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Hydrogel–Collagen/PVA as Artificial Cartilage for Osteoarthritis Application

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Osteoarthritis (OA) is a disease of degeneration in the joints involving the cartilage, the lining of joints, ligaments, and bones, causing pain and stiffness in the joints. In 2013, cases of OA reached 30% in the community aged 40-60 years. In addition, OA is the number five disease that causes defects in women in developed countries. This study aims to determine the variation of the variation of PVA in the hydrogels of the characteristics of AC and to understand the optimal composition of hydrogels / PVA. Artificial cartilage (AC) is the latest technology to replace damaged knee cartilage. One candidate for artificial cartilage is polyvinyl alcohol (PVA) hydrogel with a variety of collagen concentrations: PVA (80:20, 70:30, 60:40, 0: 100) using the freeze thawing method, freeze at -80°C for 18 hours and 6 hours at room temperature. The swelling ratio based on the results obtained by the percentage of swelling depends on the increase. The results of compressive strength test show the results which are the literature on the composition variation of 60:40 with the number 14.64 MPa. in the composition variation 60:40 shows the best sample because the literature collection is 4.31MPa. The FTIR test showed that the interaction between the – OH group and C = O formed showed the synthesis of the successful hydrogels / PVA. The SEM test was carried out on the best sample, namely at a concentration of 60:40 with a pore size of 52.225 μm and with a thickness of 1.98 μm. Based on the results of compressive characterization and tensile strength showed the best samples and met the requirements of artificial cartilage at a concentration variation of 60:40.

Keywords: Artificial cartilage; Osteoarthritis; hydrogel-collagen / PVA

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In Indonesia, cases of osteoarthritis have reached 30% in people age 40-60 years. This figure continues to increase up to 65% in elderly people more than 61 years [1]. Based on data (WHO) in 2013, an estimated 9.6% of males and 18% of women aged more than 60 years had symptomatic osteoarthritis, and 80% of those people had limitations in the move and 25% could not do activities Daily [2]. In addition, according to (WHO, 2008) Osteoarthritis (OA) is the number five disease that causes lifelong disability (Years of Life Disability) in women and developed countries [2]. However, in developing countries, OA ranks to nine causes of lifelong disability. One of the main causes of OA is excess weight that can cause the joints to withstand heavier loads against fatty tissues that will produce proteins that can damage and create inflammation in the joints. OA is caused by several factors such as: Age factor, genetic, obesity, joint injury, exercise, anatomical anomaly, metabolic disease, joint inflammatory diseases, and excessive physical activity is the risk factor of osteoarthritis [3]. Healing methods in patients with osteoarthritis can be done with 2 methods of therapy, namely pharmacological therapy (using drugs) such as, analgesic (pain reliever), anti-inflammatory drugs (NSAIDS), and analgesic (outside drugs). Analgesic drugs in the form of ointments, gels, creams, or injections with low doses. The use of these medications in the long term can have risks and side effects such as toxicity to the kidneys, and Gastrointestinal bleeding (bleeding in the gastro-intestinal tract). Injections in OA also have side effects of bone injecting because the chemicals used are acidic so that it can harm the bones. Non-pharmacological therapy is a life-style change therapy including physical therapy, rehabilitation therapy, weight loss therapy. This therapy is done to improve the ability of the drug and to reduce the side effects of the drug so that people do not get worse. Patients with OA tend to be elderly so as to do non-pharmacological therapy have their own limitations. This method is only done for early stage patients and cannot be used in advanced stage patients. But to be remembered is OA there is no cure to restore to the initial function.

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The only treatment is to minimize the side effect of the drug by cartilage (a total knee replacement). The change of cartilage is one of the tissue engineering that can provide an alternative solution for the repair and regeneration of articular cartilage [4].

Biomaterials that can be used as hydrogel with cartilage replacement function are type II collagen and Polyvinyl Alcohol (PVA). Collagen is the primary protein in composing extracellular matrix components and is the most widely found protein in the human body. Type II collagen is found in many cartilages. The principle is about 90-95% of the cartilage and forming a crossed-out primary component [5]. Collagen has a weakness that is low mechanical properties that require the addition of a polymer material that has biocompatibility properties and high mechanical properties. Hydrogel/PVA is one of the synthetic polymers with the advantages of good hydrophility and biocompatibility, not toxic and has strong mechanical properties, as well as good. stability [6]. Polyvinyl Alcohol (PVA) itself is a synthetic polymer that has properties resistant to fats and solvents, emulsifiers and adhesives that are good, nontoxic and have bending properties as well as biodegradable or easily described naturally [7].

In this study, by conducting hydrogel-collagen/ PVA synthesis is used as a material to form a hydrogel that resembles the original cartilage tissue using a variation of type II-PVA collagen composition (80: 20, 70:30, 60:40, 100:0) performed To know the characteristics of the mechanical properties by conducting tensile strength test to know i.e. tensile strength of artificial cartilage, press force test to know the mechanical properties of the press force of artificial cartilage, test Fourier Transform Infra-Red to know the function groups that exist in artificial cartilage, test site to know the uptake of water in artificial cartilage, degradation test to know the level of degradation in artificial cartilage, and SEM test to know Thickness and pore of artificial cartilage.

MATERIALS AND METHODS

The materials used for the manufacture of artificial cartilage tissue engineering are collagen, PVA, 0, 01M acetic acid, and phosphate buffered saline (PBS).

Collagen Solution Synthesis

Collagen Solution Synthesis by preparing 1.125 gr of type II collagen material and 7.5 ml of citric acid is stirred slowly with magnetic stirrer to a homogeneous solution. Then the solution is stored at a temperature of 4 °C.

Manufacturing hydrogel/PVA

The manufacture of hydrogel/PVA can be done by preparing tools and materials first. The required tools are the measuring cups, digital scales, petri cups, beaker glass, spatulas and magnetic stirrers. Material PVA Molecular weight 8.5 X 105 weighing 6,125 gr. The next step by adding 0.01 M acetic acid 17.5 ml is then heated to a temperature of 90 º C and stirred slowly with magnetic stirrer for 90 minutes. When the solution is already homogeneous and the solution becomes clear, the hydrogel-PVA synthesis ends.

RESULT AND DISCUSSION

An Artificial Cartilage-based hydrogel-collagen/PVA is made by dissolving collagen with acetic acid until late by adding a PBS (phosphate buffered saline) solution using magnetic stirrer and inserted in the freezer at 4 ºC for 24 hours. PVA is collapsed with acetic acid for ± 1.5 h with a temperature of 90 º.

Solution of collagen in mixed with solution of PVA with concentration variation (collagen: PVA 80:20, 70:30, 60:40, 0:100) using magnetic stirrer until both solutions become homogeneous. The hydrogel-collagen/PVA sample uses the freeze tawing with a variation in collagen/PVA concentrations (80: 20, 70:30, 60:40, 0:100). After that the sample is in freeze with a temperature-80 °c and is allowed at room temperature for 24 hours so that the final result is shaped leaflet and transparent. The sample is shown in Figure 1 below:

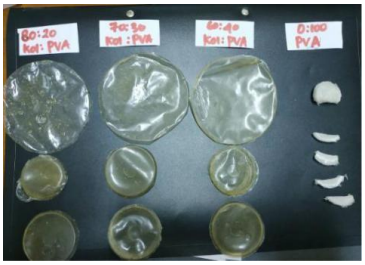

Figure 1. The Synthesis of Artificial Cartilage Hydrogel-Collagen/PVA.

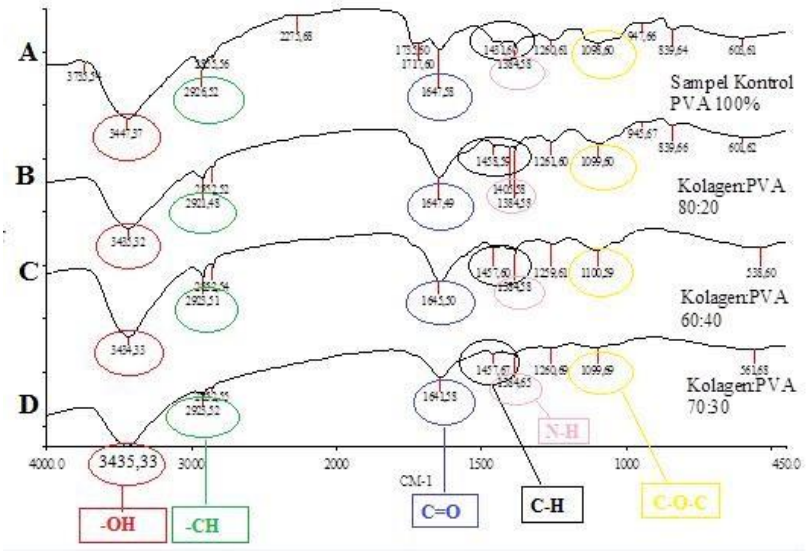

Figure 2. Spectra FTIR Artificial Cartilage Hydrogel-Collagen/PVA with Variation of Collagen: PVA; A (80:20), B (70:30), C (60:40), D (0:100).

Samples that have been made are then tested using FTIR to determine the function group contained in the sample, SEM test is done to find out the morphology of the surface, thickness and porosity of the sample, test site to know the degree Sample development, compressive strength and tensile strength test to determine the mechanical PHISYCS properties owned by the sample. Some of these tests were conducted to determine the potential of Artificial Cartilage-based collagen/PVA as a substitute for artificial cartilage.

Fourier Transform Infra-Red (FTIR) Test Results – Artificial Cartilage-based hydrogel-collagen/PVA

The result of FTIR spectrum artificial cartilage hydrogel-collagen/PVA, each has a cluster representing collagen and PVA. In hydrogel-collagen/PVA There are a number of clusters detected by FTIR, the cluster is -OH, -CH, C = O, C-H, N-H. The results of FTIR artificial cartilage hydrogel-collagen/PVA test can be seen in Figure 2.

As described in the literature [8], on the Spectra FTIR freeze dried hydrogel-collagen/PVA which shows the peak-OH (stretching) on the sample experiencing stretching vibration at the peak of the sample control (D) in the range of numbers 3100-3700 cm^{-1}, mainly the wavelength is 3447.37 cm^{-1}, concentration variation of collagen-PVA (A) 80:20 was in 3435.32 cm^{-1}, concentration variation (B) 70:30 was in 3435.33 cm^{-1} and the concentration variation (C) 60:40 was in 3434.33 cm^{-1}. At the same time, the intermolecular hydrogen bond interaction between the PVA and collagen molecular chains causes the-OH peak of the PVA stretching shifts to the lower number of waves after inserting the collagen.

The C-H cluster stretching located in the range of 2800-3100 cm^{-1} is indicated on the number of waves 2921.41 cm^{-1}, (B) 2923.52 cm^{-1}, (C) 2923.51 cm^{-1} and (D) 2926.52 cm^{-1}, C = O cluster stretching 1700-1600 cm^{-1} shown in the number of waves (A) 1647,49 cm^{-1}, (B 1645.50 cm^{-1}, (C) 1641.58 cm^{-1} and (D) 1647.58 cm^{-1}, in each sample. Stretching C = O, which comes from the collagen and the crystal area of PVA. Infrared Spectra indicates the presence of multiple networks with hydrogen bonds between the Koldan PVA.

The C-H bending cluster located at a range of 1450-1565 cm^{-1} was shown on the number of waves in the sample (A) 1458.59 cm^{-1}, (B) 1457.67 cm^{-1}, (C) 1457.60 cm^{-1}, and (D) 1431.60 cm^{-1} on each sample. The N-H cluster is located at A range of 1300-1800 cm^{-1} at the present on the number of waves in the sample (A) 1384.58 cm^{-1}, (B) 1384.65 cm^{-1}, (C) 1384.65 cm^{-1}, (D) 1384.58 cm^{-1} in each, the sample is an amide group III of type II collagen. In the C-O-C absorption cluster, the range of 1,100-1.005 cm^{-1} is a carbohydrate cluster in the present on the number of waves, such as Table 4.1 (A) 1099.60 cm^{-1}, (B) 1099.69 cm^{-1}, (C) 1100.59 cm^{-1}, and (D) 1098.00 cm^{-1} in each sample [9]. This research, the role of hydrophilic groups is essential for structural formation during hybridization preparations. Hybridization is a combination of two or more materials, each material has a different nature. But from merging two or more of these materials demonstrates the distinctive characteristics of each material. In this FTIR test show the characteristic of the cluster – OH to the Khasan of the PVA and the cluster C = O peculiarities of collagen. If there is a cluster – OH and C = O are formed showing the synthesis of hydrogel-collagen/ PVA successfully.

Degradation of Artificial Cartilage-based Hydrogel-Collagen/PVA

The degradation test is performed as a simulation when artificial cartilage is incorporated into the human body. On the degradation test of Artificial Cartilage collagen/PVA After the sample testing was soaked in PBS solution but very fast damage, within 5 hours the sample began to change shape and was destroyed. It can be seen in Figure 3a (which shown degraded samples) and Figure 3b (which shown dissolved samples). According to Wang, et al [8], these are due to enzymatic hydrolysis due to the process of hydrolysis with the help of the enzyme found in collagen, so that the degradation increases and slowly the sample is degraded perfectly, it causes Loss of samples when immersed in a PBS solution. In pure collagen, the hydrogel is very easy to degrade, but the reduction happened after the addition of PVA material into the hydrogel. This is because PVA cannot be degraded by proteases, and the main cause of degradation due to collagen enzymolysis.

Table 1. Wavenumber FTIR of Hydrogel-Collagen/PVA.

Sample Col-PVA	Wavenumber (cm^{-1})					
	-OH	C-H (*stretching*)	C-H (*Bending*)	N-H	C-O-C (*Stretching*)	C=O
A (80:20)	3435,32	2921,41	1458,59	1384,58	1099,60	1647,49
B (70:30)	3435,33	2923,52	1457.67	1384,65	1099,69	1645,50
C (60:40)	3434,33	2923,51	1457,60	1384,58	1100,59	1641,58
D (100:0)	3447,37	2926,52	1431,60	1384,58	1098,00	1647,58

Figure 3a. Degraded samples.

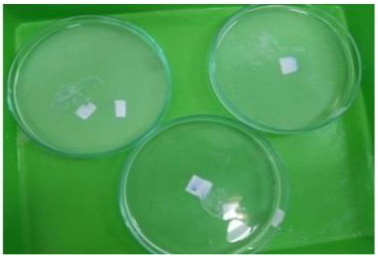

Figure 3b. Dissolved Samples.

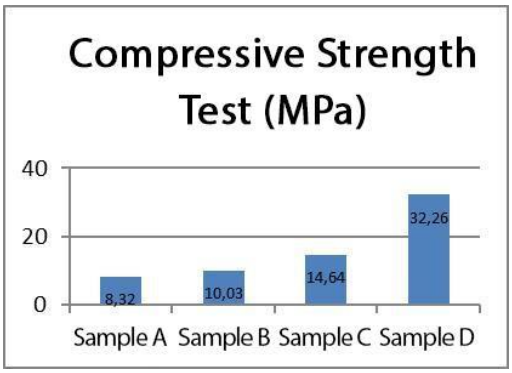

Figure 4. Compressive Strength of Hydrogel-Collagen/PVA A (80:20), B (70:30), C (60:40), D (0:100).

Compressive Strength of Artificial Cartilage Hydrogel-Collagen/PVA

Compressive strength data can be seen the higher the concentration of PVA, the compressive strength value is increasing. However, samples with varying concentrations of collagen: PVA 80:20 and 70:30 are not eligible for artificial cartilage with the compressive strength value being too low. Whereas in the 0:100 control samples passed the requirement limit for artificial cartilage, so on the control samples also ineligible for artificial cartilage. Compressive strength value of artificial cartilage hydrogel-collagen/ PVA According to the literature is 14.5 ± 3.3 MPa. The value of compressive strength test results from the above four samples that approached the criteria as artificial cartilage in sample 60:40 in the results of compressive strength test with a number 14.64 MPa which is the best result for artificial cartilage as seen Figure 4.

Tensile Strength of Artificial Cartilage-based Hydrogel-Collagen/PVA

At a concentration variation of 60:40, tensile strength was 4.31 MPa. Based on the tested samples it appears that the higher the concentration of PVA then the compressive strength higher. This is because PVA has good mechanical strength and has high flexibility [10]. The higher the tensile strength then the elongation of the hydrogel is smaller. In Figure 5 shows that on hydrogel-collagen/PVA the concentration variation of 60:40 has the highest chart height because the sample is thicker so that the sample becomes elastic. The compressive strength on the control sample is the highest as well as with tensile strength. In the variation 60:40 and 100:0, the sample were very thin. The PVA role demonstrates a more significant effect on mechanical properties than collagen. The result of tensile strength in Table 2 was is in accordance with Figure 5 and the research of Obeid et.al [11].

Table 2. Nilai Tensile Strength of Hydrogel-Collagen/PVA.

Code	Variation	Material	Tensile Strength
A	(80 : 20)	Col : PVA	2,46 MPa
B	(70 : 30)	Col : PVA	3,14 MPa
C	(60 : 40)	Col : PVA	4,31 MPa
D	(100 : 0)	Col : PVA	11,54 MPa

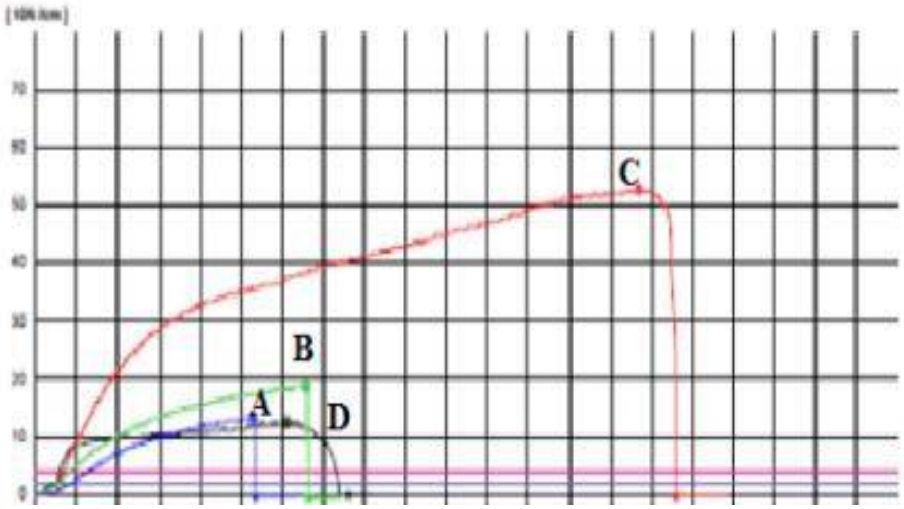

Figure 5. Tensile Strength of Hydrogel-Collagen/PVA Graph
A (80:20), B (70:30), C (60:40), D (0:100).

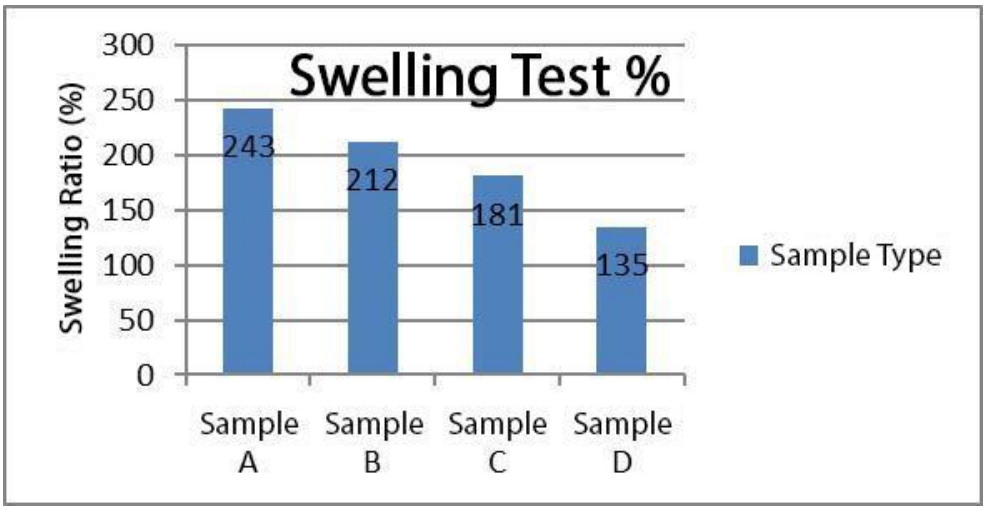

Figure 6. Swelling Capacity of Hydrogel-Collagen/PVA A (80:20), B (70:30), C (60:40), D (0:100).

Swelling Capacity of Artificial Cartilage-based Hydrogel-Collagen/PVA

The phenomenon of site degrees relates to density. High density is caused by the PVA molecule that fills the hydrogel cavity. In addition, the number of cross bonds formed is also more and more, resulting in water will be difficult to diffuse into the hydrogel. The hydrogel is described as hydrophilic so it absorbs bodily fluids but insoluble. The appropriate site rate for the Artificial Cartilage application is 109% [12]. In this research, get close to the ideal results in sample control D with a fairly high figure difference of 135% as seen in Figure 6.

Morphological Property using Scanning Electron Microscope (SEM) of Artificial Cartilage-based Hydrogel-Collagen/PVA

This test is done with a variation of the concentration of 60:40 because it is the best sample of the mechanical test that is test tensile strength and compressive strength test. In addition to testing the pore diameter and surface structure on the sample, the SEM test can also see the thickness size of the sample. Results of the SEM test indicating the diameter of the pore and the surface structure of the hydrogel-collagen/PVA sample can be found in Figure 7.a. The size of pore diameter in the hydrogel-collagen/PVA sample is shown in Figure 7a with a different diameter of 30 µm, 42.4 µm, 54.8 µm, and 89.1 µm with an average pore diameter of 52.225 µm is a fairly large pore diameter, but Hydrogel has an open pore and interconnected structure. The diameter distribution of pores were between 50 and 100 µm, which is suitable for the permeation of nutrients, oxygen, and waste from cells [13].The size of the pore according to the literature instead of cartilage < 20 µm [14].This can be seen in Figure 7a which indicates that there is an irregularity of pore dispersion in the sample, there are many small pores scattered with some large pores seen in the SEM image, it shows The irregularities of pore-sprawl can affect mechanical properties [6].The thickness of artificial cartilage-based hydrogel-collagen/PVA can be seen in Figure 7b.

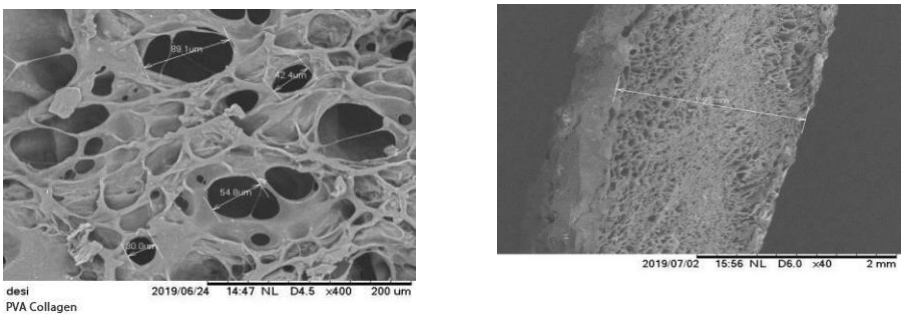

Figure 7a. Pore Size Result by SEM of Artificial Cartilage-based Hydrogel-Collagen/PVA with concentration variation 60:40 7b. Thickness of Artificial Cartilage-based Hydrogel-Collagen/PVA by SEM (400X magnification).

According to previous research in human conducted by Obeid et al [11], the thickness standard of the human cartilage was 3.23 ± 0.63 mm. In this study the thickness was 1.98 mm which was not meet with the standard. The effort to improve the thickness could be by explore temperature variation in the process of freeze thawing to produce smaller pore size. The smaller pore size possibly to increase the required thickness, add material volume.

CONCLUSION

1. Compressive Strength and Tensile Strength value showed that the higher PVA concentration the better mechanical properties will be. Degradation test results occur very quickly. Within 5 hours the sample began to change shape and was destroyed due to collagen enzymolysis.

2. The best composition of hydrogel-collagen/PVA as artificial cartilage in this research was found in the PVA concentration variation of 40 wt% with a compressive strength value of 14.64 MPa and a Tensile Strength value of 4.31 MPa. This value was indicating that the result almost in line with the standard of compressive strength and tensile strength which were 14.4 ± 3.3 MPa and 4 MPa, subsequently.

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