Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):655-658

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preparation of collagen wound-healing membranes

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ABSTRACT

Collagen can be extracted from the fresh pigskin through enzymatic hydrolysis. Collagen, together with various Chinese herbal extracts including Callicarpa bodinieri, Glycyrrhiza uralensis, and Zanthoxylum nitidum, are used to prepare collagen wound-healing membranes. In order to make drugs well dispersed in the membrane, Polyvinylpyrrolidone (PVP) is used as the dispersant to prepare drug dispersions by applying solvent evaporation-fusion method. The optimal procedure to prepare the membrane is studied and concluded by experimenting with different proportions of humectant, drugs and dispersants.

Keywords: Collagen, wound-healing membrane, *Callicarpa bodinieri*, *Zanthoxylum nitidum*, *Glycyrrhiza uralensis*.

INTRODUCTION

Skin defect resulting from wound is one of the most common clinical symptoms. Early wound closure is the key to the treatment of skin defects. Some previous studies used plant extract for wound-healing [1][2][3]; while some used plant extract along with collagen [4]. Collagen has excellent biocompatibility and cell compatibility properties. It is also a good hemostatic and it can promote wound healing. Membranes made of collagen are natural, non-toxic, bio-degradable and have good mechanical properties. Therefore, they are ideal materials for medical purposes.

Callicarpa bodinieri can stop bleeding, eliminate stasis and diminish inflammation [5]. *Zanthoxylum nitidum* can reduce swelling, ease pain, stimulate blood circulation, eliminate stasis, reduce secretion, and promote wound healing [6]. *Glycyrrhiza uralensis* can diminish inflammation, fight against ulcer and virus, and it mediates other medicines [7]. *Callicarpa bodinieri*, *Zanthoxylum nitidum* and *Glycyrrhiza uralensis* are widely used for external application.

In this paper, the novel techniques to prepare collagen wound-healing membranes by adding *Callicarpa bodinieri*, *Zanthoxylum nitidum* and *Glycyrrhiza uralensis* into collagen are studied, and thus a better medical product is provided.

EXPERIMENTAL SECTION

2.1. Reagents and instruments

Collagen (laboratory-prepared); Soluble starch (AR); Glutaraldehyde (AR); Propanetriol (AR); Sodium carboxymethyl cellulose (CP); Polyvinyl pyrrolidone (AR); Absolute ethyl alcohol (AR); Mannitol (AR); TDZ5-WS low speed multi-pipe automatic balancing centrifuge (Xiang Yi Centrifuge Instrument Co., Ltd); DF-101S Series

Thermal-arrest Magnetic Stirrer (Zhengzhou Great Wall Industrial and Trading Co., Ltd); SHZ-C Series Multiple-usage Vacuum Pump with water circulation (Gongyi Ying Yu Hua Instrument Co., Ltd); DHG-9070A Series Heating and Drying Oven (Shanghai Jing Hong Laboratory Instrument Co., Ltd).

2.2. Extraction of collagen protein

First a knife was used to remove residues of fat from the selected fresh pigskin. Then the pigskin was put into a reaction flask with 10% Na_2CO_3 solution in order to be degreased. Then by proteinase hydrolysis, centrifuge separation and oven drying, the collagen powder was obtained [8]. With respect to two variables in the proteinase hydrolysis: time and temperature, a series of experiments were performed. Specifically, membranes were prepared (according to the later steps) under different scenarios and their mechanical properties were tested and compared. It turned out that the optimal time and temperature were: 45°C, 70 minutes. Collagen made under this optimal condition was sent to the Analysis and Test Center, Chengdu Branch of Chinese Academy of Sciences. By GPC test with polyethylene glycol as a standard sample, the relative molecular weight of the optimal collagen was obtained as: weight-average molecular weight = 18,520, number-average molecular weight = 2,850, and polydispersity index = 6.50.

2.3. Extraction of traditional Chinese herbs

Firstly, *Callicarpa bodinieri*, *Zanthoxylum nitidum*, and *Glycyrrhiza uralensis* were crushed into coarse particles of approximately $3mm^3$. Then three equally weighted *Callicarpa bodinieri* were sampled and soaked in 75% ethanol solution7, 15 and 30 days, respectively. Then the three liquids were separately vacuum filtered. The filtrates were mixed together, vacuum distilled at 70°C, and the extract in paste form was gained. The extract was further dried in a drying oven at the temperature of 60°C, and became power. Similarly, the above method was applied to obtain *Zanthoxylum nitidum* and *Glycyrrhiza uralensis*'s powder exacts.

2.4. Determination of the amount of humectant

Adding humectant in the solution while preparing collagen wound-healing membrane can make the dried membrane soft, not easy to break, and moisturizing. Frequently used humectants are glycerol, propylene glycol, lactic acid, and etc. In this paper, glycerin was used as the humectant and single factor experiment was applied. As listed in Table 1, all the substances listed were mixed together with water in each scenario. The volume of glycerol was the only factor that changed over five scenarios. Then a sticky collagen blending was acquired for each scenario. Each blending was spread flat on a clean, dry template to form a liquid layer of uniform thickness and smooth surface. Then it was dried in a constant temperature oven. Peeling if off, one collagen membrane for each scenario was obtained.

2.5. Determination of the dispersant and its dosage

An experiment was designed to compare the dispersion effects of drugs using two different reagents: polyvinyl pyrrolidone (PVP) and mannitol. Furthermore, the amount of PVP to be used was studied by single factor experiment method.

Our experiment applied solvent evaporation-fusion method to prepare drug dispersions. Solvent evaporation-fusion method is to dissolve Chinese herbal extracts in a solvent and then mix it with fused dispersant. By using this method, the components of the herbal extracts can be blended at molecular state with the dispersant at a relatively low temperature, so that in the later evaporation process the aggregation of the herbal components can be prevented by the dispersant. So a solid solution can be formed and the drugs it contains can have a high degree of dispersion.

Specifically, the experiment was carried out in the following steps: firstly 95% ethanol solution was added into the mixture of Chinese herbal extracts and dispersant. Then it was stirred evenly and then heated to 70°C until the dispersant fused. After it was cooled down, the solid drug dispersion was obtained [9]. The drug dispersion was further added into the previous mixture of collagen solution as in Table 1, No.4, and it was stirred evenly in a water bath at 40°C. Then a viscous collagen wound-healing liquid was obtained. Similarly as before, collagen wound-healing membranes were prepared through these liquids and how well the drugs are dispersed in the membranes were studied for each of the two dispersant we used.

2.6. Experiment on determining the amount of drugs

Different amounts of drugs were experimented with and membranes were made according to the above method. Then the dispersion degrees of drugs in these collagen wound-healing membranes were compared.

RESULTS AND DISCUSSION

3.1. Determination of the amount of humectant

The results from experimenting with the amount of humectant are shown in Table 1. Comparing these membranes' tensile strength, moisturizing effect, and viscosity under five scenarios listed, it was found that the membrane had the optimal effects when the volume of glycerol was 2ml.

Table 1 Assessment of collagen membranes with different volume of glycerol

No.	1	2	3	4	5
Collagen (g)	0.1	0.1	0.1	0.1	0.1
Sodium carboxymethyl cellulose (g)	9.0	9.0	9.0	9.0	9.0
Starch (g)	0.1	0.1	0.1	0.1	0.1
Glutaraldehyde (ml)	3.0	3.0	3.0	3.0	3.0
Glycerin (ml）	0	1.0	1.5	2.0	2.5
Membrane's comprehensive performance	bad	general	general	good	general

3.2. Determination of the dispersant and its dosage

The results from experimenting with different kinds of dispersants and various dosages are shown in Table 2. It turned out that when the mannitol was used as the dispersant, a larger amount of it must be used in order to achieve the same dispersion effects compared to the case when PVP was used. So PVP was chosen finally to be the dispersant. Additionally, it was found that the larger the amount of PVP was, the more dispersed were the drugs; but the more viscous and less tough the membranes would become. When the amount of PVP was between 0.1g and 0.2g, we obtained the optimal membranes. Therefore the final amount of PVP was set to be 0.1g.

Table 2 Comparison of dispersion effects in the membranes using different dispersants

Dispersant No.	Mannitol			PVP			
	1	2	3	4	5	6	7
Herbal extracts (g)	0.2	0.3	0.2	0.3	0.3	0.3	0.3
Dispersant (g)	0.2	0.2	0.1	0.05	0.1	0.2	0.3
Dispersion effects	better	bad	best	better	best	best	better

3.3 Determination of the final amount of drugs

According to the dispersion degrees of drugs in these collagen wound-healing membranes, the final amount of drugs was set to be 0.3g.

CONCLUSION

In this paper, experiments were designed and the optimal techniques were studied in order to prepare collagen wound-healing membranes through adding the extracts from *Callicarpa bodinieri*, *Zanthoxylum nitidum* and *Glycyrrhiza uralensis* to collagen. In order to improve the degree of dispersion of drugs in the membrane, the drugs were first made into a solid drug dispersion. By various experiments, it is concluded that the optimal method to produce the solid dispersion is to: firstly, add 0.1g PVP and 95% ethanol solution into 0.3g of extracts from *Callicarpa bodinieri*, *Zanthoxylum nitidum* and *Glycyrrhiza uralensis*; secondly, stir it and heat it to 70°C until fusion. The optimal method to prepare the collagen wound-healing membranes is to: firstly, mix 0.1g collagen, 0.1g starch, 2.0ml glycerol, 0.4g drug dispersion, and 3.0ml glutaraldehyde together and stir them at room temperature; secondly, add 9.0g aqueous solution of sodium carboxymethyl cellulose to the mixture, and heat it in a water bath at a constant temperature of 40°C, so that a viscous liquid was obtained; thirdly, spread the liquid flat on a clean, and dry template to form a liquid layer of uniform thickness and smooth surface; fourthly, dry the liquid in the oven at 60°C and then peel it off to obtain the collagen wound-healing membrane.

The novel collagen wound-healing membrane is a natural medical material that has good properties of biocompatibility and cell adaptability. Furthermore, it can help stop bleeding, fight bacteria, ease pain, and reduce swelling. All these advantages make it an ideal substitute of the traditional wound dressing.

Acknowledgments
The authors would like to acknowledge YF Bai for assistance with the experiments and M Zhu for valuable discussion. This research was supported by the Scientific Research Fund of the Education Department of Sichuan Province (Grant No. 11ZA095).

REFERENCES

[1] C Das; S Dash; DC Sahoo; AK Sahu; R Hota; D Rout. *J. Chem. Pharm. Res.*, **2011**, 3(5), 15-19.
[2] MLA Bastos; RLS Houly; LM Conserva; VS Andrade; EMM Rocha; RPL Lemos. *J. Chem. Pharm. Res.*, **2011**, 3(4), 213-222.
[3] AA Attama; PF Uzor; CO Nnadi; CG Okafor. *J. Chem. Pharm. Res*., **2011**, 3(3), 718-724.
[4] MK Babu; OS Prasad; TEGK Murthy. *J. Chem. Pharm. Res*., **2011**, 3(3), 353-362.
[5] P Pan; QS Sun. *J. Shenyang Pharmaceutical University*, **2006**, 23(9), 565-567.
[6] JF Zhou; FJ Qin; J Feng; LT Wei. Lishizhen M*edicine and Materia Medica Research*, **2012**, 23(1), 19-20.
[7] JX Su; Y Zhou; WJ Yan; WP Liu. *Animal Husbandry and Feed Science*, **2010**, 31(8), 111-113.
[8] ZB Shu; LJ Yuan; JF Hu; CH Cheng; H Yang. *J. Sichuan Normal University* (Natural Sciences), **2008**, 31(6), 739-741.
[9] SJ Qiao; MZ Jin; L Yang. *J. Guangdong Pharmacy College*, **2007**, 23(3), 263-265.