Application in Wound Healing

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Wound healing requires careful, directed, and effective therapies to prevent infections and accelerate tissue regeneration. In light of these demands, active biomolecules with antibacterial properties and/or healing capacities have been functionalized onto nanostructured polymeric dressings and their synergistic effect examined. In this work, various antibiotics, nanoparticles, and natural extract-derived products that were used in association with electrospun nanocomposites containing cellulose, cellulose acetate and different types of nanocellulose (cellulose nanocrystals, cellulose nanofibrils, and bacterial cellulose) have been reviewed. The impact of these combinations in wound healing are here examined and explored.

cellulose | cellulose acetate | nanocellulose | biomolecules functionalization | natural-extracts | nanofibrous dressings | antimicrobial surfaces | tissue regeneration

1. Introduction

In wound care, infections are a major concern, since they delay the healing process, leading to tissue disfigurement or even patient death. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are the most common bacteria that are isolated from chronic wounds, being *S. aureus* usually detected on top of the wound and *P. aeruginosa* in the deepest regions. They can express virulence factors and surface proteins that affect wound healing. The co-infection of *S. aureus* and *P. aeruginosa* is even more problematic, since the virulence is increased; both bacteria have intrinsic and acquired antibiotic resistance, making the clinical management of these infections a real challenge [1]. In fact, the World Health Organization considers *P. aeruginosa* as one of the organisms in urgent need for novel, highly effective antibacterial strategies that combat its prevalence. Multiple strains of *S. aureus*, including methicillin-resistant and vancomycin-resistant strains, have been identified as high priority microbes in the fight against antimicrobial resistance build up [2]. In addition to the above, other microorganisms, such as beta-hemolytic streptococci, and mixtures of Gram-negative species, such as *Escherichia coli* and *Klebsiella* strains, are also present in wounds. Bacterium native to human skin such as *Staphylococcus epidermidis* (Gram-positive), may also turn pathogenic when exposed to systemic circulation in the wound bed [3]. Therefore, immediate care of open wounds is pivotal in preventing infection [4]. To treat this problem, new alternatives of wound dressings have emerged with incorporated bioactive agents that are capable of fighting these infections and accelerating the healing process.

2. Application in Wound Healing: Synergistic Effect with Specialized Biomolecules

The performance of bioactive dressings processed via electrospinning is dependent on the polymer or polymer blends properties (i.e. hydrophilicity and hydrophobicity), drug solubility, drug-polymer synergy, and mat structure. Antimicrobial agent-loaded electrospun mats have shown superior performance to films produced by other techniques, in regard to water uptake (four to five times superior), water permeability, drug release rate, and antibacterial activity [5].

Drugs, nanoparticles, and natural extracts (Table 1) are some of the antimicrobial agents that have been incorporated in nanofibrous dressings, in order to reduce the risk of infection [6]. These compounds have been used for their anti-inflammatory, pain-relieving, vasodilation, and antimicrobial features [7].

Table 1. Examples of compounds incorporated in electrospun nanostructures containing cellulose or its derivatives.

Subtract	Drugs	Nanoparticles	Natural Extracts	Ref.
Cellulose	Tetracycline hydrochloride (TH) Ciprofloxacin (CIF) Donepezil hydrochloride (DNP)	Silver NPs (AgNPs) Zinc oxide NPs (ZnONPs)	Bromelain	[8][9][10][11][12][13][14]
CA	TH Ferulic acid (FA) Ibuprofen (IBU) Ketoprofen (KET) Amoxicillin Thymoquinone (TQ) Silver salt of sulfadiazine (SSD)	Silver Titanium dioxide Zinc oxide Copper	Cinnamon (CN); Lemongrass (LG); Peppermint (PM) Rosemary; Oregano Thymol Zein Asiaticoside (AC) Curcumin (Curc) Acid gallic Gingerol Garlic extract	[15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31][32][33][34]
CNC	TH	ZnO AgNPs	Thymol	[35][36][37][38][39][40][41]
BC		Soy protein particles	Tragacanth gum (TG)	[42][43][44]

Subtract	Drugs	Nanoparticles	Natural Extracts	Ref.
		Graphene oxide (GO)		

Several researchers claim that producing cellulose-based electrospun mats is a big challenge due to its highly crystalline structure, long chain length, increased rigidity, and strong inter- and intramolecular hydrogen bonding [45]. Selecting a proper solvent, adding other complementary polymers, or converting cellulose into its derivatives can facilitate this task. The solvents or solvent systems most used for cellulose are the ionic liquids (ILs), aqueous alkali/solvents (NaOH/urea), and polar aprotic solvents in combination with electrolytes (DMAc/LiCl); however, these are not very volatile, not being completely removed during electrospinning and, thus, limiting the use of cellulose in large scale productions. A proper solvent system is also very important in attaining appropriate viscosity levels, required for a successful electrospinning process. In fact, this is such an important processing parameter that to guarantee proper polymer solubilization, heaters have been placed within the electrospinning apparatus generating a new system, the melt-electrospinning (minimize the viscosity of spinning dopes) [46]. The option of transforming cellulose into its derivatives, such as cellulose acetate (CA), cellulose acetate phthalate (CAP), *ethyl cellulose* (EC), carboxymethyl cellulose (CMC), hydroxypropylcellulose (*HPC*), among others, is by far the most recurrent alternative to reduce the complexity of processing cellulose via electrospinning. Besides, most of these derivatives require different pHs for solubilization, which is a great advantage for biomedical applications [47].

Modifications have been proposed to increase the effectiveness of immobilized drugs, natural compounds, peptides, or other biomolecules within a cellulose-based nanostructured surface. For example, Nada et al. activated CA by introducing azide functional groups on the residual -OH groups of the polymeric chains, enhancing the release kinetics of capsaicin and sodium diclofenac from the electrospun mat and, thus, promoting patient relief [48]. To confer biocidal properties to CA nanofibers, Jiang et al. modified their surface with 4,4'-diphenylmethane diisocyanate (MDI). This resulted in a 100% inactivation of *S. aureus* and a 95% of *E. coli* within 10 min of exposure, and complete death after a 30 min contact [49]. Nano complexes with cellulose nanocrystals (CNCs) were developed with cationic b-cyclodextrin (CD) containing curcumin by ionic association and used in the treatment of colon and prostate cancers [50]. Nanocellulose has also contributed to the development of new and more efficient strategies for these biomolecules' delivery. The three -OH groups that were present in each individual glucose unit originate a highly reactive structure, which allows interaction with other molecules or with enzymes and/or proteins, contributing to overcome the low solubility of most drugs in aqueous medium [51]. Besides, the -OH groups can also be tailored by physical adsorption, surface graft polymerization, and covalent bonding to further improve the performance of the biomolecules. As a consequence of the bonds established, strong polymer-filler interactions are generated, significantly increasing the mechanical properties of material [52]. Nonetheless, the in vivo behavior of nanocelluloses is still little explored. Studies have reported that its toxicity depends on the solution concentration and its surface charges. In recent literature, nanocelluloses have not shown any toxicity at concentrations lower than 1 mg/mL; however, there are studies that reveal a concentration-dependent apoptotic toxicity of cellulose nanofibrils (CNFs) at 2–5 mg/mL. Additionally, anionic nanocelluloses, e.g., carboxymethylated-CNF, have been reported to be more cytotoxic than cationic nanocelluloses, e.g.,

trimethylammonium-CNF [53]. Toxicity effects might arise from the diversity of chemical structures and properties between cellulose types and sources. Among nanocelluloses, bacterial cellulose (BC) is considered to be the most biocompatible and has already been applied in wound dressings [54]. Still, its electrospinnability is very challenging for the same structural reasons of cellulose [45].

The incorporation of BC into synthetic and natural polymers has been carried out to enhance their morphological features as well as physicochemical and biological performances. A wide variety of polymers, such as chitosan, polycaprolactone (PCL), polyethylene oxide (PEO), ethylene vinyl alcohol (EVOH), polyvinyl alcohol (PVA), polylactic acid (PLA), polyacrylonitrile (PAN), polyester, silk, and zein, have been blended with BC and processed by electrospinning. Functionalization with 3-aminopropyl triethoxysilane (APS) has been attempted to further enhance cell attachment and antibacterial properties of BC-containing electrospun membranes for wound healing. BC membranes grafted with two organosilanes and acetyled have also shown an improved moisture resistance and hydrophobicity [55]. Naeem et. al even synthetized in situ BC on CA-based electrospun mats in a process known by self-assembly to produce a new generation of wound dressings [56].

Even though CNF has already been applied as a reinforcing agent in many polymeric composites via electrospinning, no reports have been found regarding the incorporation of biomolecules along its fibers [57].

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