

RESEARCH ARTICLE

Effect of collagen-chitosan hydrogel formula combined with platelet-rich plasma (A study of pH, viscosity, and swelling test)

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ABSTRACT

Platelet-rich plasma (PRP) contains growth factors that speed up a healing process. The liquid form of PRP is difficult to be applied, so it needs material as a carrier of collagen. Collagen can be used as carrier materials and capable to activate growth factors and increase the concentration of the PRP to become a gel. Collagen often losses its shape and size due to degradation when exposed to body fluids. In the selection of materials, collagen can synthesize with chitosan. Collagen-chitosan hydrogel has potential as scaffold. Many formulations for proper hydrogel applied in the oral cavity need to pay more attention to several conditions, such as a neutral pH, high viscosity and ideal swelling. The aims of this study was to examine the influence of various formulations of collagen-chitosan hydrogel PRP against pH, viscosity, and swelling. The study samples consisted of 25 collagen-chitosan hydrogel formulations. The samples were divided into five groups: 100/0; 75/25; 50/50; 25/75; 0/100. The first stage of the test was pH, viscosity and swelling measurement of the collagen-chitosan hydrogel to determine which ones matched the criteria. Collagen-chitosan hydrogel formulations were added with PRP with a ratio of 1:1. The second stage was to measure the pH, viscosity, and swelling measurement to see the results of the physical parameters of the hydrogel after mixed with PRP. The observation data were analyzed by t-test for pH and viscosity, while two-way ANOVA and post hoc LSD for swelling test. The results showed that collagen-chitosan hydrogel formulations of 25/75 and 0/100 met the criteria of pH, viscosity, and swelling. The pH of both groups showed no significant difference ($p>0.05$), but the viscosity and swelling variables showed significant difference ($p<0.05$). To conclude, collagen-chitosan hydrogel formulations mixed with PRP had no effect on pH, but had an effect on viscosity and swelling test. The collagen-chitosan hydrogel formulations mixed with PRP which matched the criteria were 25/75 and 0/100.

Keywords: chitosan; collagen; hydrogel; platelet-rich plasma

INTRODUCTION

Regenerative technology to treat infra-bony bone damage uses platelet-rich plasma (PRP).¹ Platelet-rich plasma is a source of growth factors capable of supporting soft tissue healing, encouraging a faster healing process and proven to increase bone formation and density.² A disadvantage of PRP is easily gets resorbed by the body.

Platelet-rich plasma in liquid form conduces to the loss of PRP into the oral cavity.³ Platelet-rich plasma requires a carrier that is collagen.^{3,4} Collagen in PRP can be a safe alternative, stimulate the release of growth factors from platelet granules, increase the consistency of PRP into gelation, and

be able to reduce PRP clot retraction.^{2,3} Collagen often losses shape and size because of degradation of bodily fluids. Collagen needs to synthesized with chitosan to increase its mechanical strength.⁴

Collagen-chitosan hydrogel is more resistant to enzymatic degradation, denaturation, and compressive strength. Collagen-chitosan hydrogel has potential as a scaffold to support cell therapy and vascularization of damaged tissue.⁵ In fact, hydrogel formulation is determined by the ratio of collagen-chitosan concentration, and these are the ones used: 100/0; 75/25; 50/50; 25/75; 0/100.⁶ Making the right hydrogel formulation for oral cavity application requires attention to the detail

of the requirements, including neutral pH, high viscosity and ideal swelling.⁷ Hydrogels have the same pH value in the oral cavity, from 6.2 to 7.6⁸ to avoid irritation in the oral cavity.⁹ The appropriate viscosity value of gel preparations according to the requirements is 2000-4000 cps.¹⁰ The more amount of chitosan molecules, the higher of viscosity.¹¹ The low elasticity of collagen makes it unable to maintain the porous structures, and the amount of chitosan is increased, the solubility decreases.¹² Swelling value that can be used to cover wound applications is between 100%-200%.¹³

From the description, we had a problem whether different collagen-chitosan hidrogel formulations mixed with PRP had effect on pH, viscosity, and swelling. This research aimed to examine the effect of various collagen-chitosan hydrogel formulations mixed with PRP on pH, viscosity, and swelling.

MATERIALS AND METHODS

This research was a pure experiment and had been approved by the ethics committee of the Faculty of Dentistry, Universitas Gadjah Mada with registration number 001643/KKEP/FKG-UGM/EC/2018. The study sample was human peripheral blood which fulfilled the inclusion criteria: having normal platelet count and not suffering from bleeding disorder.

The hydrogel was made from a mixture of chitosan and collagen powder (fish collagen) which were registered with pharmaceutical grade. Chitosan was put into a beaker glass containing 100 ml of water over a magnetic stirrer at a speed of 3000 rpm, stirred until dispersed 1%, and 1 % acetic acid was added. Hydroxypropyl methylcellulose and collagen were prepared by weighing them on a digital scale. The collagen powder was added into the chitosan, then stirred at a speed of 3000 rpm until homogeneous. The hydroxypropyl methylcellulose powder was added into the solution at 3000 rpm using a homogenizer until homogeneous. Once homogeneous, the preparation was left for 15 minutes at a room temperature and put into a cooling machine.

The following is the comparison of the collagen chitosan (100/0; 75/25; 50/50; 25/75; 0/100).⁷

One hundred mL of donor's blood was taken, then every 9 mL of blood was put in a vacutainer tube containing 1 mL of sodium citrate 3.8%. Each tube was centrifuged for 10 minutes at 1200 rpm. The first centrifugation (EBA 20 Hettich) produced two layers: the upper layer contained platelet-poor plasma (PPP), and the lower layer contained red blood cells. Platelet-poor plasma was separated using 3 way stopcock (Onemed), followed by the second centrifugation for 10 minutes at 3500 rpm. The second centrifugation produced two layers, namely the upper 2/3 layers were PPP and 1/3 the bottom layer was PRP. Platelet-rich plasma obtained was then collected in one syringe and mixed until homogeneous PRP was obtained.

Hydrogels were measured using a pH meter, by dipping electrodes into the hydrogel, until the device showed a constant pH value. The number indicated by the pH meter was the value of the pH of the preparation.¹⁰ Neutral pH ranged from 6.2 to 7.6.⁸ The collagen-chitosan hydrogel formulation that met the criteria of having neutral pH was then examined for viscosity.

Using the Rheosys viscometer, 2 mL of the base of the hydrogel was placed on top of a cylindrical container, then the viscosity was measured by a viscometer equipped with a spindle (2.0/30 mm cone plate) at a speed of 10 rpm for 30 seconds. It was then followed by scale reading.¹⁴ The viscosity value of the gel preparations that met the requirements was 2000-4000cps.¹⁰ The formulation of collagen-chitosan hydrogel that met the criteria of pH and viscosity was then examined for swelling.

Swelling was measured by a tea bag method using distilled water at 220 °C. Tea bags made of nylon fabric had a pore size of ±300 mesh. The dry hydrogel weight was labelled as W0. Nylon bags that had been filled with hydrogels were immersed into distilled water at a room temperature at every 10-minutes interval. Then the nylon bag was lifted and hung somewhere while left to drip for 15 minutes and then labelled as Wt. Soaking, lifting,

and weighing were repeated within 125 minutes.¹⁵ The water absorbed by the hydrogel (water uptake) was expressed as swelling ratio (%), as defined in the following:

$$\text{Swelling Ratio (\%)} = \{(Wt-W0)/W0\} \times 100\%^{16}$$

All the measurements were conducted using electronic microbalance (Adam AAA 250 L) with a precision of ± 0.4 g. Swelling value reached 100%.¹³ The collagen-chitosan hydrogel formulation was added to the PRP with a ratio of 1:1.¹⁷ The second stage included measuring pH, viscosity, and swelling to see the result of the hydrogel's physical parameters after PRP was mixed in the same way as above.

The first step was to test the physical parameters of the collagen-chitosan hydrogel formulation with ratios of 100/0; 75/25; 50/50; 25/75; 0/100 to get collagen-chitosan hydrogel

formulation that fit the criteria. The second stage was to test the physical parameters of the collagen-chitosan hydrogel formulations according to the criteria, which had been added with PRP with a ratio of 1:1.

RESULTS

The collagen-chitosan hydrogel formulations included into the first stage were 25/75 and 0/100. In the second stage, the results of the pH, viscosity, and swelling of the collagen-chitosan hydrogel formulations according to the criteria, in which PRP had been added with a ratio of 1:1, are described as follows:

Table 4 Result of *post hoc least significant difference* (LSD) test swelling of collagen-chitosan hydrogel mixed with PRP formulations 25/75 and 0/100 in observation time of 25, 50, 75, 100, and 125 minutes.

Table 1. The mean and standard deviation pH of collagen-chitosan hydrogel mixed with PRP formulations 25/75 and 0/100

| Group of collagen-chitosan hydrogel formulations with PRP | n | $\bar{x} \pm$ SD pH of collagen-chitosan hydrogel with PRP | p |
|---|---|--|-------|
| 25/75 | 5 | 7.04 \pm 0.123 | 0.110 |
| 0/100 | 5 | 7.25 \pm 0.222 | |

p = no significant difference (p>0.05)

Table 2. The mean and standard deviation of viscosity of collagen-chitosan hydrogel with PRP formulations 25/75 and 0/100

| Group of collagen-chitosan hydrogels with PRP formulations | n | $\bar{x} \pm$ SD viscosity of collagen-chitosan hydrogels with PRP (cps) | p |
|--|---|--|--------|
| 25/75 | 5 | 2304.13 \pm 175.33 | 0.037* |
| 0/100 | 5 | 2583.27 \pm 176.95 | |

p = significant difference (p<0.05)

Table 3. The mean and standard deviation of swelling of collagen-chitosan hydrogel mixed with PRP formulations 25/75 and 0/100 in observation time of 25, 50, 75, 100, and 125 minutes

| Group of collagen-chitosan hydrogels with PRP | n | $\bar{x} \pm$ SD swelling of collagen-chitosan hydrogel with PRP | | | | |
|---|---|--|------------------|------------------|------------------|------------------|
| | | 25 minutes (%) | 50 minutes (%) | 75 minutes (%) | 100 minutes (%) | 125 minutes (%) |
| 25/75 | 5 | 167.8 \pm 13.4 | 155.0 \pm 16.5 | 149.0 \pm 17.0 | 126.0 \pm 13.6 | 121.4 \pm 21.4 |
| 0/100 | 5 | 142.2 \pm 9.6 | 130.6 \pm 7.7 | 125.4 \pm 8.1 | 116.6 \pm 8.3 | 107.8 \pm 12.5 |

Table 4. Result of LSD test swelling of collagen-chitosan hydrogel mixed with PRP formulations 25/75 and 0/100 in observation time of 25, 50, 75, 100, and 125 minutes

| | 25/75 25 m | 25/75, 50 m | 25/75, 75 m | 25/75, 100 m | 25/75, 125 m | 0/100, 25 m | 0/100, 50 m | 0/100, 75 m | 0/100, 100 m | 0/100, 125 m |
|--------------|---------------|----------------|----------------|-----------------|-----------------|----------------|----------------|----------------|-----------------|-----------------|
| 25/75, 25 m | - | 0.143 | 0.034* | 0.000* | 0.000* | 0.005* | 0.000* | 0.000* | 0.000* | 0.000* |
| 25/75, 50 m | | - | 0.488 | 0.002* | 0.000* | 0.143 | 0.007* | 0.001* | 0.00* | 0.000* |
| 25/75, 75 m | | | - | 0.010* | 0.003* | 0.432 | 0.038* | 0.009* | 0.001* | 0.000* |
| 25/75, 100 m | | | | - | 0.594 | 0.066 | 0.594 | 0.944 | 0.279 | 0.040* |
| 25/75, 125 m | | | | | - | 0.020* | 0.289 | 0.643 | 0.578 | 0.120 |
| 0/100, 25 m | | | | | | - | 0.183 | 0.057 | 0.005* | 0.000* |
| 0/100, 50 m | | | | | | | - | 0.547 | 0.110 | 0.011* |
| 0/100, 75 m | | | | | | | | - | 0.310 | 0.046* |
| 0/100, 100 m | | | | | | | | | - | 0.310 |
| 0/100, 125 m | | | | | | | | | | - |

Between the two groups according to the observation time, the significance value was 0.000 ($p < 0.05$), indicating that the time of observation attributed to a significant difference in swelling. The result of the analysis between the groups of hydrogel formulations showed a significance value of 0.000 ($p < 0.05$), indicating that the hydrogel formulation group had a significant difference in swelling. The result of the interaction analysis at the time of observation and the hydrogel formulation group showed a significance value of 0.576 ($p > 0.05$), indicating that the interaction between the observation time and the hydrogel formulation group had no effect on swelling. The difference between each group of treatment was then followed by LSD test.

DISCUSSION

Making the right hydrogel formulation for oral cavity application requires attention to the details of requirement, including neutral pH, high viscosity and swelling, with regard to the ideal condition to make the right dosage consistency.⁷ The pH of collagen-chitosan hydrogels in the formulations of 100/0, 75/25, 50/50, 25/75, and 0/100 showed that the pH fell into the criteria for neutral pH of the oral cavity, which is 6.2 – 7.6.⁸ All the groups were then tested for their viscosity. The viscosity of the collagen-chitosan hydrogels met the viscosity

criteria if the value was between 2000-4000 cps,¹⁰ and they were formulations 25/75 and 0/100. This way, formulations 25/75 and 0/100 could continue to swelling test. The swelling results of formulations 25/75 and 0/100 met the swelling criteria, i.e. between 100%-200%,¹³ hence the two formulations met the criteria for ideal hydrogels.

Collagen-chitosan hydrogel formulations 25/75 and 0/100 were added to PRP in a ratio of 1:1.¹⁷ The purpose of measuring the pH, viscosity and swelling in the second stage was to see the result of the hydrogel's physical parameters after PRP mixing. Independent t test showed no significant difference of the pH values between formulation 25/75 and formulation 0/100, because all the hydrogel formulations made and PRP had to have a neutral pH that matched the pH of the oral cavity.⁹ Measurement of pH was conducted to determine the suitability of the hydrogel with the pH of the oral cavity to avoid irritating the oral cavity and to determine the safety of the hydrogel. Ideally, the hydrogel preparations have a pH value is the same as the pH of the oral cavity. Physiology pH value of PRP ranges from 7.46 to 7.48.¹⁸

Independent t test result indicated significant differences in the viscosity between formulation 25/75 and formulation 0/100. The addition of chitosan to collagen increased its viscosity.¹¹ Increasing the ratio of collagen and decreasing the

ratio of chitosan in the hydrogel slowed down the gelation reaction and decreased viscosity.⁶ The addition of chitosan could increase its physical properties through additional amino groups that function to increase crosslinking density and strengthen hydrogel.⁵ The viscosity values of gel preparations that meet the requirements are between 2000-4000 cps.¹⁰

The means of the swelling results of formulation 25/75 and formulation 0/100 met the criteria of swelling between 100%-200%.¹³ The two-way ANOVA test result on the swelling between groups of observation times showed that there were significant differences because the hydrogel was sensitive to water, so the initial immersion of the hydrogel would lead to high water absorption in seconds. The longer the absorption, the lower the capacity.¹⁹ The process of water absorption runs continuously, accompanied by the opening of hydrogel pores. Hydrogel will swell until hydrogel tissue is fully filled by water molecules.¹⁹ Hydrogel will reach equilibrium when it experiences a saturation phase. The saturated phase occurs when a sample is unable to absorb water solution optimally. Hydrogel pore causes water to enter hydrogel to diffuse slowly into water.²⁰

Two-way ANOVA test result on swelling between the groups of 25/75 and 0/100 hydrogel formulations showed significant differences. The trend of decreasing percentage of swelling occurs because of the increasing formulation of chitosan. A higher chitosan formulation indicates that hydrogel pores are increasingly smaller, thus there is less liquid entering the hydrogel.²¹ The low elasticity of collagen causes it to not maintain porous structures. Chitosan has a higher elasticity that can help maintain porous structures.¹² The ability of collagen to expand is due to the presence of polar amino composition in collagen, allowing it to bind with water. The swelling process is fast due to the uneven surface of the collagen particles. Uneven and wavy surface area can expand the contact surface with water and allow immediate hydration.²²

The collagen-chitosan hydrogel formulations mixed with PRP, i.e. 25/75 and 0/100, were

included as hydrogels which met the criteria, but the hydrogel formulation of only chitosan and PRP (formulation 0/100) had a disadvantage with regard to chitosan being not osteo-inductive and not having sufficient ability for bone formation.²³ Chitosan lacks mechanical strength as it is insoluble in water and has less optimal hemostatic.²⁴ Chitosan hydrogels can be modified to create a beneficial osteogenic microenvironment by incorporating extracellular matrix components (ECM) in bone tissue. Collagen is the most common ECM protein in bone tissue because it can become a place for the attachment of hydroxyapatite crystals.²⁵

The advantages of collagen-chitosan hydrogels can increase endothelial cell differentiation and angiogenesis, and encourage the formation of capillary tissue in the in vitro test. In addition, these hydrogels are more resistant to enzymatic degradation, denaturation, and compressive forces so collagen-chitosan hydrogels have the potential as scaffold to support cell therapy for damaged or diseased tissue vascularization.⁵

This study concluded that the formulation of collagen-chitosan hydrogel with PRP had no effect on pH, but there was an effect on viscosity and swelling test. The formulations of collagen-chitosan hydrogel with PRP which matched with the criteria were 25/75 and 0/100.

CONCLUSION

The formulations of collagen-chitosan hydrogel mixed with PRP has no effect on pH, but has an effect on viscosity and solubility test. The formulations of collagen-chitosan hydrogel mixed with PRP which matched the criteria were 25/75 and 0/100.

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