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Generation and imaging of patient customized implants

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Abstract
Personalized medicine is the development of individual solutions and therapies tailored to the specific disease pattern of a patient. To enable patient customized medical solutions 40 partners of the Aachen Research Cluster "innovation medical technology in.nrw" are investigating a new generation of biomedical devices and systems. The subproject *Patim* addresses non-invasive monitoring techniques to observe dynamic changes in tissue engineered cardiovascular implants.

1 Introduction
Over the last decade, remarkable progress has been made in the field of cardiovascular tissue engineering. The approach to combine cell seeding with natural or synthetic scaffolds to create implants with proper mechanical, structural and functional properties could be shown particularly applicable to small calibre vascular grafts in pre-clinical [1] and clinical studies [2]. Small calibre substitutes are used for peripheral and cardiac revascularization procedures to overcome the shortcomings of currently available synthetic grafts, including thrombus formation resulting in poor patency rates, infection and lack of growth and remodelling potential. To minimize the risk of life- or limb-threatening conditions, these grafts are clinically evaluated as model for arteriovenous (AV) shunts [3].
Tissue engineered implants undergo remodelling processes within the construct itself and with the surrounding host tissue. Haemodynamic forces act at *ex vivo* generated neovascular tissue and lead to the degradation of scaffold material and synthesis of extracellular matrix proteins. During this maturation process changes at the cellular and molecular level can adversely affect the anatomy and function of the graft. To obtain insights into such processes, extensive animal experiments have to be performed, requiring the sacrifice several animals at each observation time. However, the obtained results represent the state of the implant at that specific time point and do not depict the dynamic longitudinal remodelling process. Such changes still remain hidden due to the lack of non-invasive monitoring possibilities. For the clinical translation of these highly innovative tissue engineered implants online and longitudinal monitoring is crucial to record inter-individual processes and to counteract potential pathological changes at an early stage.

The aim of our work is the generation and imaging of patient customized cardiovascular implants. Therefore functional and molecular imaging techniques, such as magnetic resonance imaging (MRI) and positron emission tomography (PET) are combined to gain continuous information about the functional and cellular state of the implant. Furthermore, a targeted therapy is developed to reduce or specifically destroy activated macrophages. The online monitoring will enable the delivery of such therapy at the earliest detection of an inflammatory process at the site of the implants.

2 Methods

2.1 Visualization of cells and textile scaffold by MRI
To enable MR imaging, the incorporation of contrast agents into textile scaffolds and / or endothelial cells used for the *ex vivo* colonization of the grafts appears a simple and suitable method to provide enhanced visibility for tissue- engineered implants. Ultrasmall superparamagnetic iron oxide nanoparticles (USPIO) are favourable cell markers, since they present strong T2 and T2* contrast, and therefore allow sensitive and long-term tracking of labelled cells. However, conventional USPIO without surface functionalization show only low cell internalization rates, so that high USPIO concentrations or transfection agents are required to effectively label the cells. These

labelling conditions often come with decreased cell viability and reduced proliferation of labelled cells, which can drastically affect the *ex vivo* colonization of the implant. In our studies we therefore used flavin mononucleotide (FMN)-coated USPIO (FLUSPIO), which show highly efficient and specific receptor-mediated uptake by endothelial cells. FLUSPIO are approximately 100 nm in diameter and possess a T2 relaxivity which is comparable to clinically used T2 contrast agents. Cell internalization is mediated by the riboflavin carrier protein (RCP) and can reach high concentrations of up to 0.7 pg per metabolically active cell, without inducing cytotoxic adverse effects [4]. Consequently, it is possible to colonize implants *ex vivo* with FLUSPIO-labelled cells and monitor localization, as well as cell survival and replacement by means of MRI. Another favourable property of this novel non-polymeric FMN-coating is that it renders the USPIO fluorescent, which facilitates sensitive fluorescence tracking of the labelled cells e.g. by immunohistochemistry.

Besides cells and cell-containing scaffolds, also textile materials can be labelled with USPIO. Textile structures are commonly used as co-scaffolds in tissue-engineered vascular grafts [3] to enhance the mechanical properties of the constructs. Ideally, the bioresorbable textile component provides temporary mechanical stability until new tissue has been synthesized, organized, and cross-linked into a stable structure by seeded cells. In such setups, USPIO can be used to monitor the degradation process of the textile structures in which they are initially embedded during the fiber spinning process [5].

2.2 Labeled molecular probes addressing physiological processes using PET

Radionuclide-containing molecular probes are routinely used in nuclear medicine to non-invasively unfold information on pathological and physiological processes applying tomographic techniques, like PET. Such molecular insights are also highly desired from engineered tissues both before and after implantation. Regarding tissue-engineered vascular grafts, two sub-tissues are defined by the endothelial layer and the extra cellular matrix. Focusing on the endothelium, several crucial molecular processes were identified. In a first step, imaging inflammation was chosen as a target. Hence, several specific inhibitors of the cyclooxygenase-2 (COX-2) have been developed, based on an indometacin derivative. COX-2 is an intracellular enzyme highly overexpressed in inflammation processes. In an early test phase, several non-radioactive analogs were synthesized and screened using a cell uptake assay and FACS analysis. For this assay, two cell lines, HUVEC and transfected HEK, were used. The latter cell line is specially designed to express COX-2 in high amounts exclusively by induction with tetracycline, thus representing an ideal test cell line. Indometacin was chemically modified by conjugation on the carboxylic acid of the indol moiety. Derivatives were designed and synthesized with different polarity and labelling possibilities. Basically, I-124 and F-18 labelling shall come to operation, enabling PET measurements. Within the labelling strategy, also fast “click chemistry” has been applied. Cell uptake studies including blocking experiments have been conducted with all radiolabelled potential COX-2 inhibitors.

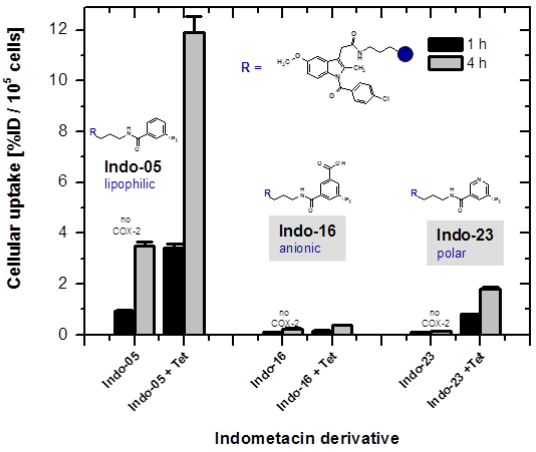

Image 1 Cellular uptake of potential COX-2 inhibitors labeled with radioiodine in the transfected human embryonic kidney cell line HEK hCOX-2Δ for incubation times 1 and 4 hours. COX-2 is exclusively expressed in the presence of tetracycline (Tet). Uptake without Tet reflects unspecific cell accumulation. Best specific/non specific uptake was observed for the polar indometacine derivative Indo-23

As a first result, some of these compounds were found to possess good specificity, paired with a minimum of unspecific cell retention (Image 1). In a next step, these promising candidates will be investigated both in a suitable rodent inflammation model, as well as in engineered vessel tissues growing in a bioreactor applying a µ-PET scanner dedicated to analyse whole body radioactive probe kinetics in small animals (Image 2). These experiments are expected to give new molecular insights in the function and viability of the endothelial cell layer of engineered tissue grafts.

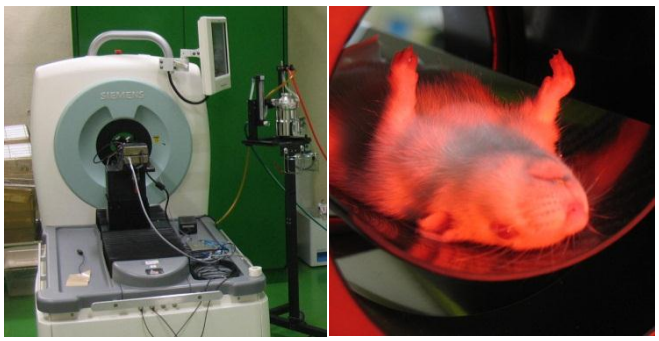

Image 2 INVEON µ-PET facility for non-invasive tomographic detection of molecular probes labeled with positron emitting nuclides in rodents and small animals

2.3 Suppression of inflammation

In chronic inflammation, frequently caused by an implant, activated macrophages play an important role. They ac-

cumulate at the site of inflammation and secrete effector molecules that damage the ambient tissue.

One of our concepts aim to develop a targeted therapy to reduce or destroy activated macrophages. For this, we use "cytolytic fusion proteins" (CFPs), the next generation of what is known in literature as immunotoxins. CFPs are chimeric proteins that combine a specific binding activity and a cytotoxic component in one molecule. So it comprises two important characteristics for an effective compound: the specificity, targeting specific cells by binding to surface markers and thereby delivering the active ingredient (the effector or toxin) to its site of action. We have extended the concept of CFPs to a novel and highly improved first-in-class therapeutic principle: the Human Cytolytic Fusion Proteins (HCFPs).

The binding is accomplished by an antibody or an antibody fragment. Instead of being derived from bacterial or plant, the effector proteins now are of complete human origin. We focus on the development and production of cytolytic fusion proteins that have a single chain antibody fragment (scFv) as the binder and a toxin of human source representing the 4th generation of immunotoxins. The CFPs will be produced recombinantly using special yeast which is already a well-established host for the industrial production of pharmaceuticals. This yeast in particular has some important advantages compared to other expression systems, making it very attractive for a large scale production of CFPs.

In our specific case, the binding part of CFP is a single chain antibody fragment (scFv) that specifically targets a receptor molecule on the surface of activated macrophages. The cytolytic compound is a human enzyme selected from the class of proteases, RNAses or Kinases, e.g. Angiogenin, Granzyme B or DAPKinase 2.

3 Conclusion

Significant progress has been made in recent years in the field of tissue engineering. The importance of online monitoring techniques is generally recognized in the field. While new approaches towards this goal have been proposed for in vitro processes [6], there is no noninvasive imaging modality to visualize the behavior of implants post-implantation. The goal of the *Patim* network is the development of imaging markers to be integrated into tissue engineered constructs, and molecular imaging methods with PET-MRI to monitor the graft's behavior after implantation. As a consequence of the capability of monitoring an implant online, inflammation processes can be detected and targeted therapies developed within the consortium to destroy activated macrophages can be optimally delivered to the patient at an early stage.

4 Acknowledgment

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5 References

[1] Koch. S.: Fibrin-polylactide-based tissue-engineered vascular graft in the arterial circulation. Biomaterials, 2010

[2] Shin'oka. T.: Endothelial cell seeding of polytetrafluoroetylene vascular grafts in humans: a preliminary report. J Vasc Surg, 1987

[3] Peck. M.: The Evolution of Vascular Tissue Engineering and Current State of the Art. Cell Tissues Organs, 2012

[4] Jayapaul. J.: FMN-coated fluorescent iron oxide nanoparticles for RCP-mediated targeting and labeling of metabolically active cancer and endothelial cells. Biomaterials. 2011

[5] Slabu, I.: Investigation of superparamagnetic iron oxide nanoparticles for MR-visualization of surgical implants. Curr Pharm Biotechnol. 2012

[6] Kreitz. S.: Nondestructive Method to Evaluate the Collagen Content of Fibrin-Based Tissue Engineered Structures Via Ultrasound. Tissue Engineering Part C Methods. 2011