

Smart Bandage System: A Combination of Chitosan, Nanoparticles and Electric Stimulation

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Abstract

Diabetes mellitus, the world's fastest growing chronic disease, still affects more than 420 million people around the world. For years, many patients continue their lives with these diabetic wounds or lose their limbs mostly due to infection, against all countermeasures taken which are a long and painful treatments. For such chronic wounds, passive dressing treatments are accepted to be not very effective. In this study, a dressing system was developed for the improvement of healing time in chronic and diabetic wounds. The designed system has combined the effectiveness of bioelectric stimulation and the clinical effects of chitosan to accelerate wound healing process. The dressing system was obtained by bringing these two systems together. The designed chitosan films form the inner surface layer of dressing, while the electro stimulation circuit electrodes are at the ends of the dressing. When sending current pulses to the tissue surface during treatment current could pass through the chitosan films. Therefore, the current flowing through chitosan films was also measured in this study.

Akıllı Pansuman Sistemi: Kitosan, Nanopartiküller ve Elektrik Stimülasyon Kombinasyonu

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Özet

Dünyanın en hızlı artan kronik hastalığı olan şeker hastalığı, halen dünya çapında 420 milyondan fazla insanı etkilemektedir. Uzun ve ağrılı bir tedaviyle birçok hasta yıllardır diyabetik yaralarla hayatına devam etmekte ve çoğunlukla hastalar enfeksiyon nedeniyle uzuvlarını kaybetmektedir. Bu tür kronik yaralar için pasif pansuman tedavilerinin çok etkili olmadığı kabul edilmektedir. Bu çalışmada, kronik ve diyabetik yaralarda iyileşme süresinin iyileştirilmesi için bir pansuman sistemi geliştirilmiştir. Tasarlanan sistem, yara iyileşme sürecini hızlandırmak için biyoelektrik stimülasyonun etkinliği ile kitosanın klinik etkilerini birleştirmiştir. Bu iki sistemin kombinasyonu ile pansuman sistemi elde edilmiştir. Tasarlanan kitosan filmler sargının iç yüzey katmanını oluştururken, elektro stimülasyon devresi elektrotları sargının uçlarındadır. Tedavi sırasında doku yüzeyine akım darbeleri gönderirken akım kitosan filmlerden geçebilir. Bu nedenle bu çalışmada aynı zamanda kitosan filmlerinden geçen akım ölçülmüştür.

1. Introduction

The skin is the largest organ of the human body. Major acute injuries can be fatal due to the area covered all over the body. Wound dressings play a big role in treating these kinds of deformations of skin [1]. Chronic wounds include, but are not limited, to diabetic foot ulcers, venous leg ulcers, and pressure ulcers. They are a challenge to wound care professionals and consume a great deal of healthcare resources around the globe [2]. Chronic wounds can be classified as vascular ulcers (e.g., venous and arterial ulcers), diabetic ulcers, and pressure ulcers (PUs) [3].

In a study it was indicated that, the most commonly used models and approaches for the investigation of diabetic wound healing are largely insufficient for the development of novel therapeutics. To overcome these barriers, scientists must consider more innovative tools and approaches for diabetic wound healing [4]. The aim of our study is to design a new wound care system that improves the approach of wound management.

In studies about chronic wounds, the absence of a significant reduction in wound size within 2-4 weeks of treatment showed that the dressing material used was ineffective. Each wound has different features. There are hundreds of materials

on the market as dressing material, making it difficult to choose suitable dressings. The benefits of wet dressings in wound healing have been revealed many times [5]. Wounds in a moist environment heal faster than wounds exposed in the open air. Since the physical structure of chitosan creates a moist environment, chitosan films create a suitable environment on the inner surface of the dressing [1]. In order for the dressing to be a positive factor in wound healing, it must have some if not all these properties: The wound surface must be moist, and the moist environment must be maintained. The antibacterial properties of the inner surface of the dressing should protect the wound against bacteria, infections, fungi, etc. It should absorb wound fluids and reduce necrosis in the wound. It should prevent dehydration on the wound surface. It should have a flexible and smooth surface. It should be non-toxic. It should be biocompatible and biodegradable [5]. Nowadays, with the increasing need of wound treatment, dressing types have become increasingly complex. For this reason, the designed dressing system is a bit different than the normal dressing. The designed dressing system was created by combining two different systems. The first system is electric stimulation, which has a significant contribution to wound healing as it helps collagen matrix formation in wound healing processes and increases epithelialization [6]. In one study it was reported that the electrodynamic field orientates and stimulates the migration of electric charged cells such as fibroblasts, keratinocytes and macrophages and stimulates angiogenesis [7]. In our study the aim of the designed electronic stimulation device is to increase the activation energy in wound healing mechanism by applying current at certain intervals. With the stimulation circuit, the current and frequency values can be adjusted in accordance with the size of the wound and the individual, so it could provide the most optimal treatment. The designed

system was named smart dressing system as it's going to provide a personalized treatment. The second system uses chitosan. Chitosan increases the granulation of the wound with its accelerating effect for the treatment of open and deep wounds. It accelerates the formation of fibroblast layer, which increases the healing rate due to its hemostatic effect [8]. Due to its antimicrobial activities, resistance to environmental conditions, adherence nature, antifungal and excellent oxygen permeability, chitosan is used as excellent membrane for wound and burn dressings.

Nanotechnology is widely applied in many areas including wound healing [9]. Innovation in wound dressings include nanoparticles [10]. Based on these studies; nanoparticle-based chitosan films were obtained. In order to actively use these factors on the dressing surface, chitosan hydrogel films were obtained. The main purpose of the system is to shorten the healing time in chronic wounds. The dressing types used today do not cover all the features that should be in an ideal dressing. By combining these two systems, the dressing design could serve as an ideal dressing for wound healing.

2. Methods and Materials

In this study, MCP4725 DAC, adjustable voltage amplifier card MT36082-24V, Op-amp (LM741), electrodes (TMS electrodes), power supply (battery), and an atmega microcontroller were used for the design of the electro stimulation circuit. Chitosan, acetic acid, lactic acid, boric acid, agar agar, polyvinyl alcohol and silver nanoparticles were used to make the chitosan films. The outline of the study is given in figure 1.

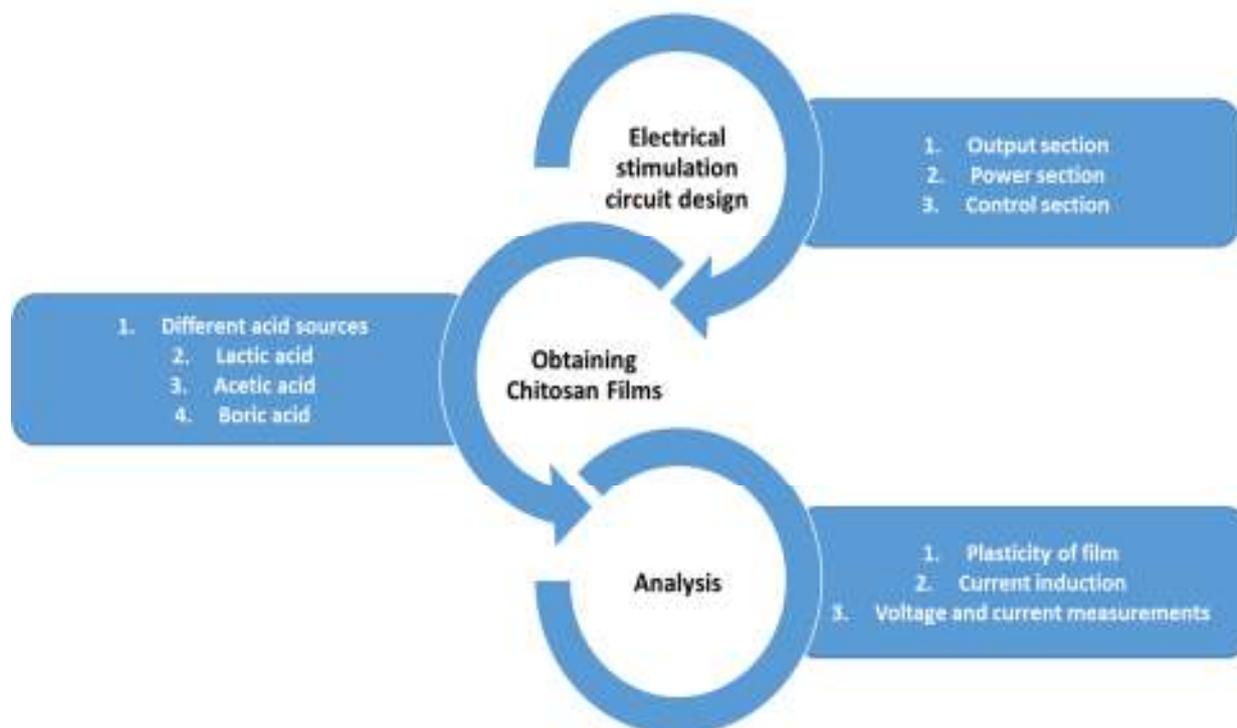


Figure 1. Study outline

2.1. Electrical Stimulation (ES) Circuit Design

A typical electrical stimulation design consists of several blocks. The stimulation circuit consists of a Control section, Isolation, Digital Analog Converter (DAC), Output Section, Electrodes and Power Supply. The control section consists of Atmel microcontroller and is responsible for producing a low amplitude electrical pulse sequences as well as controlling the pulses frequency. The isolator is used to protect the

microcontroller by separating it from other circuit elements. DAC is used to convert digital signals from the microcontroller to an analog signal [11,12]. The Output section is the last module in a typical ES device. The output stage has the task of transferring energy to tissues that will safely stimulate the produced low amplitude pulses. The output section is designed by taking inspiration from the HOWLAND a voltage to current converter circuit containing a general-purpose operational amplifier (LM741). The HOWLAND circuit is shown in figure 2.

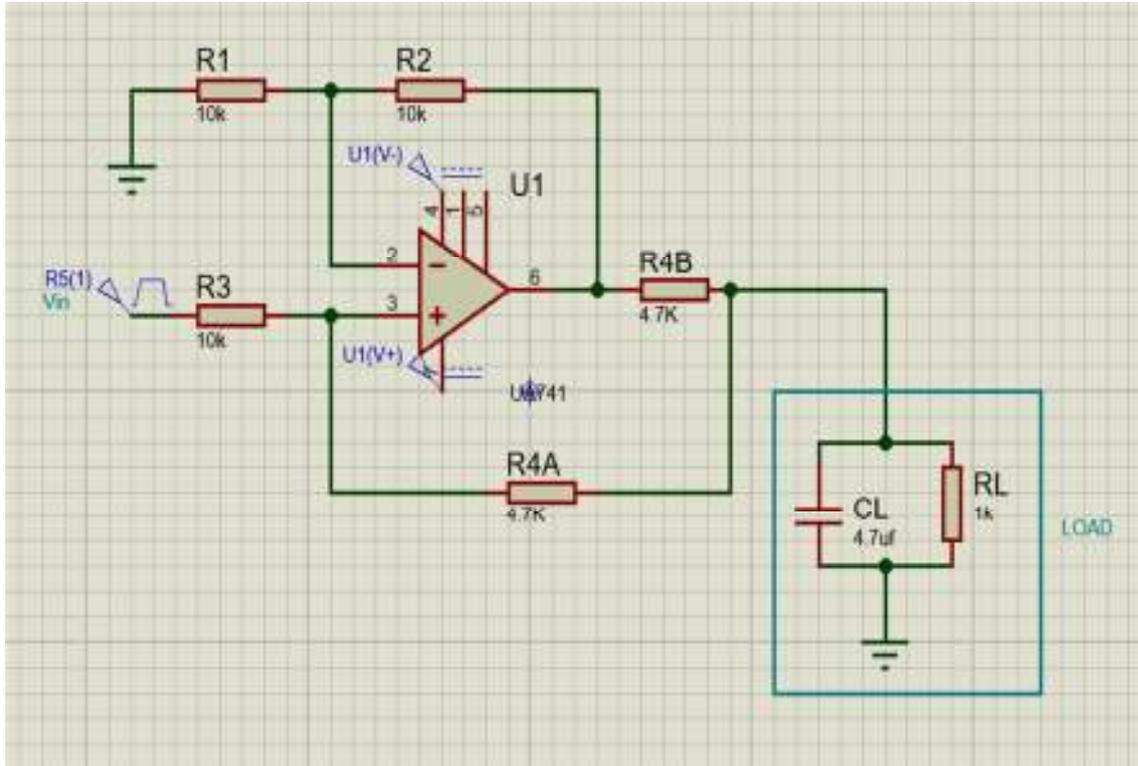


Figure 2. Howland circuit

2.1.1. Output Section - Howland Circuit Topology

The Howland current source is an op-amp based Voltage Controlled Current Source (VCCS), whose output is controlled by matching resistors of both positive and negative feedbacks. In the Howland structure, the output current is briefly calculated by the following formula:

$$I_{out} = \frac{-V_{in} \times R_2}{R_{4B} \times R_1}$$

$R_1 = R_2 = R_3 = 10k$ ohms, $R_{4A} = R_{4B} = 4.7k$ ohms, $V_{in} = 5v$. According to the above formula $I_{out} = 1$ mA. In order to be sure of the results, the circuit was simulated after the circuit was constructed.

2.2. Obtaining Chitosan Films

Biocompatible synthetic polymers were added to increase the chemical and mechanical stability of the chitosan films. Experimental groups were prepared as 1, 2, 3, 4, 5 and were also branched within themselves.

2.2.1. Preparation of The Experimental Groups

In the 1.1 experimental group 1 gram of chitosan was mixed with 100 ml of distilled water at 90 °C at 400 rpm in a mechanical mixer for 30 minutes. Then the obtained chitosan solution was sterilized in an autoclave at 121 °C for 45 minutes. Then 25 ml of acetic acid was added to the chitosan solution. The obtained solution was stirred on magnetic stirrer overnight. The solution, which was stirred overnight, was poured into petri dishes and it left to dry at room temperature. In the 1.2 group 0.5 g chitosan was added to 25 ml distilled water and mixed for 30 minutes in magnetic stirrer. The prepared solution was autoclaved, for 15 minutes at 121 °C and 1 atm pressure, sterilized. The obtained solution was divided into 2 groups. 1 ml of acetic acid was added to the 1.2a solution and 1 ml of lactic acid was added to the 1.2b solution. These solutions were then mixed in magnetic stirrer again. The solutions obtained were poured into petri dishes and it left to dry at room temperature. The remaining groups went through the same solution preparation processes. Silver Nanoparticle solution was prepared only for Experiment 4th and 5th groups. After the drying process of 4th experimental group solutions, nanoparticles were added with spray. Nanoparticles were added in certain ratios in the 5th experimental groups. The current values passing through the

films obtained after the drying process of all solutions were measured.

2.2.2. Preparation of Silver Nanoparticle Solution

To prepare the solution, 49 ml of distilled water was added on 1 ml of 20 μm AgNP. The solution was then stirred in the mixer for 15 minutes. The silver nanoparticle solution was sprayed over 4.1 and 4.2 group films. The physical properties of the films were examined after drying. The 3.1 group chitosan film and 4.2 group chitosan film are shown in figure 3 and figure 4 respectively.



Figure 3. 3.1 group chitosan film



Figure 4. 4.2 group chitosan film

Table 1. Chitosan experimental groups

	Chitosan	Acetic Acid	Lactic Acid	Boric Acid	Agar Agar	PVA	20 μm Ag Np
1.1	1g	25ml	-	-	-	-	-
1.2	0.5 g	-	-	-	-	-	-
1.2.a	0.5 g	1ml	-	-	-	-	-
1.2.b	0.5 g	-	1ml	-	-	-	-
2.1	0.5g	1ml	-	-	-	-	-
2.1.a	0.5g	1ml	-	-	-	-	-
2.1.b	0.5g	1ml	-	1g	-	-	-
2.1.c	0.5g	1ml	-	-	1,2g	-	-
2.1.d	0.5g	1ml	-	-	-	-	-
2.2	0.75g	1ml	-	-	-	-	-
2.2.a	0.75g	1ml	-	-	-	-	-
2.2.b	0.75g	1ml	-	(%) 1ml	-	-	-
2.2.c	0.75g	1ml	-	-	1.2g	-	-
2.2.d	0.75g	1ml	-	-	-	-	-
2.3	0.5g	-	1ml	-	-	-	-
2.3.a	0.5g	-	1ml	-	-	-	-
2.3.b	0.5g	-	1ml	(%) 1ml	-	-	-
2.3.c	0.5g	-	1ml	-	1.2g	-	-
2.3.d	0.5g	-	1ml	-	-	-	-
2.4	0.75g	-	1ml	-	-	-	-
2.4.a	0.75g	-	1ml	-	-	-	-
2.4.b	0.75g	-	1ml	(%) 1ml	-	-	-
2.4.c	0.75g	-	1ml	-	1.2g	-	-
2.4.d	0.75g	-	1ml	-	-	-	-
3	0.75g	-	-	-	-	-	-
3.1	0.75g	0,5ml	-	-	-	1 ml	-
3.2	0.75g	-	0.5ml	-	-	1 ml	-
4.1	0.75g	1ml	-	-	-	-	-
4.2	0.75g	-	1ml	-	-	-	-
5.1	0.75g	1ml	-	-	-	-	8ml
5.2	0.75g	-	1ml	-	-	-	8ml

2. Results

The electrostimulation circuit was designed to send current pulses at a frequency of 100Hz. The current pulses can go up

to 1 mA. As can be seen in figure 5 and 6 the values produced by the designed circuit and the values given by the circuit during simulation are very close to each other. The voltage drop in the actual circuit prototype can be attributed to the non-ideal circuit component

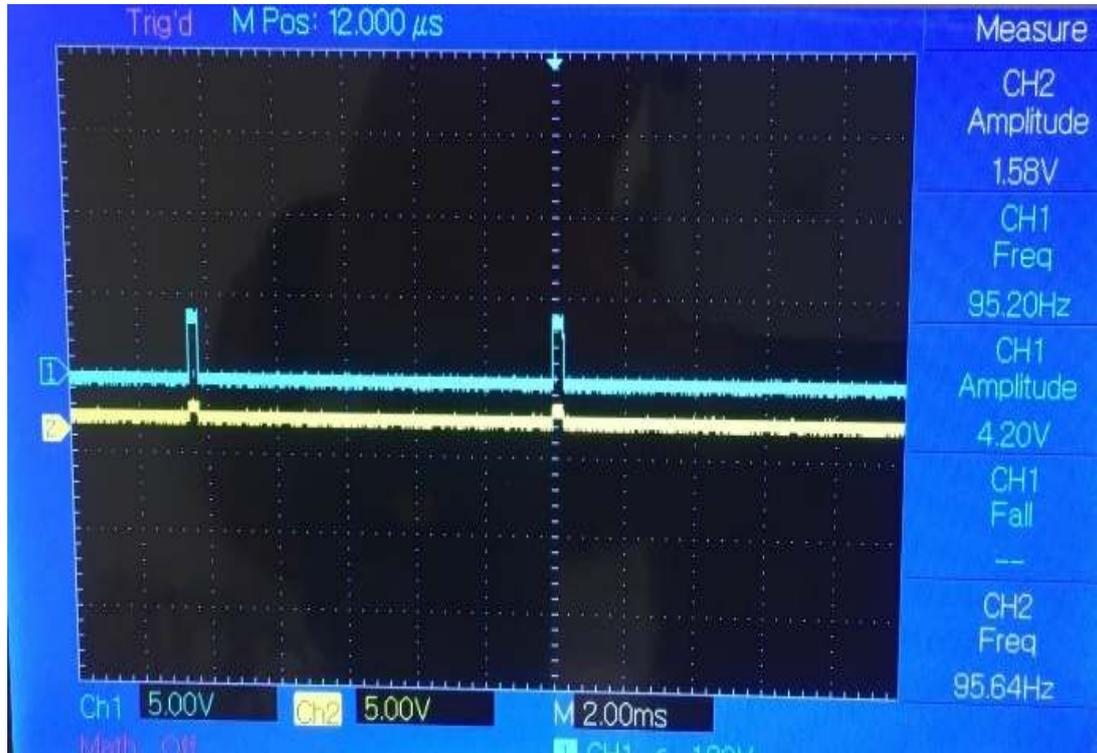


Figure 5. The input and output voltage pulses of the circuit prototype

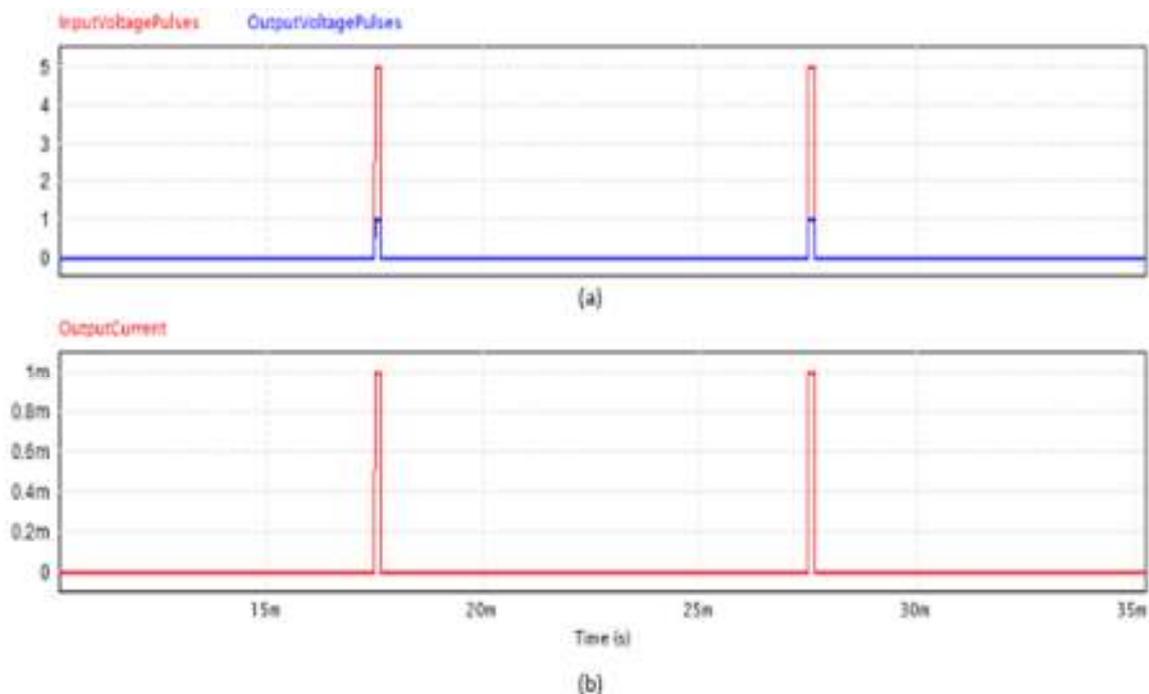


Figure 6. a) The input and output voltage pulses in the simulation. b) The output current pulses in the simulation. The current pulses can go up to 1 mA.

The designed system electro stimulation circuit electrodes were at the ends of the dressing and were to send current pulses to the tissue surface. As the pulse accepted to be sent to the tissue, it was observed that current could pass through

the chitosan films. Related with the current statement current passing through chitosan film surface were measured. The data obtained from this measurement can be seen in table 2, table 3, table 4 and table 5.

Table 1. Solutions conductivity

Experimental group	Current (mA)
1.1	0.895
1.2a	0.94
1.2b	0.94
3.1	0.8
3.2	0.435
4.1	0.45
4.2	0.565
5.1	0.605
5.2	0.605

Table 2. Solutions conductivity values standard deviation

Experimental group	Current (mA)
1.1	0.021213
1.2a	0
1.2b	0
3.1	0.042426
3.2	0.615183
4.1	0
4.2	0.304056
5.1	0.219203
5.2	0.219203

The 1.1, 1.2a chitosan-acetic acid and 1.2b chitosan-lactic acid group solutions had current of a 0.895 mA, 0.94 mA and 0.94 mA, passing through, respectively. After the drying process, the currents were 0,945 mA, 0,95 mA and 0,95 mA, respectively.

In the 2nd experiment group, subgroups of 2.1 2.2 2.3 and 2.4 were prepared using agar agar. After the solutions were dried, current values could not be obtained from the films.

In the 3th experiment group, chitosan films were obtained with 3.1 (chitosan-acetic acid- PVA) and 3.2 (chitosan-lactic acid- PVA). The group solutions conductivity was 0.8 mA and 0.435 mA, respectively. After the solutions dried, the conductivity changed to 0 mA and 0.185 mA, respectively.

Table 3. Films conductivity

Experimental group	Current (mA)
1.1	0.945
1.2a	0.95
1.2b	0.95
3.1	0
3.2	0.185
4.1	0.44
4.2	0.855
5.1	0
5.2	0.03

Table 4. Films conductivity values standard deviation

Experimental group	Current (mA)
1.1	0.007071
1.2a	0
1.2b	0
3.1	0
3.2	0.007071
4.1	0.452548
4.2	0.06364
5.1	0
5.2	0

4th experiment group included subgroups of 4.1 and 4.2, films were obtained after the solutions were dried. After the drying process of 4th experimental group solutions, nanoparticles were added with a spraying. The experiment group solutions had the conductivity of 0.45 mA and 0.565 mA, respectively. After the solutions dried, the conductivity changed to 0.44 mA and 0.855 mA, respectively.

In the 5th experiment group, chitosan films were obtained with 5.1 (chitosan-acetic acid- AgNP) and 5.2 (chitosan-lactic acid- AgNP) solutions. The experiment group solutions conductivity was 0.605 mA and 0.605 mA, respectively. After the solutions dried, the conductivity changed to 0 mA and 0.03 mA, respectively.

While the first group did not lose its conductivity property after the drying processes the other groups did. The second group is not concluded since most of the group films couldn't be obtained. The differences in films conductivity can be seen in figure 7 and figure 8.

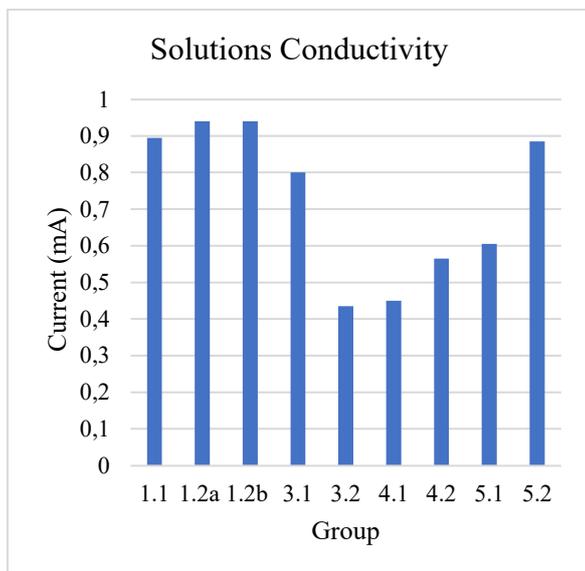


Figure 7. Solutions conductivity

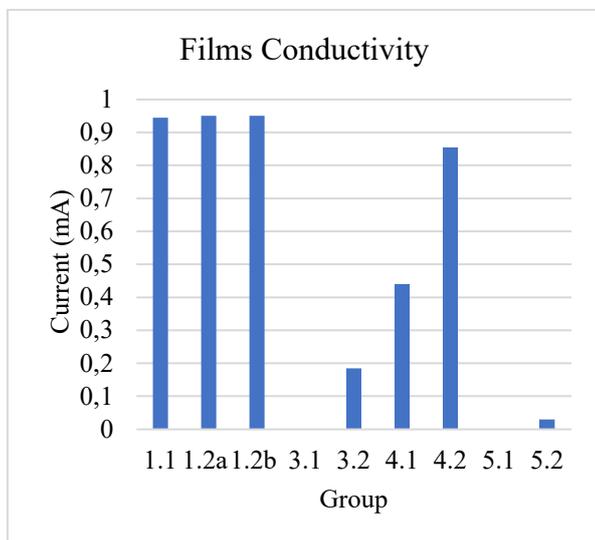


Figure 8. Films conductivity

Even there was no analyzes held for mechanical stability but with empirical check it can be said that chitosan films containing acetic acid were dry and had more mechanical stability and on the contrary the films containing lactic acid were flexible and preserved their moist structure.

3. Discussions

Many studies have advocated the use of ES therapy in conjunction with standard wound care [13,14] ES is defined as the application of electrical current through electrodes placed on the skin either near or directly on the wound [15]. Bioelectric dressings are emerging as a useful method of delivering ES to the wound site. However, studies of these specific modalities are lacking. Most of the devices used for ES are bulky and need to be operated by experts in this field. In one study a bioelectric wound dressing, named the PosiFect RD® DC device, has been used in treating pressure and venous ulcers. This dressing contains a miniature electrical circuit delivering a microcurrent to the wound bed for a minimum of 48 hours and has shown promise in treating

these chronic wounds [16,17]. In other work, the patented Modular Adaptive Electrotherapy Delivery System (MAEDS) was developed. The system is lightweight, flexible, battery-powered disposable device. The system with the control circuit can provide a sustained delivery of controlled reliable electrotherapy to effectively minimize infection and maximize healing [18].

In our study a few important points were taken into consideration when designing the electro stimulation system. These; It is not to cause any harm to the patient's body. A maximum of 1 mA is given from the electrodes of the electro - stimulation circuit to the tissue. This value does not create any negative effects for the patient.

The designed device has been prepared comfortably and conveniently so as not to hinder the daily life of the patient. The skin surface is modeled by parallel connection of a capacitor and resistor. The resistance of the wound surface is considered to be equal to 1KOhm. Using this information, the current and voltage values to be produced by the circuit designed were calculated.

It has been determined that the values produced by the designed circuit and the values given by the circuit during simulation are very close to each other.

In the second part of our study, it was aimed to obtain chitosan films and to use these films in the most appropriate way in wound healing. In this study, the dissolution of chitosan powder homogeneously and its physical and mechanical properties in different acidic environments were investigated. In the histological examinations performed in the studies we referenced, it was confirmed that chitosan films increase the epithelization rate and that organize the collagen accumulation in the dermis. We think that chitosan films with support materials (cotton etc.) can be a suitable dressing type. In one of the studies we examined, cotton fabric was covered with chitosan and polyethylene glycol (PEG) and then freeze-dried. When the coated fabric was examined by scanning electron microscopy (SEM), it was observed that the fabric had a porous structure. The porosity of the produced material was 54-70% and the pore size was between 75-120 μm . Cotton fabric was used as a support material for the chitosan and PEG layer, and a very thin light structures were obtained. In the studies we referenced, it was determined that chitosan dissolves homogeneously in acidic environments due to its cationic structure. Considering that Chitosan molecules may have different behaviors in different acidic and basic environments. Acetic acid, lactic acid, boric acid, PVA, silver nanoparticle, and agar agar were used in our study. In a study the freeze-thaw process was chosen to increase biocompatibility and achieve porous structure in prepared bio nano-composites. The hydrogel prepared in the mentioned study has optimum mechanical properties in both of the dry and wet state [19]. By taking the mentioned study as an example, in the 2nd Experiment group dry chitosan films with mechanical strength were obtained using acetic acid. Chitosan-lactic acid films were obtained also but with a wet and flexible structure. In one study chitosan was crosslinked with collagen to obtain the chitosan films [20]. In our study chitosan was crosslinked with polyvinyl alcohol (PVA) to obtain the chitosan films. In one study chitosan-alginate hydrogel was obtained. The gel had stability to it due to the presence of alginate, but in order to increase the

mechanical strength of the material, polyvinyl alcohol, and polyethylene oxide, which are environmentally compatible synthetic polymers, have been added [21]. In our study the first experiment was carried out by using some information from this study. Chitosan solutions were left to dry for one week, thus chitosan films were obtained. In the second experimental group, it was aimed to shorten the drying time by increasing the solution density. However, using agar made the films too dry and could not be obtained from the petri dish. One study examined several excellent polyvinyl alcohols (PVA) / polymers that blend hydrogel using different types of crosslinking to obtain suitable polymeric coating materials. The review explores different polymers which have been blended previously in the literature with PVA as wound dressing, showing the feasibility, property change, and purpose which are behind the blending process with PVA [22]. With reference to the mentioned study, the third experiment group chitosan films were obtained by adding PVA. In one study chitosan-silver nanoparticles (chitosan-AgNP) and chitosan-silver-graphene oxide (chitosan-Ag-GO) nanocomposite were used as a new generation of antimicrobial agent synthesized by the hydrothermal method. As a result, it has been proven that chitosan films containing nanoparticles have antibacterial, antimicrobial, and antifungal effects [23]. Using this study as a reference, experiments of the fourth group were carried out. 20 μm Ag-NP were added to the chitosan solution before carrying out the drying process.

In one study, boric acid helped the chitosan dissolve and contributed to the formation of the film. Because boric acid has a weak pH and then it can be easily dissolved in acidic solutions with pH under 6 [24]. We believe that combining the smart electrical stimulation system with chitosan films can serve as an ideal dressing for wound healing and shorting the healing time without hindering the daily life of the patient.

4. Conclusion

The designed ES system can send pulses of current up to 1mA to the wound. The current value and the pulse type as well as the frequency of the pulses can be adjusted with user interface for optimum treatment. The system is designed to be comfortable and to be powered by small batteries.

There are studies about increasing the functions of chitosan film forms and to expand their use. In this study, physical structures of chitosan hydrogel film forms were investigated. Recently it has emerged that the chitosan film form can be used in wound treatment. And at the same time, natural polymeric hydrogels such as chitosan have a wide area in tissue engineering, as they are extracellular matrix-like structures. Chitosan hydrogel films have been prepared due to its antimicrobial properties, good adhesion to mucosal surfaces and supporting epidermal cell growth. As the cationic chitosan contains an amino group that can react, it can easily react with anionic ions. The amino groups in chitosan are protonated at acidic pH and deliver positive chitosan chain. Since most biological cell surfaces are anionic, electrostatic interactions occur on the wound surface with the cationic structure of chitosan and this interaction increases wound healing. The antibacterial effect of chitosan

depends on its cationic structure. With respect to the cationic structure of chitosan, it can be easily dissolved in acidic solutions with pH under 6. The use of weak acetic acid and lactic acid helped the chitosan dissolve and contributed to the formation of the film. Synthetic and biocompatible polymers were used to strengthen the mechanical properties of the films. In the 1st experiment group, chitosan-acetic acid and chitosan-lactic acid films were dried at room temperature for 1 week. It took time for the solutions to pass into film form because the solutions were filled in the Petri dishes at a height of 1 cm. In the 2nd experiment groups 2.1 2.2 2.3 and 2.4 agar was used. Because of the agar the films could not be obtained. In the 3rd experiment group, film forms were obtained with chitosan-acetic acid-PVA and chitosan-lactic acid-PVA. After the solutions were dried, current and voltage values could not be obtained from the films with the acetic acid-PVA group. 4th experiment group 4.1 and 4.2 films were obtained after the solutions were dried. Films obtained with acetic acid were dry films with mechanical strength. The films obtained with Lactic Acid were flexible and preserved their moist structure. In the 5th experiment group, chitosan films were obtained with chitosan-acetic acid and chitosan-lactic acid solutions. It has been determined that it is not the preparation methods but the type of the used acid that cause the change in the mechanical properties of the films.

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