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ON ENDOVASCULAR METHODS FOR CELL TRANSPLANTATION

- exploring the selective intra-arterial and trans-vessel wall routes

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Cover art is a 3D reconstruction of CNS vasculature of a rabbit

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Reality, life, experience, concreteness, immediacy ...
– William James

ABSTRACT

Background: Cell based transplantation methods are a pivotal part of the emerging field of regenerative medicine. These transplantation methods could possible produce curative treatments where, previously, expectations of such feats were low. Diseases for which treatments are evaluated are in such diverging physiological systems as *e.g.* the CNS with ischemic or traumatic injuries, the endocrine system with type I diabetes mellitus, or musculo-skeletal system with dystrophies, hematological system with leukemia and cardio-vascular system with ischemic heart disease among others. Transplantation methods for cells span from open surgical/percutaneous, over intravenous, to specific intra-arterial methods. The method for delivery of cells is in fact an important part of the translation of cell based therapies to clinical practice. With that said, the use of endovascular techniques opens attractive routes of transplantation that needs to be thoroughly studied in order to achieve maximum efficacy.

Methods: We have utilized a model of traumatic brain injury in the rat where cell transplantations have been performed by selective intra-arterial methods and compared to intravenous administration. Analysis of engraftment has been performed by immunohistochemistry and cell characterization has been performed by microarray and RT-qPCR. Further, we have developed the Extroducer catheter system, a "nano"-catheter aimed for trans-vessel wall technique passage, in simulator, *ex vivo*, *in vivo* in rat and with full clinical integration in rabbit and swine. Long term follow-up studies have been performed both in rat (14 days) and rabbit (5, 30 and 80 days).

Results and Conclusions: We first show that selective intra-arterial methods increase engraftment levels up to fifteen-folds higher compared to intravenous controls. However, not all cell systems are found to be optimal for intra-luminal transplantation methods. Some of the factors limiting engraftment were thus explored within the cell systems themselves. These findings indicated that lack of engraftment might be dependent on integrin expression and endothelial interactions. For cells that lack the capacity of diapedesis and especially for more specific niche cell systems, such as insulin producing cells in the pancreas, the Extroducer can create direct parenchymal access via the endovascular route. Thus, the Extroducer system, developed within this thesis, offers a working channel between the proximal end of an endovascular catheter and the parenchyma of any organ of the body. Development and testing verified integration with clinical routine catheters. No long term adverse events were observed in the rat or the rabbit following the trans-vessel wall passage. In conclusion, endovascular intervention can provide a number of conceptually different methods for cell transplantation.

LIST OF PUBLICATIONS

This thesis is based on the following four papers which will be referred to in the text by their roman numerals.

I. **Lundberg J**, Le Blanc K, Söderman M, Andersson T and Holmin S
Endovascular Transplantation of Stem Cells to the Injured Rat CNS
Neuroradiology (2009).

II. **Lundberg J**, Södersten E, Sundström E, LeBlanc K, Andersson T, Hermanson O and Holmin S
Targeted Intra-arterial Transplantation of Stem Cells to the Injured CNS is More Effective than Intravenous Administration - Engraftment is Dependent on Cell Type and Adhesion Molecule Expression
Submitted

III. **Lundberg J**, Jonsson S and Holmin S
New Endovascular Method for Transvascular Access of Arteries and Veins: Developed in Simulator, in Rat and in Rabbit with Full Clinical Integration
PloS ONE (2010)

IV. **Lundberg J**, Jonsson S and Holmin S
Long Term Follow-up of the Endovascular Trans-Vessel Wall Technique for Parenchymal Access in Rabbit with Full Clinical Integration
Submitted

Also by this author but not part of thesis

Lundberg J, Karimi M, von Gertten C, Holmin S, Ekström TJ and Nordqvist A-C
Traumatic Brain Injury Induces Relocalization of DNA-methyltransferase 1
Neuroscience Letters 457 (2009)

Asplund M, Thaning E, **Lundberg J**, Sandberg-Nordqvist A C, Kostyszyn B, Inganäs O and von Holst H
Toxicity Evaluation of PEDOT/biomolecular Composites Intended for Neural Communication Electrodes
Biomed Mater. (2009)

LIST OF ABBREVIATIONS

CNS	Central Nervous System
CT	Computerized Tomography
dpi	days post injury
DSA	Digital Subtraction Angiography
ECM	Extra Cellular Matrix
FACS	Fluorescent Activated Cell Sorting
hMSC	Human Mesenchymal Stem Cells
hNPC	Human Neural Precursor Cells
ICAM-1	Inter Cellular Adhesion Molecule 1
IHC	ImmunoHistoChemistry
JAM	Junctional Adhesion Molecule
MRI	Magnetic Resonance Imaging
rNPC	Rat Neural Progenitor Cells
PBS	Phosphate Buffered Saline
PECAM-1	Platelet/Endothelial Cell Adhesion Molecule 1
PET	Positron Emission Tomography
SCA	Subclavian Artery
SMA	Superior Mesenteric Artery
TBI	Traumatic Brain Injury
TIPS	Transjugular Intrahepatic Portosystemic Shunt
VCAM-1	Vascular Cell Adhesion Molecule 1
VLA-4	Very Late Antigen - 4

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INTRODUCTION

Results from cell transplantation research have received interest and attention both from a clinical, a scientific and a public point of view. This thesis discusses new transplantation methods for different cell systems. In the introduction, different cell based therapies are presented followed by an overview of pathological conditions wherein several cell based strategies are implemented. Thereafter the delivery of cells in broad terms, and then specifically by endovascular technique compared to surgical technique, is presented. Ending the introduction is a general description of the active process of diapedesis as it is understood for immunological cells, followed by the broad aims of the thesis and then the succinct aims of the thesis in bullet points. The thesis includes numerous and quite divergent methods and we provide a short summary of the methods and methodological considerations in this text. More detailed descriptions of the methods used are found in respective paper. Finally, results and a general discussion followed by conclusions from the work presented are provided. The main results are also all presented in the articles comprising this thesis.

Cell based therapies

Cell based strategies are sought after as a way of repairing or to facilitate self renewal in pathological organ systems that have little or no regenerative capacity of its own. The plethora of different diseases in organ systems that might have a regenerative capacity, but is limited through physiological processes, is almost boundless (Bajada *et al.* 2008). Cell based therapies have been successfully used in the clinical practice for distinct pathological conditions during a relative long period of modern medicine. One of the broadest success stories are the transplantation of cells to patients suffering from hematological diseases (Buckner *et al.* 1974; Thomas *et al.* 1975; Thomas *et al.* 1975; Slavin *et al.* 1998). Hematological stem cell transplantation has also been expanded to comprise autologous transplantations following chemotherapy of solid tumor forms (Childs *et al.* 2000). Following the isolation of stem cell lines from human blastocysts (Thomson *et al.* 1998) and other adult sources such as the central nervous system (CNS) (Johansson *et al.* 1999), bone marrow (Bruder *et al.* 1997), multi-lineage mesenchymal (Pittenger *et al.* 1999), adipose tissue (Zuk *et al.* 2001) among others, new cell based approaches to disease treatment can be envisioned. The potential for *in vivo* expansion of these cells followed by transplantation, re-implantation and/or tissue engineering becomes possible (Vacanti *et al.* 1999). These findings open up possibilities for strategies aimed at ameliorating disease burden, or in the long run, obtaining curative goals through cell therapies in a clinical setting. Proposed treatments

can broadly be divided into stimulation of an endogenous population or transplantation of cells (Lindvall *et al.* 2006), be them homologous, from a donor or across the xeno-barrier. The wider implications of the prospect of cell based treatments are summarized in a review article where the coining of the effort and/or subject of Regenerative Medicine is presented (Daar *et al.* 2007).

The CNS has attracted attention since the potential of restored function could be very valuable for patients. Particularly since pathological conditions in the CNS can be severely disabling. Cell based therapies are in clinical trials in *e.g.* Parkinsons Disease (Freed *et al.* 2001; Gordon *et al.* 2004), ischemic stroke (Kondziolka *et al.* 2000; Bang *et al.* 2005) and spinal cord lesions (Sykova *et al.* 2006). Outside the CNS, other clinical trials with cell based therapies aimed at *e.g.* muscle dystrophy (Gussoni *et al.* 1997; Miller *et al.* 1997), ischemic heart disease (Stamm *et al.* 2003), graft versus host disease (Le Blanc *et al.* 2004; Ringden *et al.* 2006) and type I diabetes mellitus (Scharp *et al.* 1991; Shapiro *et al.* 2000; Korsgren *et al.* 2008) have yielded promising results. So far, many of the cell therapies are still in trials since both safety and effects must be thoroughly evaluated.

In many areas of pre-clinical, and to some extent clinical research, cell based therapies deliver positive results. However, as with the definition of stem cells/progenitor cells (Potten *et al.* 1990) the field of cell based therapies are a very heterogeneous one (Bajada *et al.* 2008). One feasible way of applying taxonomy to this field is by discussing different basic components in the treated diseases. The first example would be in pathological conditions with a definable population of cells being defect. Examples of diseases with certain cell types being depleted are Morbus Parkinson - dopamine producing cells, (Lindvall *et al.*), muscle dystrophy -satellite cells of the muscles (Gussoni *et al.* 1997) and type I diabetes - insulin producing cells (White *et al.* 2001). Such specialized cells might be easier to replace than the second general idea of cell transplantation wherein attempts of transplantation is aimed at more intricately functioning physiological systems. The complexity increases steeply when transplanted cells must differentiate into subpopulations of cell and/or interact within networks (*e.g.* the CNS). An example of that would be the transplantation of neural progenitor cells with the aim of full neural integration (Nikolic *et al.* 2009). A third strategy is to exert effects on existing cells/organs through transplantation but without functional integration. This strategy might explain some of the results from studies aimed at integration of cells, but without engraftment in the target organ, albeit with positive functional results observed (Borlongan *et al.* 2004). One explanation for that phenomenon is that in more complex situations, such as following CNS insults, some of the reported beneficial effects might be associated with immune modulation or local secretion of growth factors, thereby rescuing cells from apoptosis. Examples of

immune modulation could be Fas-ligand expressing cells (Ghio *et al.* 1999; Nagata 1999; Lee *et al.* 2008) whereas secretion of growth factors could be exemplified by an over-expression of IGF-1 from transplanted mesenchymal cells (Haider *et al.* 2008). Modulation of the immunological response by cell transplantation has also been shown to favorably treat graft versus host reactions in clinical practice (Le Blanc *et al.* 2004; Ringden *et al.* 2006). The concept of transplantation of cells serving as self renewing, local, biologically active, pharmacological factories are attractive for many parts of regenerative medicine (Amar *et al.* 2003). Local, self-sustaining, treatments that are only affecting niche parts of organs have many benefits that might include, but are not limited to, higher local concentration, less risk of adverse events and customization to different pathological conditions.

Delivery of cells

For cell transplantation, different percutaneous techniques assisted by modern imaging are viable through minimal invasive methods (Bale *et al.* 2007) and most parts and locales of the human body can be reached with that approach. On the other hand, for organs with less accessible anatomical location, parenchymal access can be associated with significant surgical risks (Villiger *et al.* 2005; Ben-Haim *et al.* 2009). In situations where engraftment rate after intraluminal cell administration is low and when a high anatomical specificity is required, such as the scenario when replacing a distinct cell type, direct puncture of the parenchyma might be preferable. For CNS applications this can be done with stereotactic needle puncture or in a combination with open surgery (Hagell *et al.* 2002; Wennersten *et al.* 2004).

Direct parenchymal access can also be achieved by endovascular technique. An example of this is a system that adds a possibility to, via large veins, administrate cells to the heart parenchyma (Thompson *et al.* 2003). The design of that system, requiring a large diameter catheter and without a closure device for the penetration site makes it usable only in large vessels on the venous side, more specifically, in the coronary sinus of the heart (Thompson *et al.* 2003; Siminiak *et al.* 2005). Other organs that might be difficult to reach, such as the CNS and the pancreas, are not reachable by the transvenous technique due to the design of that system requiring a large catheter diameter. Furthermore, venous navigation to most parts of the CNS and the pancreas, and to certain parts of the heart, is very difficult due to more unpredictable venous anatomy and venous valves.

Insulin producing cells are today transplanted by a hybrid method with percutaneous access to the portal vein and then intra-luminal cell release in the bloodstream. The concept of the intra portal transplantation is considered superior to open surgical techniques, due to the un-acceptable risk of adverse events (Kandaswamy *et al.* 1999;

Humar *et al.* 2000). The risk-analysis naturally differs substantially for different surgical procedures and transplantations, both with respect to organs and cells. Risks with portal vein transplantation include portal vein thrombosis, hemorrhages, and transient increase of transaminase values (Shapiro *et al.* 1995; Ryan *et al.* 2001). A continuous work of reaching a balance between the risk of bleeding after the procedure and portal vein thrombosis is of utmost importance for portal vein transplantations and results are improving steadily in clinical trials. Further adding to the risk side of the comparison is the need for immunosuppressant treatment after transplantation. The potential benefit of the procedure must also, as in the case with diabetes and insulin producing cells, be compared to the standard treatment of insulin injection and or pumps. Inclusion criteria for portal vein transplantation of insulin producing cells are thus already subject to immunosuppressive therapy for other previous transplantations, patients with unstable glycemia, unawareness hypoglycemia, or progressive chronic complications despite intensive insulin treatment (Bertuzzi *et al.* 2006). Simultaneously it has been suggested that it would be of great benefit if insulin producing cells could be transplanted directly to the parenchyma of the pancreas. Advantages with pancreas as the target locale are *e.g.* the possibility of mimicking the physiological release of insulin and due to a more hospital micro-environment for the insulin producing cells; the pancreas has a higher oxygen tension compared to the liver (Merani *et al.* 2008).

Many different strategies of cell based therapies are being evaluated in both pre-clinical and clinical trials. As previously stated, this thesis pertains to the delivery methods of cells to the different organs. One of the larger obstacles that have been observed is that the lung acts as a kind of clearance filter for the intravenous cell infusion, resulting in pulmonary trapping (Barbash *et al.* 2003; Fischer *et al.* 2009). An intra-arterial selective approach would possibly result in higher transplantation efficiency in certain conditions. The versatility of cell suspensions must not be underestimated and limit the way of thinking when considering treatments with cell based approaches (Nikolic *et al.* 2009). The cell suspensions can easily be handled and administrated through tubing and catheters, thus providing the possibility to by-pass the lung and selectively reach designated target vessels/parenchyma with a first passage effect. Those possibilities of cell handling forms the basis for this thesis on catheter based strategies for cell transplantation.

Endovascular treatments are continuously providing a third option to open surgical or percutaneous approaches. From the establishment of the Seldinger technique (Seldinger 1953) and the first use of digital subtraction angiography (DSA) (Meaney *et al.* 1980) to the modern interventional lab with 3D road maps (Soderman *et al.* 2005) and CT like capacities of the C-arm (Soderman *et al.* 2008) the path has been long but rapidly progressing. The driving force up until today, that has made the leap ward style of

improvements possible, is both rapid developments in computational power and material sciences. The arteries and veins can today be regarded as "internal routes" for navigation, diagnosis and intervention. The shift from open surgical options is for example illustrated by the patients that used to undergo thoracotomy and that now are being referred for percutaneous coronary intervention or the established coiling of intracranial aneurysms instead of open neurosurgical operation.

Scaling from bench to bedside

As this thesis hopes to illustrate; the rapid development of endovascular technique has implications on cell transplantation methods as well. To illustrate these implications as opposed to organ transplantation, one can visualize a liver transplantation. The wound in the abdominal wall must at least be big enough for the liver to go into the patient. This severely limits the possibility of minimal invasive transplantation of organs. On the other hand, the versatility of cell suspensions could make intra-luminal techniques the natural way of access. In experimental trials, open surgical options are used in e.g. rodent models for transplantation with positive results. This presents a limitation of scalability for clinical translation. For instance, when evaluating pre clinical CNS transplantation schemes in rodents one or two burr holes are established and cells are transplanted. One or two injections in the rat brain covers a relatively large volume but when scaling that to the human brain following *e.g.* a middle cerebral artery ischemic event, a large number of percutaneous trajectories would be required to cover a human brain volume corresponding to the experimental situation. The migratory capacity for transplanted mesenchymal cells after stereo-tactical transplantation has been shown to be around two millimeter over 14 days (Chen *et al.* 2001). Furthermore, the mechanical neuronal injury and the risk for intracerebral hemorrhage would increase with each injection trajectory. In brain stimulation procedures, the literature is somewhat divergent; the risk for hemorrhage could be as high as 5% per injection (Ben-Haim *et al.* 2009). The easier clinical scalability of endovascular access comes in the terms of cells delivered to a larger volume of tissue by taking advantage of the already existing vascular system. If an ischemic stroke occurs due to a vessel occlusion at some point, it would be very tempting to intraluminally disperse cells from the same point via the vascular system in order to reach the affected parenchyma. A normal cell dose for a human adult would probably at least be hundredfold higher than in the rodent and needs to spread out over a vastly larger volume thus requiring many injection trajectories. The average human brain weight is quoted at around 1400 grams as opposed to the adult rat at 2 grams to give some sense to the scale proportions.

Endovascular intervention is not without risks either, exemplified by the risk of adverse events reported at 0 to 4.0%, commonly reported at 0.5%, in different cerebral

interventions (Raymond *et al.* 1997; Cognard *et al.* 1998; Ng *et al.* 2002; Murayama *et al.* 2003; Gonzalez *et al.* 2004; Cronqvist *et al.* 2005). Added to the risk of the procedure *per se* is the risk of cell transplantations. The risk of intra-arterial transplantation has already been documented in humans for up to 24 months without severe complications, albeit in a small material (Sykova *et al.* 2006). Other pre-clinical studies with intra-arterial coronary injections performed in healthy dogs revealed micro-infarction of the heart parenchyma (Vulliet *et al.* 2004) whereas that has not been observed in clinical studies (Stamm *et al.* 2003). Factors that might be limiting are the size of the cells injected versus the size of the capillary system, the proportion of shunts in the microcirculation, the stickyness of the cells and the amount of cells administrated. Many of the cells have a much larger diameter (10 up to 70 µm) as opposed to the capillaries 5 to 8 µm (Chien *et al.* 1975). The shunting zones in the microcirculation could potentially lead also large cells to the post-capillary venules where diapedesis usually occurs (Tuma 2008). The main reason for leukocyte adhesion/diapedesis through venules is the usually restricted expression of adhesion molecules on venular but not arteriolar or capillary endothelium (Tuma 2008). In intravital microscopy studies, adipose mesenchymal cells have been shown to act as embolic material (Furlani *et al.* 2009). This risk could be speculated on to be lower than the comparable trauma of the surgical methods although a final risk assessment requires a randomized clinical trial.

Leaving the bloodstream - diapedesis

One limiting factor to specific intra-arterial and intravenous transplantations is that an intra-parenchymal approach yields a higher efficacy. It has been shown that when performing transplantations to a rodent stroke model and comparing intravenous, intra-ventricular and intra-striatal injections, the highest efficacy in sheer number of cells were obtained with the intra-striatal route (Jin *et al.* 2005). Further limiting the selective intra-luminal approach is the speculation that some cell systems, such as insulin producing cells, appear incapable of leaving the bloodstream (Hirshberg *et al.* 2002).

In all implementations aimed at intra-luminal administration (intra-luminal encompassing both intravenous and selective intra-arterial administration) the ability of the cells to leave the bloodstream, or perform diapedesis (Fulton *et al.* 1957) is fundamental. The diapedesis function has previously been studied predominantly in immunological cells (Fulton 1957), it is in fact the active process whereby cells leave the bloodstream. The diapedesis of immunologically active cells has been thoroughly studied since the discovery of the significant multistep, ordered, cross-talk procedure of leukocyte-endothelial cell interaction both *in vitro* and *in vivo* (Butcher 1991; Springer 1994).

The barricades limiting the cells from haphazardly leaving the bloodstream are many. All blood vessels contain an endothelium and several organized barriers. In the CNS for instance, tight junctions are located between endothelial cells, predominantly to permit the conservation of the water fraction of the blood stream. The liquid pressure gradient composed of the blood pressure can be broken down to one force directed with the axis of the laminar flow of blood and one force aimed perpendicular to the flow on to the wall (Glagov *et al.* 1992; Fay 1994), thereby providing an evolutionary rationale for sealing the blood stream tightly. Situated underneath the endothelial cells and forming their structural base sits the basal layer; a specific protein structure composed of extra cellular matrix (ECM) proteins abundant with expression of elastin, laminin and collagen type I, III and IV (Mayne 1986). Collagen and elastin are the major structural components of blood vessels of all sizes throughout the mammal body. The cross-banded fibrils, visualized by electron microscopy in the tunica media and tunica adventitia formed by type I and type III collagen provide the tensile strength and comprise probably 80 to 90% of the total collagen present. The other major structural protein component in elastic arteries is elastin; the protein that provides the elastic component of the blood vessels (Mayne 1986). Around most capillaries, pericytes also further seal the blood stream. These cells are less abundant in the post capillary venules where most of the diapedesis occurs. As a general note the post capillary venules are the most "leaky" part of the vascular tree. It should be noted that blood vessels are not merely the plumbing in the mammal body, it is in all respects a vividly living part of the organism reorganized as a response to stress and demand (Gibbons *et al.* 1994) and among other things has a self-generating electro potential towards the bloodstream that is important for the clotting cascade (Danon *et al.* 1976).

As previously mentioned, in a diverging literature it has been speculated that some cells totally lack the function of exiting the blood stream, thereby limiting intra-luminal based techniques. Even though insulin producing cells appears incapable of leaving the bloodstream, they are still transplanted through the portal vein (Hirshberg *et al.* 2002). The mechanism of action is believed to be microembolization of the cells to the liver parenchyma in the low pressure system that the portal vein constitutes (Lehmann *et al.* 2007). It has been speculated that arterial blood from the hepatic artery pushes pack into the portal vein during the mixing of the two flows which would stop the cells and create advantageous conditions for engraftment. The cells found functioning in the livers of transplanted patients are, however, situated as plaques in the hepatic artery tree thus adding yet another hurdle to the understanding of portal vein transplantation. The hypothesis is that the cells cannot perform diapedesis but are instead primarily forming a mural thrombus after, with low probability, being displaced against the blood flow into the arterial tree and then encapsulated by endothelial cells. The plaque formation

can thereby provide capillary ingrowth, an absolute requirement for endocrine function (Korsgren *et al.* 2008). Such a hypothesis would also shed further light on the low efficacy of portal transplantations methods. Nevertheless, the portal vein approach to transplantation is today the golden standard since the existing open surgical options of total pancreatic transplantation carries a mortality risk of 10% during the first year of follow-up (Kandaswamy *et al.* 1999; Humar *et al.* 2000).

The process of diapedesis is basically divided into tethering, rolling and stopping of leukocytes prior to diapedesis into inflamed tissue. It is thought of as a multistep procedure involving complex crosstalk between cells in the bloodstream and the endothelial cells. On the endothelial side intra-cellular adhesion molecule -1 (ICAM-1) (Dustin *et al.* 1986; Rothlein *et al.* 1986), vascular cell adhesion molecule -1 (VCAM-1) (Elices *et al.* 1990; Pulido *et al.* 1991) and junctional adhesion molecule –A (JAM-A) have all been implicated to play crucial roles. Interestingly, all of these receptors are part of the Ig-super family. Leukocytes capacity for homing and diapedesis is pivotal for the development of the inflammatory response to injury and starts as a tightly controlled up-regulation of endothelial E- and P-selectins that stimulate the leukocytes (Vestweber *et al.* 1999). These leukocytes then respond by activation of G protein-coupled receptors that increase the affinity for endothelial VCAM-1 and ICAM-1 (Muller 2003). Interaction with VCAM-1 is maintained through the heterodimer CD29 and CD49d forming the cell surface antigen Very Late Antigen – 4 (VLA-4) (Elices *et al.* 1990) and the CD11aCD18 heterodimer interacting with ICAM-1 (Meerschaert *et al.* 1995). VCAM-1 and ICAM-1 have previously been shown to be up-regulated on the endothelium as a response to inflammation (Dustin *et al.* 1986; Pulido *et al.* 1991). This interaction starts the diapedesis itself wherein the leukocytes "crawl" through, either a para-cellular or a trans-cellular pathway interacting with both PECAM-1 and/or CD99 or members of the JAM family of proteins (Petri *et al.* 2006). Thus significant crosstalk is needed to initiate and execute the active process of diapedesis.

VCAM-1 is also implicated both as a model of treatment for immunological modulation of the inflammatory responses following CNS insults and as surface antigen for cell treatment of CNS insults. In one study, fluorescence activated cell sorting (FACS) was performed for the expression of CD49d and identifying it as one of the important factors for directing diapedesis (Guzman *et al.* 2008). This finding has received a lot of interest from different fields, among other it has been shown that VCAM-1 expression has a critical role in transplantation of cells in dystrophic muscle (Gavina *et al.* 2006). Another utilization of VCAM-1 is the selective blocking by the monoclonal antibody drug Natalizumab used in multiple sclerosis, thereby inhibiting diapedesis for immunologically active cells (Stuve *et al.* 2008). VCAM-1 blockade has

also been tested for neuroprotective effects following ischemic events in pre-clinical trials with disappointing results (Justicia *et al.* 2006).

Broad aims of the thesis

This thesis over-arching aims are all connected to different optimization methods for cell based transplantational techniques. The first hypothesis tested in I and II are that a selective intra-arterial approach has benefits over intravenous administration. When performing study II and realizing that all cells where not optimal for intra-luminal approaches, a more detailed analysis of cells were included in that study broadening the thesis to include analysis of the molecules involved in the active diapedesis process. The aims of part of this thesis are therefore to compare different cell system in engraftment levels to the CNS and to further illuminate the active process of the cells that leave the bloodstream in order to achieve a reasonable engraftment. During the development of the specific intra-arterial approach utilized in I and II, the interesting finding that all cell systems were not optimal for intra-luminal approaches, a problem shared by other cells such as insulin producing cells, made it clear that yet another approach was desirable. Hence, in an attempt for providing a third method for minimal invasive transplantations, the development of the trans-vessel wall technique was initiated as described in III. This device pertains to cells that lack the possibility to leave the bloodstream and subsequent aim of that part of the thesis is to test the possibility of using the system to achieve parenchymal access and then test the possible long-term effects of such an intervention as performed in IV. It was originally intended for CNS applications, however, the aims where then broadened to "hard-to-reach" organs in the entire body. A *caveat* at this point is that the purpose of the studies compiled in this thesis is, in part, not regenerative medicine since the primary outcome of none of the studies actually measures regeneration. Nevertheless, an integrated part of translation of cell based therapies to the clinical practices is in our opinion the method of delivery of cells to the recipient. Therefore the use of endovascular means needs to be thoroughly studied in order to facilitate the maximum efficacy of transplantation methods. Characterization of not just regenerative capacity of the cells themselves seems important but also the capacity of the cells to reach the proper site of engraftment where any transplantation effect can be maximized.

AIMS OF THE THESIS

i. To investigate if it is possible to transplant cells to the CNS by selective endovascular technique.

ii. To compare selective intra-arterial with intravenous methods of cell delivery to the CNS.

iii. To test different cell systems in the same transplantation setting and subsequently investigate if any differences arise with engraftment.

iv. To investigate if it is possible to use trans-vessel wall technique methods for cell transplantation and substance administration with standard clinical catheters and angiographical equipment without hemorrhagic or thrombo-embolic complications.

v. To study the long term effects of implanted device through the vessel wall.

METHODOLOGICAL CONSIDERATIONS

This thesis comprises a significant amount of methodology development. To avoid repetition and lengthy descriptions, all steps are not included in this text. A detailed description of materials and methods are provided in the original articles and manuscripts. Herein short descriptions of commonly used methods and somewhat lengthier descriptions about non-standard methods are outlined. Further, advantages, disadvantages and methodological considerations behind some of the experiments are presented.

Cell preparation (Paper I and II)

All human cell isolations were approved separately by the Southern Regional Ethics Committee of Stockholm. All animal handling and protocols for rat cell isolation were approved by the Northern Regional Ethics Committee of Stockholm.

All cell systems transplanted were isolated/generated in the laboratories of Prof. Katarina Le Blanc (human mesenchymal stem cells, hMSC), Associate Prof. Erik Sundström (human neural progenitor cells, hNPC) and Associate Prof. Ola Hermanson (rat neural progenitor cells, rNPC) through cooperation between the research groups that participated in the transplantation studies.

hMSC were isolated from bone marrow taken from the iliac crest of healthy volunteers, as previously described (Grinnemo *et al.* 2006). hNPC were derived from the subcortical forebrain of human first trimester embryos as previously described (Joseph *et al.* 2009). Generation of rNPCs was carried out as previously described (Akesson *et al.* 2007). Prior to transplantation, the cells were mechanically dissociated and prepared to a final concentration of 50000 cells per µl. Different cell concentrations were also tested ranging between 10000 cells per µl to 100000 cells per µl (un-published results).

Animal management (Paper I through IV)

A total number of 463 animals have been utilized in the laboratory work, not counting animals used for cell isolation. Of these animals, 434 were rats, 28 were rabbits and 2 were swine. The vast majority of the animals have undergone multiple operations. In close collaboration with the animal handling facilities, the utmost care has been taken to reduce the suffering and provide stimulation to each and every animal. The majority of rats have not been included in published papers due to preliminary studies establishing models and negative results. All animal studies were conducted according to Karolinska Institutet guidelines of animal experiments on small rodents and rabbits

or the guidelines of swine handling. All studies were approved by the regional ethics committee for animal research in Stockholm, Sweden. Reduction of numbers of animals, replacement by simulations and refinement in experiments has been thoroughly considered during planning and execution of all studies.

It is the author's firm belief that in order to test the hypotheses of the studies performed for this thesis, the use of live animals is a necessity. The possible benefits of the studies performed, in the mind of the author, justifies the studies and the subsequent designs of the same. The author deployed reasoning from the utilitarian school for this position albeit reaching different conclusions than the ones of Peter Singer (Singer 1976).

Small Animal TBI and Transplantation procedures (Paper I and II)

The TBI model was chosen since we, within the group, had previous experience with it (Holmin *et al.* 1997). The surgery can be performed quickly and gives rise to minimal behavior alterations thus lowering the detection threshold for ischemic adverse events. The endovascular route for cell transplantation was modified from a model for ischemic stroke; the middle cerebral artery occlusion model (MCAO) (Longa *et al.* 1989). A separate model was developed and evaluated for MCAO followed by repeated surgery for transplantation (un-published results).

One day following TBI, the animals were re-anesthetized and neck explorations were performed. Transplantations immediately after the TBI during the same anesthesia were also performed (un-published results). The transplantation procedure for intra-arterial administration included ligation of the ipsilateral external carotid artery. Thereafter, via the stump of the external carotid artery, cannulation of the internal carotid artery was performed with a nitinol alloy catheter (Nitinol superelastic tube with outer diameter 0.193 mm ± 0.0127 mm, inner diameter 0.104 ± 0.0127 mm and length 300 mm (Tube NiTi SE 508, ground surface, Euroflex GmbH, Germany) connected to a microsyringe. After securing access to the vascular system, the nitinol-catheter was advanced in the internal carotid artery, passing the origin of the pterygopalatine artery towards the skull base. The use of the Nitinol tubing was chosen to mimic clinically available micro catheters and was the smallest possible fabrication of tubing with pushability at that time. The use of polyethylene tubing was also considered but that approach was never used since, for a more selective intra-arterial approach, the origin of the pterygogopalatine artery must be passed. Polyethylene is less rigid and thus less maneuverable than nitinol. Otherwise half of the cell suspension would be administered to the masseter muscles.

Cell suspensions were then infused during two minutes. Blood flow in both the common carotid and internal carotid artery was preserved during the entire

transplantation procedure and only the external carotid artery remained ligated after surgery. For intra-venous transplantation, the nitinol catheter was inserted into the ipsilateral internal jugular vein via a neck incision and advanced through the superior thoracic aperture past the subclavian vein. The neck approach for intravenous administration was chosen to mimic the surgical trauma of the intra-arterial administration. Two hours post-operatively, animals were scored according to the Bederson scale for stroke classification (Bederson *et al.* 1986). The scoring was performed to provide clinical evaluation of a possible adverse ischemic event following the intra-arterial transplantation.

Computer simulations and endovascular simulator (Paper III)

Design simulations of the Extroducer were performed in COMSOL Multiphysics software (Comsol AB, Sweden). Designs were then tested in a system, scaffolded by iron tubing (ID 250 µm), on rat aorta *ex vivo,* cut longitudinally and hanging in free air between two connecting points on a cork plate. The cork plate was connected to a loading cell 1004-300 (AB Svenska våg, Sweden) which in turn was connected to a weight instrument LD5208 (AB Svenska våg, Sweden) giving real time output to a computer recording the force applied in the perpendicular direction of the inside of the vessel. Hereby forces could be measured for vascular penetration with the grinded nitinol tip and different depth limiting collars. Ratio calculations could be performed between penetration by the distal portion and different depth limiting designs. Important information could be gained from these experiments and further, the use of animals could be minimized. The *ex vivo* setup also provided a more controlled environment compared to *in vivo* to be able to utilize a large number of repetitions in trials with different prototype designs.

An *ex vivo* simulation environment of the human vascular tree was constructed to test 1700 mm long superelastic nitinol alloy tubes, with diameters as described under prototype manufacturing. A simulator of the human femoral artery, iliac artery, aorta, coeliac truncus and hepatic artery was constructed by connecting plastic tubing of decreasing size and mounting them on a plate. The distal end corresponding to the hepatic artery was then introduced in rat carcasses through the distal end of the abdominal aorta for *ex vivo* testing. Standard 6 F introducer, guiding catheter; Envoy (Cordis, USA) and micro-catheter; Prowler Plus (Cordis, USA) were used to navigate within the simulator. The nitinol tubes were deployed inside the standard clinical guide- and micro-catheter systems. Herein we tested the maneuverability of the system and if enough driving pressure could be applied to penetrate vessel walls.

Extroducer prototype manufacturing (Paper III, IV and un-published data)

Several designs were tested but the only design tested in vivo was constructed in a similar fashion throughout all the papers. Briefly, we started with sharpening a tip from a super-elastic nitinol tube 1700 mm long with outer diameter 0.193 mm ± 0.0127 mm, inner diameter 0.104 ± 0.0127 mm (Tube NiTi SE 508, ground surface, Euroflex GmbH, Germany) in a faceted manner and connected the tube to a 25 µl micro syringe. A depth limiting collar was constructed by copper and cyanoacrylate glue and then formed into shape giving an added radius of 35 to 40 µm compared to the rest of the prototype. For construction of detachment zones, we applied a 3 µm thick parylene layer through gas polymerization in vacuum (ParaTech Coating AB, Sweden). Directly proximal to the depth limiting collar, a small insulation defect was cut through the parylene. We achieved electrolysis detachment of prototypes by applying 8 (rabbit) to 10 V (swine) tension through a cathode needle placed in the hind limb and applying the other end to the prototype, making it the anode. This resulted in a circumferential dissolution of the catheter where the cut in the parylene was made. The distal penetration end was then separated from the proximal access portion of the nitinol tube thereby leaving the detached distal penetration end through the vascular wall. The distal penetrating tip thus provides the exit strategy for the intervention.

The construction of the depth limiting collar was, in its entire construction and final result developed within the group. The largest problems in engineering in such a small scale are the magnitude of forces that are included in "visualization" of how it "should" work in relation to standard endovascular interventional procedures; here simulations were also helpful. The hollow detachment zone was also developed within the group, based on the electrolysis detachment of the initial Guglielmi detachable coils (Guglielmi *et al.* 1991).

Small Animal trans-vessel wall testing (Paper III)

In the rat we tested a 300 mm long nitinol prototype that was introduced through the rat tail artery within a plastic PTFE-190 Sub-Lite wall tubing (SUBL) with outer diameter of 482± 0.025 µm and inner diameter of 330± 25 µm (AgnTho's, Sweden). The plastic tubing was used to minimize damage to the vascular tree by the sharp penetrating tip.

A new method for endovascular arterial access was developed with introduction of catheters via the medial tail artery. A small longitudinal incision was cut on the ventral part of the tail through the skin and the fascia overlying the artery. A ligature was used to secure the PTFE-190 tube containing the nitinol tube and then the catheter system was blindly navigated up through the aorta. Two significant advantages can be

achieved with tail artery access in comparison with the standard access via the femoral artery; the possibility to perform repeated interventions and the added possibility to use a more rigid system that would otherwise be difficult to navigate proximal to the aortic bifurcation.

For observation and usage of the Extroducer prototypes, open surgical preparation of either the common carotid artery via a small mid-line incision medially on the neck, or the subclavian artery via an axillary exploration, were performed. To maximize surgical access and navigational success-rates, both the major and the minor pectoral muscles were cut.

For all prototypes, tests to exclude vasospasm as a potential hemostatic cause were performed by soaking the perforated vessel with papaverin (Recip AB, Sweden) and observing it for 90 minutes. Mechanical manipulation and cannulation through the lumen of the detached Extroducer was also performed to stress-test the system for a possible hemorrhage.

Rabbit endovascular intervention (Paper III and IV)

Several different procedures were performed on rabbits. Trans-vessel wall technique Extroducer perforations were initially performed with fluoroscopical, angiographical and simultaneous open microsurgical monitoring. Thereafter, perforations were performed with fluoroscopical and angiographical monitoring only, followed by methylene blue- and contrast agent injection through the nitinol tube. During the later phase of testing, the procedures were finished by detaching the distal Extroducer portion by electrolysis and removing the proximal catheter system.

All large animal angiography and surgery was performed with a Philips XD20 angiographical equipment (Philips medical system, Netherlands). Without the resolution of the used system or a similar system, the navigation and subsequent testing of the Extroducer would not have been possible. Nitinol in these small dimensions is fairly hard to visualize but the addition of radiological markers at this stage, *e.g.* tungsten would have even further complicated the prototype manufacturing process.

In planning the large animal trials all sorts of custom, or by hand manufactured materials for facilitating the endovascular intervention with the Extroducer prototype, were avoided to simulate the potential clinical application of the system and the integration of the developed system with clinical standard material. The femoral artery of the anesthetized rabbit was exposed surgically and a 5 French Introducer was inserted in the artery (Terumo, USA). The artery is too small for a percutaneous approach since it is almost impossible to palpate through the skin. Under fluoroscopic and angiographic control, a 5 French Envoy guiding catheter (Cordis Corporation, USA) or a 4 French Vertebral guiding catheter (Terumo, USA) was navigated to

different parts of the vasculature of the rabbit. A Prowler Plus microcatheter (Cordis Corporation, USA) or Renegade high-flow micro-catheter (Boston Scientific, USA) was inserted within the Envoy guiding catheter and together with a Transend Platinum Tip guidewire (Boston Scientific, USA) navigated under angiographic control to the microvasculature (0.5 – 1 mm in lumen diameter) in different parts, of the rabbit. After having reached the desired target location, the guidewire was withdrawn from the microcatheter and replaced by the Extroducer system protected within a PTFE-190 Sub-Lite tubing (AgnTho's, Sweden). The distal penetration tip of the Extroducer was pushed out of the protective PTFE-190 tubing and deployed through the arterial wall. This was performed both under direct visual control with surgical microscope (Carl Zeiss AB, Sweden) and under high magnification fluoroscopic control with subsequent digital subtraction series.

For the acute experiments, the use of a larger guiding catheter allowed for contrast injections through the guide catheter. When performing the long term follow up studies, a smaller guide catheter was used to minimize trauma to the femoral artery, thereby facilitating vascular closure. The downside to that strategy was that it became impossible to perform contrast injections through the guide. The Renegade high-flow was then a better choice than the Prowler Plus microcatheter since contrast injections were possible even with the Extroducer inside the lumen of the micro-catheter.

Swine endovascular intervention (Un-published data)

Two swines were included in this preliminary proof of concept study. Several interventions were performed in the same swine. Interventions included methylene blue- and contrast agent injection of up to 200 µl through the nitinol tube at a flow rate of 2 to 5 microliters per second. We obtained XperCT images and reviewed these using the XD20 system XperCT Highdose program and soft tissue algorithms (Philips Medical Systems, the Netherlands) (Soderman *et al.* 2008). The XperCT does not give optimal images but they are good enough for anatomical charting of the pancreas in swine. The obvious advantage with this strategy was that the same equipment could be used without moving the swine.

The femoral artery of the anesthetized swine was exposed surgically and a 5 French introducer was inserted (Terumo, USA). Under fluoroscopic control, a 5 French Envoy guiding catheter (Cordis Corporation, USA) or a 5 French Cobra guiding catheter was navigated to the vasculature of the pancreas. A Renegade micro-catheter (Boston Scientific, USA) was inserted within the Envoy guiding catheter and together with a Transend Platinum Tip guidewire (Boston Scientific, USA) navigated under angiographic control to the microvasculature. After having reached the desired target, the guidewire was withdrawn from the microcatheter and replaced with the Extroducer

system protected within a PTFE-190 Sub-Lite wall tubing. Thereafter, the Extroducer was deployed through the arterial wall, injections with methylene-blue and contrast were performed and the distal penetrating tip of the Extroducer was detached as previously described. Thus, in principle, the same methods were used in this setting as in the rabbit, but larger introducers and guide catheters made it significantly easier to perform the interventions.

The swine is known to be notoriously hard to perform endovascular interventions in due to a strong tendency of vasospasm. Even so rabbit are not usable for pancreas access creation since the pancreas of the herbivore is almost nonexistent as opposed to the swine with more humanoid organ characteristics.

Microarray and RT-qPCR (Paper II)

Cellular mRNA were extracted from cell suspensions with Qiagen RNeasy kit (Qiagen, Sweden) according to the manufacturer's protocol and then analyzed by microarray and RT-qPCR. Microarray was used for comparison between human cell systems. Thereafter RT-qPCR was used to confirm results from the microarray and to include the rNPC in the analysis.

Tissue handling, Histochemistry and IHC (Paper I through IV)

Coronal 14 μm cryosections of the brain and the other organs were taken using a Leica cryostat (CM 3000, Leica Instruments GmbH, Nussloch, Germany). HuN, Human Nuclear antigen, a protein found in human cell nuclei but not in rodent nuclei, were used as primary antibodies (MAB1281, Chemicon International, Temecula, CA, USA) for human cell detection, whereas BrdU antibodies (Dako A/S, Denmark) were used for detection of transplanted rodent cells. VCAM-1 or ICAM-1 antibodies were used for adhesion molecule detection.

The brain of each animal was screened for transplanted cells. This was performed by analyzing cryosections from the whole brain with immunohistochemistry. Cryosections were taken from the rostral to the caudal part of the brain with a 2 mm interval. For analyzing the number of cells in the perilesional zone, six different sections with 140 μm intervals, starting from the lesion center, were analyzed. All transplanted cells in the whole ispi- and contralateral hemisphere of each analyzed section were counted. For histological analysis of adverse effects by the transplantation such as thromboembolic complications, Hematoxylin and Eosin staining were performed according to Mayers protocol (Bancroft 1996).

For evaluating implanted devices we performed histochemical analysis of blocks of tissue containing the detached tips. The appropriate tissue was dissected with

fluoroscopic guidance. The tissue blocks were then mounted in plastic and cut by specialized ground cutting technique providing the possibility of leaving the metal tip *in situ*. Staining was performed with van Geeson and toulene blue on grind cut blocks and then visualized by light-microscopy.

Statistical analysis (Paper I, II)

Different groups were analyzed with parametric ANOVA followed by Bonferroni corrections for comparison of different groups (Paper I). In paper II, we changed statistical method to a non-parametric method using Kruskal-Wallis analysis for comparisons between all groups. This was followed by analysis for differences between selective i.a. and i.v transplantation by Mann-Whitney U-test for the three cell systems with subsequent Bonferroni correction for each comparison (C = 3). One can argue that non-parametric analysis should have been used in the first paper as well since small samples were used. We have, prior to the writing of this thesis re-analysed the data by the non-parametric methods used in paper II and a significance was reached also with that method.

Microarray p-value for signal was generated as an internal control in Beadstudio (Illumina Inc., USA) through internal controls randomly placed on the chip. A p-value < 0.05 was considered significant in all studies.

In paper III and IV, no control groups were used since they are all concerned with method development. For statistical analysis, the trans-vessel wall technique should be compared to other transplantation methods.

RESULTS AND DISCUSSION

In this thesis three different cell transplantation methods are evaluated. The first two are the selective intra-arterial method and the intravenous route, these are compared for efficacy. The third method is the trans-vessel wall technique by using the Extroducer; an endovascular catheter system developed within this thesis for penetrating the vessel wall from the inside to out, thereby creating a working channel to extravascular tissue.

First, we show that, for certain cell types, the selective intra-arterial method is superior to the intravenous one after TBI in the rat. This was measured by the level of engraftment of hMSC at one and five days following TBI, without thrombo-embolic complications (Paper I). Selective intra-arterial transplantation method for rNPC after TBI in the rat is also superior compared to intravenous method. We were, however, not able to engraft hNPC, *ceteris paribus*, thus indicating that diapedesis and engraftment is an active process and that different cell systems have different capabilities to engraft following intraluminal delivery (Paper II). We show, by indirect methods, that CD29CD49dVCAM-1 interactions might be one of the factors with impact on engraftment in an intra-luminal transplantation setting (Paper II). Further, we show that it is possible to perform minimally invasive parenchymal injections by trans-vessel wall technique by the development of the Extroducer (Paper III). The Extroducer is shown to have no adverse long term effects on the blood vessels up to 3 months following interventions (Paper IV) and it is feasible to use the system as a novel approach for transplantation of e.g. insulin producing cells to the pancreas or other cell types to organs that are difficult or risky to reach by traditional methods. The trans-vessel wall technique thereby adds a new possibility when transplanting cell populations without the necessary properties of diapedesis (un-published data). The establishment of a working channel to the extravascular tissue, by endovascular method, also open up several other possible applications.

Selective intra-arterial method versus intravenous method (Paper I, II and unpublished results)

We established a model for selective intra-arterial transplantation that we applied to a model of TBI in the rat (Feeney *et al.* 1981). We compared the selective intra-arterial method to intra-venous methods for different cell systems in the same TBI setting (Paper I and II). Several different variables were tested such as cell concentrations, days post injury for transplantation, time for infusion, level of immunosuppressant drugs etc. The majority of transplantation experiments were not successful, from the start and many different variables were tested before robust engraftments could be reached.Too

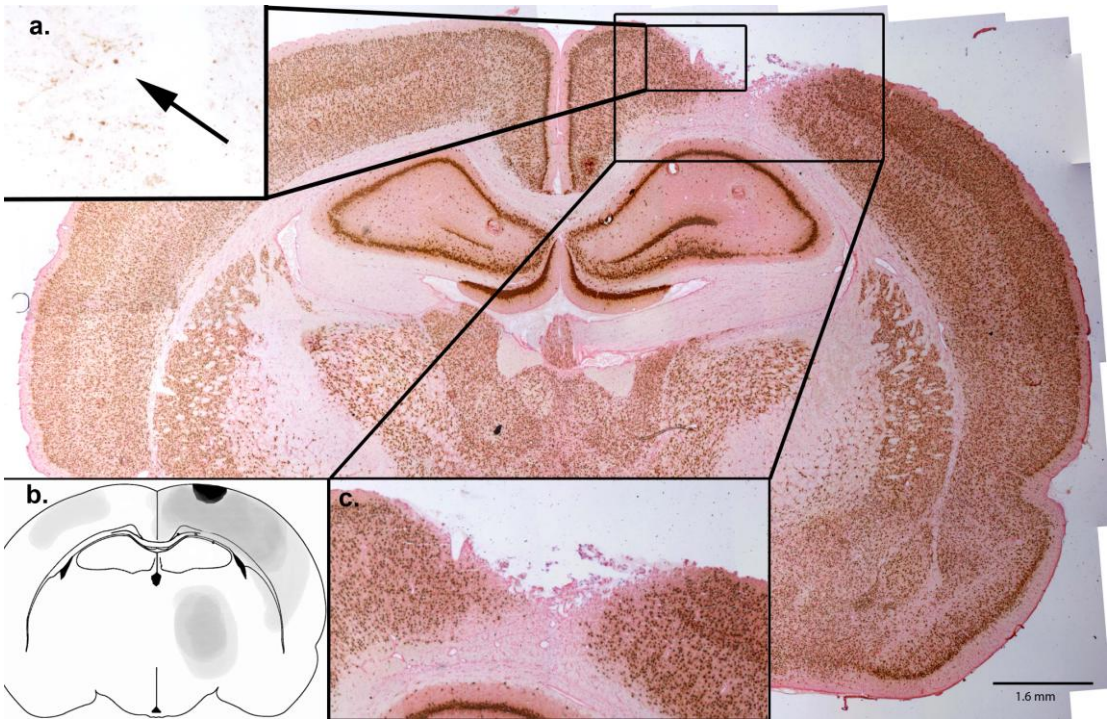

Fig 1. *In the background is a reconstructed image showing a coronal section of the rat brain 2.5 mm posterior to bregma stained with GFAP (red) and NeuN (Brown) five days after traumatic brain injury. In blow-up a. a low magnification single staining with HuN (human nuclear antigen, MAB 1281) without any counterstaining, along the peri-lesional zone is shown. Brown dots represent HuN positive, transplanted cells (arrow indicates an example of a positive cell). In blow-up b. the black area represents the contusion zone and the grey areas represent primary localization of engrafted mesenchymal stem cells. In blow-up c. a magnification of the injury area itself is presented.*

few cells had a dire impact on the success of transplantation; 200.000 (unpublished results) and 500.000 hMSCs did only result in very low levels of engraftment. The failure of low cell numbers can obviously be interpreted as an indication that the efficacy of the intra arterial method in this setting is quite low although it is still superior to intravenous alternatives.

Results of engraftments levels were obtained through IHC methods by counting engrafted cells in sectioned brains (Fig 1). We found that engraftment levels of rNPCs were more than five-fold higher than in the control group ($p=0.034$) and hMSC were more than fifteen-fold higher than the in control group in absolute values ($p=0.007$), with a large spread within the intra-arterial groups (Fig 2) (Paper II). Few studies compare selective intra-arterial and intravenous methods but recent clinical data suggest an advantage for the selective intra-arterial route (Sykova *et al.* 2006). A known problem with all intravenous methods is the fact that the lung acts as a kind of

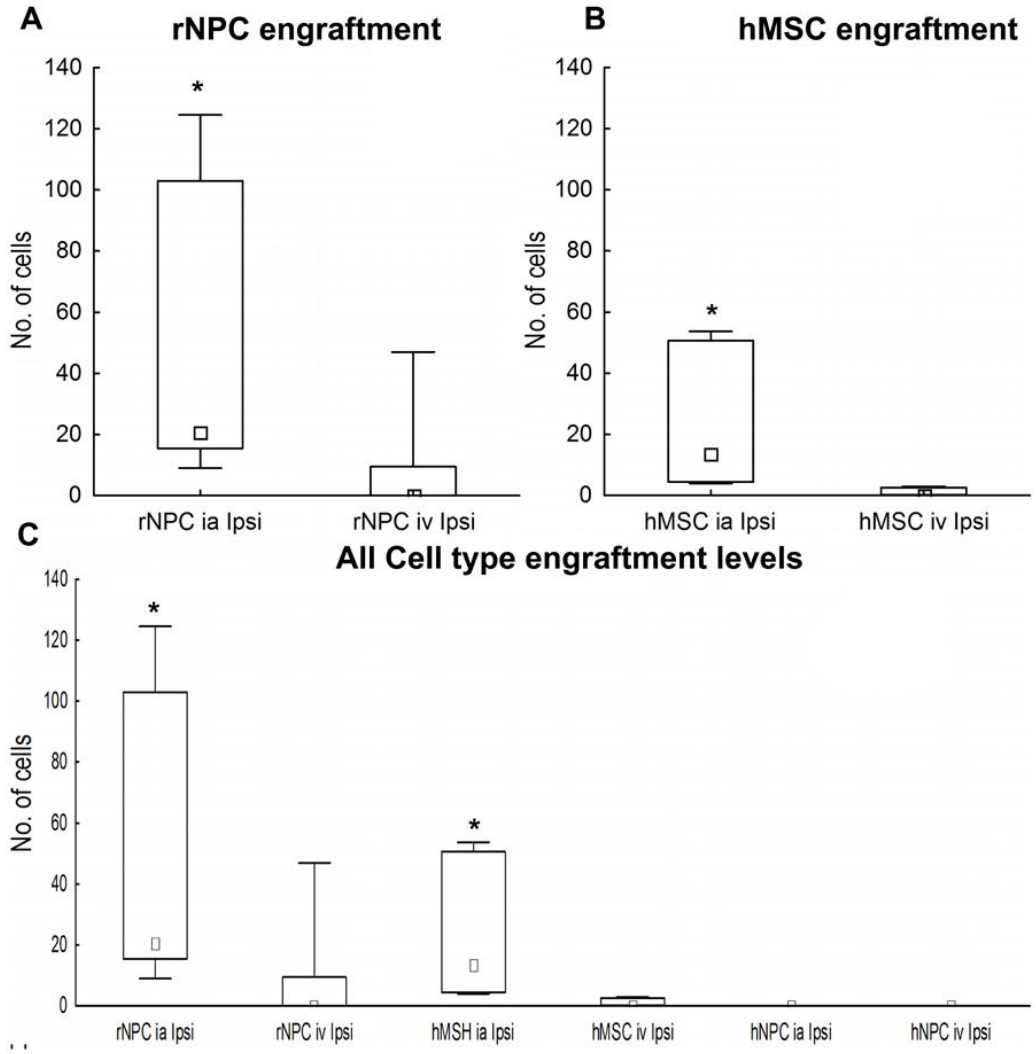

Fig 2. *Engrafted cells were counted per section and is reported with median (marker), quartiles (box) and max – min (whiskers). In A a significant difference between engraftment levels per section following selective intra-arterial and intravenous transplantation in the ipsilateral hemisphere of the rNPC group. In B a significant difference between engraftment levels per section following selective intra-arterial and intravenous transplantation in the ipsilateral hemisphere of the hMSC group and finally in C a panel of all engraftment levels per section in the ipsilateral hemispheres of all groups. * marks p < 0.05*

clearance filter during the first passage (Barbash *et al.* 2003; Fischer *et al.* 2009). Following intravenous cell infusion, the blood transports the cells after venous passage to the right ventricle and then through the lung where up to 80% of cells are trapped during the first passage (Fischer *et al.* 2009). Thus as low as 20% of the cells transplanted might be ejected for the first total body distribution through the aorta. Of all the blood leaving the left ventricle of the heart, only somewhere between 1.8 to 8.5

percent of the blood actually reaches the brain of the rat (Pannier *et al.* 1973) leaving only 0.01 to 1.7 percent of the initially transplanted cells to reach the brain in the first passage. All cells not distributed to the brain are then again re-transported for another passage through the lung, acting as a filter. This phenomenon has been studied *e.g.* by PET for mesenchymal stem cells (Ma *et al.* 2005). This situation can readily be changed by placing a micro-catheter in the arteries supplying the organ of interest. An interesting finding in the present study is that our hMSCs predominantly were found in the spleen with few transplanted cells in the lung at 24 hours post injection, a bio-distribution phenomenon, from the lung to the spleen, previously described in rat following intravenous hMSC transplantation (Detante *et al.* 2009). To increase the understanding of the role of cell line properties for engraftment, we conducted a study with transplantation of different cell lines through either intra-arterial or intravenous routes. This analysis showed that there were dramatic differences between the different cell lines; hNPCs did not engraft at all after intra-luminal delivery whereas there was a significant difference between the hMSCs and the rNPCs using the same transplantation method. Noteworthy is the large variability in the engraftment levels of the latter two cell lines. One important factor that might contribute to this is that neither hMSC nor rNPCs are defined homologous cell lines, succinctly there can be important variations within the cell systems transplanted. The remarkable finding that no engraftment was obtained following hNPC transplantations is even more noteworthy since the hNPCs have previously been robustly transplanted by open surgical technique (Wennersten *et al.* 2004; Akesson *et al.* 2007).

Gene expression profiling of cell systems (Paper II)

After discovering that there were differences in engraftment capability between the different cell lines, the opportunity to investigate the bio-molecular basis of said differences presented itself. We started with characterizing and confirming earlier results (Clausen *et al.* 2007) that our TBI model leads to up-regulation of VCAM-1 expression in the endothelium. That result may suggest that the injured CNS parenchyma could provide cues for diapedesis and migration of engrafted cells in similar ways as immunological cells respond to inflammatory cues (Butcher 1991; Springer 1994). As a screening method, we started by performing microarray on the human cells. rNPCs were not included in the microarray analysis due to problems with cross-species comparisons in the microarray chips used. hMSCs showed a broad expression of integrins, commonly expressed by immune cells, that are important for diapedesis through the vessel endothelium and subsequent migration into the parenchyma. Specifically, analysis of the heterodimers forming receptors for ICAM-1 and VCAM-1 were analyzed, based on previous work indicating CD49d expression as

important for successful intra-vasal transplantations (Guzman *et al*. 2008). Thus, probably the most interesting finding was the CD49d signal of 68 in hMSC as opposed to 0.4 in hNPC (p=0.0047). This was then confirmed with RT-qPCR data from all cell lines with average CD49d mRNA levels that were highest in the hMSC (0.98) followed by the rNPC (0.0057) and finally a dwindling finding in hNPC (0.0012). CD29 and CD49d forms a heterodimer named very late antigen -4 (VLA-4) which was expressed in falling order in hMSC, rNSC and hNPC (Fig 3). The difference in mRNA levels between rNPC and hNPC is not large but might reflect larger differences in protein translation. Further studies at the protein and functional level are required to elucidate the importance of CD49d for diapedesis of these cell systems. CD11a mRNA was detected in hNPC (0.0029) albeit with low CD18 mRNA (0.00025), suggesting that CD11a-CD18-ICAM-1 interaction may be dispensable for engraftment. In contrast, hMSC displayed high CD18 mRNA levels (0.43) but no detectable CD11a expression, suggesting neglectable ICAM-1 dependent engraftment in the hMSCs.

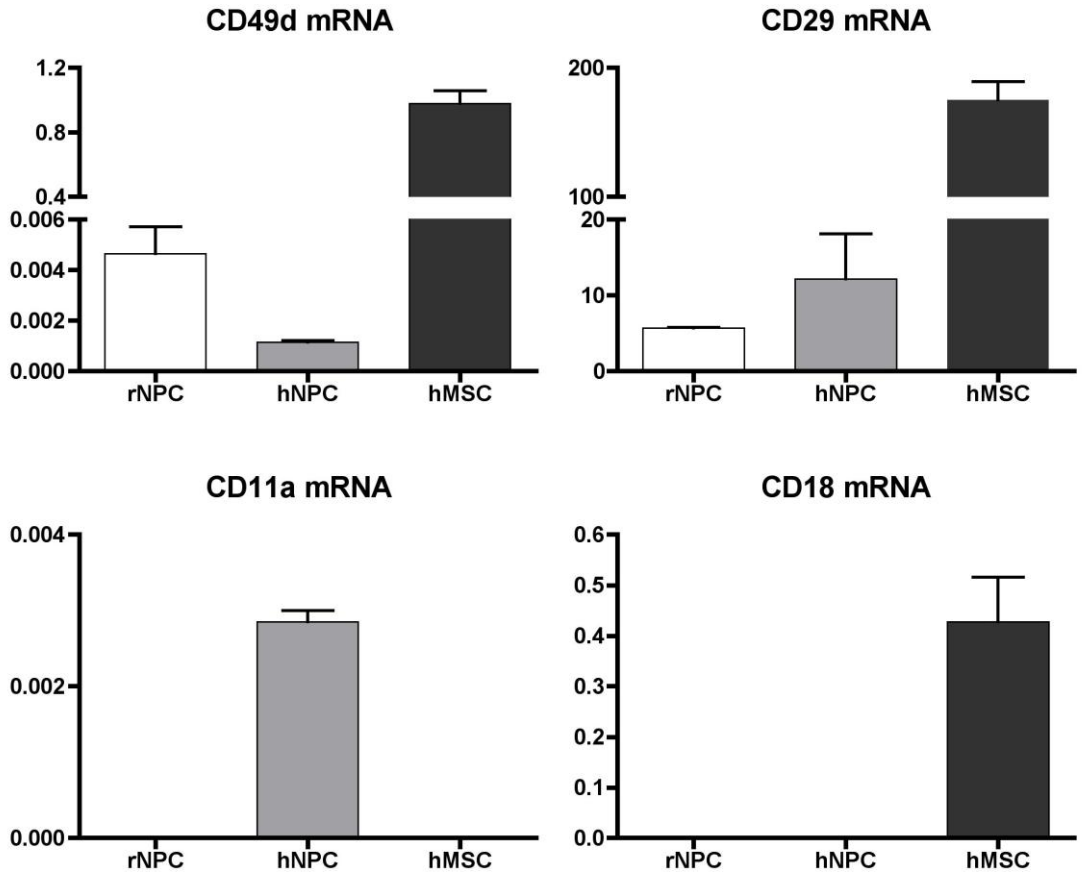

Fig 3. *Bars represent relative levels of integrin CD49d, CD29, CD11a and CD18 mRNA expression in rNPC, hNPC and hMSC compared to respective endogenous TBP mRNA levels. Error bars represent the distribution between the biological replicates.*

Finally, both our own findings regarding hNPCs and other previously known cells incapable of diapedesis, such as insulin producing cells (Hirshberg *et al.* 2002), shows the apparent need for surgical techniques. In some organs that are hard to reach and/or when surgical technique comes with a high risk of adverse events for the patients, the need for an alternative strategy becomes apparent. Thus, we also initiated the development of the trans-vessel wall approach.

Computer simulations and Extroducer design (Paper III)

An endovascularly based system that could penetrate the vessel wall would, in instances where the target parenchyma is either hard to reach or carries a significant surgical risk, be a method with both the merits of accurate placement and reduction of patient risk. Further, it would solve the problem for certain cells to leave the bloodstream.

The development of the Extroducer started with conceptual testing of exit through an arterial wall *in vivo*, more specifically in the tail artery of the rat. This was performed with a sharply cut nitinol tube acting as both introducer and Extroducer. This showed that no hemorrhage occurred with the device still in place, whereas when the penetrating device was retracted, the expected bleeding occurred. Thus the design with a hollow detachment zone was initiated where the distal portion of the penetrating catheter would be left in place as a plug in the vessel wall. For accurate placement of the detachment zone in relation to the inner vascular wall, a depth limiting collar was added to the design. After that, several new designs were simulated before returning to *in vivo* testing.

After detachment of the distal tip, the inner lumen would be open and could potentially cause hemorrhage in the target tissue. We performed flow calculations through different radii of a distal tip at a pressure of 200 mmHg. Fluid mechanics states that the flow rate out of the Extroducer lumen varies as the fourth power of the radius. Consequently, the flow rate quickly becomes small as the lumen radius is reduced. In practical terms, this gives an auto-sealing effect up to around a 100 µm radius where flow of water with a perfect axial entry in the tube is in the range of 100 µl per second at physiological blood pressures. When introducing coagulation in the model at low and/or turbulent flow it becomes increasingly hard to establish a maximum diameter with persisting absence of flow, but it could be up to 150 µm.

We then performed *ex vivo* tests on longitudinally cut aortas coupled to a loading cell for force measurements with the Extroducer system scaffolded by iron tubing. Initial, perpendicular, penetration by the sharp tip was achieved at 11 gram. Full force to penetrate the vessel wall with intrusion depth limiting collars was then applied, again

augmented by scaffolding iron tubing. We evaluated several different types and sizes of intrusion depth limiting collars for optimal performance in relation to catheters and the vessel wall. The optimal collar was additionally tested and we found that when using the final design, the vessel wall gave away at 45 gram. Therefore with regular plastic catheter scaffolding, instead of iron scaffolding, of the Extroducer system, it is impossible to overshoot the intrusion depth limiting collar past the vessel wall prior to bending the nitinol tube body.

Integration of the Extroducer system with clinical routine guides, wires and catheters was then tested in our simulator environment with an artificial human vascular tree connected to rat carcasses. Navigations through the simulator system were all successful with full compatibility towards the clinical setting.

Extroducer in vivo testing - small animals (Paper III)

After computer simulations and the *ex vivo* testing, the next part of the study was short term testing in rat by creating arterial access from the medial tail artery and performing the Extroducer trans-vessel wall technique passage in either the subclavian or carotid artery. Two different stages of the procedure was tested; first the trans-vessel wall technique passage per se with surgical microscope monitoring of hemorrhage or other adverse events, and thereafter the deployment of the distal penetrating tip through the vascular wall and retracting the proximal part of the system. No cases of intra-operative hemorrhage or intra-luminal thrombosis occurred. Thus, the vascular penetration procedure was uneventful and the vessel wall completely sealed around the Extroducer, thereby preventing leakage of blood.

The second group with deposited Extroducer tips also showed absolute hemostasis during the primary intervention. Fourteen days post intervention, this group showed no signs of pain or discomfort. No signs of dissection of the vessels or impairment of blood-flow distal to intervention sites were observed and macroscopical analysis of the organ supplied by the vessel, showed no infarcts. The flow simulations, indicating absence of blood-flow through the interior lumen, was also tested *in vivo* by cannulating the deployed distal tip of the prototypes with a nitinol mandrel. This was done to reassure that even when removing possible clotting inside the prototype, it still prevented bleeding from inside the vessel to the extravascular space. Furthermore, no signs of delayed hemorrhage were detected.

Extroducer in vivo testing - large animals (Paper III)

After successful trials in small animals, the Extroducer system was tested in large animals. An adaptation towards clinical use was that these prototypes were

manufactured from a longer nitinol tube, 1700 mm *vis-à-vis* 300 mm that was used in the rat. We evaluated the prototypes in the rabbit together with standard clinical catheters and angiographical equipment.

The Extroducer prototypes within the microcatheters were visible at high magnification fluoroscopy and thereby maneuvered into the subclavian artery (SCA) (Fig 4). The rabbit SCA was chosen since it is close to the intended target vessel size of 0.5 to 3 millimeters, the SCA is fairly easy to access in order to perform simultaneous open surgical monitoring and it had been used in the rat. A slight amount of pressure was required on the protecting plastic catheter to advance the system through the microcatheter to the desired vessel wall, thereafter the Extroducer was gently advanced out through the vessel wall to the extra-vascular space. Hemorrhages were neither observed by simultaneous direct observation through a surgical microscope, nor by high resolution angiographical series (DSA), during and after the intervention (Fig 4). Further, no thromboembolic complications or vascular dissections were observed using high resolution DSA. No navigational problems were encountered with respect to Extroducer prototype integration with clinical catheters.

Finally, electrolysis detachment of the distal Extroducer distal tip was tested in rabbit. We chose electrolysis since it was the easiest way of performing detachment in our hands. Our design was based on the work of the first detachable coils (Guglielmi *et al.* 1991). An important difference compared to the detachment zone in coils was, however, that we needed a hollow detachment zone which required additional development. After navigation to the designated intervention site and after methylene blue or contrast agent had been deposited in the extravascular space, a tension of 8V was applied and the distal tip was then detached after, on average, five minutes (range three to nine minutes). This was also un-eventful without observation of hemorrhage from around the body of the distal tip or through the inner lumen. The procedure was successfully performed both with simultaneous microscopical monitoring via surgical access, and with fluoroscopical/angiographical guidance solely.

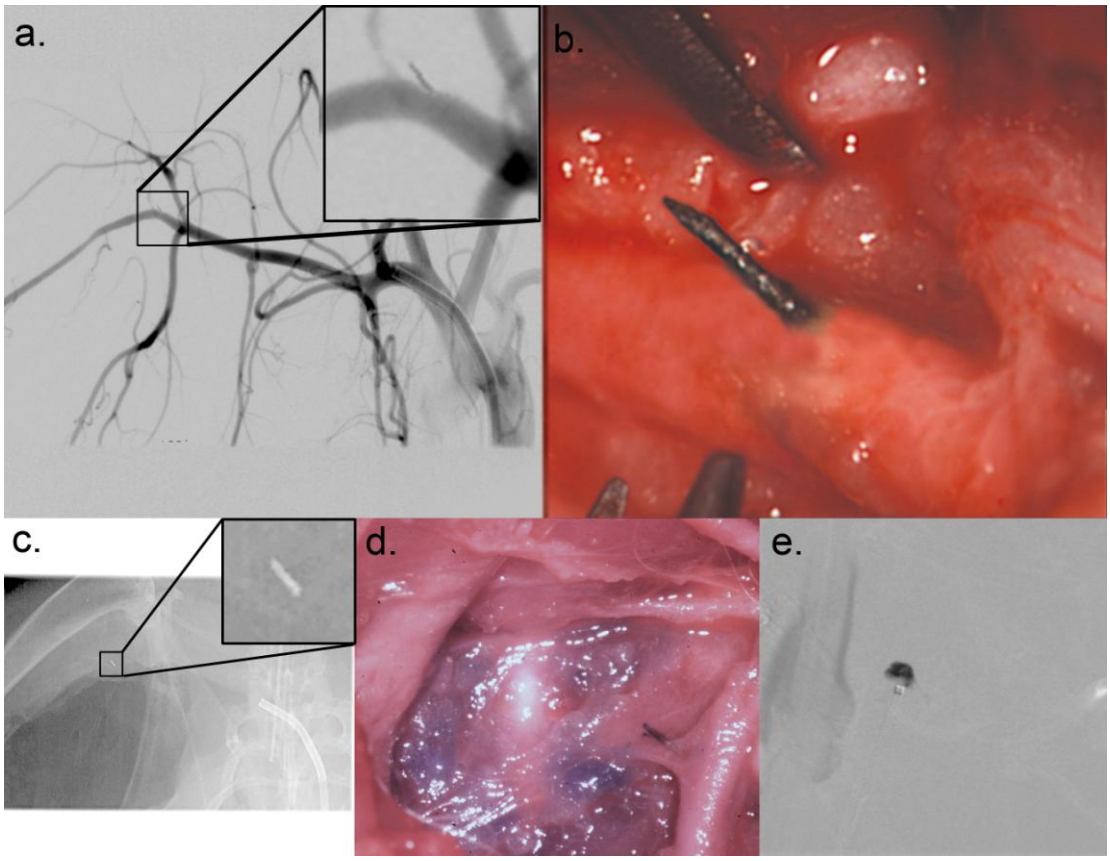

Fig 4. *For full control over the procedure in the large animal trials, both a surgical microscope and high resolution angiographical series was used. In a. digital subtraction angiogram showing a detached Extroducer tip without hemorrhage, dissection or thromboembolic complications. In b. photograph showing the microsurgical view of the detached Extroducer tip. In c. x-ray image showing the detached Extroducer tip with guide catheter. In d. photograph from post-operative dissection showing the detached Extroducer tip with methylene blue injected in the surrounding tissue. In e. digital subtraction angiogram showing an extra vascular injection of 25 µl contrast agent through the Extroducer system.*

In a previous work describing a method for penetrating large veins (Thompson *et al.* 2003), that system design required a much larger catheter and also lacked a method for sealing the vessel wall, thus making penetration through the arteries impossible. This severely limits the use of that system to their testing vessel, i.e. the sinus coronarius of the heart, whereas the Extroducer is applicable in both arteries and veins of any sizes down to approximately 0.5 mm in diameter. Another system, in which vessel perforations are performed, is the trans-jugular intrahepatic portacaval stent shunt (TIPS) technique (Richter *et al.* 1990). That system also does not have the requirement of sealing the vessel wall when finishing the procedure, since a patent blood flow

through the stent is the preferred result. On the contrary, thrombosis of the stent might be considered the main problem (Merli *et al.* 1998) which requires rigorous follow-up.

Thus, the Extroducer system is unique in the ways that it permits safe exit of both arteries and veins and that it is usable in vessels with large dimensions as well as in the microvasculature with inner lumen diameters down to approximately 0.5 mm.

Extroducer testing with long time follow up (Paper IV)

In the long term follow up, the end points five days, one month and three months after the deployment of the device was selected. No stenosis or late hemorrhagic complications were noted in any animals. No alterations in behavior or other measures of discomfort were noted either.

We started with a total number of 25 primary trans-vessel wall interventions in 17 animals. Working channels were established in 100% of the cases without acute hemorrhagic or thrombo-embolic events, verified by DSA. Six of the interventions were aborted due to distal tip detachment failure with our hand-made relatively primitive electrolysis detachment method (leading to euthanasia of 3 animals and 3 that we were able to salvage to stay in the study). Two animals were euthanized by order of the veterinarian during the follow-up period, one because of a non-related pulmonary infection (but included and analyzed by histology) and the other since it fulfilled the behavioral abortion point criteria after 24 hours (excluded from the study). That animal was then autopsied and was found to have an embolic occlusion of the internal carotid artery without association to the Extroducer placement, but rather associated with the vascular navigation. The final distribution of followed up animals were as follows; two at the five days end point with only histological analysis of one, five at the 30 days end point and six at the 80 days end point with histological analysis of one (with pulmonary infection) resulting in a total number of 19 detached Extroducer tips.

In the follow up DSA we also found that four of 19 (21 %) of the detached tips were no longer placed through the vessel wall but had instead been “pushed” or “migrated” through the endothelium to the extravascular space immediately adjacent to the penetration site. No vascular stenosis or other adverse reactions were observed around those tips. Also for the rest of the tips, that were located through the vessel wall, no adverse reactions were detected. During initial testing, five prototypes, detached precisely in the rotator plan of the humero-scapular joints in the SCA, were dislodged into the lumen of the vessel with the depth limiting collar pointing distally in the flow of blood. This phenomenon occurred due to the fact that the anterior extremities of the rabbit were flexed in a certain position during anesthesia and that the five Extroducer tips were detached exactly in the plane of movement of the joint. Thus, the detached tip

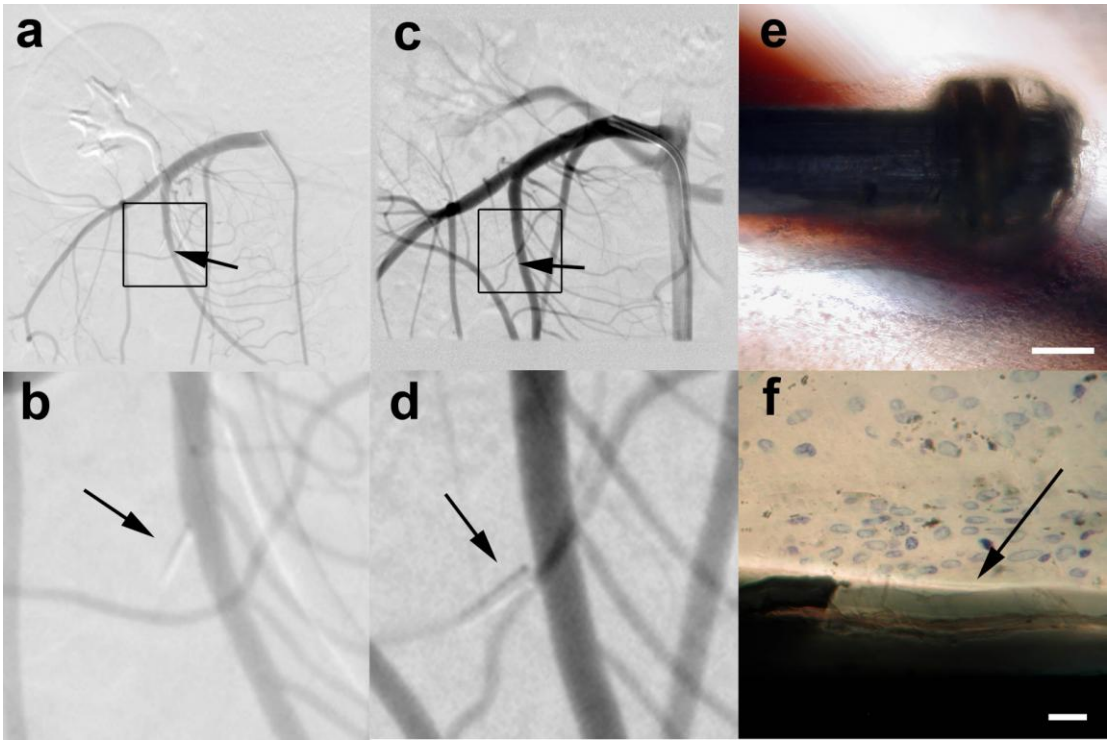

Fig 5. *In a. the initial follow up angiogram directly following detachment in the Superior Mesenteric Artery (SMA) is shown with a square marking the blow-up in b. Arrows indicate the detached distal tip. In c. an SMA angiogram, performed 80 days after the intervention in the same animal, is shown with a square indicating the blow-up in d. Arrows indicate the detached distal tip. In e. a microphotograph of a histological van Geeson and toulene blue staining prepared by grind-cutting with the detached tip in situ, is shown. Scale bar = 100 µm. The blow-up in f shows the parylene coating surrounding the detached tip which is marked by an arrow. Note that no fibrous response or inflammation is observable. Scale bar = 4 µm.*

was pushed into the vessel immediately when changing the position of the extremity during wake up. For all other prototypes, detached within other segments (millimeter more proximal or distal) of the SCA and in all other vascular segments tested in the body, this phenomenon was not observed.An important endpoint was biocompatibility of the detached tips. The nitinol alloy used was selected for its many advantageous properties. Nitinol is a nickel/titanium alloy with both memory and super elastic properties (Adler *et al.* 1990). These special properties are the foundation for the use of nitinol in stent fabrication in clinical practice. Thus, the excellent biocompatibility of nitinol (Castleman *et al.* 1976) has been extensively studied, especially with respect to vessel wall interactions in the use of stents (Stoeckel *et al.* 2004). However, the compatibility of nitinol when placed through arterial walls have, to our knowledge, not been studied. The parylene, used as coating in our device, is also FDA approved and CE marked when used in pacemaker electrodes. To evaluate interaction between the deposited tips and the endothelium, we performed histological analysis with the

prototypes *in situ* by a specialized grind-cutting technique and then consulted an external, independent, evaluator with expertise in the field of titanium implants. This evaluation showed full biocompatibility with a very small fibrotic capsule (< 1 µm) formed around the detached distal tips (fig 5). No ongoing inflammation was observable around any of the distal tips. Around one (of the total number 14 left in place) of the day five animal implants, three macrophages were indentified in the area of the detached distal tip. Apart from those three macrophages, no other signs of inflammation were observed. The endothelia showed no signs of alterations adjacent to the deposited tips. In conclusion, the biocompatibility of the distal tips were all comparable to titanium implants. The interactions of nitinol with the interior of the vascular wall and the extra vascular space has not been as extensively studied since stents most often are positioned inside the vascular lumen with direct contact only with the bloodstream and the endothelium. Therefore, the present histological analysis in this new application and new position of nitinol, adds important knowledge about biocompatibility for possible future applications.

The prototypes excluded due to failed detachment were analyzed by sweep electron microscopy (SEM), since monitoring of electric current gave limited prognostic information about the failure to detach. We found that, in failed detachments, large amounts of chloride were observed by surface spectroscopy (6-10 wt %) indicating titanium chloride ion formations in a passive layer, thus providing current transmission without electrolysis. Titanium chloride molecules can provide a surface area that lets electron pass through but without formation of soluble titanium or nickel ions. This problem is probably due to the simple technique used for creating the insulation defect in our hands. However, numerous available solutions for detachment are available on the market that can easily be integrated with the trans-vessel wall technique in an industrialized manufacturing process.

A model for multiple interventions in rabbit was also established. Application of tissue glue directly after retracting the femoral introducer, made it possible to, at all time-points, to establish new endovascular access in the same femoral artery. This has implications for endovascular research with the rabbit for numerous research projects.

Extroducer proof of concept study with pancreas access (Un-published data)

After initial testing of the concept and long term follow, up a preliminary feasibility study was designed to address a known clinical problem; the inherit risk of adverse event (Villiger *et al.* 2005), low efficacy (Korsgren *et al.* 2008) and sub-optimal micro-environment in the liver (Merani *et al.* 2008) of the portal vein approach to transplantation of insulin producing cells. A swine model was chosen and we started by acquiring anatomical information through XperCT, a method of obtaining CT images

with the C-arm of the angiographical equipment (Fig 5) (Soderman *et al.* 2008). After initial planning, we performed DSA series as road maps (Smart Mask™) to navigate the micro catheter to the chosen point of intervention. When reaching tip to tip with the micro catheter, the Extroducer was gently pushed out through the vessel wall thereby creating a working channel between the proximal end of the catheter and the pancreas parenchyma, usable for e.g. transplantation of insulin producing cells (fig 6). No haemorrhage or embolic complications were observed when creating access to the parenchyma. Vasospasm was observed and could be reversed with intra-arterial infusion of the calcium-channel blocker nimodipin.

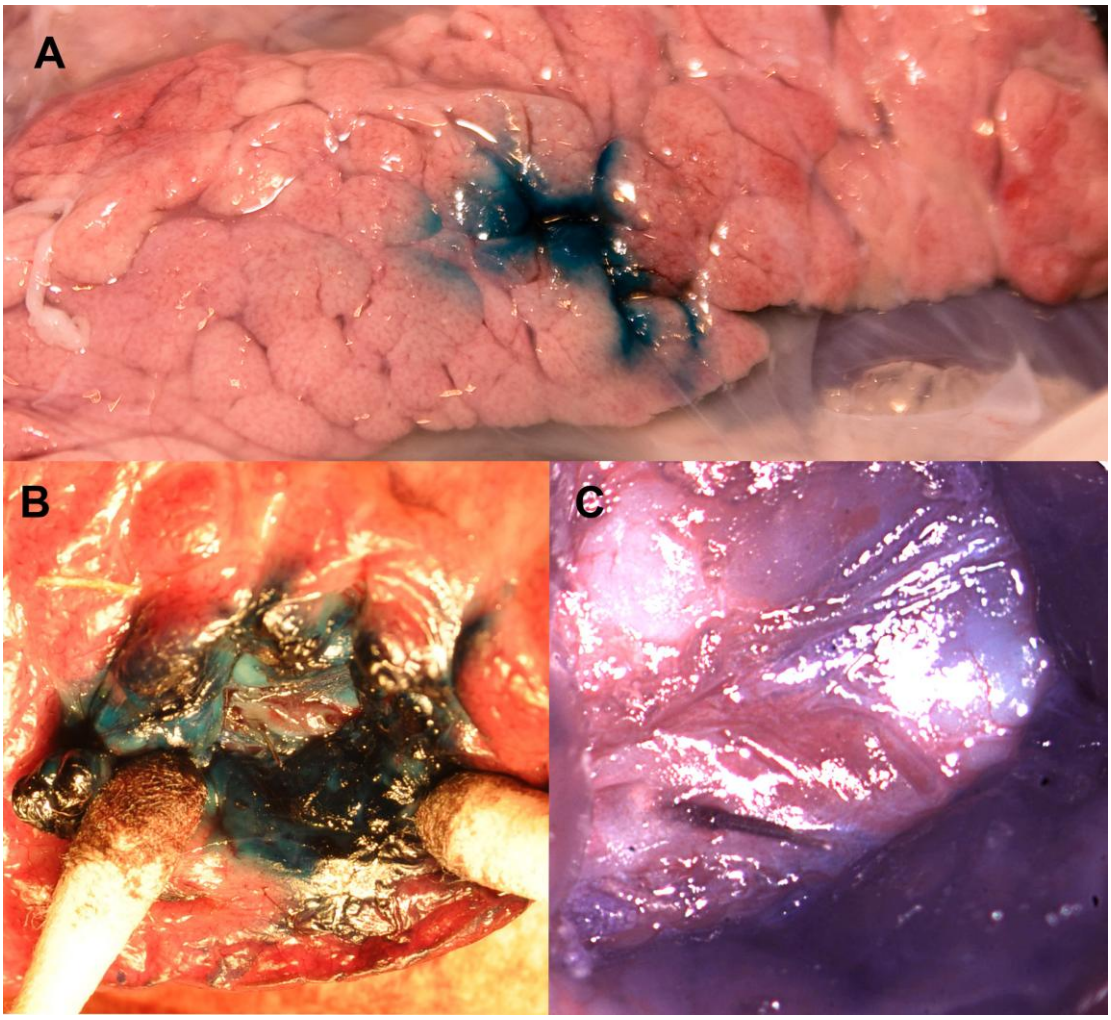

Fig 6. *Following the interventions, cadaver dissections were performed. In A, an overview from the dissection shows small fragment of blue colour on the outside of the pancreas followed by higher magnification of the pancreas itself in B and finally in C, an operating microscope view to illustrate the placement of the detached distal tip in the vessel.*

We performed injections of a blue coloured dye (methylene-blue) to mark parenchymal access which was confirmed by subsequent autopsy. Dissections of the swine showed the deposited methylene blue located in the middle of the pancreatic parenchyma (fig 6). Further the detached distal parts of the Extroducer were observed in the vessel walls within the pancreas, surrounded by methylene blue staining (fig 6). In addition, during the intervention, a contrast agent was injected through the Extroducer system to demonstrate that the deposited contrast was not washed out with the blood stream thereby confirming extra vascular access to the pancreas (fig 6).

GENERAL DISCUSSION

The rationale for the studies comprising this thesis is that when translating cell based regenerative medicine to patients, the actual route of transplantation will be very important. For operative techniques there is a problem with scalability and for intravenous techniques there is a problem with efficacy. We show that by selective intra-arterial methods it is possible to increase the level of cerebral engraftment with certain cell types with six to fifteen fold yields. Further, not all cells are optimal for intra-luminal transplantation. It is, for example, known that insulin producing cells lack the capability to perform diapedesis (Hirshberg *et al.* 2002) and we show that hNPC, a cell system previously transplanted by open surgical means (Wennersten *et al.* 2004) also lacks the capability to perform diapedesis. For these cell systems, and other applications, we have developed the Extroducer as a tool to establish a direct, minimal invasive working channel with parenchymal access in organs that are difficult or risky to reach with traditional techniques.

The first passage of cells delivered through selective intra-arterial approaches, compared to systemic intravenous delivery, results in a higher local concentration, shorter blood stream exposure and less mechanical stress factors before cells reach the target site. These factors could be of importance for successful engraftment. Supporting that hypothesis are the present results showing significantly higher total cerebral uptake of cells after intra-arterial compared to intra-venous administration. The next supporting fact is the higher uptake of cells in the ipsilateral hemisphere after intra-arterial administration and by the absence of difference between the hemispheres after intravenous transplantation. Future studies are needed for elucidating molecules responsible for diapedesis, e.g by direct methods such as knock ins, of for example CD49d, in non-functioning cell systems and knock down or blocking in other cell systems.

The absence of adverse effects in transplanted animals suggests that in the short term, the selective intra-arterial transplantation method is safe for delivering even high concentrations of cells. It has been reported ischemic events following intra-arterial approaches to the heart in dogs (Vulliet *et al.* 2004) thus indicating the need for thorough studies prior to translation into clinical practice. However, in clinical studies on intracoronary infusions (Stamm *et al.* 2003) and in spinal cord artery infusions (Sykova *et al.* 2006) no embolic events have been recorded. Connected to the need for safety studies, it could be argued that the higher engraftment rates in our intra-arterial groups would be a consequence of microembolization of cells. In that scenario we would, however, have detected ischemic histological changes and localization of the transplanted cells within arterioles and capillaries, which we did not.

For all intra-luminal approaches, this thesis shows that a thorough investigation must be performed to clarify if the cells actually can perform diapedesis prior to choosing transplantation strategy. hNPCs has previously been shown to have an impact on neurological outcome when transplanted with open surgical techniques, but in their present form they seem unsuitable for intra-luminal transplantation. Therefore, based on the findings in this thesis, considerations should be made that CD29CD49dVCAM-1 interaction is one of the best studied crosstalk mechanisms on how immunological cells leave the bloodstream to perform homing to disease ridden tissue where inflammation occurs (Elices *et al.* 1990). This line of reasoning is supported by other studies implicating VCAM-1 interaction in both ischemic stroke and muscle dystrophy pre-clinical studies (Gavina *et al.* 2006; Guzman *et al.* 2008). An interesting comparison can be made regarding diapedesis in cell systems aimed for transplantation compared to immunological cells. In such an exercise it could be considered blatantly ignorant to immediately dismiss the fylogenetically conserved mechanism of the immunological system and suggest a hitherto unknown method of diapedesis for these cells. Applying Occam's razor to the hypothesis of diapedesis for cell transplantation, two conclusions can potentially be made; i) the process of diapedesis is most probably an active process for cell systems transplanted, since there are no known inactive ways of diapedesis and ii) the most likely system for diapedesis crosstalk should be found within the same systems that are used by the immunological system, meaning that proteins are highly likely to come from the Ig-super family such as VCAM-1. The other, more complex, explanation would be that a hitherto unknown system for diapedesis exists. That it also unlikely since mutations in such a system would lead to genetical diseases that should be known, but without reasonable explanations. A research program dedicated on endovascular transplantation of different cell systems in different diseases should include a variety of diseases and cell systems to increase the understanding of both cell-endothelium interactions and the effects on target niches.

When designing both pre-clinical and clinical cell transplantation studies, consideration should be taken to how the cells are hypothesized to reach their designated targets. The first option might be open surgical techniques for small niche locations in a target parenchyma. Downsides to open surgical/percutaneous techniques are *e.g.* impracticalities of accruing the desired extent of target tissue volume, especially if the volume is relatively large, such as following a major CNS insult. Further, the patient risk for adverse events might be unacceptable in relation to a potential benefit, in particular for "difficult to reach" organs such as the CNS, the pancreas and/or the heart. If the disadvantages of open surgical/percutaneous techniques are unacceptable, intra-luminal options could be explored. For intra-luminal approaches, the concept of how transplanted cells are presumed to leave the blood

stream becomes an issue with several potential solutions. For a cell system without the necessary features for diapedesis, one could use knock-in methods to provide the necessary adhesion molecule set up, otherwise intra-parenchymal injections must be considered. For knock-in methods, an intra-arterial approach would probably still have benefits in efficacy over intravenous methods through the first passage effect and by avoiding pulmonary trapping. As previously discussed an interesting purely academical calculation when performing intravenous cell transplantations could result in such a low cell dose to the brain as 0.01 to 1.7% after intravenous injection. Assuming 80% of cells are trapped, in the first passage of the lung (Barbash *et al.* 2003; Fischer *et al.* 2009). This can readily be changed by placing a micro-catheter in vessels supplying the target parenchyma with blood thereby providing a chance for all cells to perfuse the target parenchyma.

For more discrete functioning cells, an intra-parenchymal injection might be even more attractive for reasons such as shielding the cells from the exposure to the bloodstream and accurate anatomical placement. In difficult to reach organs, minimally invasive direct parenchymal transplantation could be performed by the trans-vessel wall technique described within this thesis. That technique might, however, not be suitable for treating a large ischemic lesion in humans. On the contrary, in discrete lesions where only a niche cell needs to be replaced, such as in type I diabetes, where the cells do not possess properties for diapedesis, the Extroducer could potentially really show its worth. The next natural step is transplantation of insulin producing cells to swine before planning for clinical trials.

The Extroducer is not limited to cell transplantation. Its main design is to provide a working channel by endovascular technique to the parenchyma in various, otherwise, inaccessible organs. Through that working channel, other procedures such as local chemotherapy-, irradiation-, growth factor administration, tissue sampling, electrophysiological diagnostics and thermo-therapy becomes possible. Further, combined with optical spectroscopic analysis, it might even be possible to perform infra-light histological analysis of tissue via the Extroducer.

General conclusions

In the articles compiling this thesis we first show that selective intra-arterial administration is a safe way, with a short follow up time, to increase engraftment levels compared to intravenous delivery. However, not all cell systems are optimal for intra-luminal transplantation. These factors might be dependent on integrin expression and endothelium interactions. For cells that lack the capacity to perform diapedesis, and especially for more specific niche cell systems in organ systems that are difficult to reach, we have also developed a system for trans-vessel wall parenchymal access. The

Extroducer system has been evaluated both for long term effects and feasibility for pancreas access. *Ergo*, endovascular intervention should provide a number of methods for efficient and safe cell transplantation in current and future clinical practice. The transplantation method must be decided on a disease to disease, cell to cell and patient to patient manner.

CONCLUSIONS

i. It is possible to transplant cells to the CNS by selective intra-arterial technique and it results in significantly increased engraftment compared to intravenous cell administration.

ii. All cell systems are not optimal for intra-luminal approaches. Possibly this is dependent on integrin expression of the cell systems transplanted and the cross-talk with the endothelium in the CNS parenchyma.

iii. CD29CD49d and VCAM-1 interactions appear pivotal for creating CNS parenchymal engraftment after intra-luminal cell delivery.

iv. The Extroducer system renders parenchymal cell transplantation and substance administration possible by trans-vessel wall technique, using standard clinical catheters and angiographical equipment, without hemorrhagic- or thrombo-embolic complications.

v. The distal tips, provided for securing the vessel wall defect, cause no adverse events with up to three months follow-up.

vi. It is conceptually possible to use the Extroducer system for transplantation of insulin-producing cells to the pancreas of large animals with full clinical integration.

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