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ORIGINAL RESEARCH

Biocompatibility of Silicone Elastomer Incorporated with Chitosan: Morphology, Mechanical, Biodegradation Assessment and the Potential for Injectable Biomaterials

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Abstract

One of the major concerns associated with the use of silicone material is microorganisms and fungal growth which can result in degradation of the material, inflammation, and chronic infection. Thus, the development of antimicrobial silicone elastomer is becoming necessary. The aim of this study was to evaluate the effect of adding different concentrations of chitosan particles into the silicone matrix. The samples were characterized using a Universal Testing Machine (UTM), Scanning Electron Microscopy (SEM), MTT Assay, antibacterial and hydrolytic material degradation for a month. The addition of 50% chitosan recorded the highest value in the pore area of 29.282 with the widest zone of bacterial inhibition of 6.4 ± 0.4 mm as well as the highest% cell viability of 80.08 ± 1.21%, the furthermore, the shortest lifetime predicted from biodegradation test was around 36 weeks.

KEYWORDS:

1 | INTRODUCTION

Low back pain is one of the diseases that result from degenerative disc disease (DDD), which is the movement of the nucleus pulposus through the annulus fibrosus, which causes compression of the nerves in the spine. In addition to the effects of the natural aging process, environmental and genetic factors such as Hernia Nucleus Pulposus (HNP), improper sitting and spinal accidents also affect DDD, causing mechanical failure of the IVD (intervertebral disc). These factors influence each other, so environmental and genetic influences will accelerate the aging process[1].

Intervertebral disc (IVD) is a spinal cushion formed from fibrous cartilage. The IVD plays an important role in receiving compressive loads on the body and distributing them across each other's body[2]. As time passes, the intervertebral disc will undergo a natural aging process often called DDD (Disk Degeneration Disease)[3]. The cause of DDD is due to degeneration or damage to the nucleus pulposus in the IVD. As a result, the hydrostatic pressure on the inner surface of the annulus fibrosus will often decrease, causing tears, cracks or holes due to repeated loading. Currently, surgery is the most common treatment for nucleus pulposus degeneration. However, this will lead to increased stress and loss of mobility in the adjacent intervertebral disc, accelerating degeneration[4]. Surgery for degeneration is not curative, because the surgical procedure involves removing a portion of the spine that presses on the spinal nerves[5]. The high cost of treatment and the ineffectiveness of surgical procedures for spinal degeneration diseases necessitate the discovery of new methods that are faster, easier and more affordable for this treatment, such as the injectable method. The injection method used is the process of introducing biocompatible materials as IVD substitutes, such as alginate, poly(vinyl alcohol) or chitosan[6]. Chitosan is a polymer derived from chitin that is very abundant in nature. Chitosan has good biocompatibility, antimicrobial, and biodegradable properties with low toxicity. Chitosan also has high crystallinity, rigid form, so it can be used in various biomedical applications[7]. Previous research has reported that adding chitosan to PDMS (polydimethylsiloxane) could reduce the surface tension and increase the tensile strength of the material[8]. Another advantage of adding chitosan to PDMS is improving their mechanical properties. Since hardness and brittleness are the main weaknesses of chitosan, which limit its use as a replacement material for spinal cushions, mixing chitosan with PDMS can make the material softer and more elastic[9]. Other research has shown that adding chitosan to natural rubber latex can increase the heat resistance and improve the mechanical properties of natural rubber[10]. In addition, there is one type of silicone rubber, namely RTV 585, which has a curing temperature at room temperature[11]. This can be used as a base to use RTV 585 silicone rubber as an injectable material.

2 | EXPERIMENTAL PROCEDURE

The Materials used in this research include chitosan dissolved in STPP (sodium tripolyphosphate) and vanillin solution. RTV 585 silicone rubber is used in the synthesis of RTV 585 silicone rubber/Chitosan hydrogel. Aquades and 1% CH3COOH solution can be used to dissolve chitosan.

The process starts with preparing the chitosan solution and silicone rubber RTV 585, which is weighed in weight percentage. The chitosan solution is put into the mold together with silicone rubber RTV 585 in a composition without mixing, 10, 20, 30, 40, 50% chitosan. Then the stirring process was carried out for 30 minutes at room temperature using a mechanical stirrer. The next stage is the casting process in the mold and 3 g of curing catalyst is added. Subsequently, SEM EDX tests were performed to determine the cross-sectional morphology and chemical composition in the composite, as well as MTT assay tests using BHK (Baby Hamster Kitnap) 21 fibroblast cells, antibacterial using S.mutans bacteria, and material degradation tests using PBS solution (phosphate buffered saline).

3 | RESULT AND DISCUSSION

Morphological analysis was used to determine the cross-sectional morphology of the composite, which was observed using SEM at 500x magnification. Figure 1 shows that there are pores in Silicone Rubber RTV 585.

Figure 1 shows the SEM results of the RTV 585/chitosan silicone rubber composite which has pores due to entrapped air and the solution remaining in the RTV 585 silicone rubber, without the addition of chitosan the percentage of pore area is 5.91%. With the addition of 50% chitosan, there are pores with the largest % surface area, namely 29.28%. This is due to differences in the density of the solution, the results of which will affect the mechanical properties of the composite[12].

Morphological results from SEM tests show irregular pore shapes. Figure 1 shows the micromorphology of Silicone Rubber RTV 585, where the higher the composition of the chitosan solution and the addition of a curing catalyst, the more pores/air voids there are and the lack of a vacuum method to remove air trapped in the composite. These air voids can affect the mechanical properties of the composite[13].

Energy Dispersive X-ray (EDX) tests were performed on all samples. This test was performed to determine the amount of nitrogen in each sample so that the effect of adding chitosan on the composition level of each sample could be seen

From the EDX test, it can be concluded that the addition of chitosan solution increases the amount of nitrogen in the test sample. So this can be seen as the presence of chitosan in the sample, in addition, the addition of chitosan solution can increase the

TABLE 1 Composite composition of Silicone Rubber RTV 585/Chitosan

Addition of Chitosan	Nitrogen wt %
Without Chitosan	-
Chitosan 10%	0.57
Chitosan 20%	0.67
Chitosan 30%	0.73
Chitosan 40%	1.49
Chitosan 50%	1.56

amount of nitrogen in the composite. The results obtained showed that the lowest elemental composition was the addition of 10% chitosan, which was 0.57 wt%, and the highest was the addition of 50% chitosan, which was 0.16 wt%.

TABLE 2 The Effect of Addition of Chitosan in the Compressive Strength of Composite

No	Addition of Chitosan (%)	Compressive Strength (MPa)
1	0% Ch	1.93 ± 0.41
2	10% Ch	1.87 ± 0.88
3	20% Ch	1.68 ± 0.61
4	30% Ch	1.57 ± 0.74
5	40% Ch	1.18 ± 0.58
6	50% Ch	0.92 ± 0.88

Based on Table 2 , it can be seen that the specimen without the addition of chitosan has the highest compressive strength value of 1.93 ± 0.41 Mpa. While the smallest compressive strength value is possessed by the specimen with the addition of 50% chitosan of 0.92 ± 0.88 Mpa. It is found that there is a decreasing trend so that it can be concluded that the higher the chitosan composition, the lower the compressive strength value. This decrease in compressive strength is due to the presence of voids in the sample. Defects such as voids in the composite can affect the distribution of the load carried out by the matrix, if there is a stress concentrated in the area around the defect, the mechanical properties of the composite will decrease[14].

In the human nucleus pulposus, in vivo pressure is between 0.46 Mpa - 1.36 Mpa in sitting conditions and in standing conditions it is between 0.5 Mpa to 0.85 Mpa. While the highest pressure that can be received by the nucleus pulposus is recorded at 2.3 Mpa[15]. Based on Table 2 , the composite with the addition of 40% chitosan and 50% chitosan meets the criteria to be a substitute material for nucleus pulposus replacement.

TABLE 3 Hydrolytic Degradation after Four Weeks

Komposisi (%)	Week 1 (% weight loss)	Week 2 (% weight loss)	Week 3 (% weight loss)	Week 4 (% weight loss)
Without Chitosan	0.48 ± 0.07	0.77 ± 0.08	0.96 ± 0.05	1.14 ± 0.20
Chitosan 10%	0.63 ± 0.04	0.89 ± 0.22	1.03 ± 0.23	1.26 ± 0.22
Chitosan 20%	0.77 ± 0.05	1.11 ± 0.01	1.39 ± 0.04	1.67 ± 0.03
Chitosan 30%	1.11 ± 0.04	1.57 ± 0.44	1.85 ± 0.33	2.26 ± 0.36
Chitosan 40%	1.87 ± 0.15	2.42 ± 0.89	2.91 ± 0.86	3.52 ± 0.70
Chitosan 50%	2.00 ± 0.20	2.60 ± 0.46	3.15 ± 0.50	3.84 ± 0.27

Table 3 shows the effect of adding chitosan to the Silicone Rubber RTV 585/chitosan composite on the hydrolytic degradation test. It can be seen from the results of the biodegradation test that the weight loss of the composite material will increase along with the addition of chitosan composition to the Silicone Rubber RTV 585.

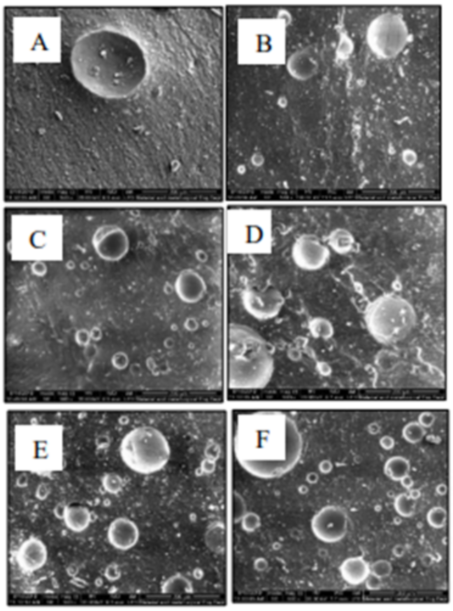

FIGURE 1 Comparison of SEM photographs with 500 (F) 50% Chitosan

Based on the data in Table 3 , the sample with a composition without the addition of chitosan has the lowest weight loss, which is 0.48% in the first week, 0.77% in the second week, 0.96% in the third week, and 1.14% in the fourth week. Meanwhile, the sample with a composition of 50% chitosan has the highest weight loss, which is 2.00% in the first week, 2.60% in the second week, 3.15% in the third week, and 3.84% in the fourth week. Based on the data obtained, the estimated lifetime calculation of the composite can be done using linear regression. The prediction graph can be seen in Figure 2 .

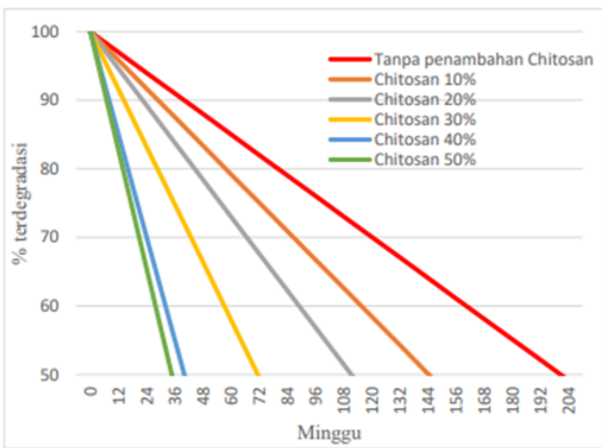

FIGURE 2 Lifetime prediction of Silicone Rubber RTV 585/ Chitosan

The lifetime prediction of composite materials can be seen from the amount of sample weight loss at a certain time. When the weight loss reaches 50%, it can be said that the material is not functioning properly. It was found that the test sample without the addition of chitosan had the longest lifetime, which was 204 weeks. The lifetime of the test sample decreased with the addition of chitosan composition. The sample with the shortest lifetime was obtained in the 50% chitosan sample with an age of 36 weeks. The sample lifetime can be seen in Table 4 .

TABLE 4 Hydrolytic Degradation after Four Weeks

Komposisi (%)	**Week 1 (% weight loss)**	**Week 2 (% weight loss)**	**Week 3 (% weight loss)**	**Week 4 (% weight loss)**
Without Chitosan	0.48 ± 0.07	0.77 ± 0.08	0.96 ± 0.05	1.14 ± 0.20
Chitosan 10%	0.63 ± 0.04	0.89 ± 0.22	1.03 ± 0.23	1.26 ± 0.22
Chitosan 20%	0.77 ± 0.05	1.11 ± 0.01	1.39 ± 0.04	1.67 ± 0.03
Chitosan 30%	1.11 ± 0.04	1.57 ± 0.44	1.85 ± 0.33	2.26 ± 0.36
Chitosan 40%	1.87 ± 0.15	2.42 ± 0.89	2.91 ± 0.86	3.52 ± 0.70
Chitosan 50%	2.00 ± 0.20	2.60 ± 0.46	3.15 ± 0.50	3.84 ± 0.27

Silicone rubber RTV 585 material is reported to be a polymer material that has a low degradation rate. Silicone rubber RTV 585 obtained from chemical synthesis containing Silicon (Si) elements tends not to be easily degraded by the body[12],[16],[17]. The degradation of the silicone rubber RTV 585/chitosan composite is caused by the hydrolysis of acetic acid which reacts with phosphate ions in the phosphate buffered saline solution contained in the synthesized chitosan. Acetic acid is hydrolyzed so that it increases the degradation of chitosan, which also causes a decrease in pH. This is what causes the mass loss ratio to become faster[18],[19]. Therefore, the composite degradation process does not occur in the composite forming material but rather in the increase in the viscosity of acetic acid and the reduction of crosslink bonds using vanillin (4-hydroxy-3-methoxybenzaldehyde)[20], causing the material to become brittle. In this case, the composite degeneration media in this study, namely phosphate buffered saline (PBS), has ions such as NaCl and Na2HPO4 so that this media can stimulate weight reduction of the Figure 3 shows that there were no significant macro visual changes from the first week to the fourth week in the composite sample.

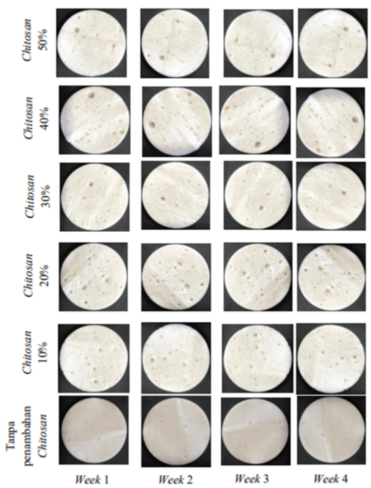

FIGURE 3 Macro-visual of the sample after Hydrolytic Degradation

The MTT assay test was conducted to determine the development of cells when given composite material which was influenced by the addition of chitosan percentage.

In Table 5 , it can be seen that the development of BHK 21 Fibroblast cells obtained increased cell viability with the addition of chitosan. In the composition without the addition of chitosan, the cell viability obtained after calculating was 10.30%, in

TABLE 5 Cell Viability Calculation Results

Addition of Chitosan	Cell Viability (%)
Without Chitosan	10.30
Chitosan 10%	18.64
Chitosan 20%	29.17
Chitosan 30%	40.59
Chitosan 40%	51.93
Chitosan 50%	80.08

the composition of 10% chitosan addition, the cell viability was 18.64%, in the composition of 20% chitosan addition, the cell viability was 29.17%, in the composition of 30% chitosan addition, the cell viability was 40.59%, in the composition of 10% chitosan addition, the cell viability was 51.93%, and in the composition of 50% chitosan addition, the cell viability was 80.08%. During the MTT assay cell viability measurement, the lowest percentage was in the specimen without the addition of chitosan, which was 10.30% and the largest percentage of cell viability was in the specimen with 50% chitosan, which was 80.08%. Composite silicone rubber RTV 585/ chitosan without the addition of chitosan with the addition of chitosan showed increasing cell viability, this indicates that chitosan has good biocompatibility in the mammalian body[21],[22]. It has also been reported that the nature of silicone rubber material does not allow the growth of both tissue and bacteria so that it becomes a bioinert material[16],[17]. In this study, the cell viability value changed with the addition of chitosan, as shown in Figure 4 . Chitosan is a material that has good biocompatibility, where several journals report that chitosan when composited with other materials will increase good cell viability, this is influenced by the cells used in the study and the type of testing carried out[23] [24]. The deacetylation process will cause the removal of acetyl groups from chitin molecules, resulting in chitosan with a high degree of chemical reactivity from amino groups[25]. This is what causes when Silicone Rubber RTV 585 is composited with chitosan into a controlled substrate, cells will spread on the surface of Silicone Rubber RTV 585/chitosan and do not experience damage to cell morphology. It has been shown that some proteins from chitosan remain in the sample or are secreted by cells. The addition of chitosan plays an important role in cell adhesion and spread on the composite. This shows that Silicone Rubber RTV 585 mixed with chitosan will cause adhesion and development in fibroblast cells[26]. The value of cell viability against mammalian cells will increase along with the addition of chitosan levels and its antibacterial properties will increase. In addition, other studies have reported increasing biocompatibility properties[22],[27],[28]. The results of cells before and after planting samples can be seen in Figure 4 .

4 | CONCLUSION

In this study, the influence of chitosan addition on the properties of Silicone Rubber RTV 585/chitosan composites was investigated, focusing on morphology, mechanical performance, hydrolytic degradation, and biocompatibility. The SEM analysis revealed that increasing chitosan content led to a higher number and size of pores in the composite, with the 50% chitosan sample exhibiting the most significant pore area. This morphological change contributed to a decline in compressive strength, as observed in the mechanical testing, where the compressive strength decreased with increasing chitosan content, reaching a minimum for the 50% chitosan sample. The hydrolytic degradation test showed a corresponding increase in weight loss and a decrease in the estimated lifetime of the composites, with the 50% chitosan sample having the shortest predicted lifespan. In contrast, the addition of chitosan significantly enhanced the biocompatibility of the composite, as evidenced by the increasing cell viability in the MTT assay, with the highest cell viability observed in the 50% chitosan sample. These results suggest that while chitosan improves the biocompatibility of Silicone Rubber RTV 585 composites, it also impacts their mechanical and degradation properties. Therefore, optimization of chitosan content is essential to balance biocompatibility with the desired mechanical and degradation characteristics for specific biomedical applications, such as soft tissue engineering.

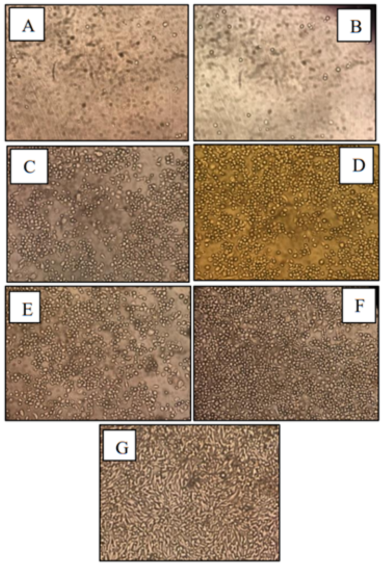

FIGURE 4 Effect of Chitosan Addition to RTV 585/Chitosan Silicone Rubber Composite on Cell Viability (A) Without Chitosan Addition, (B) 10% Chitosan, (C) 20% Chitosan, (D) 30% Chitosan, (E) 40% Chitosan, (F) 50% Chitosan, (G) Control Cell

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