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## One-step production of laser-induced graphene via CO<sub>2</sub> laser on agarose–lignin membranes

Beatriz S Machado<sup>1</sup> , Maria Morais<sup>1</sup> , Tomás Pinheiro<sup>1</sup> , Jonas Deuermeier<sup>1</sup> , Vasco Teixeira<sup>1</sup> , Daniela Nunes<sup>1</sup> , Rodrigo Martins<sup>1</sup> , José M Inácio<sup>2</sup> , Elvira Fortunato<sup>1</sup> and Henrique V Almeida<sup>1,\*</sup>

<sup>1</sup> CENIMAT|i3N, Department of Materials Science, School of Science and Technology, NOVA University Lisbon and CEMOP/UNINOVA, 2829-516 Caparica, Portugal

<sup>2</sup> Stem Cells and Development Laboratory, iNOVA4Health, NOVA Medical School|Faculdade de Ciências Médicas, NOVA University Lisbon, 1169-056 Lisbon, Portugal

\* Author to whom any correspondence should be addressed.

E-mail: [hmmv.almeida@fct.unl.pt](mailto:hmmv.almeida@fct.unl.pt)

**Keywords:** laser-induced graphene (LIG), agarose–lignin biopolymers, biocompatible membranes, electroactive materials, cost-effective functional materials, sustainable bioelectronics

Supplementary material for this article is available [online](#)

### Abstract

Laser-induced graphene (LIG) is a highly promising material for bioelectronics due to its excellent electrical conductivity, high surface area and biocompatibility. Nevertheless, the functionalization of biocompatible substrates with LIG is essential to propel the use of LIG-derived technologies forward in bioengineering. This study demonstrates the successful fabrication of LIG on agarose–lignin membranes using a single-step CO<sub>2</sub> laser process. Membranes with 3 or 5 wt.% agarose, and 0.25 or 0.5 wt.% lignin were characterized for thickness and swelling degree to assess their behavior in a human-mimicking media. The LIG was comprehensively studied, measuring electrical and sheet resistance, and by employing techniques such as Raman spectroscopy, scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectroscopy (EDS), and x-ray photoelectron spectroscopy (XPS) to evaluate graphitization efficiency and investigate its physicochemical characteristics. Electrical measurements revealed that the lowest sheet resistance achieved was equal to  $139 \pm 2 \Omega \text{ sq}^{-1}$ , with lower laser speeds (below  $76.2 \text{ mm s}^{-1}$ ) and higher power settings (above 2.5 W) leading to improved conductivity. SEM analysis revealed a three-dimensional porous structure with pore sizes ranging from nanometers to micrometers, contributing to enhanced electrical conductivity and suitability for bioelectronic applications. EDS mapping further identified carbon, oxygen, and sodium. XPS analysis provided detailed insights into the chemical states of carbon, indicating high-quality graphene formation. The integration of LIG with these flexible, biocompatible membranes highlights their potential for use in bioelectronic devices, including wearable sensors and implantable medical technologies. These findings underscore the potential of agarose–lignin-based LIG as a scalable, eco-friendly platform for future bioelectronic innovations.

### 1. Introduction

Graphene is composed of sp<sup>2</sup>-bonded carbon atoms arranged in a two-dimensional hexagonal lattice in a honeycomb-like structure and has an astonishing plethora of applications, mainly due to its wide range of properties [1]. This material has a high surface area, high electrical and thermal conductivity,

a Young modulus of approximately 1 TPa, which jointly with its high fracture strength translates into great compressive and tensile stiffness. Graphene also presents good optical and electronic properties, large adsorption capability of water and other hydrophilic compounds, among many other characteristics [2–6]. Regarding applications, graphene has gained recognition in the areas of biosensing, tissue engineering,

and wearable electronics [7]. Methods for graphene production include mechanical exfoliation, chemical vapor deposition and reduction of graphene oxide (GO) [8, 9]. However, despite presenting advantages, these processes are complex, cost-, energy- and time-consuming, besides requiring high processing temperatures, presenting low efficiency and generating large volumes of chemical waste, which hinder the scaling up of graphene production [9, 10]. Therefore, there is an urgent need for alternative, cost-effective, and scalable graphene production methods.

Laser-induced graphene (LIG) was serendipitously discovered in 2014 when polyimide (PI) was struck with a CO<sub>2</sub> infrared laser under ambient conditions, producing three-dimensional structures of graphene while attempting to pattern GO on PI [11]. Initially, the process was limited to PI and polyetherimide (PEI), but it was later found that other polymers, like sulfonated poly(ether-etherketone) (SPEEK), polysulfone (PSU) and polyethersulfone (PES), could also be converted into LIG because of their similarities with PI and PEI [12]. LIG is formed through a photothermal process in which a laser beam irradiates the polymer surface, generating extremely high localized temperatures. These temperatures break specific carbon bonds, and when the temperature decreases, the carbon atoms recombine to form graphitic structures, while the other elements are released in gas form [9, 10, 13].

LIG is an incredible substitute for conventional graphene forms since it presents several attractive features, such as being a sustainable material, time- and cost-effective fabrication process, having a high surface area, good electrical and thermal conductivity [10, 14]. Besides this, the morphology, chemical composition, and surface properties of LIG are tunable by varying production factors, including the carbon precursor, the type of laser, laser power, speed, and wavelength. Furthermore, variations in these properties allow tuning the physicochemical properties of LIG, which jointly with the atmospheric laser engraving conditions, play a crucial role in shaping the electrical, mechanical, and surface characteristics of the resulting LIG [13, 15, 16]. The LIG fabrication process allows graphene synthesis and patterning to be completed in a single step, eliminating the need for expensive cleanroom equipment, chemical treatments, or complex post-processing. Additionally, the process can be carried out in ambient air without requiring solvents or other complex external conditions. The geometry of LIG patterns can also be easily modified using computer software, making it an ideal solution for customizable and scalable production with high productivity, low energy consumption, and eco-friendliness [10, 17]. In regards to applications, several LIG-based

devices have been reported, such as nanogenerators [18], energy storage devices [19–21] and sensors [22–26].

Recent research has demonstrated that naturally occurring carbon-based materials, such as wood, cellulose, lignin, coconut shells, and potato surfaces, can be successfully transformed into LIG using a CO<sub>2</sub> infrared laser under ambient conditions [27, 28]. Specifically, biopolymers rich in aromatic units, such as lignin, are easily converted into graphitic structures.

Lignin accounts for 25%–30% of the total plant biomass, found abundantly in the cell wall of plants, providing them with compressive strength and stiffness and protecting them against pathogens. Presently, lignin is a by-product of lignocellulosic biorefineries, with more than 60% going to waste. Chemically, it is a complex, heterogenous, and hydrophobic aromatic biopolymer, in which the primary elements comprise phenylpropanoid units, namely syringyl, guaiacyl, and p-hydroxyphenyl. Additionally, lignin is abundant, environmentally friendly, biodegradable, and biocompatible. This biopolymer also possesses antimicrobial and antioxidant properties, as well as ease of functionalization, making it increasingly attractive for biomedical applications [29–31]. Organosolv lignin and alkali lignin, among other types, are widely studied for their diverse structural and functional properties. Organosolv lignin is extracted using organic solvents, like ethanol or methanol, under acidic conditions, producing a high-purity (around 93%), sulfur-free lignin [32, 33]. In contrast, alkali lignin is typically obtained via the Kraft process, using alkaline solutions such as sodium hydroxide, which can introduce sulfur and other impurities in lignin structure (around 30% pure) [33]. Organosolv lignin generally possesses a lower molecular weight (ranging between 600 and 1600 Da) compared to alkali lignin (within the range of 3000 Da) as well as a lower polydispersity, with these attributes contributing to its homogeneity and making it highly suitable for biomedical applications and bioelectronics [34]. Specifically, organosolv lignin's purity enhances its biocompatibility, reducing the risk of adverse reactions and improving its integration with biological tissues [32, 33].

Biopolymers present themselves as ideal candidates for LIG precursors due to their diversity, abundant carbon content, biocompatibility, and availability, paving a new way into the sphere of graphitization by laser irradiation [10, 13]. Inside this category, a multitude of biopolymers can be used as a template for LIG production, such as agarose.

Agarose is a cost-effective, biocompatible, and versatile polysaccharide sourced from marine red algae, characterized by the presence of repeating monomeric units of agarobiose,

a disaccharide composed of D-galactose and 3,6-anhydro-L-galactopyranose [35]. It displays notable characteristics such as thermo-reversible gelation and significant swelling behavior, tunable mechanical properties, and attractive physiochemical features to be used in bioengineering. Alongside, agarose has a prominent water uptake capacity, poroelastic structure and is biologically inert, making it suitable for a wide range of applications in areas such as tissue regeneration, self-healing hydrogels and wound dressing [36]. Beyond this, agarose-based biomaterials have exhibited a remarkable electro-responsive behavior, offering potential applications in fields such as artificial muscle development [35, 36]. LIG scribed directly onto agarose–lignin membranes provides the mechanical flexibility necessary for soft tissue integration. Unlike traditional rigid electronics, which often lead to mechanical mismatches with biological tissues, LIG's ability to flex and conform makes it ideal for applications in wearable sensors and implantable devices designed to interface with the skin, brain, or heart [37]. This adaptability enhances comfort and functionality in bioelectronic devices that require seamless interaction with soft, dynamic biological environments [36].

While agarose exhibits excellent properties and numerous applications, it has only been explored as a precursor for LIG synthesis in a previous work from our group [38], a master thesis that enabled the execution of the work presented herein. However, the two works present significant differences. Firstly, the application focus of the two studies sets them apart, as the thesis focuses on cardiomyocyte culture and maturation, whereas the work presented in this document casts a wider network, focusing on the development of sustainable and suitable materials for a broad range of cutting-edge bioelectronic devices namely for wearable sensors and implantable devices, thus propelling innovations in medical technology and wearable electronics. Another notable difference is the biological component involved. The first study employs human-induced pluripotent stem cell-derived cardiomyocytes to evaluate how the substrates support cell metabolism, morphology, and overall functionality. On the other hand, this manuscript is more centered on material characterization and physicochemical properties. It thoroughly investigates the correlation between the substrate composition and laser engraving conditions and the electrical conductivity and structural and chemical properties of the LIG. In sum, the study presented here explores the fabrication of agarose–lignin membranes and their conversion into LIG through CO<sub>2</sub> laser irradiation. Moreover, a comprehensive characterization of the biopolymeric membranes and LIG produced on these substrates under varying laser parameters of power (2.5 to 5.5 W) and speed (38.1 to 76.2 mm s<sup>-1</sup>)

is provided. Electrical resistance and sheet resistance measurements alongside analytical techniques such as Raman spectroscopy, scanning electron microscopy (SEM) coupled with energy-dispersive x-ray spectroscopy (EDS), and x-ray photoelectron spectroscopy (XPS) are employed to assess the effective formation of LIG on the surface of the agarose–lignin membranes. This study documents the production of LIG from biopolymeric membranes, marking an initial step toward developing agarose–lignin substrates for bioelectronics applications, especially in flexible, biocompatible sensors and wearable medical devices.

## 2. Experimental section

### 2.1. Materials

The agarose, alkali lignin (C<sub>3</sub>OH<sub>25</sub>ClN<sub>6</sub>, ≥97%, CAS: 8068–05-1), and glycerol (C<sub>3</sub>H<sub>8</sub>O<sub>3</sub>, 100%, CAS: 56–81-5) were obtained from Sigma-Aldrich. The acetic acid (CH<sub>3</sub>COOH, ≥99.8%, CAS: 64–19-7) and sodium tetraborate decahydrate, hereafter referred to as borax (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O, ≥99.5%, CAS: 1303–96-4) were procured from Honeywell. All chemical compounds were used without further purification, and Milli-Q water was also used throughout the experiments.

### 2.2. Production of agarose–lignin membranes

The production of the membranes, illustrated in figure S1, started by mixing a solution of acetic acid at 5% (v/v) with the required amount of lignin (0.25 or 0.5 wt.%) in a glass beaker with a magnetic stirrer. Afterward, this suspension was left stirring at room temperature (RT) for 24 h. Then, this dispersion was placed on a stirring plate at 550 rpm, and 0.5 mL of glycerol, followed by 0.125 g of borax, were added, being left to stir for 2 h. Then, agarose (3 or 5 wt.%) was added while stirring and the suspension was heated to 90 °C. Solvent evaporation during stirring was minimized by covering the beaker with aluminum foil throughout the production of all the membranes. Immediately after, the fully solubilized mixture was evenly poured into a 6×6 cm mold made with tape on top of a 10×10 cm glass substrate. Then, the membranes were left to dry inside a fume hood for several days under ambient conditions. Optical images of the six flexible membranes are presented in figure S2 and their corresponding composition is listed in table 1.

#### 2.2.1. Characterization of the membranes

The thickness of the dry membranes was measured using a digital micrometer (Mitutoyo, resolution 1 μm). Five measurements were carried out in five different areas of the film for each one of the six membranes to ensure statistical coherence. Swelling studies were performed by cutting 1×1 cm squares of

**Table 1.** Composition of the control (AGB1 and AGB2) membranes and of those with lignin (AGBL1, AGBL2, AGBL3, AGBL4).

| Membrane | Agarose<br>concentration (wt. %) | Lignin<br>concentration (wt.%) | Borax (g) | Glycerol (mL) |
|----------|----------------------------------|--------------------------------|-----------|---------------|
| AGB1     | 3                                | 0                              |           |               |
| AGB2     | 5                                | 0                              |           |               |
| AGL1     | 3                                | 0.25                           | 0.125     | 0.5           |
| AGL2     | 3                                | 0.50                           |           |               |
| AGL3     | 5                                | 0.25                           |           |               |
| AGL4     | 5                                | 0.50                           |           |               |

**Table 2.** Nomenclature of each lasing condition having as a base the combination of the parameters in study, laser power (W) and scanning speed ( $\text{mm s}^{-1}$ ).

| Condition | Parameters<br>( $\text{W/mm s}^{-1}$ ) | Condition | Parameters<br>( $\text{W/mm s}^{-1}$ ) |
|-----------|----------------------------------------|-----------|----------------------------------------|
| C1        | 2.5/38.1                               | C15       | 2.5/63.5                               |
| C2        | 3/38.1                                 | C16       | 3/63.5                                 |
| C3        | 3.5/38.1                               | C17       | 3.5/63.5                               |
| C4        | 4/38.1                                 | C18       | 4/363.5                                |
| C5        | 4.5/38.1                               | C19       | 4.5/63.5                               |
| C6        | 5/38.1                                 | C20       | 5/63.5                                 |
| C7        | 5.5/38.1                               | C21       | 5.5/63.5                               |
| C8        | 2.5/50.8                               | C22       | 2.5/76.2                               |
| C9        | 3/50.8                                 | C23       | 3/76.2                                 |
| C10       | 3.5/50.8                               | C24       | 3.5/76.2                               |
| C11       | 4/50.8                                 | C25       | 4/76.2                                 |
| C12       | 4.5/50.8                               | C26       | 4.5/76.2                               |
| C13       | 5/50.8                                 | C27       | 5/76.2                                 |
| C14       | 5.5/50.8                               | C28       | 5.5/76.2                               |

the produced membranes and weighing them. Three squares for each sample were cut to ensure statistical coherence. Afterwards, the samples were submersed in PBS solution ( $\text{pH} = 7.4$ ) at RT for 120 min. At different time points: 10, 30, 60, 90, and 120 min, the membranes were taken out of the PBS solution, carefully wiped with paper to remove surface moisture, and then weighed again. The swelling degree (SwD) was calculated according to equation (1):

$$\text{Swelling degree (\%)} = \frac{W_w - W_d}{W_d} \times 100 \quad (1)$$

where  $W_w$  is the weight of the wet film and  $W_d$  is the weight of the dry film.

### 2.3. LIG production

In order to obtain the conversion of these membranes into LIG, a  $10.6 \mu\text{m}$   $\text{CO}_2$  infrared laser (VLS3.5 Laser Platform, Universal Laser Systems) was employed. A preliminary study of the parameters that led to the formation of LIG was performed by varying the speed and power. As for the defocus amount and pulses per inch (PPI), they were kept constant at 8 mm and at 1000, respectively, throughout the LIG synthesis, as they did not constitute variables in this work. For LIG fabrication, first, matrixes with  $2 \times 2$  mm squares were drawn in the Adobe Illustrator software and then exported to the laser software. These squares were scribed on the substrate using raster mode under

a nitrogen flux. This preliminary study was undertaken equally on the six membranes. Each distinct conjugation of laser power and scanning speed tested is hereafter referred to as a lasing condition and is presented in table 2.

### 2.4. LIG characterization

The electrical resistance of each  $2 \times 2$  mm LIG square of every matrix scribed in each membrane, was preliminarily evaluated using a digital multimeter by placing each probe tip on each diagonal of the specific square. Five measures were made on each square. The  $2 \times 2$  and  $5 \times 5$  mm LIG squares on each agarose–lignin membrane, graphitized under condition C6, were inspected with a Leica M80 Optical Stereo Microscope at  $1.25\times$  magnification, using a two-light, medium-intensity setting. Inspection was focused on the  $5 \times 5$  mm squares, which showed membrane damage under this condition. The sheet resistance values were determined by engraving  $5 \times 5$  mm LIG squares, under the conditions C1–C5, cutting these squares from their respective membranes, and by patterning droplets of silver ink on each corner of the LIG squares, followed by the resistivity measurements using a high-performance Hall effect measurement system (HL5500, BioRad/Nanometrics). The presented sheet resistance values are the average of the values obtained using three LIG squares engraved on different parts of each membrane.

The chemical and structural characterization was performed with an in Via Qontor confocal Raman microscope (Renishaw) equipped with a 532 nm laser (green) operating at 5 mW, with a 50× magnification objective lens (Olympus) to focus the laser beam, with 30 s exposure time. For each one of the two LIG conditions that yielded the lowest sheet resistance, measured in each agarose–lignin membrane, six spectra were obtained from different areas of the LIG square. Furthermore, it is possible to calculate the crystallite size ( $L_a$ ) of the LIG samples using the generalized Tuinstra and Koenig equation, proposed by Cançado *et al* [38]:

$$L_a = (2.4 \times 10^{-10}) \lambda^4 \left( \frac{I_D}{I_G} \right)^{-1} \quad (2)$$

where  $\lambda$  is the wavelength of the laser and ( $I_D/I_G$ ) the ratio of the D and G peak intensities.

The morphological studies of the LIG scribed under the conditions C4 and C5 in the AGL1 membrane formulation were performed using a SEM (Regulus SU8220, Hitachi). The thickness and average pore size were measured using the ImageJ software. Five measures were made in each sample for the thickness measurements and ten measurements were made in each sample for the pore size measurements. As the membrane composed of 3 wt.% agarose and 0.25 wt.% lignin (AGL1) joint with the lasing conditions C4 and C5 led to LIG with improved electrical properties, these samples were further evaluated using EDS and XPS. Their conductivity was also estimated. First, an elemental analysis was performed by EDS to identify the chemical elements and their abundance. The resistivity can be calculated if the values of the sheet resistance measurements along with the thickness of the LIG layers are known through equation (3):

$$\rho = R_{\text{sheet}} \times t_{\text{LIG}} \quad (3)$$

where  $R_{\text{sheet}}$  is the sheet resistance and  $t_{\text{LIG}}$  is the thickness of the LIG. Having the resistivity, the electrical conductivity ( $\sigma$ ) can be calculated as the inverse of the resistivity ( $\rho$ ):

$$\sigma = \frac{1}{\rho}. \quad (4)$$

The quantitative and qualitative surface analysis, aimed at estimating the elemental composition and identifying the types of chemical bonds and chemical states present in the samples, was performed using a Kratos Axis Supra with a monochromated Al K $\alpha$  source. The Al K $\alpha$  source was running at 150 W and detailed scans were recorded with 10 eV pass energy. The software CasaXPS was used for data analysis, whereas the software Origin was used for data analysis.

## 2.5. Statistics

GraphPad Prism 7 software was used for statistical analysis. Results are expressed as the mean value  $\pm$  standard deviation, with the replicates ( $n$ ) of each set of measurements depending on the type and the accuracy of the measurements, varying between  $n = 3$  and  $n = 10$ . Shapiro–Wilk test was used to test for normality and Levene test was used to test for equal variance on all quantitative data sets. Two-way ANOVA was used to determine statistical significance within a data set and Turkey HSD post-hoc test was used to determine if there were significant differences between groups. Differences were significant when \*:  $p < 0.05$ ; very significant when \*\*:  $p < 0.01$ ; highly significant when \*\*\*:  $p < 0.001$ ; extremely significant when \*\*\*\*:  $p < 0.0001$ .

## 3. Results and discussion

### 3.1. Characterization of the agarose–lignin membranes

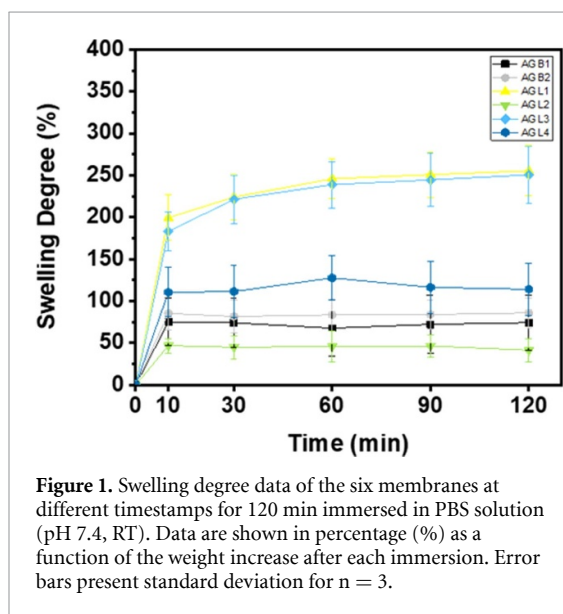
The results of the membranes' thickness measurements are presented in table 3, ranging from  $120 \pm 10 \mu\text{m}$  (AGL2) to  $176 \pm 25 \mu\text{m}$  (AGL3), with the standard deviation of the measurements performed on AGB1 and AGL3 being the highest, while AGL2 and AGL1 present the lowest standard deviations. This observation indicates that the latter membranes are more suitable for applications where uniform and homogenous materials are needed.

The swelling ability of a biopolymeric material after absorbing water of a buffer solution is a crucial property in biomedical applications because its hydration level affects not only its surface properties and mobility, but also its mechanical properties, and the nutrient and metabolite mechanism of infiltration through the biopolymeric matrix [39]. This unique characteristic of the biopolymers depends on various factors, such as thickness, drying process, thermodynamic compatibility, polymer relaxation time, interaction with the functional groups of the biopolymer and crosslinker chain structure and chemical properties [40]. The results presented in figure 1 show that all of the membranes absorbed the PBS at different percentages with minor variations, meaning that there were minor batch-to-batch variations. PBS (pH  $\sim 7.4$ ) was chosen because it matches the osmotic pressure and ion concentration present on the human body as well as the pH of the surrounding media, since both are highly influential in the SwD, which demonstrates the possibility of these membranes to be used in a plethora of applications, from tissue engineering to implantable devices [41].

Upon closely analyzing figure 1, it is evident that AGL1 exhibits the highest values of SwD, while AGL2 shows the lowest values over the same time span.

**Table 3.** Thickness measurements of all membranes, using a digital micrometer ( $\pm 1 \mu\text{m}$ ).

| Sample | Thickness ( $\mu\text{m}$ ) |
|--------|-----------------------------|
| AGB1   | 126 $\pm$ 19                |
| AGB2   | 167 $\pm$ 10                |
| AGL1   | 132 $\pm$ 10                |
| AGL2   | 120 $\pm$ 10                |
| AGL3   | 176 $\pm$ 26                |
| AGL4   | 134 $\pm$ 16                |

**Figure 1.** Swelling degree data of the six membranes at different timestamps for 120 min immersed in PBS solution (pH 7.4, RT). Data are shown in percentage (%) as a function of the weight increase after each immersion. Error bars present standard deviation for  $n = 3$ .

After 120 min, SwD values range from  $41 \pm 14\%$  for AGL2 to  $256 \pm 30\%$  for AGL1. The most significant increase in SwD occurs within the first 10 min, after which it stabilizes for all membranes except AGL1 and AGL2, which only reach stabilization after 30 min of immersion in PBS. Furthermore, an important observation is that membranes with lower lignin content, specifically AGL1 and AGL3, exhibit the highest SwD values compared to both the controls and membranes with double the lignin content. Lignin is considered a hydrophobic aromatic biopolymer. However, this compound has hydrophobic aromatic rings and hydrophilic hydroxyl and carboxyl groups [30, 32], so when the lignin-containing membranes were immersed in PBS, there was the formation of  $\text{COO}^-$  ions derived from the ionization of the carboxyl groups present in the biopolymer, since the  $\text{pK}_a$  of the carboxyl group is 4.5 and the pH of the solution is 7.4. Besides this, the hydroxyl groups also ionize in PBS, originating  $\text{OH}^-$  ions, given their  $\text{pK}_a$  of 4.5 [42]. When this happens, counterions will enter the membranes, altering the osmotic pressure and inducing PBS transfer from the surrounding media to the membrane, generating swelling. Hence, there is a heightened hydrophilic character of the membrane induced by the introduction of 0.25 wt.%

of lignin into the formulation of AGL1 and AGL3. Besides this, plasticization diminishes the interaction between lignin particles, thus allowing for a higher dispersibility of this compound that acts as a filler inside the crosslinked matrix [43]. Upon increasing the lignin concentration to 0.5 wt.% in AGL2 and AGL4, it is hypothesized that the hydrophobic nature of lignin intensifies due to the increased presence of lignin particles in the matrix. Consequently, there are more interactions among these particles, especially because the glycerol concentration was not adjusted, potentially leading to the formation of agglomerates that repel water, thus accentuating the membrane's hydrophobic character [44, 45]. This suggests a threshold has been reached concerning the concentration of lignin. When looking at the controls, the behavior is similar to what is observed in the membranes with the higher lignin content, with the low plateau of SwD reached. However, it has to be highlighted that this does not mean that the characteristics of these latter four films are equal for LIG conversion.

### 3.2. $\text{CO}_2$ LIG synthesis

The six biopolymeric membranes were used for a preliminary study of the parameters that led to the formation of LIG. For this, the laser parameters scanning speed and laser power were varied to assess their influence on the properties of the graphitic structures. The graphitization of a matrix having twenty-eight squares per membrane was performed, where in each column the laser power varied from 2.5 to 5.5 W (5%–11% of maximum operational value) and in each line the scanning speed, in rast mode, varied from 38.1 to 76.2  $\text{mm s}^{-1}$  (3% to 6% of maximum operational value). Regarding the nitrogen flux, it was also kept constant throughout the irradiation because LIG present on the surface of lignin-rich materials necessitates preparation in a nitrogen atmosphere to inhibit oxidation [17, 28].

Figure 2 shows that at the highest speed and lowest power, highlighted by the red rectangular line (C22), only partial LIG formation occurred, as indicated by the gray color of the correspondent LIG square. This phenomenon happened in AGL1 as well as in AGL2, AGL3, and AGL4. However, in the controls AGB1 and AGB2, the homologous square was not graphitized, which implies that at high scanning speeds and lower powers, the laser's energy is insufficient to break chemical bonds, impeding the rearrangement of carbon atoms and inhibiting LIG formation.

Particularly for AGB1 and AGB2, in addition to the latter explanation, agarose is an aliphatic compound, and when compared to aromatic precursor compounds, aliphatic precursors lack the

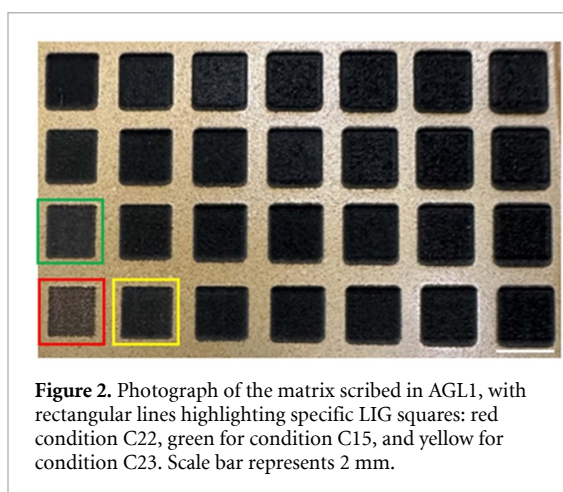


**Figure 2.** Photograph of the matrix scribed in AGL1, with rectangular lines highlighting specific LIG squares: red condition C22, green for condition C15, and yellow for condition C23. Scale bar represents 2 mm.

inherent aromatic ring structures with  $sp^2$  hybridized carbon bonds necessary for efficient graphitization. Consequently, aliphatic precursors necessitate a higher degree of laser exposure to attain graphitic structures comparable to those achieved by their aromatic counterparts [9]. If the scanning speed remains constant and the power increases by 0.5 W (C23), or if the laser power remains constant and the scanning speed decreases by  $12.7 \text{ mm s}^{-1}$  (C15), both decreases account for 1% of variation, the LIG squares are almost completely graphitized, as indicated by their dark-gray color. Again, this phenomenon happened in AGL1, AGL2, AGL3, AGL4, and in the control membranes as well. For this reason, it can be deduced that these membranes are very sensitive to laser power and scanning speed variation, since 1% of variation is enough to promote the transition from partial to complete graphitization of LIG, for both parameters. The findings align with existing literature, showing that increasing laser power enhances graphitization efficiency but increases the probability of material ablation, while reducing power can result in incomplete graphitization. Similarly, lower scanning speeds improve graphitization up to a threshold, beyond which ablation occurs, whereas higher speeds decrease energy exposure, leading to incomplete graphitization [9]. Hence, identifying the optimal balance between these two parameters is imperative to attain a successful and high-quality graphitization. Lastly, it is noteworthy that visible graphitization was attained with just a single laser scan for all the agarose-based membranes, which has never been done before to the best of the authors' knowledge.

### 3.3. LIG characterization

#### 3.3.1. Electrical resistance measurements

The electrical resistance of the six membranes was a starting point in the LIG characterization studies to evaluate which lasing conditions would yield

the highest-quality graphitic structures. Once the optimal conditions were determined, they were selected for all subsequent studies. The electrical resistance measurements were made using a digital multimeter. The results are depicted in figure 3, in which is discernible that for the six membranes, when laser power increases from 5.0 W to 5.5 W, for all scanning speeds (conditions C7, C14, C21, and C28), there is a sudden rise in the electrical resistance values, with two exceptions in the graphic referent to the AGB2 membrane. Lin *et al* [11] translated this phenomenon into the existence of a threshold from which higher laser powers stop improving the LIG quality and start being detrimental to the quality of the graphene, causing excess defects in the atomical structure of graphene and ablation.

Moreover, a clear generalized tendency is present: the orange points, which represent the scribing of the LIG at the lowest scanning speed ( $38.1 \text{ mm s}^{-1}$ ) for different laser powers (conditions C1–C7), are the ones to yield graphitic structures with the lowest electrical resistances. Conversely, the purple points, which represent the scribing at the highest scanning speed ( $76.2 \text{ mm s}^{-1}$ ) for different laser powers (conditions C22–C28), are the ones that yielded graphitic structures with the highest electrical resistance. A lower degree of crystallization obstructs current flow stemming from less organized 2D layers, which indicates that the electrical resistance is inversely proportional to graphene quality [46]. As reducing scanning speed led to lower electrical resistance values, the lowest scanning speed was chosen to carry out the studies.

#### 3.3.2. Sheet resistance measurements

Based on the electrical resistance analysis, which identified that the optimal graphitic structures were achieved at a scanning speed of  $38.1 \text{ mm s}^{-1}$ , further sheet resistance measurements were conducted at this specific speed for various lasing powers. Although the preliminary tests were performed with  $2 \times 2 \text{ mm}$  squares, squares with  $5 \times 5 \text{ mm}$  were scribed using different laser power to facilitate their handling and increase the reliability of the Hall effect measurements. Condition C7 led to membrane ablation and thus was excluded from these and subsequent studies. Additionally, contrary to what was observed in the  $2 \times 2 \text{ mm}$  squares, the  $5 \times 5 \text{ mm}$  squares scribed under C6 presented low integrity, an indication of membrane ablation during LIG production. This observation led to the exclusion of these samples from the sheet resistance studies. The  $2 \times 2$  and  $5 \times 5 \text{ mm}$  squares scribed under the remaining lasing conditions presented similar optical properties. As can be seen in figure S3, this phenomenon occurred in the four agarose–lignin membranes but not on the control membranes. This observation is

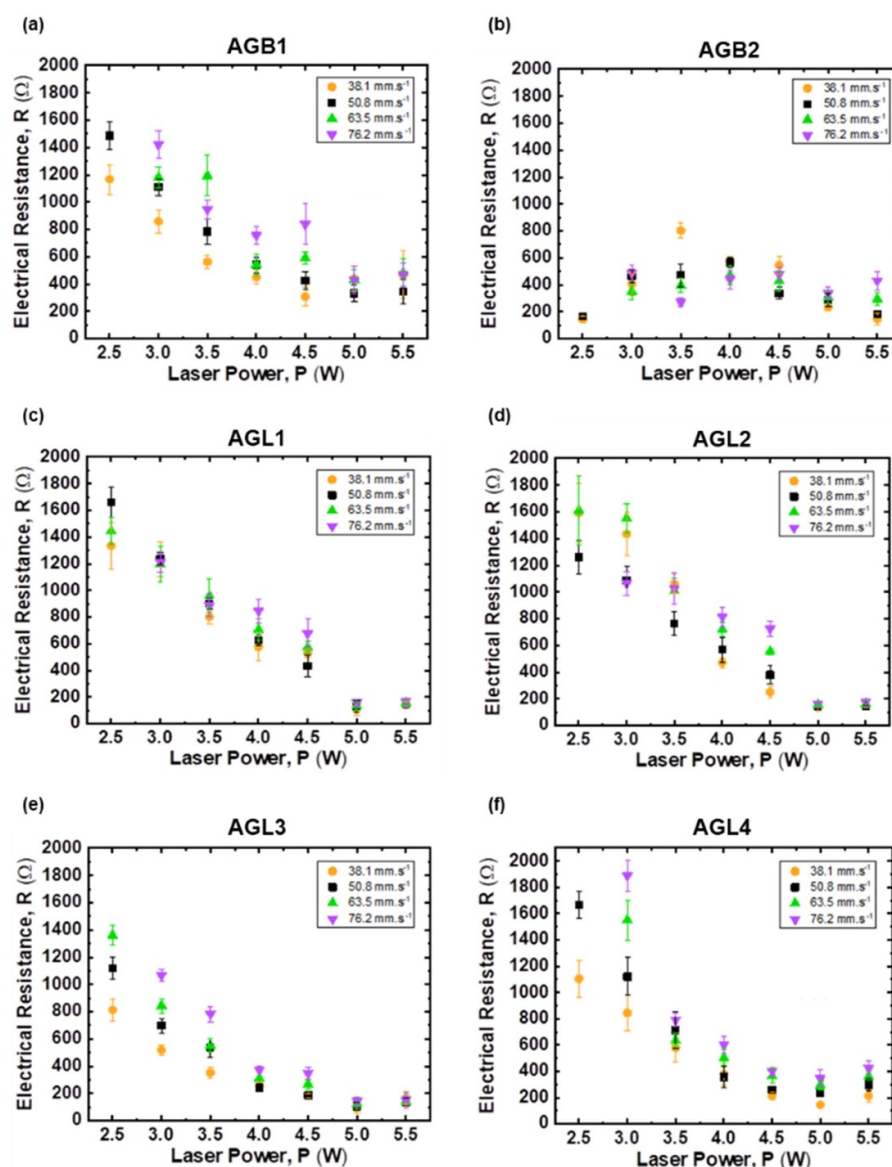


**Figure 3.** Electrical resistance mean values of the samples (a) AGB1, (b) AGB2, (c) AGL1, (d) AGL2, (e) AGL3, and (f) AGL4 for different laser powers under different scanning speeds. Error bars present standard deviation for  $n = 5$ .

associated with agarose's aliphatic nature, characterized by the absence of aromatic rings, which translates into higher thermal resistance to laser exposure. In this scope, herein, it is reported for the first time in polysaccharide membranes that the dimensions of the pattern have an impact on the integrity of the substrate. The results of the sheet resistance measurements are depicted in figure 4.

In figure 4(a), the agarose concentration was fixed at 3 wt.% to examine the effect of varying lignin concentrations on the electrical properties of AGB1, AGL1, and AGL2. When examining the data for AGL1 and AGL2, an exponential decrease is noticeable as the power augments, from condition C1–C5, being that the lowest sheet resistance obtained was  $144 \pm 3 \Omega \text{ sq}^{-1}$ , meaning that for the same agarose

concentration, and for the condition C5, the sheet resistances are similar, although the decreasing behavior is a lot more accentuated in AGL1. In figure 4(b), the agarose concentration was set at 5 wt.%, enabling a comparative study between AGB2, AGL3, and AGL4. In this graph, AGB2 does not exhibit a consistent trend, while AGL3 and AGL4 demonstrate an exponential decrease, akin to the behavior observed for AGL1 and AGL2. Notably, the lowest sheet resistance values were recorded under condition C5, with AGL3 reaching  $276 \pm 15 \Omega \text{ sq}^{-1}$ , and AGL4 achieving  $304 \pm 51 \Omega \text{ sq}^{-1}$ . This indicates that, for the same agarose concentration and under condition C5, the sheet resistances are comparable, although the rate of decrease is significantly more pronounced in AGL3. Furthermore, it is evident across both figures that the

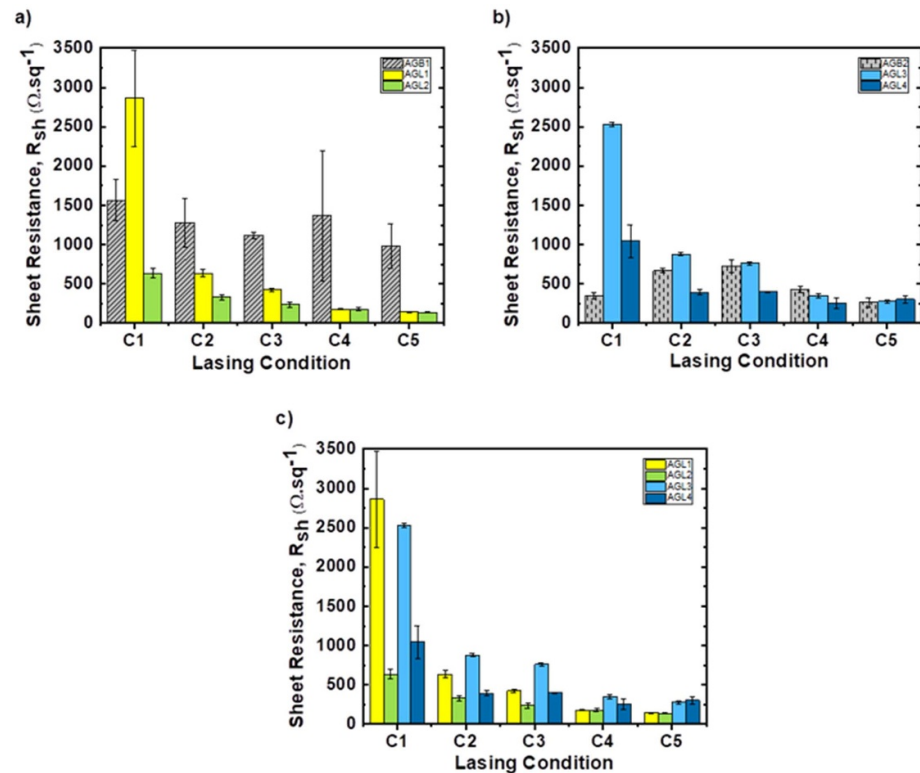


**Figure 4.** Comparison of the sheet resistance mean values between (a) membranes having 3 wt.% of agarose, (b) membranes having 5 wt.% of agarose, and (c) membranes having different lignin concentrations, graphitized under conditions C1–C5. Error bars present standard deviation for  $n = 3$ .

incorporation of lignin into the agarose membranes substantially affects the LIG electrical properties by decreasing the sheet resistance, which translates into a more crystalline material. Finally, figure 4(c) explores the optimal agarose concentration for the two lignin concentrations, comparing AGL1, AGL2, AGL3, and AGL4. By analyzing this graphic in conjunction with the other two, there is evidence that points to the fact that AGL1 and AGL2 are better formulations than AGL3 and AGL4, as a 3 wt.% agarose concentration is better for LIG conversion when compared to a 5 wt.% concentration, since the LIG produced in the membranes with higher agarose concentration exhibited almost twice the sheet resistance of that produced resorting to the membranes with 3 wt.% agarose.

Conditions C4 and C5 were then selected for the subsequent studies as they were the two lasing conditions that yielded LIG with the lowest sheet resistance values for all the membranes under study. As for the agarose concentrations, none was excluded, even if there was evidence that 3 wt.% agarose was better for LIG production, since the structural studies are of paramount importance to determine in depth the quality and properties of the LIG graphitized in the different membranes.

### 3.3.3. Raman spectroscopy

Raman spectroscopy is a widely used technique to characterize LIG as it provides structural and electronic information, such as the number of layers, the stacking order, disorder, presence of edge and grain boundaries, and charge carrier concentration [47]. The characteristic bands in the Raman spectra of multilayer graphene and graphite are the D, G, and 2D bands. The D band, known as the defect band, represents defects in the graphene lattices, being present at around  $1350 \text{ cm}^{-1}$ . The G band represents the degree of graphitization of the sample and is present at around  $1580 \text{ cm}^{-1}$ . The 2D band is known as the second order of the D band, and it not only represents the degree of crystallinity but also the number and arrangement of graphene layers, being present at  $2700 \text{ cm}^{-1}$  [48]. Analyzing figure 5, it is noticeable that for every membrane, under both lasing conditions (C4 and C5), visible graphitization is present since these three characteristic bands of carbonaceous materials are present.

Additionally, the second-order bands, D + D' and D + D', were resolved in all spectra, which indicates not only that graphitization took place, but that the graphene is of high-quality [49]. Examining the

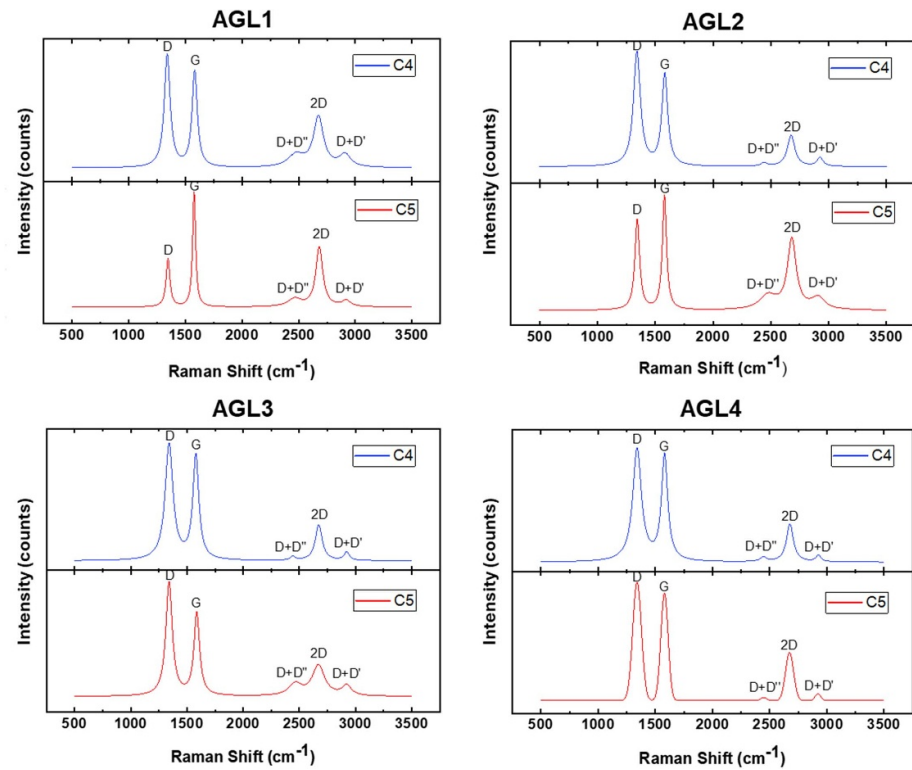


**Figure 5.** Raman spectra of LIG graphitized under the conditions C4 (blue line) and C5 (red line), with its characteristic bands of carbonaceous materials, for the membranes (a) AGL1, (b) AGL2, (c) AGL3, and (d) AGL4.

In the Raman spectra corresponding to AGL1 and AGL2 membranes, illustrated in figures 5(a) and (b), respectively, some notable differences are observed between the two conditions. Under condition C4, the LIG exhibits a lower graphitization quality, i.e., lower crystallinity, higher disorder in its structure and higher defect density, as indicated by the D bands being more intense than the G bands. This is also supported by the lower intensity of the 2D bands as well as the overall higher full width at half maximum of the bands corresponding to samples produced under condition C4 when compared to those of condition C5. Analysis of the  $I_D/I_G$  ratios presented in table 4 corroborates these observations.

The  $I_D/I_G$  ratio correlates the intensities of the D and G bands and is indicative of the density of lattice defects [50]. For AGL1, the  $I_D/I_G$  ratio decreases from 1.17 to 0.43 when transitioning from condition C4 to C5. Similarly, for AGL2, the ratio decreases from 1.27 to 0.45 when transitioning between the same conditions. This decrease indicates that for condition C4, the substrate was converted into an amorphous carbon, whereas for condition C5, the same material was converted to a more crystalline graphene for both membranes. The values for the  $I_{2D}/I_G$  ratio are also presented in table 4. This ratio gives information about the stacking of the graphene layers, being inversely proportional to the character of graphene; in

**Table 4.** Raman band intensity ratios and respective crystallite sizes of the LIG produced under the conditions C4 and C5, for the four membrane samples AGL1 to AGL4.

|      | Condition | $I_D/I_G$ | $I_{2D}/I_G$ | Crystallite size (nm) |
|------|-----------|-----------|--------------|-----------------------|
| AGL1 | C4        | 1.17      | 0.56         | 16.42                 |
|      | C5        | 0.43      | 0.53         | 45.13                 |
| AGL2 | C4        | 1.27      | 0.34         | 15.07                 |
|      | C5        | 0.45      | 0.58         | 42.91                 |
| AGL3 | C4        | 1.12      | 0.33         | 17.12                 |
|      | C5        | 1.35      | 0.45         | 14.21                 |
| AGL4 | C4        | 1.12      | 0.38         | 17.23                 |
|      | C5        | 1.16      | 0.44         | 16.60                 |

particular  $I_{2D}/I_G > 3$  is a typical feature of monolayer graphene [50]. Transitioning from condition C4 to C5, for both AGL1 and AGL2, results in a moderate increase in the  $I_{2D}/I_G$  ratio in AGL2, which points to the fact that the stacking remains practically unchanged between the conditions, with multilayer stacking of graphene sheets observed in both cases. In this transition, the crystallite size, which can be calculated as per Cançado *et al* [38], also prominently increases. Therefore, for both membranes, an increase in laser power results in higher quality graphene. This finding is consistent with the sheet

resistance results, as the lowest value was recorded for C5, indicating that the graphene converted under this condition has superior electrical and structural properties. However, the Raman spectra for AGL3 and AGL4, depicted in figures 5(c) and (d), respectively, suggest a different pattern. For both conditions, the D bands are more intense than the G bands, the 2D bands are not very intense, and all the bands present a high full width at half maximum, which points to a high defect density among the graphite lattice. Furthermore, the  $I_D/I_G$  ratio increases when transitioning from condition C4 to C5 for these membranes, which is accompanied by a decrease in crystallite size, suggesting early ablation in these membranes or that the graphitization process was not efficient when compared to AGL1 and AGL2. Moreover, when looking at the sheet resistance values, specifically for AGL4, the sheet resistance increases from  $255 \pm 62 \Omega \text{ sq}^{-1}$  (C4) to  $304 \pm 51 \Omega \text{ sq}^{-1}$  (C5), further supporting the hypothesis of substrate damage. As for the  $I_{2D}/I_G$  ratio, it is also noticeable that the graphene is multilayer for both membranes under both conditions.

The Raman results align with the sheet resistance measurements when comparing the four membranes, as AGL3 and AGL4 exhibited nearly double the sheet resistance values compared to AGL1 and AGL2 under condition C5. This indicates that the formulations with 5 wt.% agarose concentration are the least effective for LIG conversion. Overall, the sheet resistance measurements and Raman spectroscopy data, indicate that the best formulation is AGL1 as the samples produced under both lasing conditions C4 and C5, yielded the LIG with fewer defects, translating into the lowest sheet resistance and the best  $I_D/I_G$  and  $I_{2D}/I_G$  ratios.

### 3.3.4. SEM imaging of LIG

The equilibrium of power and scanning speed is fundamental during LIG formation. Outside the optimal range, either ablation occurs, or no LIG formation happens [51]. It is, therefore, essential to study the topology of samples formed under different laser irradiation conditions, particularly using SEM. In this scope, SEM analysis was conducted to gain deeper insights into the surface morphology and microstructure of the samples. The SEM images illustrated in figure 6 showcase the intricate details of the LIG porous structures. These porous structures serve to increase the surface area and promote the penetration of electrolytes into the active material, ultimately enhancing their performance by augmenting sensitivity, improving permeability, and enhancing flexibility and elasticity for bioelectronics and wearables [11, 52, 53].

SEM images (figure 6) show a 3D porous LIG layer with an open pore structure, created by the release of gas during laser scribing [10], exhibiting

hierarchical porosity, with smaller mesopores within the macropores walls. In the LIG graphitized under condition C4, the pore size distribution ranges from 0.26 to  $14.16 \mu\text{m}$ , with an average pore size of  $5.72 \mu\text{m}$  whereas the LIG graphitized under condition C5, presents a pore size distribution from 3 nm to  $10.06 \mu\text{m}$ , with an average pore size of  $1.16 \mu\text{m}$ . Pores having a size between 2 and 50 nm are considered mesopores and above 50 nm, are considered macropores instead. Increasing the laser power generally enhances the rate and volume of gas release, leading to a broader pore size distribution and also leading to smaller pore size [12, 54]. This is in accordance with the SEM results, since the LIG scribed under C5 has more 0.5 W of laser power compared to C4. Condition C4, characterized by a pore size range from 0.26 to  $14.16 \mu\text{m}$  with an average of  $5.72 \mu\text{m}$ , suggests a relatively moderate laser power. This moderate energy promotes more consistent gas release, resulting in larger, more homogeneous pores, as the energy is sufficient to ablate the material and create significant pore structures without causing excessive fragmentation. In contrast, condition C5 exhibits a broader pore size distribution ranging from 3 nm to  $10.06 \mu\text{m}$ , indicating higher variability in the diameter of the pores. The increased laser power under C5 may lead to smaller pores through more aggressive ablation and finer restructuring, while still allowing for larger pores where energy accumulates, resulting in rapid and uneven gas release. These findings indicate that the LIG structure can be controlled by changing the laser power. The extent of material ablation and the rate of gas release during laser irradiation vary significantly with different laser settings, leading to distinct pore size distributions.

The sheet resistance measurements show that under C4, the LIG has a higher sheet resistance compared to C5. This outcome is likely attributed to the complex interaction between pore structure and electrical pathways. Although the LIG scribed under condition C4, which has larger pores, offers a more consistent graphene network, it might also result in more disconnected electrical pathways. On the other hand, the smaller pores in the LIG scribed under condition C5 create more contact points, thereby connecting electrical pathways and enhancing overall conductivity [55, 56]. Another important factor to be considered is the stacking of graphene layers and their thickness, so cross-sectional SEM images of mechanically cleaved samples are illustrated in figure S4. From these images, the thickness of each sample can be measured, being that the LIG scribed under C4, figure S4(a), has a mean value of  $299 \pm 30 \mu\text{m}$ , whereas the LIG scribed under C5, figure S4(b), has a mean value of  $414 \pm 83 \mu\text{m}$ . Therefore, the LIG scribed under C4 is thinner and more homogenous than the LIG scribed under C5, which

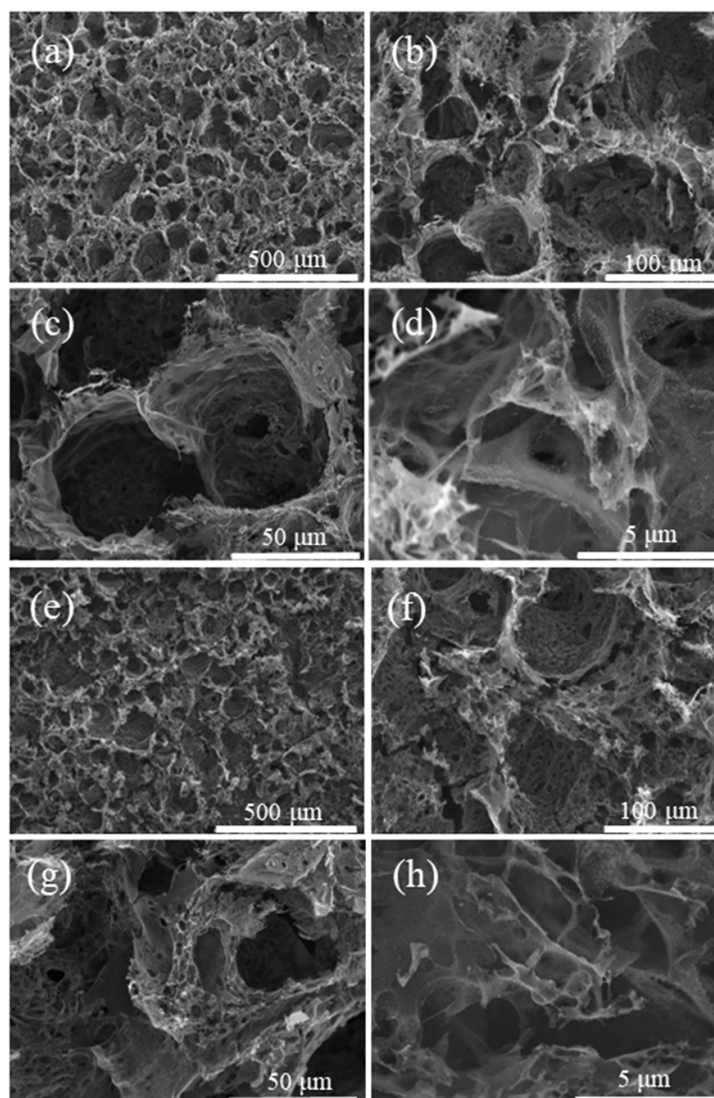


**Figure 6.** SEM images of the LIG morphology in AGL1 scribed under condition (a)–(d) C4 and (e)–(h) C5, in different scale bars of (a), (e) 500  $\mu\text{m}$ ; (b), (f) 100  $\mu\text{m}$ ; (c), (g) 50  $\mu\text{m}$ ; (d), (h) 5  $\mu\text{m}$ .

can be perceived by the variability of the thickness of each sample and by the visual analysis of the images.

### 3.3.5. EDS mapping of LIG

EDS mapping of the LIG graphitized on membrane AGL1 under conditions C4 and C5 was carried out to identify the elemental composition as well as to detect any impurities the samples might present (figures S5 and S6, respectively). This analysis revealed the presence of three major elements: carbon, oxygen, and sodium. The presence of oxygen can be attributed to the cleavage of C–O–C and C–O bonds in the original aromatic structure of lignin, as well as any bonds that remained unbroken [57]. Additionally, oxygen may originate from the graphitization process, where it is present in the released gases, some of which could have been trapped within the LIG. As for sodium, it is a contaminant, and

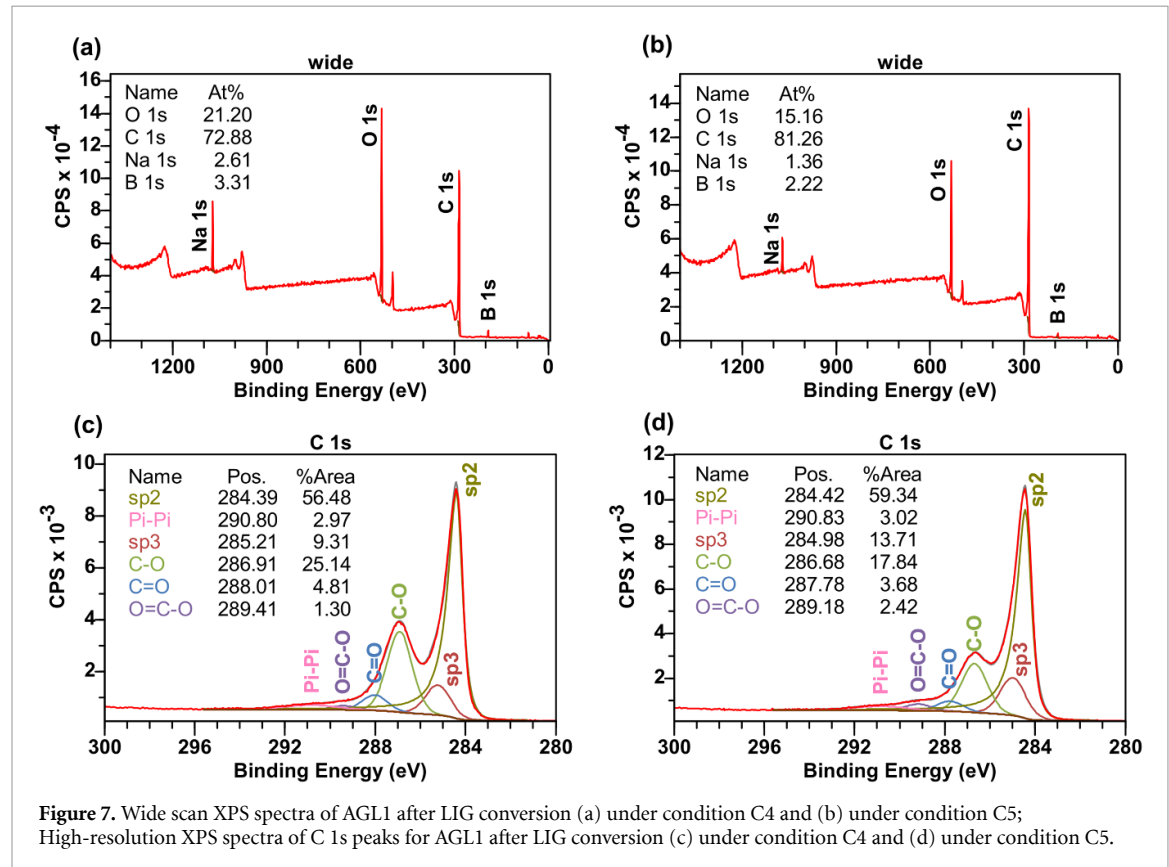
its presence can be mainly attributed to the sodium hydroxide used in the Kraft method to obtain the alkali lignin used in the formulations of the membranes. Both tables S1 and S2 which have the descriptive elemental composition of the samples in atomic concentration, portray that there are no significant differences between the atomic concentration of the same element in both samples. Vestigial elements sulfur (S), calcium (Ca) and potassium (K) can also be found in the samples. Boron is in non-considerable concentrations.

### 3.3.6. Electrical conductivity

The electrical conductivity of a material is a property that measures how efficient the electrical transport of charges is through that same material. This property was assessed in the LIG layer for both conditions, C4 and C5, in the AGL1 membrane. Analysis

**Table 5.** Sheet resistance values, thickness, resistivity, and conductivity of the LIG graphitized under conditions C4 and C5 for the AGL1 membrane.

| Properties                                                       | Condition    |              |
|------------------------------------------------------------------|--------------|--------------|
|                                                                  | C4           | C5           |
| Sheet resistance $R_{\text{sheet}}$ ( $\Omega \text{ sq}^{-1}$ ) | $180 \pm 5$  | $144 \pm 3$  |
| LIG thickness, $t_{\text{LIG}}$ ( $\mu\text{m}$ )                | $299 \pm 30$ | $414 \pm 83$ |
| Resistivity, $\rho$ ( $\Omega \text{ m}$ )                       | 0.05         | 0.06         |
| Conductivity, $\sigma$ ( $\text{S m}^{-1}$ )                     | 20.00        | 16.67        |

**Figure 7.** Wide scan XPS spectra of AGL1 after LIG conversion (a) under condition C4 and (b) under condition C5; High-resolution XPS spectra of C 1s peaks for AGL1 after LIG conversion (c) under condition C4 and (d) under condition C5.

of table 5 reveals a correlation between the degree of graphitization and the thickness of the LIG, being that a higher degree of graphitization corresponds to a thicker LIG layer. This observation aligns with previous discussions, which suggested that LIG scribed under C5 has a more crystalline structure when compared to C4. This difference in crystallinity implies a higher degree of graphitization for the LIG converted under C5. These observations are also in agreement with the literature [43].

Given the sheet resistance values ( $R_{\text{sheet}}$ ) and the thickness of the layers ( $t_{\text{LIG}}$ ) of the LIG graphitized under both conditions, in AGL1, the resistivity ( $\rho$ ) of the LIG can be estimated using equation (3). From the resistivity values present in table 5, it is evident that LIG graphitized under condition C5 has a higher resistivity of  $0.06 \Omega \text{ m}$  compared to  $0.05 \Omega \text{ m}$  under condition C4. This difference is due to the thickness of the LIG layers, which is directly proportional to the resistivity, though the difference is minimal at just

$0.01 \Omega \text{ m}$ . With the resistivity values for both conditions, the corresponding conductivity can be calculated using equation (4), as conductivity is inversely proportional to resistivity. As shown in the table, and as expected, the LIG scribed under condition C4, with a conductivity of  $20.00 \text{ S m}^{-1}$ , is more conductive than the LIG scribed under condition C5, which has a conductivity of  $16.67 \text{ S m}^{-1}$ .

### 3.3.7. XPS analysis

XPS analysis was conducted on the AGL1 membrane after laser irradiation, under the conditions C4 and C5, which yielded LIG with the lowest sheet resistance values. Figures 7(a) and (b) show the survey scans of conditions C4 and C5 with the respective elemental quantification and (c) and (d) the C 1s emissions of the conditions C4 and C5, respectively.

Besides carbon and oxygen, sodium and boron were detected, the first being associated with the processing method of the lignin (kraft) included

in the membranes and the second being a main component of the fire-retardant sodium tetraborate decahydrate included in the membranes to mitigate their thermal degradation. The deconvolution of the C 1 s peak resulted in six components at 284.4 eV ( $sp^2$ -hybridized C–C bond), 290.8 eV ( $\pi-\pi^*$  bond),  $285.1 \pm 0.1$  eV ( $sp^3$ -hybridized C–C bond),  $286.8 \pm 0.1$  eV (C–O bond),  $287.9 \pm 0.1$  eV (C=O bond), and  $289.3 \pm 0.1$  eV (O=C–O bond). The results represented in figure 7 indicate that the laser condition C5 leads to more efficient graphitization, which is evidenced by the higher area of the peak corresponding to the  $sp^2$  carbon bonds and the lower density of C–O bonds due to the breakage of C–O bonds during the photothermal conversion, attributed to the transformation of the substrate into LIG [58], corroborating the Raman measurements.

#### 4. Conclusions and future perspectives

This work successfully demonstrated the production of flexible biopolymeric membranes composed of agarose (concentration between 3 and 5 wt.%) and lignin (concentration between 0.25 and 0.5 wt.%) in four distinct formulations. It was shown that these membranes could be effectively converted into laser-induced graphene (LIG) using a single-step laser irradiation process with a low-cost CO<sub>2</sub> laser, leading to an optimized condition (C5 - 4.5 W and 38.1 mm s<sup>-1</sup>).

The inclusion of lignin was found to enhance the graphitization process, as evidenced by the improved quality of LIG when comparing the agarose–lignin membranes with the control membranes based on the Raman spectroscopy and electrical properties studies. The LIG scribed on the agarose–lignin membranes exhibited favorable sheet resistance values, with the lowest recorded at  $139 \pm 2 \Omega \text{ sq}^{-1}$ . Raman spectroscopy revealed bands characteristic of graphitic materials, with the lowest  $I_D/I_G$  ratio of 0.43, indicating an optimal balance between defects and graphitization, and identifying AGL1 as the best formulation for LIG production. Scanning electron microscopy analysis revealed a three-dimensional hierarchical porous structure in the LIG, featuring macro and mesopores with sizes ranging from 3 nm to 14.16  $\mu\text{m}$ , and LIG layers with thicknesses between 298.63 and 414.11  $\mu\text{m}$ . Energy-dispersive x-ray spectroscopy mapping reveals the presence of sodium, an impurity coming from the processing method of lignin (kraft) and oxygen, signifying the cleavage of C–O–C and C–O bonds, as well as unbroken bonds and the trapping of gases within the LIG. A minimum resistivity of 0.05  $\Omega \text{ m}$  and a maximum conductivity of 20.00 S m<sup>-1</sup> were achieved in this work using the AGL1 membrane graphitized under condition C4.

This work presents a significant advancement in bioelectronics by developing conductive, biocompatible materials from natural sources. The LIG production on agarose and lignin biopolymers provides a sustainable, eco-friendly alternative to synthetic materials used in flexible electronics. This work not only establishes a foundation for future research in bioelectronics, where conductivity and biocompatibility are crucial, but also paves the way for broader applications in tissue engineering fields and bioelectronic devices, driving innovation in medical technology.

#### Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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#### ORCID iDs

Beatriz S Machado  <https://orcid.org/0009-0000-7773-2849>

Maria Morais  <https://orcid.org/0000-0002-3527-7857>

Tomás Pinheiro  <https://orcid.org/0000-0002-8346-4198>

Jonas Deuermeier  <https://orcid.org/0000-0002-2764-3124>

Vasco Teixeira  <https://orcid.org/0009-0007-1047-0128>

Daniela Nunes  <https://orcid.org/0000-0003-3115-6588>

Rodrigo Martins  <https://orcid.org/0000-0002-1997-7669>

José M Inácio  <https://orcid.org/0000-0002-9790-8335>

Elvira Fortunato  <https://orcid.org/0000-0002-4202-7047>

Henrique V Almeida  <https://orcid.org/0000-0001-9123-4475>

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