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Review article

Layer-by-layer assembly: A versatile approach for tailored biomedical films and drug delivery

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ABSTRACT

Layer-by-layer (LbL) assembly has revolutionized the field of biomedical engineering by enabling the precise design and fabrication of thin multilayer films with diverse functionalities. This article provides a comprehensive review of the applications of LbL assembly in drug delivery, antimicrobial action, wound healing, and tissue engineering. The LbL technique involves the sequential adsorption of oppositely charged materials onto a substrate, facilitating the incorporation of different chemical species through electrostatic interactions and other driving forces. This approach offers remarkable control over film properties such as porosity, mass, and thickness, and provides the flexibility to incorporate multiple components within the film structure. In drug delivery applications, LbL-produced films have demonstrated exceptional potential for controlled and sustained release of therapeutic agents, minimizing dosing frequency and improving patient compliance. Studies successfully report incorporated antimicrobials, anticancer agents and growth factors into LbL assemblies, demonstrating their effectiveness in targeted drug delivery and combating microbial infections. In addition, LBL assembly has emerged as a promising approach for wound healing strategies. By incorporating bioactive molecules and growth factors, these films promote tissue regeneration, angiogenesis and accelerated wound closure, thereby improving the overall wound healing process. In the field of tissue engineering, LbL-produced films provide a versatile platform for constructing bioactive structures that mimic the extracellular matrix and support cell attachment, proliferation, and differentiation. This versatile approach has significant implications for the development of tissue substitutes and regenerative therapies. This review also emphasizes the influence of LbL assembly methods on film properties, including thickness and porosity, and highlights the effect of various parameters such as pH, solvent, ionic strength, and temperature on film formation.

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1. Introduction

Drug delivery systems (DDS) are a useful tool in the pharmaceutical approach to controlling drug bioavailability. It allows the drug concentration profile to remain constant within the therapeutic index and reduces adverse side effects, which is convenient for the patient and therapeutic success [1–3]. The combination of dosage form and route of administration knowledge must therefore be carefully evaluated in DDS. The former refers to the technology used to deliver the drug into the body, such as tablets, nanoparticles, microparticles and microneedles, among others, which, depending on the route of administration, are used as oral, nasal, topical, transdermal, rectal, vaginal, parenteral, or ocular. This ensures maximum efficacy, safety, and reliability of the drug [4–6].

Layer-by-layer (LbL) deposition technology has enormous potential for use in DDS [7–9]. The first paper on LbL was published by Iler (1966) who demonstrated the technique of constructing LbL films deposited on the glass surface using silica and alumina to obtain uniform thickness films [10]. However, it was not until 1991 that Decher & Hong produced several alternative multilayer arrays achieving a thickness of 170 nm [11]. Since then, it has become a versatile, simple, and inexpensive technique. The thin layers are built up by polymer bonds based on hydrophilicity, van der Waals forces, hydrogen bonding, covalent bonding, host-guest and bispecific interactions [12]. LbL assembly has been used to coat organic and inorganic materials such as nano- and microparticles, multilayers, stents, nanotubes, graphene, DNA, and proteins. Several techniques are described in the literature and the most common are dipping, spraying and spin coating for drug delivery systems [13]. The advantages of this technique are the avoidance of organic solvents, drastic temperatures and the possibility of a range of pH values and ionic forces to stabilise the formulation [14].

LbL is also referred to collectively as ‘multilayer’, ‘biomaterial’, ‘film’, ‘assembly’ and ‘membrane’, and demonstrates these properties with applications in various types of materials such as pharmaceutical, electrotonic and environmental. The aim of this review is to focus on LbL for tissue engineering, wound healing, antimicrobial and anti-cancer applications, among others. LbL allows the loading of chemical and biological molecules to promote controlled and sustained drug release.

To understand the literature profile, the papers published in the last 10 years were examined. The research was limited to papers published in English and covering the period 2012–2022. The study was conducted with LbL AND drug delivery*, LbL AND multilayer*, and LbL AND assembly, LbL AND film, LbL AND membrane. Fig. 1 shows the number of scientific articles published using Pubmed Medline data (https://www.ncbi.nlm.nih.gov/pubmed/). It was observed that the number of articles published on LbL in the pharmaceutical field has remained stable in recent years.

The number of papers maintained in all the terms studied provides information that is an understudied topic compared to other DDSs. According to the research, the use of the word “LbL” is higher due to several applications in other areas that are not the focus of this research. In order to analyse the inclusion of the terms “membrane”, “multilayer” and “film”, a much smaller number of papers was observed. It emphasizes that this methodology is new and still has the potential to be explored. In addition, when looking at the papers, the authors do not show a consensus regarding the terms and the characteristics of each area of application of the LbL technique. In addition, a search was carried out in Scopus (https://www.scopus.com) from 2012 to 2022, using the keywords mentioned above. LbL AND drug delivery*, LbL AND multilayer* and LbL AND assembly, LbL AND film, LbL AND membrane yielded 687, 1,363, 2,848, 2,367, and 894 papers, respectively. The number of papers was slightly higher compared to the previously evaluated platform.

The VOSviewer software version 1.6.16 [15] was used for data analysis to examine a bibliometric map of this study in the world using the Scopus database. The combined searched terms were “layer-by--layer” and “drug delivery” AND “assembly” or “multilayer” or “membrane” or “film” of extracted documents as the abstract and keywords. A total of 3399 papers were found since 2022 and the resulting map is shown in Fig. 2. The map highlights the terms “tissue engineering”, “biocompatibility” and “controlled drug delivery” as the central concepts of all the relationships identified. VOSviewer organised the map elements into five clusters. The largest (red) cluster includes terms related to hydrogels, biocompatibility, nanofibers, biodegradable polymers, chemistry, morphology and controlled drug delivery. The green cluster relates to topics of drug formulation, in vivo studies and human studies. The blue cluster relates to non-human studies and unclassified drugs. The yellow cluster refers to tissue engineering and scaffolds, and the smallest cluster (purple) depicts the only item about drug-delivery systems.

In this paper, our objective is to review the state of the art of LbL multilayer films-composed drug delivery systems, including the properties of materials and potential controlled release profile.

2. Layer-by-layer multilayer film

LbL multilayer films involve the development of ultra-thin structures. LbL assembly requires the alternating adsorption of materials onto

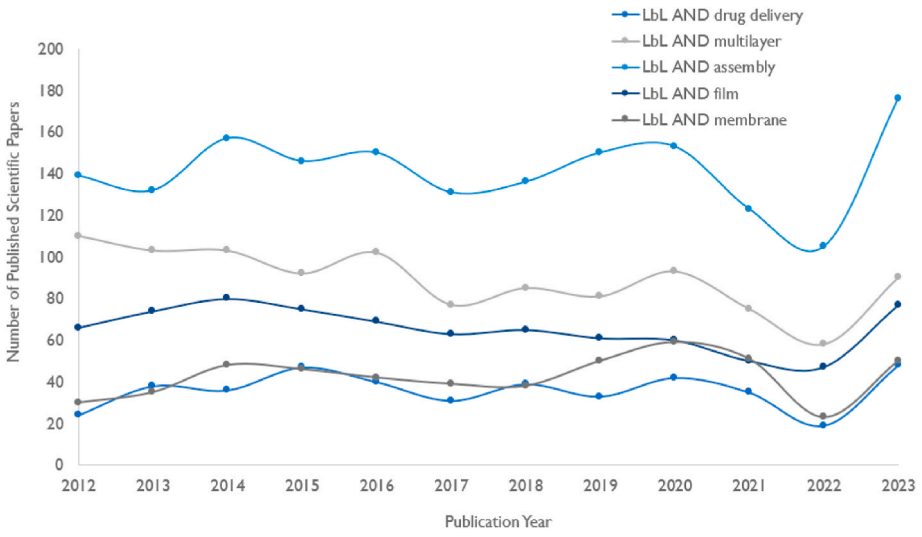

Fig. 1. Scientific papers indexed in the Pubmed Medline database, published yearly since 2012 until August 7th, 2023.

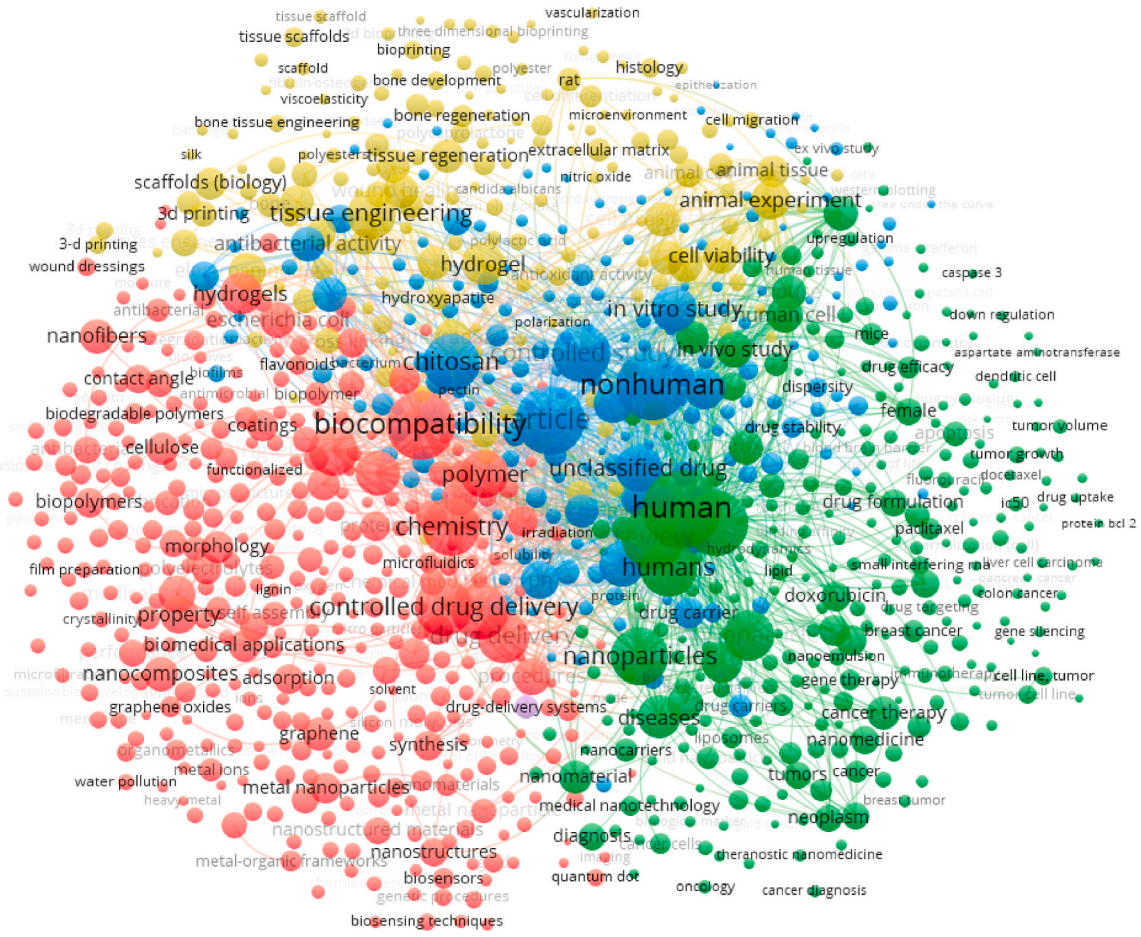

Fig. 2. Bibliometric map of published works from 2022, obtained using VOSviewer software version 1.6.16 [15], searching ["layer-by-layer" and "drug delivery"] AND ["assembly" or "multilayer" or "membrane" or "film"] as the combined keywords, recorded from the Scopus database (August 7th, 2023).

the substrate surface. The layers must consist of chemical species that allow the formation of intermolecular interactions, such as electrostatic (Fig. 3). In addition, other driving forces enable LbL formation, such as hydrogen bonding, covalent bonding, hydrophobic, van der Waals, host-guest and bio-specific interactions [16–18].

LbL assembly is a versatile, simple, economical technique that allows the control of porosity, mass and thickness, in addition to being environmentally friendly [19–21]. This method has numerous advantages, being compatible for large-scale production, and allowing the incorporation of several components. Thus, potentially obtaining an effective, functional, and intelligent system [20,21].

LBL technology has been widely applied in several areas, such as pharmaceutical and biomedical areas [22], in the production of drug delivery systems [23], production of biosensors [24], tissue engineering [25], as dressings [26], cell regeneration [27], and self-repairing materials [28].

LbL assembly methods are extremely important as they determine the characteristics of the process, namely: manual intervention, scalability, and time. In addition, these methods also influence the physicochemical properties of the films, such as thickness, interactivity, and homogeneity. These properties directly affect the performance of the product and make it application specific [29].

Other factors that can affect film properties, particularly thickness and porosity, are pH, solvent, polyelectrolyte concentration, ionic strength, and temperature [14,30,31]. It has been observed that there is a direct relationship between ionic strength and pH with the thickness of the films, and as the variables increase, so does the thickness of the film [32]. With regard to temperature, it is noted that this factor is critical to the solubility of the materials used to make the films [30,31].

Of the existing methods for producing films, the LbL method is considered to be the most suitable for the production of drug release films [33–35]. This is due to the controlled release of the drug, the lack of a requirement for high temperature and/or pressure, and the lack of restrictions on the size or shape of the substrate [16,36]. In addition, this method allows the manufacturer to modulate the properties of the films, allowing the development of films with two layers, one mucoadhesive and the other capable of controlling the release of the drug [34,37,38].

A number of materials can be used in the manufacturing of films. The most widely used are polyelectrolytes, including synthetic polymers such as poly-L-lysine, poly(sodium 4-styrenesulfonate), poly(acrylic acid), polyethyleneimine, poly(diallyl dimethyl ammonium chloride) and natural-based polymers such as chitosan, hyaluronic acid, and alginate [17,39]. Some uses of these polymers for the production of the films can be seen in Table 1.

In this way, LbL demonstrates its wide applicability and production forms, thanks to its ability to formulate with different types of materials, whether polymeric or not, thus confirming its specificity in the formulation of new nanocomposites; such as: carbon nanotubes and nanoparticles [40], liposomes [41], proteins [42], and deoxyribonucleic acid (DNA) [43].

3. Production methods

3.1. Dipping

Dipping stands out for its advantages such as cost effectiveness and

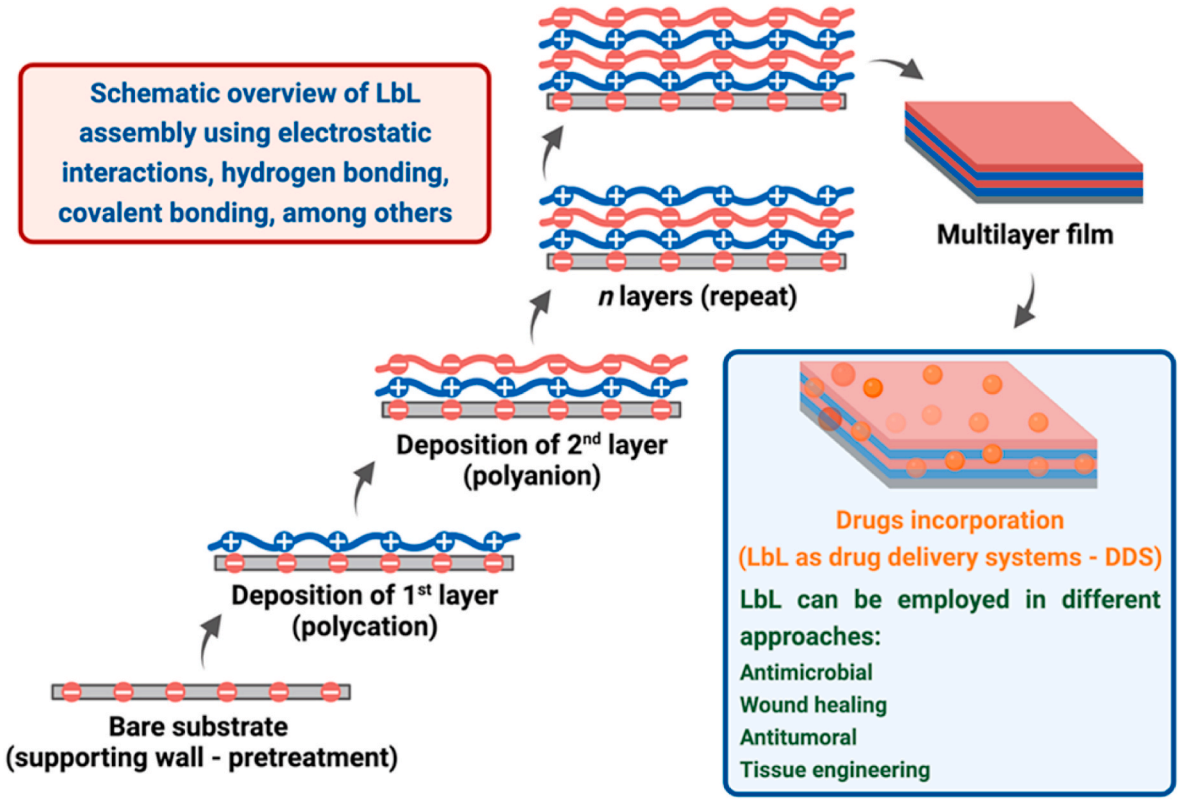

Fig. 3. LbL assembly process by alternating adsorption of materials through electrostatic interactions for drug incorporation.

scalability for mass production [62,63]. However, this method is time consuming compared to others and has as a manufacturing conditions the immersion time, the number of assembly cycles, and the speed of immersion and extraction [62–64].

The preparation of films by this method requires the use of a polyanion, a polycation and a substrate with ionic charges on the surface. In this method, the substrate is immersed in a solution of polyelectrolytes with opposite charges to those present on the substrate. After adsorption of the first layer, the substrate is washed to remove excess polyelectrolyte and to avoid cross-contamination. The substrate is then immersed in the second polyelectrolyte solution and washed. This deposition cycle is repeated until the desired number of layers is achieved [64,65].

Given the advantages of this method, coupled with the analysis of studies on LbL confirms that this methodology is commonly used. As in the work of Neto et al.. (2021), where films of chitosan, hyaluronic acid, and Rose Bengal were prepared to evaluate the antimicrobial capacity and drug delivery properties. The pH of the polyelectrolyte solutions and the degree of deacetylation of chitosan were adjusted. Effective films were obtained against *Escherichia coli* (pH 4.5) and showed controlled release of Rose Bengal (pH 7.2) [48].

Other works have demonstrated the application of controlled release by this method utilizing a film with montmorillonite, poly-L-lysine, and vancomycin for the treatment of bone infections. The developed films showed drug release, biocompatibility, and high bactericidal activity against *Staphylococcus aureus* demonstrating its effectiveness and efficiency for several functionalities [49].

3.2. Spraying

The spray LbL method consists of alternating or simultaneous spraying of oppositely charged polyelectrolyte aqueous solutions onto a substrate in a vertical position [66,67]. This method was introduced by Schelenoff, Dubas, and Farhat (2000), who fabricated films with poly (styrenesulfonate) and poly(diallyldimethylammonium chloride) by dipping or spraying for comparison [68].

Spray deposition of LbL is a simple, effective, versatile, economical, and rapid technique compared to traditional techniques such as dipping [66,69]. This method has the ability to overcome practical limitations of the dipping method, such as long times for complete adsorption of the film and the ability to coat small areas [70].

It has also been observed that the films produced by this technique have smoother characteristics and thicknesses, generally 50 %–70 % of those obtained by dipping [71]. In addition, a different molecular architecture is observed compared to the dipping method, which makes it necessary to carry out surface morphology characterisations and study the possible applications of the films [67,70].

When analyzing the studies using the spray LbL method, the work of Criado et al.. (2017) described the development of films using alginate, chitosan, and iron oxide nanoparticles. The authors observed that the cross-linked alginate and chitosan films showed a reduction in roughness and an increase in Young's modulus. However, when compared to films with alginate, chitosan and nanoparticles, an increase in roughness and elastic modulus was observed, as well as an increase in the adhesion values of the films [72].

In another study, coatings were prepared with polyethyleneimine and tannic acid complexed with iron ions, which showed antibacterial activity and contact killing of *E. coli*. Coatings incorporating silver nanoparticles were also prepared and showed enhanced antibacterial activity. In summary, the spray LbL method was found to be effective, rapid, and of high industrial viability [52].

3.3. Spin

The spin LbL method is a process in which a substrate is mounted on a rotating support at a constant speed and the material is added by dripping, which spreads out due to centrifugal force. Another method is to drop the solution onto the substrate and then rotate it. Whatever the

Table 1
Refers to the most frequent use of polyelectrolytes for film production by LbL.

Drug	Polymer	Methodology	Application	References
Bone morphogenetic protein 2	Poly(β-amino ester), Dextran Sulphate, Chitosans	Dipping	Bone regeneration	[42]
Methylene blue	polyethyleneimine (PEI)-grafted chitosan, polyacrylic acid	Dipping	Drug-delivery	[44]
Fibroblast growth factor 2	Chitosans; Alginate	Dipping	Future wound dressing	[45]
5-fluorouracil or moxifloxacin HCl	Chitosans; Sodium alginate	Casting	Treat colon cancer	[46]
Acyclovir	Iota-carrageenan, Hydroxypropyl methylcellulose e Polymethacrylates (Eudragit® RS PO and Eudragit® S100)	Solvent casting	Prevent genital herpes	[34]
Ibuprofen	Poly(ethyleneimine); Heparin; Chitosan	Dipping	Resolve implant infections during the implantation period and improve the corrosion resistance of magnesium alloys.	[47]
Rose Bengal	Polyethylenimine; Chitosan; Hyaluronic acid	Dipping	Films as antimicrobial surfaces and in drug delivery.	[48]
Vancomycin	Montmorillonite, poly-l-lysine	Dipping	Treatment of bone infections	[49]
Atenolol; Propranolol; Theophylline; Ibuprofen; Ketoprofen	Chitosan; Sodium alginate	Dipping; Spraying	*In vitro* bioinspired that mimics the key natural characteristics of the physiological mucus layer.	[50]
Fluorescein isothiocyanate; Ovalbumin; Doxorubicin hydrochloride	Chitosan, Hyaluronic acid; Alginic acid; Tannic acid	Electrostatic interaction	Anticancer treatment	[51]
Fulvestrant	Chitosan; Sodium alginate (ALG);	Dipping	Drug- delivery	[52]
Insulin	Nafion poly(allylamine hydrochloride) poly(ethyleneimine) poly(diallydimethylammonium chloride)	Dipping	Films with an insulin release control system	[53]
Emodin	Poly(ethylenimine); Poly(vinyl sufonate)	Dipping	Drug- delivery transdermal	[54]
Lysozyme	Cellulose acetate nanofibrous; N-[(2-hydroxy-3-trimethyl-ammonium) propyl] chitosan chloride; Sodium alginate	Dipping	Antibacterial	[55]
Dexamethasone	Poly(methacrylic acid); Poly(acrylamide); Poly(ethylene oxide)-*block*-poly(ε-caprolactone) micelles	Dipping	Drug delivery and induction of human mesenchymal stem cells differentiation into osteoblasts	[56]
Methotrexate	poly(allylamine hydrochloride)-dextran microgel; hyaluronic acid; poly(lactic-*co*-glycolic acid)	Spin	Drug-delivery	[57]
Doxorubicin	Poly(N-vinylpyrrolidone)-*b*-poly(N-isopropylacrylamide)	Dipping	Drug-delivery	[58]
Lysozyme	Poly(β-*l*-malic acid), and chitosan	Dipping	Drug-delivery	[59]
Recombinant human basic fibroblast growth factor				
Insulin	Poly(methyl methacrylate); glucose oxidase; Insulin; positively charged 21-arm poly[2-(dimethylamino)ethyl methacrylate]	Dropped and extended	Drug-delivery and diabetes treatment	[60]
Neurotrophin; Nerve growth factor; Lysozymes	Polyethyleneimine (PEI); Dextran sulphate; Heparin; Gelatin type B	Dipping	Neurotrophin-releasing	[61]

method, the multilayers are formed by alternating solution addition, drying, and washing [73].

This method has the advantage of fast production, more uniformity, stratified, organised, and thinner layers due to greater control of the thickness by changing the speed of the carrier and the concentration of the solution. In addition, when using this technique for small molecules, a greater influence on the morphology of the film has been demonstrated, as this presents greater mobility compared to larger molecules [74,75].

However, this method has disadvantages when high ionic strengths of polymer solutions are used in combination with low rotation speeds, causing the solution to be thicker at the point of application than at the edges. In addition, this technique does not work very well on irregular surfaces, as the distribution of the solution is impaired [29].

Considering the advantages presented, the spin LbL method is widely used in works such as that of Stana et al.. (2017), in which *N, N, N*-trimethylchitosan and alginic acid nanofilms were prepared to obtain a uniform encapsulation with better control of the release of pentoxifylline, an anti-inflammatory drug used in the treatment of chronic venous ulcer. The films produced have been shown to have two positive pharmacotherapeutic effects: a contribution to wound healing and a reduction in local inflammation [76].

In addition to this study, the work of Lai and co-workers (2018) used the spin method to fabricate films with chitosan, gelatin, and simvastatin incorporated onto a titanium substrate. The aim of the study was to realize the controlled release of simvastatin for the stimulation of bone formation. The results showed that the resulting film promoted osteoblastic differentiation and inhibited osteoclast formation to combat osteoporosis. In addition, the titanium substrate improved the biocompatibility of the film and provided cell adhesion [77].

3.4. Brushing

The LbLmethod by brushing is a technique in which a substrate is fixed to a support wall where it is brushed with a polyelectrolyte solution and then washed to form the layers [36]. This method has the advantage of speeding up film production, reducing raw material waste, and being a more dynamic process than static dipping [78].

The brushing LbL method is a method that is rarely found in published studies compared to the other aforementioned techniques. Among the existing studies is that of Iqbal et al.. (2022) [79], who used brushing to produce tannic acid and collagen nanofilms for the development of human muscle fibers. This method is relatively versatile and simple, being able to produce fibers at acidic pH with surfaces with aligned topography.

Furthermore, Li et al.. (2021) [78] used the brushing method to prepare membranes using poly(allylamine hydrochloride), sodium lignin sulphonates, and glutaraldehyde. In the study, the membrane showed satisfactory separation performance for trivalent and tetravalent anionic salts and exhibited stability in separation performance. It was

also found that the ultra-thin separation layer and hydrophilicity enabled an increase in permeate flux. Thus, the brushing LbL methodology is effective for the development of films and membranes, both economically and rapidly.

The above-mentioned methods, that can be used for the LbL assembly production, are schematically represented in Fig. 4.

4. Applications

4.1. Antimicrobial

Antimicrobial resistance occurs when microorganisms undergo changes when exposed to antimicrobial agents. According to the World Health Organization (WHO), antimicrobial resistance is one of the top ten global threats to public health and requires multisectoral action to achieve the Sustainable Development Goals (SDGs) [80]. In the face of this problem, one alternative approach to solve this is the use of new technologies and the development of new products. One of the existing alternatives is the production of films with antimicrobial activity using the LbL method, which allows the controlled delivery of a drug [74].

Several studies have applied the LbL method to the delivery of these antimicrobials, as evaluated by Taketa et al.. (2021). In this study, different films were prepared using hyaluronic acid (HA), alginate (ALG), and chitosan (CHI). The films were incorporated with silver and evaluated for antimicrobial activity using *Staphylococcus aureus* and *Candida albicans* strains. The results showed that the HA/CHI films were able to support higher silver loading compared to the ALG/CHI films. In addition, HA/CHI films were able to completely inhibit the growth of microorganisms, with a greater inhibition halo observed against the growth of *Staphylococcus aureus*. This higher activity is related to the presence of hyaluronic acid molecules, which favour the bioavailability of silver [81].

Additionally, Saracogullari et al.. (2021) studied CHI-based films prepared using tannic acid (TA), poly (acrylic acid) (PAA), ciprofloxacin (CP), and bovine serum albumin (BSA). It was found that the PAA/CHI/BSA film showed no anti-adhesion behaviour, whereas TA/CHI/BSA showed higher resistance to protein adsorption. The PAA/CHI/CP film had higher drug release capacity and antibacterial activity compared to TA/CHI/CP. It was found that pH was influenced the extent of layer association, drug release, and antibacterial activity of the developed films [82].

Neto et al. (2021) also observed the development of films by the dipping method, using Rose Bengal (RB), HA, and CHI, with different degrees of deacetylation and varying solution pH. Antibacterial activity against *Escherichia coli* was observed, with the antibacterial capacity being favoured in films assembled at pH 4.5 using chitosan with a lower degree of deacetylation. Films assembled at pH 7.2 showed no antibacterial activity but had a higher RB loading capacity [48].

Roupie et al. (2021), prepared films by the dipping method using chondroitin sulphate A (CSA), poly-L-lysine (PLL), and nisin Z, an antimicrobial peptide. They evaluated the antimicrobial activity of the films with and without nisin Z against *Staphylococcus aureus*. It was observed

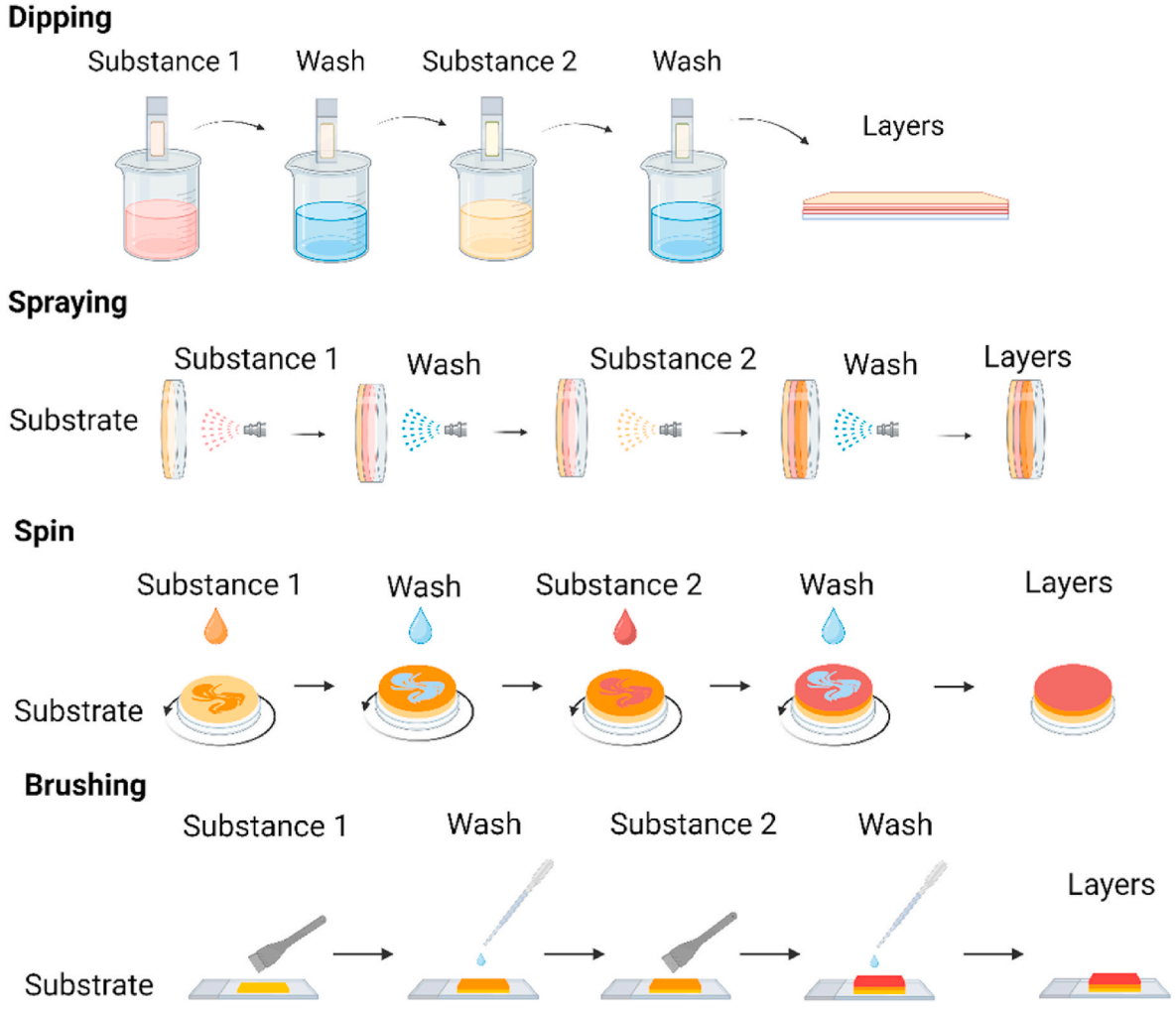

Fig. 4. Methods for the production of layer-by-layer films.

that only the films with nisin Z showed activity against the strain (60 % inhibition after 24 h and 92.5 % after 48 h of loading). It was verified that the films loaded for 48 h reached the optimal bactericidal concentration against the *Staphylococcus aureus* strain [83].

In a study by Souza et al. (2022), films were developed using gelatin and chondroitin sulphate, with and without the ionic liquids. The study evaluated the antimicrobial activity of the films against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. No antimicrobial effect was observed in the films without ionic liquids. However, when incorporated, a high antimicrobial capacity was observed, capable of preventing the adhesion and growth of microbial cells. This activity was attributed to the ammonium moieties present in the films interacting with the microbial cells, leading to bacterial adhesion and, after cell attachment, to bactericidal activity [84].

In addition to the cited studies, work was found that produced films and evaluated the antimicrobial activity of structures such as carbon nanotubes. Aslan et al. (2012) created a film using PLL, poly (L-glutamic acid) (PGA), and single-walled carbon nanotubes. This study found that the films were effective against *Escherichia coli* and *Staphylococcus epidermidis*, with an inactivation rate of 90 % after 24 h of incubation. Thus, the analysis of the developed studies confirmed that the LbL assembly was effective and allowed an alternative drug delivery approach to contribute efficiently to microbial inhibition [85].

4.2. Wound healing

Skin wounds are usually caused by several factors, such as accidents, burns, medical procedures, etc. It is important that healing occurs quickly to avoid possible infection. This happens through the inflammation, re-epithelialisation, and remodelling phases. One of the technologies used to improve and accelerate the healing process has utilized LbL films. This method allows targeted and multi-drug delivery, providing potential efficient and safe treatment [86].

Several papers have used the LbL method to form films that aid in wound healing. These include that of He et al. (2019), in which films were developed *in situ* with CHI and heparin (HE). The films were prepared using a shear flow guided LbL self-assembly methodology, which allowed the formation of the layers directly on the wound surface of diabetic mice. In this study, the CHI/HE film demonstrated effective suppression of inflammation and promoted wound healing. In addition, CHI/HE was found to induce accelerated formation of vascular structures and HE was found to support the revascularisation process and tissue regeneration. Finally, the films were found to be air permeable, customisable, and aid in chronic wound healing [87].

In a study by Maver et al. (2019), films were developed by the spin method using ALG, carboxymethyl cellulose (CMC) incorporated with lidocaine (LID), and diclofenac (DCF), which have analgesic and anti-inflammatory properties, with the aim of supporting skin regeneration. In the work, an initial rapid release, followed by a sustained release of the evaluated drugs was observed. The biocompatibility test showed that the materials used to produce the membrane were biocompatible and had no toxic effect on keratinocytes and fibroblasts. In addition, the film promoted the growth of cells, mainly skin fibroblasts, and was considered suitable for use in wound treatment [88].

In a study by Mandapalli et al. (2016), CHI, ALG, and polyethylene glycol (PEG) hydrogels were used to fabricate films with pirfenidone (PFD) incorporated into these drug films. PFD has anti-fibrotic and anti-inflammatory properties and is used to aid in the healing of excisional wounds. In the study, it was observed that wounds treated with the PFD-loaded films showed greater wound healing at day 9 compared to the PFD-loaded hydrogel, commercial povidone-iodine gel, and LbL film without drug. In addition, wounds treated with hydrogel and film were found to have less inflammation. Finally, it was found that PFD controlled collagen production by suppressing TGF-β, a protein that controls cell proliferation and differentiation, PFD was rapidly absorbed into the circulation after topical application using LbL-produced films [89].

Another study by Zhao and colleagues (2022) evaluated films produced by the spray method using polymethyl methacrylate (PMMA), phenylboronic acid-grafted γ-PGA (PBA-PGA), and polyvinyl alcohol (PVA), in addition to encapsulating an anti-inflammatory tripeptide (KPV) and epidermal growth factor (EGF), with the aim of aiding the healing of diabetic wounds. It was found that the three-layer film with the encapsulated substances (KPV and EGF) showed the best results for long-term wound healing, with inhibition of scar formation, angiogenesis (promoting the recovery of blood flow to the wound), increased collagen production, reduced inflammation, being non-toxicity, and showing biocompatibility. It also showed good transparency, flexibility, and glucose responsiveness [90].

4.3. Drug delivery

Drug delivery systems are an effective means of controlling the concentration of a drug in the blood and providing greater bioavailability of a therapeutic agent. For the drug to be effective, it is essential that the concentration be within the therapeutic range of action, and this often requires frequent administration to the patient. Therefore, one way to reduce the number of daily doses required by an individual is to incorporate drugs into systems that provide a controlled and sustained release. One of the existing methods to achieve this control is the production of films using the LbL method [16,36].

When analysing the literature, several works were found that focused on drug delivery using the LbL method. Among them is that of Lu et al. (2019), in which films were prepared by the dipping method using CHI and HA (with and without functionalisation). The films were loaded with an aspirin derivative synthesised from methyl anthranil trisulphide and acetyl salicyl chloride (ACS14). The study found that the films prepared with the functionalised materials showed better stability. It was also observed that the films swelled at acidic pH values and shrank at alkaline conditions. To evaluate the release of ACS14 from the films, the study was carried out at pH 6.5 and 7.5, with greater drug release at pH 6.5 for 3, 5 and 21 days [91].

In the study by Paker and Senel (2022), films were prepared by the dipping method using CHI grafted with polyethyleneimine, polyacrylic acid, and methylene blue (AM). The results showed that the pH and strength of the ionic solution significantly affected the loading of the drug into the film, with pH 9.0 being observed to allow rapid and high loading of AM. When the release profile of AM was evaluated, it was found that higher release occurred at low pH values. It was also found that the strength of the ionic solution influenced the loosening of the film structure, resulting in faster drug release [44].

In addition to these studies, work was found that focused on drug delivery to both the vaginal and oral mucosa. For example, in the study by Pacheco-Quito et al. (2022), vaginal films were developed using the LbL casting method with iota-carrageenan (iota-CG), hydroxypropyl methylcellulose (HPMC), acyclovir (AC), and the polymethacrylates Eudragit RS PO (ERS) and Eudragit S100 (ES), for protection against the genital herpes virus. Among the films produced, the iota-CG/HPMC with ERS/ES showed the best results, against the virus. In addition, controlled release of the drug and a mucoadhesive retention profile up to 192 h were observed [34].

Pilicheva et al. (2020) [92] prepared films, with and without cross-linking, using CHI and casein (CAS) incorporated with benzydamine hydrochloride (BZ). The results showed that the films with double crosslinking in the chitosan layer, using glutaraldehyde and sodium tripolyphosphate, exhibited higher drug loading and mucoadhesiveness. When the release profile of BZ was evaluated, it was found that the films showed a prolonged release of the drug, albeit slowly.

In addition to the aforementioned studies, it is possible to use the LbL method for drug delivery for cancer treatment, Xu et al. (2019) [52] developed films by the dipping method using CHI and nanocapsules loaded with fulvestrant. The surface of the nanocapsules were coated by

the LbL method using the biopolymers ALG and CHI. It was found that the films exhibited stability, well-defined structure, and high loading capacity. The drug release profile showed that at pH 7.4 the drug was efficiently bound, with a release of 38 % in 120 days, while at pH 5.0 a faster release of fulvestrant was observed.

In the study by Janardhanam et al. (2022) [46], films were prepared by the dipping method using ALG and CHI loaded with 5-fluorouracil (5FU) or moxifloxacin HCl (MF). The films produced were incorporated into enteric-coated capsules for targeted delivery to the colon. The films with MF were thicker than those with 5FU. In the release study, the MF film showed no initial burst release of the drug, whereas the 5FU film displayed an initial burst release followed by controlled release. In addition, the MF film was found to have linear pharmacokinetic profile, unlike 5FU, which was found to be non-linear.

4.4. Tissue engineering

Tissue engineering has been growing over the years and several papers have been published in this field. Tissue engineering aims to solve problems related to tissue loss or organ failure in patients. Thus, the concept of tissue engineering involves the implantation of a bioactive structure into individuals with the aim of replacing damaged tissues and restoring their function. One way to achieve this goal is through the use of LbL, which allows for the controlled delivery of therapeutic agents, such as growth factors, that promote tissue regeneration [93].

Among the works developed for tissue engineering is a study by He et al. (2012) [94], in which devices were fabricated by the dipping method using the biodegradable polyurethane (PU) substrate and the materials type I collagen (Col) and chondroitin sulphate (CS). In terms of surface morphology, the PU substrate was found to have a smoother surface after LbL assembly. In addition, it was observed that the hydrophilicity was modified and increased after the assembly. Therefore, it is observed that PU/Col/CS is a chondrogenic mimicking environment, which presents potential for use in cartilage tissue engineering.

Another study by Zhang et al. (2019) [95], fabricated multi-structured vascular coatings containing HE and CHI on PU/decellularized scaffold substrate (DCS). The coatings were prepared using the LbL dipping method. Cell attachment and proliferation analysis was performed at 4, 8, 24, and 48 h on PU/DCS and HE–CH–5/PU/DCS samples. It was found that endothelial progenitor cells grew rapidly in HE–CH–5/PU/DCS, whereas they proliferated slowly in PU/DCS. It was also found that He-Ch-5/PU/DCS coatings had a long clotting time *in vitro* and maintained the long-term permeability of surgical arteries. In view of these results, the coating produced was deemed as a potential treatment for diseased or damaged blood vessels.

In addition to these studies, Amaral et al. (2021) [96] produced films using the LbL method employing regenerated cellulose nanofibers (NFCR) and poly(globalide) (PGl). The films produced were intended to be used for keratinocyte cell growth for application in skin tissue engineering. The cellular metabolic activity of cultured keratinocytes was evaluated on pure PGl and NFCR/PGI films after 1, 3, and 7 days. The results showed that the films with PGl alone displayed an extremely low metabolic activity, but a much higher activity was observed for NFCR/PGI, demonstrating the role of the NFCR layer in the metabolic activity of the bilayer film. Therefore, the potential use of these materials for skin tissue engineering was supported.

In a study by Dash et al. (2022) [97], films were fabricated using the spin LbL method on a 316L stainless steel substrate. The films were prepared using Col, poly (y-glutamic acid) (y-PGA), CHI, vancomycin (VA), and strontium containing bioactive glass (SrBG). In the study, the cell viability rate in MG63 cells was evaluated and it was observed that the Col/y-PGA/VA and Col/y-PGA/SrBG films, with lower concentrations of VA and SrBG, showed a cell viability rate close to and higher than 85 %, respectively. Thus, the materials used were found to have enhanced biological activity for tissue engineering applications. In addition, it was found that Col/y-PGA/VA/SrBG films exhibited greater cell proliferation, hydrophilicity, biocompatibility, improved mechanical properties, and great potential for orthopaedic application.

4.5. Future perspectives

The current scenario of LbL studies shows a growing trend in various areas, especially in biomedical applications. Future prospects include approaches aimed at optimizing LbL techniques, since parameters such as pH, solvent and ionic strength can significantly affect the film properties. In addition, future studies are expected to focus on brushing and spraying techniques to produce films for clinical applications, since a lower prevalence of these techniques was observed compared to other LbL construction approaches. It is also expected in-depth studies related the biocompatibility of developed materials for their use in regenerative medicine. With regard to antimicrobial applications, it is hoped that further in vivo and clinical studies are carried out to prove the effectiveness of such materials. In addition to using the LbL methodology to produce films, other applications have been exploited with potential for growth.

The LbL technique can be used as a coating for other types of materials, such as nanoparticles and nanofibers, forming multiple layers on the surface, enhancing various actions, especially drug delivery [17]. In the study by Ma et al. (2021) [98], silk fibroin nanofibers were produced by the electrospinning method, with multiple layers prepared with chitosan and polydopamine. As a result, it was observed that with the increase in the number of layers, the hydrophilicity and tensile modulus improved. Another factor observed was the sample with 5 bi-layers of chitosan and polydopamine which showed antibacterial activity of 98 %, so the material produced is a potential product for the biomedical area. Another study that addressed this issue is the one by TU et al. (2019) [99], in which nanofibrous mats were produced using silk fibroin coated by the LbL technique with carboxymethyl chitosan. The results revealed that the LbL-coated nanofibrous mats showed greater thermostability and improved mechanical properties, as well as increased antimicrobial activity. This approach is considered a promising strategy for use in wound dressings. Song et al. (2013) [100] described the modification of polyacrylonitrile nanofibrous mats using silver ions to be positively charged so that they could be linked to ovalbumin to develop layer-by-layer composites. The produced films were morphologically characterized confirming the coating while keeping the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* without the risk of cytotoxicity.

Composite films based on hydroxypropyl chitosan and soy protein isolate were produced by solution casting, and evaporation process [101]. The hydroxypropyl chitosan showed a lower swelling ratio with an increase of the soy protein isolate content, and could support the attachment of proliferation of L929 cells, with hemocompatibility and cytocompatibility, opening prospects of their use as skin wound dressings. The increased content of protein isolate could promote a faster healing and skin regeneration.

5. Conclusions

The study highlighted the efficacy and significant impact of multilayer films produced using the LbL method in the healthcare sector. This versatile approach allows the use of different polymers and the incorporation of therapeutic molecules, enabling targeted and controlled drug delivery with reduced patient side effects. LbL assembly has been demonstrated to be an efficient technique for the fabrication of tailor-made biomedical films with a wide range of applications in drug delivery systems, wound healing, tissue engineering, and beyond.

The reviewed studies demonstrated the potential of LbL-produced films to achieve controlled drug release, antimicrobial properties, and tissue regeneration, offering promising opportunities for personalized medicine and improved patient outcomes. The scalability and environmental friendliness of the method makes it suitable for large-scale

production, and attractive for commercial applications. Moreover, its adaptability to incorporate different materials further enhances its potential to create innovative nanocomposites, opening new avenues in biomedical research.

The current scenario of LbL studies shows a growing trend in various applications, including antimicrobial activity, tissue engineering, dressings, and drug delivery. However, challenges remain in optimizing LbL assembly techniques and further research is required to achieve precise and reproducible film properties, as parameters such as pH, solvent, and ionic strength can significantly affect film properties. Further research and optimization of LbL techniques is warranted to achieve precise and reproducible film properties.

In summary, layer-by-layer assembly is a powerful tool that is driving advances in biomedical engineering. Its ability to customize film functionalities, coupled with its ease of fabrication, positions it as a transformative technology with the potential to address complex biomedical challenges and pave the way for novel therapies and regenerative medicine. Continued interdisciplinary efforts will undoubtedly lead to exciting breakthroughs, broadening the horizon of biomedical applications, and providing efficient solutions for various diseases.

Authors contributions

Victoria L. S. dos Santos, Rayssa C. Araújo, Erika S. Lisboa, André M. Lopes, Ricardo L. de Albuquerque-Júnior, Juliana C. Cardoso, Eliana B. Souto and Patricia Severino contributed for sample prospection, data collection, data analysis and interpretation of results; Cristina Blanco-Llamero, Eliana B. Souto, Ronny Priefer and Patrícia Severino contributed for the revision of the first drafted English version. Ricardo L. de Albuquerque-Júnior, Juliana C. Cardoso, Cristina Blanco-Llamero, Eliana B. Souto, Tanvi A. Deshpande, Henning O. W. Anderson, Ronny Priefer and Patrícia Severino contributed for the validation of results, discussion, draft and final version of manuscript preparation, and management of the project. All authors approved the final version of the manuscript.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

All authors agreed with the final version of this manuscript and with the current submission.

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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