Clinical and Experimental Health Sciences

Preparation of Chitosan-Polyvinyl Prolidone (PVP) Hydrogels with Fibroblast Growth Factor (FGF) and Investigation of in Vitro Characteristics

Murat Dogan

Sivas Cumhuriyet University, Faculty of Pharmacy, Department of Pharmaceutical Biotechnology, Sivas, Türkiye.

Correspondence Author: Murat Dogan
E-mail: mdogan@cumhuriyet.edu.tr
Received: 17.07.2021 **Accepted:** 21.10.2021

ABSTRACT

Objective: In this study, it was aimed to make in vitro characterization of the formulations by preparing hydrogel formulations using chitosan, a biocompatible and natural polymer, and PVP, a synthetic polymer. In addition, the effects of hydrogels containing FGF on the proliferation of keratinocyte cells were investigated.

Methods: Within the scope of the study, hydrogels with different properties were prepared and their water absorption capacity, and viscosities were examined. In addition, the hardness, adhesiveness, cohesiveness, and elasticity properties of hydrogels were investigated. The 3 – (4,5-dimethyl-2-thiazolyl) – 2,5-diphenyl tetrazolium bromide (MTT) test was applied to evaluate the toxicity of hydrogels on keratinocyte cell lines.

Results: It was observed that hydrogel formulations have high water absorption capacity and suitable viscosity values. In addition, the mechanical characterization results showed that the hydrogels have suitable mechanical properties. According to the results of in vitro cell culture studies, it has been observed that hydrogels stimulate the proliferation of keratinocyte cells.

Conclusion: Results showed that the mechanical properties of hydrogels containing FGF are suitable for application and according to the results of in vitro cell culture studies, hydrogels can be used in wound healing studies because they increase keratinocyte cell proliferation.

Keywords: Chitosan, In vitro characterization, Hydrogel, Cell proliferation.

1. INTRODUCTION

Wound is defined as the deterioration of tissue integrity due to physical factors, chemical factors, heat, surgery or spontaneously. Disruption of tissue integrity causes increased fluid loss, infection, hypothermia, immunity and changes in body image (1). Growth factors and cytokines are widely used in wound healing. Transforming growth factor beta (TGF-β), epidermal growth factor (EGF) and fibroblast growth factor (FGF) are growth factors used in wound treatment (2). FGF used in this study is produced by mast cell, lymphocytes, endothelial cells and keratinocyte cells. FGF is effective on keratinocyte, endothelial and fibroblast cells (2). The mechanism of action of FGF is that it creates mitogenic and chemotactic effects on keratinocytes and fibroblasts, and stimulates angiogenesis, granulation and epithelialization (3). When FGF is used only, it cannot maintain its stability due to immune system cells, natural barriers and various enzymes. Therefore, it shows low efficiency and requires continuous application. It is important to give FGF in a formulation to eliminate the stability problem and to work towards this purpose. Hydrogels are widely used for wound and burn treatment. Hydrogels have a three-dimensional network structure formed by the combination of hydrophilic molecules cross-linked by covalent bonds or held together by intramolecular physical interactions (4, 5). Hydrogels can absorb large amounts of water or biological fluids. The high hydrophilicity of hydrogels is due to the presence of hydrophilic moieties such as amino, carboxyl, amide and hydroxyl groups along the backbone of the polymer (6). In the swollen state, hydrogels have a soft and rubbery structure and closely resemble living tissues (7). Chitosan is widely used in the preparation of hydrogel formulations due to its superior properties (8). The absorption-enhancing feature of chitosan provides its use in mucosal administration, drug delivery systems, and formulation of hydrophilic macromolecular drugs (9). PVP, which is a synthetic polymer, can be used alone, or by adding it to another polymer or forming a copolymer with polymers, and it is used in various fields. PVP is a non-toxic, biodegradable, biocompatible, hydrophilic polymer with high gelling capacity as well as good complexing ability. It has a widespread use in the pharmaceutical industry (10, 11).

2. METHODS

2.1. Preparation of Chitosan-PVP Hydrogel Formulations

The ionic gelation method was used in the preparation of hydrogel formulations (12). Medium molecular weight

DOI: 10.33808/clinexphealthsci.972758

(400 kD) chitosan was used while preparing the hydrogel formulations. The amounts of FGF, chitosan and PVP used in the formulations are indicated in Table 1. Hydrogels prepared according to these concentrations have important effects on physicochemical parameters such as adhesion to the application site, flexibility and cohesiveness. Chitosan was weighed on a precision balance and 1% concentration of glacial acetic acid was added to the chitosan in the beaker into sterile bi-distilled water and mixed with a magnetic stirrer. In a separate beaker, the determined amount of PVP was dissolved in a magnetic stirrer. The two solutions were then mixed in such a way that the volumetric association ratio was 3:1 (Chitosan: PVP). The mixing process was continued for two hours to form a homogeneous structure. Hydrogels were maintained in an ultrasonic water bath for 15 minutes to remove air bubbles. Hydrogels were maintained under UV light for 10 minutes in order for crosslinking and polymerization reactions to take place. The prepared hydrogels were stored in amber colored glass bottles at +4 °C until used in in vitro studies.

Table 1. *Hydrogel formulations and components*

Samples	FGF amount (mg)	Chitosan (% w/v)	PVP (% w/v)
A1	10	3	5
A2	20	3	10
A3	30	3	20
A4	10	2	5
A5	20	2	10
A6	30	2	20

2.2. Measurement of Viscosity and Water Absorption Capacity of Hydrogels

After the air bubbles were removed in the ultrasonic bath, the viscosity of the hydrogels at room temperature was measured using the Brookfield model viscometer. The tests were repeated 3 times for each formulation. A gram sample of the hydrogel, whose water absorbing capacity was to be measured, was weighed and lyophilized. Lyophilized hydrogels were placed in appropriate petri dishes. Then, 1 ml of PBS (pH 7.4) was added to the tared petri dish with the hydrogel at intervals of 15 minutes, and when the gel reached saturation, the excess liquid was removed from the hydrogel, and the tared petri dish was weighed again. The experiment was continued until the hydrogel reached a constant weight.

2.3. Investigation of Mechanical Properties of Hydrogels

Mechanical characterization studies were performed to determine the suitability of the hydrogel for physiological conditions in its application. The mechanical properties of the hydrogels were determined with the TA.XT Plus texture analyzer (12, 13). Before the test, the hydrogels in 50 ml standard bottles were maintained at room temperature for about two hours to reach room temperature. After the hydrogels were brought to room temperature, the weight and height calibrations of the device were made and the measurement was started. The bottle containing the hydrogel is fixed to the device to be measured. The probe of the device was immersed in the hydrogel at a speed of 2mm/s for 15 mm and, after being pulled back to the surface of the hydrogel for 2 seconds, the probe was dipped into the hydrogel a second time and pulled back to complete the test.

2.4. MTT Cell Viability Assay

MTT cell viability assay was performed on the HaCaT cell line. Before starting the MTT test, cells were seeded in 96-well plates in a sterile cabinet (14, 15). In this process, fetal bovine serum (FBS) was used as DMEM medium containing L-glutamine-streptomycin. Before starting the experiment, 5 ml of medium was added to a 25 ml flask, then keratinocyte cells were seeded and kept in an incubator (37 °C) overnight. Then, the medium in the flask was poured and washed 2 times with PBS and 1 ml of Trypsin EDTA was added to the flask and pipetted several times. The cells were then removed from the incubator. Cells were plated with 96 wells and 100 µl of medium was added to each well. Adhesion of cells to the wells occurred in approximately 24 hours. Then, our pre-prepared samples were applied to the wells. 50 µg hydrogel containing 5 µg FGF was added to each well by pipetting in 25 µl medium in a 500 µl eppendorf tube. Then, the 96-well plate was placed in the incubator and incubated for 24 hours. After 24 hours, the samples were taken into a sterile cabinet and 10 µl of MTT solution (room temperature) was added to each well. After the plate was kept in the incubator overnight, 50 µl of the SDS solution was added to each well and the plate was removed from the oven. After six or eight hours, the crystals on the plate were homogenized and the absorbance values were read at 550 nm and 690 nm wavelengths on the microplate ELISA reader. Cell viability rates were calculated using these results compared to the control group.

Statistical Analysis

The results of these studies were statistically evaluated using GraphPad Prism 8 ANOVA followed by Newman–Keuls multiple comparisons test. A value of $p<0.05$ was considered statistically significant. The results were expressed as the mean and ± standard deviation (SD) values.

3. RESULTS

3.1. Results of Mechanical Characterization Study of Hydrogels

The water absorption capacity, viscosity and mechanical properties of Chitosan-PVP hydrogels were given in Table 2. The water absorbing capacities of the hydrogel formulations are between 0.474±0.010 and 0.986±0.012 g. A3 formulation containing chitosan (3%) and PVP (20%) showed the highest water absorption capacity. The viscosity values of the hydrogel formulations varied between 22.800±4424 and 43200±3574 cPs. A3 formulation containing chitosan (3%) and PVP (20%) showed the highest viscosity value. A4 hydrogel formulation containing chitosan (2%) and PVP (5%) showed the lowest viscosity value.

Table 2. *Mechanical characterization results of hydrogels*

Samples	Water absorption capacity (g±SD)	Viscosity (cPs ±SD)	Adhesiveness (N.mm)	Cohesiveness	Elasticity (N.mm)
A1	0,772±0,008	30800±2100	0,086±0,003	0,840±0,009	0,842±0,009
A2	0,864±0,009	39400±3200	0,094±0,002	0,820±0,007	0,810±0,014
A3	0,986±0,012	43200±3574	0,099±0,002	0,850±0,011	0,795±0,006
A4	0,474±0,010	22.800±4424	0,067±0,002	0,800±0,013	0,973±0,009
A5	0,534±0,007	24600±1426	0,071±0,001	0,790±0,011	0,946±0,013
A6	0,598±0,013	26300±1590	0,074±0,002	0,810±0,010	0,910±0,016

Hydrogels are widely used due to the unique properties of its. In order for hydrogels to be applied, they should have significant properties such as high adhesive and cohesive properties, water absorption capacity and viscosity within certain values, and high elasticity and bio-adhesive properties (16, 17). Hydrogels should have high adhesiveness and water absorbing capacity in order to adhere to the wound area for the desired time and to keep that area moist. When these features are provided, a more effective treatment can be provided (18, 19). According to the mechanical characterization results of the hydrogel formulations, the A3 formulation had the highest adhesiveness (0.099±0.002 N.mm) and cohesiveness values (0.850±0.011, Table 2). In addition, the A4 formulation showed the lowest adhesiveness (0.067±0.002), while the A5 formulation had the lowest cohesiveness values (0.790±0.011). The elasticity values of the hydrogels range from (0.795±0.006) to (0.973±0.009) N.mm. According to the results, it was observed that the elasticity values of the hydrogels decreased depending on the concentration of chitosan and PVP used. By looking at the adhesive and cohesiveness results of the hydrogels, it was observed that the viscosity and water absorption capacity of the hydrogels increased depending on the concentration of chitosan and PVP used ($p<0.05$). According to the results obtained, it was observed that the changes in the amount of FGF did not have a significant effect on the mechanical properties, water absorption capacity and viscosity values of the hydrogels ($p>0.05$).

3.2. In vitro Cell Viability (MTT) Results of Hydrogels

In in vitro studies on wound and burn treatment, human keratinocyte and fibroblast cell lines are generally used to calculate and evaluate cell viability and proliferation (14, 20). In this study, keratinocyte cell lines were used to observe and evaluate the efficacy of hydrogel samples in the treatment of wounds or burns and their potential to increase cell proliferation. Cell viability results of hydrogels are given in Figure 1. According to the results obtained, it can be said that hydrogels containing FGF create significant differences on cell viability. It was observed that hydrogels containing 30 mg of FGF (A6: 108.600±1.417 % and A3: 105.800±1.651 %) showed the two highest cell viability. A1 (91.920±1.457 %) and A4 (94.120±2.647 %) formulations showed the lowest cell viability. According to the results, it was observed that the concentration of chitosan and PVP did not make a significant difference on cell viability ($p>0.005$). It was observed that hydrogels containing high amount FGF had higher cell viability than the control group and other formulations. It was observed that cell viability rates of hydrogels containing FGF were significantly different from the control group ($p<0.05$).

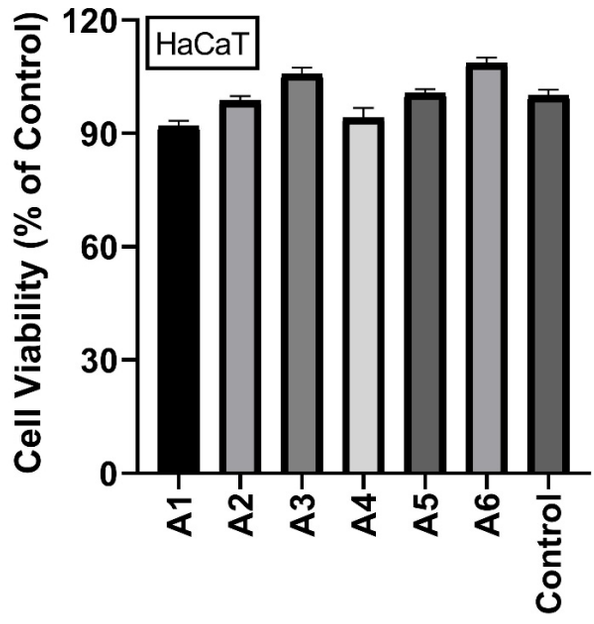

Figure 1. *Keratinocyte (HaCaT) cell viability result of hydrogel samples. The cell viability of the control group was determined as 100 %.*

4. DISCUSSION

Wounds and burns are the most common health problems in daily life. Treatment processes are also very arduous for both those who apply the treatment and the patient (21, 22). There are many drugs and medical support products for treatment (23, 24). In this study, as an alternative to the existing treatment options, a growth factor with known efficacy was planned to be formulated and applied via a suitable carrier system. It can be said that hydrogels are suitable for application in terms of their mechanical properties. In particular, the A3 formulation containing 30 mg of FGF has high water retention and viscosity, which shows that it is more suitable for application compared to others. In addition, it was observed that the A3 and A6 formulations increased the viability of keratinocyte cells more than the control group and other hydrogels. In this case, considering all

the features of the A3 formulation, it can be said that it is the most suitable hydrogel sample in terms of use and in order to shed light on future studies. have two important properties. First, the hydrogel must have suitable physicochemical properties, to be biocompatible and non-toxic. In order to meet these conditions, the polymers to be selected should have appropriate properties. Secondly, the active substance in the hydrogel is required to have bioactive properties suitable for its desired use and to maintain its effectiveness after being placed in the delivery system. In this study, it can be said that hydrogels are suitable for application in terms of their mechanical properties. In particular, the A3 formulation containing 30 mg of FGF has high water retention and viscosity, which shows that it is more suitable for application compared to others. In addition, it was observed that the A3 and A6 formulations increased the viability of keratinocyte cells more than the control group and other hydrogels. In this case, considering all the features of the A3 formulation, it can be said that it is the most suitable hydrogel sample in terms of use and in order to be beneficial for future studies.

5. CONCLUSION

According to the results of the characterization and cell culture studies of the hydrogels, it has been observed that the hydrogels containing FGF increased keratinocyte cell viability. In addition, it can be said that hydrogels have suitable viscosity and water retention values. Considering the adhesive strength, which is very important in terms of adhesion to the application area, it can be said that hydrogels prepared using high concentrations of chitosan and PVP have higher adhesive strength and are therefore more suitable for application. The meaningful results obtained in this study will be beneficial to the scientific literature by shedding light on future scientific studies.

Acknowledgments

In this study, the facilities and devices of xxxxxx xxxx, xxxxxxxx Department Research Laboratory and Medicine Faculty Cancer Research Center were used.

Funding

This study was performed by own means without the need for any funding.

Conflict of Interests

The author declare that they have no conflict of interest.

REFERENCES

[1] Alsarra IA. Chitosan topical gel formulation in the management of burn wounds. Int J Biol Macromol. 2009; 45: 16-21

[2] Camara FV, Ferreira LJ. Hydrogel synthesis, characterization and applications. Nova Science Publishers. Inc; 2012.

[3] Cretu A, Gattin R, Brachais L, Barbier-Baudry D. Synthesis and degradation of poly (2-hydroxyethyl methacrylate)-graft-poly(ε-caprolactone) copolymers. Polym Degrad Stab. 2004; 83: 399-403.

[4] Hajimiri M, Shahverdi S, Kamalinia G, Dinarvand R. Growth factor conjugation: Strategies and applications. J Biomed Mater Res. 2015; 103A (2): 819-838.

[5] Hamedi H, Moradi S, Hudson SM, Tonelli AE. Chitosan based hydrogels and their applications for drug delivery in wound dressings: A review. Carbohydr Polym. 2018; 199: 445-460.

[6] Huang S, Fu X. Naturally derived material-based cell and drug delivery systems in skin regeneration. J Control Release. 2010; 142: 149-159.

[7] Kim TH, Lee H, Park TG. Pegylated recombinant human epidermal growth factor (rh EGF) for sustained release from biodegradable PLGA microspheres. Biomaterials. 2002; 23: 2311–2317.

[8] Michael J, Majcher H, Todd H. Functional Biopolymers, Polymers and Polymeric Composites: A Reference Series (1st ed). Applications of Hydrogels. 2019; 453-491.

[9] Minoura N, Koyano T, Koshizaki N, Umehara H, Nagura M, Kobayashi K. Preparation, properties, and cell attachment/growth behavior of PVA/chitosan-blended hydrogels. Mater Sci Eng. 1998; 6: 275-280.

[10] Mogoşanu DG, Grumezescu AM. Natural and synthetic polymers for wounds and burns dressing. International Journal of Pharmaceutics. 2014; 463: 127-136.

[11] Streubel A, Siepmann J, Bodmeier R. Gastroretentive drug delivery systems. Expert Opin. Drug Deliv. 2006; 3 (2): 217-233.

[12] Zhou HY, Chen XG, Kong M, Liu CS, Cha DS, Kennedy JF. Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system. *Carbohydr Polym.* 2008; 73: 265-273.

[13] Andrews GP, Laverty TP, Jones DS. Mucoadhesive polymeric platforms for controlled drug delivery. Eur. J. Pharm. Biopharm. 2009; 71: 505-518.

[14] Wolf NB, Kuchler S, Radowski MR, Blaschke T, Kramer KD, Weindi G, Influences of opioids and nanoparticles on in vitro wound healing models. European Journal of Pharmaceutics and Biopharmaceutics. 2009; 73: 34–42.

[15] Singh S, Sharma B, Kanwar SS, Kumar A, Lead Phytochemicals for Anticancer Drug Development. Front. Plant Sci. 2016; 7: 8973-8985.

[16] Benamer S, Mahlous M, Boukrif A, Mansouri B, Youcef SL. Synthesis and characterisation of hydrogels based on poly(vinyl pyrrolidone). Nucl Instrum Methods Phys Res. 2006; 248: 284-290.

[17] Berger J, Reist M, Mayer JM, Fel O, Peppas NA, Gurny R. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications, European Journal of Pharmaceutics and Biopharmaceutics. 2004; 57, 19-34.

[18] Cheung RCF, Bun Ng T, Wong JH, Chan WY. Chitosan: An Update on Potential Biomedical and Pharmaceutical Applications. Mar. Drugs. 2015; 13: 5156-5186.

[19] Croisier F, Jérôme C. Chitosan-based biomaterials for tissue engineering. Eur Polym J. 2013; 49: 780-792.

[20] Das N, Bera T, Mukherjee A. Biomaterial Hydrogels for Different Biomedical Applications. Int J Pharm Bio Sci. 2012; 3(3): 586-597.

[21] Fan L, Yang H, Yang J, Peng M, Hu J. Preparation and characterization of chitosan/gelatin/PVA hydrogel for wound dressings. Carbohydr Polym. 2016; 146: 427–434.

[22] Jayakumar R, Prabaharan M, Kumar TS, Nair SV. Novel Chitin and Chitosan Materials in Wound Dressing. Biomed. Eng. 2009; 1-18.

[23] 23. Kean T, Thanou M. Biodegradation, biodistribution and toxicity of chitosan. Adv Drug Deliv Rev. 2010; 62: 3-11.

[24] 24. Mohamed RR, Abu Elella MH, Sabaa MW. Synthesis, characterization and applications of N-quaternized chitosan/poly(vinyl alcohol) hydrogels. Int J Biol Macromol. 2015; 80: 149-161.

How to cite this article: Dogan M. Preparation of Chitosan-Polyvinyl Prolidone (PVP) Hydrogels with Fibroblast Growth Factor (FGF) and Investigation of in Vitro Characteristics. Clin Exp Health Sci 2022; 12: 418-422. DOI: 10.33808/clinexphealthsci.972758