Aus der Klinik für Allgemein-, Viszeral- und Kinderchirurgie

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First steps in the development of a foam-based carrier-system for an intraperitoneal foam chemotherapy

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Zusammenfassung

Die Peritonealkarzinose bleibt aufgrund ihrer schlechten Prognose und eingeschränkten Behandlungsmöglichkeiten weiterhin eine ernstzunehmende chirurgische Herausforderung. Bisherige Applikationen lokaler intraperitonealer Chemotherapien sind auf die Anwendung von Flüssigkeiten und Aerosolen beschränkt, welche Einschränkungen im Bereich der Medikamentenverteilung und Eindringtiefe aufweisen. Daher ist es notwendig, unter Einbeziehung physikalischer Überlegungen ein neues innovatives Behandlungskonzept zu entwickeln. Hierzu kann ein mit Medikamenten beladener Schaum, der sogenannte Foam, zum Einsatz kommen, der aufgrund seiner einzigartigen physikalischen Eigenschaften bestehende Einschränkungen potenziell umgeht.

Ziel dieser Arbeit war es zu klären, ob die Foam Anwendung praktisch durchführbar und klinisch anwendbar ist. Zu diesem Zweck erfolgt zunächst die Entwicklung zwei unterschiedlicher Trägermedien bestehend aus Wasserstoffperoxid und Bicarbonat. Im Rahmen von in-vitro Studien erfolgte die Untersuchung der Verträglichkeit und Zytotoxizität beider Medien an humanen Kolonkarzinomzellen. Da die in-vitro Untersuchungen insgesamt eine bessere Verträglichkeit und Überlegenheit des Bicarbonat Foam zeigten, wurde dieser nach Abschluss der in-vitro Untersuchungen für die weitere in-vivo Studie am Schweinemodell verwendet. Dabei wurde nach Etablierung eines Kapnoperitoneums im Rahmen einer diagnostischen Laparotomie ein extraperitoneal entwickelter Bikarbonat Foam in die Bauchhöhle geleitet. Im Rahmen der Nachuntersuchung erfolgten regelmäßige laborchemische Kontrollen. Am 7. postoperativen Tag wurden Gewebeproben zur histopathologischen Auswertung entnommen und eine Autopsie der Versuchstiere durchgeführt.

Insgesamt war es mittels beider Trägermedien technisch möglich, einen Foam zu kreieren. Der Bikarbonat Foam zeigte sich insgesamt gut verträglich, und es bestand zu keiner Zeit der Verdacht auf lokale oder systemische Nebenwirkungen, Komplikationen, relevante laborchemische Veränderungen oder Veränderungen der Vitalparameter. Es zeigten sich lediglich laborchemische Veränderungen in den Calcium- und Kaliumwerten. Zudem zeigte sich kein Hinweis auf eine Überhitzung der Bauchhöhle oder andere unmittelbare Nebenwirkungen. Zusammenfassend sind unsere Ergebnisse daher sehr erfolgversprechend und unterstützen die klinische Anwendbarkeit von Foam-basierten Therapien im Rahmen der Peritonealkarzinosebehandlung. Obwohl diese Studie viele grundlegende Fragen in Bezug auf Foam Applikation beantwortet, sind aufgrund der kleinen Fallzahl von Versuchstieren weitere Studien erforderlich, um eine abschließende Bewertung dieses innovativen Konzeptes zu ermöglichen.

Abstract

Peritoneal metastasis remains one of the key challenges in surgical oncology. Despite extensive attempts in improving current technology for intraperitoneal chemotherapy (IPC) in the treatment of peritoneal metastasis, limitations in drug tissue penetration as well as drug inhomogeneity have been observed in both liquid and aerosol-based instillations. To overcome these restrictions, new physical concepts must be applied. In this regard, the use of foam for IPC is an innovative, revolutionary concept. Foam displays some advantages over aerosol and liquid applications, including higher drug-carrying capacity and increased local drug availability following foam degradation.

This doctoral thesis serves as a pilot study which investigates the applicability of foam-based carrier systems, their in-vitro cytotoxic effects, as well as in-vivo challenges regarding foam application. The initial steps of this study included the creation of two different foam carrier systems, one containing hydrogen peroxide and the other bicarbonate. Next, the in-vitro effect and viability on HT-29 colon cancer cells was tested for both carrier systems.

Based on this preliminary data, the bicarbonate carrier system was assessed as superior to the hydrogen peroxide system and subjected to in-vivo testing. Subsequently, three swine were anaesthetised and placed in a laparoscopic setting. During the procedure, extra abdominally created foam was directed into the abdominal cavity under visual control. Abdominal expansion was measured throughout the procedure. Follow-up assessment included regular postoperative blood count and serological measurements, as well as histological analysis and postmortem autopsy. Following foam-based intraperitoneal chemotherapy, no intra- or postoperative complications, systemic adverse effects, postoperative macroscopic changes or morbidity were observed.

Furthermore, no overheating of the abdominal cavity was detected. Blood pressure and vital parameters showed no significant changes following foam application. Serum parameters remained within range, with the exception of changes in Calcium and potassium values. Our data indicate that foam-based intraperitoneal chemotherapy is feasible with both hydrogen peroxide and bicarbonate- based systems. While according to our findings, bicarbonate carrier systems seemed superior, their use is associated with potential electrolyte disturbances which may require close clinical monitoring. While first results are promising, the power of this study is limited due to the small number of experimental animals. Thus, further studies are required to confirm our findings and help build a more comprehensive understanding of this innovative new concept.

Abbreviations

ADI	Acceptable daily intake
ALT	Alanine Aminotransferase (ALT)
ALP	Alkaline Phosphatase (ALP)
°C	Degrees Celsius
CO_2	Carbon dioxide
CRS	Cytoreductive surgery
DMEM	Dulbecco's modified Eagle's medium
FAO	Food and Agriculture Organization of the United Nations
FBIC	Foam based intraperitoneal chemotherapy
FBS	Fetal bovine serum
FER	Foam expansion rate
GMM	Gibbs-Marangoni mechanism
H&E	Haematoxylin and Eosin staining
HINAT	Hyperthermic nano aerosol therapy
HIPEC	Hyperthermic intraperitoneal chemotherapy
HT-29	Human colorectal cancer cell line
IPC	Intraperitoneal chemotherapy
KJ	Kilojoule
kV	Kilovolt
LDH	Lactate dehydrogenase
MTS	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N_2	Nitrogen
NOAEL	No-Observed-Adverse-Effect Level
O_2	Oxygen
PH	Potential of hydrogen
PIPAC	Pressurized intraperitoneal aerosol chemotherapy
PM	Peritoneal metastases
SEM	Scanning electron microscopy
TCA cycle	TriCarboxylic Acid cycle, or Krebs cycle
WHO	World Health Organization.

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3. Results

1. Introduction

1.1 Introduction to the field

Even after decades of clinical and experimental research, the treatment of peritoneal metastasis (PM) remains a significant surgical challenge with poor prognosis and median survival rates of only a few months (1, 2). In fact, only a highly selective group of patients with isolated PM can be considered for a surgical therapy (3). In those selected cases, a combination of cytoreductive surgery (CRS) with hyperthermic intraperitoneal chemotherapy (HIPEC) can offer a potentially curative PM therapy (4 – 7). However, since most PM patients do not qualify for CRS and HIPEC, only systemic intravenous chemotherapies (IVC) or intraperitoneal chemotherapies (IPC) are offered as a palliative approach. Due to low local drug availability, IVC is considered to have only a limited effect on PM (8, 9).

While IPC is considered to improve local drug availability compared to intravenous delivery, it also displays major technical and prognostic limitations e.g. increased risk of local complications caused by IPC devices, inhomogeneous drug distribution and limited drug penetration into affected tissues (10 – 13). These limitations have also been demonstrated in a more recent IPC application via aerosol formation, called pressurized intraperitoneal aerosol chemotherapy (PIPAC) (14 – 17). However, as opposed to liquid or aerosol-based applications, foam has not been widely studied as a potential drug carrier in IPC. However, foam possesses unique features which may result in superior drug effects in the peritoneal cavity, and therefore improve current IPC applications.

To evaluate whether foam-based intraperitoneal chemotherapy (FBIC) could be a feasible tool for IPC, it is necessary to study the most suitable chemical carriers for such a system. Thus, it is of utmost importance to investigate the cytotoxicity of these carriers by means of in-vitro studies, analyse the structural stability of the designed foam and ultimately test its characteristics in a suitable in-vivo model. In this pilot study, the feasibility of the technical application, safety aspects and changes in the postoperative bloodwork based on the collected in-vitro and in-vivo data will be examined. Moreover, we will focus on possible clinical challenges and unforeseen side effects which may occur. This work focuses on two main substances, including sodium bicarbonate and hydrogen peroxide. Especially hydrogen peroxide has previously displayed high levels of antitumoral activity, which has recently been the subject of oncologic research (18 – 22).

1.2 Current view on peritoneal metastasis

PM develops when malignant cells from primary gastrointestinal or gynaecological tumours disseminate in the peritoneal cavity. Following dissemination, the cells eventually adhere to the peritoneal wall.

Thus, PM can be therefore viewed as a stochastic event depending upon several additional factors which either encourage or diminish the adhesive process. The process of peritoneal tumour dissemination and metastasis starts with cancer cell detachment from the primary tumour, which can either occur spontaneously or iatrogenic during surgery. These malignant cells are transported and disseminated with the peritoneal fluid, which is a suitable environment for disseminated cells.

This may explain the clinical observation as to why metastatic formation within the peritoneum seems dominant over systemic, hematologic seeding. Following dissemination and transport within the peritoneal cavity, tumour cells can finally adhere to the peritoneal wall. This adherence initiates local growth and invasion into the stoma where malignant cell clusters can further proliferate and form a new colony of metastatic tumour cells.

1.2.1 Cancer cells in the peritoneal fluid

Spontaneous detachment of single cells or cell clusters is enhanced by intestinal and osmotic pressure as well as down regulation of cell-to-cell adhesion molecules (23, 24). Indeed, cancer cells undergo an epithelial to mesenchymal transition and reduce the amount of different adhesive molecules. For instance, E-cadherin is considered one of the key molecules which enables strong cell-to-cell contacts between epithelial cells (25). In ovarian cancer patients, ascites samples were shown to contain single cells and loose clusters or spheroid aggregates. A variety of changes regarding their surface proteins have been observed and cell clusters seem to not only contain cancer cells but also fibroblasts (26, 27).

Until now, we know that dissemination of cancer cells does not always occur spontaneously. For instance, a higher presence of cancer cells has been observed in the lavage of patients postresection following colorectal oncologic surgery (28). Thus, surgical manipulation can encourage cancer cell dissemination. Accordingly, an extensive meta-study has demonstrated that patients with more “free-floating” cancer cells in the peritoneal cavity following surgery suffered from higher recurrence rates and an overall poorer survival (29). For gynaecological cancer entities, a similar effect has been described (30 – 32).

More studies have been conducted and different cancer entities were compared, including gastric and colorectal cancers. These studies suggest that it is not merely the amount of free-floating cancer cells which determines the recurrence rate, but that these free-floating cancer cells have distinct, intrinsic potential to establish PM (32).

1.2.2 Spreading of cancer cells in the peritoneal cavity

Peritoneal recurrence is either local, which corresponds to the location of the primary tumour, or disseminated within the peritoneal cavity.

Efforts have been made to identify factors that might increase and enhance tumour dissemination. In fact, a wide range of factors could be attributed to this effect, including excess abdominal fluid, e.g. ascites or mucous (33). Bowel movement, diaphragmic movement and gravity have shown to direct peritoneal fluid in a clockwise direction (34). In addition to these physical factors, peritoneal tissue at locations with assumed higher fluid absorption rates tend to more frequently infiltrated by PM. This also includes surgical entrance sites (33).

1.2.3 Cancer adhesion to the peritoneal wall

The superficial layer of the peritoneum is a rather non-adhesive surface which facilitates organ movement (35). While this first layer should discourage any cancer cells from adhesion, several factors can favour the interaction with loose cancer cells. Some of these possible factors have been described, e.g., ICAM-1, VCAM-1, mesothelin and hyaluronate (23 – 25). Factors excreted by cancer cells such as TNF and INF-3 can further induce adhesive interactions with underlaying mesothelial cells (36).

Both the ability to adhere to as well as the ability to finally break through the mesothelial layer helps floating cancer cells form metastases, further enabling the endurance of these cells in the peritoneal cavity (37). It has already been observed that sites of the peritoneal layer displaying ruptures and discontinuity are favourable locations for PM. This phenomenon is frequently observed in surgical site metastasis (33, 38, 39). Preferentially, cancer cells adhere to disruptions of the mesothelial barrier (39). Even when scar tissue is covering these mesothelial disruptions, these scars are preferred sites for metastases (39). Histological analysis has confirmed mesothelial layer disruptions at sites of peritoneal nodules (40). In-vitro experiments have further confirmed that ovarian cancer cells isolated from ascitic fluid seem to adhere preferably at the extracellular matrix or even to plastic rather than to mesothelial cells (41).

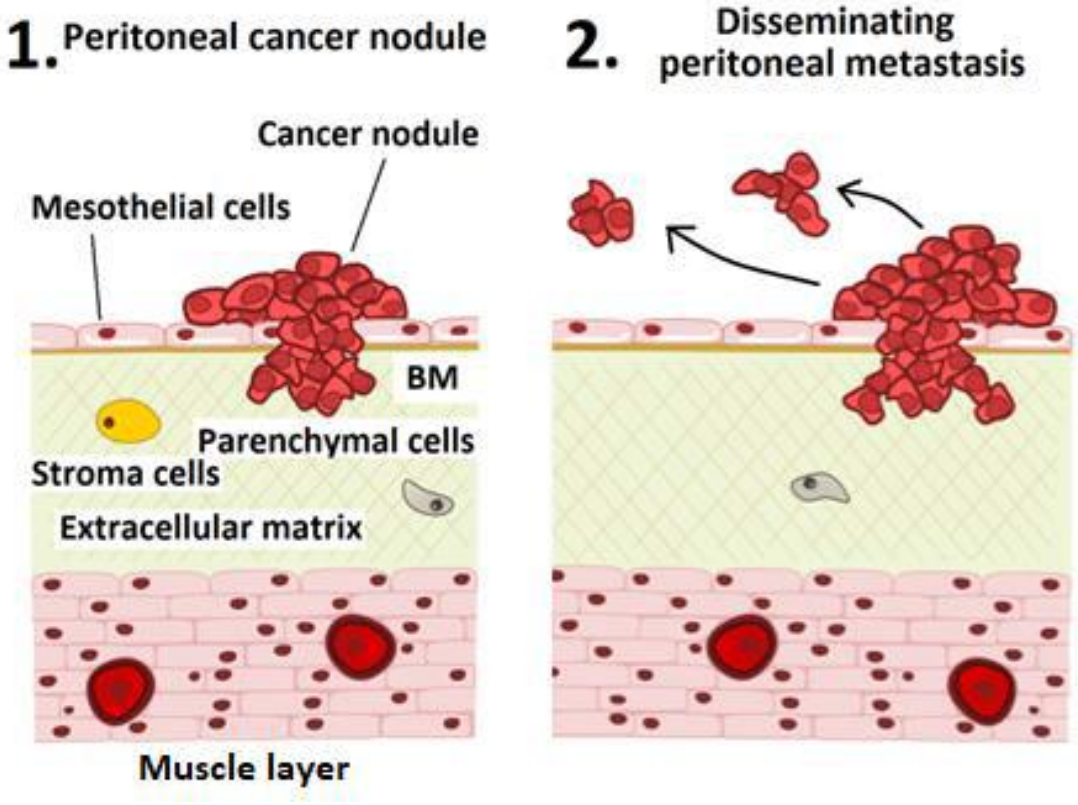

Figure 1. Model of invasive PM

Continuous horizontal spread from the initial nodule via disseminating clusters. 1) Single invasive cancer nodule within the peritoneal tissue. 2) "Free-floating" multicellular cancer clusters detaching from the "mother" nodule (from own illustrations).

1.3 Potential discriminative cytotoxic effects of physical principles

IPC attempts to improve cytotoxic effects by increasing local drug availability within target tissues. To a significant degree, the assumed discriminative nature of the therapy is based on the idea that applied chemotherapeutics tend to target malignant cells by accumulation and other principles (42 – 44). When performing IPC using liquid solutions, the applied chemotherapy passes through the peritoneal surface by mere particle diffusion (45 – 47). PM extent in patients qualifying for IPC is limited to the peritoneal cavity (48, 49). Interestingly, we observe PM cases with extensive intraperitoneal spread, yet without any distant metastasis, indicating extraordinary cancer cell biology. There seems to be an extracellular interaction between tumour invasion and cellular matrix properties which favors a horizontal tumour spread rather than a vertical spread (50, 51). While horizontal tumour spread is clearly more visible during laparoscopy, the vertical spread can be assessed following histological examination of PM nodules. As depicted in figure 1, during its local growth, a single peritoneal nodule further invades the peritoneal tissue. However, most of the peritoneal surface which falls victim to this invasive tumour growth, shows extremely low cell density. In fact, much of this first layer is comprised of extracellular matrix, collagen, and other fibres (52, 53).

A few hundred micrometres deeper in this layer, we either find primary extracellular matrix (adventitia) or cell rich tissue, in most cases in the shape of smooth muscle fibres. An increased cellular toxicity within this thin layer could help target PM more efficiently.

1.4 Current and alternative carrier systems for intraperitoneal chemotherapy

Liquids are the current carrier systems which provide IPC. Various forms of liquid installations have been examined, described in literature, recommended, and later dismissed (11, 54, 55). Basically, an active therapeutic compound is dissolved in a water-soluble chemotherapy. However, alternative compounds e.g. radioactive isotopes can also be used. The applied chemotherapy can be administered as a monotherapy or in combination with other chemotherapeutic substances. However, the efficiency of these “lavage-only” procedures have been disputed and are now often performed based on individual consideration. One of the best known and most applied types of IPC is hyperthermic intraperitoneal chemotherapy (HIPEC).

HIPEC is an additional therapeutic procedure that is usually applied during or shortly after cytoreductive surgery (CRS) (55, 56). During CRS, macroscopically visible cancer nodules are surgically removed until CC0 (complete macroscopical resection of the cancer) is achieved. Once CRS is performed, the HIPEC procedure is then conducted to potentially eliminate residual single tumour cells or smaller cell clusters which could otherwise cause tumour recurrence within the peritoneal cavity (57, 58).

The ultimate goal of combining CRS with HIPEC is to offer a curative treatment by eliminating all malignant cells. At the same time, it is understandable that this ideal outcome might not be achievable in all patients who undergo this procedure, and therefore the potential complications associated with the procedure must be carefully considered (58, 59). The challenge is to correctly distinguish patients who might benefit from the procedure from those who do not (58). Therefore, when considering a case, the first question should be whether full cancer resection is technically achievable. While surgeons might realize that some patients are unsuitable, they may still pursue CRS with HIPEC, despite knowing that full tumour resection is key for a good clinical outcome (60 – 62). In fact, patient selection plays a crucial part in overall outcome and survival, since many patients, especially those with extensive PM, should be exempt. However, IPC may still be considered in some cases of extended PM as it may slow down local PC progression.

1.4.1 Hyperthermic intraperitoneal chemotherapy (HIPEC)

In HIPEC, a heated water-based chemo solution is introduced and kept in the abdominal cavity for a duration of 30 - 90 minutes, depending on the protocol (63 – 65).

The solution is either applied during open surgery or following closure of the abdominal wound via carefully placed tubes during CRS. A continuous or intermittent in - and outflow is established to allow optimal heat and chemo transport. The applied chemo solution is dissolved in several litres of physiological saline solution.

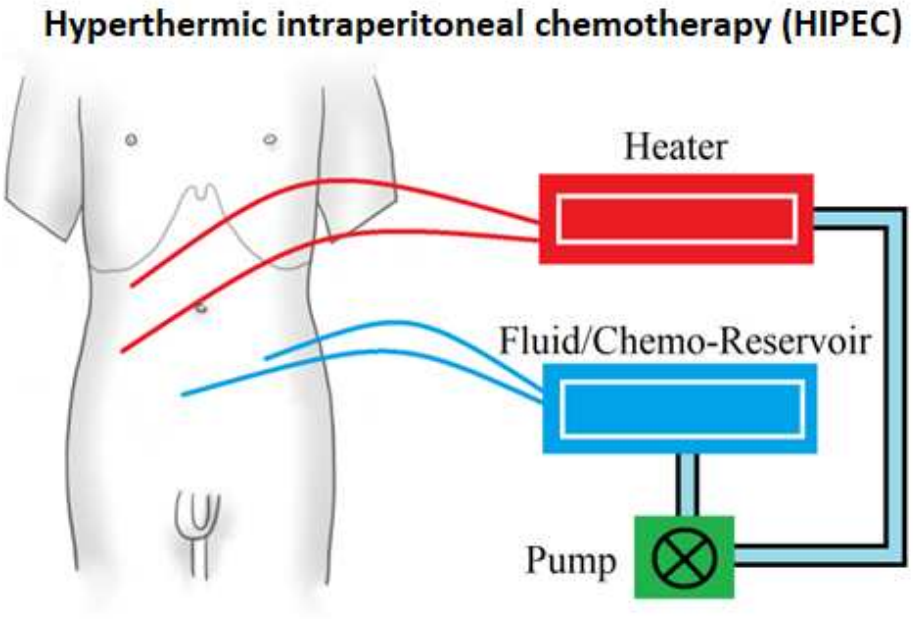

Figure 2: HIPEC model

The therapeutic fluid is directed into the abdominal cavity via tubes previously placed at distinct intraabdominal locations. Some tubes are used to redirect the fluid back to a reservoir from which it is again pumped through the heating system and back into the abdomen. A continuous chemotherapeutic flow is established which allows for continuous heat transfer (from own illustrations).

1.4.2 Pressurized intraperitoneal aerosol chemotherapy (PIPAC)

Pressurized intraperitoneal chemotherapy (PIPAC) is an alternative therapeutic approach used to deliver chemotherapy into the abdominal cavity. For this purpose, chemotherapeutic solutions e.g., doxorubicin, oxaliplatin or cisplatin are dissolved in either physiological saline or 5% glucose solution.

In each case, the volume of the applied solution equals less than 500 ml. This means that the volume applied in PIPAC is much smaller than the volume applied in HIPEC, which in turn leads to higher initial chemotherapeutic concentrations (47). Since the applied volume is lower, the spatial distribution of the chemo solution is further improved by aerosolization in the laparoscopic setting. Several initial studies have demonstrated some distribution inhomogeneity during the PIPAC procedure (17, 66). However, the local chemotherapeutic drug concentration is still improved due to the application mechanism used (17), resulting in some peritoneal tumour regression in the palliative setting (67).

Clinical and experimental studies have been performed to fully understand both the potential and limits of PIPAC (68, 69). Moreover, further studies have been conducted to determine the option to combine PIPAC with other therapeutic features which could possibly increase efficiency (70 – 72). It is important to emphasize that the indication for PIPAC differs from that for HIPEC. PIPAC is used in the palliative setting, whereas CRS with HIPEC represents a curative approach. Despite its use in the palliative setting, PIPAC therapy also exhibits restrictive inclusion criteria, such as a minimum Karnofsky-Index of 50%, intact gastrointestinal passage, as well as absence of distant or extra abdominal metastasis (73).

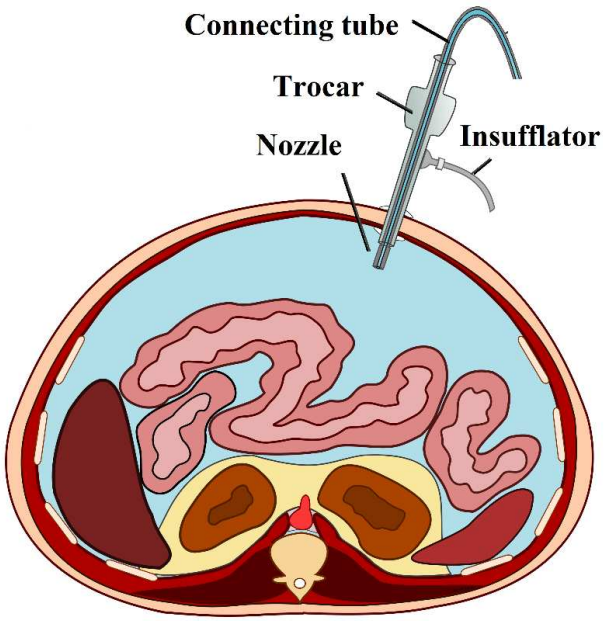

Figure 3: Model of pressurized intraperitoneal chemotherapy (PIPAC)
The therapeutic chemo solution is injected into the abdominal cavity during laparoscopy using a spray-device (micropump®). The created aerosol floats within the cavity and sediments onto organ surfaces and the abdominal wall, covering the peritoneal surface with a thin chemotherapeutic drug layer (from own illustrations).

1.4.3 Hyperthermic intraperitoneal nano aerosol therapy (HINAT)

Hyperthermic intraperitoneal nano aerosol therapy (HINAT) is a concept described and intended for IPC. However, due to its complexity, novelty and challenges in controlling applicational features, HINAT has not yet been tested in a larger clinical setting (74). Its principle is based on delivering a highly concentrated or even crystallized nano chemotherapeutic aerosol (75), which is basically transported into the abdominal cavity using a constant gas flow. The continuous gas flow carries the aerosol particles into the abdominal cavity. Due to their physical features and small size, they are not subjected to immediate gravitational sedimentation (75). To facilitate final intraperitoneal sedimentation of these small aerosol particles, an electrostatic precipitation device is used. However, the actual control and management of sedimentation within the abdominal cavity remains challenging.

Additionally, the carrying capacity of this system may be limited to only a few drugs, including doxorubicin (75).

1.4.4 Foam based intraperitoneal chemotherapy

FBIC application has been first described by Schubert et al. 2020 as a preliminary concept (76). In their paper, the authors outline the basic idea of this approach and investigate its potential for improving current liquid drug delivery. Despite some promising findings, this initial research project was very limited in size and covered only a few relevant aspects. However, this study gave important first insight into this novel concept and provided a foundation to further develop FBIC. In fact, one of the major observations made by Schubert et al. 2020 (76) was that there is no sedimentation process of dissolved particles in the foam. This result seems to be trivial since any separation of components would be against the second law of thermodynamics and the principle of entropy.

However, the confirmation of chemical homogeneity and thus, stability of drug concentration within the fluid used in FBIC was still very important. Under some circumstances, there can be particle and component inhomogeneity within a medium. Furthermore, it has been shown that an increase in drug concentration is possible without increasing the total drug dosage (76). This is a similar approach which is used in PIPAC, and which can explain the higher local drug availability and penetration rates into the tissue (47). However, in 2020 Schubert et al. also presented some challenges in the initial preparation of their study investigating this innovative concept. Since a rather high level of toxicity of the hydrogen peroxide compound was observed by Schubert et al. (76), it was necessary to conduct extensive testing and literature research for potential components in foam creation.

There is some indication that hydrogen peroxide can be a suitable carrier system for FBIC. Hydrogen peroxide has demonstrated some specific antitumoral activity. Both endogenously produced and exogenously added hydrogen peroxide display an antitumour effect (77). In fact, in terms of applicability, there is some clinical use of hydrogen peroxide in different medical fields. However, the delivery of hydrogen peroxide into solid tumours or on large internal body surfaces seems to be much more challenging than its common use as a surface applicant in the treatment of skin cancer (77). Nevertheless, hydrogen peroxide has recently been tested in the treatment of solid tumours, among other substances (78, 79). While its various antitumoral effects are increasingly understood and appreciated (80), the implementation of a potentially widely applicable hydrogen peroxide solution for oncologic purposes remains challenging (81). Applications are still limited due to challenges in dermal applications for skin cancer or small injections in breast cancer (82 – 86).

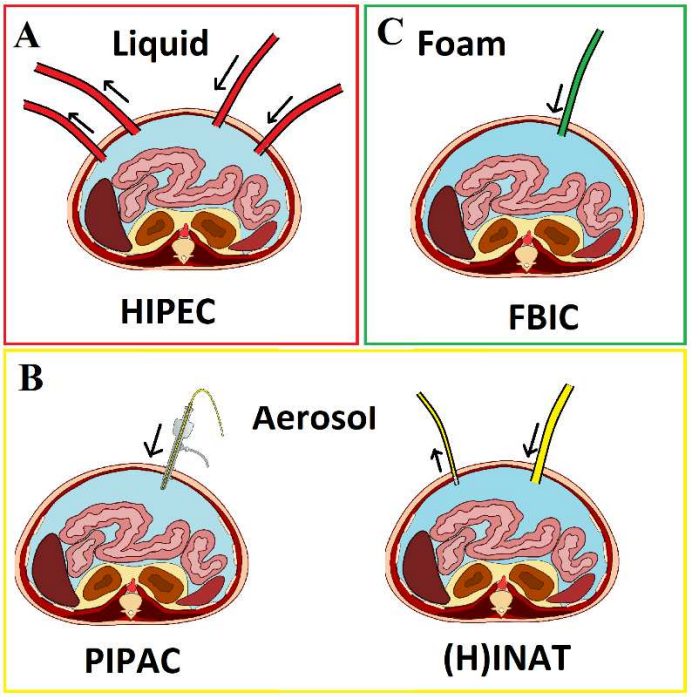

Figure 4: Different modes of intraperitoneal chemotherapy delivery (IPC)

A) Delivery of liquid intraperitoneal chemotherapy in HIPEC with CRS. B) Versions of intraperitoneal aerosol chemotherapy: On the lower left, conventional Pressurized intraperitoneal aerosol chemotherapy (PIPAC). On the lower right: Hyperthermic intraperitoneal nano aerosol therapy (HINAT) as presented by Göhler et al. (2018) with extraperitoneal aerosol generation. C) Intraperitoneal foam application (from own illustrations).

1.5 Underlying physical principles for our hypotheses

With the exception of a recent ex-vivo box model (76), foam has not been investigated as a drug carrier for intraperitoneal chemo applications despite displaying some unique characteristics. By using chemotherapeutic agents, FBIC could be a technically feasible option for PM treatment. First data on this concept indicates that drug tissue concentrations following FBIC exceeds results achieved by liquid or aerosol-based IPC (76). Moreover, slow degradation of applied foam allows for extended drug contact time with the peritoneum and an overall longer chemotherapeutic exposure.

This is an interesting feature, since the duration of contact time of a chemotherapeutic drug with the peritoneum is a relevant factor for drug availability and efficiency. In fact, foam can present other advantages over both aerosol and liquid applications. Foam exhibits a different expansion pattern than liquids and gas when applied into a cavity: In contrast to gas, foam displays a higher drug-carrying capacity (87). In foam, more than 95% of the actual volume after expansion is air, which means that even a low total drug dosage dissolved in the initial foam fluid can expand and create high drug concentrations (76).

A similar concept has been introduced by the application of aerosol chemotherapy as an alternative to liquid solutions. Aerosol chemotherapy has shown to achieve much higher drug concentrations than regular liquid solutions (47). However, aerosol chemotherapy also displays its own limitations. The increase in drug concentration goes along with spatial inhomogeneity which can be observed in aerosol chemotherapy (16, 66). The unique characteristics of foam might therefore improve PM response to IPC. The concept of Schubert et al. contained hydrogen peroxide and taurolidine as its major components. Both reagents had displayed cytotoxic properties which could potentially treat PM without the addition of chemotherapeutic agents (69, 77, 88 – 90).

However, more data is required to further evaluate if foam might be a feasible carrier for IPC and whether it could achieve increased drug penetration and more homogenous drug distribution than conventional liquids or pressurized aerosol. To the best of our knowledge, until now, no in-vivo testing of FBIC has been conducted or published in peer-reviewed literature. This pilot study is the first to present preliminary in-vivo data on FBIC, giving important insight on this novel concept's potential, challenges, and possible limitations. Hopefully, this study will encourage further research on FBIC's full potential.

1.6 Physical and chemical characteristics of foam as a carrier-medium

For further planning and analyses, it is important to focus on the basic definition of foam and its characteristics. Foam can be defined as a dispersion of gas in a liquid medium, in which the volume of the gas component is predominant (91). Mobile foams are generally unstable and gradually disintegrate. The time scale on which this occurs can vary tremendously: While short-lived foams stabilized by small surface molecules can disintegrate in a few seconds, foams stabilized by polymers or surfactants can remain stable for hours or days.

Basically, there are two typical foam structures, including spherical foam and polyhexagonal foam (figure 5). Spherical foam consists of spherical gas bubbles in a predominantly high liquid surrounding. This type of foam is usually generated immediately after foam formation. Polyhexagonal foam, as indicated by its name, consists of polyhexagonal gas bubbles (91). The volume of liquid between these gas bubbles decreases and subsequently the foam becomes "drier". In a static foam column, foam formation stops at some point. The drainage caused by gravitational force leads to a decrease in fluid volume in the foam. At the same time, the wall between the bubbles reorganizes and collapses, causing disintegration of the foam. This leads to two phenomena: 1) change in foam structure from spherical to polyhexagonal, and 2) decrease in foam volume. The kinetics of foam disintegration is irregular and cannot be perfectly timed. There can be periods with no detectable changes which are then followed by gradual decay or sudden foam collapse (92).

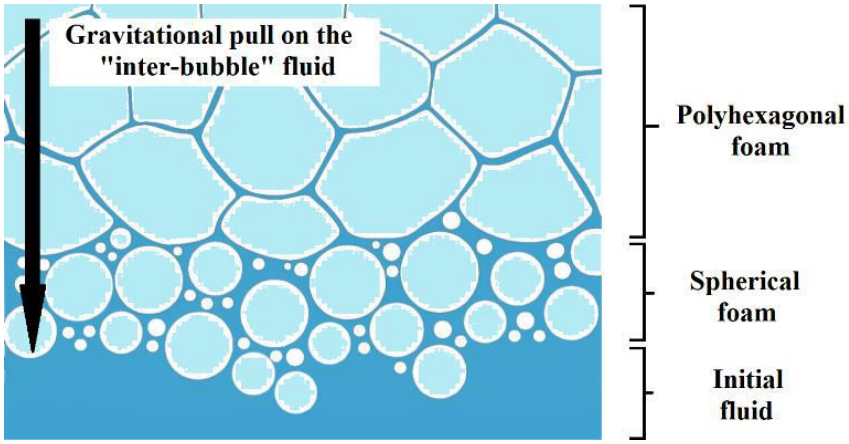

Figure 5: Foam transformation and remodeling

Model of a mobile foam gradually separating into three main sections. Gravitational pull causes drainage of liquid between bubbles, which further amplifies the gradual transition from spherical to polyhexagonal foam (from own illustrations).

1.6.1 Stabilization and disintegration mechanisms

Pure liquids do not foam because their films cannot be stabilized. In fact, an “active substance” is required to stabilize the foam surface. This active substance determines whether the resulting foam will be mobile or immobile based on the concentration and type of the stabilizer. For instance, polymers tend to create more stable and enduring foams (93). However, stability and breakdown are also influenced by a variety of other mechanisms.

1.6.2 Gibbs-Marangoni mechanism

One of the main mechanisms by which foam is stabilized is the Gibbs-Marangoni Mechanism (GMM) illustrated below (figure 6). As the film (represented by the bubble wall) expands, the occupancy of the interface is reduced by surfactant. Thus, the surface tension increases. This increased gradient leads to the transport of fluid and surfactant to the area of increased tension and instability. In summary, the effect demonstrates a mass transfer along an interphase between two phases due to the surface tension gradient. The surfactant molecules drag the fluid along and thereby further increase film thickness. A sufficient surfactant concentration is needed within the medium for the effect to take place (93).

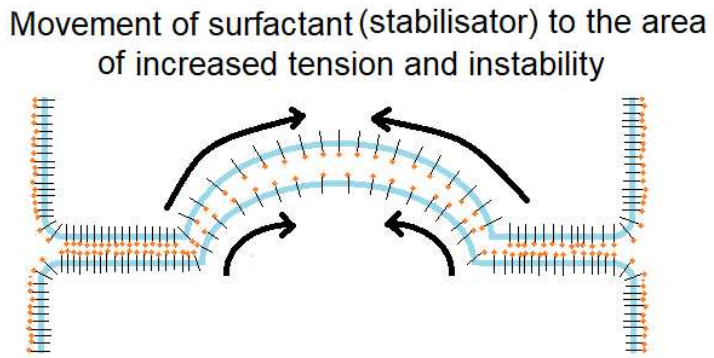

Figure 6: Model of the Gibbs-Marangoni mechanism

The movement of surfactant molecules along the line of highest tension increases film thickness after initial thinning and drags fluid along (from own illustrations).

1.6.3 Foam surface viscosity and elasticity

Interactions between molecules in the absorbed surfactant layers cause movement of the neighbouring molecules. While being two-dimensional, surface viscosity can be considered similar to the "normal" three-dimensional viscosity of a substance. When surfactant molecules have stronger interactions with each other, the corresponding films also display a higher surface viscosity and increased elastic properties (94). These effects, namely higher elasticity, and viscosity, entirely stabilize the films (bubble wall) and foam (93). However, there are multiple other relevant effects that influence the balance of foam degradation and stability.

These include electrostatic effects, van der Waals forces and slow down-drainage of the fluid. While some of these effects are more relevant, some are also less relevant for the overall stability of the foam. One of these crucial effects is called the down-drainage effect, which has been visualized in figure 5. The down-drainage effect "dries up" the upper levels of the foam. A related effect is called marginal generation, which describes a tendency in foams that areas with lower liquid content tend to move upwards. This phenomenon further dries up the "upper" areas of the foam (95). It is noteworthy that specific procedural settings are relevant for some of these factors, as they can significantly impact foam stability.

In fact, one phenomenon which can be highly relevant in foam creation but can be disregarded in our study is the evaporation of the solvent in open space (95), which usually occurs at lower humidity levels, meaning less than 99% humidity. This effect describes surface evaporation of the liquid which causes further thinning of the top films of the foam. However, in our model this effect is not considered relevant because the abdominal cavity displays a humidity level of basically 100%, which is either already present at the beginning of the procedure or established shortly after creating a continuous capnoperitoneum (96).

1.6.4 Ostwalt ripening

Another important process in the increase of the size of the bubbles and foam transformation is the Ostwald ripening. Small bubbles tend to submerge into larger ones because smaller bubbles are thermodynamically less stable (97). These larger bubbles tend to carry less fluid based on their characteristics with regards to size, form, and hydrophobic tendencies (98, 99).

1.7 Biological and chemical challenges of foam-based carriers

It is important to consider that IPC application is more than infusing chemotherapy into the abdominal cavity. In contrast to intravenous drug applications, a locoregional drug administration always bears its own challenges and difficulties. To ensure a successful IPC procedure, these challenges must be mastered. In fact, depending on how IPC is delivered, different aspects must be considered. At first glance, only few limitations seem to be associated with a fluid lavage as performed during HIPEC.

In most cases, the heated chemotherapeutic solution is directed into the abdominal cavity via previously placed tubes, whereas in some minor cases, the drug solution is poured into the abdominal cavity as an “open” HIPEC. In contrast, in PIPAC procedures we see limitations regarding the volume and solubility of the applied chemotherapeutic drug in the aerosolized carrier solution, as well as concerns with drug distribution and inhomogeneity during application (66, 87). One major challenge is that some drugs do not dissolve within a small volume or show signs of destabilization in the initial solution (87). While in PIPAC, we observe a rather physical process of aerosolizing the fluid compound, the application of foam is a far more complex endeavour. The creation of a foam which can carry different chemotherapeutic drugs is a challenging process which extends far beyond simple solubility. It is therefore crucial to identify highly relevant criteria and concerns beforehand to achieve a successful outcome.

1.7.1 The significance of foam creating capacity

Depending on the reagents, the applicational device and mode of delivery, the foam can expand within the applicational device or within the abdominal cavity. Overall, total foam expansion, which corresponds to the maximum foam volume, depends on the initial foam solution and the foam expansion rate (FER). The FER equals the volume of the “finished” foam divided by the volume of the initial foam solution used for foam creation. In general, a FER between 2-20 to 1 points to a low expansion foam. Accordingly, a a higher expansion foam would display a higher ratio of between 20 - 200 to 1. Much higher expansion ratios are considered at levels over 200 to 1 (100). In fact, various factors can impact the FER. Total foam expansion is limited when no additional air is added to the foam fluid, meaning the number of reagents is also limited.

Basically, this means that even in a setting with an optimal foam stabilator, a chemical self-expanding foam can only gain as much additional volume as the volume of the gas that is released during the chemical reaction. The foam will not surpass the total volume of its components, namely air and fluid, a finding which is self-evident (101). There are limits to achieving the maximal possible FER that could otherwise be reached under ideal circumstances.

Some of these potentially interfering factors are discussed in the following paragraphs. Beside the ideal maximum expansion, there are other relevant factors to be considered which interfere with reaching the ideal maximum expansion when creating the reagents of the foam fluid. These include the solved chemotherapeutic reagents, potential buffers to neutralize derived pH (potential of hydrogen) levels and other pharmacological stabilizers that may be applied during the process.

1.7.2. Suitable chemical expansion systems

While there are numerous chemical expanders, we aim for a self-expanding foam solution in this study. During this process, gas is released after initial activation of the foam fluid. The most logical choice to conduct this experiment is to opt for a non-reactive and non-toxic gas. For this purpose, carbon dioxide (CO_2), Nitrogen (N_2) and even Oxygen (O_2) could be potential choices. In terms of foam creation, two chemical reactions come to mind that are also commonly witnessed in the medical setting, namely the reaction of citric acid with sodium bicarbonate and the catalysation and reduction of hydrogen peroxide.

Hydrogen peroxide is a well-known antiseptic used to treat various biological surfaces (82, 102 - 104), whereas sodium bicarbonate combined with citric acid is probably best known for its use in effervescent tablets (105). Citric acid is an intermediate in the TCA cycle (tricarboxylic acid cycle, or Krebs cycle), a central metabolic pathway inherent to animals, plants, and bacteria (106). Citrate synthase catalyses the condensation of oxaloacetate with acetyl-CoA to form citrate. Then, citrate acts as the substrate for aconitase and is converted into aconitic acid.

The cycle ends with the regeneration of oxaloacetate (106). This series of chemical reactions is the source of two-thirds of food-derived energy in higher organisms. Because of its additional use as a nutritional supplement used in flavouring and its preservative qualities in food and beverages, especially in soft drinks, citrate has been denoted within the European Union by the E number E330 (107). Based on many experimental data from animals as well as the experience obtained following its nutritional use in humans, citric acid is known for its low acute toxicity.

The No-Observed-Adverse-Effect Level (NOAEL) for repeated dose toxicity in rats has been estimated at 1200 mg/kg/d (108). This dose is quite high and would correspond to a daily dose of 90 grams (g) for a 75 kilogram (kg) person. Citric acid is neither considered carcinogenic, reprotoxic or teratogenic. The NOAEL for reproductive toxicity for rats was estimated to be around 2500 mg/kg/d (108). This would correspond to a daily dose of 187g for a 75 kg person. There is no data to indicate in-vitro or in-vivo mutagenicity. Irritation, especially when affecting the eyes, the respiratory pathways and the skin, has been described as a major toxicological hazard when handling citric acid; this assessment has been confirmed by a series of reports related to eye and skin irritation (109).

Minor gastrointestinal disturbances, including diarrhoea, indigestion, nausea or “burning” were experienced by 22 out of 81 patients taking potassium citrate in water and 7 out of 75 patients ingesting solid potassium citrate for the treatment of renal calculi (108). Ingestion of potassium citrate solutions on possibly more than one occasion in one case and 200–400 ml over 5–7 days in two other cases, caused abnormal heart rhythms, which were attributed to elevated potassium levels rather than to the citrate intake (108, 110). Injection of citric acid in rats, mice and rabbits has been associated with adverse effects on the nervous system, lung, spleen and liver which were in part attributed to acidosis and calcium deficiency (108).

Volunteers given oral doses of potassium or magnesium citrate corresponding to approx. 4.7g of citric acid did not suffer from any overt gastrointestinal effects (108). Injection of large volumes of citrated blood during transfusion may lead to hypocalcaemia and changes in blood composition with concomitant nausea, muscle weakness, breathing difficulties and even cardiac arrest. No animal studies are available for acute dermal or inhalation toxicity. In general, citric acid is a strong chelating agent, and its dietary uptake may interfere with its biological availability, absorption, and excretion of metals. Moreover, loss of superficial enamel and teeth erosion as well as local irritation result from frequent ingestion of citric acid in beverages including natural fruit juices; moreover, citric acid fumes were reported to affect teeth (111 – 113).

The average daily intake of citric acid from natural sources in the diet and food additives was estimated at about 40 mg/kg for women, 130 mg/kg for infants and 400 mg/kg for individuals on slimming diets; maximum daily intake is reported at 500 mg/kg (108). No formal acceptable daily intake (ADI) level has been specified for citric acid and its common salts by the Joint FAO/WHO Expert Committee on Food Additives or the EC Scientific Committee for Food. As previously mentioned, a first attempt has been made to study the possibility for foam-based intraperitoneal application (76).

The first applied carrier system which could fulfil some of the major criteria was hydrogen peroxide. This initial choice for hydrogen peroxide seemed logical because this substance has been used in the clinical setting for decades. However, hydrogen peroxide has lost some of its clinical use today, as it has been associated with toxics effects which could interfere with wound healing and tissue regeneration (114), despite its long-term use in wound decontamination and disinfection.

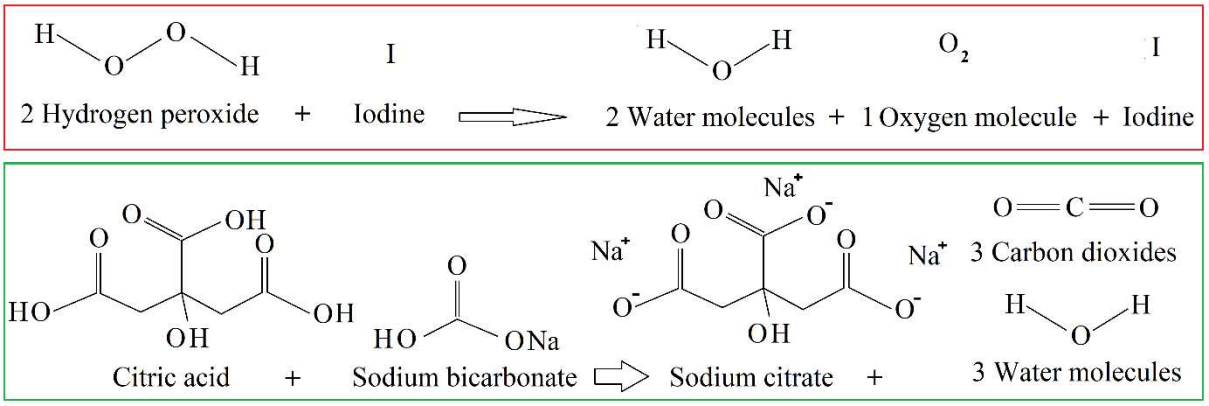

Figure 7: Reaction formula and chemical structure of reagents in hydrogen peroxide and bicarbonate-based foam.

Red frame: Hydrogen peroxide foam is a catalysed reaction with no additional active reagents beside hydrogen peroxide. Oxygen is the air compound of the foam. Green frame: The bicarbonate foam is a classical chemical reaction. Carbon dioxide is the air compound of the foam.

1.7.3. Biocompatibility and pH neutrality of the carrier solution

While the reagents of both carrier systems have been used in the medical setting and on biological surfaces, there are still concerns regarding an intraperitoneal application. We must be aware that applying a considerable volume of foam on a large biological surface can be critical. The contact surface of the reagents and the peritoneal surface extends any surface associated with wound disinfection. Therefore, an in-vitro followed by an in-vivo testing of both systems is essential to evaluate their biocompatibility.

Naturally, this includes that the carrier system has an “optimal” pH for its ultimate use on peritoneal tissue. In fact, this means that the carrier system should not expose the organism to a toxic foam with a pH level that is far different from the body pH. Besides local effects of the carrier, there should only be limited systemic effects, if any. While local toxicity might be tolerated or even beneficial to some degree as it interacts with the tumour microenvironment, a general systemic toxicity should be avoided.

1.7.4. Controlling foam formation

The concept of a chemical-based foam follows the idea of a “self-expanding solution”. Following activation, this solution is supposed to expand and transform into foam. There are also alternatives to this concept, as foam can also be produced by mere physical means. However, foam produced by physical means displays a set of its own numerous challenges, which are too extensive to be covered in this section. With regards to the “self-expanding solution” and foam expansion, it is important to note that there are two separate, feasible applicational settings. In the first setting, the primary expansion of the foam occurs within the abdomen.

In the second setting, the foam is produced extra-abdominally and the already expanded foam is then directed into the abdominal cavity. When looking at these settings, the question arises as to whether there is a way to control the foam formation process. Such management would require reliable reproduction of foam creation and expansion with only minimal variations regarding its reaction speed and maximum expansion rate. Rapid, unexpected expansion of a therapeutic foam can cause different surgical challenges which may interfere with procedural safety. Additionally, the feasibility of procedure itself, the correct functioning of the applicational device as well as the optimal distribution of the applied drug can be jeopardized. Once the initial reaction is set on, the creation of an FBIC may turn into an unmanageable process. The products of the reactions and their interaction with the local environment must be considered as well as the speed at which the reaction occurs. In sum, the reliability of the foam formation process must be assessed, and its proper operation must be ensured.

1.7.5 Pharmacological compatibility

From a chemical and pharmacological perspective, it is crucial to assess if the foam is compatible with a particular therapeutic substance dissolved in the initial solution, or whether such a substance can be added during the foam creation process and then directed into the abdominal cavity. While a wide range of substances are available for intraperitoneal chemotherapy, compatibility might not be achieved for every substance. In fact, classical chemotherapeutic drugs such as doxorubicin, cisplatin, mitomycin C and others already used compose just one group of potentially applicable drugs (115, 116).

In the future, more complex substances like antibodies could also play a significant role (117). Even the application of new checkpoint inhibitors is feasible and should be considered for future intraperitoneal application. Regardless of the type of therapeutic agent, neither the foam itself nor single components or reagents of the foam should show any signs of reaction to or changes in the therapeutic agent, or any interference with its biological target.

Thus, taking these considerations into account, potentially suitable therapeutic substances for a particular carrier system are limited. The reagents must be limited in their reactive threshold, the product and energy output. This means that the combined reaction of the reagents should be limited in effect as not to cause excessive heat associated instability of the therapeutic compound. At the same time, reagents should not be so reactive as to impact the chemotherapeutic agent. Ultimately, each applicable substance must be subjected to thorough investigation. While the optimized constellation for such a reaction has been outlined here, it is safe to assume that the numbers of applicable substances are limited. Analysing the effects of the "carrier-system" is a challenging task requiring enormous efforts, which cannot be covered in this study. It may therefore be impossible to find a carrier which fulfils all of these premises. However, we believe that there must be a manageable solution which fulfils most criteria in an acceptable manner.

1.7.6 The use of taurolidine in foam creation

The foam stabilization effects of proteins are well known (118). However, other substance classes like detergents can also enhance and stabilize foam. One of these proposed components by Schubert et al. is taurolidine (76). Taurolidine is a substance that has detergent characteristics which could support foam creation and stabilization. In fact, taurolidine has been described as a component of the hydrogen peroxide-based foam. However, it is unclear if this component is a required component for foam creation and whether its use is even advantageous. Prior to the study of Schubert et al. (76), taurolidine had already been identified as a cytotoxic agent with reported antitumoral effects on various cancer cell lines (69, 119 – 120). In clinical applications, it is mainly used due to its antibacterial qualities. Some of these clinical applications include peritoneal lavage in children or in patients with peritoneal dialyses who display signs of peritonitis (122, 123). In some cases, taurolidine has also been used to block central venous catheters (124 – 126). Although its disinfecting effects are well established, its antitumoral effects remain somewhat controversial. In-vitro and animal data mostly support taurolidine's tumoricidal abilities (127, 128), however some studies contradict this effect in the in-vivo setting.

1.8. Applicational and clinical challenges of foam based-carriers

In the previous segment, the biological and pharmacological considerations and challenges of FBIC have been discussed. Yet, there are also numerous applicational challenges that must be considered. We have outlined and emphasized that foam can be produced either intra- or extraperitoneal. The next step is to investigate how foam could be delivered into the abdominal cavity. The most obvious approach is to use a "standard" operative procedure.

One feasible option is the application of extraperitoneal created foam which, after expansion outside of the abdomen, is directed into the abdominal cavity. This application can be combined with a limited abdominal laparoscopy prior to foam application.

A second option would be to inject extraperitoneal foam using a microneedle (for example Verres-needle), a peripheral venous catheter place in the abdominal cavity or an intraperitoneally placed central-line. This type of application does not allow for prior visual check of the intraperitoneal cavity including detection of potential advanced adhesions, or observation whether full expansion is possible. In this case, visual control can only be conducted via ultrasound or computer tomography. Both presented concepts of intraperitoneal or extraperitoneal foam creation are conceivable.

1.8.1 Air-trapping and foam generation

There are numerous technical challenges in FBIC application. These include expansion pressure and the energy for the actual foam delivery in case of extraperitoneal foam creation. During laparoscopy, the capnoperitoneal pressure is around 12 - 15 mmHg. This pressure is both continuous during laparoscopy and required to ensure adequate expansion of the peritoneal cavity. At the same time, if foam creation occurs extraabdominal, a pressure build- up is needed to push the foam into the peritoneal cavity. Moreover, even if the foam is delivered from extraabdominal, the question arises as to whether further expansion of the foam could occur within the abdominal cavity.

Therefore, we must ask if a quantification of “delivered” extraperitoneal foam is possible. Can the contact with the peritoneum and flow through the tubing system cause significant degradation of the foam? This effect could lead to air trapping within the abdominal cavity, resulting in local but large air compartments that would prohibit the local peritoneum to come into full contact with the chemotherapy carrying foam.

1.8.2 Reaction onset

Reaction onset is a notable challenge. Some reagents are available as a liquid solution or in their “pure” dry form. The physical state of an applied reagent is a relevant factor since some reagents may not be completely dissolved in the required amount due to their limited solubility in water (87). Another important factor is reaction control, which must be further studied and optimized for possible clinical application. The key question is whether the entire foam fluid should be activated at once or if the reaction should be modulated and gradually set on like in a titration process. Another relevant aspect is the previously mentioned effect of “air-trapping”, which might hinder the local peritoneum to come into full contact with the chemotherapy carrying foam.

Therefore, the actual question is the following: Does expansion of the peritoneal cavity prior to foam delivery cause trapped air pocketing? And if so, will such pocket formation result in areas that are unaffected by foam application?

1.8.3 Inhomogeneity and reagents contact area

While no substance inhomogeneity has been observed within the foam fluid when they are combined (76), the inhomogeneity within the initial foam compound remains a concern. However, it is possible that the foam expansion pushes some parts of the reagent's solution into another area, which means that both reagents cannot reach sufficient contact. Depending on the delivery system, this foam expansion can separate reactive ingredients from each other.

1.8.4 Clinical challenges

There are different sets of challenges for each stage of the experiment. At the beginning (Stage 1), study design and planning, foam composition, biological and chemical aspects are major concerns. Some of the aspects regarding technical application which also covers the actual implantation of foam into the peritoneal cavity (Stage 2) have also been listed and explained. However, there are different considerations at the later stage of the experiment. These are especially centred around the subjects of best postinterventional care, safety, and monitoring (Stage 3 and 4). Equally, potential long-term effects of FBIC must be considered.

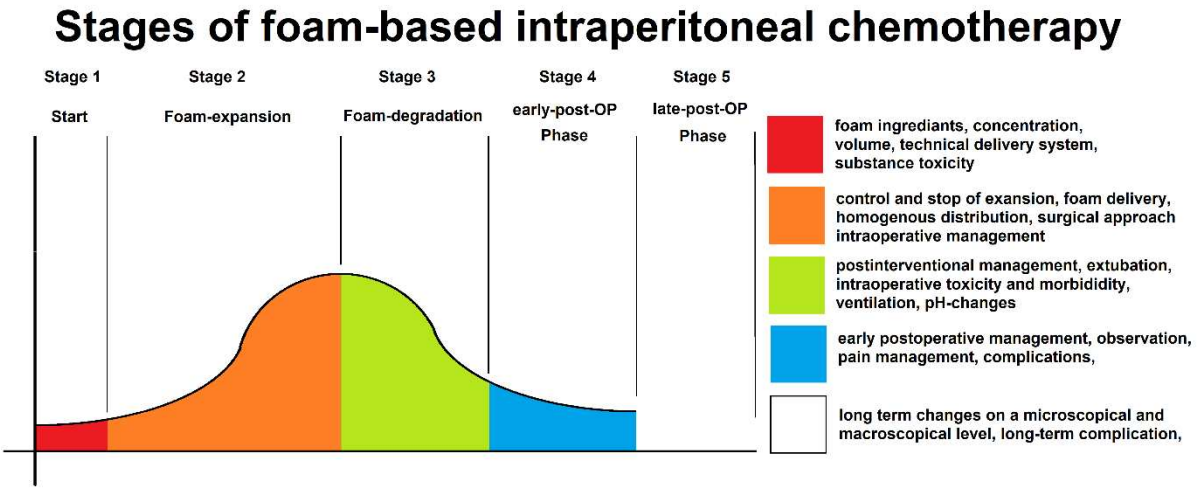

Figure 8: Challenges of foam-based intraperitoneal chemotherapy (FBIC) at different stages during the procedure

The extent of challenges that must overcome further underline the complexity of the experimental design, organisation, and execution. The X-axis represents a non-linear timeline from the preparation and start until the end of the procedure (stage 4) as well as long-term effects (stage 5). The Y-axis is a simplified visualization of the idealistic foam expansion at each stage. A list of challenges and potential related complications has been noted for each stage of the procedure (from own illustrations).

1.8.5 Intraoperative challenges of the early and late postinterventional stage

As previously mentioned, multiple applicational approaches for FBIC are conceivable. The goal is to apply the foam in the best "manageable and controlled" manner during the in-vivo part of the experiment. There are two options to do this. In the first option, the foam will be created extra abdominal and then directed into the abdominal cavity. The second option is to directly apply the reagents onto the peritoneum, meaning that foam creation occurs within the peritoneal cavity. In the second option, the operator is much less able to control the pressure build-up in the abdominal cavity. A pressure control system as currently used during laparoscopy would be beneficial to prevent critical pressure build-up. Similar control systems regarding ventilation are currently used in anaesthesiology. In the case of a critical pressure build-up, the placed trocars must be opened to release excess foam, or the system stops any further foam insufflation. However, at this time, there is no compatible control system for foam insufflation. With respect to these considerations, it seems more reasonable to apply an extra abdominal foam delivery system. In laparoscopic procedures, the abdominal cavity expands and a laparoscopic cavity of approximately 4 litres is created at 12 - 15 mmHg to create a workspace for diagnostic or surgical procedures.

Following the procedure, most of the air is released and only low residual air volumes remain trapped in the abdomen. At this time, it remains unclear whether the applied foam should be removed from the abdominal cavity or if it will partially collapse and only the air component will be released at the end of the procedure. The quantification of the foam-volume is another significant challenge. The exact quantification of foam in a self-propelled (meaning self-expanding) foam system which is directed through a tubing system to reach its ultimate destination is extremely difficult.

We know that the stability of foam can vary and is influenced by many factors. Based on yet unpublished data we also know that depending on the qualities and diameter of the tubing system, part of the foam collapse and creates a liquid film. These are some of the aspects which further complicate the calculation of the applied volume. Beside challenges regarding the surgical, applicational and anaesthesiologic management, there are further obstacles down the road, such as discomfort, systemic toxicity, and local tissue damage. With the exception of some organ damage, most systemic complications, including electrolyte imbalances, following the application of the reagents should be detectable in the early postoperative phase. However, it is important to remember that any potential harm to tissues can occur at any stage. In our model, we intend to perform an autopsy and histological examination on postoperative day 7. The timepoint was chosen based on the consideration to detect early changes, potential indications of perforation and the ability to reassess the abdominal cavity at an early stage in case of an emergency.

Yet, changes such as scaring, or adhesions can occur beyond the seventh postoperative day. Since changes that could occur after this observation period are not assessed, it is important to consider that our observation of complications is limited to a set time scope.

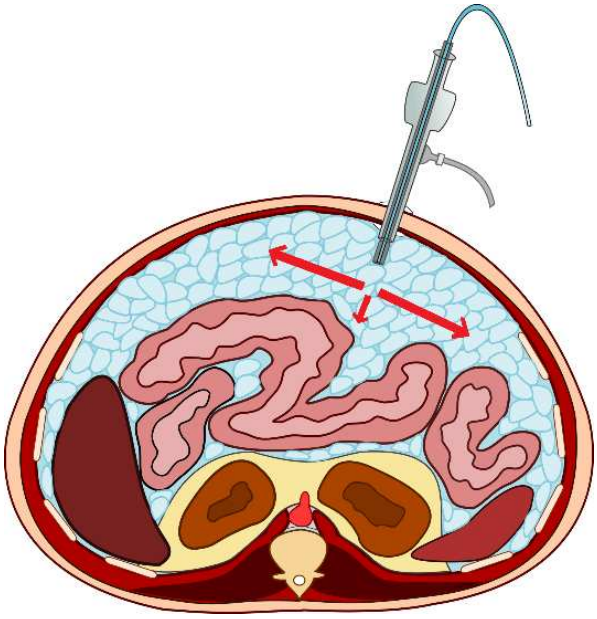

Figure 9: Cross-sectional illustration of FBIC in an in-vivo model

Assumed expansion and pressure distribution demonstrated in this trocar-guided model. The trocar is used for insufflation following extra-abdominal foam creation (from own illustrations).

1.9 Aim of this study

Based on the vast number of the previously described, existing challenges, the overall complex task ahead, the current state of knowledge and the very limited available data, this study intends to illuminate the most relevant aspects of FBIC. The purpose of this study is to test the feasibility of the FBIC concept and to establish a basic applicational setting which can be referenced in future studies. The intention is to explore a potential carrier system and investigate its application in terms of safety, technical challenges, and inherent characteristics by using a large animal model. We understand this is a difficult task, and that due to the complexity of this endeavour, not all aspects related to the FBIC concept may be extensively covered.

In fact, it is our intention to move current scientific knowledge and understanding toward new horizons, and to explore this promising and innovative concept in a courageous manner by identifying potential major challenges and concerns. Based on the considerations already expressed in the introduction section, we have established a list of questions that must be subjected to further analysis. Some of these questions cannot be answered by using only one model, but in fact require different models to grasp all relevant aspects.

Therefore, this study is divided into two parts. The first part of the experiments requires a laboratory setting for in-vitro and ex-vivo testing. The second part is based on a large in-vivo animal trial which aims to validate or reject some of the preliminary data gathered in the first part. The results collected in this study allow to build a basic understanding of this novel concept and establish the required foundation to enable further in-vitro and in-vivo experiments in this field. The major aims of the study can be listed as follows:

1) We intend to analyse the in-vitro toxicity of the two foam-based carrier-systems hydrogen peroxide and bicarbonate. Specifically, we aim to establish which system is more toxic in an in-vitro model. Therefore, we must ask:
   - *Is the cytotoxic effect of both carrier systems in-vitro the same or is there a detectable difference?*

2) We intend to analyse the physical expansion of the two foam-based carrier-systems to evaluate which system has a higher expansion ratio. Additionally, we intend to explore the extent of the exothermic reaction of both foam-based systems and to investigate which system has a higher heat production:
   - *Are the expansion ratios identical in both systems or can we observe a difference?*
   - *Do both systems express the same thermodynamic energy output following reaction initiation?*

3) We intend to investigate if the use of taurolidine is mandatory and whether it displays toxic qualities. For this purpose, we analyse its role in foam expansion, investigate its in-vitro effects at different concentrations and compare them to a well-known chemotherapeutic agent:
   - *How does the foam expansion ratio differ?*
   - *At the observed concentration, does taurolidine display associated in-vitro cytotoxicity?*

4) We intend to evaluate if laparoscopically applied foam is a safe by identifying intraoperative parameters which extend beyond known tolerance levels:
   - *Are there any temperature changes during FBIC application?*
   - *Are there any changes in vital signs during FBIC application?*
   - *Are there any disbalances in the gasometrical data during the application?*

5) We intend to evaluate some technical aspects of the procedure for later reference:
   - *Are there any relevant changes in the periumbilical diameter during the procedure?*
   - *Is the abdominal cavity accessible after FBIC application?*

- *Can swine be extubated after foam insufflation, or must evacuation occur beforehand?*

6) We intend to assess if the swine can be successfully extubated and whether any clinical signs of distress are observed:
   - *Do swine behave differently in the postoperatively period?*
   - *Are any changes in behaviour observed?*

Finally, a postoperative evaluation must be performed, including some basic laboratory workup, to evaluate potential critical changes in serum parameters routinely monitored during the postoperative phase. These include blood count and serum parameters related to liver and kidney function:

- *Are there any pathological changes in the blood workup?*
- *If so, are these changes persistent throughout the 7-day observation period?*

We aim to identify whether any specific structural changes on the macroscopical or microscopical level can be observed after 7 days:

- *Are there any noteworthy macroscopic intraperitoneal changes?*
- *Are there any noteworthy microscopic changes on the peritoneal tissue?*

2. Methods

2.1 Foam expansion, composition and technical analysis

2.1.1 Composition of hydrogen peroxide-based foam

The ratio of foam ingredients for the hydrogen peroxide foam was based on the previous description by Schubert et al (76). To create FBIC, a solution of taurolidine (Taurolin® Ringer 0.5%, Berlin-Chemie AG, Berlin, Germany), hydrogen peroxide (30% hydrogen peroxide solution, Chempur, Piekary Śląskie, Poland), human serum (from human male AB plasma, Sigma-Aldrich; Merck KgaA, Darmstadt, Germany) and 12 mm potassium iodide (Sigma-Aldrich; Merck KgaA) was used. The initial liquid solution consisted of 0.045% taurolidine, 22.8% hydrogen peroxide, 12.5% human serum. No additional chemotherapy was added at this point. The reaction was initiated by adding the potassium iodide to the prepared solution.

2.1.2 Composition of bicarbonate-based foam

The ratio of foam ingredients to create the bicarbonate-foam were mathematically and experimentally determined. The basic chemical reaction was analysed as presented (figure 7). The components of the foam are citric acid (Sigma-Aldrich, St. Louis, USA) and sodium bicarbonate (Sigma-Aldrich, St. Louis, USA). Both components are used in dry form and equal amounts in mol (1:1). No additional chemotherapy was added at this point. Since the components were present in dry form, the reaction was initiated by adding physiological saline to the solution.

2.1.3 Expansion analysis and temperature development

Experiments were performed in a standard graduated cylinder with a total volume of 150 ml. In this comparative analysis for both foams, both cylinders were equally filled with a total of 5 ml of initial foam fluid. The hydrogen peroxide foam consisted of 3.8 ml hydrogen peroxide (30%), 0.45 ml Taurolidine (0,5%) 0.15 ml -1 molar potassium-iodide (PJ) and 2.4 ml protein solution (10% solution). Instead of using human serum plasma, dried swine albumin (albumin from porcine serum, lyophilized powder, Sigma-Aldrich, St. Louis, USA) was used for both foams. The bicarbonate foam consisted of 2g of sodium bicarbonate and citric acid mixture (at a 1:1 molar ratio) plus 0.45 ml taurolidine (0,5%), 2.05 ml physiological saline and 2.4 ml protein solution (10%). Temperature sensors (Digital thermometer, Fisherbrand TM Traceable, Pittsburgh, USA) were placed at the centre of the cylinder at the 10 ml mark. After initiation of the reaction, both the expansion and temperature of the created foam were measured.

2.1.4 Taurolidine effect on maximum foam expansion

Experiments were performed in the same graduated cylinder with a total volume of 150 ml. In the comparative analysis for both foams, both cylinders were equally filled with a total of 5 ml of initial foam fluid.

The hydrogen peroxide foam and the bicarbonate foam consisted of the previously described components with taurolidine (A) and without taurolidine (B). After initiation of the reaction, maximum foam expansion was measured.

2.2. Human HT-29 cell line for viability and cytotoxicity measurement

The human colorectal cancer cell line HT-29 was obtained from CLS (Cell Lines Service GmbH, Eppelheim, Germany) and cultured in DMEM (Dulbecco's modified Eagle's medium; Sigma-Aldrich) supplemented with 10% heat-inactivated fetal calf serum (Fisher scientific, Schwerte, Germany), 2 mmol/l glutamine, 100 IU/ml penicillin, and 100 µg/mL streptomycin (Sigma-Aldrich, Sigma-Aldrich, St. Louis, USA) at 36 Degrees Celsius (°C) in a humidified 5% CO_2/air atmosphere. HT-29 cells were seeded at a density of 1.4 x 10^5 cells per well in 24-well plates (TC Plate 24 Well, Standard, F, Sarstedt AG & Co. KG, Germany) and incubated for 48 hours at 36°C with 5% CO_2.

2.2.1 In-vitro viability following hydrogen peroxide and bicarbonate foam

An MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay was performed according to the manufacturer's instruction (Promega GmbH, Mannheim, Germany) with modifications. Briefly, the medium was removed from each well and replaced by 0.3 ml of fresh DMEM. 60 µl of CellTiter 96® AQueous One Solution Reagent was added to each well and absorbance was measured on a microplate reader (Tecan, Basel, Switzerland) at 490 nm after 1 hour of incubation at 37°C. The percentage of proliferation was determined for all groups.

To investigate the effect of hydrogen peroxide and bicarbonate foam on tumour cells, the present medium was aspirated from each well and replaced with 200µl of regular medium. For each group, the following substances were additionally added: For the control group, 300 µl medium of regular medium was added. The second group was treated with 7.36µl oxaliplatin (Medoxa, medac GmbH, Wedel, Germany) per well for 45 minutes. The oxaliplatin concentration in each well was 0.24 mg/ml, approximately corresponding to a common HIPEC amount of 960 mg/4 litre (480mg/m²).

For the hydrogen peroxide group, 300µl of foam fluid was added according to the previous description without the protein solution. For the bicarbonate group, different volumes of foam fluid were added according to the previous description without the protein solution. The wells were therefore filled with either 10 µl, 25 µl, 50 µl, 100 µl, 150 µl, 200 µl, 250 µl and 300 µl of the bicarbonate foam solution. The exposure time was 1 hour at 36°C with 5% CO_2. After this period, the content of the wells was aspirated, and 0.5 ml of fresh medium was added. Cells were incubated for 48 hours under the same conditions and an MTS proliferation assay was performed.

2.2.2 In-vitro cytotoxicity of taurolidine as a component for foam

In this study, we analysed the cytotoxicity of different taurolidine concentrations (Taurolin® Ringer, Berlin-Chemie AG, Germany), which were dissolved in medium. For one hour, cells were exposed to medium containing taurolidine at the following concentrations: 0.045%, 0.06%, 0.09%, 0.135% and 0.18%, respectively. One group was treated with oxaliplatin (Medoxa, medac GmbH, Wedel, Germany). The oxaliplatin concentration in each well was 0.24 mg/ml (=0.6mmol/L), which corresponds to 960mg/4 litre (480mg/m² body surface), the concentration used in HIPEC applications.

Untreated cell cultures were used as control. Taurolidine-mediated cytotoxicity in colon cancer HT-29 cells was determined by release of lactate dehydrogenase (LDH) into the supernatant using the CyQuant LDH Cytotoxicity Assay. (ThermoFisher Scientific, Waltham, US). LDH is a cytosolic enzyme present in many different cell types. Plasma membrane damage releases LDH into the cell culture media. Extracellular LDH in the media can be quantified by a coupled enzymatic reaction. Then, the product of this reaction can be measured at 490nm.

HT-29 cells were grown in initial wells for 24 hours and LDH assay was performed according to the manufacture's instruction. To obtain maximum LDH activity, cell lysis reagent was added to three wells which had been exempt from any substance treatment and incubated for 45 minutes at 37°C. Spontaneous LDH activity was assessed in cells exempt from any reagent or lysis procedure. Then, 50µl of supernatant from each well was transferred to a 96-well plate and 50µl of LDH substrate was added to the supernatant. The plate was incubated at room temperature for 30 minutes without exposure to light. After that, a stop reagent solution was added and absorbance was measured at 490nm on a microplate reader (Tecan, Basel, Switzerland). The percentage of cytotoxicity was calculated using following formula: % cytotoxicity = (compound - treated LDH activity – spontaneous LDH activity) ÷ (maximum LDH activity – spontaneous LDH activity) x 100%.

2.3 Experimental set-up

The study included three, approximately 50 kg, female, 65-day-old swine of Polish large white breed (domestic swine by local pork supplier, Zerniki Wielkie). In-vivo swine experiments were conducted at the external research facility of the peritoneal and pleural surface malignancies research group, University Hospital Düsseldorf. The external research and operation facilities were located at the Department of Veterinary Surgery, University of Environmental and Life Sciences in Wroclaw, Poland. The experiments were approved by the local ethics committee and board on animal welfare at the Wroclaw University of Environmental and Life sciences.

2.3.1 Housing and husbandry

Swine were housed in pairs in pens with dimensions of 1.8m of width and 2.5m of length. Floors were made of concrete and covered with sawdust. Swine were housed at 18–20°C, which was considered room temperature maintained by air conditioning, and relative humidity of 60–75%. Pens were cleaned twice daily. Swine were fed a balanced diet (90.44% dry weight) containing 14.7% of protein, 3.1% of fat, 4.7% of crude protein, 6.06% of ash, 0.5% of salt (NaCl), 1.05% of calcium, 0.77% of phosphorus, 0.62% of lysine, 0.24% of methionine, 0.3% of cysteine, 0.48% of threonine, 0.183% of tryptophan, vitamin A (13 243 IU/kg), vitamin D3 (2 000 IU/kg), vitamin E (81.65 mg/kg), vitamin B1 (4.11 mg/kg), vitamin B2 (7.16 mg/kg), niacin (vitamin B3, 50.22 mg/kg), vitamin B5 (24.29 mg/kg), vitamin B6 (6.11 mg/kg) and vita- min B12 (36 µg/kg), and had unlimited access to water. Food and water were given automatically. Food was restricted for 12 h and water was restricted for 4 h before anaesthesia. Experiments were performed at 10 a.m. For environmental enrichment, soft balls, rope and wood logs as well as radio music were provided. All animals received humane care in compliance with the 8th edition of the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health [129].

2.3.2 Laparoscopic in-vivo swine model

The study included three 65-day-old swine. A laparoscopic approach was used for the procedure and swine received total anaesthesia for the laparoscopic setting. For this purpose, the swine were premedicated with an intramuscular injection of midazolam (0.3 mg/kg, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (9 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. Analgesia was performed with propofol at 1mg/kg. Swine were intubated and further anaesthesia was continued with isoflurane 1%. Additional analgesia was provided with fentanyl 2µg/kg and crystalloid fluid at 0.2 - 0.3 µg/kg/min.

Swine were placed in a supine position. An infra-umbilical mini laparotomy was performed and another at about 8 cm distance to the first one. A 10 mm trocar (Kii®Balloon Blunt Tip System, Applied Medical, Rancho Santa Margarita, CA, USA) was inserted through the infra-umbilical trocar while a 5 mm trocar was placed at the other side (figure 11). The abdominal cavity was insufflated with CO_2 to maintain a capnoperitoneum (Olympus UHI-3 insufflator, Olympus medical life science and industrial divisions, Olympus, Shinjuku, Tokyo, Japan). An initial diagnostic check-up was conducted using laparoscopic imaging via a 5 mm camera system (Karl Storz 5mm/30° Laparoscope/Tuttlingen, Germany). After visual confirmation that no anomalies were present, the “foam-insufflation” tube of the foam generating system was introduced into the 10 mm trocar. Correct placement was confirmed using laparoscopic visual imaging.

The laparoscope was then removed, a temperature probe was inserted through the trocar and the CO_2 from the capnoperitoneum was evacuated. Another temperature probe was placed onto the abdomen from the outside and fixed with adhesive tape.

2.3.3 FBIC application systems

Based on the experience of the previous models and additional unpublished data, an optimized in-vivo setting was created. This setting might partially differ from the previous form and settings of the ex-vivo model experiments. The experimental setting in the following part was adapted according to the results of the previous in-vitro data. Based on the presented results, the bicarbonate carrier system was assessed as superior to the hydrogen peroxide system. Therefore, the application system was conceived for this carrier system. The proposed foam application system consisted of a reaction chamber (Gaswaschflasche, Duran 500ml, Carl Roth, Karlsruhe, Germany) containing the dry components of the reaction (300mg of sodium bicarbonate and citrate acid at a molar ratio of 1:1).

The reaction chamber had a swirling magnet and was placed onto a magnetic stirrer device (7" Magnetic Hotplate Stirre, 4E Lab Healthcare, Guangzhou, China). The device was turned on at the beginning of the experiments. The chamber had a guided exiting site and an entrance site. The exiting site was connected to a plastic tube (foam-insufflation tube) which was then placed into the trocar (figure 10). The plastic tube had an external diameter of 10 cm and internal diameter of 8 cm. The entrance site was connected to another glass chamber (liquid – prereaction chamber) of the same type. This liquid – prereaction chamber contained the liquid components of the reaction (physiological saline 500 ml 0.09% and 25g porcine albumin (albumin powder, Sigma-Aldrich, St. Louis, USA). On the entrance site, the fluid chamber was connected to a regular air compressor, which allowed for modulation of stream and application pressure as needed. A bacterial filter system (Cytiva Whatman HEPA-Vent Filter, Fisher Scientific, Schwerte, Germany) was placed between the compressor and the following glass chamber.

2.3.4 Foam application process

After initial setup of the foam application system and preparation of the surgical field, the experiment was initiated by starting the air compressor. The compressed air filled the first chamber and directed the fluid-serum solution into the reaction chamber (figure 10). The reaction immediately initiated and continued. The magnet swirling device further enhanced the homogenous reaction of the components. The created foam was directed into the abdominal cavity through the exiting tube. The duration of the reaction was approximately 5 minutes. The insufflation of the abdominal cavity was stopped at the surgeon's empiric evaluation of sufficient „filling ".

A small foam sample was collected from the small trocar exit for further analysis. The abdominal expansion was measured throughout the procedure. Swine remained under total anaesthesia for approximately 30 minutes following initial insufflation. Information from the temperature sensors was collected and periodic arterial blood gas analysis were performed. After 30 minutes, swine were extubated and monitored for another 7 days.

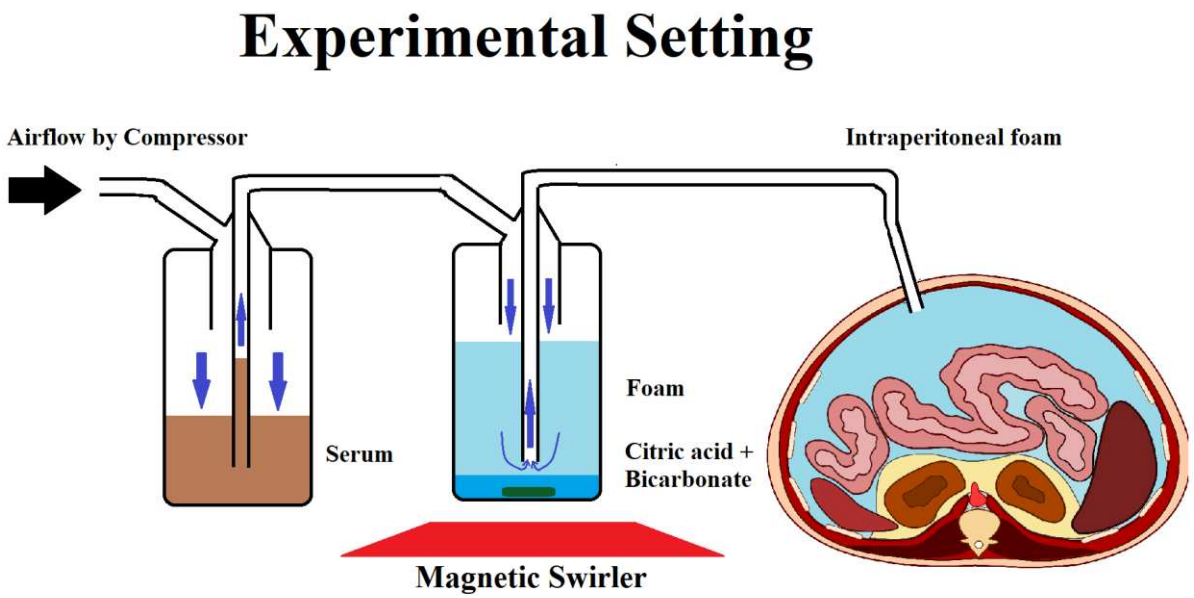

Figure 10: Experimental outline of foam based intraperitoneal chemotherapy (FBIC) application in an in-vivo model

Left: Initial chamber with ingredients dissolved in physiological saline solution (serum, chemotherapy). The fluid was pumped into the next chamber by regulated air compressor. Middle: This is the reaction chamber. The inflow of the fluid from the initial chamber activated foam generation. The pressure from the incoming fluid line prohibited the backflow of foam and directed the foam into the exiting line. The created foam was constantly directed into the abdominal cavity trough this exiting line (from own illustrations).

2.3.5 Postoperative monitoring

One operative procedure was performed per day. All swine were kept together and monitored for behaviour changes, feeding habits, indication of pain, and surgical site infection for a total of 7 postoperative days. At postoperative days 1, 3 and 7 (1d, 3d, 7d), blood samples were collected for blood count and serological measurements. On the last postoperative day (7d), an autopsy was performed.

Figure 11: Experimental view of FBIC application in an in-vivo swine model
Disinfected operative field appears red due to the use of iodine disinfectant. Surgical entrance site was visible: a large 10mm trocar was placed periumbilical, and a small 5 mm trocar was placed epigastric. Gas insufflation and subsequent foam insufflation were directed through the 10mm trocar. Both trocars were also used for laparoscopic intraabdominal visualization. Additional temperature sensors were placed to monitor temperature development during the procedure (from own gallery).

2.4 Postoperative examinations

2.4.1 Euthanization

Swine were premedicated with an intramuscular injection of midazolam (0.1 mg/kg, Midanium 5 mg/ml, WZF Polfa S.A., Poland), medetomidine (0.02 mg/kg, Cepetor 1 mg/ml, CP-Pharma Handelsgesellschaft, Germany) and ketamine (8 mg/kg, Ketamina 100 mg/ml, Biowet Puławy sp. z o.o., Poland) mixture. Then, they were euthanized with an intravenous injection by Sodium Pentobarbital with pentobarbital (50mg/kg with 12 mg/kg, Morbital 133.3 mg/ml + 26.7 mg/ml, Biowet Pulawy Sp. z o.o., Poland), according to recommendations (129). Swine cadavers were placed in a supine position. A median laparotomy was performed, and the intraabdominal cavity was thoroughly examined. Tissue samples were retrieved from multiple sites within the abdominal cavity, including the following: stomach, small intestine, liver, and multiple sites of the parietal peritoneum.

2.4.2 Microscopic analysis of peritoneal tissues

Samples were taken to the University of Düsseldorf and subjected to further analysis there. Following sample retrieval, the material was fixed in 4% buffered formalin with a pH of 7.2 - 7.4 for 48 hours. Following rinsing in running water, the material was dehydrated in an alcohol series and embedded in paraffin. The 7µm thick slides were then stained using haematoxylin and eosin (HE) staining. The material was analysed and documented with photographs using the Nikon Eclipse 80i microscope (Nikon Instruments Inc., New York, USA) and Nis-elements AR software.

2.4.3 Statistical data analyses

Cell and ex-vivo experiments were independently performed in triplicates. Statistical analyses were performed with GraphPad Prism (GraphPad Software Inc., version 8.0.2 (263)). Student t-test was used to compare independent groups. Descriptive statistics included mean, median and percentiles. Probability (p) values were considered as follows: *p<0.05 and **p<0.005, and #p>0.05, with a p-value <0.05 considered to be statistically significant.

2.4.4 Ethical approval

The human cancer cells were commercially acquired. Thus, according to the laws of the ministry of Science and Higher Education in Poland, no further ethical approval was required for the use of human cancer cells. For the in-vivo swine experiments, the approval of the local Board on Animal Welfare was obtained (UCHWALA NR 029/2021/P1), as according to Polish and European Union law.

2.4.5 Graphic design

For the graphics provided, multiple graphic programs were used. These programs include Inkscape 1.0.1,2020, GNU, USA and programs provided by Windows Office 2019, Microsoft.

3. Results

3.1 In-vitro and ex-vivo experiments

3.1.1 Temperature curve in both foam systems

The foam formation process was observed in both groups of hydrogen peroxide and bicarbonate-based foam. However, while the hydrogen peroxide foam showed an exotherm reaction, the bicarbonate foam displayed an endotherm reaction. Mean temperature levels for the hydrogen peroxide increased up to approximately 32°C from an initial temperature of around 23 – 24°C, which corresponds to room temperature. The elevated temperature remained stable for an extended period. After approximately 15 minutes, it fell below 30°C. Finally, after approximately 50 minutes, the room temperature was achieved (figure 12A). For the bicarbonate foam, mean temperature levels decrease to approximately 17 °C from an initial temperature of around 22°C, corresponding to room temperature. The reduced temperature remained stable for a shorter time (figure 12B). In the bicarbonate foam, the minimum temperature change was reached at around 2 minutes while in the hydrogen peroxide foam, the maximum temperature was reached after 5 minutes. In the bicarbonate foam, the initial room temperature was again reached after about 20 minutes.

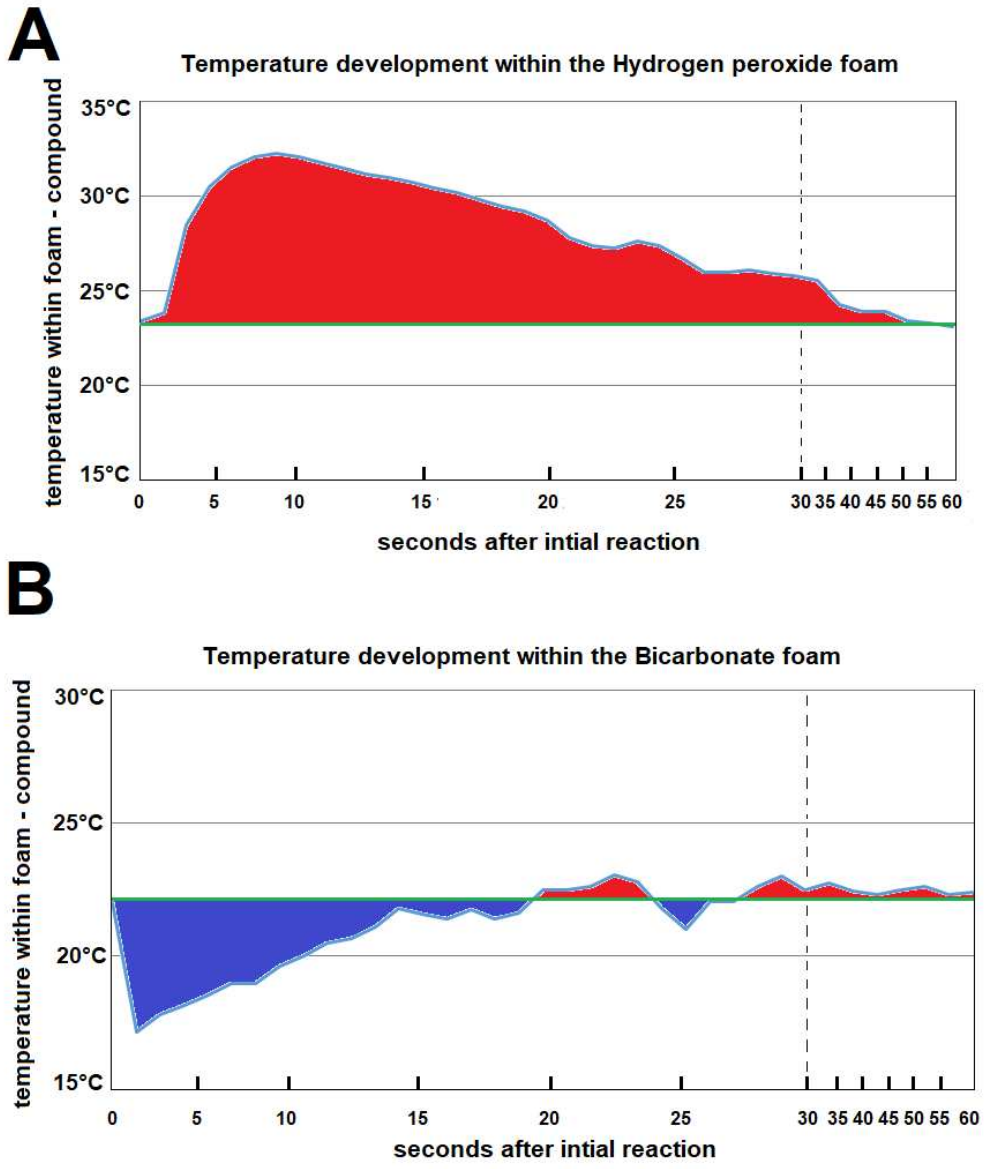

Figure 12: Exo- and endothermic reaction of hydrogen peroxide-based foam (A) and bicarbonate-based foam during expansion (B).

Hydrogen peroxide shows an exothermic reaction while bicarbonate foam shows an endothermic reaction. The return to the initial temperature is faster in the bicarbonate foam while heat build-up in the hydrogen peroxide foam remains stable for a longer time.

3.1.2 Foam expansion in both foam systems

The formation of a temporarily stable foam was possible. With bicarbonate foam, the foam reached its maximal volume at around 5 minutes into the experiment, whereas in the hydrogen peroxide foam, there seemed to be a delay in foam expansion. Maximum foam expansion was reached at around 5 to 10 minutes into the experiments (figure 13). While the bicarbonate foam rapidly degraded following initial expansion, the hydrogen peroxide foam did not collapse following initial expansion. Bicarbonate foam created an overall higher volume of foam which was nearly double that of hydrogen peroxide.

However, following the collapse of the bicarbonate foam at around 10 minutes into the experiments, both foams showed a similar collapse pattern. The volume of the produced bicarbonate foam was approximately 20 times greater than that of the initial foam fluid. The volume of the produced hydrogen peroxide foam was approximately 10 times greater than the initial foam fluid.

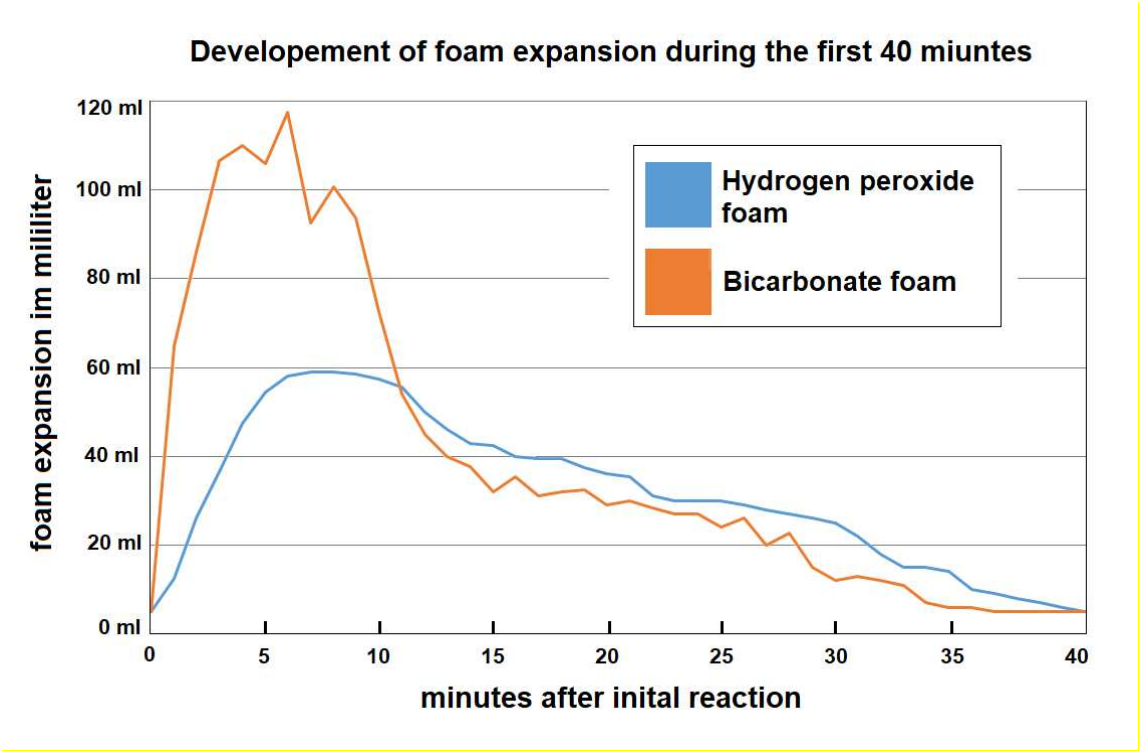

Figure 13: Volume expansion of hydrogen peroxide-based and bicarbonate foam after starting the initial reaction

Hydrogen peroxide seemed more predictable with less volume fluctuation than bicarbonate foam. The initial foam expansion was higher in bicarbonate foam, however degradation 10 minutes into the reaction is similar in both foam systems.

3.1.3 In-vitro viability after hydrogen peroxide vs. bicarbonate foam application

The in-vitro study of both foams showed significant differences in cytotoxicity. Hydrogen peroxide foam significantly reduced viability of colon cancer cells compared to untreated controls ($p < 0.0001$). While reduced viability was also noted in the oxaliplatin ($p < 0.001$) and bicarbonate group ($p < 0.05$), the extent was less notable than in the hydrogen peroxide group (figure 14). Also, the massive increase in bicarbonate foam concentration only slightly increases the effect on the cells. While the effect of the dose increase is still significant (10µl vs. 300 µl with $p<0.001$), the viability level is much better when compared to hydrogen peroxide. To further explore the effect of the witnessed exuberant cytotoxicity on peritoneal tissue, an additional test was performed on peritoneal tissue.

For this purpose, fresh post-mortem peritoneal tissue was covered with hydrogen peroxide foam for a total exposure time of 30 minutes before the foam was washed off. An untreated peritoneal sample was used as control. Beside macroscopical whitening of the tissue following hydrogen peroxide foam treatment, additional microscopical changes were observed. The superficial peritoneal layer, which mainly consisted of the mesothelium, was detached, and partially disrupted. This effect was already macroscopically detectable when the hydrogen peroxide treated sample was compared to the untreated control (figure 15 A - C).

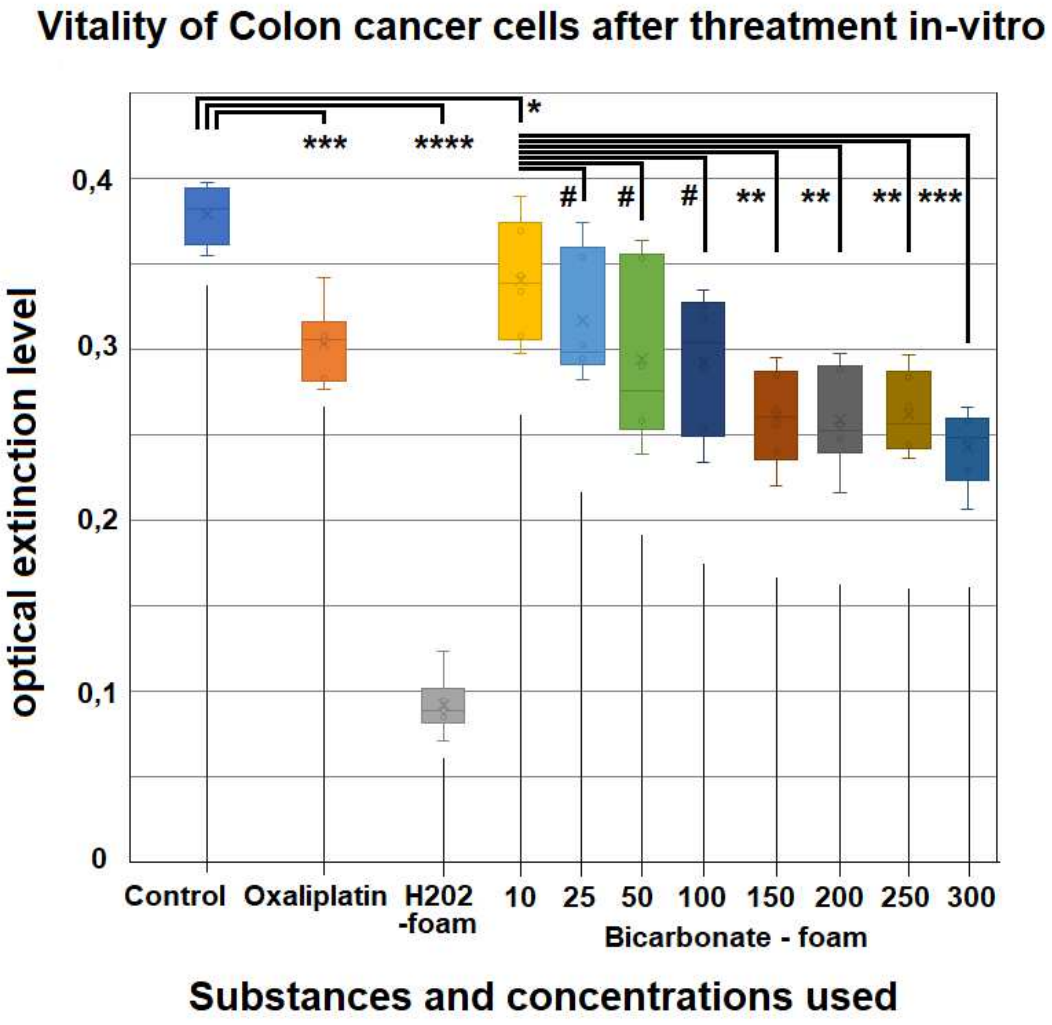

Figure 14: Vitality of in-vitro HT-29- colon cancer cells

Vitality of in-vitro HT-29 colon cancer cells following a 45-minute treatment with Oxaliplatin, H_2O_2-foam and bicarbonate foam at increasing concentrations. Significance level at # = >0.05, * = <0.05, ** = <0.01, *** = < 0.001, **** = 0.0001.

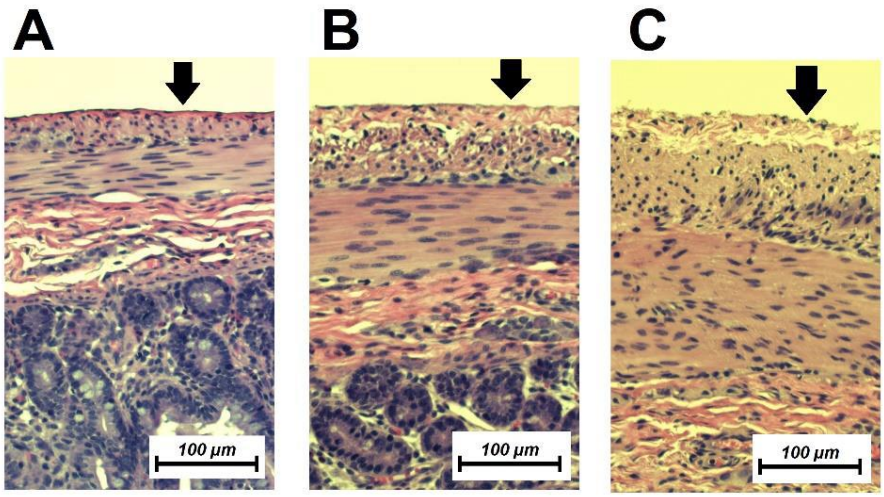

Figure 15: Foam exposure to the peritoneum

The superficial peritoneal layer exposed to bicarbonate foam (B) and hydrogen peroxide foam (C). Untreated peritoneum was used as a control (A). Following hydrogen peroxide application, detachment and partial disruption of the peritoneal surface is observed (C).

3.1.4 Taurolidine evaluation in terms of expansion and cytotoxicity

The data from the comparative measurement of foam expansion are similar for both foam systems with or without taurolidine. The current data does not demonstrate any relevant, observable effect upon taurolidine addition. The overall and maximum foam expansion does not significantly differ regardless of taurolidine (figure 16). The results concerning maximum expansion are the same for the hydrogen peroxide and the bicarbonate foam with or without taurolidine. Therefore, if we remove taurolidine (at 0.5% concentration) from the list of initial reagents, we can further reduce the volume of the initial foam fluid by approximately 9%.

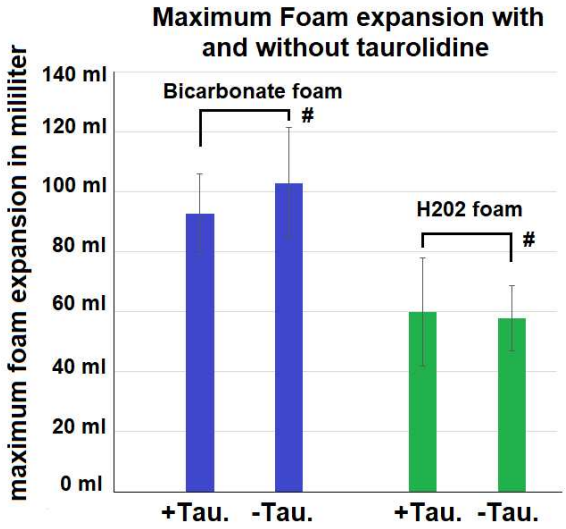

Figure 16: Maximum foam expansion

Maximum foam expansion of bicarbonate (blue) and hydrogen peroxide (green/H_2O_2) based foam measured in a 150 ml cylinder with and without addition of taurolidine. Blue columns: bicarbonate foam. Green columns: H_2O_2 foam. Significance level: # = $p>0.05$.

The data from taurolidine's cytotoxicity measurement indicate increased cytotoxicity in the HT- 29 cell line with increased taurolidine concentrations (figure 17). The cytotoxicity measured by LDH significantly increased from 0.045% to 0.18%. At a concentration of 0.135% and beyond, cytotoxicity is comparable to the in-vitro effect of oxaliplatin. Thus, taurolidine seems to display some cellular cytotoxicity.

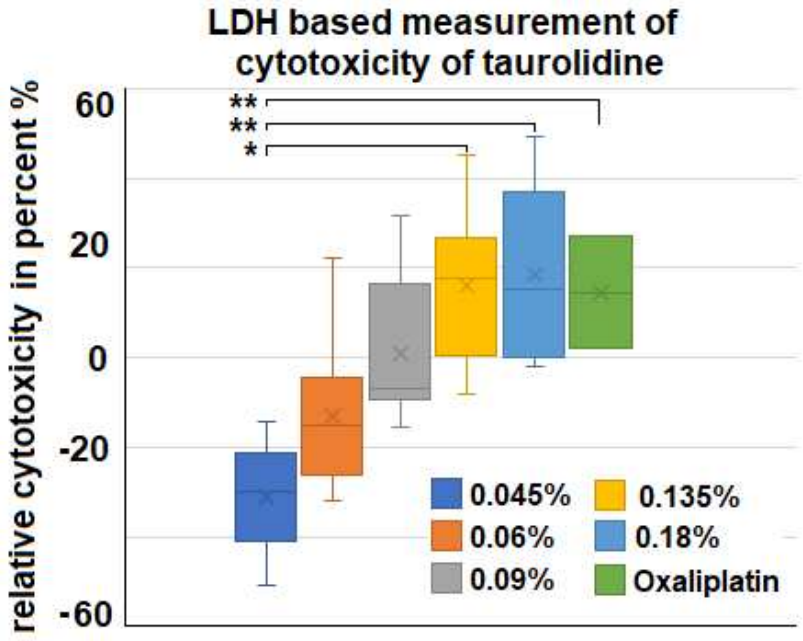

Figure 17: Cytotoxicity meassurements

In-vitro cytotoxicity of colon cancer cells (HT-29) following treatment with taurolidine and oxaliplatin for 60 minutes. Significance level at # = >0.05, * = <0.05, ** = <0.01.

3.2 Results of the in-vivo experiments

3.2.1 Applicability and safety concerns regarding FBIC

Overall, the in-vivo experiments were successfully conducted. No intra- or postoperative complications were detected. All animals survived surgery and the postoperative follow-up. No major complications or morbidity were noted. No postoperative macroscopical changes were observed after final cadaver autopsy (figure 27). No major applicational challenges occured during the operation. Extensive data was collected and no major anesthesiologic issues were detected during recovery. All swine ate and drank adequatly following the procedure. No pain or behavioral changes were observed during recovery.

3.2.2 Surgical and anesthesiologic procedures

The trocars were successfully placed in all three swine. Diagnostic laparoscopy did not display any pathologies or adhesions within the abdominal cavity. After removing the laparoscopic camera, temperature probes were placed at the described locations. CO_2 within the capnoperitoneum was evacuated by opening both trocar exits. Foam was directed into the

cavity by introducing the tube into the 10 mm trocar. Foam insufflation was continued. At some point, foam exited the small trocar at which time the exit of the small trocar was closed. The already rapid increase in abdominal diameter indicated to stop further insufflation and remove the insufllation tube. Around 15 - 20 minutes after insufflation, the camera was inserted into the abdominal cavity for visual control.

The abdominal cavity was accessible via camera. There was no visual interference from the intraabdominal foam. A large extent of the foam appeared to have collapsed. After a total of 30 minutes, the trocars were removed under visual control and the mini-laparotomy was sutured. No surgical problems or complications were observed during or after surgery. No major complications related to anesthesiology were observed. No anaphylcatic reaction was observed, no indication of cardiovascular collapse, no issues regarding intubation or extubation of the swine were detected. No general hypothermia or respiratory distress were observed. All relevant changes of investigated parameters were observed within the first 5 minutes of foam insufflation. As presented further below, some of these parameters remained unaffected until the end of the procedure. All swine were successfully extubated after 30 minutes.

3.2.3 Development of central body, abdominal cavity and skin temperatures

Data from the temperatures probes were successfully collected during the experiments. In the abdominal cavity, a rapid temperature reduction was observed during insufflation of the bicarbonate foam. The medium temperature dropped down to 20.6° C within the first 5 minutes of the procedure, but then steadily increased (figure 18 A.). 15 minutes into the procedure, the temperature was above 35°C. The skin temperature on the abdomen decreased during the procedure and reached its medium low at 15 minutes into the procedure. At that point, it was at 32.5°C. After that it slowly increased to the initial temperature at around 35.4°C.

The central body temperature remained stable during the entire procedure and did not change significantly. Capnometry showed an increase of the expiratory CO_2 level (figure 18 B). This increase happened within the first 15 minutes and reached a plateau for the remaining procedure. The initial mean level of 74 mmHg CO_2 increased to a mean level of around 94 - 95 mmHg CO_2.. A similar increase was detected for the arterial CO_2 levels, which is described at a later point. The respiratory rate increased from a baseline of 35 to over 44 within the first 5 minutes of the procedure (figure 18C). It remained elevated for 10 minutes and then decreased. After this, the respiratory rate remained stable at a higher global level of around 40/minute.

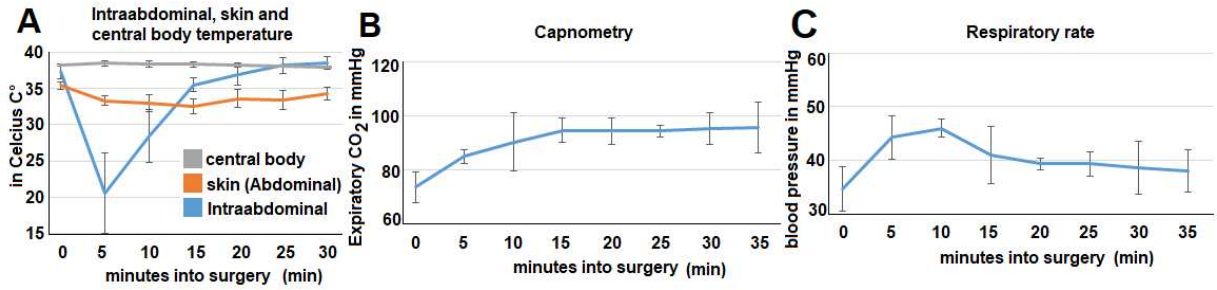

Figure 18: Intraoperative data from temperature probes, capnometry and respiratory rate

A. Data from the three temperature probes at distinct locations: skin probe on the abdomen, intraabdominal probe and central body temperature (oesophageal probe). B. Expiratory CO_2-levels in the capnometry in mmHg. C. Respiratory rate during the operative procedure.

3.2.4 Results of heart rate and blood pressure measurements

The heart rate decreased from a baseline of 123 ± 6 beats per minute down to 95 ± 6 beats per minute within the first 5 minutes of the procedure. After 20 minutes into the procedure, the heart rate again slowly increased to the initial level (figure 19). At this point, the heart rate remained stable. Blood pressure increased from the start of foam insufflation and reached its peak at 134 ± 12 mmHg at 10 minutes into the procedure. After this, the pressure continuously decreased. This was observed for the systolic, diastolic and mean blood pressure.

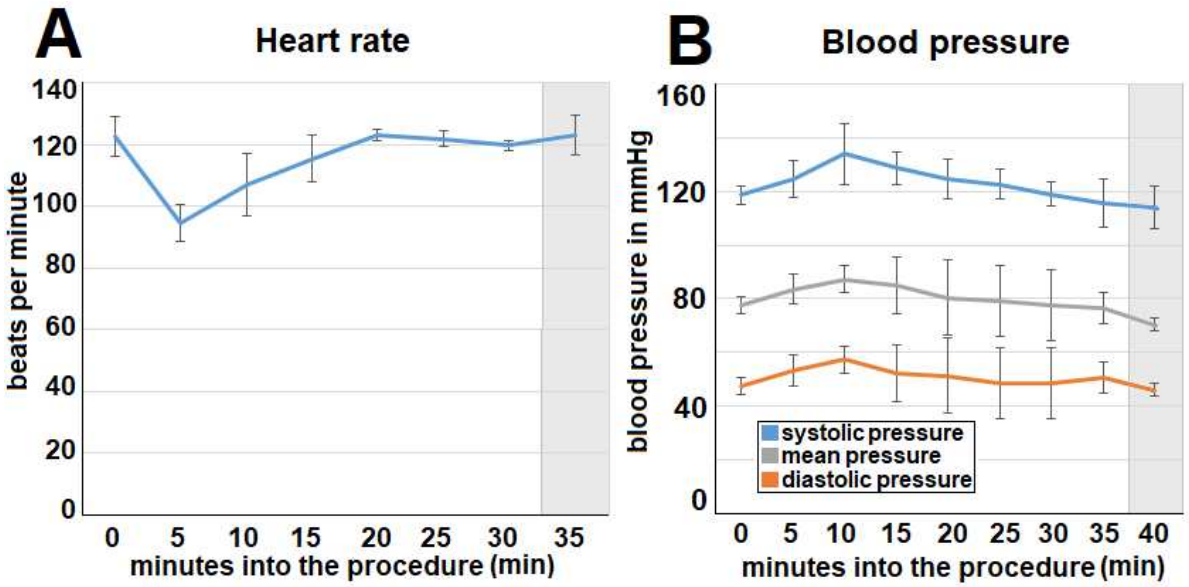

Figure 19: Intraoperative data on heart rate and blood pressure via invasive measurement

A. Mean heart rate from the three swine with standard deviation. B. Mean blood pressure from the three swine with standard deviation. Extubating phase and the following postoperative monitoring are marked in grey.

3.2.5 Development of periumbilical diameter during foam application

Diameters were measured for each stage of the procedure. The initial periumbilical diameter varied around 68 cm, 72 cm and 74 cm. During CO_2 insufflation, the periumbilical diameter increased. The applied insufflation pressure was between 12 - 14 mmHg. After laparoscopic examination, evacuation of CO_2 and insufflation with foam, the periumbilical diameter again increased and surpassed values attained during CO_2 insufflation (figure 20). The diameter increased to a maximum of 82 cm, 83 cm and 84 cm at which point foam insufflation was stopped. The mean periumbilical diameter continuously decreased 5 minutes into foam insufflation. At the end of the procedure, the periumbilical diameter was 77 cm, 78 cm and 79 cm, respectively, and therefore still above the initial diameter. 15 minutes into the procedure, a laparoscopic view was obtained by introducing the camera into the abdomen (figure 21). While a partial collapse of foam was witnessed, the peritoneal surface appeared to be partially covered by foam within the visual field.

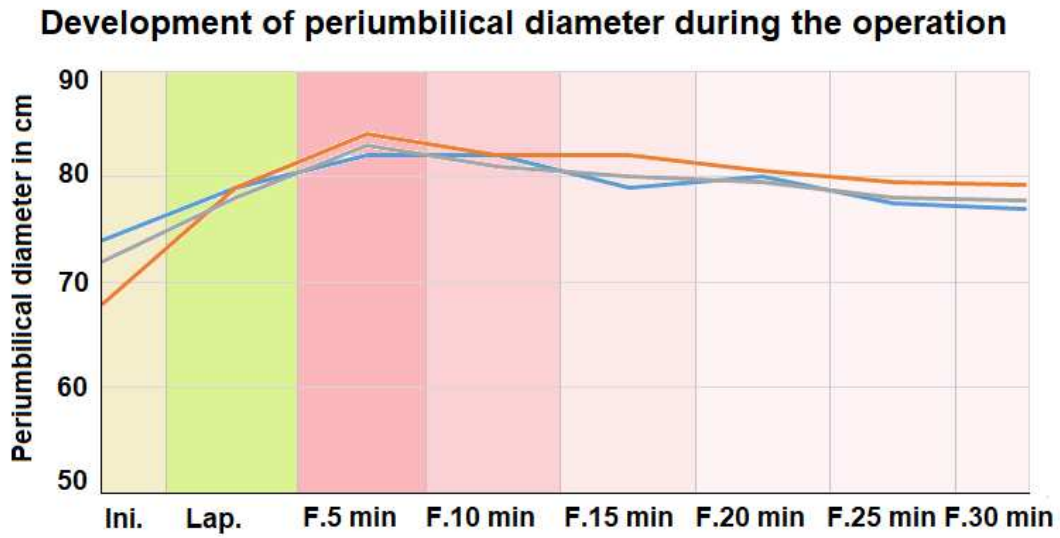

Figure 20: Different stages of abdominal expansion in experimental animals

Initial diameter before the procedure. Lap.) Diameter during laparoscopy for diagnostic purposes under 12-14 mmHg. F.) Diameters after foam insufflation within 5 to 30 minutes into the procedure.

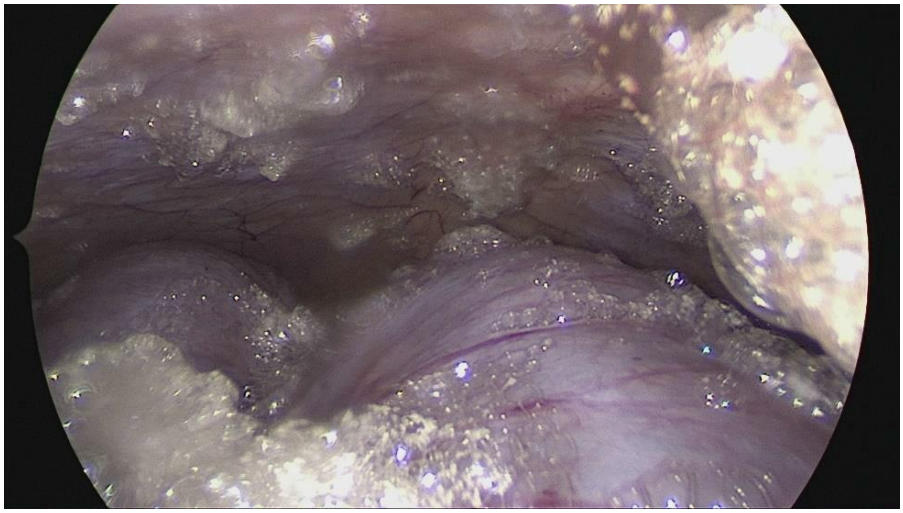

Figure 21: Laparoscopic view of the abdominal cavity via the central trocar

Partial collapse of the insufflated foam is visible with some foam still covering the visceral and parietal peritoneum (15 - 20 minutes into the procedure).

3.2.6 Development of intraoperative gasometrical parameters and electrolytes

Arterial blood gas analysis was collected from all swine before initiating the treatment and 30 minutes into the treatment. 30 minutes into the procedure, pH levels decreased. The decrease ranged from a pH of 7.23 ± 0.027 to 7.1 ± 0.03. At the same time, an increase in pCO_2-levels ($p>0.05$) and a decrease in the pO_2 level ($p<0.05$) was detected. Additionally, electrolyte changes were observed (figure 22 A - C). While the sodium serum levels seemed constant 145.3 mmol/l ± 2.3 (0 min) versus 146.3 mmol/l ± 2.08 (30 min), the potassium serum levels were significantly ($p<0.05$) lower (30 min) with 3.4 mmol/l ± 0.1 versus 3.87 mmol/l ± 0.058 (0 min) (figure 23 A - C). Moreover, the serum calcium level seemed to be significantly ($p<0.05$) reduced after initial levels at 1.42 mmol/l ± 0.117 versus 0.93 mmol/l ± 0.168 after 30 minutes.

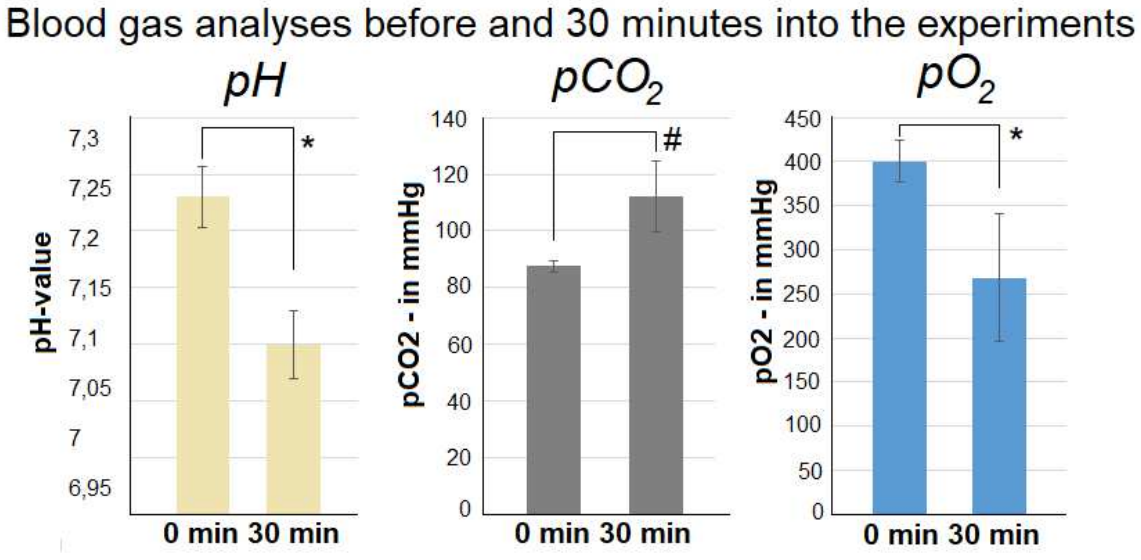

Figure 22: Intraoperative blood gas analyses

Blood samples were retrieved just prior to foam application and 30 minutes into foam application. Mean and standard deviation of measured pH, pCO_2 and pO_2 -levels are indicated. *= p< 0.05, # = p> 0.05.

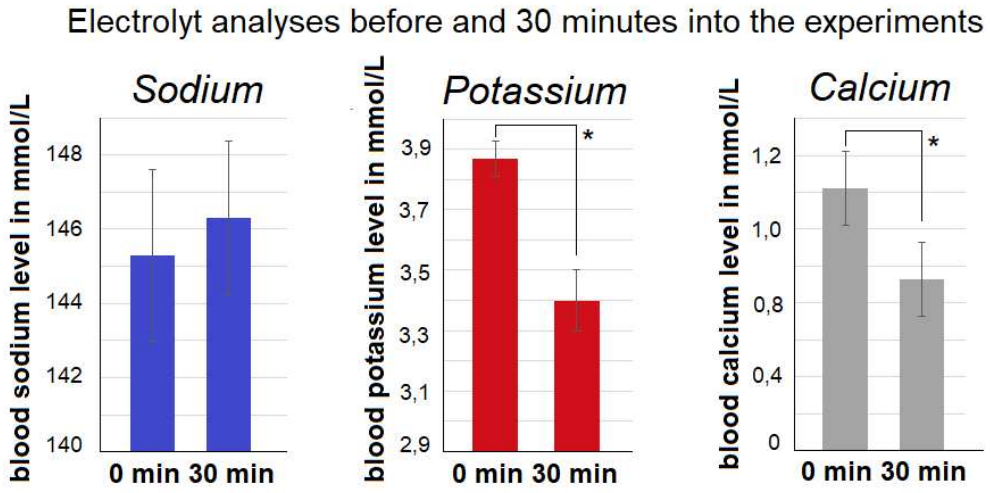

Figure 23: Intraoperative electrolyte analyses

Blood samples were retrieved just prior to foam application and 30 minutes into the procedure. Mean and standard deviation of measured sodium (Na^+), potassium (K^+) and calcium (Ca^{2+}) levels are indicated. *= p< 0.05, # = p> 0.05.

3.2.7 Development of intraoperative and postoperative blood count

The red blood count was collected from the intraoperative measurements at 0 and 30 minutes into the procedure. Furthermore, data was collected on postoperative days 1, 3 and 7. The red blood cell count seemed unchanged and remained within the reference levels above 5 million/µl intra- and postoperatively. White blood cell count remained at the upper reference level which is around 22.000 /µl. While no significant changes were observed, there seemed to be a peak in the values around the third postoperative day (figure 24). The postoperative platelet count showed great variation between day 1 (5.5 ± 1.6) $x10^6$/µl, day 3 (4.7 ±0.8) $x10^6$/µl and day 7 (5.8 ± 1.3) $x10^6$/µl. However, the mean levels remained approximately close to the mean reference level for the platelet count which is around 3 - 7 $x10^6$/µl.

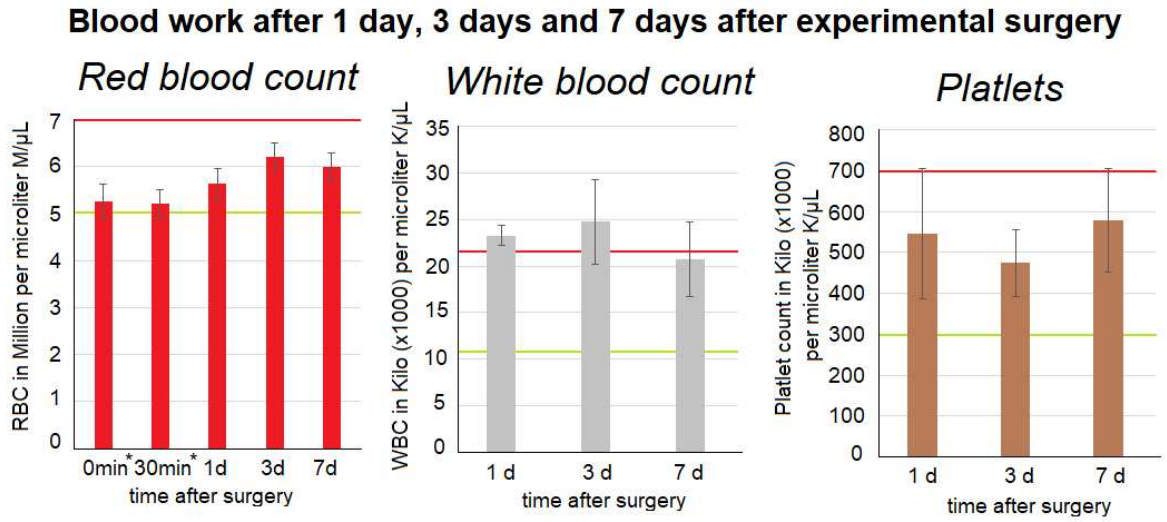

Figure 24: Postoperative blood analysis of red and white blood cells as well as platelets

Listed are red blood cell counts (left) including intraoperative measurements at 0* and 30* minutes into surgery as well as white blood cell counts (middle) and platelet counts (right). Medium and standard deviation are presented. Red and green lines indicate normal reference levels (95% Interval - red: upper limited and green: lower limit) for the parameters.

3.2.8 Development of postoperative serum parameters

Kidney related parameters remained mostly stable. The creatinine level did not significantly change ($p<0.05$) between day 1 (0.7 ± 0.1 mg/dl) and day 7 (0.77 ± 0.15 mg/dl), although a slight mean increase was noted. The maximum reference level for creatinine is at 2.1 mg/dl, but this was never reached (figure 25). The blood urea levels seem to peak around day 3 with 13.7 ± 5.1 mg/dl but decreased again on day 7 to 7 ± 1 mg/dl. The maximum reference level for blood urea was at 30mg/dl, which was also never reached. In contrast, the white blood count was slightly above the upper reference level. The levels of C-reactive protein remained below the upper reference limit of ≤ 0.4 mg/dl, which means the swine did not show any signs of general inflammation.

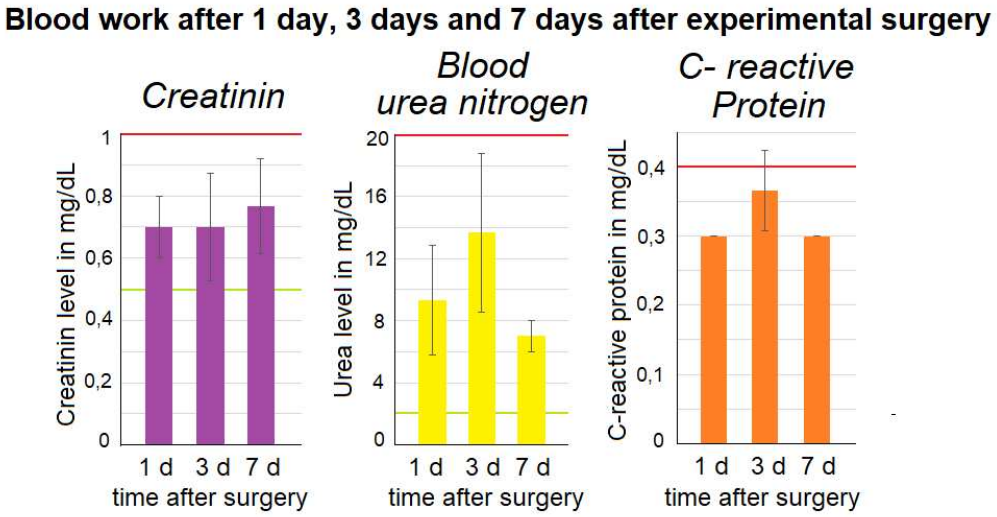

Figure 25: Postoperative blood analysis of creatinine, blood urea level and C-reactive protein
Medium and standard deviation are presented. Green lines indicate lower limit and red lines the upper reference levels for the parameters.

3.2.9 Development of postoperative liver parameters

The blood levels of two liver enzymes were measured. One was alanine aminotransferase (ALT) and the other was alkaline phosphatase (ALP) (figure 26). The mean ALT level was at 81.7 ± 13.3 U/L for day 1, 85.7 ± 23.6 U/L for day 3, and 87.7 ± 28.1 U/L for day 7. The ALT level was stable during these 3 days of measurement but still higher than the reference level of 9 - 43 U/L. No levels of ALT were available from before the procedure, so that no comparison with preoperative levels was possible. The ALP level was at 284.7 ± 89.2 U/L for day 1, 302 ± 43.2 U/L for day 3 and 260.7 ± 51.7 U/L for day 7. For all 3 days of measurements, the levels of ALP were close to the upper reference level which is at 294 U/L. There was no indication of significant changes of the ALP level during the observed interval. No preoperative levels were available for ALP, so no comparison with preoperative levels was possible.

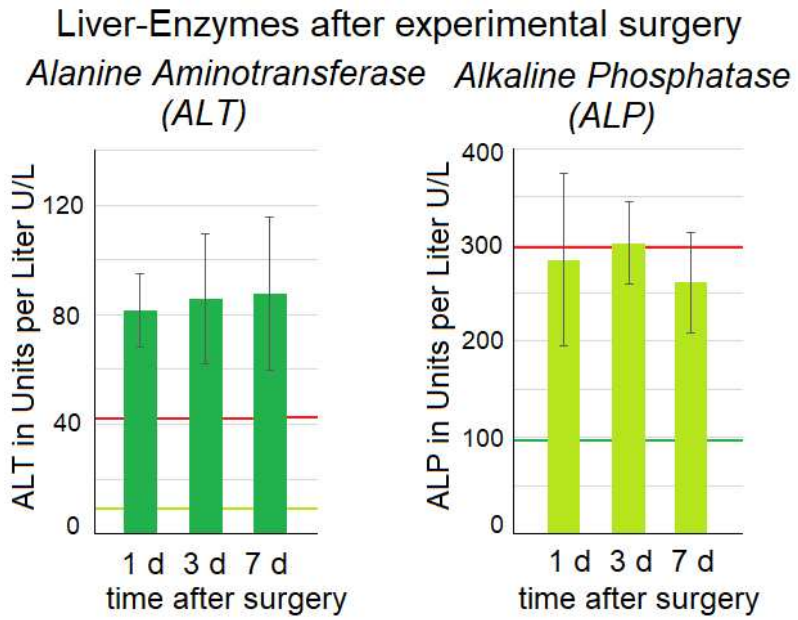

Figure 26: Postoperative blood analysis of liver enzymes

Listed are alanine aminotransferase (ALT) and alkaline phosphatase (ALP). Mean and standard deviation are presented. Green lines indicate the lower limit (95% confidence interval) and red lines the upper reference levels for the analysed parameters.

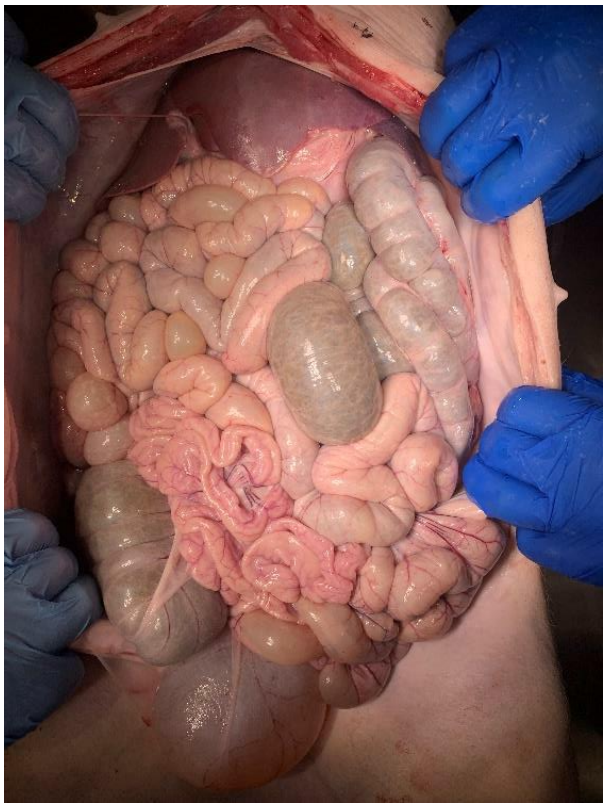

Figure 27: Median laparotomy on postoperative day 7

Following extensive exploration, no macroscopical pathologies, adhesions or organ perforation were observed (from own gallery).

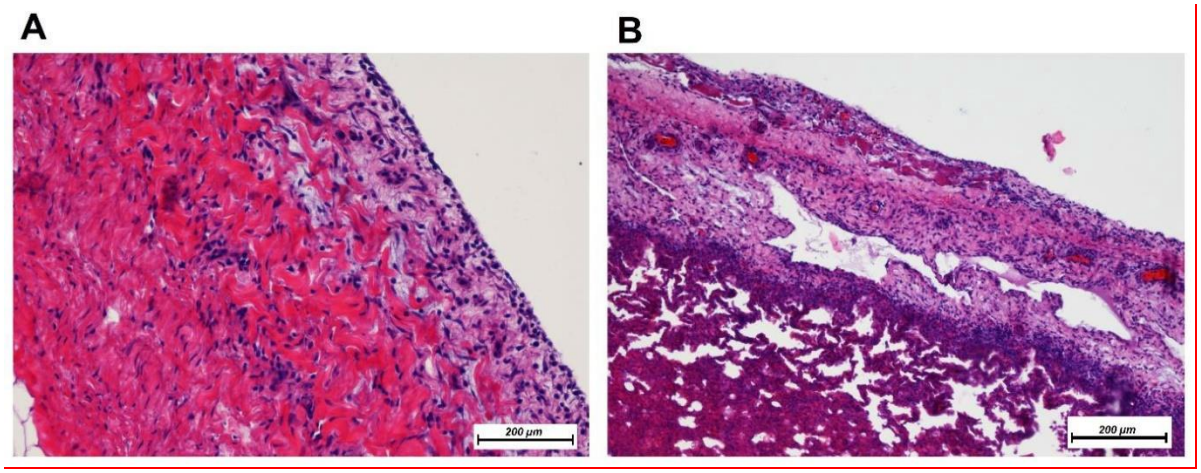

Figure 28: Histopathological analysis of peritoneal tissue. Samples from the parietal peritoneum (A) and the visceral peritoneum of the small intestine (B) on postoperative day 7. No specific changes are detectable on the peritoneum. No disruption of the peritoneal surface is observed.

4. Discussion

Intraperitoneal administration of anticancer drug solutions is an established method in PM treatment. By local installation of chemotherapeutic solutions, the solved active anticancer substances are directly brought into contact with cancer nodules in the peritoneal cavity. However, this concept displays relevant limitations. These include inhomogeneous drug distribution and limited penetration into the peritoneal tissues, which in turns causes limited drug penetration into cancer nodules. This observation has been described for liquid installations as well as aerosol-based systems that deliver IPC (15 – 17). Despite some extensive attempts in improving current technology, these limitations are still observed (130 – 132). Even novel substances have been investigated for intraperitoneal delivery, but they also display limitations in drug tissue penetration (133). Therefore, modified technical applications have been suggested to improve IPC (130, 131). Especially the use of physical means to improve these limitations has been of particular scientific interest (131, 134 – 137). In fact, this doctoral thesis provides an example of one of these physical concepts. Foam displays some unique characteristics which, to the best of our knowledge, have not been studied in terms of drug carrier potential for intraperitoneal chemotherapeutic applications beyond the study presented by Schubert et al (76).

4.1. Discussion of in-vitro data

The presented in-vitro data indicates that there are other possible options beside peroxide-based foam for FBIC. However, we must realize that there are a number of relevant factors that must be considered in these models and taken into account in the overall evaluation of foam-based carrier systems. Our data indicates that a bicarbonate foam system may be preferable to the previously proposed hydrogen peroxide-based foam (76).

In comparison, the cytotoxicity levels of the bicarbonate/citric acid solution are far lower than those of a hydrogen peroxide solution. While we do not have data from the in-vivo model, cytotoxicity levels of hydrogen peroxide could be highly destructive for the peritoneum, an observation based on the results of our HT-29 cell culture experiments. This assumption is not only supported by effects on the cell-lines, but also the histological effects on the peritoneal tissue itself. Structural changes on the microscopic and macroscopic level were observed when hydrogen peroxide was exposed to peritoneal tissues.

This observation is in accordance with our current knowledge of hydrogen peroxide solutions which can cause wound irritation and other unwanted side effects despite its antiseptic properties (138 – 140). This has been one key factor as to why hydrogen peroxide solutions are probably less commonly applied in body cavities (139, 141 – 143), along with an associated potential risk of pulmonary embolism (144, 145). While hydrogen peroxide has been used as a negative contrast for radiographic procedures for fistulas and stab wounds (146, 147), its effects following accidental intraperitoneal use have only been described in limited cases (148).

Even from a pharmacological point of view, the use of hydrogen peroxide might be problematic. In contrast to bicarbonate with citric acid, hydrogen peroxide is a highly reactive substance. Therefore, its use could lead to unprecedented effects once the foam carrier is combined with other substances such as the chemotherapeutic agent or more complex molecules. A potential unwanted reaction with another solved agent could diminish the drug effect, inactivate the drug, or cause unwanted molecular and structural changes which could cause further side effects or an increased unwanted toxicity.

As observed in our study, hydrogen peroxide foam expansion is exothermic while bicarbonate foam expansion is endothermic. The exothermic reaction of hydrogen oxide could provide further thermodynamic energy by increased heat build-up, leading to further unwanted co-reactions with the chemotherapeutic agent. In contrast, the citric acid used in the bicarbonate foam is a biological product which is part of the regular cell cycle as previously discussed (106). Related aspects on toxicity and its current use have been extensively discussed (107). Another relevant aspect is the potential for foam generation as described by the FER. This FER seems to be higher in bicarbonate foam which reduces the amount of required initial volume.

One negative aspect when using bicarbonate and citric acid is that the compounds must be present in a “dry” form. In hydrogen peroxide foam, the solubility of the reagents in water is not a relevant factor, which is in stark contrast to sodium bicarbonate and citric acid.

Both compounds have a finite solubility which limits the ability to concentrate the initial foam solution with these reagents. For sodium bicarbonate, it is estimated to be around 96g per litre (in water) at 20°C (149). For citric acid, the available data indicates a solubility in water of 64.3% at 30°C (150). Another relevant aspect are the biproducts of this reaction. The reaction product of the bicarbonate and citric acid combination is CO_2, which is an inert gas. This can cause increased CO_2 levels in the blood and expiratory air. In fact, our data from the capnometry and CO_2 blood analysis support this assumption. On the other hand, the reaction of hydrogen peroxide produces O_2, which could also be a cause for concern.

In a certain setting, pure O_2 can be inflammable, and this risk might be clinically relevant. Additionally, O_2- embolism has been described with the application of hydrogen peroxide in deeper tissue or when ingested (144, 145). Interestingly, data on temperature development revealed that these two reactions are quite different in terms of thermodynamics. Hydrogen peroxide reacts with potassium iodide in an intense exothermic manner, which generates heat that warms up the foam while the bicarbonate and citric acid combination causes an endothermic reaction. Therefore, the produced foam cools down. These effects were observed both in the ex-vivo study as well as the in-vivo swine experiment. Foam generation for both systems peaks very fast. In the peroxide foam, there is a small delay before the maximum peak is attained.

When comparing the in-vitro toxicity of both proposed foams, we can observe a far higher toxicity rate in hydrogen peroxide-based foam compared to the bicarbonate foam. This remains true even after massively increasing exposure to each well, which indicates that a bicarbonate foam carrier system may be more appropriate for potential clinical use. As for taurolidine, our data suggests that it is not a necessary substance for foam generation. Our presented data does not support the notion that taurolidine has an obligatory role as a critical reagent. On a cellular level, higher dosages of taurolidine seem to exhibit cytotoxicity in the LDH assay. However, this effect cannot be observed for lower concentrations, and is therefore concentration dependant.

This does not take into account any potential systemic toxicity for these indicated levels. When looking at foam expansion with or without taurolidine, no significant difference can be observed for both foam systems. Therefore, there is no clear requirement to use taurolidine as an additional feature as previously described (76), which is especially relevant because taurolidine displays its own toxicity, specifically on the liver (151). However, this toxicity does not seem to limit its experimental in-vivo (152, 153) or clinical use in the abdominal and pleural cavities (154, 155). It is noteworthy to consider that removing reagents from the equation helps reduce the complexity of the overall system.

In regard to taurolidine, we must also consider that our observations were focused on maximum foam volume peaks rather than the development of foam expansion during time.

4.2. Discussion of in-vivo data

Based on the results of the previously mentioned data, a bicarbonate foam system was used for the in-vivo model. This study is the first to successfully conduct an in-vivo foam application into the peritoneal cavity. The surgical intervention and foam delivery was performed without any surgical or technical problems or major obstacles. However, some technical challenges must be addressed. One major aspect was the quantification of the applied total foam volume. With the device that delivers externally created foam, the total volume of the foam delivered could neither be quantified nor estimated. The abdominal expansion was used to indirectly measure both the abdominal expansion as well as the limits of actual foam insufflation. Additionally, vital parameters and inspiration pressure from the mechanical ventilation system were used for further evaluation. Using the periumbilical diameter was an option to estimate total abdominal expansion. This concept is not totally new as it has been previously applied in different studies on intraabdominal pressure (151 – 153). The provided data indicate that at the peak of foam delivery, the measured diameter surpassed the diameter during laparoscopic capnoperitoneum before foam insufflation. The pressure of the laparoscopic capnoperitoneum reached levels of around 12 - 15 mmHg. However, no data was available on intraabdominal pressure reached during foam insufflation, especially at its peak.

Only indirect signs of potential clinically relevant pressure build-up were detectable e.g., an increase in blood pressure with a simultaneous drop in heart rate. It remained unclear how the pressure and / or serum electrolyte imbalances caused by foam may be responsible for this observation. In fact, similar in-vivo observations on vital parameters during abdominal gas-insufflation for laparoscopy have already been described (156 – 161). It is noteworthy that there is both a continuous and spontaneous decrease of the periumbilical diameter. This could indicate a rapid CO_2 absorption from the abdominal cavity.

Furthermore, it is important to note that a decrease in the periumbilical diameter allowed extubation of the swine 30 minutes into the procedure. This indicates that the applied foam levels likely did not limit the mechanical respiratory function, which had been one major concern because the applied intraabdominal extension could have interfered with diaphragm movement and ventilation (162, 163). In this regard it is important to mention that in swine as opposed to humans, the volume relation between lung cavity and abdominal cavity is leaning far more toward the abdominal cavity. Hence, swine are less likely to compensate as they have a relatively smaller total lung capacity.

Another interesting observation is that the decrease in abdominal cavity size following foam insufflation as indicated by the periumbilical diameter does not seem to correspond with the rate of foam disintegration.

Around 15 - 20 minutes into the procedure, the abdominal cavity can be visualized using optical systems available for laparoscopy. The collapse of the applied foam is faster than CO_2 absorption, which cause the formation of large pockets of air which can then be used for visual imaging. In fact, visual imaging can still detect foam at distinct locations of the abdominal cavity, including the abdominal wall. This is an important finding since the abdominal wall basically represents the “dome”, meaning the uppermost part of the peritoneal surface which must be accessed. The most likely explanation for the observed decrease in periumbilical diameter after its peak level is the continuous reabsorption of CO_2, albeit other causes, such as accidental leaking from the abdominal cavity through the trocars, are excluded.

Since we did not observe any accidental leakage, the reabsorption of CO_2 is the most likely explanation, because even if the foam collapsed on its own, CO_2 would still be trapped in the abdominal cavity. Data from capnometry and blood pCO_2 levels further support this hypothesis. Moreover, similar in-vivo data were gathered from a clinical trial performed by Blobner M. et al. (164), which explored the effects of the capnoperitoneum on postoperative CO_2 homeostasis. Theoretically, it should also be possible to calculate additional CO_2 exhalation to quantify total abdominal CO_2 volume. When we look at the measured CO_2 levels in the capnometry, we observe that they remain constant during the procedure following insufflation. One relevant observation are changes in the blood electrolyte distribution. The drop in serum pH could be explained by increased blood CO_2 levels. We also see a decrease in potassium levels, which is a common finding in decreased pH levels (165 -167).

In terms of other electrolytes, sodium levels seem unaffected while calcium levels are significantly decreased. This is not a total surprise because the citrate component of the applied intraperitoneal foam is known to react with the calcium in the blood (168, 169). This observation could be potentially independent and may not relate to secondary effects. We have previously explained and emphasized the known and potentially relevant complications and interactions of citric acid. The potential symptoms following the administration of citric acid and its sodium salts in “toxic” or high quantities are typical of a calcium ion deficiency.

The potential symptoms of calcium ion deficiency include increased general activity, hyperpnea, vasodilatation of peripheral vessels, salivation, muscle twitching, clonic and tonic convulsions, cyanosis, and Cheyne-Stokes respirations (170, 171). These were among some of the major concerns regarding postoperative systemic and local complications. While we

detected lower calcium blood levels, we did not observe associated calcium deficiency in this study. The analyses of postoperative blood work revealed no relevant effects on red and white blood cell count or platelets. Based on our data, including CRP and white blood count, no indication for infections or inflammation were noted.

This is a highly relevant observation, as the applied substances could have induced local inflammation, perforation or scar formation on such a vast surface. This observation is in contrast to substances like barium sulfate, which although safe and non-toxic for oral intake, can cause severe inflammation when leaving the endoluminal cavity and entering the peritoneum (172 – 174). We must be aware that hydrogen peroxide could potentially cause significant macro and microscopic damage on post-mortem tissue of the peritoneum. Following autopsy of the experimental animals, no intraabdominal macroscopical pathologies or signs of "changes" of the peritoneum were observed for bicarbonate foam.

This was further supported by histopathological analyses of peritoneal tissues at distinct locations. Based on the presented data, we can assume that the systemic effects of FBIC with the presented bicarbonate carrier system could be more relevant and potentially more critical than the actual local toxic effects on the peritoneum. While systemic effects are not desirable, the fact that there is no adverse reaction of the peritoneal surface to the applied foam can be considered a positive, promising finding. Foam displays some advantages over aerosol and liquid applications. It expands differently than gas or liquids and offers a higher drug-carrying capacity than gas. Thus, even with a low total drug dosage, high drug concentrations can be created, as more than 95% of the actual foam volume is composed of air (76, 175).

Additionally, local foam degradation could further increase local drug availability. Aerosol chemotherapy has already shown much higher drug concentrations than regular liquid solutions. However, this increase in drug concentration is not without its consequences, and aerosol chemotherapy displays increased inhomogeneity compared to liquid applications [16, 17]. FBIC could be a technically feasible option for PM treatment using doxorubicin, oxaliplatin or other chemotherapeutic agents. At this point, we know that peritoneal penetration depth of chemotherapy is higher compared to liquid or aerosol-based IPC (76), especially when comparing penetration levels at more peripheral locations.

This observation is in line with the previously, extensively discussed underlying mechanisms of foam creation and degradation. Foam degradation itself could prolong and ensure extended drug contact time with the peritoneal tissue, and thus in consequence improve tissue penetration rates (176). This is an interesting feature, as increased contact time of a chemotherapeutic drug with the peritoneum enhances drug availability and efficiency (177).

Both the histological data and conducted swine autopsy did not provide further information on possible complications.

While all tissue samples were solely retrieved from the intraabdominal cavity, no significant or pathological changes were observed. While it can be assumed that from an applicational and technical perspective, the delivery of intraperitoneal foam as a vehicle to carry chemotherapeutic substances is feasible, it is important to consider that this assumption is based on a limited amount of in-vivo experiments performed. Despite some limitations, our data indicate that the major safety concern following FBIC with a bicarbonate carrier system are potential electrolyte disturbances which may require close clinical monitoring. Regarding local effects of FBIC, no adhesions or intraperitoneal complications were detected.

In fact, shortly after foam application, it is possible to perform a control laparoscopy. There still seem to be some challenges in total foam delivery, evaluating effective monitoring and measuring the total inflow volume. The unique characteristics of foam might significantly improve PM response to IPC. In fact, foam containing hydrogen peroxide and taurolidine has demonstrated cytotoxic properties in our study which may be sufficient to treat PM without adding chemotherapeutic agents. However, further studies are required to closely evaluate the clinical applications of taurolidine and hydrogen peroxide foam in PM treatment.

Our data indicate that foam might serve as a possible carrier for IPC with the benefit of increased drug penetration and more homogenous drug distribution than conventional liquids and pressurized aerosol. However, further research is required to assess its potential in IPC applications. To the best of our knowledge, no clinical experience for foam-based applications in IPC has been previously collected or published in peer-reviewed literature. While this study presents preliminary data, it gives important insight into the potential of FBIC to improve PM treatment and encourages further studies to evaluate FBIC's full efficacy and biodistribution.

4.3 Limitations

The key limitation of this work is the limited number of swine used for this study, which in turn affects the extent of in-vitro and ex-vivo data. Not all performed in-vitro and post-mortem data were presented in this work, as it would have exceeded the format of this dissertation. Moreover, this work was designed to outline the first steps in the development of a foam-based carrier-system for FBIC. Thus, the aim of this study was quite specific and targeted toward a particular scientific question. Since this study was limited in scope, it was not possible to cover all relevant issues. Therefore, many relevant questions remain unanswered, and a new set of questions now arise based on the provided data.

This presented work must be regarded as an in-vivo pilot study which aimed to provide important preliminary data. For further assessment of clinical impact, applicational safety and evaluation of intra- and postoperative complications, a larger number of experimental animals is required. To gain a more comprehensive understanding of this novel concept, a more extensive level of effect analyses of carrier systems on a cellular level is necessary with respect to possible pharmacological interactions. Extensive cell analyses should also include other cancer cell lines, as well as fibroblasts and “mesothelial-like” cells. In this study, we primarily focused on the classic HT-29 cell line for the overall evaluation of this concept. However, testing on the other above mentioned cell lines should also be included. The collected vital parameters from the in-vivo swine model are certainly more accurate than data that could have been obtained in a potential mouse or rat model. At the same time, there might be relevant anatomical and physiological differences which may cause complications that have not been considered here and could result in different outcomes in humans. More data must be collected to draw a clearer picture of this novel concept.

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