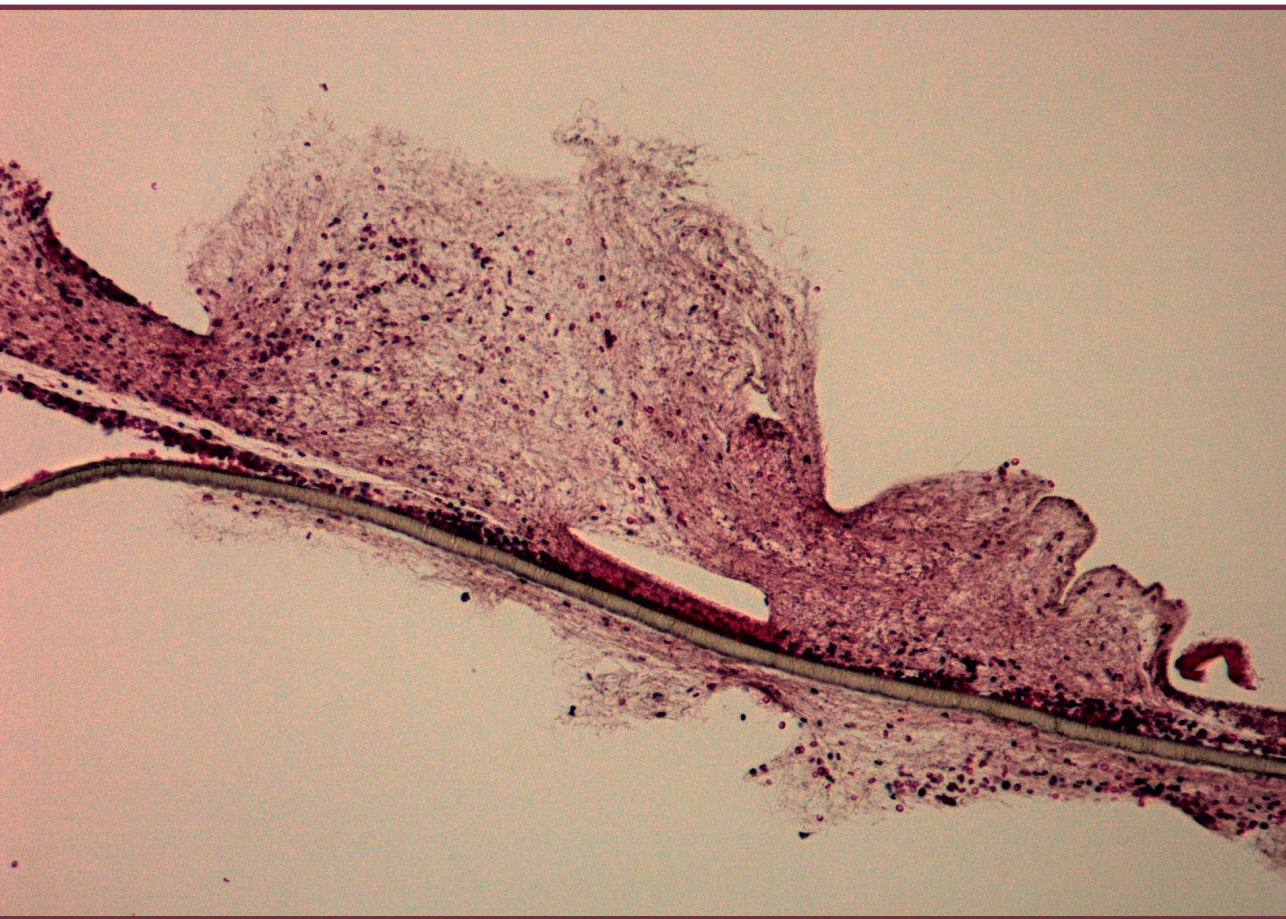
RIGA TECHNICAL UNIVERSITY

Karina Egle

# DEVELOPMENT OF AUTOLOGOUS FIBRIN MATRICES FOR MEDICAL APPLICATION

Summary of the Doctoral Thesis



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**RIGA TECHNICAL UNIVERSITY**
Faculty of Natural Sciences and Technology
Institute of Biomaterials and Bioengineering

**Karina Egle**
Doctoral Student of the Study Programme "Chemistry, Materials Science and Engineering"

# DEVELOPMENT OF AUTOLOGOUS FIBRIN MATRICES FOR MEDICAL APPLICATION

**Summary of the Doctoral Thesis**

Scientific supervisor
Assistant Professor Dr. sc. ing.
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"Success is the sum of small efforts - repeated day in and day out."
/*Robert Collier*/

# DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for defense at the open meeting of RTU Promotion Council on 26 August 2024, at 10.00 at the Faculty of Natural Sciences and Technology of Riga Technical University, 3 Paula Valdena Street, Room 272.

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University of Aveiro, Portugal

## DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for review to Riga Technical University for promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis has not been submitted to any other university for promotion to a scientific degree.

Karina Egle ……. (signature)
Date: ……

The Doctoral Thesis has been prepared as a collection of thematically related scientific publications completed by summaries in Latvian and English. The Doctoral Thesis unites four scientific publications. The scientific publications have been written in English, with a total volume of 76 pages, including supplementary data.

# CONTENTS



**Appendix I:** Egle, K., Salma, I., Dubnika, A. From Blood to Regenerative Tissue: How Autologous Platelet-Rich Fibrin Can Be Combined with Other Materials to Ensure Controlled Drug and Growth Factor Release. *Int. J. Mol. Sci.*, **2021**, *22*(21), 11553.

**Appendix II:** Egle, K., Skadins, I., Grava, A., Micko, L., Dubniks, V., Salma, I., Dubnika, A. Injectable Platelet-Rich Fibrin as a Drug Carrier Increases the Antibacterial Susceptibility of Antibiotic – Clindamycin Phosphate. *Int. J. Mol. Sci.*, **2022**, *23*(13), 7407.

**Appendix III** Dubnika, A., Egle, K., Skrinda-Melne, M., Skadins, I., Rajadas, J., Salma. I. Development of Vancomycin Delivery Systems Based on Autologous 3D Platelet-Rich Fibrin Matrices for Bone Tissue Engineering. *Biomedicines*, **2021**, *9*(7), 814.

**Appendix IV:** Egle, K., Dohle, E., Hoffmann, V., Salma, I., Al-Maawi, S., Ghanaati, S., Dubnika, A. Fucoidan/Chitosan Hydrogels as Carrier for Sustained Delivery of Platelet-Rich Fibrin Containing Bioactive Molecules. *Int. J. Biol. Macromol.,* **2024**, *262* (1), 129651.

# GENERAL OVERVIEW OF THE THESIS

## Introduction

It is still debated whether platelets can be considered as cell fragments or small non-nucleated blood cells [1], but they are known to be responsible for the activation and release of biomolecules. Platelet-rich fibrin (PRF) is an autologous material that is easily produced. It can be derived from human blood by centrifugation and is used to promote wound healing and tissue regeneration. PRF can be used in various fields of medicine, including dentistry and maxillofacial surgery [2].

The leukocytes in the PRF promote wound healing, and PRF contains growth factors that are released over time. There is a great interest in PRF antimicrobial activity, such as oral and maxillofacial surgery, plastic surgery, cardiac surgery, and dentistry [3]. The structural complexity, inhomogeneous nature, and clotting ability of PRF make its antimicrobial effect evaluation complicated. Nevertheless, most antimicrobial testing methods used are based on antibacterial agent diffusion ability in culture media. Since the most common use of PRF is in the oral and maxillofacial region, its antimicrobial activity evaluation also prevails in the oral microbiome. PRF prepared according to a specific protocol (depending on the surgical site) can ensure antibacterial and anti-inflammatory properties, and it can be a beneficial clinical tool in oral and maxillofacial surgery [4]. In the last decade, there have been few studies that combine antibiotics with PRF to provide an antibacterial effect. Based on the gathered literature regarding the antibacterial properties of PRF, it appears advantageous to integrate it with drugs to create a unified system rather than employing separate drugs and PRF individually [5].

Currently, there is a growing need for clindamycin in oral and maxillofacial surgery, where it is commonly used as a substitute for patients allergic to penicillin. Doctors claim that in addition to the bactericidal power of clindamycin, it has increased oral absorption, significant distribution in tissues (achieving a high concentration of the drug in bones), and a low level of resistance [6]. Prodrugs, inactive precursors that transform into active substances in the body, are frequently favored over the direct use of active substances in pharmaceuticals. This preference arises from their ability to enhance drug stability, solubility, and bioavailability, improving therapeutic outcomes. Clindamycin phosphate (CLP) is a prodrug of clindamycin with no antibacterial activity. CLP is reported to be rapidly hydrolyzed to the active base (clindamycin) in the blood [2]. In this PhD Thesis, the ability to convert CLP into an active form by combining it with PRF is investigated. Additionally, to explore broader possibilities of using active substances in PRF, Vancomycin hydrochloride (VANKA) was also studied. VANKA is a water-soluble tricyclic glycopeptide antibiotic [7] that prevents/destroys several gram-positive microorganisms, which are the most common pathogens. VANKA, like clindamycin, is applied in cases when penicillin is ineffective or causes allergic reactions, as well as for treating infections where other antibiotics are resistant [8], [9].

The drug's essential pharmacological characteristics can be enhanced by employing a suitable drug delivery system that allows their administration as a free molecule [10]. A drug delivery system is a technique or technological approach used to systematically and precisely

deliver therapeutic substances (such as drugs or medications) to the human body. This process entails creating and implementing systems that improve the effectiveness and safety of drug treatments. The primary objective of these systems is to finely tune the delivery of drugs to particular tissues or cells, with a concurrent effort to minimize any potential side effects [11], [12]. For example, VANKA can be encapsulated into liposomes, unique medication carriers, because they overcome the disadvantages of the peroral or intravenously administered drugs. As an alternative, polymeric microparticles from poly lactic-co-glycolic acid (PLGA) have been studied to encapsulate VANKA. Both liposomes and PLGA microcapsules have drawbacks regarding encapsulation efficiency, but encapsulation of VANKA in carrier provides high clinical benefits for the long-term use of antibiotics. Therefore, adjusting VANKA carrier composition and the drug release rate in autologous samples is essential. Current studies on drug carriers within the autologous PRF samples are limited [13].

In addition to the PRF combination with drug delivery systems that provide controlled drug delivery, PRF incorporates growth factors and cytokines that can be released directly at the administration site. Several authors described the effect of different centrifugation protocols on growth factor and cytokine release and confirmed the substantial impact of the protocols. It has been demonstrated that variations in growth factors and cytokine release patterns exist not only among different platelet concentrates but also within each type of PRF [14]. Platelet concentrate mainly contains platelets, but PRF is additionally mixed with fibrin-forming proteins that promote tissue healing. Related research in existing literature explores the combination of PRP or PRF with chitosan/fucoidan complex or gelatin/chitosan hydrogel [15], [16]. In these studies, both types of platelet concentrates are blended within a chitosan solution, and the quantity of proteins released is assessed for the resulting materials. Implementing the methodologies described in these publications would complicate medical procedures, involving multiple stages. Prior studies have explored the impact of PRF both independently and when integrated into polymer matrices, focusing on bone formation and regeneration [17]. Xu et al. incorporated granule-lyophilized platelet-rich fibrin (G-L-PRF) into polyvinyl alcohol (PVA) hydrogels, achieving sustained release of growth factors from G-L-PRF/PVA scaffolds for up to 9 days [18]. One *ex vivo* study analyzed the ability of the i-PRF matrix to be an autologous growth factor delivery system in combination with 5 collagen-based membranes. Thus, this was the first study that attempted to understand the ability and suitability of biomaterials to incorporate PRF [19].

Several fucoidan (FU) based composites have recently been established for bone tissue engineering purposes because FU increased the proliferation capacity of osteoblast-like cells and enhanced osteoblast-mediated mineral deposition [20]. FU's similarity to the human extracellular matrix and impressive qualities, like high biocompatibility and renewability, have recently intrigued researchers. It shows promise in developing regenerative medicine materials, particularly for wound treatment [21]. FU, a heparin-like molecule, enhances growth factor effects on cell proliferation, osteogenic differentiation, and mesenchymal stem cell activity [22]. Several studies [23], [24] have reported that FU can interact with growth factors (such as bFGF and TGF-β) to control their release and activity by binding and regulating signaling pathways. These are highly important in autologous PRF samples to support the release of growth factors from the matrices.

In general, the structure and properties of chitosan are similar to glycosaminoglycan (GAG), a natural polysaccharide and the main component of the extracellular matrix (ECM) [25]. Chitosan, a natural cationic copolymer, has generated significant interest for its potential in hydrogel formations. This polymer's hydrophilic nature allows it to degrade using human enzymes, ensuring biocompatibility and biodegradability, two crucial properties for medical devices. Chitosan-based hydrogels could serve as scaffolds for tissue repair success [26]. Chitosan accelerates wound healing by activating and modulating inflammatory cells and promoting granulation tissue formation. Its ability to bind negatively charged red blood cells enhances clotting, making it a crucial component in wound dressings [27].

So far, a method for the controlled release of growth factors from PRF in combination with other biomaterials has not yet been investigated, providing the possibility for longer-lasting therapy. In this study, we developed a hydrogel composed of fucoidan (FU) and chitosan (CS) due to their biocompatibility and ability to form polyelectrolyte complexes via self-assembly [28]. The formation of polyelectrolyte complexes between oppositely charged groups of chitosan and fucoidan makes the incorporation of factors possible due to electrostatic interactions [29]. Thus, fucoidan/chitosan hydrogels were used in the Thesis, investigating their potential for further use as PRF storage material.

In this PhD Thesis, the following goals were set: 1) investigating the potential of PRF`s role in converting prodrug to the active form, using clindamycin phosphate as a model drug; 2) assessing PRF`s ability to decelerate the burst release of the active substance by incorporating it in PLGA microcapsules and liposomes, using VANKA as a model drug; and 3) investigate the controlled release of bioactive molecules within PRF by incorporating them into carrier systems, using marine polysaccharide (fucoidan and chitosan) hydrogel as a model carrier system.

## Aim and Objectives

The aim of this Thesis was to develop PRF-based matrices that can provide antibacterial properties through the addition of drugs or their delivery systems, as well as ensure controlled delivery of bioactive molecules within PRF by evaluating different carriers. To achieve the goal, the following tasks were set:

1. To investigate the potential of PRF as an imitator for the conversion of the clindamycin phosphate (CLP) into the active drug form clindamycin, ensuring more effective antimicrobial properties.
2. To evaluate the influence of drug delivery carriers (PLGA microcapsules or liposomes) and their interaction with PRF matrices on the release kinetic of antibiotic drug VANKA.
3. To develop a methodology for obtaining fucoidan/chitosan hydrogel, combining it with PRF to ensure delayed release of bioactive molecules within PRF.

## Theses to Defend

1. CLP incorporation in PRF as a carrier matrix upregulates its antibacterial properties as transformation to its active form – clindamycin is ensured.
2. The release of antibiotic drug VANKA can be modeled from six to ten days, depending on the carriers (PLGA microcapsules or liposomes) used in the PRF matrices.
3. Sustained release (from 6 h to 7 days) of bioactive molecules from PRF can be achieved by combining PRF with fucoidan/chitosan hydrogel, based on the ability of fucoidan to regulate the release and activity of bioactive molecules.

## Scientific Novelty and Main Results

The scientific novelty of the Thesis lies in the dual perspective of PRF application: first, the development of an innovative hydrogel capable to modulating the release of bioactive molecules within PRF, and secondly, the development of a new drug delivery system involving controlled antibiotic or particle delivery, where the particles are incorporated into PRF and act as carriers of biologically active molecules.

A new method has been developed to encapsulate bioactive molecules within PRF in marine polysaccharide (fucoidan/chitosan) hydrogels, thereby achieving their sustained release. Environmentally friendly materials, chitosan and fucoidan, are used in this method, forming hydrogels through polyelectrolyte self-assembly. Unlike traditional drug delivery system preparation methods, this hydrogel production method does not rely on chemical crosslinkers.

Additionally, a new methodology for utilizing PRF as a carrier of biologically active molecules to enhance the antibacterial properties of drugs has been developed. PRF as a carrier extends the applications of prodrugs (e.g., clindamycin phosphate), which have fewer side effects than the active form. The obtained drug/PRF combination demonstrates the need for lower prodrug concentrations compared to the required prodrug amount without PRF. In the

future, this methodology will be further developed to enhance the antibacterial properties of drugs more broadly.

## Practical Significance

PRF properties and its combinations with various drug delivery systems and matrices were investigated, and the application of its use was found in the development of the following combinations:

1. Clindamycin phosphate with PRF as a carrier matrix for maxillofacial surgery, thus obtaining an active drug form that would provide an antibacterial effect in the postoperative period.
2. Combining PRF with various carriers (PLGA microcapsules or liposomes) of biologically active substances (such as VANKA), reducing the need for oral drug administration and adjusting the duration of biologically active substance therapy.
3. A new and environmentally friendly method of obtaining fucoidan/chitosan hydrogels combined with PRF, thus ensuring the long-term release of bioactive molecules within PRF.

## Structure and Volume of the Thesis

This Doctoral Thesis was prepared as a collection of thematically related scientific publications dedicated to the development of autologous fibrin matrices for medical applications. The Thesis unites three original publications in SCI journals and one review article.

## Publications and Approbation of the Thesis

The Thesis results are reported in three original experimental publications. One review article has been published. The main results were presented at 14 conferences.

Scientific publications

1. **Egle, K**., Dohle, E., Hoffmann, V., Salma, I., Al-Maawi, S., Ghanaati, S., Dubnika, A. Fucoidan/Chitosan Hydrogels as Carrier for Sustained Delivery of Platelet-Rich Fibrin Containing Bioactive Molecules. *Int. J. Biol. Macromol*., **2024**, *262* (1), 129651. doi: 10.1016/j.ijbiomac.2024.129651.
2. **Egle, K.**, Skadins, I., Grava, A., Micko, L., Dubniks, V., Salma, I., Dubnika, A. Injectable Platelet-Rich Fibrin as a Drug Carrier Increases the Antibacterial Susceptibility of Antibiotic – Clindamycin Phosphate. *International Journal of Molecular Sciences*, **2022**, *23*(13), 7407. doi: 10.3390/ijms23137407 (Scopus, Open Access).
3. Dubnika, A., **Egle, K.**, Skrinda-Melne, M., Skadins, I., Rajadas, J., Salma. I. Development of Vancomycin Delivery Systems Based on Autologous 3D Platelet-Rich

Fibrin Matrices for Bone Tissue Engineering. *Biomedicines*, **2021**, *9*(7), 814. doi: 10.3390/biomedicines9070814 (Scopus, Open Access).

4. **Egle, K**., Salma, I., Dubnika, A. From Blood to Regenerative Tissue: How Autologous Platelet-Rich Fibrin Can Be Combined with Other Materials to Ensure Controlled Drug and Growth Factor Release. *International Journal of Molecular Sciences*, **2021**, *22*(21), 11553.doi: 10.3390/ijms222111553 (Scopus, Open Access).

The results of the Thesis were presented at the following conferences

1. **Egle, K.,** Dohle, E., Hoffmann, V., Salma, I., Al-Maawi, S., Ghanaati, S., Dubnika, A. Exploring Platelet-Rich Fibrin Microstructure and Histology: with and without Hydrogel Carriers. *64th International Scientific Conference of RTU: Materials Science and Applied Chemistry*, Riga, Latvia**,** October 6, **2023** (oral presentation).
2. **Egle, K.,** Dohle, E., Hoffmann, V., Salma, I., Al-Maawi, S., Ghanaati, S., Dubnika, A. Fucoidan/Chitosan Hydrogels for Sustained Delivery of Platelet-Rich Fibrin Containing Growth Factors. *33rd Annual Conference of the European Society for Biomaterials*, Davos, Switzerland, 4–8 September **2023** (poster).
3. Dubnika, A., **Egle, K.,** Skadins, I., Skrinda-Melne, M., Micko, L., Grava, A., Dubniks, V., Salma, I. Development of Drug Delivery Systems Based On Autologous 3D Platelet-rich Fibrin Matrices, TERMIS-AM 2023 Annual Conference, Boston, MA, 11–14 April **2023** (poster).
4. Micko, L., Salma, I., Skadins, I., Salms, G., Dubnika, A., **Egle, K.** Platelet-rich fibrin immunological testing methodology using Elisa assay, *RSU International Research Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Riga, Latvia, 29–31 March **2023** (oral presentation).
5. Micko, L., Skadins, I., Salma, I., Dubnika, A., **Egle, K.,** Salms G. Platelet-rich fibrin antibacterial activity against *Klebsiella pneumoniae, RSU International Research Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Riga, Latvia, 29–31 March **2023** (oral presentation).
6. **Egle, K.,** Skadins, I., Grava, A., Micko, L., Dubniks, V., Salma, I., Dubnika, A. Injectable platelet-rich fibrin as a drug carrier increases the antibacterial susceptibility of antibiotic-clindamycin phosphate. *16th Annual Meeting for Scandinavian Society for Biomaterials*, Roros, Norway, 21–24 March **2023** (poster).
7. Micko, L., **Egle, K.,** Grava, A., Skadins, I., Salma, I., Salms, G., Dubnika, A. Antimicrobial activity of platelet-rich fibrin. *26*th *Congress of the European Association for Cranio-Maxillo-Facial Surgery*, Madrid, Spain, 27–30 September **2022** (poster).
8. **Egle, K.,** Dohle, E., Hoffmann, V., Salma, I., Al-Maawi, S., Ghanaati, S., Dubnika, A. Fucoidan/chitosan hydrogels as matrices for sustained delivery of platelet-rich fibrin containing bioactive molecules. *32*nd *Annual Conference of the European Society for Biomaterials*, Bordeaux, France, 4–8 September **2022** (poster).
9. Salma, I., Micko, L., **Egle, K.,** Dubnika, A. Development of protocol for obtaining autologous liquid PRF for local drug delivery systems. *32nd Annual Conference of the European Society for Biomaterials*, Bordeaux, France, 4–8 September **2022** (poster).

10. **Egle, K.,** Micko, L., Grava, A., Salma, I., Skadins, I., Dubnika, A. Study on the effect of fibrin matrice on the antibacterial activity of clindamycin phosphate. *Scandinavian Society for Biomaterials 2022. 15th Annual Meeting*, Jurmala, Latvia, 13–15 June **2022** (poster).
11. **Egle, K.,** Salma, I., Dubnika, A. From blood to regenerative tissue: how autologous platelet-rich fibrin can be used as drug carrier system. *62nd International Scientific Conference of RTU: Materials Science and Applied Chemistry*, Riga, Latvia**,** October 22, **2021** (poster).
12. **Egle, K.** Dubnika, A. Development of fibrin matrices for sustained drug delivery. *31st Conference of the European Society for Biomaterials*, Porto, Portugal, 5–9 September **2021** (poster).
13. **Egle, K.,** Salma, I., Skadins, I., Dubnika, A. Study of autologous fibrin matrices for controlled drug delivery. Scandinavian Society for Biomaterials, Jurmala, Latvia, 14 June **2021** (poster).
14. Skadins, I., Micko, L., Salma, I., Dubnika, A., **Egle K.** Antibacterial properties of platelet-rich fibrin matrices saturated with vancomycin. *Scandinavian Society for Biomaterials,* Jurmala, Latvia, June 14, **2021** (poster).
15. Dubnika, A., **Egle, K.,** Skadins, I., Salma, I. Commercial vs autologous fibrin-handling from the material point of view. *RSU International Research Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Riga, Latvia, 24–26 March **2021** (oral presentation online).
16. Skadins, I., Dubnika, A., **Egle, K.,** Dovbenko, A., Salma, I., Micko, L. Antibacterial effect of autologous matrices. *RSU International Research Conference on Medical and Health Care Sciences: Knowledge for Use in Practice*, Riga, Latvia, 24–26 March **2021** (poster).
17. **Egle, K.,** Dubnika, A. Development of fibrin matrices for controlled drug delivery. *61st International Scientific Conference of RTU: Materials Science and Applied Chemistry*, Riga, Latvia, October 23, **2020** (poster).
18. **Egle, K.** Development of autologous fibrin matrices for controlled drug delivery. *61st Riga Technical University Student Scientific and Technical Conference*, Riga, Latvia, May 22, **2020** (oral presentation).

Other scientific publications published during the research for Ph. D Thesis

1. Micko, L., Salma, I., Skadins, I., **Egle, K.**, Salms, G., Dubnika, A. Can Our Blood Help Ensure Antimicrobial and Anti-Inflammatory Properties in Oral and Maxillofacial Surgery? *International Journal of Molecular Sciences*, **2023**, *24*(2), 1073.doi: 10.3390/ijms24021073 (Scopus, Open Access).
2. Grava, A., **Egle, K.,** Dubnika, A. Enzymatically Crosslinked in situ Synthesized Silk/Gelatin/Calcium Phosphate Hydrogels for Drug Delivery. *Materials*, **2021**, 14(*23*), 7191. doi: 10.3390/ma14237191 (Scopus, Open Access).

# MAIN RESULTS OF THE THESIS

## Pathway from Patient Blood Samples to Injectable PRF

Blood is a mixture of plasma, platelets, and red and white blood cells that flow throughout the body. Blood is drawn from the patient, and by subjecting it to centrifugal force, the different components, including platelets and fibrin, naturally separate based on their densities [30]. PRF is derived from blood through a simple centrifugation process. It is widely used to accelerate soft and hard tissue regeneration. This was first described by Choukroun and his group in 2001 in France. PRF is a modification of platelet-rich plasma (PRP) and, at the same time, an autologous fibrin matrix used to improve bone regeneration and clinically used for soft tissue augmentation. Compared to other platelet concentrates, PRF is a platelet-rich fibrin clot that does not require the use of thrombin (procoagulant is used to accelerate gelation) but only centrifuged blood without any impurities. It is a new biomaterial that resembles an autologous cicatrical matrix but, at the same time, is neither a fibrin glue nor a classic platelet concentrate. In addition, PRF contains a high concentration of host immune cells, which are needed to heal wounds and reduce infections [5].

Different PRF derivatives are used today depending on the application and the desired properties. As mentioned by Wend et al., in addition to solid PRF (A-PRF), there is a clinical need to develop injectable PRF (i-PRF) matrices for various clinical procedures and to improve angiogenic potential through the ability to combine i-PRF with various biomaterials [5], [31]. Figure 1 shows the advantages of i-PRF and A-PRF.

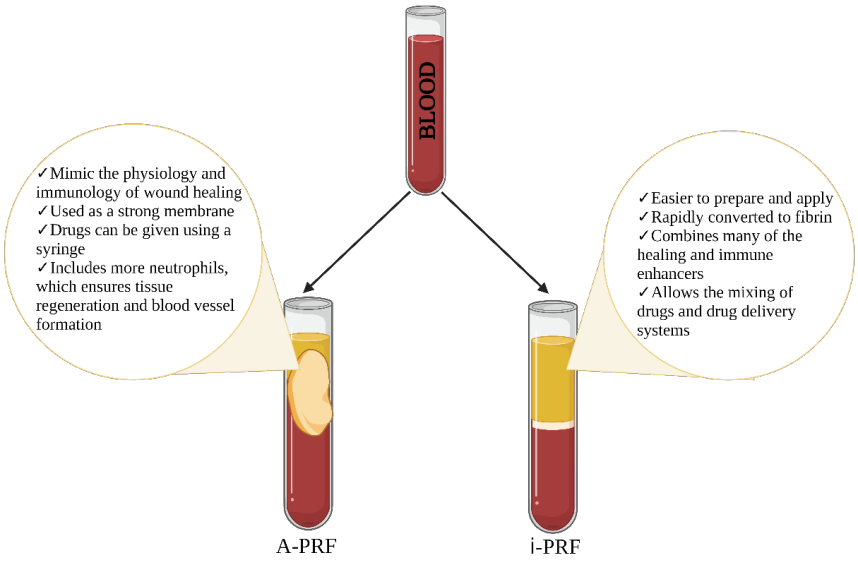


Fig. 1. Comparison of the advantages of two autologous platelet-rich concentrates i-PRF and A-PRF. Figure created with Biorender.com [5].

i-PRF is liquid injectable PRF and allows the incorporation of drugs and drug delivery systems prior to coagulation. i-PRF is a recently introduced platelet concentrate that can be easily combined with various biomaterials to improve the properties of the biomaterial. i-PRF contains not only autologous growth factors found in the blood but also cells involved in the wound healing process (Fig. 2). Panel B shows that not all elements in the blood enter the PRF layer after centrifugation.

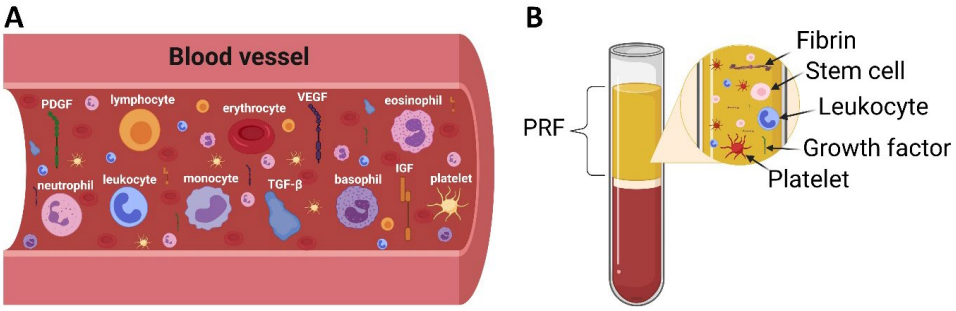


Fig. 2. Main elements of blood (A) and PRF (B). Figure created with Biorender.com [5].

PRF itself may demonstrate antibacterial activity, but it has not been relatively well studied, and there is insufficient data on what affects it. Antimicrobial activity can be defined as the destruction or inhibition of the growth of microorganisms (bacteria, fungi, and viruses) [32]. In the human body, wound healing is compromised by possible infection, especially in wounds in the oral region. Thus, the success of the use of PRF may be connected with its antimicrobial properties. Fully autologous second-generation PRF with no additives as prepared using one centrifugation may most directly reflect the antimicrobial potential of blood-derived material. Another critical and fundamental property of PRF is its ability to release various growth factors and cytokines at supraphysiologic concentrations, rendering it a significant agent in medical applications, particularly in tissue regeneration [4]. Growth factors are a heterogeneous group of proteins secreted by leukocytes and platelets with a short biological half-life rapidly eliminated from the bloodstream; they act mainly locally. Platelets are involved in hemostasis and store growth factors in alpha granules, which are activated to release these factors at the injury site [33].

Studies in the literature have shown that PRF is frequently used in combination with medications like metronidazole, clindamycin, penicillin [34], VANKA, teicoplanin, gentamicin, or amikacin to eradicate bacteria and expedite the healing process [35]. In contemporary oral and maxillofacial surgery, there is a growing requirement for clindamycin as a pharmaceutical agent. Clindamycin and VANKA are commonly considered an alternative for patients who exhibit allergic reactions to penicillin [36]. In the last decade, few studies have combined antibiotics with PRF to provide an antibacterial effect. Based on the gathered literature regarding PRF's antibacterial characteristics, an optimal approach would involve amalgamating PRF with drugs to create a unified system rather than employing distinct drugs

and PRF independently [4]. It should also be mentioned that the PRF can serve not only as a drug delivery system but also as a matrix of other materials. Scientists have tried to combine silk fibroin powder from *Bombyx mori* with Choukroun PRF, which showed the ability to prevent peri-implant defects [37]. While searching for articles on the treatment of periodontitis, it was found that PRF, in combination with other materials, can also be used to treat intrabony defects. Summarizing all available studies, it is observed that when using PRF as matrices or including it in another carrier system, there is no need to add growth factors, as PRF itself includes certain growth factors. The only thing to consider is the encapsulation of the desired drug and its interaction with other carriers that will be included in the PRF. It is also important to investigate whether the used carrier system will be able to ensure the controlled release of the growth factors in the PRF [5].

This PhD Thesis highlights the multifaceted potential of platelet-rich fibrin (PRF) in biomedical applications. The subsequent chapters will delve into specific aspects, including 1) the capacity of PRF to enhance the antibacterial sensitivity of clindamycin phosphate, 2) the influence of delivery systems on drug release from the PRF matrix, and 3) the impact of carrier systems on the release of bioactive molecules within PRF. These chapters aim to provide a comprehensive understanding of how PRF can be harnessed and optimized for targeted and effective medical interventions.

## The Potential of PRF to Stimulate CLP Antibacterial Susceptibility

Infections are one of the most common postoperative risks caused by pathogenic and opportunistic bacteria. *S. aureus* and *S. epidermidis* are gram-positive opportunistic bacteria present in the normal human microbiome [2]. Opportunistic bacteria are usually harmless when residing in the human or animal body but can cause illness or infection when conditions allow, such as a weakened immune system [38].

For the treatment of community-acquired, methicillin-resistant, and methicillin-susceptible *S. aureus* infections, clindamycin has been recommended for many years, and it can also reduce the susceptibility of methicillin-resistant *S. aureus* clinical isolates [39], [40]. Currently, there is a growing need for clindamycin in oral and maxillofacial surgery, particularly for preventing and treating jaw osteonecrosis [41]. It is widely considered an alternative for patients with an allergic reaction to penicillin [36]. Clindamycin phosphate (CLP) is a prodrug of clindamycin that has no antibacterial activity [42]. It is reported that CLP is rapidly hydrolyzed to the active base (clindamycin) in the blood [43]. The aim was to investigate the change in CLP antibacterial properties against reference culture and clinical isolates of *S. aureus* and *S. epidermidis* using PRF as a carrier matrix. Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) is one of the main ways to assess this. See the PRF_CLP sample preparation scheme in Fig. 3.

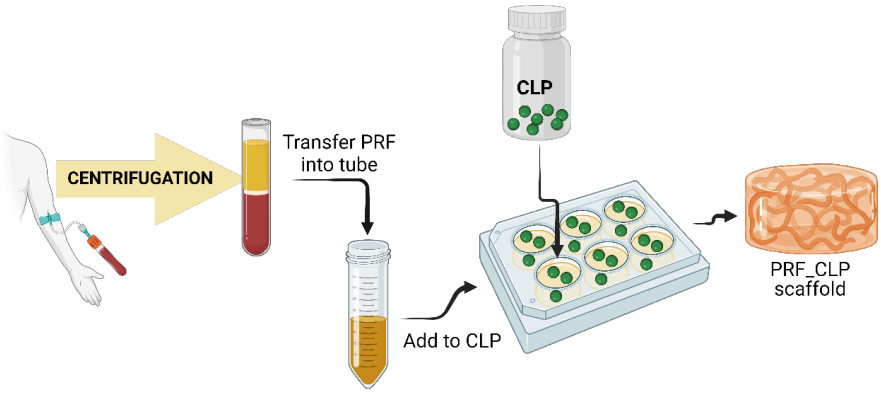


Fig. 3. CLP sample preparation scheme. Figure created with Biorender.com.

Fourier transform infrared spectrometry (FTIR) was used to examine the structural changes after combining clindamycin phosphate (CLP) with PRF (Fig. 4). Antibacterial tests were used to verify whether the combination of CLP with PRF shows an antibacterial effect and confirms the theory that in the presence of PRF, a lower concentration of CLP is required to kill and prevent bacterial growth (Fig. 5).

FTIR spectra show the interaction of CLP with PRF during the seven-day incubation period. Partial hydrolysis and conversion of CLP to clindamycin were observed. After seven days of incubation, a new bond formation (C-C at 1080 $cm^{-1}$) and a maximal increase in phosphate group absorbance over time were observed, indicating structural changes, possibly suggesting the conversion of CLP into the active drug – clindamycin.

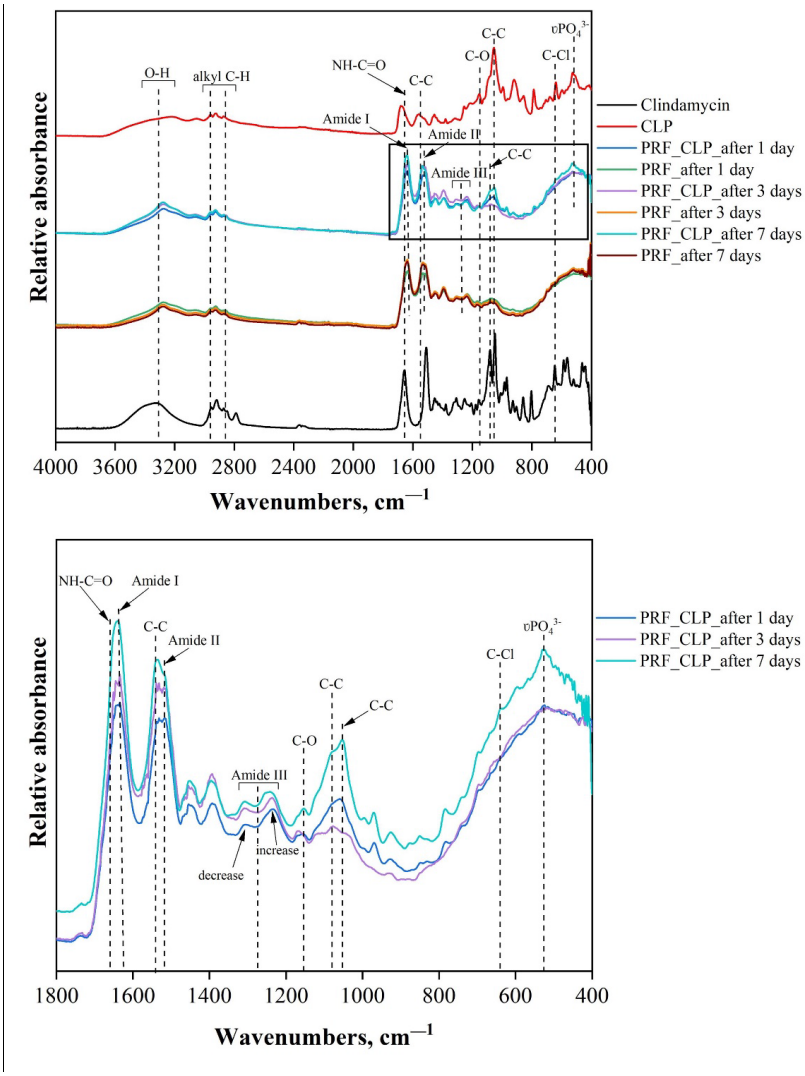


Fig. 4. FTIR spectra: **A** – PRF, PRF_CLP samples, CLP and clindamycin samples in the full spectral range; **B** – PRF_CLP samples at incubation time points in the range of 1800–400cm$^{-1}$ [2].

The composition of the blood from each donor affects the antibacterial properties of the sample, specifically, the amount of CLP required to achieve the antibacterial activity.

Comparing the MIC and MBC values of the PRF_CLP samples with pure CLP samples for all bacteria strains, a decrease in these values is observed by adding PRF to the CLP. Antibacterial tests showed that the addition of PRF enhances the antibacterial activity of CLP not only against staphylococcal reference cultures but also against clinical isolates. It can be seen that against the clinical isolates of *S. aureus* and *S. epidermidis*, higher CLP concentrations are required in PRF_CLP samples to provide a lower MBC value compared to both bacteria

reference cultures. Each donor has different blood properties (such as different white blood cell counts or vitamin D levels) that drastically affect the antibacterial effect. Studies by Schilcher et al. [44], [45] and Kuriyama et al. [46] showed that the MIC of pure clindamycin in clinical isolates against methicillin-resistant *S. aureus* (MRSA) can reach > 256 mg/L. Summarizing all the data, it can be seen that CLP with PRF is a better antibacterial material than pure CLP; and compared to the literature, we have obtained lower MIC values (ranging from 62.5 µg/mL to 145.8 µg/mL depending on the bacterial strain) than required for clindamycin (> 256 µg/mL) Depending on the bacterial strain, the concentration of the drug has to be adjusted (Fig. 5).

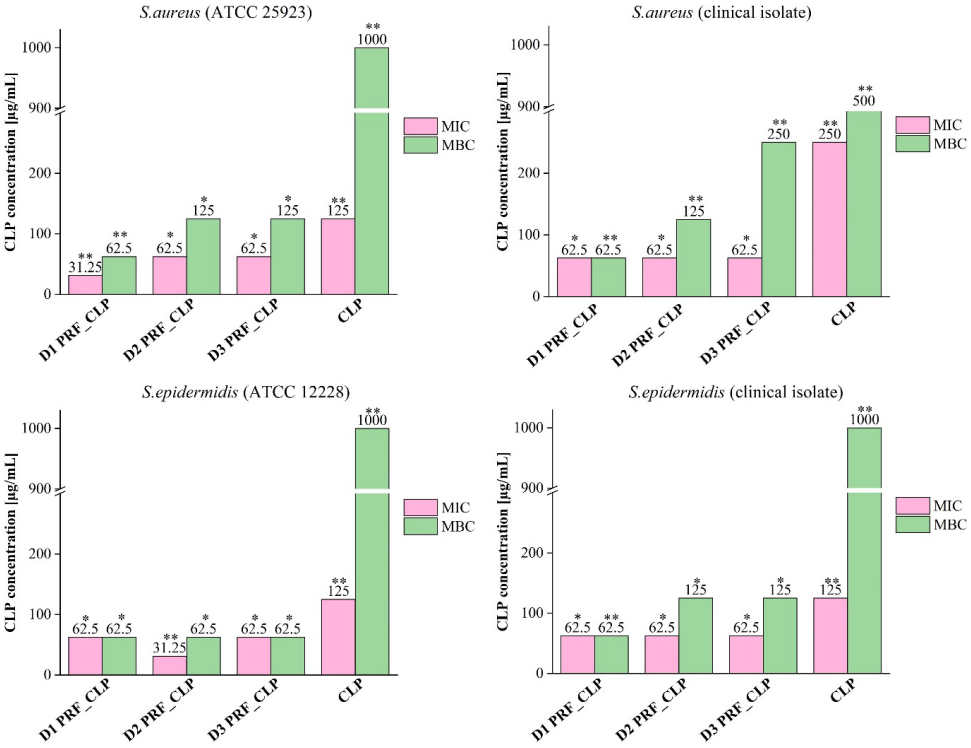


Fig. 5. MIC and MBC value differences between CLP and PRF_CLP samples against four bacteria stains (*S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 12228), *S. aureus* (clinical isolate), *S. epidermidis* (clinical isolate) for all three donors. Samples were prepared from Donor 1 blood (D1 PRF_CLP); samples were prepared from Donor 2 (D2 PRF_CLP); samples were prepared from Donor 3 (D3 PRF_CLP). *$p > 0.05$; ** $p < 0.05$ [2].

The ability of PRF to degrade naturally is considered an advantage for its use as a "warehouse" of controlled drug release systems. The release data indicated that the PRF_CLP sample can be used for a one-day local therapy, ensuring maximum CLP release (80 %) within 1 h (Fig. 6).

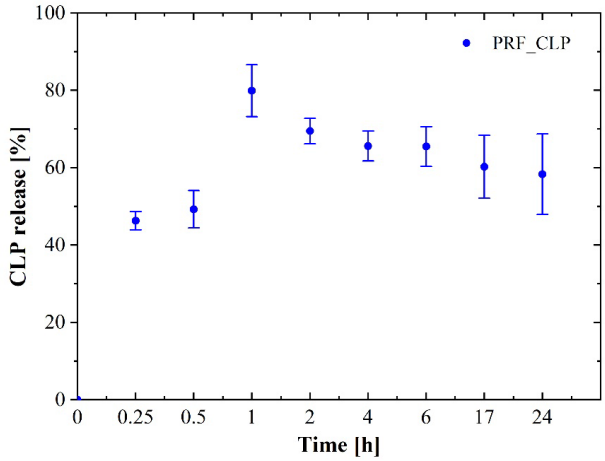


Fig. 6. CLP release from PRF matrices in Dulbecco's modified Eagle medium (DMEM); an average of the three donors' release data [2].

## Impact of Delivery System on Drug Release from PRF Matrix

Systematically used drugs, without a specific carrier, spread throughout the body, and often, the drug degradation rate is relatively short. It ensures not only a positive effect on the damaged tissue but may also induce adverse side effects on healthy tissues [47]. As observed in the previous study [2], simply mixing the drug into PRF enhances its antibacterial properties; however, the drug is released very rapidly. The aim of drug delivery systems is to achieve the highest therapeutic effect with the lowest drug concentration [48]. In this study, antibiotics VANKA encapsulated in PLGA microcapsules (PLGA_µC_VANKA) and liposomes were used. Figure 7 shows the preparation schemes of both types of delivery systems. Both developed VANKA delivery methods were incorporated into PRF matrices, testing the ability of PRF to release drugs uniformly.

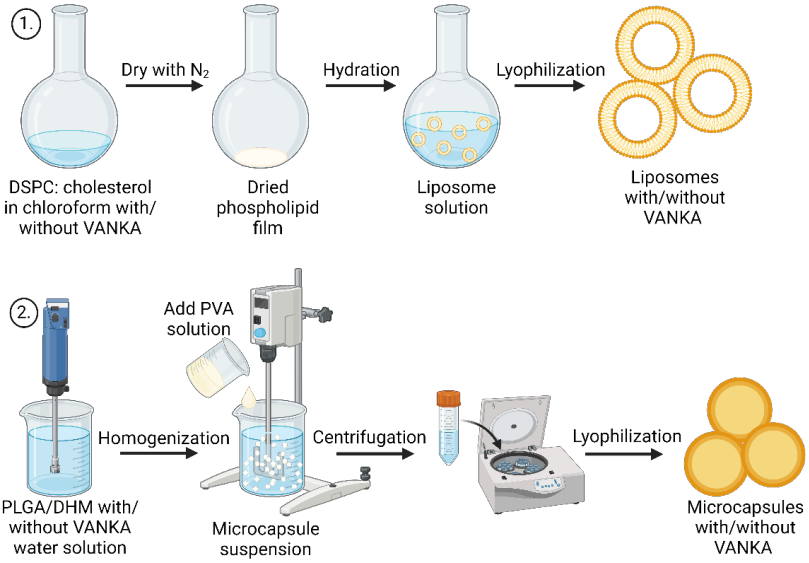


Fig. 7. Sample preparation scheme: 1) liposomes with/without VANKA; 2) microcapsules with/without VANKA. Figure created with Biorender.com.

Transmission electron microscopy (TEM) analysis showed that prepared VANKA-loaded liposomes have a heterogeneous population in which it is possible to observe a close presence of two-layer structures. Also, Fig. 8 shows that the majority of liposomes are spherical particles.

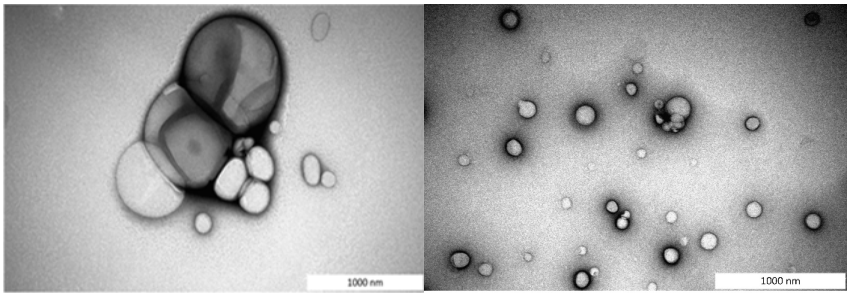


Fig. 8. TEM pictures of VANKA-loaded liposomes [13].

The release kinetics of VANKA from PRF/VANKA liposomes is higher than from VANKA liposomes without PRF matrix (Fig. 9). The increased concentration of VANKA can be explained by the fact that the $Ca^{2+}$ ions in PRF form a shell around the lipids, compressing them and thus destroying the liposomes [49], [50]. Based on the results, it can be concluded that $Ca^{2+}$ ions adversely affect the liposomes; therefore, VANKA is released faster and in higher concentrations.

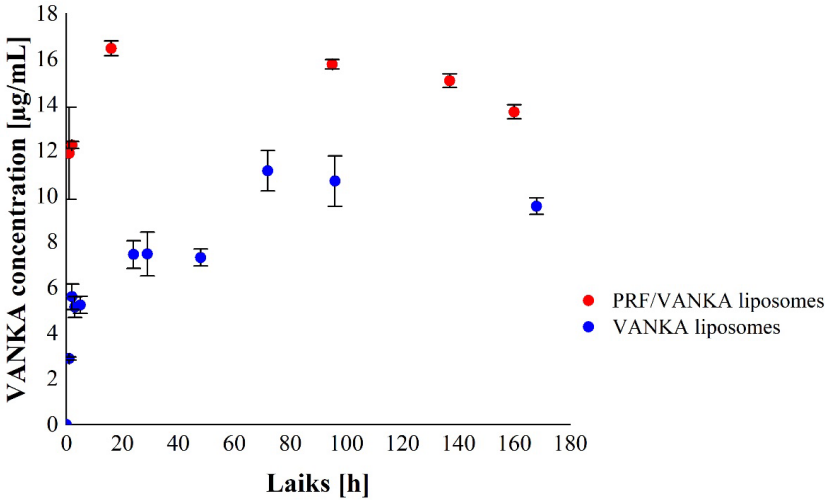


Fig. 9. Drug release from PRF/VANKA liposomes and VANKA liposomes [13].

On the other hand, scanning electron microscopy (SEM) photographs of PLGA microcapsules showed spherical particles with smooth surfaces, which indicated the absence of any drug crystal on the surface and confirmed the even distribution of the drug in the polymeric matrix (Fig. 10 B).

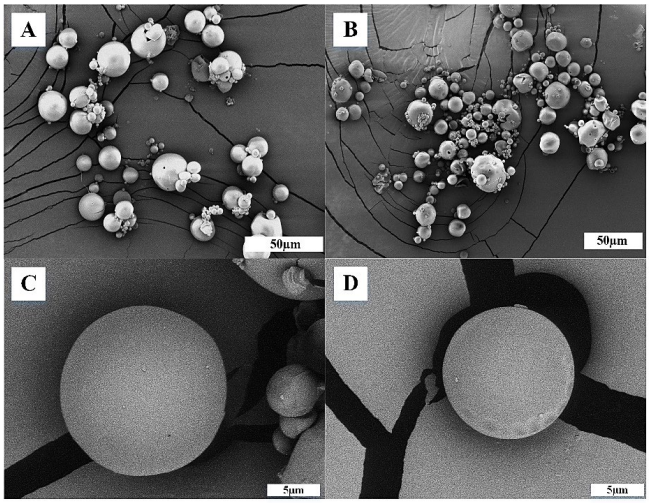


Fig. 10. SEM pictures of the surface of PLGA microcapsules without (A and C) and with (B and D) VANKA [13].

Based on the VANKA release studies from PLGA microcapsules (Fig. 11), the kinetics of VANKA release from PRF with VANKA-loaded PLGA microcapsules (PRF/PLGA_µC_VANKA) scaffolds are reduced fivefold compared to PRF with VANKA-added as free drug powder, non-encapsulated (PRF/VANKA) samples, ensuring controlled

VANKA release (from 6 to 10 days) and preventing burst release. In contrast, comparing PLGA_µC_VANKA with PRF/PLGA_µC_VANKA scaffolds, it can be observed that the VANKA release concentration is reduced twofold. This suggests that the PRF scaffold inhibits the rapid release of VANKA. Also, VANKA in PRF scaffolds without a carrier system does not ensure controlled delivery of active VANKA form at the therapeutic effect level.

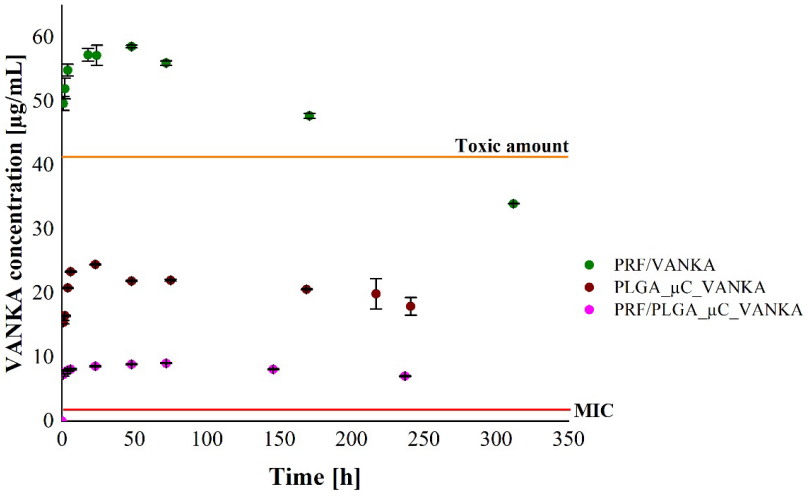


Fig. 11. Drug release from PRF/VANKA, PLGA_ µC _VANKA, and PRF/ PLGA_ µC _VANKA scaffolds [13].

The maximum duration of antibacterial effect for VANKA-containing samples (PLGA_µC_VANKA) was observed for 48 hours. For the first 24 hours, the mean diameter of the sterile area around the samples was 30 mm. For the next 24 hours, the diameter of the sterile area was reduced by 50 % (Fig. 12). As one of the possible solutions for further research, obtained samples could be placed in a bacterial suspension and incubated, preventing sample drying.

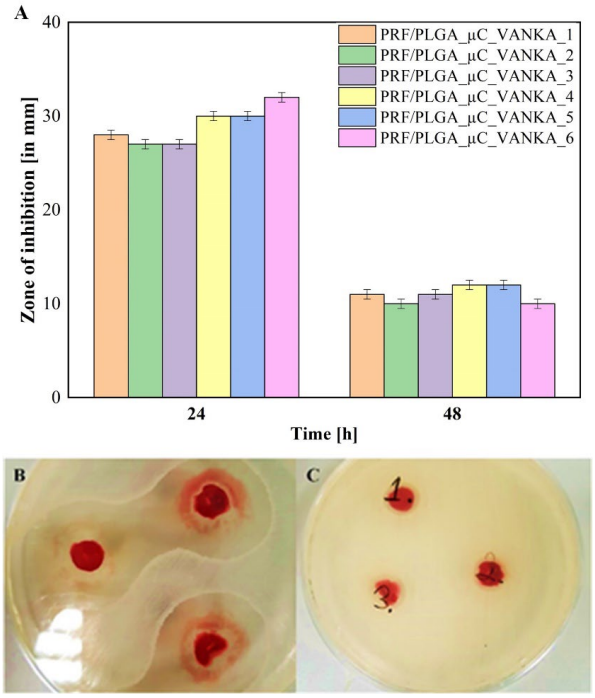


Fig. 12. Antibacterial properties of PRF/PLGA_µC_VANKA samples: (**A**) inhibition zones in mm; (**B**) sterile areas around the PRF/PLGA_µC_VANKA samples after 24 h incubation; (**C**) sterile areas around the PRF/PLGA_µC_VANKA samples after 48 h incubation. The diameter of the petri dishes is 8.5 cm [13].

Beyond its capacity for controlled drug release, PRF possesses another vital characteristic: its role in promoting healing and tissue regeneration, which is made possible by the presence of growth factors and cytokines within PRF.

## Effect of Carrier Systems on the Release of Bioactive Molecules within PRF

In this study, we developed a hydrogel composed of fucoidan (FU) and chitosan (CS) due to their biocompatibility and ability to form polyelectrolyte complexes via self-assembly [28], as well as the ability of fucoidan to bind growth factors [24]. See the FU_CS hydrogel preparation scheme in Fig. 13. To determine the optimal amount of PRF and the release kinetics of the bioactive molecules it contains, the following parameters were investigated for the hydrogels: 1) stability, deformation and flow behavior and 2) release kinetics of the bioactive molecules.

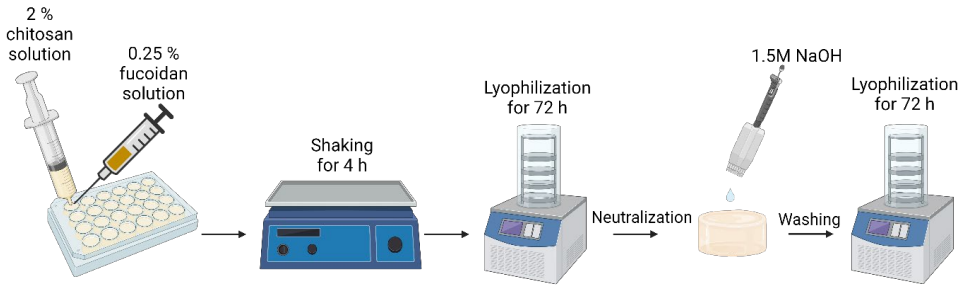


Fig. 13. FU_CS hydrogel preparation scheme. Figure created with Biorender.com [51].

Hydrogels` degradation and rheological properties were analyzed as key determinants of their mechanical behavior and susceptibility to breaking down. Thus, finding out whether the addition of PRF will be able to improve the properties of the hydrogel (Figs. 14 and 15). Then, the released amount of bioactive molecules (TGF-1, PDGF-BB, VEGF, EGF, and IL-8) from pure PRF matrix and combined with hydrogel was determined (Fig. 16).

The degradation of FU_CS hydrogels with/without PRF and pure PRF during two weeks in TRIS-HCl and citric acid was determined, and it is illustrated in Fig. 12. Based on the results, the addition of PRF to FU_CS hydrogels slowed down the degradation rates of both PRF and FU_CS hydrogels. Statistical analysis reveals no significant difference in media between FU_CS and PRF/FU_CS hydrogel samples. There is a notable gap in PRF degradation, with 87.26 ± 8.21 % in TRIS-HCl and 96.21 ± 1.71 % in citric acid within 14 days.

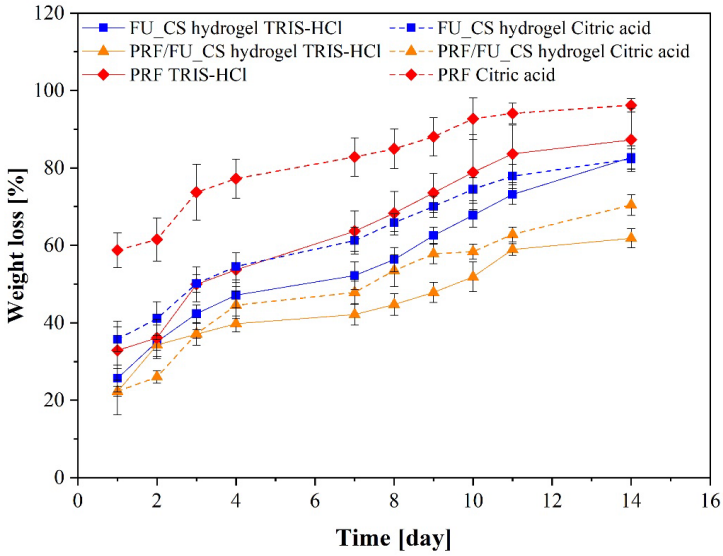


Fig. 14. Degradation degree of PRF, FU_CS and PRF/FU_CS hydrogels in TRIS-HCl (with a solid line) and citric acid (with dashed line) at 37 °C. All data is represented as average ± SD, $n$ = 3 [51].

Rheological experiments were used to explore the deformation and flow behaviors of PRF, FU_CS and PRF/FU_CS hydrogels. All samples exhibited characteristic gel-like behavior, with the storage modulus (G`) substantially exceeding the loss modulus (G``) (Fig. 15). The amplitude sweep test identified a consistent linear viscoelastic region (LVER) down to $\varepsilon \approx 2.5$ % for all samples (marked with a black line in Fig. 15 A), and G`-G`` crossing point was observed at $\varepsilon \approx 14$ % for FU_CS hydrogel, $\varepsilon \approx 28$ % for PRF/FU_CS hydrogel, and at $\varepsilon \approx 55$ % for PRF (marked with colored lines). In rheology, "ε" typically represents strain, how much a material has deformed under a certain amount of stress or force. In all frequency ranges, G` remained constant for all samples (Fig. 15 B), indicating a solid-like and stable internal structure.

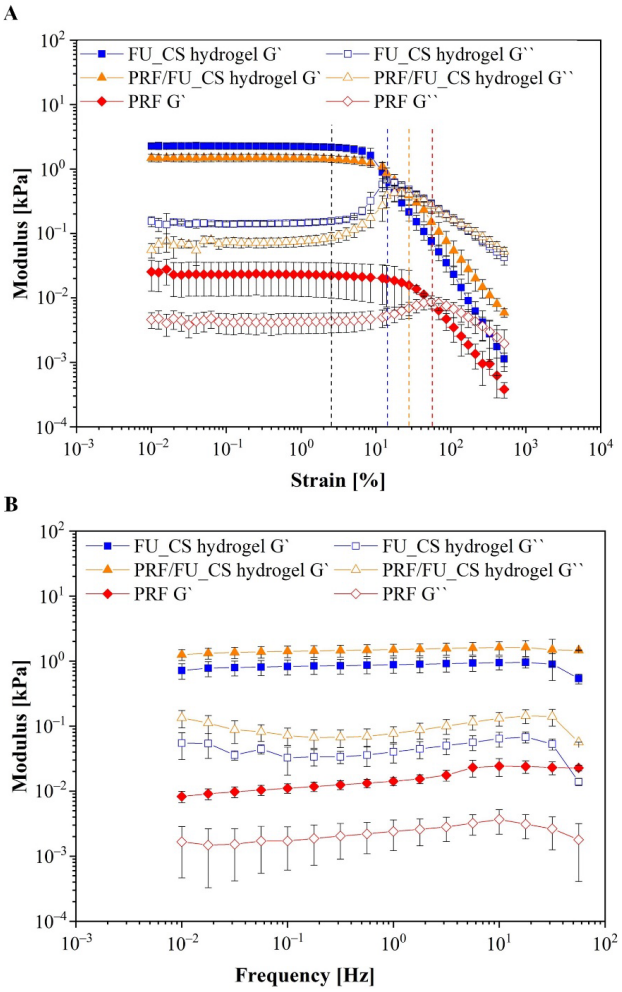


Fig. 15. Mechanical properties of scaffolds: **A** –amplitude sweep test of hydrogels with/without PRF was obtained at 1 Hz frequency; **B** – frequency sweep test of FU_CS hydrogels with/without PRF was obtained at 0.2 % strain. All data is represented as average ± SD ($n$ = 3) [51].

The data obtained show that the hydrogel, as a result of electrostatic interaction, allows the growth factors contained in PRF to be encapsulated and ensures their gradual release. For all analyzed growth factors and cytokines (TGF-1, PDGF-BB, VEGF, EGF, and IL-8), a general trend of higher released concentrations from pure PRF matrices than from PRF/FU_CS hydrogel matrices was observed. This indicates that the FU_CS hydrogel can effectively sustain the release of bioactive molecules and incorporate them into the hydrogel matrix, ensuring their long-term availability for tissue engineering purposes.

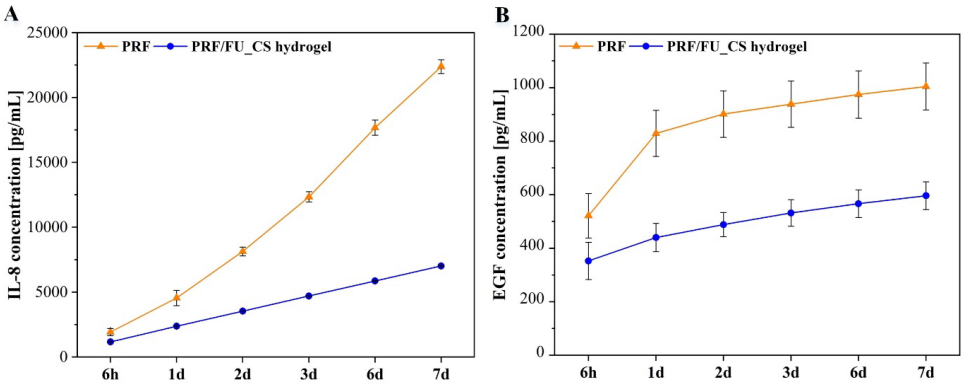


Fig. 16. Cumulative release of growth factor and cytokine. A and B show growth factor and cytokine release (IL-8 and EGF, respectively) at each time [51].

Histological studies have also shown that PRF penetrates the FU_CS hydrogel matrix, enabling the release of growth factors. After three and seven days, the comparison between unmodified PRF and PRF/FU_CS hydrogel reveals a reduction in cell count within the PRF matrix, indicating the release of bioactive molecules. The obtained data also confirm that after seven days, PRF is still incorporated into the FU_CS hydrogel structure (Fig. 17).

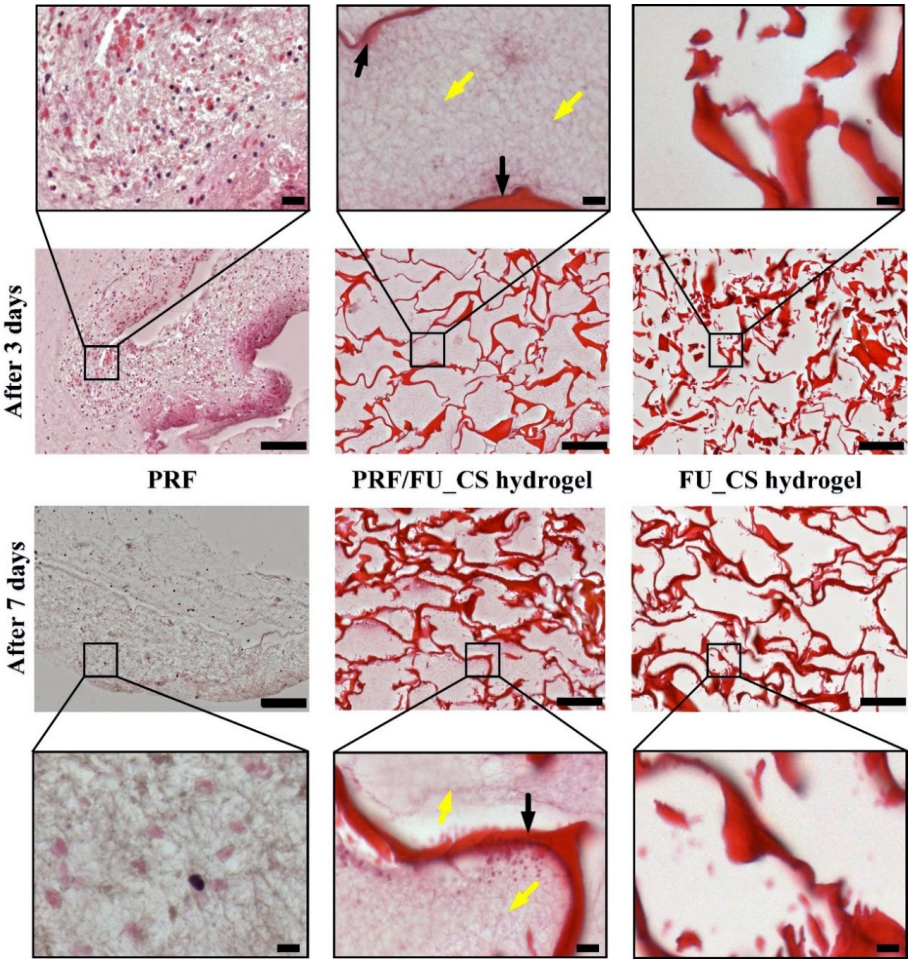


Fig. 17. Fibrin morphology after three and seven days of incubation. (Scale bar: 100 μm, increases have scale bar = 20 μm; yellow arrows show the presence of PRF and black arrows the presence of hydrogel) [51].

The use of hydrogels to modulate PRF growth factor release schedules, particularly in combination with drugs (potentially prodrugs), would offer a promising opportunity to enhance antibacterial activity. Future research should focus on optimizing drug-hydrogel interactions, exploring sustained release patterns, and assessing the capability of other drugs to convert into active forms in the presence of PRF, providing valuable insights for advanced therapeutic applications.

# CONCLUSIONS

1. The addition of PRF affects the antibacterial activity of CLP against *S. aureus* and *S. epidermidis* bacterial strains (both reference cultures and clinical isolates), resulting in lower MIC values (ranging from 62.5 µg/mL to 145.8 µg/mL depending on the bacterial strain) compared to clindamycin (MIC > 256 µg/mL).
2. The use of phosphatidylcholine liposomes as drug delivery systems in PRF matrices fails to provide a controlled release of VANKA, as their stability is influenced by the Ca2+ ions present in the PRF, thereby releasing a high concentration of the drug.
3. The use of PLGA microcapsules as a drug delivery system in PRF matrices can ensure the controlled release of VANKA for six to ten days.
4. The FU_CS hydrogel, created through electrostatic interactions, enables the encapsulation of PRF bioactive molecules (TGF-1, PDGF-BB, VEGF, EGF, and IL-8) and ensures their gradual release over seven days.

# REFERENCES


1. Garraud, O.; Cognasse, F. Are Platelets Cells? And if Yes, are They Immune Cells? *Front. Immunol.* **2015**, *6*, 1–8, doi:10.3389/fimmu.2015.00070.
2. Egle, K.; Skadins, I.; Grava, A.; Micko, L.; Dubniks, V.; Salma, I.; Dubnika, A. Injectable Platelet-Rich Fibrin as a Drug Carrier Increases the Antibacterial Susceptibility of Antibiotic – Clindamycin Phosphate. *Int. J. Mol. Sci.* **2022**, *23*, 7407, doi:10.3390/ijms23137407.
3. Jasmine, S.; A., T.; Janarthanan, K.; Krishnamoorthy, R.; Alshatwi, A.A. Antimicrobial and antibiofilm potential of injectable platelet rich fibrin – a second-generation platelet concentrate – against biofilm producing oral staphylococcus isolates. *Saudi J. Biol. Sci.* **2020**, *27*, 41–46, doi:10.1016/j.sjbs.2019.04.012.
4. Micko, L.; Salma, I.; Skadins, I.; Egle, K.; Salms, G.; Dubnika, A. Can Our Blood Help Ensure Antimicrobial and Anti-Inflammatory Properties in Oral and Maxillofacial Surgery? *Int. J. Mol. Sci.* **2023**, *24*, 1073, doi:10.3390/ijms24021073.
5. Egle, K.; Salma, I.; Dubnika, A. From Blood to Regenerative Tissue: How Autologous Platelet-Rich Fibrin Can Be Combined with Other Materials to Ensure Controlled Drug and Growth Factor Release. *Int. J. Mol. Sci.* **2021**, *22*, 11553, doi:10.3390/ijms222111553.
6. Sancho-Puchades, M.; Herráez-Vilas, J.M.; Berini-Aytés, L.; Gay-Escoda, C. Antibiotic prophylaxis to prevent local infection in Oral Surgery: use or abuse? *Med. Oral Patol. Oral Cir. Bucal* **2009**, *14*, E28–33.
7. Yang, Z.; Liu, J.; Gao, J.; Chen, S.; Huang, G. Chitosan coated vancomycin hydrochloride liposomes : Characterizations and evaluation. *Int. J. Pharm.* **2015**, *495*, 508–515, doi:10.1016/j.ijpharm.2015.08.085.
8. Liu, J.; Wang, Z.; Li, F.; Gao, J.; Wang, L.; Huang, G. Liposomes for systematic delivery of vancomycin hydrochloride to decrease nephrotoxicity: Characterization and evaluation. *Asian J. Pharm. Sci.* **2015**, *10*, 212–222, doi:10.1016/j.ajps.2014.12.004.
9. Pumerantz, A.; Muppidi, K.; Agnihotri, S.; Guerra, C.; Venketaraman, V.; Wang, J.; Betageri, G. Preparation of liposomal vancomycin and intracellular killing of meticillin-resistant Staphylococcus aureus (MRSA). *Int. J. Antimicrob. Agents* **2011**, *37*, 140–144, doi:10.1016/j.ijantimicag.2010.10.011.
10. Upputuri, R. T. P.; Kulandaivelu, K.; Mandal, A. K. A. Nanotechnology-Based Approach for Enhanced Bioavailability and Stability of Tea Polyphenols – A Review. In *Studies in Natural Products Chemistry*; 2016; pp. 399–410.
11. Tyle, P. *Drug Delivery*; Hillery, A., Park, K., Eds.; CRC Press, 2016; Vol. 77; ISBN 9781482217728.
12. Saeed Jan, M.; Alam, W.; Shabnam, M. Fundamentals Applications of Controlled Release Drug Delivery. In *Drug Development and Safety [Working Title]*; IntechOpen, 2023.
13. Dubnika, A.; Egle, K.; Skrinda-Melne, M.; Skadins, I.; Rajadas, J.; Salma, I. Development of Vancomycin Delivery Systems Based on Autologous 3D Platelet-Rich Fibrin Matrices for Bone Tissue Engineering. *Biomedicines* **2021**, *9*, 814, doi:10.3390/biomedicines9070814.

14. Micko, L.; Salma, I.; Skadins, I.; Egle, K.; Salms, G.; Dubnika, A. Can Our Blood Help Ensure Antimicrobial and Anti-Inflammatory Properties in Oral and Maxillofacial Surgery? *Int. J. Mol. Sci.* **2023**, *24*, 1073, doi:10.3390/ijms24021073.
15. Mirjalili, F.; Mahmoodi, M. Controlled release of protein from gelatin/chitosan hydrogel containing platelet-rich fibrin encapsulated in chitosan nanoparticles for accelerated wound healing in an animal model. *Int. J. Biol. Macromol.* **2023**, *225*, 588–604, doi:10.1016/j.ijbiomac.2022.11.117.
16. Rao, S. S.; Venkatesan, J.; Yuvarajan, S.; Rekha, P. D. Self-assembled polyelectrolyte complexes of chitosan and fucoidan for sustained growth factor release from PRP enhance proliferation and collagen deposition in diabetic mice. *Drug Deliv. Transl. Res.* **2022**, *12*, 2838–2855, doi:10.1007/s13346-022-01144-3.
17. Rastegar, A.; Mahmoodi, M.; Mirjalili, M.; Nasirizadeh, N. Platelet-rich fibrin-loaded PCL/chitosan core-shell fibers scaffold for enhanced osteogenic differentiation of mesenchymal stem cells. *Carbohydr. Polym.* **2021**, *269*, 118351, doi:10.1016/j.carbpol.2021.118351.
18. Xu, F.; Zou, D.; Dai, T.; Xu, H.; An, R.; Liu, Y.; Liu, B. Effects of incorporation of granule-lyophilised platelet-rich fibrin into polyvinyl alcohol hydrogel on wound healing. *Sci. Rep.* **2018**, *8*, 14042, doi:10.1038/s41598-018-32208-5.
19. Al-Maawi, S.; Herrera-Vizcaíno, C.; Orlowska, A.; Willershausen, I.; Sader, R.; Miron, R.J.; Choukroun, J.; Ghanaati, S. Biologization of Collagen-Based Biomaterials Using Liquid-Platelet-Rich Fibrin: New Insights into Clinically Applicable Tissue Engineering. *Materials (Basel).* **2019**, *12*, 3993, doi:10.3390/ma12233993.
20. Lu, H.-T.; Lu, T.-W.; Chen, C.-H.; Lu, K.-Y.; Mi, F.-L. Development of nanocomposite scaffolds based on biomineralization of N,O-carboxymethyl chitosan/fucoidan conjugates for bone tissue engineering. *Int. J. Biol. Macromol.* **2018**, *120*, 2335–2345, doi:10.1016/j.ijbiomac.2018.08.179.
21. V. K., A.D.; Udduttula, A.; Jaiswal, A.K. Unveiling the secrets of marine—derived fucoidan for bone tissue engineering – A review. *Front. Bioeng. Biotechnol.* **2023**, *10*, 1100164, doi:10.3389/fbioe.2022.1100164.
22. Park, S.; Lee, K.W.; Lim, D.-S.; Lee, S. The Sulfated Polysaccharide Fucoidan Stimulates Osteogenic Differentiation of Human Adipose-Derived Stem Cells. *Stem Cells Dev.* **2012**, *21*, 2204–2211, doi:10.1089/scd.2011.0521.
23. Purnama, A.; Aid-Launais, R.; Haddad, O.; Maire, M.; Mantovani, D.; Letourneur, D.; Hlawaty, H.; Le Visage, C. Fucoidan in a 3D scaffold interacts with vascular endothelial growth factor and promotes neovascularization in mice. *Drug Deliv. Transl. Res.* **2015**, *5*, 187–197, doi:10.1007/s13346-013-0177-4.
24. Zeng, H.-Y.; Huang, Y.-C. Basic fibroblast growth factor released from fucoidan-modified chitosan/alginate scaffolds for promoting fibroblasts migration. *J. Polym. Res.* **2018**, *25*, 83, doi:10.1007/s10965-018-1476-8.
25. Domalik-Pyzik, P.; Chłopek, J.; Pielichowska, K. Chitosan-Based Hydrogels: Preparation, Properties, and Applications. *Int. J. Biol. Macromol.*; 2019; pp. 1665–1693.
26. Ahmadi, F.; Oveisi, Z.; Samani, S.M.; Amoozgar, Z. Chitosan based hydrogels:

characteristics and pharmaceutical applications. *Res. Pharm. Sci.* **2015**, *10*, 1–16.
27. Levengood, S.K.L.; Zhang, M. Chitosan-based scaffolds for bone tissue engineering. *J. Mater. Chem. B* **2014**, *2*, 3161, doi:10.1039/c4tb00027g.
28. Venkatesan, J.; Singh, S.; Anil, S.; Kim, S.-K.; Shim, M. Preparation, Characterization and Biological Applications of Biosynthesized Silver Nanoparticles with Chitosan-Fucoidan Coating. *Molecules* **2018**, *23*, 1429, doi:10.3390/molecules23061429.
29. Chitosan-based polyelectrolyte complexes as pharmaceutical excipients. In *Controlled Drug Delivery*; M.A. Mateescu, P.I.-S. and E.A., Ed.; Elsevier, 2015; pp. 127–161.
30. Theodoridis, T.; Kraemer, J. *Guyton and Hall Textbook of Medical Physiology*; 2020; ISBN 9783131450715.
31. Wend, S.; Kubesch, A.; Orlowska, A.; Al-Maawi, S.; Zender, N.; Dias, A.; Miron, R.J.; Sader, R.; Booms, P.; Kirkpatrick, C.J.; et al. Reduction of the relative centrifugal force influences cell number and growth factor release within injectable PRF-based matrices. *J. Mater. Sci. Mater. Med.* **2017**, *28*, 188, doi:10.1007/s10856-017-5992-6.
32. The Free Dictionary: https://medical-dictionary.thefreedictionary.com/antimicrobial.
33. Leong, D. K. C.; Benedict, B. C. T.; Chew, K. T. L. Autologous Growth Factors: A Biological Treatment in Sports Medicine. *Proc. Singapore Healthc.* **2010**, *19*, 229–236, doi:10.1177/201010581001900309.
34. Polak, D.; Clemer-Shamai, N.; Shapira, L. Incorporating antibiotics into platelet-rich fibrin: A novel antibiotics slow-release biological device. *J. Clin. Periodontol.* **2019**, *46*, 241–247, doi:10.1111/jcpe.13063.
35. Knafl, D.; Thalhammer, F.; Vossen, M.G. In-vitro release pharmacokinetics of amikacin, teicoplanin and polyhexanide in a platelet rich fibrin – layer (PRF) – a laboratory evaluation of a modern, autologous wound treatment. *PLoS One* **2017**, *12*, e0181090, doi:10.1371/journal.pone.0181090.
36. Maestre-Vera, J. R. Treatment options in odontogenic infection. *Med. Oral Patol. Oral Cir. Bucal* **2004**, *9 Suppl*, 25–31; 19–24.
37. Jang, E.-S.; Park, J.-W.; Kweon, H.; Lee, K.-G.; Kang, S.-W.; Baek, D.-H.; Choi, J.-Y.; Kim, S.-G. Restoration of peri-implant defects in immediate implant installations by Choukroun platelet-rich fibrin and silk fibroin powder combination graft. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **2010**, *109*, 831–836, doi:10.1016/j.tripleo.2009.10.038.
38. Schmidt, T. *Encyclopedia of Microbiology 4th ed.*; Faculty Bookshelf. 260, 2019; ISBN 9780128117378.
39. Martinez-Aguilar, G.; Hammerman, W. A.; Mason, E. O.; Kaplan, S. L. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible Staphylococcus aureus in children. *Pediatr. Infect. Dis. J.* **2003**, *22*, 593–599, doi:10.1097/01.inf.0000073163.37519.ee.
40. Mohamed, M. A.; Nasr, M.; Elkhatib, W. F.; Eltayeb, W. N.; Elshamy, A. A.; El-Sayyad, G. S. Nanobiotic formulations as promising advances for combating MRSA resistance: susceptibilities and post-antibiotic effects of clindamycin, doxycycline, and linezolid. *RSC Adv.* **2021**, *11*, 39696–39706, doi:10.1039/D1RA08639A.

41. Fortunato, L.; Bennardo, F.; Buffone, C.; Giudice, A. Is the application of platelet concentrates effective in the prevention and treatment of medication-related osteonecrosis of the jaw? A systematic review. *J. Cranio-Maxillofacial Surg.* **2020**, *48*, 268–285, doi:10.1016/j.jcms.2020.01.014.
42. Li, H.; Deng, J.; Yue, Z.; Zhang, Y.; Sun, H.; Ren, X. Clindamycin hydrochloride and clindamycin phosphate: two drugs or one? A retrospective analysis of a spontaneous reporting system. *Eur. J. Clin. Pharmacol.* **2017**, *73*, 251–253, doi:10.1007/s00228-016-2161-7.
43. Borin, M. T.; Powley, G. W.; Tackwell, K. R.; Batts, D. H. Absorption of clindamycin after intravaginal application of clindamycin phosphate 2% cream. *J. Antimicrob. Chemother.* **1995**, *35*, 833–841, doi:10.1093/jac/35.6.833.
44. Schilcher, K.; Andreoni, F.; Uchiyama, S.; Ogawa, T.; Schuepbach, R. A.; Zinkernagel, A. S. Increased Neutrophil Extracellular Trap–Mediated Staphylococcus aureus Clearance Through Inhibition of Nuclease Activity by Clindamycin and Immunoglobulin. *J. Infect. Dis.* **2014**, *210*, 473–482, doi:10.1093/infdis/jiu091.
45. Schilcher, K.; Andreoni, F.; Dengler Haunreiter, V.; Seidl, K.; Hasse, B.; Zinkernagel, A. S. Modulation of Staphylococcus aureus Biofilm Matrix by Subinhibitory Concentrations of Clindamycin. *Antimicrob. Agents Chemother.* **2016**, *60*, 5957–5967, doi:10.1128/AAC.00463-16.
46. Kuriyama, T.; Karasawa, T.; Nakagawa, K.; Saiki, Y.; Yamamoto, E.; Nakamura, S. Bacteriologic features and antimicrobial susceptibility in isolates from orofacial odontogenic infections. *Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology* **2000**, *90*, 600–608, doi:10.1067/moe.2000.109639.
47. Safari, J.; Zarnegar, Z. Advanced drug delivery systems: Nanotechnology of health design A review. *J. Saudi Chem. Soc.* **2014**, *18*, 85–99, doi:10.1016/j.jscs.2012.12.009.
48. Li, J.; Wang, X.; Zhang, T.; Wang, C.; Huang, Z.; Luo, X.; Deng, Y. A review on phospholipids and their main applications in drug delivery systems. *Asian J. Pharm. Sci.* **2015**, *10*, 81–98, doi:10.1016/j.ajps.2014.09.004.
49. Hincha, D. K. Effects of calcium-induced aggregation on the physical stability of liposomes containing plant glycolipids. *Biochim. Biophys. Acta – Biomembr.* **2003**, *1611*, 180–186, doi:10.1016/S0005-2736(03)00053-1.
50. Melcrová, A.; Pokorna, S.; Pullanchery, S.; Kohagen, M.; Jurkiewicz, P.; Hof, M.; Jungwirth, P.; Cremer, P. S.; Cwiklik, L. The complex nature of calcium cation interactions with phospholipid bilayers. *Sci. Rep.* **2016**, *6*, 1–12, doi:10.1038/srep38035.
51. Egle, K.; Dohle, E.; Hoffmann, V.; Salma, I.; Al-Maawi, S.; Ghanaati, S.; Dubnika, A. Fucoidan/chitosan hydrogels as carrier for sustained delivery of platelet-rich fibrin containing bioactive molecules. *Int. J. Biol. Macromol.* **2024**, *262*, 129651, doi:10.1016/j.ijbiomac.2024.129651.

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