MEDICAL UNIVERSITY OF GDANSK

MARCELA NOWAK

Assessment of public awareness of chronic venous insufficiency and analysis of the effectiveness of various venous ulcer treatment methods.

DOCTORAL THESIS

Supervisor:

prof. dr hab. n. med. Wioletta Barańska-Rybak

Gdańsk 2021

Research was conducted in Clinic of Dermatology, Venereology and Allergology

Medical University of Gdansk

ACKNOWLEDGEMENTS

I would like to sincerely thank

My supervisor, prof. Wioletta Barańska-Rybak, M.D., PhD

For all faith and invaluable support during my work on this thesis.

Director of the Clinic of Dermatology, Venereology and Allergology,

Medical University of Gdansk,

prof. Roman Janusz Nowicki, M.D., PhD

for the possibility of conducting my research at his Clinic.

Team of the Department and Clinic of Dermatology, Venereology and Allergology,

in particular Dorota Mehrholz, M.D., PhD

for help in the planning of my research and substantive support.

To my loved ones for great support, understanding and everyday joy.

To all those who contributed to this work.

I dedicate this work to my parents without whom this work would never be written.

Table of Contents

KEYWORDS

chronic venous insufficiency, venous ulcers, epidemiology, screening, novel treatments of chronic ulcers, quality of life of patients, debridement, skin grafting, biofilm eradication

ABBREVIATIONS

CVI Chronic venous insufficiency

CVD Chronic venous disorders

DUS Duplex ultrasound

SUMMARY

Chronic venous insufficiency (CVI) is becoming a growing problem for modern medicine. CVI is a significant problem, not only from a medical point of view, but also from an economic one. Although the disease is characterized by many years of asymptomatic or poorly symptomatic course, the last stages of CVI are associated with the need for surgery or a long, painful and costly treatment of ulcers.

Thanks to a better understanding of the pathomechanisms and pathophysiology of CVI of venous ulcers, as well as advances in science, new treatment options are constantly being discovered and implemented.

In the surgical press, there are reports on the treatment of venous ulcers, however, in the dermatological press, the topic of venous ulcer treatment is rarely raised. It seems that a similar problem is identified among primary care physicians. What is more, it translates into low social awareness about CVI. Therefore, it is important to check patients' knowledge on venous disease, which will allow performing prophylactic campaigns aimed at stopping the disease progression. It is worth mentioning that there is also a lack of studies assessing the frequency of the first symptoms of CVI.

In my work, I wanted to holistically investigate the problem of venous insufficiency leading to venous ulcers. The first aim of this thesis was to determine the patients' knowledge on venous diseases, the frequency of the first symptoms, and then critical evaluation of the literature on various methods of debridement and the use of nanoparticles in the prevention and control of biofilm from chronic wounds, as well as clinical trials assessing the effectiveness of micrografts in accelerating wound epithelialization and comparison of the effectiveness of selected materials for mechanical wound cleansing, which can be performed by the patients themselves or their caregivers.

The survey was conducted on 175 patients who participated in the preventive assessment of birthmarks. The examination was performed under the supervision of a dermatologist who assessed the occurrence of the first symptoms of CVI. A questionnaire was prepared consisting of two parts, one to be completed by the patient and the other to be completed by the physician. The part for the patient included detailed questions about patients' knowledge about CVI. The survey showed that most people are unfamiliar with CVI and its first symptoms. The most important goal of health care should be to share knowledge about CVI among the society and encourage patients to undergo further diagnostics. Most of the patients surveyed in the questionnaire presented symptoms of the initial stages of CVI.

The study on the mechanical debridement of the wound was carried out on patients of the Department of Dermatology, Venereology and Allergology, in accordance with the inclusion and exclusion criteria. Each group consisted of 8 randomly assigned patients subjected to cleaning with a sterile sponge, monofilament cloth, non-woven cloth impregnated with sodium hyaluronate and phospholipids, and traditional sterile gauze, respectively. Total wound area and necrotic tissue were measured over 30 days by photographic documentation at 7-day intervals and planimetric measurements. The monofilament fiber cloth could potentially be seen as an effective replacement for sterile gauze as it provides a quick and easy-to-use method of cleansing a wound with less pain during cleansing. Patients using all 3 tested products showed greater satisfaction with treatment than patients treated with gauze. The results suggest that all these methods can be considered as they are well accepted by patients and cause less pain during the procedure, which is essential for good patient contact and adherence as well as complete clearance of the lesions.

5 patients of the Department of Dermatology, Venereology and Allergology took part in a study of the effectiveness of micrografts during which the size of the ulcer was compared on the basis of photographic documentation made on the day of surgery and 3, 7, 14 and 30 days after the procedure. The ulcer area was measured by planimetry. Skin micrografts by biopsy punch may be an effective and cost-effective alternative to accelerate epithelialization of granulation ulcerations in the lower extremities. This method can shorten wound healing and accelerate epithelialization, without the need to hospitalize the patient before and after the procedure. It can significantly reduce the cost of treating wounds in patients with chronic ulcers.

The dissertation also includes a review article, describing the current knowledge on the effectiveness of nanoparticles in eradication and preventing the formation of biofilm in chronic wounds.The works included in the dissertation contribute to the knowledge of innovative applications of various methods of chronic wound treatment and the results of the research presented in this thesis indicate the possibility of practical application of selected methods of treatment. The research included in the presented doctoral dissertation provided relevant information on the effectiveness of various wound cleansing methods prior to the application of a specialist dressing as well as the effectiveness of the use of micrografts. This is the first research on debridement that is not sponsored by pharmaceutical companies and comparing 3 different products to one another and to sterile gauze, which gives more objective results. The dissertation also provides information on the frequency of the first symptoms of chronic venous insufficiency and the possibility of using nanoparticles in the eradication of biofilm from chronic wounds. This doctoral dissertation holistically presented the problem of CVI and examined the effectiveness of selected methods of treating chronic wounds.

STRESZCZENIE PO POLSKU

Przewlekła niewydolność żylna (PNŻ) staje się coraz większym problemem dla współczesnej medycyny. PNŻ to istotny problem, nie tylko z medycznego punktu widzenia, ale także ekonomiczny. Chociaż chorobę charakteryzuje wieloletni bezobjawowy lub skąpo objawowy przebieg to ostatnie stadia PNŻ wiążą się z koniecznością wykonania zabiegu operacyjnego lub długotrwałym, bolesnym i kosztochłonnym leczeniem owrzodzeń.

Dzięki lepszemu zrozumieniu patomechanizmów i patofizjologii PNŻ owrzodzeń żylnych, a także rozwojowi nauki nowe możliwości leczenia są stale odkrywane i wdrażane.

W prasie chirurgicznej odnaleźć można doniesienia na temat leczenia owrzodzeń żylnych, jednak w prasie dermatologicznej temat leczenia owrzodzeń żylnych jest rzadko podnoszony. Wydaje się, że podobny problem identyfikowany jest wśród lekarzy podstawowej opieki zdrowotnej. Co więcej przekłada się to na niską świadomość społeczną o PNŻ. Ważne więc jest sprawdzenie wiedzy pacjentów na temat chorób żył, co pozwoli na prowadzenie działań zmierzających do zatrzymania postępu choroby. Warto wspomnieć, że brakuje również badań oceniających częstość występowania pierwszych objawów PNŻ.

W mojej pracy chciałam holistycznie zbadać problem niewydolności żylnej prowadzącej do owrzodzeń żylnych. Celem mojej pierwszej pracy było określenie wiedzy pacjentów na temat chorób żył, częstość występowania pierwszych objawów, a w kolejnych etapach krytyczna ocena literatury w temacie różnych metod oczyszczania ran oraz zastosowaniu mikrocząsteczek w profilaktyce oraz zwalczaniu biofilmu z ran przewlekłych, jak i badania kliniczne oceniające efektywność mikroprzeszczepów w przyspieszaniu naskórkowania ran oraz porównanie skuteczności wybranych materiałów

do mechanicznego oczyszczania ran, które może być przeprowadzane przez samych pacjentów lub ich opiekunów.

Badanie ankietowe zostało przeprowadzone na 175 pacjentach, którzy brali udział w profilaktycznej ocenie znamion. Badanie wykonano pod okiem specjalisty dermatologa, który ocenił występowanie pierwszych objawów PNŻ. Sporządzono kwestionariusz składający się z dwóch części, jedną do wypełnienia przez pacjenta, a drugą do wypełnienia przez lekarza. Część dla pacjenta zawierała szczegółowe pytania dotyczące wiedzy pacjentów na temat PNŻ. Badanie ankietowe wykazało, że większość osób nie jest zaznajomiona z PNŻ i jego pierwszymi objawami. Najważniejszym celem ochrony zdrowia powinno być podzielenie się wiedzą na temat PNŻ wśród społeczeństwa i zachęcenie do dalszej diagnostyki. Większość pacjentów przebadanych w badaniu kwestionariuszowym prezentowała objawy początkowych stadiów PNŻ.

Badania dotyczące mechanicznego oczyszczania rany przeprowadzono na pacjentach Kliniki Dermatologii, Wenerologii i Alergologii, zgodnie z kryteriami włączenia i wyłączenia. Każda grupa składała się z 8 losowo przydzielonych pacjentów poddanych oczyszczaniu odpowiednio: sterylną gąbką, tkaniną z włókna monofilamentowego, włókniną impregnowaną hialuronianem sodu i fosfolipidami oraz tradycyjną sterylną gazą. Za pomocą dokumentacji fotograficznej w odstępach 7-dniowych i pomiarów planimetrycznych w ciągu 30 dni mierzono całkowitą powierzchnię rany i tkankę martwiczą. Ściereczka z monofilamentowego włókna może być potencjalnie postrzegana jako skuteczny następca sterylnej gazy, gdyż zapewnia szybką i łatwą w użyciu metodę oczyszczania rany z mniejszym poziomem bólu podczas oczyszczania. Pacjenci stosujący wszystkie 3 badane produkty wykazywali większą satysfakcję z leczenia niż pacjenci leczeni gazą. Wyniki sugerują, że można rozważyć wszystkie te metody, ponieważ są one dobrze akceptowane przez pacjentów i powodują mniejszy ból podczas zabiegu, co

jest niezbędne dla dobrego kontaktu z pacjentem i przestrzegania przez niego zaleceń, jak i dla całkowitego ustąpienia zmian.

5 pacjentów Kliniki Dermatologii, Wenerologii i Alergologii wzięło udział w badaniu skuteczności mikroprzeszczepów, podczas którego porównywano wielkość owrzodzenia na podstawie dokumentacji fotograficznej wykonanej w dniu zabiegu oraz po 3, 7, 14 i 30 dniach po zabiegu. Powierzchnię owrzodzenia mierzono za pomocą planimetrii. Mikroprzeszczepy skóry za pomocą biopsji mogą być skuteczną i opłacalną alternatywą dla przyspieszenia epitelializacji ziarninujących owrzodzeń kończyn dolnych. Metoda ta może skrócić gojenie się rany i przyspieszyć naskórkowanie, bez konieczności hospitalizacji pacjenta przed i po zabiegu. Może znacznie obniżyć koszty leczenia ran u pacjentów z przewlekłymi owrzodzeniami.

W skład rozprawy wchodzi również artykuł poglądowy, opisujący obecną wiedzę na temat skuteczności nanocząsteczek w zwalczaniu i zapobieganiu powstawania biofilmu w ranach przewlekłych.

Zawarte w rozprawie prace stanowią wkład w poznanie nowatorskich zastosowań różnych metod leczenia ran przewlekłych i wyniki badań zawartych w niniejszej pracy wskazują na możliwość praktycznego zastosowania wybranych metod leczenia. Badania wchodzące w skład prezentowanej rozprawy doktorskiej dostarczyły istotnych informacji na temat skuteczności różnych metod oczyszczania rany przed zastosowaniem opatrunku specjalistycznego jak i skuteczności zastosowania mikroprzeszczepów. Są to pierwsze badania dotyczące oczyszczania rany, które nie jest sponsorowane przez firmy farmaceutyczne i porównujące 3 różne produkty ze sobą i ze sterylną gazą, co daje bardziej obiektywne wyniki. Rozprawa dostarczyła również informacji na temat częstości występowania pierwszych objawów przewlekłej niewydolności żylnej jak i możliwości

zastosowania nanocząsteczek w zwalczaniu biofilmu z ran przewlekłych. Niniejsza rozprawa doktorska w sposób holistyczny przedstawiła problem przewlekłej niewydolności żylnej i zbadała skuteczność wybranych metod leczenie ran przewlekłych.

LIST OF WORKS CONCERNING DOCTORAL THESIS

1. Marcela Nowak, Dorota Mehrholz, Wioletta Barańska-Rybak, Roman Nowicki, Chronic venous disorders - common and yet unknown - study of public awareness and primary symptoms in an assorted group of patients, *Adv Dermatol Allergol 2021; 38 (4): 585–589 https://doi.org/10.5114/ada.2021.108911*

   Impact Factor 1,837; Ministerial Summary 70,000

2. Marcela Nowak, Wioletta Barańska-Rybak, Nanomaterials as a Successor of Antibiotics in Antibiotic-Resistant, Biofilm Infected Wounds? Antibiotics. *2021; 10(8):941. https://doi.org/10.3390/antibiotics10080941*

   Impact Factor 4,639; Ministerial Summary 70,000

Summary Impact Factor: 6,476

Ministerial Summary: 140,000

DISCUSSION OF THE SCIENTIFIC PROBLEM

INTRODUCTION

Chronic venous insufficiency (CVI) is a common disease. It is estimated that in Poland it affects about 47% of females and 37% of males [1]. It is a significant problem, not only from medical point of view, yet also economical.

Although the disease is characterized by many years of asymptomatic or poorly symptomatic course, the last stages of CVI are associated with the need for surgery or a long, painful and costly treatment of ulcers. Main pathomechanisms that lead to CVI include reflux, obstruction or combination of both. Apart from those factors, also failure of calf and foot muscles (by decreased mobility or neuromuscular problems) can lead to inadequate venous return. Etiology of the disease is multifactorial and results from complex interactions of genetical and environmental factors. Among risk factors age, gender, obesity, family history and ethnicity should be mentioned [2-4].

Primary symptoms as heaviness, nocturnal cramps and aching of legs, especially during prolonged standing, can worsen the quality of everyday life. This may consequently lead to aggravation of daily activities and professional working. Nevertheless, the early symptoms can be alleviated by regular exercise, leg elevation and avoidance of prolonged steady standing.

Thanks to a better understanding of the pathomechanisms and pathophysiology of CVI of venous ulcers, as well as advances in science, new treatment options are constantly being discovered and implemented.

In the surgical press, there are reports on the treatment of venous ulcers, however, in the dermatological press, the topic of venous ulcer treatment is rarely raised. It seems that a similar problem is identified among primary care physicians. What is more, it

translates into low social awareness about CVI. Therefore, it is important to check patients' knowledge of venous diseases, which will allow for actions aimed at stopping the disease progression. It is worth mentioning that there is also a lack of studies assessing the frequency of the first symptoms of CVI.

TIMERS acronym stands for structured guidance on wound management approaches that optimize healing outcomes [5]. Acronym TIMERS, where T stands for tissue debridement, I for inflammation and infection control, M for moisture balance, E for wound edges and epithelial advancement, R stands for repair and regeneration and S for social and individual related factors. The biggest change with TIMERS guidelines was adding the need of good compliance with the patient by adding the letter R.

According to the TIMERS strategy, the first step of the local treatment of the wound is debridement, which stands for removal of devitalized tissue such as slough or necrosis. Debridement is a detrimental part of wound management, as it prepares the wound bed for the local treatment. A number of different methods are available for this, from the use of a bone spoon and ending with advanced ultrasound devices. Some of these methods are impossible to use in outpatient settings, not to mention usage by the patient or their caregivers. Modern wound cleaning products such as sterile cloths or sponges are available on the market. It is not known, however, whether these products have an advantage over the use of sterile gauze, as the testing of individual products is sponsored.

One of the products subjected to this research is a non-woven cloth impregnated with solution of sodium hyalyronate, aloe vera and phospholipids (CleanWnd). It contains short fibres enabling it to collect wound debris easily during mechanical debridement without leaving any residual lint. CleanWnd has been designed to cleanse and debride wounds with a solution of Sodium Hyaluronate and Phospholipid which is suitable for

wound cleansing at every dressing change. CleanWnd's non-woven impregnated cloth is manufactured by the needle punch method and contains short fibres enabling it to collect wound debris easily during mechanical debridement without leaving any residual lint. Hyaluronic acid is a vital compound found in extracellular matrix, that triggers wound healing and tissue repair process [6-9]. On the other hand, phospholipids act as a surfactant that eases cleansing and replaces some of the phospholipids lost by damaged tissue [10-11].

Second debridement tool tested is a sterile sponge (blue Schülke Wound Pad), that is designed to be soaked in a surfactant solution (Octenilin). Schülke Wound Pad wound dressings are made of flexible, foamed polyurethane with a coarse-grained structure and a rough surface. As those wound dressings differ by number of pores and roughness, the medium one was chosen to be compared with a cloth. It is characterized by medium pores and medium fluid permeability coefficient., which enables removal of fibrin and excessive exudate from the wound surface, for example, hyperkeratotic skin or residual dressings [12]. Sponges improve blood circulation and oxygen supply to tissues, which increases the strength of granulation and spontaneous cleansing of the wound, without the intervention of a surgeon.

Third debridement method is monofilament fiber cloth (Debrisoft Pad). Cloth is knitted by millions of angled, soft, polyester fibers attached to polyacrylate backing and sewn edges. It is suitable for allergic patients, as it contains none chemically irritant substances. Debrisoft is suitable for a rapid and atraumatic debridement with first visible results in two to four minutes [13]. Fiber composite lifts, binds and therefore ease to remove slough, including biofilm and non-viable debris [14-16]. Due to beveled fiber tips cloths effectively cleanse, while protecting new granulation tissue and epithelial cells [17].

According to the manufacturer, Debrisoft Pad should be hydrated with saline solution before use.

The final part of wound healing is epithelialization, which stands for a complete closure of the wound. In most cases, this stage is significantly prolonged due to disorders of the skin structure. For those reasons, skin transplant is performed in many cases of clean, non-infected wounds to accelerate the wound closure. This procedure significantly shortens the healing time, although in Poland it is only reserved for hospitalized patients. It is popular to use skin split extraction with Dermatome or specialized equipment for collecting very small epidermal grafts (Cellutome) [18]. Those methods, however, need to be performed by qualified surgeons in a surgical room condition. There is a method described based on single cases in which a dermatological punch is used to collect the skin [19-21]. It is a cheaper method compared to previously mentioned methods and easier to use. However, there is a lack of more research on this topic.

The dissertation also includes a review article, describing the current knowledge on the effectiveness of nanoparticles in eradication and preventing the formation of biofilm in chronic wounds. The greatest challenge when treating a chronic wound is prolonged infection, which is commonly caused by biofilm. Biofilm makes bacteria resistant to individuals' immune system and conventional treatment. That is why, new treatment options including nanomaterials are tested and implemented. Nanomaterials are particles with at least one dimension between 1 and 100 nM. Nanomaterials are promising as their mode of action is different that antibiotics as it relies mostly on direct contact, therefore it is considered less possible that bacteria will develop resistance towards them. Furthermore, they can also protect the drugs from enzymatic degradation in biofilm environment. Therefore, they can be used alone if they have antimicrobial properties or as nanocarriers of antibiotics and help them reach therapeutic concentration in the infected tissue

OBJECTIVES OF THE WORK

CVI

1. The aim of the first work was to test the public's knowledge of CVI among patients reporting for skin moles screening.
2. Determine the frequency of the first symptoms of CVI by the physician's verification of the symptoms of chronic venous disease and the confrontation of the results of the physical examination with the patient's assessment

DEBRIDEMENT

3. The main aim was to compare the efficacy of relative necrotic tissue removal after 30 days on patients subjected to debridement with sterile sponge, monofilament fiber cloth, non-woven cloth impregnated with sodium hyaluronate and phospholipids with relation to one another and to patients treated with traditional sterile gauze.

MICROGRAFTS

4. The project involved conducting an observational study on the effectiveness of micrografts using a dermatological punch to accelerate the epidermal ulcer epithelialization.

NANONOMATERIALS

5. This paper’s aim was to gather knowledge on advancements on alternative methods to treat local wound infection with the use of nanomaterials.

STUDY MATERIAL AND METHODOLOGY

Study 1

175 patients took part in the research. The group consisted of random patients who participated in preventive assessment of nevi. The examination was performed under the supervision of a specialist dermatologist who assessed the occurrence of the first symptoms of cvi. A questionnaire, which consisted of two sections has been made, one part to be completed by the patient and the other to be completed by the doctor. Part for the patient contained detailed questions on patients' knowledge on CVI. It begins with yes-no questions if patient knows the disease and thinks that it is common. Then it proceeds to complex questions: if patient can indicate from listed symptoms (leg fatigue, leg oedema, leg cramps, coldness of legs, pain of legs after walking, leg ulcers, varicose veins, vomiting, easy fatigability) the ones that refer to CVI. Same for methods of prophylaxis and treatment.

Study 2

The study on mechanical debridement was conducted on 32 patients of Clinic of Dermatology, Venereology and Allergology, according to inclusion and exclusion criteria.

Patients were randomly assigned to 4 different groups(n=8), respectively according to debridement method: sterile sponge (blue Schülke WoundPad), monofilament cloth (Debrisoft Pad), non-woven impregnated cloth (Clean Wnd) and sterile gauze debridement procedure. Data collected during the interview and treatment results collected during follow-up examinations was entered into the database. Each patient was given a number. Randomization of patients and assignment to a group was determined in order of application.

After reading the written information about the purpose and method of conducting the study, obtaining comprehensive answers to the questions asked and after signing the written consent to participate in the study, the patient was qualified for the study.

The primary end point of the study was the level of sloughy tissue removal, whereas the secondary end point was the degree of granulation of the wound. The study ended when the wound was completely debrided of necrotic tissue or after 30 days. In addition, this study assessed the impact of the applied treatment on ease of local wound care, development of ulcer infection, presence of hypersensitivity reactions, pain during surgery (VAS), cleaning time, cost-effectiveness and the rate of cleaning the wound from necrotic tissue.

During the inclusion visit, a thorough interview was collected and a physical examination performed as per the schedule. The inclusion and exclusion criteria were to be established on the basis of the anamnesis and physical examination (Tab.1)

Inclusion criteria	**Exclusion criteria**
chronic venous leg ulceration lasting at least 3 weeks before entering treatment	ulceration of a different etiology than venous
age> 18 years and <90 years	no pulse on the foot arteries (dorsal foot, posterior tibial, popliteal)
ulcer area between 100cm^2 and 200cm^2	use of immunosuppressive drugs within 30 days before the start of the study

ulceration without fistula	hypersensitivity to any component of the tested product
necrotic ulcers with exudate	pregnancy and breastfeeding
	poorly controlled diabetes
	immune disorders
	cancer
	malnutrition
	connective tissue diseases during exacerbation
	alcoholism, nicotinism or drug addiction
	lack of cooperation on the part of the patient
	participation in any other study during the 30 days preceding the study

Table 1 Inclusion and exclusion criteria

Assessment of the ulcer size using photographic documentation at intervals of 7 days was carried out. One photograph was taken before and the other after wound cleansing procedure. Surface area of consequently: ulcer as a whole, excudate+necrosis and granulation tissue were measured using planimetry tools.

Shin hygiene was carried out with the help of washing and conditioning foam cleaning, then depending on the group debridement procedure was carried out with the use of Schülke Wound Pad soaked in Octenilin liquid, monofilament cloth Debrisoft Pad soaked in saline solution, non-woven impregnated with sodium hyaluronate and

phospholipid cloth CleanWnd or sterile gauze. Consequently, decontamination of the wound with hypochlorite proceeded the application of the dressing - hydrogel with hydrocolloid and alginate (Purilon, Coloplast) as a primary and non-adhesive polyurethane foam dressing (Biatain non-adhesive, Coloplast) as a secondary dressing. The use of combination of those two dressings is advisable, for hydrocolloids with alginates aid necrosis removal, while foam dressings prevent dressing-related trauma and absorbs excessive wounds exudate, providing optimal wound healing conditions. At last compression therapy with short stretch bandages was applied.

Patients received dressings, debridement product tested and compression bandages. Furthermore, they were instructed how to use the received products and the first dressing was to be applied by the doctor participating in the study. Dressings were to be changed twice a week, once by the patient and the other time by the physician.

The patient was notified of the need to report for follow-up appointments in accordance with the protocol (every 7 days ± 1 day) or contact the doctor conducting the examination in case of adverse effects (pain, swelling, redness of the skin around the ulcer, increase in body temperature).

Study 3

The project involved conducting an observational study on the effectiveness of micrografts using a dermatological punch to accelerate the epidermal ulcer epithelialisation. The study was conducted on 5 patients. Patients were encrypted in accordance with the Personal Data Protection Act. After reading the written information about the purpose and method of conducting the study, obtaining comprehensive answers to the questions asked and after signing the written consent to participate in the study, the patient, who met the inclusion and exclusion criteria (Table 2) was qualified for the study.

Donor sites (skin of thighs) was anaesthetized using infiltration injection of *Lignocainum hydrochloricum 1%*. Recipient sites were anaesthetized using *Lignocainum* 2% gel under occlusive bandage for 30 minutes. Debridement of the recipient sites with Volkmann spoon was performed. Grants were taken with the use of 5 mm diameter skin punch (for every 2 cm^2 of recipient site, one grant was placed) from donor site. Recipient sites were prepared with another skin punch. Skin grafts were placed into the recipient sites after performing local hemostasis with sterile gauze. Mesh dressing with silver as a primary and non-adhesive polyurethane foam dressing as a secondary dressing were used for the first and every dressing change thereafter. It is important to place mesh dressing first, as it prevents adhesion of the graft to the dressing, so the dressings can be changed with no threat to the graft. At last compression therapy with short stretch bandages was applied, to restore physiological blood flow.

Primary endpoint of the study was the degree of wound epithelialisation, measured by calculating relative wound epithelialization= (wound area at day 0 - wound area at day 30) /wound area at day 0 * 100%.

Assessment of the ulcer size using photographic documentation was carried out on day of the procedure, as well as 3, 7,14 and 30 days after the procedure. Surface area of the ulcer was measured using planimetry.

Inclusion criteria	**Exclusion criteria**
chronic venous leg ulceration lasting at least 3 months before entering treatment	ulceration of a different etiology than venous
age> 18 years and <90 years	presence of signs of systemic infection (fever, acute abscess, subcutaneous tissue inflammation, leucocytosis, elevated CRP)

A fragment of the ulcer with a maximum surface area of 60 cm^2	presence of signs of infection in the wound
ulceration without fistula	use of immunosuppressive drugs within 30 days before the start of the study
no signs of infection in the wound and systemic infection	hypersensitivity to any component of the tested product
	ulceration covered with yellow or black necrosis
	pregnancy and breastfeeding
	poorly controlled diabetes
	immune disorders
	active cancer
	connective tissue diseases during remission
	malnutrition
	alcoholism, nicotinism or drug addiction
	lack of cooperation on the part of the patient
	participation in any other study during the 30 days preceding the study

Table 2 Inclusion and exclusion criteria

The statistical analysis of the results was carried out using the STATISTICA program with the use of appropriate tests depending on the nature of the distribution of the obtained data.

The research projects carried out as part of the doctoral dissertation were approved by the Independent Bioethical Committee at the Medical University of Gdansk (consent no. NKBBN/601/2019). All participants gave informed written consent to participate in the study.

The results of this doctoral dissertation were presented by the doctoral student at international conferences:

1. 5-6.03.2021 Warsaw, Poland, Interdisciplinary Aspects of Skin and Mucous Diseases, international, Assessment of effectiveness of the full-thickness skin micrograft, using a skin punch method, for accelerating the epithelialization of the wound bed, active participant, original research in english (2nd place)

2. 6-7.05.2021 European Academy of Dermatology and Venereology Spring Symposium, international, Assessment of effectiveness of the full-thickness skin micrograft, using a skin punch method, for accelerating the granulation of the wound bed, e-poster presentation

3. 29-30.05.2021 Warsaw International Medical Congress, Chronic venous disease - common and yet unknown - study of public awareness and primary symptoms in an assorted group of patients, active participant, Lifestyle Medicine and Public Health session

RESULTS

The doctoral dissertation consists of three studies, which resulted in two publications. Studies present research results on public awareness on CVI, prevalence of first symptoms of CVI, mechanical non-invasive debridement efficacy and efficiency of micrografts with the use of dermatological punch. Furthermore, results of one published review article which describes novelty within treatment of biofilm infected wounds with the use of nanomaterials are described.

Study 1

Article entitled **Chronic venous disorders - common and yet unknown - study of public awareness and primary symptoms in an assorted group of patients**, published in Advances in Dermatology and Allergology, presents frequency of first symptoms of CVD and level of public awareness on the subject.

Chronic venous disorders (CVD) are associated with great medical and socioeconomic impact, as it stands for around 362,000 sick leaves per year, representing 6.4 million of workdays lost. It is estimated that CVD constitutes 1-3 % of all health expenses [22]. Treatment of venous ulcers only in the UK costs 400-600 million pounds a year, and in the USA over 1 billion dollars a year [23]. The total social costs in the UK, France and Germany are estimated at over one billion dollars in each of these countries. Unfortunately, there is a lack of concrete reports on costs of treatment of CVD and associated conditions in Poland.

Venous skin changes and ulceration are predominant manifestations of CVD. Teleangiectasia and varicose veins are reported to be extremely common, with prevalence of about 80% and 20-64% respectively. Other characteristic symptoms include oedema and

skin changes, hyperpigmentation, eczema, atrophie blanche and lipodermatosclerosis. Primary symptoms as heaviness and aching of legs, especially during prolonged standing, can worsen the quality of everyday life. This may consequently lead to aggravation of daily activities and professional working. Nevertheless, the early symptoms can be alleviated by regular exercise, leg elevation and avoidance of prolonged steady standing.

The aim of this study was to determine both level of public knowledge on CVD and prevalence of first symptoms in a wide variety of patients. 175 patients took part in the research, where the mean age was 44. The group consisted of randomly assorted patients who participated in a preventive assessment of nevi.

As 64.57% of the patients stated, Duplex ultrasound (DUS) examination is currently a gold standard for diagnosis of CVD. It is based on a combination of ultrasound imaging and pulsed Doppler wave. Both anatomy and hemodynamic features of venous system can be examined. 62.85% of the patient claim to know what CVD is, whereas 73.14% of them claim that the disease is common. As subjective symptoms and objective signs of CVD they could mainly associate oedema (70,86%), leg fatigue (65,14%) and varicose veins (69,71%).

Most of the patients recognize phlebectomy (68,57%) and use of compression stockings (65,71%) as main therapeutical measures for CVD [24]. Compression therapy is widely used, because of its accessibility, non-invasiveness and high efficacy in increasing venous flow. Nevertheless 13,71% of the patients claim, they do not know any therapeutical measures for CVD and 24,57% of the patients stated that dietary supplementation is effective for CVD treatment. There is no evidence based premised to support this thesis. What is more, 26,29% of them point out by-pass grafting. This is obviously not a routinely recommended therapeutic measure for CVD. Unfortunately, only 26,29% of the patients

identified sclerotherapy as a highly effective measure to treat CVD. Sclerotherapy, which involves injection of liquid or foam agents to dilated veins, leads to damage of the endothelium and consequently ablation of the veins. It is an easily repeatable treatment that that has good long-term results.

As it comes to subjective symptoms and objective signs of CVD 54.86% of patients claimed to have teleangiectasie, 61,71% have pressure marks (impressions by socks in the evening), 30.86% have varicose veins. Dermatologists confirmed varicose veins in 15,43% of the patients, while 7,43% of the patients did not recognize any of the symptoms recognized by the physician. On the other hand, physicians ruled out varicose veins in 15,43% of the patients. This directly implies that further measures should be undertaken to spread the knowledge on CVD among the society.

Physicians should regularly examine both legs thoroughly. It should be an ultimate aim to share the knowledge on CVD among the society and encourage people to undergo further diagnostics. It may be easily done by general practitioners. The highest purpose of those measures would be to avoid high-cost treatment of ulcers and varicose veins.

Contribution of the PhD applicant towards the publication: creation of the concept of the paper and preparation of the methodology; coordination of the project; creation of a database; analysis of the collected data and preparation of the figures; collection and analysis of the literature; drafting of the paper; participation in the final editing of the paper to comply with the recommendations of the reviewers and the editors; person responsible for mail contact with the editors.

Study 2

Study entitled **Assessment of effectiveness of the full-thickness skin micrograft, using a skin punch method, for accelerating the epithelialisation of the wound bed,** presents preliminary results on efficiency of micrografting.

The degree of epithelialization for the group was compared and tested for statistical significance using the Pearson's chi squared test, assuming a significance level of 0.05.

The average numbers of grafts placed on the donor site was 5.6 ±0.980. Whole procedure lasted on average 39±4.301minutes. Average VAS value of the procedure was 4.4±0.927. On average 93±4.899 of the grafts were accepted on day 7, where in 3 patients all the grafts were adopted. On average, 74.272% ± 10.928 of the area of wound at day 0 has epithelialized at day 30. Moreover, in 2 patient complete closure of the wound was observed after 30 days. (Table 3, Fig.1-3). No adverse effects were recorded.

As the results met the normal distribution, and p calculated by Pearson's chi squared test was equal 0,322, and therefore is greater than 0.05, the results are significant, and do not differ by chance. The observed distribution is therefore not the same as in patients with chronic ulcers, who are treated conservatively.

Patient no.	Wound area at day 0 (cm^2) *	Time of the procedure (min)	VAS scale value (1-10)**	Wound area at day 7 (cm^2) *	Percentage of grafts accepted on day 7 (%)	Wound area at day 30 (cm^2) *	Degree of wound epithelialization at day 30*** (%)
1	13.651	35	3	11.680	100	0.000	100.000
2	5.131	30	2	4.730	75	2.702	47.339
3	51.431	55	4	37.339	90	21.802	57.609
4	6.728	35	7	3.223	100	0.000	100.000
5	9.394	40	6	8.285	100	3.155	66.415
MEAN ± standard error	17.267±8.662	39±4.301	4.4±0.927	13.051 ± 6.246	93±4.899	5.531± 4.120	74.272 ± 10.928
Confidence level (95%)	-	11.942	2.574	-	13.602	-	30.342

Table 3 Wound area status among the study group at day 0, 7, 30 and the degree of wound epithelialization, as well as time of the procedure and pain scale

*=Planimetry, ** visual analog scale for pain, ***calculated relative wound epithelialization= (wound area at day 0 - wound area at day 30) /wound area at day 0 * 100%

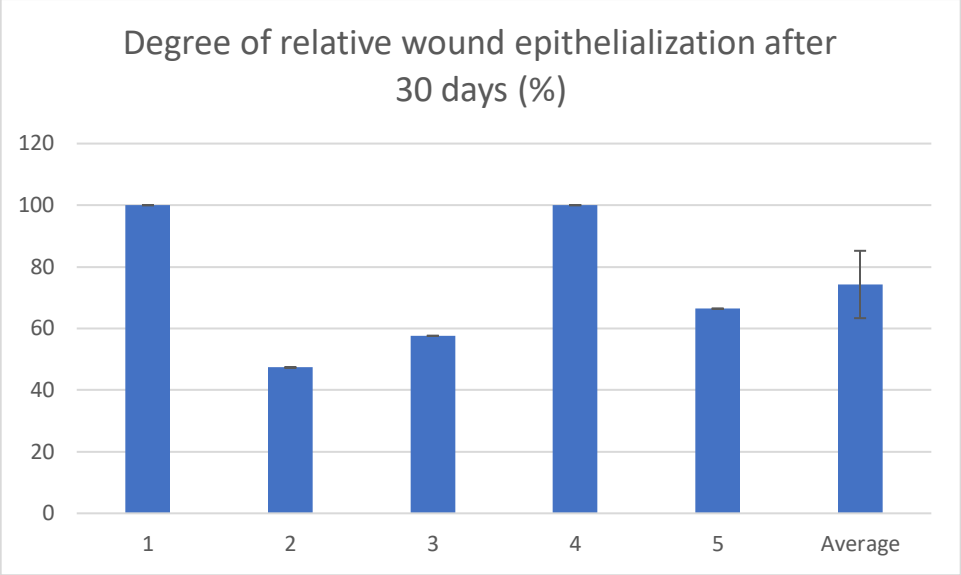

Figure 1 Degree of relative wound epithelialization after 30 days in 5 patients and avarage value

* calculated relative wound epithelialization= (wound area at day 0 - wound area at day 30)/ wound area at day 0 * 100% **Error bars indicate standard error.

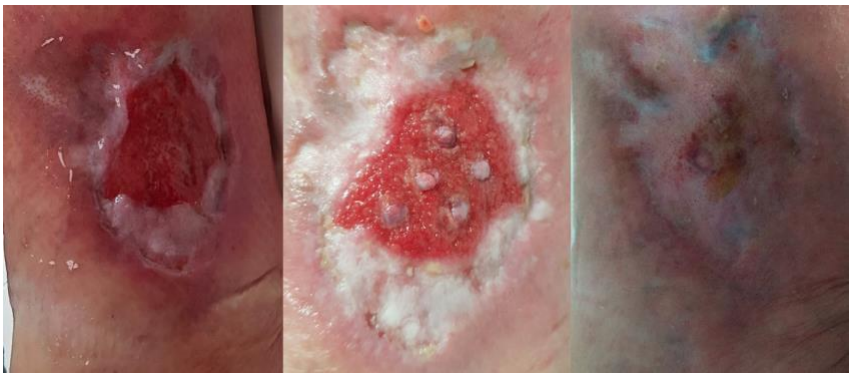

Figure 2 Patient's number 1 wound status, on the left- before the procedure at day 0, in the middle – at day 7, on the right- at day 30

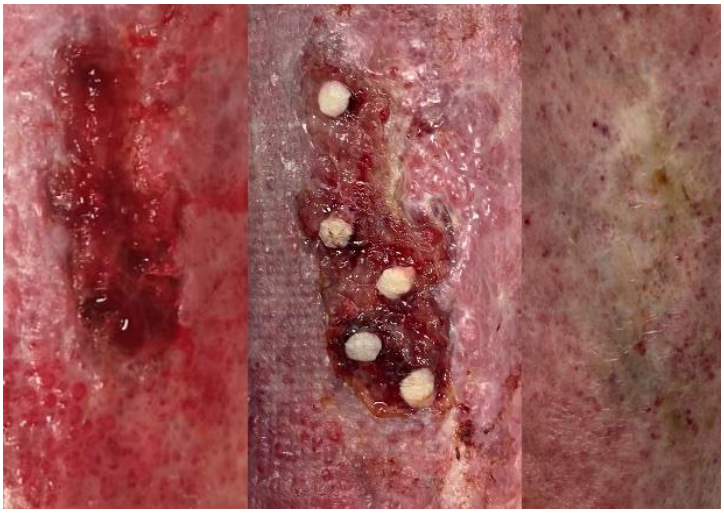

Figure 3 Patient's number 4 wound status, on the left- before the procedure at day 0, in the middle – at day 7, on the right- at day 30

The experience of the Dermatology Clinic allows us to deduce that micrografts with the help of skin punch have a very high potential to accelerate the epithelialisation of the venous ulcers. Compared to the procedure performed with the help of Cellutome and dermatome, they are cheaper and easier to perform. The cost of one procedure is approximately 10 Euros and includes two skin punches and suturing kit. In comparison, a dermatome or Cellutome skin graft cost approximately 1 000 Euros per procedure. This method can shorten wound healing and accelerate epithelialisation, without the need to hospitalise the patient before and after the procedure. It can significantly lower the expenses of wound treatment among the patients with chronic ulcers, which are becoming a growing problem for all physicians across the Europe, especially within dermatology wards.

As with every method, where tissue integrity is compromised, there is a possibility of bleeding, infection, hematoma formation, prolonged healing of both recipient and donor sites [25].[i] The risk is yet similar to the one, when perfoming a skin biopsy, which is relatively low, as the puncturing is around 4-5 mm in diameter. Aware of those complication, we maintain sterile conditions and hemostasis during the procedure. Improper transplantation or incorrectly applied dressing may also lead to the fact that

transplant will not be accepted, hence the immobilization of places where transplants have been located is so important after the procedure. The first days after skin transplantation and proper management will have a major influence on the wound healing process. This is why, we stress the importance of using compression therapy throughout the whole process. It is essential to treat the underlying condition to maintain proper blood supply to the skin. No compression therapy was used in the studies that have already been published, which could have prolonged the healing time of the ulcer.

Undoubtedly, randomised controlled trials on bigger study sample would be advised and could contribute to confirmation of the initial results. We suggest gathering 3 study groups, to get statistically significant comparison of treatment outcomes after micrografting procedure, treatment with wound dressings only and patients subjected to more conventional skin transplantation procedures, such as split-skin grafting.

Study 3

Wound debridement products and techniques - clinical examples and literature review

Among the wound cleaning technique, we distinguish (Fig. 4):

Figure 4 Among the wound cleaning techniques used in out-patients we distinguish: 1-2- monofilament cloths 3-4-sterile sponges 5-6-dressings made of poly-absorbent fiber 7-9-alginate dressings 10- collagenase ointment

Mechanical wound cleansing

Mechanical debridement is a debridement method that uses physical strength to remove necrotic tissue. These techniques are most often used for initial tissue cleansing preceding other debridement methods. The biggest problem with mechanical debridement techniques is that they are nonselective and both viable and non-viable tissues can be removed [26]. They also require premedication, as they may lead to episodic pain [27]. Special caution should be taken when treating patient who takes anticoagulants as it can be a contraindication for sharp debridement (when INR is >2.5). Such a patient should be observed longer after performed debridement procedure to manage possible bleeding [1].

Debridement with surgical tools and wet-to-dry dressings are the most commonly used form of mechanical debridement. Therapeutic irrigation (delivered by pulsed lavage or the agitation of water during whirlpool therapy) and ultrasound therapy are more sophisticated forms of mechanical debridement.

W**et to dry technique**

The "wet to dry" method involves applying gauze dressings impregnated with antiseptics or lawaseptics directly onto the wound, followed by covering the wet dressings with dry ones. This is an archaic method, although it seems that it is still commonly used in both the United States and Poland. Despite the number of disadvantages such as: low effectiveness, increasing risk of infection in the wound, pain associated with dressing change, necessity of frequent dressing change; the method is used due to the speed of application, no need for special qualifications and the wide availability of sterile gauze in treatment rooms [28].

Cleansing sponges

Modification of the wet-to-dry method are sterile sponges, which are soaked with antiseptic fluid. According to the manufacturer's recommendations, the sponge with coarse texture and rough surface is used to wash the wound from necrotic tissues, while the fine texture sponge should be placed in the wound soaked with antiseptic fluid and then covered with a dry sterile dressing (Fig. 5).

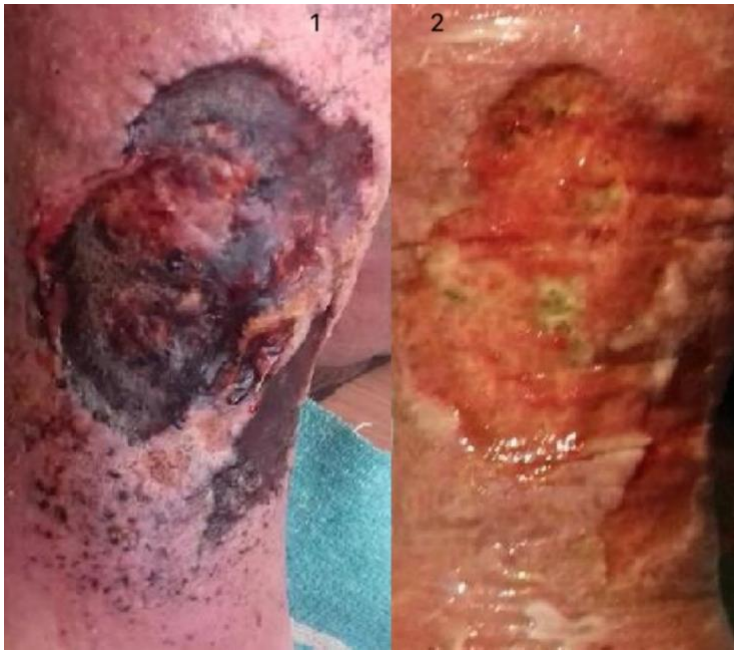

Figure 5 The effect of debridement with a sterile sponge twice a week after two weeks on Fig5.2.
55-year-old male patient with a 2-year-old venous ulcer with aboundant necrosis on left leg. The ulcer developed after erysipelas. Doppler ultrasound confirmed the insufficiency of the saphenous vein valves.

This antiseptic fluid, due to a surfactant's properties, enhances the removal of the non-viable tissue. This is a result of lowering the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. It aids in aggregating and consequently removing the necrotic tissue. Blood flow is stimulated around the wound bed which encourages the body's natural reactions to healing contaminated or infected wounds. This consequently leads to the promotion of granulation.

Cleansing with monofilaments

Another alternative for gauze is cleansing with special disposable cloths. There is a product that consists of a soft, dense nap of monofilament, 100% polyester fibres knitted to the reverse side and secured with polyacrylate. It is for single use only. Cloths are a rapid, highly effective, safe and easy method of debridement for superficial wounds containing loose slough and debris (Fig. 6).

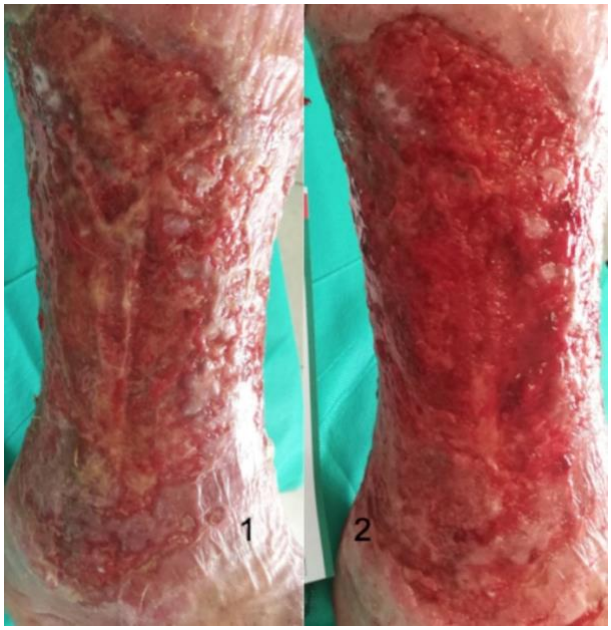

Figure 6 Fig 6.2 shows the effect of one-time debridement with a monofilament cloth.

82-year-old female patient suffering from chronic venous insufficiency known for more than 20 years. At the time presenting a two-year old exudating ulcer on a right leg with maceration of the surrounding skin.

This includes leg ulcers, pressure ulcers, diabetic foot ulcers, and post-operative wounds healing by secondary intention. It is also very effective in the removal of hyperkeratosis from the skin. Debris and exudate are actively loosened from the wound by the fibre. Skin flakes and keratoses are also removed from the surrounding skin. The fact that this procedure is significantly less painful than conventional methods is worth noting.

A monofilament debridement pad, according to the NICE (National Institute for Health and Care Excellence) Medical Technology Guidance remained cost saving in most analyses and savings ranged from £77 to £222 per patient compared with hydrogel, from £97 to £347 compared with saline and gauze, and from £180 to £484 compared with larvae depending on the assumptions included in the analysis and whether debridement took place in a home or clinic setting [29].

Cleansing with surgical instruments

The most expensive example of mechanical cleansing method is sharp tool debridement performed under general anaesthesia in operating theatre. Nevertheless, surgical debridement should be considered whenever the goal is to quickly remove large amounts of necrotic tissue [30].

In the operating theatre loads of different methods could be performed, from sharp tool to ultrasound debridement. Sterile conditions have several advantages, among those the possibility of quick and very accurate excision of all necrotic tissues without worrying about the patient's pain. It also enables physicians to simultaneously combine different methods like wound cleansing and skin grafting or negative pressure wound therapy.

An effective and simple debridement method is to remove impurities using sterile surgical instruments: Volkmann bone curette spoon, a scalpel, or tweezers. This is a quick way to get rid of necrotic tissue from a wound. Mechanical cleaning with a spoon should be an inseparable element each time the dressing is changed in the conditions of the hospital ward and the treatment room (Figs 7-8).

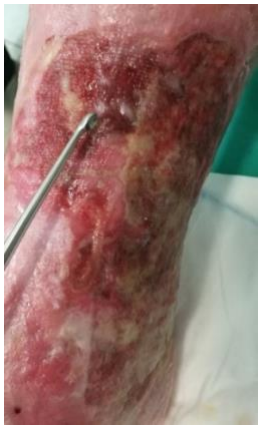

Figure 7 Surgical debridement method using Volkmann spoon. Area subjected to this form of debridement should be anaesthetized, preferably using occlusive lignocaine dressing.

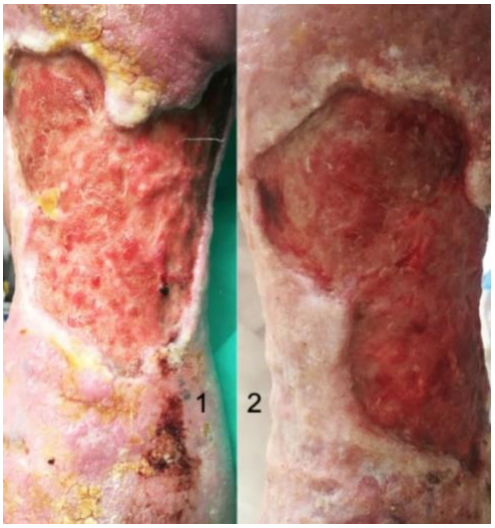

Figure 8 Fig 8.2 shows the effect of surgical debridement using Volkmann spoon, a week after the procedure.

32-year-old male patient presented with a two-year old leg ulcer and severe lipodermatosclerosis on his left leg. Ulcer had uneven raised edges. Patient has been suffering from chronic venous insufficiency and deep vein thrombosis since 5 years.

Careful use of a scalpel makes the procedure quick and painless, and the effect that we can achieve with this method often replaces long and arduous dissolution of the necrosis with the help of dressings (Fig. 9).

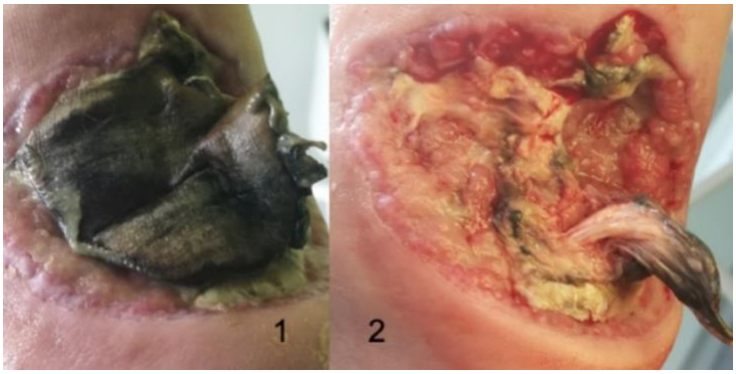

Figure 9 Fig 9.1 before and Fig 9.2 after surgical debridement of the necrosis with a scalpel. Female patient, aged 42 with advanced diabetic neuropathy since 5 years, has developed a necrotic wound at left heel after prolonged heat exposure (due to numbness she left her feet on a heater for 30 minutes)

Volkmann spoon is an indispensable tool in the clinics where wounds are processed. It allows us to remove the biofilm and yellow fibrin from the wound [31]. Patient should receive 2% lignocaine gel on the wound, embedded in an occlusive dressing for at least 30 minutes, prior to using the Volkmann spoon.

Ultrasound wound cleansing

Ultrasound debridement is a promising technology that stimulates wound healing by decreasing exudate and slough, decreasing patient's pain and dispersing the biofilm [32]. Most commonly used low-frequency ultrasound used for cleansing ranges between 20 and 60 kHz and has longer wavelengths and greater amplitude for a given input energy, which results in greater movement of molecules within tissues. Low-frequency ultrasound debridement enhances removing devitalized tissues through microstreaming and cavitational effects [33]. Ultrasound wound cleansing is more selective than previously described methods.

A randomized double-blind controlled trial has compared low-frequency low-intensity ultrasonic debridement to a sham treatment (saline mist without ultrasound) in patients with recalcitrant diabetes-related foot ulcers. Ennis et al. found that after 12 weeks of treatment 40.7% of patients who underwent LFUD had healed compared to only 14.3% in the sham treatment group [34].

Hydrosurgery

Hydrosurgery uses a high-pressure jet of sterile saline (0.9% sodium chloride) to debride wounds and, through a localized vacuum effect on the surrounding tissue, promotes cutting and aspiration of the devitalized tissue [35]. It allows to cut, remove soft tissue and reduce the bacterial load in the wound. Hydrosurgery is a highly advanced technique, that enables the operator to adjust location and depth of the cut. It is a fast and easy method for

cleansing both acute and chronic wounds. Moreover, it has been proven that it allows removal of necrotic tissue and drainage of infected tissue by reducing the bacterial load and restoring a vital wound bed [36].

A prospective controlled study comparing hydrosurgery with conventional surgical debridement carried out by Caputo et al. on 41 patients with leg ulcers has shown that time needed for debridement was 10.8 min compared with 17.7 min and thus significantly shorter for the hydrosurgery group. On the other hand, the clinical efficacy did not differ significantly between the groups. The median wound healing time was 71 days for Hydrosurgery and 74 days for conventional surgical debridement [37]. It has been stated that the average VAS score was lower in patients treated with hydrosurgery.

Negative pressure wound therapy

Negative pressure wound therapy, also known as vacuum-assisted closure (VAC) is a type of therapy that decreases air pressure on the wound below the atmospheric pressure. This can help the wound heal faster. By removing the pressure over the area of the wound, it can gently pull fluid from the wound, as well as reduce swelling, and may help clean the wound and remove bacteria (Fig. 10) [38].

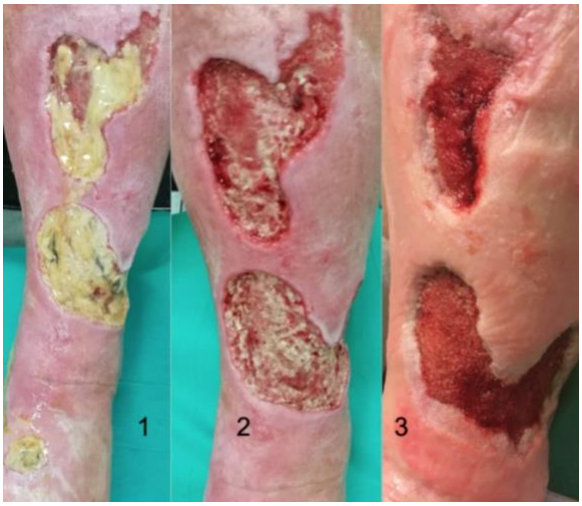

Figure 10 Fig 10.1 at the first visit, Fig 10.2 after debridement with Volkmann spoon and Fig 10.3 the effect after 3 months treatment with negative pressure system.
69-year-old female patient with deep, multiple venous ulcers filled with biofilm on left leg. Ulcers developed as a complication after the treatment of erysipela two months before.

This therapy is particularly advised if there is traumatic tissue loss, if primary wound closure is not possible or if the wound has to be left open or reopened because of an infection.

A wound vacuum system has several parts. A foam or gauze dressing is put directly on the wound. An adhesive film covers and seals the dressing and wound. A drainage tube leads from under the adhesive film and connects to a portable vacuum pump. This pump removes air pressure over the wound. It can do it all the time or in cycles. There are also sets that simultaneously enable to rinse the wound with lavaseptic [39]. The dressing is changed every 24 to 72 hours. The biggest disadvantage of this method is that during the therapy, the patient needs to carry the portable pump everywhere he or she goes.

Vacuum therapy accelerates the wound healing process due to numerous mechanisms, among which are the promotion of cell proliferation through mechanical stretching of cells, stimulation of growth of granulation tissue in the wound, increase of blood flow within the wound, removal of wound healing inhibitors found in exudate fluid.

Moreover, it leads to reduction of oedema, and reduction of bacterial colonization of the wound and prevention of cross-infection through the use of a closed system and keeping the edges of the wound closer together [40].

This method is not only used to cleansing shin wounds, yet also wounds found on the chest or abdominal area. It is widely used in patients with complicated postoperative wounds in the abdominal cavity.There are also more publications appearing that demonstrate the efficacy of negative pressure wound therapy in the head and neck. Strub demonstrated that it reduces wound infections, shortens hospital stays, and simplifies wound reconstruction strategies [41]. He also implies that VAC should be included in the wound management strategies of otolaryngologists and facial plastic surgeons. Dhir et.al. published a cohort study in which among 19 patients with a variety of complicated head and neck wounds, 84% of patients healed completely after VAC therapy [42].

This directly implies that VAC is a very efficient and promising therapy, that might soon find new indications.

Maggot therapy

Maggot therapy is a type of biotherapy that involves placement of disinfected fly larvae (*Lucilia sericata) into* a non-healing wound. The base of their action is eating out the necrotic tissue. This is the most selective method that's known, since maggots only eat away devitalised tissues.

There are numerous studies suggesting that maggot therapy is more effective and efficient in debriding chronic venous ulcers, pressure ulcers, and diabetic ulcers. Maggot therapy is also associated with a more rapid decrease in wound size and an increase in granulation tissue, making the wounds ready for surgical closure.

In his study, Sherman evaluated a cohort of 103 inpatients with 145 pressure ulcers [43]. It has been shown than within 3 weeks, maggot-treated wounds contained one-third the necrotic tissue and twice the granulation tissue, compared to non-maggot-treated wounds.

Initially, it was thought that debridement with maggots is purely mechanical. Nowadays it has been proven that maggot's excretions also contain proteolytic enzymes, which enables them to have inhibitory effect on both Gram-positive and negative bacteria including MRSA Staphylococcus aureus, Echerichia coli and Pseudomonas aeruginosa [44]. Studies performed by Brown et al. and Harris et al. showed that maggot-derived enzymes can reduce biofilms in leg ulcer patients [45]. The ammonia excreted by maggots is believed to alter the pH of the wound, which inhibits bacterial growth. Maggot therapy is beneficial for debridement, as it reduces biofilm formation and aids wound healing by regulating MMPs and infection [46]. This therapy is still considered controversial by many clinicians, however, it is a method widely accepted by prestigious wound management associations.

Nowadays, we can choose from maggots in two forms - classical, free form or closed in a special dressing [47]. Yet study performed by Blake has shown no significant difference in debriding efficiency between those two types. It is crucial to remember that use of occlusive dressings is forbidden, since it prevents oxygen exchange between atmosphere and the wound. For the same reason, use of compressive or negative pressure wound therapy is not advisable.

Among disadvantages of maggot therapy, it is very costly and it requires a proper connection between hospital and laboratory harvesting maggots. This is detrimental for proper treatment, as maggot's vitality is affected by inappropriate transport. It is also

important to strictly follow the producer's manual on when to change the dressing, since after the expiry date maggots can transform into insects.

The use of specialized dressings

There are numerous cleansing dressings on the market, which are most effective when used as a second step, after mechanical debridement. They are worth considering, because of balancing adhesion, atraumatic removal and low bioadhesion. They also provide optimal moist wound healing environment all the way across the surface of the healing wound.

Cleansing-absorbing dressing

It is a dressing whose mechanism of action is based on rinsing the wound with an antimicrobial substance - polyhexamethylene hydrochloride biguanidine, and the absorption of dead cellular elements and excess wound exudate, as well as bacteria and extracellular matrix building bacterial biofilm [48]. The dressing is built in such a way that throughout the entire application, i.e. 3 days, it maintains adequate wound pH, humidity, and reduce inflammation by reducing the concentration of metalloproteinases in the wound environment. The dressing eliminates all known local obstacles in the healing process of chronic wounds: restores the biochemical balance in the wound bed, allows removal of the necrotic load, ensures proper moist environment within the wound, destroys the biofilm and reduces the pH value to physiological level [49]. The use of the cleansing-absorbing dressing for a contaminated chronic wound allows lowering the level of active metalloproteins from the extracellular matrix in the wound bed, removing the residual necrotic layers, absorbing excessive amount of exudate and bringing proper therapeutic moisture into the wound. Furthermore, the dressing absorbs protein content, including all biofilm-building organisms, and lowers the pH to physiological value [50]. Thanks to the

PHMB content, the dressing is an excellent alternative for patients with intolerance to silver. The product is perfect to use with compression therapy, despite the large amount of fluid embedded in it. The dressing is available in two types: for deep wounds with or without pockets(a rinsing-absorbent layer is present on both sides of the dressing) and for superficial wounds (the outer layer is made of waterproof material, which allows the dressing to be used as primary and secondary one at once) The figure shows the effect of the dressing after one application on a difficult-healing wound infected with Pseudomonas aeruginosa (Fig. 11).

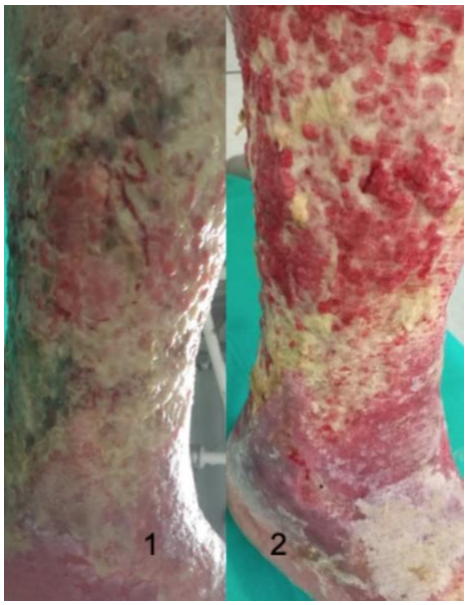

Figure 11 Fig 11.2 shows the effect of one-time application of cleansing-absorbing dressing.
84-year-old patient with encircling ulcer of right leg with abundant biofilm. Ulcer developed 5 years ago, beforehand treated unsuccessfully with gauze debridement. Patient suffers from chronic venous insufficiency and deep venous thrombosis since 25 years.

The disadvantage is the small size of the dressing and therefore the need to combine several dressings on large wounds. Groenewald in his single blind randomized trial compared wound cleansing times achieved with dextranomer dressing with standard therapy. It has been shown that bacteria and cellular debris present in the wound are taken up by capillary action and become trapped in the spaces between the beads. When the

dressing is changed, this debris will be washed away. The beads, which have a high suction pressure (up to 200 mmHg), have been claimed to reduce local tissue oedema and control odour formation [51]. The mean cleansing time for the dextranomer-treated ulcers was 6 days, compared with 15 for the control group, while the average healing time the treated ulcers was 4.4 weeks compared with 5.3 weeks for the control groups.

Dressings made of poly-absorbent fiber (Fig. 4 No. 6)

A novelty on the Polish market is a product designed for cleaning wounds in the form of a dressing made of poly-absorbent fibers. The mechanism of knitting the dressing is based on the mechanical penetration of dressing fiber into the biofilm layer covering the ulcer and its destruction during removing it from the wound [52]. The manufacturer recommends initially changing the dressing every 24 hours for a better mechanical cleaning effect on the wound, then leaving the dressing for up to 7 days. The effects of the dressing are visible on the Fig. 12., interestingly, between the changing of dressings no cleansing of the wound with a bone spoon was used.

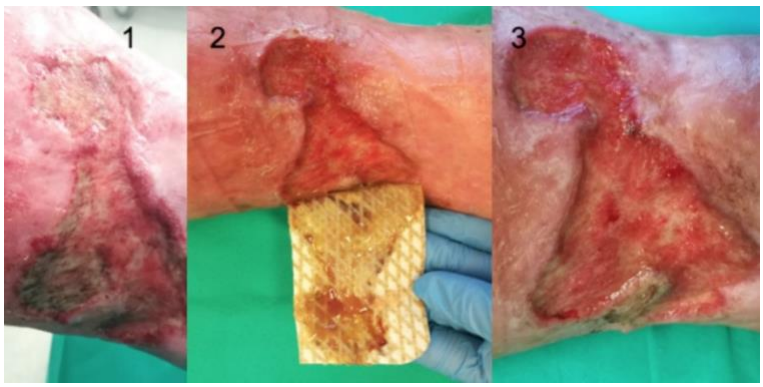

Figure 12 Fig.12.2 showing the cleansing effects of polyabsorbent fibers after one application for 3 days and Fig 12.3 showing the effect after a week.
67-year-old female patient with 9x10cm leg ulcer with uneven edges on left leg since 5 years. Patient was treated with negative pressure therapy a year ago, yet developed a severe allergic reaction with serous blisters formation, followed by their bursting. In 1978 in the same place communication trauma covered with a skin graft.

The dressing itself consists of a thin layer of fibers covered on the inside with a patented lipid-hydrocolloid layer that stimulates healing by ensuring an appropriate level of moisture and oiling the wound, as well as providing atraumatic dressing change. The con of the dressing is its low absorbency. In case of wounds with high and medium exudate, it is necessary to apply a secondary dressing because a thin layer of polyabsorbent fibers has no absorbing properties. The dressing is available in a silver-containing form and without silver, then it is possible to use antibacterial substances such as iodopovidone gel, polyhexamethylbiguanide gel or manuka honey [53].

Gethin et. al. carried out a multicenter prospective randomized and controlled study on manuka honey compared with hydrogel, performed on 108 patients with venous leg ulcers. Significant reduction of wound size after 4 weeks (34 % vs. 13 %, $p = 0.001$) was observed in a group treated with Manuka honey dressing [54].

Alginate dressings

This is a group of dressings available in the offer of almost every company specializing in the production of dressings. At the same time, they are the oldest cleansing dressings on the market. Alginates are biopolymers of natural origin obtained from marine algae [55]. As natural polymers, they are non-toxic and safe to use. They absorb the fluid from the wound and at the same time form gel and provide a physiological, moisture environment for the wound. When a water-insoluble calcium alginate fiber is placed in contact with wound exudates, the calcium ions exchange with sodium ions in the body fluid and calcium ions are released, which can act as a haemostatic agent [56].

Alginates are now manufactured as wound dressings, such as hydrogels, films, wafers, foams, nanofibers, and in topical formulations. Worth noting, there is a hydrogel containing both alginates and carboxymethylcellulose. It is primarily indicated for the

treatment of necrotic leg ulcers, pressure sores and uninfected wounds within diabetic foot. It can also be used for 1st and 2nd degree burns. The gel can be used throughout the treatment period to ensure a moist healing environment for most types of wounds.

Alginates also promote rapid re-epithelialization and granulation tissue formation. Very good effects are obtained by combining amorphous gels and alginate dressings as shown on Fig. 13.

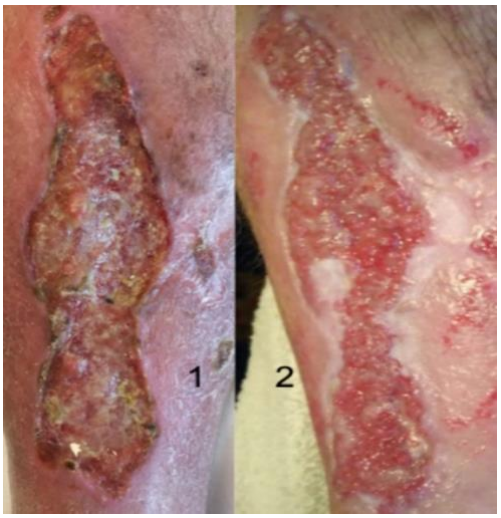

Figure 13 Fig. 13.2 shows the effect obtained by combined application of amorphous gels and alginate dressings after one month.

69-year-old female patient with exudating leg ulcer that developed a year before. Ulcer was deep and irregularly shaped with medium necrotic tissue abundance.

It is worth noting, that sometimes the dressing can smell like natural fresh algae, what can be unpleasant for the patient. There are different types of dressing, ones that dissolve completely and others that do not dissolve completely and leave flocs. Those flocs do not have to be removed completely, since, as most natural materials, they will decay themselves. It is also distinctive that it is possible that discoloration of tissues to green occurs due to single settlements.

(Fig. 4 No. 7), (Fig. 4 No. 8), (Fig. 4 No. 9),

Hydrofibre dressings

Hydrofiber dressings based on carboxymethylcellulose (CMC) can be used particularly on exuding wounds with hydrogels when the wounds are dry. The hydrofibre dressings are recognized for its therapeutic benefit in the healing process of chronic wounds, due to its autolytic properties [57]. Those innovative dressings absorb wound fluid and create a soft gel, maintaining a moist wound environment. Locks in exudate through vertically wicking, reducing the risk of maceration. It substantially minimizes pain during dressing change and while in place. Hydrofibre dressings have been designed to allow optimal fluid transport between the dressings to aid with effective exudate management. Study by Parsons et.al. has proven that hydrofibre dressing are suitable for effective exudate management, in terms of fluid handling capacity, fluid retention and low lateral fluid spread across the dressing surface [58].

The high level of absorbency and marked gelling capacity of the hydrofibre dressing offer autolytic properties conducive to local debridement which are atraumatic to the wound and therefore painless for the patient.

Enzymatic products

Enzymatic debridement base on removal of necrotic tissue through digesting and dissolving the devitalized tissue in the wound. It is a topical treatment that uses natural proteolytic enzymes or proteinsases. Proteinase activity is highly useful, since apart from debridement itself it also enhances cell migration that is fundamental for epithelialization. Some enzymes are selective towards non-viable tissue, yet some are nonselective. Enzymatic debridement could be implemented when there are contraindications for mechanical debridement. Enzymatic treatment shouldn't be used when advanced necrosis is observed and the wound is dry as they need moisture to act.

Several enzymatic debridement agents have been developed, such as trypsin, streptokinase–streptodornase combination and subtilisi [59].

Papain is a nonspecific cysteine protease derived from the fruit *Carica papaya* and capable of breaking down a variety of necrotic tissue substrates. The role of urea is to facilitate the proteolytic action of papain by altering the structure of proteins [60]. Papain is nonselective, targeting for degradation any protein containing cysteine residues (which are present in most proteins, including growth factors). Papain-urea preparations have been in clinical use for decades–particularly for pressure ulcers–and available literature indicates that these debriding systems are effective when used properly.

Collagenase ointment is another example of enzymatic debridement that is theoretically selective as it breaks down solely collagen, that is a major component of nonviable tissue in the wound [61]. It is derived from bacteria Clostridium histolyticum. Collagenase is supposed to be safe in infected wounds, yet it is most effective in physiologic pH. It removes detritus without harming the viable tissue. As a result, it enhances granulation and afterwards epithelialization of ulcer site.

Collagenase can shorten an excessive inflammatory period by down-regulating inflammatory cytokines [62]. It can also promote healing by enhancing cell migration, proliferation and angiogenesis.

Study performed by König et.al. at a group of 42 patients with chronic venous ulcers has shown that during the first 14 days the slough within the groups was reduced by almost 19% for wound pad with Ringer's solution and by 9% for ointment with collagenase, followed by an increase of 26% and 10% respectively in granulation tissue. Although the wound pad with Ringer solution appeared to be more efficient in a few cases, the general efficacy of the two products appeared to be almost the same as no statistically significant superiority of either method [63].

Another study by Waycaster and Milne concentrated on cost-effectiveness ratio that also derived for hydrogel dressing and collagenese ointment, based on the expected total costs per patient and the clinical benefit conferred, based on the number of epithelialized days occurring across a 1-year time. Patients treated with a hydrogel dressing incurred total treatment costs that were 2.7-times higher than those treated with collagenase. The clinical benefit of collagenase was 1.5-times greater when com- pared to the hydrogel [64].

Autolytic debridement

(Fig. 4 No. 10)

Autolytic debridement involves the use of moisture-donating or moisture-retentive dressings such as hydrogels, hydrocolloids or transparent films, which are placed over the wound and allow the endogenous enzymes within the wound fluid to digest and liquefy necrotic tissue. The dressing is easy to apply and is typically left in place for 2–3 days. After it is removed, the wound should be irrigated with normal saline to remove liquefied debris.

Autolytic debridement is indicated for wounds with necrotic tissue to rehydrate and soften the hard eschar depending on the slough aboundance – hydrogels for moderate or no exudate, while absorptive hydrofibers for exudative wounds [65].

Unfortunately, this technique is slower and therefore can require multiple dressing applications and irrigations for several weeks or longer. It can also be less effective within older people, especially with chronic wounds connected with compromised immune system. Autolytic debridement is also not appropriate for infected wounds or very deep cavity wounds that require packing [66].

Amorphous gels and hydrocolloids are intended to clean the wound and provide a moist environment that is favorable for healing. The dressing protects the wound from friction damage, as it provides extra fluid under the dressing. It is also almost painless to

change those dressings. For moist environment stimulates recruitment of leukocytes and consequently release of natural painkillers, it provides pain relief. Modifying the pH of the exudate favorably influences the action of sodium and calcium channels that are involved in pain response [67]. Ringer's solution is one example of those dressings, that has a dilution effect and leads to change in pH that consequently decreases the inflammatory response and reduces negative effect of pro-inflammatory components, such as metalloproteases [68].

A clinical study by Heffernan on 96 patients compared a new hydrocolloid dressing with a non-adherent dressing in the management of lacerations, abrasions and minor operations. While time to heal was similar for both groups, the patients using a hydrocolloid dressing experienced less pain, required less analgesia and were able to carry out their normal daily activities including bathing or showering without affecting the dressing or the wound [69].

A study by Schmidt et.al. conclude that, in addition to providing a moist wound-healing environment, certain hydrocolloids might contribute to the establishment and maintenance of the reducing environment necessary for energy production and hence cell division [70]. The release of hydrogen peroxide into the wound environment could conceivably contribute both to the inflammation phase of wound healing and to fibroblast proliferation and hence the granulation phase.

The increasing incidence and poor socioeconomic outcome of treatment of chronic and difficult-to-heal wounds inspired researchers to work on more efficient and cost-effective ways. Nowadays we have numerous different treatment methods, including new debridement techniques that can be easily performed by patients or their guardians. According to TIMERS strategy, debridement is the first step, that is detrimental for proper wound healing. It has been proven that adequate cleansing can influence the rate of wound

closure. The choice of the method has to be based on exudate abundance, infection presence, amount of non-viable tissue and degree of moisture of the ulcer, as well as skills of the physician and other caregivers.

Whether immediate removal of non-viable tissue is necessary, surgical or sharp tool debridement is indicated. On the other hand, those methods are not a long-term option, and should be performed by an experienced physician. Combining two or more methods often brings better clinical outcome. For instance, it is advisable to treat wound with enzymatic cleansing for a week before performing surgical debridement.

Once a wound needs two sequential episodes of sharp debridement, maintenance debridement with collagenase should be employed. This can be augmented by both using autolytic debridement in the form of secondary dressings such as foams; and/or mechanical debridement strategies as well. Foremost, patient's pain and availability should be taken into account first, when deciding on debridement method.

Comparison of the efficacy of novel non-invasive mechanical debridement methods - sterile sponge, monofilament fiber cloth and non-woven cloth impregnated with sodium hyaluronate and phospholipids in relation to traditional sterile gauze

All 32 patients enrolled to the study completed it. Characteristics of the study group at the admission has been shown in Table 4.

	Sterile gauze	blue Schülke WoundPad®	Debrisoft Pad®	CleanWnd®
Male (n)	4	4	2	5
Female (n)	4	4	6	3
Age (mean years ± SD)	73.87 ± 10.81	74.25 ± 13.53	80.37 ± 9.39	75.27 ± 15.30

Previously healed ulcer (n)	4	2	4	1
Phlebectomy (n)	0	0	2	1
Mean wound duration (months)	14.63	20.50	25.50	22.50
Median wound duration (months)	11.50	13.50	24.00	16.50
Mean wound area (cm^2± SD)	40.43 ± 30.08	38.20 ± 25.28	43.04 ± 74.53	54.86 ± 63.88
Median wound area (cm^2)	34.13	31.68	14.36	20.96
P (SW)	0.182	0.086	0.000	0.029
Clinical status of the wound bed tissue				
Necrotic tissue (mean value)	85.52%	84.58%	76.42%	91.00%
Granulation tissue (mean value)	14.48%	15.42%	23.58%	9.00%
Mean exudate aboundance (scale 0-5)	4.00	4.13	4.13	3.88
Periwound skin condition				
Hyperkeratosis (n)	5	5	1	1
Maceration (n)	3	0	5	1
Eczema (n)	1	3	5	3

Signs of inflammation in the wound (n)				
Redness (n)	0	1	1	4
Swelling (n)	2	4	3	3
Odour (n)	3	2	2	2
Healing cessation (n)	0	0	0	0
Pain within the wound (n)	0	0	2	0
Average cost of the debridement method for 30 days (zł)	3.78*	295.20*	114.30*	55.44**
n = number of patients SD- standard deviation SW- Shapiro Wilk's test * average prices in Poland, ** price in Poland after 30% refund for patients with chronic ulcers (>3months)				

Table 4. Baseline characteristics and medical history of the study groups

Mean wound area at day 0 (cm^2) was respectively 40.43 ± 30.08 for gauze, 38.20 ± 25.28 for blue Schülke WoundPad, 43.04 ± 74.53 for Debrisoft Pad, 54.86 ± 63.88 for Clean Wnd (Tab.4). Mean necrotic tissue aboundance (% of total wound area) was respectively 85.52 for gauze, 84.58 for blue Schülke WoundPad, 76.42 for Debrisoft Pad, 91.00 for Clean Wnd.

Wound area distribution in the majority of the study groups had either a distribution consistent with the normal distribution or not statistically different from the normal distribution $p > 0.05$. Anova comparative analysis of variance showed that the study groups did not differ significantly in terms of the total wound area at day 0 ($p = 0.924$). For those reasons the relative reduction in both total wound area and necrotic tissue between the study groups could have been compared.

In order to compare the efficacy of all the products, the relative reduction of total wound area between day 0 to day 30 and necrotic tissue area after first debridement procedure and between day 0 before the debridement and day 30 after the debridement was calculated.

The comparison of the reduction in wound area on day 30 compared to day 0, shows average 20.69% ± 21.93% reduction in gauze group, 23.55% ± 27.38% for Schülke Wound Pad group, 15.41% ± 25.72% for Debrisoft Pad group and 12.51% ± 17.95% for CleanWnd group (Tab. 5).

	Relative reduction [%]								
	Total wound area from day 0 to day 30		Necrotic tissue after debridement at day 0	Necrotic tissue from day 0 to day 30	Satisfaction [0-10]	Cleaning time day 0 [min]	Cleaning time day 30 [min]	VAS day 0 [0-10]	VAS day 30 [0-10]
Gauze	*Min - Max*	0 - 56.05 %	0 - 52.05 %	9.39 - 95.59%	4 - 6	4 - 10	4 - 10	4 - 8	4 - 8
	M ± SD	20.69 % ± 21.93 %	23.53 % ± 19.16 %	44.95% ± 31.47%	4.75 ± 0.89	5.63 ± 1.92	5.25 ± 2.19	6.13 ± 1.89	6 ± 1.2
	Me	11.01 %	19.11 %	43.10%	4.5	5	4	6.5	6
	p (SW)	0.082	0.483	0.513	0.018	0.011	0.001	0.024	0.534

	Min - Max	0 - 80.71 %	14.03 - 62.71 %	19.48 - 100%	7 - 9	3 - 7	3 - 7	3 - 6	2 - 4
Schülke Wound Pad®	*M ± SD*	23.55 % ± 27.38 %	34.11 % ± 17.51 %	68.38% ± 28.78%	8.13 ± 0.83	4.5 ± 1.31	4.25 ± 1.28	5 ± 1.31	3 ± 0.93
	Me	15.62 %	28.61 %	73.61%	8	4.5	4	5.5	3
	p (SW)	0.090	0.237	0.498	0.067	0.283	0.037	0.008	0.030
	Min - Max	0 - 76.83 %	22.59 - 100%	22.59 - 100%	6 - 9	4 - 7	3 - 5	2 - 7	2 - 5
Debrisoft Pad®	*M ± SD*	15.41 % ± 25.72 %	63.44 % ± 32.91 %	74.65% ± 30.95%	7.5 ± 0.93	4.75 ± 1.04	4.25 ± 0.71	4.63 ± 1.92	3 ± 1.07
	Me	7.98 %	63.77 %	90.04%	7.5	4.5	4	4.5	3
	p (SW)	0.001	0.103	0.035	0.522	0.007	0.056	0.410	0.120
Cleanwnd®	*Min - Max*	0 - 54.29 %	0 - 70.33 %	24.46 - 87.83%	6 - 9	7 - 15	7 - 15	2 - 8	2 - 8

	M ± SD	12.51 % ± 17.95 %	23.56 % ± 22.15 %	60.90% ± 23.44%	7.5 ± 1.07	11.63 ± 2.56	9.5 ± 2.73	5 ± 2.67	4.75 ± 2.31
	Me	8.87 %	20.30 %	67.08%	8	12	8	4.5	5
	p (SW)	0.003	0.143	0.327	0.120	0.796	0.030	0.066	0.373
Anova	*p*	0.782	p<0.01	0.216	p<0.001	p<0.001	p<0.001	0.484	p<0.01
	η^2	0.04	0.35	0.14	0.69	0.75	0.60	0.08	0.46

Table 5. Descriptive statistics, results of Shapiro-Wilk normality analyses and Anova analysis of variance comparisons for study groups with relation to the variability of the wound area and necrotic tissue over time and in terms of satisfaction level, wound cleaning time and pain level

Min- minimum, *Max*- maximum, *M*- mean, *SD*- standard deviation, *Me*- median, *p*- statistical significance, η^2- generalized Eta-square

Relative necrotic tissue reduction at day 0 after debridement was 23.53% ± 19.16% in gauze group, 34.11% ± 17.51% for Schülke Wound Pad group, 63.44% ± 32.91% for Debrisoft Pad group and 23.56% ± 22.15% for CleanWnd group (Tab. 5, Fig. 14)

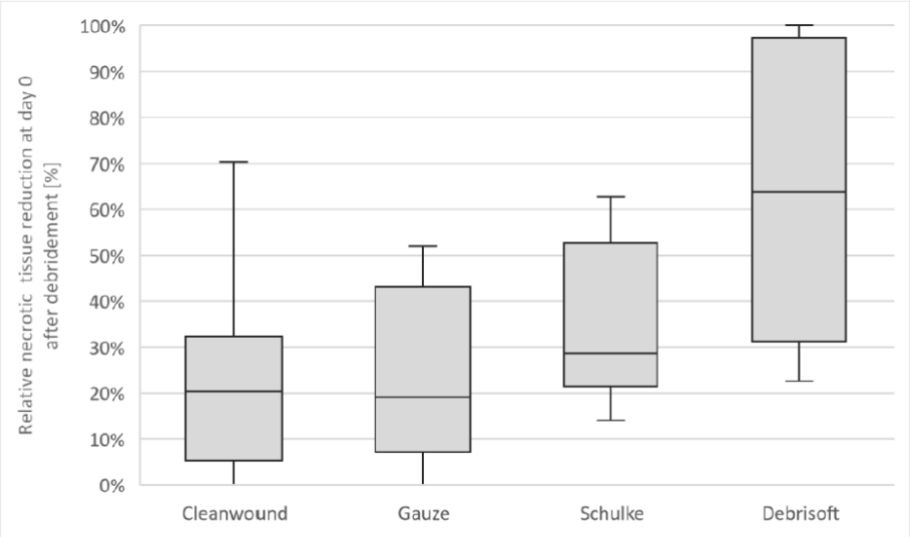

Figure 14 Distribution of necrotic tissue area reduction at day 0 after debridement

It was shown that there the differences between the groups in terms of the relative reduction of necrotic tissue after a single wound debridement on day 0 were statistically significant ($p < 0.01$; $\eta 2 = 0.35$).

Series of comparative analyses with post hoc tests, have shown statistically significant ($p < 0.05$) differences between the reduction of necrotic tissue at day 0 in the group subjected to debridement with Debrisoft Pad than in the other groups of patients subjected to debridement with other methods. (Tab. 5). On the other hand, no statistically significant differences were found in the reduction of necrotic tissue during a single cleaning between the remaining groups (Schülke Wound Pad vs CleanWnd) and in relation Schülke Wound Pad vs gauze or CleanWnd vs gauze.

Relative necrotic tissue reduction at day 30 in relation to day 0 was 44.95% ± 31.47% in gauze group (Fig.16), 68.38% ± 28.78% for Schülke Wound Pad group (Fig.17), 74.65% ± 30.95% for Debrisoft Pad group (Fig.18) and 60.90% ± 23.44% for CleanWnd group (Fig.19) (Tab. 5, Fig. 15).

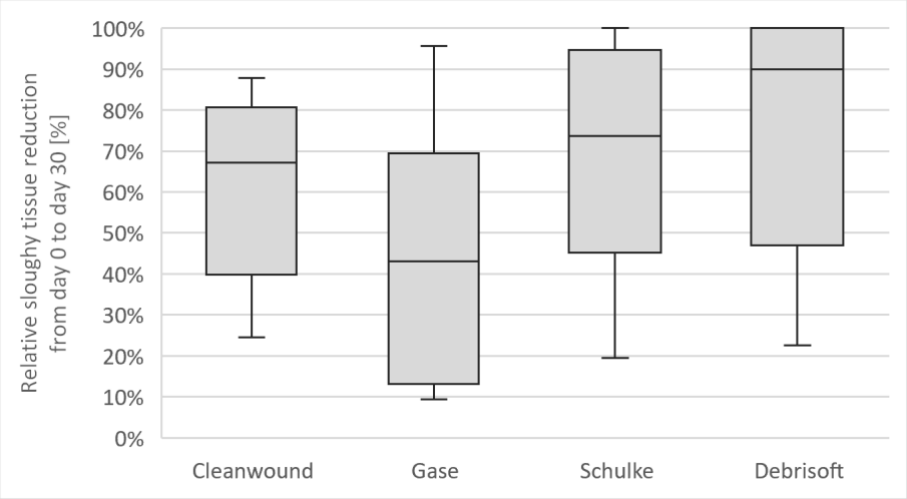

Figure 15 Distribution of necrotic tissue area reduction at day 30 in relation to day 0

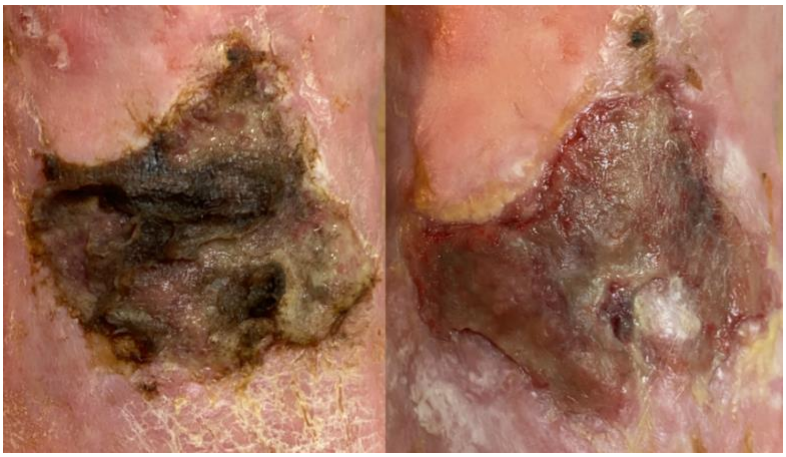

Figure 16 Patient subjected to debridement with sterile gauze, on the left- before debridement at day 0, on the right- after debridement at day 30

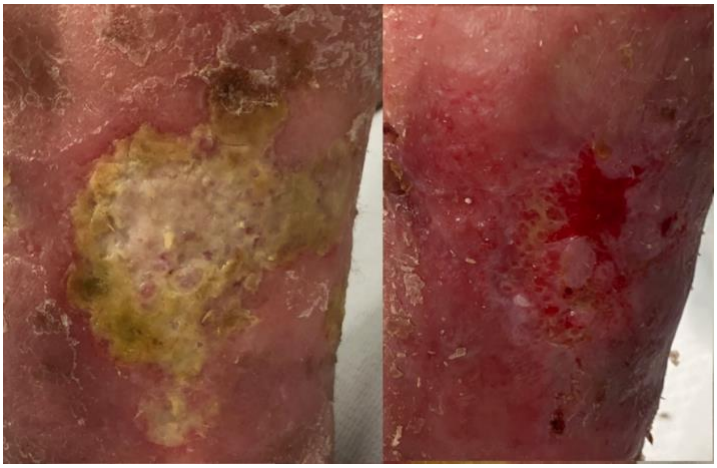

Figure 17 Patient subjected to debridement with Schülke Wound Pad®, on the left- before debridement at day 0, on the right- after debridement at day 30

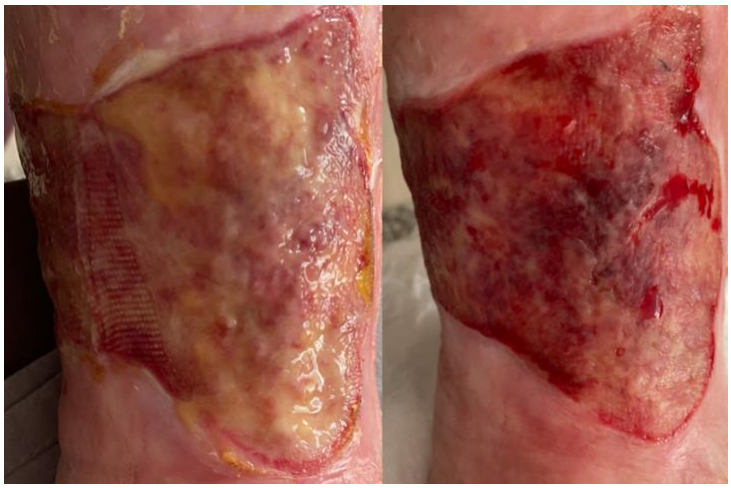

Figure 18 Patient subjected to debridement with Debrisoft Pad®, on the left- before debridement at day 0, on the right- after debridement at day 30

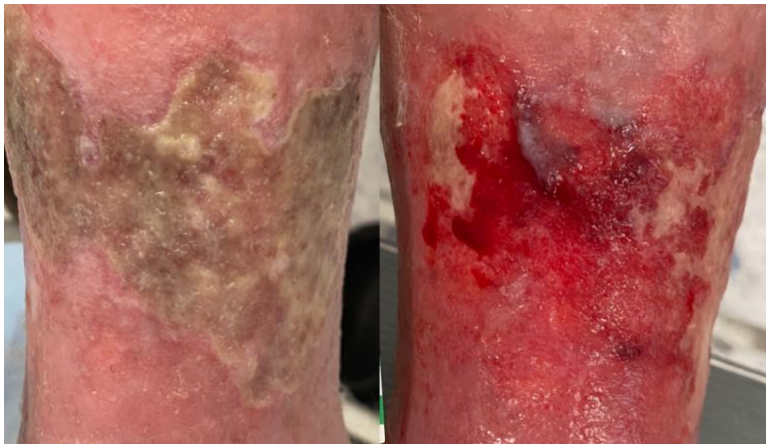

Figure 19 Patient subjected to debridement with CleanWnd®, on the left- before debridement at day 0, on the right- after debridement at day 30

According to Anova test, there was no statistical difference between the groups in the reduction of necrotic tissue over 30 days ($p = 0.216$; $\eta 2 = 0.14$), yet series of comparative analyses with post hoc tests showed that there were statistically significant $p < 0.05$ differences between the reduction of necrotic tissue over the period of 30 days in the group between the Debrisoft Pad group and the group with the gauze. There were no statistically significant differences between the other groups (CleanWnd vs gauze, Schülke Wound Pad), vs gauze, or Debrisoft Pad vs CleanWnd vs Schülke Wound Pad), which suggests that other products tested might not have been more efficient than sterile gauze in necrotic tissue reduction.

Furthermore, the correlation between the variability of the effectiveness of the new non-invasive mechanical cleansing methods at different time periods was examined in relation to the assessment of patient satisfaction, cleaning time and pain level measured in VAS scale. The correlations were calculated for the general group of test persons using a series of Pearson r correlation analyses.

The analysis of variance performed showed that the satisfaction with treatment in the study groups was statistically significantly different ($p < 0.001$; $\eta^2 = 0.69$) (Tab.5). A series of comparative analyzes using Bonferroni post hoc tests showed that patients in gauze group stated a lower satisfaction with treatment at 4.75 points ± 0.89 (in scale 0-10) than those using other methods of debridement, and the differences were significant (8.13 ± 0.83 for Schülke Wound Pad group, 7.5 ± 0.93 for Debrisoft Pad group and 7.5 ± 1.07 for CleanWnd group).

It was also shown that there were statistically significant differences between the study groups in the time of cleaning the wound at day 0 ($p < 0.001$; $\eta^2 = 0.75$) and at day 30 ($p < 0.001$; $\eta 2 = 0.60$) (Tab.5). In both cases, a series of post hoc post hoc analyses of

Bonferroni showed that the cleaning time for the CleanWnd group was longer than for the other study groups. The time of cleaning the wound in the group of people using CleanWnd in the first measurement was over 11 minutes, and in the remaining groups it did not exceed 10 minutes, on average was 5 minutes.

It was shown that the study groups differed statistically significantly in terms of the assessment of the pain scale during the procedure at day 30 ($p < 0.01$; $\eta^2 = 0.46$). Multiple post hoc Bonferroni comparisons showed that people using gauze and CleanWnd rated their pain levels higher than those using Schülke Wound Pad, and Debrisoft Pad.

A series of Pearson's r-correlation analyses showed that there was a statistically significant correlation between the level of pain in VAS scale with the relative reduction of necrotic tissue after debridement at day 0 ($p < 0.05$) and with the reduction of necrotic tissue from day 0 to 30 ($p < 0.05$). Correlations were negative which mean that people who had a greater relative reduction of the necrotic tissue after cleansing both at day 0 and day 30 in relation to day 0 had less pain intensity on the VAS scale. These correlations were of moderately strong intensity.

This study was the first one to compare 3 different non-invasive mechanical debridement methods – sterile sponge, monofilament cloth and non-woven cloth impregnated with sodium hyaluronate and phospholipids in relation to traditional sterile gauze.

The only studies on non-invasive mechanical debridement include small pilot, non-comparative studies, that suggested good results in removing sloughy, necrotic tissue after one use. The aim was to determine if there is any statistically significant difference between 3 products tested and in relation to sterile gauze, which is the first example of non-invasive mechanical method.

As it comes to limitations of the study, we admit that the study groups were rather small, as the study was conducted during COVID-19 pandemic. Undoubtedly, randomised controlled trials on bigger study sample would be advised and could contribute to confirmation of the initial results.

As the results have shown, monofilament cloth - Debrisoft Pad might be the most effective among those. This means that Debrisoft Pad could be potentially seen as an effective successor of sterile gauze, that provides a rapid and easy-to-use debridement method with lower pain level during cleaning.

Even though, that both CleanWnd and Schülke Wound Pad have not shown any statistically significant advantage over sterile gauze, when it comes to efficacy in relative necrotic tissue reduction, there were significant differences in pain level during procedure and patient's satisfaction level. Patients using Schülke Wound Pad, and Debrisoft Pad rated their pain levels in VAS lower than patients using sterile gauze. Moreover, patients using all 3 products tested stated higher satisfaction with treatment (0-10 scale) value than patients treated with sterile gauze. As ulcer treatment is unpleasant itself, it should be the highest aim to raise patient's satisfaction with the treatment. This is why, using three novel methods tested can be suggested, as they are well accepted by patients and cause less pain during the procedure, which is essential for good compliance and complete resolution of the lesions.

Relatively low price of debridement with the use of CleanWnd is also worth noticing. 30 days debridement performed twice a week is consequently approximately 3.78 polish zlotys for sterile gauze, 295.20 zl for Schülke Wound Pad, 114.30 zl for Debrisoft Pad and 55.44zl for CleanWnd. Even though, price of Debrisoft Pad is higher than sterile

gauze, patient's satisfaction and efficacy should be taken first into consideration, when choosing the debridement method.

Results of the study have not shown any statistically significant difference in relative wound area reduction from day 0 to day 30 between the groups studied. Even though this was not the main aim of the research, possibly extending the study duration till at least 3 months, simultaneously adding application of a wound pad, that accelerates epithelialization, after the wound has fully granulated, could result in obtaining more statistically significant differences in wound area reduction between different non-invasive mechanical debridement methods used.

European Wound Management Association included information about Debrisoft Pad as a mechanical debridement method in its guidelines from 2013. Surely more methods available on the market should be added to both european and international guidelines, which would meet the new TIMERS guidelines from 2019, that replaced previous TIME guidelines.

Non-invasive methods are one of those, as they enable both patient or his/her guardian or nurse to use them to bring beneficial outcome to the treatment and higher patient satisfaction. This could undoubtedly lower the costs of hospitalization of sick leaves of those patients and contribute to better life quality.

Review article

Article entitled **Nanomaterials as a Successor of Antibiotics in Antibiotic-Resistant, Biofilm Infected Wounds? published in Antibiotics,** presents current knowledge on alternative biofilm eradication and prevention.

The greatest challenge when treating a chronic wound is prolonged infection, which is commonly caused by uncontrolled bacterial growth and biofilm prevalence. Biofilm consists of microorganisms coated with a self-produced protective extracellular matrix [71]. It has a huge role in protecting the integrity of bacteria, making them resistant to conventional treatment with antibiotics. Therefore, new treatment methods, such as nanoparticles, are tested and implemented. Nanomaterials are particles with at least one dimension between 1 and 100 nM [72]. Lipids, liposomes, cellulose, silica and metal can be carriers of nanomaterials. They are very promising, as their mode of action differs from both their bulk materials and antibiotics, as it relies mostly on direct contact. They owe their properties to a large area-volume ratio. The high surface area allows heat, ions and molecules to diffuse into the particles at a higher and faster rate. Furthermore, nanoparticles usually contain core molecules and a shell that stabilizes the nanoparticle and aids their function by preventing their degradation, oxidation and by increasing their biocompatibility.

Among all, metal nanoparticles have been the most studied in relation to their antibiofilm potency in wound healing [73-74]. The most studied particles are silver nanoparticles, yet metal oxide nanoparticles such as zinc oxide, copper oxide and iron oxide also seem promising as antimicrobial treatments. Due to their small surface area, nanoparticles can penetrate biofilm and eventually penetrate to intracellular bacteria. The high surface area to volume ratio of nanoparticles allows drug loading, which can result in synergistic antibiofilm efficacy. Nanoparticles owe their antimicrobial properties to oxidative stress, formation of reactive oxygen species, metal ion release and nonoxidative mechanisms, enzymatic inhibition, DNA damage and bacteria wall disruption [75-76]. Inhibition of bacterial adhesion by nanoparticles is a key mechanism that enables them to prevent biofilm formation. In comparison to antibiotics, nanoparticles may infiltrate into

the matrix, destroy the extracellular polymer substance and eventually destroy the bacteria within the biofilm [77-78].

This review's aim was to describe in detail the mode of action of those molecules that have been proven to have antimicrobial effects on biofilm and therefore help to eradicate bacteria from chronic wounds. Nanoparticles can enhance an antibiotic's mode of action by increasing its solubility and easing its transport into the cells or can be directly bacteriostatic or bactericidal [79-80]. Research suggests that nanoparticles might either protect conventional antibiotics from degradation by pH or enzymatic activity of biofilm microenvironment or decrease biofilms resistance to the eradication. There is no data on storage, administration and mucous interaction, as well as blood clearance and long-term results, safety and side effects. Undoubtedly, randomized controlled in vivo trials are needed to state the efficiency and place of nanoparticles within novel medical treatment.

Article suggests that nanoparticles seem to be a promising treatment option for infection management, which is essential for the final stage of wound healing, which is complete wound closure.

Contribution of the PhD applicant towards the publication: creation of the concept of the paper and preparation of the methodology; collection and analysis of the literature; analysis of the collected data; drafting of the paper; participation in the final editing of the paper to comply with the recommendations of the reviewers and the editors; person responsible for mail contact with the editors.

CONCLUSIONS

1. The questionnaire study revealed that most of the people are not acquainted with CVI and its first symptoms. It should be an ultimate aim to share the knowledge on CVD among the society and encourage people to undergo further diagnostics.
2. The majority of tested patients tested in the questionnaire study have presented symptoms of initial stages of CVD.
3. Monofilament cloth could be potentially seen as an effective successor of sterile gauze, that provides a rapid and easy-to-use debridement method with lower pain level during cleaning.
4. Patients using all 3 products tested stated higher satisfaction with treatment than those treated with gauze. Results imply that all those methods could be considered, as they are well accepted by patients and cause less pain during the procedure, which is essential for good compliance and complete resolution of the lesions.
5. Skin micrografting with biopsy punch can be an effective and cost-effective alternative for boosting epithelialization of granulated leg ulcers. This method can shorten wound healing and accelerate epithelialization, without the need to hospitalise the patient before and after the procedure. It can significantly lower the expenses of wound treatment among the patients with chronic ulcers.
6. Nanoparticles may be seen as a potential successor of antibiotics in biofilm prevention and eradication.

LIST OF REFERENCES

1. Zubilewicz T., Wroński J., Michalak J., Borgis - Medycyna Rodzinna 2/2002, s. 96-100
2. Simka M., Majewski E., The social and economic burden of venous leg ulcers: focus on the role of micronized purified flavonoid fraction adjuvant therapy. Am J Clin Dermatol. 2003;4(8):573-81.
3. Guest J.F., Fuller G.W., Vowden P., Venous leg ulcer management in clinical practice in the UK: costs and outcomes. Int Wound J. 2018;15(1):29-37.
4. Wittens, C., Davies, A. H., Bækgaard, N., Broholm, R., Cavezzi, A., Chastanet, S., et.al,Editor's Choice - Management of Chronic Venous Disease: Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2015; 49: 678-737
5. Atkin L, Bućko Z, Conde Montero E, Cutting K, Moffatt C, Probst A, Romanelli M, Schultz GS, Tettelbach W., Implementing TIMERS: The race against hard-to-heal wounds. J Wound Care. 2019, 23(Sup3a):1-50
6. Brown MB, Jones SA. Hyaluronic acid: A unique topical vehicle for the localized delivery of drugs to the skin. J Eur Acad Dermatol and Venereol 2005, 19(3):308-18
7. Keen MA. Hyaluronic acid in dermatology. SKINmed. 2017, 15(6):441-448
8. Salwowska NM, Bebenek KA, Żądło DA, Wcisło-Dziadecka DL., Physiochemical properties and application of hyaluronic acid: a systematic review. J Cosmet Dermatol, 2016, 15(4):520-526
9. Olczyk P, Komosińska-Vassev K, Winsz-Szczotka K, Kuźnik-Trocha K, Olczyk K. , Hyaluronan: Structure, metabolism, functions, and role in wound healing. Postepy Hig Med Dosw. 2008, 62:651-9
10. Chhibber T, Wadhwa S, Chadha P, Sharma G, Katare OP., Phospholipid structured microemulsion as effective carrier system with potential in methicillin sensitive Staphylococcus aureus (MSSA) involved burn wound infection. J Drug Target. 2015, 23(10):943-52
11. Tsaousi G, Stavrou G, Fotiadis K, Kotzampassi K, Kolios G., Implementation of phospholipids as pharmacological modalities for postoperative adhesions prevention. European Journal of Pharmacology. 2019, 842:189-196
12. Lahnsteiner E. Case 4: Using octenilin Solution with the schülke WoundPad. J Wound Care. 2016, 25(3 Suppl):S12
13. Haemmerle G, Duelli H, Abel M, Strohal R. The wound debrider: A new monofilament fibre technology. Br J Nurs. 2011, 20(6):S35-6, S38, S40-2
14. Dissemond J, Eberlein T, Bültemann A, Riepe G, Stoffels I, Stephen-Haynes J, Roes C, Abel M. A, A purpose-designed monofilament-fibre pad for debridement of hard-to-reach wounds: Experience in clinical practice. J Wound Care. 2018, 27(7):421-425
15. Benbow M. Using Debrisoft® for wound debridement. J Community Nurs. 2011, 25(5), 17-18
16. Gray D, Cooper P, Russell F, Strindfellow S., Assessing the clinical performance of a new selective mechanical wound debridement product. Wounds UK. 2011, 7(3), 42-46.
17. Bahr S, Mustafi N, Hättig P, Piatkowski A, Mosti G, Reimann K, Abel M, Dini V, Restelli J, Babadagi-Hardt Z, Abbritti F, Eberlein T, Wild T, Bandl K., Clinical

efficacy of a new monofilament fibre-containing wound debridement product. J Wound Care. 2011, 20(5), 242-248.
18. Vinceneux Talvande B, Carvalho Lallement P, Safa G., et al. Use of Cellutome for the healing of venous or mixed ulcers. Int Wound J. 2018;**15(6),** 993–999.
19. Nordström, A., Hansson, C. Punch-grafting to enhance healing and to reduce pain in complicated leg and foot ulcers. Acta Derm Venereol 2008, ***88*(4),** 389–391.
20. Fernandez JW, Akamie RS, Casagrande EC. Punch grafts to treat lower limb intractable sores. Rev Col Bras Cir., ***44*(6),** 659–664.
21. Hjerppe A, Sane M, Huhtala H, Vaalasti A. Pinch grafting of chronic leg ulcers: a retrospective analysis of 104 patients with 169 ulcers. J Wound Care 2010; **19**: 37– 40
22. Narbutt, J., Bowszyc-Dmochowska, M., Kapińska-Mrowiecka, M., et al., Przewlekła niewydolność żylna – epidemiologia, klasyfikacja i obraz kliniczny. Rekomendacje diagnostyczno-terapeutyczne Polskiego Towarzystwa Dermatologicznego. Część I. Prz Dermatol 2018; 105: 473–485.
23. Carpentier, P., Priollet, P., Epidemiology of chronic venous insufficiency, Presse Med. 1994; 23: 197–201.
24. Leopardi, D., Hoggan, B.L., Fitridge, R.A., et.al., Systematic Review of Treatments for Varicose Veins. Ann Vasc Surg. 2009; 23: 264–276.
25. Abhishek K, Khunger N. Complications of skin biopsy. J Cutan Aesthet Surg. 2015, 8(4), 239–241.
26. Hinz P, Boenigk I, Ekkernkamp A, et.al., Rules of the surgical debridement, Tagliche Prax, 2007, 35(4), 711–719
27. Meaume S., The risks of debridement, Soins, 2011, 56(752), 42–43
28. Vowden K, Vowden P., Wound bed preparation,World Wide Wounds, 2002, Available at: http://www.worldwidewounds.com/2002/april/Vowden/Wound-Bed-Preparation.html (accessed 20.02..2020)
29. Meads C, Lovato E, Longworth L., The Debrisoft® Monofilament Debridement Pad for Use in Acute or Chronic Wounds: A NICE Medical Technology Guidance, Appl Health Econ Health Pol, 2015, 13, 583–594
30. Halim AS, Khoo TL, Mat Saad AZ, Wound bed preparation from a clinical perspective, Indian J. Plast. Surg. 2012, 45, 193–202
31. Akita S., Surgical debridement, Skin Necrosis, Springer-Verlag Vienna, 2015,1, 257-263
32. Gray D, Stang D., Ultrasound-assisted wound debridement device, Wounds UK, 2010, 6(4), 156–162
33. Ruby Chang, Y. J., Perry, J., & Cross, K. (2017). Low-frequency ultrasound debridement in chronic wound healing: A systematic review of current evidence. Plast Surg, 25(1), 21–26
34. Voigt J, Wendelken M, Driver V, et.al., Low-frequency ultrasound (20-40 kHz) as an adjunctive therapy for chronic wound healing: A systematic review of the literature and meta-analysis of eight randomized controlled trials, Int. J. Low. Extrem. Wounds, 2011, 10, 190–199
35. Ferrer-Sola M, Sureda-Vidal H, Altimiras-Roset J,et al., Hydrosurgery as a safe and efficient debridement method in a clinical wound unit, J Wound Care, 2017, 26(10), 593–599
36. Liu J, Ko JH, Secretov E, et al., Comparing the hydrosurgery system to conventional debridement techniques for the treatment of delayed healing wounds: A prospective, randomised clinical trial to investigate clinical efficacy and cost-effectiveness, Int Wound J, 2015, 12, 456–461

37. Caputo WJ, Beggs DJ, DeFede JL, et.al., A prospective randomised controlled clinical trial comparing hydrosurgery debridement with conventional surgical debridement in lower extremi-ty ulcers, Int Wound J, 2008,5, 288–294
38. Dhir K, Reino AJ, Lipana J., Vacuum-assisted closure therapy in the management of head and neck wounds, Laryngoscope,2009, 119(1), 54–61
39. Ene R, Panti Z, Albu E, et.al., Negative Pressure, a "Solution" in the Treatment of Infected Knee Prosthesis?, Maedica (Buchar), 2015, 10(1): 5–9
40. Brox-Jiménez A, Díaz-Gómez D, Parra-Membrives P, et al., A vacuum assisted closure system in complex wounds: A retrospective study, Cir Esp. 2010, 87(5), 312–317
41. Strub GM, Moe KS., The Use of Negative-Pressure Therapy in the Closure of Complex Head and Neck Wounds, Facial Plast Surg Clin North Am, 2013, 21, 137–145
42. Dhir K, Reino AJ, Lipana J., Vacuum-assisted closure therapy in the management of head and neck wounds, Laryngoscope,2009, 119(1), 54–61
43. Sherman RA., Maggot versus conservative debridement therapy for the treatment of pressure ulcers, Wound Repair Regen, 2002, 10(4), 208–214
44. Fleischmann W., Maggot Debridement. In: Surgery in Wounds, Springer,2004, 125-128
45. Telford G, Brown AP, Rich A,et.al., Wound debridement potential of glycosidases of the wound-healing maggot, Lucilia sericata, Med Vet Entomol, 2012, 26(3), 291–299
46. Gottrup F, Jørgensen B., Maggot debridement: an alternative method for debridement, Eplasty, 2011,11,e33
47. Blake FAS, Abromeit N, Bubenheim M, Li L, et.al., The biosurgical wound debridement: Experimental investigation of efficiency and practicability, Wound Repair Regen, 2007, 15(5), 756-761
48. Vowden K, Vowden K, Carville K., Antimicrobials dressings made easy, Wounds Int, 2011, 2(1), 1–6
49. Colegrave M, Rippon MG, Richardson C., The effect of Ringer's solution within a dressing to elicit pain relief, J Wound Care, 2016, 25(4), 184–190
50. Finnegan S, Percival SL., Clinical and Antibiofilm Efficacy of Antimicrobial Hydrogels, Adv Wound Care, 2015, 4(7), 398–406
51. Groenewald JH., An evaluation of dextranomer as a cleansing agent in the treatment of the postphlebitic stasis ulcer, South African Med J, 1980, 57(20), 809–815
52. Yang CY, Peng CK, Lee JS., Development of a novel composite fiber wound dressing for promoting restoration of skin architecture, In: 7th Asian-Australasian Conference on Composite Materials 2010, 2010, 2, 954-957
53. Parsons D, Bowler PG, Myles V, et.al., Silver antimicrobial dressings in wound management: A comparison of antibacterial, physical, and chemical characteristics, Wounds, 2005, 17(8), 222–232
54. Gethin G, Cowman S., Manuka honey vs. hydrogel - A prospective, open label, multicentre, randomised controlled trial to compare desloughing efficacy and healing outcomes in venous ulcers, J Clin Nurs, 2009, 18(3), 466–474
55. Lee KY, Mooney DJ., Alginate: Properties and biomedical applications, Progress in Polymer Science (Oxford), 2012,37, 106-126
56. Draget KI., Alginates BT - Handbook of Hydrocolloids (Second edition), Woodhead Publishing Series in Food Science, Technology and Nutrition, 2009, 807–828

57. Rogers AA, Rippon MG., Describing the rinsing, cleansing and absorbing actions of hydrated superabsorbent polyacrylate polymer dressings, Wounds, 2017, 13, 48–53
58. Ayello EA, Cuddigan JE., Debridement: controlling the necrotic/cellular burden, Adv skin wound care, 2004, 2, 66-75
59. Wright JB, Shi L. ,Accuzyme® papain-urea debriding ointment: A historical review, Wounds, 2003, 15,2-12
60. Falanga V., Wound Bed Preparation and the Role of Enzymes: A Case for Multiple Actions of Therapeutic Agents, Wounds, 2002, 14(2):47-57
61. Rao DB, Sane PG, Georgiev EL, Collagenase in the Treatment of Dermal and Decubitus Ulcers, J Am Geriatr Soc, 1975, 23(1), 22–30
62. Harding KG, Bale S, Llewellyn M, e.al., A pilot study of clostridium collagenase (Collagenase ABC®) ointment in the debridement of dermal ulcers, Clin Drug Investig, 1996, 11(3), 139–144
63. König M, Vanscheidt W, Augustin M, et.al., Enzymatic versus autolytic debridement of chronic leg ulcers: a prospective randomised trial, J Wound Care, 2005,14(7), 320–323
64. Waycaster C, Milne CT., Clinical and economic benefit of enzymatic debridement of pressure ulcers compared to autolytic debridement with a hydrogel dressing, J Med Econ, 2013, 16(7), 976–986
65. Gray, D., Acton, C., Chadwick P, et al. Consensus guidance for the use of debridement techniques in the UK, WoundsUK, 2011, 7,77–84
66. Jones V, Grey JE, Harding KG., Wound dressings Low adherent dressings Hydrocolloids Hydrogels Alginates, Practice, 2006, 332, 777–78
67. Quarfoot AJ, Hyla PH, Patience D, Hydrogel wound dressing, US Patent 1990, 4,909,244
68. Cuschieri L, Debosz J, Miiller P, et.al., Autolytic debridement of a large, necrotic, fully occluded foot ulcer using a hydrocolloid dressing in a diabetic patient, Adv Ski Wound Care, 2013, 26(7), 300–304
69. Heffernan A, Martin AJ. A comparison of a modified form of Granuflex® (Granuflex® Extra Thin)(*) and a conventional dressing in the management of lacerations, abrasions and minor operation wounds in an accident and emergency department, J Accid Emerg Med,1994; 11(4), 227–230
70. Schmidt RJ, Chung LY, Turner TD. Quantification of hydrogen peroxide generation by Granuflex® (DuoDERM®) hydrocolloid granules and its constituents (gelatin, sodium carboxymethylcellulose, and pectin). Br J Dermatol. 1993 129(2), 154–157
71. S.CK, G.G.M., R.S., et al. Human skin wounds: a major and snowballing threat to public health and the economy. *Wound Repair Regen.* **2009**;*17(6),* pp.763-771.
72. Buzea C., Pacheco I., Robbie K. "Nanomaterials and nanoparticles: Sources and toxicity". *Biointerphases*. **2007;** *2(4),* pp. 17–71.
73. Gold K., Slay B., Knackstedt M., Gaharwar A.K. Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles. *Adv. Ther.* **2018**;*1,* pp. 1700033.
74. Slavin YN, Asnis J, Häfeli UO, Bach H Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J *Nanobiotechnology*. **2017**; *15(1),* pp. 65.
75. Baptista PV, McCusker MP, Carvalho A,et.al., Fernandes AR Nano-Strategies to Fight Multidrug Resistant Bacteria-"A Battle of the Titans*". Front Microbiol.* 2018; *9,* pp. 1441.

76. Wang L, Hu C, Shao L, The antimicrobial activity of nanoparticles: present situation and prospects for the future. *Int J Nanomedicine.* **2017**; *12,* pp. 1227-1249.
77. AlMatar M, Makky EA, Var I, Koksal F, The Role of Nanoparticles in the Inhibition of Multidrug-Resistant Bacteria and Biofilms. *Curr Drug Deliv.* **2018**; *15(4),* pp. 470-484.
78. Gupta, A., Landis, R. F., & Rotello, V. M. Nanoparticle-Based Antimicrobials: Surface Functionality is Critical. F1000Research, 5, F1000 Faculty Rev-364.
79. Zazo H, Colino CI, Lanao JM, Current applications of nanoparticles in infectious diseases. *J Control Release.* **2018**; *224,* pp. 86-102.
80. Gao W, Chen Y, Zhang Y, et.al., Nanoparticle-based local antimicrobial drug delivery.*Adv Drug Deliv Rev.* **2018**; *127,* pp. 46-57.

1.

Original paper

Chronic venous disorders – common and yet unknown –a study of public awareness and primary symptoms in a selected group of patients

Marcela Nowak, Dorota Mehrholz, Wioletta Barańska-Rybak, Roman J. Nowicki

Department of Dermatology, Venereology and Allergology, Medical University of Gdansk, Gdansk, Poland

Adv Dermatol Allergol 2021;
XXXVIII (4): 585–589DOI:
https://doi.org/10.5114/ada.20
21.108911

Abstract

Introduction: Chronic venous disorder (CVD) is thoroughly spread across the globe. It affects about 40% of the Pol ish population. European guidelines underline that there are no data on the percentage of people who have first symptoms of chronic venous insufficiency.
Aim: To determine the frequency and pattern of first symptoms and examine public knowledge on CVD in a selectedgroup of patients.
Material and methods: Our study group consists of 175 patients who took part in preventive assessment of nevi. To determine public knowledge on CVD, we constructed a questionnaire, which consisted of two sections: one partto be completed by the patient and the other by the doctor.
Results: The median age was 41 years. From 175 patients, about 40% claimed that they do not recognize CVD. Only about half of them knew how to diagnose and treat it. Most of them associate telangiectasia and varicose veins as primary signs and symptoms of CVD.
Conclusions: Despite the fact that the disease itself is common, the level of public awareness is astonishingly low. Majority of patients tested could not associate first signs and symptoms, even though most of them had primary symptoms of the disease. In order to avoid high-cost treatment of ulcers and varicose veins, we should spread theknowledge on CVD.

Key words: chronic venous insufficiency, ulcers, varicose veins, telangiectasia.

Introduction

Chronic venous disorders (CVD) cover a wide range of pathologies ranging from small changes in the nature of reticular veins and telangiectasia not significantly affect-ing the quality of life of patients, to extensive venous ul-cers leading to severe disability. They are associated with a great medical and socioeconomic impact [1]. It is esti-mated that in Poland they affect about 47% of females and 37% of males [2]. They are a significant problem, not only from a medical point of view, yet also economical. CVD accounts for around 362,000 sick leaves per year, representing 6.4 millions of work days lost. It is estimat-ed that CVD constitutes 1–3% of all health expenses [3].Treatment of venous ulcers only in the UK costs 400–600million pounds a year, and in the USA over 1 billion dol-lars a year [4]. The total social costs in the UK, France and Germany are estimated at over one billion dollars ineach of these countries [5]. Unfortunately, there are no specific reports on costs of treatment of CVD and associ- ated conditions in Poland.

Although a holistic pathophysiology of CVD remains unknown, venous skin changes and ulceration are pre-dominant manifestations of CVD. Telangiectasias and varicose veins are reported to be extremely common, with a prevalence of about 80% and 20–64%, respec- tively [6]. Other characteristic symptoms include oedema and skin changes, hyperpigmentation, eczema, atrophie blanche and lipodermatosclerosis [7–9].

Aetiology of the disease is multifactorial and results from complex interactions of genetic and environmental factors. Risk factors include age, gender, obesity, familyhistory and ethnicity [10–12]. Older people are affected more often, with a higher prevalence among females [13].

A body mass index (BMI) greater than 30 increases therisk for CVD significantly, and so does the positive familyhistory [14].

Main pathomechanisms that lead to CVD include re-flux, obstruction or combination of both [15]. Apart from those factors, also failure of calf and foot muscles (by decreased mobility or neuromuscular problems) can lead to inadequate venous return. This obstruction can lead to venous claudication, described by the patient as burstingpain while walking, that is relieved by rest or elevation of legs.

Primary symptoms as heaviness and aching of legs, especially during prolonged standing, can worsen the quality of everyday life [16]. This may consequently lead to aggravation of daily activities and professional work. Nevertheless, the early symptoms can be alleviated by regular exercise, leg elevation and avoidance of pro- longed steady standing.

Colour Doppler ultrasound imagining, also known as triplex US, is currently the gold standard test for di- agnosis and following up CVD [17]. It enables to study both the wall and the lumen of deep and superficial veins of lower extremities and analysis of venous flow. Itshould be done systematically in comparative, bilateral way. Computed tomography of the lower extremities is widely used to study venopulmonary thromboembolism. Nevertheless it is not generally used in diagnosis of thesuperficial venous system, yet may be complementary to Doppler ultrasound scan in particular cases with an unexpected anatomic source.

The gold standard treatment option is physical ther-apy, which includes compression therapy and vascular exercises programmes, which are recommended to be started early. Compression therapy is the most efficientand cost-effective basic therapy for CVD [18]. Due to better understanding of CVD's pathomechanisms and pathophysiology of venous ulceration, new treatment op- tions are constantly being discovered and implemented[19]. In all cases the treatment is directed toward pre- vention of the retrograde blood flow and venous pooling. New types of wound dressing, surgical modalities, matrixmaterials and growth factors should all be used a supple-ment to compressive therapy.

Aim

The European guidelines emphasize the lack of dataon the percentage of population that have the first symp-toms of CVD. The purpose of the study was to determinethe level of public awareness about CVD and analysis of CVD symptoms frequency in a randomly selected groupof patients.

Material and method

One hundred and seventy-five patients took part in the research. The mean age was 44, median 41 years old. The group consisted of randomly selected patients who participated in a preventive assessment of nevi.

The participants were informed about the purpose of research and the way the test was carried out. Patients were informed about the purpose of our research and its objectives. They could ask questions concerning the questionnaire and if they did not fully understand any terms, the physician was explaining those.

Patient participation in the study was voluntary, all study participants were informed of its details andsigned informed consent for subsequent use of data foreducational and commercial purposes. All patients had the right to refuse to participate in the study.

Patients more than 18 years old, from whom we re-ceived informed consent were included in our research. Whereas patients who were unable to understand the questions in our questionnaire and from whom we couldnot obtain informed consent were excluded.

The examination was performed under the supervi- sion of a specialist dermatologist who assessed the oc-currence of the first signs and symptoms of CVD. A ques- tionnaire, which consisted of two sections has been made, one part to be completed by the patient and the other to be completed by the doctor. The patient's part contained detailed questions on patient's knowledge onCVD. It begins with yes-no questions if the patient knows the disease and thinks that it is common. Then it pro-ceeds to complex questions: if the patient can indicate from listed signs and symptoms (leg fatigue, leg oedema, coldness of legs, leg ulcers, varicose veins, vomiting, easyfatigability) the ones that refer to CVD. The same appliedto methods of prophylaxis and treatment. Patients couldchoose from compressive stockings, by-pass surgery, phlebectomy, sclerotherapy, ulcer treatment and dietarysupplements. The last complex task was to point the pro-cedure through which diagnosis of CVD can be made. Thechoice consisted of X-ray, USG duplex scan, computedtomography and complete blood count.

The other part's aim was to determine if the patient has the first symptoms of CVD and is aware of this. To both rule out situations when the patient reports falsesymptoms or does not report them, we created a simple table to be completed by the doctor. The table consistedof main primary CVD signs and symptoms (leg oedema, telangiectasias, hemosiderosis, lipodermatosclerosis and varicose veins) to be examined by the doctor.

We received an approval of the Independent Bioeth-ics Commission for Research at the Medical University of Gdansk to conduct our research.

Statistical analysis

The obtained test results were subjected to statistical analysis using the Statistica 1.2 program, determining themean values, median and standard deviation. Compat- ibility with the normal distribution was determined usingthe Shapiro-Wilk test. The probability density function

was 1. In the case of compliance with the normal distribution, a ***t***-test for independent samples was used. The***t*** test values for women and men who know/do not knowand are aware that the disease is common/uncommon were around 0.0001. Statistically significant results werethose for which $p < 0.05$. Those results directly imply thatthere were statistically relevant differences between menand women.

Results

Median age of the patients was 41, whereas mean age 44 ±15.4 years. One hundred and eight (61.75%) women and 67 (38.28%) men took part in the research, which gives 175 people in total.

62.85% know what CVD is, 72.27% of whom are women, which means that 78.70% of all women tested and only 37.31% of men tested claim to know what CVDis. 73.14% of people tested claimed that CVD is a com-mon disease, 68.75 % of whom were women, which means that only 59.7% of men, and 81.48% of women claim it is common.

As subjective symptoms and objective signs of CVDthey could mainly associate oedema (70.86%), leg fa- tigue (65.14%) and varicose veins (69.71%) (Figure 1).

As it comes to subjective symptoms and objective signs of CVD (Figure 2), 54.86 of patients claimed to have telangiectasias, 61.71% have pressure marks (im- pressions by socks in the evening), 30.86% have varicoseveins.

Dermatologists confirmed varicose veins in 15.43% of the patients, while 7.43% of the patients did not recog- nize any of the symptoms recognized by the physician. On the other hand, physicians ruled out varicose veins in 15.43% of the patients. This directly implies that furthermeasures should be undertaken to spread the knowl- edge on CVD among the society.

The examination is a key to identifying early symp- toms of CVD. Every physician should be able to notice the visible signs and take the medical history on CVD symptoms. Most of the early CVD symptoms and objec- tive signs, which physicians recognized in the randomly selected group of patients tested (Figure 3) were telan- giectasias (30.86% of the patients) and varicose veins (22.28% of the patients).

Discussion

Physicians should regularly and thoroughly examineboth legs. When strong evidence of CVD presence is vis- ible, abdomen should be examined for venous collateralspresence as well. It should be an ultimate aim to share the knowledge on CVD among the society and encourage people to undergo further diagnostics. It may be easily done by general practitioners. The ultimate purpose of

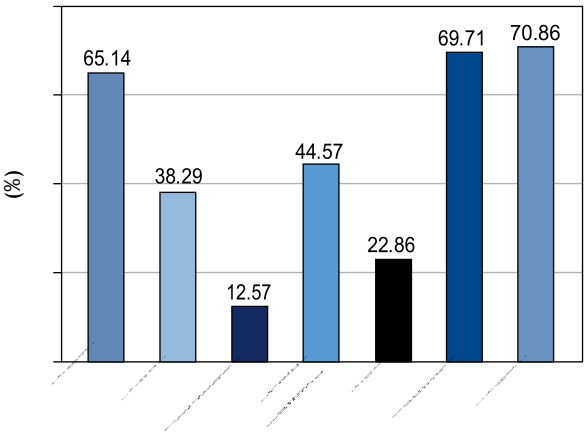

Figure 1. Signs and symptoms that patients associate with CVD

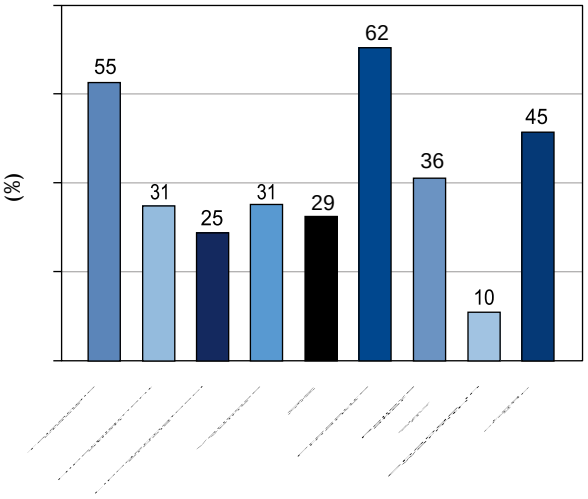

Figure 2. Signs and symptoms reported by patients

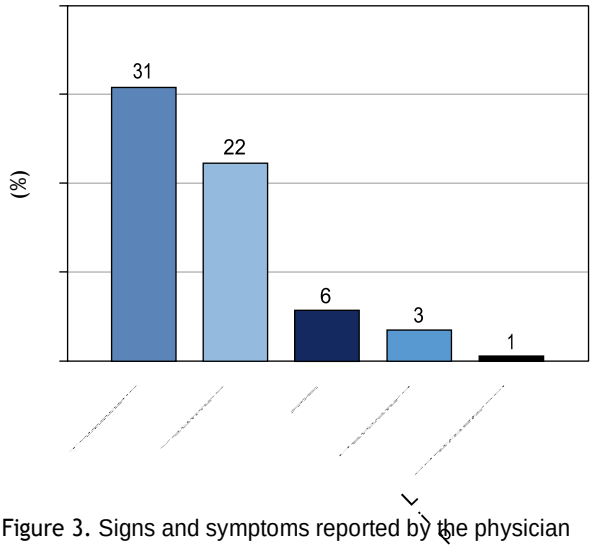

Figure 3. Signs and symptoms reported by the physician

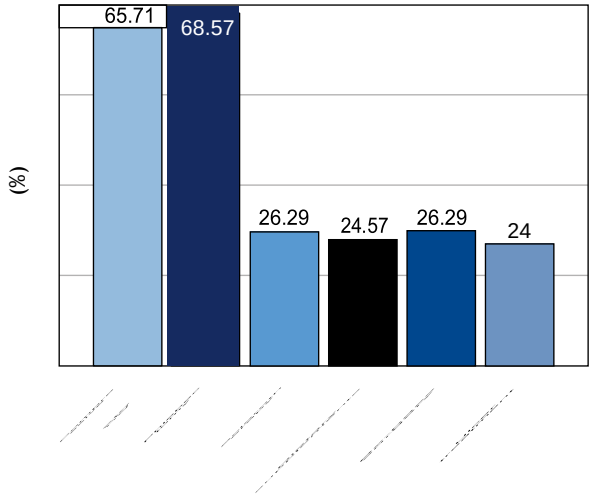

Figure 4. Therapeutic measures that patients associate with CVD

those measures would be to avoid high-cost treatment of ulcers and varicose veins.

According to 64.57% of the patients, duplex ultra- sound (DUS) examination is currently a gold standard for diagnosis of CVD [20]. It is based on a combination of ultrasound imaging and pulsed Doppler wave. Both anatomy and hemodynamic features of the venous sys- tem can be examined. Owing to DUS invention, invasivetechniques such as phlebography lost their applicability. Currently phlebography is mostly used to evaluate con- genital vein problems or to identify a vein for arterial by-pass grafting.

As seen in Figure 4, most of the patients recognize phlebectomy (68.57%) and use of compression stockings (65.71%) as main therapeutic measures for CVD. This is an uplifting conclusion since compression therapy is a significant part of conservative treatment. It is widely used because of its accessibility, non-invasiveness and high efficacy in increasing venous flow.

Nevertheless, 13.71% of the patients claim that theydo not know any therapeutic measures for CVD. 24.57% of the patients stated that dietary supplementation iseffective for CVD treatment. There are no evidence-based premises to support this thesis. What is more, 26.29% of them point out by-pass grafting. This is obviously not routinely recommended therapeutic measure for CVD.

Furthermore, surgery called phlebectomy, which was recognized by 68.57% of the patients, is a safe and effec- tive procedure that may be performed under local anaes- thesia [21]. It consists of removal or avulsion of varicose veins through small incisions.

Unfortunately only 26.29% of the patients identified sclerotherapy as a highly effective measure to treat CVD[22]. Sclerotherapy, which involves injection of liquid or foam agents to dilated veins. This leads to damage of the endothelium and consequently ablation of the veins. It is an easily repeatable treatment, which has good long- term results [23]. Not to mention that it is less invasive than surgery.

Leg elevation, physiotherapy and leg massage are highly recommended to all patients with CVD as they significantly reduce venous stasis and leg oedema.

As ulceration occurs, compressive dressings with povidone iodine or hydrocolloid dressings should be ap-plied in order to achieve wound healing [24]. Twenty-fourpercent of the patients recognised treatment of ulcers as a significant aid to manage life-threatening conse- quences of CVD.

Conclusions

Although the study revealed that most of the peopleare not acquainted with CVD and its first symptoms, themajority of tested patients presented symptoms of initialstages of CVD. It should be an ultimate aim to spread the knowledge on CVD among the society and encour- age people to undergo further diagnostics. The ultimatepurpose of those measures would be to avoid high-costtreatment of ulcers and varicose veins.

Conflict of interest

The authors declare no conflict of interest.

References

1. Szewczyk MT, Rogala J, Cwajda J, et al. Psychological and so-cial problems of patients with chronic venous disease. AdvDermatol Allergol 2007; 24: 207-10.
2. Zubilewicz T, Wroński J, Michalak J. Przewlekła niewydolnośćżylna. Od objawu i rozpoznania do leczenia. Med Rodz 2002;2: 96-100.
3. Narbutt J, Bowszyc-Dmochowska M, Kapińska-Mrowiecka M,et al. Przewlekła niewydolność żylna – epidemiologia, kla- syfikacja i obraz kliniczny. Rekomendacje diagnostyczno- terapeutyczne Polskiego Towarzystwa Dermatologicznego. Część I. Dermatol Rev 2018; 105: 473-85.
4. Simka M, Majewski E. The social and economic burden of venous leg ulcers: focus on the role of micronized purified flavonoid fraction adjuvant therapy. Am J Clin Dermatol 2003; 4: 573-81.
5. Guest JF, Fuller GW, Vowden P. Venous leg ulcer manage- ment in clinical practice in the UK: costs and outcomes. IntWound J 2018; 15: 29-37.
6. Wittens C, Davies AH, Baekgaard N, et al. Editor's Choice - Management of Chronic Venous Disease: Clinical Practice Guidelines of the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2015; 49: 678-737.
7. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. CircJ 2005; 111: 2398-409.
8. So M, Khimani N, Ngyuen M. Chronic venous insufficiency.In: Pain Medicine. Yong RJ, et al. (eds.) Springer International Publishing Cham, Switzerland 2017; 497-9.
9. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. Circ J 2014; 130: 333-46.

10. Graham ID, Harrison MB, Nelson EA, et al. Prevalence of lower-limb ulceration: a systematic review of prevalence studies. Adv Skin Wound Care 2003; 16: 305-16.
11. Jawien A, Grzela T, Ochwat A. Prevalence of chronic insuffi- ciency in men and women in Poland: multicenter cross-sec-tional study in 40,095 patients. Prz Flebol 2003; 18: 110-21.
12. Carpentier PH, Maricq HR, Biro C, et al. Prevalence, risk fac- tors, and clinical patterns of chronic venous disorders of lower limbs: a population-based study in France. J Vasc Surg2004; 40: 650-9.
13. Carpentier P, Priollet P, Epidemiology of chronic venous in-sufficiency. Presse Med 1994; 23: 197-201.
14. Beebe-Dimmer JL, Pfeifer JR, Engle JS, et al. The epidemiol- ogy of chronic venous insufficiency and varicose veins. Ann Epidemiol 2005; 15: 175-84.
15. Fernandes Abbade LP, Lastória S. Venous ulcer: epidemiol- ogy, physiopathology, diagnosis and treatment. Int J Derma-tol 2005; 44: 449-56.
16. Szewczyk M, Mościcka P, Jawień A, et al. Quality of life inpatients with leg ulcers or skin lesions – a pilot study. Adv Dermatol Allergol 2015; 12: 465-9.
17. Madureira AM. Imaging and diagnosis of chronic venous insufficiency. Cardiovasc Intervent Radiol 2009; 32: 258-9.
18. Junger M, Steins A, Zuder D, et al. Physical therapy in pa- tients with chronic venous insufficiency. VASA 1998; 27: 73-9.
19. White JV, Ryjewski C. Chronic venous insufficiency. PerspectVasc Surg Endovasc Ther 2005; 17: 319-27.
20. Neglen P, Raju S. A comparison between descending phle- bography and duplex Doppler investigation in the evalua- tion of reflux in chronic venous insufficiency: a challenge to phlebography as the "gold standard". J Vasc Surg 1992;16: 687-93.
21. Leopardi D, Hoggan BL, Fitridge RA, et al. Systematic review of treatments for varicose veins. Ann Vasc Surg 2009; 23: 264-76.
22. Rigby KA, Palfreyman SS, Beverley C, et al. Surgery versus sclerotherapy for the treatment of varicose veins. Cochrane Database Syst Rev 2004; 4: CD004980.
23. Gloviczki P, Comerota AJ, Dalsing MC, et al. The care of pa- tients with varicose veins and associated chronic venous diseases: clinical practice guidelines of the Society for Vas-cular Surgery and the American Venous Forum. J Vasc Surg 2011; 53: 2S-48S.
24. Żmudzińska M, Czarnecka-Operacz M. Management of ve- nous leg ulcers – modern wound dressings. Adv Dermatol Allergol 2006; 23: 143-8.

2.

antibiotics

MDPI

Review

Nanomaterials as a Successor of Antibiotics in Antibiotic-Resistant, Biofilm Infected Wounds?

Marcela Nowak * and Wioletta Barańska-Rybak

Clinic of Dermatology, Venereology and Allergology, Medical University of Gdansk, 80-210 Gdańsk, Poland; Wioletta.baranska-rybak@gumed.edu.pl
* Correspondence: marcela.nowak@gumed.edu.pl; Tel.: +46-79-303-64-80

Abstract: Chronic wounds are a growing problem for both society and patients. They generate huge costs for treatment and reduce the quality of life of patients. The greatest challenge when treating a chronic wound is prolonged infection, which is commonly caused by biofilm. Biofilm makes bacteria resistant to individuals' immune systems and conventional treatment. As a result, new treatment options, including nanomaterials, are being tested and implemented. Nanomaterials are particles with at least one dimension between 1 and 100 nM. Lipids, liposomes, cellulose, silica and metal can be carriers of nanomaterials. This review's aim is to describe in detail the mode of action of those molecules that have been proven to have antimicrobial effects on biofilm and therefore help to eradicate bacteria from chronic wounds. Nanoparticles seem to be a promising treatment option for infection management, which is essential for the final stage of wound healing, which is complete wound closure.

Keywords: nanomaterials; biofilm; wound management

Citation: Nowak, M.; Barańska-Rybak, W. Nanomaterials as a Successor of Antibiotics in Antibiotic-Resistant, Biofilm Infected Wounds?. *Antibiotics* **2021**, *10*, 941. https://doi.org/10.3390/antibiotics10080941

Academic Editor: Oriana Simonetti

Received: 11 July 2021
Accepted: 1 August 2021
Published: 4 August 2021

1. Introduction

1.1. Background Information

Chronic wounds are a huge burden, both for affected patients with pain and reducedquality of life and for society. They are a major challenge for healthcare, as chronic wounds stand for significant resource needs and costs for treatment. The European Wound Manage- ment Association (EWMA) distinguishes between three main categories, namely 1. initialcosts for assessment of the wound; 2. treatment of the wound and 3. care and treatment of the wound. Ragnarson et al. discovered that the weekly cost for treatment of patients with venous leg ulcers in Sweden in 2004 was estimated at between SEK 600 and 1400, depending on the size of the wound, and that the annual direct costs were between SEK 17,000 and SEK 26,500 per patient [1]. The treatment cost includes personnel resources and materials for wound dressing, of which between 65 and 69 percent of costs consist of the wound dressing itself. Guest et al. has estimated that the cost of treatment in the UK per patient in 2012–2013 was between GBP 788 (healed wounds) and GBP 4772 (unhealed wounds) per year to treat the wound and related diseases [2]. This represented 4% of thetotal expenditure on publicly funded healthcare in the UK in 2013.

The economic aspect of wound management is not only a major problem in European countries, but also worldwide. Sen et al. estimated that costs in the United States were approximately USD 50 billion per year for treatment of wounds, equivalent to 5% of their total annual spending on both Medicare and Medicaid, combined with USD 25 billion annually for the treatment of chronic wounds [3]. In addition, medical expenses in the United States associated with venous ulcers in 2007 were estimated at between USD 6391–7086 per patient and per year; in addition, these patients had a greater rate of absence from work than the control group.

The majority of chronic wounds do not heal due to a secondary infection, which alters the repair process. Moreover, most of the wounds are infected with bacteria that are resistant to commonly used antibiotics. WHO estimated that around 500,000 peopleworldwide are infected with multiresistant bacteria [4,5].

1.2. Biofilm

Prolonged healing is caused by uncontrolled bacterial growth supported by the preva- lence of biofilms, which protect integral bacteria. Biofilm consists of microorganisms coated with a self-produced protective extracellular matrix [6]. This is usually made of a mix of bacteria, fungi, algae, yeasts, microbes and cellular debris. Biofilm formation is typically clinically found in patients with nonhealing infections, such as chronic infected wounds, osteomyelitis, chronic otitis media, chronic rhinosinusitis, recurring urinary tract infections, endocarditis, lung infections due to cystic fibrosis and patients with all sorts of foreign bodies including prosthetic implants [7]. All of these infections heal slower or do not heal at all, even if treated with standard antibiotics. Notable for infected wounds is that they tend to be more sloughy and have an unpleasant odour.

Biofilm allows bacteria to survive in hostile conditions, therefore making them re- sistant to antimicrobials and immune system response, leading to prolonged infections and nonhealing wounds [8]. Bacteria within biofilm are notable for their adaptation skills,as they can withstand anoxia and nutrient limitation by altering gene expression and protein production of metabolism determining substances, therefore inhibiting metabolic rate and reducing the rate of cell division. Biofilm formation itself is divided into four stages: 1. irreversible bacterial attachment to the tissue surface; 2. microcolony formation;
3. biofilm maturation and 4. detachment or dispersion, which allows biofilm colonies to invade other areas [9] [Figure 1].

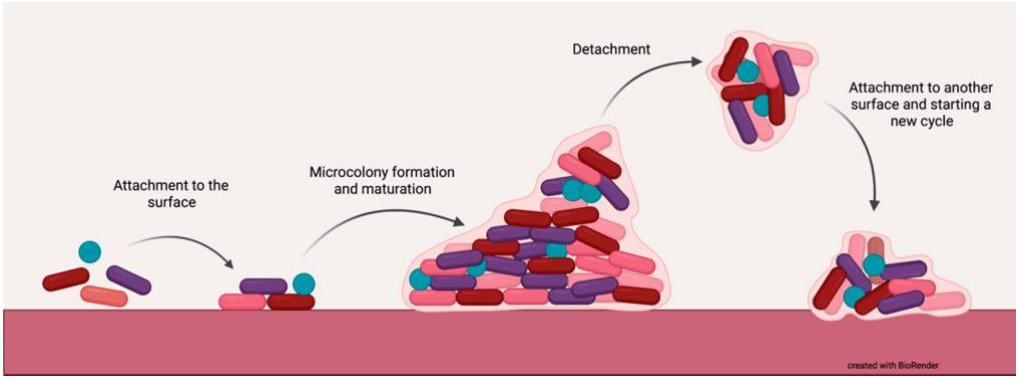

Figure 1. Cycle of biofilm formation.

It is estimated that 60–80% of chronic infections treated in hospitals are caused by biofilm abundance [10]. Biofilm infected wounds are difficult to treat as they are commonly resistant to conventional antibiotic treatment, which explains the urgency for the develop- ment of new, more effective treatment options. Until today, most of the infections caused by biofilm have been treated with a wide spectrum of antibiotics. The biggest challenge of this type of treatment is that low doses are ineffective, whereas high doses might be toxic. The main modes of action of antibiotics are namely disruption of cell wall synthesis, translation and DNA replication of bacteria. Bacteria have developed resistance mechanisms that enable them to degrade antibiotics by enzymes such as β-lactamases, acetyltransferases or aminoglycoside modifying enzymes or by efflux pumps that may result in multidrug resistance. Nowadays, almost all bacteria have developed resistance against all antibiotics that are in use. Furthermore, no new antibiotics have been discovered in the past decade. Some enzymes such as proteases, DNAse, alginate lyase, amylase and cellulase have been reported to hasten the biofilm detachment, therefore disinfecting agents containing those enzymes seem to be a promising option of treatment of chronic wounds. Innovative biofilm eradication methods are constantly tested and include application of antibiofilm nanoparti- cles (NPs) [11,12]. NPs are promising as their mode of action is different that antibiotics,

since it relies mostly on direct contact, therefore it is considered less possible that bacteria will develop resistance towards them.

1.3. Nanoparticles

NPs' applications have been studied for over 20 years. Lipids, liposomes, cellulose, silica and metal can be carriers of NPs. What is more, NMs have been known since ancient times, although without detailed knowledge of their properties [13]. There are a few examples from classical antiquity potters and glassmakers, such as the Roman Lycurgus cup of dichroic glass from 4th century CE or silver pottery from Mesopotamia from 9th century CE, where silver and copper NPs were dispersed in glassy glaze. Furthermore, NPs can be found in nature, as they are components of atmospheric pollution and are ingredients in paints, plastics, metals, ceramics and magnetic articles. The field that studies NPs is called nanotechnology and Michael Faraday is the key grounder of it. In 1857 he was the first one to describe NPs and their optical properties. The 1970s and 1980s were fundamental years for studies on NPs, then called 'ultrafine particles'.

Oddly, the properties of NPs can differ very much from the same bulk materials before their division. Their properties have been scrupulously researched and are mostly owing to their large area–volume ratio [13]. The high surface area allows heat, ions and molecules to diffuse into the particles at a higher and faster rate. When dissolved in a different medium, the interfacial layer can change the chemical and physical properties of NPs. This layer is often considered as an inseparable part of NPs and it is thought to be one of the main reasons for their activity. The interaction between NPs' surface and solvent also makes it easier for NPs to gather into suspensions and avoid floating. NPs usually contain core molecules and a shell that stabilizes the NPs and aids their function by preventing their degradation, oxidation and by increasing their biocompatibility.

Moreover, core–shell NPs may gain both electric and magnetic properties, different from their bulk derivatives, by upconverting and downconverting NPs and a shift in different wavelength spectrum emission [14]. Core–shell NPs produced from two different metals result in the formation of a core–shell structure where completely new properties are found.

NPs have been proven to possess possible dangers both to the environment and individuals treated, which are mostly caused by their high surface to volume ratio that boosts their catalytic activity. Furthermore, they attach and aggregate on the phospholipid layer and pass through the membrane. Their interaction within the cells remains unknown, yet it is unlikely that they might cross cell nucleus, Golgi apparatus or endoplasmic reticulum owing to particle size and aggregation susceptibility.

Metal NPs have been the most studied in relation to antimicrobial potency in wound healing. Among metal NMs, the most studied particles are silver NPs, yet metal oxide NPs such as zinc oxide, copper oxide and iron oxide also seem promising as antimicrobial treat- ments [15,16]. Due to their small surface area, NPs can penetrate biofilm and eventually penetrate to intracellular bacteria. The high surface area to volume ratio of nanoparticles allows drug loading, which can result in synergistic antibiofilm efficacy. NPs have antimi-crobial properties thanks to oxidative stress, formation of reactive oxygen species, metal ion release and nonoxidative mechanisms, enzymatic inhibition, DNA damage and bacteria wall disruption [17–19] [Figure 2] [Table 1]. Inhibition of bacterial adhesion by NPs is a key mechanism that enables them to prevent biofilm formation. In comparison to antibiotics, NPs may infiltrate into the matrix, destroy the extracellular polymer substance (EPS) and eventually destroy the bacteria within the biofilm.

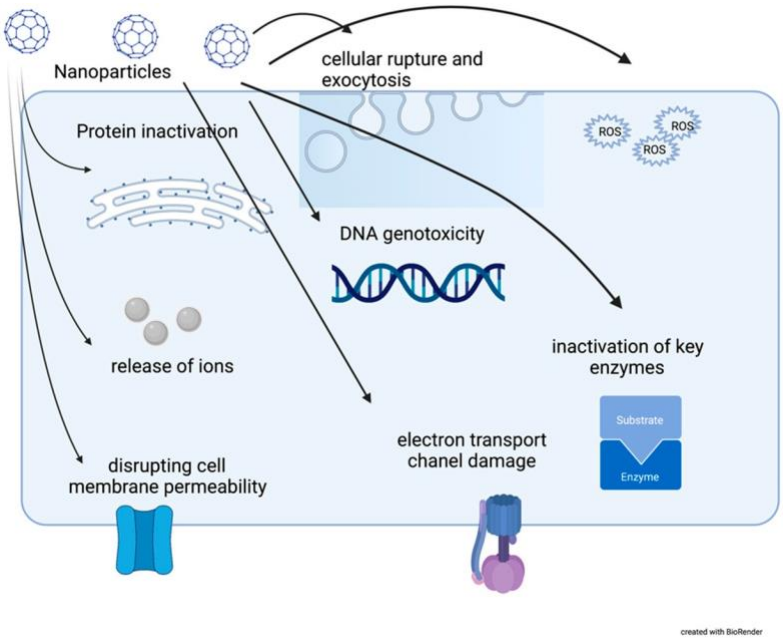

Figure 2. Nanoparticles' mode of action on bacterial cells.

Table 1. Baseline characteristics of chosen nanoparticles and nanomaterials, that are described further in the article.

Nanoparticles/ Nanomaterials	Mode of Action	Synthesis Methods	Possible Side Effects
Silver NPs	ROS generation, lipid peroxidation, inhibition of cytochromes of ETC, cell wall disruption, inhibition of cell wall synthesis, increase in membrane permeability, disruption of proton gradient resulting in lysis, adhesion to cell surface causing lipid and protein damage, ribosome destabilization, damaging DNA, disruption of biofilms	laser ablation, gamma irradiation, electron irradiation, chemical reduction, photochemical methods, microwave processing and biological synthetic methods, such as extracts from *Artemisia cappilaris*, extract from aloe vera, extract from *Acalypha indica.,* leaf extracts from *Rhizopus oryzae,* extracts of *Cocus nucifera*	Might be toxic towards keratinocytes and fibroblasts. More resistance towards AgNPs due to genetic modifications in bacteria. Ag NPs can deposit in liver, spleen, lungs and other organs and result in their dysfunction.
Gold NPs	Loss of membrane potential, disruption of respiratory chain, reduced ATPase activity, decline in subunit of ribosome for tRNA binding, bacterial membrane disruption	chemical, thermal, electrochemical and sonochemical pathways reduction by agents, biological methods using different bioreductant and capping agents such as terpenoids, phenolic compounds, proteins, polysaccharides and nicotinamide adenine dinucleotide (NAD from Citrullus lanatusrind from Plumbago zeylanica	Huge costs of production, alternative production methods should be searched, cost effectiveness is not known.
Metal oxide NPs	ROS production, disruption of membrane, adsorption to cell surface, lipids and protein damage, inhibition of microbial biofilm formation, DNA degradation, antioxidant activity	Chemical polyol method, microemulsions, thermal decomposition, electrochemical synthesis. Physical methods: plasma, chemical vapor deposition, microwave irradiation, pulser laser method, sonochemical reduction, gamma radiation, biological methods using extracts from *Caltropis procera* fruits or leaves, leaf extract of lemongrass	The high toxicity of CuO NPs causes oxidative lesions, while ZnO and TiO_2 can cause DNA damage.

Carbon nanomaterials	Inhibition of bacterial adhesion, cell membrane damage, leakage of cytoplasmic contents, higher oxygen consumption rate Graphene ROS protein dysfunction, oxidative stress, laddering of DNA, membrane damage, disturbance of the membrane permeability	Carbon nanomaterials arc discharge, laser ablation, chemical vapor deposition (CVD), ball milling, the flame procedure, solution mixing Graphene chemical reducing factors, thermal baking, photoreduction and microwave-assisted reduction	Insoluble in most solvents, might be toxic.
Mesoporous silica NPs	Inhibition of adhesion onto surfaces and thus the prevention of biofilm formation, physical damage to cell membranes, ROS production and endolysosomal burden. Mostly used as nanocarriers as they increase drug solubility, pharmakinetics and pharmadynamics, also reducing systemical toxicity.	Sol–gel process, reverse microemulsion and flame synthesis.	Toxicity, protein fouling and immunogenicity are possible.

Owing to multiple modes of antimicrobial action of NPs, they have a high potential to reduce the prevalence of multiresistant bacteria in patients with chronic wounds [20,21]. Furthermore, they can also protect the drugs from enzymatic degradation in biofilm environment [22]. Therefore, they can be used alone if they have antimicrobial properties or as nanocarriers of antibiotics to help them reach therapeutic concentration in the infected tissue [23,24].

There is still, however, a lack of data on pharmacokinetics and pharmacodynamics of NPs therapy, especially in terms of clinical trials and possible applications separately and in combination therapy with antibiotics. Possible NPs resistance and adverse effects are other challenges for NPs that await to be discovered in order to implement NPs-based treatment options for the management of nonhealing infected wounds.

This review's aim is to describe in detail the mode of action of those molecules that have been proven to have an antimicrobial effect on biofilm and therefore help to eradicate bacteria from chronic wounds [25–27].

2. Presentation of NPs

2.1. Silver NPs

Silver NPs (Ag NPs) are the most widely used and known NPs, though their exactmode of action remains unknown [28]. Multiple mechanisms have been suggested as direct interaction with the bacterial membrane inhibiting cell wall synthesis or excavation leading to cell lysis [29–31]. Silver particles have been known for their intrinsic antibiofilm properties that are owing to their surface functional groups and ion release that can interactwith biofilm.

It has been proven that Ag NPs have antibacterial activity due to continuous genera-tion of Ag^+ ions that release reactive oxygen species (ROS) [32]. Ag^+ ions are bonded to thiol-containing proteins and inhibit those that also boost ROS production [33–35]. Theyalso prevent the penetration of amines, thiols and carboxylates to biofilm [36]. Further- more, they hasten the wound healing by downregulation of metalloproteinases, which belong to the collagenase enzyme group and are essential for wound healing. Their over-expression and therefore higher load leads to underexpression of key growth factors and fibronectin [37]. By lowering the metalloproteinase secretion and enhancing cell apoptosis, NPs regulate the inflammatory reaction in the wound bed and eventually shorten the first

phase of the healing pathway. Moreover, NPs in wound dressings have been shown to control TNF-α expression that further shortens the inflammatory stage of wound healing,as they inhibit wound necrotization [38].

A study by Kalishwaralal et al., 2010 found that Ag NPs at a concentration of 100 nM prevented biofilm formation by *P. aeruginosa* and *S. epidermidis* by preventing bacterial adhesion to the surface [39]. Mohanty et al., 2012 confirmed that Ag NPs decreased *P. aeruginosa* biofilm by 65% and *S. aureus* biofilm by 88% [40]. Martinez-Gutierrez et al., 2013 showed that Ag NPs prevented the formation of *P. aeruginosa* biofilm and killed bacteria in already existing biofilm [41]. Another study showed that Ag NPs are also effective against Mycobacterium spp. biofilms [42].

Ag NPs can also boost the antibacterial effect of antibiotics. There are studies that support the synergistic effect between Ag NPs and aztreonam, ampicillin, kanamycin, streptomycin and vancomycin against *E. Coli* and *P. aeruginosa* [43]. Ag NPs with citrateand aztreonam showed antibiofilm efficacy against *P. aeruginosa*, whereas treatment with Ag NPs with ampicillin, oxacillin and penicillin inhibited and reduced MRSA biofilm by 94% [44,45].

Traditionally Ag NPs are synthesized by a chemical reduction process with the help of reducing agents in the presence of stabilizers in a suitable solvent. Recently, scientists have tried to find sustainable methods to prepare Ag NPs using plants, biological, or microbialagents as reducing and capping agents [46]. Ag NPs produced by green chemistry offer anew alternative for wound treatment without excessively polluting the environment.

Gurunathan et al. synthesized Ag NPs with leaf extract of Allophylus cobbe. Those Ag NPs have been more effective against *P. aeruginosa* and *S. aureus* biofilm when combined with ampicillin and vancomycin then when using either antibiotics or NPs separately [47]. Other studies have shown that AgNPs with rhizome extract from Rhodiola rosea signifi- cantly inhibited *P. aeruginosa* and *E. Coli* biofilm formation [48].

Ferreres et al. manufactured new metal-enzyme NPs against biofilm, that consists ofα-amylase and silver. Results are promising, as approximately 80% of *S. aureus* and *E. Coli* biofilm was eradicated [49].

2.2. Gold NPs

Gold NPs (Au NPs) as Ag NPs exhibit antibacterial and antibiofilm activity by interacting with sulfur-containing constituents in the cell membrane and leading to disruptionof the cell wall [50–52]. Au NPs cause structural damage to the biofilm, kill sessile cells and mechanically disperse the cells in the suspension. Both Au and Ag NPs have cat- alytic activity as peroxidase, glucose oxidase and superoxide dismutase altogether [53]. This activity explains how they lead to oxidative stress in bacteria by increasing ROS production [54,55]. Moreover, positively charged NPs disrupt metabolic processes and lead to perforation and leakage through negatively charged bacterial membrane [56–58].An additional advantage of Au NPs is that they inhibit intracellular ATP synthesis and tRNA binding [18,59]. Au NPs' photothermal properties enable them to inhibit biofilm formation and ablate bacteria [60]. Au NPs synthesized with Mentha piperita (peppermint) have been effective against Gram negative *E. Coli* strains, but not against Gram positive *S. aureus* [61]. Au NPs produced by Euphorbia hirta have also showed inhibition of 88% of
E. Coli strains, 86% of *P. aeruginosa* and 94% Klebsiella pneumonia [62]. Au NPs were also proven to be effective against Salmonella typhi and Enterococcus faecalis [63]. Au NPs also exhibit some antifungal properties [64–66].

Furthermore, gold is stable against oxidation, which makes it nontoxic. However, Au NPs are costly, difficult to store and do not have a high antibacterial spectrum [67].

2.3. Metal Oxide NPs

Metal oxide NPs such as iron oxide (Fe_3O_4), zinc oxide (ZnO), copper oxide (CuO), magnesium oxide (MgO) and titanium dioxide (TiO_2) are known to have antibacterial properties over both Gram positive and Gram negative bacteria [68–70]. Their mode of

action is based on ROS release through the Fenton reaction, intrinsic photocatalytic activity and the release of metallic ions [71,72].

Antimicrobial activity of NPs in general can be due to two main modes of action,namely the properties of the NPs themselves and the properties of the released metalions, both of which can have a major influence on their antibacterial activity. The rate of dissolution is crucial for toxicity, as NPs with a higher rate of dissolution usually show increased toxicity. Similarly, the smaller the NPs and the higher their surface–volume ratio, the higher the toxicity towards bacteria they have, supposedly owing to their increased mobility [73]. Metal ions are the major reason for NPs' toxicity towards bacteria, whereas NPs are helping to increase the metal ion concentration at the target place.

As already mentioned, metal ion NPs may have a different mode of antibacterial action than the free metal. Some NPs as CuO or ZnO present bactericidal mechanisms that are far different than Cu or Zn properties. This has been proven as a nonredox molecule may become redox-active as NPs and catalyze ROS production [74,75]. Earlier named CuO and ZnO NPs are able to produce ROS outside the cell and lead to cell damage by lipid peroxidation. Small NPs then can pass inside the cells and may further influence the bacterial DNA/RNA, protein, carbohydrate, lipids or ATP production and modifications. Wound management is one of the most innovative fields of medicine, where ongoing research is revolutionizing the field every day. There are numerous wound dressings that are made of nanofibers, hydrogels, hydrocolloids, alginates, gels and foams that differ from one another by their mode of action on the wound bed. Metal oxide NPs can be coated into wound dressings made of polyvinyl alcohol, chitosan, polycaprolactone or cellulose [76,77]. These dressings not only enhance the antibacterial effect of NPs, but also accelerate wound healing. These dressings would be a promising alternative for conventional treatment.

2.3.1. Zinc Oxide NPs

ZnO NPs are recognized as safe by the US Food and Drug Administration (21 CFR 182.8991) [78]. Their properties have been carefully studied, as they show the highest toxicity against multiresistant bacteria [79,80]. Their toxicity does not only depend on destroying bacterial cell wall and bacterial death, yet they also liberate ROS in the biofilm microenvironment and induce their solubility [81–84].

Zn is commonly used in cosmetics and pharmaceutical products due to its antimi- crobial, anti-inflammatory and hygroscopic properties. It has collagenolytic activity and reduces necrotic material and annihilates infections in the wound bed, stimulates epithe-lialization and hastens complete wound closure. ZnO NPs applied in Unna boots have been shown to reduce inflammation and reduce wound size [85]. Furthermore, another interesting mode of action of ZnO NPs is inhibition of bacterial kinase that, in a simple way, leads to bacteria apoptosis.

Azam et al. compared the antibacterial effects of ZnO, CuO and Fe_2O_3 NPs and showed that ZnO NPs are the most effective against Gram positive *S. aureus*, Bacillus subtilis and Gram negative *E. coli*, *P. aerogenosa*. ZnO NPs had a maximum inhibitory effect at relatively low concentration. Another experiment of Azam et al. proved that ZnO inhibited 72% more *E. Coli*, 80% *S. aureus*, 88% *P. aeruginosa* and 84% of *B. subtilis* than CuO or Fe_2O_3. A study by Beak and Wang et al. supported the initial results of Azam et al. [86].

2.3.2. Iron Oxide NPs

Aside from ROS generation through the Fenton reaction, iron oxide NPs exhibit magnetic properties [87]. By electrostatic interaction between positive NPs and negatively charged bacteria, drug resistant *S. aureus* and *E. Coli* can be trapped. Thereafter, bacteria are killed by a radiofrequency current owing to the loss of membrane potential and disruption in membrane channels [88]. Iron oxide NPs, through the Fenton reaction, produce free radicals that degrade EPS and kill bacteria within biofilm.

Another study showed that the iron oxide NPs coating nisin, activated by both electric and electromagnetic fields, increased the antibacterial efficiency of nisin that is known to be

inefficient against Gram negative bacteria. Activation of NPs led to increased permeability and local hyperthermia and resulted in synergistic antimicrobial effects on both Gram positive Bacillus subtilis and Gram negative *E. Coli* [89].

Iron oxide NPs exhibit peroxidase-like activity only at specific pH characteristics, for example for *S. mutans*, so that healthy tissues are spared [70,90].

2.3.3. Magnetite NPs

In addition to other metal oxide NPs, Fe3O4 does not show any antibacterial effect, yet it has no cytotoxic effect and has superparamagnetic properties, which makes it an idealnanocarrier [91]. Drugs that are carried with such NPs can target the biofilm at highest concentration. Due to positive charge and large surface area, it can cause mechanical disruption of the negatively charged bacterial wall [92–94].

Magnetite NPs boost the action of antibiotics against biofilm, especially penicillin, streptomycin, erythromycin, kanamycin and cefotaxime against *S. aureus* and amphotericin, and nystatin against Candida spp. biofilms has been recorded [95]. Furthermore, oleic acid magnetite NPs inhibited abundance of *S. aureus*, Saccharomyces cerevisiae, *C. tropicalis*, *C. albicans*, *C. famata*, *C. krusei* and *C. glabrata* [83].

Scientists are trying to combine naturally abundant plants with antibacterial properties and NPs. In a study where Rosmarinus officinalis oil and magnetite NPs were coated on prosthetic devices, Candida albicans biofilm was significantly reduced to approximately 2% within 72 h [96].

NPs with the use of chitosan and polypyrrole, which is a conductive polymer, were proven to inhibit formation of biofilm of *P. aeruginosa*. This particle directly inhibited the formation of virulence factors such as pyocyanine, rhamnolipids and pyroverdine and inhibited motility of bacteria [97].

2.3.4. Titanium Dioxide NPs

For a long time, titanium dioxide has been known for its antimicrobial activity and is therefore widely used as a disinfecting ingredient in cosmetics. It has been shown to be bactericidal against both Gram positive and Gram negative bacteria, making it a perfect compound for wound treatment as those are usually infected with a mix of bacteria [98].

Titanium surfaces coated with TiO_2 nanotubes by anodization route boosted antibac- terial properties [99–101]. Recent studies showed a 70% decrease in bacterial adhesion after 90 min incubation and significant inhibition of biofilm formation after 24 h of incuba- tion [102,103]. Such titanium surfaces significantly disrupt bacterial adhesion, mainly byinhibition of surface topography [104].

Several studies have shown that titanium surfaces reduce inflammation, acceler- ate bone regeneration and help platelet adhesion and activation, which makes them a promising agent in wound healing [105–108]. Addition of TiO_2 reduced inflammation and swelling around the wound site and increased thermal stability while decreasing thescaffold pore size of the material [109].

2.4. Carbon NPs

Carbon can be an ingredient of organic nanomaterials (NMs), as it has shown di- verse properties. Carbon dot is a term for various carbon NMs such as polymers, rods, sheets, fullerenes and graphene [110]. Carbon-based NMs can be synthesized separately or ennobled with metal based NMs.

2.4.1. Carbon Nanotubes

Carbon nanotubes (CNT) have an incredibly large surface area which allows high drug load and adsorption both inside the tube and on the outer surface. This eventually helps to overcome bacterial resistance mechanisms such as multidrug efflux pumps or permeability regulation and protects the antimicrobial substance from pH or enzymatic degradation. CNTs are promising since they can stabilize the drug by encapsulation and reduce the

drug's toxicity by deacceleration of its release [111]. Nanocarriers can help antibacterial agents penetrate through biofilm, reduce biofilm abundance and bacteria viability.

Isoniazid loaded chitosan+CNTs hastened the wound healing in guinea pigs with secondary bone tuberculosis initially infected subcutaneously with Mycobacterium tuberculosis. Secondary infections are even more difficult to treat than primary wound infections [112]. Those NMs were reported to decrease CD3+ and CD4+ T cell count and eventually muted the immunological response. Scientists reported 94.6% higher relative reduction in ulcers' size than with isoniazid alone, which directly implies that CNTs might boost the response of several antimicrobial agents compared to the same antimicrobials used alone.

Another study's results have shown that functionalized multiwall CNTs in polyvinyl alcohol conjugated with glucose oxidase express antibacterial properties due to generation of hydrogen peroxide from the oxidase [113]. Furthermore, it is worth noticing that CNTs yield increased wound healing rate by their ability to promote cell migration when embedded in hydrogels. The latter is widely used to produce wound dressings thanks to their biocompatibility, mechanical rigidness and hydrophilicity. A recent study testing multiwall carboxylic functionalized CNTs in fibrous hydrogels showed better healing outcomes than pure hydrogel. CNTs are thought to enhance adhesion of, for example, fibroblasts that might migrate and yield granulation of the wound bed, leading to hastened wound closure [114].

2.4.2. Graphene

Recently, graphene-based NMs have been studied for their ability to shorten wound healing time and control the local infection in the wound bed. Graphene is an allotrope of carbon that consists of a single layer of atoms arranged in a two-dimensional honeycomb lattice that gives it a large surface area [115]. Graphene is the strongest known material, harder than diamond yet more elastic than rubber. Graphene as other carbon derivatives can also be incorporated in hydrogels to facilitate better cellular adhesion and differential- ization. Due to the fact that graphene can maintain moisture within other environments, it is widely used for the production of drug carriers, biosensors, microelectromechani- cal systems (MEMS), biomimetic micro-and nanorobots and microfluidic devices [116]. Graphene-bearing Ag NPs have shown synergistic effects of both antimicrobial compounds in shortening wound healing time [117]. Furthermore, graphene can act as a photocatalyst and photodegrading agent in daylight and cleave biofilm polysaccharide linkages, disrupt biofilm and kill bacteria within it.

2.4.3. Carbon Quantum Dots

Carbon quantum dots (CQD) are the newest invention from carbon-based NMs. This group stands for zero-dimensional carbon materials. They have been proven to have antibacterial and, most interestingly, antibiofilm properties. Their role in wound healing is mainly worth noting as reducing the inflammation and promoting collagen deposition, granulation tissue development [110]. A study on CQDs embedded in chitosan dextran hydrogel showed promising results that might encourage scientists to develop new formulas with CQD and test their pharmacokinetics and bioavailability [118].

2.5. Mesoporous Silica NPs

Mesoporous silica NPs (MSNPs) are used as vehicles for antibacterial agents such as antibiotics. The main advantages of MSNPs are their high loading capacity, simple production, biocompatibility, high degree of tunability, morphology and pore diameter. They are able to shield the active particle and increase its bioavailability. Furthermore, they enable sustained release of the active substance, which helps to maintain an optimum drug level in the bloodstream. What makes them special is that they can be produced in big quantities and in a wide variety of morphologies using diverse strategies [119,120].

In this sense, they can carry more than one active substance simultaneously and act as a combined therapy.

MSNPs carrying levofloxacin showed high efficacy and high penetration within *S. aureus* biofilm, destroying it almost completely [121]. Bacterial biofilm is known to be a physical barrier for antibiotic penetration through the bacterial wall. That is why scientists are working on developing systems that enable antibacterial substances to penetrate it. MSNPs with levofloxacin and coated with concanavalin A were invented. Concanavalin A increases the efficiency of antimicrobial agents to internalize into the biofilm and eventually penetrate the mesopores and kill bacteria. Furthermore, this treatment option has been shown to have less side effects than conventional treatment for biofilm infections [122]. In another study, MSNPs with levofloxacin were grafted with third generation polycationic polypropylene imine dendrimer to provide the capability to interact with the bacterial membrane. Results confirmed that this combination provides an efficient antibiofilm effect on Gram negative bacteria [123].

3. Conclusions

Chronic wounds are a challenge for physicians worldwide, as infections of the wound bed are more and more difficult to eradicate due to antibiotic-resistant bacterial strains.

NPs seem to be a promising treatment option for infection management, which is essential for the final stage of wound healing, which is complete wound closure. NPs canenhance an antibiotic's mode of action by increasing its solubility and easing its transport into the cells or can be directly bacteriostatic or bactericidal. Research suggests that NPs might either protect conventional antibiotics from degradation by pH or enzymatic activity of biofilm microenvironment or decrease biofilms resistance to the eradication. There are no data on storage, administration and mucous interaction, as well as blood clearance and long-term results, safety and side effects. Undoubtedly, randomized controlled in vivo trials are needed to state the efficiency and place of NPs within novel medical treatment.

Author Contributions: Conceptualization, M.N. and W.B.-R.; analysis, M.N.; writing—original draft preparation, M.N.; writing—review and editing, M.N. and W.B.-R.; supervision, W.B.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding. **Institutional**

Review Board Statement: Not applicable.**Informed Consent**

Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ragnarson Tennvall, G.; Hjelmgren, J. Annual costs of treatment for venous leg ulcers in Sweden and the United Kingdom. *Wound Repair Regen.* **2005**, *13*, 13–18. [CrossRef] [PubMed]
2. Guest, J.F.; Vowden, K.; Vowden, P. The health economic burden that acute and chronic wounds impose on an average clinical commissioning group/health board in the UK. *J. Wound Care* **2017**, *26*, 292–303. [CrossRef]
3. Sen, C.K.; Gordillo, G.M.; Roy, S.; Kirsner, R.; Lambert, L.; Hunt, T.K.; Gottrup, F.; Gurtner, G.C.; Longaker, M.T. Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair Regen.* **2009**, *17*, 763–771. [CrossRef]
4. Magiorakos, A.P.; Srinivasan, A.; Carey, R.B.; Carmeli, Y.; Falagas, M.E.; Giske, C.G.; Harbarth, S.; Hindler, J.F.; Kahlmeter, G.; Olsson-Liljequist, B.; et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* **2012**, *18*, 268–281. [CrossRef]
5. Iii van Duin, D.; Paterson, D.L. Multidrug-resistant bacteria in the community: An update. *Infect. Dis Clin. N. Am.* **2020**, *34*, 709–722. [CrossRef]
6. Davies, D. Understanding biofilm resistance to antibacterial agents. *Nat. Rev. Drug Discov.* **2003**, *2*, 114–122. [CrossRef] [PubMed]
7. Bjarnsholt, T. The role of bacterial biofilms in chronic infections. *APMIS Suppl.* **2013**, *136*, 1–51. [CrossRef]
8. Harper, D.R.; Parracho, H.M.R.T.; Walker, J.; Sharp, R.; Hughes, G.; Werthén, M.; Lehman, S.; Morales, S. Bacteriophages and Biofilms. *Antibiotics* **2014**, *3*, 270–284. [CrossRef]

9. Kostakioti, M.; Hadjifrangiskou, M.; Hultgren, S.J. Bacterial biofilms: Development, dispersal, and therapeutic strategies in thedawn of the postantibiotic era. *Cold Spring Harb. Perspect. Med.* **2013**, *3*, 010306. [CrossRef] [PubMed]
10. Watnick, P.; Kolter, R. Biofilm, city of microbes. *J. Bacteriol.* **2000**, *182*, 2675–2679. [CrossRef] [PubMed]
11. Bjarnsholt, T.; Buhlin, K.; Dufrêne, Y.F.; Gomelsky, M.; Moroni, A.; Ramstedt, M.; Rumbaugh, K.P.; Schulte, T.; Sun, L.; Åkerlund, B.; et al. Biofilm formation—What we can learn from recent developments. *J. Intern. Med.* **2018**, *284*, 332–345. [CrossRef] [PubMed]
12. Vallet-Regí, M.; González, B.; Izquierdo-Barba, I. Nanomaterials as Promising Alternative in the Infection Treatment. *Int. J. Mol.Sci.* **2019**, *20*, 3806. [CrossRef]
13. Buzea, C.; Pacheco, I.; Robbie, K. Nanomaterials and nanoparticles: Sources and toxicity. *Biointerphases* **2007**, *2*, 17–71. [CrossRef] [PubMed]
14. Liu, T.M.; Conde, J.; Lipiński, T.; Bednarkiewicz, A.; Huang, C.C. Revisiting the classification of NIR-absorbing/emitting nanomaterials for in vivo bioapplications. *NPG Asia Mater.* **2016**, *8*, 295. [CrossRef]
15. Perelshtein, I.; Lipovsky, A.; Perkas, N.; Gedanken, A.; Moschini, E.; Mantecca, P. The influence of the crystalline nature of nano-metal oxides on their antibacterial and toxicity properties. *Nano Res.* **2015**, *8*, 695–707. [CrossRef]
16. Gold, K.; Slay, B.; Knackstedt, M.; Gaharwar, A.K. Antimicrobial Activity of Metal and Metal-Oxide Based Nanoparticles. *Adv. Ther.* **2018**, *1*, 1700033. [CrossRef]
17. Slavin, Y.N.; Asnis, J.; Häfeli, U.O.; Bach, H. Metal nanoparticles: Understanding the mechanisms behind antibacterial activity. *J. Nanobiotechnol.* **2017**, *15*, 65. [CrossRef]
18. Baptista, P.V.; McCusker, M.P.; Carvalho, A.; Ferreira, D.A.; Mohan, N.M.; Fernandes, A.R. Nano-Strategies to Fight Multidrug Resistant Bacteria—A Battle of the Titans. *Front. Microbiol.* **2018**, *9*, 1441. [CrossRef]
19. Wang, L.; Hu, C.; Shao, L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *Int. J. Nanomed.* **2017**, *12*, 1227–1249. [CrossRef]
20. AlMatar, M.; Makky, E.A.; Var, I.; Koksal, F. The Role of Nanoparticles in the Inhibition of Multidrug-Resistant Bacteria and Biofilms. *Curr. Drug Deliv.* **2018**, *15*, 470–484. [CrossRef]
21. Gupta, A.; Landis, R.F.; Rotello, V.M. Nanoparticle-Based Antimicrobials: Surface Functionality is Critical. *F1000Research* **2016**, *5*. [CrossRef]
22. Wang, L.S.; Gupta, A.; Rotello, V.M. Nanomaterials for the Treatment of Bacterial Biofilms. *ACS Infect. Dis.* **2016**, *2*, 3–4. [CrossRef]
23. Zazo, H.; Colino, C.I.; Lanao, J.M. Current applications of nanoparticles in infectious diseases. *J. Control. Release* **2018**, *224*, 86–102. [CrossRef] [PubMed]
24. Gao, W.; Chen, Y.; Zhang, Y.; Zhang, Q.; Zhang, L. Nanoparticle-based local antimicrobial drug delivery. *Adv. Drug Deliv. Rev.* **2018**, *127*, 46–57. [CrossRef]
25. Vallet-Regí, M.; Colilla, M.; Izquierdo-Barba, I.; Manzano, M. Mesoporous Silica Nanoparticles for Drug Delivery: Current Insights. *Molecules* **2017**, *23*, 47. [CrossRef]
26. Bassegoda, A.; Ivanova, K.; Ramon, E.; Tzanov, T. Strategies to prevent the occurrence of resistance against antibiotics by using advanced materials. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 2075–2089. [CrossRef]
27. Hemeg, H.A. Nanomaterials for alternative antibacterial therapy. *Int. J. Nanomed.* **2017**, *12*, 8211–8225. [CrossRef]
28. Burdus¸el, A.C.; Gherasim, O.; Grumezescu, A.M.; Mogoanta, L.; Ficai, A.; Andronescu, E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. *Nanomaterials* **2018**, *8*, 681. [CrossRef]
29. Yan, X.; He, B.; Liu, L.; Qu, G.; Shi, J.; HU, L.; Jiang, G. Antibacterial mechanism of silver nanoparticles in Pseudomonas aeruginosa: Proteomics approach. *Metallomics* **2018**, *10*, 557–564. [CrossRef]
30. Manikprabhu, D.; Lingappa, K. Anti-bacterial activity of silver nanoparticles against methicillin-resistant Staphylococcus aureus synthesized using model Streptomyces sp. pigment by photo-irradiation method. *J. Pharm. Res.* **2013**, *6*, 255–260.
31. Su, H.L.; Lin, S.H.; Wei, J.C.; Pao, I.C.; Chiao, S.H.; Huang, C.C.; Lin, S.Z.; Lin, J.J. Novel nanohybrids of silver particles on clayplatelets for inhibiting silver-resistant bacteria. *PLoS ONE* **2011**, *6*, 21125. [CrossRef]
32. Chernousova, S.; Epple, M. Silver as antibacterial agent: Ion, nanoparticle, and metal. *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 1636–1653. [CrossRef]
33. Eckhardt, S.; Brunetto, P.S.; Gagnon, J.; Priebe, M.; Giese, B.; Fromm, K.M. Nanobio silver: Its interactions with peptides and bacteria, and its uses in medicine. *Chem. Rev.* **2013**, *113*, 4708–4754. [CrossRef]
34. Thapa, R.; Bhagat, C.; Shrestha, P.; Awal, S.; Dudhagara, P. Enzyme-mediated formulation of stable elliptical silver nanoparticles tested against clinical pathogens and MDR bacteria and development of antimicrobial surgical thread. *Ann. Clin. Microbiol. Antimicrob.* **2017**, *16*, 39. [CrossRef] [PubMed]
35. Ramalingam, B.; Parandhaman, T.; Das, S.K. Antibacterial Effects of Biosynthesized Silver Nanoparticles on Surface Ultrastructure and Nanomechanical Properties of Gram-Negative Bacteria viz. Escherichia coli and Pseudomonas aeruginosa. *ACS Appl. Mater. Interfaces* **2016**, *8*, 4963–4976. [CrossRef]
36. Fei, J.; Zhao, J.; Du, C.; Wang, A.; Zhang, H.; Dai, L.; Li, J. One-pot ultrafast self-assembly of autofluorescent polyphenol-based core-shell nanostructures and their selective antibacterial applications. *ACS Nano* **2014**, *8*, 8529–8536. [CrossRef]
37. Wright, J.B.; Lam, K.; Buret, A.G.; Olson, M.E.; Burrell, R.E. Early healing events in a porcine model of contaminated wounds: Effects of nanocrystalline silver on matrix metalloproteinases, cell apoptosis, and healing. *Wound Repair Regen.* **2002**, *10*, 141–151. [CrossRef]

38. Kalantari, K.; Mostafavi, E.; Afifi, A.M.; Izadiyan, Z.; Jahangirian, H.; Rafiee-Moghaddam, R.; Webster, T.J. Wound dressings functionalized with silver nanoparticles: Promises and pitfalls. *Nanoscale* **2020**, *12*, 2268–2291. [CrossRef] [PubMed]
39. Kalishwaralal, K.; BarathManiKanth, S.; Pandian, S.R.K.; Deepak, V.; Gurunathan, S. Silver nanoparticles impede the biofilm formation by Pseudomonas aeruginosa and Staphylococcus epidermidis. Colloids and surfaces. *Biointerfaces* **2010**, *79*, 340–344. [CrossRef]
40. Mohanty, S.; Mishra, S.; Jena, P.; Jacob, B.; Sarkar, B.; Sonawane, A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. *Nanomed. Nanotechnol. Biol. Med.* **2012**, *8*, 916–924. [CrossRef]
41. Martinez-Gutierrez, F.; Boegli, L.; Agostinho, A.; Sanchez, E.M.; Bach, H.; Ruiz, F.; James, G. Anti-biofilm activity of silver nanoparticles against different microorganisms. *Biofouling* **2013**, *29*, 651–660. [CrossRef]
42. Markowska, K.; Grudniak, A.M.; Wolska, K.I. Silver nanoparticles as an alternative strategy against bacterial biofilms. *Acta Biochim. Pol.* **2013**, *60*, 523–530. [CrossRef] [PubMed]
43. Franci, G.; Falanga, A.; Galdiero, S.; Palomba, L.; Rai, M.; Morelli, G.; Galdiero, M. Silver nanoparticles as potential antibacterial agents. *Molecules* **2015**, *20*, 8856–8874. [CrossRef]
44. Płusa, T. Znaczenie biofilmu w kontekście narastania oporności bakterii na antybiotyki (The importance of biofilm in the context of increasing bacterial resistance to antibiotics). *Pol. Merkur. Lekarski.* **2019**, *47*, 197–202.
45. Cihalova, K.; Chudobova, D.; Michalek, P.; Moulick, A.; Guran, R.; Kopel, P.; Adam, V.; Kizek, R. Staphylococcus aureus and MRSA growth and biofilm formation after treatment with antibiotics and SeNPs. *Int. J. Mol. Sci.* **2015**, *16*, 24656–24672. [CrossRef] [PubMed]
46. Shah, M.; Fawcett, D.; Sharma, S.; Tripathy, S.; Poinern, G. Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials* **2015**, *8*, 7278–7308. [CrossRef]
47. Gurunathan, S.; Han, J.W.; Kwon, D.N.; Kim, J.H. Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Res. Lett.* **2014**, *9*, 373. [CrossRef]
48. Singh, P.; Pandit, S.; Beshay, M.; Mokkapati, V.; Garnaes, J.; Olsson, M.E.; Mackevica, A.; Mateui, R.V.; Lutken, H.; Daugaard, A.E.; et al. Anti-biofilm effects of gold and silver nanoparticles synthesized by the Rhodiola rosea rhizome extracts. *Artif. Cells Nanomed. Biotechnol.* **2018**, *46*, 886–899. [CrossRef]
49. Ferreres, G.; Bassegoda, A.; Hoyo, J.; Torrent-Burgues, J.; Tzanov, T. Metal-enzyme nanoaggregates eradicate both gram-positiveand gram-negative bacteria and their biofilms. *ACS Appl. Mater. Interfaces* **2018**, *10*, 40434–40442. [CrossRef] [PubMed]
50. Kim, J.S.; Kuk, E.; Yu, K.N.; Kim, J.H.; Park, S.J.; Lee, H.J.; Kim, S.H.; Park, Y.K.; Park, Y.H.; Hwang, C.Y.; et al. Antimicrobialeffects of silver nanoparticles. *Nanomedicine* **2007**, *3*, 95–101. [CrossRef] [PubMed]
51. Payne, J.N.; Waghwani, H.K.; Connor, M.G.; Hamilton, W.; Tockstein, S.; Moolani, H.; Chavda, F.; Badwaik, V.; Lawrenz, M.B.; Dakshinamurthy, R. Novel Synthesis of Kanamycin Conjugated Gold Nanoparticles with Potent Antibacterial Activity. *Front. Microbiol.* **2016**, *7*, 607. [CrossRef]
52. Shaker, M.A.; Shaaban, M.I. Formulation of carbapenems loaded gold nanoparticles to combat multi-antibiotic bacterial resistance:In vitro antibacterial study. *Int. J. Pharm.* **2017**, *525*, 71–84. [CrossRef]
53. He, W.; Zhou, Y.T.; Wamer, W.G.; Hu, X.; Wu, X.; Zheng, Z.; Boudreau, M.D.; Yin, J.J. Intrinsic catalytic activity of Au nanoparticleswith respect to hydrogen peroxide decomposition and superoxide scavenging. *Biomaterials* **2013**, *34*, 765–773. [CrossRef]
54. Tao, Y.; Ju, E.; Ren, J.; Qu, X. Bifunctionalized mesoporous silica-supported gold nanoparticles: Intrinsic oxidase and peroxidase catalytic activities for antibacterial applications. *Adv. Mater.* **2015**, *27*, 1097–1104. [CrossRef]
55. Zheng, K.; Setyawati, M.I.; Leong, D.T.; Xie, J. Antimicrobial Gold Nanoclusters. *ACS Nano* **2017**, *11*, 6904–6910. [CrossRef]
56. Lesniak, A.; Salvati, A.; Santos-Martinez, M.J.; Radomski, M.W.; Dawson, K.A.; Åberg, C. Nanoparticle adhesion to the cell membrane and its effect on nanoparticle uptake efficiency. *J. Am. Chem. Soc.* **2013**, *135*, 1438–1444. [CrossRef] [PubMed]
57. Lam, S.J.; O'Brien-Simpson, N.M.; Pantarat, N.; Sulistio, A.; Wong, E.H.; Chen, Y.Y.; Lenzo, J.C.; Holden, J.A.; Blencowe, A.; Reynolds, E.; et al. Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers. *Nat. Microbiol.* **2016**, *1*, 16162. [CrossRef] [PubMed]
58. Shamaila, S.; Zafar, N.; Riaz, S.; Sharif, R.; Nazir, J.; Naseem, S. Gold Nanoparticles: An Efficient Antimicrobial Agent against Enteric Bacterial Human Pathogen. *Nanomaterials* **2016**, *6*, 71. [CrossRef] [PubMed]
59. Natan, M.; Banin, E. From Nano to Micro: Using nanotechnology to combat microorganisms and their multidrug resistance. *FEMS Microbiol. Rev.* **2017**, *41*, 302–322. [CrossRef] [PubMed]
60. Ibelli, T.; Templeton, S.; Levi-Polyachenko, N. Progress on utilizing hyperthermia for mitigating bacterial infections. *Int. J. Hyperth.* **2018**, *34*, 144–156. [CrossRef]
61. MubarakAli, D.; Thajuddin, N.; Jeganathan, K.; Gunasekaran, M. Plant extract mediated synthesis of silver and gold nanoparticles and its antibacterial activity against clinically isolated pathogens. *Colloids Surf. B Biointerfaces* **2011**, *85*, 360–365. [CrossRef] [PubMed]
62. Annamalai, A.; Christina, V.L.; Sudha, D.; Kalpana, M.; Lakshmi, P.T. Green synthesis, characterization and antimicrobial activityof Au NPs using *Euphorbia hirta* L. leaf extract. *Colloids Surf. B Biointerfaces* **2013**, *108*, 60–65. [CrossRef]
63. Kuppusamy, P.; Yusoff, M.M.; Ichwan, S.J.; Parine, N.R.; Maniam, G.P.; Govindan, N. *Commelina nudiflora* L. edible weed as a novel source for gold nanoparticles synthesis and studies on different physical–chemical and biological properties. *J. Ind. Eng.Chem.* **2014**, *27*, 67. [CrossRef]

64. Bankar, A.; Joshi, B.; Kumar, A.R.; Zinjarde, S. Banana peel extract mediated synthesis of gold nanoparticles. *Colloids Surf. B Biointerfaces* **2010**, *80*, 45–50. [CrossRef]
65. Annavaram, V.; Posa, V.R.; Vijaya Lakshmi, D.; Sumalatha, J.; Somala, A.R. Terminalia bellirica fruit extract mediated synthesis of gold nanoparticles (AuNPs) and studies on antimicrobial and antioxidant activity. *Synth. React. Inorg. Met. Org. Nano Metal Chem.* **2016**, *47*, 681–687. [CrossRef]
66. Islam, N.U.; Jalil, K.; Shahid, M.; Muhammad, N.; Rauf, A. Pistacia integerrima gall extract mediated green synthesis of gold nanoparticles and their biological activities. *Arab. J. Chem.* **2019**, *12*, 2310–2319. [CrossRef]
67. Jiang, Z.; Sahar, A.; Li, X.; Robinson, S.M.; Gupta, A.; Saha, K.; Moyano, D.F.; Riley, M.A.; Rotello, V.M. Functional goldnanoparticles as potent antimicrobial agents against multi-drug-resistant bacteria. *ACS Nano* **2014**, *8*, 10682–10686.
68. Denet, E.; Espina-Benitez, M.B.; Pitault, I.; Pollet, T.; Blaha, D.; Bolzinger, M.A.; Rodriguez-Nava, V.; Briancon, S. Metal oxidenanoparticles for the decontamination of toxic chemical and biological compounds. *Int. J. Pharm.* **2020**, *583*, 119373. [CrossRef]
69. Raghunath, A.; Perumal, E. Metal oxide nanoparticles as antimicrobial agents: A promise for the future. *Int. J. Antimicrob Agents.* **2017**, *49*, 137–152. [CrossRef]
70. Gao, L.; Liu, Y.; Kim, D.; Li, Y.; Hwang, G.; Naha, P.C.; Cormode, D.P.; Koo, H. Nanocatalysts promote Streptococcus mutans biofilm matrix degradation and enhance bacterial killing to suppress dental caries in vivo. *Biomaterials* **2016**, *101*, 272–284. [CrossRef]
71. Singh, R.; Smitha, M.S.; Singh, S.P. The role of nanotechnology in combating multi-drug resistant bacteria. *J. Nanosci. Nanotechnol.* **2014**, *14*, 4745–4756. [CrossRef]
72. Kadiyala, U.; Kotov, N.A.; VanEpps, J.S. Antibacterial metal oxide nanoparticles: Challenges in interpreting the literature. *Curr. Pharm. Des.* **2018**, *24*, 896–903. [CrossRef] [PubMed]
73. Azam, A.; Ahmed, A.S.; Oves, M.; Khan, M.S.; Memic, A. Size-dependent antimicrobial properties of CuO nanoparticles against Gram-positive and -negative bacterial strains. *Int. J. Nanomed.* **2012**, *7*, 3527–3535. [CrossRef] [PubMed]
74. Meghana, S.; Kabra, P.; Chakraborty, S.; Padmavathy, N. Understanding the pathway of antibacterial activity of copper oxide nanoparticles. *RSC Adv.* **2015**, *5*, 12293–12299. [CrossRef]
75. Tiwari, V.; Mishra, N.; Gadani, K.; Solanki, P.S.; Shah, N.A.; Tiwari, M. Mechanism of anti-bacterial activity of zinc oxide nanoparticle against carbapenem-resistant acinetobacter baumannii. *Front. Microbiol.* **2018**, *9*, 1218. [CrossRef]
76. Thorasani, M.T.; Joorabloo, A.; Moghaddam, A.; Shamsi, H.; MansooriMoghadam, Z. Incorporation of ZnO nanoparticles into heparinised polyvinyl alcohol/chitosan hydrogels for wound dressing application. *Int. J. Biol. Macromol.* **2018**, *114*, 1203–1215. [CrossRef] [PubMed]
77. Li, Q.; Lu, F.; Zhou, G.; Yu, K.; Lu, B.; Xiao, Y.; Dai, F.; Wu, D.; Lan, G. Silver inlaid with gold nanoparticle/chitosan wound dressing enhances antibacterial activity and porosity, and promotes wound healing. *Biomacromolecules* **2017**, *18*, 3766–3775. [CrossRef] [PubMed]
78. Romana, D.L.; Brown, K.H.; Guinard, J.-X. Sensory trial to assess the acceptability of zinc fortificants added to iron-fortified wheat products. *J. Food Sci.* **2006**, *67*, 461–465. [CrossRef]
79. Hou, J.; Wu, Y.; Li, X.; Wei, B.; Li, S.; Wang, X. Toxic effects of different types of zinc oxide nanoparticles on algae, plants,invertebrates, vertebrates and microorganisms. *Chemosphere* **2018**, *193*, 852–860. [CrossRef] [PubMed]
80. Mahamuni-Badiger, P.P.; Patil, P.M.; Badiger, M.V.; Patel, P.R.; Thorat-Gadgil, B.S.; Pandit, A.; Bohara, R.A. Biofilm formation toinhibition: Role of zinc oxide-based nanoparticles. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2020**, *108*, 110319. [CrossRef]
81. Li, C.H.; Shen, C.C.; Cheng, Y.W.; Huang, S.H.; Wu, C.C.; Kao, C.; Liao, J.W.; Kang, J.J. Organ biodistribution, clearance, andgenotoxicity of orally administered zinc oxide nanoparticles in mice. *Nanotoxicology* **2012**, *6*, 746–756. [CrossRef] [PubMed]
82. Sondi, I.; Salopek-Sondi, B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for Gram-negative bacteria. *J. Colloid Interface Sci.* **2004**, *275*, 177–182. [CrossRef] [PubMed]
83. Dwivedi, S.; Wahab, R.; Khan, F.; Mishra, Y.K.; Musarrat, J.; Al-Khedhairy, A.A. Reactive oxygen species mediated bacterial biofilm inhibition via zinc oxide nanoparticles and their statistical determination. *PLoS ONE* **2014**, *9*, 111289. [CrossRef] [PubMed]
84. Li, Y.; Zhang, W.; Niu, J.; Chen, Y. Mechanism of photogenerated reactive oxygen species and correlation with the antibacterial properties of engineered metal-oxide nanoparticles. *ACS Nano* **2012**, *6*, 5164–5173. [CrossRef]
85. Lansdown, A.B.G.; Mirastschijski, U.; Stubbs, N.; Scanlon, E.; Ågren, M.S. Zinc in wound healing: Theoretical, experimental, and clinical aspects. *Wound Repair Regen.* **2007**, *15*, 2–16. [CrossRef] [PubMed]
86. Azam, A.; Ahmed, A.S.; Oves, M.; Khan, M.S.; Habib, S.S.; Memic, A. Antimicrobial activity of metal oxide nanoparticles againstGram-positive and Gram-negative bacteria: A comparative study. *Int. J. Nanomed.* **2012**, *7*, 6003–6009. [CrossRef] [PubMed]
87. Arias, L.S.; Pessan, J.P.; Vieira, A.P.M.; Lima, T.; Delbem, A.; Monteiro, D.R. Iron Oxide Nanoparticles for Biomedical Applications:A Perspective on Synthesis, Drugs, Antimicrobial Activity, and Toxicity. *Antibiotics* **2018**, *7*, 46. [CrossRef]
88. Chaurasia, A.K.; Thorat, N.D.; Tandon, A.; Kim, J.H.; Park, S.H.; Kim, K.K. Coupling of radiofrequency with magnetic nanoparticles treatment as an alternative physical antibacterial strategy against multiple drug resistant bacteria. *Sci. Rep.* **2016**, *6*, 33662. [CrossRef]
89. Novickij, V.; Stanevičienė, R.; Vepštaitė-Monstavičė, I.; Gruskiene, R.; Krivorotova, T.; Sereikaite, J.; Novickij, J.; Serviene, E. Overcoming Antimicrobial Resistance in Bacteria Using Bioactive Magnetic Nanoparticles and Pulsed Electromagnetic Fields. *Front. Microbiol.* **2017**, *8*, 2678. [CrossRef]

90. Benoit, D.S.W.; Sims, K.R., Jr.; Fraser, D. Nanoparticles for oral biofilm treatments. *ACS Nano* **2019**, *13*, 4869–4875. [CrossRef] [PubMed]
91. Rumyantceva, V.; Rumyantceva, V.; Koshel, E.; Vinogradov, V. Biocide-conjugated magnetite nanoparticles as an advanced platform for biofilm treatment. *Ther. Deliv.* **2019**, *10*, 241–250. [CrossRef] [PubMed]
92. Prabhu, Y.T.; Rao, K.V.; Kumari, B.S.; Pavani, T. Synthesis of Fe_3O_4 nanoparticles and its antibacterial application. *Int. Nano. Lett.* **2015**, *5*, 85–92. [CrossRef]
93. Ravikumar, S.; Gokulakrishnan, R.; Selvanathan, K.; Selvam, S. Antibacterial activity of metal oxide nanoparticles against ophthalmic pathogens. *Int. J. Pharm. Res. Dev.* **2011**, *39*, 122–127.
94. Behera, S.S.; Patra, J.K.; Pramanik, K.; Panda, N.; Thatoi, H. Characterization and evaluation of antibacterial activities of chemically synthesized iron oxide nanoparticles. *World J. Nano Sci. Eng.* **2012**, *2*, 196–200. [CrossRef]
95. Saviuc, C.; Grumezescu, A.M.; Chifiriuc, C.M.; Mihaiescu, D.E.; Hristu, R.; Stanciu, G.; Oprea, E.; Radulescu, V.; Lazar, V. Hybridnanosystem for stabilizing essential oils in biomedical applications. *Dig. J. Nanomater. Biostruct.* **2011**, *6*, 1657–1666.
96. Chifiriuc, C.; Grumezescu, V.; Grumezescu, A.M.; Saviuc, C.; Lazăr, V.; Andronescu, E. Hybrid magnetite nanoparti- cles/rosmarinus officinalis essential oil nanobiosystem with antibiofilm activity. *Nanoscale Res. Lett.* **2012**, *7*, 1–7. [CrossRef] [PubMed]
97. Khan, F.P.; Manivasagan, D.T.N.; Pham, D.T.N.; Oh, J.; Kim, -S.K.; Kim, -Y.M. Antibiofilm and antivirulence properties of chitosan-polypyrrole nanocomposites to Pseudomonas aeruginosa. *Microb. Pathog.* **2019**, *128*, 363–373. [CrossRef]
98. Bui, V.K.H.; Park, D.; Lee, Y.-C. Chitosan Combined with ZnO, TiO_2 and Ag Nanoparticles for Antimicrobial Wound Healing Applications: A Mini Review of the Research Trends. *Polymers* **2017**, *9*, 21. [CrossRef]
99. Mei, S.; Wang, H.; Wang, W.; Tong, L.; Pan, H.; Ruan, C.; Ma, Q.; Liu, M.; Yang, H.; Zhang, L.; et al. Antibacterial effects and biocompatibility of titanium surfaces with graded silver incorporation in titania nanotubes. *Biomaterials* **2014**, *35*, 4255–4265. [CrossRef]
100. Alvarez, R.; García-Martín, J.M.; Macías-Montero, M.; Gonzalez-Garcia, L.; Gonzales, J.C.; Rico, V.; Perlich, J.; Cotrino, J.; Gonzalez-Elipe, A.R.; Palmero, A. Growth regimes of porous gold thin films deposited by magnetron sputtering at oblique incidence: From compact to columnar microstructures. *Nanotechnology* **2013**, *24*, 045604. [CrossRef]
101. Izquierdo-Barba, I.; García-Martín, J.M.; Álvarez, R.; Palmero, A.; Esteban, J.; Pereze-Jorge, C.; Arcos, D.; Valler-Regi, M. Nanocolumnar coatings with selective behavior towards osteoblast and Staphylococcus aureus proliferation. *Acta Biomater.* **2015**, *15*, 20–28. [CrossRef]
102. Benčina, M.; Iglič, A.; Mozetič, M. Crystallized TiO_2 Nanosurfaces in Biomedical Applications. *Nanomaterials* **2020**, *10*, 1121. [CrossRef] [PubMed]
103. Ding, X.; Zhou, L.; Wang, J.; Zhao, Q.; Lin, X.; Gao, Y.; Li, S.; Wu, J.; Rong, M.; Guo, Z.; et al. The effects of hierarchical micro/nanosurfaces decorated with TiO2 nanotubes on the bioactivity of titanium implants in vitro and in vivo. *Int. J. Nanomed.* **2015**, *10*, 6955–6973.
104. Lorenzetti, M.; Dogša, I.; Stošicki, T.; Stopar, D.; Kalin, M.; Kobe, S.; Novak, S. The influence of surface modification on bacterial adhesion to titanium-based substrates. *ACS Appl. Mater. Interfaces* **2015**, *7*, 1644–1651. [CrossRef] [PubMed]
105. Puckett, S.; Pareta, R.; Webster, T.J. Nano rough micron patterned titanium for directing osteoblast morphology and adhesion. *Int.J. Nanomed.* **2008**, *3*, 229–241.
106. Bjursten, L.M.; Rasmusson, L.; Oh, S.; Smith, G.C.; Brammer, K.S.; Jin, S. Titanium dioxide nanotubes enhance bone bonding in vivo. *J. Biomed. Mater. Res. A* **2010**, *92*, 1218–1224. [PubMed]
107. Neacsu, P.; Mazare, A.; Schmuki, P.; Cimpean, A. Attenuation of the macrophage inflammatory activity by TiO_2 nanotubes via inhibition of MAPK and NF-κB pathways. *Int. J. Nanomed.* **2015**, *10*, 6455–6467.
108. Zhang, L.; Liao, X.; Fok, A.; Ning, C.; Ng, P.; Wang, Y. Effect of crystalline phase changes in titania (TiO_2) nanotube coatings on platelet adhesion and activation. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2018**, *82*, 91–101. [CrossRef]
109. Deepachitra, R.; Lakshmi, R.P.; Sivaranjani, K.; Chandra, J.H.; Sastry, T.P. Nanoparticles embedded biomaterials in wound treatment: A review. *J. Chem. Pharm. Sci.* **2015**, *8*, 324–329.
110. Kumar, R.; Kumar, V.B.; Gedanken, A. Sonochemical synthesis of carbon dots, mechanism, effect of parameters, and catalytic, energy, biomedical and tissue engineering applications. *Ultrason. Sonochem.* **2020**, *64*, 105009. [CrossRef]
111. Kale, S.N.; Kitture, R.; Ghosh, S.; Chopade, B.A.; Yakhmi, J.V. Nanomaterials as Enhanced Antimicrobial Agent/Activity-Enhancerfor Transdermal Applications: A Review. In *Antimicrobial Nanoarchitectonics*; Elsevier BV: Amsterdam, The Netherlands, 2017;pp. 279–321.
112. Chen, G.; Wu, Y.; Yu, D.; Li, R.; Luo, W.; Ma, G.; Zhang, C. Isoniazid-loaded chitosan/carbon nanotubes microspheres promote secondary wound healing of bone tuberculosis. *J. Biomater. Appl.* **2018**, *33*, 989–996. [CrossRef] [PubMed]
113. Santos, J.C.C.; Mansur, A.A.P.; Ciminelli, V.S.T.; Mansur, H.S. Nanocomposites of Poly(Vinyl Alcohol)/Functionalized-Multiwall Carbon Nanotubes Conjugated With Glucose Oxidase for Potential Application as Scaffolds in Skin Wound Healing. *Int. J. Polym. Mater.* **2014**, *63*, 185–196. [CrossRef]
114. Ravanbakhsh, H.; Bao, G.; Mongeau, L. Carbon nanotubes promote cell migration in hydrogels. *Sci. Rep.* **2020**, *10*, 2543. [CrossRef] [PubMed]

115. Ghosh, S.; Sanghavi, S.; Sancheti, P. Metallic Biomaterial for Bone Support and Replacement. In *Fundamental Biomaterials: Metals*; Balakrishnan, P., Sreekala, M.S., Thomas, S., Eds.; Woodhead Publishing: Cambridge, UK; Elsevier: Amsterdam, The Netherlands, 2018; Volume 2, pp. 139–165.
116. Ghuge, A.D.; Shirode, A.R.; Kadam, V.J. Graphene: A comprehensive review. *Curr. Drug Targets* **2017**, *18*, 724–733. [CrossRef]
117. Fan, Z.; Liu, B.; Wang, J.; Zhang, S.; Lin, Q.; Gong, P.; Ma, L.; Yang, S. A novel wound dressing based on Ag/graphene polymer hydrogel: Effectively kill bacteria and accelerate wound healing. *Adv. Funct. Mater.* **2014**, *24*, 3933–3943. [CrossRef]
118. Li, P.; Liu, S.; Yang, X.; Du, S.; Tang, W.; Cao, W.; Zhou, J.; Gong, X.; Xing, X. Low-drug resistance carbon quantum dots decoratedinjectable self-healing hudrogel with potent antibiofilm property and cutaneous wound healing. *Chem. Eng. J.* **2021**, *126*, 387.
119. Castillo, R.R.; Lozano, D.; González, B.; Manzano, M.; Izquierdo-Barba, I.; Vallet-Regi, M. Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery: An update. *Expert Opin. Drug Deliv.* **2019**, *16*, 415–439. [CrossRef] [PubMed]
120. Barros, C.; Fulaz, S.; Vitale, S.; Casey, E.; Quinn, L. Interactions between functionalised silica nanoparticles and Pseudomonas fluorescens biofilm matrix: A focus on the protein corona. *PLoS ONE* **2020**, *15*, e0236441. [CrossRef]
121. Pedraza, D.; Díez, J.; Barba, I.-I.; Colilla, M.; Vallet-Regi, M. Amine-Functionalized Mesoporous Silica Nanoparticles: A NewNanoantibiotic for Bone Infection Treatment. *Biomed. Glasses* **2018**, *4*, 1–12. [CrossRef]
122. Martínez-Carmona, M.; Izquierdo-Barba, I.; Colilla, M.; Vallet-Regí, M. Concanavalin A-targeted mesoporous silica nanoparticlesfor infection treatment. *Acta Biomater.* **2019**, *96*, 547–556. [CrossRef] [PubMed]
123. González, B.; Colilla, M.; Díez, J.; Pedraza, D.; Guembe, M.; Izquierdo-Barba, I.; Valler-Regi, M. Mesoporous silica nanoparticlesdecorated with polycationic dendrimers for infection treatment. *Acta Biomater.* **2018**, *68*, 261–271. [CrossRef] [PubMed]