Design and development of a novel textile composite scaffold bioreactor for application as bioartificial liver support device

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Design and development of a novel textile composite scaffold bioreactor for application as bioartificial liver support device

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Abstract

The objective of this work was to design and develop an extracorporeal liver support bioreactor dedicated to culture hepatocytes under conditions that support differentiated cell function. To address the limitations in mass transport of oxygen of currently studied bioreactors, a new design is proposed to maximize the level of oxygen supply. The design is based on a textile composite scaffold, consisting of a gas permeable teflon membrane for oxygen supply and a combination of several poly (ethylene terephthalate) (PET) woven textiles, which served to achieve structural analogies to the sinusoid, the structural unit of the liver. Several textile composite scaffolds were assembled to a parallel flow bioreactor system.

To verify the feasibility of this concept, theoretical and experimental methods were utilized. Since adequate oxygenation is a crucial issue in maintenance of differentiated hepatocyte function, supply of hepatocytes with oxygen in the proposed scaffold configuration was analyzed by a one-dimensional mathematical model based on Michaelis-Menten kinetics. The results indicated that hepatocyte aggregates in the proposed scaffold configuration can be supplied with at least a minimal oxygen partial pressure of 10 mmHg for differentiated function.

To investigate the influence of the scaffold and flow chamber geometry on blood plasma flow patterns and mass transfer characteristics, a three-dimensional computational model was developed and implemented in a numerical fluid dynamics solver. Comparison of the results from the one-dimensional to the three-dimensional computational model for oxygen partial pressure in a single cell aggregate indicated the suitability of the computational model. Subsequently, the model was scaled up to the size of a bioreactor and utilized to calculate flow patterns, oxygen partial pressure in the fluid and shear stresses on cells in a prototype bioreactor. Flow rates of 120 ml/min were calculated to be sufficient to guarantee a minimal oxygen partial pressure of 25 mmHg in the fluid. Modeled shear stresses on the cells for this flow rate were found to potentially exceed the physiological range of 5–15 dyne/cm². To protect the cells, a fine woven textile is proposed to serve as a protective interlayer between plasma flow and cell aggregates.

Biological testing of composite scaffold and housing materials revealed that utilized materials have no toxic effect on hepatocytes. Optimization of the seeding procedure for the hepatocytes resulted in a homogeneous cell distribution in a prototype bioreactor, which was constructed for biological tests. In performance tests under flow conditions realistic for clinical applications, hepatocytes demonstrated high viability after one week.
but lost relevant metabolic function indicated by albumin synthesis in the first 48 hr after seeding.

The proposed system could be shown by modeling to be a promising approach to address the restriction of currently existing devices concerning mass transfer and supply of oxygen.
ZUSAMMENFASSUNG

Ziel der Arbeit war die Entwicklung eines Bioreaktors, der als extrakorporales System zur Unterstützung der Leberfunktion eingesetzt werden kann, indem für Hepatozyten metabolische Randbedingungen geschaffen werden, welche einen differenzierten leberspezifischen Stoffwechsel ermöglichen. Um Problemen entgegenzutreten, die derzeit in der klinischen Testphase befindliche Lebereaktoren hinsichtlich des Stofftransportes von Sauerstoff haben, wurde ein neues Konzept erarbeitet, welches eine ausreichende Sauerstoffversorgung der Zellen im ganzen Reaktor sicherstellen sollte. Das Konzept basiert auf einem plattenartigen Verbundzellträger, der aus einer gaspermeablen Teflonmembran zur Sauerstoffversorgung sowie textilen Geweben aus poly (ethylene terephthalate) (PET) besteht. Im Bioreaktor sollen mehrere dieser Zellträger parallel angeordnet und mit Blutplasma überflossen werden.

Schutzschicht zwischen den Zellaggregaten und dem Blutplasma angebracht werden sollte.


Das in dieser Arbeit vorgeschlagene System erwies sich in den Untersuchungen mit den hierfür konzipierten Modellen als ein viel versprechender Ansatz, um den Problemen derzeit in der klinischen Prüfung befindlicher Leberreaktoren bezüglich Stofftransport und Sauerstoffversorgung entgegenzutreten.
1. **INTRODUCTION**

1.1. **Aim of this work**

The objective of this work was to design and develop an extracorporeal liver support bioreactor dedicated to culture hepatocytes under conditions that support differentiated cell function. The design should be derived from an analysis of potential improvement criteria of existing artificial liver support devices. It was based on a textile composite scaffold, consisting of a gas permeable teflon membrane for oxygen supply and a combination of several polyethylene terephthalate (PET) woven textiles, which served to achieve structural analogies to the sinusoid, the structural unit of the liver [1]. Several textile composite scaffolds were assembled to a parallel flow bioreactor system, consisting of two channel systems for flow of fluid\(^1\) and gas supply respectively.

To verify the feasibility of this concept, theoretical and experimental methods should be utilized. Since oxygen is known to be a crucial factor for differentiated hepatocyte function, mass transfer of oxygen in the proposed configuration should be investigated by computational modeling in order to assure appropriate supply of cells. A prototype bioreactor consisting of materials which should be assured to be non-toxic to hepatocytes should be constructed. This prototype should be utilized to develop and optimize a cell seeding process resulting in high density hepatocyte cultures. It should furthermore allow preliminary tests of the performance of the proposed system by measuring viability and differentiated function of cells cultured under flow conditions realistic for clinical application of an artificial liver support device.

This introduction identifies criteria for the use of artificial liver systems by analyzing symptoms and therapies in acute liver failure. The state of the art in artificial liver support will be reported and results of the latest clinical studies will be presented. Finally, the reasons for present deficiencies of current systems are analyzed and conclusions for the functional specifications of the proposed system are drawn.

In Chapter 2, a novel design derived from this specifications is proposed and its realization in a prototype bioreactor is described. In Chapter 3, the concept is investigated with regard to its mass transfer characteristic by modeling. Experimental tests of the concept evaluating toxicity of utilized materials, optimization of cell seeding and performance tests of hepatocytes in a prototype reactor setup are ruled out in Chapter 4.

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1. Fluid stands for blood, blood plasma, or cell culture medium. Where necessary in this work, the type of fluid is explicitly mentioned.
1. Introduction

1.2. Acute liver failure

The liver

The liver is one of the largest organs of the body, constituting approximately 1.5% - 2% of body weight. The biological performance of the liver is complex. Many of the specific processes involved with this organ’s multiple functions are not yet fully understood [2].

Receiving two-thirds of its blood from the portal vein of the gastro-intestinal tract, the liver comes in direct contact with nutrient-rich blood after it has coursed through the gut, spleen and pancreas. It is capable of temporarily storing over half of the nutrients that are absorbed by it, thereby balancing elevated levels of carbohydrates, amino acids and fats after a meal. Five to eight percent of the liver’s total weight is stored glycogen, which, like the rest of the liver’s stored nutrients, is systematically released according to biological need. The liver is therefore also essential to the body’s regulation of energy [3].

In the liver, the parenchymal cells, hepatocytes, are arranged in sheets, facing blood filled spaces called sinusoids. The blood is separated from the surface of the hepatocytes by a single layer of flattened endothelial cells with interspersed macrophage-like Kupffer cells that covers the side of the hepatocyte sheet (Figure 1.1). Small fenestrae in the endothelial layer allow exchange of molecules and small particles between the hepatocytes and the bloodstream while protecting the hepatocytes from mechanical damage by direct contact with the blood stream [4]. The metabolites supplied by the blood reach the hepatocytes via diffusion through the space of Disse. This space is formed by extracellular matrix (ECM) and contains further macrophage-like cells, the stellate cells.

At the same time, the hepatocytes remain connected with the lumen of the gut via a system of channels. Hepatocytes surround bile canaliculi (Figure 1.1) which are approximately 1 µm in diameter [3]. They secrete primary bile into the canaliculi, which ultimately discharged into the gut via bile ducts. Junctions between the cells that form these ducts, impermeable tight junctions, retain bile from returning into the blood circulation.

Considerable evidence exists that not all hepatocytes in the liver perform identical functions. Functional differentiation among cells can be determined or influenced by gradients of concentration of oxygen and other substances. The influence of differences in oxygen partial pressure on metabolic function of hepatocytes is supported by several studies with isolated hepatocytes, e.g. [5, 6]. This differentiation of functions among the cells is called metabolic zonation.
The liver’s metabolic functions can be divided into three classes:

- **Detoxification**: The liver transforms potentially dangerous metabolites and toxins into water-soluble compounds. Urea is one such compound, produced from ammonia, which is toxic to the organism in high doses.

- **Synthetic functions**: The liver synthesizes amino acids and proteins, e.g. albumin and factor V. Due to this, the liver plays an important function in regulating proper coagulation function [7], osmotic pressure [8], and enzymatic functions of the blood [9].

- **Biotransformation**: This function of the liver involves metabolic conversion of hydrophobic substances into water-soluble substances and their subsequent storage and controlled release into blood or bile. An example is the conjugation of lipid-soluble bilirubin with glucuronic acid to water-soluble bilirubin in the liver, so that it can be excreted in the bile [10].
Acute liver failure

Acute liver failure (ALF) may develop either in absence of underlying diseases (fulminant hepatic failure; FHF) or as an acute exacerbation of a chronic process (acute on chronic liver failure; AoC). Recent studies demonstrated that acetaminophen overdose is, with 38% of all cases, the most common cause of ALF, followed by idiosyncratic drug reactions (14%) and acute viral infections (4% Hepatitis A virus, 8% Hepatitis B virus). Rare causes included cancer, lymphoma, Wilson’s disease, acute ischemic liver injury, autoimmune hepatitis and Budd-Chiary syndrome (total, 19%) [11].

By definition, the most common clinical feature of ALF is hepatic encephalopathy [12], which is characterized by a status of perturbance of consciousness, delirium and spasms [13] and marked elevations of serum ammonia [12]. Additionally, often complications occur which impair dramatically the life expectancy of patients suffering from ALF. Cerebral edema is an indirect result of hepatic encephalopathy and its exact mechanism is poorly understood. Disruption of the blood-brain barrier with low molecular-weight substances and loss of autoregulation of cerebral blood flow are some of the features of cerebral edema [14-16]. Late clinical features of cerebral edema include hyperventilation, systemic hypertension, paralysis of brain stem reflexes and often lead to death.

There are numerous metabolic derangements seen in ALF cases, which are e.g. metabolic acidosis, acute pancreatitis and renal failure. Furthermore sepsis, cardiovascular, hemodynamic and respiratory complications can cause multiorgan failure, and thus increase significantly the risk of mortality.

Mortality rates of patients with ALF depend on many factors like patient age, number and nature of complications etiology and the availability of a transplant. For FHF, mortality rates range from 10 to 40% with transplantation and 50 to 90% without transplantation [17]. In the latter case, survival tends to be better in cases of viral infections and acetaminophen overdose (40%) than caused by other etiologies (10 - 20% survival) [12]. Patients with AoC develop an acute failure over the time of an underlying disease. They are mostly not eligible for high-urgency liver transplantation. Mortality rates are not explicitly given because they strongly depend on therapy of chronic ill patients before the appearance of the acute failure. However, due to the possible therapies before the development of the exacerbation mortality rates will probably be lower compared to FHF.
**1. Introduction**

*Therapy of acute liver failure*

Although spontaneous recovery from ALF is possible, the only effective therapy for patients with ALF remains a liver transplantation [18]. In Europe and North America, the survival rate 3 years after transplantation is 70 -80 % [19, 20].

However, due to expanding indications for liver transplantations, there is a great shortage of organs. In 2000, approximately 15 000 patients were on the waiting list in the US, while only approximately 2 000 were transplanted. In this period, approximately 850 patients were removed from the list due to their death.

Because of the discrepancy between the need and availability of livers for transplantation, the need for a device which is capable to substitute the patients liver function temporarily is evident. While the availability of such a bridge device will, of course, not affect the mismatch between liver need and availability, it would enhance the effectiveness of use of those donor livers that do exist, in that the patients would be in a healthier pre-transplant state. Moreover, some spontaneous recovery would make transplantation unnecessary.

**1.3. Artificial liver support: state of the art**

Methods used for artificial liver support can be classified as either non-biological or biological. The former is based, as is conventional renal dialysis, on blood detoxification; the latter utilizes viable liver tissue preparations as active components and are is therefore specified as bioartificial.

**1.3.1. Non-biologic liver support**

Since many years, it has been assumed by a group of researchers that hepatic encephalopathy is due to the accumulation of dialyzable molecules [21]. To remove these toxins, in recent years several techniques were applied. Table 1.1. lists those techniques and summarizes the function principles.

The crucial indicator for the success of an liver device is the promotion of the statistic survival rate. In this context, it is important to know that in all reports about clinical studies of artificial liver support devices, it has to be taken into account that treatment of the patients with the device mostly ends with transplantation. Consequently, survival rates not only depend from the efficacy of a liver support device, but also from regional and seasonal availability of donor organs. Therefore, especially the comparability of results of different studies is limited.
Currently two non-biological devices are under clinical investigation:

Stange et al. [22] introduced hemodialysis utilizing a highly permeable membrane impregnated with albumin, which facilitated the rapid transfer of albumin-bound substances such as bilirubin, from the patients plasma to the dialysate solution. To achieve this, the device contains three different fluid compartments, i.e., the blood circuit, the albumin circuit and an open loop dialysate circuit. Currently, a CE-approved multicenter study with this molecular adsorbent recycling system (MARS, Teraklin, Germany) is being executed. Preliminary clinical data reports a 70 % survival rate of patients (n=64) with AoC liver failure, for FHF no data is available. Treatment of patients with the device was terminated as soon as an organ for transplantation was available or native recovery occurred.

Ash et al. [26, 31] recently reported the use of a hemoabsorption system called BioLogic-DT (Hemo Therapies, USA), in which blood is circulated in a parallel plate cellulose membrane dialyzer against a suspension of finely powdered charcoal. In a clinical study, significant improvement was only observed in patients with AoC liver failure (77 % survival, n=71), but no improvement was observed in patients with FHF which were transplanted when a suitable organ was available (56 % survival, n=39) [30, 31].

Summarizing clinical results, latest non-biological device studies described above have not proven as successful an increasing in survival rates for FHF. Studies were mostly performed with patients with AoC. Hemodialysis is efficient at reducing blood ammonia levels, but not in increasing survival [21]. As well, no significant increase in survival has been observed for hemofiltration [24], sorption [32] and plasma exchange [33].
1.3.2. Bioartificial liver support

Many researchers believe, not only due to limited success of various non-biological approaches, that an effective artificial liver should not only remove toxins from the blood. It should also support and/or replace further liver functions which are also impaired or lost in ALF, i.e. biotransformation and synthesis. Therefore viable hepatocytes should be a major component of an artificial liver support system.

In recent years, three main types of bioreactor designs as housings for hepatocytes were proposed and investigated. Figure 1.2 gives an overview over the design principles. Hollow-fiber membrane systems are based on and developed from hemodialysis cartridges, containing numerous hollow-fibers made of a semipermeable material. The ends of these fibers are embedded in a resin so that the cartridge has two compartments, an intracapillary space (ICS) and an extracapillary space (ECS). In a common arrangement, hepatocytes are located in the ECS and blood or plasma is pumped through the ICS. Metabolites, toxins and nutrients reach the cells via diffusion through the membrane.

![Diagram of bioreactor design principles](image)

**Figure 1.2:** Bioreactor design principles: Hollow fiber membrane (a), plate dialyzer (b) and packed bed (c).

The packed-bed bioreactor design essentially allows the direct perfusion of hepatocytes with blood or plasma. Hepatocytes can be either microcarrier-attached, microencapsulated or located in three dimensional scaffold matrices like foams.
Plate dialyzer systems employ hepatocytes attached as monolayers on plate like scaffolds. Blood or plasma flows over those plates and is in direct or indirect contact with hepatocytes. In the latter case, hepatocytes can be embedded in gels or separated from flow by artificial membranes.

Numerous reviews summarize the application of these three principles in several devices, e.g. [30, 34]. The full range of cellular functions required in an artificial liver device has not yet been determined \textit{in vitro}. Therefore \textit{in vivo} studies of these devices reviewed e.g. in [18, 35] characterize markers of classes of liver specific metabolic functions (detoxification, synthetic functions and biotransformation).

At present, a number of clinical trials with bioartificial liver support devices are under way. Table 1.2 gives an overview about those trials and resulting survival rates. It furthermore lists biological and technical details which are important for the analysis of function of these devices in the following section.

Survival rates are strongly influenced by the fact that device-treated patients are transplanted as soon as a suitable organ is found and shipped to the transplantation center. As mentioned above, patients with FHF which are finally transplanted have a survival rate of 60-90%. Compared to that, no statistical significant data could be obtained that demonstrated the benefit of bioartificial liver support devices for FHF patients. Consequently, in spite of some encouraging results, no device has received FDA- or CE-approval up to now [30].
### Table 1.2: Overview about ongoing clinical studies of bioartificial liver support devices. Abbreviations: FHF, fulminant hepatic failure; AoC, acute on chronic liver failure; ELAD, extracorporeal liver assist device; BLSS, bioartificial liver support system; AMC BAL, Academic Medical Center bioartificial liver; MELS, modular extracorporeal liver support.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Bioreactor design principle</th>
<th>Commercial name / company</th>
<th>Cell mass and source</th>
<th>Flow rate</th>
<th>Membrane type (NMWCO) and surface</th>
<th>Ongoing study phase</th>
<th>Number of patients (indication)</th>
<th>Survival rate(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sussman [36]</td>
<td>Hollow fibre cartridge</td>
<td>ELAD / Hepatix</td>
<td>2x200 g C3A cell line</td>
<td>150-300 ml/min blood</td>
<td>70 kDa, 2 m(^2)</td>
<td>-</td>
<td>23 (FHF)</td>
<td>54 %</td>
</tr>
<tr>
<td>Millis, unpublished</td>
<td>Hollow fibre cartridge</td>
<td>ELAD / Vitagen</td>
<td>4x200 g C3A cell line</td>
<td>150-300 ml/min blood</td>
<td>70 kDa, 2 m(^2)</td>
<td>I/II Multicenter</td>
<td>25 (FHF)</td>
<td>92 %</td>
</tr>
<tr>
<td>Demetriou [37]</td>
<td>Hollow fibre cartridge</td>
<td>HepatAssist / Circe Biomedical</td>
<td>5x10(^9) cell (50g) primary porcine</td>
<td>400 ml/min plasma</td>
<td>60 kDa 0.7 m(^2)</td>
<td>II/III Multicenter</td>
<td>36 (FHF) 10 (AoC)</td>
<td>80 % 20 %</td>
</tr>
<tr>
<td>Nyberg [38]</td>
<td>Hollow fibre cartridge</td>
<td>LIVERx 2000 / Algenix</td>
<td>70 g primary porcine</td>
<td>blood, flow rate n.a.</td>
<td>-</td>
<td>I 1-center</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patzer, unpublished</td>
<td>Hollow fibre cartridge</td>
<td>BLSS / Excorp Medical</td>
<td>100 g primary porcine</td>
<td>blood, flow rate n.a.</td>
<td>n.a.</td>
<td>I 1-center</td>
<td>2 (FHF) 3 (AoC)</td>
<td>50 % 33 %</td>
</tr>
<tr>
<td>Chamuleau [39]</td>
<td>Spirally winded polyester network</td>
<td>AMC BAL / Biotest</td>
<td>100 g primary porcine</td>
<td>Plasma, flow rate n.a.</td>
<td>no membrane</td>
<td>I Multicenter in preparation</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gerlach [40]</td>
<td>Hollow fibre cartridge</td>
<td>MELS / Charite (non-profit)</td>
<td>250-500 g primary porcine</td>
<td>100 ml/min plasma</td>
<td>200 kDa 4.2 m(^2)</td>
<td>I/II Multicenter</td>
<td>8 (FHF)</td>
<td>100 %</td>
</tr>
</tbody>
</table>

\(^a\) Percent survival with or without liver transplantation
1.4. Analysis of improvement criteria of artificial liver support devices

The need for improvement of the performance of artificial liver support devices is evident as long as survival rates do not significantly exceed rates obtained without device treatment. Analysis of critical reviews on this topic reveals that important issues with potential for improvement can be divided in two groups: biological issues and issues concerning the technical aspects of the design of a bioreactor.

1.4.1. Biological criteria

*Cell mass and source*

Even in the most severe forms of liver failure, there is a certain amount of residual liver function [34]. As can be seen from Table 1.2, various investigators agree that an additional support of 5-20% of the natural liver cell mass would be sufficient to keep the patient alive, if these cells perform 100% of their *in vivo* activity also *in vitro* 5-20% liver cell mass correspond to 5x10^{9}-2x10^{10} hepatocytes and is the currently utilized cell number in all devices undergoing clinical trials.

In spite of some knowledge regarding the number of cells required, it remains still unsolved which cell source is mostly suited for practical application. In theory, normal human hepatocytes would be the ideal tissue to use. This approach is impractical due to the shortage of cadaverous human livers, as above mentioned. As an alternative, the development of a primary human hepatocyte cell line is being aggressively pursued. The human hepatoblastoma C3A cell line, which is a sub-clone of the HepG2 cell line, has been developed and clinically used by Sussman et al. [41] and Millis. A main restriction of this approach is the potential risk caused by the leakage of tumor cells and their products into the patient’s circulation. Therefore, primary porcine hepatocytes are most commonly used in devices undergoing preclinical and clinical evaluation. They are available in large amounts, but, however are, xenogenic and expose the patient to the risk of an infection with porcine endogenous retroviruses (PERV) [42]. For future applications, human stem cells are considered for therapy of liver failure. Potential sources include embryonic, progenitor or transdifferentiated stem cells [30]. The potential of those cells is that they proliferate *in vitro* and, in future, a differentiation into functional competent hepatocytes should be possible.

*Differentiation of the hepatocyte phenotype*

Independent from the utilized cell source, the basic biological problem is the stabilization of the differentiated hepatocyte phenotype in bioartificial liver support devices.
When enzymatically isolated from the liver and cultured in monolayers or suspension culture, primary hepatocytes rapidly lose differentiated function [43, 44]. Commonly accepted approaches to support differentiation include culture of hepatocytes in aggregates instead of monolayers [45], coculture of hepatocytes with non-parenchymal cells of the liver [46] and manipulation of extracellular matrix environment by coating of substrates [47-49] or embedding hepatocytes in gels that include ECM components [43, 50]. In current clinical devices, hepatocytes are utilized in aggregates, but to my knowledge, no bioactive coatings have been used. Coculture systems are not widely used and only Gerlach et al. [35] employ a mixed liver cell culture including nonparenchymal cells.

**Exposition to shear forces**

In hollow-fiber membrane devices, hepatocytes are protected from shear forces by separation from the plasma or blood stream by a membrane. The only currently clinically tested device which exposes cells to shear is the one of Chamuleau et al.[39]. The exact influence of shear forces on hepatocytes has not yet been completely clarified. Moreover, shear forces could detach cells from substrates in plate dialyzer and backed bed bioreactor configurations. Tilles et al. recently reported that hepatocytes cultured in a flat-bed bioreactor under shear stresses of 0.01-0.33 dyn/cm$^2$ have two-fold higher albumin and urea synthesis rates than under 5-21 dyn/cm$^2$ [51]. On the other hand, it is reported, that shear stress of 4.7 dyn/cm$^2$ promoted the ammonium removal and urea synthesis of hepatocytes in coculture with non-parenchymal cells [52]. Recently, Roy et al. published that a shear stress of 10 dyn/cm$^2$ did not adversely affect hepatocyte function in terms of cytochrome P450 activity [53].

**Oxygen supply**

All investigators agree on the crucial importance of oxygen supply for hepatocyte function. In fact, oxygen is required for all those reactions, which produce energy in mammalian cells. Experimental evidence supports the critical role of oxygen supply: oxygen depletion has been identified as the scale-limiting factor for hepatocyte cell cultures in hollow-fiber bioreactors [54]. In comparison to many other cell types, hepatocytes have elevated levels of carbohydrate, protein and xenobiotic metabolism and are therefore characterized by a very high oxygen consumption rate (OCR). Consequently, a poor oxygen supply may be expected to have negative effects on the energy metabolism of the hepatocytes and may compromise their viability and differentiated function. Data found in literature regarding oxygen consumption rates of differentiated and functional competent hepatocytes in culture widely vary from 10-50 nmol/sec/cm$^3$ [55, 56].
1.4.2. Technical design criteria

**Oxygen mass transfer**

High oxygen concentrations have been proven to favour maintenance of maximal insulin secretion by isolated islets of Langerhans [57]. In hollow-fiber membrane devices for bioartificial liver support, oxygen supply may be suboptimal, as many reviewers agree [35, 36, 41]. In most devices presently under clinical evaluation, oxygen supply is only achieved via oxygenation of blood or plasma flow which is separated from the cells by a membrane. To overcome this limitation, Gerlach et al. [60] and Chamuleau [39] et al. utilized special hollow-fiber compartments for gas delivery. Recent concepts that are not yet in clinical trials also employ internal membrane oxygenators mostly in flat plate geometries [50, 51, 52].

**Membrane characteristics**

Semipermeable membrane characteristics not only restrict oxygen mass transfer, but also bidirectional mass transfer of other constituents and products of detoxification, synthetic function and biotransformation. The membrane in a bioartificial liver device circuit is typically characterized by its nominal molecular weight cut off (NMWCO), which is selected both to prevent the exposure of the bioreactor cells to components of the patients immune system and to block the transport of larger xenogenic substances into the patients circulation. The aim of allowing free transport of larger carrier proteins such as albumin (~60 kDa), while preventing transport of immunoglobulins (~150 kDa), complement (>200 kDa) and viruses [30] led most groups to chose a NMWCO of 60-150 kDa. In this work, it is assumed that the whole system consists of two circuits: the patient’s blood circuit and the bioreactor circuit. The design and development of the latter is topic of this work. Immunoseparation occurs before blood or plasma enters the bioreactor circuit and will therefore not be considered.

**Homogeneity of cell distribution and flow patterns**

Geometric constraints can also affect mass transfer in bioartificial liver support devices. It has to be claimed that spacial hepatocyte distribution and fluid flow regime should be uniform at least in the physiological range of cell densities, fluid velocities and resulting shear forces [30]. In hollow-fiber devices currently used, cells are inoculated by flushing in a cell suspension. 20-55 % of the inoculated cells form aggregates in the ECS, while the rest forms monolayers on the hollow-fiber membranes [62]. The latter leads to a further resistance to diffusion of oxygen and other agents from the ICS to the ECS and consequently to channeling of mass transfer through regions of low resistance.
Dead volume ratio

A further important geometric parameter is the ratio between biological active tissue volume and flow volume, \( V_{\text{Tiss}}/V_{\text{Flow}} \) [51]. If the ratio is too low, this dead volume overloading is especially dangerous in acute liver failure patients who are already hemodynamically unstable [2, 35]. In current hollow-fiber membrane designs, \( V_{\text{Tiss}}/V_{\text{Flow}} \) can be estimated as 1, since the ECS and ICS are of the same order of magnitude. In typical plate dialyzer bioreactors cultivating cell monolayers, and consequently requiring a typical plate surface area of about 10 m\(^2\) [63], the ratio is strongly dependent on channel height. With typical channel heights of 1 mm as recently proposed in [52], a ratio of \( V_{\text{Tiss}}/V_{\text{Flow}} \) of about 0.02 results. Although the minimization of dead volume could be achieved by simply reducing the channel height, this results in a reduction in oxygen availability and increased shear stress applied to the hepatocytes.

1.5. Consequences on the design of a novel bioartificial liver support device concept

Based on the analysis of possible improvement criteria of artificial liver support devices in the previous section, design Specifications was derived on which the development of a novel design concept described in the following chapter was based:

1. Cell mass, source and distribution in the bioreactor: A novel design for a bioartificial liver support device must undergo several phases of material screening and in vitro testing. Those experiments are usually performed with reduced cell mass to keep costs for biological and scaffold materials in a reasonable scope. Furthermore, the degree of actual activity can not be assumed to be 100 %, so that even more than 5-20 % of the natural liver cell mass are necessary to keep the patient alive. Therefore, the device has to offer the capacity for an easy modular scaling to the clinically required cell mass. Independent of the utilized cell source, a simple seeding process must result in a homogenous cell distribution in the bioreactor.

2. Differentiation of the hepatocyte phenotype: Long-term retention of the differentiated hepatocyte phenotype should be achieved by cultivating cell aggregates contrary to monolayers and by the application of bioactive coatings, but not by embedding the cells in gels. The latter would cause further mass transport restrictions. A novel design should also offer the possibility of the application as coculture system not only for hepatocytes, but also for the coculture with nonparenchymal cells.
3. **Application to shear forces:** Since it still remains unclear whether and in which range shear forces promote hepatocyte function, a new system should give the option to switch between *application of and prevention from shear forces by simple design modifications* which do not affect other system specifications.

4. **Oxygen supply:** Present clinical devices seem not to meet the requirement of adequate oxygen supply, which is a crucial factor for hepatocyte function. In these devices, oxygenation occurs mainly via saturated fluids (blood, plasma or cell culture medium respectively). A novel device should therefore offer *additional pathways of oxygen mass transfer*, resulting eventually in a physiological oxygen partial pressure gradient generating a situation comparable with the metabolic zonation in the natural liver.

5. **Mass transfer and homogeneity of flow:** While transport in bioartificial liver support devices is a combination of convective and diffusional phenomena, mass transfer resistances from and to the cellular compartment often arise from diffusion resistances. In contrast, transport in the liver is achieved primarily by convection along the sinusoids with short diffusion distances (5-10 µm) across the space of Disse. The novel design concept should take into consideration this by mimicking the anatomy of the liver as closely as possible. Especially effective immunoisolation membranes should be applied before and behind the effective bioreactor chamber, in which convective fluid flow regime should be technical manipulated to achieve liver-like conditions without unphysiological diffusion barriers.

To reach a maximum performance of the biological component in the bioreactor, cells should preferably kept under homogeneous conditions regarding the supply with nutrients and agents involved in metabolic processes. This can be achieved by uniform flow conditions, i.e. spatial homogenous distribution of fluid stream in the flow volume. The design of latter is also influenced by the obligation to maximize the ratio between biological active tissue volume and flow volume $V_{\text{Tiss}}/V_{\text{Flow}}$. 

2. **PROPOSITION OF A TEXTILE COMPOSITE SCAFFOLD SYSTEM**

Based on the specifications derived from the analysis of potential improvement criteria of artificial liver support devices, a novel concept for a textile composite scaffold bioreactor system was developed. The following section describes the proposed system in detail and refer to the design specifications derived in the previous chapter.

2.1. **Mimicking the microanatomy of the liver to design a novel hepatocyte scaffold**

In analogy to the situation in the natural liver as reviewed in Chapter 1, the technical principal of the plate dialyzer was chosen and modified. As basis for the scaffold, a gas-permeable teflon (polytetrafluoroethylene, PTFE) membrane is used (Figure 2.1 A). Since woven PET textiles have been proven to be suitable for hepatocyte culture in recent studies [64, 65], on this membrane a woven polyethyleneterephate (PET) textile is fixed by clamping or ultrasonic welding. This so-called structural textile creates compartments of a size which is defined by the textile mesh size. In those compartments, hepatocytes are seeded. It is hypothesized that cells completely fill the compartments and form functional competently aggregates. The aggregate size can be controlled via the mesh size of the structural textile.

In Figure 2.1, the scaffold is shown schematically very coarsely, with textile filaments situated in a plane. In reality, those filaments are interwoven and lie one upon the other, as illustrated in Figure 2.2 a. When seeding hepatocytes into those compartments, the latter are not filled completely, as the scanning electron microscopy image in Figure 2.2 c indicates. Therefore, in this work a woven textile with a filament diameter d is assumed to induce hepatocyte aggregates in between of a height of d-1.5d (Figure 2.2b). For a characteristic filament diameter of 50 m hepatocyte tissue thickness will range between 50 and 75 m.

When stacking-up this elementary scaffold unit to a bioreactor configuration consisting of several units, a channel for blood or plasma flow (in the following called fluid flow) in analogy to the sinusoid in the natural liver, has to be kept open. Therefore, a further woven PET textile, the so-called sinusoid textile is applied (Figure 2.1 B). The following symmetrical stacking-up leads to the completion of the modular bioreactor unit (Figure 2.1 C). It consists of two channel systems for fluid flow and oxygen supply respectively.
2. Proposition of a textile composite scaffold system

A: Seeding of hepatocytes on a textile composite scaffold

B: Mimicking the sinusoids

C: Completion to a modular bioreactor unit

Figure 2.1:  
A: Hepatocytes are seeded in compartments formed by the woven PET structural textile fixed on an oxygen permeable teflon membrane.  
B: A further woven PET textile, the sinusoid textile, is applied to hold open a channel for flow of fluid when  
C: Elementary scaffold units are stacked up to a modular bioreactor unit which provides two channel systems for fluid flow and oxygen supply respectively.
2. Proposition of a textile composite scaffold system

Figure 2.2: Illustration of the deduction of the schematized textile mesh (b) from the a realistic textile (a). Choosing a aggregate height of 1-1.5 times the mesh size is confirmed by the scanning electron microscopy image of hepatocytes in such a textile mesh (c).

2.2. Discussion of the concept with respect the given specifications

The above described concept was derived from the specifications worked out in Chapter 1. In this section it is discussed, to which extent the design concept satisfies these specifications.

1. Cell source, mass and distribution

The proposed concept is independent from the utilized cell source. Depending on future research and development, primary cells, cell lines or stem cells can be employed.

Due to the modularity of the concept, customization of the required cell mass is possible by scaling up the reactor. This can be done either by increasing the number of elementary scaffold units in a bioreactor or by parallel or serial layout of several bioreactor units. Assuming that hepatocytes can be simplified as cubes with an edge length of 10 µm and that the textile mesh has an open area of appr. 75 %, a total surface of the proposed scaffold of 0.15-0.6 m² can be estimated to accommodate enough hepatocytes according to 5-20 % of the natural liver mass. This magnitude gets larger when it is taken into account, that those cells potentially do not perform 100 % of their in vivo activity.

The surface textile defines a very strictly defined arrangement of compartments with exact same size allover the elementary scaffold unit and the bioreactor units are stacked up symmetrically in the bioreactor unit. Therefore a homogeneous distribution of hepatocytes in the proposed configuration results, as claimed in the design Specifications 1.
2. Proposition of a textile composite scaffold system

2. Differentiation of the hepatocyte phenotype

In the proposed concept, hepatocytes are cultured as aggregates (design Specification 2) within the compartments formed by the structural textile. Therefore the mesh size of this textile determines the maximum aggregate size of hepatocytes.

As mentioned in Chapter 1, the formation of aggregates enhances differentiated hepatospecific cell function. Moreover aggregates are necessary to achieve the required total cell mass for artificial liver support on an area realistic for a clinical applicable bioreactor [35]. On the other hand, the use of aggregate sizes larger leads to necrosis in the interior of the aggregate due to shortage of nutrients and oxygen, and such partial necrosis must be prevented. Sizes of functional competitently hepatocyte aggregates in culture have been reported to be larger than 50 µm [66]. Liver cells isolated from newborn rats and seeded on a non-adherent plastic substratum were found to spontaneously re-aggregate and to form, within a few days, spheroidal aggregates that reached a plateau diameter of 150 - 200 µm [45, 67]. The number of cells in an aggregate formed by self assembly in suspension culture was determined to be 20 - 100 hepatocytes [60]. Thus, the mesh size of the structural textile is chosen in a range of 100 - 200 µm.

In the proposed basic arrangement illustrated in Figure 2.1, hepatocyte aggregates adhere on a gas-permeable teflon membrane made of expanded teflon. Teflon is chemically very inert [68, 69], which promises good biocompatibility of the surface. In the recent years, teflon foils were utilized for cell culture of several types of cells, for example keratinocytes [70], monocytes [71], macrophages [71] and bone marrow cells [72]. Hepatocytes cultured on teflon foils showed differentiated function monitored by ammonia removal and cytochrome P-450 metabolism [50, 73].

A disadvantage caused by the inertness of the teflon foil is the difficulty of chemical functionalization of the surface. As mentioned in Chapter 1, several coatings are promising to initiate and enhance the differentiated function of hepatocytes. To allow the application of such coatings in the proposed concept, an additional woven PET textile can be employed between the inert teflon membrane and the structural textile. (Figure 2.3 left). The mesh size of this so-called surface textile was chosen so that hepatocytes with a characteristic size of about 10 µm adhere on the PET and cannot pass the mesh in general. The surface textile PET can be surface-modified with sulfonate groups, which render it negatively charged. The charged surface can electrostatically interact with a construct that has a positively charged anchor and a bioactive tail. The development of this bioactive coating was subject of a separate thesis [49].
2. Proposition of a textile composite scaffold system

Figure 2.3: Left: An additional PET textile, the surface textile can be employed between the teflon membrane and the structure texture in order to allow the application of bioactive substances via chemical bindings to the surface textile. Right: The optional woven PET Disse textile prevents hepatocytes from shear stresses caused by fluid stream and can serve as culture substrate for nonparenchymal cells.

3. Exposition to shear forces

In the natural liver, hepatocytes are separated from blood stream by a single layer of flattened endothelial cells and Disse’s space. Since they are not in direct contact with the blood stream, hepatocytes are prevented from being mechanically damaged by shear forces. As claimed in the design Specification 3, the new system should offer the option of applying shear forces or not. This can be achieved by the optional application of a further woven PET textile, which is in analogy to the Disse’s space in the natural liver attached between the hepatocytes and the blood stream (Figure 2.3 right) and can therefore be called “Disse textile”. It has the same specifications as the surface textile in order to ensure that hepatocytes cannot leave the compartment formed by the structural textile and the Disse textile. At the same time it allows transport of nutrients and metabolites from the fluid to the hepatocytes. An estimation of acting shear forces in the proposed system is made by a computational fluid dynamic investigation in Chapter 4.

To simulate the presence of endothelial cells between the sinusoids and the Disse space in vitro, the Disse textile could also serve as substrate for seeding of nonparenchymal endothelial cells (Figure 2.3 right). Heterotypic cell interaction between parenchymal cells and nonparenchymal neighbors has been reported to modulate cell growth and differentiation [74]. In vitro, cocultivation of hepatocytes and nonparenchymal cells has been used to modulate the hepatocyte phenotype [46].
4. Oxygen supply

The proposed concept is distinguished by the utilization of a gas-permeable teflon membrane, which serves as internal membrane oxygenator. In contrary to all devices undergoing clinical tests, except the one by Gerlach et al.[39], here hepatocytes are supplied with oxygen in two ways, as claimed in design Specification 4:

First, oxygen is supplied by flow of oxygenated fluid through the bioreactor. This pathway is limited by the solubility of oxygen in fluid and by the fact that oxygen partial pressure in the fluid decreases by consumption as the fluid flows through the bioreactor geometry. Consequently, hepatocytes at the outlet regions experience a lower oxygen partial pressure as those at the inlet regions, which could eventually result in oxygen partial pressure gradient generating a situation comparable with the metabolic zonation in the natural liver.

A second pathway for oxygen supply does not depend upon position in the bioreactor. Oxygen as gas can be conducted convective through the gas channels of the bioreactor in high concentrations so that the transport process to the hepatocytes is only limited by diffusion characteristics through the membrane. This results in a uniform oxygen partial pressure at the interface between the teflon membrane and the hepatocytes along the entire surface of the bioreactor geometry. Oxygen transport and concentration profiles in the proposed configuration are investigated by use of models for mass transport fiber in Chapter 3.

5. Mass transfer and homogeneity of flow

The proposed design offers, as sought in design Specifications 5, a convective form of mass transfer at the sinusoid textile channels with short diffusion distances to the hepatocyte tissue through the Disse textile, if utilized. The height of the sinusoid channels is determined by the filament diameter of the textile (cf. Figure 2.2). This allows control of the ratio \( V_{\text{Tiss}}/V_{\text{Flow}} \), which is typically of the order of magnitude of 1/3 in the proposed configuration.

The sinusoid textile not only keeps open the flow channel, but also influences the flow distribution by its spatial extended regularity. It creates a velocity profile that has components not only parallel to the structural textile which harbours the cells, but also perpendicular. The benefits of this sinusoidal textile are investigated by a computational fluid dynamic model in Chapter 3.
Restriction of the proposed system

As described in Chapter 1, hepatocytes surround bile canaliculi, into which they secrete bile. This bile is ultimately discharged into the gut via bile ducts. A restriction of the above proposed composite scaffold bioreactor system is obvious in this context. Hepatocytes form bile duct-like structures in aggregates [75], however bile cannot be removed from the canaliculi in this configuration. This leads to deteriorated metabolic functions [76] and malfunction after up to 14 days in culture [67]. Consequently, the proposed configuration is in any case only suited for a short-term treatment of acute liver failure. Bioreactor modules have to be exchanged after a certain time of treatment, which has to be determined under clinical application.

2.3. Realization of a prototype bioreactor for concept testing

The complete design of the proposed textile composite scaffold bioreactor is based on a complex system of numerous microscopic layers of alternating oxygen supply channels, textile composite scaffolds and flow channels kept open by further textiles. Aim of prototype testing, which is described in Chapter 4, was to concentrate on the verification of the benefit of certain design specifications. It was investigated if and how it is possible to reach a homogeneous cell distribution in the entire bioreactor (design Specification 1, investigated in Chapter 4.2), and if cells form aggregates and show differentiated function (design Specification 2, investigated in Chapters 4.2 and 4.3 respectively), which is only possible under the prerequisite of appropriate oxygen supply (design Specification 4, investigated in Chapter 3). Therefore the concept was reduced on some features of the proposed system. As described in detail in the next section, the prototype consisted of only one textile composite scaffold layer, oxygen supply channel and flow channel. This allowed direct access to the scaffold to analyze homogeneity and morphology of cell distribution and simplified construction of this first prototype bioreactor. The textile composite scaffold itself was chosen to include the surface textile between teflon membrane and structural textile (cf. Chapter 2.3, left). This was meant to allow the application of bioactive coatings as discussed in Chapter 1.

2.3.1. Materials, Methods and assembly

The elements of the prototype bioreactor are shown in Figure 2.4 in the design drawings with dimensions. The prototype bioreactor consists of four plates (100x100x4 mm, Figure 2.4a) of polycarbonate (PC, Bayer, Germany).
2. Proposition of a textile composite scaffold system

Figure 2.4: Design drawing of the prototype bioreactor (Sefar AG, Rueschlikon, Switzerland).
In two of the plates, rhombic-alike cavities as displayed in Figure 2.4b were milcut representing the flow chamber and the gas chamber respectively. At the border of the cavities, a 4 mm wide recess with a depth of 0.125 mm was milcut in order to fix membrane and textiles on it. Chamber inlets and outlets were realized by drilling holes of 2 mm diameter in the 4 mm-edge of the plates as also showed in Figure 2.4b. In this holes, stainless steel tubes were plunged on which silicone hoses with connectors to the external periphery were plugged.

Figure 2.5 shows the principle of the assembly of the bioreactor plates to the prototype unit. On the bottom disc (without cavity), the disc with the cavity for the gas chamber was put, with a silicone gasket in between. On top of this plate, the teflon membrane, the surface textile and the structural textile were fixed by a custom-made adhesive film (Sefar AG, Switzerland) on the border recess. The flow chamber disc and the cover disc were put on this configuration, with silicone gaskets in between the discs.

**Figure 2.5:** Assembly of the four bioreactor plates to the prototype bioreactor. The image section beneath shows the structure of the textile composite scaffold which is fixed with adhesive films on the border recess of the gas chamber disc.
Figure 2.6a shows a photograph of the opened prototype bioreactor. In this picture, the PET textiles are fixed on the border recess of the bottom side of the flow chamber disc in order to visualize both the transparent teflon membrane and the PET textile scaffold. The discs were fold up in direction of the arrows along the dashed line and clamped into a chassis. This construction consisted of a bottom chassis and an upper chassis, in between which a pressure disc was fixed. The pressure disc was pressed by screws towards the bioreactor discs lying on the bottom chassis and therefore sealed up the bioreactor gas and flow chambers.

Figure 2.6: Realization of the prototype bioreactor: The four plates were fold up in direction of the arrows along the dashed line (a) and clamped into a chassis.
2.3.2. Integration of the prototype bioreactor in a flow path

For biological tests with hepatocytes in the bioreactor described in Chapter 4.3, it was necessary to exactly adjust flow rates through the flow chamber and monitor and control temperature and pH of the medium in the experiment. Therefore a commercially available system (Cell Pharm (CP) 1500, Unysin, USA) was chosen, in which the prototype bioreactor was implemented. The flow path of the CP 1500, which is originally designed as device for antibody production in hollow fiber membrane bioreactors, was modified with regard to its utilization in the biological testing experiments. The resulting flow path is depicted in Figure 2.7.

![Figure 2.7: Flow chart of the experiments with prototype bioreactor integrated in UnisynCP 1500.](image)

Cell culture medium from the medium reservoir was pumped with flow rates between 20 and 100 ml/min through probes for temperature and pH. After passing an oxygenator, medium flowed through the bioreactor flow chamber and back into the medium reservoir. Temperature and pH probes were connected to a PC. Temperature was kept constant at 37°C by controlling the heater around the medium reservoir with CP 1500 software. pH in the sodium bicarbonate-buffered medium was controlled to be constant at pH 7.4 by regulating the air/CO₂ mixture with which medium was oxygenated. Air also flowed through the gas chamber. Air hoses were sealed by sterile filters (0.45 m) in order to avoid contamination of the flow path by invasion via the gas conduits.
3. Modeling of mass transfer characteristics

Aim of this chapter was to analyze to what extent the proposed textile composite scaffold bioreactor system design could satisfy the specifications regarding oxygen supply of hepatocytes (*Specification 4*), the overall mass transfer and homogeneity of flow (*Specification 5*) and the appropriate resulting shear forces on the cells (*Specification 3*) as described in Chapter 2. Therefore, the following modeling strategy has been selected:

1. A one-dimensional model for oxygen supply of hepatocytes in an elementary scaffold unit was developed (Chapter 3.1). As explained below in detail, it was derived from a already successfully established formalism based on Michaelis-Menten kinetics and therefore served as reference for the further development of computational models.

2. Taking flow of fluid in the three-dimensional bioreactor system into account, mass transfer of oxygen is no longer only governed by diffusion, but also by convection of oxygen dissolved in flowing fluid. Therefore, a formalism was utilized that was able to describe general mass transfer in a three-dimensional bioreactor geometry and was implemented into a computational fluid dynamic solver algorithm (Chapter 3.2). In order to estimate its potential, results of this numerical model were compared to results obtained with the one-dimensional model's reference results (Chapter 3.3).

3. By scaling up the model to the scale of an entire realistic flow chamber it was possible to make predictions about oxygen supply of hepatocyte aggregates in different geometric locations of the flow chamber (Chapter 3.4) and to calculate a minimum flow rate required to appropriately supply oxygen to all cells. Furthermore, an estimation of shear forces on the cells in a prototype bioreactor (cf. Chapter 2.3) was done. This prototype was utilized for the following biological testing experiments which are described in Chapter 4.

4. Finally modeling was utilized to investigate the influence of flow of fluid with the calculated minimum required flow rates through a section of a flow channel modulated and kept open by a sinusoid textile (Chapter 3.5). Therefore flow patterns, shear forces on the cells at the cell-fluid interface, and oxygen concentration profile in the fluid were analyzed.
3. Modeling of mass transfer characteristics

3.1. One-dimensional model for oxygen supply

In the proposed textile composite scaffold bioreactor, hepatocytes are seeded in compart-
ments built up by woven textiles as shown in Figure 2.1 in the previous chapter. In this
configuration, oxygen is supplied to the hepatocytes from two sides: On the side of the
gas-permeable teflon membrane, oxygen from the oxygen supply channels passes the
membrane on which hepatocyte aggregates adhere and diffuses into the aggregates. Sec-
ondly, oxygen is provided to the cells from the side of the oxygenated cell culture
medium from where it also diffuses into the hepatocyte aggregate. While diffusing, oxy-
gen is consumed by the cells in a manner that is described by Michaelis Menten kinetics.
The consequence is that in the cell aggregates a characteristic oxygen partial pressure
attunes, which is determined by the oxygen concentrations at the boundaries, the diffu-
sion properties of oxygen in hepatocytes and the oxygen consumption rate of cells.

Below a certain critical oxygen partial pressure $P_c$ in the cells, hypoxic damage occurs
and respirations ceases. This would lead to malfunction of the proposed bioreactor sys-
tem. To calculate oxygen partial pressure profiles in hepatocyte aggregates in the pro-
posed configuration, a mathematical model utilizing Michaelis-Menten kinetics was
developed from a well-established formalism which has been successfully utilized to
predict oxygen profiles in metabolic active cells enclosed within immunoisolation
devices consisting of semipermeable membranes by Colton and coworkers [77] and in
recent time to calculate oxygen transfer to cultured hepatocytes in microchannel paralle-
plate bioreactors by Yarmush, Toner and coworkers [78].

3.1.1. Theoretical analysis of oxygenation of a hepatocyte aggregate

Problem formulation

We consider one-dimensional oxygen diffusion in Cartesian coordinates through a layer
of hepatocytes in the proposed configuration. A hepatocyte aggregate in a so-called ele-
mentary scaffold unit is confined by two textile filaments representing a compartment
generated by a woven textile fabric, the structural textile (cf. Chapter 2.1). The oxygen
partial pressure profile $p(O_2)$ in the layer of hepatocytes (solid curve in Figure 3.1) is a
function of the distance from the middle plane of the hepatocyte aggregate $x$ which is
considered positive in direction of the teflon membrane. It depends from the oxygen par-
tial pressures at the cell-fluid interface ($P_{Pl}$) and the cell-membrane interface ($P_{TE}$)
respectively. The dashed line depicts the critical oxygen partial pressure $P_c$ below which
hypoxic damage occurs and respiration ceases. $L$ is the thickness of the aggregate.
3. Modeling of mass transfer characteristics

Figure 3.1: Schematic diagram of a hepatocyte aggregate between two textile filaments on a teflon membrane, overflown by fluid. The solid curve is a possible oxygen partial pressure profile. $P_{Te}$ and $P_{PL}$ are the oxygen partial pressures at the cell-membrane interface ($x = L/2$) and at the cell-fluid interface ($x = -L/2$). The dashed line depicts the critical oxygen partial pressure $P_c$ below which hypoxic damage occurs and respiration ceases. $L$ is the thickness of the aggregate.

The species conservation equation for oxygen in the aggregate is

$$D \frac{d^2 c}{dx^2} = V$$

(1)

where $D$ is the effective diffusion coefficient of oxygen in the cell layer, $V$ is the local oxygen consumption rate per unit volume in the aggregate. The local concentration of oxygen in $c$ is linearly related to the local oxygen partial pressure $P$ by

$$c = \alpha P,$$

(2)

where $\alpha$ is the Bunsen coefficient of oxygen solubility. Use of partial pressures instead of concentrations eliminates need for partition coefficients between adjacent phases (medium, cells and teflon membrane) because the partial pressures at equilibrium are equal across an interface:
3. Modeling of mass transfer characteristics

\[ D \alpha \frac{d^2 P}{dx^2} = V \]  

The oxygen consumption rate per unit volume \( V \) is assumed to follow Michaelis-Menten kinetics,

\[ V = V_{\text{max}} (1 - \epsilon) \frac{P}{K_m + P} \]  

for \( P > P_c \), where \( V_{\text{max}} \) is the maximum oxygen consumption rate per unit volume of hepatocytes, \( 1 - \epsilon \) is the viable cell fraction in the cells and \( K_m \) is the Michaelis Menten half saturation constant. Below \( P_c \), results of the mathematical model formalism are not relevant.

The boundary conditions for the hepatocyte aggregate in the proposed configuration are expressed in terms of the oxygen partial pressures \( P_{Te} \) and \( P_{Pl} \) at the cell-membrane interface and the cell-fluid interface, respectively:

at \( x = L/2 \)

\[ P = P_{Te} \]  

and at \( x = -L/2 \).

\[ P = P_{Pl} \]

\( P_{Te} \) and \( P_{Pl} \) are shown in Figure 3.1. Since \( P_{Te} \) and \( P_{Pl} \) are not equal in general, the problem is not symmetric at \( x = 0 \).

**Oxygen partial pressure profile**

Assuming that the entire hepatocyte aggregate is exposed to \( P > P_C \), Equation 3 can be solved with boundary conditions, Equation 5 and Equation 6. This leads to an expression for the oxygen partial pressure in the aggregate:

\[ P(x) = \frac{P_{Te} + P_{Pl}}{2} + (P_{Te} - P_{Pl}) \frac{x}{L} - \frac{V}{2D \alpha} \left[ (\frac{L}{2})^2 - x^2 \right] \]

The first two terms on the right-hand side of Equation 7 represent the oxygen profile in absence of consumption, the last term represents the symmetrical reduction in local oxygen partial pressure resulting from oxygen consumption by the hepatocytes.
**Maximum supportable cell aggregate thickness**

According to the species conservation equation, Equation 3, at x = L/2

\[
D \alpha \frac{dP}{dx} = N_{Te},
\]

where \(N_{Pl}\) is the oxygen flux generated by the diffusion of oxygen through the teflon membrane. Differentiation of \(P(x)\), Equation 7, leads to

\[
\frac{dP}{dx} = \frac{P_{Te} - P_{Pl}}{L} + \frac{V(P)}{D \alpha} x,
\]

which can be combined with Equation 8 to a quadratic expression in L,

\[
-\frac{V(P)}{2D \alpha} L^2 + \frac{N_{Te}}{D \alpha} L - (P_{Te} - P_{Pl}) = 0,
\]

the positive root of which is

\[
L_{max} = \frac{N_{Te}}{V(P)} + \left[\left(\frac{N_{Te}}{V(P)}\right)^2 - \frac{2(P_{Te} - P_{Pl})D \alpha}{V(P)}\right]^{0.5}
\]

This allows estimations of the maximum supportable cell aggregate thickness \(L = L_{max}\), in which \(P > P_C\) and consequently hepatocytes are supplied with sufficient oxygen.

### 3.1.2. Calculations

**Parameters**

Using the equations derived in the previous section, now the oxygen profiles in hepatocyte aggregates in the proposed textile composite scaffold configuration are calculated. Values given in literature for the oxygen consumption rate \(V_{max}(1 - \varepsilon)\) of hepatocytes differ over a wide range. The first values come from Nyberg et al. [56]. HepG2 line cells are entrapped within a gel that is introduced into the lumen of a hollow fiber cartridge. Using information detailed within this article, \(V_{max}(1 - \varepsilon)\) of 10.0 nmol/sec/cm\(^3\) was calculated. Since the cells were entrapped, this value is likely to be diffusion-limited. Recent measurements of microcarrier-attached HepG2 cells within a stirred tank found an average value of 25.0 nmol/sec/cm\(^3\) [56]. This estimate could be metabolically lim-
3. Modeling of mass transfer characteristics

ited, because hepatocyte cell lines are metabolically restricted compared to primary hepatocytes. Foy et al. measured the oxygen consumption rate of primary rat hepatocytes monolayers [80]. From this results, a value of even approximately 50.0 nmol/sec/cm³ can be calculated.

In view of the above, calculations in this work were performed for values of $V_{\text{max}}(1-\varepsilon)$ of 10, 25 and 50.0 nmol/sec/cm³.

**Oxygen partial pressure boundaries at cell-membrane and cell-fluid interface**

Oxygen transport characteristics from the medium side to the hepatocytes depend on homogeneity of oxygen partial pressure profile in the medium. In many *in vitro* situations cell culture medium acts as diffusion barrier for oxygen, e.g. in cell culture dishes where oxygenation solely occurs from the air above the medium. In case of the proposed bioreactor configuration, the medium is not static, but flows above the cells. It is recirculated, or exchanged respectively, in a circuit which also includes devices for media oxygenation. For this reason, in this section fluid is considered to be well mixed and consequently provide a fixed value of oxygen partial pressure, namely 40 mmHg [77, 80].

From the side of the teflon membrane, oxygen diffusion must be taken into account. In the membrane, no oxygen consumption occurs. Consequently, the oxygen profile between $P_{\text{ext}}$ and $P_{\text{Te}}$ is linear. The flux can be expressed by

$$N_{\text{Te}} = \frac{P_{\text{Te}} - P_{\text{ext}}}{R_{\text{Te}}},$$

where $R_{\text{Te}}$ is the diffusive mass transfer resistance of the teflon membrane of a given thickness $L$ which can be expressed as

$$R_{\text{Te}} = \frac{L}{D_{\text{Te}} \alpha_{\text{Te}}}.$$  \hspace{1cm} (13)

The oxygen flux $N_{\text{Te}}$ itself depends on the pressure gradient and the oxygen concentration gradient respectively. To estimate $P_{\text{Te}}$, a characteristic value was utilized corresponding to a typical cell culture situation on this teflon membrane [81].

Under the presumption that external oxygen partial pressure in an incubator $P_{\text{ext}}$ is 159.6 mmHg (21 % $O_2$) and a given thickness of the teflon membrane of 25 m as well as other parameters listed in Table 3.1, $P_{\text{Te}}$ was calculated as 137 mmHg.
3. Modeling of mass transfer characteristics

3.1.3. Results

**Oxygen partial pressure profile in hepatocyte aggregates**

Oxygen partial pressures as function of distance from the center of the aggregate on the proposed configuration in the elementary scaffold unit were calculated for the above-mentioned maximum oxygen consumption rates. Cell aggregate thickness \( L \) was set 50 m, which corresponds to typical filament diameter of a commercially available woven PET textile.

Figure 3.2 shows the distance from aggregate center versus the oxygen partial pressure for the three hypothetical oxygen consumption rates. For all consumption rates, oxygen partial pressure does not fall below \( P_c \) which is illustrated as dashed line in this figure.
3. Modeling of mass transfer characteristics

Figure 3.2: Calculated oxygen partial pressure \( P(x) \) as a function of the distance \( x \) from the center of the cell aggregate. The curves correspond to three maximum oxygen consumption rates \( V_{\text{max}}(1 - \varepsilon) \) of 50 \( (a) \), 25 \( (b) \) and 10 nmol/sec/cm\(^3\) \( (c) \) respectively. The dashed line represents the critical oxygen partial pressure.

**Maximum supportable cell aggregate thickness**

In the proposed configuration of the textile composite scaffold, the size of hepatocyte aggregates is determined by the so-called structural textile in two ways. First, the maximum width of aggregates is given by the mesh size. Since the configuration is perfused from above by fluid, we assume that hepatocytes that are atop the structural textile, are flushed away at least on a long-term time scale. For this reason, the second geometric parameter of the structural textile which influences the size of hepatocyte aggregates is the textile filament diameter. As explained in Chapter 2, this filament diameter determines the cell layer thickness, which was estimated to be 1 to 1.5 times the filament diameter.

To make possible an optimization of the employed woven textiles on the requirements of hepatocytes for oxygen, the maximum supported aggregate thickness was calculated using Equation 11 and the parameters listed in Table 3.1. Maximum supportable cell layer thickness varied from around 300 m to slightly more than 50 m corresponding to inserted values of \( V_{\text{max}} \) from 10 to 50 nmol/sec/cm\(^3\) (Figure 3.3).

The grey sector represents the range of potential thickness of an aggregate cultured in compartments created by a textile with 50 m filament diameter (cf. Figure 2.2). In case
of an actual cell layer thickness of more than 75 \( \text{m} \), the aggregate could not be supplied with enough oxygen (i.e. oxygen partial pressure would fall below \( P_c \)) under the assumption of a high oxygen consumption rate of 50 nmol/sec/cm\(^3\).

![Figure 3.3](image)

**Figure 3.3:** Calculated Maximum supportable cell layer thickness \( L_{\text{max}} [\text{m}] \) as function of maximum oxygen consumption rate \( V_{\text{max}} [\text{nmol/sec/cm}^3] \). The grey sector represents the range of potential cell layer thickness in compartments created by an structural textile with 50 \( \text{m} \) filament diameter according to Figure 2.2.

3.1.4. Discussion

Taking into account that \( P_c \) is between 0.1 mmHg [82, 83, 85] for metabolically active cells and 10 mmHg [84] for hepatocytes in particular, calculated oxygen partial pressure profiles demonstrate that no hypoxic damage to the cells occurs in the proposed configuration when the hepatocyte cell layer thickness is supposed to be 50 \( \text{m} \).

If the cell layer thickness exceeds 50 \( \text{m} \) and a oxygen consumption rate of 50 nmol/sec/cm\(^3\) is supposed, the oxygen partial pressure in the aggregates would not be above \( P_c \) in the entire cell aggregate, as the results of calculations of maximum supportable cell layer thickness demonstrate. However, as discussed above, oxygen consumption rates of hepatocytes given in literature vary over a wide range, of which 50 nmol/sec/cm\(^3\) is the maximum found. It is therefore well possible, that real consumption rates are lower and could possibly allow even thicker cell layers than 75 \( \text{m} \). On the other hand, cell layer thickness could technically very simply be controlled by applying...
structural textiles with smaller filament diameters which would avoid insufficient oxygenation even in case of high oxygen consumption rates.

A problem arises with high oxygen partial pressures at the cell-teflon membrane interface: oxygen partial pressures of 137 mmHg as attained at the teflon membrane are non physiological for liver cells, which is in the natural liver in contact with arterial blood having a maximum of not above 100 mmHg [80]. This could lead to toxic effects on the cells caused by hyper oxygenation. Farris measured oxygen toxicity to hepatocytes. From this results it can be calculated, that hepatocytes cultivated under an atmosphere of 722 mmHg die after 6 to 10 days, but show no toxic reactions when cultured under 151 mmHg, which indicates that the oxygen partial pressures in the proposed bioreactor system is not harmful to hepatocytes and could even be increased if necessary.

The values of $N_{\text{Tc}}$ and $P_{\text{Tc}}$ used and listed in Table 3.1 are valid for conditions of a standard cell culture incubator. A relatively simple possibility to manipulate the maximum supportable cell aggregate thickness is given by application of an overpressure in the oxygen supply channels of the textile composite scaffold bioreactor. According to Equation 11 this would lead to an increase of either oxygen flux generated by the teflon membrane and oxygen partial pressure at the cell-membrane interface.

As a conclusion of the analysis presented in this section, it can be seen that the results of the one-dimensional model of the elementary scaffold unit indicate the suitability of the proposed textile composite scaffold to ensure appropriate oxygen supply to the hepatocytes. It therefore seems to satisfy the design Specification 4 concerning the oxygen supply.

In the above described model, fluid is supposed to be well mixed in terms of having a uniform oxygen partial pressure. In the reality of the proposed bioreactor system, oxygen-saturated fluid enters the bioreactor at the inlet and the partial pressure away from it is a function of convective and diffusional mass transfer processes under conditions of oxygen consumption by the hepatocytes. The above-depicted one-dimensional model is not able to allow for modeling of those processes. To overcome these restrictions of the one-dimensional model and to allow predictions about mass transfer under fluid flow conditions, homogeneity of flow and resulting shear stresses, in the following sections a three-dimensional numerical computational fluid dynamic model is set up and employed.
3. Modeling of mass transfer characteristics

3.2. Three-dimensional models for mass transfer: Theoretical background, materials and methods

In this section, the theoretical background of mass transfer as it occurs in the proposed bioreactor system is explained. Since in this case flow processes are involved, mass transfer must be described by the more general Navier-Stokes formalism including diffusional and convectional mass transfer. This formalism is the basis of a commercially available fluid dynamic solver which is introduced later in this section. This solver is able to numerically solve the set of relevant mass transfer equations for a given flow volume geometry and defined boundary conditions and is therefore used in the following sections to attain further predictions of mass transfer and related phenomena.

3.2.1. Theoretical background of mass transfer

The mass and momentum conservation equations describing general fluid flows (the Navier-Stokes equations) are, in Cartesian tensor notation [86]:

\[
\frac{1}{\sqrt{g}} \frac{\partial}{\partial t} (\sqrt{g} \rho u_j) + \frac{\partial}{\partial x_j} (\rho \vec{u}_j) = s_m
\]

(14)

\[
\frac{1}{\sqrt{g}} \frac{\partial}{\partial t} (\sqrt{g} \rho u_i) + \frac{\partial}{\partial x_j} (\rho \vec{u}_j u_i - \tau_{ij}) = -\frac{\partial p}{\partial x_i} + s_i
\]

(15)

where t is time, \( x_i \) are the Cartesian coordinates (i=1,2,3), \( u_i \) is the absolute fluid velocity component, \( \vec{u}_j \) is the relative velocity between fluid and local coordinate frame, \( p \) is the pressure, \( \rho \) is the density, \( \tau_{ij} \) are the stress tensor components, \( s_m \) is the mass source, \( s_i \) is the momentum source and \( \sqrt{g} \) is the determinant of the metric tensor (repeated subscripts denote summation).

The calculations in the following sections are performed under the assumption that the regarded fluid is a Newtonian fluid. This is correct for blood plasma and cell culture medium and can be assumed also for blood, since its dynamic viscosity is approximately constant in a range of shear rates between 1 1/sec and 1000 1/sec [87]. For laminar Newtonian flow, the stress tensor components can be expressed by the following equations:

\[
\tau_{ij} = 2 \mu s_{ij} - \frac{2}{3} \mu \frac{\partial u_k}{\partial x_k} \delta_{ij}
\]

(16)
where $\mu$ is the molecular dynamic fluid viscosity and $\delta_{ij}$, the Kronecker delta, is unity when $i = j$ and zero otherwise.

$s_{ij}$, the rate of strain tensor for laminar Newtonian fluids, is given by:

$$s_{ij} = \frac{1}{2}\left(\frac{\partial u_i}{\partial x_j} + \frac{\partial u_j}{\partial x_i}\right)$$  \hspace{1cm} (17)$$

When flows are no longer laminar, but turbulent, models of turbulence to determine the Reynolds stresses and turbulent scalar fluxes. These models comprise additional differential or algebraic equations that relate the unknowns of Equation 14 and Equation 15 to selected ensemble-averaged properties of the turbulence field and also provide a framework for calculating these properties. The most prominent example of a turbulence model is the well known $\kappa$-$\varepsilon$ model, comprising transport equations for the turbulent kinetic energy $\kappa$ and its dissipation rate $\varepsilon$.

For turbulent Newtonian flows, $u_i$, $p$ and other dependent variables, including $\tau_{ij}$, assume their ensemble averaged values (equivalent to time averages for steady-state situations):

$$\tau_{ij} = 2\mu s_{ij} - \frac{2}{3}\mu\frac{\partial u_k}{\partial x_j}\delta_{ij} - \rho\bar{u}_i \bar{u}_j'$$  \hspace{1cm} (18)$$

with

$$-\rho\bar{u}_i \bar{u}_j' = 2\mu s_{ij} - \frac{2}{3}\left(\mu\frac{\partial u_k}{\partial x_j} + \rho k\right)\delta_{ij}$$  \hspace{1cm} (19)$$

where the $u'$ are fluctuations about the ensemble average velocity, $\mu_t$ is the turbulent viscosity and the overbar denotes the ensemble averaging process. The rightmost term in the above represents the additional Reynolds stresses due to turbulent motion. These are linked to the mean velocity field via the turbulence models.

Molecular diffusional fluxes of mass can be assumed to obey Fick's law. Accordingly, mass transfer of each constituent $m$ of a fluid mixture, whose local concentration is expressed as a mass fraction $\rho m_m$, is assumed to be governed by a species conservation equation of the form:

$$\frac{1}{\sqrt{g}}\frac{\partial}{\partial t}\left(\sqrt{g}\rho m_m\right) + \frac{\partial}{\partial x_j}\left(\rho \bar{u}_j m_m - F_{m,j}\right) = s_m$$  \hspace{1cm} (20)$$
where $F_{m,j}$ is the diffusional flux component and $s_m$ is the rate of production or consumption due to chemical reaction. For laminar flow, $F_{m,j}$ is determined by Fick’s law ($D_m$ is the molecular diffusivity of constituent $m$, $c_m$ is the local concentration of a constituent expressed by a mass fraction),

$$F_{m,j} = \rho D_m \frac{\partial c_m}{\partial x_j} ,$$  

(21)

whereas for turbulent flow the diffusional flux component is determined by ensemble averaged values:

$$F_{m,j} = \rho D_m \frac{\partial c_m}{\partial x_j} - \bar{\rho} u_j \bar{c}_m ,$$  

(22)

The rightmost term, containing the concentration fluctuation $\bar{c}_m$, represents the turbulent mass flux and is given by

$$-\bar{\rho} u_j \bar{c}_m = \frac{\mu_t}{\sigma_{m,t}} \frac{\partial c_m}{\partial x_j} ,$$  

(23)

where $\sigma_{m,t}$ is the turbulent Schmidt number and $c_m$ the local concentration of a constituent expressed by a mass fraction.

3.2.2. Materials and methods: Implementation of models into a computational fluid dynamic solver

For the model design of scaffold and bioreactor geometries, a computer aided design (CAD) program (Unigraphics, V 15) was used. Geometries were exported as solids (.stl files). Meshing of solids and therefore segmentation into finite volumes was realized by utilizing an automatic mesher (Pro*AM, V 3.1).

Meshed geometries were imported in a numerical fluid dynamic solver (STAR-CD, V 3.100a), where boundary conditions could be defined and solution algorithms could be defined. To reduce computing time, STAR-CD simulations were executed on stardust of ETH Zurich, a HP-Superdome multi-purpose parallel computer providing a total memory of 48 gigabyte, powered by 48 550-MHz 4-way superscalar processors.

The software STAR-CD numerically solves the system of Equations 14-23. The main options available in STAR-CD for general turbulence applications are variants of the well known $\kappa-\varepsilon_D$ model, all comprising transport equations for the turbulent kinetic
3. Modeling of mass transfer characteristics

energy $\kappa$ and its dissipation rate $\varepsilon_D$. Where necessary, turbulent calculations were performed with the linear low Reynolds number $\kappa$–$\varepsilon_D$ model. In this linear model, in which general transport equations for $\kappa$ and $\varepsilon_D$ are solved everywhere, including the near-wall regions, there is a linear relationship between Reynolds stresses and strains.

In StarCD, materials with different characteristics like a fluid (i.e. fluid) and a solid (i.e. a cell layer) in which the constitutive equations are solved separately have to be formally divided by a baffle. Baffles are defined as surfaces that are impermeable or permeable and are for the purposes of the calculations considered to be of infinitesimal thickness. The two surfaces are treated as walls, with appropriate boundary conditions conditions to be defined on. In the case of mass transport through a baffle, a porous baffle has to be defined in which the convective transport characteristic is linked to the local pressure drop $dp$ across its infinitesimal thickness $L$ by the Ergun equation:

$$\frac{dp}{L} = \frac{150 \mu (1 - \varepsilon)^2 u}{\varepsilon^3 D_p^2} - \frac{1.75 \rho (1 - \varepsilon) u^2}{\varepsilon^3 D_p}$$

(24)

$D_p$ is the mean diameter of hypothetical particles in a porous medium and $\varepsilon$ is the volume porosity. From this equation, the following expressions for the permeability coefficients $\alpha_p$ and $\beta_p$ can be derived:

$$\alpha_p = \frac{1.75 \rho (1 - \varepsilon)}{\varepsilon^3 D_p}$$

(25)

$$\beta_p = \frac{150 \mu (1 - \varepsilon)^2}{\varepsilon^2 D_p^2}$$

(26)

Errors in the solution domain can be caused by the mesh spacing, irregularity and non-orthogonality. STAR-CD has an implemented residual error estimate method. The residual at a particular cell and iteration can be understood as an imbalance in the transport equations resulting from the constitutive equations arising from incomplete solution. The residuals are normalized in an appropriate way to produce the estimate of the absolute magnitude of the error, with the same physical dimensions as the variable in question. For the Navier-Stokes equations, the error is estimated for the velocity field, with the error in pressure distribution incorporated into the same estimate. The error estimate in temperature, turbulence dissipation and other scalars can be included, depending on the nature of the flow.
In STAR-CD the course of the calculations can be followed and controlled by monitoring values of all the dependent variables at a user-specified cell. If a steady-state solution is being sought, then a necessary condition for achieving it is that the variation in the monitoring values which corresponds to the residual should be less than some small, user-specified tolerance which is recommended to be between 0.01 to 0.001. [86]

3.3. Three-dimensional computational model for mass transfer: Model setup and verification

This section describes a three-dimensional model of a scaffold section which was set up analogous to the section of the so called elementary scaffold unit in the one-dimensional model. In this first computational model, fluid was assumed to be stationary, i.e. no fluid flow occurs (fluid velocity \( v_{Pl} = 0 \)), so that oxygen transport only occurs via diffusion in fluid, but not via convection of the latter.

Predictions of both models were compared in order to estimate the three-dimensional numerical model’s potential to be extended to larger geometry sections in which then the additional feature of fluid flow and therefore convectional mass transport in the bioreactor system is of further interest.

3.3.1. Model setup and calculations

The elementary scaffold unit is as a part taken out of the proposed configuration where the hepatocyte aggregate is confined by textile filaments representing a compartment generated by a woven textile fabric, the so called structural textile, and a oxygen permeable teflon membrane (Figure 3.4 left). The elementary scaffold unit geometry for the three-dimensional numerical model consists of a fluid volume in which fluid resides and a layer of hepatocytes, a hepatocyte aggregate (Figure 3.4 right). This geometry was meshed by finite volumes measuring 5x5x2 \( \mu \text{m} \). At the interface between fluid and hepatocytes, in a layer of 10 \( \mu \text{m} \) thickness in each volume, the mesh was refined by a factor 2.

The presence of oxygen was realized in the model as a scalar parameter characteristic for each finite volume, representing the mass fraction concentration of oxygen. In the flow volume, generally characterized by \( v_{Pl} \), the dynamic viscosity of fluid \( \eta \), the density of fluid \( \rho \) and the diffusion constant of oxygen \( D_{Ox,Pl} \), diffusional and convectional mass transport of oxygen was allowed in general, while in the hepatocytes only diffusional mass transport was applied (\( D_{Ox,Hep} \)). To model the oxygen consumption of hepatocytes, a scalar oxygen sink was programmed in a fortran user-subroutine and implemented in
3. Modeling of mass transfer characteristics

the solution algorithm. Analogous to the first order Michaelis-Menten kinetics used in the one-dimensional model, again a first order catalytic process was assumed.

In the proposed textile composite scaffold bioreactor oxygen is supplied to the hepatocytes from two sides:

- From the fluid, which is saturated with oxygen outside the bioreactor and has therefore an characteristic initial oxygen partial pressure \( p(O_2,_{\text{Pl}}) \) when entering the bioreactor at the inlet.
- From the teflon membrane. This membrane divides the cell volume from the gas reservoir with a defined oxygen partial pressure \( p(O_2,_{\text{ext}}) \). As already argued in the previous section and calculated in the Equations 12 and 13, dependent from the membrane thickness \( L \), the pressure gradient over the membrane \( \Delta p \) and the oxygen permeability of the membrane, a oxygen partial pressure \( p(O_2,_{\text{Te}}) \) as boundary condition at the interface between teflon membrane and hepatocytes can be assumed.

The fluid reservoir (Figure 3.4 right, red; 200x200x50 \( \mu \text{m} \)) and the cell aggregate (Figure 3.4 right, green; 100x100x50 \( \mu \text{m} \)) were modeled as separate volumes. The volumes were divided by a porous baffle as an internal boundary condition for the calculation of the solution of the conservation equations. High numbers of \( \alpha_P \) and \( \beta_P \) (cf. Equations 25 and 26) were chosen for the porous baffle, representing a high porosity, while the diffusion resistance of the baffle was set zero. This accommodates the fact that hepatocytes represent a solid phase and mass transfer of oxygen in the aggregate occurs only by diffusion, but not by convection.

![Figure 3.4](image)

*Figure 3.4:* Setup of the model of an elementary scaffold unit implemented in StarCD (right), taken out as a section from the proposed textile composite scaffold bioreactor system (left). Fluid (dark) and hepatocyte (light) are modeled as separate volumes.
In order to make predictions of a simple computational model comparable with the results of the above presented one-dimensional model, no fluid flow was applied (velocity of fluid \( v_{Pl} = 0 \)) and the presence of the sinusoid textile, as described in Chapter 2.1, was neglected. To refer to the fact that the elementary unit is surrounded by congeners units, symmetrical boundary conditions were applied on the four surfaces laterally delimiting the fluid volume.

The numerical values for the above mentioned characteristic quantities are listed in Tables 3.1 and 3.2. Results were generated by utilizing a StarCD steady state calculation of the constitutive equations with maximum 5000 iterations.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Reference/remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Velocity of fluid, ( v_{Pl} )</td>
<td>0</td>
<td>m s(^{-1})</td>
<td>-</td>
</tr>
<tr>
<td>Dynamic molecular viscosity, ( \mu )</td>
<td>( 6 \times 10^3 )</td>
<td>Pa s</td>
<td>[88]</td>
</tr>
<tr>
<td>Density of fluid, ( \rho )</td>
<td>1025</td>
<td>kg m(^{-3})</td>
<td>[81]</td>
</tr>
<tr>
<td>Permeability coefficients of the baffle, ( \alpha_p, \beta_p )</td>
<td>( 1 \times 10^{30} )</td>
<td>-</td>
<td>i.e. no convective flow</td>
</tr>
</tbody>
</table>

a. Since most currently tested devices work with plasma as fluid, in the following calculations for the dynamic viscosity and density of fluid values of blood plasma were utilized.

**Table 3.2:** Values of parameters used additionally to Table 3.1 to calculate the oxygen profiles in hepatocyte aggregates in the proposed textile composite scaffold configuration using the 3 dimensional numerical model for an elementary unit described above.

### 3.3.2. Results

Calculations were automatically terminated after 2189 iterations because the error estimation and convergence criterion of a maximum residual tolerance of 0.001 was satisfied. Figure 3.5 a shows the graphical visualization of the resulting oxygen partial pressure profile on the surface of the three-dimensional model of the elementary unit. The oxygen partial pressure in the fluid reservoir seems to stay approximately constant around 40 mmHg, while in the oxygen consuming hepatocyte layer a drop down to around 15 mmHg occurs, before the partial pressure rises to 137 mmHg, which corresponds to the fixed boundary conditions at the interface between the hepatocytes and the teflon membrane.
Figure 3.5: Results of oxygen partial pressure profiles in the three-dimensional model at the surface (a) and along the middle axis A-A of the geometry in the fluid volume (b) and the hepatocyte volume (c). Results of the three-dimensional model (3D-calc., solid lines) were compared to those of the one-dimensional model (1D-calc., dashed lines).
3. Modeling of mass transfer characteristics

The slab A-A in Figure 3.5 a represents the middle axis of the geometry, along which scalar sensors were defined in the post processing in order to monitor the oxygen partial pressure profile along this line. The results are shown in Figure 3.5 b and c respectively. Curves in the diagrams were fitted by polynomials of degree 5.

In the fluid reservoir, an oxygen partial pressure drop down to 36.7 mmHg at the cell-fluid interface occurs in the three-dimensional model (Figure 3.5 b, solid line). The dashed line shows the progression in the one-dimensional model, where oxygen partial pressure was fixed at 40 mmHg by definition as a boundary condition. Data of the three-dimensional model was fitted by polynomials of degree 5, which leads to the artefact of an increasing oxygen partial pressure at the distance of -50 m from the fluid cell interface.

In the hepatocyte volume (Figure 3.5 c, solid line) the oxygen partial pressure profile along the middle axis of the three-dimensional model falls to a minimum of 12 mmHg at a distance of 13.5 \( \mu \text{m} \) from the cell-fluid interface, before it reaches the value of 137 mmHg, fixed by the boundary condition at the cell-teflon membrane interface. In comparison to that, the minimum of the one-dimensional model (Figure 3.5 c, dashed line) does only fall to 15 mmHg and is localized at 14 \( \mu \text{m} \) from the cell-fluid interface.

3.3.3. Discussion

The comparison of the results of the three-dimensional and the one-dimensional model shows a small quantitative difference in the hepatocyte volume. The deviation is probably and apparently due to the fact, that in the three-dimensional model diffusional mass transfer of oxygen in the fluid can also be contemplated. This represents a more realistic situation and leads to a lower oxygen partial pressure at the cell-fluid interface and consequently to a lower minimum of oxygen partial pressure in the hepatocyte volume.

However, the results of the different models show satisfying qualitative conformity in terms of the existence of a minimum in the oxygen partial pressure which does not fall below a critical value for appropriate hepatocyte oxygen supply. This indicates that diffusional transport and consumption of oxygen in the bioreactor are as well described in the three-dimensional computational model as in one-dimensional the model based on the established Michaelis-Menten kinetics. Therefore, the model was extended to describe also features of convectional mass transfer mediated by fluid flow in geometries representing larger sections the proposed bioreactor system (Chapter 3.4) and including more structural details (Chapter 3.5).
3.4. Integral model of mass transfer in the bioreactor chamber: Oxygen supply and shear forces.

For the model of the elementary scaffold unit in the last section, an uniform oxygen partial pressure in fluid of 40 mmHg was supposed which only diminished in direction from the center of the flow volume to cell-fluid interface. Since it has to be assumed that oxygen partial pressure in fluid is reduced by consumption of oxygen by the cells when fluid flows through an entire bioreactor chamber e.g. as described in Chapter 2.3, this supposition is only true for regions close to the fluid inlet of the bioreactor chamber.

Aim of this chapter consequently was to analyze oxygen partial pressure in the fluid flow in the entire hypothetical bioreactor consisting of the proposed textile composite scaffold (cf. Chapter 2.1) implemented in a realistic flow chamber geometry (cf. Chapter 2.3). Furthermore predictions about shear forces on cells in the prototype bioreactor utilized for biological testing (Chapter 4.3) were made.

For this purpose, the modeled part of the proposed bioreactor system was scaled up from the above described units including one or several cell compartments, to an entire, macroscopic and realistic flow chamber.

Model setup and calculations

Figure 3.6a shows the geometry of the bioreactor flow chamber as housing for the proposed textile composite scaffold system. The chamber has an overall length from inlet to outlet of 80 mm, a height of 4 mm and a maximum width of 40 mm in the middle of the chamber. It rejuvenates at the inlet and outlet, and narrows into openings of 2 x 2 mm through which fluid flows in and out of the configuration.

This chamber design was chosen in order to achieve a homogeneous flow distribution in terms of an uniform flow rate per volume over a large portion of the flow chamber and thus to avoid dead space where insufficient oxygen fluid exchange leads to inadequate supply of hepatocytes with oxygen from the fluid side. Similar designs of flow chambers can be found in the field of tissue engineering as bioreactors to cultivate skin, cartilage and other tissues [88] and recently for hepatocytes [78].
3. Modeling of mass transfer characteristics

Figure 3.6: Model geometry of the flow chamber designed to investigate oxygen mass transfer and shear stresses under fluid flow conditions. The left part (b) shows the fine structure of the model which is distinguished by the existence of a flow volume in the center, confined by the cell volumes and the teflon membranes. The ratio of volumes corresponds to the proposed scaffold design.

The chamber in the model was simplified compared to the originally proposed configuration having only one flow channel with 2.3 mm height, representing the total volume of all microscopic flow channels (Figure 4.6.b) according to proposed bioreactor design. The hepatocyte cell compartments housed in the textile meshes were also simplified and represented by two layers of 0.85 mm height under the maintenance of the ratio between active cell volume and flow volume $V_{\text{Tiss}}/V_{\text{Flow}}$. Since it could be assumed that at least in the middle of this layer oxygen partial pressure would reach zero, oxygen consumption rate of cells was only considered zero order in order to guarantee the total oxygen consumption in the system was equal at least zero order to that in the proposed system (Figure 2.1). The height of 0.85 mm of the cell layer corresponds to a number of single textile composite scaffolds (consisting of teflon membrane, structural textile, sinusoid textile and structural textile and membrane again, cf. Figure 2.1) of 10-12, depending of filling height in the structural textile (cf. Figure 2.2). The total cell mass in the model bioreactor chamber can therefore be estimated to be approximately 5 g, which means that 10 bioreactors would be needed to reach the minimum required cell mass of 50 g.

In the model of the elementary scaffold unit in the last section, the oxygen partial pressure at the cell-teflon membrane interface could be assumed to be uniform in the
entire model. The teflon membrane therefore was replaced by a boundary condition on which the oxygen partial pressure $P_T$ was fixed. In this model, the oxygen partial pressure in the fluid volume is dependent from the location in the flow chamber, which leads to different oxygen concentration profiles in the hepatocyte aggregates. Since in terms of Fick's law the mass transfer of oxygen through the membrane $dm$ is proportional to the concentration gradient $dC$ over a distance $dL$,

$$dm \propto \frac{dC}{dL},$$  (27)

this leads to different values for the oxygen partial pressure on the cell-teflon membrane interface in different regions of the flow chamber. The presence of the membranes was taken into consideration in the model by having only two oxygen permeable membrane layers of 25 µm thickness, confining the hepatocyte aggregate volumes and the modeled flow chamber geometry on top and beneath (Fig. 4.6b). Since these model membranes represent the total membrane area which is 10-12 fold larger, the permeability of teflon for oxygen was defined 10 fold compared to the value given in Table 3.1.

In the computational model, the membrane volumes were separated from the cell volumes by porous baffles of infinitesimal thickness allowing for diffusional transport of oxygen through them. The membrane volumes themselves were also defined to only allow transport of oxygen by diffusion, but not convection. Hepatocytes and flow volumes were defined as described in the previous section and meshed by finite volumes measuring 0.57 x 0.57 x 0.57 mm, while the membrane volumes were reduced to a height of 0.0025 mm in the membrane layer.

Inlet velocities in the model were defined based on the following estimation:

In natural liver, approximately 1000 ml blood per minute is brought into contact with hepatocytes. As referred in Chapter 1, only 5-20% of the natural liver cell mass, corresponding to 50-200 g, are supposed to be sufficient to keep a patient alive. For this fraction of cell mass, under physiological conditions a blood flow rate of 50-200 ml/min would be appropriate. Since only 10% of the minimum required cell mass is housed in the model reactor chamber, this flow rate would be reduced to 5-20 ml/min.

This estimation is based on the assumption that hepatocytes in an artificial environment of a bioreactor achieve the same metabolic performance as in the natural liver. Since this can not be generally presumed, in this work 30 - 120 ml fluid are assumed to be lead through the bioreactor and brought into considerably effective contact with hepatocytes. In the above described flow chamber geometry, this corresponds to inlet velocities of 0.05 - 0.2 m/s. Reynolds numbers therefore range between 18 and 73, which indicates the laminar character of flow.
Table 3.3: Values of parameters used additionally to Table 3.1 and 3.2 to calculate the oxygen mass transfer and shear stresses under fluid flow conditions.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Unit</th>
<th>Reference/remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow rates through the model bioreactor chamber</td>
<td>30, 60, 120</td>
<td>ml min(^{-1})</td>
<td>-</td>
</tr>
<tr>
<td>Reynolds numbers(^a) at the inlet corresponding to flow rates (dV/\text{dt}) of 30, 60 and 120 ml/min</td>
<td>18.1, 36.3, 72.5</td>
<td>-</td>
<td>i.e. laminar flow</td>
</tr>
</tbody>
</table>

\(^a\) \(Re = \frac{\rho v D}{\mu}\), where \(D\) is a characteristic length for the flow volume geometry, here the height.

The numerical values for the above mentioned characteristic quantities are listed in Tables 3.1, 3.2 and 3.3 respectively. Results were generated by utilizing a StarCD steady state calculation of the constitutive equations with maximum 5000 iterations.

3.4.1. Results

Errors in the calculations were estimated for the velocity field and the scalar mass concentration of oxygen. For all inlet velocities, calculations were terminated after 5000 iterations where the residuals for the velocities were minor to \(10^{-5}\), while the residual of oxygen mass remained approximately 0.005. Since as termination criterion was set that all residuals had to be less than \(10^{-3}\), the calculations were not terminated automatically. However, this can be assumed as sufficient for convergence (cf. 3.2.1).

Flow patterns in the model. As argued above, the flow volume in this three-dimensional model for oxygen mass transfer is a result of simplifications and does not reflect the exact geometry of a flow channel in the proposed bioreactor system. The numerous microscopic flow channels kept open by the so-called sinusoid textiles are taken together in the model, resulting in a macroscopic flow volume in which the presence of a textile is neglected. The sinusoid textile in the microscopic chambers acts as a diffusor and homogenizes flow rates per volume over the entire flow chamber. Therefore, it was investigated to what extent the simplification in the model reduces the homogeneity of flow rates per volume and the transport of oxygen in the fluid.

Figure 3.7 shows the flow regime in the middle plane of the model flow chamber. The absolute velocities of fluid over the majority of the flow chamber are found to exist in a small range, indicated by a relatively homogeneous color distribution for all velocities.
Due to the magnification of the cross section in the middle of the chamber, the magnitudes of the absolute velocities decrease to 1 -2% of the inlet velocities. Peclet numbers\(^1\) for oxygen range between 0.1\(\times 10^6\) and 0.4\(\times 10^6\), depending on inlet velocities.

For all inlet velocities, it can be seen quantitatively in the vector profile of the velocity field (Figure 3.7 b) that no eddies caused by inlet phenomena influence the distribution of fluid in the model flow chamber.

\[ Pe = \frac{Lv}{D_{Ox,Pl}} \]

\(^1\) The Peclet number \( Pe = \frac{Lv}{D_{Ox,Pl}} \) is a dimensionless number which gives the ratio between mass transport by convection and diffusion of an agent in a fluid, depending on a characteristic length \( L \), the fluid velocity \( v \) and the diffusitivity of this agent in the fluid, here e.g. \( D_{Ox,Pl} \).

Oxygen mass transfer. Results of the calculations of oxygen mass transfer in the bioreactor chamber are displayed in Figures 3.8 and 3.9. Oxygen partial pressure profiles at the cell-fluid interface are graphically visualized over the entire bioreactor in Figure 3.8 for flow rates of 30 ml/min (a), 60 ml/min (b), 120 ml/min (c) and the same flow rate but locked internal membrane oxygenator (d), i.e. oxygen is transferred in the bioreactor only via oxygenated fluid. Qualitatively, for all inlet velocities a decrease of oxygen partial pressure as a function of distance from the inlet can be found. Due to the geometry of

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Figure 3.7: Flow patterns in the model chamber, visualized in the middle plane for flow rates of 30, 60 and 120 ml/min respectively. Velocity magnitudes are depicted color coded in (a), while the lower part (b) shows the velocity vector profile.
the chamber and the resulting flow profile described in the previous section, the oxygen partial pressure profile shows concentric rings around the inlet, the radius of which is diminished near the wall zones. The latter is due to lower velocity magnitudes near the walls which are defined as no-slip boundary conditions.

![Figure 3.8: Oxygen partial pressure in fluid at the cell-fluid interface for 30 (a), 60 (b) and 120 ml/min (c). If the internal membrane oxygenator is locked (d), even for a flow rate of 120 ml/min a rapid decrease of oxygen partial pressure can be observed, which indicates the importance of the teflon membrane.](image)

To give a qualitative overview about oxygen partial pressure under the flow rates of 30, 60, and 120 ml/min respectively, it is shown in Figure 3.9 against the distance from the inlet along the symmetry middle axis of the flow chamber B-B' in Figure 3.6. The dashed line marks the critical oxygen partial pressure at the cell-fluid interface. Under-shooting of this value (25.5 mmHg) leads to an undershooting of the critical oxygen partial pressure $P_C$ in the cell volume of the model. This would mean that hepatocytes are not supplied with oxygen appropriately and lose differentiated function.

For flow rates of 30 ml/min (Figure 3.9, a) and 60 ml/min (Figure 3.9, b), the oxygen partial pressure in the medium falls below the critical value at a distance of 27.5 mm and 41.5 mm respectively. Only for 120 ml/min does the partial pressure stay above the critical value along the symmetry middle axis in this part of the whole flow chamber, except in the outlet region.
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Figure 3.9: Oxygen partial pressure profiles along the middle axis of the bioreactor, as a function of distance from inlet. Flow rates are 30 (a), 60 (b) and 120 ml/min (c) and with locked the internal membrane oxygenator (d) at 120 ml/min. The dashed line marks the critical oxygen partial pressure at the cell-fluid interface, which is explained in detail in the text.

Figure 3.10: Simple model for a hepatocyte to calculate shear stresses. A force $F$ acting parallel to the base plain with total surface area $A$ leads to a shear stress of $\tau = F/A$. 
Shear stresses in the prototype bioreactor. To estimate shear stress on hepatocytes induced by flow within the model flow chamber, a simple model was set up (Figure 3.10). Specially, a hepatocyte cell was described by a cube with edge length $d$ of 10 $\mu$m resulting in a total surface of this base plain of $A=100 \, \mu m^2$. A shear force $F$ acting parallel to this plane of the cell leads to a shear stress of $\tau=F/A=G(a/d)$, where $G$ is the shear modulus.

![Figure 3.11](image)

**Figure 3.11:** Calculated shear stresses on the cell layer at the cell-fluid interface, caused by flow with flow rates of 30 (a), 60 (b) and 120 ml/min.

The shear stresses resulting from flow rates from 30, 60 and 120 ml/min respectively are depicted in Figure 3.11. For all flow rates, shear stresses at the inlet and outlet areas have
their maximum, according to the maximum in fluid velocities. In the middle of the flow chamber, where velocity vectors are parallel according to Figure 3.7, shear stresses have a maximum of approximately 3.5 dyn/cm² for a flow rate of 30 ml/min, approximately 6.5 dyn/cm² for 60 ml/min and approximately 12 dyn/cm² for 120 ml/min.

3.4.2. Discussion
Peclet numbers for oxygen in the model range between $0.1 \times 10^6$ and $0.4 \times 10^6$, depending on inlet velocities. This means that mass transport of oxygen in the model chamber is clearly dominated by convective processes and that diffusional transport of oxygen from the inlet into the chamber plays no important role in the model chamber. In contrast to the model here, in the originally proposed configuration, cross sections are locally reduced by the presence of the sinusoid textile. As seen in Chapter 3.5, this has an enhancing effect on local flow velocities, and the Peclet number will therefore increase. Consequently, mass transport will even more be dominated by convection.

The illustrated oxygen partial pressures in the fluid flowing through the model chamber are therefore due to the oxygen sink in the cell volume corresponding to consumption of oxygen by hepatocytes in the bioreactor. The higher the flow rate, the more oxygen is transferred into the bioreactor via flow per unit time and the more oxygen remains available in the fluid even in the outlet regions of the flow chamber. In the case of a closed internal membrane oxygenator and a concurrent high flow rate of 120 ml/min, oxygen partial pressure in the fluid decreases even more rapidly than with low flow rates and opened membrane. This indicates the importance of the internal membrane oxygenator for supply of hepatocytes in the bioreactor. Without it, oxygen transport would only occur via fluid flow. This would lead to conditions in the bioreactor chamber, under which hypoxic damage would occur and hepatocyte respiration would cease.

The detected decrease of oxygen partial pressure in the fluid from inlet to outlet represented an analogon to the natural zonation of the liver. The oxygen partial pressure in blood in rat livers has been reported to grade from the periportal zone (60 mmHg) to the perivenous zone (35 mmHg) [3].

The model calculations indicate that in the proposed bioreactor configuration, a flow rate of at least 120 ml/min is necessary to appropriately supply 5 g hepatocyte mass with oxygen. To support the minimal required cell mass of 50 g hepatocytes, consequently 1200 ml oxygenated fluid were necessary, which is nearby the physiological flow rate of approximately 1000 ml/min in the natural liver.

To illustrate the consequences of the results achieved for oxygen partial pressure in the model flow chamber on hepatocytes located in the proposed bioreactor configuration, the oxygen partial pressure profile in the cell volume was calculated utilizing the
one-dimensional model set up in Chapter 3.2. Oxygen partial pressure in the hepatocyte aggregates located in a structural textile compartment is shown against the distance from the cell fluid interface at a distance of 20 mm from the outlet on the symmetry middle axis B-B of the flow chamber in Figure 3.12. The dashed line shows the critical oxygen partial pressure under which hypoxic damage occurs to the cells.

**Figure 3.12:** Oxygen partial pressure profiles in the hepatocytes at conditions at the cell-fluid interface resulting from flow rates of 30 (a), 60 (b) and 120 ml/min (c) and for a situation where the internal membrane oxygenator is active, but no oxygen in fluid (d).

Under the conditions caused by inlet velocities of flow rates of 30 ml/min (Figure 3.12a) and 60 ml/min (Figure 3.12b), the slices of hepatocytes undersupplied with oxygen are approximately 25 µm and 15 µm respectively thick. As predicted, for a flow rate of 120 ml/min (Figure 3.12c), oxygen partial pressure is higher than $P_C$ over the entire cell
layer. For the same velocity, but locked oxygenator, at this location in the chamber no oxygen at all could be supplied to the cells.

Part d of this figure shows a profile which occurs when the internal membrane oxygenator is active, but no further oxygen supply from the fluid side occurs. This situation could be realistic far away from the inlet at low flow rates of smaller than 30 ml/min as well as for non-oxygenated fluid. In this situation, only cells approximately 10 m from the cell-membrane interface are supplied with enough oxygen.

The model assumes that all single hepatocyte layers in the originally proposed textile scaffold bioreactor configuration can be taken together under conservation of $\frac{V_{\text{Tiss}}}{V_{\text{Flow}}}$. The large volume of all hepatocytes taken together in this model causes higher oxygen fluxes per surface area through the cell-fluid interface and could therefore result in lower oxygen partial pressure at the interface as in the proposed configuration would be found. This would mean that in the originally proposed configuration, the oxygen partial pressure would not be reduced in the same extend. However, this effect could eventually be compensated by the fact that also fluid volumes of the proposed configuration are taken together in this model resulting to a larger oxygen reservoir as in the proposed model.

The magnitude of the minimum flow rate depends on certain factors. For the oxygen consumption rate of hepatocytes, the highest value found in literature (50 nmol/sec/cm$^3$) was assumed to be true. Regarding the fact that even primary hepatocytes could be metabolically limited after the process of separating them from a whole liver and culturing them in a non-physiological environment, this value could be clearly reduced. Furthermore $\frac{V_{\text{Tiss}}}{V_{\text{Flow}}}$ (cf. Chapter 1) was chosen in the model assuming that in the real textile composite scaffold configuration the compartments build up by the structural textile were completely filled with cells. If this is even possible for technical and biological reasons will be investigated in Chapter 4. However, less hepatocytes in the bioreactor would lead to less total oxygen consumption and therefore cause a decrease of the minimum required flow rate for sufficient oxygen supply per bioreactor unit, but would increase total number of bioreactors necessary to achieve minimal required cell mass.

Statements in literature about effects of shear stress on hepatocytes vary. On the one hand, shear stress below 5 dyn/cm$^2$ seem to promote differentiated function [51], on the other hand it has been reported that also shear stresses of 10 dyn/cm$^2$ do not adversely affect hepatocyte function for at least 12 hours [53]. For low flow rates of 30 and 60 ml/min respectively, shear stresses most probably would not affect hepatocytes, but oxygen transport would be the limiting factor. If the flow is increases to 120 ml/min to get better oxygen supply, the resulting shear stress could eventually exceed a physiologically reasonable magnitude. As long as the literature is not clear in the point of shear
3. Modeling of mass transfer characteristics

stresses, a balance between the appropriate flow rate and reasonable shear stresses is difficult to achieve.

A possibility to generally prevent cells from shear stresses is the application of the Disse textile, as depicted in Chapter 2. The latter represents an additional layer of a fine meshed textile between the hepatocytes and the fluid flow in the bioreactor chamber and which could optionally be utilized as scaffold for a coculture system with non parenchymal cells. This textile would act as an additional transport barrier for agents from the fluid stream to the cell compartments and would have effects on oxygen mass transfer which had to be investigated in detail.

The high flow rates needed to avoid a decrease of oxygen partial pressure in fluid flow away from the inlet could be avoided by a general modification of the proposed textile scaffold bioreactor, here only described in brief:

Instead of piling up the scaffold units symmetrically around the flow channel as showed in Chapter 2, one side of the flow channel could be confined only by an oxygen permeable membrane as depicted in Figure 3.13. From this side, oxygen would diffuse directly into the fluid flow, potentially keeping oxygen partial pressure in fluid constant over the entire bioreactor chamber.

Figure 3.13: Design modification of the proposed textile scaffold bioreactor system as approach to keep oxygen partial pressure constant over the entire flow chamber by direct internal oxygenation of fluid flow.
3.5. Effect of the sinusoid textile on mixing and shear stresses

For the calculations of this section, the scale of the geometry of the computational model was refined again from the macroscopic flow chamber to the microscopic scale of a few cell aggregates in the proposed microstructured textile composite scaffold. Structural detail was added by taking into consideration that flow channels are kept open by a textile, the so-called sinusoid textile. This textile has an influence on flow of fluid through the channels. It modifies the flow regime characterized by its velocity vector profile by mixing the fluid which could have an influence on oxygen partial pressures in the flow chamber. Furthermore, the textile constricts the cross section of the flow channels leading to an increase of velocity magnitude at the strictures. This causes shear stresses on the cells in the upper layer of the hepatocytes which could potentially exceed physiological values unobjectionable for the cells. Therefore beyond velocity vector profile and oxygen partial pressure profile also shear stresses were investigated in this section.

3.5.1. Model setup and calculations

In order to include the spacial regularity of the flow-volume embedded textile, now the concept of the elementary scaffold unit discussed in Chapter 3.3 is extended by regarding four such units on both sides of the flow volume (Figure 3.14). Around the flow volume, measuring 700 x 700 x 100 µm, cuboids of 200 x 200 x 100 µm were attached, representing the cell volume, the hepatocyte aggregate housed in the compartments of the structural textile (Figure 3.14, top). The geometry of a textile with filament diameter of 50 µm and a mesh size of 100 µm was subtracted from the flow volume, as signified in Figure 3.14, down. The resulting geometry was meshed by a finite volume model using automatic meshing functions of StarCD. Specifications for the baffles, separating flow and cell volumes, as well as the formalism to define oxygen as a consumable scalar parameter are identical to the above described model.

Fluid flow was included in this model by defining an inlet surface through which fluid enters the flow volume with a defined inlet velocity \( v_{\text{in}} \). According to the calculations for the entire bioreactor in the previous section, the flow rate was defined to be 120 ml/min. The inlet velocity \( v_{\text{in}} \) was therefore defined to be \( 0.2 \times 10^{-2} \text{ m/s} \), which was the velocity found in the majority of the flow chamber for a flow rate of 120 ml/min.
To refer to the fact that the elementary unit is surrounded by congeners units, symmetrical boundary conditions were applied on the surfaces laterally delimiting the fluid volume except the inlet surface. Results were generated by utilizing a StarCD steady state calculation of the described model with 5000 iterations. The model was run laminar and turbulent, the latter with an $\kappa$-$\varepsilon_D$ model using the default settings of Star-CD.

The numerical values for the above mentioned characteristic quantities are listed in Tables 3.1, 3.2, and 3.3 respectively. Results were generated by utilizing a StarCD steady-state calculation of the constitutive equations with maximum 5000 iterations.
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3.5.2. Results

Errors in the calculations were estimated for velocity and the scalar mass concentration of oxygen. For all inlet velocities, calculations were terminated after 5000 iterations where the residuals for the velocities were smaller than $10^{-5}$, while the residual of oxygen mass remained approximately 0.006. Since as termination criterion was set that all residuals had to be smaller than $10^{-3}$, the calculations were not terminated automatically. However, this can be assumed as sufficient for convergence (cf. Chapter 3.2.1).

**Flow patterns.** Figure 3.15 shows the results of the calculations of the flow patterns in the above described model. Velocity magnitude in a section perpendicular to the cell-fluid interface (Figure 3.15a) is depicted color coded in Figure 3.15b for the model with sinusoid textile (left) compared to the model without textile (right).

Velocities in the model without textile stay constant at the value of the inlet velocity, only at the boundary conditions at the wall velocity decreases. In the flow volume with sinusoid textile on the other hand, an increase of velocity up to 0.006 m/s can be detected in the turbulent (not shown) as well as in the laminar calculations. From those maximum velocities in the described flow volume geometry with textile, a Reynolds number of 290 can be calculated. The flow is therefore still laminar, and thus only results for the laminar calculations will be presented in the following.

**Oxygen partial pressure profile.** Oxygen partial pressure at the cell-fluid interface (Figure 3.16 a) is depicted color coded in Figure 3.16 for the model with sinusoid textile (Figure 3.16 b) and without textile (Figure 3.16 c) under fluid flow conditions.

Oxygen partial pressure at the interface in presence of the textile was approximately 35 mmHg by mean, indicated by the color of the majority of the interface surface (Figure 3.16 b). On the entire surface, it reached a maximum of approximately 35.7 mmHg were the filament of the sinusoid textile came nearest to the interface and at the borders of the interface surface. A minimum of 34.8 mmHg was observed where the distance between filament and interface surface had its maximum.

In absence of the sinusoid textile in the model, oxygen partial pressure was approximately 37 mmHg over the majority of the surface, while a maximum of approximately 37.4 mmHg was only observed at the boundaries of the interface surface.
Figure 3.15: Velocity magnitude (b) and vector profile (c) of fluid flow in a section of the described model, with (left) and without (right) textile in the flow channel.
Figure 3.16: Oxygen partial pressure in a section plane at the cell-fluid interface (a) in models with textile (b) and without textile (c).
Shear stresses. In the proposed textile composite bioreactor configuration, hepatocytes form aggregates confined by the structural textile and are perfused from above by fluid. Flow of fluid leads to shear forces on the upper layers of cell aggregates. This can have, related to the magnitude of the shear stresses resulting from this forces, positive or negative effects on differentiated function of hepatocytes. Design Specification 3 accommodates this by claiming that shear stress on hepatocytes should be kept in a physiological range by technical measures.

Shear stresses at the cell fluid interface are depicted color coded in Figure 3.17 for the model with sinusoid textile (Figure 3.17 b) under fluid flow conditions.

Shear stresses at the cell-fluid interface (Figure 3.17 a) in presence of the textile ranged between 2 dyn/cm$^2$ and approximately 32 dyn/cm$^2$ (Figure 3.17 b). The maximum was reached were the filament of the sinusoid textile came nearest to the interface, while a minimum was observed where no underlying filament restricted the open cross section for flow.

Figure 3.17: Calculated shear stresses in a section plane at the cell-fluid interface (a) in the model with textile (b).
3.5.3. Discussion

In the model without the sinusoid textile, oxygen partial pressure under flow conditions over the majority of the cell-fluid interface was with approximately 37 mmHg found to be slightly higher than in the corresponding static model (36.7 mmHg) discussed in Chapter 3.3. The reason for this could be that under flow conditions, oxygen partial pressure decrease caused by diffusion of oxygen in the cell volume was reduced by convective transport of oxygen in those areas. However, this small difference of only 1% in oxygen partial pressure would barely make a significant difference measurable in biological experiments.

Minimum values of oxygen partial pressure in the model with sinusoid textile were lower as predicted values for the model without textile. This decrease could be explained by the fact that the presence of the sinusoid textile diminishes the total volume available for fluid. Consequently, the amount of oxygen present in the flow volume decreases, so that in equilibrium a lower oxygen partial pressure at the interface attunes. However, this decrease to approximately 95% of values calculated for the model without the textile can be assumed not to influence oxygen partial pressure profile in the cell aggregates greatly.

Furthermore, oxygen partial pressure patterns at the cell-fluid interface were not as homogeneous as in the model without textile. The pattern reflects the presence of the textile and therefore indicates the mixing effect on fluid. This is supported by the velocity vector profile in the textile model in Figure 3.15. The non parallel velocity vector components in direction to the cell-fluid interface lead fluid, the oxygen partial pressure of which was not reduced by consumption of oxygen by the cells, from the center of the flow volume to the interface. The fluid is mixed and local elevation of oxygen partial pressure at the interface is the consequence.

It is evident and can be also be seen in the velocity profile that absolute velocities in the textile model increased where the open cross section for fluid flow was reduced by the textile, which led to an elevation of shear stresses on the cell-fluid interface. Compared to the values in the literature described above, the values of up to about 34 dyn/cm² most probably exceed the physiological range and would disturb differentiated cell function. A reduction of shear forces on hepatocytes could be achieved by the application of a further textile as proposed in Chapter 2. The latter represents an additional layer of a fine meshed textile between the hepatocytes and the fluid flow in the bioreactor chamber.

Based on the analysis presented above, it can be said that the presence of the sinusoid textile, which is necessary to keep open a flow channel for fluid flow in the proposed bioreactor configuration, has positive effects on oxygen mass transport by mixing the
3. Modeling of mass transfer characteristics

fluid. However, the increase of oxygen partial pressure due to this mixing seems to be very small. It can therefore not be expected that a precise increase of maximum supportable cell layer thickness would result. Elevation of shear forces is an undesired but inevitable effect which can be avoided by the application of the Disse textile, described in Chapter 2.

3.6. Summary and conclusions

The one-dimensional model for oxygen mass transfer and consumption derived from an established Michaelis-Menten model in the first section of this chapter indicated, that in the proposed textile composite scaffold bioreactor system hepatocyte aggregates can be appropriately supplied with oxygen and therefore hypoxic damage of cells can be avoided in general. Utilizing a three-dimensional model implemented in a numerical solver algorithm, these results could be verified and thus indicated the potential of the model to be extended to the situation of fluid flowing through the configuration. It could be shown that the sinusoid textile, which keeps open a channel for fluid flow, mixes fluid, but does not clearly increase oxygen partial pressure boundary conditions at the cell-fluid interface. Enhanced shear stresses caused be acceleration of fluid in strictures between the filaments and cell-fluid interface could exceed physiological range and necessitate the application of a further fine meshed textile to separate fluid flow from cell compartments.

Scaling up the model to dimensions of a realistic flow chamber geometry as described in Chapter 2.4 allowed prediction of oxygen supply of hepatocytes not only at the inlet area where oxygen partial pressure in fluid flow actually could supposed to be constant, but also away from the inlet, where partial pressure could be reduced due to consumption of oxygen by the cells. Under the assumptions of completely filled cell compartments and the highest oxygen consumption rate found in literature, a minimum required flow rate of 120 ml/min through the bioreactor configuration was found to appropriately oxygenize 5 g of hepatocytes. To reach the minimum required cell mass for liver support (50 g), therefore 10 bioreactor modules should be operated serial or parallel in clinical use, resulting in a physiological fluid exchange rate of 1200 ml/min.

As a conclusion it can be stated that the presented results indicate that the proposed textile composite scaffold bioreactor could satisfy the specifications regarding oxygen supply of hepatocytes (*Specification 4*), the overall mass transfer and homogeneity of flow (*Specification 5*) as well as resulting shear forces on the cells (*Specification 3*) described in Chapter 2.
Shear stresses acting on cells in the prototype bioreactor system when applying calculated minimum flow rate probably exceeded physiological range. This minimum flow rate is calculated under the presumption that several layers of hepatocytes in the bioreactor result in a total cell mass of 5 g per reactor module. In the prototype bioreactor described in Chapter 2.3, only one layer of cells is present. Flow rates in the biological testing experiments could therefore be reduced, avoiding high shear stresses on cells without application of the additional Disse textile. The biological experiments are described in the next chapter.
4. Biological testing

This chapter deals with the testing of the biological feasibility of the textile composite scaffold bioreactor system proposed in this work. The first section describes the experiments and results of toxicity testing of employed materials for scaffolds and bioreactor housing and their influence on hepatospecific functions. In the second section, a study to optimize the seeding process of hepatocytes on the scaffolds in order to achieve high density cultures in terms of filled structural textile compartments is reported. The third section finally describes preliminary tests of the biological performance of the prototype bioreactor under clinically realistic flow conditions over 1 week.

4.1. Composite scaffold material screening

The materials employed for scaffolds and bioreactor housing could potentially have toxic effects on hepatocytes. This could restrict adhesion on scaffolds, viability of attached cells and energy metabolism of living cells. Furthermore, materials could affect cell specific functions by causing a dedifferentiation of hepatocytes. Those aspects are investigated in this section for the materials of the proposed textile composite scaffold bioreactor system.

4.1.1. Materials and Methods

Scaffolds and housing materials

As reference scaffold, a gas permeable membrane of expanded teflon (TM) was used (bioFOLIE 25®, IVSS, Germany), which has been proven to enhance differentiated function of hepatocytes, as discussed in detail in Chapter 2.2. TM reference was utilized in form of 60 mm dishes with this membrane ground (Petriperm®, IVSS, Germany). It is as a basis part of the proposed textile scaffold bioreactor described in Chapter 2. As a further, more common control tissue culture polystyrene (TCPS) 60 mm dishes (Primaria, Becton Dickinson, USA) which were collagen coated (type I, 120 g/ml, Serva, Germany) were utilized.

In order to investigate potential toxic effects of PET textile scaffolds in the proposed configuration, woven PET textile fabrics (Sefar, Switzerland) were employed. Open textile scaffolds (Pore size 200 and 300 μm, open area approximately 55%, wire diameter 50 μm, plain weave pattern) where utilized for investigation of toxic characteristics of woven scaffolds meant to serve as the structural textile as described in Chapter 2.1, while dense textile scaffolds (Pore size <5 μm, open area 1%, filament diameter 34 μm, twill
weave pattern) were analyzed as surface textiles on which cells directly adhere. Textile samples were cut to the same size of the Petriperm dishes and clamped in these dishes in contact to the membrane. The resulting textile composite scaffolds, named as Petex 5, Petex 200 and Petex 300, generated culture situations analogous to the proposed scaffolds described for the novel bioreactor system, where hepatocytes adhere on the teflon membrane, housed in compartments created by the structural textile (cf. Petex 200 and Petex 300) or are cultured alternatively on a dense PET textile meant as an anchor for bioactive coatings respectively. 

Bioreactor housing extraction experiments were carried out in 24 well plates (NUNC, Nalge Int., Denmark). Materials investigated for the housing were polycarbonate (PC, Bayer, Germany) and polystyrene (PS, Bayer, Germany). As positive control, PVC (Portex, SIMS, United Kingdom) was used. As a reference material, TCPS, either collagen coated or not, was utilized.

**Isolation and culturing of hepatocytes**

Hepatocytes were isolated by a two-step collagenase perfusion method [89], modified as described in [90]. Briefly, the liver was perfused in situ with calcium-free, bicarbonate-buffered (pH 7.4) Hank’s balanced salt solution (HBSS, Amimed, Switzerland) at 37°C for 5 min. The liver was then carefully removed and perfused with a recycling solution of oxygenated buffer containing Ca^{2+} (5 mM) and collagenase (100 U/ml, type I CLS, Worthington Biochemical, USA) for 10 min. After disruption of the liver capsule, the cells were isolated and suspended in Williams Medium E (WME, Amimed, Switzerland). After the suspension was filtered through a Cellerctor tissue sieve (Bellco, USA) with the sequential use of 280 and 190 m mesh size screens, the hepatocytes were centrifuged 2 times for 2 min at 350 rpm (20g). The supernatant, mainly containing non-parenchymal cells and cell debris, was removed, and the pellet was washed twice with ice-cold WME. Typically, 82-92% of the isolated hepatocytes were viable by a trypan blue dye exclusion test.

For toxicity tests of materials, cells were subsequently seeded on scaffolds prepared in 60 mm dishes as described above at a cell density of 0.5, 1 and 2x10^6 cells/ml WME supplemented with penicillin (100 U/ml), streptomycin (0.1 mg/ml), insulin (100 nM), dexamethasone (100 nM) and 10% fetal calf serum, 2 ml suspension per dish, resulting in 3.5, 7 and 14x10^4 cells/cm². The cells were allowed to attach for 3 h in a 95% air-5% CO₂ atmosphere at 37°C in an incubator. Subsequently, the medium containing non-attached cells was replaced by fresh serum free medium supplemented with antibiotics and hormones. Hepatocytes were maintained up to 5 days in culture with daily exchange of medium before protein, neutral red and MTT tests were assessed as described beneath. Results are presented in Chapter 4.1.2.
For bioreactor housing material tests, cells were seeded in 24-well plates without textile scaffolds, at a density of $1 \times 10^6$ cells/ml in the same medium, 0.5 ml/well, resulting in 2.5x$10^5$ cells/cm$^2$. The cells were also allowed to attach for 3 h under the same conditions as described above, but after removing the medium containing nonattached cells, 0.5 ml material extracted medium was added. This was obtained by extracting 10 pieces of 1 cm$^3$ PC, PS or PVC per 100 ml serum free medium supplemented with antibiotics and hormones for three days before the start of experiments in the same incubator used for cell culture. Results are presented in Chapter 4.1.2.

**Total protein**

Total protein assay was utilized to quantify cell mass attached on the scaffolds. Protein mass approximately corresponds to cell number, dependent on the degree of differentiation of a cell [91]. Total protein was determined by a commercially available bicinchoninic acid (BCA) protein assay kit (Pierce, USA). At the end of the experiments, medium was removed completely from the samples and 1 ml of a 1% triton-X100 in PBS solution was added for 2 h in order to remove the hepatocytes from the samples and to lyse them. Each 25 1H$_2$O and lysate per sample were transferred in 96 well plates (NUNC, Nalge, Denmark). As standard, bovine serum albumin (BSA), was used in concentrations of 1500 g/ml, 1000 g/ml, 750 g/ml, 500 g/ml, 250 g/ml, 125 g/ml and 25 g/ml in 1% triton solution. Reagents A and B of the assay were mixed 50:1 and 200 l of this solution were added to the samples in the 96 well plate. Photometric examination at $\lambda=550$ nm in a spectrometer (Ultramark BioRad, USA) was performed after 30 min incubation at 37°C. Data processing was done by the standard software MikroPlate Reader 5 (Molecular Devices Corp., USA).

**Neutral Red test (NR)**

Viability of cells attached on the samples was measured by the neutral red test [92]. Living cells actively take up this inert dye and accumulate it in the lysosomes. The amount of accumulated dye can be determined by spectroscopy.

NR solution (Sigma, USA) was diluted 1:60 in PBS and 600 l (24 well plate: 150 l) were added to the samples and incubated for 30 min at 37°C. Subsequently, cells were fixed by adding 600 l (24 well plate: 150 l) Karnovsky solution (4% paraformaldehyde and 5% glutaraldehyde in PBS) for 5 min. Samples were rinsed with PBS, PBS was removed and 1.2 ml (24 well plate: 500 l) lysis solution (0.4%HCl in isopropanol) were added for 15 min for color developing. Optical densities were measured at $\lambda=540$ nm in a spectrometer (Uvikon 820, Kontron Instruments, Italy).
4. Biological testing

**MTT test**

MTT test was also utilized to investigate viability of cells [93]. MTT dye, a tetrazolium salt, is reduced to formazan in cells by mitochondrial succinate dehydrogenase, which is only active in viable cells, and the activity depends on grade of differentiation and metabolic activity of cells. Therefore, energy metabolism of viable cells attached on the scaffolds was assessed by this test. Formazan has a maximum of absorbance in the range of $\lambda=550-600\text{ nm}$ and can be spectroscopically quantified.

500 l MTT solution, 5 mg/ml MTT (Sigma, USA) in PBS (pH 7.4) were added to the samples in the dishes (24 well plate: 125 l) and incubated for 20 min at 37°C. After removing the solution, the same amount of lysis solution (90% ethanol, 10% HEPES-NaCl) was added. Further 15 min later (room temperature) optical densities were measured at $\lambda=560\text{ nm}$ in a spectrometer (Uvikon 820, Kontron Instruments, Italy).

**Albumin ELISA**

Liver-specific function of hepatocytes was investigated by albumin synthesis, which is an indicator for the differentiated protein synthetic function of hepatocytes [94]. Medium supernatants were analyzed with a sandwich enzyme-linked immunosorbent assay (Sandwich-ELISA; sandwich: albumin is fixed between two antibodies).

Medium supernatant was collected each day, stored at -20°C and defrosted shortly before the analysis. 96 well plates (NUNC-Immuno Plate MaxiSorp Surface, Nalge, Denmark) were coated with 50 l rabbit anti rat albumin (ICN Biomedicals, Germany) diluted 1:250 in PBS. After 2 h at room temperature the antibody was removed and the samples were blocked with 350 l/well BSA/PBS overnight at 5°C.

As a standard, a set of samples of 5 to 65 ng/ml rat serum albumin (RSA, ICN Biomedicals, Germany) in PBS were utilized. Standards and medium supernatant samples, which were diluted 1:500, 1:1000 and 1:2000 respectively, were incubated in the prepared 96-well plate (50 l/well) for 2 h at room temperature. After washing the samples 3 times with 200 l PBS/well for 2 min, samples were incubated for further 2 h at room temperature with 50 ml/well peroxidase conjugated rabbit anti rat albumin (ICN Biomedical, Germany), 1:5000 in 3%BSA/PBS. Samples were then washed again 3 times with 200 l PBS/well for 2 min, before 50 l/well color developer substrate (10 ml substrate buffer, 4 mg ABTS and 6 l H$_2$O$_2$) was added. After 20-40 min in the dark, samples developed green color and were measured at $\lambda=415\text{ nm}$ with a spectrometer (Ultramark BioRad, USA). Data processing was done by the standard software MikroPlate Reader 5 (Molecular Devices Corp., USA).
4. Biological testing

4.1.2. Results

Toxicity tests of materials

Results of 24 h short-term toxicity tests of textile composite scaffold systems compared to TM reference and collagen coated TCPS are shown in Figure 4.1. After 24 h, on dense PET textiles (Petex 5) protein, which corresponds to the total cell mass in the well, was on average 20% lower than on TM reference, where 0.881 mg per dish were found. On open PET composite scaffolds protein was in the same range (Petex 200) and slightly higher (Petex 300) than on TM reference. On TCPS, both with and without collagen coating, less hepatocytes attached, approximately 70% of TM reference on average.

Figure 4.1: Toxicity tests, Protein, NR/Protein and MTT/Protein ratio, of potential materials for utilization in the proposed textile composite scaffold bioreactor on hepatocytes after 24 h. 100% protein is equal to 0.881 mg/well (n=3, *: n=6).

While viability as indicated by the NR uptake test (NR/Protein ratio) on open PET textile composite scaffolds was on average not much lower than on TM reference, on collagen coated TCPS and on Petex 5 it was higher. On these samples, a similar tendency was found for MTT/Protein ratio, indicating enhanced energy metabolism of hepatocytes, approximately 10 - 15% higher. On dense PET textile composite scaffolds, energy metabolism was found to be restricted compared to a TM reference, by means of sub-
stantially lower MTT/Protein ratio levels, 50 - 75% compared to TM reference, but comparable to those of hepatocytes on collagen coated TCPS.

The following experiments were performed to determine potential long-term toxic effects of materials on hepatocytes. After 1 day, the same trend as described above was detected (Figure 4.2): cell attachment on scaffolds, by measurement of total protein, was not different on open and dense PET textile scaffolds as well as on collagen coated TCPS and TM reference. Both a higher NR/Protein and MTT/Protein ratio signal were found for collagen coated TCPS and Petex 5, indicating higher viability and enhanced energy metabolism. After 5 days, no substantial differences in viability and energy metabolism could be detected for all scaffolds. At the same time, total protein was found to be on average slightly higher on open and dense PET textile composite scaffolds than on TM reference.

Figure 4.2: Total protein, NR/Protein and MTT/Protein ratio after 24 h and 5 days of hepatocyte culture on sample scaffolds. 100% protein is equal to 0.819 mg/well. (n=3)
4. Biological testing

Liver specific functional tests

Figure 4.3 shows the results of albumin synthesis on scaffolds over 4 days. On Petex 5 and collagen coated TCPS, initial values are on a lower level on day 1 compared to the TM reference and Petex 200, where 260 - 280 ng per dish and day were measured. While on these scaffolds a slight increase of albumin synthesis is indicated on day 2, on TM reference and Petex 200, synthesis generally decreases over 4 days. However, on day 3 and 4 no substantial differences between all samples can be detected, and absolute values are threefold lower compared to TM reference on day 1.

![Figure 4.3: Albumin synthesis of hepatocytes cultured on PET textiles over 4 days (n=3).](image)

A detailed comparison of scaffold materials concerning albumin synthesis is shown in Figure 4.4, where daily albumin synthesis in % of the albumin synthesis measured on the TM reference is depicted. As expected, due to the similarity of surfaces of the teflon membrane and the open Petex 200 scaffold, no variation between this and the 100% TM reference value can be detected. On Petex 5, albumin synthesis is lower than on Petex 200 on day 1, but approximately reaches this level on the subsequent days. However, average albumin synthesis on Petex 5 is higher than on collagen coated TCPS over all 4 days.
4. Biological testing

**Figure 4.4:** Detailed comparison of albumin synthesis on different scaffold materials: TM reference (100%), Petex 200, Petex 5 and collagen coated TCPS. (n=3)

**Bioreactor housing material screening**

Results obtained from the extraction experiments of the potential bioreactor housing materials polycarbonate (PC) and polystyrene (PS) are shown in Figure 4.5. For each of the functional parameters Protein, NR and MTT respectively, no substantial differences were detected after 5 days between PC, PS, and TCPS references. The positive control of PVC extraction cultured hepatocytes shows clearly lower levels of these markers and therefore indicated toxicity of PVC to hepatocytes, which is typically attributed to extraction of plasticizers in the PVC.
4. Biological testing

Figure 4.5: Influence of potential housing materials for the bioreactor chamber on functional hepatocyte parameters Protein, NR and MTT (n=3).

4.1.3. Discussion

As outlined in Figure 4.1, total protein measurements of hepatocytes on all PET textile composite scaffolds showed that the presence of a textile PET mesh, either dense, i.e. covering the TM as a surface textile, or open, i.e. only acting as a structural textile creating compartments on the remaining TM surface, did not restrict cell adhesion on the samples. Obvious is the increase of viability and energy metabolism on collagen coated TCPS and Petex 5 after 24 h compared to TM reference¹. On collagen coated TCPS, this increase could possibly be explained by positive biologic surface signalling effects of the extracellular matrix protein collagen. Notably, this effect was present even though the dense Petex 5 was not coated with collagen and it showed similar effects on hepatocytes. The reason for this remains unclear, it could potentially be due to the surface structure of Petex 5, which can be described in terms of porosity and roughness. Ranucci et al. [95]

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¹ Since it was not aim of this section to demonstrate the benefit of the structural textile on the TM, but only to show that the materials are not toxic, no t-tests were performed in order to substantiate significance.
recently reported that hepatocyte function depends on pore size of collagen foams. Enhanced function was found when pore sizes were smaller than characteristic cell sizes. In the short-term experiments, a decrease in MTT/Protein ratio was detected on open PET textiles (Petex 200 and Petex 300 respectively) after 1 d, indicating reduced viability on these scaffolds (Figure 4.1). In the long-term experiments carried out in the second series, this effect was not confirmed. MTT/Protein ratio on Petex 200 was found to be on the same level as on TM reference (Figure 4.2). It therefore remains unclear whether negative short-term effects on energy metabolism have to be expected on open textile composite scaffolds or not. The difference between the two experimental series could be also due to variabilities in different primary rat hepatocyte populations.

While NR/Protein and MTT/Protein ratio are after 5 d on the same level for all tested scaffolds, after this time higher total protein can be detected on Petex 5 and Petex 200. Although this is only 20% on average, with a standard deviation of 5-10%, this could be due to production of extracellular matrix or be interpreted as a sign of proliferation, which for highly differentiated hepatocytes typically follows a process of dedifferentiation [97]. The latter possibility is supported by the fact that albumin synthesis, which is a specific function of differentiated hepatocytes, on PET textile composite scaffolds decreases from day 3 onward constantly. It reached, with 60 - 80 ng per dish and day on average, a level on day 4 which is only approximately 25% of TM reference on day 1. On the other hand, even on TM reference this level is similar on day 4, and even lower on collagen coated TCPS, while for those samples no increased total protein was found.

An observation which contradicts the finding of enhanced viability and energy metabolism on collagen coated TCPS and Petex 5 after 1 day is that on those samples lower albumin synthesis rates were found compared to the TM reference and Petex 200 at the same time. TCPS and Petex 5 increase on day 2, and then follow the trend of TM reference and Petex 200 to decrease and reach the low level of them on day 4. As discussed above, this could be due to variabilities of different hepatocyte populations or surface signalling effects of biologic nature mediated by collagen or mechanical character caused by porosity and roughness of dense textile composite scaffolds. At least, it can be said that no dramatic impairment concerning albumin synthesis of hepatocytes cultured on the materials proposed for the novel textile composite scaffold bioreactor design can be detected in comparison to TM reference. The latter is known to have the potential to promote differentiated hepatocyte function [48].

More clear results were obtained investigating potential bioreactor housing materials in extraction experiments. When hepatocytes were cultured on TCPS in PC and PS extracted medium for 5 days, total protein, NR and MTT were found on the same level as cultured under non-treated medium which means, that no toxic effects of those materials
were observed. In comparison, those parameters were reduced to approximately 30-40% when culturing hepatocytes in PVC extracted medium, which is well known for its cell toxicity. As a conclusion from this section it can be drawn, that long-term and short-term toxicity tests of hepatocytes on potential scaffold materials and in medium extracted to potential housing materials showed no toxic effects on cells. The materials are therefore suited for application in the proposed textile composite scaffold bioreactor design. However, further work has to be done to optimize long-term differentiated hepatocyte function on the scaffolds, e.g. by modifying scaffold surfaces with biomimetic coatings [49].

4.2. Seeding optimization

In this section, investigation of the seeding process of hepatocytes on textile composite scaffolds as proposed in this work is described. The aim was to evaluate methods which enhance the yield, i.e. the ratio of attached cells on the samples to inoculated cell number, in order to guarantee a possibly economic handling with the available cell mass. Those methods can be divided in two approaches:

First, treatment of the surface of the scaffolds can modify adhesion characteristics for cells. Therefore scaffolds were treated by sonication and vacuum in order to optimize wetability of the textiles. Furthermore, scaffolds were treated with oxygen plasma in order to modify their hydrophilicity and with it cell adhesion characteristics. These scaffold treatment tests were performed on small test scaffolds produced as inlays for 24 well plates.

Second, homogenization of cell suspension inoculated on the samples should guarantee that cells sedimenting from cell suspension form a uniform distribution on the scaffold. These scaffold seeding tests were either performed on small test scaffolds which were agitated while cells sedimented and on large scale bioreactor scaffolds, assembled in the prototype design described in Chapter 2.3.

4.2.1. Materials and Methods

Cell culture

Seeding experiments were performed using the cell line HepG2. Immortalized cell lines like HepG2 represent a more homogeneous starting material than primary hepatocytes, which can show notable variabilities between different isolations concerning viability and adhesion characteristics. Furthermore, since cell division of such cells is practically unlimited and thus a steady supply of fresh cells is ensured, problems with the availabil-
ity of primary rat hepatocytes in large amounts were circumvented and unnecessary animal experiments were avoided.

For the cultivation of HepG2 cells, a minimum essential medium (MEM) was prepared in quantities required for one week, with 10% FBS, 1% L-glutamine, 1% nonessential amino acids, 1% Na-pyruvate and 1% penicillin (100 U/ml) / streptomycin (100 U/ml). All ingredients and medium were obtained from GibcoBRL, Switzerland and were stored at 4°C. HepG2 cells were maintained in 200 ml flasks (Nalge Nunc International, Denmark), filled with 15 ml medium, under incubator condition at 37°C in a humidified atmosphere of 95% air - 5% CO₂. Medium was changed each second day. The culture was split when cells covered 70% to 90% of the cultivation area. For this, cells were trypsinized with Trypsin-EDTA (GibcoBRL, Switzerland) for 10 to 15 min at 37°C and 5% CO₂ and visually inspected until cells detached from the flask. The enzyme was then quenched with fresh medium and cells were centrifuged at 800 rpm for 5 min at room temperature. Supernatant was removed and cells were resuspended in 5ml fresh medium. Viability of cells was determined by trypan blue exclusion and was typically between 92-95%. 1 ml of cell suspension was transferred into a new 200 ml flask prefilled with 14 ml medium. The rest of the cells were available for scaffold experiments.

Scaffolds for surface treatment and seeding tests
For the scaffold treatment and scaffold seeding experiments, woven PET textile fabrics (Sefar, Switzerland) of mesh sizes 200 μ and 5 μ were cut into circular pieces of 14 mm diameter and washed for 15 min in a sonicator at 60°C in acetone, ethanol and deionized water to remove potential contaminants on the fibers and within the pores. The fabrics were air dried at room temperature and autoclaved for 1 h at 121°C and 1 bar.

To investigate the influence of enhancing hydrophilicity of the scaffold surface on the number of attached cells, PET scaffolds were plasma-treated-treated. Plasma is generated by gas and microwaves or radio frequency. The atmosphere of plasma is made of free electrons, high reactive radicals, ions, UV-ray and various different excited particles, depending on the gas used. A main feature of plasma treatment, whereby samples become more hydrophilic, is an oxidation reaction [97]. Plasma modification leads to an enhancement of adhesion properties and does not cause toxic effects or does not influence disadvantageously the polyester [98]. In addition surface treatment has a cleaning-effect, which typically favorably affects adhesion properties [99]. Since surface tension of plasma-treated samples decreases as a function of time, transferring into medium directly after plasma treatment is essential. This so-called "hydrophobic recovery" is caused by adsorption of hydrocarbon molecules from the atmosphere, the reorientation
of functional groups on the surface and the diffusion of additives or chains of low molecular weight within the polymer [100].

Sterile PET scaffolds were plasma-treated for 5 min in oxygen plasma at 0.5 mbar and transferred into the wells of a 24-well plate, which did not affect their sterility. Plasma was generated by radio frequency (50 kHz, 100 W) using a Plasma Cleaner/Sterilizer (PDC-32G-2, Harrick, USA).

Plasma-treated and untreated scaffolds of mesh sizes of 5 m (surface textile, at the bottom of the well) and 200 m (structural textile, on the surface textile) were transferred into the wells of a 24-well plate (Costar, Switzerland). In order to fix the structural textile on the surface textile at their outer borders, a cylindrical stainless steel ring (dimensions: height 5 mm, outer diameter 15 mm, inner diameter 11 mm, washed and sterilized as described above for the scaffolds) was utilized. This is illustrated in Figure 4.6. Wells were then filled with 0.5 ml cell-free medium.

![Figure 4.6: Scheme of the scaffold experiments setup in 24 well plates. The structural textile was fixed on the surface textile (lying at the bottom of the well) by a stainless steel ring.](image)

As a reference scaffold, untreated scaffolds assembled in this way and incubated at 37°C in a humidified atmosphere of 95% air — 5% CQ for at least 10 h were utilized and named as standard scaffolds in the following.

To optimize wetting before seeding cells on the scaffolds, scaffolds in cell-free medium were vacuum and sonication-treated. Plates with PET scaffolds in the above described assembly were stored 1 h in the vacuum of a desiccator, coupled to a ejector water jet pump. Plates with sonication-treated PET scaffolds were exposed 5 min to sonication treatment at room temperature in a sonicator.
Cell-free medium was removed from the prepared standard, vacuum, sonication, and plasma-treated scaffold samples and 0.5 ml/well cell suspension of variable concentrations (0.5, 1, 2 and 5x10^6 cells/ml) were added and plates were transferred into the incubator. Supernatant containing nonattached cells was removed after 4 h and 0.5 ml/well cell-free medium was added.

In order to investigate the influence of agitating the samples while cells sediment on the scaffolds on resulting cell density and distribution, seeded standard scaffolds were circularly agitated on a lab shaker (100rpm) for 15 min and 1 h respectively. Again, supernatant containing nonattached cells was removed after 4 h and 0.5 ml/well fresh medium was added. Results were compared to those of standard-treated scaffolds under static conditions.

**MTT test**

MTT test was used to quantify attached number of viable cells on the scaffolds in these experiments [93]. This is possible for an established cell line such as HepG2 because metabolic activity of cells is approximately uniform.

Medium over the seeded PET scaffold was exchanged versus 1 ml of fresh medium and 0.1 ml of 5 mg/ml MTT in PBS, after which incubation for 3 h at 37 \( \degree \)C followed. Supernatants were removed, followed by thoroughly mixing in 1 ml of dimethyl sulfoxide (DMSO) to lyse the cells and solublize the dye. Samples of this liquid were transferred into the wells of a 96-well plate and read in an automated microplate spectrophotometer (Rainbow, SLT Labinstruments, USA) at 560 nm. In order quantify number of adherent hepatocyte cells on seeded PET scaffolds, an MTT-HepG2 cell standard curve was prepared by inoculating 1 ml of six different HepG2 cell concentrations into the wells of a 24-well plate (Costar, Switzerland). As a reference value 1 ml of medium was inoculated into three wells. After 4 h of incubation at 37\( \degree \)C in a humidified atmosphere of 95% air — 5% \( \text{CO}_2 \), medium was removed and nonadherent cells were counted, in order to evaluate adherent cell numbers in the wells. MTT-assay of adherent cells was performed, in order to examine corresponding optical density (OD) values measured at 560nm.

**Scanning electron microscopy studies**

After removal of the medium, seeded PET scaffolds were fixed in 1 ml of a glutaraldehyde / PBS (3/97) (v/v) solution at 4\( \degree \)C for 1 h. Glutaraldehyde (Fluka Chemie AG, Switzerland), used for protein linking, serves for the fixation of cell shape and cell localization. After fixation, samples were dehydrated and dried by an hexamethyldisilazane (HMDS, Fluka Chemie AG, Switzerland) drying procedure [101].
After removal of the fixation solution, seeded scaffolds were washed once in PBS and dehydrated in an ethanol series. Ethanol solutions were prepared by diluting in PBS. The ethanol series consisted of one 10 min change in 50%, one 10 min change in 75%, two 10 min changes in 95% and three 10 min changes in 100% ethanol solutions. 100% ethanol was exchanged for a 1:1 mixture of 100% ethanol and HMDS for 30 min, after which 1:1 mixture was replaced with pure HMDS for 30 min.

HMDS was removed and samples were air-dried, after which PET scaffolds were sputtered (SCD004 Sputter Coater, Balzers, Liechtenstein) by silver or gold at 0.05 mbar and 15 mA for 10 min. Samples were stored in a desiccator before as well as after sputtering. Sputtered scaffolds were observed under a Hitachi S-2500C SEM at 15 kV acceleration voltage.

**Bioreactor scaffold seeding tests**

The center of interest in the bioreactor seeding experiments was to determine a possibly simple handling to achieve homogeneous high density cultures on the scaffolds in the bioreactor. Therefore, scaffolds were neither plasma surface-treated nor sonication or vacuum-treated. They were assembled into the prototype bioreactor unit as described in Chapter 2.3. Since for these bioreactor scaffold seeding tests only the distribution but not the function of cells was assessed, the lower part of the prototype bioreactor was simplified by leaving out the teflon membrane for oxygenation and fixing the scaffolds directly on the lower plate of the bioreactor (cf. Figure 4.7).

For bioreactor seeding experiments, the prototype bioreactor was γ-sterilized (7-10 kGy, Studer Kabelwerke, Switzerland), prefilled with 10 ml cell-free medium and incubated for 1 h at room temperature. Up to four times with an interval of 4 h, 10 ml HepG2 cell suspension (1.5x10^6 cells/ml) was inoculated into the bioreactor either by a roller pump (automated seeding) or using a syringe (manual seeding).

The principle of automated bioreactor seeding is illustrated in Figure 4.7. 60 ml of a HepG2 cell suspension, containing 1.5 x 10^6 cells/ml, was pumped for 30 min at a flow rate of 2 ml/min from the reservoir through the bioreactor and recycled back to the reservoir, after which pump was switched off. The inlet and outlet of the bioreactor were sealed and incubation for 1 h at 37°C in a incubator followed at static conditions. Non-adherent cells were removed by flushing with cell-free medium for 30 min at a flow rate of 0.42 ml/min from the medium reservoir through the bioreactor into a waste bottle.
The manual bioreactor seeding includes the manual inoculation of the cell suspension using a syringe. The bioreactor was prefilled with 10 ml cell-free medium before 15 ml of a HepG2 cell suspension containing $1.5 \times 10^6$ cells/ml were manually inoculated in one direction using a syringe (one-direction seeding) or alternate in two directions (two-direction seeding) using two syringes, coupled to the inlet and outlet respectively (Figure 4.8). In the two-direction seeding process, the cell suspension was homogenized by slow push-pull injection with both syringes. After cell inoculation, the inlet and the outlet of the bioreactor were sealed and transferred for 1 h in an incubator. Nonadherent cells were removed after 1 h by flushing the bioreactor with fresh cell-free medium. This process was repeated up to 4 times in order to achieve high density cultures on the bioreactor scaffold.
After 10-12 h the prototype bioreactor was opened and the textile scaffold was carefully removed for SEM analysis in order to investigate the cell distribution over the entire bioreactor scaffold. Since only qualitative cell distribution of HepG2 in the bioreactor chamber was meant to be investigated, no quantitative MTT tests were performed.

Figure 4.8: Principal of one-directional (left) and two-directional (right) manual bioreactor seeding process.

Table 4.1 gives an overview about the performed tests reported in this chapter (Chapter 4.2). It summarizes the aim of the tests, utilized scaffolds and applied treatment methods and seeding methods and analysis. Results of these tests are reported in the subsequent section.
4. Biological testing

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Table 4.1: Overview about the performed tests reported in this section, regarding aim, utilized test scaffolds, applied treatment methods and seeding methods and analysis.

4.2.2. Results

Scaffold treatment tests

The left-hand parts of Figures 4.9 and 4.11 show the yield, i.e. the ratio of attached cells on the samples to inoculated cell number, on treated scaffolds as a function of the cell number that was inoculated on the samples, quantified by MTT test. A maximum of yield was found when a cell suspension of 1-2x10^6 cells/ml was seeded, corresponding to a total seeded cell number of 0.5-1x10^6 cells on the samples for both scaffolds treated in vacuum or sonication to remove air bubbles from the meshes (Figure 4.9) as for plasma-treated scaffolds to enhance hydrophilicity (Figure 4.11). This finding is supported by the total attached cell numbers on the scaffold, represented in the right-hand parts of Figures 4.9 and 4.11. Increase of inoculated cell number did not lead to a further increase of attached cells in the same extent.

In this range of inoculated cell number, for vacuum-treated scaffolds, a yield of approximately 80% was found, higher than on sonication-treated samples (approximately 75%) and standard-treated scaffolds (60-70%) (Figure 4.9, left). For higher inoculated cell numbers, the differences in attached cells became smaller and disappeared for 2.5x10^6 inoculated cells, where all yields are only about 30%. Figure 4.10 qualitatively illustrates the results of this experimental series with SEM analysis. Cells attached on the surface textile in the compartments of the structural textile. Only a few cells adhered on the structural textile itself. Cell density seemed to increase from standard over sonication to vacuum-treated scaffolds, but compartments seem not to be completely filled in all cases.
4. Biological testing

Figure 4.9: Influence of vacuum and sonication treatment of scaffolds before seeding with hepatocytes in 24-well plates, quantified by MTT test. No agitation during seeding was applied. 100% is the total inoculated cell number. (n=3, Standard: n=6)

Figure 4.10: SEM images of standard, vacuum and sonication-treated PET scaffolds. No agitation during seeding was applied. $2.5 \times 10^6$ HepG2 cells were seeded on the treated scaffolds and fixed for SEM after 12 h.
4. Biological testing

Plasma treatment led to clearly higher yields of inoculated cells, 80-90%, compared to standard treatment in the range of low inoculated cell numbers (0.25-0.5x10^6 cells) (Figure 4.11, left).

**Figure 4.11:** Influence of plasma treatment of scaffolds before seeding with hepatocytes in 24-well plates, quantified by MTT test. No agitation during seeding was applied. 100% is the total inoculated cell number. (n=3, Standard: n=6)

**Figure 4.12:** 2.5x10^6 HepG2 cells seeded on standard-treated and plasma-treated PET scaffolds. No agitation during seeding was applied. 2.5x10^6 HepG2 cells were seeded on the treated scaffolds and fixed for SEM after 12 h.
For higher inoculated cell numbers of 1-2.5x10^6 cells, no clear difference could be detected between plasma-treated and standard scaffolds. In Figure 4.12, SEM images of cell seeded standard (left) and plasma-treated (right) scaffolds are shown. While on standard samples, cells formed aggregates between the structural textile, which seem to have a three dimensional extension, cells on plasma-treated scaffolds not only form more flat, two dimensional configurations similar to monolayers, but also adhered on the top of the filaments of the structural textile.

**Scaffold seeding tests**

Figure 4.12 shows the influence of agitation during seeding on yield as a function of cell number. Again, highest measured yields were found in the range of 1-2x10^6 cells/ml suspension, resulting in a total seeded cell number of 0.5-1x10^6 cells on the nontreated standard samples (Figure 4.12, left). In this range, on standard scaffolds the highest yields were found (65-70%), while on scaffolds agitation during for 15 min and 60 min respectively a lower yield was found (45-58%). For low (0.25x10^6 cells) and high inoculated cell numbers (2.5x10^6 cells) no difference in yield was found. Total attached cell number increased when the cell number was increased up to 1x10^6 cells and seemed to reach a plateau for higher inoculated cell numbers (Figure 4.12, right).

![Figure 4.13](image)

*Figure 4.13:* Influence of agitation during seeding (motion for 15 min and 1 h respectively) of HepG2 cells on PET scaffolds in 24-well plates, quantified by MTT test. Samples were not surface treated. 100% is the total inoculated cell number. (n=3, Standard: n=6)
The seeded samples were analyzed by SEM. The differences in cell density were remarkable, densities on all scaffolds between the center of the scaffolds and the border (Figure 4.14). In the center, a clearly higher cell density was found as well as for static and agitated samples (Figure 4.14, left row) and cells not only attached on the surface textile in the meshes, but also on the structural textile. At the border of the scaffolds (Figure 4.14, right row), cells only formed aggregates in the compartments created by the surface textile.

Figure 4.14: SEM images of agitated seeded HepG2 cells on PET scaffolds (motion for 15 min and 1 h respectively, 2.5x10^6 cells were seeded on each sample. Samples were not surface treated. Images were obtained in the center (left row) and the border (right row) of the scaffold after 12 h.
**Bioreactor seeding tests**

Since the aim was only to optimize cell distribution on the bioreactor scaffold, for these experimental series scaffolds were not surface treated. Scaffolds that were seeded in the bioreactor after assembling them as described in Chapter 2.3, but without teflon membrane for oxygenation, were investigated by SEM in order to make qualitative statements about homogeneity of resulting cell distribution on the scaffolds. Images were taken of various regions on the scaffold, namely at positions which are defined in Figure 4.15 as inlet, outlet, border and middle.

![Figure 4.15: Definition of 4 positions on the bioreactor textile composite scaffold where SEM images were acquired: Inlet, Outlet, Border, Middle.](image)

When seeding the scaffolds automatically by pumping cell suspension unidirectionally through the bioreactor chamber, high cell densities were found near the inlet, where cells even transgressed the compartments given by the structural textile (Figure 4.16 A). In areas farther apart, i.e. in the middle, at the border and at the outlet of the flow chamber, clearly lower cell densities were found and compartments were not filled (Figure 4.16 B, C and D).
Figure 4.16: SEM analysis of a bioreactor scaffold seeded with hepatocytes with the (unidirectional) automated seeding method. (Conditions cf. Chapter 4.2.1)

Figure 4.17: SEM analysis of a bioreactor scaffold seeded with hepatocytes with the unidirectional manual seeding method. (Conditions cf. Chapter 4.2.1)
Also when seeding the scaffold unidirectional not by constant pumping, but by single slow injection of cell suspension using a syringe, high cell densities were found near the inlet, but at the border and at the outlet of the flow chamber lower cell densities were found and compartments were not filled. Figure 4.17 shows high density transgressing cells near the inlet (A) and lower densities at the remaining areas (B,C,D). In contrast, the utilization of bidirectional manual seeding led to homogeneous cell distribution over the entire bioreactor scaffold. This is illustrated in Figure 4.18. Aggregates were found to be filled completely to the level of 3/2 filament diameter (cf. explanation in Chapter 2), which is depicted by a rotated image of representative cell compartments on a bidirectional manually seeded bioreactor scaffold in Figure 4.20.

Figure 4.18: SEM analysis of a bioreactor scaffold seeded with hepatocytes with the bidirectional manual seeding method.
4. Biological testing

4.2.3. Discussion

The maximum yield at intermediate inoculated cell numbers of 0.5-1x10⁶ cells was independent of scaffold treatment and motion of the scaffolds while seeding. It therefore seems to represent a special characteristic of the cells. For high concentrations, conditions concerning oxygen and nutrient supply from medium seem to be suboptimal for such an amount of cells. This could lead to death of a high number of cells which are then removed with supernatant when medium is changed. The low yield at low inoculated cell numbers could not be explained in this model and remained unclear in this work.

Although the yield at high concentrations is lower than for intermediate concentrations, the total cell number attached to the scaffolds could be slightly increased by increasing the number of inoculated cells in the range of 1-2.5x10⁶ cells per sample. This is important when seeding primary hepatocytes on the scaffolds desiring a possibly high attached total cell mass in only one seeding step.

The higher yield on sonication and vacuum-treated scaffolds compared to standard-treated scaffolds can be explained by the removal of air bubbles from the textile. The consequence is a higher wetability of the samples and therefore a higher probability for cells to reach the textile surface and attach on it. This interpretation is supported by the enhancing effect of plasma treatment on cell attachment. Plasma treatment is known to increase hydrophilicity of scaffolds, which apparently increases yield. For plasma treat-
ment, this effect acts not only on the surface textile, but also on the structural textile. The following attachment of cells on the latter is not wanted in the context of desired filled compartments (cf. Chapter 2). For an application of plasma treatment on bioreactor scaffolds, this method had to be modified by treating only the surface scaffold, but not the structural textile.

Agitation during seeding, meaning shaking of scaffolds while seeding, was meant to homogenize cell suspension over the scaffold and therefore lead to higher yields and uniform cell densities over the entire sample scaffolds. In contrast, higher yield was found on samples which were not agitated. The reason for the latter could be that constant mechanical stresses as caused by shaking the samples over a period of time of 1 h damage the cells and lead to death. Furthermore, homogeneity of cell distribution was not observed in SEM analysis of seeded scaffolds. This could be due to geometric constraints of the samples in the experiments. Agitation on a circular shaker probably led to circular flow characterized by annular streamlines along which cells moved and therefore caused inhomogeneous cell distribution on the agitated samples. Therefore, the agitation during seeding method on small test samples as inlays for 24-well plates turned out not to be an appropriate method to investigate influence of cell suspension homogenization on scaffold cell distribution.

As well as for automated as for manual bioreactor scaffold seeding, the main problem was the inhomogeneous cell distribution on the scaffold when seeding with unidirectional cell suspension flow. The high cell densities near the inlet with aggregates overgrowing the structural textile compartments found are not wanted, because in the originally proposed bioreactor configuration, microscopic flow channels kept open by the sinusoid textile would surely be occluded. Furthermore, sufficient oxygen supply via medium and internal membrane oxygenator could not be guaranteed in the inlet region where the huge tissue amounts are located. Therefore, unidirectional seeding methods seem not to be suitable for bioreactor scaffold seeding, although they would represent a very easy handling method for clinical use.

The desired result of high density homogeneously distributed cell aggregates in the structural textile was finally achieved by the above described bidirectional manual seeding process, featuring slow push-pull motion of the cell suspension in the bioreactor chamber by two syringes coupled to inlet and outlet. Push-pull movement was only applied for 2 min, so that negative effects as observed in agitation during seeding experiments were not expected. From this homogenized cell suspension, cells sedimented on the scaffold, resulting in an also homogeneous distribution on the scaffolds even after up to 4 iterations of this process.
4.3. Bioreactor performance tests

In this section, tests of the biological performance of the prototype bioreactor under clinically realistic flow conditions over 1 week are described and discussed. To monitor viability of cells, enzymes were measured which are released when cells die. Liver specific differentiated function was investigated by measuring secretion of albumin into the medium in the flow\(^1\) path.

4.3.1. Materials and methods

*Scaffolds and Bioreactor*

Scaffolds and membranes were prepared and assembled in the bioreactor and the bioreactor unit was implemented in the Unisyn 1500 Flow path as described in Chapter 2.3.

*Cell culture and seeding*

Primary rat hepatocyte cultures were prepared as described in Chapter 4.1.1. Cells were seeded on the bioreactor scaffold using the bidirectional manual seeding method described in Chapter 4.2.1 with 15 ml cell suspension of a concentration of \(3 \times 10^6\) cells/ml suspension and one or two iterations of this process. Since the volume of the bioreactor chamber is 10 ml, 3 and \(6 \times 10^7\) cells respectively were seeded in the bioreactor. For cell sedimentation on the scaffolds, the bioreactor was transferred into an incubator for 4 h when seeded once before medium containing nonadherent cells was exchanged by carefully flushing the chamber with 15 ml medium in a syringe. When seeding twice, the bioreactor was transferred to the incubator for only 3 h, before filling in the second charge which flushed out at the same time nonadherent cells. Removed cells were counted using the trypan blue method in order to quantify nonattached viable and dead cells. After an addition 3 h the bioreactor was flushed again with 15 ml cell-free medium and connected to the Unisyn CP 1500 flow path with a medium reservoir of 700 ml medium. Medium was not changed during the runtime of 7 days. Medium temperature and pH were controlled by the system to be constant at 37\(^\circ\) C and pH=7.4. Three basic experimental series were carried out, each consisting of two experiments:

- In the first series, the *setup with deactivated internal membrane oxygenator* (Chapter 4.3.2) and \(3 \times 10^7\) cells in the reactor, the internal membrane oxygenator was deactivated by sealing the oxygen supply. Flow rate was set 100 ml/min.

† In the second series, in the *setup with activated internal membrane oxygenator* (Chapter 4.3.2), oxygen supply was realized by pumping air through the supply chan-

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1. In this chapter, flow of fluid through the bioreactor is flow of cell culture medium.
4. Biological testing

nels. The flow rate in these experiments was also 100 ml/min, and again 3×10⁷ cells were in the reactor. Aim of these first two trials was to compare viability and hepatospecific function of hepatocytes cultured in a setup with and without activated internal membrane oxygenator.

In the third series, to investigate the performance of high density cultures (Chapter 4.3.2), twofold seeding was done, resulting in 6×10⁷ cells in the reactor. To investigate if even such an amount of cells can be supplied with oxygen via the membrane, the internal membrane oxygenator was activated while reducing the flow rate to only 20 ml/min.

3 ml medium samples were taken at 1, 4, and 24 h and then each 24 h after connecting the bioreactor to the flow path in order to determine enzyme release of cells and albumin synthesis.

**Lactate dehydrogenase (LDH)**

LDH is an enzyme which is a constituent of cells cytoplasm. Destruction of the cell membrane, i.e. cell death, is followed by release of LDH. LDH is unstable, with a half-life depending from medium concentration [102]. Furthermore, cells also release LDH in small amounts in healthy state. LDH is not liver specific. LDH assay was performed following [103].

**Aspartate aminotransferase (AST)**

AST is also not liver specific. This enzyme is released by cells when the plasma membrane is destroyed. AST assay was therefore also utilized in order to quantify general cell death and was performed following [103].

**Alanine aminotransferase (ALT)**

ALT is a liver specific enzyme which is released by hepatocytes when the plasma membrane is destroyed. ALT assay was therefore utilized in order to quantify hepatocyte cell death and was performed following [104].

**Normalization of enzyme analysis results**

In order to determine the number of dead cells, as quantified by LDH, AST and ALT, enzyme content of 10⁴, 10⁵ and 10⁶ cells was determined after destroying their plasma membrane by crushing cells in a plunger.

**Albumin ELISA**

Albumin ELISA was utilized to investigate liver specific hepatocyte function as described in Chapter 4.1.1.
4. Biological testing

4.3.2. Results

After removal of the supernatant containing nonattached cells, 96.1% +/- 0.7% of seeded cells remained in the bioreactor unit. Of those which came out, 61.6% +/- 21.5% were dead, as measured by trypan blue exclusion. Number of dead cells in the system after 7 days was quantified by comparing LDH, AST and ALT values of day 7 with normalized enzyme analysis results. It was found that in all experimental series between 0.04-2.05% of cells, which remained in the system when connecting the bioreactor to the flow path on day 1, died during the runtime of 1 week.

Setup with deactivated internal membrane oxygenator

![Graphs showing LDH, AST, ALT, and albumin synthesis levels over runtime.]

Figure 4.20: Setup with deactivated internal membrane oxygenator: Cumulated LDH (a), AST (b) and ALT (c) measurements and albumin synthesis (d) for two experimental series (w and ). Mean values (x) are connected.
When cells were only supplied with oxygen via flow with a flow rate of 100 ml/min, LDH was found to be relatively constant after an initial increase in 48 h over 7 days in both experimental series (Figure 4.20a). The absolute values of those independent experiments differed by approximately 100 mU/ml, resulting in a mean of approximately 200 mU/ml over both series. The liver specific enzyme ALT (Figure 4.20c) showed the trend to increase over the first 48h, followed by a decrease to a level of 15 mU/ml by average of both experiments. AST, which is not liver specific, showed by mean a constant increase over 7 days (Figure 4.20b), ranging between 75 and 100 mU/ml. However, some data points, especially of the first series ( ), do not fit in this trend.

Albumin synthesis could only be detected in the first 48 h, increasing from 50 ng/ml to approximately 150 ng/ml. After this time, no further albumin was secreted in the medium, which was, as mentioned, not changed over the 7 days of the experimental series (Figure 4.20d).

Setup with activated internal membrane oxygenator

The two following experimental series were carried out under the same flow conditions and with the same cell number, but with activated internal membrane oxygenator. This means, as described above in Chapter 4.3.1, that the air was pumped through the oxygen supply channels (cf. Chapter 2.3). One series was terminated after 96 h due to a yeast contamination.

LDH was found on a similar level as before of around 150 - 200 mU/ml by mean, remaining in this range over the complete experiment time (Figure 4.21a). Also ALT was relatively constant over time, but was now slightly lower, measuring only 10 mU/ml by mean of both experiments (Figure 4.21b). The increase of AST over time detected in the two series with locked internal membrane oxygenator could not be found in this series with activated oxygenator (Figure 4.21c). After an initial increase of approximately 20-40 mU/ml, AST remained on this level, which is substantially lower than in the series before.

Synthesis of albumin (Figure 4.21d) occurred, as in the series with deactivated oxygenator, only in the initial phase, after more than 48 h no increase of albumin level, which was with approximately 150 - 200 ng/ml slightly higher than before, could be found.
4. Biological testing

Figure 4.21: Setup with activated internal membrane oxygenator: Cumulated LDH (a), AST (b) and ALT (c) measurements and albumin synthesis (d) for two experimental series (w and x). Mean values (x) are connected

Performance of high density cultures

Two additional series were carried out with a two-fold number of cells seeded in the reactor, activated internal membrane oxygenator and a flow rate which was reduced to 20 ml/min Absolute values of the measurements were normalized on the amount of cells seeded in the reactor with onefold seeding as in the experiments before (3x10^6 cells). LDH (Figure 4.22a) and ALT (Figure 4.22b) were found to be relatively constant on a level of around 300 mU/ml (LDH) and 15 mU/ml (ALT) respectively, comparable to the values of the series before.
AST (Figure 4.22c) in these series showed a similar progression as described in the series with locked oxygenator and high flow rate. It constantly increases in the first two days and then reaches a plateau at approximately 150 mU/ml.

In one series (w), a slight increase of albumin was found at least up to 120 h, while in the other series, albumin was relatively constant around 100 ng/ml (Figure 4.22d). In average, an albumin level results that slightly increases in a range of 100 - 150 ng/ml. This is in the same range as the absolute values in the experiments described above.

Figure 4.22: Setup with activated internal membrane oxygenator, reduced flow rate (20 ml/min) and twofold seeding (high density cultures): LDH (a), AST (b) and ALT (c) measurements and albumin synthesis (d) for two experimental series (w and ). Mean values (x) are connected.
4. Biological testing

4.3.3. Discussion

Cells remaining in the bioreactor after removing supernatant containing nonattached cells are more than 95% of cells initially seeded. This indicates good biocompatibility and the ability to promote formation of hepatocyte aggregates attached to the textile composite scaffold. In all experimental series, only a maximum of approximately 2% of attached cells in the reactor was found to die over 7 days, as indicated by LDH, ALT and AST. This satisfyingly low number indicates the ability of the system to keep hepatocytes alive over a period of time comparable to potential clinical treatment periods.

In the setup with deactivated internal membrane oxygenator, the absolute values of LDH of the two independent series differ by average by 100 mU/ml, which is 50% of the mean. This is explained by the fact that variabilities in different primary hepatocyte populations can lead to substantial differences of independent experiments under otherwise identical conditions. However, in these experimental series the trend of both series matches. It indicates that in the initial phase after removing the supernatant containing nonattached cells and connecting the bioreactor unit to the flow path, a few cells could die, while after 48 h no additional cells seem to die. This finding could be distorted by the instability of LDH with a similar degradation rate as the rate of appearance from dying cells. This is supported in the series without oxygenator by the finding that AST, which is, like LDH, not liver specific, increases over seven days and indicates therefore the death of additional cells. Hepatocytes on the other hand seem only to die in the first 48 h, releasing the liver specific enzyme ALT.

Activation of the internal membrane oxygenator does not decrease LDH substantially, but leads to lower AST values on average, indicating that less cells died. At the same time, ALT is lower as in the setup with deactivated oxygenator. This allows the supposition, that by activating the oxygenator the viability of hepatocytes can be enhanced and indicates the benefit of the internal membrane oxygenator.

Differentiated function of hepatocytes measured by albumin synthesis could only be detected in the first 48 h after connecting the reactor unit to the flow path when cells were cultured in the setup with deactivated internal membrane oxygenator and a flow rate of 100 ml/min In those 2 days, maximum albumin production rates were calculated to be 1.16 g albumin per 10^6 cells per day. These values are fivefold lower than those obtained in a similar flat plate bioreactor setup obtained by [105], and nearly 30 fold lower than those which can be obtained in collagen double gel configuration under perfusion conditions by [61], but in the same range as the values obtained by Nyberg et al. in a hollow fiber membrane bioreactor [106]. Designs of the flat plate bioreactors are discussed in detail in Chapter 5. Activation of the internal membrane oxygenator did not change this, average maximum level of albumin in the medium remained at 150 ng/ml.
This indicates that in spite of an enhancement of viability of hepatocytes by the activation of the internal membrane oxygenator, hepatospecific functions of cells could not be conserved over a clinical relevant period of time in the proposed configuration.

The reduction of the available amount of oxygen was done by reducing the flow rate of oxygenated plasma from 100 ml/min to 20 ml/min and doubling the number of cells in the system at the same time (*Performance of high density cultures*). No substantial changes in absolute values and time course of LDH, AST and ALT were found (per $3 \times 10^7$ cells). This indicates that the internal membrane oxygenator is able to supply cells even under the mentioned conditions with enough oxygen so that viability remains on a high level. Albumin concentration showed the trend to increase slightly over the entire 7 days, but these also represent low synthesis rates and did not exceed the maximum value of 150 ng/ml after 7 days as well. This could indicate a positive influence of reduced shear stresses due to diminished plasma velocities at the cell-plasma interface. However, the total shear stresses are much lower as calculated in the computational model due to a larger flow volume and resulting lower absolute velocities in the prototype bioreactor compared to the principal propose textile composite scaffold bioreactor system proposed in this work.

A potential reason to question the obtained results concerning measured albumin synthesis is the fact, that albumin in the circuit could be lost due to adsorption of albumin to polymer surfaces in the bioreactor and hoses of the flow path. Albumin loss has also been detected in hemodialysis [107, 108], where a albumin loss in the patients circuit of 3-6 g during a 24 h treatment was found. Furthermore it is known that protein adsorption on polymer monolayers is in the order of magnitude of g/cm$^2$ [109]. In the presented experimental setups, 700 ml medium were circulated, containing 220 g/l bovine serum albumin. This is a substantially higher concentration as the rat albumin concentration achieved by hepatocytes synthesis. Therefore it could be assumed, that most of adsorbed albumin in the circuit is bovine albumin and measurements of rat albumin have not been affected substantially.

As a summarizing conclusion, it can be stated that a high percentage of cells seeded in the bioreactor attaches to the textile composite scaffold and remains viable up to 7 days, indicated by relatively low and constant enzyme levels. The beneficial effect of the internal membrane oxygenator predicted from the calculations in Chapter 3 could be indicated by presenting lower enzyme levels in experimental series with activated oxygenator compared to oxygenation only via medium flow, however it should be noted that viability was high in all cases. Differentiated function monitored by albumin synthesis could not be found after day 2 in perfusion culture. To prolong this period, and so to provide the necessary prerequisite for a successful clinical application, the system has to
be improved not using not only technical, but also biological approaches. The application of bioactive coatings as discussed in Chapter 2 and presently under investigation by [49] as well as the optimization of culture media and the utilization of alternative cell sources providing less sensitive high differentiated cells are such promising approaches.
5. SUMMARY AND RECOMMENDATIONS

5.1. Summary

Aim of this work was to design and develop a novel extracorporeal liver support bioreactor dedicated to culture differentiated hepatocytes. From the analysis of potential improvement criteria of existing devices, 5 specifications for the development of the novel concept were derived (Chapter 1).

The concept was based on a textile composite scaffold (Chapter 2), which served to achieve structural analogies to the functional unit of the liver. Several textile composite scaffolds were assembled to a parallel flow bioreactor system. The proposed concept eases scaling of the reactor and is independent of cell source, as sought in design Specification 1. It should promote differentiation of hepatocytes by culturing size-defined cell aggregates in compartments created by the structural textile and the possibility to utilize bioactive coatings coupled to the chemically well-defined PET surface textile (Specification 2). Due to its assembly characterized by small flow channels kept open only by a small sinusoid textile, it also allows for a large ratio of cell volume to flow volume of 1/3, as claimed in design Specification 5.

Modeling of mass transfer characteristics (Chapter 3) using an one-dimensional model, based on diffusive oxygen transport and Michaelis-Menten kinetics for oxygen uptake, indicated that hepatocyte aggregates in the proposed scaffold configuration can generally be supplied with sufficient oxygen for differentiated function (Chapter 3.1), as claimed in design Specification 4. Extension to three-dimensional mass transfer analysis considering also flow of fluid and therefore convective transport in the bioreactor required the implementation of models into a computational fluid dynamic solver (Chapter 3.2). Comparison of the results of the one-dimensional and three-dimensional computational model for oxygen partial pressure in a cell aggregate in an elementary scaffold unit indicated the suitability of the computational model (Chapter 3.3). Subsequently, the model was scaled up to the size of an entire realistic bioreactor and utilized to calculate flow patterns, oxygen partial pressure in the fluid and shear stresses on cells in a prototype bioreactor (Chapter 3.4). The decrease of oxygen partial pressure in the fluid from inlet to outlet represented an analog to the natural zonation of the liver, as claimed in design Specification 4. It was found that with a minimum required flow rate of 120 ml/min, conditions can be achieved that permit cells that reside even near the outlet of the bioreactor flow chamber would be supplied with sufficient oxygen. Modeled shear stresses at the cell-plasma interface in the prototype bioreactor and in an extended elementary scaffold unit including the sinusoid textile in the flow channel (Chapter 3.5)
were found to exceed the physiological range and therefore potentially necessitate an additional sinusoid textile between cells and flow. This possibility was claimed in design Specification 3. The sinusoid textile was, as expected, found to mix the fluid and therefore contributed to achieve uniform flow conditions (claimed in design Specification 5). No evidence was found that it significantly elevated oxygen partial pressure in the cell aggregates.

Biological testing (Chapter 4) of composite scaffold and housing materials (Chapter 4.1) revealed that utilized materials have no toxic effect on hepatocytes. Seeding optimization (Chapter 4.2) of hepatocytes on the proposed textile composite scaffold resulted in homogeneous cell distribution in terms of uniformly filled structural textile compartments over the entire bioreactor scaffold, achieved by a simple seeding process, as claimed in design Specification 1. In prototype bioreactor performance tests under flow conditions realistic for clinical applications, hepatocytes demonstrated high viability, approximately 98%, after one week (Chapter 4.3). Relevant metabolic function indicated by albumin synthesis rates comparable to findings of other groups could only be found in the first 48 h after seeding hepatocytes in the bioreactor.

As a conclusion of the results summarized above it can be seen that the proposed concept satisfies the design specifications derived for this work. It is therefore promising to overcome deficiencies in devices currently under clinical investigation, specially concerning mass transfer and supply of hepatocytes with oxygen. Biological long term function in terms of differentiated function of hepatocytes in the proposed prototype system over more than 2 days could not be demonstrated. To achieve long term hepatospecific function in bioartifical liver devices, in future work not only technical, but also biological approaches should be pursued, as discussed below.

5.2. Recommendations

Results of theoretical oxygen mass transfer in this work point out the importance of this topic for extracorporeal liver support devices in general. Even in the proposed concept, which differs from conventional hollow fiber membrane systems by an additional pathway of oxygen supply via an internal membrane oxygenator, restrictions due to limitation of oxygen were found. With physiological flow rates, only small cell mass could be supplied with sufficient oxygen. As a consequence, a number of 10 proposed bioreactor modules would be needed in order to reach minimum required cell mass of 50 g hepatocytes. The minimum required cell mass is estimated under the assumption, that these cells are performing with 100 % efficiency of equivalent liver tissue mass. Since none of the devices working with 50-200 g cell mass in current clinical studies could demon-
strate unequivocal clinical benefit, this seems not to be so and could therefore represent a further reason for failing of these devices beyond insufficient oxygen mass transfer.

To address the oxygen mass transfer limitations effectively, recently two groups published new designs for hepatocyte bioreactors, which include the utilization of internal membrane oxygenators, similar as proposed in this work. Some results of this groups were already mentioned in Chapter 4.3.3 and compared to our results, subsequently the design principles are briefly summarized.

Bader et al. designed a flat membrane bioreactor, in which primary porcine hepatocyte monolayers were cultured embedded in a matrix of 10-20 m collagen [61]. Culture medium flowing through the bioreactor chamber was oxygenated via a gas permeable PTFE membrane. Cells were additionally supplied with oxygen from oxygen supply channels via a second PTFE membrane. In order to reach the cells, oxygen has to pass not only the membrane, but also the collagen layers and an additional microporous membrane on the culture medium side. Albumin synthesis in this system was 30 fold higher even after 18 days in culture as in the first 48 h detected in the bioreactor designed in this work. Also urea synthesis and ammonia removal were found on a high level [110].

The approach of Yarmush, Toner et al. [105] is also the utilization of a flat-plate bioreactor, which offers a low fluid compartment channel height of 550 m minimizing the priming volume of blood plasma and includes a oxygen permeable polyurethane membrane for oxygenation of flowing plasma. Primary porcine hepatocytes were found to synthesize albumin for a period of 4 days with a rate fivefold higher as found in our device. The internal membrane oxygenator for blood plasma flow both in mathematical models [79] and experiments demonstrated it s ability to supply hepatocyte monolayers with enough oxygen even with low flow rates of approximately 1 ml/min [51], creating low shear stresses, which do not affect hepatocyte function [53].

Regarding 5-30 fold lower albumin synthesis rates in our work compared to the above summarized studies, an important approach for further work in this project is consequently the optimization of biological issues of hepatocyte performance. Potential approaches for biological optimization are improvement of cell culture media by growth factors and application of bioactive coatings. Furthermore, application of coculture systems with non-parenchymal cells and utilization of newly developed functional competent hepatocyte cell lines and in bioreactors should be of interest. Subsequently, a brief overview issues is given.

**Growth factors and hormones in cell culture media**

An approach to achieve long-term maintenance of differentiated primary hepatocytes is the addition of various growth factors to the cell culture medium. In recent years, influ-
ence of hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor alpha (TGFalpha) was investigated [111]. HGF and EGF were shown to increase cellular calcium in rat hepatocytes [113]. It was found that mature adult hepatocytes can enter into clonal growth under the influence of HGF, EGF and TGFalpha in a serum free medium, and that after population expansion, the proliferating hepatocytes can return to mature hepatocyte phenotype in this medium and the presence of Matrigel (see below) [113]. Recently, Michalopolous et al. [114] reported the establishment of culture conditions that allow cultivation of human hepatocytes in the presence of HGF and EGF for at least 4-5 weeks, monitored by hepatocyte specific gene expression.

Hormonally defined media [115, 116] are known to stabilize hepatocyte morphology, survival, and liver-specific function, but are, however, inapplicable to bioartifical liver devices because of the exposure of patients to these non-physiological components.

Coatings

In order to generate an environment in which controlled differentiated hepatocyte function is possible, several coatings for utilized culture system were investigated. Most of them consisted of or at least included ECM molecules, which should influence hepatocyte function via cellular signalling. Freshly isolated rat hepatocytes cultured on low density laminin or fibronectin (1 ng/cm²) maintained high levels of albumin gene expression, but entered a state of proliferation for higher densities. Also collagen was widely used as surface coating, as summarized in [117]. In recent years, it was furthermore sucessfully used as three-dimensional matrix to culture hepatocytes, which are capable of cytochrome P450 expression [118] and bilary excretion [119]. It was also shown that hepatocytes in Matrigel, which is extracted from the Engelbreth-Holm-Swarm mouse sarcoma, embedded in sandwich-configuration, maintain their polarity and function [43]. Crude liver membrane fractions and extracellular matrix components as substrata were proven to regulate differentially the preservation and inducibility of cytochrome P-450 isoenzymes in cultured rat hepatocytes [48]. Recently a biomimetic surface coating consisting of an anchoring group (polylysine), a protein repellent spacer (polyethylene glycol) and different peptide signalling groups was suggested by [49].

A different approach from utilizing ECM molecules was suggested by Akaike et al. A synthetic glycopolymer coating, which stimulates asialoglycoprotein receptors of hepatocytes, was able to induce tissue reconstruction in primary rat hepatocytes [120] and to regulate proliferation stage of hepatocytes [47].
Hepatocyte cell lines and stem cells

Since it is difficult to proliferate primary hepatocytes in culture (cf. Coculture Systems) to obtain a sufficient cell mass with differentiated function, alternative cell sources are investigated for use in bioartificial liver devices. Existing cell lines can be classified in two groups, tumor-derived cell lines and immortalized cell lines, depending on their origin.

Cell lines derived from hepatic tumors, such as Hep G2 [121], or a subclone of it, C3A [37], have already been used in clinical trials [122], also cf. Table 1.2. Despite particular success in demonstration of markers of differentiated function, the risk of transmitting oncogenic substances or cells into the patients circulation remains. Efforts to improve the control and safety led to the usage of immortalized cell lines, derived from primary hepatocytes, which have the ability to immortalize spontaneously or can be achieved by genetical engineering. Examples for immortalized cell lines under investigation are HepZ [124] and HepLiu [125].

Furthermore, stem cells are being considered as cell source for bioartificial liver devices. It is hoped that such cells can proliferate in vitro and differentiate into fully functional hepatocytes. Again, different approaches are being pursued, which are distinguished by the use of adult liver stem cells [125] and embryonic stem cells [126].

An extensive overview about cell lines and stem cells for artificial liver support devices is given in [29, 34, 42, 127].

Coculture systems

Heterotypic cell interaction between parenchymal cells and nonparenchymal neighbors has been reported to modulate cell growth, migration and differentiation. An extensive overview about various approaches to the cocultivation of primary hepatocytes and non-parenchymal cells is given in [74]. In particular, coculture with Kupffer cells was found to regulate cytochrome P450 activity in rat hepatocytes [73]. Hepatocytes in coculture with 3T3 fibroblast cells maintained differentiated function monitored by the secretion of albumin over 18 days and formed canalicular systems. Recently it was shown that it is even possible to induce differentiation of liver epithelial (stem-like) cells into hepatocytes induced by coculture with hepatic stellate cells [129].

Although the concept of cocultures has not yet been applied to a clinical bioartificial liver device, it merits consideration. Potential approaches to implement cocultures are schematized in [64] and in this work (cf. Chapter 2.2).
6. Literature


6. Literature


6. Literature


6. Literature


6. Literature


[87] vilastic scientific inc, PLASMA VISCOSITY AND BLOOD VISCOELASTICITY, vilastic scientific inc, Austin, TX, USA 2001.


6. Literature


# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>ALF</td>
<td>Acute liver failure</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
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<tr>
<td>AoC</td>
<td>Acute on chronic liver failure</td>
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<td>AST</td>
<td>Aspartate aminotransferase</td>
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<tr>
<td>BSA</td>
<td>Bovine serum albumin</td>
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<td>CE</td>
<td>Conformit europ enne</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<tr>
<td>ECS</td>
<td>Extracapillary space</td>
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<tr>
<td>FDA</td>
<td>Food and drug adminstration of the USA</td>
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<tr>
<td>FHF</td>
<td>Fulminant hepatic failure</td>
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<tr>
<td>HMDS</td>
<td>Hexamethyldisilazane</td>
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<tr>
<td>ICS</td>
<td>Intracapillary space</td>
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<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
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<tr>
<td>NMWCO</td>
<td>Nominal molecular weight cut off</td>
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<td>OCR</td>
<td>Oxygen consumption rate</td>
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<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>Polycarbonate</td>
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<td>PS</td>
<td>Polystyrene</td>
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<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PVC</td>
<td>Polyvinylchloride</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electrone microscopy</td>
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<tr>
<td>TCPS</td>
<td>Tissue culture polystyrene</td>
</tr>
<tr>
<td>TM</td>
<td>Teflon membrane</td>
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<td>WME</td>
<td>Williams medium E</td>
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C U R R I C U L U M  V I T A E

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