

# Layer-by-Layer Assembly for Biofunctionalization of Cellulosic Fibers with Emergent Antimicrobial Agents

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**Abstract** Coating with polyelectrolyte multilayers has become a generic way to functionalize a variety of materials. In particular, the layer-by-layer (LbL) technique allows the coating of solid surfaces to give them several functionalities, including controlled release of bioactive agents. At present there are a large number of applications of the LbL technique; however, it is still little explored in the area of textiles. In this review we present an overview of LbL for textile materials made from synthetic or natural fibers. More specifically, LbL is presented as a method for obtaining new bioactive cotton (as in cellulosic fibers) for potential application in the medical field. We also review recent progress in the embedding of active agents in adsorbed multilayers as a novel way to provide the system with a “reservoir” where bioactive agents can be loaded for subsequent release.

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## 1 Introduction

### 1.1 *Antimicrobial Textiles*

The number of textiles functionalized for antimicrobial activity has increased considerably over the last few years. Antimicrobial textiles were first created to prevent damage to textiles under adverse environmental conditions during their storage or use. Textiles are widely used in daily life and there has been a growing need to develop associated finishes that can offer improved protection to users, for example, from microbes (bacteria, fungi) that could pose a threat to health. Hence, there is a pressing need to develop functionalized textiles that are resistant to microbes, especially for use in healthcare activities. Synthetic antimicrobial compounds used in textile articles are very effective against a wide range of microorganisms. However, the continuous use of antimicrobial compounds can lead to bacterial resistance and desensitization of users, as well as produce a negative environmental impact [1–4].

To minimize these risks, there is currently a high demand for antimicrobial textiles produced with environmentally friendly, nontoxic natural compounds. The low incidence of adverse effects of natural compounds, compared with their synthetic counterparts, has led to such textiles being explored as attractive and promising alternatives [5–7].

Cotton is the textile substrate most widely used in the health sector. In the form of cellulose fibers [3, 4], it is known for its versatility, natural comfort, softness, breathability, and ability to absorb moisture [8]. Cotton is often used to make a

variety of clothing for industrial and biomedical purposes. Because of its properties, cotton is also ideally suited as wound dressing material. In fact, cotton gauze is still the most commonly used textile for wound dressing in hospitals; however, new products have emerged for wound healing and for protection against entry of bacteria. For exudative wounds, there are a range of absorptive products (including various hydrophilic foam dressings, hydrogels, and alginates) that can absorb up to 20 times their weight [9] and can be formulated in combination with cotton substrates. At the same time, cotton is prone to act as host for the growth and development of microorganisms, making biofunctionalization with antimicrobial agents a necessity.

## ***1.2 Current Functionalization Processes in Textile Materials***

Coating is an important technique for adding value to technical textiles and a generic method of surface functionalization. In its most generic form, coatings involve polymer layers that are applied directly to one or both surfaces of a given fabric. A number of nanotechnologies have received special attention in the textile industry for fiber or fabric modification and to endow the substrates with new properties that are revealed at the nanoscale, especially multifunctional properties [10] that include antimicrobial activity to reduce the risk of microorganism transmission.

Several processes are available for coating textile materials, depending upon the requirement of the end product. The most significant processes include techniques such as sol-gel, which is a wet process that is broadly employed in the textile field and involves a simple pad or dip coating; magnetron sputter coating, which is one of several physical vapor deposition methods; plasma, which is a suitable technique for modifying the structure and topography of the surface; and others involving composite and hybrid systems [11].

These methods have a number of disadvantages, the most significant being the need for expensive solvents and equipment and the requirement for multistep and complex processing. In addition, under certain conditions it is necessary to use high temperatures, and the success of modification is often dependent on the substrate topography. It is apparent that new strategies are needed and the LbL technique, in particular, is as an attractive option because of its relative simplicity and efficiency.

The prerequisite for the success of LbL coating is the presence of a minimal surface charge on the substrate. In such cases, the LbL technique provides coatings on the surface of textiles to enable a wide range of functionalities [12]. Despite its technological appeal, the LbL deposition process has not been extensively implemented in the textile industry, particularly for natural fibers. The main reason for the slow introduction of LbL for natural fibers is their unique characteristics, including surface chemical heterogeneity, which complicates the application of such coatings.

## 2 LbL Assembly Technique

LbL is a simple and versatile method that can provide new types of coatings for textile materials. It was proposed by Decher and collaborators in the early 1990s [13] and the interest it has generated since then can be judged by the growing number of published papers on the subject.

LbL assembly can be applied on many kinds of charged surfaces [14], mainly to deposit multilayers of controlled architecture and composition from aqueous solutions. Electrostatic interactions are the main driving force for assembly within the neighboring layers of polyelectrolytes. Generally, LbL assembly proceeds as follows: (1) a charged substrate is immersed in a solution of an oppositely charged colloid that adsorbs as the first monolayer; (2) a washing cycle removes excess and unbound material; and (3) the coated substrate is submerged again to deposit a second layer to form a bilayer structure [15]. These cycles can be repeated as often as needed, and crosslinking is often applied to convert the obtained LbL multilayers into surface-bound hydrogels [16]. The number of deposition cycles and the type of polyelectrolyte used in the LbL construction allow full control of the thickness and roughness of the multilayered film [17]. Usually, multilayered films based on electrostatic interaction are affected by environmental conditions such as, pH, polyelectrolyte concentration, nature of solvents, and ionic strength [14, 18, 19].

LbL materials can be selected from a large variety of materials other than polyelectrolytes: small organic molecules, polymers, natural proteins, inorganic clusters, clay particles, and other colloids. In such cases, LbL can open new possibilities because a myriad of component combinations can be formulated in single devices with designed features and architectures. Importantly, the resulting functions might not be associated with those of the native (substrate) material. Surface functionality, for example, can be controlled directly by choosing appropriate polyelectrolyte combinations. This allows modification of electrical, optical, magnetic, physicochemical, and biological properties of the materials. Multilayer coating with bioactive, natural polyelectrolytes has become a new process for surface biofunctionalization.

Advantages of the LbL technique are that the process is inexpensive, relatively fast and simple, does not require sophisticated equipment and precise stoichiometry, or rely on complicated chemical reactions to deposit successive layers [20]. Another advantage of LbL deposition is its insensitivity to the size and shape of the substrate in producing comfortable coatings. This means that an LbL assembly can be realized not only on planar substrates, but also on substrates with different shapes and curvatures. For example, Caruso et al., (1998) demonstrated LbL deposition on a spherical template. After template dissolution, microcapsules were obtained [21]. Theoretically, LbL substrates can be of any size, shape, topography, or topology, and no stoichiometric control is necessary to maintain surface functionality and avoid propagation of defects [22].

In LbL deposition, pH can be used to adjust the strength of interlayer bonding and, therefore, multilayer thickness. As stated before, this requires the presence of a minimal surface charge, which, if absent, can be induced by different means [23].

Most knowledge about the LbL method has been developed in the field of drug delivery systems, allowing the creation of sophisticated materials and the production of capsules as carriers of drugs that can be released gradually. A major challenge in drug delivery is to produce controlled, sustained or triggered release systems for small encapsulated drug molecules. A discussion of these processes can be found in a review article by Wohl and Engbersen [24].

Several biomedical engineering studies have reported the creation of multilayer coatings by the LbL technique involving synthetic and natural polyelectrolytes [25]. Related multilayers have a characteristic low packing density to facilitate diffusion of bioactive agents, which in most cases are embedded throughout the multilayer. In fact, various parameters such as pH, ionic strength, temperature, light, and chemical or electrochemical stimuli have been used to tune the release and/or retention of bioactive agents within the multilayers. This allows control of drug dose, and delivery on demand with reduced toxicity and increased efficacy [16].

Using the concept of diffusion from multilayers, several authors have immobilized more than ten different water-soluble proteins in multilayers, ensuring the inhibition of protein denaturation [26–28]. Appropriate preservation of the functional characteristics of given compounds have ensured good results for effective incorporation of proteins and drugs between the multilayers, and their diffusion and subsequent release [29–33].

Several researchers have reported bioactive proteins, peptides, hormones, growth factors, and drugs that can be directly integrated into LbL architectures, without any covalent bonding with a polyelectrolyte and while maintaining their native structures and activities [29, 34–41]. The strategy described in all these efforts can be valuable in the application of a variety of drugs/bioactive agents. This opens a route for substrate functionalization via multilayers with embedded bioactive agents, in which the multilayers act as a reservoir for bioactive agents that can be gradually released and controlled.

A recent review provides an exhaustive account of the potential uses of the LbL method in biomedical engineering [42]; however, the technique is still in its infancy for textile applications. In recent years, researchers have used the LbL process to modify the surface of textile fabrics to impart or improve numerous surface properties, including UV protection [43, 44], hydrophobicity/hydrophilicity [45, 46], flame retardancy [47–49], and antimicrobial activity [50–54].

Our aim in this review is to assess the feasibility of employing the LbL method to obtain functionalized cellulose (cotton) with antimicrobial properties by incorporating bioactive agents between the layers for subsequent controlled release. This approach is supported by the work of Caridade et al., who studied the production of thick membranes by LbL assembly of chitosan and alginate and the control of membrane permeability to bioactive agents [55].

### 3 LbL in Textile Materials

The LbL process has been widely used to create multilayer films on various substrates. However, it has not been extensively employed for textile fibers. Textile fibers present some unique challenges for LbL assembly, such as the chemical heterogeneity and irregular shape of their surfaces [56]. Because LbL is a relatively new method for material functionalization, there are only a few reports concerning its adoption for textiles. For simplicity, this review divides textile substrates into two categories, synthetic and natural fibers.

#### 3.1 *LbL Coatings on Synthetic Fiber Materials*

Synthetic fibers play an important role in the textile industry. There are many different types of synthetic fibers, but the most used are polypropylene, polyethylene, polyester, polyamide (nylon), and polyvinyl alcohol. A great disadvantage of some synthetic fibers is low hydrophilicity. This affects the LbL process because the fiber surface is not easily wetted. Table 1 shows a summary of the state of the art in application of the LbL method for textile materials involving synthetic fibers, mainly polypropylene, polyester, and polyethylene terephthalate (PET). The deposition of several layers endows the textile material with several features; however, attempts to impart antimicrobial properties to synthetic textiles have been introduced only recently.

#### 3.2 *LbL Coating of Cellulose-Based Textiles*

Cotton, as one of the most commonly used materials in textiles, is suitable for medical usage, especially for wound dressings as a result of its high liquid absorbency and hygienic nature. However, cotton is characterized by its heterogeneity, which causes problems in conventional coatings but creates an opportunity for application of the LbL technique. Currently, only a few reports exist on the application of the LbL method to cotton. In this section we present the most important developments in terms of functionalized cotton using the LbL technique. A special note on antimicrobial cotton obtained by LbL is discussed at the end of this section.

There are many early reports on the application of the LbL technique to cellulosic fibers, mainly wood fibers. Hyde et al. reported application of LbL to cotton substrates. They found that the cationization process produced a cotton surface capable of supporting polyelectrolyte films via LbL deposition. They observed that the LbL deposition process is more dependent on the nature of the polyelectrolytes than on the nature of the original substrate; the analyses revealed

**Table 1** Summary of main milestones in LbL application to synthetic fibers in textiles

Author (year)	Substrate	Polyelectrolytes	Notes
Polowinski (2005) [57]	Polypropylene	PAH/PAA	The dyeing technique allows the type of external layers deposited in succession to be identified. The LbL method deposits layers of polymeric complexes, not only onto polypropylene, but also onto other textile materials with smooth surfaces.
Dubas et al. (2006) [58]	Nylon	PDADMAC/anionic scarlet dye	The LbL of PDADMAC/anionic scarlet dye has a high dependence on the number of layers, salt concentration, and concentration of chemicals but is almost independent of dipping time.
Polowinski (2007) [59]	Polypropylene, polyester	PAH/PAA	The LbL method was used to deposit thin polymeric layers on textile fabrics. A necessary condition for using this method is a smooth surface on the fibers in the fabric.
Jantas et al. (2007) [60]	Polyester	PAA/PVP	The surface of fibers in the fabric becomes smoother after depositing PAA/PVP nanolayers.
Polowinski (2007) [61]	Polypropylene	PAA/PDAMA/PAH Nanoparticles: Au, Pt, Ag	The LbL method is a convenient way of depositing colloidal particles of silver, gold, or platinum onto textiles.
Stawski et al. (2009) [62]	Polypropylene	PAH/PAA	Deposition of successive polyelectrolyte layers fails to provide complete coverage of the modified surface.
Park et al. (2009) [63]	Nylon 6	Alginate sodium salt and chitosan	The morphology of a polyelectrolyte multilayer coated on nylon 6 fibers was uniform and smooth. The surface morphology, stiffness, and hydrophilicity of the system was controlled by regulating the number of polyelectrolyte nanocoats.
Carosio et al. (2011) [48]	PET	Silica nanoparticles	PET fabrics were coated with silica nanoparticles. This study demonstrates the ability to impart flame retardant behavior using a water-based, environmentally friendly protective coating.
Martin et al. (2013) [65]	Non-woven PET	MB/chitosan/polyCD (cyclodextrin polyelectrolyte)	The aim of this work was to develop an antibacterial multilayer coating activated with methylene blue (MB). The authors prepared two types of samples, MB-free and MB-loaded.

(continued)

**Table 1** (continued)

Author (year)	Substrate	Polyelectrolytes	Notes
Martin et al. (2013) [64]	Non-woven PET	Chitosan/polyCTR-beta CD (beta cyclodextrin polymer)	This work developed the formation of a multilayered coating onto a PET textile support in order to obtain reservoir and sustained release properties towards bioactive molecules.

*PAH* poly(allylamine hydrochloride), *PAA* poly(acrylic acid), *PDADMAC* poly(diallyldimethylammonium chloride), *PVP* poly(vinyl pyrrolidone), *PDAMA* poly(diallylamine-co-maleic acid), *PET* polyethylene terephthalate, *PSS* poly(sodium styrene sulfonate), *MB* methylene blue

conformal and uniform coating of the cotton fibers [66]. From this work, there is indication that various functionalities can be developed for a given substrate, depending on the combinations of polyelectrolytes used. It is noteworthy that the nature of the substrate in LbL deposition has a relatively minor role, allowing the LbL to be applied smoothly onto cotton. Other publications report methods of analysis of samples obtained by the LbL technique [67]. Studies were also carried out to unveil the influence of physical parameters such as pH, concentration, ionic strength of polyelectrolytes, and cationization level of the substrate [56, 68]. In general, it was concluded that the deposition process was not significantly influenced by the degree of cotton cationization. In contrast, physical parameters were found to have a major influence on the success of LbL coating.

Protection against UV irradiation has been approached by using LbL coating of cotton fabrics [43, 44, 69, 70]. High UV protection factors were obtained as well as good resistance to washing, revealing the stability of the layers obtained by LbL on cotton. In fact, durability of the coating is often associated with the LbL technique. Another important property often reported for cotton is that of hydrophobicity [45, 71]. In this case, LbL coating is an easy method for fabrication of hydrophobic cotton fabrics.

More recently, several authors have used the LbL technique to coat cotton with specific polymers in order to enhance its flame retardancy properties. The studies showed that flame retardant coatings can be readily applied to textile fabrics for commercial and industrial applications [47–49, 72].

Providing antimicrobial properties to textiles is an effective way to prevent disease transmission in applications involving consumer and healthcare markets. Many textiles are treated to afford protection against bacteria, fungi, and other related microorganisms. During the past few years, several studies have aimed at functional antimicrobial modification via LbL assembly onto cotton fibers. Using *N*-halamine polyelectrolyte deposition on cotton fibers via LbL, cotton textiles were obtained with antimicrobial properties and potential application as medical textiles [73, 74]. A similar effect was obtained in our work group using chitosan and alginate as polyelectrolytes [52, 53, 75]. Other approaches for obtaining antimicrobial cotton have used chitosan [50, 51], copper [76], and ZnO [70] nanoparticles. In



these works, the nanoparticles were coated via LbL onto cotton fabric to form a nanocoating.

The LbL technique also offers new opportunities for the preparation of functionalized biomaterial coatings and the possibility of incorporating bioactive molecules between the layers [77–79]. Peptides, proteins, and active agents adsorbed or embedded in multilayer films have been shown to retain their biological activities [41], whereas covalent attachment to the active agents can reduce or even destroy their biochemical activity [79]. Thus, with the LbL technique, active agents can be directly integrated into the architecture without the need for covalent bonding.

Based on this concept, Gomes et al. investigated methods for the functionalization of cotton with polyelectrolyte multilayer films that incorporated a bioactive amino acid (L-cysteine, L-Cys). In this work, the strategy was based on the use of multilayer films as reservoirs of L-Cys [80]. These kinds of systems are promising for use in biomedical textiles, specifically for wound dressings. Cotton fiber is the basis of many wound dressings, and wound dressings containing antibiotics have been developed for the inhibition of wound infection [81–83]. Note that the continuous use of antibiotics has resulted in multiresistant bacterial strains; consequently, there is an urgent for alternatives to antibiotics.

## 4 Antimicrobials of the Future: Antimicrobial Peptides

Antibacterial resistance is a natural biological phenomenon that occurs in microorganisms and is potentiated by indiscriminate use of antibacterial agents. If the microorganism becomes resistant to a particular antibacterial agent, when an infection occurs, the effect of the antibacterial agent is reduced or nullified. Therefore, it is urgent that new antibacterial agents are discovered and used. Recently, a large group of low molecular weight natural compounds that exhibit antimicrobial activity were isolated from animals and plants, resulting in a new generation of antibacterial agents, named antimicrobial peptides (AMPs).

AMPs are promising agents because they are natural compounds [84, 85]; have a broad spectrum of action [86, 87]; exhibit high activity, even at low concentrations [87, 88]; have a low tendency to develop resistance as a result of their different mechanism of action [84, 85, 89–91]; act quickly and efficiently against bacterial agents [87, 91]; are generally of small size; and have low mammalian toxicity [87, 91]. The ability of AMPs to kill multidrug-resistant microorganisms has gained considerable attention and clinical interest. An alternative approach to wound healing with AMPs is related to the function of AMPs in removing destructive proteases from the wound. These proteases cause considerable destruction of growth factors and connective tissue proteins during the prolonged inflammatory phase of a chronic wound [92]. The 2010 review paper by Gouveia refers to AMPs for the first time as promising antimicrobial agents for textiles [93]. AMPs can be incorporated into textiles to produce nontoxic antimicrobial textiles. AMPs

produced in bacteria, insects, plants, invertebrates, and vertebrates are important components of the natural defenses of most living organisms. AMPs exhibit potent ability to kill a broad range of microorganisms, including Gram-negative and Gram-positive bacteria, fungi, and viruses [90, 94, 95].

#### ***4.1 AMPs: Characterization and Classification***

Currently, databases (e.g., <http://aps.unmc.edu/AP>) list over 2,400 AMPs. Their characterization is complicated as a result of their great diversity, but can be simplified if based on their secondary structure. AMPs are mainly grouped into four classes:  $\beta$ -sheet,  $\alpha$ -helical, loop, and extended peptides [96]. There are AMPs with positive [97] and negative [98] charges, or they can be amphipathic molecules (with both hydrophobic and hydrophilic regions). Some have sequences of less than 10 amino acids, whereas others contain nearly 100 amino acids [99]. In general, the AMPs are described as small molecules containing 12–50 amino acids with a cationic charge between +4 and +6 (as a result of the presence of the amino acids lysine and arginine) [91, 100]. Anionic AMPs generally have a net charge in the range of  $-1$  to  $-7$ , as a result of the presence of glutamic and aspartic acids. AMPs are mainly cationic and interact with membranes in a general mechanism that involves interaction between charged residues of the peptides and anionic components of the membrane surface.

#### ***4.2 Mechanisms of Action***

The AMPs in bacteria can cause disruption of the membrane, resulting in lysis or pore formation, allowing efflux of essential ion and nutrients. In this case, the AMPs are transported into the cell and inhibit DNA and RNA synthesis, inhibit ribosomal function and protein synthesis, and target mitochondria [101]. Many models of antimicrobial action at the membrane level have been proposed. Models that have greater acceptance in the scientific community include the carpet model, toroidal pore, and barrel-stave [88].

AMPs also possess antiviral properties. They inhibit viral fusion and egress, thus preventing infection and viral spread via direct interaction with the viral membranes and host cell surface molecules [101]. These properties, combined with the broad range of activity and short contact time required to induce killing, have led to the consideration of AMPs as novel therapeutic agents.

### 4.3 Applications of AMPs

AMPs applications are not limited to the development of new drugs, because there are also other medical, environmental, and industrial applications. Some potential medical applications of AMPs include:

- Prevention and treatment of eye disease, and in antimicrobial coatings for contact lenses [102]
- Antimicrobial coating on polymeric materials, such as implants and catheters, for prevention of bacterial colonization and biofilm formation on the surfaces of the implants [88]
- Functionalization of biomedical materials (e.g., in heart valves) and other textile materials such as socks for diabetics, gauze for chronic wounds, etc. [88]
- Wound healing, treating fibrosis, acne, Crohn's disease [90]

Recently, our research group found AMP applications in textiles, particularly in cotton gauzes for wound dressings [95, 103]. Incorporation is the most popular method for preparing immobilized AMPs on a variety of surfaces and retaining their ability to kill bacteria [104, 105]. Gomes et al. (2015) developed a new strategy for the biofunctionalization of cotton by incorporation of AMPs, with possible application in medical textiles such as wound dressings. This study demonstrated that cotton functionalized with chitosan/alginate loaded with AMPs is effective against pathogenic bacterial strains. The results demonstrated antimicrobial activity as well as low cytotoxicity. The main advantage of using AMPs is a result of their natural existence, meaning that they are well tolerated by the human body and that low concentrations are required. In addition, their synergistic biocide mechanism of action is effective against multiresistant bacteria.

## 5 Concluding Remarks and Future Prospects

Successful deposition of multilayers onto the surfaces of textile fibers, especially those based on cellulose, by the LbL technique can open opportunities for the development of functional textiles in a broad range of applications. The LbL technique in textiles is entirely new and is a simple and effective method, with a strong possibility of industrial implementation.

Bioactive agents can be directly embedded between polyelectrolyte layers without the need for covalent bonding, which facilitates the development of strategies to produce antimicrobial cotton by using AMPs in polyelectrolyte multilayer films. These coatings on cellulose surfaces are new and potentially useful as antibacterial materials in a wide variety of biomedical applications. In addition, LbL deposition allows easy fabrication of multimaterial films, in which different layers carry different functionalities or control the quality and quantity of active

agents. Overall, the reviewed work lays the groundwork for scale-up in cellulose-based materials and opens new avenues towards the development of nontoxic and safe biomedical textiles.

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