Optimization of electrospinning parameters and degradation properties for Gelatin/CMC nanofibers

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Abstract. This research aimed to enhance strength of Nanofiber like Biopolymer as raw material for characterization which is popularly used in food and cosmetic industry and as medical material because it did not hazardously affect human body. This study employed Nano fiber characterization with Electrospinning Method by using gelatin/CMC, common and inexpensive biopolymer with cellulose that could strengthen Nanofiber. The fabrication of fiber scaffold by electrospinning method produced nano-fiber by dissolving gelatin in organic solvent, 2,2,2-trifluoroethanol, and dissolving CMC in DI water. The design of experiment was surface responds and 3 factors were analyzed including 1) gelatin concentration varied from 8-12\%, CMC concentration in range of 0.6-1.0\% and 3) mixture percent of CMC in range of 10-30\%. After the test of tension, the highest value was 13.99Mpaderived from gelatin with 10.64\% concentration, CMC with 0.81\% concentration and mixture of CMC with 17.79\% concentration. After that, the work piece obtained from mixing CMC with concentration at 10, 20 and 30\%, 10\% Gelatin and 0.80\% CMC as constant due to highest volume of product from such 3 values, was tested for decomposition compared with pure-scaffold gelatin. The findings revealed that decomposition rate of CMC mixture at 20\% concentration was slowest and it could be found that the result of tension testing and decomposition testing was consistent. That is, 10\% Gelatin, 0.8\% CMC and 20\% CMC mixture gave the best value in both experiments. Additionally, it was obvious that pure-scaffold gelatin took only 1 hour to totally decompose while CMC mixture at 10\%, G91 that degraded entirely in 24 hours, CMC mixture at 30\%, G73 degraded in 48 hours, CMC mixture at 20\%, G82 degraded in 54 hours to decompose entirely with CMC mixture. It means that using CMC which is cellulose derivative as ingredient in scaffold gelatin could strengthen it better.

1. Introduction
Electrospinning is a simple but highly effective technique to use degradable synthetic polymer like PLA, PGA, PLGA, and PCL as well as natural polymer for biological and medical purposes because natural polymers can go along with human body better [1]. It is a popular technique to apply fibers such as cosmetic skin mask, military protective clothing, Nano sensor, Application in life science and tissue. Electrospinning’s principle is dependence on electric potential difference. Biopolymer will be induced and become miniature fibers to supporting areas [2].
This study examined best tension of mixing gelatin and carboxymethylcellulose at different proportion with Electrospinning technique of artificial skin. Design of Experiment (DOE) method was used as an instrument to find proper level of factors affecting tension [3]. This is because natural polymer obtained from collagen found in skin, flesh and bone of animals is biodegradable, despite being hydrophilic polymer, but it cannot be mixed and characterized with electrospinning method. Therefore, this research used a solvent that could be dissolved with gelatin which was 2,2,2 trifluoroethanol. Carboxymethylcellulose, CMC was employed to strengthen artificial because carboxymethylcellulose (CMC) is cellulose derivative. Normally, cellulose fiber with high alpha-cellulose or high-quality cellulose was required. Additionally, it is applied in various industries such as laundering, paint, glue, textile, paper, ceramic, food and medicines as CMC is colorless, odorless, and unharmed to humans and well soluble.

Furthermore, the study was conducted to change crosslinking to enhance strength of mechanical properties. Accordingly, crosslinking was employed with temperature use or Dehydrothermal treatment (DHT). DHT is a physical process by giving high heat in vacuum condition (Normally, temperature higher that 140 Celsius Degree is required) [4]. The entire process will remove water from fibers resulting in condensation and crossing of bonds which can strengthen fibers.

2. Experimental procedure

2.1 Materials
Powdered gelatin from Porcine Skin, 180 G Bloom, TypeB from Fluke Analytical and 2,2,2-trifluoroethanol (TFE)(purity 99.0%) from Sigma-Aldrich were used. To prepare gelatin, gelatin was dissolved in TFE and stirring in room temperature for 6 hours with concentration of 6.64, 8.00, 10.00, 12.00, 13.36% and powdered CMC from Sigma-Aldrich was dissolved with distilled water with concentration 0.46, 0.60, 0.80, 1.00, 1.14 % and stirring in 70° C temperature for 30 minutes. After preparation, substances were characterized with electrospinning method by mixing CMC in gelatin in proportion of 3.18, 10.00, 20.00, 30.00, 36.82% and irritating the mixture in room temperature for 30 minutes.

2.2 DHT crosslinking
Scaffolds were crosslinked with DHT method by desiccating scaffold of gelatin and CMC with silica gel in vacuum box for 2 days. After that, the scaffold was inserted into vacuum oven in 140 C° temperatures for 48 hours.

2.3 Electrospinning
Gelatin/CMC was prepared to be characterized with electrospinning method by adding the substance in 5-ml syringe with needle spinneret #20 or 0.9-mm. hold. 10 KV high voltage power supply was employed and substance flow rate was controlled with syringe pump at 0.8ml/h. The substance was injected on supporting foil with distance from needle tip to foil sheet was 13 centimeters [5].

2.4 Experimental design
Enhancing efficiency of tension of Scaffold with Central Composite Designs (CCD) of Response Surface Mythology (RSM) with Minitab 17 Program depended on 3 factors including gelatin concentration (A), CMC concentration (B) and ratio of mixing CMC in gelatin (C) [6]. Table 1 showed Parameters for experimental design and the general regression equation to predict the effects of factors on responses is defined as

\[ Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_i^2 X_i^2 + \sum_{i=1}^{3} \sum_{j>i} b_{ij} X_i X_j + \ldots \ldots \] (1)

Where Y was the response variable (impact strength), b_0 was the constant, \( \sum \) was the residual (error) term, b_i was the linear coefficients, b_i^2 was the quadratic coefficient, b_{ij} was the interaction coefficient and X_i was the dimensionless coded independent variable.
Table 1. Parameters for experimental design.

| Factor | Variation | Low(-1) | Medium(0) | High(+1) |
|--------|-----------|---------|-----------|----------|
| Gelatin concentration (%), (A) | 8 | 10 | 12 |
| CMC concentration (%), (B) | 0.60 | 0.80 | 1.00 |
| Ratio of mixing CMC in gelatin (%), (C) | 10 | 20 | 30 |

2.5 Degradation of Gelatin-CMC scaffolds

Lysozyme from chicken egg white (Bio Basic Inc.) concentration was 31.2 u/ml (0.1 mg/ml PBS buffer). After that it incubated in a thermostatted oven at 37 °C for 54 hr. The sample incubated is controlled in buffer without enzyme. The sample started to remove and rinse with purified water to remove the enzymes, dried and weighed in 0.5, 1, 1.5, 24, 48 and 54 hours consecutively. We calculated the degree of enzymatic degradation from the following equation [7, 8]:

\[
\text{Weight remain (\%)} = 100 - \left(\frac{(W_0 - W_f)}{W_0}\right) \times 100
\]

Where: \(W_0\) is initial weight of the scaffold
\(W_f\) is final weight of the scaffold

3. Results and Discussion

According to table 2, it indicated different result of tension from experiment of Response Surface Mythology (RSM) in each experiment

Table 2. Experimental design and results

| Run | Gelatin concentration (%), (A) | CMC concentration (%), (B) | Ratio of mixing CMC in gelatin (%) (C) | Response |
|-----|-------------------------------|-----------------------------|----------------------------------------|----------|
| 1   | 8                             | 0.6                         | 10                                     | 7.787    |
| 2   | 12                            | 0.6                         | 10                                     | 9.824    |
| 3   | 8                             | 1                           | 10                                     | 4.357    |
| 4   | 12                            | 1                           | 10                                     | 11.384   |
| 5   | 8                             | 0.6                         | 30                                     | 7.606    |
| 6   | 12                            | 0.6                         | 30                                     | 8.493    |
| 7   | 8                             | 1                           | 30                                     | 5.701    |
| 8   | 12                            | 1                           | 30                                     | 11.533   |
| 9   | 6.64                          | 1                           | 20                                     | 1.352    |
| 10  | 13.36                         | 1                           | 20                                     | 8.112    |
| 11  | 10                            | 0.46                        | 20                                     | 8.331    |
| 12  | 10                            | 1.14                        | 20                                     | 7.883    |
| 13  | 10                            | 1                           | 3.18                                   | 13.201   |
| 14  | 10                            | 1                           | 36.82                                  | 13.142   |
| 15  | 10                            | 0.8                         | 20                                     | 13.702   |
| 16  | 10                            | 0.8                         | 20                                     | 13.552   |
| 17  | 10                            | 0.8                         | 20                                     | 13.775   |
| 18  | 10                            | 0.8                         | 20                                     | 13.651   |
| 19  | 10                            | 0.8                         | 20                                     | 13.676   |
| 20  | 10                            | 0.8                         | 20                                     | 13.624   |
3.1. Analysis of Variance (ANOVA)

Table 3 showed the result of analysis indicated that factors affecting experimental response included gelatin concentration (%), (A) provided that P-Value was 0.001 and CMC Concentration (%), (B) with P-Value of 0.019. These two factors’ p-value was less than 0.05 and P-Value of Lack-of-Fit was 0.761 which was more than 0.05. It means that the regression equation was highly accurate. R-sq(adj) was 99.86% indicating that the experiment was substantially equitable and it could be accepted. For Response Surface Mythology (RSM), the regression equation of this experiment was given also.

| Source            | DF | Adj SS  | Adj MS  | F-Value | P-Value |
|-------------------|----|---------|---------|---------|---------|
| Model             | 9  | 253.738 | 28.193  | 6634.12 | 0.000   |
| Linear            | 3  | 54.136  | 18.045  | 4246.24 | 0.000   |
| A                 | 1  | 53.973  | 53.973  | 12700.29| 0.001   |
| B                 | 1  | 0.162   | 0.162   | 38.18   | 0.019   |
| C                 | 1  | 0.001   | 0.001   | 0.25    | 0.630   |
| Square            | 3  | 185.447 | 61.816  | 14545.89| 0.000   |
| A*A               | 1  | 144.311 | 144.311 | 33957.77| 0.001   |
| B*B               | 1  | 55.995  | 55.995  | 13176.09| 0.005   |
| C*C               | 1  | 0.470   | 0.470   | 110.70  | 0.012   |
| 2-Way Interaction | 3  | 14.155  | 4.718   | 1110.25 | 0.000   |
| A*B               | 1  | 12.339  | 12.339  | 2903.40 | 0.000   |
| A*C               | 1  | 0.688   | 0.688   | 161.83  | 0.001   |
| B*C               | 1  | 1.128   | 1.128   | 265.51  | 0.022   |
| Error             | 10 | 0.042   | 0.004   |         |         |
| Lack-of-Fit       | 5  | 0.014   | 0.003   | 0.51    | 0.761   |
| Pure Error        | 5  | 0.028   | 0.006   |         |         |
| Total             | 19 | 253.781 |         |         |         |

Regression Equation in Uncoded Units

\[
\text{Result} = 13.6646 + 1.9880 \text{A} - 0.1090 \text{B} - 0.0088 \text{C} - 3.1644 \text{A*A} - 1.9712 \text{B*B} - 0.1807 \text{C*C} + 1.2419 \text{A*B} - 0.2932 \text{A*C} + 0.3756 \text{B*C}
\]

3.2 Analysis of response surface

Analyzing effects of 3 factors including gelatin concentration (%)(A), CMC concentration (%)(B) and CMC concentration (%), (B) on tension by drawing Three-dimensional plots according to Figure 1, the researcher found effects of tension from interaction between gelatin concentration(A) 10%, and CMC concentration (B) 0.8%. However, impacts of tension from interaction between ratio of mixing CMC in gelatin(%), (C) for 10-30% and gelatin concentration (%), (A) or CMC concentration (%), (B) slightly affected tension.
Figure 1 Impacts of tension from co-factors of (a) gelatin concentration (A) vs CMC concentration (B), (b) gelatin concentration (A) vs ratio of mixing CMC in gelatin (C), (c) gelatin concentration (A) vs ratio of mixing CMC in gelatin (C)

3.3 Experiment Analysis
Values of factors for setting to obtain highest response were 10.64% concentration of gelatin (A), 10.64%, 0.810% concentration of CMC (B), ratio of mixing CMC in gelatin (C) for 17.79% and needed response value was 13.99 MPa according to Figure. Confidence Interval of mean was found that the possibility to obtain mean of response in this range was 95% and prediction interval was discovered that possibility to obtain response in this range was 95% also.

Multiple Response Prediction
Variable Setting
|       | A         | B         | C         |
|-------|-----------|-----------|-----------|
| Value | 10.645    | 0.810     | 17.791    |

| Response | Fit           | SE Fit      | 95% CI       | 95% PI       |
|----------|---------------|-------------|---------------|--------------|
| Tensile (MPa) | 13.9961       | 0.0262      | (13.9377, 14.0545) | (13.8396, 14.1527) |

3.4 Degradation of Gelatin-CMC scaffolds result
This study analyzed the result from Analysis of response surface with degradation method to make a comparison between Gelatin pure scaffolds and Gelatin-CMC scaffolds. Three best values were as follows in the table 4.

Table 4 Blending composition of gelatin/CMC

| Samples | Concentration of Gelatin (A) | Concentration of CMC (B) | Ratio of mixing CMC in gelatin (C) |
|---------|------------------------------|--------------------------|------------------------------------|
| G100    | 10%                          | 0.8%                     | 0%                                 |
| G91     | 10%                          | 0.8%                     | 10%                                |
| G82     | 10%                          | 0.8%                     | 20%                                |
| G73     | 10%                          | 0.8%                     | 30%                                |

From the test, it was found that G100 which was Gelatin pure scaffolds could degrade in 1 hour when compared with G91 that degraded entirely in 24 hours, G73 degraded in 48 hours, G82 degraded in 54 hours with mixture of CMC as shown in figure 2.

Figure 2 Result of Degradation of Gelatin-CMC scaffolds testing
4. Conclusions
According to the test result, maximum tension was 13.99 Mpa originated from concentration of gelatin at 10.64%, concentration of CMC at 0.81% and mixture of CMC at 17.79%. The result of testing degradation between Gelatin-CMC scaffolds including G100, G91, G82, G73 compared with pure scaffolds showed that G82 had slowest degradation rate as a result of best tension. It also was found that gelatin with pure scaffolds could degrade entirely in 1 hour while G91 that degraded entirely in 24 hours, G73 degraded in 48 hours, G82 degraded in 54 hours with mixture of CMC. It means that using CMC which was cellulose derivative as ingredient of scaffold gelatin could strengthen the scaffold gelatin substantially. So that adding CMC in appropriate ratio can help decaying especially G82. The result of decaying conforms to the result of tension from the analysis result of Response Surface Mythology (RSM).

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