FACULTY OF TEXTILE ENGINEERING TUL

DEVELOPMENT OF DOUBLE-LAYERED NANOFIBROUS MATERIALS FOR THE FORTIFICATION OF INTESTINAL ANASTOMOSIS

Ing. Markéta Klíčová

SUMMARY OF THE DOCTORAL THESIS

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Abstract

Severe and often life-threatening postoperative complications, such as anastomotic leakage and peritoneal adhesions, significantly impact the outcomes of colorectal surgery. Currently, there are no available materials on the market capable of effectively preventing such complications. This thesis responds to the needs of clinical practitioners lacking such materials on the market and addresses this gap by aiming to develop a unique multi-layered nanofibrous material designed specifically to cover intestinal anastomoses. The theoretical section of the thesis introduces readers to the complexities of postoperative complications in colorectal surgery and the current state of development in the field of nanofibrous materials. Two review studies not only summarize existing knowledge but also establish connections between publications, leading to the selection of suitable fabrication methodologies and the identification of biodegradable materials for further development. Large-scale electrospinning and electrospraying was chosen, acknowledging the limitations of low-scale lab devices in hindering commercialization. Synthetic polymers, particularly biodegradable polyesters like polycaprolactone (PCL), emerge as key players in the production of antiadhesive nanofibers. The experimental section evaluates the impact of different sterilization methods on the PCL nanofibrous material. This aspect, often overlooked in the literature, becomes a focal point with notable interest and citations in subsequent scientific articles. After verifying the possibility of covering anastomoses using nanofibers, the development of materials continued by optimizing the surface weight for effective anastomotic healing. The consistent integration of material development with the requirements and discoveries of clinical practitioners during the initial implantations led to the establishment of further requirements for fibrous scaffolds. The developed materials included multilayered fibrous structures with a hydrophilic inner layer and an antiadhesive outer layer as well as scaffolds inspired by the superhydrophobic lotus leaf. Additionally, nanofibrous drug delivery systems based on PCL and antibiotics gentamicin sulfate were explored. The functionality of the materials was examined in an experimental model mimicking the complex healing of colonic anastomosis in pigs. The three-week observation period concluded with evaluations of peritoneal adhesions and anastomotic healing. Novel scoring system Intestinal Wall Integrity Score was introduced for evaluating the healing of the intestinal wall at the site of anastomosis. This thesis contributes novel insights and innovative solutions to the field of gastrointestinal surgery, laying the foundation for the development of materials that can significantly reduce severe postoperative complications.

Keywords

gastrointestinal anastomosis; anastomotic leakage; postoperative adhesion; nanofibers; electrospinning; electrospraying; biocompatibility; biodegradability; superhydrophobic surface

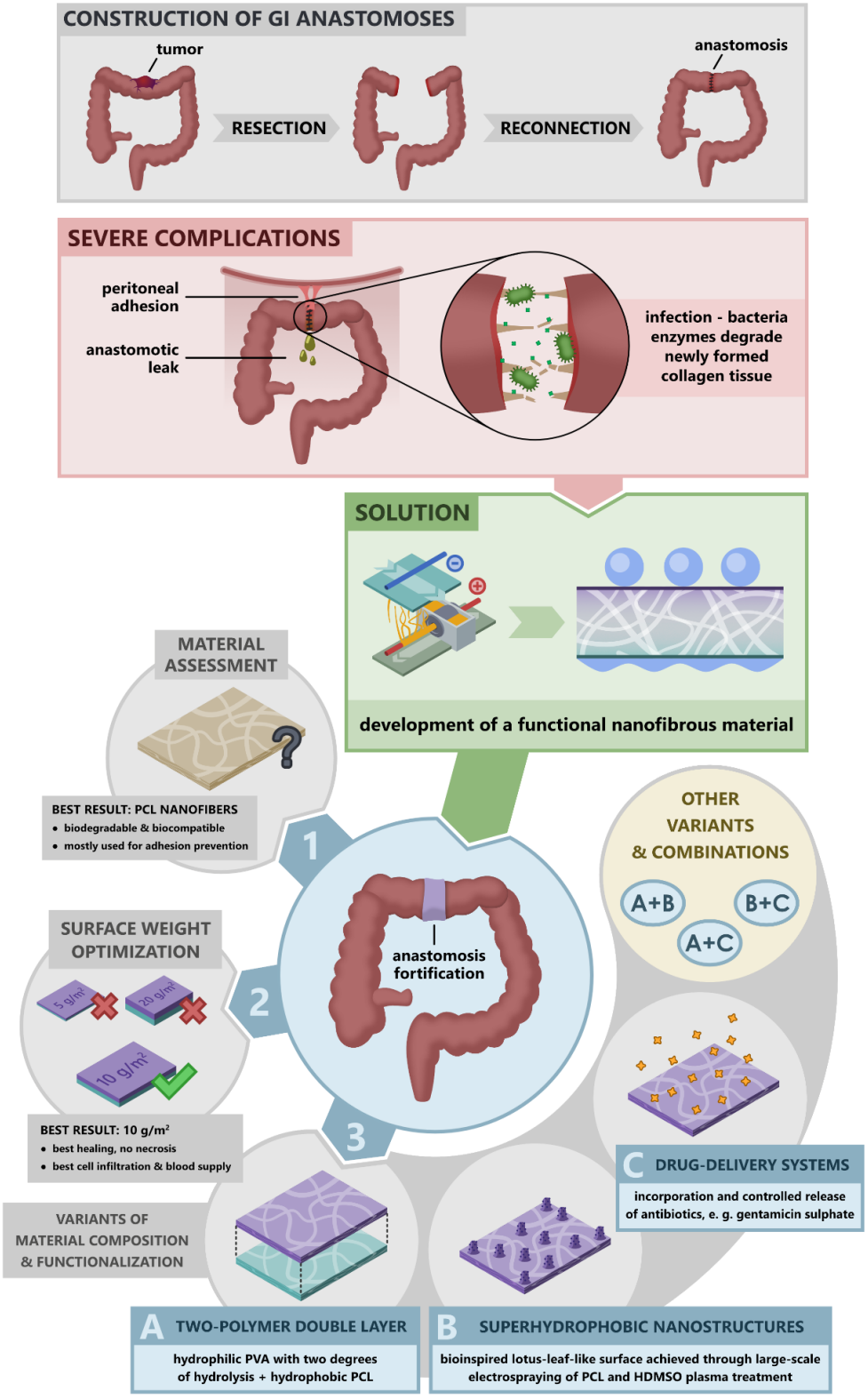
Abstrakt

Závažné a mnohdy život-ohrožující pooperační komplikace, kterými jsou zejména anastomotický leak a peritoneální adheze, významně ovlivňují výsledky kolorektální chirurgie. V současné době neexistují dostupné komerční materiály, kterými lze efektivně preventovat zmíněné komplikace. Tato disertační práce reaguje na požadavky klinických lékařů, kterým takový material na trhu chybí a představuje vývoj unikátního vícevrstvého nanovláknitého materiálu navrženého speciálně pro krytí intestinálních anastomóz. Teoretická část práce uvádí čtenáře do složitostí postoperativních komplikací při kolorektální chirurgii a aktuálního stavu vývoje na poli nanovlákenných materiálů. Dvě přehledové studie nejen shrnují stávající znalosti, ale také identifikují souvislosti mezi publikacemi, a tak vedly k výběru vhodné metodiky výroby a identifikaci biodegradabilních materiálů pro vlastní vývoj. Byla zvolena metoda výroby materiálů pomocí electrospinningu a electrosprayingu na zařízení typu Nanospider. Bezjehlová technologie přípravy byla vybrána právě na základě podrobné literární rešerše, která ukazuje, že laboratorní metody vedou k přípravě nehomogenních vrstev, omezeným možnostem charakterizace, a také zpomalují následný transfer do komerční praxe. Syntetické polymery, zejména biodegradabilní polyestery jako polykaprolakton (PCL), jsou nejpoužívanějšími vstupními materiály při výrobě antiadhezních nanovláken. Experimentální část zkoumá vliv různých sterilizačních metod na planární nanovlákenné materiály na bázi PCL. Vliv sterilizace, který je v literatuře často opomíjený, se stává stěžejním bodem s významným zájmem a citovaností v následných impaktovaných publikacích. Po ověření možnosti krytí anastomóz pomocí nanovláken byla pokračováno ve vývoji materiálů optimalizací plošné hmotnosti pro efektivní hojení anastomóz. Konzistentní provázání vývoje materiálů s požadavky a objevy klinických lékařů během prvotních implantací materiálů, vedlo ke stanovení dalších požadavků na vlákenné scaffoldy. Vývoj tak zahrnoval přípravu vícevrstvých vlákenných vrstev s hydrofilní vnitřní vrstvou a antiadhezivní vnější vrstvou, stejně jako představení hierarchických vlákenných materiálů, které jsou inspirovány nativním superhydrofóbním povrchem lotosového listu. Kromě toho byly zkoumány nanovlákenné systémy pro řízené uvolňování léčiv na bázi PCL a antibiotika gentamicinu sulfátu. Funkčnost materiálů byla zkoumána v experimentálním modelu simulujícím složité hojení kolorektální anastomózy u prasat. Třítýdenní pozorovací období bylo zakončeno vyhodnocením levelu peritoneálních adhezí a úspěšnosti hojení anastomózy. Nový skórovací systém (Intestinal Wall Integrity Score) byl představen pro hodnocení hojení střevní stěny v místě anastomózy. Tato disertační práce přináší nové poznatky a inovativní řešení v oblasti gastrointestinální chirurgie, a pokládá základy pro vývoj materiálů, které mohou významně snížit závažné pooperační komplikace.

Klíčová slova

gastrointestinální anastomóza; anastomotický leak; pooperační adheze; nanovlákna; electrospinning; electrospraying; biokompatibilita; biodegradabilita; superhydrofobní povrch

Graphical abstract



CONTENT

1. INTRODUCTION

Colorectal surgery and bowel resections stand as fundamental curative modalities for colorectal cancer patients today. Despite advancements in surgical techniques, postoperative complications, particularly anastomotic leakage and peritoneal adhesions, remain prevalent and significantly impact patient outcomes. Patients experiencing anastomotic leakage face elevated morbidity, mortality, the necessity of diverting stomas, and compromised eligibility for adjuvant oncological treatments, thereby affecting their long-term survival. The imperative to mitigate the occurrence of anastomotic leakage underscores the critical need for innovative solutions, which constitute the primary focus of this thesis.

The proposed nanofibrous materials represent a promising avenue in addressing these challenges. Within the framework of this doctoral thesis, nanofibrous materials were meticulously developed to tackle severe complications in abdominal surgery, particularly following intestinal resection. Given the seriousness and persistence of these complications in surgical practice, numerous discussions have revolved around developing materials to prevent anastomotic leaks and peritoneal adhesions, yet no material has achieved comprehensive success in this regard. This underscores the complexity inherent in developing effective preventative measures.

The thesis endeavors to fill this gap through the development of a novel nanofibrous material to prevent peritoneal adhesions and anastomotic leakage. It represents a pioneering application of nanofibrous materials, particularly polycaprolactone (PCL), in fortifying intestinal anastomoses in a large animal model. This intervention not only demonstrated safety with zero mortality and morbidity but also facilitated efficient, facile manipulation without necessitating additional fixation – a crucial requirement in surgical materials.

Advanced characterization methods were employed to precisely understand their structure, composition, and performance. These efforts contributed to the optimization of surface weight, a critical factor influencing the material's functionality. Through systematic experimentation, a surface weight of 10 g/m^2 was identified as the optimal balance, offering sufficient cohesion with minimal thickness. This optimization not only ensures practicality in application but also signifies a nuanced approach to material engineering, where the interplay of structural elements is fine-tuned for maximum efficacy.

The developed materials showcase a biomimetic lotus-leaf-like surface, achieved through a combination of large-scale electrospinning and electrospraying of PCL functionalized via hydrophobic plasma treatment. Additionally, the layering of hydrophilic polyvinyl alcohol (PVA) and hydrophobic PCL through electrospinning forms dual-sided nanofibrous patches.

These patches, tested on pigs, not only effectively prevented anastomotic leakage but also offered insights into the distinctions between *in vitro* and *in vivo* testing, opening avenues for further exploration. Furthermore, the optimization of nanofibrous drug delivery systems, particularly with the critical concentration of gentamicin sulfate, showcases the versatility of these materials for broader applications in postoperative care. The findings from these endeavors not only present a significant leap in material development but also contribute to advancing the field of tissue engineering.

While these breakthroughs mark significant strides, the author acknowledges that the intricacies of anastomotic healing mechanisms remain incompletely understood. The concluding chapters of this thesis outline avenues for future development, recognizing the persistent openness of many chapters in this evolving narrative. Nevertheless, these introduced advancements in nanofibrous research hold the potential not only to transform surgical procedures but also to catalyze innovations with broader implications across diverse medical domains (such as wound healing).

The synthesized findings and outcomes pertaining to these nanofibrous materials are presented in this dissertation, supported by a series of publications in peer-reviewed journals. The conceptual framework of this thesis interlaces these publications, offering a cohesive narrative that synthesizes their findings. This comprehensive approach aims to advance the field of tissue engineering, providing novel solutions to improve the outcomes of intestinal anastomosis procedures.

2. GOALS OF THE THESIS & PH.D. STUDY OUTCOMES¨FET

The overarching objective of this dissertation is to pioneer the development of biocompatible nanofibrous materials, strategically designed for the coverage of intestinal anastomoses and the prevention of severe postoperative complications, specifically anastomotic leakage (AL) and peritoneal adhesions (PA). The specific goals were delineated as follows:

1. Comprehensive Reviews - Understanding the Principles:

a. Exploration of nanofibrous materials for preventing postoperative adhesion.
b. Insightful analysis of intestinal healing mechanisms.

2. Development and Production of Nanofibrous Materials Aligned with Clinical Requirements:

a. Dynamic collaboration with the medical team led by Prof. Václav Liška, ensuring adaptability to current needs of clinical surgeons.
b. Modification of material properties based on ongoing *in vivo* testing results.
c. Streamlining production processes and enhancing material functionalization for repeatability.

3. Thorough Characterization of Nanofibrous Materials:

a. In-depth morphological characterization.
b. Precise measurement of surface properties.
c. Evaluation of the functionalization of the fibrous layers.
d. *In vitro* testing to evaluate cytocompatibility of the developed materials.

4. Contribution to Research Funding and Research Subtask Management:

a. Active participation in project proposal submissions.
b. Endeavors to establish additional collaborations within the professional community.
c. Supervision of master's theses.

5. Presentation of Results:

a. Authoring impacted publications on material production and characterization.
b. Presentation of results at conferences.

This dissertation endeavors not only to address pressing issues in tissue engineering but also to contribute to the advancement of clinical practices through the development of innovative nanofibrous materials. The outlined goals encapsulate a holistic approach, from comprehensive research and development to active collaboration with medical practitioners and the dissemination of findings within the scientific community.

3. UNVEILING THE CHALLENGES IN INTESTINAL ANASTOMOSES AND THE NEED FOR INNOVATIVE SOLUTIONS

The surging interest in innovative medical solutions has naturally given rise to new prospects for utilizing nanofibrous scaffolds. Nonetheless, these advancements come with continuously growing expectations and requirements. One specific requirement, stemming directly from clinicians in clinical practice, is the creation of biocompatible nanofibrous materials for covering intestinal anastomoses. This imperative need serves as the key focus and primary objective of this thesis. Intestinal anastomoses, surgical connections of intestinal tissues, are an integral part of surgical procedures for the removal of a portion of the intestine due to colorectal tumors, autoimmune diseases, intestinal blockages, *etc*. Intestinal anastomosis refers to the surgical connection or joining of two segments of the intestine, typically after a resection or removal of a diseased portion of the intestine. Intestinal anastomoses belong among the most common procedures performed in abdominal surgery, yet they lead to numerous and often life-threatening postoperative complications. These complications primarily include anastomotic leak (AL) and peritoneal adhesions (PA) as shown in Figure 1.

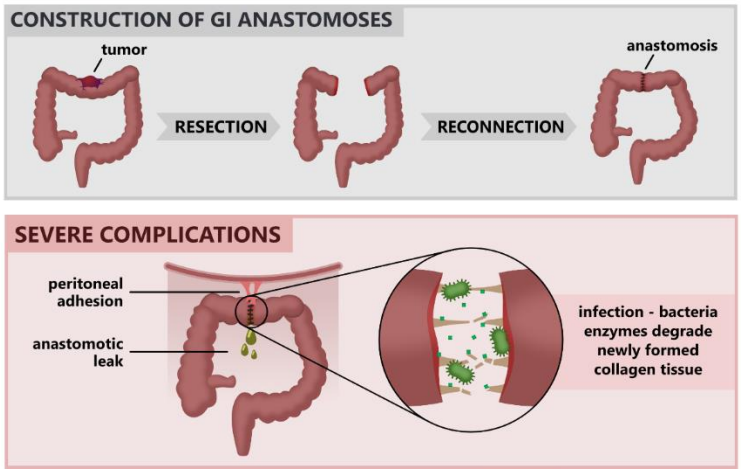


Figure 1: Visualization of the surgical procedure and potential postoperative complications. Author's own picture.

Anastomotic leak refers to the unintended leakage of intestinal contents into the abdominal cavity through the created anastomosis. Despite the modern medical efforts, AL occurs very frequently, with studies reporting an incidence of 8-20%.[1–3] This postoperative complication leads to high postoperative morbidity and mortality, tumor recurrence, a significant reduction in the patient's quality of life (establishment of a stoma), prolonged hospital stay, and subsequent costly care (up to 2.5 times higher costs compared to patients without complications).[4]

Peritoneal adhesions manifest as strands of connective tissue with diverse characteristics, ranging from delicate membranes to robust and occasionally vascular bonds that interconnect different structures within the abdominal cavity. Their severity varies and can be observed following various surgical interventions, injuries, or inflammations in the abdominal region. Over an extended period, these adhesions may contribute to persistent abdominal discomfort, intestinal obstruction, infertility, and significantly reduce the quality of life after surgery.[5] Moreover, PA pose a long-term risk, potentially leading to gastrointestinal passage blockage, even many years after the initial surgery.[6]

Healing of anastomoses: The recovery process for a meticulously constructed anastomosis in both the small and large intestines typically spans approximately three weeks. Nevertheless, even beyond this timeframe, the healing mechanisms persist, leading to the maturation of the anastomosis.[7] The highest risk of anastomotic leakage occurs during the early postoperative phase, specifically between the 5th and 8th days following surgery, when the newly formed tissue is particularly delicate, as indicated in the literature.[8] Conversely, the risk of anastomotic strictures tends to increase at a later stage, with complications potentially arising years after the surgical procedure.[9] Achieving physiological healing of intestinal anastomoses relies on critical factors such as adequate blood supply and oxygenation, a technically proficient suture, a suture free from tension, and optimal contact with the surrounding healthy peritoneal surfaces.[10,11] An in-depth understanding of the intricate processes involved in anastomotic healing was indispensable for the material research undertaken in this thesis. Thus, a review study exploring the current knowledge of anastomotic healing was collaboratively prepared with clinical surgeons. This comprehensive study, delving into the nuanced principles of healing, has been successfully published. It stands as one of the notable commented articles, with its results elaborated in ***Journal Article: B. Review About the Intestinal Healing and Prevention Suggestions for Diminshing Postoperative Complications.***

3.1 The Role of Local Preventive Materials in Postoperative Complications: State-of-the-Art

In gastrointestinal surgery, the role of local prevention of postoperative complications is of paramount importance. The use of various materials to protect and fortify intestinal anastomoses aims to minimize the occurrence of complications such as anastomotic leakage and the formation of peritoneal adhesions. These measures seek to enhance the integrity and healing of surgical sites, reduce the risk of postoperative morbidity and mortality, and facilitate quicker patient recovery.

Despite a multitude of materials being studied, the quest for an optimal solution that effectively balances the promotion of healing and the prevention of complications continues.

Commercialized Products

Currently, there are no commercially available materials for local coverage of intestinal anastomoses that would prevent the occurrence of anastomotic leaks and peritoneal adhesions. Given the severity of both complications, intensive worldwide research into material solutions is underway, and a variety of products have been tested.

Among the most evaluated materials are fibrin patches and tissue adhesives. For example, Tachosil®, a collagen patch serving as a matrix for tissue glue and other active ingredients, is available on the market. In a study by Nordentoft *et al.*[12], Tachosil® was proven to be safe for covering intestinal anastomoses; however, the results showed no effectiveness in preventing anastomotic leaks. Tissue adhesives can be based on cyanoacrylate, glutaraldehyde, fibrin, and polyethylene oxide hydrogels. These preparations exhibit disadvantages such as high cost, complicated preparation for surgeons, or even the risk of *in vivo* toxicity.[13]

Besides various stitching techniques, surgical anastomoses can also be connected using staplers or surgical staplers. To reduce bleeding after the use of staplers, a product called Gore® Seamguard® has been introduced to the market, promising a decrease in the incidence of anastomotic leaks. The structure of Gore® Seamguard® is based on a synthetic copolymer of polyglycolic acid and trimethyl carbonate. However, in the largest randomized studies, no decrease in the incidence of anastomotic leaks was demonstrated with the use of fibrin adhesives or Gore® Seamguard®.[14]

Other commonly used materials for creating a local antiadhesive barrier are based on hyaluronic acid and are used in gynecological surgeries.[15,16] However, based on literature research and consultation with clinical physicians, these materials have not been approved for use in the small and large intestine. The product Seprafilm™ (Genzyme, Cambridge, MA), made from hyaluronic acid and carboxymethylcellulose, is considered by some physicians to be too fragile and rapidly degradable. The product Interceed™ (Gynecare, Somerville, NJ), based on cellulose, may help reduce adhesions; however, the presence of blood is a contraindication for use, and blood must be completely removed before application, complicating the surgical process.[17] In general, commercially available products have not yet proven capable of reliably preventing peritoneal adhesions.[18,19]

Research Approaches without Commercialization

In the existing literature, diverse research methodologies have been identified, each contributing to the development of fibrous materials aimed at adhesion reduction. This field of inquiry is of utmost importance, emphasizing the need for a comprehensive understanding of ongoing research activities. The culmination of information gathered throughout the Ph.D. study resulted in the production of a detailed review on antiadhesive materials, featured as one of the commented articles (see ***Journal Article: A. Review about Antiadhesive Nanofibrous Materials***). Here, I would like to briefly summarize that the developed products have certain limitations, and their use for medical applications is still restricted.

Tang *et al.*[20] created an antiadhesive material based on natural biopolymers agarose and collagen. Antiadhesive films were crosslinked with glutaraldehyde to support mechanical behavior. According to the presented results, the materials exhibit antiadhesive behavior both *in vitro* (fibroblasts and adult stem cells obtained from adipose tissue) and *in vivo* (rabbit model). However, despite the widespread use of glutaraldehyde as a crosslinking agent, its toxicity and adverse effects on human health have been described.[21,22]

Sun and Tan[23] also focused on antiadhesive materials for preventing secondary tissue damage during cover replacement, using a combination of alginates with gelatin. However, alginates have disadvantages, such as unsuitability for dry wounds, low stability, and mechanical properties.[24] In a study by Zhao *et al.*[25], the preparation of a material containing the antifibrotic mitomycin-C was described. The structure of the resulting fibers was of the core-shell type. The resulting structure led to a reduction in tendon adhesion, showing the potential use of nanofibers for preventing tissue adhesions. However, the intricate management of mitomycin-C release, the difficulty in achieving a completely uniform distribution in core-shell systems, and the potential adverse effects on the human body present noteworthy challenges. Consequently, it is my assertion that the market introduction of such a product would be unattainable. Other studies used non-degradable polymers, such as polytetrafluoroethylene[26] or polyvinylidene fluoride[27]. Although these materials have been shown to be antiadhesive, their use is significantly limited due to their biologically non-degradable nature.

According to the literature review, the developed fiber materials were mostly produced by needle electrospinning on a laboratory scale, so repeatability of conducted tests on large samples was not ensured in any case. Further disadvantages and reasons for unsuccessful commercialization are critically evaluated and presented with all due respect to other scientific teams in ***Journal Article: A. Review about Antiadhesive Nanofibrous Materials***.

3.2 The Role of Nanofibrous Local Preventive Materials: Research Approach Within the Thesis

Nanofibrous scaffolds have emerged as a cornerstone in tissue engineering, mainly attributable to their remarkable resemblance to the intricate architecture of the natural extracellular matrix.[28] Electrospinning has revolutionized the fabrication of these ultrafine fibers, enabling precise control over their composition, alignment, and morphology.[29] With diameters ranging from a few nanometers to micrometers, nanofiber scaffolds offer an idealized environment for cell attachment, proliferation, and differentiation. Their high surface area-to-volume ratio and customizable porosity facilitate efficient nutrient exchange and support vascularization, essential for tissue integration and regeneration. By incorporating biologically active molecules, these nanofibrous structures can be further tailored to guide specific cellular responses, thereby advancing the restoration of diverse tissue types and holding great promise for the future of regenerative medicine and implantology.[30]

In the realm of tissue engineering, the potential of nanofibers to meet the diverse demands of medicine is immense. However, navigating the path from research to clinical trials is a formidable journey. The intricacies of this endeavor require a comprehensive approach that anticipates and responds to the dynamic needs of both the medical field and the market. Throughout my research, I aimed for consistency and close collaboration with medical professionals, maintaining a humble understanding of the entire process and acknowledging the inherent risks of failure.

The unique properties make nanofibers highly suitable for the creation of tissue-engineered scaffolds that support the regeneration and function of damaged tissues. **An ideal material for anastomosis fortification should meet several key criteria.** Firstly, it should be biocompatible, meaning it must not induce an inflammatory reaction and/or cause any adverse effects. Additionally, it should be biodegradable to gradually dissipate as the tissue heals, eliminating the need for re-operation for material removal. The ability for sterilization, as well as the stability of structural and chemical properties post-sterilization, is also crucial for surgical applications. The material should also replicate the natural curvature of the tissue, contributing to better adaptation and functionality. Material strength is paramount for effective fortification of intestinal anastomosis. The advantage lies in easy manipulation during surgical procedures, quick application without unnecessarily prolonging the operation. Adhesiveness on one side ensures the material adheres ideally without the need for additional fixation.

In conclusion, it is noteworthy that, despite over two centuries since the inception of the first intestinal anastomosis, there is still an absence of commercially available materials capable of reliably preventing both associated complications. This dissertation addresses this gap by introducing a nanofibrous planar material designed to fortify intestinal anastomoses, providing a potential solution to the longstanding challenge. The extensive research and development of this nanofibrous covering have been conducted in collaboration with the esteemed team led by Prof. MUDr. Václav Liška, Ph.D., at the Biomedical Center of the Faculty of Medicine, Charles University in Pilsen. Rigorous testing on a large animal model, integral to the Czech Health Research Council (AZV) project *NU20J-08-00009*, has been undertaken (see Figure 2). The material's properties have been continuously refined through active engagement and constructive feedback obtained in close cooperation with clinicians practicing in the medical field. As part of this collaborative initiative, firsthand observations in operating rooms were facilitated during the application of nanofibrous materials on the small and large intestines of animals.

Throughout this research, it became evident that the exceptional intrinsic properties of nanofibers, characterized by a large specific surface area, porosity, and a structure resembling the native extracellular matrix, were insufficient in isolation to prevent both complications. Consequently, additional functionalization of the materials was necessary to enhance their efficacy, aligning with the well-established benefits of nanofibers in tissue engineering. The subsequent theoretical chapters delve into the methods of electrospinning and electrospinning, utilized approaches for the development of biomimetic materials and drug delivery systems in this thesis. It was discovered during our research, that these approaches may hold promise for the local prevention of postoperative complications.

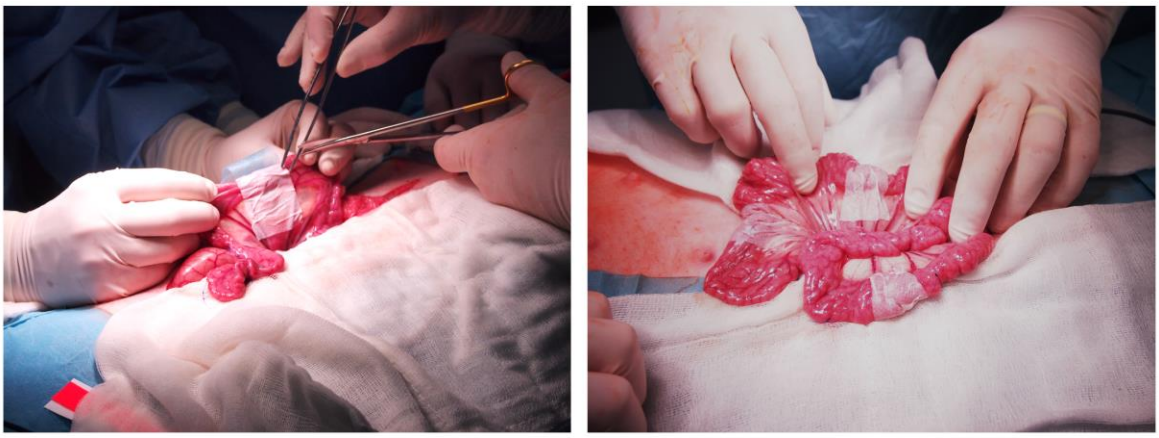


Figure 2: First application of nanofibrous material on intestinal anastomosis in a large animal model – collaborative research with the Biomedical Center of the Faculty of Medicine, Charles University in Pilsen. Polycaprolactone nanofibers placed around the anastomosis. Author's own pictures.

3.3 Electrospinning

3.3.1 Rationale for the Chosen Fabrication Method: Introduction to Electrospinning

Among various fabrication methods, electrospinning stands out as a prominent technique for creating nanofibrous structures. Electrospinning utilizes an electric field to draw charged fibers from a liquid polymeric solution, resulting in materials (or specifically scaffolds for biomedicine) with unique properties. Over the last two decades, electrospinning has become a cornerstone in nanotechnology research and development, primarily due to its economic viability, straightforward equipment operation, and process versatility.

This technique has attracted considerable attention from researchers and industries alike. Its adaptability allows for the fabrication of nanofibrous scaffolds using a wide range of synthetic and natural polymers or their combinations. Researchers can tailor the morphology of electrospun scaffolds by adjusting key parameters, providing control over fiber structure and orientation. This versatility makes electrospinning a preferred method for producing nanofibrous materials for various applications in tissue engineering and beyond. In addition, electrospinning has gained popularity in the field of drug delivery due to its ability to directly incorporate drugs into nanofibers and control the release profile.

For laboratory-scale investigations, researchers have access to numerous commercially available electrospinning apparatuses. These tools offer flexibility for exploring different materials and optimizing conditions for specific applications. On an industrial scale, specialized machinery demonstrates the scalability of the electrospinning process, emphasizing its role in large-scale production. Notably, the large-scale electrospinning method is well-established at our Department for Nonwovens and Nanofibrous Materials (Figure 3). The original patent about large-scale electrospinning arose from the team led by Prof. Jirsak, who introduced the Nanospider of the first generation based on a cylindrical spinner. This development showcases the department's expertise and contribution to advancing electrospinning technologies. The access and understanding of the principles behind this process led to the clear selection of electrospinning as the fabrication method for scaffolds in this thesis. This strategic choice sets this study apart, as the producibility and homogeneity of the process establish the complexity of characterizations and dictate the material's future usage and possibilities.

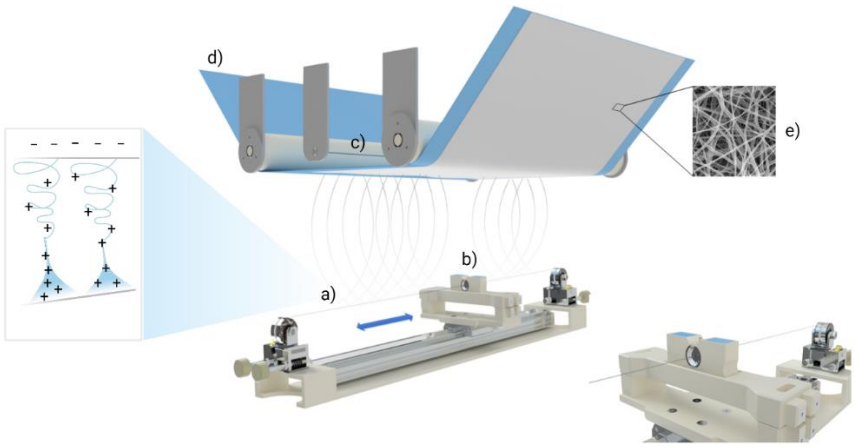


Figure 3: The needleless electrospinning Nanospider technology: a) a spinneret (steel wire), Taylor cones are formed along the charged wire; b) a cartridge for polymer solution; c) a collector (steel wire); d) a supportive textile material for nanofibrous collection; e) randomized nanofibrous planar structures.

3.4 Complexity of the Research

The realm of biotechnological research is inherently complex and multifaceted, requiring a harmonious integration of various disciplines. A successful biotech research endeavor entails not only the adept utilization of specialized terminology but also a comprehensive understanding of the fundamental principles underlying intricate processes, such as surgical procedures. The collaboration between materials engineering, cell biology, and the insights of clinical practitioners becomes paramount, emphasizing the need for a delicate balance between these diverse fields.

To maximize the potential for success throughout the my research and developed, I meticulously adhered to a structured procedure analogous to the development process of novel medical materials. The novel medical material development process is a meticulously structured sequence that involves several stages designed to ensure that only safe and effective medications are brought to the market. The main stages for scaffold development are depicted in *Figure 4*. While the exploration of Clinical Trials and Regulatory Approvals is a critical aspect of the medical material development process, it is important to note that this stage was not within the defined goals and scope of my thesis. The focus of this research endeavor primarily centered around the earlier stages, encompassing scaffold design, development, fabrication, and material characterization, as well as *in vivo* animal studies using large animal models. The aim was to lay a robust foundation for potential future advancements and applications in clinical trials and lay the foundational groundwork for potential future advancements and successful product development.

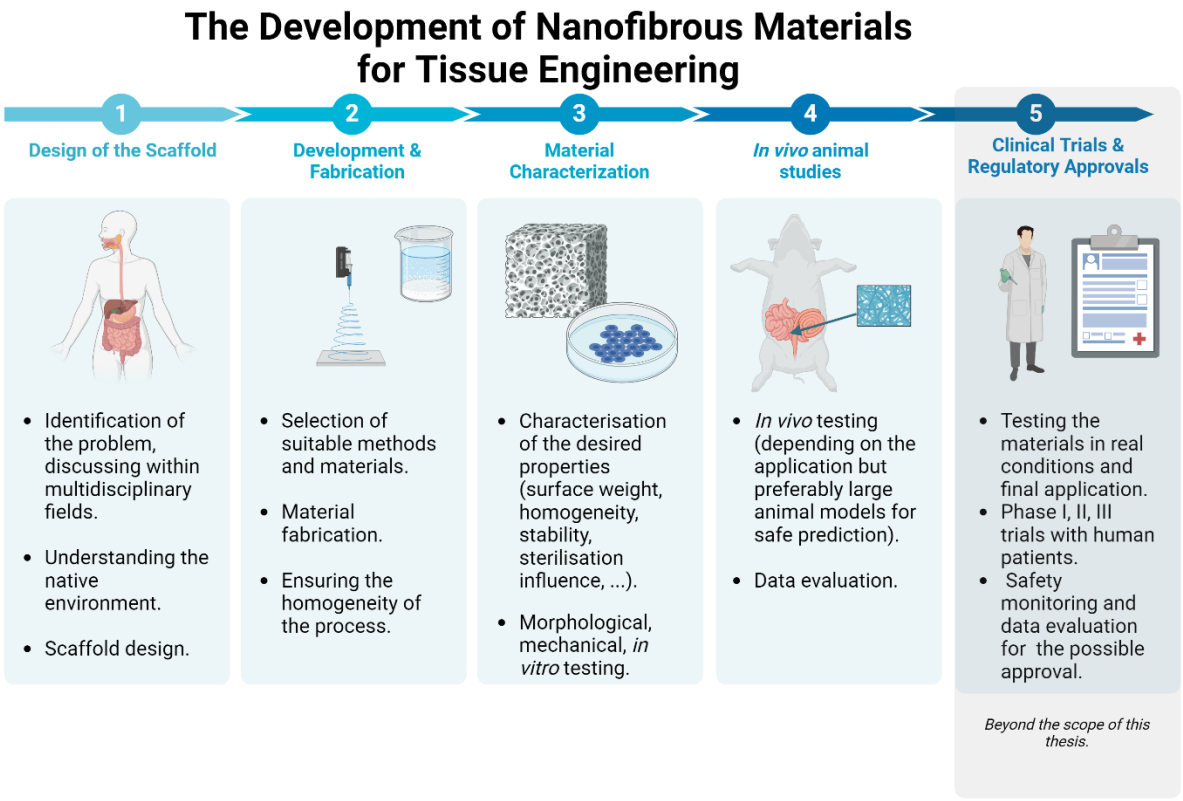


Figure 4: Stages of nanofibrous material development for clinical practise with a focus on the development of nanofibrous material for sealing gastrointestinal anastomoses. Based on the author's gained knowledge during the nanofibrous development consistently supported by official U.S. Food and Drug Administration (FDA) guidelines for drug development[31]*. Author's original picture, graphics created with BioRender.com.*

4. THE LIST OF PUBLISHED AND FURTHER DISCUSSED STUDIES

The thesis provides a comprehensive commentary on nine impactful articles, elucidating the intricate details of the experimental design, methodologies employed, and the profound outcomes derived from a series of systematic investigations. The first two articles, pivotal in shaping the foundation of this research endeavor, stand as review studies. These studies played a crucial role in advancing our understanding of the intricacies surrounding the healing mechanisms of intestinal anastomoses. Additionally, they shed light on the current landscape of antiadhesive nanofibrous materials, unveiling both their promises and the inherent shortcomings. With due respect to the collective efforts of other researchers in the field, these studies meticulously analyze the reasons behind certain setbacks, offering valuable insights for future endeavors. Subsequently, the following seven articles delve into the continuous research trajectory focused on the development of nanofibrous materials. Each article serves as a testament to the persistent pursuit of innovation, providing detailed accounts of the methodologies employed and the subsequent results obtained. Notably, these articles often accompany *in vivo* studies, offering a glimpse into the practical implications of the developed materials. The synthesis of these articles contributes to the evolving narrative of this research, laying the groundwork for further inquiries and advancements in the realm of nanofibrous materials for surgical applications.

THEORY SECTION

Klicova, M.; Rosendorf, J.; Erben, J.; Horakova, J. Antiadhesive Nanofibrous Materials for Medicine: Preventing Undesirable Tissue Adhesions. *ACS Omega* **2023**, *8* (23), 20152–20162. https://doi.org/10.1021/acsomega.3c00341.

- Impact Factor 2023: 4.1
- Publisher: ACS Omega
- Quartile: Q2
- Number of Citations: 1

Rosendorf, J.; **Klicova, M.**; Herrmann, I.; Anthis, A.; Cervenkova, L.; Palek, R.; Treska, V.; Liska, V. Intestinal Anastomotic Healing: What Do We Know About Processes Behind Anastomotic Complications. Front. Surg. 2022, 9, 904810. https://doi.org/10.3389/fsurg.2022.904810.

- Impact factor of the journal: 1.8
- Publisher: Frontiers in Surgery
- Quartile: Q3
- Number of citations: 7

EXPERIMENTAL SECTION

Horakova, J.; **Klicova, M.**; Erben, J.; Klapstova, A.; Novotny, V.; Behalek, L.; Chvojka, J. Impact of Various Sterilization and Disinfection Techniques on Electrospun Poly-ε-Caprolactone. *ACS Omega* 2020, *5* (15), 8885–8892. https://doi.org/10.1021/acsomega.0c00503.

- Impact factor of the journal: 4.1
- Publisher: ACS Omega
- Quartile: Q2
- Number of citations: 32

Rosendorf, J.; Horakova, J.; **Klicova, M.**; Palek, R.; Cervenkova, L.; Kural, T.; Hosek, P.; Kriz, T.; Tegl, V.; Moulisova, V.; Tonar, Z.; Treska, V.; Lukas, D.; Liska, V. Experimental Fortification of Intestinal Anastomoses with Nanofibrous Materials in a Large Animal Model. *Sci. Rep.* 2020, *10* (1), 1134. https://doi.org/10.1038/s41598-020-58113-4.

- Impact factor of the journal: 4.6
- Publisher: Nature Scientific Reports
- Quartile: Q2
- Number of citations: 14

Rosendorf, J.; **Klicova, M.**; Cervenkova, L.; Horakova, J.; Klapstova, A.; Hosek, P.; Palek, R.; Sevcik, J.; Polak, R.; Treska, V.; Chvojka, J.; Liska, V. Reinforcement of Colonic Anastomosis with Improved Ultrafine Nanofibrous Patch: Experiment on Pig. *Biomedicines* 2021, *9* (2), 102. https://doi.org/10.3390/biomedicines9020102.

- Impact factor of the journal: 4.7
- Publisher: Biomedicines
- Quartile: Q2
- Number of citations: 7

Klicova, M.; Klapstova, A.; Chvojka, J.; Koprivova, B.; Jencova, V.; Horakova, J. Novel Double-Layered Planar Scaffold Combining Electrospun PCL Fibers and PVA Hydrogels with High Shape Integrity and Water Stability. *Mater. Lett.* 2020, *263*, 127281. https://doi.org/10.1016/j.matlet.2019.127281.

- Impact factor of the journal: 3
- Publisher: Materials Letters
- Quartile: Q3

- Number of citations: 20

Rosendorf, J.; **Klicova, M.**; Cervenkova, L.; Palek, R.; Horakova, J.; Klapstova, A.; Hosek, P.; Moulisova, V.; Bednar, L.; Tegl, V.; Brzon, O.; Tonar, Z.; Treska, V.; Lukas, D.; Liska, V. Double-Layered Nanofibrous Patch for Prevention of Anastomotic Leakage and Peritoneal Adhesions, Experimental Study. *In Vivo* 2021, *35* (2), 731–741. https://doi.org/10.21873/invivo.12314.

- Impact factor of the journal: 2.3
- Publisher: In Vivo
- Quartile: Q4
- Number of citations: 7

Klicova, M.; Oulehlova, Z.; Klapstova, A.; Hejda, M.; Krejcik, M.; Novak, O.; Mullerova, J.; Erben, J.; Rosendorf, J.; Palek, R.; Liska, V.; Fucikova, A.; Chvojka, J.; Zvercova, I.; Horakova, J. Biomimetic Hierarchical Nanofibrous Surfaces Inspired by Superhydrophobic Lotus Leaf Structure for Preventing Tissue Adhesions. *Mater. Des.* 2022, *217*, 110661. https://doi.org/10.1016/j.matdes.2022.110661.

- Impact factor of the journal: 8.4
- Publisher: Elsevier
- Quartile: Q1
- Number of citations: 19

Klicova, M.; Mullerova, S.; Rosendorf, J.; Klapstova, A.; Jirkovec, R.; Erben, J.; Petrzilkova, M.; Raabová, H.; Šatínský, D.; Melicherikova, J.; Palek, R.; Liska, V.; Horakova, J. Large-Scale Development of Antibacterial Scaffolds: Gentamicin Sulfate-Loaded Biodegradable Nanofibers for Gastrointestinal Applications. *ACS Omega* 2023, *8* (43), 40823–40835. https://doi.org/10.1021/acsomega.3c05924.

- Impact factor of the journal: 4.1
- Publisher: ACS Omega
- Quartile: Q2
- Number of citations: 0

5. JOURNAL ARTICLE: A. REVIEW ABOUT ANTIADHESIVE NANOFIBROUS MATERIALS

5.1 Introduction of the Article

The article[32] rigorously reviews the vast array of scientific research dedicated to developing functional nanofibrous barrier materials and touches upon the conspicuous lack of their commercial availability, despite their proven potential. It critically analyzes the challenges that have hindered the transition from lab-scale production to viable commercial products. These include the limitations of needle electrospinning, which, while enabling the verification of polymeric solution spinnability, suffers from low yield and efficiency — an impediment for large-scale production. The article seeks to bridge the gap between material science and clinical application, presenting advances in electrospinning techniques for the fabrication of versatile nanofibrous structures capable of mitigating undesirable tissue adhesions. This review elucidates diverse approaches of material's development and characterization and situates them in context as it could be seen in the *Figure 5*.

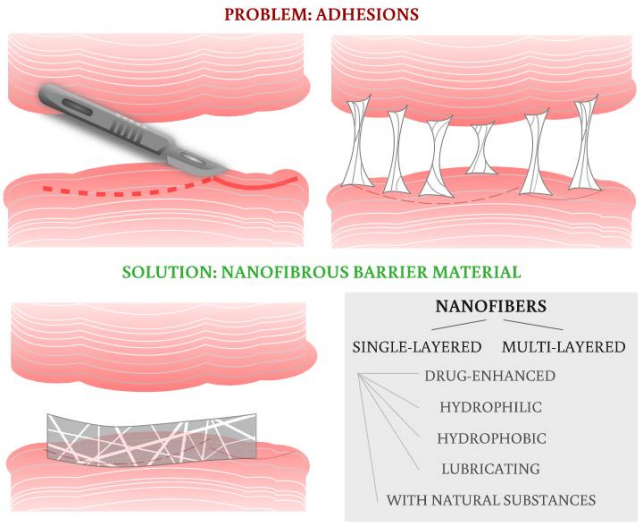


Figure 5: Graphical abstract.[32]

5.1.1 Key Highlights/Findings

1. ***Criticall Analysis of the Currently Used Fabrication Methods:*** This review rigorously analyses the vast array of scientific research dedicated to developing functional nanofibrous barrier materials and touches upon the conspicuous lack of their commercial availability, despite their proven potential. It critically analyzes the challenges that have hindered the transition from lab-scale production to viable commercial products. These include the limitations of needle electrospinning, which, while enabling the verification of polymeric solution spinnability, suffers from low yield and efficiency — an impediment for large-scale production, see Figure 6 and Figure 7. The discussion extends to the

necessity for semi-industrial and industrial scalability of production, which is presently deficient but critical for moving towards practical applications. The main barriers identified include low efficiency in needle electrospinning processes and the absence of controlled environmental conditions, factors which compromise repeatability and reliability of the process.

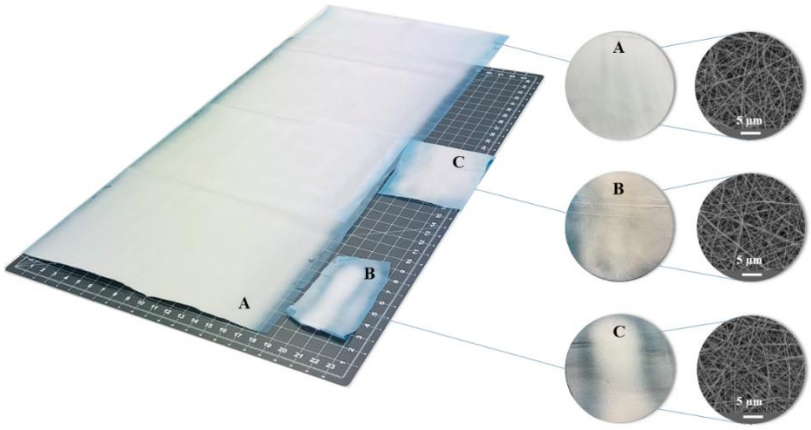


Figure 6: Difference between needleless electrospinning (A) and needle electrospinning nanofibrous products (B and C). Comparison of the homogeneity on the macroscopic (inhomogeneous) and microscopic view (homogenous). The microscopy (SEM) images show a similar structure in all products without revealing the specific weight/thickness inconsistency across the whole surface.[32]

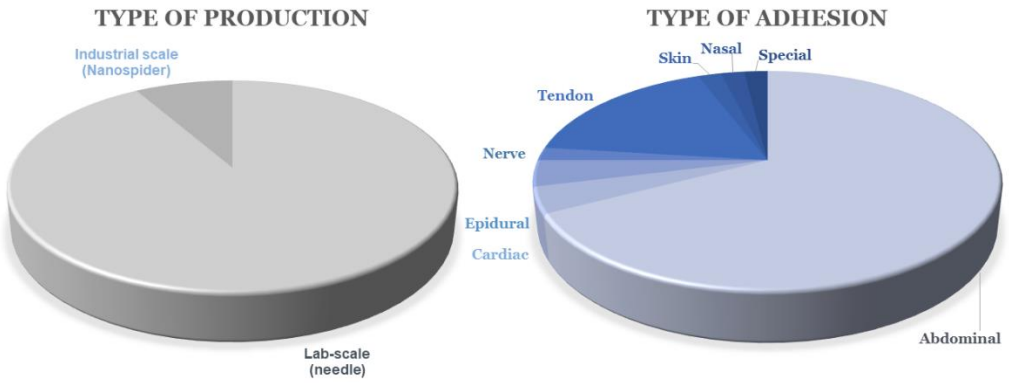


Figure 7: Methods for production of nanofibrous antiadhesive barriers (left). Prevented types of adhesions via nanofibrous materials (preclinical research only; right). [32]

2. ***Review of Results from Animal Testing to Date:*** This article also scrutinizes the testing methodologies used, recognizing the prevalence of small animal model testing due to the inherently low productivity of needle electrospinning. It emphasizes the importance of expanding tests to larger models that more accurately predict human responses, ensuring both biocompatibility and relevance for clinical use.
3. ***Pointing out the Lack of Information on Sterilized Materials:*** Additionally, attention is drawn to the potential of incorporating actives into nanofibrous systems and the need for characterizing nanofibers post-sterilization to comprehend material behavior changes upon sterilization.
4. ***Highlighting Inconsistencies in Relation to the Appropriate Surface Wettability:***

Importantly, it identifies a discrepancy in the literature regarding the optimal material hydrophilicity/hydrophobicity for preventing tissue adhesions. Looking forward, the review acknowledges a growing focus on lubricating nanofibers — a novel approach posited to inhibit cell attachment completely and reduce friction between tissues, potentially offering a more effective way to combat postoperative adhesions.

5.2 Integration into the Overall Conceptual Framework

Understanding the current landscape of literature and methodologies is paramount in the trajectory of our research. The review article "Antiadhesive Nanofibrous Materials for Medicine" serves as a vital bridge between existing approaches and the innovative strides made in our nanofibrous material development for intestinal anastomotic healing. Highlighting the significance of large-scale electrospinning, a technique integral to our trials, is essential. Not only does it ensure reproducibility and ease of transfer, but it also aligns with our commitment to real-world applicability beyond small animal model testing. The choice of PCL, a well-established biodegradable polyester, echoes promising outcomes observed in similar research domains (see *Figure 8*). Synthetic polymers are the most frequently used for the production of antiadhesive nanofibers, and among them, biodegradable polyesters are preferred, with polycaprolactone playing a leading role. It is clear that the majority of the reviewed research focused on preventing postoperative adhesions was concentrated on the use of PCL, highlighting its prominence in the field. Furthermore, our Department has a robust background in the development of PCL scaffolds for wound healing, which have already progressed through clinical trials, ensuring a wealth of experience to draw upon. These strategic choices and linkages reflect the nuanced and deliberate path we tread in pushing the boundaries of nanofibrous materials for biomedical applications.

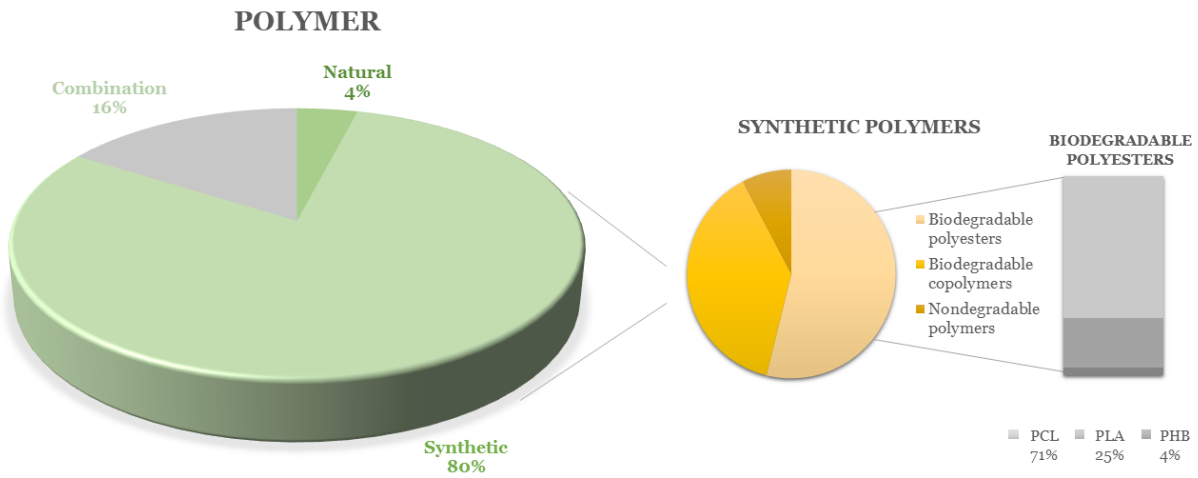


Figure 8: Graphical overview of used polymers for antiadhesive nanofiber production.[32]

6. JOURNAL ARTICLE: B. REVIEW ABOUT THE INTESTINAL HEALING AND PREVENTION SUGGESTIONS FOR DIMINSHING POSTOPERATIVE COMPLICATIONS

6.1 Introduction of the Article

This review[33] deals with the complexities of intestinal anastomotic healing and seeks to shed light on the processes that may lead to complications such as anastomotic leaks, strictures, and fistulae following colorectal surgery. Despite advancements in colorectal surgery, anastomotic leakage remains a significant postoperative concern with potentially life-threatening consequences. We aim to review and discuss current known pathophysiological mechanisms and identify emerging mechanisms that require further study. Additionally, we address the limitations of current clinical knowledge regarding the healing processes of intestinal anastomoses. The ultimate goal of this review is to guide future research directions and improve the understanding of anastomotic wound healing to help develop better preventative strategies for related complications.

6.1.1 Key Highlights/Findings

1. ***Role of Blood Supply:*** The paper acknowledges that adequate blood supply is crucial for both the healing process and tissue vitality. The use of indocyanine green can help assess blood circulation quality to the anastomotic site and potentially modify surgical plans to improve outcomes.
2. ***Impact of Bacterial Infection:*** We emphasize the association between anastomotic leaks and microbial infections, particularly with bacterial strains like Pseudomonas Aeruginosa or Streptococcus Faecalis. Infections contribute to complications by producing enzymes like collagenases that break down connective tissue critical for healing.
3. ***Complexity of Wound Healing:*** Wound healing within the intestine is complex, involving various layers of the organ, diverse cell types, and differing healing capacities (e.g., peritoneum vs. mucosa), which complicate the understanding of healing processes.
4. ***Emerging Pathophysiological Mechanisms:*** We suggest that knowledge about intestinal anastomotic healing is limited and cite multiple emerging mechanisms, such as the potential role of human collagenase activators like matrix metalloproteinase 9 in degrading the extracellular matrix.
5. ***Healing Differences:*** We emphasize that healing of intestinal anastomoses cannot be directly compared to cutaneous wound healing due to the different organ characteristics and environmental factors within the human body.
6. ***Research Gaps:*** The paper points out there's a substantial lack of comprehensive studies on healing at the cellular metabolism level and across the different gastrointestinal layers, making it difficult to fully understand the process and develop targeted interventions.
7. ***Necessity for Advanced Research:*** Employing advanced laboratory methods to study metabolic and proliferative changes in the context of physiological and pathological conditions is crucial for progress in clinical visceral surgery and in creating preventive measures for anastomotic complications.

6.2 Integration into the Overall Conceptual Framework

The objective is to comprehend how anastomoses heal and why, for the past 200 years, we have grappled with severe postoperative complications. Understanding these mechanisms aids in designing better and more functional materials. This review is crucial not only for delineating the intricacies of surgeries but also for informing material development. As our insights indicate, we underscore the link between anastomotic leaks and microbial infections, particularly involving bacterial strains such as Pseudomonas Aeruginosa or Streptococcus Faecalis. Infections contribute to complications by generating enzymes like collagenases that degrade connective tissue crucial for healing. In response to these challenges, our development focuses on nanofiber drug delivery systems for targeted administration of specific antibiotics to the intestinal anastomosis, thereby reducing the antibiotic dosage compared to oral or intravenous applications. We further highlight the complexity of anastomotic healing and the misconception of equating intestinal healing to cutaneous healing.

7. JOURNAL ARTICLE: C. STERILIZATION METHOD VERIFICATION FOR PCL NANOFIBERS: EVALUATING EFFICACY AND EFFECTS

7.1 Introduction of the Article

The article[34] "Impact of Various Sterilization and Disinfection Techniques on Electrospun Poly-ε-caprolactone" discusses the importance of sterilization for biomaterials used in medicine, highlighting the difference between sterilization and disinfection as visualised in *Figure 9.* It underlines the necessity for sterile materials for clinical practice, achieved through aseptic fabrication or terminal sterilization. Low-temperature sterilization methods such as ethylene oxide and gamma irradiation are preferred for thermosensitive materials like polycaprolactone, with an emphasis on the implications of these methods on the material's properties and medical staff safety.

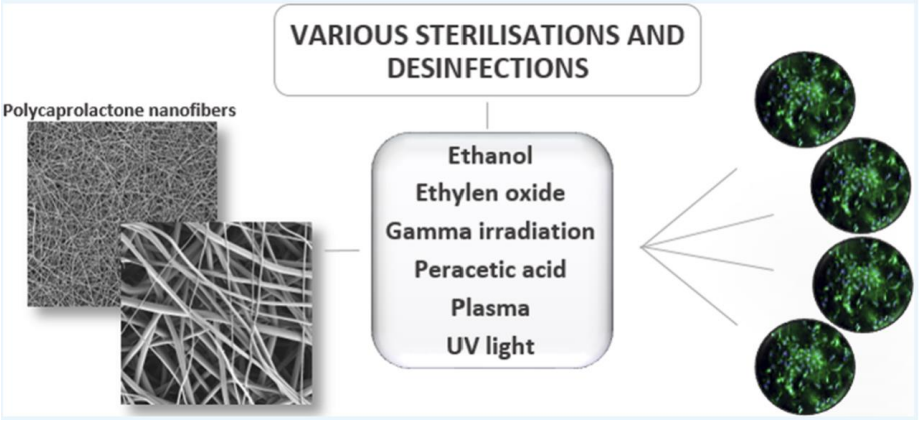


Figure 9: Graphical abstract.[34]

7.1.1 Key Highlights/Findings

1. ***Hydrogen Peroxide Plasma Sterilization:*** This method is not suitable for nanofibrous PCL sterilization as it leads to the complete loss of the fibrous structure, particularly if the temperature during the cycle exceeds 55 °C.
2. ***Ethylene Oxide (EtOx) Sterilization:*** EtOx treatment is appropriate for PCL sterilization. The study found no changes in fibrous morphology or molecular weight after sterilization with EtOx. However, there was a delayed fibroblast proliferation rate for EtOx-sterilized samples compared to other methods. Despite this, EtOx sterilization was found to be suitable as it did not induce cytotoxic effects.
3. ***Gamma Irradiation:*** This method did not alter the morphology of electrospun PCL when a dose of 29 kGy was used. Fibroblasts seeded on gamma-irradiated samples displayed the highest level of viability and cell density compared to other techniques. The improved cell interactions were attributed to an increase in surface wettability post-irradiation.

4. ***Peracetic Acid (PAA) Sterilization:*** PAA sterilization had no impact on the fibrous morphology and molecular weight of the PCL. Fibroblast proliferation on PAA-sterilized mats was lower compared to gamma-treated samples but higher than on EtOx-sterilized materials.
5. ***Ethanol Soaking and UV Irradiation Disinfection:*** Both methods left the morphology, molecular weight, and thermal properties of the electrospun PCL unchanged (*Figure 10* shows the fiber morphology as an example). Ethanol soaking was found to be more efficient than UV irradiation in terms of higher cell viability and cell number per area.

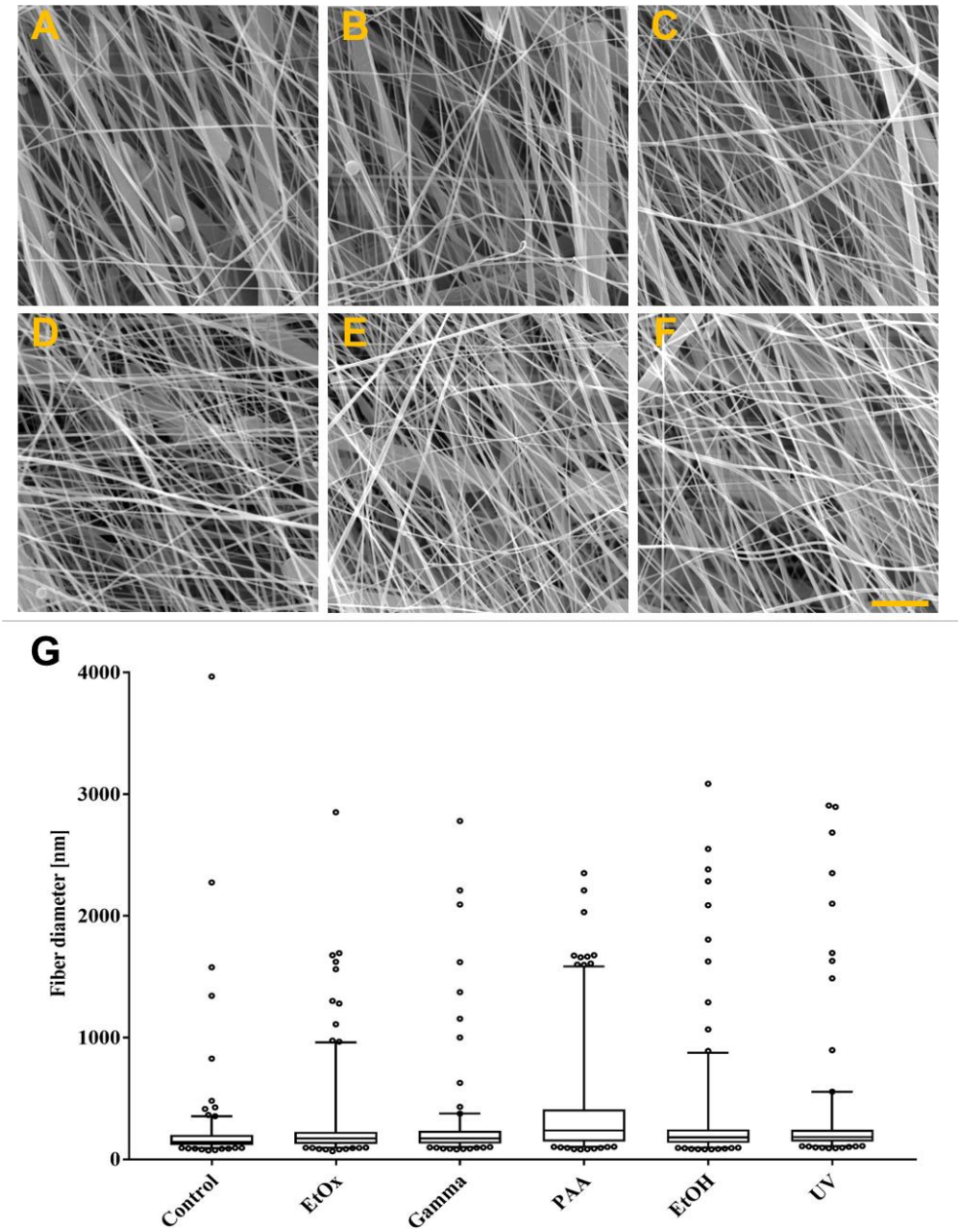


Figure 10: SEM pictures of electrospun PCL after sterilization by ethylene oxide (A), gamma irradiation (B), peracetic acid (C), disinfected by ethanol (D), UV irradiation (E), non sterile control (F), scale bar 5 µm. Box plot graph of fiber diameter characteristics (G).[34]

6. ***Overall Material Compatibility:*** The most suitable methods for the sterilization of electrospun PCL with a molecular weight of 45,000 g/mol were identified as EtOx and gamma irradiation. These methods did not harm the essential properties of the PCL, preserving both the micro- and nanoscale fibrous structure. *Figure 11* shows the fluorescence microscopy images of stained mouse fibroblast cultured on sterilised materials.

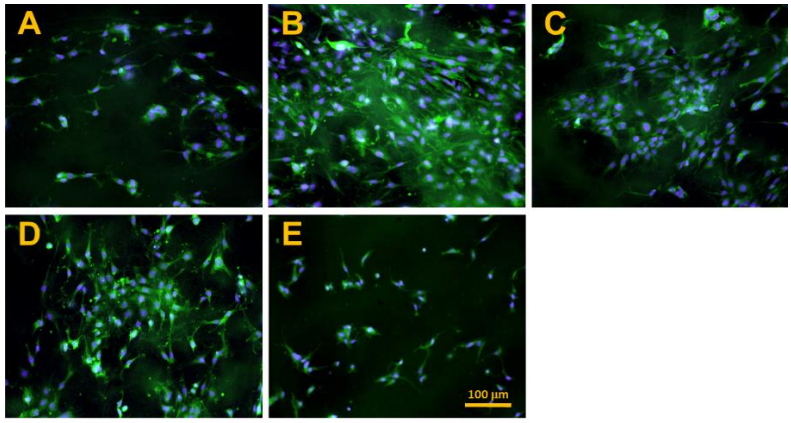


Figure 11: Fluorescence microscopy pictures of fibroblasts after 7 days of culturing on nanofibrous PCL samples sterilized by ethylene oxide (A), gamma irradiation (B), peracetic acid (C), disinfected by ethanol (D), UV irradiation (E), scale bar 100 µm.[34]

7.2 Integration into the Overall Conceptual Framework

This research not only reinforces the extensive use of PCL in medical applications, as highlighted in the previous review on antiadhesive materials, but also underscores the distinctive attributes of electrospun nanofibrous materials. It emphasizes the critical need for meticulous evaluation of their response to various sterilization techniques, particularly focusing on electrospun PCL, given its low melting point, which necessitates low-temperature sterilization methods. An often overlooked aspect is the resilience of resulting nanofibrous layers to sterilization, a crucial consideration for *in vivo* testing and subsequent applications. Unfortunately, many materials remain untested post-sterilization, leaving substantial uncertainties about their „true“ properties. This knowledge gap, as previously indicated in the review on antiadhesive materials, can lead to misinterpretations in studies, particularly since sterilization methods can significantly alter surface properties such as wettability. This study ensures a comprehensive understanding of the properties of PCL nanofibers post-sterilization, providing a solid foundation for our subsequent development and application in the broader context of creating functional nanofibrous patches for intestinal applications.

8. JOURNAL ARTICLE: D. IMPLANTATION OF PCL AND PLCL NANOFIBERS IN LARGE ANIMAL MODELS: A FIRST COMPREHENSIVE DESCRIPTION

8.1 Introduction of the article

The paper[19] titled "Experimental fortification of intestinal anastomoses with nanofibrous materials in a large animal model," explores the use of biodegradable nanofibrous materials for the reinforcement of surgical connections in the intestines (anastomoses) in a large animal study using pigs. This experimental research investigates whether nanomaterials based on PCL or copolymer of L-lactic and caprolactone (PLCL) can be safety used on the large animal intestines after anastomosis construction. The study includes the development of a new scoring system for assessing peritoneal adhesions and reports on clinical outcomes, histological assessments, and material handling. The findings are intended to contribute to better postoperative outcomes by fortifying anastomoses and minimizing adhesion formation using these nanofabrics.

8.1.1 Key Highlights/Findings

1. ***Safety of Nanofibrous Scaffolds:*** The use of polycaprolactone and polylactic acid with polycaprolactone nanofibrous scaffolds for reinforcing anastomoses in the gastrointestinal tract of pigs was found to be safe. There were no indications that these materials caused adverse effects or systemic disturbances.
2. ***Material Handling:*** The nanomaterials used were easy to manipulate and apply during surgeries. The application is shown in *Figure 12*.

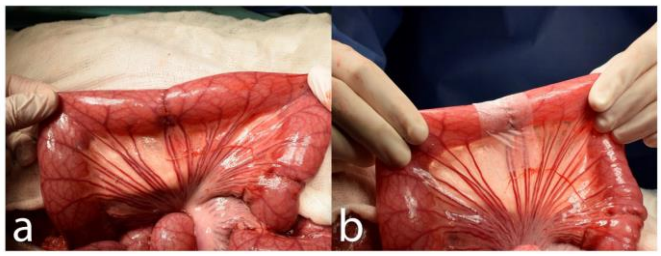


*Figure 12: Reinforcing the end-to-end anastomosis on the small intestine in a pig model: (**a**) constructed anastomosis; (**b**) the PCL nanomaterial applied to the site of anastomosis partially covering the mesentery.*[19]

3. ***Mortality and Morbidity:*** There were no deaths or major complications within the experimental groups, indicating a strong safety profile for the nanofibrous materials.
4. ***Anastomotic Leakage and Stenosis:*** No cases of anastomotic leakage or stenosis (narrowing of the intestinal opening) were reported among the study subjects across all groups.
5. ***Peritoneal Adhesion Scores:*** The study introduced a new scoring system for peritoneal adhesions, which did not show a significant difference in adhesion levels between the control group and those with applied nanofibrous materials.

6. ***Tissue Healing:*** Histological analysis suggested that the application of nanofibrous materials did not interfere with the healing of anastomoses, as measured by parameters like collagen fiber content and inflammatory cell infiltration (*Table 1*).

*Table 1: Summary of the most important results for each group. *Each animal was assigned a score equal to the average of all segment scores in that animal (i.e. 24 segment scores per animal; result theoretically ranging from 0 to 2). Mean and Standard error of the mean (SEM) stated in the table were then calculated from these animal averages.*[19]

	Control Group (n = 8)	PCL Group (n = 8)	PLCL Group (n = 8)	*p*-value between groups (test)
Material fibre thickness	—	325 ± 36 nm	2047 ± 585 nm	—
Material thickness	—	49 ± 5 nm	53 ± 6 nm	—
Macroscopic signs of anastomotic stenosis (count; %)	0; 0%	0; 0%	0; 0%	—
Macroscopic signs of anastomotic leakage (count; %)	0; 0%	0; 0%	0; 0%	—
Mean PAAS score per segment (0–2) (mean ± SEM across pigs)*	0.479 ± 0.086	0.823 ± 0.171	0.688 ± 0.070	0.715 (repeated measures ANOVA)
Incomplete re-epithelisation (count; %)	0; 0%	0; 0%	0; 0%	—
Volume fraction of vWF positive cells [%] (mean ± SEM)	2.22 ± 0.10	2.16 ± 0.16	2.38 ± 0.12	0.690 (repeated measures ANOVA)
Volume fraction of collagen fibres [%] (mean ± SEM)	15.51 ± 2.10	15.67 ± 2.36	11.87 ± 1.91	0.740 (repeated measures ANOVA)
Volume fraction of MAC387 positive cells [%]:				0.550 (two-way ANOVA)
• stitch not in sample (n: mean ± SEM)	8: 0.38 ± 0.09	8: 0.46 ± 0.19	8: 0.21 ± 0.06	
• stitch in sample (n: mean ± SEM)	7: 0.80 ± 0.23	5: 0.67 ± 0.16	6: 0.70 ± 0.27	

8.2 Integration into the Overall Conceptual Framework

This study, focused on testing PCL and PLCL nanofibrous materials on large animal models, significantly contributes to the overarching conceptual framework of the thesis. One of its primary merits lies in its ability to bridge the gap between laboratory development and actual surgical practice. By utilizing large animal models, specifically pigs, the study provides a realistic platform for assessing the materials in conditions that closely resemble human surgical scenarios. The application of the nanofibrous materials does not extend the duration of surgical operations. This revelation is crucial, as it emphasizes the materials' seamless integration into existing surgical protocols, aligning with the practical requirements of surgical teams. A notable advantage highlighted by the study is the standalone application of the materials. The absence of additional substances, such as tissue adhesives, simplifies the surgical process and establishes the nanofibrous materials as self-sufficient in addressing the targeted surgical challenges. This finding enhances the materials' appeal for surgeons seeking straightforward and efficient solutions. While the study did not reveal substantial improvements in healing or the prevention of complications, its significance lies in dispelling doubts about the feasibility and safety of applying nanofibrous materials directly in surgical settings. This outcome is invaluable for our future material research, providing a clear path for refining and optimizing nanofibrous patches for intestinal anastomoses.

9. JOURNAL ARTICLE: E. OPTIMIZATION OF PCL NANOFIBROUS MATERIALS FOR ENHANCED IMPLANTATION PERFORMANCE

9.1 Introduction of the Article

The paper[35] titled "Reinforcement of Colonic Anastomosis with Improved Ultrafine Nanofibrous Patch: Experiment on Pig" addresses the challenge of anastomotic leakage following colorectal surgical procedures. To tackle this issue, we developed an ultrafine nanofibrous patch made of polycaprolactone, a material known for its biocompatibility and mechanical properties. The experimental study was conducted on pigs, where the nanofibrous patch was used to reinforce colonic anastomoses, with the aim of improving the healing process and minimizing the risk of leakage. The findings from the study were hopeful, revealing no significant leakage and increased collagen levels in the experimental group, suggesting a potential for the patch to be used in enhancing anastomotic healing.

9.1.1 Key Highlights/Findings

1. ***Discovered limits in needleless electrospinning:*** Identified the lower threshold for surface weight achievable with the Nanospider technique and PCL polymer. The material was tailored to a weight of 10 g/m² to expedite degradability and enhance support for the healing process. The morphology of the resulting fibers could be seen in *Figure 13*.

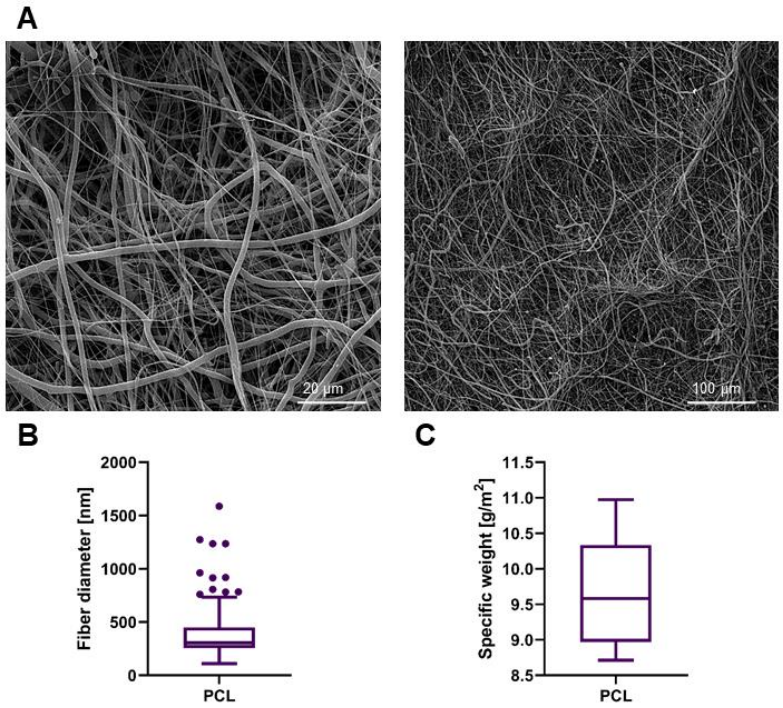


Figure 13: The SEM images of the electrospun PCL planar layer, scale bars 20 µm and 50 µm (A). The boxplot of fiber diameters (n=500) (B). The calculated value of specific weight of the nanofibrous layer (n=10) (C).[35]

2. ***Material Characteristics:*** The polycaprolactone nanofibrous material in lower surface weight was described as easy to manipulate and apply without needing extra fixation during surgical procedures. *In vitro* assays confirmed the cytocompatibility and cell growth on the planar nanofibrous scaffold, as it can be seen in *Figure 14*.

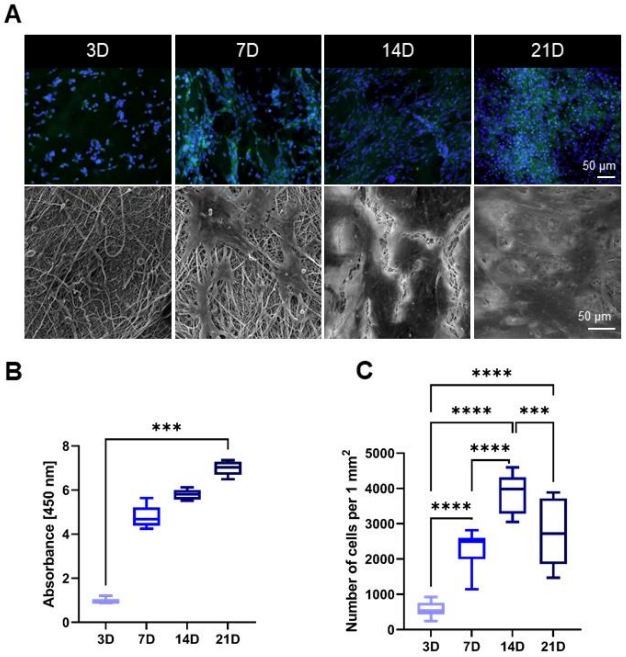


*Figure 14: The fluorescence microscopy images (blue cell nuclei and green actin cytoskeleton) and the SEM images of the cells on the PCL scaffold after 3, 7, 14 and 21 days of the in vitro testing, scale bars 50 µm (A). The result of the colorimetric CCK-8 assay after the same time period, Kruskal-Wallis ***p<0,0004. (B). Counted number of the cells on the surface of PCL materials per 1 mm2, ordinary one-way ANOVA, ***p<0,0006, ****p<0,0001 (C).*[35]

3. ***Clinical Observations:*** Throughout the observation period, all animals survived with no major health complications. The experimental material did not lead to any adverse reactions or prolong procedure times.
4. ***Weight Gain:*** A higher percentage of animals in the Experimental group (with patch) gained over 5% of their body weight compared to the Control group (without patch), suggesting that the patch may contribute to improved postoperative recovery.
5. ***Anastomotic Healing:*** There were no macroscopically visible pathological reactions to the nanofibrous material in the abdominal cavity, and no macroscopic signs of anastomotic leakage were observed in any of the animals.
6. ***Histological Assessment:*** Histologically, there were no signs of full-thickness defects (anastomotic leakage) in either the Control or the Experimental group, and the integrity of the intestinal wall appeared normal in all specimens.
7. ***Collagen Formation:*** There was a significantly higher volume of collagen in the Experimental group, which could indicate enhanced healing strength of the anastomosis reinforced with the PCL patch.

9.2 Integration into the Overall Conceptual Framework

This study is essential for advancing our research as it establishes the ideal surface weight for materials that are not only electrospinnable using selected methods but also applicable around the intestine during surgical procedures. Additionally, these materials need to strike a balance — they should be stable enough to handle yet possess the lowest possible surface weight to promote proper healing. This ensures improved healing outcomes, characterized by a lack of necrotic tissue and a higher collagen content compared to the initial material (PCL, 20 g/m^2). Despite its positive effects on healing, the material did not influence peritoneal adhesions. Consequently, our ongoing research will focus on functionalizing one side of the material to prevent the occurrence of adhesions.

10. JOURNAL ARTICLE: F. DOUBLE-LAYERED NANOFIBROUS MATERIALS: DESIGNING VARIATIONS IN WETTABILITY ON EACH SIDE

10.1 Introduction of the Article

This study[36] presents the development of a novel double-layered planar scaffold fabricated through needleless electrospinning, offering distinct characteristics on each of its sides. The scaffold is composed of a hydrophobic polycaprolactone fibrous layer and a hydrophilic poly(vinyl alcohol) fibrous layer, where the latter can have either a low (PVA_L) or high (PVA_H) degree of hydrolysis. The unique design addresses the challenge of PVA's rapid solubility in water by coupling it with a water-stable PCL layer, thus avoiding the need for cytotoxic cross-linking agents and preserving biocompatibility. This innovative combination yields a scaffold with high shape integrity and water stability (as visualised in *Figure 15*), which shows promise in various medical applications, including wound dressings and adhesion prevention. The study evaluates the morphology, wettability, and biocompatibility of the scaffold, with a focus on the adhesion and proliferation of mouse fibroblasts.

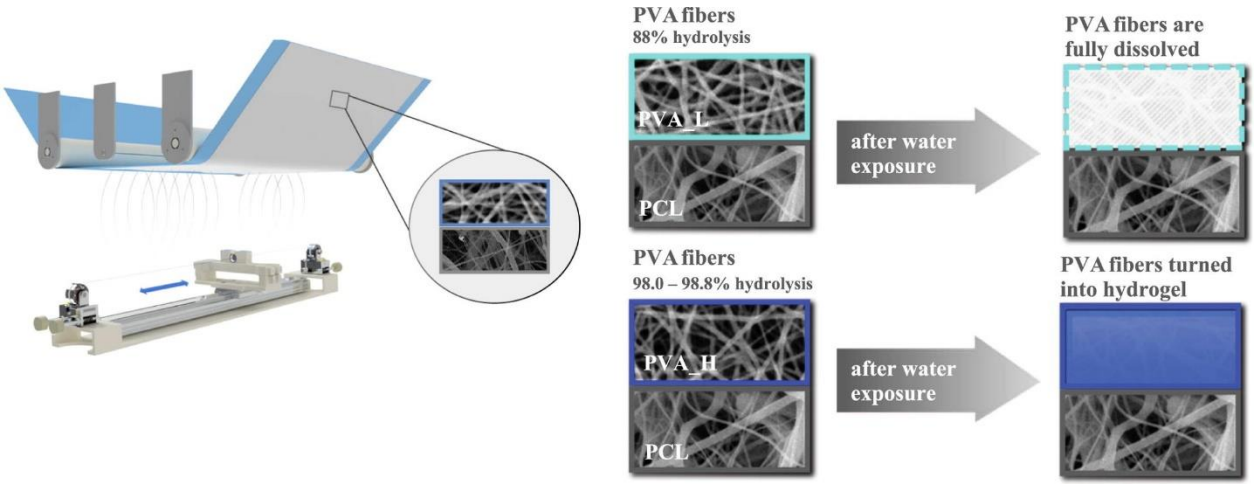


Figure 15: Graphical abstract.[36]

10.1.1 Key Highlights/Findings

1. ***Scaffold Design:*** The paper describes a double-layered scaffold with a unique combination of materials—one layer of hydrophobic polycaprolactone fibers and one layer of hydrophilic poly(vinyl alcohol) fibers, where PVA is available in both low and high degrees of hydrolysis (PVA_L and PVA_H respectively).
2. ***Water Stability:*** It is demonstrated that the PCL layer retains its structural integrity in the presence of water, whereas the PVA layer has varying responses—PVA_L dissolves while PVA_H forms a hydrogel-like structure, yet the overall shape of the scaffold is preserved due to the PCL layer's stability. The morphology and wetting properties of the scaffolds are shown in *Figure 16*.

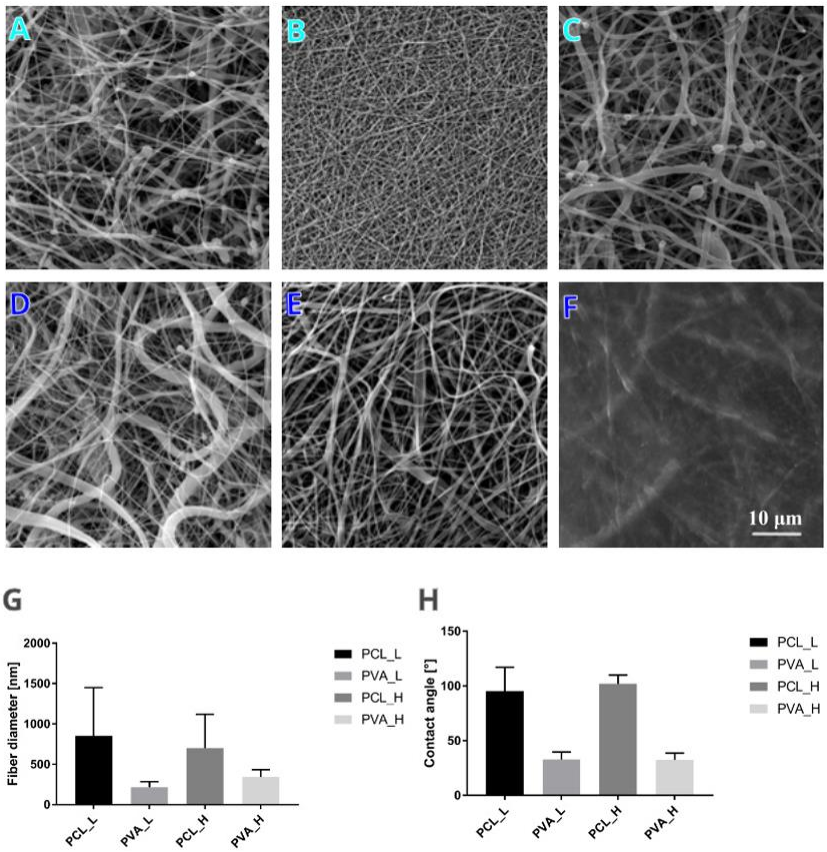


Figure 16: The SEM morphology images of PCL_L (A), PVA_L (B), PVA_L after water exposure (C), PCL_H (D), PVA_H (E) and PVA_H turned into gel after water exposure (F). Scale bar 10 µm. The graphs show the measured values of fiber diameters (G) and contact angles (H).[36]

3. ***Cytocompatibility and Cell Response:*** Through *in vitro* testing with 3T3 mouse fibroblasts, the study reveals differences in cell proliferation and adhesion on PCL versus PVA surfaces. Notably, PCL fibers promote cell growth, while PVA layers show limited cell adhesion which could be beneficial for applications requiring reduced cell attachment (see *Figure 17*).

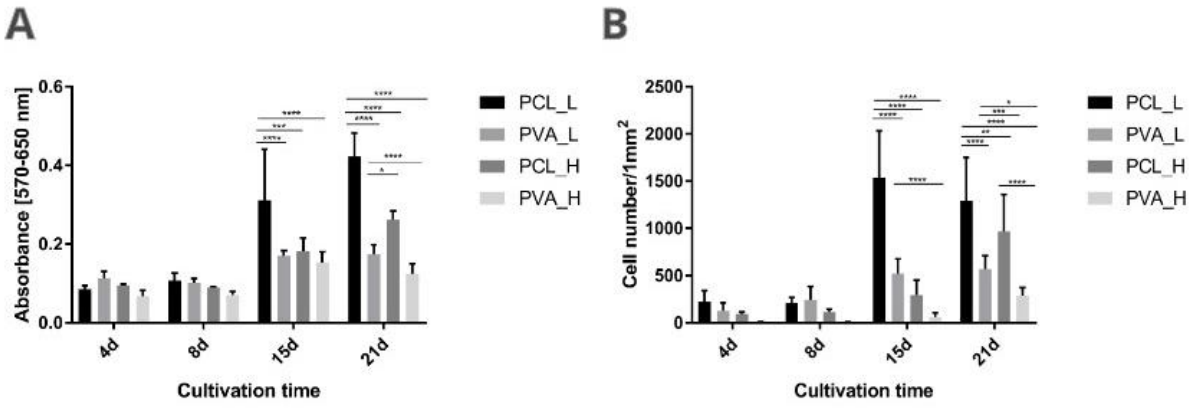


*Figure 17: Graphs of fibroblast metabolic activity during 21 days experiment (A) and number of calculated cells on the surface (B). * denotes p<0.0332, ** p<0.0021, *** p<0.0002, **** p<0.0001 (two-way ANOVA, Bonferonni).*[36]

10.2 Integration into the Overall Conceptual Framework

After confirming the feasibility of applying nanofibers around intestinal anastomosis, further consideration was given to "enhancing" the material with a hydrophilic side that would adhere to the anastomosis, while the other side remained antiadhesive (hydrophobic) to prevent the formation of peritoneal adhesions. The material was developed in two variants (hydrophilic sides based on PVA's with different rates of solubility), and nanofibrous scaffolds were subsequently tested in animal models to verify their functionality, as described in the following commentary.

11. JOURNAL ARTICLE: G. *IN VIVO* ASSESSMENT OF PVA/PCL DOUBLE-LAYERED MATERIAL PERFORMANCE

11.1 Introduction of the Article

Our study[37] investigates the effectiveness of double-layered nanofibrous materials in enhancing the healing of intestinal anastomosis and preventing peritoneal adhesions. These are common and serious complications in colorectal surgery. The study uses an experimental model on pigs to test patches made of polycaprolactone and polyvinyl alcohol with two degrees of hydrolysis, namely PVA_L (in the manuscript denoted as PVA2) and PVA_H (in the manuscript denoted as PVA1) applied to intentionally created defective anastomoses. The primary outcomes examined include the healing process at the anastomotic site and the extent of peritoneal adhesions, with a focus on providing a complex assessment of anastomotic integrity and healing (see *Figure 18*). The findings indicate that while the nanofibrous materials prevented anastomotic leakage, they may have delayed the overall healing process and increased adhesion formation, suggesting that further research and material optimization are needed before clinical application.

11.1.1 Key Highlights/Findings

1. ***Prevention of Anastomotic Leakage:*** The double-layered nanofibrous patch successfully prevented anastomotic leakage in the tested pigs. Histological assessments confirmed that there were no microscopic signs of anastomotic leakage.
2. ***Inflammatory Response and Healing Quality:*** The PCL/PVA_H group showed a higher inflammatory reaction, which could imply an active healing process. However, the histological assessment of the specimens suggested inferior healing quality when the material was applied, compared to control specimens (animals that did not receive the patch).
3. ***Peritoneal Adhesions:*** The materials used for the patch were pro-adhesive, with a suggestion that both materials contributed to an increased formation of adhesions at the site of the anastomosis and in its vicinity.
4. ***Intestinal Wall Integrity:*** Using a new semiquantitative scoring system developed by the researchers, the integrity of the intestinal wall at the site of the anastomosis was found to be significantly lower in the experimental groups compared to the control group.
5. ***Material Displacement:*** The study found that the nanofiber material remained fully attached at the application site in the majority of animals in the PCL/PVA_H group, while partial displacement occurred in the PCL/PVA_L group but still covered the defect.
6. ***Impact on Clinical Outcomes:*** No significant negative effects on clinical outcomes were observed postoperatively in the animals that received the nanofibrous patches.

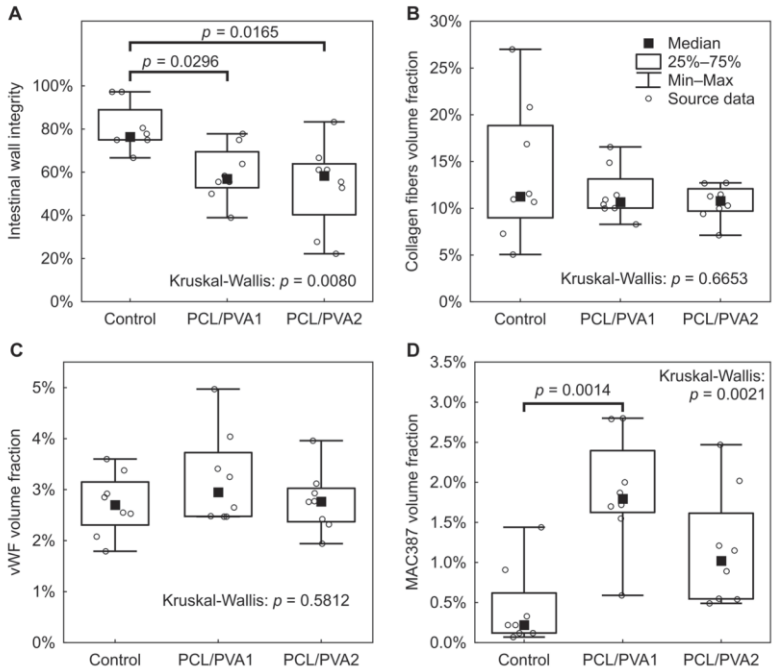


Figure 18: Results of histological evaluation in box plot graphs. A) Anastomosis deficiency score; B) comparison of collagen fibers volume fractions; C) comparison of vWF positive cells volume fractions; D) comparison of MAC387 positive cells volume fractions.[37]

11.2 Integration into the Overall Conceptual Framework

In this research, the outcomes of implanting double-layered nanofibrous materials based on PVA and PCL polymers are described. Although the development of these materials directly stemmed from the clinical requirements of medical professionals, the final results revealed significant shortcomings such as:

- ***Impaired Healing:*** The healing process at the anastomotic site was found to be inferior when the patch was applied compared to the control group. This suggests that the materials may have interfered with the normal healing process of the intestine.
- ***Increased Inflammatory Response:*** In the group treated with the PCL/PVA_H material, there was a higher inflammatory reaction compared to the control. While inflammation can be a part of the normal healing process, excessive inflammation may indicate a prolonged or altered healing phase.
- ***Pro-adhesive Nature:*** Despite the intention to prevent peritoneal adhesions, both materials tested in the study led to a pro-adhesive response, resulting in a higher extent of adhesions, which can be problematic in the long term.
- ***Partial Material Displacement:*** Although most of the material remained attached to the tissue, there were instances of partial displacement. This could potentially lead to less effective coverage of the anastomotic defect.
- ***Potential for Anastomotic Strictures:*** The development of anastomotic strictures is a possible outcome, which could be detrimental to the function of the gastrointestinal tract.

Unfortunately, these conclusions point to the unsuitability of this material for the intended application. While we believe that our future research will not follow the same path, these findings can still contribute to scientific discourse. The citation count of the article on material development indicates that other researchers are using the acquired knowledge, and the material may find utility in different applications, potentially in surface healing of skin wounds, where drugs can be introduced into PVA to accelerate the healing process. In future developments, I will revisit the knowledge that PCL nanofibers with decreased surface weight exhibited good performance. The emphasis will be on refining the antiadhesive side of the materials, as detailed in subsequent chapters.

In conclusion, the experiment with double-layered nanofibrous patches (PCL/PVA_L and PCL/PVA_H) on pigs indicated that the materials effectively prevented anastomotic leakage at the site of intestinal surgery. However, these patches did not improve and in some cases delayed anastomotic healing as measured by a novel scoring system, and they increased the likelihood of peritoneal adhesion formation. Future research is necessary to optimize these materials for clinical use, possibly with alterations to their properties to reduce adhesion formation while maintaining their preventive effects against leakage.

12. JOURNAL ARTICLE: H. DEVELOPMENT BIOINSPIRED HIERARCHICAL MATERIALS FOR PREVENTING POSTOPERATIVE TISSUE ADHESIONS

12.1 Introduction of the Article

The paper[38] presents the development of a biocompatible nanofibrous material intended to serve as an anti-adhesive barrier to prevent the formation of postoperative tissue adhesions. The material is inspired by the hierarchical structure of the superhydrophobic lotus leaf (see *Figure 19*), and it is produced using a combination of needleless electrospraying and electrospinning technologies with polycaprolactone. Furthermore, the material's surface hydrophobicity is enhanced via cold plasma modification with hexamethyldisiloxane.

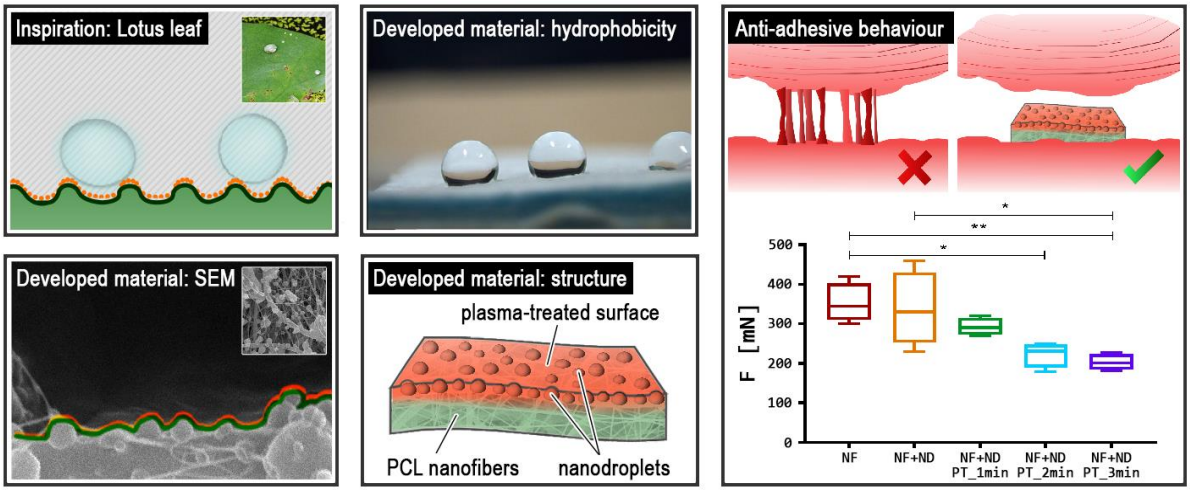


Figure 19: Graphical abstract.[38]

12.1.1 Key Highlights/Findings

1. ***Innovation in Anti-adhesive Barrier Design***: The material mimics the nanostructure of the lotus leaf to achieve an anti-adhesive effect. This bio-inspired design is a novel approach in the development of biomedical barriers and holds promise in reducing unwanted tissue adhesion post-surgery.
2. ***Dual Wettability and Hierarchical Structure***: The fabrication process results in a material that presents dual wettability—hydrophobic on one side, due to the plasma-treated nanodroplets, and more hydrophilic on the other side, due to the untreated PCL nanofibers. This hierarchical design could be crucial in achieving the desired anti-adhesive properties while maintaining biocompatibility.
3. ***Surface Characterization and Stability***: Comprehensive characterization of the material, including SEM analysis of morphology, contact angle measurements for wettability, and FTIR analysis for chemical composition, provides evidence of the successful creation of the HMDSO layer on the material surface. The temporal stability of these surface modifications is an important finding for the potential longevity of the material in clinical applications as presented in *Figure 20*.

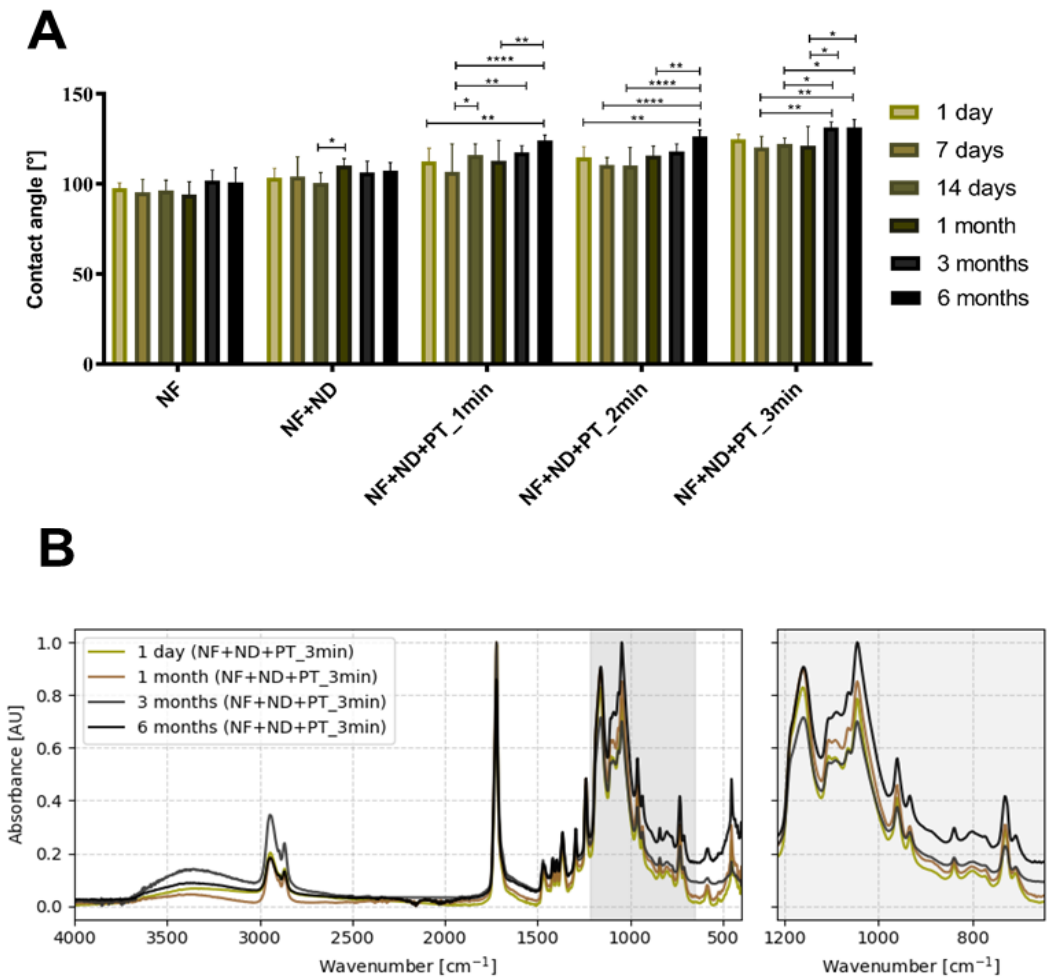


*Figure 20: Measured contact angle of tested materials in period of six months (n=10); * p<0,0332, ** p<0,0021, *** p<0,0002, **** p<0,0001 (ANOVA, Bonferroni) (A); FTIR structure revealing the presence of characteristic siloxane group during 6 months observation (B).*[38]

4. ***Cytocompatibility and Cell Behavior***: *In vitro* cytocompatibility tests confirmed the non-toxic nature of the materials using 3T3 mouse fibroblasts. The study includes tests for cell adhesion and proliferation, suggesting that while the material is designed to prevent tissue adhesion, it does not adversely affect cellular activity, which is important for healing and integration into the body.
5. ***Ex Vivo Anti-adhesive Efficacy***: *Ex vivo* testing with native tissue is used to confirm the material's anti-adhesive properties as shown in *Figure 21*, which are important for predicting *in vivo* performance while minimizing animal testing.

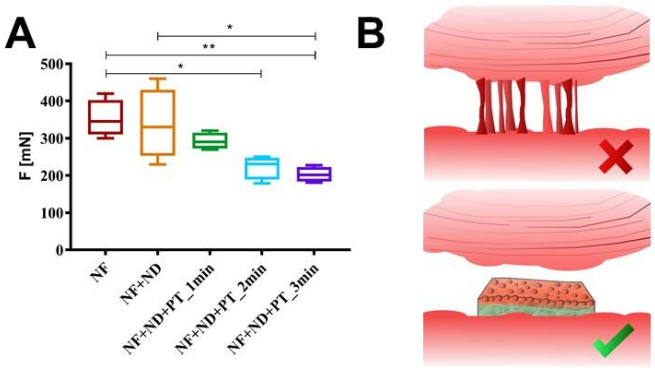


Figure 21: Results of peel test 90° with all fabricated materials, hydrophobic treatment led to decreased adhesion to intestinal tissue (A). Material serves as a potential barrier prevention of the undesired tissue adhesions (B).[38]

6. ***Potential for Scale-up and Industrial Production***: The use of needleless electrospinning for the production process highlights the potential for scaling the fabrication.

12.2 Integration into the Overall Conceptual Framework

This research paper signifies an advancement within the overarching scope of my thesis about nanofibrous materials for preventing peritoneal adhesions. Our exploration of optimal nanofibrous materials began with single-layered PCL nanofibers, showcasing successes in certain aspects but also revealing challenges, particularly in adhesiveness , and their inability to prevent peritoneal adhesions. Pure PCL single layers exhibit notable adhesivity to the intestine and could serve as the adhesive side; however, the other side requires treatment to prevent adhesions with other tissues. Subsequent research highlighted the potential efficacy of developing a material with significantly different wettabilities on each side. However, challenges emerged during *in vivo* animal studies, where dynamic conditions led to delaminations of the layers with different chemical compositions. Motivated by these shortcomings, our subsequent investigations embraced a nature-inspired approach to functionalize PCL while maintaining a consistent chemical composition and preventing unwanted delamination inside the *in vivo* models. Drawing inspiration from the nanostructure of the lotus leaf, we developed a biomimetic material with the potential to prevent unwanted tissue adhesion post-surgery. The scalability, biocompatibility, and functionalization of this biomimetic material align with our vision for advancing surgical practices and improving patient outcomes. In parallel, the ongoing manuscript preparation for the successful *in vivo* pig testing represents the next crucial phase in our research. In our forthcoming manuscript, not yet published, the introduction of the lotus-leaf effect to the material has shown promising results in delimiting adhesion creation. Thus the promise of our biomimetic material as an anti-adhesive solution becomes more evident, offering hope for enhanced surgical practices and improved patient outcomes.

13. JOURNAL ARTICLE: I. NANOFIBROUS DRUG-DELIVERY SYSTEMS RELEASING ANTIBIOTICS TO PREVENT ANASTOMOTIC LEAKAGE

13.1 Introduction of the Article

The paper[39] presents research on the development and characterization of biodegradable nanofibrous scaffolds loaded with gentamicin sulfate (GS), an antibacterial agent, for potential gastrointestinal applications. Various nanofibrous layers with different concentrations of GS were fabricated using electrospinning techniques. These materials were designed to be biocompatible, with the aim of providing antibacterial properties to prevent infections that can impede wound healing in gastrointestinal tissues. The study involves evaluating the nanofibers' morphology, chemical composition, absorption capabilities, and antibacterial efficacy, as well as their impact on cell viability and proliferation as visualised in *Figure 22*. The research findings could have broader implications for medical applications beyond the gastrointestinal focus presented.

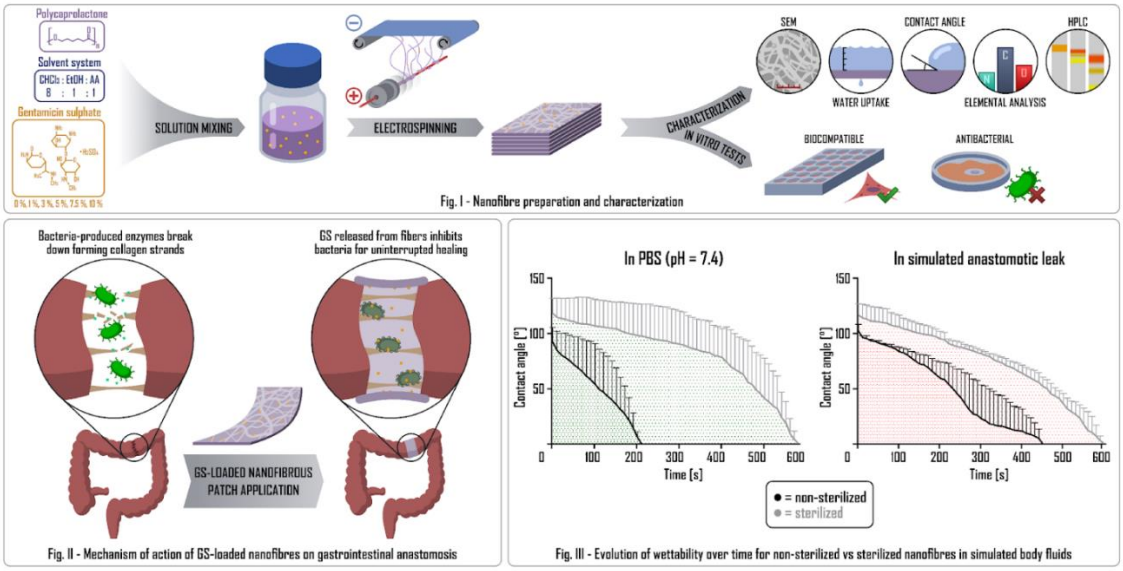


Figure 22: Graphical abstract.[39]

13.1.1 Key Highlights/Findings

1. ***Development of Antibacterial Nanofibers:*** The research successfully developed biodegradable nanofibrous materials loaded with gentamicin sulfate, designed for application in gastrointestinal surgeries to prevent postoperative complications.
2. ***Cytocompatibility:*** The nanofibers were proven to be cytocompatible, meaning they were non-toxic to mouse fibroblast cells, which is crucial for any material intended for medical use. The materials supported cell growth without significant differences among tested samples, and cell adhesion was not negatively impacted by the presence of the antibiotic and shown in *Figure 23*.

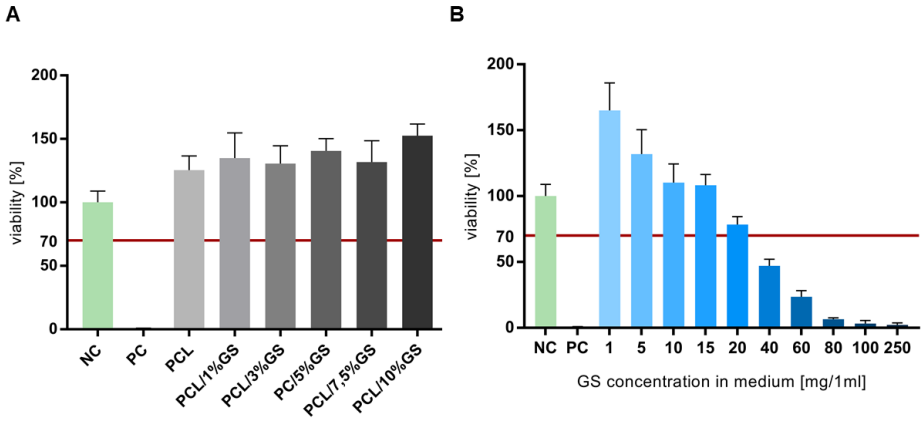


Figure 23: Graph of cytocompatibility of the developed and tested materials; PC and NC stands for positive (cytotoxic), resp. negative (cytocompatible) controls (A). The cytocompatibility of pure GS in the cell media. The cytotoxic concentration was found (20 mg/1 ml) is the limit of cytocompatibility of GS in DMEM cell media (B).[39]

3. ***Antibacterial Effectiveness:*** The materials exhibited antibacterial properties with a distinctive inhibition of growth for both Escherichia coli and Staphylococcus gallinarum, with the antibacterial effect increasing with higher gentamicin sulfate concentrations as it could be seen in *Table 2*.

Table 2: Measured inhibition zones of model bacteria strains.[39]

Material	Inhibition zone (*E. coli*)	Inhibition zone (*S. gallinarum*)
PCL	0 ± 0	0 ± 0
PCL/1%GS	6.4 ± 0.5	12.3 ± 0.5
PCL/3%GS	7.6 ± 0.1	12.5 ± 0.4
PCL/5%GS	7.9 ± 0.7	12.8 ± 0.5
PCL/7.5%GS	8.7 ± 0.8	13.0 ± 0.9
PCL/10%GS	10.0 ± 0.6	15.5 ± 0.5

4. ***Sterilization and Concentration Impact:*** The study considered the effect of sterilization on the materials. It was found that the inhomogeneous distribution of gentamicin sulfate at higher concentrations (e.g., PCL/10%GS) may impact the uniformity and could affect absorption measurements (see *Figure 24*).

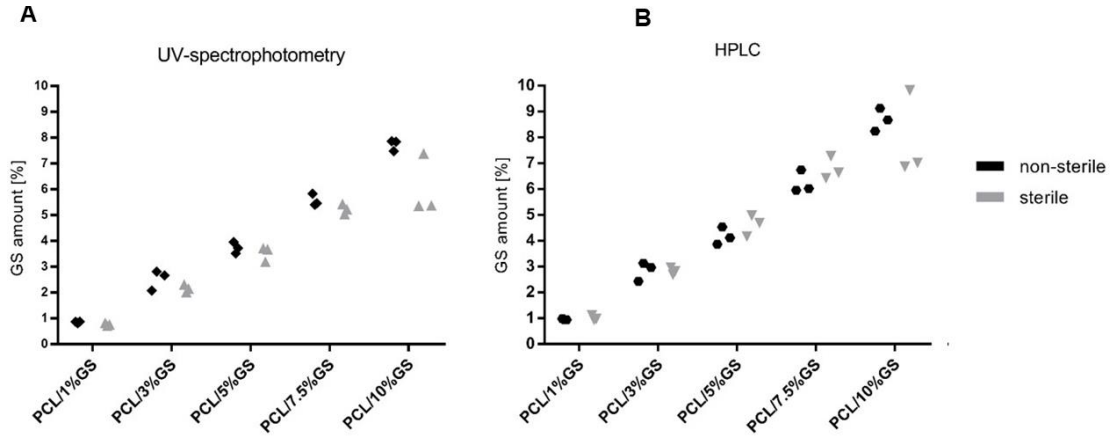


Figure 24: The measured amount of GS in the nanofibrous materials via UV-spectrophotometry (A) and HPLC (B).[39]

5. ***Absorption Dynamics:*** The wettability and absorption behavior of the nanofibrous materials were examined, showing that though initially appearing non-wetting, over time the test liquids fully soaked into the materials, indicating a capacity to deliver hydrophilic drugs like gentamicin sulfate.
6. ***Industrial Production Viability:*** Importantly, the study addressed the scalability of production, a step often overlooked in research, by utilizing a needleless electrospinning technique suitable for large-scale production.

13.2 Integration into the Overall Conceptual Framework

In previous studies, it has been not only established that PCL is a suitable polymer for covering intestinal anastomoses but also demonstrated that one side of the planar nanofibrous material can be successfully modified with lotus leaf-inspired structures, leading to a reduction in adhesion formation. Another milestone was, therefore, to verify the feasibility of incorporating a selected antibiotic (gentamicin sulfate) into the PCL layers. This research is based on the fact that an infection at the site of the anastomosis, caused by bacterial strains producing substances leading to the degradation of newly formed tissue, prompted the exploration of homogeneously incorporating antibiotics into PCL layers. We have confirmed our ability to achieve this and understand their properties both pre- and post-sterilization. These materials will undergo further testing by using *in vivo* models (the study has commenced, and results are pending evaluation and impact assessment). In the future, we plan to incorporate a broader range of antibiotics to prevent specific infections in individual patients.

Conclusion

Within the scope of the doctoral thesis, nanofibrous materials were developed with the potential to prevent severe complications in abdominal surgery, particularly following intestinal resection. As these complications are serious, often life-threatening, and **prevalent for over two centuries**, there have been numerous discussions on the development of materials for preventing anastomotic leaks and peritoneal adhesions and their commercialization. However, **none of the previously developed materials have been successful** in preventing either or both complications to date; many have not withstood large randomized studies or have not been recommended for application in gastrointestinal surgery. From these data, it is evident that the successful development of such materials is a challenging discipline.

The doctoral thesis involved the development of a nanofibrous material with an antiadhesive side to prevent the occurrence of peritoneal adhesions and a material with antibacterial properties for preventing anastomotic leakage. With respect to other research endeavors and to the best of our knowledge based on current scientific literature, this represents the first application of nanofibers on a large animal model for this indication. Main highlights and contributions to the scientific community are:

I. Introducing the comprehensive overview encapsulating the progress and the state-of-the-art in fabricating nanofibrous materials that meet the challenging criteria needed to address postoperative complications effectively.

II. Completion of the current understanding and knowledge gaps surrounding the process of intestinal anastomotic healing and its associated complications.

III. Pioneering application of nanofibrous materials, prepared through homogeneous needleless electrospinning, for the reinforcement of intestinal anastomosis in a large animal model.

IV. Introduction of an innovative approach involving the layering of hydrophilic (PVA) and hydrophobic (PCL) fibrous materials using electrospinning, while maintaining scaffold stability and shape integrity even with the use of a water-soluble polymer.

V. Verification of the impact of surface weight on anastomotic healing.

VI. Introduction of an innovative method for the preparation of biomimetic hierarchical fibrous materials using needleless electrospinning.

VII. Optimization of the preparation of PCL fibrous drug delivery systems, determination of the critical concentration of gentamicin sulfate in fibrous systems.

VIII. Introduction of novel Intestinal Wall Integrity Score (IWIS) in the collaboration with clinical doctors.

Throughout the entire research, strict adherence to consistent experimentation and technologically feasible integration of all approaches was maintained. This approach ensures the future possibility of integrating antibiotics into biomimetic hierarchical fibrous materials. It is anticipated that, at a certain stage of the research, the culmination of insights will lead to the creation of a single functional layer capable of preventing both anastomotic leakage and peritoneal adhesions. Consistency and the potential for future development of a unified nanofibrous layer were emphasized throughout the research. A carefully selected methodology for evaluating properties, such as characterizing nanofibrous layers based on crucial medical parameters, such as measuring the contact angle (a pivotal parameter for cell adhesion), verifying cytocompatibility (important for preventing undesirable toxic reactions in the human body), measuring adhesion directly to native intestinal tissue (*ex vivo* liver tests), and more, was also a focal point.

Due to the separate development of the materials, their potential application in various contexts is more versatile. Undesirable adhesion formation occurs not only in gastrointestinal tract surgeries but also in gynecological, tendon and pelvic surgeries. Antibacterial nanofibrous materials, on the other hand, hold potential applications in scenarios where bacterial infection is a concern (e.g., skin or oral cavity). Antibiotic-loaded nanofibrous systems act locally, leading to lower antibiotic concentrations *in vivo*, reduced side effects and organism burden, and a lower risk of bacterial resistance development against the specific antibiotic.

This thesis has yielded a collection of innovative processes culminating in the development of biodegradable fibrous materials designed to prevent severe postoperative complications, meeting the direct needs expressed by clinicians in clinical practice. The collaborative efforts with the Biomedical Center have not only resulted in the successful preparation of the AZV NU20J-08-00009 project but have also led to the creation of nine impactful publications and other outcomes poised for further dissemination in scientific journals. The concepts presented in this dissertation establish robust foundations for a new trajectory in clinical prevention, specifically targeting anastomotic leaks and postoperative adhesions.

Future Perspectives

In this doctoral thesis, the novel fibrous materials for covering intestinal anastomoses after surgeries were introduced. Throughout the research, collaboration with clinical practice and physicians was established, and the ongoing development of current possibilities was continuously monitored. There is still a void in the market when it comes to readily available commercial solutions for severe postoperative complications. I believe that the presented thesis has offered new directions for development and defined the necessary properties of such solutions. However, the path to real clinical practice is still long. In the future, it is essential to test the final material on a significant sample of large animal models, evaluate preclinical studies, and subsequently validate the use of the developed solution for real clinical practice through clinical tests on human patients. We plan to continue this collaboration and have applied for another AZV project, which will be led by the author of this thesis, Ing. Markéta Klíčová, and MUDr. Jáchym Rosendorf, Ph.D.

Within the development of new materials, new observations in improving anastomotic healing were also carefully monitored. There is a widely held belief within the scientific community that exerting **influence on the intestinal bacteriome or preventing an elevation in the collagenolytic activity during the healing of anastomosis can markedly decrease the likelihood of anastomotic leak in colorectal surgery**. Recently, a newly identified risk factor has surfaced, characterized by a highly specific mechanism. This involves an infection occurring at the anastomotic site, triggered by bacterial strains that produce substances leading to the degradation of newly formed tissue during the healing process. These active substances encompass both bacterial collagenases and human endogenous collagenase activators. This cascade ultimately results in heightened collagen degradation within the newly formed tissue, leading to a decrease in the mechanical strength of the intestinal anastomosis and, consequently, the occurrence of a leak. In response to this knowledge, **the development of nanofibrous materials with incorporated antibiotics has been initiated**, and we plan to verify **the possibility of incorporating inhibitors of certain metalloproteinases, especially MMP9**.

The prospective application of a specialized anti-collagenolytic version of the material for **personalized elective procedures**, guided by the preoperative assessment of the patient's intestinal microbiome from stool samples, holds the promise of preventing anastomotic leakage in a manner directed towards a specific pathogen and **uniquely tailored to each patient**.

Additional Information Regarding the Thesis

Guidelines of the Rector of the Technical University of Liberec No. 4/2023: Utilization of Artificial Intelligence in Teaching and Creative Work as of September 01, 2023:

The directive issued by the Rector of the Technical University of Liberec, No. 4/2023, dated September 01, 2023, accepts and encourages the use of artificial intelligence tools in education, the composition of university qualification papers, and creative work. The Technical University of Liberec outlines fundamental principles and rules for the application of artificial intelligence tools in this directive. In this doctoral thesis, tools such as ChatGPT 3.5 and Jenni AI, specifically designed for scientific writing, were employed. However, all impacted publications, which are commented upon, were prepared without the use of artificial intelligence. Despite the use of these tools in composing the doctoral thesis, all scientific ideas, conclusions, and conceptualizations belong to the author, Ms. Markéta Klíčová.

Additional Outcomes & Achievements of the Author

LIST OF PUBLICATION

- **Klicova, M.**; Rosendorf, J.; Erben, J.; Horakova, J. Antiadhesive Nanofibrous Materials for Medicine: Preventing Undesirable Tissue Adhesions. ACS Omega 2023, 8 (23), 20152–20162. https://doi.org/10.1021/acsomega.3c00341.
- **Klicova, M.**; Klapstova, A.; Chvojka, J.; Koprivova, B.; Jencova, V.; Horakova, J. Novel Double-Layered Planar Scaffold Combining Electrospun PCL Fibers and PVA Hydrogels with High Shape Integrity and Water Stability. Materials Letters 2020, 263, 127281. https://doi.org/10.1016/j.matlet.2019.127281.
- **Klicova, M.**; Oulehlova, Z.; Klapstova, A.; Hejda, M.; Krejcik, M.; Novak, O.; Mullerova, J.; Erben, J.; Rosendorf, J.; Palek, R.; Liska, V.; Fucikova, A.; Chvojka, J.; Zvercova, I.; Horakova, J. Biomimetic Hierarchical Nanofibrous Surfaces Inspired by Superhydrophobic Lotus Leaf Structure for Preventing Tissue Adhesions. Materials & Design 2022, 217, 110661. https://doi.org/10.1016/j.matdes.2022.110661.
- **Klicova, M.**; Mullerova, S.; Rosendorf, J.; Klapstova, A.; Jirkovec, R.; Erben, J.; Petrzilkova, M.; Raabová, H.; Šatínský, D.; Melicherikova, J.; Palek, R.; Liska, V.; Horakova, J. Large-Scale Development of Antibacterial Scaffolds: Gentamicin Sulfate-Loaded Biodegradable Nanofibers for Gastrointestinal Applications. ACS Omega 2023, 8 (43), 40823–40835. https://doi.org/10.1021/acsomega.3c05924.

- Horakova, J.; **Klicova, M.**; Erben, J.; Klapstova, A.; Novotny, V.; Behalek, L.; Chvojka, J. Impact of Various Sterilization and Disinfection Techniques on Electrospun Poly-ε-Caprolactone. ACS Omega 2020, 5 (15), 8885–8892. https://doi.org/10.1021/acsomega.0c00503.
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- Erben, J.; **Klicova, M.**; Klapstova, A.; Háková, M.; Lhotská, I.; Zatrochová, S.; Šatínský, D.; Chvojka, J. New Polyamide 6 Nanofibrous Sorbents Produced via Alternating Current Electrospinning for the On-Line Solid Phase Extraction of Small Molecules in Chromatography Systems. Microchemical Journal 2022, 174, 107084. https://doi.org/10.1016/j.microc.2021.107084.
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ACTIVE PARTICIPATION IN CONFERENCES

International Conferences

- **Klicova, M.**, Puchrík, M., Rosendorf, J., Liska, V., Pálek, R., Horáková, J. Vliv strukturace biodegradabilních polymerních materiálů na růst buněk a potenciální prevenci pooperačních adhezí. XIV. B.m: Národní knihovna České republiky. International conference Bioimplantologie 2023. ISBN 978-80-11-03134-3 (2023).
- **Klicova, M.,** Klapstova, A., Rosendorf, J., Palek, R., Liska, V., Krejcik M., Horakova, J. Lotus leaf inspired nanofibrous materials for prevention of tissue adhesions. 14th International Conference on Nanomaterials - Research & Application, October 19 - 21, 2022 / OREA Congress Hotel Brno, Czech Republic, EU. In: NANOCON 2022 – Abstracts. Brno: TANGER Ltd. ISSN: 2694-930X (2022).
- **Klicova, M.,** Rosendorf, J., Krejcik, M., Horakova, J. Hydrophobic Nanofibrous Materials for Prevention of Postoperative Tissue Adhesions. Proceedings of the 8th World Congress on New Technologies (NewTech'22). Publication Date: 07, 2022. ISBN: 978-1-990800-11-5. ISSN: 2369-8128 (2022).
- **Klicova, M.**, Klapstova, A., Krejcik, M., Mullerova, J., Erben, J., Rosendorf, J., Palek, R., Liska, V., Horakova, J. Antiadhesive Nanofibers for Novel Applications in Gastrointestinal Surgery. In: NANOCON: NANOCON 2021 - Abstracts. Brno: TANGER Ltd. ISBN 978-80-88365-00-6 (2021).
- Müllerová, S., Klapstova, A., Petržílková, M., Satinsky, D., Raabová, H., Rosendorf, J., Palek, R., Liska, V., **Klicova, M.** Biodegradable Nanofibrous Materials for Fortification of Gastrointestinal Anastomoses. In: NANOCON: NANOCON 2021 - Abstracts. Brno: TANGER Ltd. ISBN 978-80-88365-00-6 (2021).
- Oulehlová, Z., **Klicova, M.**, Klápšťová, A., Krejčík, M., Liška, V., Rosendorf, J., Pálek, R., Červenková, L. Planar Polymeric Nanofibrous Patches For Sealing The Gastrointestinal Anastomoses. 47TH Textile Research Symposium, Czech Republic. ISBN 978-80-7494-473-4 (2019).
- **Klicova, M.**, Horakova, J., Klapstova, A., Volesky, L., Liska, V., Rosendorf, J. Hydrophobic ultrafine hyaluronic acid nanofibers. Proceedings of the 5th World Congress on New Technologies (NewTech'19) Lisbon, Portugal – August, 2019 Paper No. ICNFA 151 DOI: 10.11159/icnfa19.151 (2019).

National Conferences

- **Klicova, M.**, Puchrík, M., Rosendorf, J., Liska, V., Horakova, J. Biodegradabilní kompozitní materiály pro prevenci peritoneálních adhezí. Laboratorní a klinické aspekty regenerativní medicíny. B.m.: České vysoké učení technické v Praze. ISBN 978-80-01-07052-9 (2022).
- **Klíčová, M.**, Müllerová, S., Rosendorf, J., Pálek, R., Liška, V., Horáková, J. Antibakteriální nanovlákenné vrstvy pro prevenci infekcí z důvodu kolorektálního leaku. Biomateriály a jejich povrchy XV. B.m.: České vysoké učení technické v Praze. ISBN 978-80-01-07023-9 (2022).
- **Klicova, M.,** HORÁKOVÁ, J. Nanomateriály v tkáňovém inženýrství a regenerativní medicíně. In: *36. Lékárnické Dny*. Liberec: SOLEN, s.r. o. ISBN 78-80-7471-370-5 (2021).
- **Klicova, M.**, Hujer, J., Erben, J., Liška, V., Rosendorf, J., Pálek, R., Calamari, E., Feng, D., Novak, R., Ingber, D., Horáková, J. Biodegradabilní polyesterové materiály pro nové

biomedicínské aplikace. In: *Biomateriály a jejich povrchy XIV*. B.m.: České vysoké učení technické v Praze. ISBN 978-80-01-06872-4 (2021).

- **Klicova, M.**, Erben, J., Klápšťová, A., Liška, V., Rosendorf, J., Pálek, R., Ingber, D., Novak, R., Calamari, E., Feng, D., Horáková, J. Polyesterová nanovlákna pro nové aplikace v regenerativní medicíně. České vysoké učení technické, Praha. ISBN 978-80-01-06754-3 (2020).
- **Klicova, M.**, Horáková, J., Klápšťová, A., Krejčík, M., Rosendorf, J., Pálek, R., Liška, V. Nanovlákenné materiály pro prevenci pooperačních adhezí. České vysoké učení technické, Praha. ISBN 978-80-01-06625-6 (2019).
- **Klicova, M.**, Horáková, J., Voleský, L., Liška, V., Rosendorf, J., Klápšťová, A., Jirkovec, R. Plazmatická modifikace hydrofilních nanovlákenných povrchů. České vysoké učení technické, Praha. ISBN 978-80-01-06471-9 (2018).

INVITED LECTURES

International Presentations

- Forbes Summit 30pod30, Bratislava, Slovakia (2021).

National Presentations

- Presentation of current research and nanotechnologies at the charitable event „Žárovky" (2021).
- Presentation of research for the Israeli Ambassador during his visit to the Liberec region (2019).
- Lecture at the annual conference of the Regional Innovation Strategy of the Liberec Region (RIS3) on the topic: Nanofibrous covers for reinforcing intestinal anastomoses (2018).

SIGNIFICANT AWARDS IN THE FIELD OF SCIENCE AND RESEARCH

International Awards

- Young Innovator of The Year Audience Choice Award. Falling Walls Lab, Berlin (2018).

National Awards

- Minister of Education, Youth and Sports Award for outstanding students and graduates in the study program and for exceptional student achievements (2023).
- Sanofi Award for the best pharmaceutical research in the Czech Republic (2023).
- Werner von Siemens Award for leading the best diploma thesis (2022).
- 1st Place in the Student's competition at the conference Biomateriály a jejich povrchy, oral presentation of the interim results of the doctoral thesis (2020).
- 2nd Place in the Student's competition at the conference Biomateriály a jejich povrchy, oral presentation of the interim results of the doctoral thesis (2019).

- EIT Health National Winner - competition in the field of business and Innovation (2019).
- TOP 10 Diploma Theses in the Czech Republic. Werner von Siemens Award (2019).
- Young Scientist Award. ERN (Euroregion Neisse-Nisa-Nysa) Award (2018).
- Governor's Award for exceptional diploma thesis results in Liberec Region (2018).
- National Winner of Falling Walls Labs - international competition of innovators and young talents (2018).
- 1st Place in the Student's competition at the conference Biomateriály a jejich povrchy, oral presentation of the interim results of the doctoral thesis (2018).

PEDAGOGICAL ACTIVITIES

Supervision of Theses: In the academic year 2020/2021, I supervised the master thesis of Ing. Senta Müllerová *(Incorporation of antibiotics into biodegradable nanofibrous layers for new medical applications)*. The thesis won the 1st place in the Werner von Siemens 2021 Price for the best diploma thesis in Czechia, received the 2nd place in the poster section during the international conference NANOCON 2021, was selected among the TOP 8 theses by Contipro, and received recognition from the governor of the Liberec region for outstanding work. In the academic year 2018/2019, I supervised the master thesis of Ing. Zuzana Oulehlová *(Development of planar nanofibrous layers for new applications in gastrointestinal surgery)*. The thesis won the 1st place in the national Contipro competition for biomaterial research. I also supervised the theses of Ing. Pavel Děkan and Ing. Kristýna Manhartová, focusing on the development of fibrous materials for medical applications.

Lectures at the Faculty of Textile Engineering, Technical University of Liberec: In the academic years (2019/2020, 2020/2021, 2021/2022, and 2022/2023), I lectured on the subject Tissue Engineering for Czech (full-time and distance learning) and international students. For teaching purposes, I developed new interactive lecture materials suitable for both in-person and online instruction.

MEDIA PRESENTATIONS OF THE RESEARCH

Since 2018, I have regularly provided media appearances for Czech media (ČT 1, ČT 24, Forbes, MF Dnes, Hospodářské noviny, and others) where I describe the current state of development, research possibilities, and studies at TUL. These appearances amount to hundreds of recordings.

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CURRICULUM VITAE

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EDUCATION	
9/2018 – now	DOCTORAL STUDY IN TEXTILE TECHNOLOGY AND MATERIALS ENGINEERING
	Faculty of Textiles, Department of Nonwovens and Nanofibrous Materials at the Technical University of Liberec.
1/2020 – 3/2020	INTERNSHIP AT HARVARD MEDICAL SCHOOL
	Wyss Institute for Biologically Inspired Engineering at Harvard University under the supervision of Prof. Donald E. Ingber (founding director at Wyss Institute). Focus: Organs-on-chip, namely production and incorporation of nanofibrous materials into microfluidic chips for dynamic cell cultivation.
9/2016 – 6/2018	MASTER STUDIES with HONOURS
	Faculty of Mechatronics, Informatics and Interdisciplinary Studies at the Technical University of Liberec.

WORK EXPERIENCE	
3/2023 – now	HEAD OF BIOLOGICAL APPLICATIONS; IQS nano s.r.o.
3/2021 – 2/2023	REPRESENTATIVE HEAD OF RESEARCH AND DEVELOPMENT; Nanopharma, a.s.
3/2018	STUDY INTERNSHIP, Contipro a.s.
6/2015 – 12/2015	ASSISTANT SCIENTIFIC WORKER, Institute for Nanomaterials, Advanced Technologies and Innovations (CxI), Technical University of Liberec

LANGUAGE SKILLS	
English	C1 level (advanced) according to CEFR
German	B2 level (intermediate)

RECOMMENDATION OF THE SUPERVISOR

FAKULTA TEXTILNÍ TUL

Disertační práce: **Development of Double-layered Nanofibrous Materials for Fortification of Intestinal Anastomoses**

Autorka: **Ing. Markéta Klíčová**

HODNOCENÍ ŠKOLITELE

Předložená disertační práce je koncipována jako soubor komentovaných článků, což hodnotím velmi pozitivně vzhledem k vysoké publikační aktivitě autorky. Je autorkou celkem 12 odborných článků dle Web of Science, které již byly citovány celkem 126x k dnešnímu dni. Pro disertační práci byly vybrány publikace týkající se tématu disertační práce, které čítají 9 publikací. Ve 4 pracích je Markéta Klíčová prvoautorkou, v ostatních článcích je uvedena na druhém místě (v jednom případě na třetím místě) pořadí autorů, což svědčí o aktivním zapojení na všech výstupech, které jsou v práci komentovány. Pořadí článků je logicky řazeno, představuje cestu, kterou se ubíral její výzkum. Všechny výstupy jsou velmi jasně a přehledně zpracovány v kapitole Goals of the Thesis & PhD Study Outcomes. Na začátku práce jsou uvedeny i 2 review články, které shrnují řešenou problematiku. Na tyto teoretické články navazuje 7 originálních prací, ve kterých se autorka zabývá vývojem nanovlákenných materiálů se specifickými vlastnostmi. Všechny zmíněné články prošly recenzním řízením a byly publikovány v impaktovaných časopisech (ACS Omega, Nature Scientific Reports, Materials Letters, Materials & Design). Komentáře ke všem článkům velmi jasně popisují hlavní přínosy pro řešenou problematiku, což činí práci velmi přehlednou a čtivou.

Markéta Klíčová prokázala schopnosti samostatné i týmové práce. Během celé doby řešení spolupracovala s týmem Biomedicínského centra pod vedením prof. Lišky. Společné úsilí o vývoj nanovlákenného materiálu pro aplikace v břišní chirurgii vyústily ve společný projekt obou pracovišť: Prevence střevního anastomotického leaku a pooperačních adhezí pomocí nanovlákenných biodegradabilních materiálů, který byl řešený v letech 2020-2023.

Během doktorského studia Markéta odvedla úspěšně 5 diplomových prací, z nichž 2 byly oceněny (firma Contipro, cena Siemens).

Kromě práce na samotném tématu disertační práce bych ráda vyzdvihla další oblasti, ve kterých Markéta Klíčová excelovala. Jedná se především o prezentaci Technické univerzity ve veřejném sektoru formou mediálních výstupů. Její působení je velmi inspirativní pro další mladé vědce a vědkyně. Dále oceňuji Markéty podíl na výuce předmětu Materiály pro tkáňové inženýrství. Markéta zpracovala podklady pro studium formou interaktivních prezentací, tento předmět si díky tomu zasluhuje sále větší pozornost studentů nejen Fakulty Textilní, ale i Fakulty Mechatroniky.

Studentka byla aktivní i v navazování mezinárodních spoluprací – během studia vycestovala do USA na Wyss Institute (Boston, Harvard Medical School), účastnila se několika zahraničních konferencí s orálními prezentacemi. Na poli české i zahraniční vědy byla vyznamenána řadou ocenění jako např. Falling Walls Lab, cena ministra školství, mládeže a tělovýchovy pro vynikající studenty a absolventy, cena Sanofi.

Vzhledem k výše vyjmenovaným superlativům vztahujících se k výstupům studentky není pochyb o mém jednoznačném doporučení k obhajobě.

Kontrola plagiátorství proběhla dne 14.3.2024, byla zde nalezena relevantní podobnost s 9 dokumenty s mírou shody 1-25%. Vzhledem ke koncepci práce – komentované publikované články se jedná o využití shodného mnohoautorského článku v závěrečných pracích členů týmu Biomedicínského centra / školitelky, případně shoda se samotnými články. Plagiátorství tedy nebylo pozorováno.

Předložená práce splňuje všechny požadavky pro udělení titulu Ph.D.

Navrhuji, aby práce Ing. Markéty Klíčové byla přijata k obhajobě.

doc. RNDr. Jana Horáková, Ph.D.

V Liberci dne 14.3.2024

OPPONENTS' REVIEWS

FAKULTA ZDRAVOTNICKÝCH STUDIÍ TUL

Oponentský posudek na dizertační práci Ing. Markéty Klíčové nazvané „ Development of Double-layered Nanofibrous Materials for the Fortification of Intestinal Anastomoses

Předložená dizertační práce si klade za cíl připravit nanovlákenný materiál, který by na jedné straně bránil peritoneálním srůstům a na druhé straně prosakování anastomozy, což patří mezi časté a dosud špatně ošetřené problémy v kolorektální chirurgii. Z tohoto hlediska je předložená práce velmi aktuální a její výsledky posunuly některé hranice znalostí těchto problémů a jejich možného řešení. Navíc se zabývá nejen přípravou nových materiálů v laboratorních podmínkách (jsou jich denně po světě připravovány tisíce), ale rovnou se zamýšlí nad jejich převedením do velkovýroby, což posouvá přínos této práce ještě dál.

V předložené práci je velmi podrobně a chronologicky popsána cesta, kterou se ing. Klíčová vydala k dosažení svého cíle. Nejprve si udělala podrobnou rešerši problémů na poli střevních anastomoz v podobě dvou publikovaných review. Získané poznatky potom přetavila do série pokusů, jejichž výledky postupně publikovala v přiložených dalších sedmi publikacích. Tyto výsledky začínají prvotními jednoduššími studiemi, které postupně přidávají na své komplexnosti. Na všech publikacích pracovala v týmu s praktikujícími lékaři, takže její výzkum hned od začátku řešil zásadní a praktické problémy operujících chirurgů. V jednom článku se i podrobně zabývá různými typy sterilizace, což je velmi významný ale současně velmi opomíjený krok při aplikaci v laboratořích vyrobených materiálů.

Ing. Klíčová svojí sepsanou dizertační prací, kde komentuje výsledky svých spoluautorských publikací, jasně ukazuje, že má o problematice anastomoz a možnostech jejich zkvalitnění přehled a je nejen schopna navrhnout a připravit nanovlákenné materiály, ale je i schopna na nich udělat základní pokusy na buňkách v laboratoři i se účastnit pokusů na zvířatech, kde se také nové materiály použily.

Co se týká formy předložené dizertační práce, tak práce je velmi čtivá a přehledná. Jednotlivé publikace jsou jasně komentovány a uspořádány. Práce obsahuje i velký počet vlastních kvalitních obrázků, které zjednodušují pochopení textu, což jako pedagog velmi oceňuji. Čtení práce jsem si velmi užila, protože je psaná formou detektivního příběhu a až do konce jsem byla napnutá, jak to dopadne.

V práci komentované publikace jsou na vysoké úrovni, o čemž svědčí názvy časopisů, ve kterých byly přijaty i počty citací, které již získaly. Vždy se jedná o práce s větším počtem autorů, protože jde o mezioborové téma, ale ing. Klíčová hraje vždy velmi důležitou roli, ať již v pozici prvního autora (4x), tak korespodujícího (4x) nebo se významně podílí na hlavní myšlence a jejím finálním zpracování.

V závěrečné části práce – Conclusion – ing. Klíčová shrnuje separátně poznatky a přínosy jednotlivých publikací, což dělá velmi pěknou a přehlednou formou. Já bych ale její potenciál ještě rozvinula a očekávala bych ještě takové globální zamyšlení nad všemi publikacemi dohromady, protože se to samo nabízí. To je tak jediná lehce negativní připomínka k celé práci, která je jinak výborná.

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Předložená disertační práce obsahuje spousty dat i poznatků, které jsou součástí 9 publikací, které jsou v práci patřičně komentovány a utvářejí ucelený soubor výsledků na dané téma. Studentka jednoznačně prokazuje předpoklady k samostatné vědecké práci, a proto tuto práci doporučuji k přijetí k obhajobě.

Z komentářů a otázek, které jsem si při čtení poznamenala, vybírám:

1) V druhém přiloženém review B, které se zabývá tématem, jak zabránit postoperativním komplikacím, a které je podle mě striktně lékařsky zaměřené, by mě zajímal podíl studentky. Kterou částí textu se konkrétně zabývala nebo jaká přesně byla její úloha v tomto review?
2) V přiloženém článku C, který se týká sterilizačních metod, se zmiňuje o sterilizaci pomocí peroctové kyseliny (PAA). Podle různých analýz tato sterilizace nemá negativní vliv ani na nanomateriál ani na buňky. Mně osobně dokonce připadají výsledky na buňkách lepší než pro etanol. Jsou ještě nějaké další, v publikaci neuvedené důvody, proč ji autoři ale nedoporučují používat. Jestli jsem dobře vyčetla z článku, tak sterilizace ethylen oxidem se prováděla v Praze a gamma zářič se používal v Brně, tak proč nepoužít PAA, kterou může mít každý ve své laboratoři?
3) Zajímal by mě vlastní názor studentky na otázku související s článkem D – proč si myslí, že tyto nově vytvořené materiály při hojení nepomohly?
4) V rámci článku E se připravily nové „lehčí" materiály a měla jsem pocit, že je to z důvodu snazší (rychlejší) odbouratelnosti. To se ale zřejmě nestalo. Ale nikde jsem si nepřečetla komentář, proč to tak je, proč se to ani v tomto případě neodbouralo. Může to studentka okomentovat?
5) V článku F se popisují nové dvouvrstevné materiály, které se testují na stabilitu ve vodném roztoku po dobu 24 h, ale pak při pokusech s buňkami se nejkratší časový interval použije 4 denní. Jak vypadá stabilita vrstev při těchto delších inkubacích – 4, 8,15 a 21 dní? Tady u toho článku mě překvapuje, že buňky kultivované 4 a i 8 dní na površích vůbec nevykazují žádnou proliferaci, i když v předchozím článku buňky na PCL rostly hned od začátku. Je pro to nějaké vysvětlení?
6) V rámci článku H se připravují hydrofobní materiály, které mají zamezit srůstům (jinými slovy adhezi buněk), ale i přesto, že mají kontaktní úhel větší než 105 °C, tak na ně buňky adherují a rostou, což není běžné. Čím si to studentka vysvětluje?
7) A závěrečná otázka týkající se budoucnosti výzkumu studentky. V práci píše, že se bude zabývat vývojem nanovlákenných materiálů s inkorporovanými antibiotiky a různými inhibitory enzymů. Nemá studentka obavu z rezistence a nemá nápad, jak zabránit růstu bakterií ještě jiným způsobem?

V Liberci dne 2. 5. 2024

prof. RNDr. Marie Hubálek Kalbáčová, Ph.D.

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This is evaluation report of PhD thesis of Mrs. Marketa Klicova titled "Development of Double-layered Nanofibrous Materials for the Fortification of Intestinal Anastomoses"

The quality of the research presented here is of the highest academic standards from all points of view:

- The scientific question is clearly stated and addressed by well planned experiments.
- Scientific problem is of very high importance.
- Data analysis and interpretation is valid and does not allow any ambiguities.
- The thesis is of very high scholarly presentation.
- The results presented in this PhD thesis already resulted in **twleve** (12) **publications** in journals, 4 of them as a first author. This is excellent achievemen which is of the highest standards and fully comparable to any top University in Europe.

In detail:

The thesis presents an innovative approach to developing nanofibrous materials aimed at fortifying intestinal anastomoses. The application of these materials in gastrointestinal surgery to reduce severe postoperative complications such as anastomotic leakage and peritoneal adhesions is both original and highly relevant. The integration of biodegradable polymers and the exploration of novel nanofibrous drug delivery systems mark significant advancements in the field.

The theoretical sections are well-researched and comprehensive, effectively setting the stage for the experimental work. The literature reviews on the complexities of postoperative complications and the current state of nanofibrous materials are thorough and provide a solid foundation for the research hypotheses and experimental design.

The choice of large-scale electrospinning as fabrication methods is appropriate and well-justified. The experimental design and methodology are robust, covering extensive evaluations of material properties, biocompatibility, and functionality.

The data presentation is clear, and the analysis is generally sound. The graphical abstracts and figures are informative and effectively illustrate the research outcomes.

The development of double-layered nanofibrous materials demonstrates high innovation, especially with the incorporation of a hydrophilic inner layer and an antiadhesive outer layer. The practical applications of this research are evident, with potential significant impacts on

surgical practices and patient outcomes. Further exploration into the commercial viability of these materials would be an excellent addition.

The thesis is well-structured, scientifically rigorous, and makes a notable contribution to the field of biomedicine and tissue engineering. The research addresses a critical need in gastrointestinal surgery, offering innovative solutions that could transform current practices.

To sum up, this is an excellent piece of work, very carefully done. The results were already published in major journals. I can fully recommend candidate to receive PhD title which she fully deserves.

RNDr. Martin Pumera, Ph.D.

Distinguished Professor of Chemistry

Group Leader

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2017, 2018, 2019, 2020, 2021 Highly Cited Researcher | ERC-StG 2009 Awardee | #203 the Most Cited Scientist in the World in 2019

Editor-in-Chief: Applied Materials Today (Elsevier; Impact Factor 8.0)

Editorial Board Member: ACS Nano, Small, Mater. Today, Chem Eur J; PCCP; Electrochem Commun; Electroanalysis;

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Francesca Gazzaniga, PhD
Assistant Professor of Pathology
Harvard Medical School

April 26th 2024

To Whom It May Concern:

Below is my report for Marketa Klicova's thesis.

a) **Evaluation of the Ph.D. Thesis for the given field**- The purpose of this thesis is to develop new materials that significantly reduce complications from colorectal surgery. Prevention of anastomotic leakage and peritoneal adhesions and scalability of the materials were the focus of this thesis. Given that there are currently no commercially available materials to prevent anastomotic leakage, this thesis addresses a major shortcoming of the field. By extensive literature search, the author noted that many materials that have been tested in preclinical settings are not easily scalable to levels needed to be effective in the clinic. Therefore, she chose to focus her studies on easily scalable materials, an important decision for translating findings to the clinic. This thesis is addressing a major shortcoming in the field, has answered important questions with medical relevance and identified new potential strategies to reduce complications from colorectal surgery.

b) **Comments on the problem-solving procedure, the methods used and the achievement of the stated objective.** The author took a logical and linear approach to solving the shortcomings of current preclinical materials that prevent anastomotic leakage. Firstly, she focused on commercially large-scale techniques with biocompatible materials to develop her patches. Secondly, tested various sterilization techniques to ensure her materials could be sterilized without being damaged. Thirdly, she asses the optimal surface weight for her patches. Fourthly, she focused on reducing adhesion by developing double layer patches. Finally, she incorporated antibiotics into her patches to further reduce the risk of infection upon surgery. During each of these prototypes, the author performed controlled studies in vitro to assess toxicity and in vivo to assess function. She identified a scalable technique to make nanofiber patches that do not cause toxicity in vitro, are safe in a large animal model, and can deliver antibiotics. This work has set the essential information for larger scale studies to investigate the efficacy of these patches in preventing anastomotic leakage and peritoneal adhesions.

c) **An opinion on the results of the Ph.D. Thesis and the importance of the author's specific contribution.** The importance of this Ph.D. Thesis is that the author has identified scalable nanofiber patches that are non-toxic. Importantly, the author assessed the effect of different sterilization techniques, an essential step in translating these findings to the clinic, optimized the surface weight, designed a novel double layer patch to reduce the potential for peritoneal adhesions, and assessed the patch's ability to deliver antibiotics. The results from this thesis are essential for translating this type of material to the clinic and set the stage for large-scale studies to assess the efficacy of these patches in large animal models and eventually human studies.

d) **Other statements concerning mainly the evaluation of the method, clarity of structure, layout and the language level of the Ph.D. Thesis.** The thesis is clearly and logically written. However, I think citations should be added to the Introduction, Conclusion, and Future perspectives sections.

e) **Comments on the student's publications,** The student has been highly productive during her thesis. She has written a comprehensive first author review of antiadhesive nanofibrous materials. Has three first author papers in which she developed a double layer nanofibrous patch, optimized the patch to reduce peritoneal adhesions, and optimized the patch for antibiotic delivery. These papers show her comprehensive understanding of the shortcomings of the field and highly controlled and innovated approaches for addressing these shortcomings. She has multiple middle author papers, a testament to her collaborative nature and dedication to the research which optimized electrospinning techniques, sterilization procedures, and assessed toxicity and function in animal models.

f) **Unambiguous statement whether he recommends the PhD. thesis for defense.** Yes I recommend the Ph.D thesis for defense.

Sincerely,

Francesca S. Gazzaniga
Principal Investigator, Department of Pathology, MGH
Assistant Professor of Pathology, Harvard Medical School