Poly L-Lactic Acid (Plla)-Collagen Coating Chitosan as a Spring-Loaded Silo Candidate for Gastroschisis

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Abstract

Gastroschisis is one of the birth defects with organ conditions that come out of the abdominal cavity. The handling of gastroschisis is conducted by the staged closure method or wrapping the organ that comes out to be inserted slowly using gravity and a spring-loaded silo.

To overcome this problem, a research study was conducted to find candidates for spring loaded silos with the combination of 5% PLLA, collagen with various concentrations (1, 0.75, 0.5, and 0.25)%, and 1% chitosan which was expected to reduce the risk of side effects in infants with gastroschisis such as infections and microbes. It can also improve the mechanical properties of the spring-loaded silo membrane. Based on the FTIR test, it showed the functional groups of PLLA (Ester), Collagen (Amide), and Chitosan (Amine).

The SEM results showed that the overall pore value was 4.42-6.67 µm, thus it can meet the goretex pore size with a pore size range of 0-25 µm. The results of the tensile strength test, the UTS value on the abdominal wall was 2 - 9.2 MPa, thus the K2 sample with a value of 8.56 MPa has met and the value of the Elasticity Modulus of the linear alba layer on the abdominal wall was 23 - 335 MPa.

The results of the contact angle test showed that the PLLA-Collagen chitosan coating samples were better on hydrophilic properties than the PLLA-Collagen samples. Cytotoxicity test results showed that the percentage of living cells was above 70% thus the membrane was non-toxic.

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Introduction

Reported gastroschisis has increased over the past 25 years from 0.1 cases per 10,000 to 1 case per 10,000 live births in developed countries, and from 3 to 5 cases per 10,000 births in developing countries.[1] Indonesia was one of the developing countries that have a high risk of giving birth to babies with gastroschisis. In the 2010-2012 period, to be precise, at Sanglah Hospital Bali, there were 37 cases of infants with gastroschisis.[2]

Gastroschisis is a congenital defect in the anterior abdominal wall of infants (generally located lateral to the right side of the umbilicus) resulting in evisceration of the contents of the abdominal cavity such as intestines, right gastric canal, and vaginal canal, thereby increasing the risk of infection and injury.[3] This disorder is associated with maternal age <20 years, maternal malnutrition, low economy, smoking, alcoholic beverages, drugs such as non-steroidal anti-inflammatory drugs and acetaminophen in the first trimester. This is because several types of drugs are categorized as teratogens, namely substances contained in drugs that if consumed by pregnant women can trigger birth defects. Hence, it can increase the risk of gastroschisis, because it can affect the fetus, placental function, and cause very strong uterine muscle contractions. This can occur due to damage to blood vessels thus blood vessels constrict and reduce the supply of oxygen and nutrients to the fetus.[4]

Several solutions needed in handling this Gastroschisis case are primary closure and

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staged closure. Staged closure with spring loaded silo can be used in cases of swollen bowel and there is a lot of bowel outside the body. Staged closure aims to wrap the contents of the abdominal cavity out, allowing gravity to help the intestines slip into the stomach and reduce intra-abdominal pressure. Gastroschisis with the staged closure method using silos resulted in lower infection rates (including sepsis, central line infection, pneumonia) and necrotizing enterocolitis (NEC) when compared to primary closure.[5]

Thus, other alternative materials are needed, one of them is Poly-L-Lactic Acid (PLLA). PLLA is a synthetic polymer thus in its application PLLA is less biocompatible when compared to natural polymers. PLLA as a biodegradable material has good mechanical properties and biocompatibility, but low hydrophobicity and low thermal stability. Besides that, PLLA has problems due to its rigidity, easy fracture, and low crystallization rate.[6] Chitosan is a natural biopolymer that has biocompatible, non-toxic, bioactive properties, does not cause immunological, anti-bacterial, fungi static, and anti-tumoral reactions. The added chitosan coating can increase biocompatibility and anti-bacterial so that it protects the intestines from bacterial contamination.[7] Collagen is widely used in hernioplasty applications because it can fight infection, cause minimal inflammatory reactions, biodegradable, biocompatible, low immunogenicity, and increase proliferation, adhesion, migration, and differentiation of host cells.[8] The addition of collagen can increase the mechanical strength of the silo because it increases the Ultimate Tensile Strength value.[9] Research by Widiyanti et al., 2014 showed the need to improve the composition of additional collagen material thus it can increase the mechanical strength of the silo.[10]

By combining PLLA, collagen, and chitosan, it is hoped that it can reduce the risk of side effects in infants such as microbial infection and can improve the mechanical properties of the silo. To test the resulting silo, FTIR test, SEM morphology test, tensile strength test, contact angle test, and cytotoxicity test (MTT assay) are needed.

Materials and methods

The main ingredients used in this study were Poly-L-Lactic Acid (PLLA) (Mn = 100,000-125,000 Da) from PolySciTech, chloroform from Merck (USA), and chitosan from crab shells acetylated more than 80% (Mw = 430,000 Yes). Additional materials used as variations in this study were collagen and glutaraldehyde.

Methods of Synthesis

Spring-Loaded Silo Membrane Fabrication

The manufacture of spring-loaded silos began with dissolving the synthetic polymer Poly-L-Lactic Acid (PLLA) in a cotton-like shape into the solvent, namely chloroform. Then after 14 hours of dissolving in the solvent, the solution will look clear, thus indicating that the solution was homogeneous. After that, the process of forming a spring-loaded silo which was molded using a petri dish was soaked for a while with NaCl solution thus the spring-loaded silo sample can be released from the mold.

Spring-Loaded Silo Coating Process with Collagen and Chitosan

The collagen coating process began with cleaning the sample with aquades to remove adhering dirt. Subsequently, collagen solutions with different concentrations were made (1, 0.75, 0.5, and 0.25% w/v). Each concentration was dissolved in 0.1M acetic acid solution and stirred for 3 hours at room temperature. Then, the samples were soaked in a collagen solution that had been formed for 1 hour in an incubator at 37°C.

After that, the chitosan solution was prepared by dissolving it in 1% acetic acid solution and stirring until homogeneous. The chitosan coating process was performed using the dip coating method which was dissolved in 1% acetic acid for 30 minutes at 27°C and dried at 30°C.

Methods of Characterization

Fourier Transform Infrared (FTIR)

The sample will be tested at a wave number of 500-4000 cm-1. Characterization using FTIR was carried out on a cuvette filled with sample pieces with a size of 1 mm^2 and KBr and inserted into the FTIR instrument. The result of the FTIR test was a graph showing the wave number (cm-1) and transmission (%).

Scanning Electron Microscope (SEM)

The sample preparation procedure for the SEM test started from cutting the sample into 10 mm size. The sample was placed in a sample container then coated with gold. Furthermore, the morphological samples were observed with a

magnification of 500-2000X

Tensile Strength

The sample was cut in the shape of a dog bone, then clamped at each end in the top-down position of the machine. After that, one end of the membrane was pulled up by the machine. Thus, the required magnitude of force was obtained until the sample was disconnected.

Contact Angle

The contact angle test was conducted by dripping ± 20 L of distilled water using a pipette on a spring-loaded silo sample on a glass slide. This test was repeated 5 times and then measured using ImageJ software.

Cytotoxicity (MTT Assay)

Cytotoxicity test was conducted with a 96-well microplate for the sample testing container. The cut samples were placed into plates and incubated for 24 hours at 37°C and 5% CO_2. Furthermore, MTT 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide reagent was added and incubated again at 37°C. After incubation, purple formazan crystals formed on the bottom were added and dissolved with 200 L DMSO for 30 minutes. Viability was observed with optical density using ELISA Multi plate Reader at a wavelength of 570 nm.

$$\%\ \text{Living Cell} = \frac{\text{OD treatment} - \text{OD media}}{\text{OD cell} - \text{OD media}} \text{x} 100\% \quad (1)$$

Results

Spring-loaded silo Poly L-lactic acid (PLLA)-Collagen with chitosan coating has been successfully made with four variations of composition PLLA (5% w/v), Collagen (1%, 0.75%, 0.5% and 0.25% w/ v) and 1% chitosan, and there is the addition of 0.05M glutaraldehyde to the chitosan. The control solution (K0) is PLLA 5% Collagen 1%. Treatment solution is (K1) PLLA 5% Collagen 0.75% with 1% Chitosan coating. Treatment solution is (K2) PLLA 5% Collagen 0.5% with 1% Chitosan coating. While the treatment solution (K3) is PLLA 5% Collagen 0.25% with 1% Chitosan coating and the addition of 0.05M Glutaraldehyde. The results of the synthesis of Spring-loaded silo samples with variations of PLLA, Collagen, and Chitosan showed that the samples were elastic, smooth, flat, and transparent.

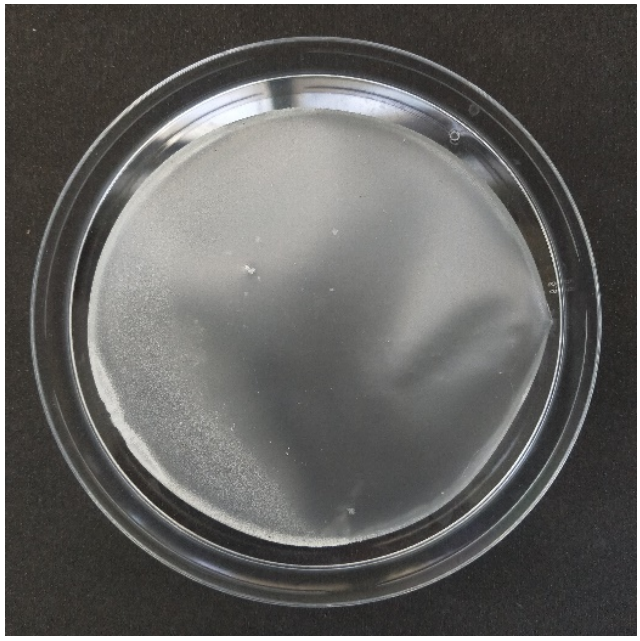

Figure 1. Spring-Loaded Silo Membrane.

Fourier Transform Infrared (FTIR) Results

The results of the FTIR functional group test showed that the spring-loaded silo sample already contained the functional groups of PLLA (Ester), Collagen (Amide), and Collagen (Amide). The FTIR spectrum was shown in Figure 1.

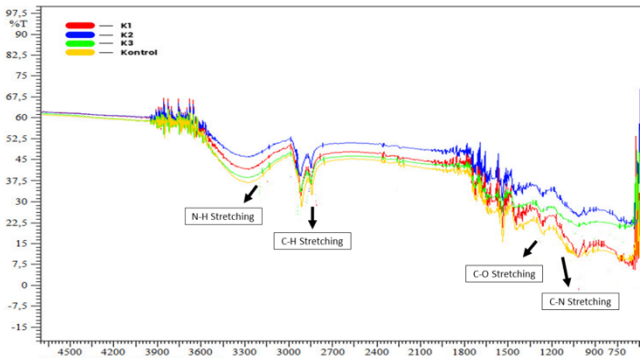

Figure 2. FTIR spectrum of a spring-loaded silo sample.

Based on Figure 2, the FTIR functional group test proves the content of chitosan, collagen, and PLLA in the spring-loaded silo samples. The visible absorption band between 3176.90 to 3177.86 cm^{-1} represents the free primary amino group (-NH2) at the C2 position of glucosamine which is the main group present in chitosan. The functional group (C-H stretching) at a wave number of 2847.05 cm^{-1} is a characteristic of polysaccharides. We did not find an absorption band at 1550 cm-1 (N-H bending)

which is a typical N-acetyl group in chitosan this may be due to overlapping with other bands. The functional group Amide II (C-N stretching) on collagen is at a transmittance of 1267.25 to 1269.57 cm-1. PLLA has identical functional groups derived from esters (O=C-O). In this study, the ester functional group (C=O stretching) which is generally found at a wave number of 1750 cm^{-1} is not found. However, stretching C-O bonds can be found at wave numbers 1149.62 to 1177.59 cm^{-1}.

Scanning Electron Microscope (SEM) Results

The test was conducted at 2000x magnification of the spring-loaded silo sample, in order to obtain a morphological picture showing the presence of pores in each sample. Hence, pore measurements were conducted using ImageJ software and the results of the SEM test were shown in Table 1.

Sample		Thickness (µm)	Pore Size (µm)
K0	PLLA 5%-Kol 1%	0.09	6.67±0.69
K1	PLLA 5%-Kol 0.75%-Chit 1%	0.11	6.38±1.11
K2	PLLA 5%-Kol 0.5%-Chit 1%	0.08	6.12±1.19
K3	PLLA 5%-Kol 0.25%-Chit 1%-GA 0.05 M	0.07	4.42±1.33

Table 1. Result of SEM test.

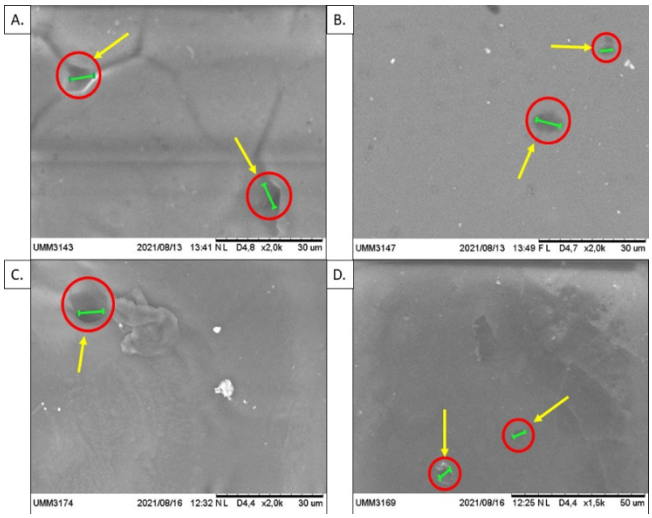

Figure 3. Morphological results of spring-loaded silo samples (A) Sample K0; (B) Sample K1; (C) Sample K2; (D) Sample K3.

Morphologically, it can be seen if the sample has pores as shown in Figure 2. In Table 1, samples K0, K1, K2, and K3 experienced a decrease in pore size. The resulting pore size in the K0 sample was 6.67±0.69 µm, the pore size in the K1 sample was 6.38±1.11 µm, the pore size in the K2 sample was 6.12±1.19 µm, and the pore size in the K3 sample was 4.42±1.33 µm. With an overall pore size of 4.42-6.67 µm which met the Goretex pore size with a size range of 0-25 µm.[11]

Tensile Strength Results

Based on Table 2, it can be seen that the concentration of collagen and chitosan coating and the addition of glutaraldehyde affected the Ultimate Tensile Strength (UTS) and Elasticity Modulus values in the spring-loaded silo samples. The UTS value in the K0 sample was 7.53 MPa, the K1 sample was 11.3 MPa, the K2 sample was 8.56 MPa, while in the K3 sample was 13.94 MPa. The tensile strength value on the abdominal wall was 2 - 9.2 MPa [14] thus the K2 sample with a value of 8.56 MPa has fulfilled this value range. The modulus of elasticity in the K0 sample was 240 MPa, the K1 sample was 442 MPa, the K2 sample was 543.99 MPa, while the K3 sample was 646.79 MPa. The modulus of elasticity of the linear alba layer on the abdominal wall was 23 - 335 MPa.[12] The current handling of gastroschisis is still using a urine bag and a blood bag. Each of these materials is still not in accordance with abdominal wall standards. Blood bag has a tensile strength value of 13.11 MPa,[13] while based on the tensile strength test that we have conducted on urine bags, it is 9.75-12.59 MPa.

Sample		UTS (MPa)	Elasticity Modulus (MPa)
K0	PLLA 5%-Kol 1%	7.53	240
K1	PLLA 5%-Kol 0.75%-Chit 1%	11.3	442
K2	PLLA 5%-Kol 0.5%-Chit 1%	8.56	543.99
K3	PLLA 5%-Kol 0.25%-Chit 1%-GA 0.05M	13.94	646.79

Table 2. Result of tensile strength test.

Contact Angle Result

The sample is declared hydrophilic if the value of the degree of contact angle is <90° and more hydrophobic if the value is >90°.[14] Figure 4 showed that the treatment sample which was K1 with a value of 74.4°, K2 with a value of 71.97°, and K3 with a value of 70.21° has a lower degree of contact angle compared to the degree of control angle (K0) which has a value of 83.69°. Based on these results, it can be concluded that the spring-loaded silo treatment sample PLLA-Collagen chitosan coating is better for hydrophilic

properties than the control sample PLLA-Collagen.

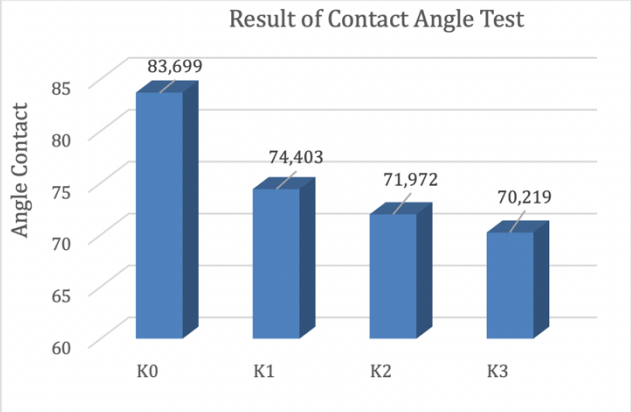

Figure 4. Result of contact angle test.

Cytotoxicity Results (MTT Assay)

The cytotoxicity test used Baby Hamster Kidney (BHK-21) cells. Based on Figure 5, the value of cell viability in sample K0 was 92%. The sample K1 was equal to 71.89%. The sample K2 was equal to 80.99%. While the sample K3 was 72.47%. All the results of the cytotoxicity test showed non-toxicity. This was indicated by the percentage of living cells that were above 70%.[15] *Chitosan* has been recommended as biomaterial for biomedical applications primarily due to its *biocompatibility.*[16]

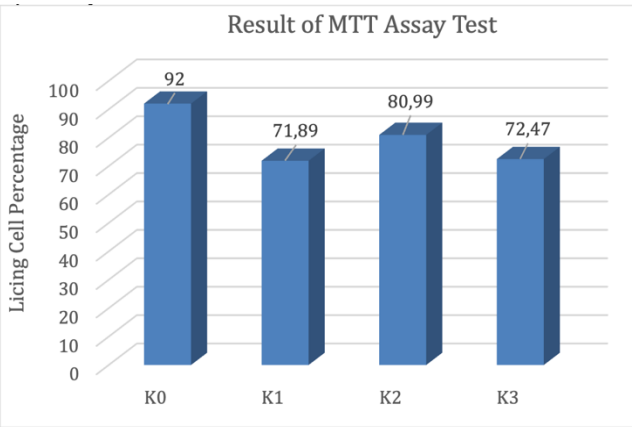

Figure 5. Results of the percentage of cell viability of spring-loaded silo samples.

Conclusions

Spring-loaded silo membrane samples with variations of PLLA, collagen, and chitosan showed a smooth, flat, and transparent sample surface. FTIR test results examined that the sample contains functional groups of PLLA (Ester), collagen (Amide), and chitosan (Amine). The results of the characterization of the spring-loaded silo membrane was revealed with FTIR, SEM, tensile strength, contact angle, and MTT Assay tests. FTIR test showed the functional groups of PLLA (Ester), Collagen (Amide), and Chitosan (Amine). The SEM results showed that the overall pore value is 4.42-6.67 µm, thus it can meet the goretex pore size with a pore size range of 0-25 µm. The results of the tensile strength test, the UTS value on the abdominal wall is 2 - 9.2 MPa, hence the K2 sample with a value of 8.56 MPa has met and the value of the Elasticity Modulus of the linear alba layer on the abdominal wall is 23 - 335 MPa. The results of the contact angle test showed that the PLLA-Collagen chitosan coating samples were better on hydrophilic properties than the PLLA-Collagen samples. Cytotoxicity test results showed that the percentage of living cells was above 70% thus the membrane was non-toxic.

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Declaration of Interest

The authors report no conflict of interest.

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