Supporting information

AI-Driven Optimization of PCL/PEG Electrospun Scaffolds for Enhanced *In vivo* Wound Healing

Katarina Virijević[1], Marko N Živanović[1*], Dalibor Nikolić[1], Nevena Milivojević[1], Jelena Pavić[1], Ivana Morić[2], Lidija Šenerović[2], Luka Dragačević[3], Philipp J. Thurner[4], Manuel Rufin[4], Orestis G. Andriotis[4], Biljana Ljujić[5], Marina Miletić Kovačević[6], Miloš Papić[7], Nenad Filipović[8, 9]

[1] Institute for Information Technologies, University of Kragujevac, Kragujevac 34000, Serbia
[2] Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade 11000, Serbia
[3] Institute of Virology, Vaccines and Sera "Torlak", Belgrade 11000, Serbia
[4] Institute of Lightweight Design and Structural Biomechanics, TU Wien, Wien 1060, Austria
[5] Department of Genetics, Faculty of Medical Sciences, University of Kragujevac, Kragujevac 34000, Serbia
[6] Department of Histology and Embryology, Faculty of Medical Sciences, University of Kragujevac 34000, Kragujevac, Serbia
[7] Department of Dentistry, Faculty of Medical Sciences, University of Kragujevac, Kragujevac 34000, Serbia
[8] Faculty of Engineering, University of Kragujevac, Kragujevac 34000, Serbia
[9] BioIRC - Bioengineering Research and Development Center, Kragujevac 34000, Serbia

[*]Corresponding author: Marko N Živanović, senior research associate
marko.zivanovic@uni.kg.ac.rs

Data mining research

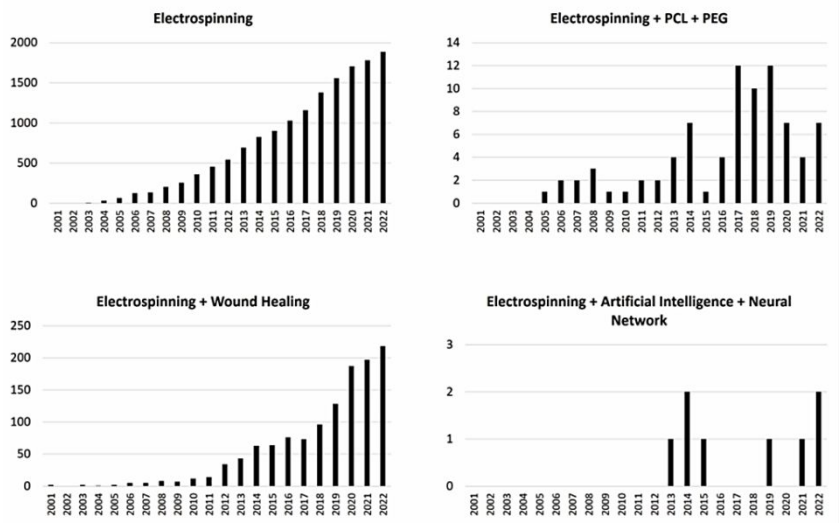


Figure S1. Publication Trends in "Electrospinning", "Electrospinning + PCL + PEG", "Electrospinning + Wound Healing" and "Electrospinning + Artificial Intelligence + Neural Network" Research (2001-2022)

Table S1. Synonyms and Related Terms for Electrospinning in Research (2001-2022)

Item search	Nr of Items 2001-2022
Electrospinning + AI + NN + Wound Healing	0
Electrospinning + AI + NN + PCL	2
Electrospinning + AI + NN + PEG	0
Electrospinning + AI + NN + PCL + PEG	0
Electrospinning + Wound Healing + PCL	228
Electrospinning + Wound Healing + PEG	23
Electrospinning + Wound Healing + PCL + PEG	7

Neural network input/output data

To facilitate a deeper understanding of the ANN model presented in the paper, in the supplementary section, we will provide the complete code required for its replication within the MATLAB environment. In the Table S2 presents the structure of the input file, which contains all the values needed to train such a neural network (this table is an example of the input data structure and contains only 2 rows of input data, all the data from the experiment were used to train the network).

Table S2. Input data structured for ANN – CSV data file

PCL	PEG	CHCL3	DMF	Q [ml/h]	U [kV]	Diameter [nm]
0.1700	**0**	**1**	**0**	**0.800**	**20**	**1029**
0.1800	**0**	**1**	**0**	**0.750**	**15**	**890**
…	**…**	**…**	**…**	**…**	**…**	**…**

MATLAB functions:

Importing the data in MATLAB from a structured CSV table (example was presented in Table S2):

```
data = readmatrix("Import_data.csv");
x = data(:, 1:6);  %import input data
y = data(:, 7);    %import target values
m = height(y);     %get no rows
```

Visualization of the data:

```
%Plot: IN1[PCL] vs OUT
plot(x(:,1),y, 'o')
xlabel('In [%]')
ylabel('Out - Diameter[nm]')
title('Plot: IN1[PCL] vs OUT')
%--
%Plot: IN2[PEG] vs OUT
plot(x(:,2),y, 'o')
xlabel('In [%]')
```

```
ylabel('Out - Diameter[nm]')
title('Plot: IN2[PEG] vs OUT')
%--
%Plot: IN3[CHCL3] vs OUT
plot(x(:,3),y, 'o')
xlabel('In [%]')
ylabel('Out - Diameter[nm]')
title('Plot: IN3[CHCL3] vs OUT')
%--
%Plot: IN4[DMF] vs OUT
plot(x(:,4),y, 'o')
xlabel('In [%]')
ylabel('Out - Diameter[nm]')
title('Plot: IN4[DMF] vs OUT')
%--
%Plot: IN6[Flow] vs OUT
plot(x(:,5),y, 'o')
xlabel('In [mL/h]')
ylabel('Out - Diameter[nm]')
title('Plot: IN6[Flow] vs OUT')
%--
%Plot: IN7[Voltage] vs OUT
plot(x(:,6),y, 'o')
xlabel('In [kV]')
ylabel('Out - Diameter[nm]')
title('Plot: IN7[Voltage] vs OUT')
%--
```

Plotting results in the form of graphs are presented in Figure S2.

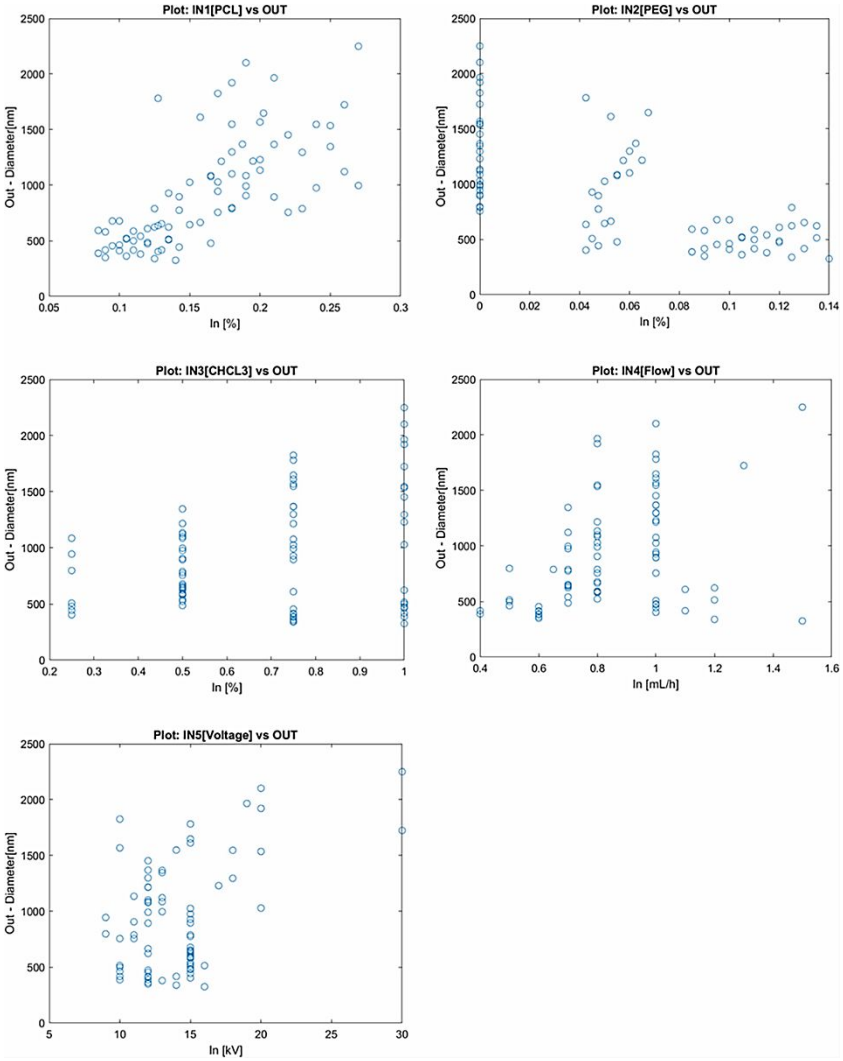


Figure S2. Basic visualization of the dependence of output data (vertical axis) on individual input data (horizontal axis).

Train an artificial neural network (ANN) for the first time:

```
xt = x';
yt = y';
hiddenLayerSize = [10]; % The number of neurons in the hidden layer is chosen
based on experience
net = fitnet(hiddenLayerSize); %setup hidden layer size
net.divideParam.trainRatio = 70/100; %Divide input data for training set
net.divideParam.valRatio = 20/100; %Divide input data for validation set
net.divideParam.testRatio = 10/100; %Divide input data for testing set
[net, tr] = train(net, xt, yt); %Training network
view(net);
```

Performance of the ANN network

```
yTrain = net(xt(:,tr.trainInd));
yTrainTrue = yt(tr.trainInd);
%RMSE of predicted output for trainig set
sqrt(mean((yTrain - yTrainTrue).^2))

yVal = net(xt(:,tr.valInd));
yValTrue = yt(tr.valInd);
%RMSE of predicted output for validation set
sqrt(mean((yVal - yValTrue).^2))

yTest = net(xt(:,tr.testInd));
yTestTrue = yt(tr.testInd);
%RMSE of predicted output for test set
sqrt(mean((yTest - yTestTrue).^2))
```

Optimize the number of neurons in the hidden layer

```
for i = 1:1:60
    %defining the architecture of the ANN
    hiddenLayerSize = i;
    net = fitnet(hiddenLayerSize);
    net.divideParam.trainRatio = 70/100;
    net.divideParam.valRatio = 20/100;
    net.divideParam.testRatio = 10/100;

    %traning the ANN
    [net, tr] = train(net, xt, yt);

    %determing the error of the ANN
    yTrain = net(xt(:,tr.trainInd));
    yValTrue = yt(tr.valInd);
    yTrainTrue = yt(tr.trainInd);
    yVal = net(xt(:,tr.valInd));

    %RMSE of predicted output for trainig set
    rmse_train(i) = sqrt(mean((yTrain - yTrainTrue).^2));
    %RMSE of predicted output for validation set
    rmse_val(i) = sqrt(mean((yVal - yValTrue).^2));
end
```

Select optimal number of the hidden layer

```
plot(1:60, rmse_train); hold on;
plot(1:60, rmse_val); hold off;
```

The results of the optimization of the number of neurons in the hidden layer are presented in Figure S4 of this paper. The best prediction is obtained with 8 neurons in the hidden layer, i.e. the network is neither overfitting and underfitting. The next step is retraining the network with this number of neurons in the

hidden layer and analyzing the performance of such a system. The code and how to train the network has already been explained, so further code will be shown to visualize the results of the ANN prediction model:

Visualize the prediction from the final ANN model

```
plot(yTrainTrue, yTrain, 'x'); hold on;
plot(yValTrue, yVal, 'o');
plot(0:2500, 0:2500); hold off;
```

The prediction results are satisfactory and the graphic representation is given in the methods of this paper in Figure S5.

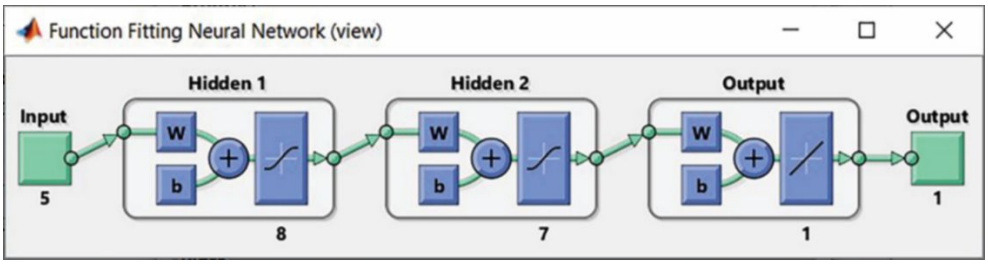


Figure S3. Schematic representation of the neural network

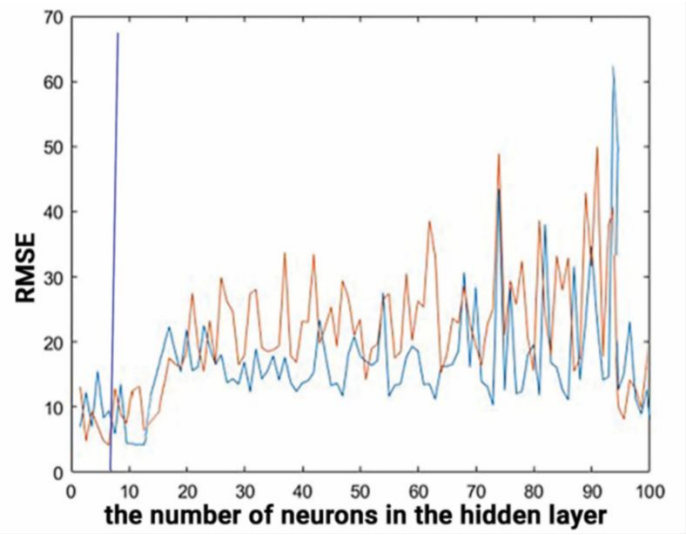


Figure S4. Graph of RMSE relation between training data set (blue line) and validation data set (orange line) depending on the number of neurons in the hidden layer

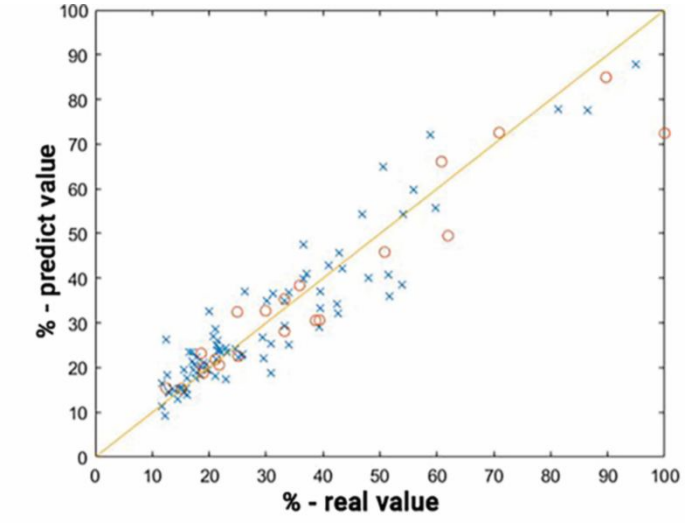
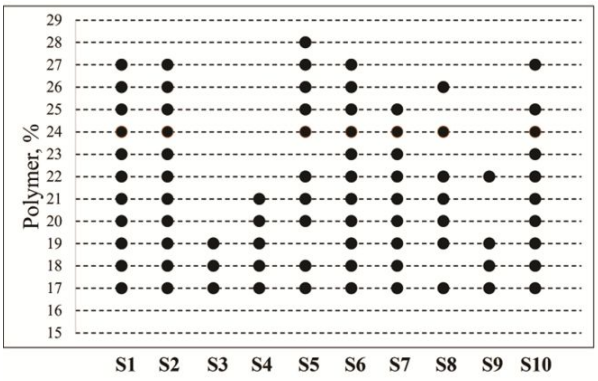


Figure S5. Visual representation of ANN precision; the horizontal axis represents the percent of the real result, and the vertical axis represents the percent of ANN prediction; the training set (blue dots), prediction set (orange dots)

All electrospinning-compatible polymer-solvent combinations



Polymer series	Substance	Solvent
Series 1	PCL	$CHCl_3$
Series 2	PCL	$CHCl_3$:DMF = 1:1
Series 3	PCL	$CHCl_3$:DMF = 1:3
Series 4	PCL	$CHCl_3$:DMF = 3:1
Series 5	PCL:PEG = 1:1	$CHCl_3$
Series 6	PCL:PEG = 1:1	$CHCl_3$:DMF = 1:1
Series 7	PCL:PEG = 1:1	$CHCl_3$:DMF = 3:1
Series 8	PCL:PEG = 3:1	$CHCl_3$:DMF = 1:1
Series 9	PCL:PEG = 3:1	$CHCl_3$:DMF = 1:3
Series 10	PCL:PEG = 3:1	$CHCl_3$:DMF = 3:1

Scheme S1. Electrospinning-Ready Polymer and Solvent Combinations. "Substance" is PCL or PCL combined with PEG. The substance is dissolved in mass concentrations from 17 to 28% in $CHCl_3$ or a combination of $CHCl_3$ and DMF.

Fiber Distribution for all polymer combinations

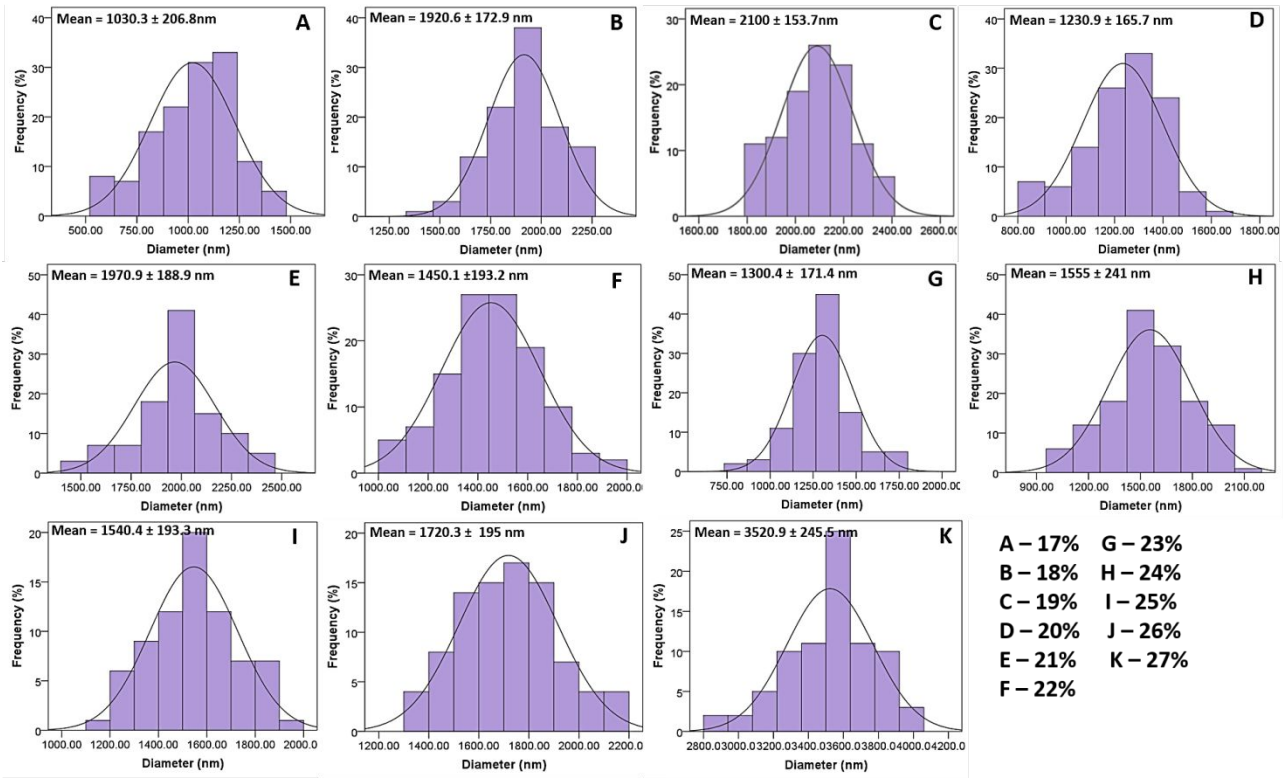


Figure S6. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 1**: PCL in $CHCl_3$. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25% J) 26% K) 27%

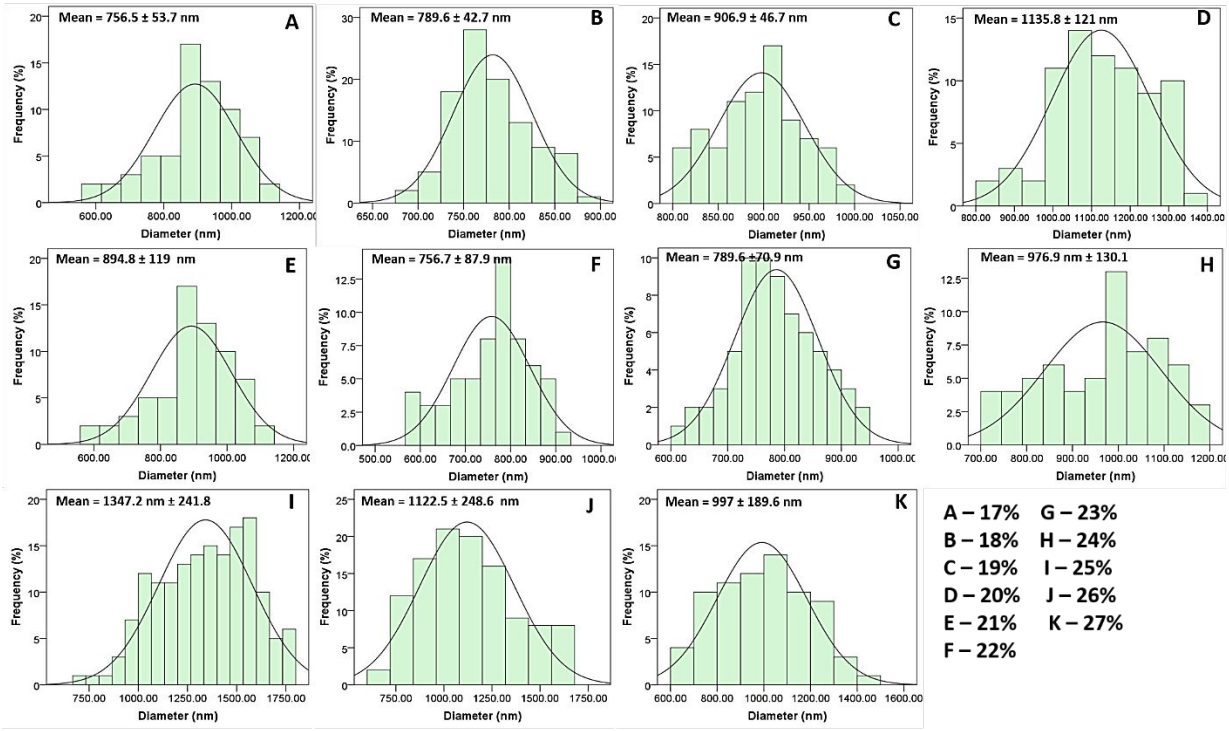


Figure S7. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 2**: PCL in $CHCl_3$:DMF=1:1. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25% J) 26% K) 27%

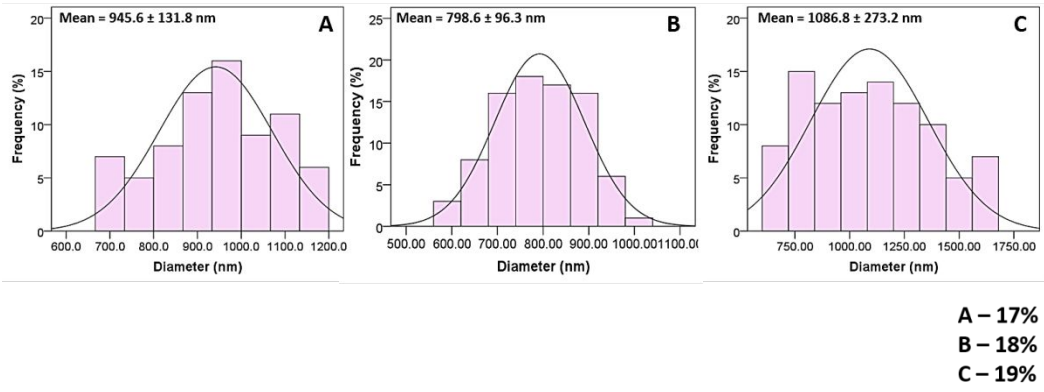


Figure S8. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 3**: PCL in $CHCl_3$:DMF=1:3. A) 17% B) 18% C) 19%

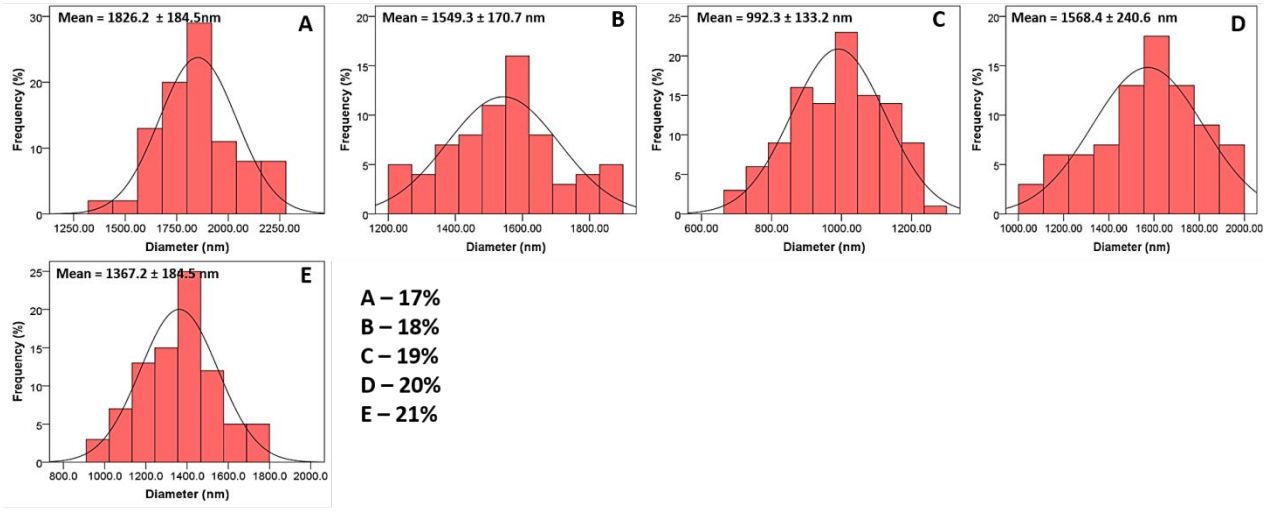


Figure S9. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 4**: PCL in $CHCl_3$:DMF=3:1. A) 17% B) 18% C) 19% D) 20% E) 21%

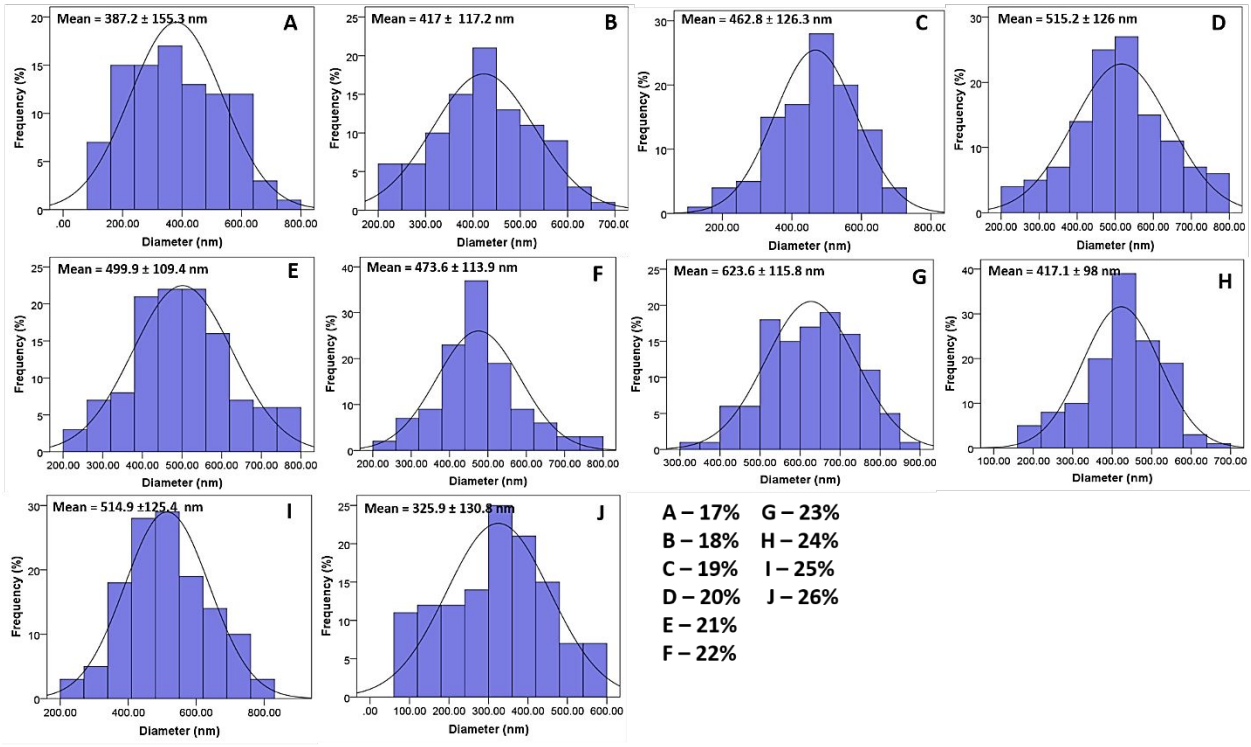


Figure S10. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 5**: PCL:PEG=1:1 in $CHCl_3$. A) 17% B) 18% C) 20% D) 21% E) 22% F) 24% G) 25% H) 26% I) 27% J) 28%

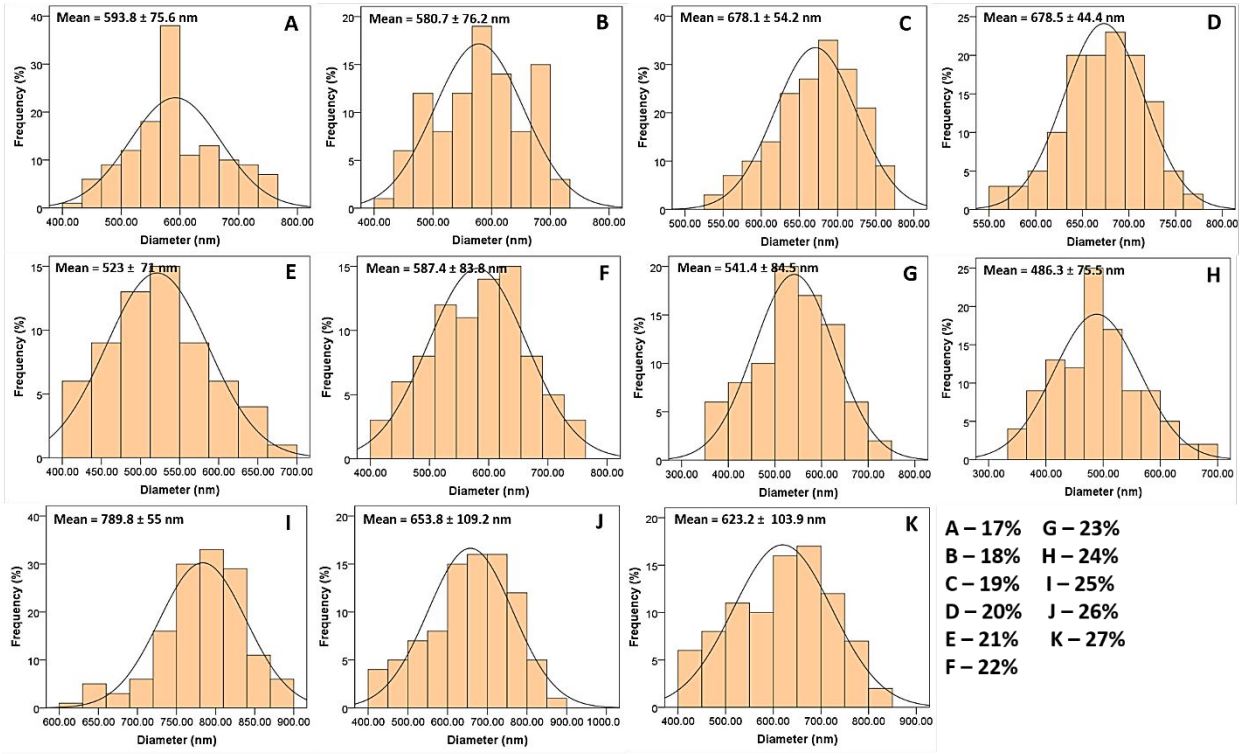


Figure S11. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 6**: PCL:PEG=1:1 in $CHCl_3$:DMF=1:1. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25% J) 26% K) 27%

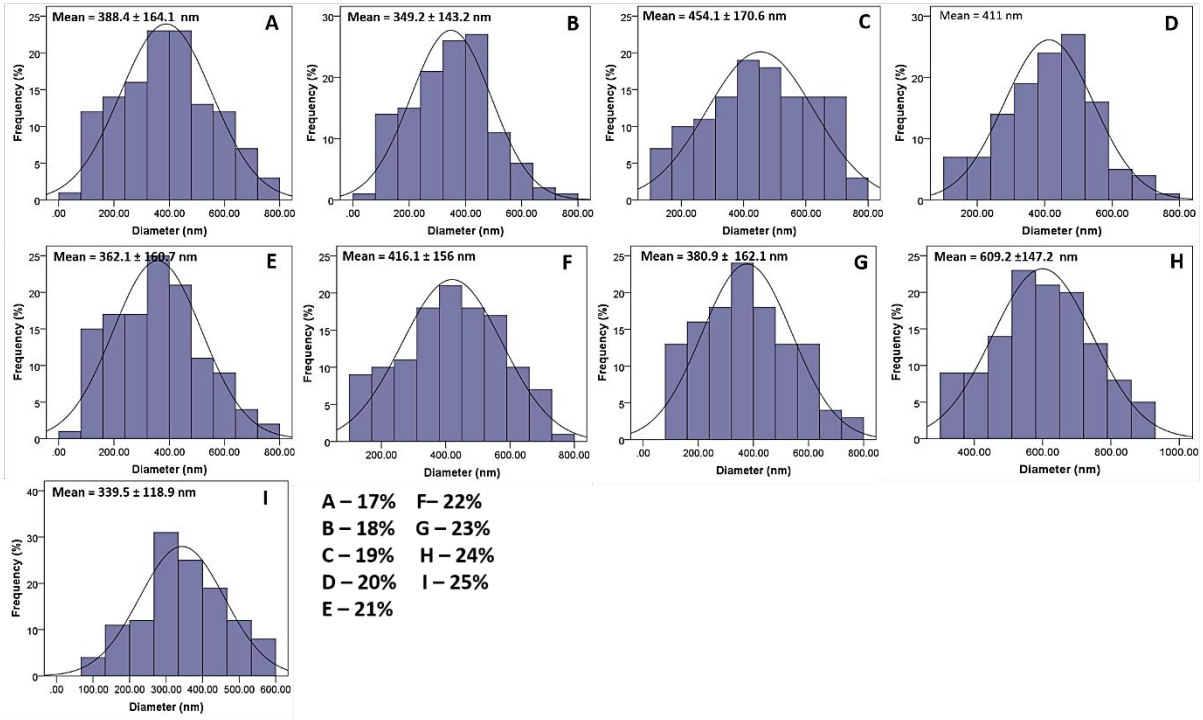


Figure S12. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 7**: PCL:PEG=1:1 in $CHCl_3$:DMF=3:1. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25%

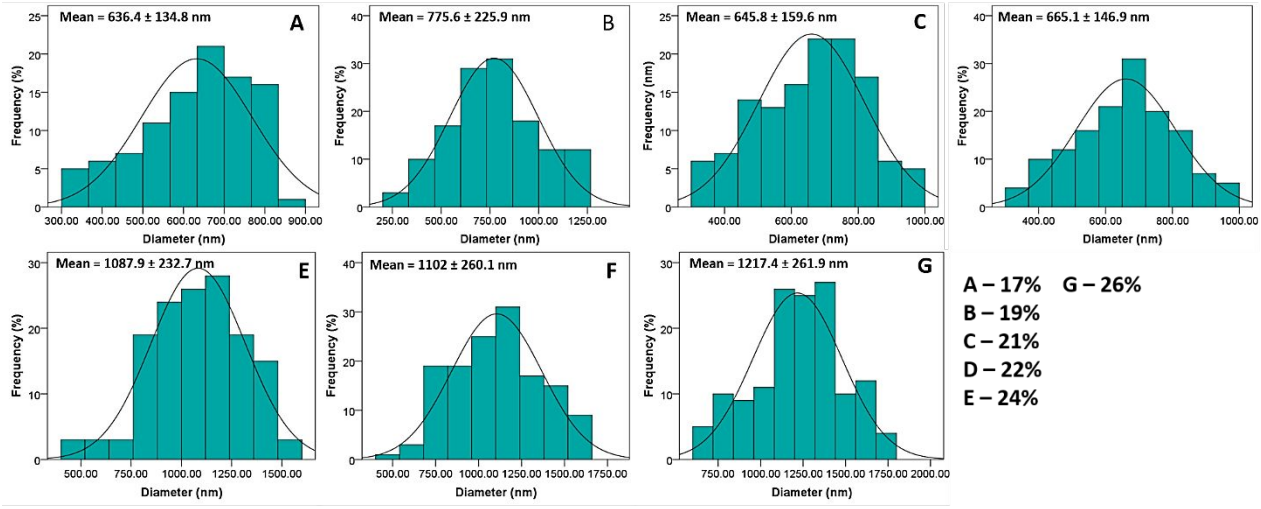


Figure S13. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 8**: PCL:PEG=3:1 in $CHCl_3$:DMF=1:1. A) 17% B) 19% C) 20% D) 21% E) 22% F) 24% G) 26%

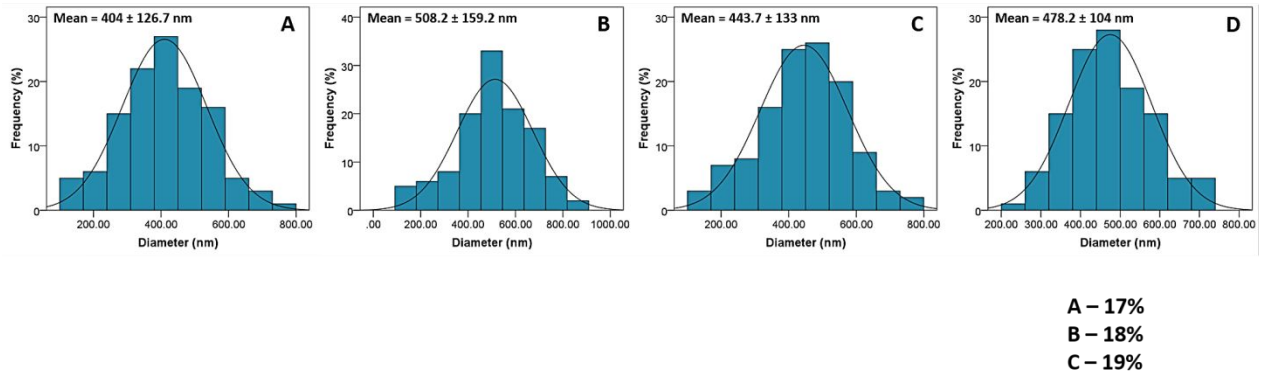


Figure S14. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 9**: PCL:PEG=3:1 in $CHCl_3$:DMF=1:3. A) 17% B) 18% C) 19% D) 22%

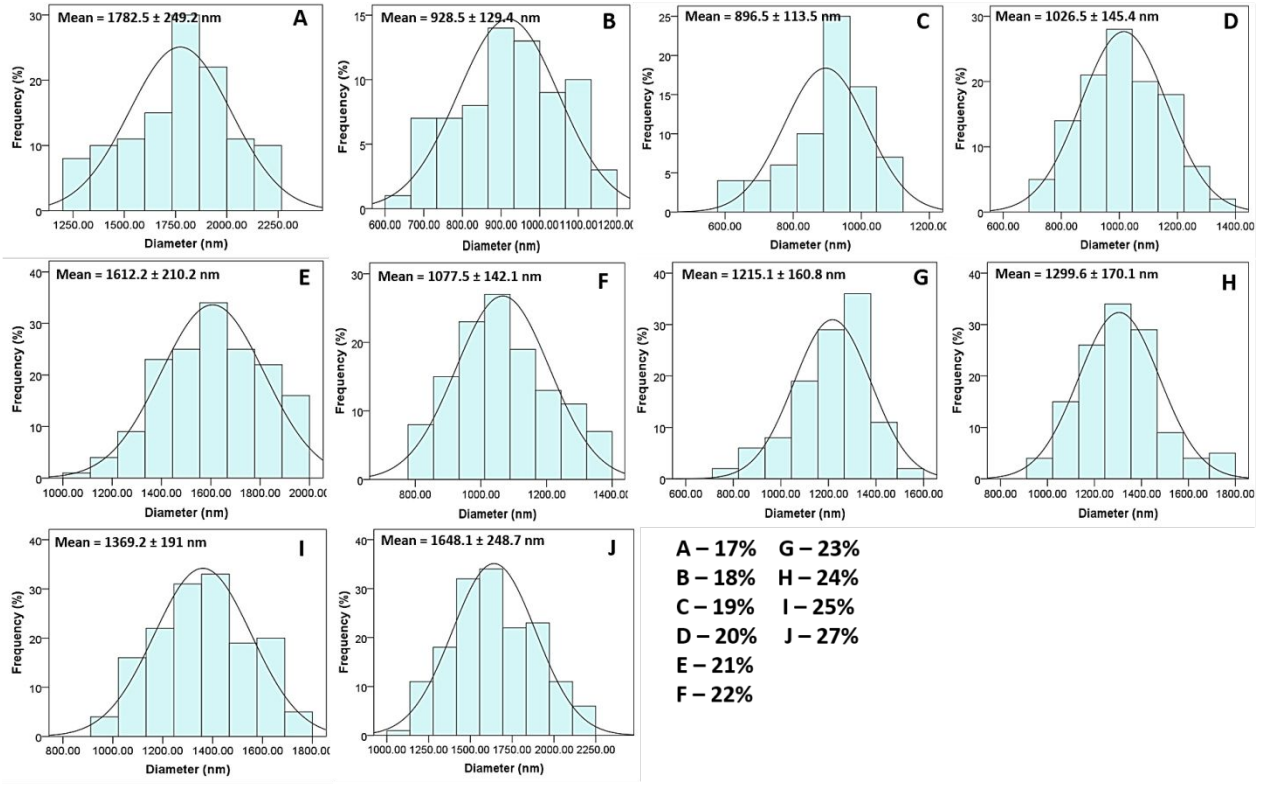


Figure S15. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 10**: PCL:PEG=3:1 in $CHCl_3$:DMF=3:1. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25% J) 26%

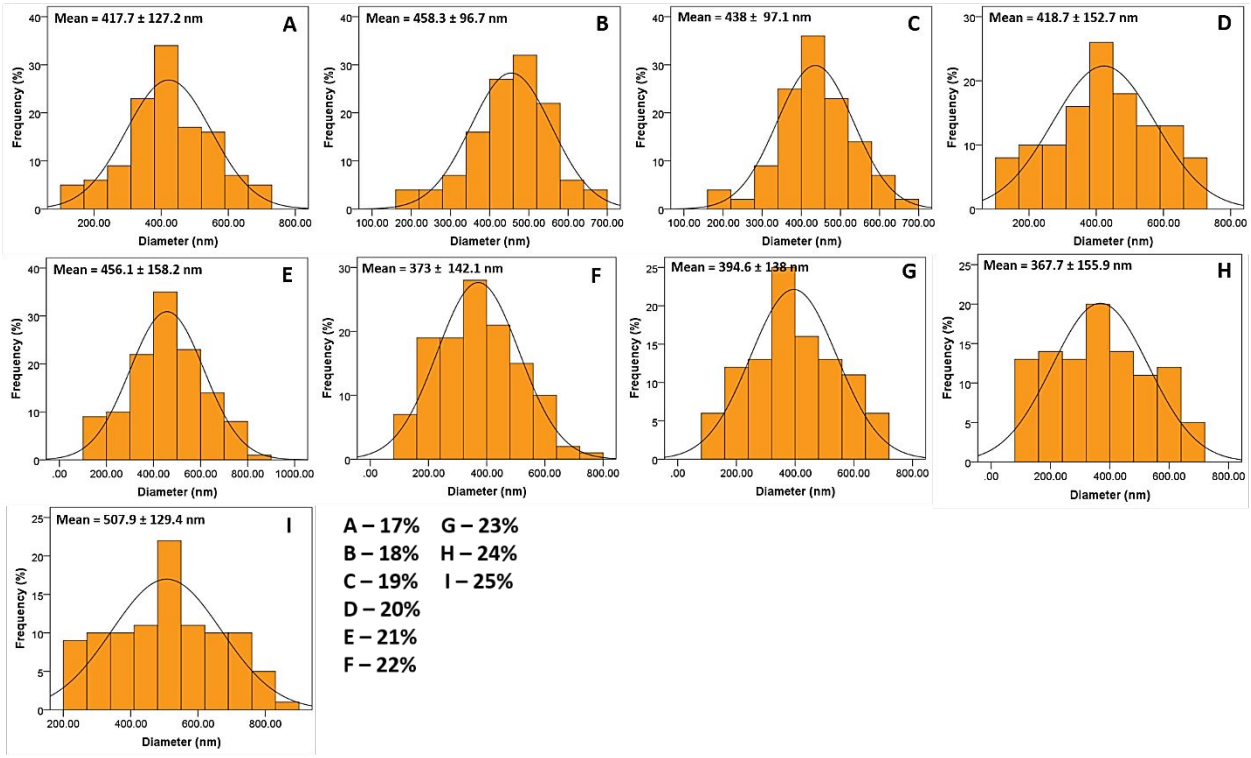


Figure S16. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 15**: PCL:PEG=1:3 in $CHCl_3$:DMF=3:1. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25%

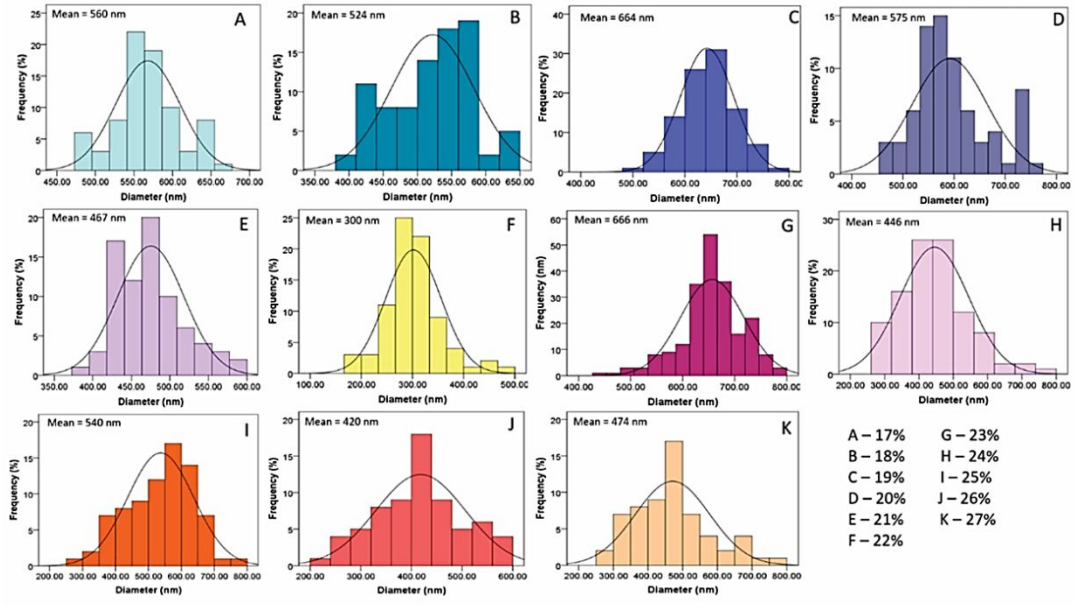


Figure S17. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 16**: PCL:PEG=7:3 in $CHCl_3$:DMF=7:3. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22% G) 23% H) 24% I) 25% J) 26% K) 27%

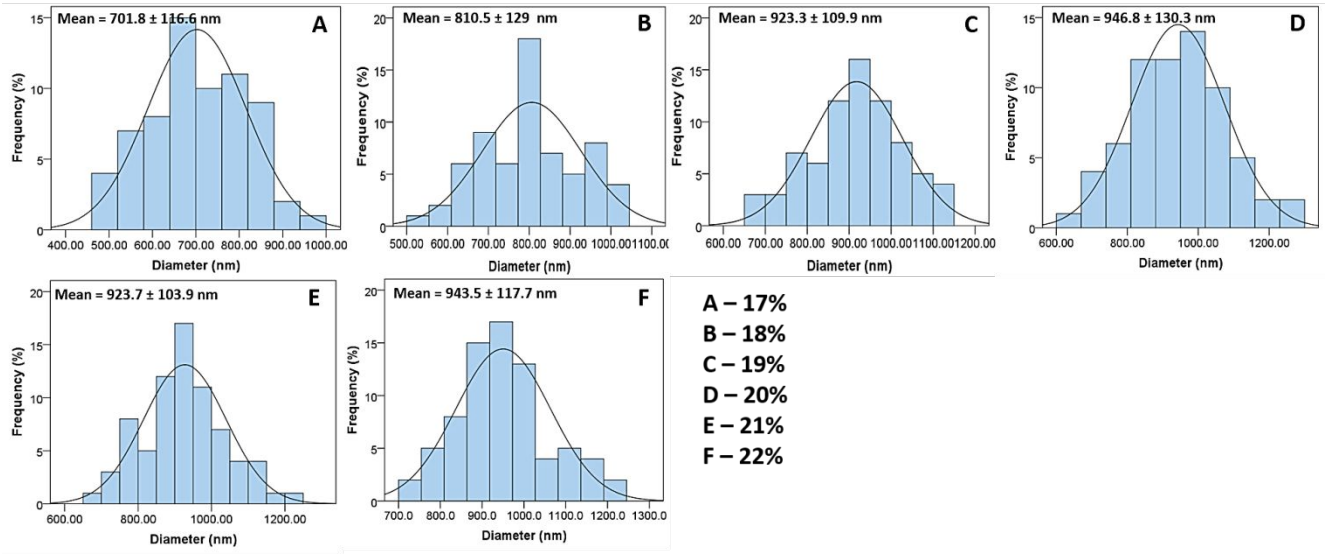


Figure S18. Fiber Diameter Distribution Analysis of Electrospun-Derived Scaffold for **Series 17**: PCL:PEG=3:1 in $CHCl_3$. A) 17% B) 18% C) 19% D) 20% E) 21% F) 22%

Microbiological evaluation of tested samples

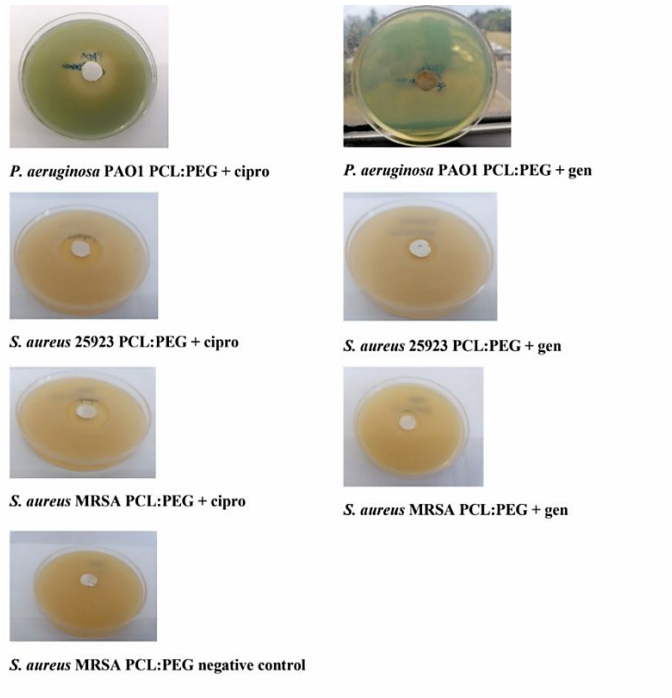


Figure S19. The action of scaffolds bearing antibiotics on selected strains of bacteria by disk diffusion method.

In vivo estimation (Chick embryo chorioallantoic membrane (CAM) assay)

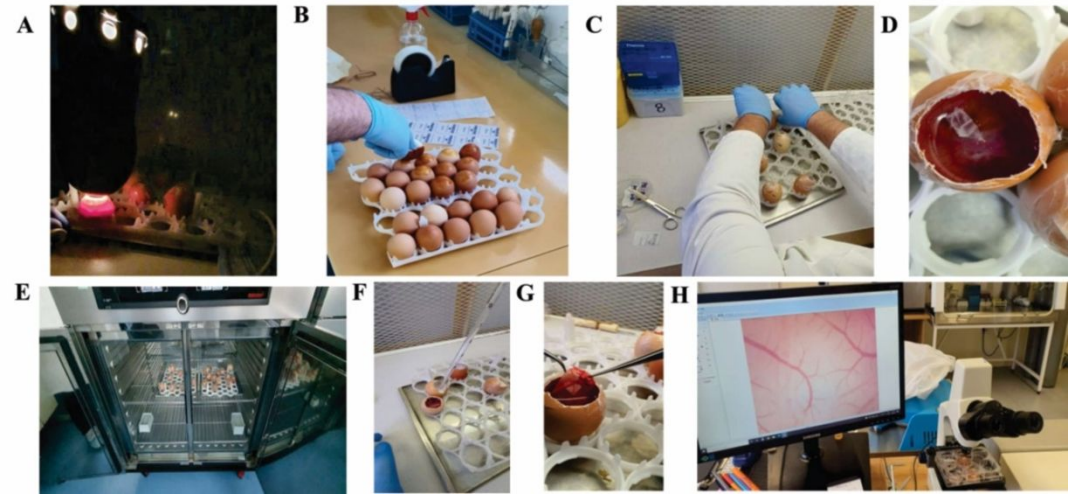


Figure S20. Chick Embryo CAM Assay Procedure: A) Egg selection B) Egg disinfection with 10% of iodine solution C) Inoculation and preparation for scaffold insertion D) Egg's incubation E) Daily monitoring of embryo development and possible contamination F) Sacrifice of treated embryos and fixation with 4% PFA G) CAM membrane preparation H) Image capture and evaluation of blood vessels