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## Comparative Characterization of Chitosan/Gelatin/Geothermal Silica Biocomposites in Two-Dimensional Film and Three-Dimensional Scaffold Forms

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# Comparative Characterization of Chitosan/Gelatin/Geothermal Silica Biocomposites in Two-Dimensional Film and Three-Dimensional Scaffold Forms

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**Abstract.** Scaffold, a template resemble an extracellular matrix, contributes a necessary part in tissue engineering to accommodate the growth of cells. In the development of scaffold made from organic materials such as chitosan and gelatin, researchers have done various ways to modify its properties and one of them is by incorporate it with inorganic materials. This research explored the potential of silica derived from geothermal power plant waste as a biocomposite material for scaffold. Biocomposites with two-dimensional (2-D) film form were prepared by simple drying process at room temperature and ambient pressure, while three-dimensional (3-D) scaffold form were fabricated by freeze-drying. The obtained biocomposites were characterized by Fourier Transform Infra-Red (FTIR) spectroscopy and Scanning Electron Microscopy (SEM). In addition, swelling and degradation tests were also performed on the films and scaffolds. The results showed that there are interactions between each component in chitosan/gelatin/geothermal silica biocomposites and the addition of geothermal silica decreases the swelling and degradation rates of the biocomposites. These results indicate that geothermal silica has a high potential to be used as an additive for controlling the physical properties of chitosan/gelatin scaffolds.

## 1. Introduction

In the regeneration process of damaged tissue, implanted scaffolds have an essential role. Tissue damage causes the loss of cells and native extracellular matrix (ECM). In order to support cell growth in the healing process, scaffolds should be properly designed to resemble the functions of ECM and provide a supportive environment [1]. A great number of 2-D films and 3-D scaffolds have been developed utilizing a wide range of biomaterials to promote tissue regeneration. The biomaterials have to possess the capability to support cell attachment and nutrient supply. In addition, they need to show good biocompatibility and biodegradability [2].

Scaffolds are made of natural or synthetic materials, or a combination of both in hydrogel [3], composite [4], thin film [5] or lyophilized forms [6]. Recently, development of organic scaffolds incorporating inorganic materials has gained much interest. The purpose of this blending is to overcome the certain limitations of stand-alone organic scaffolds; such as, high degradation rates and overly high solubility in physiological conditions [7].

One common organic-based scaffold is chitosan/gelatin. Chitosan is a natural polysaccharide derived from chitin, a major component in the exoskeleton of crustaceans. Chitosan is obtained by the partial deacetylation of chitin, and is a promising biomaterial for various medical applications; such as, bone grafting, wound dressing, and drug delivery systems, due to its low cost, resource abundance, anti-microbial activity, low toxicity, biocompatibility, and biodegradability [8]. The structure of chitosan



resembling glycosaminoglycan and the polysaccharide's hydrophilic nature makes it an attractive material as a scaffold for tissue engineering; especially since the monomeric unit of chitosan, N-acetyl glucosamine, is an essential compound in wound healing [9].

Gelatin, an anionic natural polymer, is one of the blending components for chitosan to improve the biological activity of the scaffolds by improving cell adhesion and migration via the Arg-Gly-Asp (RGD)-like sequence [10]. Chitosan and gelatin form polyelectrolyte complexes that increase the bond stability between the components [11]. On the other hand, gelatin is known to have a hydrophilic nature that indirectly affects the degradation rate of the resulting scaffold. In order to control the degradation rate of chitosan/gelatin scaffolds, methods of forming blends with other polymers have been studied extensively.

Silica sludge or geothermal silica with more than 50 % amorphous silica content in amorphous structure is obtained as an unutilized side product of PT. Geo Dipa Energi Dieng geothermal power plant. Amorphous silica has bioactive characteristics and has an ability to assist the bone-forming process by stimulating cell mineralization [12]. The purpose of this research is to explore the viability of using the geothermal silica as a silica source to modify the properties of chitosan/gelatin scaffolds. In particular, the research focuses on the fabrication of 2-D films and 3-D scaffolds to better understand the fundamental characteristics of the biocomposites.

## 2. Experimental section

### 2.1 Materials

Chitosan powder was purchased from CV. Naturindo Perkasa. Gelatin was purchased from a local supplier. Geothermal sludge was provided by PT. Geo Dipa Energy, Dieng, Central Java, Indonesia. Acetic acid ( $\text{CH}_3\text{COOH}$ ) 98 %, hydrochloric acid (HCl) 37 %, and sodium hydroxide (NaOH) pellets were purchased from Merck.

### 2.2 Procedures

**Table 1.** Composition of chitosan/gelatin/geothermal silica blended scaffolds

2-D films		3-D Scaffolds	
Sample	Chi/Gel/Si Ratio	Sample	Chi/Gel/Si Ratio
A1	1:1:0	B1	1:1:0
A2	1:1:1	B2	1:1:0.5
		B3	1:1:1
A3	1:1:2	B4	1:1:1.5
		B5	1:1:2
		B6	1:1:2.5

#### 2.2.1 Geothermal Silica Purification

The purification of geothermal silica was conducted based on previous experiment with slightly modification [4, 13, 14]. Silica sludge was washed with demineralized water, dried in an oven at 60 °C, subjected to size reduction by using a mortar grinding machine, and sieved through a 60-mesh sieve. The purified silica was prepared by alkali extraction followed by acidification steps as described below. Twenty grams of the dried silica sludge was dissolved in 800 ml of 1.5 N NaOH solution, gradually heated to 90 °C, and stirred at 300 rpm for hour. The solution was left to cool down and subsequently filtered through filter paper. The filtered sodium silicate solution was titrated with 2 N HCl to form silica

gel. The gel was stored for 18 hours, filtered, and continuously washed until reaching pH 7. Finally, the neutralized gel was dried in an oven at a temperature of 60 °C prior to subsequent use.

### 2.2.2 Preparation of Scaffolds

In order to fabricate 2-D and 3-D scaffolds, chitosan (1 % w/v) and gelatin (1 % w/v) were dissolved in 60 mL of 1 % acetic acid solution in water. After a gel was formed, silica powder (1 % w/v) was added to the solution and stirred for 2 hours at ambient temperature until the solution was homogeneously mixed. The 2-D films were fabricated by drying the solution at room temperature and ambient pressure in an 8.5 cm diameter polyethylene petri dish until a thin layer of film was formed. For 3-D scaffold preparation, the polymer mixture was poured into a 9.5 cm diameter glass petri dish and chilled overnight at -20 °C. It was subjected to freeze-drying until dry biocomposite sponges were obtained. These were stored in a vacuum container prior to subsequent use. A series of blended biocomposites were prepared by changing the amounts of silica in the chitosan/gelatin/silica blend within the range of 1:1:0 to 1:1:2.5 according to the ratios shown in Table 1.

### 2.2.3 Characterization

The components of geothermal silica were analyzed with an Energy Dispersive X-ray (EDX) instrument (Shimadzu Co., Japan) by detecting X-ray spectra emitted from materials as a response to bombardment with a focused electron beam. The molecular interaction between components in a scaffold was observed with Fourier Transform Infra-Red (FTIR) spectrometer (ABB MB3000) by using KBr method. FTIR measurements were performed in the spectra range between 4000 and 400 cm<sup>-1</sup>. Morphology and porosity of the scaffolds were observed by Scanning Electron Microscopy (SEM: JEOL JSM-6510 LA) after coating the specimens with a platinum layer.

### 2.2.4 Swelling Study

The scaffold swelling test was performed by soaking a dry sample of known weight into pure water or Phosphate Buffered Saline (PBS) at room temperature for one hour. The sample was then removed and water on the sample surface was removed by absorbing with tissue paper. The degree of swelling was calculated using the Eq. 1:

$$\text{Degree of Swelling} = \frac{W_s - W_d}{W_d} \times 100\% \quad (1)$$

Where,  $W_d$  and  $W_s$  are the weights of dry and swollen samples, respectively. All data are the average of triplicate samples.

### 2.2.5 Degradation Test

The biocomposite degradation test was similar to the swelling test, but instead of 1 hour, the samples were soaked in pure water (H<sub>2</sub>O) or PBS for 4 days and weighted every 24 hours. The degradation ratio was calculated using the Eq. 2 [4]:

$$\text{Degradation ratio} = \frac{W_0 - W_1}{W_0} \times 100\% \quad (2)$$

where,  $W_0$  and  $W_1$  are the weights of initial and final samples, respectively. All data are the average of triplicate samples.

### 3. Results and discussion

#### 3.1 Silica Purification

The elemental composition of purified silica was analyzed by EDX Spectroscopy. The results are shown in Table 2. The result of the EDX analysis indicates a relatively high purity of silica (95.58 %) after several steps of the washing process. Thus, geothermal silica has potential as a blending component for biocomposites [13].

**Table 2.** Elemental composition of purified geothermal silica by EDX Analysis

Element	Atomic %
Si	95.58
S	1.63
Al	1.39
Fe	0.91
Ca	0.38
K	0.08
Cu	0.02
Zn	0.01

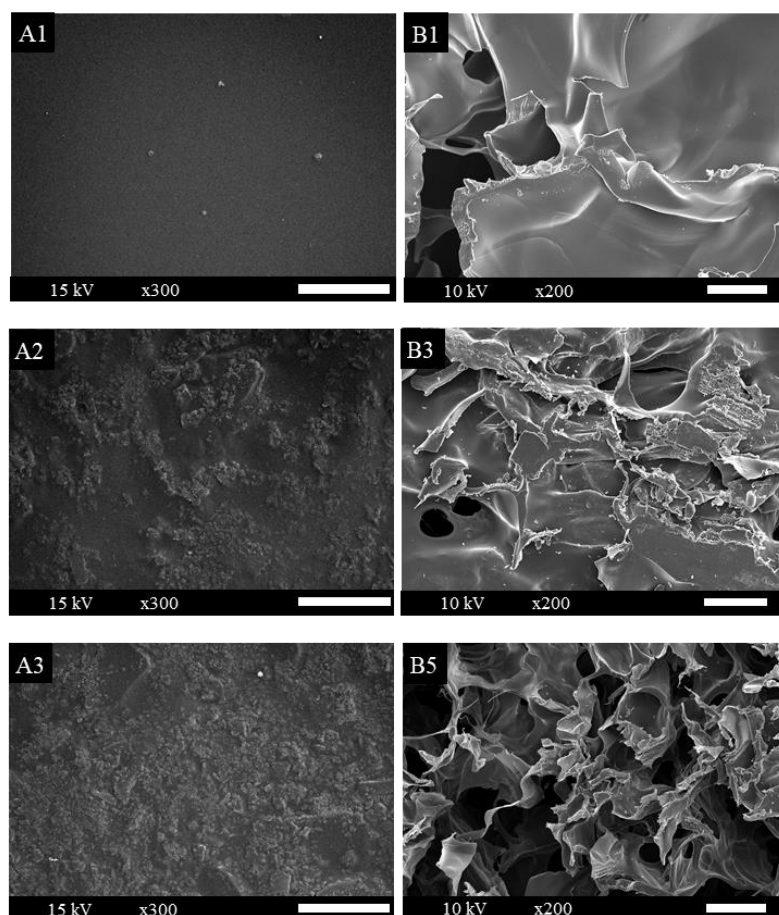
#### 3.2 Morphology

The surface morphology of chitosan/gelatin/silica 2-D films and 3-D scaffolds was observed by SEM as shown in Figure 1. The SEM result revealed that the 2-D films show a rough surface with homogeneous silica distribution, and no interconnected pore was observed. The surface roughness of the films has the tendency to increase along with the amount of silica contained. Silica attached on the film surface are apparent and may provide better properties for cell adhesion on the films. Silica attached on the film surface may assist bone forming process by stimulating cell mineralization [12].

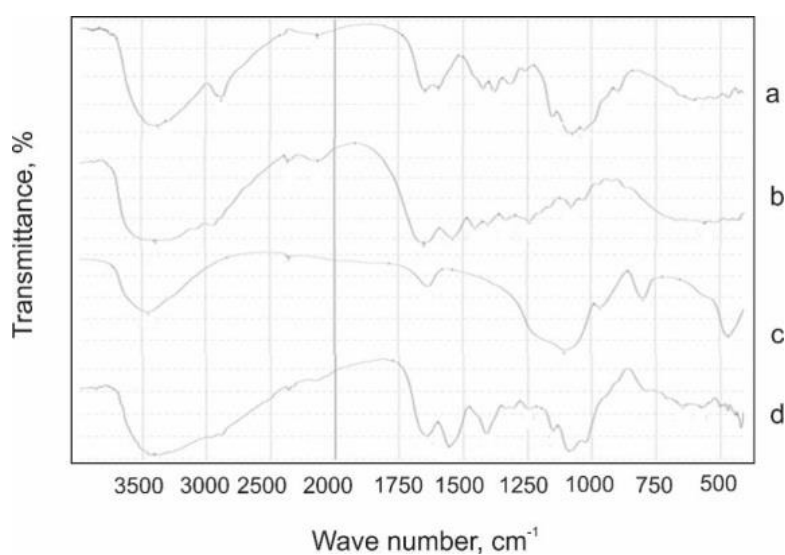
Contrastingly, interconnected pores formed in the 3-D scaffolds. By increasing the amount of silica in biocomposites, the pore size decreases. Pore diameters in chitosan/gelatin/silica=1:1:2 are in the range around 100 to 150  $\mu\text{m}$ . The pore size of scaffold could affect cell penetration and distribution through the scaffold which is important to promote better vascularization and tissue formation [15]. Physically, higher composition of silica for the equal total mass would produce a more fragile biocomposite due to smaller number of silica binding polymers. The interconnected pores contribute to the nutrient transfer and cell infiltration: essential roles expected for tissue engineering applications. By changing the amount of silica contained in scaffolds, optimal morphology and physical properties can be achieved.

#### 3.3 Chemical structure of the biocomposites

FTIR spectra of the 1:1:1-chitosan/gelatin/geothermal silica composite along with its pure components are shown in Figure 2. Peaks at  $1634\text{ cm}^{-1}$  and  $1557\text{ cm}^{-1}$  in the spectrum of chitosan are assigned to the presence of  $\text{NHCOCH}_3$  (amide I) and  $-\text{NH}_2$  groups (amide II), respectively. Peaks at wavenumbers  $2884\text{ cm}^{-1}$  and  $3400\text{--}3500\text{ cm}^{-1}$  represent  $-\text{CH}_3$  and  $-\text{OH}$  groups. A tapered peak at  $1411\text{ cm}^{-1}$  indicates symmetrical deformation and a peak at  $1090\text{ cm}^{-1}$  indicates the vibration of amine groups. In contrast, the characteristics of gelatin are evident by the peaks at  $3294\text{ cm}^{-1}$ ,  $1500\text{--}1200\text{ cm}^{-1}$ , and  $1550\text{ cm}^{-1}$  corresponding to N-H group,  $\text{CH}_2$  deformation, and the deformation of N-H by alpha helix, respectively. A silanol (Si-OH) group in silica spectra is shown at the peak of  $1635\text{ cm}^{-1}$ , while the existence of Si-O-Si is shown at  $1100\text{ cm}^{-1}$ . The interaction of silica with chitosan and gelatin leads to the friction on N-H group that overlap with  $-\text{OH}$  group at the peak of  $3411\text{ cm}^{-1}$ . Meanwhile, a peak at  $647\text{ cm}^{-1}$  demonstrates the Si-O-C bond of the blended scaffold [15].

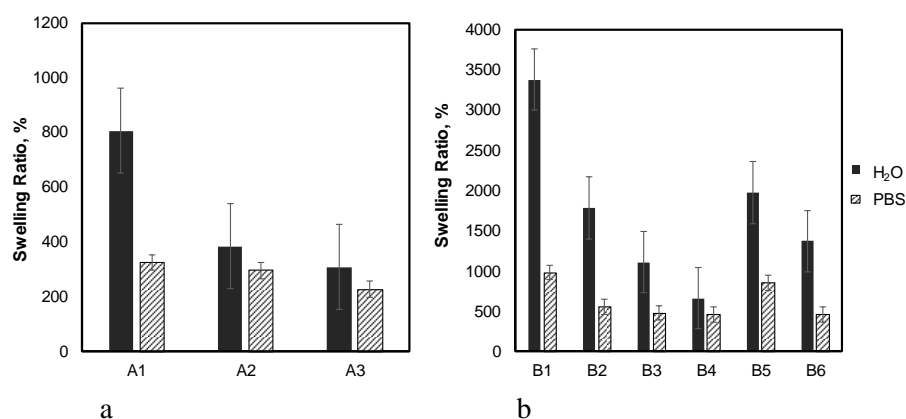


**Figure 1.** SEM images of 2-D films of chitosan/gelatin/silica = 1:1:0 (A1), 1:1:1 (A2), 1:1:2 (A3) and 3-D scaffolds of chitosan/gelatin/silica = 1:1:0 (B1), 1:1:1 (B3), and 1:1:2 (B5). Scale bar: 100 μm



**Figure 2.** FTIR spectra of (a) chitosan, (b) gelatin, (c) geothermal silica, and (d) 1:1:1 blended biocomposite

### 3.4 Swelling properties

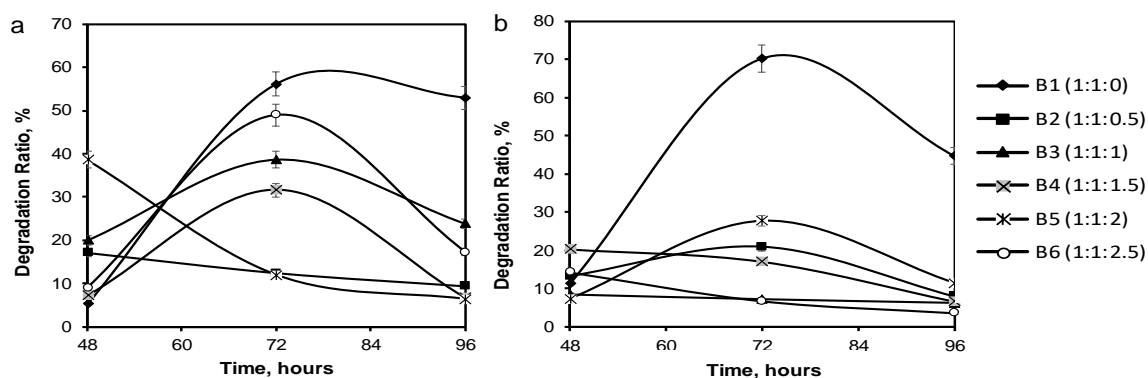


**Figure 3.** The degree of swelling in H<sub>2</sub>O and PBS versus silica composition in 2-D films (a) and 3-D scaffolds (b). Silica content is 0 (A1, B1), 0.5 (B2), 1 (A2, B3), 1.5 (B4), 2 (A3, B5), and 2.5 (B6) as shown in Table 1.

The degree of swelling versus the composition of geothermal silica in 2-D films and 3-D scaffolds are shown in Figure 3. The swelling test was performed for 1 hour to determine the absorption ability of biocomposites in H<sub>2</sub>O or PBS. The addition of silica to the biocomposites was found to considerably reduce the degree of swelling. This decreasing trend can be ascribed to the interaction between silica and chitosan's positive charge that reduces the number of free amine groups in chitosan. The amine group can form hydrogen bonds with H<sub>2</sub>O as the swelling media, thus the reduction of free amine groups leads to a lower degree of swelling.

Meanwhile, the difference in the degree of swelling in H<sub>2</sub>O and PBS is caused by a higher ion concentration of PBS that leads to lower osmotic pressure, resulting in a lower absorption capability of the biocomposites. This low swelling ratio is preferred in the application of tissue engineering to avoid increasing mean pore size of the 3-D scaffolds.

### 3.5 Degradation rate



**Figure 4.** The degradation ratio of 3-D scaffolds as a function of time in (a) H<sub>2</sub>O and (b) PBS

The results of the degradation test are shown in Figure 4. A significant difference was observed in the degradation rate of the scaffolds with silica incorporated compared to the scaffold without silica. The addition of silica significantly decreased the degradation rate of the scaffolds. The maximum degradation ratio was achieved after 72 hours of immersion.

#### 4. Conclusion

In this study, blended scaffolds were synthesized using chitosan, gelatin and silica. The addition of silica from a geothermal source was aimed at controlling the degradation rate of the scaffolds. FTIR analysis showed interactions between three blended components, while SEM analysis of the 3-D scaffolds showed the formation of interconnected pores that play important roles in tissue engineering. The results of swelling and degradation analyses indicated that the addition of silica reduces the degree of swelling leading to scaffolds with a low degradation rate. In conclusion, geothermal silica has high potential for improving the properties of organic scaffolds, particularly by controlling the degradation rate of the scaffolds.

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