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Design of 3D Multi-Layered Electrospun Membranes Embedding Iron-Based Layered Double Hydroxide for Drug Storage and Control of Sustained Release

Mariana P. Figueiredo^{a,b,c}, Géraldine Layrac^b, Anne Hébraud^b, Lionel Limousy^{c,d},
Jocelyne Brendle^{c,d}, Guy Schlatter^{b,*}, Vera R. L. Constantino^{a,*}

^a IQ-USP, Departamento de Química Fundamental, Instituto de Química, Universidade de São Paulo - USP, Av. Prof. Lineu Prestes 748, 05508-000, São Paulo, São Paulo, Brazil.

^b ICPEES, Institut de Chimie et Procédé pour l'Energie, l'Environnement et la Santé, CNRS UMR 7515, Université de Strasbourg, 25 rue Becquerel, 67087 Strasbourg Cedex 2, France.

^c Université de Haute-Alsace, IS2M, CNRS UMR 7361, 3b. Rue A. Werner, F68100 Mulhouse, France.

^d Université de Strasbourg, France.

*corresponding authors: Guy Schlatter, guy.schlatter@unistra.fr; Vera R. L. Constantino, vrlconst@iq.usp.br.

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ABSTRACT

Nowadays polymer dressings are expected to possess multiply functions. Besides acting as physical barriers, dressings may provide for the injured tissue species able to turn wound healing process faster and painless. In this way, dressings can be designed aiming to enable the release of drugs. The possibility to modulate drug release kinetics is a desired characteristic to be achieved in order to turn drug delivery systems adequate to specific treatments. However, hydrophilic drugs and hydrophobic polymers incompatibility hinders such modulation and a long-term release cannot be achieved efficiently. Here we present the design of poly(lactic acid) (PLA) membranes containing iron-based Layered Double Hydroxide (LDH) particles able to storage a hydrophilic anionic drug (derived from the non-steroidal anti-inflammatory naproxen). LDH particles are excellent candidates to compose multifunctional composites. They may present diverse biocompatible compositions, possess an elevated encapsulation capacity and tends to promote drugs sustained release by its own, besides assisting tissues regeneration process. Nanofibrous membranes were prepared by the combination of electrospun PLA and electrosprayed LDH as alternated layers (approach A) and also by both technics performed at the same time (approach B). In approach A, by varying the thickness of the PLA fibrous layers, it was possible to easily modulate the drug release rate. Half of drug content was released after 1, 4 and 17 days for the membranes containing the thinnest, the intermediate and the thicker PLA layers, respectively, and after 56 hours for the membrane prepared by the approach B. Naproxen release was kept for 18 days for the thinnest membrane, 59 days for the membrane prepared by the approach B and 66 days for the thicker membranes. We believe that this work can inspire the development of new functional membranes with tunable drug release profile thanks to the versatile electrospinning and electrospraying techniques.

Keywords: electrospinning; electrospraying; layered double hydroxides; drug release modulation; functional membranes

1. INTRODUCTION

The design of composite membranes allowing the precise control of the release kinetics of drugs emerged this last decade for advanced applications in wound dressing, tissue engineering and nanomedicine.[1,2] Especially, in the case of hydrophilic drugs, the controlled delivery over time is an important issue which has to be taken into account in order to deliver the optimal amount of therapeutics to a targeted diseased area while avoiding burst release and undesirable side effects due to overdose. Furthermore, when hydrophilic drugs are embedded in a hydrophobic insoluble polymer membrane, long-term release is more difficult to control due to drug-polymer incompatibility.[3] In this case, partitioning of the drug and the polymer matrix might occur, leading to either burst release or encapsulation of the drug within the matrix. Nanofibrous membranes embedding drugs elaborated from the process of electrospinning have attracted researchers for such applications.[4,5] Indeed, electrospun nanofibrous membranes mimic the extracellular matrix components, enabling cells growth and differentiation. Furthermore, reports have shown that cell-fibrous interactions lead to a normal cells phenotypes and gene expression; and fibers possess high surface area/volume ratio and high porosity, which creates nutrient gradients.[6] Among the drug vectors which could be embedded in polymeric membranes, Layered Double Hydroxides (LDHs) have been identified as excellent candidates.[7,8] LDHs are structured bidimensional materials with general formula

$[M^{2+}_{(1-x)}M^{3+}_x(OH)_2](A^{n-})_{x/n} \cdot zH_2O$, formed by the stacking of positively charged layers composed by di (M^{2+}) and trivalent (M^{3+}) metal cations.[9] The M^{2+} and M^{3+} ions are distributed in the centre of octahedrons which are formed by six coordinated hydroxide anions, as shown in **Figure 1**. Structure neutrality is guaranteed by the presence of hydrated anions (A^{n-}) between the layers. These materials are commonly abbreviated by $M^{2+}_R M^{3+} - A^{n-}$, where R is the molar ratio between M^{2+} and M^{3+} . LDH matrix by its own is commercialized as the antacid TALCID™, composed of magnesium and aluminium cations and intercalated with carbonate anions.[10] Thanks to the biocompatibility of LDHs compositions (verified *in vitro* and *in vivo* [11–17]), their effective interactions with biological membranes, high encapsulation efficiency, and capacity to prolong drug release, these materials are promising to be applied as vehicles for drug storage and delivery.[18–20] In this way, many species such as aminoacids,[21] anti-inflammatories [22] and cancer drugs [23] have been intercalated between the LDHs layers. Recently our group showed that LDHs may interfere positively in tissue regeneration, being able to conduct the formation of different types of collagen according with the layer composition.[16,17] Furthermore, the combination of the LDH matrix with polymers has been used to prepare mimetic bone substituents, conferring mechanical strength and remodeling facilities by stress shielding.[24,25] The release of species such as healing cells activator from LDHs layers has improved even more bone regeneration process.[26]

Therefore, electrospun membranes embedding LDHs may form interesting implantable functional composites with drug release properties, acting as supports for damaged tissues due to surgery interferences and improving tissues regeneration. To the best of our knowledge, the only approach proposed in the literature consists in dispersing LDHs in the polymer solution which is further electrospun leading

consequently to a membrane in which LDH nanoparticles are inside the matrix of fibers.[27] Besides, in this case, LDHs compositions presented only Al^{3+} as trivalent cations, an exogenous metal. Valarezo et al.[27] reported the release of amoxicillin pre-encapsulated into the LDH layers of composition Zn_2Al , itself encapsulated into poly(ϵ -polycaprolactone) (PCL) fibers. While homogeneous fibers were obtained for LDH content of 3 % wt, the formation of beads was observed for a higher LDH content of 5 and 7 % wt, showing the limitation of the possible amount to be loaded in the polymer composites. The slowest drug release rate was observed for the membrane presenting 3 % wt of LDH amoxicillin, extending for more than 20 days. Other works reported the intercalation of non-steroidal anti-inflammatory drugs into LDHs of composition Mg_2Al or Mg_3Al . Yang et al.[28] applied flurbiprofen acetyl as model drug, poly(lactic-co-glycolic acid) (PLGA) as polymeric matrix and 5 % of LDH-drug concentration relative to the polymer. Heterogeneous fibers diameter due to the presence of beads were observed for the LDH-PLGA nanofibers. After 5 days, less than 40 % of the drug was released. The work of Tammaro et al.[29] showed that PCL fibers containing Mg_3Al LDHs intercalated with diclofenac anions also exhibited a slow release of the drug (50 % after 240 days). Gao et al.[30] reported the intercalation of ibuprofen or ketoprofen into the LDH of composition Mg_2Al and composites formed with PCL polymer. It was verified that fibers diameter tends to increase when the LDH amount in the composite increases. After 5 days, 44 and 48 % of ibuprofen were released from composite membranes containing 5 and 10 % wt of LDH, respectively. However, for the same period of time and same LDH content, only 25 and 20 % of ketoprofen were released. Miao et al.[31] also reported the pre-encapsulation of anions derived from ibuprofen into the Mg_2Al LDH for preparation of composites based on poly(lactic acid) (PLA) and PCL electrospun fibers containing 5 % wt of LDH. Despite the hydrophobicity of both

polymers, ibuprofen release from PCL was faster than from PLA fibers: approximately 40 % and less than 10 % after 2 h, respectively. This behavior was attributed to the poor chain flexibility of PLA. Although a slow release is desired most of the time in medicine's formulation, it is interesting to find mechanisms to modulate the drug release rate from the carrier, in order to adjust the formulation according with the treatment of different diseases, with different bioactive species and administration routes. Examples of a desired faster drug release include the administration of extra dose insulin during meals[32,33] and anti-inflammatories to immediate relief of low or moderate pain.[34] On the other hand, a sustained drug release is intended when long-term drug circulation and treatments are necessary, such as in the administration of basal doses insulin unrelated to meals [33,35] and postoperative antibiotics,[36] anaesthetics[37] and anti-inflammatories,[34] to avoid infections and severe chronic pains. In this way, the same work presented a strategy in order to increase and modulate the hydrophilicity of the PLA fibers based on the addition of poly(oxyethylene-b-oxypropylene-b-oxyethylene) (Pluronic) into the processed formulation, leading to a faster drug release.

Figure 1. Schematic representation of the LDH structure.

The studies above mentioned showed that the LDH concentration in the polymer solution is limiting, since its increase tends to the formation of beads and, consequently, non-homogeneous fibers. Furthermore, hydrophobicity of most biocompatible polymers such as PLA, PLGA and PCL, which can be easily processed by electrospinning, hinders drug release in aqueous media.[38] Therefore, the development of strategies in order to overcome the polymers hydrophobicity and modulate drug release rates is desirable to expand the possible administration routes of electrospun membranes.

The present work is inspired by our previous results for which composite scaffolds elaborated from the combination of electrospinning and electrospraying processes show interesting properties due to the presence of both nanofibers and microparticles components. For example, when electrosprayed particles are simultaneously or alternately deposited with electrospun fibers, scaffolds with various patterns can be obtained.[39,40] Hybrid scaffolds with PCL fibers and hydroxyapatite particles can also be envisaged for bone regeneration application.[41] Furthermore, it was also demonstrated that scaffolds entrapping hydrophilic particles in a hydrophobic

fibrous mesh allow the modulation of the hydrophilicity/hydrophobicity balance. Such property was exploited to fabricate fibrous membranes allowing the controlled release of a drug.[42]

Here, two kinds of membranes having different structures and made of PLA electrospun nanofibers and drug-loaded-LDH electrosprayed microparticles were elaborated in order to modulate the kinetic of drug release while avoiding the use of any hydrophilic adjuvant. Multilayer membranes with LDH particles in sandwich between PLA fibrous layers of various thicknesses were studied and compared with membranes in which the LDH particles were entrapped in the fibrous PLA mesh. PLA was chosen as polymer due to its biocompatibility, good mechanical properties and performance for fibers formation. Aluminum accumulation has been verified in different tissues and has been associated to neurodegenerative diseases.[43–45] Therefore, in order to turn the drug delivery devices more promising for biological applications, half of the Al^{3+} content was substituted by Fe^{3+} cations (endogenous) in the LDHs composition. Naproxenate was applied as a model hydrophilic drug and was pre-encapsulated into LDHs layers. In order to compare the effect of the organic anions regarding the interaction between LDHs particles and fibers, similar membranes were prepared with LDH precursors intercalated with Cl^- anions.

2. Experimental section

Poly(D,L-lactide) (PLA) (MW 180 kDa, L = 95%, NatureWorks 7000D). N,N-dimethylformamide ($\geq 99.8\%$), dichloromethane ($\geq 99.8\%$), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) (99 %), aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$) (99 %), iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) (98 %), sodium chloride (NaCl) (99.5

%), sodium hydroxide (NaOH) and sodium naproxenate (NaNAP) ($\text{NaC}_{14}\text{H}_{13}\text{O}_3$) with purity $\geq 98\%$, were purchased from Sigma-Aldrich. Ethanol absolute was purchased from Carlo Erba. All products were used as received.

2.1. LDH preparation

LDHs, intercalated with NAP and Cl^- anions, were prepared following the procedure previously reported,[17] with some changes and adapted as follows.

2.1.1. Synthesis of the $\text{Mg}_4\text{FeAl-Cl}$ sample

The LDH intercalated with Cl^- anions, abbreviated by $\text{Mg}_4\text{FeAl-Cl}$ LDH, was prepared under N_2 atmosphere by the co-precipitation method at constant pH equal to 10.5, by adding under mechanical stirring at 900 rpm and 50°C concomitantly a mixture of a $0.05\text{ mol}\cdot\text{L}^{-1}$ solution of $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$, $\text{FeCl}_3\cdot 6\text{H}_2\text{O}$ and $\text{AlCl}_3\cdot 6\text{H}_2\text{O}$ (to obtain a $\text{Fe}^{3+}/\text{Al}^{3+}$ molar ratio equal to 1) and a 0.2 mol L^{-1} NaOH solution into 200 mL deionized water. After addition, the mixture was aged under stirring for 1 h at the same synthesis temperature. The obtained solid was recovered by filtration, washed with 2 L of deionized water and lyophilized for 24 h at 200 mbar and -50°C (in a Thermo Savant ModulyoD equipment). The $\text{Mg}_4\text{FeAl-Cl}$ LDHs used to test the stability of the particles suspension were prepared by a similar procedure, with exception of the mechanical stirring rate, kept at 400 rpm.

2.1.2. Intercalation of NAP into the Mg_4FeAl LDH by ion exchange method

Starting from the remaining slurry of the $\text{Mg}_4\text{FeAl-Cl}$ LDH synthesis, not submitted to washing procedure, the excess of Cl^- ions was removed by centrifugation with 4 cycles of 3 min each at 10000 rpm. For the preparation of approximately 0.5 g of

the LDH intercalated with NAP, abbreviated by $\text{Mg}_4\text{FeAl-NAP}$, the precursor was resuspended in 20 mL of an aqueous solution of NaNAP in a molar ratio $\text{NAP} / (\text{Fe}^{3+} + \text{Al}^{3+})$ equal to 3. The suspension was kept under N_2 atmosphere and vigorously stirring with stir bar at 200 rpm for 24 h at 80 °C. The solid was washed with deionized water by filtration under reduced pressure and then dried by freeze drying in the same conditions applied for the $\text{Mg}_4\text{FeAl-Cl}$ LDH.

2.2. Preparation of suspensions and solution

2.2.1. Preparation of LDH suspension to be electrosprayed

The LDH suspension used to prepare the LDH-PLA membranes has been optimized for efficient electrospraying of the smallest aggregates (see Supporting Information and **figures S1-S3**). LDHs-ethanol suspensions (1% wt) were previously submitted to ultrasonication (Branson Digital Sonifier equipment) at 40 % amplitude for 30 min (2 seconds on / 2 seconds off) just before the electrospraying procedure.

2.2.2. Preparation of PLA solution to be electrospun

A 10 % wt PLA solution was prepared by dissolving the beads in a mixture of dichloromethane and dimethylformamide in weight ratio equal to 3:2 overnight under slow stirring with a magnetic stirring bar.

2.3. Preparation of the composite membranes

Two approaches are proposed for the preparation of composite membranes. The approach A consists in the fabrication of multi-layered membranes by alternating electrospinning PLA fibers and electrospraying LDHs particles. In approach B, LDHs

electrosprayed particles are regularly dispersed in the PLA electrospun fibrous membrane.

Figure 2. Preparation of the composite membranes in the case of a) approach A with variable and adjustable thickness of the PLA fibrous layers and b) approach B with simultaneous PLA electrospinning and LDH electrospaying.

2.3.1. Electrospun PLA and electrospayed LDH: building up a delivery membrane (Approach A)

Electrospinning and electrospaying experiments were conducted with a home-made setup previously described [42] and allowing the deposition on a drum collector rotating at 125 rpm. All solutions or suspensions were electrospun or electrospayed at a temperature $T = 22 \pm 1^\circ\text{C}$ and a relative humidity $RH = 40 \pm 5\%$. Working distance between needle and collector was equal to 16 cm for all samples. For all electrospinning and electrospaying steps, an alternating translation movement (see **Figure 2**, amplitude of the movement = 7 cm, average velocity of the movement = 2 cm/s) of the needle was imposed in order to enlarge the deposition leading to the fabrication of membranes having a width of 11 cm.

In approach A, the membranes contain 7 layers made from the deposition of 4 PLA electrospun fibers taking in sandwich 3 LDHs electrosprayed particles (**Figure 2a**). Three different PLA layer thicknesses were prepared by changing the production time of the electrospun layers. Applied voltages, flow rates and production times are given in **Table 1**. Membranes containing electrosprayed $\text{Mg}_4\text{FeAl-NAP}$ particles are called $\text{NAP-A}_{\text{thin}}$, $\text{NAP-A}_{\text{med}}$, $\text{NAP-A}_{\text{thick}}$ and the membranes with electrosprayed $\text{Mg}_4\text{FeAl-Cl}$ are called $\text{Cl-A}_{\text{thin}}$, Cl-A_{med} , $\text{Cl-A}_{\text{thick}}$. In order to compare the samples, the total electrospraying time was the same for each membrane and equal to 78 minutes.

Table 1. Applied parameters for preparation of the membranes

Approach	Method	Voltage on the needle (kV)	Voltage on the collector (kV)	Flow rates (mL.h ⁻¹)	Production Time (min)
$A_{\text{thin}}, A_{\text{med}},$ A_{thick}	Electrospraying	25	0	6	3 layers
	Layers 2, 4, 6				26 min/layer
A_{thin}	Electrospinning	16	-5	2.8	4 layers
	Layers 1, 3, 5, 7				15 min/layer
A_{med}	Electrospinning	16	-5	2.8	4 layers
	Layers 1, 3, 5, 7				22 min/layer
A_{thick}	Electrospinning	16	-5	2.8	4 layers
	Layers 1, 3, 5, 7				30 min/layer
B	Electrospinning	16		2.8	
	Electrospraying	28	0	6	78 min

2.3.2. Simultaneous PLA electrospinning and LDH electrospaying (Approach B)

In approach B (**Figure 2b**, **Table 1**), the membranes were prepared by simultaneous deposition of electrospun PLA and electrosprayed LDH aiming to contain the same total amount of LDH as applied in approaches A (total electrospraying time equal to 78 min). For both needles an alternating translation movement (amplitude of the movement = 7 cm, average velocity of the movement = 2 cm/s) was imposed in order to enlarge the deposition and allowing thus the fabrication of membranes having a width of 11 cm. Samples were abbreviated as Cl-B and NAP-B, indicating the application of B approach and LDH particles containing Cl⁻ and NAP anions, respectively.

2.3.3. Pristine PLA membrane

As a control, pristine PLA membrane was electrospun for 2 hours. Therefore, it contained the same polymer amount as in the membranes prepared according with approach A_{thick}.

2.4. NAP release tests

Naproxen release experiments were conducted in triplicate from 3 cm x 10 cm membranes. The membranes were accommodated in closed glass flasks containing 70 mL of saline aqueous solution (0.9 % wt NaCl); the pH value was equal to 5.55 ± 0.01 . The release media was magnetically stirred at 130 rpm and the assays were performed in a climatized room at 22 °C. At specified time, 3 mL of saline solution were collected and replaced with an equivalent amount of dissolution medium. NAP concentration was determined by UV–visible absorption spectrophotometry at a maximum absorption (λ_{max}) equal to 230 nm. After the first released NAP aliquot measured, accumulative

NAP weight (m^{NAP}_i) released was corrected through the equation (1), where C_i is the NAP concentration (mL L^{-1}) in such a time i , considering the removed aliquots (3 mL) for measurement in anterior times k and then replaced by the same volume of saline solution in order to maintain the flasks volume equal to 70 mL.

$$m^{\text{NAP}}_i = 0.07C_i + 0.003 \sum_{k=1}^{i-1} C_k \quad (1)$$

2.5. Materials characterization

X-ray diffraction (XRD) patterns of powdered LDHs were obtained on a Bruker D8 Advance A25 equipment with variable slits mode, using Cu anode ($\lambda = 1.518 \text{ \AA}$), scan range $1.5\text{--}70^\circ(2\theta)$ and scan step of $0.02^\circ(2\theta) / \text{s}$.

Fourier transform infrared (FT-IR) spectra of powdered LDH samples and of the composites were recorded in the $4000\text{--}400 \text{ cm}^{-1}$ range on a Bruker spectrophotometer, model α , by attenuation total reflection (ATR) mode with acquisition step of 4 cm^{-1} and 512 scans.

Fourier transform Raman (FT-Raman) spectra of the LDH samples were recorded in an FTRaman Bruker FRS-100/S spectrometer using 1064 nm exciting radiation (Nd:YAG laser Coherent Compass $1064\text{--}500 \text{ N}$), a Ge detector, laser power of 100 mW , acquisition step of 4 cm^{-1} , and 2048 scans.

Chemical elemental analyses of carbon, hydrogen, and nitrogen were recorded on Perkin Elmer-CHN 2400.

Inductively coupled plasma atomic emission spectroscopy (ICP-AES) analyses of metals were performed in duplicate on Spectro Analytical Instrument.

Images by scanning electron microscopy (SEM) of the membranes were obtained in a VEGA3SEM equipment using samples deposited on a copper tape and coated with

gold using a Q150RS Quorum equipment. The LDHs micrographs were obtained in a XL30 FEG Philips equipment depositing the powders in a copper tape and covering samples with carbon.

Mechanical tensile measurements were performed with a TA Instruments Discovery HR-3 hybrid rheometer at room temperature and applying a velocity of $50 \mu\text{m s}^{-1}$. The normal force sensor of the rheometer was used to record the tensile force. Samples were analysed in quintuplicate and were prepared cutting $3 \times 1.5 \text{ cm}$ rectangles. The mechanical tests were carried out at room temperature ($T = 21 \pm 2^\circ\text{C}$) and humidity ($\text{RH} = 50 \pm 20\%$) without environmental control.

Thermogravimetric analysis (TGA) data were recorded on TA instrument, Q5000 model, using platinum crucible and a heating rate of $10^\circ\text{C min}^{-1}$, from room temperature to 1000°C , under synthetic air flow of 25 mL min^{-1} .

Membranes thicknesses were determined using a Schut digital micrometre. Six measurements in different points were performed in samples with $3 \text{ cm} \times 10 \text{ cm}$ area.

Contact angle measurements were performed with static deionized water drop on the membranes using a DSA 25 tensiometer.

Absorbance in the ultraviolet–visible spectral region was measured on a Shimadzu UV-2600 spectrometer.

3. Results and discussion

3.1. Mg₄FeAl-Cl and Mg₄FeAl-NAP LDHs characterization

LDH samples intercalated with Cl⁻ anions or NAP were first fully characterized. **Figure S4 (Supplementary Information)** presents the XRD patterns of Mg₄FeAl-Cl (precursor) and Mg₄FeAl-NAP samples. Precursor LDH diffractogram displays the characteristics peaks (narrow and well resolved) related to the LDH phase.[9] On both diffractograms peaks are narrow revealing a structural organization. After NAP intercalation, the shift of the (00l) peaks to small angles region is observed, which indicates that the interplanar distances are higher, endorsing the organic anion intercalation. Moreover many (00l) harmonic peaks are visualized for the hybrid LDH. The presence of the residual Mg₄FeAl-Cl phase in the Mg₄FeAl-NAP sample is evidenced by the permanence of the (113) reflection, normally displaced to small angles region and not visualized for single phases LDHs intercalated with bulky anions, once it is dependent of the c crystallographic parameter. Mixture of phases is commonly observed for NAP intercalation by ion-exchange reaction in MgAl LDHs and also by coprecipitation method.[17,46–48]

FT-IR spectra of the LDH materials are present in **Figure S5**. The band at 1361 cm⁻¹ is assigned to the antisymmetric stretching of the carbonate anions (contaminant), while the broad bands below 700 cm⁻¹ are assigned to the metal-OH translation.[9] For the Mg₄FeAl-NAP sample, the band at 1230 cm⁻¹ is attributed to the in plane bending of the C-H bounds in the naphthalene ring. The bands at 1392 and 1545 cm⁻¹ can be assigned to the stretching modes of the COO⁻ group.[46]

FT-Raman spectra of the LDHs are presented in **Figure S6**. For the precursor phase, the less intense band at 1062 cm⁻¹ is assigned to the symmetric stretching of

adsorbed carbonate anions.[49] The band at 540 cm^{-1} is assigned to metal-OH translation modes. For the $\text{Mg}_4\text{FeAl-NAP}$ sample, the most intense bands at 1632 and 1416 cm^{-1} are attributed to C–C ring vibrations while the band at 1386 cm^{-1} is assigned to CH_3 deformation.[17]

TGA curves of the $\text{Mg}_4\text{FeAl-Cl}$ and $\text{Mg}_4\text{FeAl-NAP}$ materials are shown in **Figures S7** and **S8**, respectively. Three and four main mass loss events are observed, for the precursor and the hybrid LDH sample, respectively. For both compounds, the first and second events, occurring from room temperature up to $180\text{ }^\circ\text{C}$ and $230\text{ }^\circ\text{C}$ for the precursor LDH and for the hybrid material, respectively, are associated to the release of adsorbed and intercalated water molecules. For the $\text{Mg}_4\text{FeAl-Cl}$ sample, the third event, that occurs between $180 - 600\text{ }^\circ\text{C}$, is assigned to the matrix dehydroxylation. The residual mass corresponds to 59.5 % wt. For the $\text{Mg}_4\text{FeAl-NAP}$ LDH, the third and fourth mass loss events correspond to the overlapping matrix dehydroxylation and NAP decomposition. The smaller residual mass (46.4 % wt) observed, in comparison with the precursor material, agrees with NAP presence.

SEM micrographs of the $\text{Mg}_4\text{FeAl-Cl}$ and $\text{Mg}_4\text{FeAl-NAP}$ samples are present in **Figure S9**. For both samples, a sand rose morphology and many sets of 2D plates are visualized. The great similarity between the sample's morphology suggests that the chloride-exchange is a topotactic process, in which the final lattice is determined by the lattice of the pristine LDH.

The elemental chemical composition of the $\text{Mg}_4\text{FeAl-Cl}$ and $\text{Mg}_4\text{FeAl-NAP}$ LDHs, as well as the amount of water obtained by TGA experiments, are gathered in **Table 2**. Molar ratio between the metals are close to the nominal values. Naproxen anions were intercalated in appreciable amounts (36.17 g of NAP for 100 g of hybrid).

Table 2. Elemental chemical composition of Mg₄FeAl-Cl and Mg₄FeAl-NAP LDHs.

Sample	Mg/Al	Mg/Fe	Fe/Al	Mg / (Fe + Al)	% H ₂ O	wt % C	wt % NAP
Mg ₄ FeAl-Cl	4.32	4.08	1.06	2.10	13.0	-----	-----
Mg ₄ FeAl-NAP	4.36	4.18	1.04	2.14	14.4	26.51	36.17

3.2. 3D Composite membranes by PLA electrospinning and LDH electrospaying

Membranes micrographs are presented in **Figure 3**. Regarding the multi-layered membranes obtained from approach A, some LDH particles are exposed on the surface of the membranes containing the thinnest PLA layers (Cl-A_{thin} and NAP-A_{thin}). Increasing the PLA thickness, fewer particles are visualized on Cl-A_{med} and NAP-A_{med} surfaces and no particles are observed for the Cl-A_{thick} and NAP-A_{thick} samples. The surface aspects of the Cl-A_{thick} and NAP-A_{thick} samples are similar to the one of the pristine PLA membrane, indicating that 30 min of PLA electrospinning is enough to completely cover the last layers containing LDHs particles. Less particles aggregation is observed for the membranes prepared from approach B by simultaneous PLA electrospinning and LDH electrospaying (Cl-B and NAP-B samples) compared to the particles distribution on surface of the thinnest membranes and to the surfaces aspect of the last deposited LDH layers (L), abbreviated by Cl-A_{thick}L and NAP-A_{thick}L samples. Indeed, in the approach A, a large number of particles is electrospayed at the same time, favoring the formation of aggregates on the mat, as was seen when electrospaying on a flat collector (**Figure S1** in SI) whereas in approach B, only few particles are electrospayed before new fibers land on the mat and hide them, hindering the formation of aggregates.

Figure 3. SEM micrographs of pristine PLA membrane and PLA membranes containing Mg_4FeAl LDHs intercalated with Cl^- and NAP anions, prepared by alternating 4 electrospun PLA layers with 3 electrosprayed LDH layers (Approach A) with different PLA thickness (A_{thin} – electrospinning of each layer performed for 15 min, A_{med} – 22 min and A_{thick} – 30 min) and prepared by both electrospinning and electrospraying at the same time (Approach B). Samples were analyzed applying BSE detector, 5 kV and work distance around 10 mm.

FT-IR spectra of the membranes containing Cl-LDH (**Figure 4a**) and NAP-LDH (**Figure 4b**) particles have been obtained. All bands observed for the pristine PLA (**Figure S10**) are present in the spectra related to the composites, whose assignment was made as follows.[50] The bands at 2994 and 2945 cm^{-1} are attributed to the antisymmetric and symmetric stretching of the $-\text{CH}_3$ group, respectively. The band at 1751 cm^{-1} is assigned to the stretching of the $-\text{C}=\text{O}$ group. Antisymmetric $-\text{CH}_3$ bending frequency is observed at 1452 cm^{-1} , while the corresponding symmetrical mode appears at 1361 cm^{-1} . The bands at 1261, 1184, 1129, 1084 and 1043 cm^{-1} are related to vibrational modes of the C-O-C group. Finally, the band at 867 cm^{-1} is related to the stretching of the O-CH- CH_3 portion and the band at 753 cm^{-1} to the wagging of the α - CH_3 group. For the membranes whose SEM images show higher levels of exposed particles, the bands at 3430 cm^{-1} , in the spectra for Cl-A_{thick}L and Cl-B, and at 3433 cm^{-1} , for the NAP-A_{thick}L and NAP-B samples, respectively, are attributed to the stretching of the -OH group presented in the LDH layers and to intercalated and adsorbed water molecules. Bands below 700 cm^{-1} , attributed to the metal-OH stretching, are also present in the spectra of the A_{thick}L and B samples. Additionally, for the NAP-A_{thick}L sample, the bands at 1547, 926 and 473 cm^{-1} are assigned to vibration modes of NAP anions.[17]

Figure 4. FT-IR spectra of membranes containing a) Cl-LDHs particles and b) NAP-LDH particles.

Table 3 presents the membranes thickness. A non-significant difference was observed for the membranes prepared by the same approach, differing by the presence of Cl-LDH or NAP-LDH particles, indicating a similarity in particles distribution during electrospraying method, independent of the nature of the LDH. In agreement with the architecture of the membranes, according with the consecutive increase in PLA layers thickness from A_{thin} to A_{thick} , it was observed the corresponding increase in thickness, from $61 \pm 24 \mu\text{m}$ for Cl- A_{thin} and $53 \pm 21 \mu\text{m}$ for NAP- A_{thin} to $116 \pm 14 \mu\text{m}$ for Cl- A_{thick} and $120 \pm 16 \mu\text{m}$ for NAP- A_{thick} , respectively. Comparing the membranes prepared by B approach (78 min of PLA deposition) with the ones prepared by A_{med} approach, in which the PLA fibbers content is slightly higher (88 min of PLA deposition as compared to 78 min for B), a decrease in thickness is observed for Cl-B and NAP-B samples. It may indicate a better particles-PLA fibers accommodation when electrospinning and electrospraying are performed at the same time.

Static water contact angles (SCA) for the pristine PLA and LDH-PLA membranes, as well as for the last LDH electrospayed layer of the A_{thick} samples (Cl-

$A_{\text{thick}}L$ and $NAP-A_{\text{thick}}L$) are compiled in **Table 3** (see also **Figure S11**). Comparing the SCA value of the pristine electrospun PLA ($129^\circ \pm 5^\circ$) with the values for the Cl-LDH-PLA samples, it is observed a decrease in the hydrophobicity to $124^\circ \pm 6^\circ$ for the Cl- A_{thick} sample and a more pronounced decrease for the other Cl-LDH-PLA samples. When NAP-LDH particles are present in the composites, the decrease in hydrophobicity compared to the pristine electrospun PLA membrane is less pronounced, showing the property of NAP anions in conferring higher hydrophobicity to the LDHs.

Table 3. Membranes average thickness, static water contact angle and mechanical properties obtained by tensile tests for PLA membrane and PLA_LDH composites.

Sample	Membranes Thickness (μm)	Contact Angle ($^\circ$)	Yield stress (MPa)	Yield strain (%)	Stress at break (MPa)	Strain at break (%)	Young modulus (MPa)
PLA	148 ± 22	129 ± 5	1.9 ± 0.1	2.9 ± 0.1	3.1 ± 0.2	70 ± 1	98 ± 5
Cl- A_{thin}	61 ± 24	121 ± 4	1.5 ± 0.1	3.3 ± 0.3	2.1 ± 0.2	82 ± 4	74 ± 6
Cl- A_{med}	102 ± 22	121 ± 5	1.5 ± 0.2	2.8 ± 0.6	2.5 ± 0.2	82 ± 6	82 ± 7
Cl- A_{thick}	116 ± 14	124 ± 6	2.1 ± 0.1	3.0 ± 0.3	3.9 ± 0.2	100 ± 3	109 ± 6
Cl-B	86 ± 26	121 ± 5	1.5 ± 0.2	2.8 ± 0.9	2.8 ± 0.3	79 ± 6	76 ± 12
Cl- $A_{\text{thick}}L$	-----	121 ± 5	-----	-----	-----	-----	-----
NAP- A_{thin}	53 ± 21	126 ± 5	0.7 ± 0.4	2.6 ± 0.8	1.4 ± 0.9	42 ± 5	39 ± 28
NAP- A_{med}	92 ± 19	127 ± 5	1.8 ± 0.1	4.6 ± 0.5	2.4 ± 0.1	100 ± 2	85 ± 2
NAP- A_{thick}	120 ± 16	127 ± 6	2.0 ± 0.2	2.7 ± 0.7	3.6 ± 0.1	52 ± 2	96 ± 5
NAP-B	99 ± 33	125 ± 5	1.7 ± 0.1	3.1 ± 0.1	2.5 ± 0.1	81 ± 3	84 ± 1
NAP- $A_{\text{thick}}L$	-----	127 ± 5	-----	-----	-----	-----	-----

Table 3 presents mechanical properties (Young modulus, yield stress and strain and stress and strain at break) of the membranes obtained by tensile measurements. Stress-strain curves can also be seen in **Figure S12**. Although LDH-fibers interactions might change the mechanical behaviour, it is shown that the amount of incorporated LDH is not enough to significantly affect the tensile properties.

Technological medical devices, such as multifunctional dressings, require a deep understanding of the materials structure in order to tune materials properties and explain their behaviour once applied. LDHs were previously fully characterized and a high drug load was verified. Physicochemical properties of the nanofibers membranes were investigated. The interaction degree among PLA nanofibers and LDHs particles responded to the applied approach and resulted in mechanical properties improvements. The different membranes presented a particular superficial morphology, in which modifications in the thickness of the polymeric diffusional barrier are expected to affect the drug release profile, discussed below.

3.3. Kinetic of NAP release: effect of the membranes morphology

Drug released amount was calculated from the calibration curve shown in **Figure S13**. Initial release, highlighted in **Figure 5a**, shows that after 5 min more than 5 % of NAP was released and probably LDH particles present in the membrane's surface were responsible by the initial burst, with exception of the NAP-A_{thick} sample, whose initial drug release was delayed for about 2 hours. Later release profile, better visualized in **Figure 5b**, shows a considerable release rate difference among the samples. Half of NAP amount was released after 24, 56, 96 and 408 h (17 days) of experiment for the NAP-A_{thin}, NAP-B, NAP-A_{med} and NAP-A_{thick} composites, respectively. The release rate can be correlated with the degree of LDH particles exposure to the saline solution media. Besides, in order to reach the LDH particles inside the structure, the aqueous media has to penetrate through the PLA layers, which are hydrophobic. From A_{thin} to A_{thick}, the thickness of the PLA layer increases, increasing the penetration time inside the membrane and slowing the drug release. Naproxen was released over 18 days for the

NAP-A_{thin} sample and for longer time for the other samples: 59 days for the membrane prepared by the approach B and 66 days for both NAP-A_{med} and NAP-A_{thick}, after which it reached a plateau.

Figure 5. Drug release profile: naproxen release percentage as a function of time for the different composites. a) Semi-log scale. b) Linear scale.

In order to get more insight in the release mechanisms, the naproxen release profiles of the different membranes were fitted by the power law Ritger-Peppas model[51] (**equation 2**) which is currently used to describe the drug release process from matrices in general and polymeric systems:

$$\frac{M_t}{M_\infty} = Kt^n \quad (2)$$

Where M_t is the drug amount released after a time t and M_∞ is the drug amount at the equilibrium state. **Figure 6** presents the curves of the model as well as the obtained kinetic parameters (constant K and release exponent n) and linear correlation coefficients (R^2). Drug release from all devices was well fitted by the Power Law model, with R^2 values greater than 0.98. The low n values obtained for NAP-A_{thin}, NAP-A_{med} and NAP-B membranes (respectively 0.28, 0.23 and 0.26), smaller than 0.45, suggests a Fickian type release with dominant diffusion process.[51–53] In the case of NAP-A_{thick} membranes, n value is larger and equal to 0.59, characterizing an anomalous

transport. It must be mentioned that such model gives only an overall behavior of the membrane rendering difficult an exact description of the drug release mechanisms and especially the lag-time observed for the NAP-A_{thick} membranes. The release behavior of LDH materials (i.e. no associated to a polymer) is often related to a Fickian diffusion, which is based on ion exchange process.[54,55] However, in our case, the multilayer structure of the membranes induces a complex sequential flow through the PLA nanofibrous layers and then, through the LDH electrospayed layers. The latter being the domains where the drug is initially released by intra-LDH-particles and inter-LDH-particles diffusion. Considering the architecture of the NAP-A_{thick} membranes, it is possible to propose that initially the release medium permeates the pores of the PLA hydrophobic mesh of the external layer. Then, after this first step, exchange of NAP by Cl⁻ saturating the LDH layers allows the release of the drug through the porous layer of PLA towards the external medium. The drug release delay was not evidenced for all other membranes because some LDH particles were immediately exposed to the release medium as shown in **Figure 3** for the membranes containing the thinnest PLA layers (i.e. NAP-A_{thin}, NAP-A_{med}, NAP-B).

Figure 6. Experimental points and Ritger-Peppas model (solid lines) of Naproxen release percentage as a function of time for the different composites in log-log scale.

In order to better understand the dissimilarity among the dissolution profiles for the membranes prepared by the different approaches, the “difference factor” (f_1) and the “similarity factor” (f_2), described by **equations 3** and **4**, respectively, were calculated and are indicated by the *Agência Nacional de Vigilância Sanitária* (The Brazilian Health Regulatory Agency) [56] and by the FDA [57] to compare tested and reference formulations. Where, N is the number of dissolution time points and Rt and Tt are the dissolution values (percentages in our case) for the reference and the tested system at a time t , respectively. The factor values are shown in **Table 4**. Dissolutions profiles are considered equivalents if f_1 is between 0 and 15 and if f_2 is between 50 and 100, as observed among the NAP-A_{med} and NAP-B membranes, while all other membranes show a different profile, compared two by two.

$$f_1 = 100 \left(\frac{\sum_{t=1}^N |R_t - T_t|}{\sum_{t=1}^N R_t} \right) \quad (3)$$

$$f_2 = 50 \log \left[100 \left[1 + \frac{1}{N} \sum_{t=1}^N (R_t - T_t)^2 \right]^{-0.5} \right] \quad (4)$$

Table 4. “Difference” (f_1) and “similarity” (f_2) factors calculated for the composites compared two by two.

Rt	NAP-A _{thin}	NAP-A _{med}	NAP-B	NAP-B	NAP-B
Tt	NAP-A _{med}	NAP-A _{thick}	NAP-A _{thin}	NAP-A _{med}	NAP-A _{thick}
f_1	28.4	44.7	27.7	10.0	49.5
f_2	33.2	32.1	39.9	60.4	27.7

4. Conclusions

The development of 3D multi-layered electrospun membranes containing drug loaded iron-based LDH particles was achieved with the aim of forming multifunctional

dressings by different approaches. In approach A, a faster, an intermediate and a slower NAP release were observed for the thin, medium and thick PLA layer thickness, respectively. Therefore, drug release could be modulated by simply varying the preparation parameter. Compared to the membranes prepared by the approach A, approach B involved only one step preparation and the membrane presented an intermediate NAP release profile. The developed membranes fulfilled important characteristics expected for a technological drug delivery device, since the prepared drug delivery composites were able to modulate and keep NAP release for more than two months. Furthermore, membranes architecture can inspire the development of even more interesting and functional devices based on electrospinning and electrospraying techniques.

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