Research Article

Preparation and Evaluation of Wound-Healing Emulsions Based on Chlorhexidine, Cetrimide and Polyglycolic Acid Suture Fibres

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ABSTRACT

Wound-healing involves a complex interaction of cells and processes which can be improved using appropriate wound-dressing materials. Antiseptics like chlorhexidine and cetrimide are often used together as good wound-dressing preparation, effective against both aerobic and anaerobic micro-organisms. There is need for more effective wound-healing preparations accompanied with the advantage of minimal scar formation. The aim of this study was to develop and evaluate wound-healing emulsions containing chlorhexidine, cetrimide and polyglycolic acid microfibers. The emulsions were formulated by measuring and mixing fixed portion of chlorhexidine gluconate, cetrimide and polyglycolic acid microfibers with varying proportions of LABRASOL® and paraffin oil. In vitro evaluations such as dilution test, pH, viscosity, post-formulation stability tests and self-emulsification time test were carried out on the emulsions. In vivo wound-healing activities were studied using burn wound model and wound percentage contraction was investigated. The emulsions were mostly oil-in-water type on dilution, the pH was found to be between 3.4 and 3.8, and viscosity was in the range of 4.5 - 10.0 mPa.s whereas self- emulsification time was within range of 6.32 - 17.65 s and emulsions showed good organoleptic properties. The emulsions showed about 95-100% wound size reductions at 21 days of post-wounding application with minimal scar formation. The chlorhexidine/cetrimide/ polyglycolide-loaded emulsion is a novel formulation that can improve wound- healing.

Keywords: Wound-healing, polyglycolic acid suture, emulsions, chlorhexidine gluconate, cetrimide.

INTRODUCTION

Burn wounds are defects in skin and can be due to thermal damage and healing of the damage is a necessary physiological and physico-chemical response. It follows sequential steps of inflammation and tissue repair, which are complex but organized physiological processes of epithelialization, formation of tissues and its remodeling[1].

Wound-healing proceeds through a complex and highly ordered series of overlapping, sequential events that involve numerous cell types, growth factors, cytokines, hormones and enzymes [2,3,4]. Optimal wound-healing requires rapid hemostasis, inflammation, mesenchymal cell differentiation, proliferation and migration to wound site, suitable angiogenesis, re-growth of epithelial tissue over wound surface (re-epithelialization), appropriate synthesis, cross-linking and alignment of collagen to provide strength and integrity to the healing tissue [5,6]. These phases and their biophysiological functions usually occur in the proper sequence, at specific time intervals, and continue for a specific duration at effective and optimal intensities.

Wound care and maintenance involves a number of measures including dressing and administration of painkillers, use of anti-inflammatory agents, topical systemic antimicrobial agents and wound-healing drugs. To facilitate healing, dressings are applied to try to protect the wound from contamination and keep the wound surface moist to maintain the integrity of the cells present in the defect.

An emulsion is defined as a biphasic system consisting of two immiscible liquids, the dispersed phase uniformly dispersed throughout the continuous phase. Emulsions are generally thermodynamically unstable systems thus an emulsifier is required to stabilize them [7]. Emulsifiers stabilize the system by forming a thin film around the globules of the dispersed phase. Oral and topical preparations can be formulated as emulsions. Self-emulsifying emulsions can be applied on wounds to manage the exudates. Self-emulsifying formulations are isotropic mixtures of oil, surfactant, co-solvent and solubilized drug [8]. These formulations can rapidly form oil-in-water (o/w) fine emulsions when dispersed in aqueous phase under mild agitation. The oil-in-water emulsified exudates formed upon application of these formulations on wounds would present a moist wound area which is beneficial since wound dressings are expected to maintain a moist environment around the wound and absorb the exudates from the wound surface[9]. The presence of oil provides a form of sustained hydration. Emulsification process may be associated with the ease with which water penetrates the oil-water interface with formation of liquid crystalline phase

resulting in swelling at the interface, thereby causing greater ease of emulsification [10]. At the initial phase of wound healing, there is formation of fibrin clot which can be facilitated by the inclusion of biodegradable polymers (e.g polyglycolic acid) and this may form cell adhesion domains within the wound environment. Cetrimide, in combination with chlorhexidine, is often used for disinfection and dressing of wounds. Polyglycolide/ polyglycolic acid, a biodegradable thermoplastic polymer has also been explored in wound dressing, tissue engineering and controlled drug delivery [11]. The research question arises, "Can we develop a more effective wound healing preparations based on cetrimide/ chlorhexidine/ polyglycolic acid microfibers with minimal scar formation?" There is definitely a need for an effective wound-dressing in the acceleration of wound healing and minimization of scar formation.

The aim of this study was to develop and evaluate burn wound-healing emulsions containing chlorhexidine, cetrimide and polyglycolic acid microfibers for faster healing with minimal scar formation.

MATERIALS AND METHODS

MATERIALS

Chlorhexidine gluconate, cetrimide, polyglycolic acid suture fibre (Fabricado, Mexico), n-propyl alcohol, LABRASOL® (Caprylocaproyl macrogol-8 glycerides) (Gattefosse, France), paraffin oil.

METHODS

Preparation of antiseptic mixture.

A fixed combination antiseptic mixture was prepared containing 0.3%w/w, 3%w/w and 2.84%w/w of chlorhexidine gluconate, cetrimide and n-propyl alcohol, respectively, in aqueous medium. The solution was mixed effectively and a stock antiseptic obtained.

Micronization of polyglycolic acid suture fiber

Polyglycolic acid suture was reduced into bits by cutting with a hand blade and thereafter milled using a mixer with rotating blades. The milling was done with the initially reduced suture suspended in aqueous medium.

Preparation of emulsion

A 5 ml quantity of the antiseptic solution was transferred into a 250 ml beaker followed by addition of 10 ml of paraffin oil and 10 ml of LABRASOL® and made up to 100 ml with distilled water. The mixture was homogenized at 5000 rev/min for 5 min. A 20 mg amount of the polyglycolic acid suture was weighed out and added to the mixture and stirred continuously to ensure uniform dispersion. The resulting emulsion was thereafter transferred to a container and labeled as batch 1. Various batches were produced via the above procedures using varying volumes of antiseptics, paraffin oil and LABRASOL® and a constant weight of polyglycolic acid suture. This is presented in Table 1.

Evaluation of the emulsions

Determination of emulsion type

A 1 ml volume of the emulsion was transferred into a 10 ml measuring cylinder. The emulsion was made up to 10 ml using distilled water. The resulting solution was observed for any separation, creaming, cracking or instability. This procedure was carried out on all the batches at room temperature (25 °C).

Determination of pH of the emulsions

Twenty millilitre volumes of emulsions were transferred into 100 ml beakers. The calibrated pH meter was rinsed in distilled water and the electrode end dipped into the emulsion. The pH was read and recorded for each batch of emulsion. The pH of the formulation gives an indication of the level of H^+ or OH^- ions present and this may affect the healing process.

Table 1: Formula for the emulsions

Batch	Composition in 100 ml (ml)			
	Antiseptic solution (chlorhexidine gluconate, 0.015g, cetrimide, 0.15g, and n-propyl alcohol, 0.142g per 5ml stock)	Paraffin oil (ml)	LABRASOL® (ml)	Polyglycolic acid suture fibres (mg)
1	5	10	10	20
2	10	10	10	20
3	15	10	10	20
4	5	10	15	20
5	5	20	10	20
6	5	20	15	20
7	5	30	10	20
8	5	30	15	20
9	5	15	15	20
10	5	25	10	20
11	5	25	15	20

Determination of viscosity

The viscosity of each batch of emulsion was determined using a Rotational viscometer with Roto number 1 and speed of 60 rev/min and the various viscosities were recorded in milli Pascal seconds,mPa.s.

Centrifugation test

A 3 ml volume of each batch of emulsion was collected and transferred respectively into small plastic bottles and centrifuged at 4000 rpm for 5 min. The samples were observed for phase separation.

Self-emulsification time

The magnetic stirrer beaker assembly was used to assess self-emulsification behavior of the batches. A 1ml volume of each batch of emulsion was introduced into a beaker

containing 250 ml of distilled water at 37±1 °C under mild stirring. The time for the formulation to form a homogenous mix upon dilution was taken as the self emulsification time.

***In vivo* study**

Evaluation of wound-healing activities using burn wound model.

The wound-healing activities of the formulations were studied using burn wound model [12]. The rats were anaesthetized prior to creation of the wounds, with 1 ml of intravenous Ketamine hydrochloride (10 mg/kg body weight). The dorsal fur of the animals was shaved with an electric clipper and the area of the wound to be created was outlined. Burn wounds were induced by applying a heated metal plate on the dorsal area of the animals for 30 s. The animals were divided into five groups of 5 animals each. The Group 1 animals were treated with a formulation containing paraffin oil, LABRASOL® (1:1 volume ratio) and distilled water, and considered as a control without antiseptic component. Group 2 animals were treated with emulsion preparation (Batch 1) containing 5 ml of antiseptic solution, 10 ml of LABRASOL®, 10 ml of paraffin oil and 20 mg of polyglycolic acid suture fibre while animals of Group 3 were treated with emulsions (Batch 11) consisting of antiseptic solution (5 ml), LABRASOL® (15 ml), paraffin oil (25 ml) and polyglycolic acid suture (20 mg). Animals from group 4 and group 5 were treated with DERMAZINE® cream (reference standard) and normal saline respectively for control. The topical treatment began immediately after wound creation by applying 1 ml of the emulsion to the wound site, and this continued every 48 h for 3 weeks. The measurements of the wound areas were taken on 3rd, 6th, 9th, 12th, 18th and 21st day following the initial wound using a meter rule and a pair of divider. The relative wound size reduction was calculated using Equations 1 and 2

$$Percentage\ wound\ contraction\ (\%) = \frac{healed\ wound\ size}{total\ wound}\ x\ 100 \text{ ----------1}$$

$$Relative\ wound\ size\ reduction(\ \%) = \frac{Ao - At}{Ao}\ x\ 100 \text{ --2}$$

Where A_o and A_t are the wound size at initial time and time "t", respectively

RESULTS AND DISCUSSION

Evaluation of the emulsions

Emulsion type

The type of emulsion was determined using dilution method. Emulsions are broadly divided into two types, oil-in-water (O/W) and water-in-oil (W/O) emulsions. Among the identification tests for emulsions, dilution method is mostly used [13]. On dilution, the resulting solution was found to be homogenous, some formed homogenous mixture spontaneously while others creamed at first. The resulting solutions were cloudy. Table 2 below gives a detailed description of the behavior of the emulsions on dilution. Formation of a homogeneous mixture on addition of water means it is an oil-in-water type of emulsion.

Acidity of the emulsions

The pH of the formulations was in the range of 3.4 and 3.8. The pH within a wound plays an important role in wound healing as infection control, antimicrobial activity, oxygen release, angiogenesis, protease activity and bacterial toxicity is improved. Higher alkaline pH in both acute and chronic wounds contributes to low healing rate while higher healing rate is the case for wounds with a neutral or acidic pH. When wound begins to heal, pH moves from the alkaline to neutral or acidic. Acetic acid, boric acid, ascorbic acid, alginic acid, and hyaluronic acid create this acidic environment which is required to facilitate wound healing [14,15]. According to the results of the experiment, the formulations have pH values of 3.4-3.8 which is in the acidic range and this may be beneficial in wound healing. Moreover, the acidic nature of the emulsion would accelerate the biodegradation of the polyglycolic acid micro sutures and improve the degradative effect of esterase enzymes *in situ* in the wound site.

Viscosity of emulsion

The viscosity of the emulsion was found to be in the range of 4.5 mPa.s - 10 mPa.s (Table 3). The maximum viscosity of 10 mPa.s was seen in batch 8 while the minimum viscosity of 4.5 mPa.s was observed in batches 2 and 3. The increase in viscosity of the batches corresponded with increases in volume of paraffin oil. Rheological properties of emulsions is controlled by the volume concentration of the dispersed phase, particle sizes of the disperse phase, viscosity of the continuous phase, nature and concentration of the emulsifying system [16]. Paraffin oil is the dispersed phase in this study, it is seen that emulsions with higher proportion of paraffin oil had higher viscosities. The volumes of the paraffin oil and viscosities of the formulations affect the self-emulsification times of the test formulations.

Table 2: Dilution behavior of the emulsions

Batch	Observation	Emulsion Type
1	Homogenous	o/w
2	Homogenous	o/w
3	Homogenous	o/w
4	Creamed at the bottom	w/o
5	Did not mix spontaneously,	w/o
6	Mixed slowly	o/w
7	Homogenous	o/w
8	Mixed slowly and homogenous	o/w
9	Homogenous	o/w
10	Homogenous	o/w
11	Homogenous	o/w

Table 3: Viscosity data of the formulations

Batch number	Vol. of paraffin oil in 100 ml of emulsion	Viscosity emulsion (mPa.s)
1	10	4.9
2	10	4.5
3	10	4.5
4	10	5.7
5	20	4.9
6	20	6.5
7	30	8.3
8	30	10.0
9	15	5.7
10	25	5.5
11	25	8.7

Effect of centrifugation

The emulsions separated into 3 distinct layers when centrifuged. The stability of an emulsion can be assessed in terms of the physical outlook of the emulsion, the physical and chemical stability of the emulsion components [17,16]. Although the emulsions separated on centrifugation (external stress), they were redispersed spontaneously on mild shaking. Originally, the disperse oil globules are kept suspended and dispersed within the emulsion by the stabilizing effect of the surface-active agent (Labrasol®), moreover, the kinetic energy of the continuous phase maintains this suspension against the sedimentation force of gravity. However, increasing this force by application of high centrifugal forces may have suppressed the Brownian motion of continuous phase molecules and pushed the disperse phase to the bottom or top of the container depending on relative densities.

Self-emulsification time

The self-emulsification time of the emulsions was in the range of 6.32 - 17.65 s (Table 4). Self-emulsification time is an important index for the assessment of the efficiency of emulsion formulation. Emulsions that emulsifies completely in less than 1 min are known to have increased stability. Emulsions should disperse completely and rapidly when subjected to aqueous dilution under mild agitation [18,19]. In this present study, all formulations emulsified in less than 20 s. Batch 10 (5ml antiseptic solution, 25 ml paraffin oil, 15 ml LABRASOL®, 20 mg polyglycolic acid suture) formed a dilute homogenous emulsion in a short time (6.32 s) compared to other formulations, thus it can be inferred that it has the highest self-emulsification capacity. The self emulsification capacity is an important factor in the process of handling the exudates coming out of the wounds. However, it is important to note that the study was done using 250 ml of distilled water whereas in the wound environment, the exudates come out in small 'pumps' at a time. Furthermore, the study involved distilled water whereas the actual wound would contain macromolecules, chemical mediators, factors and other substances some of which can be distributed within the continuous phase, disperse phase or align at the interfacial area with the surface-active agents. These bio-interfacial activities play crucial role in the overall wound-healing process. If the biological substances adsorbing at the oil-water interface significantly affect, negatively, the functionality of the LABRASOL® surface-active agent, then *in situ* emulsification of exudates may occur at a slower rate. Microemulsion properties such as phase behavior and stability are greatly influenced by the properties of the interfacial films such as interfacial tension, spontaneous curvature (H_o) and film rigidity. Since the oil-water interfacial tension (Υ_{ow}) is the work required to increase the area of an interface by unit amount, then the formation of microemulsions requires Υ_{ow} to be significantly low [20]. Any factor or entity around the wound area that increases the interfacial tension would reduce emulsification and vice versa.

Table 4: Self-emulsification time of formulations

Batch Number	Self-emulsification Time (s)
1	8.15
2	6.80
3	7.81
4	12.16
5	12.37
6	17.65
7	14.87
8	15.34
9	11.26
10	6.32
11	11.39

Percentage (%) wound size reduction

At 3 days post-application of the emulsions, the DERMAZINE® cream showed the greatest wound size reduction compared with others (Table 5). After 12 days, the wound size reduction was significantly increased in batch 11 (selected) of the formulation in the order of batch 11 emulsion > DERMAZINE® cream > Normal saline > formulation of water, LABRASOL® and paraffin oil alone > batch 1 emulsion. Generally the emulsions showed about 95-100 % wound size reduction at 21 days of post-operation. The results suggested that the emulsions with antiseptic agents significantly improved wound-healing compared with the blank control. The antiseptic/ polyglycolic acid based emulsion provided enhanced antimicrobial activities, acidic environment and biodegradable, 'contained' micro-environment required for wound-healing. Figure 1 captures changes in wound gross morphology during the healing process through 21 days study.

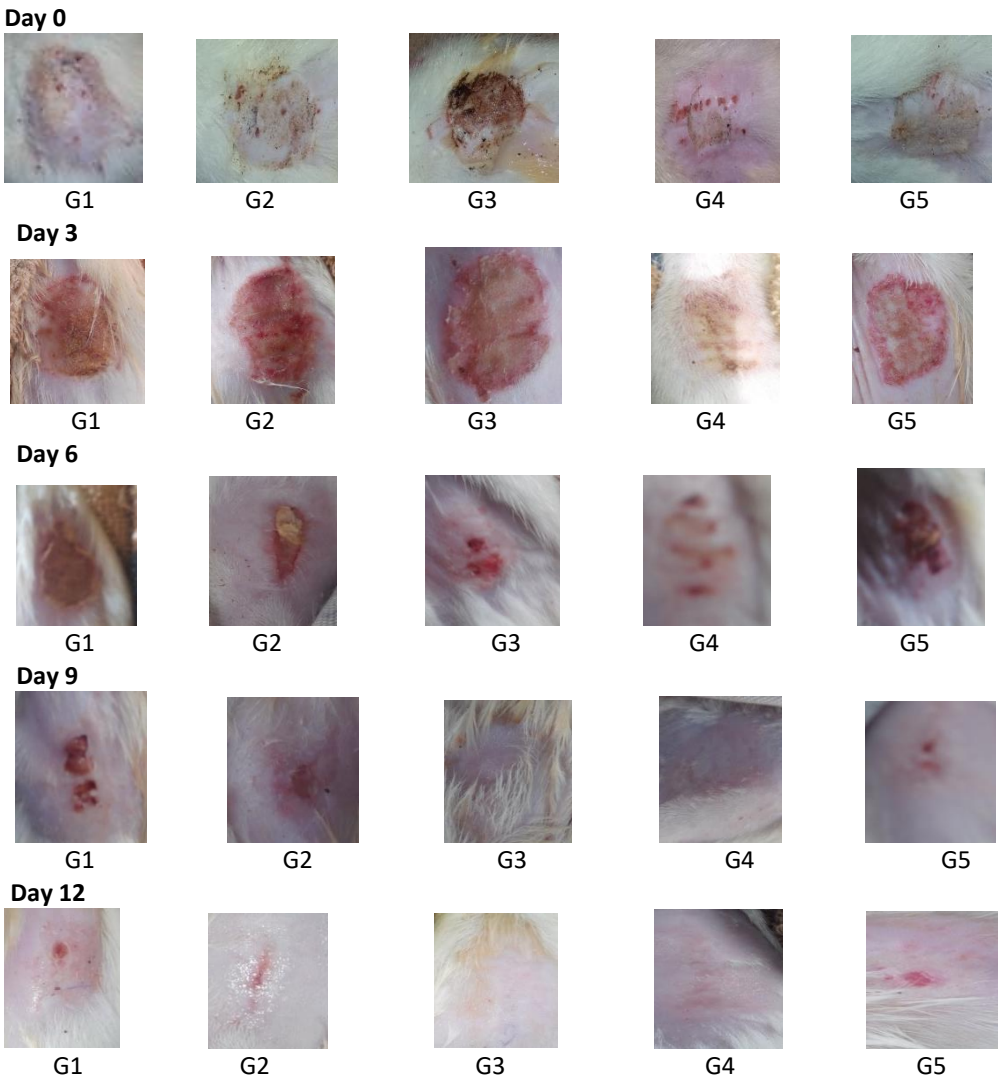
After skin is injured, micro-organisms that are normally sequestered at the skin surface obtain access to the underlying tissues. The unrestricted microbial access may lead to contamination, colonization or invasive infection of underlying tissue. Inflammation is a crucial part of the wound-healing process, and is important to the removal of contaminating micro-organisms. In the absence of effective anti-infective mechanisms, however, inflammation may be prolonged, since microbial clearance would be incomplete. Both bacteria and endotoxins can lead to the prolonged elevation of pro-inflammatory cytokines such as interleukin-1 (IL-1) and TNF-α and prolong the inflammatory phase. If this continues, the wound may enter a chronic state with retarded or failed healing. The presence of antimicrobial agents in some of the emulsions may have reduced that period of inflammation, thereby facilitating completion of healing. Apart from the presence of anti-microbial agents, the inclusion of suture microfibers may provide domains for cell adhesion including biofilms which usually contain

replicating microorganisms (eg. *Staphylococcus aureus*, *Pseudomonas auruginosa*, β-hemolytic streptococci) and degradation of the microfibers can open up any adhering biofilm, thereby exposing the microorganisms inside to the harsh activity of the antimicrobial agent.

Table 5: Percentage (%) wound size reduction of rats on days 3, 6, 9, 12, 18 and 21 post- wounding.

Percentage wound size reduction (%)					
Days	**Grp 1**	**Grp 2**	**Grp 3**	**Grp 4**	**Grp 5**
3	33	28	26	39	33
6	34	25	39	47	39
9	62	43	73	79	77
12	78	55	95	89	81
18	91.5	83	100	100	92
21	100	95	100	100	95

Grp 1- animals treated with a formulation containing water, Labrasol® and paraffin oil, **Grp 2**- animals treated with emulsion preparation containing 5 ml of antiseptic solution, 10 ml of Labrasol, 10 ml of paraffin oil and 20 mg of polyglycolic acid suture fibres, **Grp 3** – animal treated with emulsions containing antiseptic solution (5 ml), Labrasol® (15 ml), paraffin oil (25 ml) and polyglycolic acid suture (20 mg), **Grp 4** – animals treated with Dermazine® cream (reference standard), **Grp 5** – animals treated with normal saline



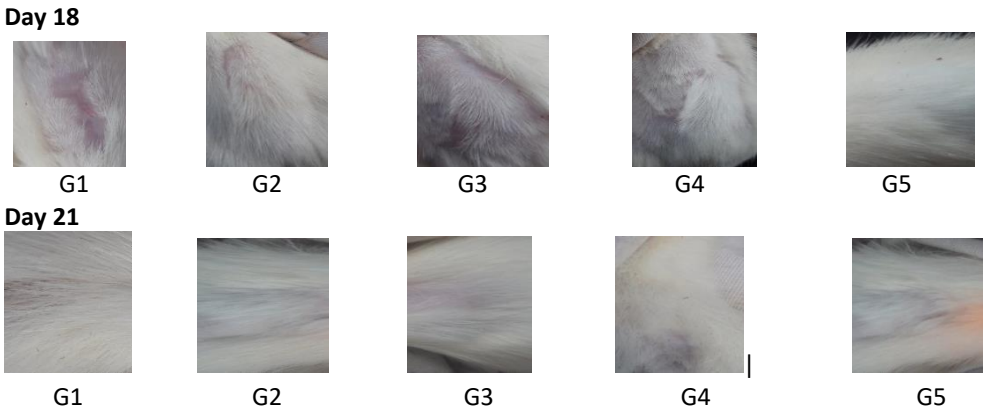


Figure 1: Representative gross morphological results at days 0, 3, 6, 9, 12, 18 and 21 post-wounding using digital camera. G1-G5 represents Groups 1-5

CONCLUSION

In conclusion, the antiseptic wound-healing emulsions were stable and within optimum pH for wound-healing. Physicochemical behaviour of the formulations relevant to their effect on wound-healing was studied. The emulsion batch comprising antiseptic solution (5 ml), Labrasol® (15 ml), paraffin oil (25 ml) and polyglycolic acid suture (20 mg) significantly ($p < 0.05$) improved the wound-healing process compared with the negative control. Thus, it is a potential wound-dressing application with excellent physico-chemical qualities and improved healing activities in wound care. There is a possibility of minimal scar formation associated with the preparations after complete healing of the wound. Further studies are required to understand the effects of the emulsions on other forms of wounds and tissues.

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