Applications of Pullulan-Based Biomaterials

Subjects: Materials Science, Biomaterials

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Polysaccharide polymers have been used to fabricate wound dressings. The applications of biopolymers, such as chitin, gelatin, pullulan, and chitosan, have greatly expanded in the biomedical field due to their non-toxic, antibacterial, biocompatible, hemostatic, and nonimmunogenic properties. Most of these polymers have been used in the form of foams, films, sponges, and fibers in drug carrier devices, skin tissue scaffolds, and wound dressings. Currently, special focus has been directed towards the fabrication of wound dressings based on synthesized hydrogels using natural polymers. The high-water retention capacity of hydrogels makes them potent candidates for wound dressings as they provide a moist environment in the wound and remove excess wound fluid, thereby accelerating wound healing. The incorporation of pullulan with different, naturally occurring polymers, such as chitosan, in wound dressings is currently attracting much attention due to the antimicrobial, antioxidant and nonimmunogenic properties. Despite the valuable properties of pullulan, it also has some limitations, such as poor mechanical properties and high cost. However, these properties are improved by blending it with different polymers. Additionally, more investigations are required to obtain pullulan derivatives with suitable properties in high quality wound dressings and tissue engineering applications.

Keywords: pullulan ; chitosan ; hydrogel

1. Introduction

The wound healing process is a complex and dynamic process of overlapping phases, and specific conditions are needed to support healing. The main goals of wound management are to provide a physical barrier against bacterial infections and to maintain an optimum moist environment, allowing the healing process to be accelerated [1][2][3]. The wound area must be covered with an ideal wound dressing in order to prevent the dressing from failing to function [4]. An ideal wound dressing material should have several specific properties, including: (a) biocompatibility; it is essential that the dressing not create any toxicity in the wound environment; (b) high absorption capacity of exudate; large amounts of wound fluids need to be removed, as wound exudates promote a high risk of bacterial colonies and growth; (c) adequate water vapor permeation rate; an optimal moisture level should be maintained in the wound, as a high level of water vapor permeation rate dehydrates the wound too quick and can cause scars, while a low level of water vapor permeation rate leads to excess exudates, thereby increasing the risk of bacterial infections; (d) good physical barrier; bacterial penetration must be prevented; (e) antimicrobial activity; microorganisms must not be able to grow under the dressing; and (f) non adhesive properties; adhesiveness of the wound dressing is most likely to increase the risk of further injuries upon removal [4][5][6]. Wound dressing materials are fabricated from naturally derived or synthetic polymers, or from a combination of the two. Wound covering materials are mostly produced as films, sponges, hydrocolloids, and hydrogels [7]. Until now, naturally derived polymers have received enormous attention in biomedical, pharmaceutical, and medical applications due to their biocompatibility and biodegradability properties [8]. There are still research challenges to developing multifunctional and cheap wound dressings through simple green synthesis approaches, as the dressings should show biocompatible, biodegradable, mucoadhesive, hemostatic, and bactericidal properties along with their main focus as wound dressings and drug delivery devices. Of the various types of wound dressings that have been fabricated, polysaccharide types have several advantages; for example, along with the above-mentioned properties, the hydrophilic groups on their polymers create a three-dimensional crosslinked network.

Naturally occurring polymers are greatly employed in the design and fabrication of wound dressing due to their similarity with the extracellular matrix (ECM) and nonimmunogenic properties that are detected with synthetic polymers [9][10]. Polysaccharides are a class of natural polymers made up of monosaccharide units and their derivatives [11]. Polysaccharides consisting of just one kind of monosaccharide unit are referred to as homopolysaccharides or homoglycans, while those containing more different types of monosaccharide units are called heteropolysaccharides or heteroglycans [12]. The main advantages of polysaccharides are their chemical properties; these properties are similar to heparin, providing the polymers with good hemocompatibility and making them less costly, in general, than other polymers

[13]. Studies have shown that polysaccharides act as immunomodulatory materials to regulate inflammatory activities in wounds [14][15].

2. Applications of Pullulan-Based Biomaterials as Wound Dressings and Skin Tissue Engineering Scaffolds

Currently, pullulan composites with different biopolymers, such as chitosan, chitin, gelatin, and collagen, have gained considerable importance and have been used to develop films, sponges, and hydrogels for wound dressings, skin tissue scaffolds, and drug delivery devices. Considering the beneficial properties of pullulan and other polymers, such as chitosan, synthesis of hydrogel composites using these polysaccharides will greatly enhance wound healing.

Duceac et al. recently fabricated a chitosan-pullulan composite with tunable pore size and targeted properties for drug delivery applications. The fabricated composite structures consisted of a core of chitosan covered with different forms of modified pullulans, that is, one contained carboxyl groups and the other contained aldehyde groups. The researchers demonstrated that the two types of materials produced possessed different physical and biological properties [16]. The chitosan-TEMPO oxidized pullulan beads were formed by physical bonds, while the chitosan-periodate oxidized pullulan beads were produced by chemical linkage. The researchers demonstrated that the two different composites had high antibacterial activities. Hemocompatibility studies of the composites showed mild coagulation as a result of low amount of free amino acid groups on the surface of the chitosan composites; this occurred because the amino groups are involved in ionic or covalent interactions with the carboxyl or aldehyde oxidized pullulan. The hemostatic property of a material is a characteristic regarding the biological activity and its applications. It is very important for fabricated wound dressings to show hemostatic actions. Their findings showed that these composites could not only act as drug delivery devices but also as modern wound dressings with excellent properties [16].

In addition, they observed that TEMPO-oxidized beads (CPT) had the best bactericidal activity, which could be explained by the higher antibiotic incorporation in their network. The obtained results proved that drug incorporated beads exhibited antibacterial activity. The hydrogel beads showed distinct inhibition area, which confirmed the drug release and antibacterial activity against *Staphylococcus aureus*. The functionalized beads, CP and CPT, had higher inhibition zones (14.33 mm and 18.66 mm, respectively), and chitosan beads had the smallest inhibition zone (11 mm). Their findings demonstrated that surface functionalization of pullulan led to higher drug encapsulation efficiencies.

Emam et al. synthesized polyvinylpyrrolidone (povidine)-bound iodine (PI) loaded in pectin/carboxymethyl pullulan hydrogel. Carboxymethyl pullulan was first prepared through etherification reaction in an alkaline pH of aqueous/organic solution. Pullulan was suspended in isopropanol and 1M NaOH was added dropwise; it was then magnetically stirred for 60 min, followed by the dropwise addition of monochloroacetic acid in the reaction mixture at 70 °C for 5 h. Synthesized carboxymethyl pullulan was crosslinked with pectin, with glutaraldehyde used as the crosslinker, to obtain pectin-carboxymethyl pullulan hydrogel. Polyvinylpyrrolidone (povidine)-bound iodine (PI) acted as an antiseptic reagent against skin infections and wound healing. It was demonstrated that the release of PI from the hydrogel matrix was highly efficient as a result of good swelling ability of the composite network. The hydrogel could be used as a wound dressing for treating skin injuries and as a drug delivery device [17]. **Figure 1** illustrates the synthetic pathway of pectin/carboxymethyl pullulan hydrogel.

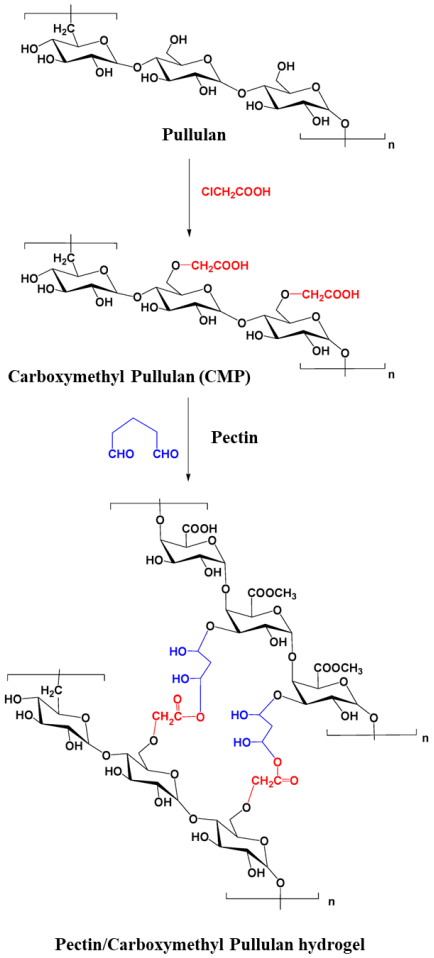

Figure 1. Preparation of pectin/carboxymethyl pullulan hydrogel. Reproduced from [17], with permission from MDPI, 2022.

The antimicrobial activities of the synthesized pectin/carboxymethyl pullulan hydrogel were investigated, including the release of polyvinylpyrrolidone (povidine)-bound iodine (PI), against two pathogens, *Escherichia coli* and *Candida albicans.* It was confirmed that the biological activity of released PI from the hydrogel was highly effective. Studies showed that the inhibition areas of released PI from the hydrogel samples were 19 mm and 20 mm for *Escherichia coli* and *Candida albicans*, respectively (**Figure 2**) [17]. In addition, the antimicrobial activity of pure PI was 25 mm for both pathogens. The low inhibition zones of the released PI could be related to the concentration of PI in the released hydrogel samples. However, the activity of PI was not affected after it was released, except the low PI concentration, which led to decreased activity in the inhibition zone.

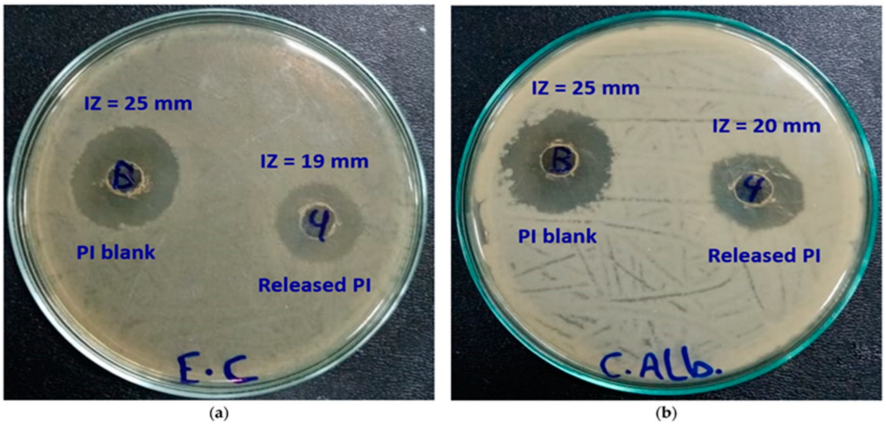

Figure 2. Antimicrobial activity of pure PI (left) and released PI (right) against (**a**) *Escherichia coli* (**b**) *Candida albicans*. Reproduced from [17], with permission from MDPI, 2023. PI: Polyvinylpyrrolidone (povidine)-bound iodine.

Priya et al. synthesized 10% pullulan hydrogel with no crosslinkers and evaluated its wound healing efficiency in daily topical administration. Their findings showed faster healing of wounds when the hydrogel was administered topically. They explained that the fast healing was due to the controlled release and availability of the therapeutic agent at the wound site as well as the antioxidant and energy generating properties of pullulan. Pullulan, being a biodegradable polysaccharide polymer, could also be a source of energy for cells, such as fibroblasts, which are actively involved in the healing process.

Furthermore, the increases in the rate of wound closure and the decreases in the healing time with pullulan treated wounds could result from its hydroscopic nature, which facilitated bacterial dehydration, inactivated them, and reduced their surface area. Dehydration of wound fluid may improve cells and tissues oxygenation, promoting wound healing. Their histological examination demonstrated improved growth of fibroblasts and epithelialization in wounds treated with pullulan [18]. This clearly supports pullulan as a potent material for wound healing.

Chen et al. fabricated a pullulan/collagen hydrogel with tunable, suitable biomechanical properties and improved biocompatibility for wound treatment and regeneration. In this research, they compared the efficacy of the synthesized hydrogel with two marketed wound dressings, Promogran™ (55% collagen and 45% oxidized regenerated cellulose) and Fibracol ® Plus (90% collagen and 10% alginate) [19]. They used a mouse excisional wound model and dressed the wounds with the commercial dressings and the synthesized pullulan/collagen dressing (TauTona wound dressing, TWD) alongside untreated control wounds, then investigated the healing process. Their findings demonstrated that pullulan/collagen hydrogel dressings enhanced collagen architecture and alignment and accelerated healing in murine wounds after 14 days compared to the other commercial dressings. At postoperative days, PODs 10 and 12, the area of the wound treated with the synthesized hydrogel was significantly reduced compared to control wounds. The sizes of wounds treated with Promogran™ and Fibracol® Plus were not significantly different from the pullulan/collagen dressing. The percentage of the wound sizes at PODs 10, demonstrated that the wounds treated with pullulan/collagen dressing had smaller wound size than Promogran™ and Fibracol® Plus. They further proved that pullulan/collagen treated wounds demonstrated a significant decrease in macrophages, lymphocytes, and overall tissue response, which accelerated wound repair compared to Promogran ™. Finally, their studies showed that stromal cells derived from adipose tissues seeded in the developed hydrogel promoted healing in murine burn model, reduced time of wound closure, decreased scaring and developed collagen network [19][20]. The pullulan/collagen hydrogel demonstrated clinical feasibility and ease of use. Recently, pullulan/collagen hydrogel dressing has been manufactured by the TauTona group in Redwood City, Canada, and has been referred to as TauTona wound dressing (TWD) [19].

Nicholas et al. investigated the efficacy of pullulan/gelatin scaffolds on skin regeneration. They fabricated a cost-effective pullulan/gelatin hydrogel with suitable mechanical properties for skin substitutes and cells, such as fibroblasts and keratinocytes, that were grown in the hydrogel. The excisional wounds treated with hydrogels exhibited less macrophage infiltration, decreased inflammation, and improved angiogenesis after 14 days of post mouse-skin biopsy compared to the control. Their findings suggested that the pullulan/gelatin hydrogel could be suitable in skin wounds with a high level of inflammation, such as chronic wounds and burns [21].

Biomedical sponges are soft and flexible materials with a highly interconnected porous network. The high swelling rate of these scaffolds and fast hemostatic ability can make them suitable for preventing the accumulation of unwanted wound fluids. In addition, sponges with high water content provide a moist wound environment and protect it from bacterial infection. Wang et al., developed succinyl pullulan/carboxymethyl chitosan sponges as a potential wound dressing. Succinyl pullulan (pullulan-COOH) was prepared by mixing succinic anhydride and an aqueous solution of pullulan. Succinyl pullulan and carboxymethyl chitosan were mixed and crosslinked with 1-ethyl-3-(3-dimethylami nopropyl)-carbodiimide/N-hydroxy succinimide (EDC/NHS) and the sponges were obtained. The crosslinker introduced amide bonds between the carboxyl groups present in pullulan-COOH and amino groups in carboxymethyl chitosan, which has been confirmed to be non-toxic. They demonstrated that the sponges maintained a good, moist environment that significantly reduced the wound area. Histological evaluation revealed that the sponges promoted proliferation of the fibroblast and improved epithelialization [22]. Further wound dressing materials based on pullulan are summarized in **Table 1**.

Table 1. Additional pullulan-based hydrogels wound dressings.

Application	System Used	Drug/Growth Enhancing Factor(s)	Cell Types Treated	Reference
Wound healing	**Pullulan film**	**-**	**Rat skin cells**	[23]
Wound dressing	**Keratin/pullulan/PVA hydrogel membrane**	**Cefotaxime**	**Incision on male SD rat skin cells**	[24]
Wound dressing and antibacterial effect	**Carboxymethyl pullulan hydrogels**	**Gentamicin**	**In vitro drug release studies in phosphate buffer saline solution**	[25]
Wound healing	**Hyaluronic/pullulan/ grafted-Pluronic F127 hydrogel**	**Curcumin**	**Female rat skin cells**	[26]

Application	System Used	Drug/Growth Enhancing Factor(s)	Cell Types Treated	Reference
High oxidative stress wound dressing	Pullulan hydrogels	Mesenchymal stromal cell	Anterior and posterior full thickness transverse incisions on skin cells	[27]
Wound dressing	Pullulan/dopamine hydrogel	-	In vitro studies of sheep blood	[28]
Wound healing	Pullulan/chitosan composite nanofibers	Tannic acid	NIH 3T3 mouse embryonic fibroblast cells	[29]
Wound dressing and antioxidant effect	Pullulan/bacterial cellulose hydrogel	Vitamin C and E	In vitro studies in phosphate saline buffer	[30]
Early cutaneous wound healing	Pullulan/collagen composite hydrogels	-	Full thickness skin cells incisions	[31]
Wound dressing	Collagen/pullulan hydrogel	Polydatin	Wistar rat skin cells	[32]
Wound healing	Cholesterol bearing pullulan nanogels	Prostaglandin E1	Full thickness defect on rat dermal cells	[33]
Wound dressing and drug delivery system	Pu-g-p(AA-co-IA) hydrogel film	Ampicillin	In vitro drug release study in phosphate buffer saline solution	[34]

Pu-g-p(AA-co-IA) = Pullulan grafted poly(acrylic acid-co-itaconic acid); PVA = Polyvinyl alcohol.

Over the past few years, pullulan has been reported to have applications in vascular engineering, bone tissue engineering [35], and skin tissue repairs. Tissue engineering is a recently growing field that assists in the regeneration and repair of injured tissues and potentiates patients' wound healing process. Hydrogel as a skin substitute greatly depends on the material from which it is developed. The non-toxic, nonimmunogenic, non-mutagenic, and antioxidant properties of pullulan have shown it to be a suitable material for skin regeneration applications. Pullulan methacrylate hydrogels have promising potentials in the production of cell-laden microscale tissues to incorporate cells in a three-dimensional environment [16][36]. Research has shown that cells encapsulated in pullulan methacrylate hydrogel possessed excellent viability, proliferation, and accelerated the repair of wounds in rats and mice [37]. Pullulan/collagen hydrogels can be used as skin scaffolds to accelerate wound healing due to their excellent mechanical properties, such as porosity and pore size [38][39]. These hydrogel scaffolds can easily replicate skin architecture and promote encapsulation of stem cells and elements of wound healing for the restoration of skin tissues.

Pullulan scaffolds have demonstrated potential antioxidant properties which can be of great importance for skin regeneration. The antioxidant property protects the stromal cells from oxidative damage [40]. Atila et al. reported a 3D electrospun pullulan-cellulose acetate scaffold which had excellent cytocompatibility, as the cells could easily adhere, spread, and grow on the hydrogel scaffolds. As such these scaffolds had great potential for skin tissue engineering applications [41]. Pullulan significantly promoted cell proliferation and enhanced cell adhesion. Therefore, pullulan and its composites could be potent materials in skin tissue engineering applications.

Recently, Younas et al. developed a multifunctional pullulan microneedle patch loaded with chitosan/fucoidan nanoparticles for differential release of moxifloxacin, lidocaine, and thrombin. Chitosan and fucoidan were used to synthesize moxifloxacin nanoparticles with a diameter of 258.0 ± 10.86 nm and surface charge 45.1 ± 3.9 mV. Lidocaine (LH), thrombin (TH), and moxifloxacin nanoparticles (MOXNP) were then encapsulated in a 30% (*w*/*w*) pullulan-based microneedle patch. Their findings demonstrated that the microneedle patch achieved rapid hemostasis/analgesia and sustained antibacterial activity. The patch facilitated the rapid release of thrombin and could offer efficient coagulation. Their results proved that the pullulan microneedle patch was highly biocompatible with combined hemostatic, analgesic, and prolonged antibacterial effects. Therefore, the multifunctional patch based on polysaccharides (pullulan, chitosan, and fucoidan) can be used for high-quality wound healing [42]. The researchers investigated the mechanical strength of the pullulan-based microneedles and claimed that the microneedles both with and without drug encapsulation exhibited outstanding mechanical properties. The blank microneedle (MN) and the drug loaded sample had significant displacement at 2.27 N/needle and 2.73 N/needle, respectively. In addition, they reported that the combined polysaccharides developed microneedle patch had high biocompatibility. Transdermal drug delivery is a modern delivery system for therapeutic agents possessing systemic side effects. Pullulan-based dissolving microneedles have been utilized for transdermal delivery of small and large bioactive molecules [43].

The hemocompatibility of pullulan is one of the important criteria for its applications in skin tissue engineering and wound management. Baron and co-workers fabricated a hemostatic wound dressing based on dialdehyde pullulan and dopamine. The developed multifunctional cryogels were prepared by a series of combinations of hemi(acetal) and Schiff base interactions. The assessment of hemostatic effect was performed based on the blood clotting index (BCI). They prepared three different samples of cryogels. The first cryogel sample was based solely on dialdehyde oxidized pullulan (PO). The two dialdehyde oxidized pullulan/dopamine cryogels were prepared based on the mechanism of dopamine incorporation. The first oxidized pullulan/dopamine cryogel sample was fabricated by in situ loading of dopamine followed by lyophilization (POD), and the second oxidized pullulan/dopamine sample was obtained by post-incorporation of dopamine (POD1). The obtained results demonstrated that BCI values of the oxidized pullulan (PO) and oxidized pullulan/dopamine (POD1) hydrogel samples were <50 % (**Figure 3**) which was attributed to better blood clotting ability of the hydrogels. In addition, they observed lower blood clotting indices of the cryogels with increased oxidation of pullulan [44]. Hence, hemostatic wound dressings can help to reduce blood loss in chronic and acute wounds and fasten wound healing [28][32][44]. They further observed that periodate-oxidation pullulan could form stable hydrogels due to the hemi(acetal) interactions, and also that dopamine interacted with the aldehyde groups, resulting in improved mechanical stability of the hydrogels networks. Therefore, hemostatic activity and mechanical stability of pullulan-based hydrogels suggested that they can be promising materials for wound dressings and skin tissue scaffolds.

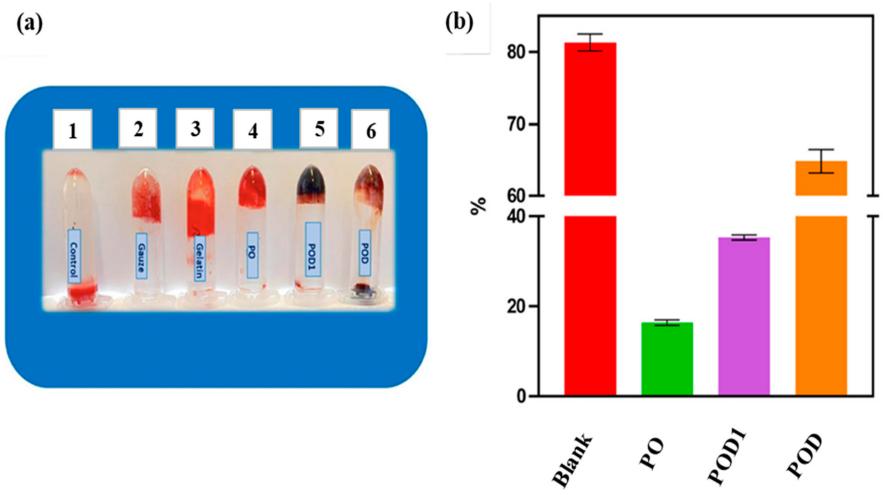

Figure 3. Results of in vitro blood coagulation: (**a**) Photographs of blood clotting. 1: control; 2: medical gauze; 3: gelatin; 4: PO (oxidized pullulan); 5: POD (oxidized pullulan/dopamine cryogel prepared by in situ loading of dopamine; 6: POD1 (oxidized pullulan/dopamine cryogel sample prepared by post-incorporation of dopamine); (**b**) Blood clotting index of the cryogel samples incubated in whole blood for 10 min. Reproduced from [28] with permission from MDPI, 2023.

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