International Journal of Advanced Technology and Engineering Exploration, Vol 11(111)
ISSN (Print): 2394-5443 ISSN (Online): 2394-7454
http://dx.doi.org/10.19101/IJATEE.2023.10102055

Research Article

Evaluation of Gelatin-PCL scaffold using polynomial model for skin tissue engineering

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Received: 02-August-2023; Revised: 20-February-2024; Accepted: 21-February-2024

Abstract
The mechanical behavior of the scaffold plays a crucial role in tissue engineering applications. This research aimed to fabricate a gelatin/polycaprolactone (PCL) scaffold for use in tissue engineering and evaluate the stress-strain relationship of the scaffold to analyze the shear modulus of the material. The scaffold was fabricated by blending gelatin with polycaprolactone and using the salt leaching technique to create a porous structure under various gelatin and PCL conditions. Uniaxial compression tests and uniaxial tension tests were conducted to determine the mechanical properties of the gelatin/PCL scaffolds. Data on applied pressure and displacement were used to establish the relationship between engineering stress and strain. The deformation characteristics of the scaffold include significant and non-linear behavior. The calculation of the shear modulus is based on a hyperelastic model that generates curves closely aligned with the engineering stress and strain curves. The polynomial model, a type of hyperelastic model, can predict the deformation behavior of materials under non-linear deformation. The constants of the polynomial model were analyzed to determine the shear modulus. The shear modulus of the gelatin/PCL scaffolds was found to range between 3.22 megapascals (MPa) and 60.00 MPa. The GP 82 scaffold exhibited the highest compression test value at 19.60 MPa, while the GP 91 scaffold showed the highest tension test value at 60.00 MPa. The GP 64 scaffold displayed the lowest value at 3.22 MPa. This polynomial model can predict the mechanical behavior of non-linearly deformed scaffolds, making it applicable for tissue engineering applications.

Keywords
Gelatin, Polycaprolactone, Salt leaching technique, Shear modulus, Hyperelastic model.

1.Introduction
Tissue engineering research is widespread today due to the need to use skin tissue in patients who have lost skin from burns and accidents. When the skin is damaged so large and deep that the body cannot repair itself. Skin replacement tissue is therefore a part in helping the skin recover. However, this skin replacement tissue is still costly [1, 2]. Producing skin replacement tissue with its properties similar to real skin was challenging due to the complex structure of skin, such as flexibility, breathability, and softness. Nowadays, researchers have devised ways to produce artificial or synthetic dermatitis and cultured cells that can be biodegradable to transplant the wound area.

Thus, it becomes possible to repair damaged skin by using skin substitutes, known as "scaffold". Scaffold is collagen, a widely used natural polymer. It is used in tissue repair and regeneration, which has biological compatibility and can be mixed with other synthetic polymers to help increase the scaffold's properties. Because in previous research, it was found that the pure gelatin scaffold was lack of the strength properties comparable to real skin. Therefore, there was a need to add synthetic polymers into the mixture to enhance the desired properties of the synthetic skin. Commonly used biodegradable polymers for blending such as carboxymethyl cellulose (CMC), agarose etc [3]. In this research, skin substitutes containing gelatin and gelatin/polycaprolactone (PCL) were studied in various ratios in order to have properties similar to real skin. The forming process

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by salt leaching method is simple, uncomplicated, inexpensive and can be used as skin replacement.

Gelatin is a natural polymer derived from pig skin and bones. Gelatin contains collagen, which is a constituent of the skin, acts as a moisture retainer and is non-toxic to cells. Gelatin has high elastic properties and biocompatibility but is disadvantaged by its low strength [4–7]. Therefore, it must be improved to increase strength. Increasing the strength of a scaffold often involves using materials with higher stiffness, which may compromise flexibility. Conversely, enhancing flexibility may come at the cost of reduced strength. Achieving an optimal balance is challenging due to the inherent trade-off.
PCL is a synthetic polymer. It has good biocompatibility, good organic polymer compatibility, and good biodegradability, and can be used as cell growth support materials. PCL is inter-compatible with many conventional plastics and can be completely degraded in 6-12 months under natural environment. In addition, PCL also has good shape memory temperature control properties and is widely used in drug carriers and tissue engineering scaffold construction [8–11]. In this research, PCL was used in combination with gelatin to strengthen the scaffold.

Trifluoroethanol (TFE) is a colorless, and volatile liquid. It is a fluorinated alcohol, meaning it contains fluorine atoms in its molecular structure. TFE is widely used in various fields, including chemistry and biochemistry, due to its unique properties. One of the notable characteristics of TFE is ability to induce and stabilize alpha-helical structures in peptides and proteins. This property makes it a valuable solvent for studying the secondary structure of biomolecules. TFE is also utilized in organic synthesis as a solvent and as a reagent. Its fluorinated nature imparts specific properties to the compounds it is used with, making it useful in certain chemical reactions [12]. In this research, TFE was used as a solvent to dissolve gelatin and PCL together, and the properties of TFE allowed both components to blend seamlessly into a uniform mixture.

There have many methods for forming polymer materials [13] such as salt leaching technique [14], electrospinning technique [15–17], three-dimensional (3D) printing technique [18–20], freeze-drying techniques [21] etc. One of the popular molding processes is salt leaching technique. It is a process that creates a polymer to form a pore structure by using salt Sodium Chloride (NaCl), which can determine the size of the pore by the size of salt. It is suitable for the size of cells that needs to be cultured. In addition to the structural properties, swelling is also an important factor in determining the suitability of cell cultures. Swelling refers to a measure of the ratio of water absorption both before and after in the cell structure. Another feature required is the degradation of scaffold in order to realize the appropriate usage time in the form of artificial skin by examining the rate of degradation for different time periods so that the best ingredients can be achieved [22].

The objective of this research was to find the optimal ratio between gelatin and PCL that has similar strength properties to the skin and can be used as a cell culture structure for rejuvenation of damaged skin. The scaffold strength analysis uses the theory of hyperelastic materials to determine the scaffold strength in various ratios. The strain energy function will be used to predict the deformation behavior of the material in order to obtain a constant of scaffold. Then, it can be utilized for designing the artificial skin for patients in the future.

This paper is organized as follows: Section 2 discusses related work. Section 3 elaborates on the methodology. Sections 4 and 5 present the results and discussion, respectively. Finally, the paper concludes in Section 6.

2.Literature review

The research involved the composition of the scaffold molding material, the substrate forming method, and the analysis article for the material constant in the case of large deformation, based on a mathematical model of hyperelastic material. The related work was summarized as under.

Singh et al. combined particulate leaching and freeze-drying techniques to produce matrices comprising of polyethylene glycol diacrylate (PEGDA) and gelatin methacryloyl (GelMA), characterized its pore size and the interconnectivity of porous structure. Their investigation revealed that the presence of larger pores altered the matrix morphology, thereby affecting its mechanical strength, swelling behavior, and degradation rate. This study highlighted the potential of particle leaching methods combined with freeze drying to tune matrix properties to achieve specific spherical sizes. This was critical for a variety of applications. Moreover, the analysis of compression modulus indicated a significant influence of the porogen leaching method on scaffold

mechanical properties, with the addition of gelatin further accelerating the degradation process [23].

Gautam et al. developed an electrospun nanocomposite scaffold for bone tissue engineering using gelatin, PCL, and nanohydroxyapatite (nHAp). The electrospun nanofibers of gelatin blended with PCL were subsequently treated with nHAp for varying durations. Field emission scanning electron microscopy (FESEM) was employed to assess fiber diameter and morphology of the fabricated nanocomposite scaffold. In vitro analysis of the electrospun nanocomposite scaffold indicated its potential suitability for bone tissue engineering applications [24].

El et al. investigated the preparation of scaffolds with antimicrobial and antioxidant properties using gelatin (gel) and PCL polymer by incorporating oregano oil through electrospinning technique. The addition of oregano oil altered the morphological characteristics of the observed fibers which investigated by scanning electron microscopy. Analysis of weight changes using heat and X-ray showed minimal alterations in the properties of the PCL/gel scaffold upon oregano oil incorporation. Cell toxicity analysis demonstrated the biocompatibility of the PCL/gel/oil scaffold. Thus, the PCL/gel/oil scaffold was considered a promising material for wound dressing applications [25].

Weizel et al. researched materials with high flexibility for human soft joint cartilage, utilizing two substitute materials, Hydrogel ChondroFiller liquid and Oxidized Alginate-Gelatin (ADA-GEL). They employed a method of deformation analysis considering both compression and tensile data simultaneously to ensure reliable parameter estimation. The unconditional response of soft cartilage was the best represented by the Ogden model, while both soft cartilage and ADA-GEL exhibited comparable fits with both Ogden and Mooney-Rivlin models. It was found that the Mooney-Rivlin model was the most suitable for constructing the unconditional response model of ADA-GEL. Therefore, it was recommended to use both the Mooney-Rivlin and Ogden models for material response analysis [26].

Yang et al. introduced a developed neo-Hookean model for materials with circular pores. They utilized the shrimali-lefevre-lopez (SLL) model to predict the highly effective hyperelastic responses of materials with closed-cell porous material. The research findings demonstrated that the neo-Hookean model accurately assessed the high-flexibility properties of materials with porous structures in all deformation conditions tested. Additionally, the neo-Hookean model complemented the SLL model in predicting various effective properties under specific deformations [27].

Khaniki et al. investigated flexible structures capable of undergoing large reversible deformations and deformations under various loads. They developed and utilized multiple nonlinear elastic models to accurately predict material deformation and large-scale stresses, recognizing the limitations of linear elastic models. The hyperelastic models were proven to be suitable for capturing the mechanical behavior of biological tissues, leading to significant outcomes when combined with different continuum mechanics formulations. Consequently, they presented a comprehensive analysis of the mechanics of hyperelastic structures with focusing on the application of various strain energy function [28].

Wang et al. proposed a straightforward composite process by combining melt-electrospinning writing (MEW) with solvent electrospinning (SE) to fabricate customizable nanostructured scaffolds for bone tissue engineering. The incorporation of gelatin nanofibers into the scaffold rendered its hydrophilicity and slightly enhanced its mechanical strength. When compared to MEW/PCL scaffolds, the composite MEW/SE scaffolds exhibited superior cell adhesion performance, leading to increased cell proliferation and enhanced osteogenic potential. Research findings indicated that gelatin nanofibers did not hinder cell infiltration but instead promoted 3D growth of bone cells. The combined using of MEW and SE presented a promising approach for producing well-designed hierarchical nanostructured scaffolds aimed at facilitating new bone formation [29].

Wang et al. conducted research on a modified hyperelastic structural model derived from the Yeoh model. Their aim was to enhance the accuracy of predictions for multiaxial deformations of highly flexible polymeric materials while retaining the benefits of the original Yeoh model. The modified model included an additional correction term compared to the original model. This correction term was formulated as a composite function based on a power function derived from the principal stretches, which were obtained from the corresponding residual strain energy which predicted by the Yeoh model for material deformation [30].

Jebur et al. conducted experimental tests on rubber using Ogden's model, Mooney-Rivlin model, Neo Hooke model, and Arruda-Boyce model. The parameters for simulating material responses were obtained for each model using the finite element method (FEM) to enable comparison. These models were commonly employed in rubber research, and it was utilized to predict tensile test curves. The study suggested that these hyperelastic models could accurately describe and predict the behavior of elastomeric rubber [31].

Abasalta et al. investigated the use of electrospinning to produce core-shell nanofibers with the objective of controlling the drug release pattern through the shell layer. Core-shell nanofibers composed of CMC/PCL were fabricated using coaxial electrospinning technique, and using polynomial models to predict the average core-shell fiber diameter of CMC/PCL nanofibers by adjusting three variables appropriately. Testing involved determining the voltage-pressure ratio, CMC concentration, and PCL concentration. The minimum core-shell diameter of the synthesized CMC/PCL core-shell fibers had to undergo specific characterization analysis using FTIR, XRD, SEM, and TEM [32].

Sheikhzadeh et al. investigated the fabrication and enhancement of PCL/xanthan electrospun nanofibers through experimental design. They employed a composite design, focusing on electrospinning parameters such as the PCL/xanthan blend ratio, applied voltage, flow rate, and needle tip to collector distance. The study assessed the significant impact on responses. The nanofibers' morphological characteristics were examined by using scanning electron microscopy. The researchers predicted the optimal values of the necessary independent variables for producing nanofibers with desired properties through polynomial equations and response surface plots [33].

Guerreiro et al. investigated electrospun nanofibers of PCL/gelatin, which were considered as potential drug delivery systems aimed to enhance the efficacy of treatment for superficial skin wounds. The advantageous properties of PCL and gelatin, such as biocompatibility, biodegradability, mechanical strength, and parameters for blending in the hybrid structure were identified. Predictive models for the behavior of nanofibers were developed based on the ratio of PCL in the solution. The performance of the nanofibers was evaluated by measuring the fiber diameter, mesh thickness, and mesh permeability. Statistical analysis demonstrated the significance of the model, and independent validation which confirmed the predicted response under the most suitable conditions [34]. It had been identified that there was a potential for scaffolds composed of gelatin and PCL to be used in constructing cell-nurturing structures for the skin. Among the various shaping methods available, the preference was salt leaching due to its simplicity and non-complex. The polynomial model was employed to predict the deformation behavior and determine the strength of the scaffold. The utilization of this polynomial model with multiple variables allowed for accurate and precise computation of the scaffold's strength.

3.Methods

In this research, we studied and developed the skin scaffold using gelatin substrate and PCL as an ingredient to enhance the dermal scaffold properties. To find suitable ratios for applications in tissue engineering. As well as to find the material constant using the hyperelastic theory. Researchers have carried out research and experimented with the following steps. *Figure 1* shows the research process. This started with research and related theory studies, experimental design, equipment preparation, molding, testing of various specimens, material constant calculation. Until analysis and conclusion of the experiment.

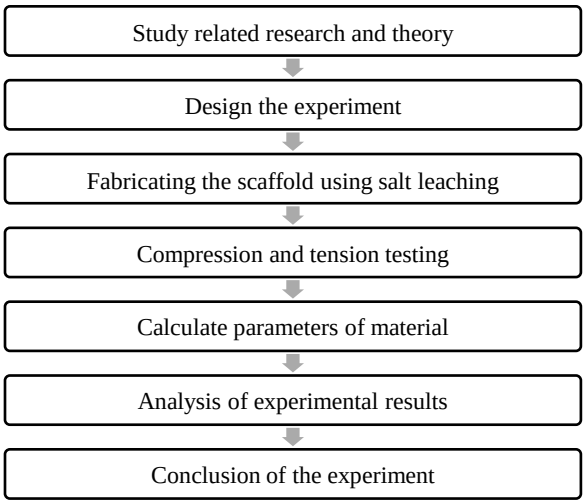


Figure 1 Procedures of research

3.1Material and fabrication of scaffold

Type A gelatin was purchased from Bio Basic Inc. with a yellow powder and derived from Pork skin. Its bloom number was 238-282 and its viscosity was 38-50 mps. PCL was purchased from Sigma-Aldrich

with molecular weight of 45,000 g/mol. TFE was purchased from Sigma-Aldrich. NaCl was purchased from Bio Basic Inc. Gelatin and PCL were dissolved in a TEF solvent and then prepared as a mixture at various ratios as shown in *Table 1*.

Table 1 The various ratios of Gelatin and PCL

condition	Gelatin		PCL		coding
	%	g.	%	g.	
1	100	6	0	0	GP100
2	90	5.4	10	0.6	GP91
3	80	4.8	20	1.2	GP82
4	70	4.2	30	1.8	GP73
5	60	3.6	40	2.4	GP64

3.1.1Fabrication of gelatin/PCL scaffold

Salt leaching techniques have been used to shape porous cell structures for cell culture. Forming was done by pouring the polymer powder in the solvent. The polymer powder was then heated to a temperature higher than the polymer's melting point for a suitable time to form a solution. When it formed a solution, it was mixed with a powdered salt that has been graded at different ratios using inorganic salt NaCl. Then, it was poured into a mold suitable for forming the cell scaffold. After that, the mixture in the mold was heated in an oven until dry. When the specimen dried, the salt was leached away by rinsing with deionized (DI) water over appropriate intervals and heated again in an oven to form a porous internal structure which suitable for cell culture. This process was called solvent-casting or salt-leaching technique [35–38].

Salt leaching technique was used to fabricate gelatin/PCL scaffold which was low cost and easily molded. Briefly, gelatin and PCL were prepared in form of a solution by using a concentration of 30%/wt by using TFE as a solvent to dilute gelatin and PCL, then combined both solution with a specified ratio. After that, stirred it for 30 minutes by heating at 50^{o}C. When the solution was mixed together, added NaCl to the solution. Next, poured the mixture into the mold and dried it at 50^{o}C for 48 hours. When the scaffold was dried, removed it from the mold and rinsed it with DI water for 15 minutes for each. In order to remove the salt from the scaffold to produce the porous structure. Then, Leaving at room temperature for 72 hours until dry. After that, dehydrothermal (DHT) crosslinking was used to strengthen the scaffold structure by heating at 140 ^{o}C under vacuum for 48 hours. Then, keep the gelatin/PCL scaffold in humidity controlled. The fabrication process was shown in the *Figure 2*.

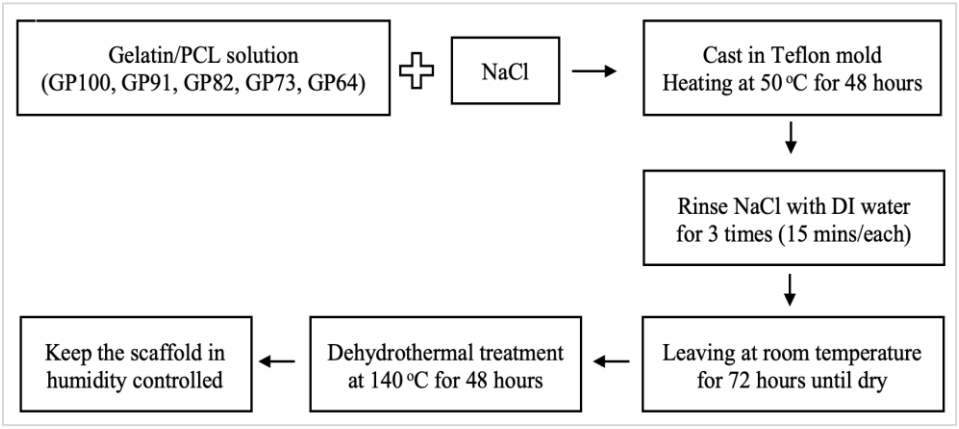


Figure 2 Fabrication of gelatin/PCL scaffold by salt leaching technique

3.1.2Structure of scaffold

Scanning electron microscope (JSM 5910 LV) was used to examine the internal structure of scaffold. It was used to study the overall surface of cells or the skin of the object being studied. In which the electron beam would shine on the surface of the scaffold [39, 40] (*Figure 3*).

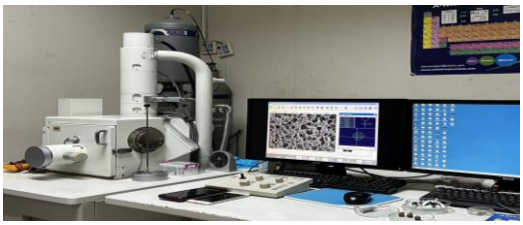


Figure 3 Scanning Electron Microscopy (SEM): JSM 5910 LV

3.2Hyperelastic material

The behavior of porous structural materials was often described by the hyperelastic model which were commonly used in mathematical equations [41–43]. It could be found using the strain energy function (W) and required that this type of material was very little compression behavior. Therefore, we could make assumptions about materials that incompressible Material. Apply the experimental results obtained under the uniaxial tension or uniaxial compression to find the coefficient of the strain energy function.

From the above, hyperelastic materials could be used the strain energy function to describe the behavior of materials that were in the form of deformation. Finding the relation between engineering stress and engineering strain in uniaxial tension or uniaxial compression. It could be written as follows Equation 1.

$$\sigma = 2\left(\lambda^2 - \frac{1}{\lambda}\right)\left(\frac{\partial W}{\partial I_1} + \frac{1}{\lambda}\frac{\partial W}{\partial I_2}\right) \quad (1)$$

Principle stretch (λ) could be found from ε + 1, where ε was Engineering Strain and I_i was Principle Invariants which was a function that could be calculated from principle stretch as shown in the Equation 2.

$$I_1 = \lambda^2 - \frac{2}{\lambda},\ I_2 = \frac{1}{\lambda^2} + 2\lambda,\ I_3 = 1 \quad (2)$$

From the Equation 1, σ was the equation of stress and strain in the form of a tensor (Cauchy stress tensor). That was to say, the cross-sectional area where the load was changed. But in this research, we would consider the fixed cross-sectional area. Therefore, the equation of relation between engineering stress and engineering strain must be used instead. In the case of tension test or compression test, there was the following Equation 3.

$$T = 2\left(\lambda - \frac{1}{\lambda^2}\right)\left(\frac{\partial W}{\partial I_1} + \frac{1}{\lambda}\frac{\partial W}{\partial I_2}\right) \quad (3)$$

where T is Engineering Stress
λ is Principle Stretch
W is strain energy function

3.3Strain energy function

Materials with hyperelastic deformation behavior needed to find the equation to show the stress and strain relation. In order to describe nonlinear elastic deformation behavior written in strain energy functions. The strain energy function was divided into 2 types. One of type was phenomenological model. It was a model that represents behavior from observation. Developed from the stress and transformation relation without considering the structure within the molecule. Another type was physical based model. It was a model that represents physical behavior, which was developed from a microstructure using kinetic theory or statistical mechanics theory. Assumptions from molecular chain motions in each element [44, 45].

The research used the polynomial model which was in the phenomenological model to find the strength of the scaffold. Because this model was a model that was suitable for scaffold materials and it has multiple constants [46–49], allowing the curve to be well adapted to the deformation behavior of the material. The model of polynomial could be written in the form of strain energy function as follows Equation 4.

$$W = \sum_{i+j=1}^{N} C_{ij}\,(I_1 - 3)^i (I_2 - 3)^j \quad (4)$$

When N = 2

$$W = C_{10}(I_1 - 3) + C_{01}(I_2 - 3) + C_{11}(I_1 - 3)(I_2 - 3) + C_{20}(I_1 - 3)^2 + C_{02}(I_2 - 3)^2 \quad (5)$$

The above equation is a popular model used to predict deformation behavior. Because the number of terms used is appropriate, it includes the first invariant, I_1 and second invariant, I_2. From the strain energy function Equation 5, substitute it into the stress-strain Equation 3, as follows.

$$T_{11} = 2\left(\lambda - \frac{1}{\lambda^2}\right)\left(C_{10} + C_{11}\left(2\lambda + \frac{1}{\lambda^2} - 3\right) + C_{20}\left(\frac{4}{\lambda} + 2\lambda^2 - 6\right) + \frac{C_{01} + C_{02}\left(4\lambda + \frac{2}{\lambda^2} - 6\right) + C_{11}\left(\frac{2}{\lambda} + \lambda^2 - 3\right)}{\lambda}\right) \quad (6)$$

Therefore, Equation 6 is a mathematical polynomial model for predicting the deformation behavior of a material. From the constant of the polynomial equations, they can calculate the shear modulus from the following Equation 7.

$$\mu = 2(C_{10} + C_{01}) \quad (7)$$

Where μ is Shear modulus

The Polynomial model was particularly suitable for describing the hyperelastic behavior of soft biological tissues. It had been widely used in finite element analysis to simulate the mechanical response of these materials under various loading conditions.

3.4Geometry and condition test

In the compression test, it could find the deformation behavior of the material where the force acting was perpendicular to the cross-sectional area. The specimens were molded in accordance with

American Society for Testing and Materials (ASTM) D3574 type C [50], which required a thickness of not less than 10 mm and the cross-sectional area was not less than 100 mm2. In the tension test, the specimen was a dog bone in shape with a compressive cross-sectional area was not less than 4 mm2 as shown in the *Figure 4.*

For the tension tests and compression tests, the universal testing machine (UTM) was used to test the scaffold as shown in the *Figure 5*. The loading condition which applied to the machine was a constant deformation rate of 5 mm/min. The data obtained from the compression and tension tests represented force and displacement, displayed on a graph in the X-Y coordinate system. The horizontal axis represented the displacement, while the vertical axis represented the applied force.

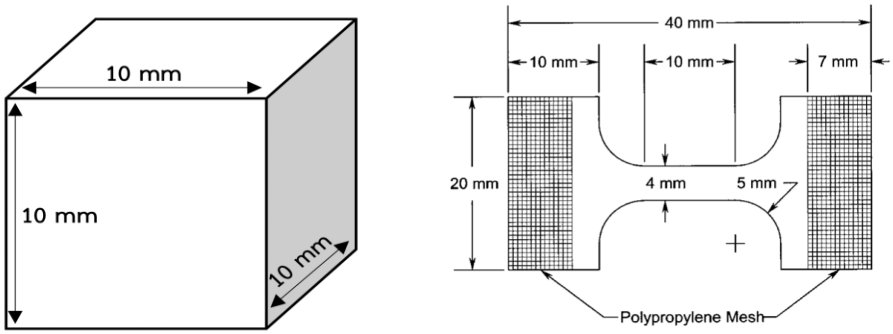


Figure 4 The specimen size of compression test and tension test

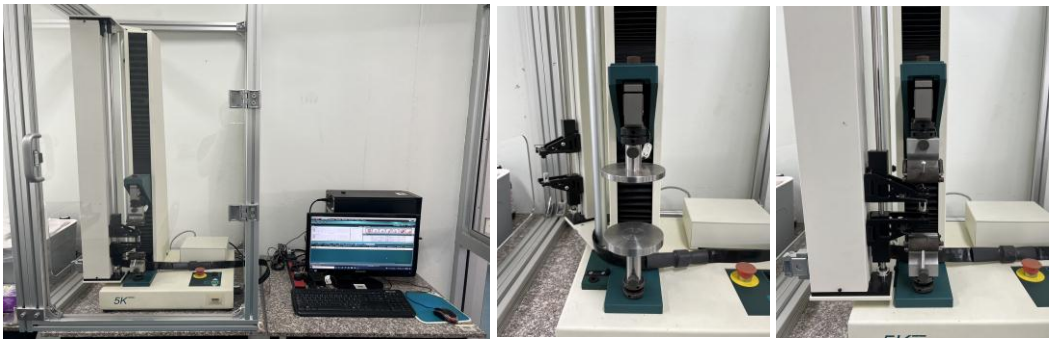


Figure 5 Universal testing machine (UTM)

4.Results

4.1Internal structure of scaffold

The structures were examined using a scanning electron microscope with a magnification of 60x to evaluate the surface structure and pore size of the scaffold which could be shown in *Figure 6.*

The SEM images, it was found that the structure of scaffold which contained gelatin and PCL components shown porous structure and had continuous porosity which was suitable for tissue cell culture. The cell walls become thicker when the GP82 with the strength of the structure increased because the cell walls were thicker. Whereas, the other ratio between GP100 and GP64 had a thinner wall.

4.2Identification of constitutive parameter

Form the compression tests and tension test, the data showed force and distance relation which could be changed to the engineering stress and engineering strain relation by using the cross-sectional area that received force and thickness of scaffold. In the relationship between compressive force and deformation of the gelatin/PCL scaffold, it was found that the relationship was non-linear deformation behavior. In the initial range of the relationship (0-12% strain), the curve occurred a relatively steep slope because a significant force was required to initiate scaffold deformation. In the subsequent range of the relationship (12-30% strain), the slope of the curve decreased compared to the initial range because a constant force applied to the scaffold could lead to continued deformation. In the final range of the relationship (30-50% strain), the slope of the curve increased again as a higher force was required to

cause additional deformation and further scaffold deformation. All of these represented the deformation behavior under compression of the gelatin/PCL scaffold, as shown in *Figure 7 (a).* In the relationship between tensile force and elongation of the gelatin/PCL scaffold, a similar non-linear behavior was observed. However, the curve exhibited a single curve pattern from the initiation of the tensile force until the test specimen was separated. This was illustrated in *Figure 7 (b).*

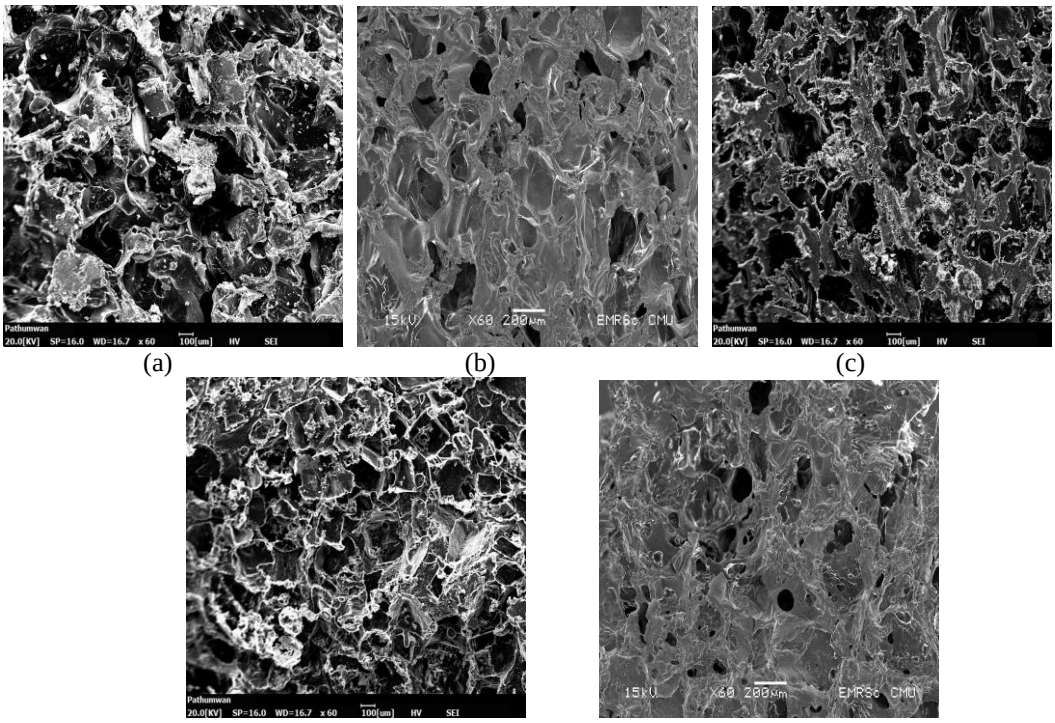
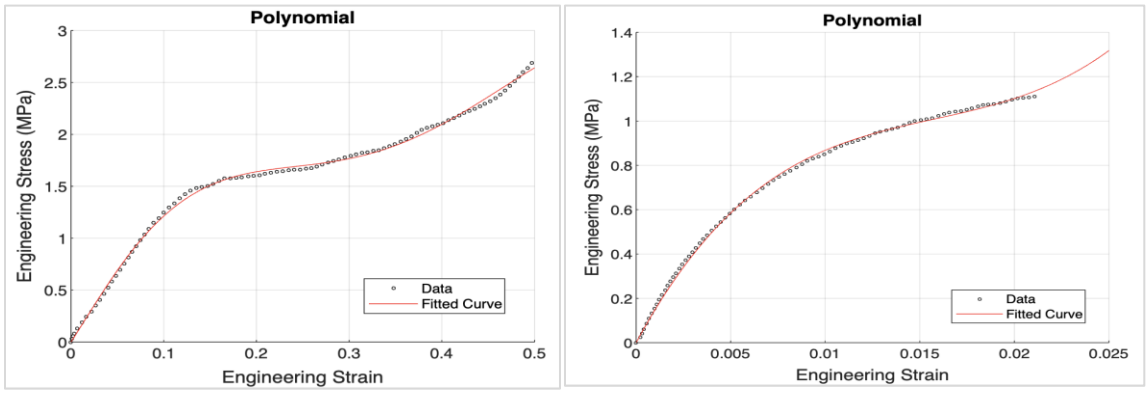


Figure 6 SEM images of gelatin/PCL scaffold (a) GP100 (b) GP91 (c) GP82 (d) GP73 and (e) GP64



(a) (b)

Figure 7 Curve fitting of gelatin/PCL scaffold (a) Compression test (b) Tension test

Based on the compression test using the Polynomial model to adjust the curves to deformation behavior at 50% strain and used to find the shear modulus. The results showed that the addition of PCL to the scaffold increased the strength. The GP82 scaffold showed the highest shear modulus, which was equal to 19.60 mega pascal (MPa) and after grade point (GP82), the strength decreased significantly with increasing amount of PCL. The GP64 scaffold showed the lowest shear modulus which equal to 3.22 MPa. When GP82 scaffold has the highest shear modulus corresponding to the thick-walled scaffold,

thus making GP82 scaffold the strongest. From the results of tension test, it was found that the ratio between gelatin and PCL at GP91 scaffold showed the highest shear modulus which was 60.00 MPa when compared to GP100 scaffold with significantly different. The gelatin/PCL scaffold at GP64 occurred the lower shear modulus which was 4.20 MPa. This result showed that increasing PCL in scaffold affected to the scaffold strength especially in GP91 but slightly improved in GP64 scaffold. Therefore, only a certain amount of PCL could improve the scaffold mechanical structure which was found in the mixture of GP91 scaffold. From *Table 2*, it was observed that the shear modulus of compression increases when the PCL was introduced into the gelatin/PCL scaffold at GP91 and GP82. However, the value decreased when additional PCL was incorporated into the GP73 and GP64. As for the shear modulus of tension, it increased when the PCL was added to the gelatin/PCL scaffold at GP91, similar to the shear modulus of compression. However, it decreased when more PCL was added into GP82, GP73, and GP64.

Table 2 Result of initial shear modulus and modulus of elasticity at each gelatin/PCL ratio

Gelatin/PCL ratio	Shear modulus of compression (MPa)	Shear modulus of tension (MPa)
GP100	4.58	56.00
GP91	14.06	60.00
GP82	19.60	31.60
GP73	3.26	25.60
GP64	3.22	4.20

5.Discussion

In this section, we would compare and analyze the research findings to understand their significance and meaning.

SEM images of gelatin/PCL scaffold as shown in figure 5, it was evident that the internal structure of the scaffold exhibited a sponge-like pattern, resulting from the size of salt crystals introduced during the salt leaching process. This porous structure served as a residence for the skin cells to proliferate and disseminate. In this research, upon incorporating an increased amount of PCL into the scaffold, the internal structure became stronger. The porous structure became more pronounced and distinct compared to the GP100 scaffold. The cell walls were thickest as shown in the GP82 scaffold. However, the addition of PCL led to a noticeable thinning of the cell walls. Consequently, this led to a reduction in the overall structural strength. Therefore, it could be concluded that PCL has the potential to enhance the structural strength, but only certain compositional ratios contributed to the overall robustness of the structure.

The biological implications of a scaffold's structural changes were significant, particularly in the context of tissue engineering and regenerative medicine. Scaffolds played a crucial role in providing a 3D framework for cells to adhere, proliferate, differentiate, and ultimately contributing to the formation of functional tissues. Changes in the scaffold's structure could influence the surface properties that affected cell adhesion. An optimal scaffold structure promoted cell attachment, spreading, and facilitating cell proliferation. Structural modifications could enhance or inhibit these processes, by impacting the overall effectiveness of the scaffold in supporting tissue growth. The mechanical properties of a scaffold, such as its stiffness and elasticity, impacted cellular behavior. Structural changes that enhanced or mimicked the mechanical characteristics of the native tissue could positively influence cell responses and tissue development.

The thickness of cell walls and the porous structure of a scaffold could significantly impact various biological processes, including cell growth, tissue regeneration, and other cellular activities. The thickness of cell walls in a scaffold could affect cell adhesion. Thinner cell walls might provide a more conducive environment for cell attachment and spreading, facilitating better initial adhesion. This was crucial for cells to migrate into and populate the scaffold. A scaffold's porous structure allowed cells to migrate and proliferate within the 3D space. Well-designed porosity provided pathways for nutrient and oxygen diffusion, aiding in cell migration and distribution throughout the scaffold.

The Polynomial model is a hyperelastic model that can effectively fit non-linear deformation behaviors. From the results, this model demonstrated a high level of accuracy with R2 = 0.99. This was due to the fact that the polynomial model had five independent variables, enabling it to accommodate a wide range

of curves, which was a significant advantage over other hyperelastic models. For instance, when compared to other hyperelastic models such as the Blatz-Ko model, which had only an independent variable, the polynomial model's versatility in handling multiple curve adjustments was notable. From the curve fitting of gelatin/PCL scaffold as shown in *Figure 6*, it could be observed that the adjustments made by the Polynomial model could effectively predict the deformation behavior of the scaffold for both compression and tensile testing. The addition of PCL into the gelatin scaffold significantly impacted the internal structural strength of the scaffold, enhancing the characteristics of the open-cell structure. This resulted in thicker and more complete cell walls when compared to the structure of a pure gelatin scaffold. When the gelatin/PCL scaffold was subjected to uniaxial compression, the GP82 scaffold exhibited the highest shear modulus of compression, while for tensile testing, the GP91 scaffold demonstrated the highest shear modulus of tension. These outcomes were influenced by the gelatin component, which affected the scaffold's flexibility. When an excessive amount of PCL was added, it led to an increase in the structural strength of the scaffold. However, this increase in strength came at the expense of reduced flexibility in the scaffold's structure. As a result, the shear modulus decreased when incorporating PCL content exceeding 30% of the gelatin/PCL scaffold.

The results showed that the use of the polynomial model was better at predicting deformation behavior compared to other models, such as the Blatz-Ko model, which can only predict early-stage deformation. The salt leaching technique for fabrication was a practical and cost-effective option compared to other fabrication methods like freeze-drying technique, making it a good choice for fabricating scaffold.

5.1Limitations

The limitations of this research start with the issue of small and thin-sized test specimens, making them susceptible to ambient humidity and wind forces, thus necessitating the control of the laboratory environment. Secondly, the fabrication of the specimens using the salt leaching method in the salt removal step might result in residual salt remaining within small portions of the specimens. The last one was scaffolding preparation of the gelatin/PCL solution, it was essential to use the TFE solvent to ensure the homogeneous blending of both gelatin and PCL components.

A complete list of abbreviations is summarised in *Appendix I.*

6.Conclusion and future work

Gelatin/PCL scaffold had interconnected porous structure. The GP82 scaffold shown the highest porosity. According to the compression test and tension test, polynomial model could adjust the stress-strain curve of scaffold. The gelatin/PCL scaffold that could support the maximum force which could be found in GP82 with significant difference compared to GP100 scaffold. The salt leaching technique was one of the techniques that could fabricate the gelatin/PCL scaffold for tissue engineering with simple and uncomplicated processes. The Polynomial model could be used to predict the mechanical behavior of the gelatin/PCL scaffold for the benefit before using in the tissue transplantation on the patient. There are various other types of hyperelastic models, such as the Ogden model, Yeoh model, and Gent model, each with its own specific form of the strain energy function and material constants. For future research on different parameters to validate the method, we suggest the Mooney-Rivlin model which contain more parameters to predict the scaffold mechanical behavior for better results. For further research, these models can be chosen based on the specific material properties and desired accuracy of the analysis.

Acknowledgment

We would like to thankful of department of advanced manufacturing technology, faculty of engineering, Pathumwan Institute of Technology, Thailand and department of Mechanical Engineering, Faculty of Engineering, Chiang Mai University, Thailand.

Conflicts of interest

The authors have no conflicts of interest to declare.

Data availability

Not applicable.

Author’s contribution statement

All authors contributed equally to the study’s conception and design, analysis, interpretation of results, and manuscript preparation.

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Appendix I

S. No.	Abbreviation	Description
1	3D	Three-Dimensional
2	ADA-GEL	Alginate-Gelatin
3	ASTM	American Society for Testing and Materials
4	CMC	Carboxymethyl Cellulose
5	DI	Deionized
6	DHT	Dehydrothermal
7	FEM	Finite Element Method
8	FESEM	Field Emission Scanning Electron Microscopy
9	GelMA	Gelatin Methacryloyl
10	GP 82	Grade Point
11	MEW	Melt-Electrospinning Writing
12	MPa	Mega Pascal
13	NaCl	Sodium Chloride
14	nHAp	Nanohydroxyapatite
15	PCL	Polycaprolactone
16	PEGDA	Polyethylene Glycol Diacrylate
17	SE	Solvent Electrospinning
18	SLL	Shrimali-Lefevre-Lopez
19	TFE	Trifluoroethanol
20	UTM	Universal Testing Machine