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Review article

Advances and perspectives in use of semisolid formulations for photodynamic methods

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ABSTRACT

Although nearly 30 years have passed since the introduction of the first clinically approved photosensitizer for photodynamic therapy, progress in developing new pharmaceutical formulations remains unsatisfactory. This review highlights that despite years of research, many recurring challenges and issues remain unresolved. The paper includes an analysis of selected essential studies involving aminolevulinic acid and its derivatives, as well as other photosensitizers with potential for development as medical products. Among various possible vehicles, special attention is given to gelatin, alginates, poly(ethylene oxide), polyacrylic acid, and chitosan. The focus is particularly on infectious and cancerous diseases. Key aspects of developing new semi-solid drug forms should prioritize the creation of easily manufacturable and biocompatible preparations for clinical use. At the same time, new formulations should preserve the primary function of photosensitizers, which is the generation of reactive oxygen species capable of destroying pathogenic cells or tumors. Additionally, the use of adjuvant properties of carriers, which can enhance the effectiveness of macrocycles, is emphasized, especially in chitosan-based antibacterial formulations. Current research indicates that many promising dyes and macrocyclic compounds with high potential as photosensitizers in photodynamic therapy remain unexplored in formulation and development work. This review outlines potential new and previously explored pathways for advancing photosensitizers as active pharmaceutical ingredients (APIs).

1. Introduction

Two of the most significant challenges facing the 21st century medicine include the battle against cancer and the escalating issue of antimicrobial resistance. Inconsiderate pharmaceutical policies in infection treatment and animal farming practices have led to an increased risk of developing resistance mechanisms among both bacteria and fungi. Considering the scale and pace of these processes, the World Health Organization (WHO) declared in the "Antimicrobial Resistance Global Report on Surveillance" that we are inevitably approaching the "post-antibiotic era" [1]. Sally Davies, former Chief Medical Officer of the United Kingdom, likened the challenge of combating therapy-resistant microbes to an impending climate catastrophe [2]. From a statistical perspective, in Europe alone, over 33,000 cases of excessive deaths due to incurable bacterial and fungal infections occur annually. According to J. O'Neill's report, this number globally reaches up to 700,000, and by 2050, it may exceed 10 million [1]. In recent years, there has been a rapid development of antimicrobial resistance among microorganisms. Increasingly, multidrug-resistant (MDR) pathogens are isolated from both hospital and open healthcare setting patients. Even though a century has passed since the discovery of the first antibiotic, medicine still lacks a universal remedy for bacterial and fungal infections [3]. Furthermore, drugs and medical products that once demonstrated high bactericidal or bacteriostatic efficacy are becoming obsolete due to microorganisms' ability to develop new resistance mechanisms [3–5]. On the other hand, concurrently, the medical world must confront the escalating incidence of tumors, including malignant tumors. According to the latest World Cancer Report: Cancer Research for Cancer Prevention, cancer remains a leading cause of premature deaths, ranking first or second in 134 out of 183 surveyed countries within the age group of 30–69. In the additional 45 countries, it stands as the third leading cause of death in the same age bracket. Out of the total

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15.2 million premature deaths recorded in 2016, a notable 29.8 %, equivalent to 4.5 million cases, were attributed to neoplastic diseases [6]. WHO's alarming assertion suggests that sustaining elevated rates of human papillomavirus (HPV) infection [7], viral hepatitis [8], and tobacco smoking [9] could lead to a significant increase in cancer cases in the next two decades. Projections indicate that doctors may diagnose up to 60 percent more cancer cases. Furthermore, a staggering 80 % of new cases will occur in low-income countries [6].

Both issues pose immense medical, social, and economic challenges for the world. This makes it necessary and valuable to search for new, alternative methods of therapy. A promising and versatile solution could be the application of anticancer photodynamic therapy (PDT) and photodynamic antimicrobial therapy (PACT). Although targeted at different diseases, these therapies are based on the same chemical and physiological foundations [10–12]. The constant challenge lies in the continuous development of pharmaceutical formulations that facilitate easy administration by medical staff and are acceptable to patients. This review focuses on evaluating progress in this field in recent years concerning semi-solid drug forms and the development of nanotechnology for future formulation advancements.

2. Potential and limitations of PDT – An overview

Photodynamic therapy (PDT) is an established modality to treat both oncological and non-oncological diseases [13–19,173]. In oncology, PDT is considered one of the possible anticancer therapies, alongside chemotherapy, radiotherapy, surgery, and immunotherapy, etc. and it can be used as a standalone modality or with combination with other types of treatments [16,17]. PDT is also an emerging modality for treatment of bacterial infection [18,20–26].

PDT relies on photosensitizer (PS) which is a molecular entity capable of producing highly cytotoxic species upon light absorption [27,28]. Typically, cytotoxic species are produced upon reaction of the excited state PS with molecular oxygen leading to formation of reactive oxygen species (ROS). Two types of ROS can be generated: singlet oxygen $^1O_2$, i.e. excited state of oxygen formed through energy transfer from excited PS (Type II mechanism), or superoxide anion-radical $O_2^{\cdot-}$, formed upon electron transfer from photoexcited PS to O_2 (Type I mechanism) [29,30]. The simplified mechanism of PDT has been shown on Fig. 1.

Superoxide radical can further react with water of hydronium cation to produce other ROS, such as hydroxyl radical $OH^{\cdot}$, hydrogen peroxide H_2O_2, and others. Recent findings however, indicate that type I mechanism may be more complex, and $O_2^{\cdot-}$ may be a side product in Type I mechanism rather than actual cytotoxic species [29,30]. It should be emphasized that, as noted by Hamblin and Abrahamse, the impact of oxygen concentration on the dominance of type I or type II mechanisms in the vicinity of a PS may be illusory [31]. On the other hand, there is no doubt that the availability of an adequate amount of oxygen is essential for the majority of PSs [32]. However, there is a small group of PSs that exhibit a kind of "photodynamic reaction" even in a hypoxic environment. As indicated by the aforementioned authors, this type of reaction can be classified as a type III reaction. Compounds such as some representatives of psoralens, tetracycline, or inorganic salts belong to this category. These mentioned PSs typically react with crucial elements of bacterial cell, such as DNA, RNA, or ribosomes, without requiring the presence of oxygen in the environment, forming so-called photoadducts [33]. The key component for PDT is a PS which should be able to generate ROS in excited state. Since ROS photosensitization requires close proximity between excited-state PS and O_2, then PSs featuring the high quantum yield of the long-lived triplet excited state (with the lifetime of hundreds of μs) are typically used for PDT. Moreover, for anticancer therapeutic application PS must be excited after its localization in the tumor, thus it must absorb light with the deep tissue penetration. Since tissue strongly absorbs visible light (with wavelength below 650 nm), the optimal wavelength for PS is 650–900 nm (therapeutic window). The 900 nm limit is due to the water absorption between 900–1000 nm [34]. Considering the potential opportunities and limitations for photodynamic therapy (PDT), the source and type of light used play a crucial role. Although in the early experiments with PDT, natural white light was predominantly used, it was quickly replaced by light of specific wavelengths corresponding to the absorption maximum of a given photosensitizer [35,36]. A significant milestone in the development of PDT was the introduction of lasers, which allowed for effective control over the level of necrosis [37]. However, lasers are characterized by relatively high production costs and limited availability. Further advancements were made with the introduction of LED light, though it cannot be considered monochromatic. Combining different wavelengths to optimize treatment can be highly beneficial, especially in terms of light penetration through patient tissues. In the case of using the photosensitizer Photogem, three different wavelengths and their combinations were utilized. The focus of the research was to

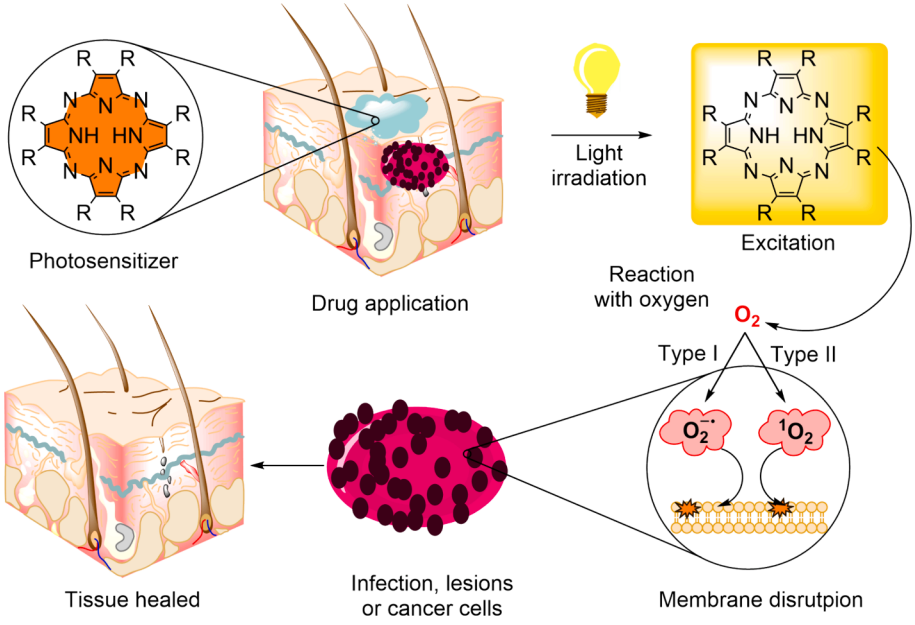


Fig. 1. Simplified mechanism of PDT.

determine the difference in the potential to induce necrosis in tissues. It was found that using a wavelength of 630 nm can induce necrosis to a depth of 1.08 mm, corresponding to a threshold dose of about 86 J/cm^2. A significantly smaller necrosis depth was observed with the 600 nm wavelength, only 0.4 mm, with a higher threshold dose of 134 J/cm^2. The 660 nm wavelength practically did not cause necrosis, suggesting a very high or infinite threshold dose. The research on the synergistic effect of using different wavelengths proved particularly interesting. The combination of 630 nm and 660 nm wavelengths resulted in a reduction of the 630 nm threshold dose to approximately 50 J/cm^2. This suggests that although the 660 nm wavelength alone does not cause necrosis, it enhances the effectiveness of the 630 nm wavelength, leading to deeper necrosis than previously anticipated. On the other hand, the combination of 630 nm and 600 nm wavelengths, both of which individually induce necrosis, resulted in a necrosis depth of 0.9 mm, indicating a strong synergistic effect between them [35]. A beneficial and synergistic effect was also observed when combining two different light sources. Applying laser therapy at the beginning followed by PDT with milder radiation can stimulate the tissue healing process. In dentistry and antibacterial treatment, photobiomodulation (PBM) combined with PDT is used, utilizing a wavelength corresponding to the photosensitizer's absorption maximum and accelerating tissue regeneration. Although the current paradigm favors red light due to its tissue penetration, there are studies suggesting that the use of other wavelengths and combinations could have clinical justification [38,174]. However, further research is still needed to understand the mechanisms responsible for synergistic effects and to develop optimal therapeutic protocols that take these phenomena into account [38,39].

There are numerous PSs reported in the literature which have been examined for PDT, but there are only few which are clinically approved. In fact, they are classified in several ways, most commonly into three generations, but also into porphyrinoids and non-porphyrinoids, what implies also further classification of various classes of porphyrinoids. According to the classification based on generations, the first generation includes derivatives of natural hematoporphyrin, the second one is related to modified and synthetic PSs that possess enhanced spectral properties, commonly shifted towards red region (such as chlorins or phthalocyanins), and the third generation is associated with use of additional molecules and systems that provide enhanced targeting and selectivity, such as liposomes. The porphyrinoids themselves, are also commonly divided into the subclasses, based on the parent structure of particular porphyrin derivative: porphyrins, chlorins, bacteriochlorins, phthalocyanines and so on. Besides, the non-porphyrinoid class may for example include thiazines, xanthenes and curcumins. The classes approved for clinical use involve mainly porphyrins and chlorins, as well as aminolevulinic acid (5-ALA) and its derivatives, serving as precursors for biosynthesis of protoporphyrin IX (PpIX). Other groups, including bacteriochlorins, pheophorbides, phthalocyanines, texaphyrins, thiazines hypericin and polypyridyl rhutenium complexes, are currently under clinical trials, although for some of them the clinical trials were terminated. These are broadly discussed in various review articles cited in this paper [14–18,27–29,34,40,41]. The representative classes of PSs are presented in Fig. 2.

Lifetime of both excited state PS and ROS is relatively short (ms at most) [42,43]. Therefore the therapeutic action of PS is restricted to the very small volume, in practice to the cell where PS is localized. Thus, PDT is potentially highly localized therapy since PS is non-toxic until it is photoexcited, and the light can be selectively delivered only to the targeted sites. For the tumor treatment PDT potentially offers safer therapy without side effects caused by many chemotherapeutic agents [44], and less invasive than surgery. To develop the full potential of PDT several requirements should be met. To avoid undesirable side effects, PS must be selectively delivered to the target cells. In case of antitumor PDT several approaches have been developed to improve tumor localization. For example, enhanced permeability and retention (EPR) effect causes a selective accumulation of nanoparticles in tumor, thus

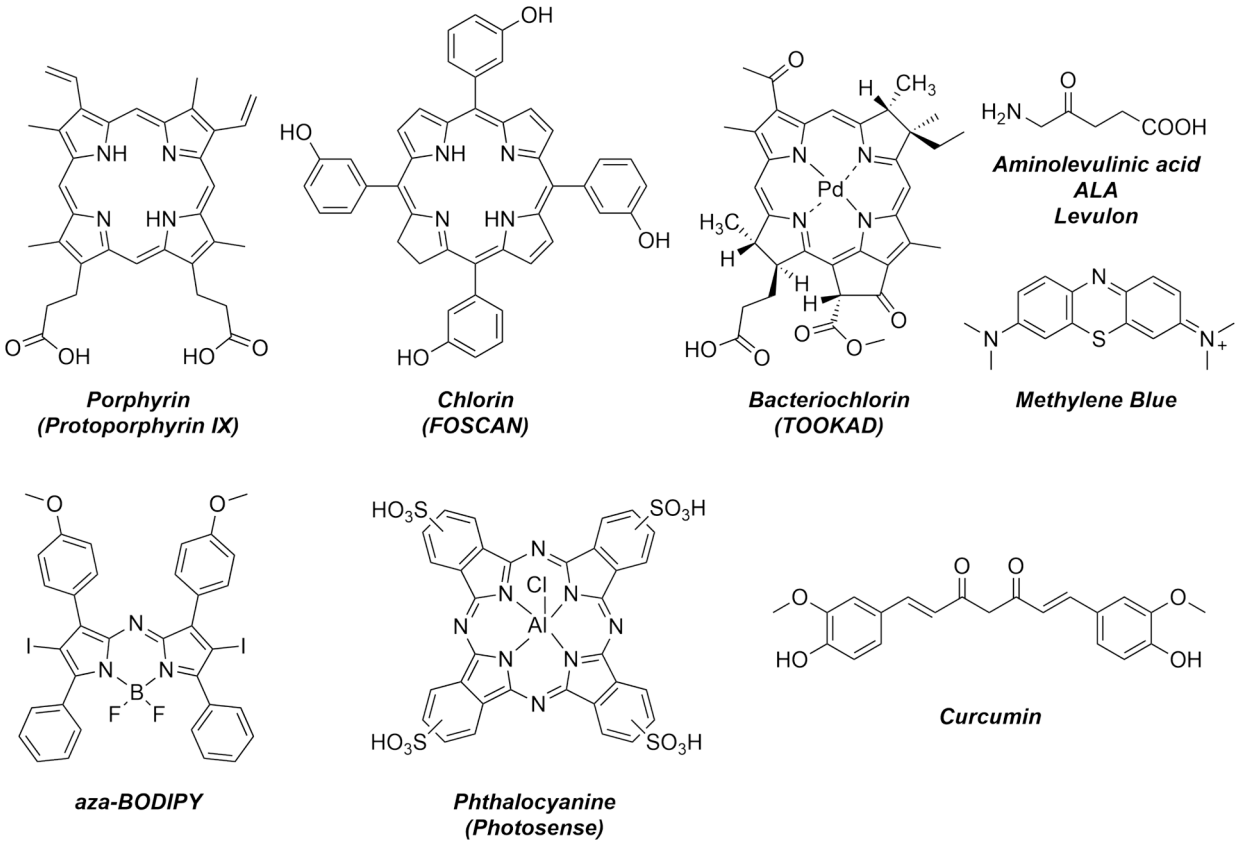


Fig. 2. Representative PSs used in PDT.

formulation of PSs into nanomaterials improved accumulation in the target tumors [45,46]. Another strategy relies on attaching a targeting unit, i.e. molecular fragments, which due to the specific tumor biology and biochemistry are selectively taken up by tumor cells [47]. Targeting units include: cancer-specific antibody [48], peptides [47], sugar [49], aptamers [50],folic acid [47], besides other molecules, receptors of which are overexpressed on the tumor cells. The effectiveness of PDT toward tumors is associated not only with inducing cell death by direct destruction of their components, but also with disruption of their neovasculature and induction of immune responses *via* activation of various signalling pathways. As stated before, there are several mechanisms of cell death itself triggered by photodynamic processes, of which the greatest impact is exerted by apoptosis and necrosis, often related to the location of PS accumulation, and immunogenic cell death (ICD), associated with immune responses [51,52]. The apoptotic processes are often initiated upon accumulation of PS in mitochondria, what results in disrupting the mitochondria membrane by generated ROS and releasing the cytochrome *C* into the cytoplasm. This further induces activation of caspases, as well as mitochondrial apoptosis-inducing factor (AIF), that initiate cell destruction. Additionally, damaging the mitochondria may lead to destruction of anti-apoptotic proteins what constitutes another factor that either weakens the anti-apoptotic response of the cell, or can induce apoptosis itself. On the other hand, necrosis is related to the accumulation of PS in cellular membrane and destroying it by gerenated ROS, what results in loss of membrane integrity, leakage of ATP and cell lysis. Moreover, the ICD is triggered upon release of signaling molecules, known as damage-associated molecular patterns (DAMPs) by damaged or dying cells, that become then recognized as malignant ones and targeted by the immune system, especially by T cells [51–53]. The potential improvement of antitumor PDT may be associated particularly with immunotherapy and its ability to either bind to growth factors overexpressed by tumors, such as epidermal growth factor receptor (EGFR) to achieve better targeting, or improve the immune response due to significantly impairing the immunosuppressive environment provided by tumors. Another possible improvement is combining the PS with moieties able to emit specific light upon irradiation with X-rays, or using the PS that is prone to activation by X-rays itself. While X-rays have high permeability through the tissues, it may pave the way for effective PDT toward deeper located tumors. More strategies that intend to improve the clinical capabilities of PDT are associated with providing more oxygenated tumor environment, since various types are known for being typically hypoxic due to huge oxygen consumption [51,54].

Analysing semi-solid formulations designed for PDT, several issues need to be clearly addressed. Firstly, almost all formulations for surface application are characterized by increased density and the presence of molecules responsible for gelling. Although this reduces the ability of substances to freely diffuse into the target tissue, a significant portion of the generated reactive oxygen species (ROS) may not attack pathogenic cells or tumors. Instead, they may interact with the cross-linked structure of the environment. This necessitates the design of a formulation that will release the PS into the surrounding tissues in an easy but at the same time-controlled manner. The released PS should also accumulate in the target tissue, rather than remain solely in the *stratum corneum* or diffuse into the bloodstream. Simultaneously, the impact of the formulation on the lifetime of the excited state is poorly understood. The density and optical properties of the formulation itself can also modify the light parameters reaching the site of action [55,56]. In many cases, these factors may have a moderate impact on the overall effectiveness of therapy but ignoring them could be a mistake.

Another potential limitation of PDT is long circulation of PS in human body, which causes light-sensitivity long after the therapy is over (up to month) [57]. One of the strategy to overcome this barrier is formulation of a “activatable PSs”, i.e. PSs which are photochemically inactive and non-toxic even upon excitation [28,58]. At the target site, PS is activated by a chemical reaction induced by specific tumor microenvironment (TME). Thus, PS are activated upon protonation (taking advantage from lower pH characteristic of the tumor environment) [59], or by reaction catalyzed by enzymes overexpressed in the cancer cells (for example, proteases) [60]. The significant limitation, often referred as Achilles’ heel of PDT remains the depth of light penetration through tissue [61]. Light, even in the near-IR spectral window penetrates tissue less efficiently than, for example X-ray used for radiotherapy, thus in practice, application of traditional PDT is often restricted to tumors or other diseases located on or few millimeters beneath the surface of the tissue [61]. To address this problem “self-illuminating” PSs has been proposed. In this version of PDT a PS is connected to the chemi- or bioluminescent molecule, which is excited by a chemical and biochemical reaction and subsequently transfers the excited state energy to PS [62]. In other approaches, Cherenkov radiation, i.e. light generated by positrons or electrons emitted by radioactive nuclei, has been used to excite PS to achieve a depth-independent activation of PDT [63]. Exceptionally interesting photodynamic process trigger seems to be a Cherenkov radiation, which can be considered as UV optical light emitted during radioactive reaction. Such kind of light emitted inside the body may repurposing the first generation of PSs. There are reports on using of eosin or chlorin e6 in Cherenkov radiation triggered PDT [64,65]. However, there are some problems have to be solved. The ability to excite the PS in the *in vivo* have to be confirmed. The question is that different cancer cells enable to accumulation of radionuclide in needed amount? Is the possibility of scattering, or quenching emitted light by the cancer cells/tissue? Going further and beyond the formal PDT, activation of sensitizer by other sources of energy, i.e. ultrasound (sonodynamic therapy) [66–68,68–70], and low-intensity X-ray [61] also was tested as a mean to overcome shallow depth limitation of classical PDT. Similar method to PDT is photothermal therapy (PTT) mentioned here. Briefly, PTT is based on PS and light with appropriate wavelength as well. The difference is the structure of PS, which are mostly gold or silver nanostructures activated with NIR light. Interaction of light and PS leads to photon energy conversion into the heat causing local hyperthermia. The temperature locally rises to the values that enable ablation of PS surrounding cells [71,72].

3. Semi-solid forms of drugs — examples and importance for PDT

Semi-solid dosage forms of drugs are currently, alongside oral formulations, one of the most widely used forms of administering medicinal substances. They predominantly allow for localized drug delivery and exhibit a consistency between liquid and solid states. Among the most representative examples of semi-solid drug forms are creams, ointments, gels, and pastes [73,74]. From a historical perspective, these are also some of the oldest forms of drugs, with their origins dating back to ancient times. Their roots can be traced practically to early antiquity, as evidenced by the Ebers Papyrus from around the 15th century BCE, which presents the first recipes for medicinal ointments [75]. Ancient Romans and Greeks also prepared ointments based on lard and olive oil [76], while Ayurvedic preparations contained clarified butter (Ghee) [77]. With the development of medicine and pharmacy, bases and matrices that did not have animal origins and exhibited significantly better durability and stability began to be utilized. Currently, in accordance with the widely accepted pharmacopoeial definition, ointments are a form intended primarily for application on the skin or mucous membrane to induce local or systemic effects achieved through transdermal penetration of medicinal substances. A characteristic feature of ointments is the dissolution, suspension, or emulsification of the medicinal substance in a suitably selected, single or multicomponent base [74]. From the perspective of application in PDT, special attention should be paid to the site of action of the delivered substance. Semi-solid drug forms administered topically may have either epidermal character (the action site of which is the epidermis) or endodermal character (the target site of the active substance is the dermis). The medicinal product for topical application containing 5-ALA, produced in for of a gel – Ameluz – is available on the European market. [78]. The most common

type of gels used in the pharmaceutical industry, including Ameluz, are hydrophilic gels (also known as hydrogels) [79,80]. The basic components of hydrogels include water, glycerol or propylene glycol, in which gelling agents such as carbomers, poloxamers, cellulose derivatives, or aluminum-magnesium silicates are dissolved. In the case of Ameluz, water serves as the solvent, while xanthan gum, Polysorbate 80, and soy-derived phospholipids contribute to the formulation. Xanthan gum is composed of glucose, mannose, and glucuronic acid, along with partially esterified acetic and pyruvic acids. It is obtained through the fermentation of carbohydrates by *Xanthomonas campestris* bacteria. The gum is capable of forming pseudoplastic solutions, with viscosity strongly dependent on mechanical factors such as mixing. It exhibits minimal sensitivity to pH, temperature, and the addition of salts, making it highly chemically and physically stable. Resistant to acidic environments, it does not react to enzymatic action or changes in temperature. Its versatility is evident in its ability to withstand both high temperatures and freeze–thaw cycles, rendering it suitable for various industrial processes [81]. However, attention should be paid to limitations arising from the development of such formulations. The use of hydrogel as a vehicle restricts its application mainly to hydrophilic substances. A significant majority of new PSs with desirable properties, such as high efficiency in generating singlet oxygen, the ability to form long-lived excited states, high photostability, non-toxicity in the absence of light, or low production costs, exhibit very limited solubility in water. Therefore, it would be highly desirable to develop formulations based either on non-polar solvents or utilizing modern carriers such as liposomes, invasomes, micelles, nanocapsules, microspheres, etc [82–84]. A straightforward solution may be the development of semi-solid drug forms based on hydrophobic gels (also known as lipophilic gels or oleogels) [85]. In this case, the carrier typically consists of liquid paraffin with the addition of polyethylene and oils gelled with silica. An alternative approach to the formulation challenge has been presented by the manufacturers of the Alacare preparation in the form of a medicinal patch containing 8 mg of 5-ALA. In this case, the embedded carrier in the outer protective layer is a copolymer of acrylate and vinyl acetate (Duro-Tak 387–2353/87–2353) [86]. These patches are left on the skin for a duration of 4 h, after which the skin is subjected to a brief exposure to red light – necessary to induce the photodynamic effect.. The use of the patch aims primarily to prolong the contact of the active substance (in this case, the PS) with the skin. The substrates in the patches are designed predominantly to adhere easily to skin [87]. Despite the exceptional convenience of this dosage form, it has some limitations. The most significant one is the inability to apply it to mucous membranes. The manufacturers of LEVULAN® KERASTICK® have presented an alternative pathway in the development of their product, which includes a powdered medicinal substance and a liquid solution. LEVULAN® KERASTICK® is a prescription medicine used on the skin with blue light irradiation (BLU-U® PDT) for the treatment of minimally to moderately thick actinic keratoses of face, scalp, or upper arms [88]. The use of a solution remains the most cost-effective and definitely the simplest solution, although not suitable for every medical purpose. Simultaneously, the development of drugs in the form of solutions for external application seems particularly interesting, especially in the case of bacterial infections in postoperative wounds or those associated, for example, with diabetic foot ulcers. An intriguing and, at the same time, underexplored perspective could involve the introduction of photosensitizing substances into solutions of modern antiseptics containing polyhexanide biguanide (PHMB) and/or betaine, which are very important in the treatment of infected wounds.

Considering the significance of formulations in relation to PDT, we can refer to two primary aspects: changes in the pharmacokinetic parameters of the PS and the optical properties of the formulations themselves [89]. The appropriately chosen vehicle can lead to the accumulation of the active substance in a specific target tissue [90]. Selecting the right excipients during the formulation process can thus be crucial and influence therapy parameters. Extended or shortened retention times, as well as penetration into deeper or shallower skin layers, are possible. The use of semi-solid drug forms in PDT also has its drawbacks and limitations. For instance, thicker formulations may slow the release of the photosensitizer, prolonging the required pre-incubation time. Simultaneously, a formulation that is too thick may remain on the surface, while a lighter one might penetrate too deeply into layers where light penetration and potential PDT effectiveness are limited [90]. Therefore, researchers need a flexible approach and thoughtful consideration of the substance delivery strategy right from the PS design stage [91–93]. It is important to remember that the optical properties of the formulations, such as transparency, scattering, and absorption, play a crucial role in the activation of photosensitizers. For this reason, gel formulations seem particularly promising because it is relatively easy to achieve forms with high transmittance and neutral optical properties [93,94]. Moreover, with the help of the prepared skin optical clearing gel, the refractive index inhomogeneity between skin tissue components can be attenuated, and the light scattering effect within the skin tissue is potentially reduced [95]. This phenomenon can be utilized, for example, in the development of PDT for the treatment of melanoma [96–98].

The already mentioned modifications of photosensitizer pharmacokinetics are inextricably linked to the selection of appropriate drug forms. Enhancement of PS penetration and retention may be achieved by the proper choice of the PS and its form in the formulation [99]. The selection of PS depends on their application. For anti-cancer treatment lipophilic agents with little or no charge (either positive or negative) should be chosen. For antimicrobial use PSs should have evident cationic charges, and usually more charges improve targeting Gram-negative bacteria. The choice of PS agents with strong absorbance in the deep-red spectral region increases their efficiency as light penetration increases with wavelength. In case of anti-cancer applications, long wavelength (far red/near-infrared) absorption bands of PS enable good tissue penetration of the exciting light. In case of antimicrobial purposes this feature does not play such important role as the treatment refers mainly to topical areas [99]. Covalent attachment of PSs to ligands demonstrating some affinity for neoplasia or to receptors expressed on specific tumors [100] enable to direct them to the target place. The targeting vehicles help control localization of PSs and include monoclonal antibodies [101], peptides [102], proteins such as transferrin [103], epidermal growth factor [104] and insulin [105], LDL [106], epidermal growth factor peptide [Meyers 2015], somatostatin [102], folic acid [107] and many others. The emergence and development of nanotechnology contributed to use of nanocarriers, solubilization, encapsulation and delivery of PSs to both tumors [108] and microbial cells [109]. The planar conjugates of PSs that are necessary for light absorption tend to be hydrophobic and prone to aggregation. Liposomes, micelles, nanoemulsions can be composed of lipids or amphiphilic polymers that self-assemble into delivery vehicles for PSs Sadasivam et al., 2013 Jan 1 [cited 2024 Aug 19];5 [110].. These nanovehicles cause a significant increase in photochemical efficiency, they allow PSs to be better taken up by cells and to be more selectively delivered to tumors or other target tissues [111].

4. Advances in *in vivo* and clinical research using creams and gels containing 5-ALA

4.1. Cancerous and pre-cancerous lesions

Treatment of certain carcinomas with 5-ALA became a versatile approach due to much easier administration towards malignant cells and the selectivity and specificity of PpIX biosynthesis rather than heme, resulting in high concentration of an active PS inside the cells that require treatment. Such formulations can be particularly effective against various types of malignant or premalignant lesions, either inside oral cavity or on the body surface [112]. The standard 5-ALA concentrations vary mainly from 7.8 % to 20 %, whereas the majority of methyl

aminolevulinate (MAL) formulations have 16 % concentration [113–133]. Those formulations are often similar to, or based on commercial ones (Metvix®, Ameluz®), although they may be enhanced with additional factors in order to improve the efficacy of PpIX production, the photodynamic effect itself, as well as reduce the number of repetitions to maintain the desired effect. The non-commercial formulations may be based on block polymers of ethylene oxide and propylene oxide (such as Lutrol/Pluronic F127) and poly(acrylic acid) (Carbopol) [118,113–115], on lanolin and pentetic acid ointment [117], on Sepigel base (polyacrylamide with C-13-14 isoparaffin and ethoxylated fatty alcohols) [121] or simple dissolution in Vaseline [126] or Lipobase cream [132]. The formulations described in the literature, with emphasize on gel or cream form are presented in Table 1. The improvements may include an addition of calcipotriol (a synthetic vitamin D derivative), as stated by Yang et al. and Piaserico and Fargnoli [114,130], or utilize other derivatives of the active compounds, such as phosphate salt of 5-ALA [132]. The protocol itself may be also enhanced by splitting the irradiations into smaller intervals followed by short breaks in order to restore the PpIX inside the tissue [113,115]. The occlusive application may as well enhance the efficiency, compared to non-occlusive one, as stated by Meierhofer et al. [128].

4.2. Non-cancerous lesions

The gel or cream formulations used for non-cancerous lesions usually were less concentrated, when compared to cancer-related ones, oscillating mainly around 5–10 % of 5-ALA. The formulations with particular gel or cream form are presented in Table 2 [124,134–142]. Interestingly, the enhancements included into the formulations were associated with the use of nanoethosomes as transdermal vesicles, as proposed by Zhang et al. [139]. The nanoethosomes showed superior accumulation of PpIX and better photodynamic activity than the control 5-ALA gel. Similarly, they tested an addition of hyaluronic acid into the 5-ALA gel and reported increase of 5-ALA accumulation and photodynamic effect itself, although they were lower in comparison to nanoethosomes. Similar one, commercially available hyaluronic acid (HA)-enhanced gel formulation was tested by Huang et al. who reported its activity in the treatment of photoaging lesions, indicating the beneficial role of HA and its possibility to use in younger patients [140]. HA was also tested in combination with chitosan to form nanogels loaded with 5-ALA and methotrexate [142]. Wang et al. used such formulation for the combined therapy of psoriasis. Nanogels appear to increase pharmacokinetic parameters such as drug uptake, PpIX conversion and ROS generation. Moreover, the presence of specific psoriasis-targeted methotrexate

Table 1
In vivo and clinical effects of 5-ALA and MAL PDT on pre-cancerous and cancerous lesions.

Disease	Formulation	Light source, wavelength, and intensity	Preincubation time, PDT repetitions	Number of patients, Complete response rate	Recurrence rate, follow-up	Ref.
Oral proliferative verrucous leukoplakia	20 % 5-ALA gel	635 nm diode laser, 100 J/cm^2	1.5 h; 4 times	1; 100 %	0 %, 12 months	[113]
Oral precancerous lesions	20 % 5-ALA gel[a] 20 % 5-ALA gel[b]	640 nm custom LED source, 100 J/cm^2	3.5 h; 3 times 2.5 h; 4–6 times	6; 67 % 6; 67 %	0 %, 20 weeks[c] 0 %, 20 weeks[c]	[114]
Oral leukoplakia	20 % 5-ALA gel	635 nm diode laser, 100 J/cm^2	1.5 h; 2–5 times	5; 60 %	60 %, N.P.	[115]
Lip florid papillomatosis	10 % 5-ALA cream	635 nm diode laser, 120 J/cm^2	3 h; 3–5 times	4; 100 %	25 %, 6 months	[116]
Oral precancerous lesions	20 % 5-ALA cream 10 % MAL cream	630 nm custom LED source, 12 J/cm^2	Time N.P.; repetitions N. A.	9; 100 % 11; 100 %	0 %, 20 days[c] 0 %, 20 days[c]	[117]
Cervical intraepithelial neoplasia grade I	6 % 5-ALA gel	635 nm laser with optic fiber, 200 J/cm^2, 520–2250 s[d]	4 h; 3 times	30; 80 %	0 %, 6 months	[118]
Actinic keratosis	16 % MAL cream	628 nm diode laser, 100 J/cm^2	3 h; 1–2 times	62; 97 %	5 %, 7 years	[119]
Basal cell carcinoma	8 % 5-ALA gel 16 % MAL cream	635 nm custom LED source, 37 J/cm^2, time N.P.	3 h; 2–3 times	138; 93 % 143; 92 %	7 %, 12 months 8 %, 12 months	[120]
Actinic keratosis	10 % 5-ALA gel	630 nm commercial LED source, light dose N.P., 600 s	2 h; 1–3 times	15; Complete response N. P.	Recurence rate N.P., 3 months	[121]
Actinic keratosis	8 % 5-ALA gel	635 nm commercial LED source, 37 J/cm^2	3 h; 1–2 times[e]	30; 93 %	0 %, 6 months	[122]
Actinic keratosis	8 % 5-ALA gel 16 % MAL cream	Wavelength N.A., daylight, light dose N.A.	30 min; 1 time	52; 80 % 52; 77 %	20 %, 9 months 32 %, 9 months	[123]
Vulvar intraepithelial neoplasia grade I	5 % 5-ALA gel	590–760 nm halogenic lamp, 120 J/cm^2	3 h; 10 times	1; 100 %	0 %, 8 years	[124]
Cervical intraepithelial neoplasia grade I				2; 100 %	0 %, 2–3 years	
Cervical intraepithelial neoplasia grade III				2; 100 %	0 %, 2–4 years	
Basal cell carcinoma	12 % 5-ALA gel	630 nm laser, 350 J/cm^2	3 h; 2 times	82; 94 %	4 %, 3–76 months	[125]
Basal cell carcinoma	20 % 5-ALA ointment	638 nm, custom laser, 48 J/cm^2	4 h; 1–4 times	50; 87 %	0 %, 36 months	[126]
Squamous cell carcinoma	10 % 5-ALA gel	Wavelength N.P., commercial LED source, 37 J/cm^2	3 h; 2–4 times	12; 100 %	0 %, 4 weeks	[127]
Actinic keratosis	8 % 5-ALA gel	635 nm commercial LED source, 37 J/cm^2	3 h; 1 time	43; 72 %[f] 43; 36 %[g]	20 %[f], 6 months 49 %[g], 6 months	[128]
Basal cell carcinoma	8 % 5-ALA gel	635 nm commercial LED source, 37 J/cm^2, time N.P.	3 h; 2 times	31; 74 %	0 %, 12 months	[129]
Actinic keratosis	16 % MAL cream	Wavelength N.A., daylight, light dose N.A.	30 min; 1 time	36; 64 %[a] 36; 58 %[b]	0 %[a], 3 months 0 %[b], 3 months	[130]
Actinic keratosis	8 % 5-ALA gel	Wavelength N.A., simulated daylight, 14–24 J/cm^2	30 min; 2 times	12; 85 %	N.P., 3 months	[131]
Actinic keratosis	10 % 5-ALA gel 10 % MAL gel 18 % 5-ALA phosphate gel	630 nm commercial LED source, 37 J/cm^2	3 h; 2 times	15; 83 % 15; 86 % 15; 84 %	N. P., 12 weeks N. P., 12 weeks N. P., 12 weeks	[132]
Cervical intraepithelial neoplasia grade I-III	20 % 5-ALA gel	635 nm commercial LED source, 80–100 J/cm^2	3.5 h; 4–6 times	183; 90 %	2 %, 12 months	[133]

N.P. – Not provided, N.A. – Not applicable, [a]With calcipotriol, [b] Without calcipotriol, [c]Animals were euthanized after this period, [d]Each patient had the time calculated individually, [e]Per single area, [f]Occlusive 5-ALA application, [g]Non-occlusive 5-ALA application.

Table 2
In vivo and clinical effects of 5-ALA and MAL PDT on non-cancerous lesions.

Disease	Formulation	Light source and PDT parameters	Preincubation time, PDT repetitions	Number of patients, Complete response rate	Recurrence rate, follow-up	Ref.
Photoaging lesions	5 % 5-ALA cream	630 nm, commercial LED source, 60 J/cm^2, 600 s	2 h, 2 times	10, N.P.	N.A., 2 months	[134]
		550–570 nm intensed pulse light, 60 J/cm^2, time N.P.		10, N.P.	N.A., 2 months	
Lichen planus	10 % 5-ALA cream	635 nm diode laser, 100 J/cm^2, 1000 s	3 h, 3 times	7, 71 %	0 %, 6 months	[135]
Facial flat wart	10 % 5-ALA gel	630 nm, commercial LED source, 70–120 J/cm^2, 1200 s	3 h, 3 times	12, 89 %	0 %, 24 weeks	[136]
Lichen sclerosus	5 % 5-ALA gel	590–760 nm halogenic lamp, 120 J/cm^2, 600 s	Time N.P., 10 times	102, 87 %	0 %, 12 months	[137]
Cutaneous wart	10 % 5-ALA cream	630 nm custom light source, 75 J/cm^2, 1020 s	3 h, 3 times	13, 85 %	0 %, 4 months	[138]
Squamous cell hyperplasia	5 % 5-ALA gel	590–760 nm halogenic lamp, 120 J/cm^2, 600 s	3 h, 10 times	2, 100 %	0 %, 2–6 years	[124]
Genital warts				1, 100 %	0 %, 5 years	
Lichen sclerosus				2, 100 %	0 %, 2–5 years	
Hypertrophic scars	2 mg/mL 5-ALA gel	635 nm laser, 5 J/cm^2, 600 s	6 h, 4 times	4, response rate N.P.	Recurrence rate N.A., follow-up N.A.	[139]
Photoaging lesions	2 % 5-ALA gel	633 nm commercial LED source, light dose N.P., 1200 s	40 min, 3 times	6, 67 %[a]	Recurrence rate N.A., 4 weeks	[140]
Alopecia aerata	5-ALA gel, concentration N.P.	630 nm commercial LED device, 37 J/cm^2, 450 s	3 h, 6 times	1, 100 %	Recurrence rate N.A., 4 months	[141]
Psoriasis	10 % 5-ALA gel	635 nm commercial laser, 7 J/cm^2, 175 s	4 h, 7 times	8, response rate N.A.	Recurrence rate N.P., 7 days	[142]

N.P. – Not provided, N.A. – Not applicable, [a]Assessed by physicians.

resulted in effective treatment of the lesions [142].

4.3. Bacteria and fungi related diseases

PDT utilizing 5-ALA gels or creams for treatment of bacterial diseases, such as acne vulgaris, is also a versatile approach. Although the majority of formulations were basic 5-ALA gels, some enhancements were also utilized. The formulations with an emphasis on gel or cream form are presented in Table 3 [143–149]. An interesting approach was presented by Kwon et al., who used an unusual 5-ALA ester – 3-butenyl-5-aminolevulinate. The PpIX precursor appeared to be effective for PDT application and presented good outcomes which can be associated with better transmembrane transport of the hydrophobic ester rather than that of more hydrophilic 5-ALA itself [143]. Although the occurrence of 5-ALA gel-mediated PDT of bacterial and fungal infections was rare, its usage may provide a convenient form of either monotherapy or as an adjuvant therapeutic protocol, possessing the potential to efficiently combat particularly resistant microbes and difficult-to-treat stages of various diseases.

5. New semi-solid formulations against cancer and microorganisms

5.1. Based on alginates

Alginates are constructed of 1,4-linked-β-mannuronic acid and α-L-guluronic acid and finally form hydrogels, which can be stabilized with Ca^{2+} ions [151]. Hydrogels bring meaningful advantages to the pharmaceutical formulations such as high loading and high stability. However, some problems with such drug-cargo system have been noted. One of them is slow diffusion of large molecules outside the hydrogel. On the other hand, hydrogels are likely to modify.

Table 3
In vivo and clinical effects of 5-ALA and MAL PDT on bacterial and fungal infections.

Disease	Formulation	Light source and PDT parameters	Preincubation time, PDT repetitions	Number of patients, Complete response rate	Recurrence rate, follow-up	Ref.
Acne vulgaris	1.5 % 3-butenyl 5-ALA	Wavelength N.A., daylight, light dose N.A.	Time N.P., 84 times	46, 58 %	0 %, 12 weeks	[143]
Acne vulgaris	5 % 5-ALA cream	633 nm, commercial LED source, 36–108 J/cm^2	2 h, 3 times	12, 86 %	0 %, N.P.	[144]
		590–1200 nm intensed pulse laser, 15–17 J/cm^2	2 h, 3 times	12, 76 %	0 %, N.P.	
Acne vulgaris	20 % 5-ALA gel	Wavelength N.P, commercial LED source, 37 J/cm^2	1.5 h, 2 times	23, 74 %	0 %, 12 weeks	[145]
Acne vulgaris	5 % 5-ALA gel	630 nm commercial LED source, 25 mJ[a]	2 h, 2–6 times	35, 70 %	0 %, 6 months	[146]
MRSA-infected wounds	10 % 5-ALA gel	635 nm commercial LED source, 25 J/cm^2	3 h, 1 time	8, 100 %	Recurrence rate N.A., 14 days[b]	[147]
Acne vulgaris	5 % 5-ALA gel	633 nm commercial LED source, 9 J/cm^2	1.5 h, 4 times	23, 96 %	0 %, 12 weeks	[148]
	10 % 5-ALA gel			23, 70 %	0 %, 12 weeks	
Acne vulgaris	16 % MAL cream	635 nm commercial LED source, 20 J/cm^2	1.5 h, 2 times	14, 74 %	0 %, 20 weeks	[150]
			1.5 h, 4 times	12, 85 %	0 %, 20 weeks	
Hyperplastic candidiasis	20 % 5-ALA gel	628 nm LED laser, 100–200 J/cm^2	2 h, 3 times	10, 10 %[c]	Recurrence time N.P., 8 weeks	[149]
				10, 30 %[d]	Recurrence time N.P., 8 weeks	

N.P. – Not provided, N.A. – Not applicable, [a]Energy dose not specified, [b]Animals were euthanized after this period, [c]Without nystatin, [d]With nystatin.

5.1.1. In antimicrobial applications

Application of alginate-PS conjugates in bacteria photoinactivation to prevent plant diseases has been studied. Liu et al. have presented alginate oligosaccharide-PpIX conjugate, where thio-linker was used [152]. The conjugate is inactive under light exposure. The shape of obtained conjugate was characterized by transmission electron microscopy (TEM) as core–shell type. The core is formed by PS and the shell by polymer. Such construction provides additional benefit – the photostability of macrocycle is increased and it is kept in an inactive state, able to work when it is needed. However, the S-S bond between alginate and PS is efficiently decomposed by glutathione (GSH), which is secreted by an infected plant. In this way thiolated PpIX is released. Its photoactivity against *Escherichia coli* and *Staphylococcus aureus* increased 2-fold in comparison to pure PpIX. Interestingly, system used to treat infections with *Erwinia carotovora* has shown dependency between the bactericidal activity and kinetics of GSH secretion [152].

Covalently bounded PS with sodium alginate was the basis of wound dressing proposed by Guo et al. [153]. Modified alginate was linked with phenylboronic acid as well, which enabled to form cross-bond with calcium ions to prepare aerogel. Phenylboronic group worked also as targeting tool via forming bond between boronic ester and glycoproteins or polysaccharides expressed on the bacteria surface. In the *in vitro* model authors reported 90 % reduction in *S. aureus* growth of developed system [153]. Nevertheless, it is relatively low value when compared to other data [154–156,20,21,24], due to quick hemostasis and high biosafety the *in vivo* experiments have been made. Trials were performed on mouse model of *S. aureus* infected wounds and showed that application of a new tool accelerates the curing process including bacteria elimination [153].

Chitosan/alginate hydrogel seems to be an ideal platform for transport of PSs into the wound. Ji et al. synthesized an iridium complex with high ability to form singlet oxygen under light irradiation. Obtained PSs have been loaded into hydrogel composed of chitosan and sodium alginate. In *in vitro* studies, designed system showed high activity against *S. aureus* and *Pseudomonas aeruginosa*. Simultaneously, on the H22 cell the biosafety was indicated. The developed material was subjected to treat a wound in *in vivo* mouse model, which resulted in total *S. aureus* eradication [157].

An interesting approach to wound dressing in form of foam has been developed over the years by the team of prof. Tønnesen. They have prepared six formulations of alginate based foam, including curcumin as a PS and hydroxycyclodextrins or low-molecular-mass poly(ethylene glycol) (PEG400) as solubilizing agents. It was determined that PS was homogeneously distributed within foam volume and it was released as a monomer. Unfortunately, curcumin was susceptible to photodecomposition in foam, which forced the scientists to look for a direction. The designed foam can be sterilized with γ radiation which does not impact formulation ingredients and PS. In the *in vitro* photoactivity evaluation against microbes it turned out that formulated curcumin is highly photoactive against *Enterococcus faecalis* at a relatively low light dose (9.7 J/cm^2 resulted in >6 log reduction in bacterial growth). Interestingly, against *E. coli* at over 2-fold higher light dose (29 J/cm^2) dependently on solubilizer there was no activity (cyclodextrins) and negligible activity (<1 log for PEG400) [158,159]. Similar results were reported for foam with poloxamer (Pluronic 127) as a solubilizer and tetrakis(4-hydroxyphenyl)porphyrin (THPP) [160]. Unfortunately, the antimicrobial activity of developed foam with porphyrin has not been provided. It is noteworthy that the simple and handy formulation works efficiently as a wound dressing. Promising results concerning the use of hydrogels as cargo for PSs were also reported by Feng et al. They have modified alginate hydrogel based on keratin to prepare composite scaffolds. Moreover, keratin materials are known to provide excellent hemostasis parameters. The methylene blue (MB) as PS contributes to antibacterial activity [161]. Sharma et al. have introduced to wound dressing both pectin and carboxymethyl cellulose next to sodium alginate and PS. As PSs chlorin e6 (Ce6) and MB were used. The film containing Ce6 revealed ca. 3 log reduction in MRSA growth which was comparable to free Ce6. Interestingly, MB loaded film showed only ca. 1 log reduction in bacterial growth. Authors have linked such observation with low binding ability of Ce6 by film components. The developed films showed high biocompatibility (no influence on HaCaT cells growth) [162].

Particularly resistant to the treatment are infections of bacteria forming so-called biofilm. Biofilm is well-organized compact structure of numerous bacterial cells. For ideal visualization it is also called “city of microbes” [163]. The mentioned structure with “special needs” in terms of treatment protocol can be efficiently combated with curcumin-loaded nanoparticles built from chitosan and sodium alginate. It was observed that functionalized NPs were highly active against *Streptococcus mutans* biofilm (3logreductionofbiofilmgrowth). Interestingly, mucoadhesive film composed of the same ingredients has not shown any antimicrobial photoactivity. However, it may be considered as a transporting platform for other drugs [164].

Alginate as a base for forming hydrogels with excellent parameters to cure chronic wounds seems to be a perfect PS cargo system for maximal enhancement of the treatment efficacy.

5.1.2. In anticancer applications

Yang et al. proposed an approach based on multimodal combined therapy (MCT) in cancer treatment. They analyzed the tumor microenvironment (TME) and observed an increase in the concentration of H^+ ions, H_2O_2, and GSH, as well as a significantly reduced level of molecular oxygen, which resulted in decreased susceptibility to ferroptosis in cancerous tissues. As a potential response to this challenge, they developed a nanoplatform consisting of gold combined with N-acetylcysteine (NAC) and 4-mercaptobenzoic acid (MBA) – $Au_{25}(NAMB)_{18}$ nanoclusters complexed with Cu^{2+} and coated with cross-linked sodium alginate and hyaluronic acid polysaccharide (HA). The obtained nanoparticles exhibited increased release of Cu^{2+} in acidic environments. The released ions then underwent a reduction process to Cu^+ in the presence of excess GSH. This facilitated the generation of hydroxyl radicals, inducing cuproptosis, a form of programmed cell death. Additionally, irradiation induced an increase in temperature (photothermal therapy, PTT) and production of reactive oxygen species (PDT). Importantly, this multifaceted approach demonstrated strong anticancer efficacy both in *in vitro* studies (with an 81.4 % reduction in cell growth) and in *in vivo* experiments (resulting in an 85.7 % inhibition of tumor growth) conducted on HepG2 and H22 cells, respectively [165].

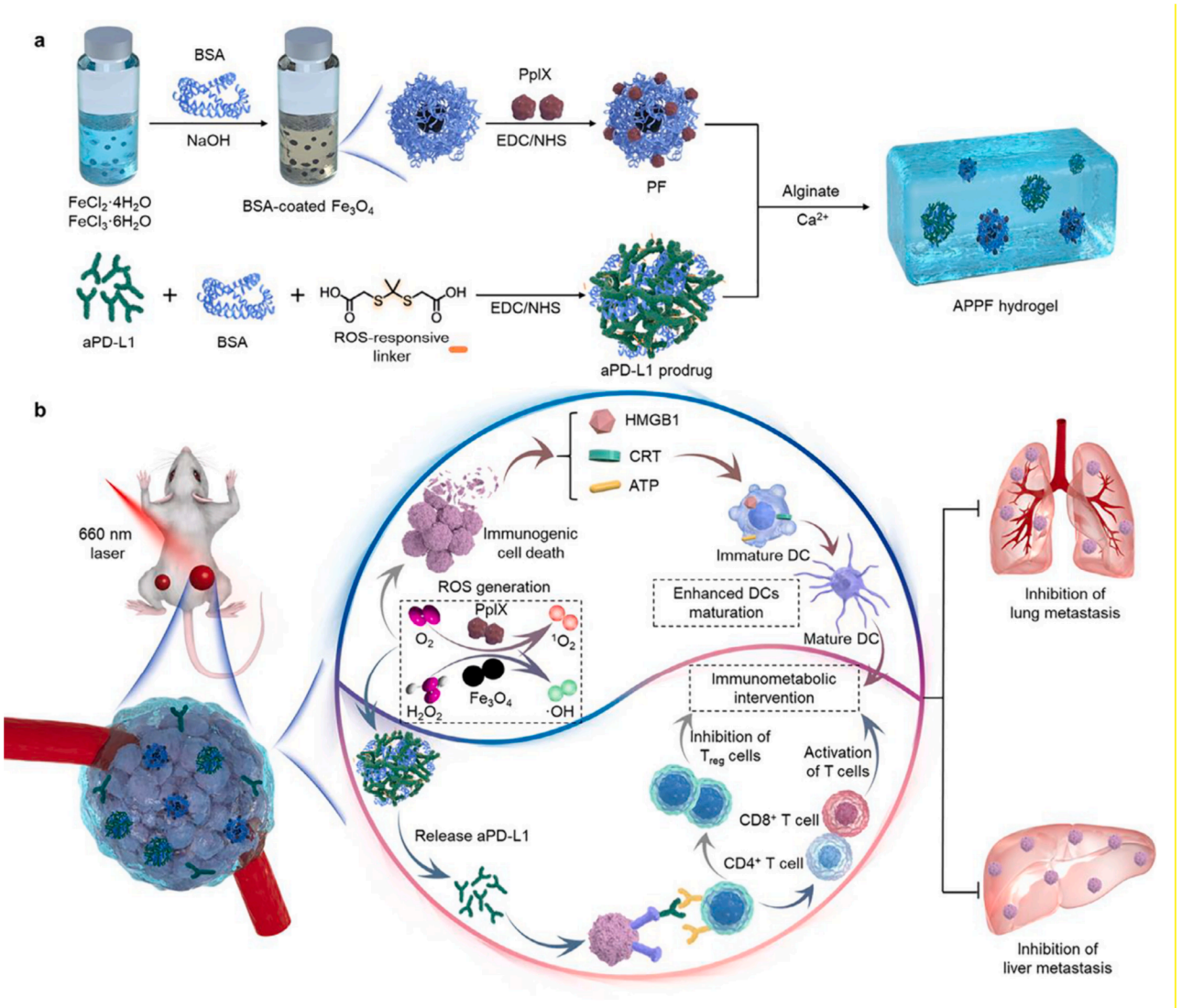
Liu et al. have proposed hydrogel based on alginate bearing PS, manganese oxide and proenzyme nanoparticles. This bright concept focuses on: 1) PDT via PpIX as PS; 2) manganese oxide under irradiation provides molecular oxygen which is crucial for PDT in hypoxic environment of tumors and 3) proenzyme nanoparticles composed of deoxyribonuclease I are connected with N-hydroxysuccinimide by $^1O_2$-sensitive linker. The released by singlet oxygen enzyme induces cell death and inhibits neutrophil extracellular traps responsible for tumor metastasis. In brief MnO_2 and PpIX were immobilized in bovine serum albumin, enzyme was inactivated by conjugation and finally both were implemented in the Ca^{2+} cross-linked alginate. The reported results of activity evaluation are very promising. The high activity of the designed platform was confirmed against 4 T1 cells in *in vitro* model after irradiation with 660 nm light (reduction of the living cells by over 90 %). Simultaneously, the high biocompatibility and low toxicity in the dark conditions was observed. In *in vivo* experiment on mouse model, activity against breast cancer and its lung metastases was evaluated. No significant body weight changes between the treated and control groups were observed. High activity was noted for the designed tool in comparison to each component used separately. It’s worth noting that only the use of the discussed nanosystem prevented the occurrence of lung metastases. [166].

Sun et al. have tested another strategy to overcome disadvantageous TME – hypoxia. Oxygen level within a tumor can be increased using bio-oxygen pump, such as cyanobacteria S.2973. A near infrared (NIR)-

activated PS (indocyanine green, ICG) was introduced to the mesoporous silica, which together with cyanobacteria were gelled by sodium alginate and Ca^{2+} ions. After administration of the developed drug, red laser was used for irradiation. This enabled to produce oxygen by bacteria and did not turn on cascade reaction following photodynamic effect (killing bacteria by formed ROS as well). Further a laser at 808 nm induced producing ROS by ICG. Experiments *in vitro* confirmed the high activity of silica loaded ICG. This allowed to perform *in vivo* model tests with 4 T1 cells inoculated to mice as a tumor model. No significant differences in body weight of mice were observed in comparison to the control group. For the group treated with the designed drug the lowest tumor weight and the highest tumor inhibition growth were noted. Interestingly, no side effects caused by bacteria introduced onto body were observed [167]. Although obtained results are very promising, the proposed approach has to be deeply investigated in other tumor models.

Ding et al. from the same group designed also a complex pro-drug hydrogel based on sodium alginate and containing PpIX as PS for PDT, Fe_3O_4 for chemodynamic therapy (CMT) and antibody aPD-L1 for inducing immunogenic cell death. Antibody is covalently bound with ROS responsible linker with bovine serum albumin. After deposition in cancer cell the antibody is triggered by both PDT under light irradiation with 660 nm and via chemodynamic mechanism linked to Fenton reaction – formation of hydroxyl radical and hydrogen peroxide by iron oxide. Besides the triggering role of PDT and CMT they work against cancer separately and what is most important they can work in parallel. Therefore, in *in vitro* experiments high biosafety in the dark was indicated, whereas under light irradiation the cascade of reaction was turned on resulting in high activity against 4 T1 cells. Even better results were achieved in *in vivo* mice model. In the group treated with the designed platform primary tumor volume was significantly reduced, whereas in the others, tumor volume increased even 12 fold. Interestingly, in the same cured group volumes of distant tumors were significantly reduced as well [168]. It could be concluded that proposed prodrug could pave the way to indicate new direction in the photodynamic method development (Scheme 1).

Zhou et al. have combined activity of glucose oxidase, prodrug tirapazamine and semiconducting polymer, Poly[2,6-(4,4-bis-(2-ethylhexyl)-4H-cyclopenta [2,1-b;3,4-b′]dithiophene)-alt-4,7(2,1,3-benzothiadiazole)] (PCPDTBT) as a PS. All active ingredients were mixed with alginate. The nanoplatform was designed for direct injection into the tumor, where with endogenous Ca^{2+} chelation of all agents was reached. In tumor, hydrogel slowly released its ingredients. Glucose oxidase contributed to oxygen production for PDT. What important, formed molecular oxygen was *in situ* consumed by PS to produce singlet oxygen



Scheme 1. A prodrug hydrogel with tumor microenvironment and near-infrared light dual-responsive action for synergistic cancer immunotherapy. [168] Licensed by Elsevier (license number 5718250987267).

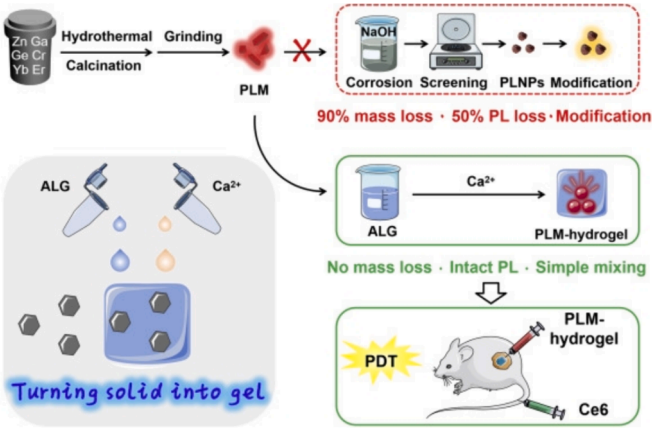
– active factor against cancer. This enabled to maintain hypoxic condition within TME necessary for activating prodrug – tirapazamine. In biological activity evaluation *in vitro* inhibition of cell viability at a level of ca. 70 % was noted. In *in vivo* model the tumor was completely eradicated and did not return in 14 days post-treatment. It was indicated that such approach is not ideal. It cannot be used when tumors are located deeply due to low tissue penetration of 808 nm light and the need of injection into tumor [169]. Therefore, the development of such treatment protocol has to be continued.

Multimodal approach was also presented by Pillarisetti et al., who chemically conjugated alginate and PS – pheophorbide and adipic acid hydrazide to form nanogel. The formulation contained a conventional anticancer drug – doxorubicin. In this way the PDT and chemotherapy were combined to treat model cell lines of breast cancer and melanoma. The designed tool, multi-stimuli responsive alginate nanogel (MSAN) responded both to pH change and to TME. In the lowered pH release was more efficient. The biological activity was also evaluated. Intracellular ROS production was observed. The treated cell lines BF16F10 and 4T1 were effectively killed by the MSAN [170]. Similar approach presented Du et al., who prepared alginate modified liposomes bearing folate as a targeting factor. In such nanoparticles, hypocrellin B as PS was located in the outer lipid membrane. Experiments *in vitro* showed reasonable reduction of HeLa (human cervical cancer) cells [171].

Yang et al. have proposed combination of chemotherapy, PDT, and gene therapy of cancer. Additionally their nanocomposite provides nanoprobe for magnetic resonance (MR) and computed tomography (CT) imaging of tumors. Firstly, Fe_3O_4 core was bound with Au and such nanocrystals were covered by silica shell. The doxorubicin – chemotherapeutic agent and Ce6 – PS were loaded into mesoporous channels. The obtained core/shell was covered layer-by-layer with alginate/chitosan polyelectrolyte. Obtained multinanosystem finally was combined by electric attraction with P-glycoprotein (P-gp) small hairpin RNA – sensitizing agent of drug resistant cancer cells (by down regulation of P-pg level). Prepared "nano-truck" was opened up in acidic conditions of TME and released all transported "cargo". The designed tool inhibited drug-resistant MCF-7/ADR cells growth by over 60 % vs. monotherapy. Experiment *in vivo* established high potential of this nanocomposite in reducing tumor volume. Authors concluded that this combined therapy revealed neither synergistic nor additive effect. It has to be emphasized that mouse body weight did not differ significantly from control. What important, dark toxicity of the designed tool has been evaluated and no toxic effects were noted. Moreover, it was observed that hemolysis did not appear, thus nanocomposite can be administered intravenously [172].

The crucial PDT limiting factor is the excitation of PS with light. This phenomenon is necessary to turn on cascade of processes leading to combat cancer. The problem is with deeply located tumors inside the body where light has difficulty to penetrate tissues [131,132,48]. Revolutionary approach is to develop the nano-sized "bulb". Sun et al. have proposed a smart persistent luminescence material (PersLum) based on the core composed of ytterbium, erbium, chromium and so on. Such core has been dispersed in sodium alginate to form hydrogel. Obtained PersLum excited with UV light emitted red luminescence. In the presence of previously activated PersLum material, Ce6 was efficiently forming singlet oxygen. The *in vitro* model proved its high activity against 4 T1 cells reducing their growth about 82 %. Simultaneously, authors have reported that "the bulb" was inactive in the absence of Ce6. Such good results encouraged researchers to perform experiments in *in vivo* model. Interestingly, PersLum administered in mice was able to turn on even after seven days. The protocol of the treatment was as follows: administration of Ce6 to the body, after 2 h (distribution time) activated PersLum was injected directly into tumor. 16-Fold reduction in tumor volume in comparison to the control was noted. It should be emphasized that "the bulb" showed no toxicity. No differences in body weight and no histopathological changes within control group were observed. The idea of such approach seems brilliant, nevertheless, that there are some difficulties have to be solved. First at all, the question is "will the system provide enough photons to excite PS inside the whole volume of tumor?". The second problem is pharmaceutical technology – PersLum obtained via "turning solid into gel" strategy had an irregular morphology, which is an obstacle to clinical use. Moreover, the pathway of elimination of the developed material has to be found. All these factors can limit use of PersLum in comparison to the interstitial PDT, where light is provide in the laparocsopi way through fiber optics (see Scheme 2).

Alginate component bound to PS can act not only as a water-solubilising group but also as a targeting factor. It is widely known that the scavenger receptor-A on tumor-associated macrophages easily recognizes polyanions like alginates. Thus, Tang et al. synthesized conjugate of sodium alginate and zinc(II) phthalocyanine derivative. The obtained conjugate significantly improved water solubility of phthalocyanine. The reasonable selectivity of such tool to SR-A-positive HepG2 cell line was observed, whereas no activity was observed against SR-A-negative HepG2 cell line. In *in vivo* evaluation on mouse model, increased deposition of conjugate in the liver was noted. What is promising, the 87 % reduction in tumor growth was achieved. Simultaneously, no toxic effects were observed. The important relationship was reported: when bigger alginate (1331.5 kDa vs. 41.2 kDa) component was used, the selectivity and treatment response were significantly worse [176]. Nanoformulation based on chitosan/alginate with antibodies directed against the death receptor (DR5) proposed by Abdelghany et al. showed high selectivity. It was reported that antibody itself activates the caspase 8 pathway leading to the cell death. The formed particles are 560 nm in diameter and could be loaded with 9.1 μg of N-methylpyridyl porphyrin derivative (TMP) per each 1 mg of formulation. The cargo system transports efficiently and preferentially the PS into HCT116 cells showing up-regulation of the DR5 receptors at their surface. Obtained results clearly indicated that under light irradiation after proper incubation time cancer cells growth was significantly inhibited [177]. The interesting PS delivery tool was designed by Manimaran et al. They have developed microneedles composed of carboxymethyl cellulose, sodium alginate and polyethylene glycol making them dissolvable. The proposed composition of needles with practical mechanical parameters allows penetration of buccal mucosa model to a depth of 300 μm. The applied needles dissolve within 30 min. Thus, PS – ICG was effectively delivered from the dissolvable microneedle patch to the tumor and after IR-light activation ROS were formed. The potential of such approach was evaluated on oral carcinoma FaDu xenografted tumor model in athymic nude mice. What promising, significant decrease of tumor volume in comparison to the control group was noted [178]. Gomez et al. introduced curcumin as a PS into chitosan/alginate



Scheme 2. Turning solid into gel for high-efficient persistent luminescence-sensitized PDTherapy [175]. Licensed by Elsevier (license number 5718251405773).

nanoparticles. According to performed studies it was concluded that activation of nanoparticles with UV light (10 J/cm^2) contributes to significant decrease of viable hyperproliferating HaCaT cells – human keratinocyte (unusual proliferation induced by tumor necrosis factor-α glycoprotein) [179] (see Fig. 3).

5.2. Gelatin

Gelatin is a natural, biocompatible, biodegradable and multifunctional biopolymer containing widely used mixture of peptides. It is obtained as a result of transformations of a naturally occurring parent protein – collagen. The necessary condition to produce it is the destruction of cross-links between polypeptide chains. It is also required to break some polypeptide bonds [142]. The molecular weight of gelatine is highly dependent on its origin and method of manufacturing. For the most part, particle size remains in the range of 40 to 90 kDa, although numerous deviations from this norm are noted.

Gelatine is (or, in some countries, was) used in pharmacy, among others, as a drug per se for hypovolemia and hypotension [143]. Gelatin also occupies a very important position in drug formulation. It is a component of pharmaceutical capsules, ointments, cosmetics, tablet coatings and emulsions [144]. In addition, it is a stabilizer for numerous vaccines, including vaccines against measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria and tetanus toxin [145]. Due to many years of experience in the use of gelatine and its well-known safety profile, including, above all, lack of toxicity, it is an excellent agent for biomedical purposes. Therefore, new medical technologies are being developed based on this mixture of peptides. Among other things, the use of gelatin heart valve substitutes and the development of blood substitutes based on it were considered. Gelatin-based gels are also characterized by very good biocompatibility. However, it should be strongly emphasized that the original gelatin, as it rapidly dissolves in an aqueous medium, is not suitable as a modified release carrier. Structural modifications are necessary to use it for this purpose. First of all, its crosslinking is crucial. The increase in crosslinking may have a beneficial effect on its mechanical and thermal stability, and as a result it can regulate the release period of bioactive molecules [145]. There are many studies on gelatin-based hydrogels. They reveal common and characteristic features for all types of hydrogels, such as the ability to incorporate large amounts of water and swell while maintaining their original structure. Typical of gelatin-based hydrogels, however, is their thermolability. They belong to the so-called “physical gels” created by forming hydrogen bonds. Gelatin dissolves at a temperature above 40 °C, while gelling occurs when the temperature drops below 30 °C. Together with the temperature decrease, the original spatial arrangement of collagen triple helices returns. Their restructuring allows for the creation of a stable three-dimensional structure characteristic of gelatin hydrogels [146]. Thanks to the already mentioned crosslinking, they are able to keep their structure relatively stable for a longer period of time [145]. It is also possible to chemically modify the gelatin to provide a scaffold for tissue engineering. For example, the modified methacrylated gelatin hydrogel is activated by visible light in the air or in an aqueous environment. Under the influence of the trigger, rapid cross-linking and gelation occurs at the target site. It provides a very promising basis for the growth and differentiation of bone marrow-derived cells, resulting in the intensification of TGF-b3-induced chondrogenesis [147].

The discussed advantages of gelatin as a cheap, easily available and biocompatible material naturally attracted the attention of researchers associated with PDT, both directed against microorganisms and cancer.

5.2.1. In antimicrobial applications

When using PACT in bacterial and fungal infections, the greatest limitation is the delivery of light to the site of infection. Mainly spot irradiation is possible, therefore the therapy of generalized infections is very limited. For that reason, the treatment of superficial infections, including skin infections, wounds, ulcers, boils, diabetic foot, and inflammation associated with acne vulgaris, is particularly promising. The skin, as the largest human organ, constantly in contact with the outside world, is particularly vulnerable to abrasions and contamination. For infections caused by some Gram-positive bacteria, including *S. aureus*, it is possible to use their innate ability to produce matrix metalloproteinase (MMP) enzymes, especially gelatinase (MMP-2 and MMP-9). In addition, they are markers of infection for some strains of bacteria. This property allows for targeted delivery of the drug substance

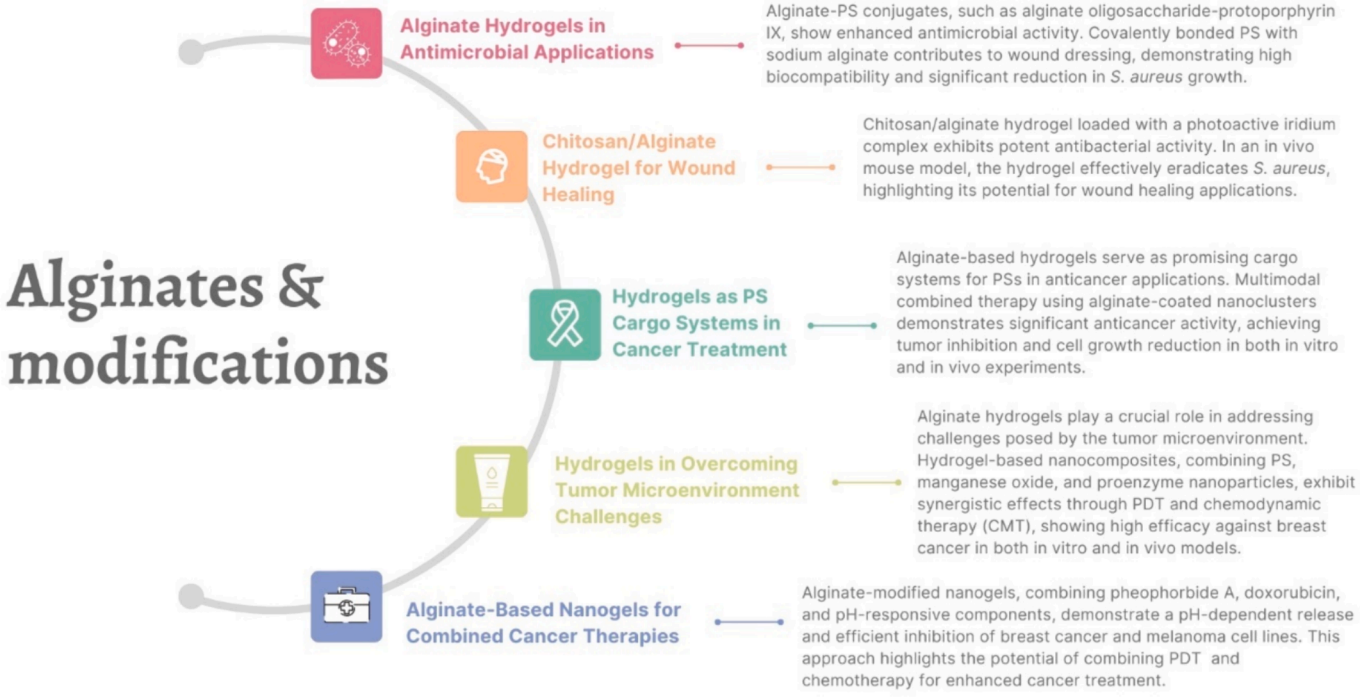


Fig. 3. The application of alginates and their modifications in formulations used against microorganisms and cancer.

to the site of infection. The active substances incorporated into the gelatin matrix of the hydrogel will not be released until they come into contact with the infected tissue. Such formulation was developed e.g. for vancomycin, in which antibiotic molecules have been bound with gelatin nanoparticles [180]. Under the influence of gelatinase, the drug is released, which allows to achieve the desired concentration *in situ* and at the same time to reduce systemic side effects. An analogous technology was developed by Han et al. for photodynamic/photothermal therapy (PDT/PTT) [181]. In this case, a combination of gelatin nanogel with CuS nanodots – G@CuS was prepared. Light with a wavelength of 808 nm was used to induce photothermal activity. It should be noted that Cu^{2+} ions themselves exhibit antibacterial properties due to their ability to cause damage to the integrity of the bacterial membrane and disrupt the enzymatic function of the cell [182,183]. Also, the oxidation–reduction reactions taking place at the time of conversion of Cu^{2+} to Cu^{+} play a significant role in the antimicrobial potential of copper. On the other hand, the oxidative stress caused by Cu^{2+} ions is not inert to mammalian cells and may cause a cytotoxic effect on eukaryotic cells [181]. The use of a gelatin matrix in the experiment made it possible to avoid this effect due to the release of copper ions only under the influence of *S. aureus* enzymes. The selectivity of this model was confirmed by a control study conducted with *E. coli* bacteria, which do not produce gelatinase and at the same time are more sensitive to the action of copper ions (the mechanism determining the increased activity has not yet been fully investigated). In this case, marginal activity of the CuS nanodots was observed. Irradiation of the sample with NIR light caused the CuS and G@CuS nanodots to eradicate the bacteria. This result was observed even with G@CuS, which was previously less effective against *E. coli*. The results of the *in vitro* tests were confirmed in an *in vivo* study in mice. The duration of therapy for a wound superinfected with *S. aureus* was 9 days. For the phosphate-buffered saline (PBS), gelatin and CuS particles, the rate of wound healing in comparison to control probe was 27 %, 41 % and 51 %, respectively, and the wounds were still covered with thick scars. Almost complete cure (approx. 87 %) was obtained for the combination of nanogel and CuS. For this group, visually, there was almost complete healing and a small wound remained. Histopathological tests for the PBS and nanogel groups showed still intensive colonization of the wound by *S. aureus* bacteria. In the CuS and G@CuS groups, due to the Cu^{2+} treatment, the number of bacteria decreased, while *S. aureus* in the G@CuS+IR group was completely eliminated [181]. Also, the morphological image of the tissue looked much better, which indicated the synergistic effect of PACT with gelatin. The positive effect of gelatin on wound healing has also been confirmed in previous (not related to PDT) experiments [184,185].

An analogous, though more extensive, approach was presented by Wang et al. They used a gelatin gel combination containing Cypate-conjugated antimicrobial peptides (AMP-Cypates/CA), liposome-encapsulated perfluorodecalin (Fl), and recombinant type III collagen + gelatin (GC) – GCACF [186]. The antibacterial peptide is a relatively new agent with a broad spectrum of activity and low resistance. It can be used in monotherapy, but it exerts particularly promising effects as an adjuvant agent [182]. Therefore, it is also applied in PDT/PTT. The type III collagen used in the formulation is also characterized by greater biocompatibility and limited immunogenicity. Also here, as in the study of Han et al., the specificity of the therapy was ensured by the activation of the gel by the bacterial enzymes gelatinase, hyaluronidase and esterase. The wound environment is usually characterized by reduced perfusion in relation to healthy tissue (usually < 10 mmHg), which makes healing difficult and, at the same time, reduces PTT/PDT activity. In this case, Fl was used as an oxygen transfer agent in form of solution. An important issue, however, is its very limited solubility in water – in order to overcome this challenge, it has been enclosed in a liposomal carrier. Under the influence of light radiation, the MIC90 value decreased from 8 μM to 4 μM, which the authors explain by the photothermal and photodynamic effect of AMP-Cypates and its cooperation with the intrinsic activity of the tested conjugate. Nevertheless, the importance of these results from the perspective of clinical practice should be critically assessed. This study also confirmed a stronger antibacterial effect against *S. aureus* bacteria, which confirms the role of metalloproteinases in the delivery of the active substance. The combination of GCACF with irradiation provided a very good bactericidal effect in *in vitro* tests. A mouse model confirmed the effectiveness of the combination of GCACF and light *in vivo*. Interestingly, Fl alone, despite the hypothetical increase in oxygenation of the wound environment, did not give any significant effects. Only GCACF led to the complete resolution of the infection and good construction of normal tissue within a 10-day follow-up [186]. Very good results were also obtained by combining a gel based on gelatin and recombinant type III collagen with the peptide (GKRWWKWWRR)2KGGK, which also belongs to the family of antibacterial photodynamic peptides. It was combined with Ce6 to obtain an effective formulation against *S. aureus*. The maximum effect was achieved again only after irradiating the system with a red light of 660 nm. In this way, a MIC90 value of around 3.2 μM without photo irradiation and < 0.1 μM with a short light treatment was achieved [187]. This experiment also confirmed the results in an *in vivo* mouse model. However, it should be noted that a parallel trial evaluating efficacy in strains that do not produce gelatin-degrading enzymes has not been conducted. Notably, the same research group also developed a gelatinase-responsive antibacterial photodynamic nanocomposite (GRAPN) using Ce6, gold nanoclusters, and polycationic antimicrobial peptide (Ce6-GKRWWKWWRRPLGVRGC). In this case, an additional test with Gram-negative *E. coli* was carried out. As expected, this strain was less responsive to treatment [188].

An important potential area of using PDT is dentistry [174]. Many PDT-based materials are already commercially available and used both in the treatment of neoplastic lesions and in the treatment and prevention of bacterial infections. The key aspect is the search for biocompatible and mucoadhesive materials suitable for easy adaptation for dental purposes. The use of hydrogel matrices seems to be particularly promising. Their spatial 3D structure, ease of modification and flexibility make them a perfect carrier supporting tissue regeneration and platforms for drug delivery. They are suitable for the treatment of oral and maxillofacial diseases, such as periodontal diseases, caries, pulp diseases, oral cancer, and mucosal diseases [79,189]. Promising examples include the use of a chitosan/gelatin based biomimetic scaffold combination. It was the basis for dental pulp stem cells as a potential element of reconstructive medicine [190]. Gelatin-based hemostatic agents, considered as an effective and important part of bleeding control, are also being intensively developed [191]. Chang et al used a gelatin-HA base as an element of an innovative technique called guided tissue regeneration (GTR). On its foundation, periodontal regeneration membranes combined with PDT were developed. GTR has a huge and already used potential in periodontium regeneration in clinics [192,193]. This method is a regenerative surgical technique performed to cover adjacent soft tissues and to integrate bone. Since the growth of soft tissues definitely precedes the development of periodontium and bone, the use of the appropriate GTR membrane allows for sustainable development and prevents the inward overgrowth of gingival tissues. The study achieved a great fixation index (above 89 %) for the hydrogel membrane combined with temoporphyrin. On one hand, a very good antibacterial effect was achieved for short-term irradiation, but the killing effect on L-929 cells was negligible (survival rate of about 73 %). It should be emphasized that the use of photoactive gelatin-HA membranes significantly reduced the pro-inflammatory gene expression. Good biocompatibility and lack of cytotoxicity predestine this platform for potential use in clinical practice [194]. He et al. used a gelatin-based Ce6-loaded mesoporous polydopamine nanoparticles (GelMAc/MPDA@Ce6), NIR light-activated model to combat bacterial infections. They paid particular attention to the photobiomodulation (PBM) effect associated with PDT. Moreover, PDT can have a bactericidal activity, on the other hand, PBM improves tissue regeneration. Importantly, PBM is based on the use of red light or NIR, which enables effective connection

with compounds from the chlorin group [10,195]. Simultaneous performance of both protocols allows for achieving synergy and optimize medical results. An exceptionally beneficial effect of PBM is the activation, stimulation of migration and proliferation of fibroblasts, which transform into myoblasts. An additional benefit from PBM materials is the mobilization of tissues for the production of proteins such as collagen I and fibronectin. In the case of this study, it was crucial to modify the matrix with a catechol motif. The synthesized formulation was able to adhere very well to both tissues and implants. The introduction of the PS was intended to limit the growth of bacteria. Unfortunately, the obtained material showed great potential as a component of PBM, but its antibacterial potential is moderate, because it was not possible to obtain a reduction above 1 log (calculations of the review authors, results in the source publication given in percentage values) [196].

In recent years, pioneering research has also been carried out on the use of a gelatin matrix in the treatment of diabetic foot and related complications. Diabetic foot is a disease associated with damage to blood vessels and nerves resulting from progressive and/or poorly controlled diabetes. Its far-reaching consequences are disorders associated with neuropathy (e.g. sensory disturbances), the formation of ulcers and wounds difficult to treat, and, as a result of the two previously mentioned factors – infection. Circulatory disorders and high exposure to injuries and abrasions resulting from impaired surface sensation mean that the human body has a clearly impaired ability to fight infections. The perfusion of injuries is limited, and at the same time the migration of lymphocytes to the site of infection is hindered. This makes pathological bacterial flora very easy to expand and develop. As a result, it is necessary to apply expensive and sophisticated methods of treatment, combined with extensive antibiotic therapy. At the same time, the risk of developing antibiotic resistance and septic shock or gangrene, increases. In recent years, a significant growth in the number of diabetic foot cases has been observed, which is associated with the progress of civilization diseases such as obesity. Treatment costs for diabetic foot patients are rising and their quality of life is declining [197–200]. Ding et al. developed a hydrogel based on the metabolite itaconate, 4-octyl itaconate (4OI) and modified black phosphorus (BP). Under laser irradiation, the 4OI–BP-entrapped hydrogel enables rapid gelation, forming a membrane on wounds, and offers high PTT and PDT efficacy to eliminate bacterial infection. As 4OI itself is an antioxidant, its dark activity can be observed to alleviate endothelial damage and promote neovascularization. In the case of the analyzed study, the bactericidal effect was also only a supplement to the medical effect. It was possible to achieve a reduction of *E. coli* and *S. aureus* with 92.27 % and 96.69 %, respectively. Although this is a significant outcome, it should be emphasized that it is far from the required 3 log reduction necessary to consider an agent as having antibiotic activity. However, a promising anti-inflammatory result and a general therapeutic effect confirmed in an *in vivo* animal model were obtained. The formulation also showed a significant proangiogenic effect (the key factor in the treatment of diabetic foot). 4OI was able to activate the KEAP1–Nrf2 signalling pathway, increased the expression level of Nrf2, and promoted the expression of downstream target proteins: heme oxigenase (HO-1), vascular endothelial growth factor (VEGF), and fibroblast growth factor 2 (FGF2) [201].

A valuable supplement to diabetic foot therapy is the use of nitric oxide (II), the deficiency of which plays an important role in the pathogenesis of vascular diseases. NO is a vasodilating factor that improves the functioning of blood vessels and, as a result, increases perfusion. Moreover, it stimulates the angiogenesis process and improves tissue oxygenation and nutrition. These properties lead to better wound healing and faster reduction of infections. Additionally, some studies indicate that higher levels of NO reduce inflammation by modulating cytokines and other inflammatory mediators [202,203]. It is possible to use the synergistic effect between the properties of NO and PTT and PDT [204]. Wang et al. proposed a model using a hydrogel containing gelatin and polylysine and introduced iron/tannic acid (FeIIITA) composite nanoparticles. FeIIITA exerted a photothermal effect, and at the same time polylysine caused a bactericidal effect. The ability of the system to generate NO was confirmed by fluorescent staining. In the case of this formulation, again, the bactericidal effect was only an adjuvant, considering that an inhibition of 83.28 % for *E. coli* and 77.90 % for *S. aureus* was obtained [205]. A comparable effect of up to 89.4 % inhibition was achieved against *S. aureus* in a 3D-printed artificial skin prototype. A matrix consisting of cationic conjugated poly(phenylene vinylene) derivative (PPV) and gelatin/alginate/hyaluronic acid was used to produce the novel formulation. The use of PPV in relation to commercially used verteporfin is characterized by lower cytotoxicity and preferential interaction with the matrix.

5.2.2. *In anticancer applications*

The development of gelatin as a carrier for anti-cancer substances is, in the vast majority of cases, significantly limited to use as a nanoplatform modifying the release of the drug or extending its retention time in the body. This is especially important when, in order to be effective, the drug must circulate in the body for a minimum period of time and at the same time remain at a certain concentration [206]. The simplest solution is usually to increase the dose, but a huge problem occurs when the drug has a narrow or very narrow therapeutic window. In this case, the development of drug delivery platforms is necessary. As Foox and Zilberman pointed out, there are several mechanisms by which the release of active agents from drug delivery systems to the surroundings can be controlled: i) diffusion; ii) swelling followed by diffusion (mostly in hydrogels); iii) diffusion and degradation (erodible systems); iv) hydrolysis of the covalent bond when the drug is covalently bonded to the biodegradable polymer (pendant chain systems); v) osmotic pressure; and vi) externally or self-regulated systems [207]. For example, a hydrogel containing *cis*-platin – a conventional anti-cancer drug – was developed. It consisted of gelatin and polyvinyl alcohol in various concentrations. This model allowed to achieve the effect of prolonged release. As a result, the application of a formulation containing a small amount of chemotherapy agent allowed to achieve effectiveness comparable to high doses of the drug administered intraperitoneally [208]. Most of the research conducted in the field of combining PDT with gelatin focuses on using it as an component that forms nanoparticles modifying the properties of the PS itself. Its non-toxicity and ability to achieve EPR effect, resulting in efficient passive accumulation in solid tumors, is primarily exploited. Gelatin particles have an additional advantage due to the presence of free amino groups that can be modified. Moreover, gelatin can be combined with other molecules, which modifies the properties of the delivery platform. From the perspective of using PDT, such combination with polylactic acid (PLA) seems to be valuable due to its preferential interaction with hydrophobic chemical compounds. Many of the PSs available on the market are strongly or moderately hydrophobic. This system was used, among others, to design a formulation of Hypocrellin derivatives in PDT directed against human breast adenocarcinoma (MCF-7), human gastric sarcoma (AGS) and mouse specific Dalton's lymphoma (DLA). It was effectively captured by cancer cells, and its effectiveness in both free and incorporated forms was comparably high [209]. It is also possible to obtain formulations based on pure gelatin nanoparticles in combination with phthalocyanines. Carvalho et al. used chloroaluminum(III) phthalocyanine (Photosense), which was selected as potentially suitable and effective PS for PDT due to the presence of diamagnetic metal as the central atom. At the same time, it is characterized by high hydrophobicity and a tendency to form inactive aggregates in water. The combination with nanoparticles overcame these application barriers, but the formulation efficiency was tested only against one commercial cell line – J774A-1. The study succeeded in achieving a relatively high encapsulation efficiency of EE=76.0 ± 1.5 %, but broader efficiencies against other cancer lines and in different conditions, such as hypoxia, seem to be necessary [210]. Gelatin can also be used to prolong the circulation of nanoparticles in

the blood. This approach has been used in combined PTT/PDT. In this case, the formulation contained a combination of gelatin, gold nanoparticles and Polyethylene glycol methyl ether (PEG-CH) and IR780 (aheptamethine cyanine molecule). The use of such arrangement effectively increased the uptake by HeLa cells (about two fold increase). The combination of IR780 with gold nanoparticles additionally allowed to increase thermal conversion by about 10 % even over several irradiation cycles [211]. An innovative approach to use gelatin nanoparticles in PDT was presented by Xu et al. who combined zinc(II) phthalocyanine (ZnPc) and up-conversion nanoparticles of NaGdF4:Yb, Er,Mn@NaGdF4:Yb (UCNPs). In the encapsulation process, ZnPc molecules have been wrapped in the interlaced net structure of the peptide chain from gelatin, forming UCNPs@gel-ZnPc nanocomposite. The introduction of manganese ions made it possible to excellently increase the secondary emission of red light, which is necessary for ZnPc activation. At the same time, the obtained formulation proved to be highly biocompatible. A clear relationship was observed between the concentration and efficiency of the nanoplatform, where the highest efficiency was achieved for 800 μg/ml, with a maximum of about 90 % reduction in cell viability [212]. It should be emphasized that the use of a gelatin-based matrix does not always bring the desired results. In case of some combinations with PSs, the phenomenon of formation of aggregates may be observed, which results in quenching the formulation fluorescence and limiting the formation of ROS. This phenomenon was observed, among others, for films containing the porphyrin/chitosan and porphyrin included in chitosan/gelatin system. However, it turned out that the dye was bound to the membrane of cancer cells (HeLa) to a very small extent, which indicated rapid internalization by endocytosis [213]. The film formulation also allowed for achieving the effect of controlled release, which occurred only with a subtle decrease in pH from 7 to 6. This is particularly interesting because a more acidic pH is characteristic of cancer cells [214] (Fig. 4).

5.3. Chitosan

Chitosan is a cationic polymer, obtained by deacetylation of chitin, commonly occurring in nature [181]. Chitosan is biocompatible and biodegradable polymer widely used in biomedical field and drug delivery systems in the form of hydrogels, sponges, microspheres, nanoparticles, and thin films. The application results from its several interesting properties such as anti-inflammatory, antimicrobial, antifungal, antitumor, anticancer, anti-diabetic, wound healing and antioxidant activity [182,183]. Chitosan can promote platelet agglutination and act as haemostatic agent. Moreover, it can also promote the regeneration of the skin, muscles, and nerves [183,184]. Additionally, to the important intrinsic properties of chitosan belong mucoadhesion and capability of promoting penetration of drugs into skin [182]. These features have made it a popular component for development of topical semisolid preparations, including hydrogels. Chitosan is poorly soluble in neutral and basic media and it is insoluble in water and most organic solvents. Therefore, its chemical derivatives are synthesized to improve the solubility and expand applications [181,185]. It is also a promising material for the incorporation of PSs and therefore semisolid formulations based on chitosan are becoming more and more popular in PDT and PTT [186].

5.3.1. In antibacterial applications

Bayat et al. formulated chitosan hydrogels bearing ZnPc-colistin conjugate as an antibacterial agent for application in PACT. The system was intended for treating wounds induced by MDR Gram-negative bacteria, mostly *P. aeruginosa*. As the use of the majority of PSs is limited due to their poor water solubility and their incapacity to disrupt the outer bacterial membrane, the novel zinc(II) phthalocyanine-colistin (ZnPc-Col) conjugate was synthesized and incorporated into chitosan hydrogel to overcome the drawbacks. A solution of zinc(II) phthalocyanine tetra-aldehyde ZnPcTa (from 10-20 % w/w) and glutaraldehyde (15–25 % w/w) as cross-linker in 1 ml acetic acid was mixed with a 3 % (w/v) chitosan solution to obtain various hydrogels. The hydrogel microstructure varied depending on the content of ZnPc-Col and glutaraldehyde. It was evaluated with spectroscopic methods, scanning electron microscopy (SEM), and rheological measurements. For testing the photosensitizing activity 1,3-diphenylisobenzofuran was applied as a singlet oxygen chemical quencher. The proportions of ZnPc-Col and glutaraldehyde in formulations affect the pore size of the hydrogel, viscoelastic properties, and singlet oxygen production ability. For the hydrogel H-3 with the highest amount of glutaraldehyde, lower singlet oxygen production was noted, whereas in disk diffusion assay against *P. aeruginosa* the best antibacterial efficacy was observed for the

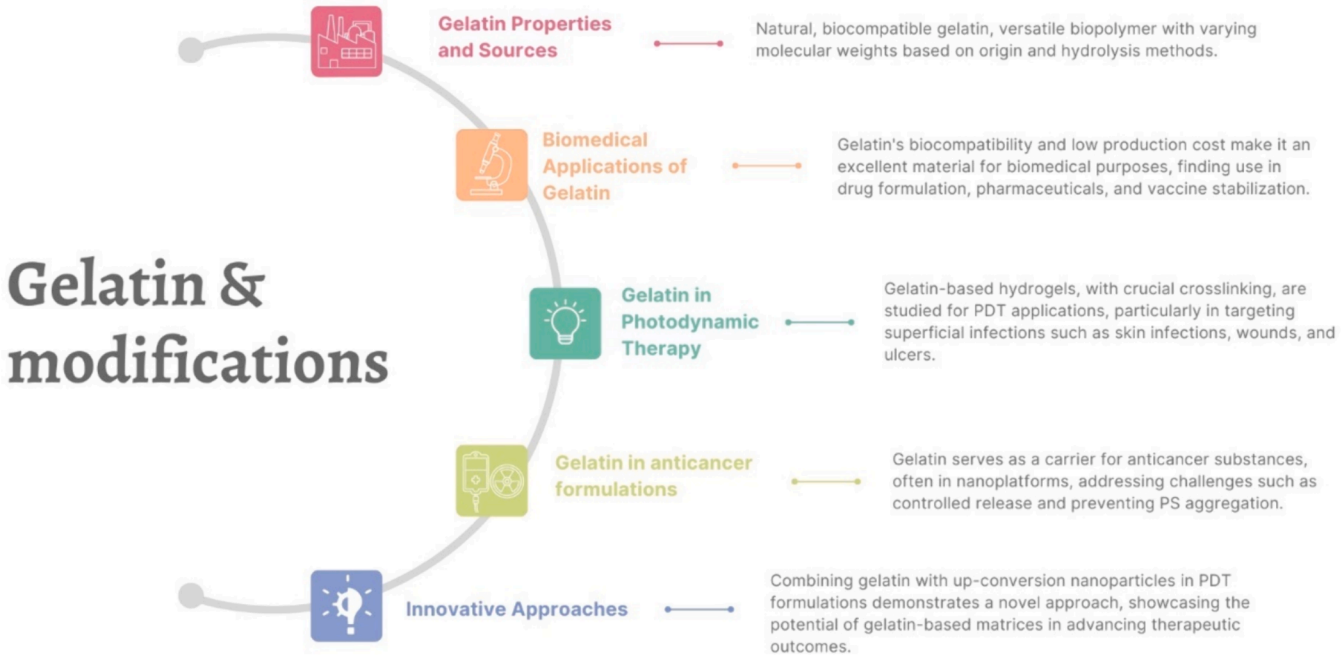


Fig. 4. The properties of gelatin and its applications for biomedical purposes and cancer treatment, including PDT.

hydrogel H-1. Moreover, substitution of ZnPc with bulky cyclic polypeptides at peripheral positions prevented aggregation and resulted in higher production efficiency of $^1O_2$ in comparison to ZnPcTa. Additionally, permeability of phthalocyanine through outer membrane of Gram-negative bacteria was improved. For these reasons, the designed chitosan hydrogel formulation of ZnPc-colistin conjugate can be considered as promising hydrating dressings [215]. Bayat 2020 et al. continued their research on chitosan-based hydrogel matrix with.

a phthalocyanine PS. They developed a chitosan matrix containing zinc(II) tetraamino-phthalocyanine cross-linked with glutaraldehyde (ZnTAPc) for loading difloxacin hydrochloride. Difloxacin hydrochloride was selected as a fluoroquinolone drug to evaluate the drug release features of the tested hydrogels. Photodynamic hydrogels were prepared by combining difloxacin-chitosan solution with ZnTAPc and glutaraldehyde as a gelator 2.0 % and 4 % w/w of the ZnTAPc were used with respect to chitosan polymer. Hydrogels with difloxacin hydrochloride (6.67 % w/w) (ZnTAPc/Dif/CS) and without the drug (ZnTAPc/CS) were formulated. As a result of reaction of NH_2 groups of ZnTAPc and chitosan with aldehyde groups of the glutaraldehyde an imine bond was formed which is necessary in the cross-linking process. Insertion of ZnTAPc increased its solubility in physiological medium. The amount of ZnTAPc and pH medium affected the difloxacin release which was faster at pH 7.4 than pH 5.8. The prepared hydrogels appeared to effectively generate singlet oxygen under irradiation with low energy light and can be considered good candidates for chemo-PDTherapy [216].

A formulation containing toluidine blue (TBO) as PS for use against microorganisms has also been developed. The researchers designed a hydrogel on the basis of chitosan and different concentrations of a gelling agent – hydroxypropyl methylcellulose. TBO with chitosan and TBO alone were used as controls for examination of the PACT efficacy of the novel hydrogel formulation. Its efficacy was tested *in vitro* against the biofilms of *S. aureus* and *P. aeruginosa.* The penetration level of TBO into viable *S. aureus* biofilms was examined with confocal scanning laser microscopy (CSLM). Approximately 1 log reduction in viable counts was observed in *S. aureus* biofilm cells incubated with TBO, whereas the mixture of TBO and chitosan revealed a significant antimicrobial effect (3.5 log reduction) under a light dose of 20 J/cm^2. Although incorporation of hydroxypropyl methylcelluslose (HPMC) affected the chitosan hydrogel properties and increased its hardness, viscosity and bioadhesion, it inhibited TBO diffusion into the biofilm and led to reduced antimicrobial effect on both *S. aureus* and *P. aeruginosa*. After light irradiation a longer retention time was necessary to improve bactericidal efficacy. The tested formulations were prepared for optimization of the content of HPMC in hydrogel and identification of its influence on their mechanical properties and the antimicrobial effect. The improved bactericidal effect of chitosan in the designed hydrogel formulation decreased with the increase of the HPMC concentration. Therefore, development of the optimal topical formulation will concern the selection of the proper proportions of components to achieve the desired release rate and the mechanical properties, which will have impact on clinical efficacy and the ease of application [217]. Peng et al. developed formulations of chitosan hydrogels with TBO to evaluate their mucoadhesive property and antimicrobial efficacy of PACT. They applied increased (HPMC) content (0.25 % HMPC in F-1 formulation and 0.5 % HPMC in F-2 formulation), which led to the increased mucoadhesiveness. They also developed a simple *in vitro* 3D gingival model mimicking the oral periodontal pocket to culture the biofilms of *S. aureus*, *Aggregatibacter actinomycetemcomitans*, and *Porphyromonas gingivalis* and subjected them to irradiation [218]. Firstly, they compared the cell survival fraction of *S. aureus* biofilms at the illumination site and non-illumination site after PACT. The survival rate at the non-illumination site remained 10^3 colony-forming units (CFU)/ml even with the increased total light dose (108 J/cm^2). They observed an essential light-dose dependent decrease of the *S. aureus* survival at the illumination site. Next, they increased the number of irradiation sites treated with F-2 from two to four. *S. aureus* biofilm can be completely killed by illumination with a light dose of either 16 or 27 J/cm^2 per site with 2 h post-incubation. The total light dose higher than 54 J/cm^2 was found necessary for a complete eradication of *S. aureus* as a light dose of at least 27 J/cm^2 seemed to be required for each side. Similarly, the survival of the periodontal strains in both biofilms treated with F-2 gradually decreased in a light-dose dependent manner with 0.5 h of post-incubation with the investigated gel formulation. A total light dose irradiation higher than 32.4 J/cm^2 was required to achieve a considerable decrease in survival rate of periodontal biofilms. In case of the 2 h of post-incubation with F-2, it turned out that viability of both periodontal biofilms notably declined in comparison to control at various light doses. Complete killing of studied strains was achieved for the total light dose increased to 54 J/cm^2 for *A. actinomycetemcomitans* and to 108 J/cm^2 for *P. gingivalis*. A substantial drop in the viability of both biofilms compared to control at various light doses was observed for the 2 h of post-incubation with the novel hydrogel. The developed chitosan hydrogel formulation containing TBO potentiated the PACT efficacy against the periodontal biofilms and was improved by light irradiation [218].

Research on infections caused by *Cutibacterium acnes*, a cause of common acne, is still necessary. Frade et al. studied a chitosan gel effectiveness against the bacteria in biofilm and planktonic forms, comparing it with MB in PACT. Various MB concentrations were tested, and red light irradiation followed. PACT using chitosan gel achieved planktonic bacterial reduction at 12.5 μg/ml MB, and a 1.9 log reduction in biofilm with 75 μg/ml MB. Chitosan hydrogel (0.25 %) showed synergism with PACT in planktonic phase, causing bacterial reduction. While the tested formulation alone slightly reduced biofilm, aqueous solution-mediated PACT had no significant effect. Chitosan, a promising MB delivery component, demonstrated antimicrobial activity against *P. acnes* in suspension and biofilm. The synergistic effect of chitosan and PACT was observed in planktonic phase, making chitosan an interesting candidate for topical use in antimicrobial PDT. Rheological properties indicated high skin adhesiveness, unaffected by MB incorporation [219].

Mai et al. developed a hydrogel-based nanodelivery system for antibacterial phototherapy and skin regeneration ability with potential application in treating burn infection. The scientists embedded porphyrin PS sinoporphyrin sodium (DVDMS) and poly(lactic-co-glycolic acid) (PLGA)-encapsulated basic fibroblast growth factor (bFGF) nanospheres in carboxymethyl chitosan (CMCS) – sodium alginate to obtain a hybrid hydrogel formulation. The nanosystem was designed to intelligently deliver bFGF and DVDMS to the burn wound site and accelerate its healing. The biological stability of DVDMS was improved and bFGF was slowly released. The novel nanohybrids enabled *in vivo* fluorescence imaging, which can help tracking the hydrogel *in situ*. Encapsulation of DVDMS did not cause any change in its spectral patterns compared to those of the free form, and the hydrogel formulation did not affect the physical characteristics of DVDMS. The rheological properties of the new formulation were appropriate and $^1O_2$ yields were high. Light irradiation (30 J/cm^2, 5 min) of the examined system at the concentration 10 μg/ml resulted in eradication of almost 99.99 % of *S. aureus* and MDR *S. aureus in vitro*. Moreover, thanks to KEGG database, analysis changes in multiple signalling pathways were noted in MDR *S. aureus* after PACT, which considerably affected the genes/pathways involved in protein synthesis and oxidative stress. Novel formulation combined with PACT led to successful inhibition of bacteria growth and promotion of wound healing as increase of different regenerative factors and decrease of some proinflammatory factors in the burn wounds were observed. Improvement in healing process resulted also from higher collagen deposition and rapid epithelialization. The designed system due to its good biocompatibility and effectiveness in killing bacterial cells seems to be a portable, light-triggered, antibacterial theranostic platform of a promising potential for treating burn infections [220].

An interesting modification of PDT involves the development of formulation containing very stable black phosphorus (BP), which

exhibits photodynamic activity. Mao et al. prepared BP-based chitosan hydrogel for PDT integrated with stimulation of skin regeneration and bacteria-infected wound healing. 2D few-layer black phosphorus nanosheets have recently gained attention due to their ability to produce $^1O_2$ under the irradiation of visible light to kill both Gram-negative (*E. coli*) and Gram-positive *(S. aureus)* bacteria [221]. The investigated hydrogel was obtained via a simple electrostatic interaction between the BPs and chitosan. The formulation revealed not only the antibacterial efficacy but it also stimulated the formation of fibrinogen and triggered PI3K/Akt and ERK1/2 signalling pathways which contribute to skin homeostasis, enhance cellular proliferation and differentiation [222]. Fibrinogen plays an important role in defense against bacterial infections and in tissue repair, supports incoming leukocytes and is a reservoir for growth factors. It can accelerate incrustation by the coagulation of platelets and fibrin, and wound healing [223]. The use of the investigated chitosan hydrogel resulted in elimination of 98.90 % *E. coli* and 99.51 % *S. aureus* within 10 min under simulated visible light. Additionally, the killing effect turned out to be repetitive and the antibacterial efficacy reached up to 95.6 % and 94.58 % against *E. coli* and *S. aureus*, respectively. Moreover, application of the hydrogel led to the formation of fibrinogen and triggering phosphoinositide 3-kinase (PI3K), phosphorylation of protein kinase B (Akt), and extracellular signal-regulated kinases (ERK1/2) signalling pathways, whereas no damage to major organs in rats was observed during treatment. Therefore, it can be considered as a safe multimodal therapeutic system for sterilization and repair of tissues.

5.3.2. *In anticancer applications*

Belali et al. studied novel chitosan hydrogel as a porphyrin PS carrier for selective PDT. They developed a formulation with chitosan, cross-linked by 2,4,6-tris(p-formylphenoxy)-1,3,5-triazine TRIPOD and tetrakis(4–aminophenyl)porphyrin (NH_2-TPP) via dynamic covalent Schiff-base linkage, functionalized with folate (FA) [224]. The aim of the study was to obtain a pH-sensitive, self-healable and injectable targeted PS delivery system that was expected to overcome low solubility, subsequent aggregation and a lack of tumor selectivity. The utility of PDT in combination with CS/NH_2-TPP/FA hydrogels was investigated by the $^1O_2$ generation, cytotoxicity and phototoxicity analysis with and without irradiation. CS/NH_2-TPP hydrogel was capable of generating more $^1O_2$ in comparison to NH_2-TPP in equivalent concentration. The hydrogel network prevented self-quenching of porphyrins in hydrogel network and made the porphyrin water soluble and an effective system for singlet oxygen generation. Additionally, the targeting and PDT efficacy of the CS/NH_2-TPP and CS/NH_2– TPP/FA hydrogel were evaluated on human liver cancer cell line (HepG2) and human breast cancer cell line (MCF-7). MCF-7 cells with folate receptors were used as target cells, while HepG2 cells (FR^- HepG2) without folate receptors were used as control cells, to check the impact of folic acid on the cellular uptake of CS/NH_2-TPP/FA hydrogel. During MTT assay performed on HepG2 cells treated with CS/NH_2-TPP and CS/NH_2-TPP/FA hydrogel HepG2 cell viability was ca. 94 % at all concentrations (0.05–0.25 mg/ml). Phototoxicity of the CS/NH_2-TPP and CS/NH_2– TPP/FA hydrogels in HepG2 cells revealed a slight dose-dependence. CS/NH_2-TPP and CS/NH_2-TPP/FA hydrogels led to ca. 55 % cell death after irradiation at 660 nm with light dose of 5 J/cm^2. The same results of phototoxicities of different concentrations of CS/NH_2-TPP hydrogel were obtained for MCF-7 cells as for HepG2 cells. In case of CS/NH_2-TPP/FA hydrogels after laser irradiation for increasing concentrations of the CS/NH_2-TPP/FA hydrogels, decreased cell viability was observed, which contributed to enhanced cytotoxicity probably due to overexpressed folate receptors in MCF-7 cells. The cross-linked sponge-like hydrogel structure possessed micropores of 20–30 μm diameter and nano-layer surface with pores of 200 nm width. According to spectroscopic results the aggregation of porphyrin in aqueous media was eliminated due to its diminished stacking interaction in 3D hydrogel structure. Microporous structure of hydrogel efficiently transfers the excitation energy to the PS and contributes to singlet oxygen release, which is higher in PDT application for hydrogel (0.64) than for free porphyrin (0.5). Moreover, the CS/NH_2-TPP/FA injectable hydrogel releases more than 90 % of PS. These features make the novel hydrogel to be a promising model for the preparation of highly efficient and targeting PS delivery system [224]. Ferreira et al. developed three biopolymeric films containing porphyrin dye for localized PDT of cancer. They prepared the first film from chitosan only, the second one from chitosan (80 %) and PEG (20 %) and the third film from the combination of chitosan (80 %) and gelatin (20 %) [225]. For the experiment, except for above mentioned placebo films, systems containing porphyrin were also produced as the fourth, the fifth and the sixth film, respectively. The utility of a synthesized asymmetric porphyrin dye in these systems as PS for PDT was to be evaluated. Photochemical studies were conducted both for the dye in solution and for the dye incorporated into the three biopolymeric films. The fifth film (PS in chitosan and PEG) revealed the best fluorescence quantum yield and lifetime, whereas for the remaining two films with PS the presence of non-emissive aggregates exhibiting a strong quenching effect in the fluorescence intensity, quantum yields and lifetimes was observed. PEG turned out to act as a dispersant and prevented aggregate formation, which increased the fluorescence intensity. Singlet oxygen quantum yields were also determined and considered very promising both for the dye in solution ($\Phi_\Delta = 0.54$) and for the dye in the polymeric films. All the systems were more active after irradiation and revealed low toxicity in the dark. The release studies of the dye were performed and after a few days the dye was completely released in acidic buffer. The polymeric films were evaluated by fourier-traansform infrared (FTIR) spectroscopy and revealed strong interaction between the polymers in the different mixtures. The intracellular localization of the porphyrin in solution and in the polymeric films onto HeLa cells was determined with confocal microscopy. A “punctuate staining” pattern was noted, characteristic of markers localized on the endosomes and no dye was located in the plasma membrane as no colocalization of the porphyrin with the membrane marker could have been observed. Therefore, the dye seemed to have a very fast internalization inside the HeLa cancer cells (possibly at the endosomes) and to be rapidly internalized by endocytosis process, which enabled the destruction of cancer cells after irradiation. The drug release studies lead to the conclusion that acidic conditions (pH~6) of the medium promote the drug release from the polymeric films, which is advantageous as the environment around the cancer cells is acidic. The scientists found the system composed of the porphyrin and the chitosan/PEG film the most promising therapeutic agent for PDT.

Chitosan-based semi-solid formulations can also be utilized in the oral cavity. Garcia et al. prepared chitosan-based mucoadhesive gel for oral mucosal TBO delivery in the PDT treatment of oral cancer. The selected PS, is considered to exhibit significant photocytotoxicity in a variety of tumour cells. Taking into consideration the fact that both the properties of oral mucosa and TBO prevent the PS from reaching the target site at therapeutic concentration, the researchers attempted to assess the effectiveness of a penetration enhancer Tween 80® (TW) as a component of the novel gel to achieve the mucosal retention of TBO for the PDT [226]. The tested mucoadhesive gel formulations contained 4 % chitosan and 1 % TBO in combination with or without 5 % TW. The protocol for the photodynamic procedure embraced one hour formulation exposure, then the removal of the residual formulation and irradiation of the target site with a laser (670 nm) of a total energy of 80 J/cm^2 and a fluence rate of 300 mW/cm^2. It turned out that the number of apoptotic cells after treatment with the 4 %CH gel containing 1 %TBO and 5 %TW and laser irradiation was approximately 1.3-fold higher in comparison to treatment with gel without TW followed by laser exposure. In the release studies TW reduces TBO release from the novel formulation, but it increases TBO retention in the mucosa. As far as mucoadhesive and rheological properties are concerned, 4 %CH gels containing 5 %TW and 1 %TBO appeared to reveal adequate characteristics for oral mucosa use, although they present a slightly acidic pH. These mucoadhesive gels may be a promising delivery system in the

treatment of oral cavity cancer using PDT due to prolonging the residence time of the TBO at the target site and the penetration optimization of TBO in oral mucosa [226] (see Fig. 5).

5.4. Polyacrylic acid

5.4.1. In antimicrobial applications

Pereira et al. performed a study on 11.5 % polyacrylic acid (PAA) containing 0.3 % MB dye as a PS to investigate the efficacy of PDT against *S. mutans* present in the carious lesions [227]. They infected one hundred twenty molars with *S. mutans* and divided them into eight groups: sterile saline, PAA, MB: MB 0.3 %, PAA+MB: PAA containing 0.3 % MB, LLL: irradiation with low-level laser, PDT (MB): MB 0.3 % + laser, PDT (PAA): PAA+laser, and PDT (PAA+MB): PAA containing 0.3 % MB+laser. When comparing the results of treatment with PA and MB only, the largest percentage reductions in *S. mutans* growth from all treatment protocols were noted for PDT (MB), PDT (PAA+MB), and PDT (PAA) and were 53.62 %; 50.47 %; 46.73 %, respectively. The study findings confirmed higher efficacy in reduction of *S. mutans* counts in dentinal caries of PAA, MB, and PAA+MB applied in combination with PDT than applied without it [227].

Interestingly, the research group led by Chandna et al. proposed a novel modification by incorporating elements of nanotechnology into the traditional formulation. They developed photodynamic nanoconjugates by conjugating a PS – rose bengal (RB) with lignin derived metallic and bimetallic (silver and gold based) nanoconjugates (AuLNCs, AgLNCs and AuAgLNCs) and incorporated them into a polyacrylic acid hydrogel matrix to investigate their antimicrobial photodynamic activity [228]. The incorporations of RB or the nanoconjugates into the hydrogel contributed to an increase in the lifetime of the triplet state, which is crucial for generation of ROS and enables to decrease the necessary dose. Therefore, they exhibited good photophysical properties (ROS generation and photoluminescence). The incorporation of nanoconjugates caused also a structure change of the polyacrylic acid hydrogels. Nanocomplexes and nanoconjugates were crosslinked with the hydrogel network, which improved their structure, surface morphology and also stability. One of the most important parameters of the formulation is its ability to release active substances. As microbial infection is related with acidic environment, the pH-triggered degradation of the hydrogels was needed and the studied hydrogel revealed the capability for such release. As far as photodynamic efficacy against *Candida tropicalis* is concerned, the nanoconjugates and nanoconjugate-doped hydrogels were subjected to irradiation with green laser (2.5 mW, 525 nm, 3 min). The significant impact on the fungal cells was observed and for hydrogel-RB@AuAgLNCs the maximum killing was reported. Its mechanism can be related to the pH triggered release of the nanoconjugates from the hydrogels and singlet oxygen formation due to exposure to irradiation. Additionally, the bimetallic nanoconjugate doped hydrogel has the maximum percentage of fungal growth inhibition with very low IC_{50} value (0.1 μg/ml) and it appeared to be much better than its monometallic counterpart. The novel photodynamic antimicrobial hydrogel could be used as targeted therapeutics for wound healing and related biomedical applications [228].

5.4.2. In anticancer applications

Biocompatible hydrogels are broadly used in anticancer treatments due to their structure of cross-linked networks suitable for encapsulation of bioactive substances [229]. Besides chitosan hydrogels, PAA hydrogels and their combinations are gaining more and more applications in the development of semisolid drug formulations. Such polymer hydrogels are built of hydrophilic polymer chains forming a network able to retain a large amount of water. Their good biocompatibility makes them suitable for usage in cartilage replacement, artificial muscles and wound dressings. Moreover, the proper mechanical properties must be achieved, such as toughness and/or stretchability [230]. According to Fuhrer et al hydrogels consisting of poly(acrylic acid) (PAA) and calcium ions belong to the materials with shapeable, stretchable and self-healing behaviour resulting from the reversible and dynamic nature of the electrostatic and hydrogen bonds in their structure. These expected characteristics are related to the molar mass of the grade of PAA and the conditions of preparation [231].

The researchers also investigated the usefulness of hydrogel-based adhesive followed by PDT in oral potentially malignant disorders (OPMDs). PAA–CHI–ALA (PACA), a dry double-sided tape (DST), was formulated utilizing a blend of biopolymer polyacrylic acid (PAA), chitosan, and 5-aminolevulinic acid (5-ALA) [142]. Efforts were directed towards overcoming the limitations of 5-ALA, including low

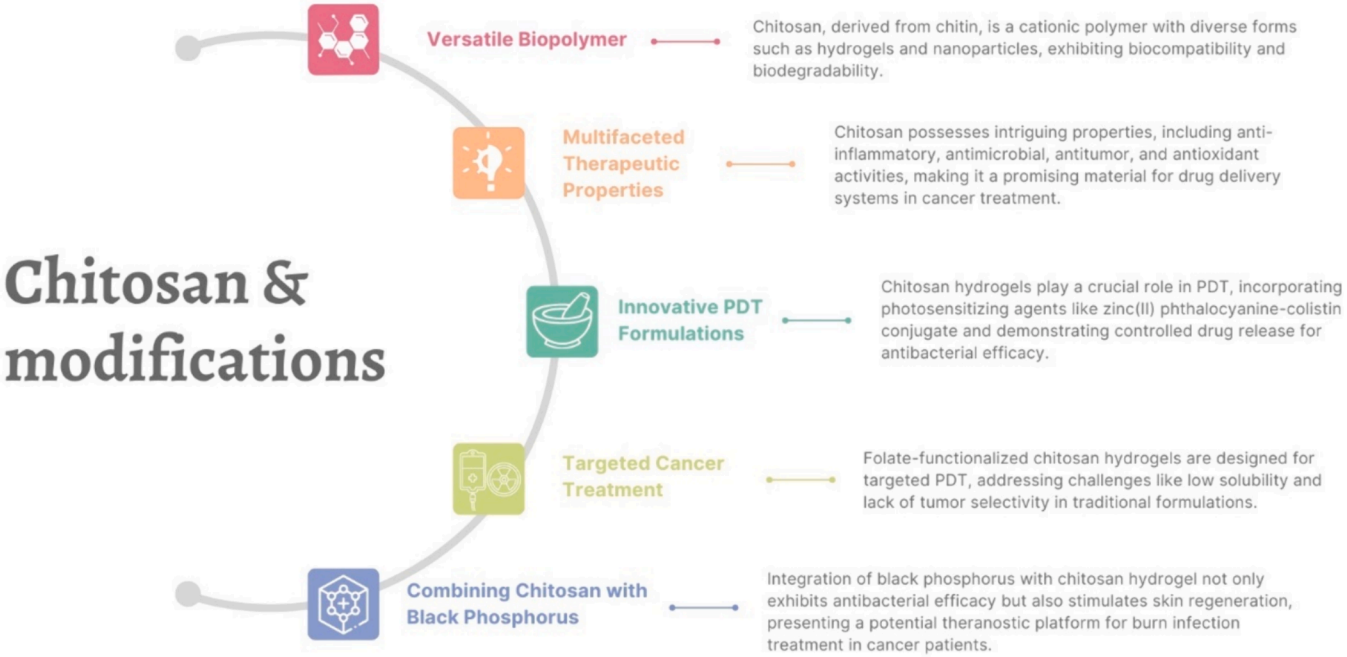


Fig. 5. The properties of chitosan and its application in formulations used against microorganisms, cancer and in combination with black phosphorus.

delivery efficiency, discomfort, and susceptibility to interference by saliva. The DST was to remove interfacial water from the surface and form temporary crosslinking to improve the adhesion properties. The dry PACA hydrogel revealed high stability and adhesive strength (62 kPa, porcine tongues in artificial saliva), which allowed for at least a 2 h PDT process. Under laser irradiation of 635 nm, PACA hydrogel-mediated PDT showed high anticancer activities in OPMDs *in vitro* and *in vivo* in a hamster oral carcinogenesis model. Moreover, a trial was conducted on 60 OPMD volunteers to demonstrate the feasibility and alleviation of patient discomfort during treatment with PACA hydrogel patch. Using diffusion-based finite-element models the spatiotemporal dynamics of 5-ALA drug delivery was studied and PACA (with the thickness 1.0 mm) in combination with PDT showed excellent therapeutic effects for OPMDs [142]. The results of the study indicated that PACA hydrogel-mediated PDT could be a patient-friendly, biocompatible treatment modality providing sustained diffusion of 5-ALA for OPMDs.

Both PAA and PEG can be applied as components of a modern formulation against tumors. Liu et al. developed a multifunctional therapy platform of PTT/PDT and radiotherapy for potential liver cancer treatment [232]. They linked arginylglycylaspartic acid (RGD) peptides and metronidazole (MN) with the copolymer chains of polyacrylic acid (PAA) and PEG to obtain degradable and compatible *in vitro* and *in vivo* RGD-PEGPAA-MN. Liquid-metal (LM) nanoparticles were coated with this modified copolymer forming RGD-PEG-PAA-MN@LM nanoparticles, which after degradation in acidic environment could together with LM target tumor cells. RGD-PEG-PAAMN@LM nanoparticles are capable of converting the photoenergy of NIR into thermal energy and generate a larger amount of ROS under NIR or X-ray irradiation. Moreover, MN as a radiosensitizer could improve the radiation sensitivity of hypoxic tumor tissues. The synergic effect of these nanoparticles on cancer reduction after the consecutive radiations of NIR and X-ray was notably higher than the single radiation. In the *in vivo* experiments on tumor bearing mice, the volume of tumor in RGD-PEG-PAA-MN@LM group at the14th day after two radiations of NIR and X-ray notably decreased and was smaller than the tumor volume in LM group. The tumor of RGD-PEG-PAAMN@LM group at the 14th day after two radiations almost vanished, which confirmed better synergistic effect of RGDPEG-PAA-MN@LM nanoparticles on photothermal conversion, photodynamics under two irradiations and their better sensitization of X-ray radiation [232] (see Fig. 6).

5.5. Poly(ethylene oxide)

5.5.1. In antimicrobial applications

De Oliveira et al. designed and investigated a pharmaceutical platform to release phthalocyanine aluminium(III) chloride (AlClPc, Photosense) for topical administration in PDT [233]. Although Photosense belongs to PSs recommended for treating skin lesions worsened by microbial infections, it exhibits a high self-aggregation tendency in the biological fluid, which poses a problem in *in vivo* direct administration. To overcome the limitation, the researchers designed a novel formulation of bioadhesive and thermoresponsive hydrogel comprising triblock-type Pluronic F127 (F) and Carbopol 934P (FCarb) to successfully deliver the PS. Carbopol 934P was introduced to the formulation to increase the F127 hydrogel adhesion on the skin, improve stability, rheological and mechanical properties, prolong the residence time of formulation in the action site. A bioadhesive thermoresponsive platform was prepared by the solid dispersion method. F127 (20 %, w/w) and AlClPc (0.05 %, w/w) were co-solubilized in ethanol until total dispersion and further evaporation of alcohol. The Carb (0.20 % w/w) was dispersed in water and finally combined with the thin film of F-AlClPc to obtain a gel or viscous solid resulting from the micellization stage. In the rheological analyses it turned out that the AlClPc exerted a low effect on the hydrogel matrix and changed the gelation temperature from 27.2 ± 0.1 °C to 28.5 ± 0.9 °C. The dermatological platform revealed rheological and textural properties at the same temperatures, whereas some differences were observed in terms of presence or absence of AlClPc in the formulation. Release of some part of the loaded PS was observed and the dermis layer was reached in 30 min, which allows AlClPc to generate ROS under irradiation with red light. The ROS generation capacity of AlClPc was maintained also after incorporation in the FCarb. The applied dermatological formulation demonstrated matrix erosion effects with the release of Pc loaded micelles. In the permeation studies the partition of the PS from FCarb platform into deeper layers of the skin was observed. In *in vitro* assays using L929 fibroblasts the low effect on cell viability and proliferation in the dark was showed. The platform did not affect healthy fibroblast cells. Moreover, the FCarbAlClPc promoted the inhibition of *S. aureus* strain. The novel platform revealed potential in the treatment of skin lesions aggravated by infections of secondary

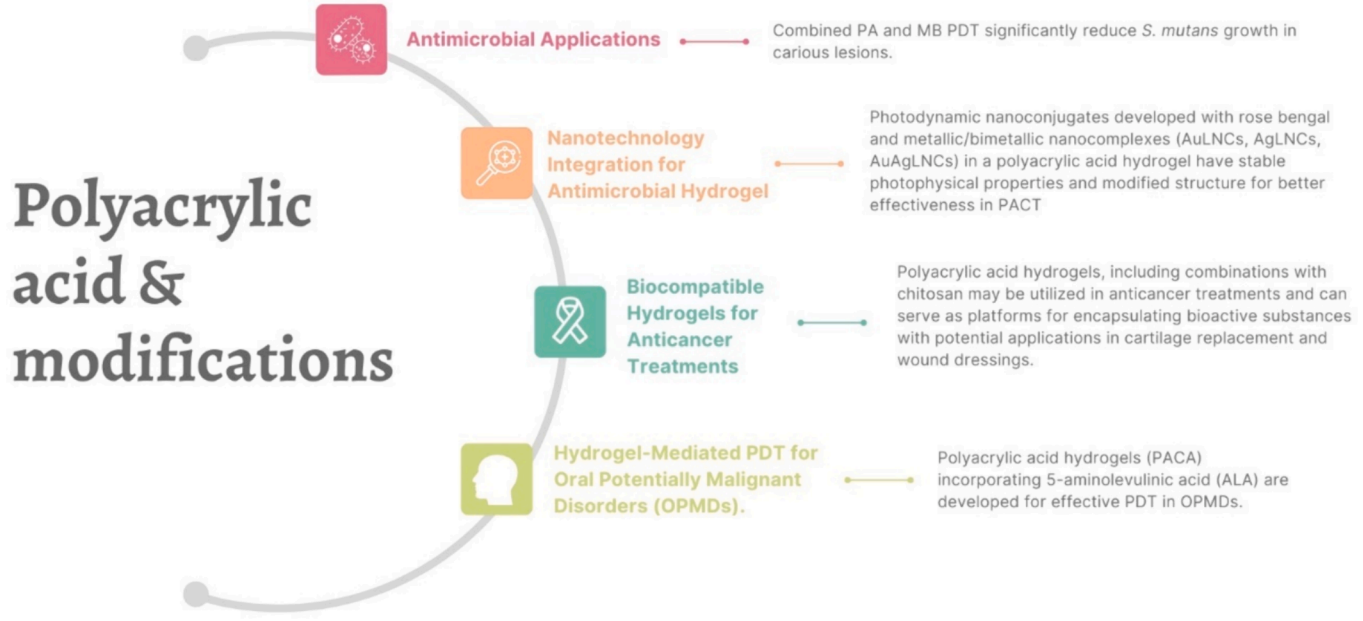


Fig. 6. The applications of polyacrylic acid and its formulations used in therapies against microorganisms and cancer.

nature [233].

In their study, Solovieva et al. investigated the antibacterial and pro-regenerative effects resulting from the photoactivation of the Photodithazine (PD)-Pluronic F127-Chitosan polymer system [234]. The researchers aimed to develop a formulation with a PS to adapt PDT for treatment of septic wounds. It was to retain longer on a wound surface than hydrophilic formulations e.g. gels. Therefore, chitosan was selected as a component of the system because of its high sorption capacity, antimicrobial and wound healing activity. As this natural polymer is often mixed with water-soluble or amphiphilic polymers to obtain hydrogel-based dressings, Pluronic 127 was chosen as the copolymer. PD was diluted with water, mixed with Pluronic 127 water solution and chitosan in 1 % acetic acid water solution to final concentrations of $2 \cdot 10^{-1}$ M of Pluronic, $2 \cdot 10^{-3}$ M of PD, $4.7 \cdot 10^{-2}$ M of chitosan (per unit), and 0.5 %wt of acetic acid. This system was administered to rat wounds infected by Gram-negative and Gram-positive bacteria. The animals were subjected 24 and 72 h after injury to PDT and exudate samples were collected before and after PDT for microbiological analysis. Autopsy tissues were also taken for microbiological testing. It turned out that the associated microflora *in vivo* was suppressed and suppurative inflammation in the wounds was inhibited. Moreover, in the morphological study the benefits of application of PD-Pluronic-Ch complex were observed. The novel system accelerated restoration of microcirculation, promoted proliferation of fibroblast and vessels and triggered collagen synthesis. Therefore, the novel formulation of gel structure may be recommended for prevention and treatment of suppurative inflammatory diseases of skin and soft tissues [234].

5.5.2. *In anticancer applications*

Poly(ethylene oxide) (PEO) is a component of a gelling agent called poloxamer. Poloxamers are a group of synthetic copolymers of PEO and poly(propylene oxide) (PPO) in the triblock arrangement [PEO]x-[PPO]y-[PEO]x, with varying numbers of monomers x and y [235]. Block copolymers are high molecular weight (several thousand Da) due to their long chains. The copolymer blocks in the monomers are chemically dissimilar (e.g., polar and non-polar), which contributes to their amphiphilic character and surface-active properties. The amphiphilicity in aqueous solution is related to the PEO hydrophilicity and the PPO hydrophobicity. The block segregation causes sol–gel phase change [236]. The transition between these phases depends on the molecular weight and composition of the Pluronic®. The tendency to obtain a gel network is greater at higher temperatures and higher concentrations of Pluronic®. Gels are formed as a result of transition from a micellar solution to a body-centered cubic crystal structure. Different Pluronic® grades have capabilities of forming rigid gel networks, e.g., F127, P407, P84. Pluronics® F127 undergoes phase change close to physiological temperature, which makes it an exceptional delivery vehicle for various active substances [237]. One of the first studies on Pluronic F-127 (a PEO-PPO surface) to assess its usability in topical anticancer formulations was performed by Miyazaki et al. in 1984 [238]. They investigated the dermal delivery of 5-fluorouracil and adriamycin. The concentration of the gelling agent influenced the apparent release of the APIs from the gels. Its increased concentration corresponded with the decrease in the apparent release rate, whereas an increase in the temperature from 30 to 44 °C of the *in vitro* dissolution test led to the improvement of the released amount of drug. The Pluronic F-127 gels appeared to be promising vehicles for application in topical drug delivery systems due to their reverse thermal gelation behaviour, safety and proper drug release characteristics.

As poloxamers are thermoresponsive and their micellization, aggregation, and gelation depend on temperature [239],Collaud et al. developed a thermosetting formulation on the basis of polyoxamer 407 to combine it with 5-ALA-mediated PDT and make use of its thermoresponsiveness. An alternative, cervix-sparing treatment for cervical intraepithelial neoplasia (CIN) was to base on the small necessary dose to diminish side effects [240]. The prepared gels are solid at body temperature and liquid at room temperature, which improves adhesion of 5-ALA to the cervix uteri and allows for a more effective release of 5-ALA-hexyl ester (HAL) and PpIX in nude mice skin. They are also known for good compatibility with other chemicals and high solubilization capacity for various drugs. After 2 h about 70 % of HAL was released, whereas from the control cream formulations less than 20 % of the drug was released. The obtained poloxamer 407 thermosetting gel became semi-solid in contact with the female genital tract. It revealed proper rheological characteristics and a satisfactory HAL release *in vitro* and a tendency to increase porphyrin accumulation in nude mice skin. The membrane diffusion method and spectrofluorimetry were used for determination of *in vitro* release-time profiles, which turned out to be correlated with the *in vivo* results.

Borghi-Pangoni et al. prepared and characterized bioadhesive and thermoresponsive system containing a natural lipophilic PS hypericin (Hyp) for local PDT of cancer and antimicrobial inactivation [241]. They developed formulation on the basis of a polymer blend composed of poloxamer 407 and carbomer 934P to overcome poor water solubility of Hyp. Rheological characteristics, gelation abilities, mucoadhesive properties and the syringeability were tested. The obtained results seemed promising. The formulation containing 20.00/0.15 (%, w/w) of Polox/Carb was selected for introduction of Hyp. The Hyp availability was assessed with the dielectric analysis. The formulation did not permeate two different types of biological barriers (rat intestinal mucosa and pig skin) and exhibited proper biological photodynamic activity. It could be suitable for local treatments of the skin and mucosa disorders. The investigated formulation revealed a suitable sol–gel transition temperature and rheological parameters, whereas its dielectric behavior resulted from its dependence of temperature and Hyp presence. Release of Hyp turned out to be rapid as it was completely available after 2 h. In addition, this system did not display permeation in two different types of biological barriers (rat intestinal mucosa and pig skin). Uric acid and irradiation with a polychromatic LED light source was used to determine singlet oxygen quantum yield. ROS originated by Hyp photoexcitation caused degradation of the uric acid. The tested system containing Hyp showed good photodynamic activity in Caco-2 cell culture model, after 30 min of light exposure. The formulation seemed to be promising for local hypericin delivery in PDT [242]. A bioadhesive and thermoresponsive platform was also prepared by Campanholi. It contained 0.5 % chlorophyll-based extract for the photodynamic treatment of primary or secondary morphological skin lesions [243]. The formulation included chlorophylls (Chl) and their derivatives from the New Zealand spinach extract as PS, Pluronic® F127 20.0 % and Carbopol® 21 934P 0.2 % (w/w) (FC). Mechanical, rheological and texture parameters were tested and found suitable for a dermatological use due to easy application and good characteristics of retention in the place of administration. The sol–gel transition temperature of the FC-Chl system was 28.8 ± 0.3 °C and exhibited body-temperature-stimulated FC-Chl gelation effects. The drug delivery ability of the biomedical platform *in vitro* and *ex vivo* studies and the cutaneous permeability through pig ear skin of the chlorophyll compounds were demonstrated. PSs reached a permeation depth of 830 μm (between the epidermis and dermis). *In vitro* release studies revealed two mechanisms obeying the zero-order and first-order kinetics models. Photodynamic activity of the system turned out to be promising because of its ability to form cytotoxic species effectively degrading uric acid. For the spinach extract and the isolated chlorophyll *a* species in ethanol similar values of the quantum yield and life time of singlet oxygen were obtained. The FC-Chl platform could be applied for the delivery of chlorophyll-based extract in combination with PDT [243].

Luo at al. developed a nanocomposite gel for intra-tumoral NIR two-photon PDT (TP-PDT) against 4 T1 murine breast cancer implanted subcutaneously [244]. They prepared a micellar thermosensitive hydrogel composed of methoxy poly(ethylene glycol)-polylactide copolymer (mPEG-PDLLA) and Pluronic (F127). For application of two-photon excitation to PDT, a NIR two-photon absorbing imidazole

derivative T1 was co-encapsulated with pyropheophorbide a (PPa), a hydrophobic second-generation PS from chlorophyll *a* derivatives, using fluorescence resonance energy transfer (FRET). mPEG-PDLLA micelles are biodegradable, biocompatible, prevent PS molecules from aggregating and enhance tumor penetration. However, as they dilute and reach other organs, side-effects or diminishing the PDT effect can appear. Therefore, such formulation is developed to prolong micelle residency in the site of injection and local concentration of PDT. The composite changes from liquid at room temperature to gel at body temperature within the tumor providing long-term retention and avoiding the initial burst release of drug from nanocarriers. The TP-PDT was activated by NIR laser light, which significantly inhibited the tumor growth (>50 % even under 1 cm thick muscle tissue) because of large generation of ROS in deep tissue. The composite hydrogel showed non-significant cytotoxicity without NIR laser irradiation. Moreover, the novel formulation revealed non-significant systemic toxicity and good biocompatibility, demonstrating potential for cancer therapy.

The research conducted by Py-Daniel et al. focused on the development of a new colloidal system for PDT drug delivery. This innovative system involved integrating chloroaluminum(III) Phthalocyanine/Pluronic Micelles (AlClPc) into Pluronic F127 micelles (F127/AlClPc) with the aim of evaluating its efficacy as an anti-tumoral agent [245]. They studied the micelle size distribution, loading efficiency and the *in vitro* ROS production as a function of DDS composition. They also evaluated the *in vitro* tumoral activity of the AlClPc loaded F127 micelles on the A549 human lung carcinoma cell line. The selected phthalocyanine derivative belonging to the second generation of PSs, which possess excellent capability of producing at high quantum yields relatively long-lived singlet and triplet oxygen states. However, due to its hydrophobicity association to drug delivery systems is necessary to promote its dispersion in physiological environment, improve the specific uptake by targeted tissues, and enhance the PDT efficiency. Formulations of Pluronic F127 micelles loaded with the PS – AlClPc, were prepared and tested for the optimum conditions for the F127 micelle formation and AlClPc incorporation. Absorption/emission spectroscopies and dynamic light scattering were performed. The micelles were formed with F127 concentrations ranging from 50 to 150 mg/ml. AlClPc molecules were encapsulated into the hydrophobic micelle core and solubilized in physiological medium (PBS pH=7.2) and their capacity of $^1O_2$ generation after LED irradiation was confirmed. The performance of F127/AlClPc micelles was tested in PDT of A549 cancer cells. Application of the F127/AlClPc formulations, at different AlClPc loadings, followed by 18 min of light irradiation (660 nm LED, fluence of 25.3 J/cm^2), exhibited high phototoxicity. A cellular damage of the irradiated area as high as 90 % was observed for dosages of AlClPc from 0.1 to 5.0 μg/ml. The developed system turned out to effectively broaden the range of application of PDT with highly hydrophobic AlClPc (see Fig. 7).

6. Miscellaneous formulations

Various formulations have been applied on the PDT field. Their versatility and beneficial properties provide great improvements for combating either bacteria or cancer. Among less popular, yet not worse formulations, there can be distinguished some other ideas. Since various materials possess similar, but also distinct behaviour, their usage can be tailored to the case. More unusual formulations have been presented in a shape of peptide-based hydrogels (different than gelatin), gels based on nanocrystals of modified cellulose, as well as diverse nanoemulsions. Utilizing peptides can constitute a good assist for the main therapeutic agent, usually binded to the peptide terminus, for example as delivery enhancement and may also serve as the precursor of the formulation itself, forming the proper formulation upon activation (such as oxidation-induced crosslinking of cysteine moieties present in the peptide or built-in methacrylate moieties prone for polymerization by generated ROS) [246,247]. Alternatively, the formation of hydrogel can be achieved by crosslinking the charged polypeptides, for example polylysine with opposite charged dipeptides [248]. Usage of natural peptides, such as fibroin extracted from silk was also presented as effective hydrogel formulation for encapsulation of porphyrin-based PS [249] The ability to form different nanoparticles, depending on the oxidation of sulphide moiety into sulphoxide ones has been also described [250]. The formation of hydrogels based on spontaneous self-assembly of shorter peptides was proven to be a versatile approach for carrying the therapeutic agents as well [251,252]. Interestingly, hydrogels can be created with rod-shaped cellulose nanocrystals. One of the methods involves oxidation of hydroxyl groups present of rings into aldehyde moieties, which can be further crosslinked by the use of amine groups present on the linkers [253,254]. Another option involves formation of hydrogel directly by oxidation of dopamine moieties, present on modified cellulose [255].

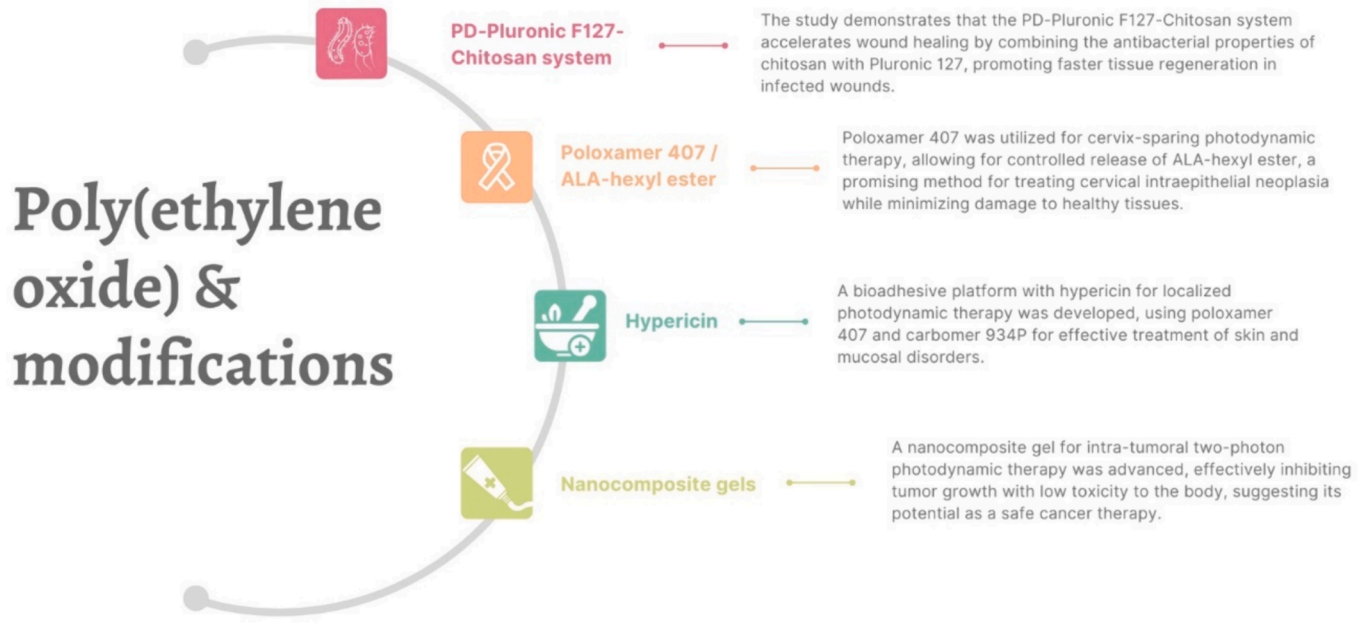


Fig. 7. The application of polyethylene oxide derivatives in combination with various substances against microorganisms and cancer.

An interesting example of a compound requiring formulation work is Parietin (PTN). As far as research on PTN is concerned, scientists concentrate on the improvement of its poor water solubility, phototoxicity against bacteria and tendency to aggregate in the biological environment. In case of pharmaceutical technology, there are no data on research on semisolid formulations of PTN. There are reports on studies that were carried out to overcome the mentioned drawbacks or to investigate biological effects. Ayoub et al [2022] prepared a complexation of PTN with (2–hydroxypropyl)-β-cyclodextrin (HP-β-CD) [256]. Later, Ayoub et al. [2023] encapsulated Parietin in poly(lactic-co-glycolic acid) nanoparticles (PTN NPs) by emulsification diffusion method. Incorporating hydrophobic PTN into PLGA preserved its photoactivity and phototoxic effect remained comparable to the free drug in organic solvents. This formulation was suitable for parenteral application [257].

7. Conclusions

The presented review summarises (outlines) new perspectives for the development of semi-solid drug forms containing PSs. Special attention must be paid to the challenges associated with designing new formulations. The ongoing issue is poor water solubility of many potential candidates for photodynamic therapy drugs. On one hand, this may indicate the need for utilizing nanocarriers in drug preparation; on the other hand, considering traditional water-free ointment bases is also possible. The usage of intrinsic properties (e.g., antibacterial activity) of some polymers in formulations appears valuable. They can be adjuvants in the action of active substances and at the same time expand their use. Moreover, developed formulations have to meet specific requirements imposed by regulatory authorities, with a particular emphasis on reproducible, predictable drug release and lack of toxic effects. Modern pharmaceutical technology opens up a huge field of possibilities, which is confirmed by the studies analyzed in this review. Thanks to new approaches further products based on PACT and PDT can be introduced to the commercial market, and thus contribute to increase their application in the daily therapy of many diseases.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used ChatGPT-4 to correct the language of the text in order to increase the readability of the article. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRediT authorship contribution statement

Daniel Zietal: Writing – review & editing, Writing – original draft, Visualization, Supervision, Conceptualization. **Beata Czarczynska-Goslinska:** Writing – review & editing, Writing – original draft. **Marcin Wysocki:** Writing – original draft. **Marcin Ptaszek:** Writing – original draft. **Łukasz Sobotta:** Writing – review & editing, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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