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Three Dimensional Tissue Models for Research in Oncology

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1. Introduction

Cancer is a leading cause of death world-wide with 7.6 million deaths in 2008 which is estimated to rise up to 11 million in 2030. The main cancer types include lung, stomach, liver, colorectal and breast cancer (World Health Organization [WHO], 2011). One of three persons in developed countries dies of cancer, and the expected survival time of newly diagnosed cancer patients (e.g. pancreatic carcinoma) is often less than five years (Kamb, 2005).

In the last three decades great advances in understanding the molecular bases of cancer biology have been made. But up to now cancer therapy is still often empirical, based on inhibiting DNA synthesis and cellular division with a high rate of clinical failure. There is an unprecedented number of new substances in clinical trials. However, the number of highly efficacious drugs approved by the regulatory authorities remains low and there is still a desperate medical need for new drugs which are successful for long-term survival of patients. Costs for bringing a drug to market are estimated to be over US$ 1 billion (Figure 1). This makes the low success rate for oncology products of about 10 percent even more disappointing (Hait, 2010). Patients, the pharmaceutical and biotechnology community invest enormous efforts to validate new therapeutics only to watch them fail in humans due to the lack of suitable model systems. Today commonly used cancer models include native human tumor cell lines (e.g. HCT116 colon) or engineered cell lines (FLT3.dependent BaF/3 cells). Cancer cell lines provide a certain degree of standardization and are easy to handle, but the establishment of cell lines is difficult and often they lack key features of the tumor they should model. Improvements for the generation of new cancer cell lines are recently made by the introduction of three-dimensional (3D) culture systems, the selection of tumor initiating cells and the use of specialized media. To create a more complex tumor environment cell lines can be grown subcutaneously (e.g. PC-3 prostate) or orthotopic (e.g. PC-3 prostate, implanted in prostate) in immuno-compromised mice (Kamb, 2005; Caponigro & Sellers, 2011). In immuno-compromised mice human cancer cell lines produce tumor-like tissues that show a slight resemblance to clinical tumors concerning histological architecture and they fail to recapitulate key aspects of human tumorigenicity such as invasion and metastasis. A more complex tumor tissue is formed after implantation of whole fragments of human

tumors into a mouse. Also mouse tumors can be implanted syngeneically (e.g. B16 melanoma), induced (radiation induced skin tumors) or genetically engineered (e.g. RIP-Tag mouse pancreatic islet) (Kamb, 2005). An advantage of mouse models is that mechanistic studies can be done in genetically engineered mouse models. As a novel strategy in the development of engineered mouse models, chimeric animals are created that arise from blastocysts which are injected with engineered stem cells that harbour tissue specific inducible oncogenes. These chimeric animals develop tumors in the context of normal tissues and could reflect clinical observations accurately such as e.g. distinct tumor regression upon anti-EGFR treatment dependent on the mutated oncogene (Zhou et al., 2010). Improvements of animal models have been made by using mice with humanized haematopoietic and immune systems or by using genetic mouse models that include tumor progression and metastasis. But the success of these models remains to be determined (Hait, 2010). In 2001 a retrospective study of drug candidates showed only a weak correlation between the responses of xenografted tumors to these drugs and the clinical outcome (Weinberg, 2006). To counteract this problem preclinical strategies and tumor models have to be developed with a robust predictive value for the clinical outcome of anti-cancer agents.

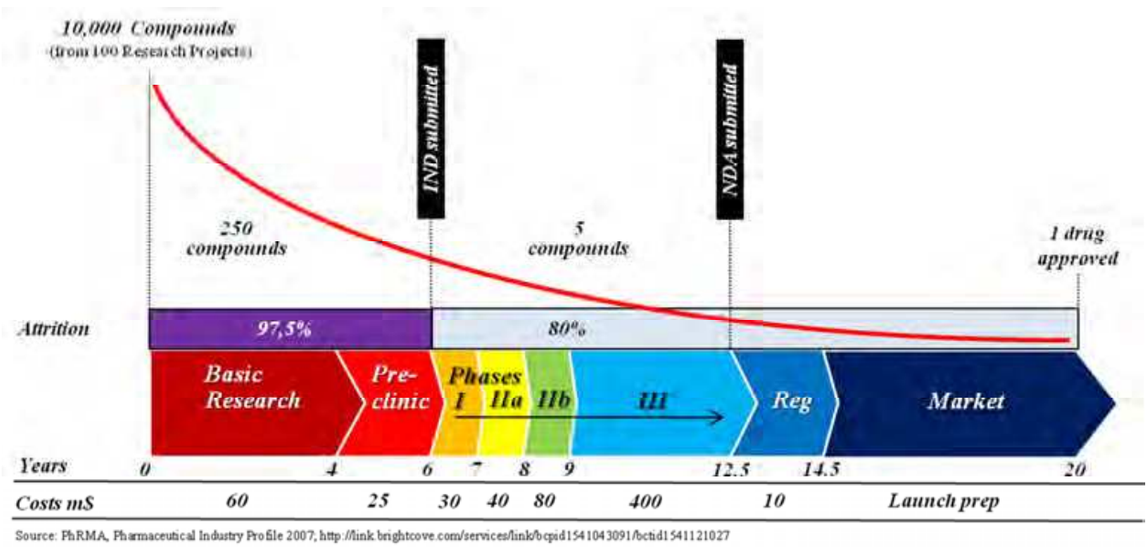

Fig. 1. Time Schedule of the Pharma Drug Development Process. There is an urgent need for novel drugs and strategies to fight cancer. However, the development of a single new anti-cancer agent is very costly (up to 1 billion US$). Moreover, only very few compounds are successful: During long lasting testing of many compounds more and more substances become selected as being ineffective until in the end one compound out of 10.000 becomes one single approved drug. Reg: Regulation.

To understand the demands tumor models have to fulfil in the first part of this chapter, we are going to give (i) a survey of the historical development of preclinical models, (ii) explain different classes of mechanisms of anti-cancer drugs including some examples and problems of cytotoxicity, and (iii) summarize the limitations that face preclinical models right now.

1.1 History of preclinical tumor models

The first cancer models were established over 60 years ago and were derived from carcinogen-induced mouse tumors, primarily sarcomas and lymphomas (Chabner & Roberts, 2005). Observed effects of mustard gas on lymphoid and myeloid cell lineages in soldiers were reinvestigated in a transplantable lymphoma model by Gilman and Philips in 1946 (Gilman & Philips, 1946; as cited in Caponigro & Sellers 2011). This contributed to the introduction of *in vitro* and *in vivo* models for first successful clinical trials with anti-neoplastic agents. In the following 30 years these transplantable models were used to determine the potential efficacy and toxicity of drugs. In 1957 Heidelberger introduced 5-fluoruracil, an inhibitor of the pyrimidine biosynthesis pathway, still a key component in many cancer treatment regimens today, based on the observation that rat hepatomas show an extremly high level of uracil uptake (Heidelberger et al., 1957; as cited in Caponigro & Sellers, 2011). Many chemotherapeutic agents used today, such as anti-mitotic vinca alkaloids, actinomycin D and platinum salts were discovered or developed during one of the first large-scale programmes of the Cancer Chemotherapy National Service Center at the US National Cancer Institute. During this program which started in 1955, mouse lymphoma models were investigated (Chabner & Roberts, 2005). In the late 1980s, a new concept for the application of preclinical models to validate novel therapeutics called NCI60 (National Cancer Institute, 60 human tumor cell lines) also included solid tumors in large-scale screening platforms. This effort required the use of new technologies, such as performing cell culture in microtiter well plates, the development of high-throughput cytotoxicity assays, and the establishment of a bioinformatic infrastructure to analyze the large data sets being generated (Caponigro & Sellers, 2011). These models provided important insights in different mechanisms of cancer cell growth and its inhibition. Recently the role of NCI60 changed more to a service screen for cancer scientists (Shoemaker, 2006). Even though up to now also many tumor cell lines were created from solid tumors, leukemia-lymphoma cell lines remain a key tumor model system reflecting the original tumor with high precision due to its detailed oncogenomic documentation. But here also some hematopoietic entities are underrepresented and resist the establishment of cancer cell lines (Drexler & Macleod, 2010). New technologies from tissue engineering should also help to generate models with immortalized primary cells that maintain differentiation.

1.2 Mechanisms of anti-cancer drugs

To optimize the application of preclinical tumor models it is important to understand the different ways and mechanisms in which anti-cancer drugs act. These mechanisms can be divided into three classes: The first class of agents improves personal therapeutic accuracy by targeting genetic dependencies, such as mutations in oncogenes responsible for cancer progression. Recent successful examples include imatinib (Gleevec/Glivec; Novartis), which targets the activated BCR-ABL gene fusion product in chronic myeloid leukaemia (CML) or in gastro-intestinal stromal tumors (GIST) (Kamb, 2005). It is also under investigation for application in other cancers as NSCLC. Other biomarkers for patients with NSCLC were determined recently and include an activating mutation in the Epidermal-Growth-Factor-Receptor (EGFR) gene which is recommended to be tested before a treatment with therapeutics such as gefitinib or erlotinib is started (Cadranel et al., 2011). Colon cancer patients with a mutation in the k-ras gene are excluded from antibody therapy against the

EGFR because the targeted pathway is constitutively activated in these patients without any influence of the receptor on this activation. Further success in targeted therapy is shown by a high clinical response rate of malignant melanoma patients (phase I) to inhibitors of B-RAF kinase activity (Bollag et al., 2010). Also the treatment with trastuzumab in metastatic breast cancer patients with amplified/overexpressed epidermal growth factor receptor 2 (HER2 or ERBB2) showed some success (Vogel et al., 2002). Furthermore mutated downstream components of the ras-dependend mitogen-activated protein (MAP) kinase signalling pathway, a major regulator of cell survival and proliferation, are attractive targets for therapeutic intervention (Fremin & Meloche, 2010). The development of imatinib was driven on by mechanistically experimenting with different engineered cancer cell lines and was confirmed in xenografted mouse experiments deducing treatment success from the inhibition of Abl-protein tyrosine kinase and PDGF receptor activation (Buchdunger et al., 1996). This indicates that also mechanistically designed experiments using cancer cell lines and animal tumor models can lead to clinical success.

Drugs of the second class of therapeutics target host-tumor interactions, such as hormones and secreted factors upon which the tumors depend. They can be developed on the basis of hormonal suppression in non-tumor bearing animals. Examples for efficacious therapies are aromatase inhibitors for the treatment of oestrogen receptor-positive breast cancer (Baum et al., 2002; Caponigro & Sellers, 2011). And recently, abiraterone, an inhibitor of CYP17A, revealed some progress in the treatment of castration–resistant prostate cancer by complete blocking of androgen synthesis. Prostate cancer depends to 80 percent on androgen the biosynthesis of which is catalyzed in the last step by the P450 enzyme CYP17A both in testes and in adrenals (Hartmann et al., 2002; Reid et al., 2010). Understanding the molecular epidemiology of human tumors should help to promote the development of agents against such host factors.

Agents of the third class of drugs often affect fundamental cellular processes on which cancer cells rely more strongly than normal cells. The target population of these therapeutic agents is often determined by using the trial-and-error method, a fact that leads to high failure rates (Caponigro & Sellers, 2011). Among these drugs of the third class there are common cytotoxic chemotherapeuticals such as nucleoside analogues, DNA-modifying chemicals and natural products with a narrow therapeutic window that were initially introduced in the 1940s (Kamb, 2005). As a therapeutic guideline, safe starting doses are estimated from animal toxicology studies escalating up to the maximum tolerated dose (MTD), which is defined as the highest dose which can be tolerated without any unmanageable side effects. In xenograft animal models the efficacy of each drug is estimated from the ratio of tumor volumes in treated animals and control animals. The toxicity is estimated from the loss of weight of the animals which should be no more than 10 percent during the course of a treatment over two weeks (Kamb, 2005). These standard animal experiments correspond only approximately to human responses to new substances (Hartung, 2009). Limitations of animal models reside mainly in the differences between animals and humans concerning body weight, life span, metabolism and drug uptake mechanisms. Results from toxicology studies of different animal species show only a concordance in the case of 53-60 percent of all tested chemicals (Schardein et al., 1985). The toxic effect in humans can be predicted correctly only for 43-63 percent of all the tested pharmaceuticals (Olson et al., 2000). In clinical trials made by the pharmaceutical industry, 20 percent of the drug candidates showed toxic effects in humans whereas these

effects were not detected in animal models (false-negative results). The number of false-positive results concerning chemicals which are not toxic in humans but could show to be toxic in animals is estimated from reproductive toxicity testing in 63 percent of all investigated drugs (Hartung, 2009). Data from phase III trials suggest that preclinical models overestimate the efficacy of candidate molecules, especially of cytotoxic agents (Caponigro & Sellers, 2011). Starting in 1990, over 10.000 compounds per year have been screened in model systems derived from eight different solid tumors. Unique patterns of cytotoxicity should help to unravel their mechanism of action (Caponigro & Sellers, 2011). Typical cytotoxic mechanisms are operated via the inhibition of microtubules and topoisomerases. Novel therapeutic mechanisms, such as the inhibition of the proteasome by bortezomib (Velcade; Millennium Pharmaceuticals) were recently introduced concerning the treatment of multiple myeloma (Adams, 2002). But attention should also be paid to inter-individual variations in drug metabolism. For instance, irinotecan is a standard drug in treatment of advanced colorectal cancer but 10 percent of the caucasian population show low enzyme activity of an important catabolic enzyme and therefore suffer from greater side effects based on the toxicity of the drug. Here also individual biomarker tests could reduce the problem of superfluous side effects caused by toxicity, because patients with this specific genotype should be treated in a different way than people with another genotype (Schilsky, 2010). However, with regard to toxicity, it has to be taken into account that cancer is mainly a disease of older persons. This augments the problem of drug-toxicity because the detoxification becomes more difficult in elderly people.

1.3 Limitations of preclinical tumor models

There is a number of typical limitations of preclinical models: Certainly, the number of models available does not reflect the number of distinct tumors. Moreover, the molecular characterization of models is often inadequate and hence the alignment to human cancers is impossible. The observed effects such as tumor growth rate reduction do not necessarily correlate with the tumor regression observed in the patient. Moreover, *in vitro* models undergo various selection pressures and biases such as the dependence on oncogenic key pathways. The preclinical models do not reflect stromal-tumor interactions. As an exception, human cells transplanted in mice exhibit stromal interactions and to a certain content angiogenesis. However, these observations still suffer from cross-species differences. Furthermore, murine models often actively reject human tumors and hence it is difficult to study tumor initiating cells in the murine organism. In contrast, the anti-tumor immunity is low to absent in immuno-compromised animals. Finally, the costly and time-consuming *in vivo* testing limits high-throughput screening of substances (Caponigro & Sellers, 2011). Tissue engineering could offer new strategies for overcoming the mentioned limitations of conventional tumor models and could even improve cost effectiveness and accuracy.

2. Overview about tissue engineering

Tissue engineering is an interdisciplinary field that applies the principles of engineering and the life sciences to the development of biological substitutes that restore, maintain, or improve tissue function (Lavik & Langer, 2004). Potentially, it can create replacement structures from biodegradable scaffolds and autologous cells for reconstructive surgery (Stock & Vacanti, 2001). According to the U.S. Department of Health and Human Services,

meanwhile 110.586 people in the USA are waiting for an organ, and due to the shortage of available donor material, an average of 20 potential candidates for organ transplantation die each day in the USA. This could be prevented with an artificial alternative for donor tissue suitable for transplantation and reconstructive surgery.

The basic conception of tissue engineering is the cultivation of cells on biocompatible scaffolds or matrices, so that they can organize and develop a tissue structure. These scaffolds can be composed of synthetic materials like hydroxylapatite (HA) and polyurethane (PU) or of biological materials like collagen or decellularized organs. The advantage of synthetic materials is the easier availability, but they are not suitable to generate all kinds of tissues. Solid materials like HA are suited very well for the culture of e.g. osseous tissue for oral and casualty surgery. Human osteoblasts build 3D structures on HA-matrices and are able to calcify without special growth factors [zellwerk.biz]. PU-matrices have the advantage of biodegradability which means that the matrix disintegrates in the body gradually and only the newly grown tissue remains. Moreover, PU scaffolds can be formed easily before implantation, so that body parts like e.g. ear cups can be reconstructed by using computer tomography (CT) data and can then be implanted after having been reseeded with cells. By using collagen/fibronectin gel skin equivalents can be cultured which resemble naturally human skin with its two-layered composition very closely (Fraunhofer IGB, Stuttgart, Germany). In this model (Figure 2), the dermis is built up by using primary dermal fibroblasts deriving from skin biopsies which are embedded into a collagen gel. This dermis is used as a basis for the epidermal ceratinocytes which are seeded into a fibronectin gel and build up the epidermis. This patent-registered skin model (Patent-Nr. EP 1 290 145B1) is accredited for the control of biocompatibility of medical products (DIN ISO 10993-5). But for dermal reconstructive surgery (e.g. in the case of burns), a graft consisting of decellularized bovine skin is used. This kind of matrix consists of collagen which is virginally structured and is therefore better suitable for being used as a tissue replacement. During the healing procedure, the body's own fibroblasts produce a collagen matrix whereas the transplanted matrix becomes resorbated (Kolokythas et al., 2008).

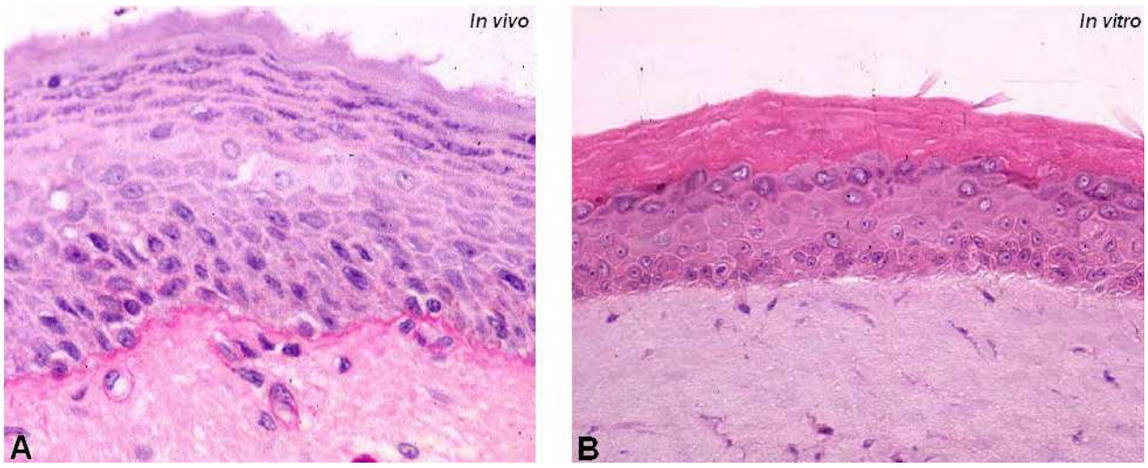

Fig. 2. Cross section of native skin (A) and skin equivalent (B), hematoxylin and eosin (HE) stain. Both samples show a two-layered construction with epidermis (upper part) and dermis (bottom). The basal lamina is seen as a thin red line between epidermis and dermis in the natural skin (A).

Due to the possibility of reseeding the matrices with autologous cells, immune responses are reduced or even prevented (Stock & Vacanti, 2001). One example is found among others in the area of cardiovascular tissue engineering: The fibrin gel for the production of heart valve scaffolds derives from the patient's blood. Autologous myofibroblasts are injected into this gel and after growing the tissue becomes lined with autologous endothelial cells (Jockenhoevel et al., 2001). Therefore no heterologous cells are needed. Another advantage of the *in vitro* grown tissue is the ability to expand in the body of the patient. This is very important in the case of paediatric surgery because implants which are able to grow do not need to be replaced and therefore no additional operations are needed (Jockenhoevel et al., 2001). For this reason tissue engineering is a very important technology in the field of regenerative medicine. Decellularized matrices are also often used for the replacement of the viscera like kidney, liver and lung due to their elasticity. Homografts deriving from a different organism (e.g. pig) and allografts deriving from an individual of the same species (human) are used for generating these matrices.

In vitro applied bioartificial human tissue models allow the generation of functional 3D cell-systems mimicking the microenvironment of potential human target tissues which eventually reflect the *in vivo* behaviour of tested cells more precisely than currently applied cell-based test systems. Their application in drug research may help to make drug development safer and more efficient and reduce research and development expenses. Additionally, they represent promising models for individualized oncologic therapy by revealing new insights into mechanisms of organogenesis and expression of malignancy (Walles et al., 2007).

3. Generation of vascularized *in vitro* tissues

Our tissue model technology is based on decellularized porcine small bowl segments and preserved tubular structures of the capillary network within the collagen matrix which is functionally associated with one small vein and artery (biological vascularised scaffold [BioVaSc]) (Mertsching et al., 2005). To obtain this biological scaffold BioVaSc (Figure 3) with a preserved feeding artery, a draining vein, and a functional capillary network for graft supply, we developed a special harvesting procedure in the pigs. Our decellularization procedure left behind a dense layer of cross linked collagen and elastin fibers evolved from the stratum compactum, the small intestinal submucosa (SIS), the tunica serosa, and the tunica muscularis externa with its arterial and venous network. HE staining was used to survey the cellular state of each processed matrix (Mertsching et al., 2005). Our decellularization process resulted in remaining acellular tubular network of 20 to 200 µm diameter (Fig. 4 A, B) connected to the venous and arterial pedicle, respectively, with a basal membrane and elastica interna. Semi quantitative DNA detection was applied as molecular marker for scaffold decellularization. Its results confirmed our histological findings. Our scaffold thickness was 0.2±0.01 mm. First clinical experience shows, that the BioVaSc is well tolerated and supports an extensive tissue maturation process following implantation (Mertsching et al., 2009). The functional reseeding of the remaining tubular structures of the vascular network within our biological scaffold with endothelial cells was the main objective of this study. Microvascular endothelial cells (mECs) were identified as the ideal endothelial cell (EC) type in respect to genetic and functional stability during isolation, proliferation and seeding in the vascular structures. To control the differentiation of the endothelial cells, we evaluated the endothelial specific markers CD31, VE-Cadherin and Flk-1 by immunohistochemistry and western blot analysis. Anyway the mECs generate an active antithrombotic surface that facilitates transit of plasma and cellular

constituents (Mertsching et al., 2009). For proper function of the endothelium, integrity is required, i.e. mainly ensured by endothelial cell-to-cell junctions. A functional vascular endothelial lining is of paramount importance to avoid graft thrombosis and failure.

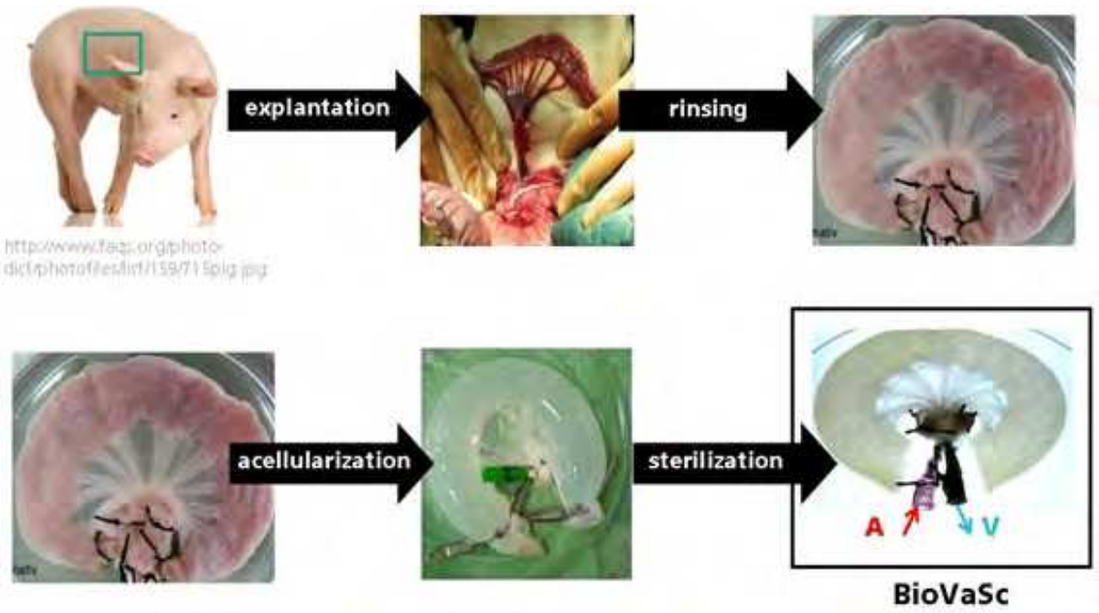

Fig. 3. Standardized method to generate a biological vascularised scaffold (BioVaSc) for the engineering of complex vascularised tissues. A part of the porcine jejunum is isolated including the feeding artery and vein and the connecting capillary bed. After intensive rinsing the cells are removed chemically. The acelluarisation process is finished after a γ-radiation to sterilize the BioVaSc.

Three weeks after endovascular seeding and permanent perfusion, the luminal surface of the supplying artery and vein is lined with a cellular monolayer staining positive for CD31, which mediates adhesion between cells that express CD31. Its expression is limited to endothelial cells, platelets, leukocytes, and their precursors and it has been shown to be highly specific and sensitive for vascular endothelial cells. Within the scaffold matrix, CD31 positive structures are unequally distributed. No positive reaction was detected in acellular controls, excluding non-specific antibody-reactions with the matrix and remaining porcine endothelial cells in the scaffold (Mertsching et al., 2009). Analogical findings were obtained for VE-Cadherin and Flk-1. VE-Cadherin is an endothelial-specific trans-membrane protein that promotes hemophilic cell adhesion mechanically connecting endothelial cells providing the structural base for interendothelial mechanical stability. It plays a morphogenic role in vascular development by participating in contact inhibition of VEGF signalling and its expression is required for the normal organization of the vasculature in the embryo (Carmeliet et al., 1999). Flk-1 is a high-affinity VEGF-2 receptor and is found on differentiated endothelial progenitor cells. It produces a positive endothelial proliferation signal during developmental blood vessel formation. Western blot analysis confirmed the immunohistochemical findings. The simultaneous expression of three specific endothelial markers on our reseeded biological scaffold indicates the successful EC seeding of the vascular structures. Vitality of vascularized scaffold was analyzed by 2`-[18F]-fluoro-2`-desoxy-glucose (FDG) positron emission tomography (PET) using a dedicated fullring scanner facilitating general graft survey with no

need for tissue dissection. As viable cells take up FDG, localized vitality can be in principle visualized by PET. To increase the resolution of detected activity distribution, additional thin layer scanning was performed. PET images showed 3D activity accumulation within the proximity of the reseeded arterial pedicle. Only radioactive background was seen in acellular controls. PET imaging allows quantification of the PET signal. Maximum uptake ratios for the reseeded biological matrix (perfused with buffer-medium) were 11±0.7 vs. 2±0.9 for the non-seeded matrix. In addition to this, PET scanning revealed the functional integrity of the endothelial vascular lining, thereby supporting our immunohistochemical morphological findings. Cellular FDG uptake can be stimulated by insulin exposure and we applied this principle as an additional test for functional cellular vitality. Insulin stimulation amplified the FDG PET uptake in reseeded matrices by a factor of 4.2±1.1. No increase was detectable in acellular controls. These findings amplify our previous findings that the reseeded matrices are populated with viable EC (Mertsching et al., 2005).

The approaches presented here enable the generation of a functional artificial vascular network in a porcine scaffold (Fig. 4 A-D). The generated biological vascularized scaffold may serve as a universal scaffold for tissue engineering.

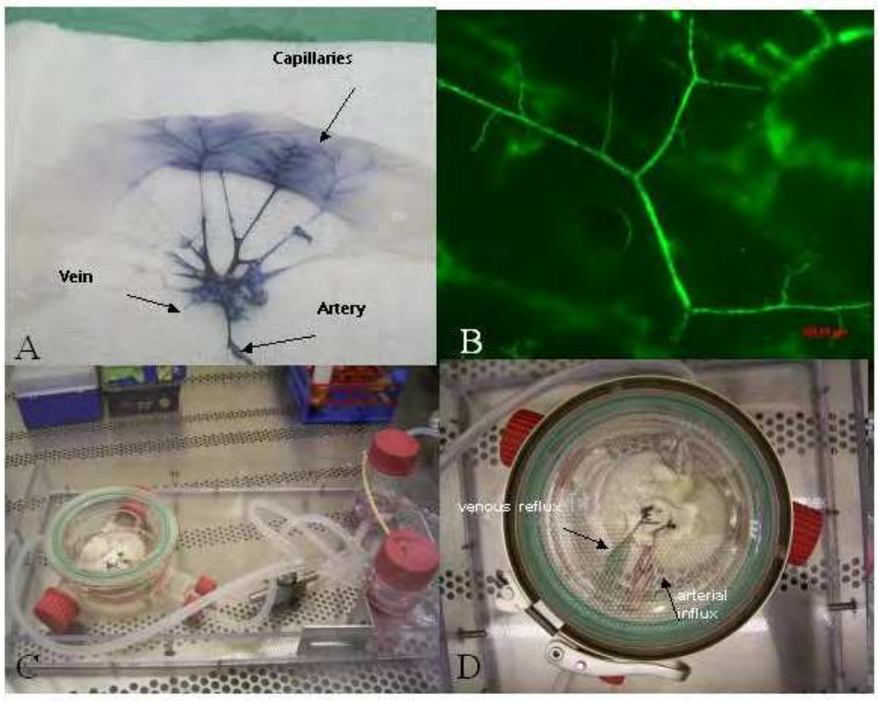

Fig. 4. A) The vascularized scaffold (BioVaSc) with blue stained capillary network. B) Seeded capillaries of the matrix with fluorescence marked liver endothelial cells. C) A bioartificial human tissue model in the specific developed bioreactor enclosed in the closed perfusion stet-up. D) 3D human vascularised tumor test system with arterial and venous access.

Pharmaceutical research is hampered by limited predictive value of routinely applied *in vitro* and *in vivo* drug screening models for clinical efficacy. In drug development, the common approach of pharmaceutical industry is to screen small-molecule libraries for function and toxicity in biochemical based or ligand binding high-throughput assays (Sundberg, 2000). In general enzymes and two-dimensional (2D) cell lines are used in those cell-based assays. The

obtained results are of limited biological relevance, since the 2D cell systems do not adequately mimic the 3D environment in healthy and tumour tissues (Sundberg, 2000).

4. Engineered tumor tissues for applied research

Our BioVaSc can additionally be populated with tumor cells to create *ex vivo* vascularized tumor-like structures. The system offers the option to administer the substances as well as nanomaterials in the arterial inflow and to study the influence of the tumor-like tissue macroscopically as well as on a cellular and molecular level. Human derived tumor cells are co-cultivated with the endothelial cells in such a way that they can form a physiological filtration barrier. The medium is conducted over capillaries seeded with endothelial cells to the tumor cells. Anti-angiogenic therapy molecules as antibodies are now tested in the system. This model offers the possibility to simulate physiological drug application and a human 3D test system to established nanomaterials/systems for cancer research/therapy.

Hackmann and Redelmeier have found out in 2006 that 92 percent of the new drugs fail in clinical studies although the animal experiments were successful (Hackam & Redelmeier, 2006; as cited in Schanz et al., 2010). And it is not least for ethical reasons that suitable alternatives should be found for animal experiments. Already established test tissues at Fraunhofer IGB are the intestine, liver, trachea and fascia models. The cultivation of the cells must take place in special bioreactors in which nutrients can be fed and metabolism products can be removed and which reflect the conditions in the human body in this way (Fig. 4 C, D). These test systems will be transferred into tumor test systems. The heterologous cellular micro-environment of a tumor can be copied by co-culture of tumor cells with stroma cells like fibroblasts. This is important, because the cancerogenesis is influenced by interactions of stroma, epithelium and components of the extracellular matrix (Micke & Ostman, 2004). The main application will be the testing of patient-specific therapies like drugs, chemotherapy, and immunotherapy. In this manner one can test new therapies and drugs outside the patient, but within human (perhaps autologous) tissue before they find use in the cancer therapy.

Why do we need tumor test systems? Up to now there are no suitable human *in vitro* models of cancer available as described in "the biology of cancer" (Weinberg, 2006). But why do we need as many "nature identical" tumor models generated from human cells? The first reason is that new therapies and new drugs are needed to fight cancer by inhibiting tumor growth, angiogenesis as well as tumor metastasis. To test the effects of many different substances on these processes of tumor development, a time-saving and standardized method is used. The widely used animal xenograft animal models show some advantages for being used as a test system e.g. the interaction between tumor and the whole living organism including blood circulation. The immune system can be studied as well as psychophysiological consequences of medication which can be analyzed during behavioural studies of the animals. But on the other hand there are many pros for the *in vitro* tumor models: the generation of such *in vitro* tumors is not as time-consuming as the breeding and fostering of animals used for xenograft transplantation, a circumstance which is very interesting for high-throughput screening of drugs and diverse therapeutical methods from a wide pool of possible cancer treatments. Moreover, *in vitro* tumor models do not contain any unrelated cells from other species which interact with the human cell population and can bias the results. And, last but not least, the *in vitro* tumor models can be standardized better than any living organism can be. Additionally, synthetic human tumors cultured in 3D do not suffer psychologically from treatment and for this reason can be supported from an ethical point of view for first drug screening.

Another important field is the so-called "personalized medicine", a very individual tumor treatment for each patient. As not only each patient but also each tumor can be seen as an individual, it is very important to individualize treatment, too. A big aim for the future is the cultivation of primary cells deriving from the special tumor of the individual patient. These primary tumor cells can be used to build up artificial tumors *in vitro* which can also be medicated *in vitro* and which can be used for analyzing the characteristic features of this special disease e.g. the genetic background of the tumor cells, their interaction with surrounding tissue cells and their responsiveness to various treatments. All these studies can be done in parallel by using an array of these *in vitro* tumor models. By using these models it could be possible to circumvent side effects of inefficient treatments and it could be possible to adjust tumor treatment within a short period of time because of the parallel investigation possibilities. For the investigation of tumor-stroma interactions these *in vitro* tumor models are also very useful. Activated cancer-associated fibroblasts e.g. are involved in tumor progression and are a possible target for anti-cancer therapies (Kalluri & Zeisberg, 2006). Dermal fibroblasts can be isolated quite easily from skin biopsies and can be employed to mimic these interactions between tumor cells and connective tissue in the human body. Also the interaction between endothelial cells and the tumor cells can be investigated in an artificial tumor especially when it is grown and cultured in a bioreactor with an artificial "blood stream". In this dynamic system it is possible to study the angiogenesis of the tumor and to identify signals between tumor cells and endothelial cells as well as to test the ability of drugs to inhibit these interactions. Also signal pathways involved in tumor development can be analyzed in these artificial tumors generated by co-cultivation of tumor cells and stroma cells as well as extracellular matrix components involved in carcinogenesis (Micke & Ostman, 2004).

5. Examples for 3D tissue models for research in oncology

An *in vitro* model of malignant melanoma was created by using of the 3D skin model developed by the Fraunhofer company (EP 01953961.8-2405). Different types of melanoma cells can be used to create skin tumors on or in this dermal equivalent.

Malignant melanomas often differ in invasiveness as well as tumor expansion. There must be various subtypes of melanomas and for this reason it is important to investigate the features of this special tumor which a single patient suffers from. To characterize the pathogenical background of different tumors and to test various medical therapies *in vitro*, this 3D skin model developed by the Fraunhofer IGB (EP 01953961.8-2405) can be used. Additionally, it can be useful in testing pharmaceutical penetration and resorption of different drugs. The test tissue was generated as described in [Walles et al., 2007]: Human primary fibroblasts derived from human foreskin (1×10^4 cells/cm^2) were seeded in a rodent collagen-I gel (isolated from rat tail) resulting in a dermal-like cell-matrix construct. Human ceratinocytes (1×10^5 cells/cm^2) were seeded on the surface of the cell-matrix construct and incubated for 5 days in cell culture medium (submerged cell culture). The "epidermal" ceratinocyte layer of the tissue surface was exposed to the air for another 12-14 days to induce ceratinization (air-lift culture). The resulting tissue was composed of a stable connective tissue rich of cells (= dermis) with a ceratinizing surface.

Now different human melanoma cell lines were inoculated into the dermal layer and showed their characteristic growth behavior in these *in vitro* models (Figure 5).

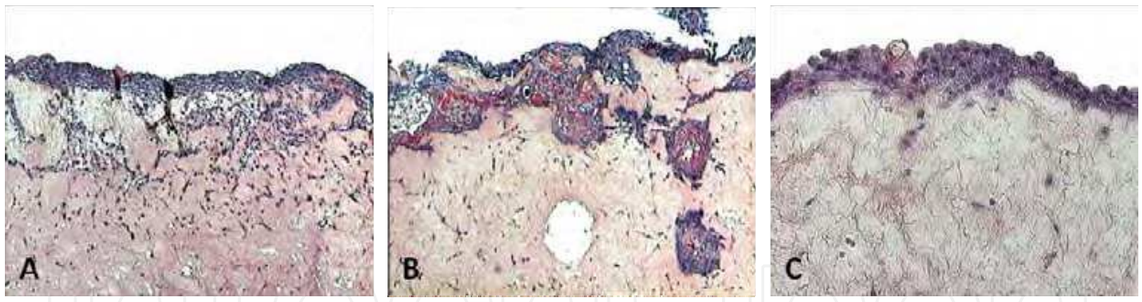

Fig. 5. Bioartificial human *in vitro* skin model for malignant melanoma spread. A) Superficial spreading melanoma (SSM) diffusely infiltrating the skin equivalent. B) Nodular melanoma (NM) forming tumor nests. C) Lentigo maligna melanoma (LMM) spreading superficially without invasion of the skin equivalent. (Adapted from Walles et al., 2007)

Additionally, an interaction between tumor tissue and endothelial cells could be shown when they were co-cultivated in this model (Walles et al., 2007). For this reason, also endothelial cell differentiation as well as angiogenesis can be analyzed with this model as well as the development of medical devices, e.g. a laser assisted diagnostic device for melanoma can be developed using such an *in vitro* model (Mertsching et al., 2008).

Because lung cancer is the most common cancer in terms of both incidence and mortality (Jemal et al., 2011) it is very important to develop new and better therapeutic approaches. An *in vitro* model of lung adenocarcinoma was created by using the BioVaSc reseeded with A549 tumor cells as well as primary dermal fibroblasts in co-culture. A549 cells are a lung cancer cell line derived from adenocarcinomic human alveolar basal epithelial cells. Results showed that the primary fibroblasts influenced the growth of the tumor cells concerning growth behavior and morphology and formed nodular structures in some areas (Wasik, 2011). Immortalized cell lines derived from tumor cells are a good basic material to build up standardized *in vitro* tumors because primary cells derived from tumor biopsies are not as uniform as these cell clones. There are different matrices which can be used as a substrate for the 3D cell culture e.g. inserts of polycarbonate.

Another important field of cancer research is colon cancer because it is the second most common cancer disease in Germany and kills about 600.000 people annually worldwide [globocan.iarc.fr]. An *in vitro* model for colon adenocarcinoma which was also created by using the BioVaSc reseeded with cells of different cancer cell lines derived from colon adenocarcinomas (Caco-2 and SW480) as well as dermal primary fibroblasts and mECs which were co-cultured with the cancer cells. In this model, the SW480 cells show nodular growth in co-culture with primary fibroblasts and exhibit a more malignant morphology than Caco-2 cells. To test the influence of substances on tumor growth and invasiveness, *in vitro* models built up from human colon carcinoma cells are in very dire need because animal xenograft models have some drawbacks especially concerning such studies of the gut: mice or rats have a very different diet compared to man and in consequence of this their gut not only a “miniature edition” but it is different in some ways compared to the human one e.g. the gut flora is very different or food does not stay as long in the gut as in humans. First models are built up by using various colon carcinoma cell lines e.g. the well-established cell lines Caco-2 and SW480. Budding results could be seen concerning the more aggressive SW480 cells which invade the matrix in monoculture and showed nodular structures in co-

culture with fibroblasts (own data, unpublished). But more read outs for tumor features of these *in vitro* "tumors" are needed e.g. proliferation quantification as well as verification of an upcoming epithelial mesenchymal transition (EMT).

6. Conclusion

Conventional preclinical tumor models were proved to be successful in some limited settings when the cell models, the human xenografts or the mouse model include mutations, amplifications or translocations of the original malignancy that was to be treated in the clinic. Also important aspects such as pharmacokinetic behaviour, tissue distribution and percentage of free compounds in plasma can be determined in conventional animal tumor models. However, there is a tendency to overestimate the real clinical impact of any compound measured by reduction of the tumor mass in xenograft models. Nowadays genetically engineered mouse models are ever more sophisticated but still habour additional unknown changes which challenge the translation to the patient (Kamb, 2005). Other drawbacks of conventional models remain, such as the insufficient number of experiments for proper statistics, the time-consuming generation of animal models and the low number of cell lines that do not represent the tumors' heterogeneity (Kamb, 2005).

Cancer is foremost a genetic disease, making the analysis of correlation between compound activity and molecular changes important (Caponigro & Sellers, 2011). Averagely about 80 genes are affected in different common tumor types as breast and colon cancer (Todaro et al., 2010) which makes clear that 60 cancer cell lines can not accurately reflect the diversity of over 100 histologically distinct tumor types. Progress in drug development requires the identification of essential and compensating functions that are specific to cancer cells and their translation in a proper tumor model.

At present many projects try to identify gene signatures in screens of large sets of cell lines that predict sensitivity and resistance to specific targeted drugs. For example, the Novartis Institutes for BioMedical Research in collaboration with other investigators assembled in the "Cell Line Encyclopedia Project" expression profiles, copy-number alterations and dose-response data of over 1000 cell lines (Caponigro & Sellers, 2011). Other promising approaches for targeted drug development are current genome-profiling projects such as the National Cancer Institute's "Cancer Genome Atlas" and the Sanger's Institute's "Cancer Genome Project" (Schilsky, 2010). However, while relating targeted anti-cancer drug efficacy and toxicity it is important to consider physiology. In organisms from *E. coli* to mouse three quarters of the individual genes are non-essential or only essential in a particular genetic or epigenetic background. This explains why tumors often not depend on the target for viability (Kamb, 2005).

Another approach for successful cancer therapy is the testing of different drug combinations in sufficiently large sets of *in vitro* models where many compounds can be tested in one cell line or one combination in many different cell lines. In some cases combination therapy could also overcome acquired drug resistance.

In future it would be a desirable goal to create *in vitro* tumor models by using primary cells derived from the patient's tumor biopsies to get to test various therapeutic approaches on autologous test systems *in vitro* before application. Therefore this personalized medicine would be the best to avoid side effects of ineffective treatments and to save time and

therefore lives because many therapeutic methods can be tested in parallel *in vitro* on their effectiveness in fighting this special tumor before being given to the patient. For successful personalized medicine further biomarkers such as k-ras in anti-EGFR colon cancer therapy have to be identified and diagnostic tests have to be co-developed with new drugs (Hait, 2010). To accelerate the development and approval of targeted cancer therapies, clinical investigators, scientists, drug developers and regulatory experts joined by the Brookings Institution and have recently proposed a novel strategy for drug and biomarker co-development called "targeted approval" (Schilsky, 2010). Tumor models with higher efficiency and accuracy should be generated with cell lines harbouring certain mutations that are targeted by drugs the impact of which is predicted by bioinformatic analysis of genomic and epigenomic screens.

It has been proven that personalized medicine could also improve cost efficiency in prescription practice: Stratification of patient population that should be treated with certain agents such as the use of anti-EGFR drugs only in k-ras wild-type colorectal cancer patients. This could save the US health care system about US$ 700 million annually (Schilsky, 2010). Not only is the development of novel drugs expensive but also the drugs themselves and the following treatment protocols. The National Institute of Health of the United States estimated the overall cost of cancer care in 2007 to US$ 219.2 billion. Cost-utility studies for cancer started initially in the 1990s and extended to 14 percent in 2007, which gives hope to improve cost-effectiveness in cancer in future (Greenberg et al., 2010).

In general new approaches have to be considered to bring more successful anti-cancer agents to market. If a strong genetic and epidemiological indication exists for any drug more caution should be applied before excluding these rashly from the clinic (Kamb, 2005). Toxicity models should balance sensitivity (false-negative rate) with selectivity (false-positive rate) (Kamb, 2005). Treatment-related deaths in Phase I oncology have fallen down to a fraction of a percentage point (Roberts et al., 2004) and therefore regulatory agencies should reconsider the criteria for advancement into the clinic to improve the throughput of drug testing in humans. However, there is a need for global regulation to judge which criteria form the basis of approval of drugs which are active but have no significant side effects (Hait, 2010).

Last but not least there is hope to improve clinical applications also due to impressive and well-coordinated ventures occurring worldwide that could go along with the generation of more complex and accurate preclinical 3D tumor models in the field of tissue engineering to fight cancer.

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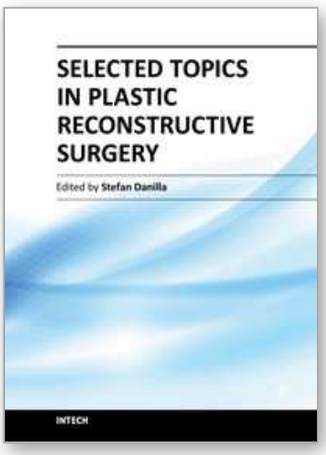

Selected Topics in Plastic Reconstructive Surgery

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Plastic Surgery is a fast evolving surgical specialty. Although best known for cosmetic procedures, plastic surgery also involves reconstructive and aesthetic procedures, which very often overlap, aiming to restore functionality and normal appearance of organs damaged due to trauma, neoplasm, ageing tissue or iatrogenesis. First reconstructive procedures were described more than 3000 years ago by Indian surgeons that reconstructed nasal deformities caused by nose amputation as a form of punishment. Nowadays, many ancient procedures are still used like the Indian forehead flap for nasal reconstruction, but as with all fields of medicine, the advances in technology and research have dramatically affected reconstructive surgery.

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