Colloid particle adhesion to cells in 3D perfusion constructs

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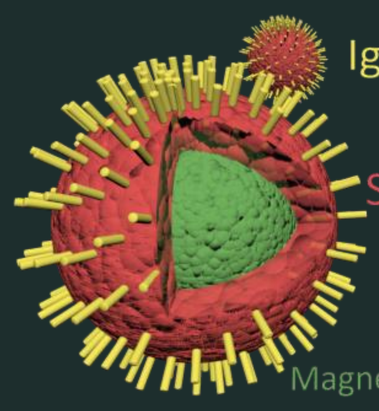
INTRODUCTION

OBJECTIVES

- Design of a method suitable for an adhesion study in artificial flow system simulating the real conditions of desired end use of created micro and nanoparticles.
- **Focus on modelling adhesion in organic tissue:** specific adhesion study of antigen-targeting nanoparticles under fluid flow conditions in human body.

AG-targeting NANOPARTICLE



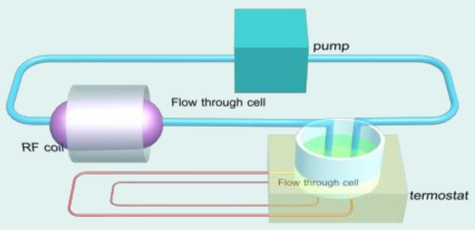
M-75 is a monoclonal antibody binding specifically to CA IX antigen overexpressed in many tumors (colorectal, lung, cervical, breast and carcinoma).

SiO_2 shell provides mechanical and structural support for nanoparticle. Moreover, the material allows for simple ammination of nanoparticles → such modified nanoparticles are then able to covalently bind to IgG-M75 using precursor ABTES. Porous silica is marked by FITC and can act as drug carrier and release the adsorbed drug "on demand" using RF heating.

Due to the presence of FeO_x core, nanoparticles are able to act as contrast agents within MRI imaging technology → tracking the 3D adhesion of nanoparticles in real time.

MEASUREMENT

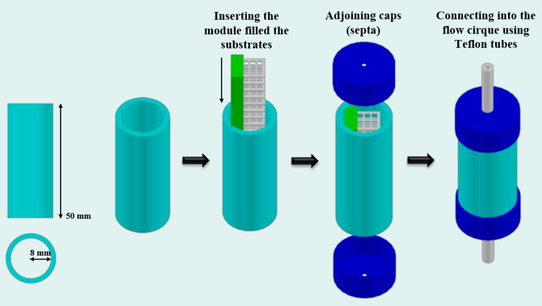
FLOW CIRCUIT

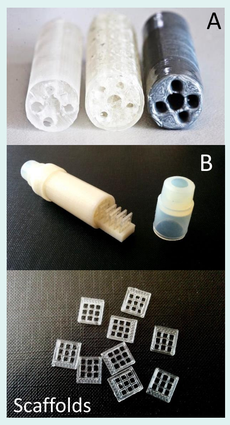


Constructed flow circuit consists of:

- inlet and outlet teflon tubes
- peristaltic pump
- termostat
- optical thermometer
- flow through cell
- MRI scanner (Bruker Desktop ICON)

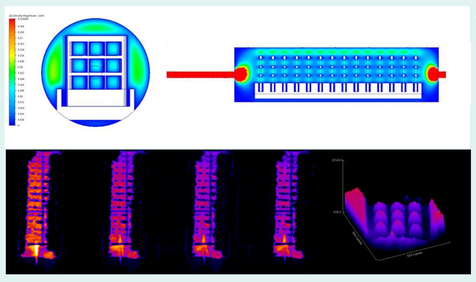
FLOW THROUGH CELL





- Structured one-piece layer (Fig. A, non-specific adhesion).
- Slide-in rack with griddle 3D scaffolds (Fig. B).

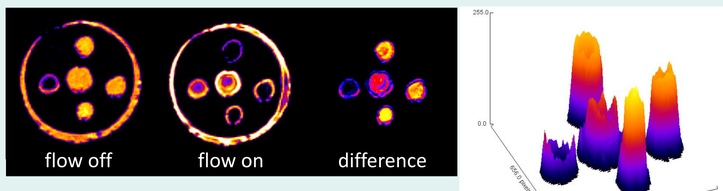
FLOW CHARACTERISTICS



CFD model of flow (flow rate 1.5 ml/min).

MRI scans of flow in the tissue model (no turbulencies visible).

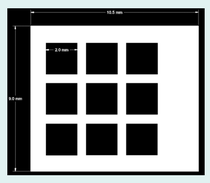
- The CFD model of flow in the cell predicts velocities in the range 10-100 µm/s (flow rate 1.5 ml/min) → such velocities are comparable to interstitial flow velocities in tissues (10s µm/s , Hompland et al., 2014).

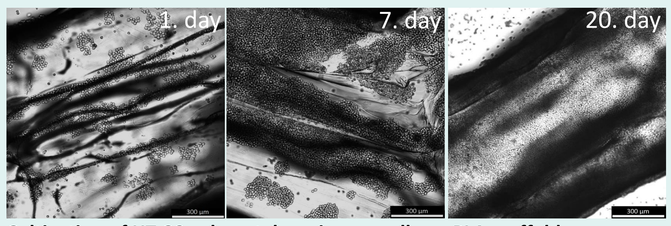


MRI scans of flow in the structured layer (loss of the intensity of the signal caused by the flow of escited spins out of measured region.

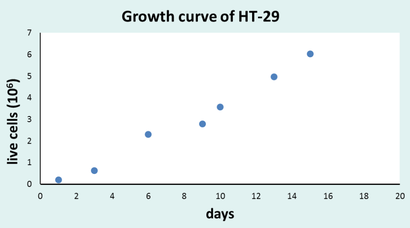
SCAFFOLDS (cell growth)

- 3D printing
- biocompatible material PLA (Poly-L-lactic acid)





Cultivation of HT-29 colorectal carcinoma cells on PLA scaffold.



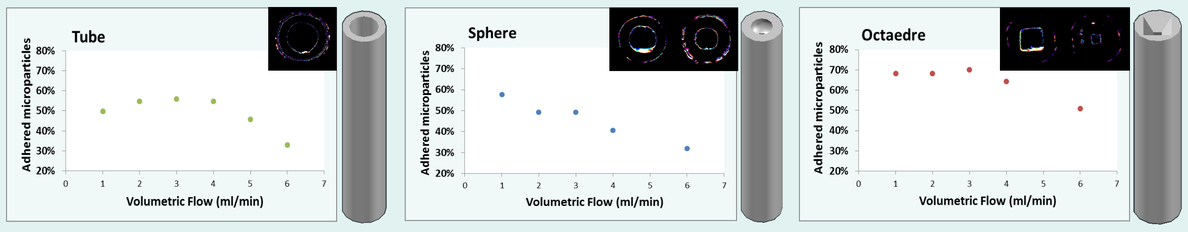
EVALUATION

- microscopy
- automatic cell counter (trypan blue staining)

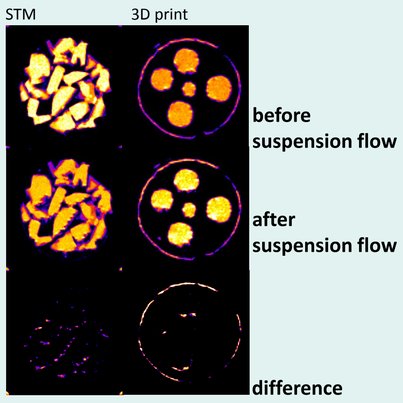
ADHESION

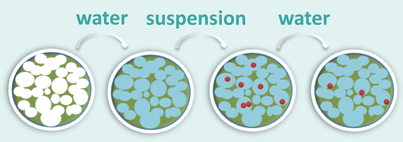
NON-SPECIFIC ADHESION

BASIC LAYERs : simple 3D void structure and Alginate/FeO_x/SiO_2 microparticles (size ≈ 60 µm)



COMPLEX LAYERs : complex 3D void structure and preparation using Solid Template Method (STM) or 3D printing (Alginate/FeO_x/SiO_2 microparticles)

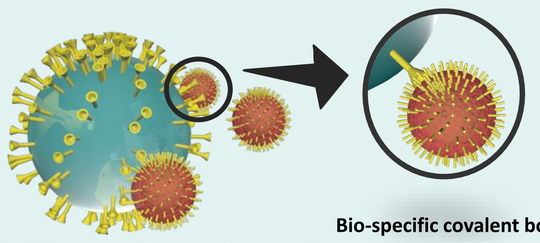




Situation in the cell during the experiment.

Method	Material of layer	Upflow Fe^{3+} c (mg/l)	Downflow Fe^{3+} c (mg/l)	Concentration difference (%)	Slice free area filled with particles (%)
STM	PDMS >3mm	4.3	2.5	43	5
3D Print	PLA	4.0	2.4	40	3
3D Print	ABS	4.0	2.5	37	2
3D Print	PDMS	4.0	2.7	33	3

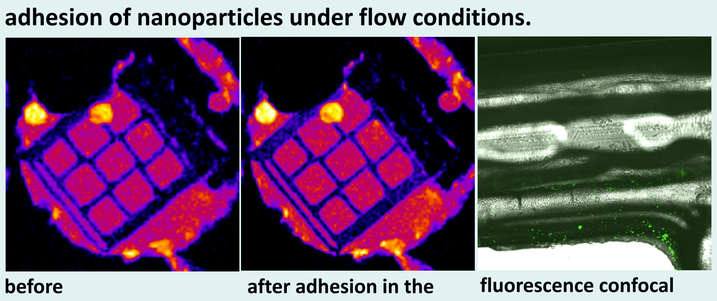
BIO-SPECIFIC ADHESION



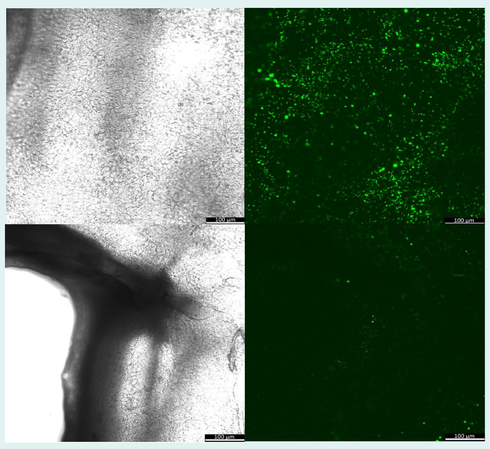
HT-29 cancer cell expressing antigen CA IX.

Bio-specific covalent bond between CA IX on the surface of cell and IgG-M75 antibody on the surface of nanoparticle.

Contrast enhancement in MRI scan on the scaffold after adhesion of nanoparticles under flow conditions.



before | after adhesion in the flow cell | fluorescence confocal microscopy of scaffold



Incubation of 3D scaffolds overgrown by HT-29 cancer cells with nanoparticles (200 µg/ml) modified by IgG-M75 antibody (top row) and by BSA protein (bottom row)

CONCLUSION

- Novel approach for studying 3D adhesion of microparticles was introduced .
- The flow trough cell designated specially to adhesion studies using MRI was designed, manufactured and completed with various 3D differentiated layers , which were also developed and characterized.
- Specific adhesion of nanoparticles modified by specific IgG-M75 antibody on HT-29 cells proved in a stationary medium and was observed also in 3D under flow conditions using MRI technology.

FUTURE

- Continuation of the study of bio-specific adhesion under flow similar to the one in humam body.

ACKNOWLEDGEMENT

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