity to both low density and high density keratin nanofibers.
Keratin obtained by discarded wool by a green approach was successfully used for the preparation of high added value coatings intended for biomedical applications.

Sub-micrometric keratin nanofibers were obtained with random orientation on plane Ti-disks by means of electrospinning deposition with stationary collector while oriented fibres were produced by means of the application of a rotating collector.

The ability of keratin to bind metal ions was exploited for fibres enrichment with antibacterial silver ions.
More information on this research on the following publications

- Ferraris, S., Truffa Giachet, F., Miola, M., Bertone, E., Varesano, A., Vineis, C., Coчис, A., Sorrentino, R., Rimondini, L., Spriano, S., *Nanogrooves and keratin nanofibers on titanium surfaces aimed at driving gingival fibroblasts alignment and proliferation without increasing bacterial adhesion*, Materials Science and Engineering C 76 (2017) 1-12
- Andrea Coчисis, Sara Ferraris, Rita Sorrentino, Barbara Azzimonti, Chiara Novara, Francesco Geobaldo, Francesca Truffa Giachet, Claudia Vineis, Alessio Varesano, Asmaa Sayed Abdelgeliel, Silvia Spriano, Lia Rimondini, *Silver-doped keratin nanofibers preserve a titanium surface from biofilm contamination and favor soft-tissue healing*, J. Mater. Chem. B, 2017, 5, 8366
- Sara Ferraris, Vincenzo Guarino, Andrea Coчисis, Alessio Varesano, Iriczalli Cruz Maya, Claudia Vineis, Lia Rimondini, Silvia Spriano, *Aligned keratin submicrometric-fibers for fibroblasts guidance onto nanogrooved titanium surfaces for transmucosal implants*, Materials Letters 229 (2018) 1–4