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# Plasma treated and nano/micro-structured PEEK substrates for adipose tissue-derived stem cell studies

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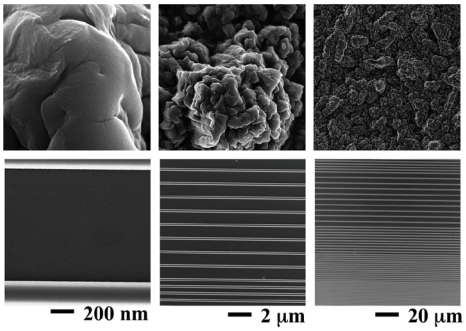
## INTRODUCTION

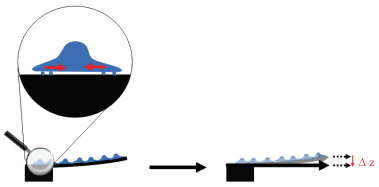
Polyetheretherketone (PEEK) gains increasing interest as biomaterial for trauma, orthopaedic and spinal implants [1]. Cell shape is known to regulate differentiation of human mesenchymal stem cells [2]. Our aim is to understand the influence of surface chemistry and morphology on stem cell differentiation. Therefore, our goal is to develop surfaces that induce alterations in stem cell shape and thus a specific stem cell differentiation. This knowledge is of potential interest for clinical applications such as implant surfaces or stem cell therapy. By means of plasma treatment and micro-structuring we modified PEEK foils and characterized adipose tissue-derived stem cells (ASC) in direct material contact.

## MICROSTRUCTURING

Commercially available medical grade PEEK foils (APITV Series from VICTREX) were mirco-structured by hot embossing. As a clinically relevant surface, we replicated a sandblasted and acid etched titanium disk into 25 µm thin PEEK foil (see figure). These roughened surfaces are applied for dental implants. According to confocal laser scanning measurements, the replica revealed the same roughness as the disk. Other model structures in the micro-meter range were obtained from a silicon master with different patterns, an example with grooves shown in the figure.



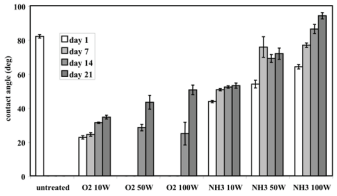

## MEASUREMENT OF CELL FORCES

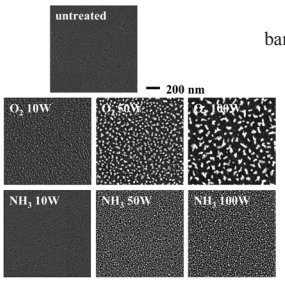



We also aim to measure cell forces on different substrates with a simple laser device setup detecting deflection changes of cell seeded cantilevers (see scheme). The system was set up with silicon cantile_vers and a fibroblast cell-line on the commercially available CANTISENS ® system for the proof of principle.
Using different PEEK substrates, with we hope to quantify the effect of chemical and topographical modification of the surface on ASC.

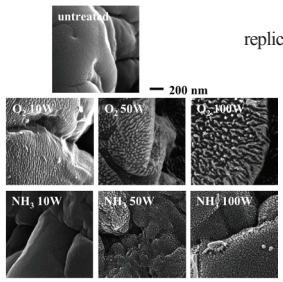
## PLASMA TREATMENT

Besides a decrease in contact angle (see figure), both oxygen and ammonia plasma treatments induced highly reproducible nano-structures on the PEEK substrates (see figure). The pillar-like structures increased in size with applied power. Oxygen plasma is more effective in surface etching than ammonia plasma. The contact angles of plasma treated PEEK substrates increased during 21 days of dry storage (see figure). In general, higher etching rates resulted in larger contact angles. The contact angle of oxygen plasma treated substrates after 21 days stayed below 50°, compared to a contact angle of 82° for untreated PEEK surfaces. Ammonia plasma treated substrates resulted in contact angles of 50°, 70° and 92° for 10 W, 50 W and 100 W plasma intensity, respectively.



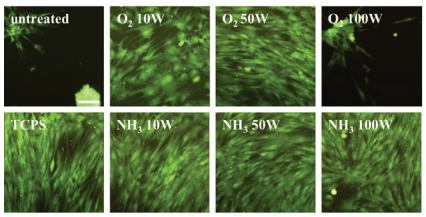

bare PEEK substrate



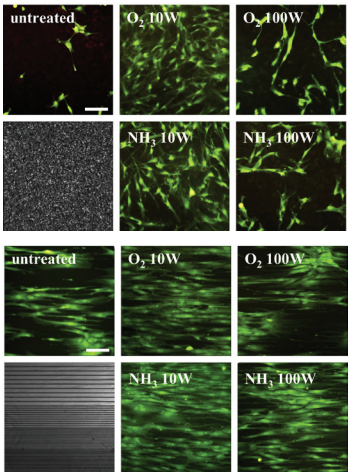

repliceted PEEK substrate

## ASC ON PEEK SUBSTRATES

ASC were isolated from liposuction-derived tissue, and tested of different PEEK substrates The cells were seeded on the different substrates at passage 4, cultured for 48 h under standard conditions and stained with Calcein-AM. Both cell types did neither grow homogeneoulsy on untreated PEEK nor on strongly oxygen plasma treated PEEK substrates (see figure). Ammonia plasma and intermediate oxygen plasma treatment had a positive effect on cell attachment and proliferation. The treatment induced similar phenotypes as on tissue culture polystyrene (TCPS). Scale bar 200 µm.




Regarding micro-structuring by hot embossing, ASC grew dense on titanium replicas treated with low oxygen and ammonia plasma intensities (see figure). ASC clearly aligned on grooved substrates, indepen-dent of groove width (2-20 µm). Notably, ASC grew more dense on the unfavorable substrates (untreated and 100 W oxygen plasma). Harsh oxygen plasma treatment resulted in aggregation of the aligned cells. Scale bars 200 µm.




## CONCLUSION AND ACKNOWLEDGEMENT

Oxygen and ammonia plasma induced higly reproducible nano-structures on PEEK foils. These pillar-like features are tuneable in size with the power of the applied plasma. We are going to characterize the nano-structuring effect of plasma treatment in more detail since it is known that nanoscale surface topography has an effect on protein adsorption and cellular response [3]. Our still preliminary results demonstrated that ASC reacted to plasma treatment and micro-structuring of PEEK substrates with different phenotypes. The aim is to investigate the stem cell differentiation potential of thus induced phenotypes into the osteogenic and adipogenic lineage.
Furthermore, we are interested in the quanitification of cell forces by the presented cantilever bending approach.

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[1] S. M. kurz and J. N. Devine (2007) *Biomaterials* ***28:****4845-69.* [2] Mc Beath et al. (2004) *Developmental Cell.* ***6****:483-95.* [3] M. S. Lord et al. (2010) *Nano Today.* ***5****:66-78.*