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Chitosan-based layered nanofibers loaded with herbal extract as wound-dressing materials on wound model studies

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ABSTRACT

In the present study, multilayer electrospun chitosan-based dressing containing Semellil extract (ANGIPARS ™) was fabricated and evaluated as wound dressing. The first layer was polyurethane nanofibers which used as a protective layer, the middle layer was chitosan nanofibers loaded with extract and third layer, chitosan mat, was applied to improve sustained release of dressing. In order to increase the stability of dressings in physiological environment genipin, was applied to crosslink the chitosan nanofibers. So the FE-SEM images indicated stability of genipin cross-linked nanofibers after immersion in PBS. In addition, extract release of the mats were considered by UV absorption in determined wavelength (268 nm). Maintaining 84% cell viability was proved that the genipin-cross-linked dressing containing extract, is nontoxic to human skin fibroblasts. Also the full-thickness wound in rat models was used to evaluate the healing effect of the extract-loaded coatings, moreover, histological performance and significant differences between groups were investigated by one-way ANOVA test. The genipin-cross-linked mats containing extract showed more improved and accelerated wound-healing properties than other samples. This result was confirmed by histological examination and these mats had significantly wound-closure percentage of about 94% after 14 days. All these results indicate that chitosan-based electrospun dressings containing Semellil Melilotus Officinalis extract, due to improved wound healing profile, could be used as a wound dressing.

Keywords: *multilayered dressing, wound healing, genipin, semellil extract, full-thickness wound.*

1. INTRODUCTION

An appropriate wound dressing will be satisfactory for preventing infection along improving healing process, also an ideal wound dressing should be biocompatible and biodegradable, with the ability to absorb exudates while avoiding the skin dryness. So many studies have been performed on creating effective skin wound coatings and the goal of all was to achieve a coating which accelerates the healing process while decrease scars which can be caused by the wound [1].

In recent decades, nanofibers are emerging in the era of medicine, and can be produced by different methods. The most used technique for producing nanofibers is electrospinning which was invented by Formhals in 1934 [2]. Electrospun nanofibers have specific advantages in wound dressing applications [3, 4]: high surface area-to-volume of nanofibers cause a salient increase in exudates absorption, porous structure of electrospun nanofiberous mats and their pore size are enough to prevent the entrance of micro-organisms, while supplies the permission of cellular respiration and gas exchange so inhibits wounds to be dried. Electrospun mats have appropriate flexibility and compatibility with wound and this affair cause protection from infection. In addition, the resemblance of electrospun nanofibers structure to extracellular matrix (such as collagen fibers) causes faster growth of healthy cells in nanofiber mats [5, 6]. Therefore, the formation of scar tissue and also the healing time are reduced.

The kind of polymer to electrospun is an important issue for coating wounds which can be impressive for wound healing process. Chitosan (Cs) is a biopolymer, which is derived from hydrolysis of chitin, and has anti-inflammatory and antibacterial effects while is not toxic, also chitosan is a biodegradable and biocompatible polymer which these properties make it a good choice for enhancement of wound healing process [7, 8]. Also using a blend solution of chitosan with other polymers such as Polyvinyl alcohol (PVA) [9, 10], Polylactic acid (PLA), Poly ethylene oxide (PEO) [11, 12] and collagen can be an effective way to facilitate chitosan electrospinning [13].

Recently, ANGIPARS™, a new phyto-therapeutic drug formulation from Semellil Melilotus Officinalis extract is introduced for wound healing applications. This herbal extract can improve the process of wound healing by increasing angiogenesis [14]. Medical effects of this extract cannot be attributed solely to one matter, however Coumarin is known as an active ingredient. Coumarin is a phenolic substance which various derivatives of its have anti-inflammatory [15], anti-cancer [16] and anti-diabetic activities [17].

In previous our study, electrospun nanofibers of Cs/PEO loaded with Semellil extract were prepared to use as wound dressing, and it was optimized in terms of structure and drug delivery [18]. In the mentioned study, a toxic cross-linking agent, glutaraldehyde, was used to crosslink chitosan nanofibers in order to prepare sustainability of chitosan nanofibers in the physiological environment. Although, the negative effect of glutaraldehyde on wound healing process, beside low tensile strength of this chitosan

based nanofibers, were major problems to use this nanofiberous mat as a wound dressing material.

Genipin, a naturally cross-linking agent which spontaneously reacts with free amino groups, is a good alternative for glutaraldehyde. Moreover, it has much lower toxicity effects (about 5000 to 10000 times) and is more biocompatible in compare with glutaraldehyde [19, 20]. Also genipin cross-linked materials have better mechanical strength [21]. Thus, in order to stabilize Semellil loaded chitosan nanofiber dressing, genipin has been applied as a cross-linking agent to improve tensile strength of dressing besides improving its biocompatibility.

In order to improve flexibility of chitosan-based mats, Polyurethane (PU) nanofibers, can be used as supporter layer for this membrane. Different types of Polyurethane have a variety of medical applications, like manufacturing of implants, wound dressings, medical devices and drug carriers, this broad use is due to their excellent physical and mechanical properties and also their acceptable biocompatibility and hemocompatibility [22-24].

There are several reports which designed electrospun multi-layer structures to improve drug release from the wound dressings [25-28]. Thus, in this study, in order to achieve sustained release of chitosan-based mat, Cs/PEO nanofibers without extract and genipin were fabricated on the facial surface of dressing. This layer could improve extract sustained release of the dressing by delaying contact between surface wound and drug loaded layer (middle layer).

Finally, multi-layer electrospun dressing included the following layers, was designed: i) PU nanofibers, ii) Cs/PEO-genipin nanofibers containing Semellil Melilotus Officinalis extract, and iii) Cs/PEO nanofibers (Table 1). This membrane was prepared and evaluated as a new dressing to improve wounds healing process (Fig. 1).

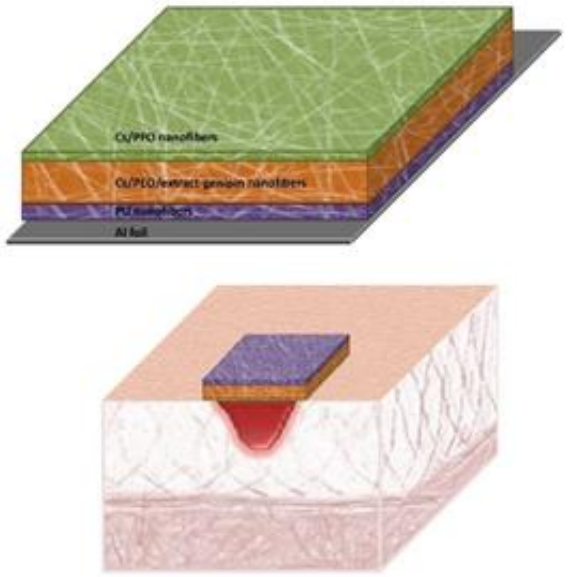


Figure 1. Schematic image of multi-layer mat containing extract (up), and its placement on wound area (down).

2. MATERIALS AND METHODS

2.1. Materials.

Polyurethane (polyether polyurethane) used from commercially available material (KU2-8670, Bay Company). Low molecular weight chitosan (Cs, degree of deacetylation 91.2 %) was supplied from Easter Group ((Dong Chen) Co., Ltd, China). Polyethylene oxide (PEO) (MW 900 kD) was obtained from Acros Organics Co. Hexafluoroisopropanol (HFIP) was supplied by Trademax Pharmaceutical, Chemicals Co. Ltd, China. Glacial acetic acid was purchased from Merck Chemical. Glutaraldehyde was purchased from Panreac (Spain). Semellil extract received from Rose Pharmed Biotechnology Co, Iran. Genipin (GP), methyl-2-hydroxy-9-(hydroxymethyl) - 3-oxabicyclonona-4, 8-diene-5-carboxylate, was purchased from Challenge Bioproducts Co. Ltd. (Touliu, Taiwan). RPMI medium and fetal bovine serum (FBS) were obtained from Gibco, USA and MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetra zolium bromide) from Sigma-Aldrich, USA. Human fibroblast (AGO-1522) were supplied by National Cell Bank of Iran, Pasteur Institute, Iran. Ketamine 10% and xylazine 2% were obtained from Alfasan, Holland.

2.2. Preparation of solutions.

PU granules were dissolved in HFIP [29] in a concentration of 3.0% (w/w) under magnetic stirring at room temperature. Chitosan, polyethylene oxide (2.5% w/w) and Semellil Melilotus Officinalis (20.0% w/w) solutions were prepared separately by dissolving in aqueous acetic acid solution (80.0% v/v) as solvent [24]. The chitosan and PEO solutions were then mixed together in weight ratio of 4:1. Afterward, Semellil solution was blended into Cs/PEO mixture in weight ratio of 50:50, 40:60, 30:70, 20:80 and 10:90. To increase stability of chitosan-based nanofibers in physiological environments, genipin solution (10.0% w/v) in methanol, was added in weight ratio of 100:1 to Cs/PEO/extract solutions to crosslink the chitosan nanofibers. All polymer solutions were magnetically stirred at room temperature to obtain homogenous solutions. Furthermore, different dissolution times were utilized to supply suitable solutions for electrospinning process.

2.3. Electrospinning of multi-layer mats.

The electrospinning processes were carried out using Electroris® (FMN, Tehran, Iran). An aluminum sheet covered rotating drum as a collector. Solutions were poured into a syringe with 18 G stainless steel needle and flow rate was varied for each layer of nanofibers.

For the electrospinning of multi-layer mat, at first, PU solution was electrospun as a protective layer (Table 1. (i)). After the PU nanofibrous mat was successfully synthesized on the collector, process was followed by electrospinning of chitosan-based solutions with different concentrations of Semellil extract (Table 1. (ii)). In the last step, a thin layer of Cs/PEO nanofibers was electrospun on these dressings as third layer (Table 1. (iii)).

To investigate the effect of cross-linking agent on improving the potential of wound coating, multi-layer mat was synthesized without genipin, which was cross linked by conventional cross-linking agent, glutaraldehyde (Table 1. (iv)). All parameters were

the same as genipin-loaded mats without adding genipin to the solution.

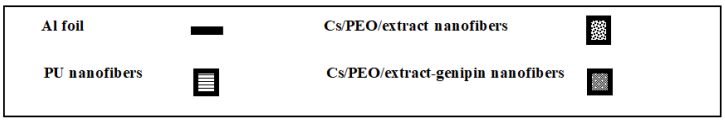
2.4. Cross linking of mats.

After the completion of electrospinning process, the mats were removed from collector, and those which had genipin exposed to water vapor while suspended in a sealed desiccator at 30 °C for 24 h [30]. Change of membranes color to dark blue was a sign for crosslinking of chitosan nanofibers. Then mats were dried at 37 °C for 24h.

The genipin-free membrane also was cross linked by exposed to vapor of 25.0% w/v aqueous glutaraldehyde solution at 37 °C for 24 h [31]. All of the samples were maintained for further analysis.

Table 1. Arrange layers in electrospun multi-layer nanofiberous mats.

Types of electrospun mat were fabricated in the study	Components of electrospun multi-layer mats	Schematic images of layers of multi-layer mats
i. protective layer	PU nanofibers	
ii. Double layer	(PU)(Cs/PEO/extract-genipin nanofibers)	
iii. Multi-layer	(PU))(Cs/PEO/extract-genipin nanofibers)(Cs/PEO)	
iv. Double-layer mat	(PU)(Cs/PEO/extract) cross linked by glutaraldehyde	



2.5. Drug release.

To investigate the release profile of dug-loaded mats, Coumarin, one of the main component of Semellil extract, was determined as an indicator of extract release. Special concentration of Semellil extract in PBS (5mg/ml) was prepared and shacked to homogenize the solution. Then the solution was filtered by filter paper and scanned for understanding the λmax of Coumarin by using UV-Vis spectrophotometer (UV-Visible (SHIMADZU)) to obtain calibration curve.

To measure the drug release from nanofibrous mats, pieces of membranes with specific size (3×3cm) were weighted and placed into 100 ml phosphate buffer saline(PBS, pH=7.5). These solutions were then incubated under stirring at 37 °C. Aliquots of samples (1ml) were collected from the release medium and replaced with fresh PBS at specific time intervals: 0.5, 1, 2, 4, 6, 8 and 24 h. The absorbance was determined by UV-vis spectrophotometer at known λmax. With the aid of calibration curve, percentage of drug releases was calculated by changing absorbance to extract concentrations.

2.6. Sustainability test for nanofibers.

To evaluate fibrous stability of cross-linked chitosan nanofibers in aqueous medium, identified sizes of electrospun mats was immersed into phosphate buffer (PBS) (pH: 7.4) at 37 °C for one day and the morphological changes were examined by FE- SEM after dried in room temperature.

2.7. Cell viability.

Cytotoxicity of nanofibrous mats was assessed by MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay, based on a procedure adapted from the ISO 10993-5 standard test method. The samples (Table 2) were placed into 96-well plate, then sterilized with UV radiation for 2h. Human fibroblast cells (AGO 1522) were seeded in wells at a density of 104 cells/well in RPMI medium supplemented with 10.0% FBS. The plate was then incubated at 37 °C for 24 h and after that culture medium of wells was removed and replaced with 100 µl MTT solution (0/5 mg/ml). Finally MTT solution was removed after re-incubated for 4 h and removed with 100 µl isopropanol to dissolve formazan crystals, formed in living cells. Absorbance of formazan solutions was detected by plate reader (AWARNESS TECHNOLOGY, Stat fax2100, USA) at wavelength of 268 nm and the significant differences between groups were conducted by using SPSS to determine the cell viability (%) of samples which statistical significances were set at $p<0.05$. Culture medium, RPMI, was utilized as negative control was defined as 100.0%.

Table 2. Samples were used in MTT assay.

Group	Samples into 96-well plate	Evaluated factor
1	PU nanofibers	Cytotoxicity of PU
2	Cs/PEO/extract-genipin electrospun mat	Cell viability of genipin cross-linked dressing
3	Cs/PEO/extract electrospun mat cross-linked by glutaraldehyde	Cell viability of glutaraldehyde cross-linked dressing
4	Culture medium (RPMI)	Negative control

2.8. Wound healing study.

30 male Wistar rats (250-300g) were randomized into 5 group consisting of 6 animals in each group. By IP injection of Ketamine and Xylazine, animals were anesthetized and then hairs on their dorsal skin region were shaved. Following this, a full-thickness incision wound (2×2 cm) was made on back of each rat. The wounds were then covered by samples coatings (Table 3) and wound dressings were replaced daily with new samples. In order to evaluate changes in wound areas, wounds were imaged using a digital camera for period of 14 days and changes in wound area were measured using Image J software. Decrease of wound area considered as wound closure percentage which is an indicator for wound healing effect of samples that were determined according to the equation as follow:

$WCR=(A_0-A_t)/A_0 \times 100\%$

Where A0 and At are the initial wound area and the wound area at time t, respectively. Results of percentages of wounds closure evaluated through one-way ANOVA test.

2.9. Histological examination.

At the appropriate time points (3th, 7th and 14th days), a rat in each group was sacrificed and wound area with surrounding tissues were excised to histopathological studies. Obtained wound

samples were fixed in 10.0% formalin, embedded in paraffin, sectioned at 5µm thickness and the wound site sections were stained with hematoxylin and eosin. Then the stained sections were observed under optical microscopy to evaluated re-epithelialization and granulation [32].

3. RESULTS

3.1. Electrospinning of PU solution.

In order to synthesize of protective layer, 3.0% PU solution was prepared and electrospun easily. FE-SEM images showed PU nanofibers without nodes, also, average diameters of PU nanofibers was 512nm±63 (Fig. 2) that was determined by Image J program. This electrospun mat was uniformly on the plate collector and the resulted scaffold could support the chitosan-based dressings.

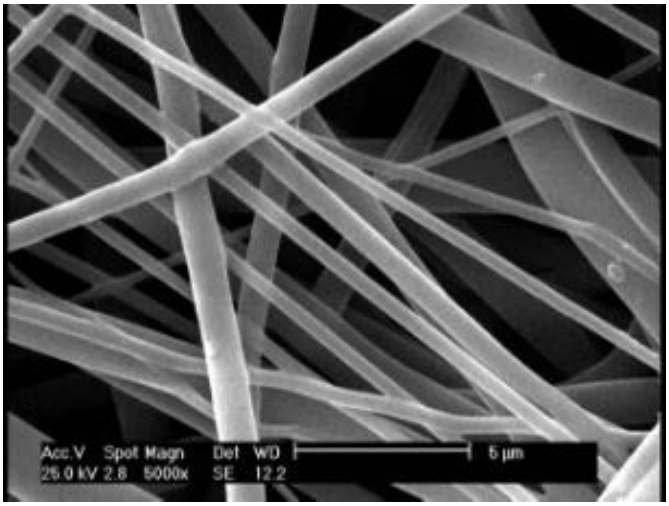


Figure 2. FE-SEM micrograph image of optimized PU nanofibers.

3.2. Electrospinning of Cs/PEO solution containing Semellil extract.

Chitosan and PEO mat containing various amount of the Semellil extract (10.0%, 20.0%, 30.0%, 40.0% and 50.0%) and (10.0% w/v) genipin were fabricated.

Whatever the concentration of extract in polymer solution increased, the viscosity of the solution increased also, so electrospinning process gets harder. The reason is that the process of extract crosslinking by genipin, or relate to hydrophilicity differences between chitosan and extracts. Thus only solutions containing 10.0%, 20.0% and 30.0% the extract concentrations could be successfully electrospun.

3.3. The drug release rate of electrospun Cs/PEO/extract-genipin mats with different ratio of extract.

The release profiles of extract with the concentrations of 10.0%, 20.0% and 30.0% loaded electrospun Cs/PEO nanofibrous mats were plotted in Fig. 3. As indicated in this image, a burst release during the first hour was seen in all samples and until fourth hour reached to its maximum value, then remained constant up to end. In a research, Kim et al. showed that burst effect of drug release from electrospun mats was caused by weak physical interactions between the drug and polymer which drug-molecules were present on the surface of nanofibers and easily released when exposed to aqueous media [33]. So burst effect in the extract release might be due to the difference in hydrophilicity of chitosan and extract that leads to the low physical interactions between them. As a result, hydrophobic extract easily placed on the surface of chitosan nanofibers and quickly released when exposed to aqueous media. In the other hand, the initial and total releases of extract enhanced by increasing extract concentration due to locating more amount of extract in the surface of the nanofibers. The maximum amount of drug release from chitosan nanofibers in optimized condition was about 30% (for samples with 10.0%, 20.0% and 30.0% extract were 18%, 21% and 30% respectively). It may be due to the trapping drug molecules between the pores of nanofibers [34]. Also, genipin can interact with amine groups in extract components and prevents its release.

From two view point, the lower moisture level of wound area beside chitosan degradation enzyme in this area it can be expected the extract release profile from dressings, were different with *in vivo* situation, whether sustained release or maximum release. In addition, the gradual destruction of non-cross-linked chitosan nanofibers in surface layer, can be caused sustained release of extract from the mats. In this study, due to maximum release of three-layer mat containing 30% extract, applied it as wound dressing for more evaluations at cytotoxicity test and animal model.

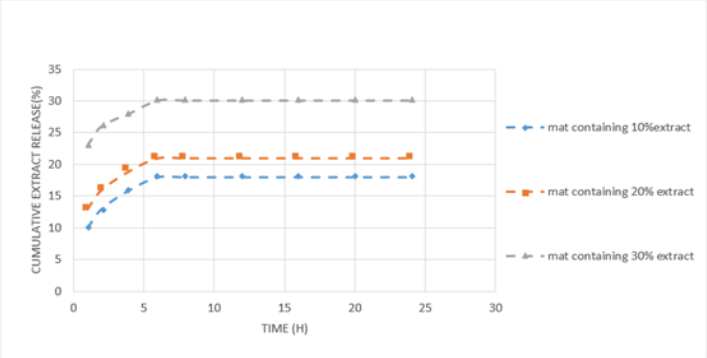


Figure 3. Extract release from electrospun mats loaded with 10%, 20% and 30% extract, during 24 h.

3.4. Morphology of drug loaded electrospun Cs/PEO mats after cross linking.

FE-SEM micrograph images of mats containing 30% extract, after cross linking in steam, has been shown in Fig. 4. As can be seen in these images, when cross linking occurred, the diameter of nanofibers increased. For example, diameter of nanofibers in double layer mat increases from 148nm to 192nm.

The electrospun mat which was cross-linked by genipin demonstrated good stability in aqueous media and its structure remained unchanged. In the multi-layer mat due to the absence of the crosslinking agent, genipin, in the third layer, chitosan fibers lost their fibrous structure, because of contact with water vapor molecules. This result was similar to study that was done by Mirzayi E, and et al. which had compared effects of structural

changes were caused by genipin and glutaraldehyde in cross-linked Cs/PEO nanofibers [30].

3.5. Cytotoxicity test.

To evaluate the cytotoxicity of multi-layer mat containing 30.0% Semellil extract, human fibroblast cells AGO was used for MTT assay. A plate reader device was used to read the absorbance of samples and results of cytotoxicity test evaluated through one-way ANOVA test. To calculate the cell viability percentage of samples, the mean absorbance was assumed 100% and absorptions of samples were divided by the mean absorbance (Fig. 5).

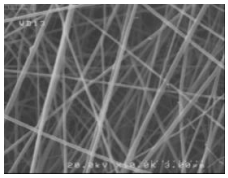
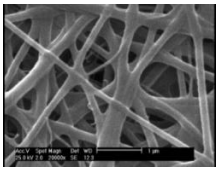
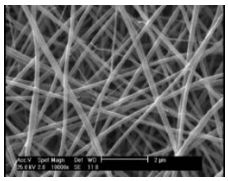
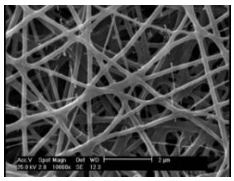
Samples descriptions	Before cross linking	After cross linking
Double layer mat: (Pu) (Cs/PEO/extract-genipin)		
Multi-layer mat: (Pu) (Cs/PEO/extract-genipin)(Cs/PEO)		

Figure 4. FE-SEM micrograph images of double layer mat ((Pu)(Cs, PEO/extract-genipin)) and multi-layer mat ((Pu) (Cs/PEO/extract-genipin)(Cs/PEO)) before and after cross linking.

With assuming P<0.05, statistical evaluation showed significant differences between groups. It means that the PU nanofibers (Table 2(sample 1)) and genipin cross-linked multi-layer mat (Table 2 (sample2)), have no significant cytotoxicity effects. Also it seems that mat cross linked by glutaraldehyde (Table 2 (sample3)) have some degree of cytotoxicity compared with the control group. Our results confirm the same results which were obtained by Sung and et al. [21] and many other studies that had compared toxicity of glutaraldehyde with genipin [36, 37].

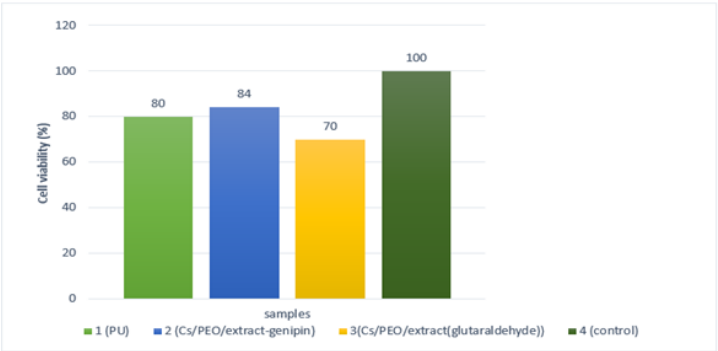


Figure 5. Cell viability percentage of samples in MTT assay.

3.6. *In vivo* test.

Fig. 6 exhibit the wound healing procedure in taken photographs of wound areas which were treated with different samples (Table 3), on the first, 7th and 14th days after injury (Fig. 6). Percentage of wound closure for each animal on days of 3, 5, 7, 9, 11 and 14 after injury were calculated and the average of these numbers as a percentage of wound closure was determined (Fig. 7). Also a significant difference between groups in different periods of times has been shown in Fig. 8.

Table 3. Different samples to evaluate wound healing in 5 groups of rats.

Group	Sample as dressing wound	Evaluated factor
A	30.0 % extract loaded multi-layer mat cross linked by genipin: (PU)(Cs/PEO /extract-genipin)(Cs/PEO)	Healing effect of three layer electrospun mat
B	30.0 % extract loaded multi-layer mat cross linked by Glutaraldehyde: (PU)(Cs/PEO/extract)	Comparing the healing effect of cross linker agent
C	30.0 % extract loaded double layer mat cross linked by Genipin: (PU)(Cs/PEO-Genipin)	Healing effect of Cs and Genipin alone
D	Commercial ointment of ANGIPARS™	Healing effect of commercial ointment
E	Physiological serum	Control

Evaluating different mats on animal models (Fig. 7) showed an improved wound healing effects in samples of A and C, compared to other dressings, in adition, significant differences were not observed in wound healing effects between A and C groups (P <0.05)(Fig. 8). This result may be due to this issue that the wound healing effects of ANGIPARS™ (the extract of Semellil Melilotus Officinalis), is related more to its oral form and using its ointment simultaneously improves the wound healing profile. Both groups of B and D showed nearly similar wound healing effects, while the E group (control) had lowest healing effect. Moreover no significant difference shown between groups of B and D (P <0.05). Ascending curves (Fig. 8) displayed a significant difference in each group by passing time, and distances between each curve from the other curves represented differences of wound healing profile between groups.

Multi-layer mats cross linked by glutaraldehyde (B sample) had fewer improving effect than similar sample cross linked by genipin (A sample), which indicate that genipin has a better effect over glutaraldehyde in wound healing process. It confirms the results of cytotoxicity test in this study and previous investigations [36, 37].

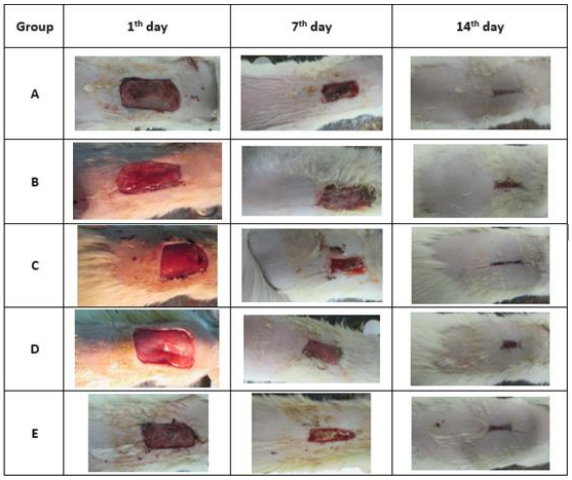


Figure 6. Photographic images from skin wounds treated with samples (A: multi-layer mats containing extract and cross linked by genipin, B: mats containing extract and cross linked by glutaraldehyde, C: Genipin cross-linked multi-layer mats without extract, D: commercial ointment, E: control) on first day, 7th day, and 14th days after injury.

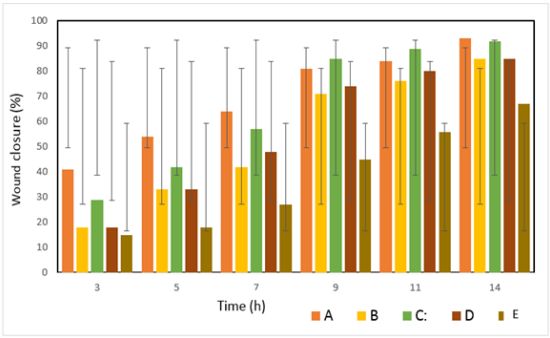


Figure 7. Average of wound closure percentage in 5 groups (A: Genipin cross-linked multi-layer mats containing extract, B: glutaraldehtde cross-linked mats containing extract, C: Genipin cross-linked multi-layer mats without extract, D: commercial ointment, E: control) during 14 days after injury. As indicated, A and C have most percentage of wound closure, and E has worst percentage in 14th day.

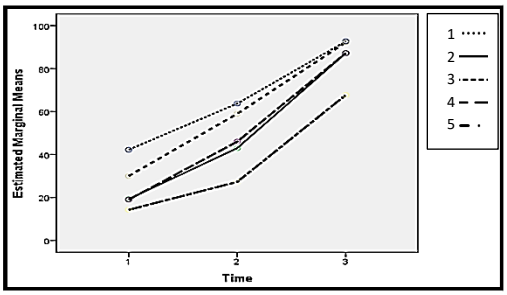


Figure 8. Graphs determine significant difference between groups in time (1: group A, 2: group B, 3: group C, 4: group D and 5: group E).

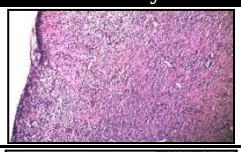
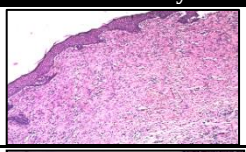
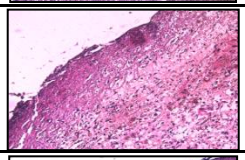
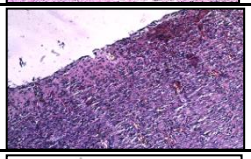
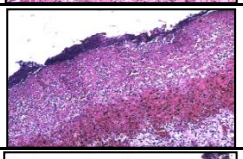
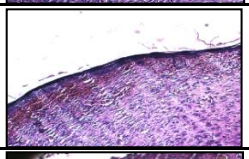
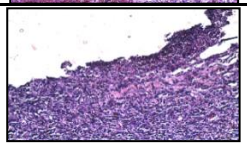
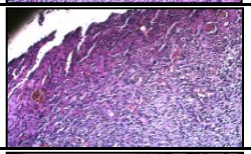
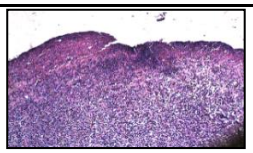
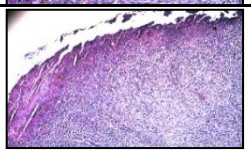
Group	7ths day	14ths day
A		
B		
C		
D		
E		

Figure 9. Representative histology (H&E staining, 100x) for obtained tissue samples of 7th and 14th days. After day 7 post-treatment all groups showed an increased cellular infiltration and angiogenesis. Also, granulating tissues are observed. In groups A and C after day 7 post-treatment, the wounds have new epidermis and a large decrease in exudate is observed. After day 14 post-treatment in A and C groups full thickness re-epithelialization and stratum corneum is seen. But in B, D and E groups after day 14th focal and disorganized re-epithelialization is seen. (A: mat containing extract, and genipin as cross linking agent. B: mat containing extract, and cross-linked by glutaraldehyde. C: mat containing genipin with no extract. D: Commercial ointment. E: physiological serum)

3.7. Histopathology test.

In order to assessed the effect of dressing samples on wound healing, Histological studies on wounds area tissues on the 3th, 7th and 14th days done. Fig. 9 shows the wound area of groups after the 7th and 14th days of treatment. As it was expected from inflammatory phase process, in all groups, abundant polymorphonuclear (PMN) cells seen on the day 3th and neovascularization and fibro-leucocyte exudates were observed, whereas no epithelial tissues were existed.

On the 7th day, PMN cells in A and C groups were reduced but in other groups there were numerous inflammatory cells in the site of wound. Fig. 9 indicated that the inflammatory phase with no epithelial tissues were seen in groups B, D and E, while by reducing the inflammatory phase in groups of A and C, deposition of collagen fibers in dermis tissues and re-epithelialization have occurred, in the other words proliferative phase has begun in A and C groups. However, the wound surface in group A was fewer than others and group E had the most wound surface. In A and C on the 7th day, the epithelium layer was forming around the wound.

The difference between samples wound healing effects is more marked on the day 14 post-treatment. A large number of infiltrated PMN cells in tissues is due to inflammatory phase in groups B, D and E. In groups A and C, PMN cells were rare and it seems that proliferative phase in these groups has finished on the 14th day. In A and C groups, full thickness re-epithelialization were observed in dermis and epidermal layers were well-organized (hair follicle, Sweat glands and deposition of collagen fibers for normal tissue) and stratum corneum were formed, while focal and disorganized re-epithelialization was demonstrated in B,D and E groups. These results were similar to research which Veleirinbo and et al. had done [38].

The wound healing and wound area reduction on the 14th day showed significant differences between groups and the least wound area were related to A and C groups.

4. CONCLUSIONS

In this study, an electrospun multi-layer mat containing Semellil Melilotus Officinalis extract was fabricated for use as a wound dressing, also to reduce cytotoxicity, genipin as a nontoxic crosslinking agent was used instead of glutaraldehyde. In the following, the extract release profile, in vitro cytotoxicity and wound healing potential in animal models of the dressings were evaluated.

Finally, the results of these study demonstrated that the multi-layer chitosan-based electrospun mat containing Semellil extract and cross linked by genipin, is a proper wound dressing. This kind of dressing can be a good alternative for ANGIPARS™ ointment, due to its better function to wound healing. In addition, using electrospun dressing containing the extract could remove undesirable color and malodor of ANGIPARS™ commercial ointment and it facilitates the local use of the drug favorably.

According to these results, using genipin instead of glutaraldehyde as cross-linking agent is suggested. Furthermore, electrospun mat cross-linked by genipin lonely can be used as a proper wound dressing or can be applied in tissue engineering.

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