A new concept for in vivo self-endothelialization of vascular prostheses by immobilized DNA-aptamers working as capture molecules for circulating endothelial progenitor cells

Hans P. Wendel[1], Jan Hoffmann[1], Angela Paul[1], Marc Harwardt[2], Jürgen Groll[2], Thomas Reeswinkel[2], Doris Klee[2], Martin Moeller[2], Tobias Walker[1], Tim Greiner[3], Gerhard Ziemer[1]
[1]Department of Thoracic, Cardiac and Vascular Surgery, University of Tuebingen, Germany, [2]Department of Textile and Macromolecular Chemistry, University of Technology Aachen, Germany, [3]Dept. of Laboratory Animal Medicine/Veterinary Service, University Hospital, Tuebingen, Germany

Statement of Purpose: Due to their insufficient biocompatibility and high thrombogenicity, small diameter artificial vascular prostheses still do not show a satisfactory patency rate. In vitro endothelialization of artificial grafts before implantation has been established experimentally years ago, but, this procedure is extremely time consuming and expensive, and therefore has never been used for routine clinical applications. This study deals with the coating of graft surfaces with capture molecules for circulating endothelial progenitor cells (EPCs), mimicking a pro-homing substrate to fish out EPCs from the bloodstream after implantation and to create an autologous functional endothelium.

Methods: Aptamers against EPCs were generated by systematic evolution of ligands by exponential enrichment (SELEX), a technique from combinatorial chemistry, which uses the ability of singled-stranded oligonucleotides to self-fold into three dimensional structures. They adapt a selective and high affine binding capacity to any protein target and can be selected from a library of 1015 starting nucleotides. We have spotted a defined aptamer onto a hydrogel coated surface, installed in a flow chamber, which allows an online detection of the attachment of EPCs from fresh human whole blood. Finally these cells were cultivated in growth factor enriched medium and fluorescence marked antibodies against CD34, CD 31, von Willebrand factor and VEGFR-2 were used to characterize the cell attachment.

Results/Discussion: Using the SELEX technology, small capture molecules (aptamers) with a high affinity to EPCs were identified, isolated and grafted onto polymeric discs using a blood compatible star-PEG coating. This step prevents any unspecific and undesired interaction between the device and the blood and enables the immobilized aptamers to specifically fish out EPCs from the blood stream. A porcine in vitro model that demonstrates the specific adhesion of EPCs and their differentiation into vital endothelial-like cells within 10 days in cell culture is presented. We suggest that the rapid adhesion of EPCs to aptamer-coated implants could be useful to promote endothelial wound healing, prevents increased neointimal hyperplasia and helps to keep the lumen of small diameter vessels open regarding a long time regeneration process.

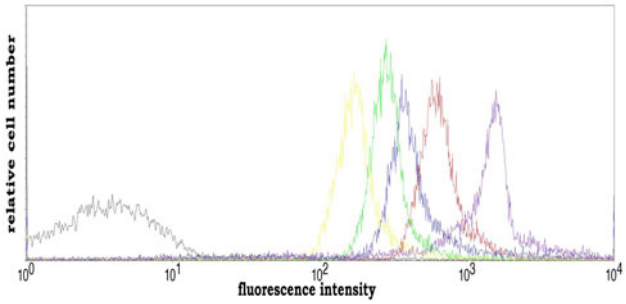

Figure 1. Binding affinity of aptamers to porcine CD31 positive cells. Flow cytometric analysis of FITC-ssDNA pool labeled cells, after successive rounds of selection. Starting from the black curve (negative control; incubation with start library), a progressive increase in fluorescence intensity can be followed during rounds of selection 1 (yellow), 3 (green), 5 (blue), 7 (red) and 9 (purple).

Conclusions: We hypothesize that future in vivo self-endothelialization of blood contacting implants by homing factor mimetic capture molecules for EPCs may bring revolutionary new perspectives towards clinical applications of stem cell and tissue engineering strategies.

References:

Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science. 1990;249:505-510.
Guo K, Wendel HP, Scheideler L et al. Aptamer-based capture molecules as a novel coating strategy to promote cell adhesion. J Cell Mol Med. 2005;9:731-736.