CHAPTER 9

Basic Biology and Animal Models in Stem Cell-Mediated Tissue Regeneration

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Summary

Tissue Engineering and related research attracts a lot of attention from both clinical and industrial aspects. The reason is the potential to alleviate a diverse variety of conditions such as degenerative diseases. However, recent research demonstrates that there is also a potential for engineered cells in fields that have not classically been associated with tissue engineering. All those applications have in common that the efficacy of the newly developed products must be tested in animal models and prove their usefulness before they can be applied in humans. A great amount of animal models are available already. Many of these animal models were developed for testing of biomaterials. These models are sometimes appropriate for the evaluation of tissue engineered products, but often the tissue engineered products require the development of new and specific animal designs. In this article we demonstrate animal models that can be used to test tissue engineered constructs and cells to replace the most relevant tissue structures and organs. Adding to this we present data to answer the question, why stem cell lines play an important role in testing engineered tissues and cells in animals.

Keywords: Animal Models, Tissue Engineering, Stem Cell Lines, Multipotent Adult Progenitor Cells

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Introduction

In recent years there has been a tremendous progress in the field of tissue engineering. The basis for that is a better understanding of stem cell biology in general. Multipotent adult stem cells seem to have a differentiation potential almost equal to that of embryonic stem cells (ESCs). This eliminates at least some moral issues and the problem of getting access to the stem cells in their organ specific niches. This function is referred to as "stem cell plasticity" and describes the ability of adult stem cells to differentiate into various lineages *in vitro* and *in vivo*. The problem of having stem cells readily available at all times, without being dependent on donors, the problem of stem cell purity and the fact that stem cells tend to differentiate *in vitro* led to the use of adult stem cell lines for the research in tissue engineering. Beside the great advantages that has been made in the molecular understanding of stem cells any result that was found in vitro has to proof efficacy in vivo before it can been applied for a therapeutic use. Although stem cell therapy and tissue engineering is patient-tailored and individualized, animal models are the gold standard and a necessary control step between bench and bedside (1).

Although there is a clear necessity for the use of animal models there are clear limitations to them as well. Galen, one of the most important anatomical and physiological researchers in the 2nd century conducted his studies almost entirely on apes and pigs. Until the beginning of the renaissance he and the following researchers extrapolated these findings to humans, which initiated many errors. This anecdote should remind us that animal models in tissue engineering have limitations. Although there have been great efforts made to apply engineered tissues and cells in animals, sometimes less effort was put in selecting the most suitable animal models.

In this article we would like to demonstrate the use of animal models in the engineering of cardiovascular, muscle, bone, cartilage and neuronal tissues. These are the fields that have drawn the most attention, because the replacement and regeneration of these tissues have the most obvious clinical application. However, new aspects of using engineered tissues are emerging and they include the use of engineered cells to specifically target tissues and to suppress the immune system in patients. All of those strategies have in common that the existing therapeutic means are lacking specificity. The specificity of the engineered cells can provide a therapy with fewer side effects.

Clearly, the use of animal models is associated with a variety of moral aspects. We would like to point out some of the moral questions that are associated with the use of animal

models in tissue engineering and put them into the perspectives of the legal context, the public opinion and the balance between human benefit and animal suffering.

Animal models in cardiovascular tissue engineering

Diseases of the heart and the blood vessels are the most frequent causes of death in the USA and Europe (www.WHO.com) (2). In this group of diseases one needs to include peripheral vascular disease, which causes much suffering due to disability, limb loss and subsequently lead to death (3-7). There is a need for the replacement of vessels using bypass grafts and to improve the blood flow in limbs that have been seized by peripheral vascular disease (8). The current concepts to tackle the problem of bypasses are the use of living grafts and prosthetic bypasses. Living grafts are obtained from the long saphenous vein (leg), A. radialis, A. thoracica interna or less often the cephalic and basilic vein (arm). This accounts for about 2/3 of the bypass operations. In the use of autologous grafts the surgeon has to rely on patient's own vasculature, whose reason for seeking help is his poor vasculature already. In 1/3 of the cases the use of prosthetic material such as polyethylene terephthalate (Dacron), "expanded" polytetrafluoroethylene ("e"PTFE) and polyurethane is necessary (9). The use of these prosthetic grafts has a worse clinical outcome compared to the use of autologous conduits. The limited access to autologous and the inferior clinical outcome are the reasons why there is a great need for engineered living grafts (10-13).

Ischemic heart disease results in myocardial infarction, which causes poor cardiac function. This has an effect on almost all the other organs, life quality in general and may even need heart transplantation. Current concepts of using stem cells include stem cell therapy, where stem cells are applied systemically or locally in the affected coronary artery and functional substitutes for the damaged heart tissue (14-17).

Animal models that are used in tissue engineering with respect to engineered cardiovascular constructs include primates, porcine, canine and rodents (rat and mouse). Primates have the advantage that they resemble humans from an anatomical and physiological aspect. Their slow growth makes them ideal for long-term studies over several years, which can be a disadvantage as well. Further ethical aspects restricts their use to experiments, where lower species can not been used (18). Baboons were used to demonstrate that endothelial cells and muscle cells proliferate on PTFE grafts in the iliac position. After 12 months 60% of the graft was covered with endothelial cells (19). Among others these

types of studies led to the development of seeded grafts, which have in contrast to unseeded grafts a less amount of amorphous protein coverage.

Pig is a popular experimental animal in tissue engineering, due to the similarities in anatomy and physiology to humans. In Cardiovascular research the fast growth of the animals is sometimes a limiting factor. Over a period of two months pigs are capable of quadruplicating their weight, which leads to mismatches in engineered constructs. There are some pig strains that have lower growth kinetics, but their cost stands against a widespread use. Pig animal models have been used for the development of tissue engineered mesh-stents comprised of fibronectin and procine smooth muscle cells. The smooth muscle cells were labelled with green fluorescent protein and one month after the implantation histological analysis revealed mild intimal proliferation and no significant increase in the labelled smooth muscle cells. Today, there is a widespread use of porcine tissue as aortic valve replacement in human. Their preparation includes protein derivitisation by formalin and other substances (20). Despite their advantage over mechanical valves of not needing high anticoagulation there exists the problem of poor durability. Glutaraldehyde has been used to reduce immunological reactions and to reduce degradation by enzymes. Porcine valves have been seeded with myofibroblasts and endothelial cells and implanted into sheep over a period of three month. Endothelial cell coverage was found.

Canine animal models have a long history in cardiovascular research. William Harvey's studies on circulation in the 17th century were performed on dogs (1). The problem of fast growth that exists in pigs almost does not exist in dogs and they are easy to work with. However, moral issues have made it more and more difficult to use dogs in western countries. An example of the successful use of resorbable scaffolds in dogs was the transplantation of a non-woven fabric sheet of polyglycolic acid (PGA) and a copopolymer seeded with cells obtained from mongrel dog femoral veins. An example of testing the long-term biostabability was the transplantation of 5mm internal diameter vascular polyurethane graft into the aorta of a canine model for 36 months (21). After explantation of the graft Seifalian and his coworkers found that the compliance of the graft was retained even after perivascular ingrowth (22). L'Heureux and his colleagues developed a tissue engineered blood vessel purely from human cells by wrapping vascular smooth muscle cells and fibroblasts around a mandrel (10). After a maturation period under pulsatile flow the construct was transplanted as interposition femoral graft into a mongrel dog. Anticoagulation and immunosuppression were used to circumvent the problem of xenotransplantation. Despite the mentioned difficulties and the lack of endothelial cells the patency was 50% after one week.

Rodents in general have the advantages of being cheap to maintain, easy to anaesthetise and there exists a variety of species with specific risk factors and stem cell lines for production of syngenic bioengineered cells and tissues. However, the biggest disadvantage in cardiovascular tissue engineering research in rodents is size. This surgical challenge led to the fact that rodents are much less popular in cardiovascular tissue engineering research than in other fields. However, rats have been successful animal models in engineered heart tissue. Examples include the peritoneal implantation of a ring-shaped three-dimensional heart consisting of cardiac myocytes, the use of temperature responsive resorbable poly (N-isopropylarylamide) sheets seeded with sheets of neonatal rat cardiomycytes and models allowing muscle regeneration to be altered from centripetal to centrifugal (23). Despite the small size of rats there have been studies in rats examining tissue engineered vessels. Endothelial cell and in another experiment smooth muscle cell seeded grafts were interposed in rat carotid artery and assessed for patency. The result of these experiments showed layers of smooth muscle cells and endothelial cells covering the lumen, whereas the control grafts excising just of the microporous, biodegradable polyurethane showed covering with clots only.

Mice being even smaller than rats present an even greater technical challenge. However, they are superb in the research of molecular and cellular mechanisms of cardiovascular tissues. Myocardial and endothelial progenitor cells isolated from bone marrow and ESCs were used to restore cardiovascular tissue function. Other cell sources include autologous and allogenic fetal cardiac myocytes, myoblasts isolated from skeletal muscle satellite cells, bone marrow stromal cells and mesenchymal stem cells. Injection of these cells in infarcted border zones in the heart led to improved myocardial function (24-25). Recent reports suggest that endogenous cardiac stem cells may be able to proliferate in the myocardium under certain circumstances (26). Experimental animal work was not only used to provide new means of treating symptomatic coronary artery disease, it was also used to show how the existing gold standard therapy, the percutaneous coronary angioplasty can be improved. One of the major complications in this intervention is restenosis, but 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase inhibiting drugs (statins) were found to be beneficial to the reduction of the occurrence of this complication. One important mechanism of this effect is their action on endothelial progenitor cells. Walters *et al*. demonstrated increased reendothelialisation of balloon injured arteries in rats and Werner *et al*. showed that statin treated animals have reduced neointima formation with concomitant increased number of circulating endothelial progenitor cells in mice (27, 28). Endothelial progenitor cells have

been shown to play a crucial role in the regeneration of the endothelium in several transgenic mouse models. Transgenic mice carrying the LacZ genes driven by an endothelial TIE2 promoter, and hence expressing ß-galactosidase only in endothelial cells, demonstrated that endothelial cells of vein grafts are regenerated from circulating progenitor cells and not migration from neighbouring mature endothelial cells (29). By further creating chimeric mice via bone marrow transplantation it was determined that of the circulating progenitor cells that regenerated the vein graft endothelium, only one third were derived from the bone marrow cells. Adding to that ApoE deficient mice presented a reduced number of circulating progenitor cells and also a reduced regeneration of the endothelium of vein grafts. (30). Given that vascular progenitor cells are found in bone marrow, vessel adventitia and circulation it is certainly plausible that there are other reservoirs of progenitor cells elsewhere in the body e.g. fat tissue. Due to stem cell plasticity adult mesenchymal-type stem cells that have routinely not been regarded as being involved in arterial regeneration seem to play a role in vessel regeneration. We have conducted a study, where a syngenic peripheral blood derived stem cell line (RM26) $CD34^-$, $Sca\text{-}1^+$ cells were injected into a mouse chronic hind limb ischemia model. We found that intravenously infused RM26 cells significantly improved the collateral blood flow (arteriogenesis) and neo-angiogenesis formation in a murine hind-limb ischemia transplant model. Although transplanted RM26 cells did not integrate into the growing collateral arteries, cells were found adjacent to local arteriogenesis, but instead integrated into the ischemic skeletal muscle exclusively in the affected limb for simultaneous tissue repair. We concluded that molecularly primed hem-/mesangioblast-type adult progenitor cells can circulate in the peripheral blood improving perfusion of tissues with chronic ischemia and extending beyond the vascular compartment (31).

Skeletal tissue repair

The mesenchymal division of stem cells (MSCs) is particularly to be focused on, regarding stem cell- mediated regeneration in bone- and cartilage- tissue. MSCs can not only be isolated from bone marrow or peripheral blood, but also from a variety of other sources like, for example, umbilical cord- blood, adipose tissue or skeletal muscle. Following their stem cell nature, they can proliferate and differentiate into different mesenchymal directions, like the osteogenic, chondrogenic or angiogenic lineage. It is because of good progress in stem cell isolation and characterisation, coupled with the ability to control proliferation and

differentiation capacity, remarking extraordinary behavior focusing immunological aspects, that researchers and physicians pin their hope on MSCs to get new approach regarding well-known questions and problems. Still, deficiency of the acquired tissue after loss for example by trauma, burning, or tumour excision or the lack of physical regeneration capacity in degenerative diseases, limits healing process and therapeutical success in the way that often unsatisfying results are obtained.

We chose the following examples to give a brief overview of the current scientific topics and possibilities in tissue engineered stem cell mediated animal models: Abdel-Hamid *et al*. (32) examined in 2005 the effects of intraarticularly injected bone marrow derived stem cells (BMSCs) on the healing process of meniscal wounds in knee-joints of adult dogs. After clinical and immunomorphological evaluation of the healing progress 12 weeks later, values showed to be statistically significantly higher for the injected menisci as compared with the non injected ones.

The healing effect of canine segmental bone defects by porous ceramic implants, consisting of hydroxyapatite and beta-tricalcium phosphate, loaded with autologous mesenchymal stem cells was examined in 1998 by Bruder *et al*. (33). The group was able to show that the amount of bone found in cell- loaded implant pores was significantly higher than in unloaded implants. The same group was able to demonstrate in 2003 by Arinzeh *et al*. (34) that even allogeneic mesenchymal stem cells loaded on hydroxyapatite-tricalcium phosphate implants, enhance the repair of critical-size segmental defects in canine femurs without immunosuppressive therapy while no adverse immune response was detected in this model.

But, not only bony animal- models combined with the use of mesenchymal stem cells are established, also the cartilage division is represented, for example by Guo *et al*. (35) in 2004. Autologous MSCs were seeded into bioceramic scaffold-beta-tricalcium phosphate to repair articular cartilage defects in a sheep model. Twenty-four weeks later they observed that the defects were resurfaced with hyaline-like tissue and an ideal interface between the engineered cartilage, the adjacent normal cartilage, and the underlying bone.

To add to this small selection a murine model of regenerating bone tissue using a combination of mesenchymal stem cells and different composite- graft types, made among others of fibrin, thrombin and collagen, follows an abridged report of our work.

With the objective to create a murine model of tissue engineered bone regeneration which is not restricted to one single cell line, we used different mesenchymal stem cells, either bone marrow or peripheral blood derived from different mouse species, like Balb/c-, p

53 -/- and CBA- mice. Knowing that angiogenesis is as important for the successful transplantation of *in vitro* generated tissues like bone- structures for the repair of large bone-defects as the bony tissue itself, we intended to generate transplantable grafts, which are composed of *in vitro* generated bone from multipotent adult progenitor cells and still maintain an angiogenic potential to ease and facilitate a successful engraftment of sustainable and viable bone tissue. After cell isolation, SV- 40 mediated immortalisation and cultivation of these fibroblast-like mononuclear cells we reached stable culture and expanding conditions. Immunophenotyping showed clonal cell lines negative for CD 31, CD 45 and positive for CD 29, CD 44, CD 73 and CD 90. Both, primary cells of the Balb/c- cell line and the p 53 -/- cells showed negative results regarding CD 34 whereas subclone 8 of the Balb/c- cell line was positive for this surface marker. RM 26/ 2-1 cells showed partly positive results as well. RT- PCR survey showed a mixed picture regarding for example endothelial section of transcription and similarities regarding among others the GATA- section, what ensured us to have a diversified spectrum of mesenchymal stem cells to examine behaviour in various scaffolds.

The shell section of the future composite graft was built by Tisseel®, Baxter Hyland Immuno Vienna, Austria, which not only enhances with its high amount of vascular endothelial growth factor (VEGF) arteriogenesis but also differentiation of progenitor cells into angiogenic direction. Tissufleece ®, also provided by Baxter Hyland Immuno Vienna, Austria, a collagen fleece of equine origin was chosen to built the bony core.

Former undifferentiated cells of all inserted mesenchymal stem cell lines showed after 17 days of cultivation in three dimensional fibrin- thrombin matrix, positivity for the endothelial surface marker CD 31, what was represented by over hundred times higher expression in fluorescence activated cell scan and immunostaining, as shown in Figure 1 and 2. After returning angiogenic differentiated cells back to two dimensional plastic bottom flask culture, they lost endothelial activity again and returned to CD 31 negativity, what is probably caused by lack of the appropriate environment.

In vitro along the osteogenic pathway pre-differentiated cells of the same different clonal lines kept up producing osteogenic products like alkaline phosphatase and osteotypical extracellular matrix components like osteocalcin for more than 28 days cultivation in the collagen matrix.

After combining the single components to a composite graft, same behaviour as under separate cultivation conditions was found. The interface between shell, the fibrin-thrombin matrix, and core, the equine collagen, contained both, CD 31 positive and osteoactive cells,

what could be explained by a migration of angiogenic differentiated cells into the core and of the predifferentiated osteoactive cells into the shell.

These findings convince of composite- grafts, made of different matrix and cellular components, linking the capability of multipotent stem and progenitor cells to differentiate along the lineage of the missing tissue, with the ability to stay in differentiating readiness, to build an indispensable connection to the vascular system, what allows cell survival, integration and a successful result in transplanting engineered tissues. This is why we propose to use both components for an extended graft- survival of transplanted tissue due to an improved microvascular system.

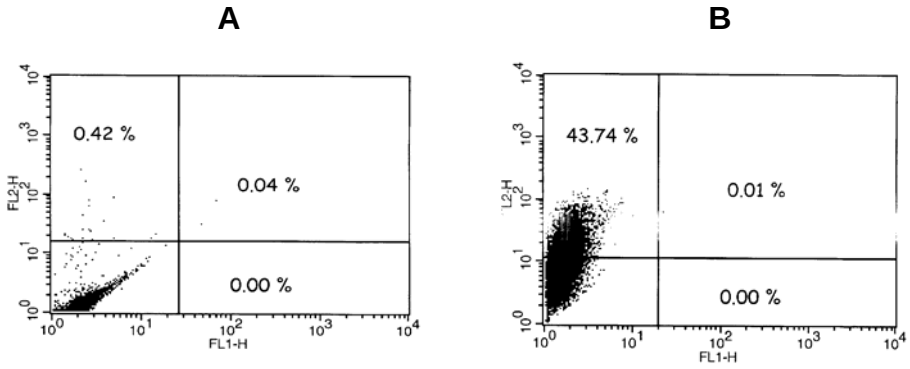
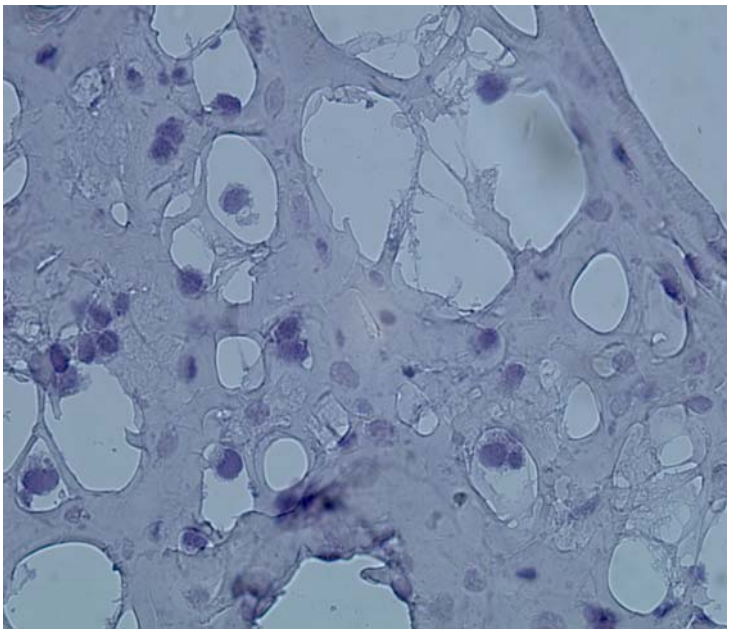


Fig. 1. FACS of Balb/ c primary cells regarding CD 31. A: two dimensional plastic bottom cultivated B: cultivated for 17 days in three dimensional fibrin-thrombin matrix.

A



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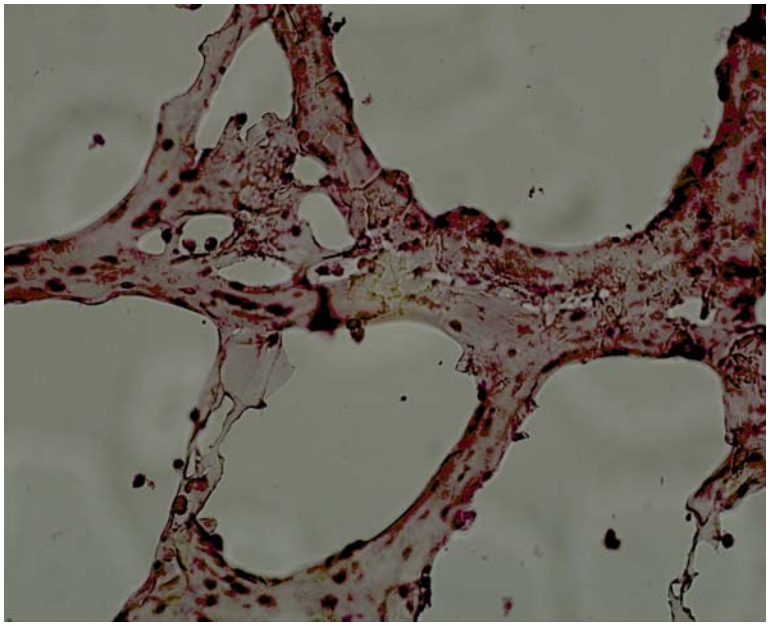

Fig. 2. Immunohistochemistry of Balb/c primary cells. A: cells cultivated for one day in three dimensional fibrin- thrombin matrix negative for CD 31. B: cultivated for 17 days in three dimensional fibrin- thrombin matrix positive for CD 31.

Nervous system repair

Diseases of the nervous system such as cerebral ischemia and the degenerative Alzheimer's disease (AD), Parkinson's disease (PD), multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) as well as injuries of peripheral nerves or the spinal cord (SCI) have been subject to intense research in the past decades. The severity of these neurologic diseases for the patients and their families but also the life-long costs that, e.g. a young SCI patient causes, leads to a high degree of public interest in the finding of an appropriate treatment. Despite these efforts and due to the complexity of the nervous system, most neurologic diseases remain incurable. However, the increasing knowledge in the biology of ESCs and adult stem cells, especially their transdifferentiability, multi-potency and regenerative potential, shifted the focus of research from medicinal treatment to a possible therapeutic use of stem cells. The findings of adult neural stem cells (NSC) in the central nervous system (CNS) of human beings and other mammals (36), and their association with repairing neural tissue, e.g. in multiple sclerosis (37), respectively their absence in degenerative brain diseases (38) were consistent with the idea of a therapeutic stem cell use. The almost insurmountable obstacle of obtaining autologous NSCs from a living donor in sufficient numbers can be evaded by the use of either heterologous ESCs or the ethically less disputed and autologous

multi-potent adult progenitor cells (MAPC) which are derived from bone marrow (39,40). Under certain culturing conditions, all three cell lines have shown to be able to develop a neural cell phenotype, express neural specific antigens and show the same electrophysiological behavior as a neural cell in a patch-clamp assay (40-42). Moreover, the different cell lines have also proven their *in vivo* differentiability. After bone marrow transplantation of labelled bone marrow derived hematopoietic stem cells into sublethally irradiated adult mice with genetically defective stem cells, the transplanted cells were distributed throughout the brain as parts of the macroglia and microglia (43). Furthermore, when injected into the lateral ventricles of healthy, neonatal mice, they were able to migrate through the brain and differentiate into astrocytes (44).

There are several animal models focusing on the spinal cord of the rat as a well suited model for the investigation of stem cell behavior in the regeneration of neural tissue. The spinal cord is either surgically transected (45), injured by compression and contusion (46-48) or demyelinated (49-52). The contusion model resembles the typical and most common injury type and clinical setting of SCI in humans the strongest whereas the transection model is best suited to assess the quality of bridging devices (53). In these animal models, ESCs as well as adult NSCs obtained from either adult or fetal spinal cord, showed survival, differentiation and functional improvement of the spinal cord after local application at the site of injury (54-56). Sasaki *et al.* reported in 2001 that bone marrow-derived stem cells were capable of repairing demyelinated axons of the spinal cord of an adult rat (57).

In order to assess the therapeutic potential of stem cells in neurodegenerative diseases, some of which are mentioned above, more specific animal models are needed. In comparison to stroke, where several types of neurons and other cerebral tissues such as vasculature are destroyed, the neurodegenerative Parkinson Disease (PD) has a very typical pattern of neuron loss. Therefore, an animal model corresponding to the specific neural degeneration is easier to develop. The neurotoxic substances 6-hydroxydopamine (6-OHDA), when injected into the median forebrain bundle (58,59), and N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), when applied systemically (60,61), are capable of inducing a PD-like pathology in adults of different species by selectively degenerating dopaminergic neurons (70). Due to the fact that MPTP application in adult, non-human primates results in symptoms and a histopathology closely resembling the ones seen in human idiopathic PD, this model is considered to be the gold-standard for the evaluation of therapeutic benefits before a clinical trial. However, for the assessment of stem cell transplantations and their possible regenerative assistance, the use of rodents is much cheaper and they react with similar symptoms to the

neurotoxins (62). To measure the efficiency of their treatment, most researchers evaluate the "rotarod" test. After destroying the dopaminergic neurons only unilaterally, the number of rotations of the animal performed ipsilateral to its lesioned side in response to a single amphetamine application is counted. A decreasing number of rotations compared to the initial value before transplantation stands for a better function of the substantia nigra (63-65).

Several stem cell lines have previously been shown to be capable of differentiating into dopaminergic neurons *in vitro*. Among these cell lines are ESCs, NSCs derived from fetal and adult brain and mesenchymal stem cells (MSCs) derived from bone marrow (66-69). Using the 6-OHDA-lesion model in rats, Bjorklund *et al.* showed in 2001 that murine blastocyst-derived ESCs are capable of differentiating into a "dopaminergic-like" neural phenotype, expressing the marker proteins neuronal nuclei (*NeuN*), tyrosine hydroxylase (*TH*), dopamine transporter (*DAT*) and aromatic amino acid decarboxylase (*AACD*). Also, the group that received treatment showed improvements in the rotarod test, seven weeks after treatment, indicating a functional effect of the newly formed neurons (63). Similar results were obtained in the same year by Li *et al.* using bone marrow derived cells in a mouse MPTP model. Four years later, in 2005, Takagi *et al.* were the first to prove functionality of dopaminergic neuron-induced embryonic stem cell transplants in a non-human primate MPTP-lesion model (64).

To date it seems as though ESCs are the most potent progenitors for neural regeneration. But, they carry the burden of high risks and inconveniences: The frequently formed teratoma-like tumors, the undisputed ethical concerns and the dependency on immunosuppression after the heterologous transplantation. Bone marrow derived progenitors have proven to be almost equally functional despite the fact that they are harder to differentiate *in vitro*. To our knowledge there have been no reports on tumorigenic processes caused by bone marrow derived MAPCs. The transplantation of *ex vivo* induced MAPCs is autologous and thus does not rely on immunosuppression.

Moral issues concerning animal models in tissue engineering

Can animal interests sometimes outweigh the human interest of carrying out animal experiments? Which human interests are vital enough to outweigh animal suffering? What are the social consequences of doing versus not doing animal experiments? Before tissue engineers start conducting an animal experiment they need to pose these and similar

questions. The act of asking such questions might be even more important than having an answer ready, since the validity of one own answer can ultimately not be proven. The choice of performing animal experiments leads to a commitment to animals. This commitment implies that animals have interests and that their interests count. If animals count morally it implies that they have rights and in this philosophical debate one of the most engaged defenders of animals rights is Tom Regan. On the other hand a critic of this idea is for example Carl Cohen (71, 72). He argues that animals can not have rights, because rights can only be attributed to members of a community. Only humans are regarded as members of such a community, since being a member of a community implies moral obligations and corresponding rights. Animals in his point of view have no obligations and therefore they have no rights. However, Cohen extends humans moral obligations to animals as they are sentient creatures.

If animal rights are not regarded as absolute as human rights are one might be able to solve the moral dilemma of performing animal experiments. In such case human rights outweigh animal rights. In cases where human benefits are moderate, while animal suffering is severe animal interests may take priority over human interests. This might lead to a practical approach for researchers in tissue engineering, where the goal of alleviating human suffering is clear most of the time: there has to be a balancing approach to every experiment. For this approach one has to take into consideration not only whether an experiment should be carried out but also how it should be carried out. Psychologically, it is probably more realistic and fair to researchers, patients benefiting from tissue engineering and even animals to accept that humans have a stronger moral obligation to humans than to animals. One part of this practical approach is to use the lowest species possible to conduct the experiment.

What does the public think about animal experiments? Studies have been carried out and found in the public opinion approval and disapproval of animal experiments based on the context. People that were asked merely whether they are for or against animal experiments were strongly opposed to it. However, when asked whether they would approve to animal experiments if the would help to alleviate life threatening diseases the majority approved to animal experiments. Furthermore, experiments on monkeys were viewed more negatively than on mice except if pediatric life threatening diseases could be treated with the knowledge gathered out of the experiments. These information can be very helpful for tissue engineering researchers. Their research must be explained in a way that layperson can understand it. They have to provide the necessary information to the public that they can put the use of animals in tissue engineering experiments into perspective. Today, where tissue engineers rarely inform

the public and hold meetings where even many expert researchers can not comprehend the implication of the data that is presented we need to make a transition to a general view among researchers that clear-cut goals and easily understandable presentation of information becomes an important aim. Open dialogue with the public and a balancing approach to animal experiments will help to answer some of the moral questions that every researcher involved in animal research should to pose.

Conclusions

Stem cell and gene therapy approaches hold much hope for the development of new tools to treat many life-threatening diseases. The linking of stem cell therapy also with selective gene therapy enhances therapeutic options for the regeneration or replacement of diseased or missing cells. Tissue specific gene expression in the context of differentiation of stem cell lines can be used in the context of stem cell recruitment and differentiation. Potential clinical applications for this new therapeutic strategy include also tissue regeneration to induce or promote tissue remodeling and wound healing.

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