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Box-Behnken Design a Key Tool to Achieve Optimized PCL/Gelatin Electrospun Mesh

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Hybrid electrospun nanofibers of polycaprolactone (PCL)/gelatin are considered as drug-delivery systems for increasing the treatment efficacy in superficial (skin) wounds. Continuous delivery of therapeutic agents, skin extracellular matrix similarity, management of wound exudate, and antimicrobial barrier effect are the major advantages of electrospun nanofibers in skin applications. Additionally, combining the favorable properties of PCL and gelatin, regarding their biocompatibility, biodegradability and mechanical performance have been revealed promising parameters to be considered for blend in hybrid structures. However, the usual optimization protocol of nanofibers' production in electrospinning is based on the observation of one-variable-at-time being this methodology expensive and time-consuming. Therefore, in this research work, a statistical model based on four input variables namely, the flow rate, the needle-working distance, the applied voltage, and the ratio of PCL in the solution, is developed to predict the behavior of nanofibers. The performance of nanofibers is monitored by measurements of fiber's diameter, mesh's thickness, and mesh's permeability. Overall, the model showed to be statistically significant (p-value < 0.05) and an independent analysis validated the predicted response for optimal condition. Finally, a delivery study is performed to evaluate the electrospun mesh performance as a drug carrier.

1. Introduction

The demand for new fiber-based drug-delivery systems, particularly as wound dressings, is explained for the specific features of these structures which are an advantage over conventional materials. Electrospun nanofibers can present diameters reaching only a few nanometres, a high surface area to volume ratio, and high porosity of the meshes.[1] This set of features is especially important during wound treatment since it allows gas exchanges with the exterior while the barrier function against pathological agents is ensured. For this reason, fiber-based wound dressing made by electrospinning demonstrated great potential compared to traditional ones since they promote hemostasis, provide absorption of wound exudate, adequate permeability, conformability to the wound bed, and no scar induction.[2] Electrospinning is an efficient and low-cost technique that has shown relevant scientific progress in the replacement of traditional manufacturing technologies to obtain nanofibers with specific morphologies and design. Moreover, the random arrangement of electrospun fibers mimics the skin extracellular matrix and therefore, has been used as topical and transdermal drug-carriers for improving regenerative therapies.[3]

Some electrospun wound dressing combines synthetic and natural polymers, namely PCL and gelatin, to conjugate in the final structure the good mechanical properties and, the high biocompatibility and biological cues.[4–7] PCL is a synthetic polymer that has been widely used in wound dressing applications due to its biodegradability, high pharmaceutical acceptance, and good mechanical properties.[8] However, depending on the process' phase to be considered, the slow degradation of PCL can be a disadvantage regarding the wound dressing applications since it not follows the natural cycle of the healing process of skin.[9] Furthermore, the efficiency of drug carriers is strongly related to the ability to create communication paths between these carriers and skin cells. This process is mediated by integrin, a protein present in the cell membrane and which works as a receptor for easy adhesion to the extracellular matrix.[4] Usually, synthetic polymers include a lower number of integrin-binding sites comparing to natural polymers.[4] For these reasons, the PCL used in wound dressing applications is usually blended with a natural polymer to increase the cell-affinity of the structure and to adjust the degradation process of nanofiber's matrix. Gelatin is one of the most used natural polymers and is obtained from partial hydrolysis of collagen. The interactions between the RGD sequence (arginine-glycine-aspartic) present in gelatin and the integrin present in cells are responsible for the enhancement of cell adhesion.[10] Therefore, the arising chemical properties as biocompatibility and biodegradability making it widely used in the biomedical field.[4,6,11]

However, the nanofibers optimization process is usually performed through the observation of each variable-at-time which is an expensive and time-consuming approach given the high number of parameters related to the electrospinning process.[12] Concerning the above mentioned, in this research work, it was produced an optimized hybrid electrospun nanofiber of

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PCL/gelatin using design of experiments (DoE). Previous studies already applied this methodology to achieve the optimal processing parameters for optimization of fiber diameter in PCL/Gelatin meshes.[13,14] When compared with the previous studies, the proposed model was designed specifically for wound dressing development and is focused in different production conditions by evaluating their interaction with other performance indicators as the thickness and the permeability of PCL/Gelatin nanofibers. Electrospun nanofibers strongly depend on the production conditions such as percentage of PCL, flow rate, distance needle-collector, and applied voltage and, for this reason, these factors were considered as the inputs of the developed model. Finally, the performance of PCL/Gelatin electrospun meshes was evaluated considering the fiber diameter, mesh thickness, and respective permeability to show the potential of this developed biomaterial for wound dressing applications.

The electrospun meshes could be used to provide a structural base to wound healing but, at the same time, they could be doped with particular agents that lead to an increase of the regeneration phenomenon and/or help with the pain management. Previous studies developed drug delivery systems for the release of anti-inflammatory drugs as ibuprofen, antibiotics, or other materials as metallic nanoparticles of zinc or silver responsible for the treatment of skin bacterial infections.[15–18] Accordingly, in this study, it was also accessed the protein release behavior of the optimized meshes for further incorporation of therapeutic agents.

Although the production and characterization of PCL/Gelatin electrospun meshes were reported in literature, is still difficult to model the production parameter tuning. For example, when the goal is to reduce the fiber diameter several approaches could be applied (e.g., reduction of the flow rate, variation of applied voltage, among others).[19,20] However, during this process there is a trade-off between the multiple parameters that must be considered to avoid droplet formation and/or non-solvent evaporation. Furthermore, optimizing the process exclusively against the fiber diameter may introduce unexpected behavior in the remaining response variables. Considering the complexity of such heuristical approach, the proposed statistical model is able to drive the production parameters to quickly obtain fibers matching specific requirements for average fiber diameter, mesh thickness and mesh permeability. Taking this into account, this is the first work designed specifically for optimization of wound dressings production and which includes a more complete analysis of PCL/Gelatin electrospun mesh since a higher number of input and output variables is considered compared with previous studies. Moreover, the release behavior of the optimized meshes was accessed as an opportunity to understand which agents fit its therapeutic effect on the meshes release profile.

2. Materials and Methods

2.1. Materials

The nanofibers produced in this work result from a polymer blend of gelatin from porcine skin (type A, 300 Bloom, 60 mesh) purchased from Sigma Aldrich (St. Louis, M.O., USA) and PCL (Mw = 50 kDa) purchased from Perstorp (Malmo, Sweden). The PCL and gelatin were dissolved in glacial acetic acid (AA), purchased from Scharlau (Barcelona, Spain) and 2% v/v of triethylamine (TEA), purchased from Sigma Aldrich (St. Louis, M.O., USA), was added to increase the solution conductivity. Moreover, the protein delivery studies were based on bovine serum albumin (BSA) (*M*w = 66.5 kDa) release profile which was purchased from GERBU (Heidelberg, Germany). Bicinchoninic acid Pierce Protein Assay Kit (BCA) purchased from Thermo Scientific (Rockford, USA) was used to assess the concentrations of the protein.

2.2. Production of PCL/Gelatin Nanofibers

Three different solutions of PCL/gelatin were prepared by blending 15 wt% gelatin and 17 wt% PCL in the volume ratio of 25:75, 50:50, and 75:25. PCL/gelatin meshes were produced using a home-made electrospinning apparatus composed of i) a syringe pump (Pump 11 Elite Series, Harvard Apparatus) operating at a flow rate range from 0.2 to 0.6 mL h^{-1}; ii) a high-voltage source (Genvolt 73 030 model) applying a voltage in a range of 10 to 15 V; and iii) a ground copper collector (10 × 10) cm covered with aluminum foil and placed at different distances from the needle tip between 10 and 15 cm. The experiments were conducted under an average temperature of 22 °C and a relative humidity of 53%.

2.3. DoE for Optimization of PCL/Gelatin Electrospun Fibers

An initial screening to determine the levels of input and output variables was followed by an analysis based on response surface method to refine the model. In this case, Box-Behnken design (BBD) is the most favorable approach to fill the requirements for predictive models with three or more input variables as considered in the present work.[21] This methodology leads to the reduction of the number of experiments to run compared to other approaches since in this case the analysis is only based on central points.[22]

The model created considers as input variables (factors) the parameters of production which influence the mesh behavior and as output variables (responses) the ones used to measure the mesh properties. Both factors and responses were identified based on preliminary experiments and previous studies.[6] Thereby, four factors were selected: % of PCL, flow rate, distance needle-collector, and applied voltage (**Table 1**).

The responses were defined regarding the final purpose of the nanofiber structure that is skin regeneration. These variables include the fiber diameter, the thickness of the structure, and its permeability. **Table 2** shows the optimal conditions expected for each response regarding the purpose of this work which is to develop hybrid electrospun fibers from PCL-gelatin blending to be used as a wound dressing with controlled protein delivery.

As aforementioned, BBD was chosen as an efficient method to find the optimal conditions based on mathematical support. Therefore, a total of 27 experiments generated through Design

Table 1. Factors and respective levels used to optimize PCL/gelatin electrospun fibers production.

Factor	Low level	Med. level	Max. level
% of PCL	25	50	75
Flow rate [mL h^{-1}]	0.2	0.6	1.0
Distance [cm]	10	12.5	15
Applied voltage [kV]	10	12.5	15

Expert 11.0 software (StateEase, Minneapolis, MN, U.S.A.) were used to build the model. Below is presented the global second-order polynomial equation (Equation (1)):

$$r_k = \alpha_0 + \alpha_A A + \alpha_B B + \alpha_C C + \alpha_D D + \alpha_{AB} AB + \alpha_{AC} AC + \alpha_{AD} AD + \alpha_{BC} BC + \alpha_{BD} BD + \alpha_{CD} CD + \alpha_{AA} A^2 + \alpha_{BB} B^2 + \alpha_{CC} C^2 + \alpha_{DD} D^2, k \in \{1,2,3\} \quad (1)$$

Where output variables are represented by r_k and k range from 1 to 3 which corresponds to the number of different responses (diameter, thickness, and permeability), α represents the coefficients' values and the letters *A*, *B*, *C*, and *D* are a representation of the four input variables, % of PCL, flow rate, distance needle-collector, and applied voltage, respectively. The software finds the coefficients' values (α) in such equations and built the model to estimate the factor's levels which leads to an optimal response of nanofibers.[23]

After finding the coefficients, the experiment was reproduced to validate the model and verify the accordance between the values suggested by the software and the experimental results obtained for three samples produced on different days.

2.4. Characterization of PCL/Gelatin Nanofibers

2.4.1. Meshes Morphology and Fiber Diameter

The electrospun mesh morphology was observed by scanning electron microscope (SEM) using a VEGA3 (TESCAN) at a high-voltage of 15 kV. Prior examination samples were coated with gold/palladium thin film, by sputtering, using the sputter coater equipment (Quorum Technologies, UK). The open-source ImageJ-Fiji software was used to measure nanofiber diameters based on the analysis of the SEM images acquired at a magnification of 3000×. The diameter corresponds to the average of 50 measurements manually acquired for each condition ($n = 3$).

Table 2. Responses and the expected tendency for optimal PCL/gelatin electrospun fibers' production.

Response	Optimal point
Diameter [nm]	200 to 300
Thickness [μm]	Maximize
Permeability [g m^{-2} per day]	Maximize

2.4.2. Mesh Thickness

The average thickness of the electrospun matrix was measured using a micrometer (Mitutoyo digital micrometer, model: QuantuMike). This feature was calculated as the average of 5 measurements in different zones of the mesh ($n = 5$).

2.4.3. Water Vapor Permeability

The permeability of electrospun mesh to water vapor was calculated based on the ASTM E96 protocol.[24] According to the protocol a glass goblet was filled with 5 mL of distilled water, the aperture is covered by the nanofiber and the samples are placed in the incubator chamber (IKA KS 4000) for 24 h at 32 °C ($n = 5$). The formula to obtain permeability is shown in Equation (2):

$$\text{Permeability} = \Delta W/(A.t)(g/m^2/\text{day}) \quad (2)$$

Where ΔW represents the difference of weights of samples before and after being placed at the incubator, A is the area of the aperture, and t the time spent in the incubator.

2.4.4. Porosity

As previously mentioned, the high porosity of the mesh promotes cell proliferation and contributes to the healing process. Porosity was calculated based on a theoretical approach described by other authors.[25] To obtain the bulk density of the two polymers combined, the calculation was performed based on the weighted sum of both bulk densities (ρ_{bulk}) using mass fraction (w) of each polymer in the electrospinning solution, as shown in Equation (3):

$$\frac{1}{\rho_{\text{bulk(PCL/Gelatin)}}} = \frac{\rho_{\text{bulk}}}{w}(\text{PCL}) + \frac{\rho_{\text{bulk}}}{w}(\text{Gelatin}) \quad (3)$$

Consequently, porosity is assessed using the $\rho_{apparent}$ and the ρ_{bulk} of PCL and gelatin as shown in Equation (4):

$$\text{Porosity} = (1 - [\rho_{\text{apparent}}/\rho_{\text{bulk}}]) \times 100\% \quad (4)$$

Where $\rho_{apparent}$ is the apparent density expressed in the unit of g cm^{-3} and ρ_{bulk}, is the bulk density also expressed in the unit of g cm^{-3}.

2.5. Protein Release Study

2.5.1. BSA Incorporation

Hybrid electrospun fibers of PCL/gelatin were produced incorporating different amounts of BSA protein (2.5, 5, 7.5, 10, and 20 wt%) while previously determined production parameters of electrospinning were maintained constant. The incorporation of BSA was performed to quantify the protein release rate and to evaluate the capacity of the structure to encapsulate therapeutic agents.

2.5.2. Model Protein Release Profile

The protein-delivery study was based on the release rate of the standard protein BSA. For protein quantification, the BCA was used considering the supplier's recommendations.[26] Therefore, samples of PCL/gelatin (control) and PCL/gelatin/BSA (sample w/BSA) with a pre-determined percentage of BSA were produced and then submerged in phosphate-buffered saline (PBS) solution prepared with a pH of 7.4. Finally, the samples were stored in falcons fulfilled with 50 mL of PBS and placed in the incubator chamber operating at a temperature of 37 °C and a stirring of 100 rpm for 24 h. During the experiment, the falcons have been sealed in order to minimize potential PBS evaporation. Six different time-points (30 min, 1, 2, 4, 8, and 24 h) were considered to perform the BSA released measurements. The protein concentration was quantified based on absorbance values measured at 562 nm using a spectrophotometer (SPECTROstar Nano, BMG Labtech). To this purpose, a calibration curve was drawn (R-squared = 0.99) and the mass of BSA for each time point was calculated using Equation (5):

$$\text{BSA mass} = ([\text{sample } w/BSA] - [\text{control}])/\text{volume} \quad (5)$$

Where the BSA mass was calculated by subtracting the mass of protein in the control nanofibers, which is related only to gelatin, to the mass of protein in nanofibers with BSA, due to the presence of both gelatin and BSA.

3. Results and Discussion

3.1. DoE for Optimization of PCL/Ge Nanofibers

3.1.1. Design of the Model

A mathematical model was developed to find the optimal values for electrospinning parameters to access the previously mentioned desirable responses. After the screening phase, different possibilities to optimize surface responses such as Composite Central Face Design or BBD were considered. In this context, BBD was chosen due to the main characteristics of the model particularly, this method revealed to be effective and also a less time-consuming approach.[21,27]

The previous set of runs suggested by the Design-Expert software, were replicated, and responses were measured (Supporting Information contains the supplementary DoE data for this paper). To verify the reproducibility of the results, the central point is replicate in three runs and the agreement for each response was evaluated.

The multiple regression Equation (6) to Equation (8) resultant from the Design of Expert 11.0 provides the influence and interaction that the chosen inputs (PCL ratio (*A*), flow rate (*B*), distance needle-collector (*C*), and applied voltage (*D*)) have on the outputs (diameter, thickness, and permeability):

$$\begin{aligned}\text{diameter} = {} & 304.00 - 124.50A + 14.08B - 23.17C - 27.75D \\ & + 0.75AB - 0.75AC - 39.00AD - 12.25BC \\ & - 80.75BD + 52.50CD + 82.17A^2 \\ & - 27.71B^2 - 19.33C^2 + 62.04D^2\end{aligned} \quad (6)$$

$$\begin{aligned}\text{thickness} = {} & 53.33 + 0.83A + 16.67B - 6.67C + 9.17D \\ & + 7.50AB - 2.50AC + 2.50AD - 2.50BC \\ & + 5.00BD + 5.00CD - 13.75A^2 - 7.50B^2 \\ & + 5.00C^2 + 1.25D^2\end{aligned} \quad (7)$$

$$\begin{aligned}\text{permeability} = {} & 1473.33 - 21.92A - 16.92B - 68.33C + 62.83D \\ & + 32.75AB + 72.50AC - 60.00AD + 37.50BC \\ & + 107.50BD + 19.50CD - 94.92A^2 - 130.17B^2 \\ & - 200.54C^2 - 173.29D^2\end{aligned} \quad (8)$$

Through the analysis of the above-demonstrated equations is possible to observe the influence of factors in each response. In this regard, according to Equation (6), solution composition, in terms of the ratio of PCL, ($\alpha_A = -124.5$) is the most influent factor in fiber diameter followed by applied voltage ($\alpha_D = -27.75$). Other studies report the influence of production factors such as applied voltage or distance, in fiber diameter of electrospun nanofibers. However, this influence is strongly dependent on external conditions such as: the environmental conditions during the production; the polymers used in the solution; or the own factor levels adopted during the experiment.[14,21,28,29] Additionally, considering Equation (7), flow rate ($\alpha_B = +16.67$) revealed to be the factor with the most significant impact in fiber thickness, followed by applied voltage ($\alpha_D = +9.17$) and distance ($\alpha_C = -6.67$). Equation (8) refers to permeability and identify distance needle-collector ($\alpha_C = -68.33$) as the most influent parameter on its response, followed by the applied voltage. To our knowledge, no studies are reporting the use of DoE to predict the influence of production conditions in the thickness and permeability of PCL/gelatin electrospun nanofibers. In general, it was possible to observe that applied voltage presents an influent behavior in the considered responses with a positive impact on fiber thickness and permeability and a negative impact on fiber diameter.

3.1.2. Analysis of Variance

Multivariate statistics can use analysis of variance (ANOVA) to measure the covariance between random variables and confirm the statistical significance of the model for all responses. The results obtained in this experiment for ANOVA analysis are summarized in **Table 3**.

A careful analysis of ANOVA table confirms the statistical significance of all models since the *p*-value, which represents the probability of obtaining a test result equal or extremer than the results observed under the null hypothesis, showed values lower than 0.05 for diameter, thickness, and permeability.[30] Besides, the Model F-value, which results from regression analysis, was 2.85 for diameter, 2.90 for thickness, and 4.19 for permeability. Lack of Fit was also analyzed for the three models, 3.35 for fiber diameter, 1.41 for thickness, and 6.77 for permeability with a percentage of occurrence of 25.18%, 48.57%, and 13.55%, respectively. The significance of this parameter is dependent if the model cannot fit the data and, therefore, a more complex model should be used. Thereby, Lack of Fit revealed to be non-significant for the three models showing a *p*-value higher than 0.05, which means that according to these

Table 3. ANOVA for response surface quadratic model (BBD).

Response	Sum of squares	df	Mean of square	F-value	p-value	R^2
Diameter [nm]	3.292E+005	14	23 514.87	2.85	0.0384	0.7686
Thickness [μm]	7258.33	14	518.45	2.90	0.0358	0.7722
Permeability [per day]	4.970E + 005	14	35 501.56	4.19	0.0087	0.8300

parameters the model fits the experimental data. Furthermore, R^2 measures the percentage of variation in response explained by predictor variables, namely how close are the data to the regression line. In this case, the R^2 is 0.7686, 0.7722, and 0.8300 for fiber diameter, thickness, and permeability respectively, suggesting that the three models can be used to predict these responses.[31,32]

3.1.3. 3D Response Surface Plots

Figure 1 shows the interaction observed between the factors studied and the responses for the most relevant situations. The interaction between variables is represented by the color intensity and contour lines. Regarding the color scale, red represents the highest interaction and blue the lowest interaction between variables. Also, contour lines characterized the level of interaction between two variables, an elliptical form represents the highest zone in 3D surface plots and so, the highest interaction between variables.[31,32]

By comparing the surface plots obtained for the three responses (Supporting Information) is possible to observe that the interaction between factors was, in general, more pronounced for the fiber permeability response. In this case, the presence of red color and the ellipticity confirm the interaction between all factors. For fiber thickness, the more evident interactions are the ones between flow rate and distance and flow rate and the applied voltage. These results agree with the previous analysis based on the coefficients of multiple regression equations. In the case of the fiber diameter output, the interaction between factors is not so obvious being the blue color the predominant. The interaction between PCL ratio and applied voltage showed the most notorious interaction, which once again is consistent with the previously observed results from the multiple regression equation.

3.1.4. Model Validation

The model was then used to predict the optimal production conditions considering the main parameters defined. Regarding fiber's diameter, the nanofibers were characterized by their diameters' distribution to ensure high similarity with the skin extracellular matrix. This property is verified for diameters at a maximum of 300 nm and for this reason, the value was fixed between 200 and 300 nm as a boundary condition for the model. Also, a common 2D electrospun mesh will not be able to significantly improve the healing process for depth wound and to avoid an excessive burst release of therapeutic agents. Therefore, mesh thickness should be maximized to obtain 3D structures.[33] Finally, to ensure the healing process, the maximum mesh permeability was favored.[1,2,5] To achieve these requirements, **Table 4** shows the levels of input variables predicted by the software for optimal production of PCL/gelatin nanofibers.

Regarding this information, **Table 5** shows the confidence interval and the obtained results from the application of the optimal conditions in the electrospun nanofibers.

The experimental results obtained for the optimal point were compared with the predicted measures and shown in Table 5. Considering a confidence interval of 95%, the results for all three responses are within the tolerance range and consequently following the model which confirms its reproducibility.

3.2. Characterization of PCL/Gelatin Nanofibers

The nanofibers produced at the optimal conditions were characterized according to the performance tests above mentioned in the previous section and reported in literature.[34,35] Table 5 summarizes the experimental results obtained for each output. Also, the theoretical calculation of porosity was included in the analysis of the performance of these nanofibers to verify the capacity of nanofibers to soak wound exudate and promote cell seeding.[2,36]

The results showed an average fiber diameter of 369 ± 158 nm which is in concordance with the predicted value, however, the experimental estimation is very close to the value of CI high. In fact, the accuracy of the model for prediction of fiber diameter was the lowest value obtained for the three responses considered. Some possible explanations could be related to the uncertainty of the manual measurements performed combined with the pixel resolution in the acquired images. Therefore, the analysis of nanofibers' morphology shown a random arrangement of nanofibers which demonstrates that the use of hybrid meshes of PCL/gelatin mimics the structure of the skin and contributes to increasing the efficiency of the healing process improving the regeneration of the cellular matrix.[2] A representation of the morphology of PCL/gelatin nanofibers and the distribution of fiber's diameters are shown in **Figure 2**.

Besides, the theoretical calculation based on the experimental measurements described in Equation (3) revealed 98% of porosity. High values of porosity are required since during the inflammatory process excess wound exudate can be produced being important to maintaining wound hydration and the required moisture levels.[36]

Also, the barrier function of the mesh should be ensured, since it avoids wound infection caused by the action of pathological external agents while favorable exchanges of oxygen and water with the external medium are allowed.[36] This ability is assessed by measuring the permeability of the mesh which shows an average value of 1474 ± 52 g m^{-2} per day. High values

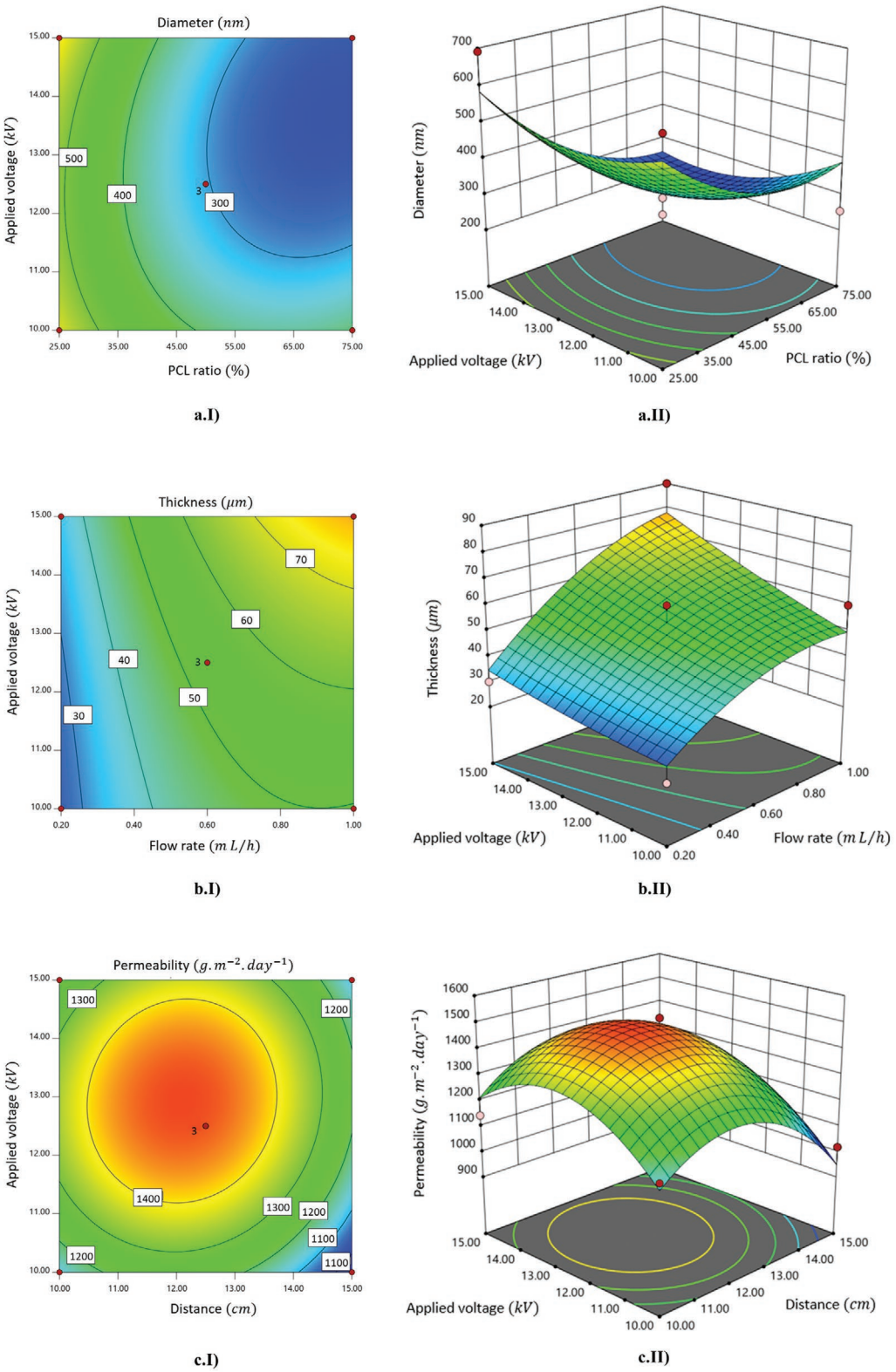

Figure 1. Contour (I) and respective surface (II) plots related to multiple regression equation recorded from a statistical model developed and which represents the interaction between different factors and its influence on the defined outputs (a-diameter; b-thickness, and c-permeability).

of water vapor permeability are mentioned as able of promoting wound healing.[37]

Finally, it is also relevant to describe that cell proliferation is enhanced by increasing the thickness of wound dressings. In

Table 4. Predicted factors (% of PCL, flow rate, distance needle-collector, and applied voltage) for optimal PCL/gelatin nanofiber production.

Factors	Level
% of PCL	50.10
Flow rate [mL h^{-1}]	0.82
Distance needle-collector [cm]	11.91
Applied voltage [kV]	14.11

this case, the nanofibers produced shown an average thickness of 65 ± 24 µm. Therefore, the treatment of deep wounds uses skin substitutes and other approaches of increased thickness.[33]

3.3. Protein Release Study

3.3.1. BSA Incorporation

BSA was used to study the release behavior of nanofibers to test their further ability to deliver therapeutic agents for wound treatment. Therefore, the maximum amount of BSA loaded was evaluated based on the analysis of previously defined responses of diameter, thickness, and permeability to ensure that the required characteristics of such variables were maintained. **Figure 3** shows the results for the set of samples of PCL/gelatin nanofibers prepared with different concentrations of BSA and produced considering the optimal conditions predicted by the model developed.

BSA incorporation experiments demonstrated that low concentrations of protein reduce the fiber diameter. However, increasing the concentrations of protein, leads to a growing trend in diameter, thickness, and permeability values, and consequently predefined limits are exceeded. This effect, previously reported in other studies using BSA, is particularly disadvantageous in fiber's diameter since as mentioned, the favorable performance is achieved for values lower than 300 nm.[38] Besides, difficulties in protein dissolution for concentrations above 7.5 wt% led to irregularities in the normal operation of electrospinning process which were probably caused by an increase in solution viscosity.[2] According to the results obtained and considering the requirements imposed, nanofibers of PCL/gelatin/BSA produced for a concentration of BSA of 2.5 wt% revealed the closer agreement with the pre-defined limits for fiber's diameter, mesh's thickness, and permeability. **Table 6** shows the results obtained for optimal concentration of BSA.

For this condition, the produced nanofibers show the average diameter of 270 ± 91 nm that is lower than 300nm and high values of the thickness 60 ± 30 µm and permeability 1789 ± 69 g m^{-2} per day that ensure the maintenance of electrospun meshes performance.

Table 5. Predicted point and experimental results for PCL/gelatin nanofiber production.

Response	Predicted	95% CI low	95% CI high	Obtained values	Validate
Diameter [nm]	279.3	179.83	378.85	369 ± 158	Yes
Thickness [µm]	70.0	55.37	84.63	65 ± 24	Yes
Permeability [g m^{-2} per day]	1427.1	1326.24	1527.96	1474 ± 52	Yes

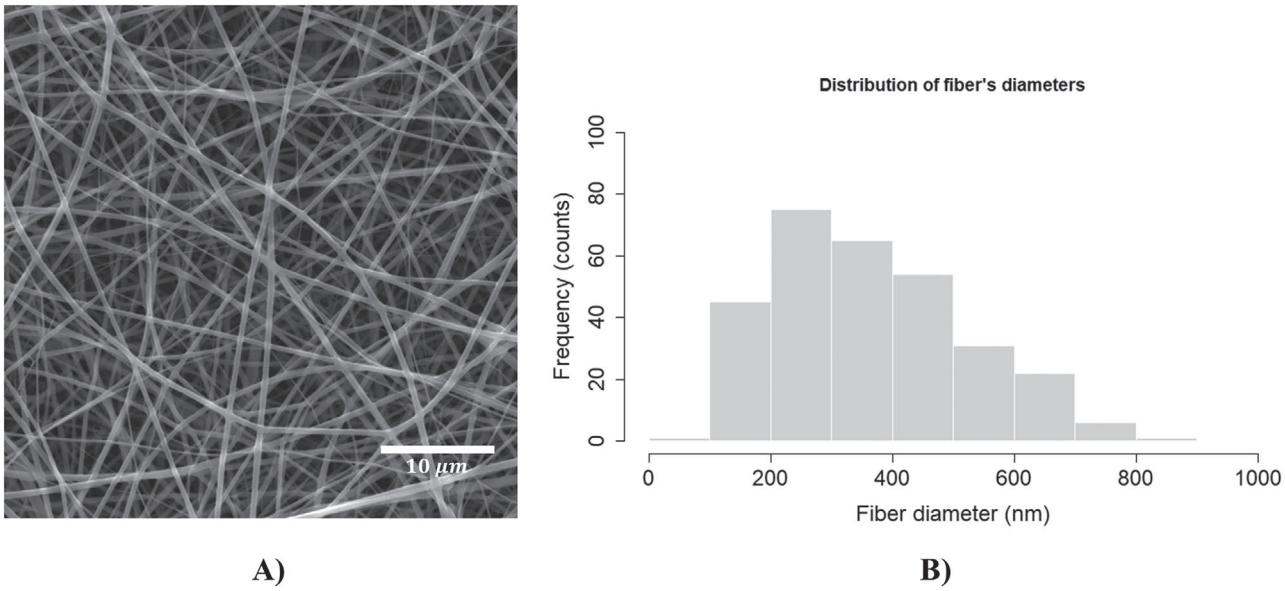

Figure 2. A) SEM image of morphological appearance and B) distribution of diameters of PCL/gelatin nanofibers produced under the same conditions of the ratio of PCL, flow rate, applied voltage, and needle's working distance (50%, 0.8 mL h^{-1}, 11.9 cm and 14.1 kV respectively).

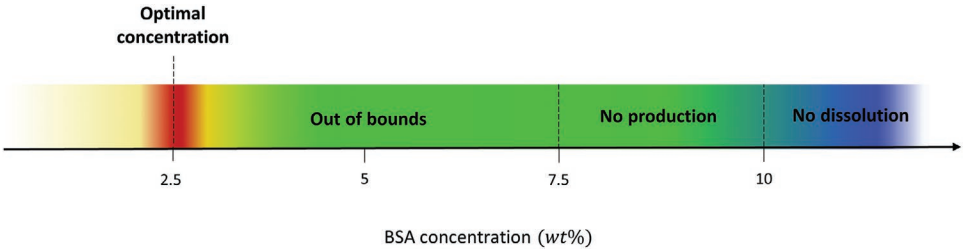

Figure 3. Representation of BSA incorporation results in PCL/gelatin nanofibers for different concentrations of protein.

3.3.2. Model Protein Release Profile

Therefore, **Figure 4** shows the obtained protein release profile for the produced hybrid nanofibers considering the electrospinning conditions previously predicted by the developed model.

By observing Figure 4 and regarding the theoretical initial amount of BSA loaded (which was nearly 10 mg per sample) it is possible to conclude that almost half of the total amount of protein was released during the first 24 h. The value of 10 mg per sample is an estimated value calculated based on the amount of BSA incorporated in the solution (2.5%), the flow rate used (0.82 mL h^{-1}), the time of deposition (10 min), and the area of the sample (100 cm^2). Besides, a burst release of BSA was observed during the first 4 h of the experiment being the protein delivered at a rate of 1 mg h^{-1}.

Several theories have been exploited to explain burst release phenomenon based on potential influencing factors as the compatibility of drugs and polymers, nanofibers morphology, or medium characteristics.[39–41] In this particular case, the burst release effect can be explained due to the presence of more charged groups in proteins comparing to PCL which leads to a migration of these compounds during the electrospinning process to the nanofiber's surface and, consequently, accelerate BSA release.[39,42]

This behavior presents an advantage when an initial therapeutic effect is required with a continuous release of drug during the first hours. However, this is a standard and quick approach used during the initial phases of characterization studies working as a comparison tool among similar analysis. For this reason, smooth changes can probably be observed when other therapeutic agents will be considered.

4. Conclusions

The present research work presents a statistical model to predict the performance of PCL/gelatin electrospun nanofibers used in wound dressing applications as drug delivery system. The performance of such nanofibers was evaluated regarding fiber's diameter, thickness, and permeability of the mesh. The production parameters used during the electrospinning process namely, the applied voltage, the needle-working distance, the flow rate, and the ratio of PCL in the solution were studied using a DoE approach. This methodology used these variables as the inputs of the model to predict the desirable response. The global model showed statistical significance ($p < 0.05$) for all the outputs. Also, statistical analysis releveled that 76.86%, 77.22%, and 83% of the dataset is explained by the model for the three performance parameters above-mentioned, respectively. Besides, independent experiments validate the optimal point predicted by the model. Finally, the protein release study suggested the capacity of PCL/gelatin mesh to be used as a drug carrier to treat skin wounds taking into account the release profile obtained. This study showed a constant release of the therapeutic agent from the polymeric meshes produced during the first hours of action and a release profile compatible with final proposal for wound dressings. Although electrospinning is usually described as an unstable and multi-variable dependent process, it was possible through these indicators to demonstrate the model robustness under the same production conditions. Additionally, the methodology applied to this research work demonstrated to be a strong alternative to decrease the time spent during the optimization process which is described as one of the major limitations to the use of electrospinning in an industrial scale. As a final result, the mathematical model developed statistically suggests and describes the interaction between the production parameters and the responses evaluated. This particularity becomes useful to better understand the key influencers in the process and which conditions are critical for each response. Overall, the DoEs showed to be a valuable tool for the optimization of electrospun mesh properties.

Table 6. PCL/gelatin/BSA nanofibers' characteristics produced at the optimal point for 2.5 wt% of BSA.

Diameter (nm)	Thickness (μm)	Permeability (g m–2 per day)
270 ± 91	60 ± 30	1789 ± 69

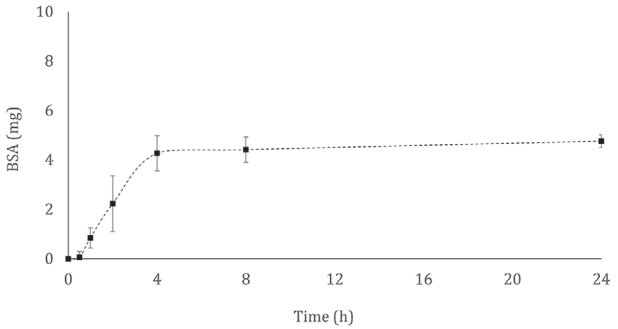

Figure 4. BSA release profile in PCL/gelatin nanofibers produced by electrospinning considering the conditions defined by the developed model.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

Research data are not shared.

Keywords

design of experiments, drug delivery systems, electrospinning, wound dressing

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