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ORIGINAL ARTICLE

Fabrication and Evaluation of Transdermal Microneedles for a Recombinant Human Keratinocyte Growth Factor

Rekombinant İnsan Keratinosit Büyüme Faktörü için Transdermal Mikro İğnelerin Üretimi ve Değerlendirilmesi

Melbha Starlin CHELLATHURAI*, Vivien Wang Ting LING, Vijayaraj Kumar PALANIRAJAN

UCSI University, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Kuala Lumpur, Malaysia

ABSTRACT

Objectives: Microneedle transdermal patches are a combination of hypodermic needles and transdermal patches used to overcome the individual limitations of both injections and patches. The objective of this study was to design a minimally invasive, biodegradable polymeric recombinant human keratinocyte growth factor (rHuKGF) microneedle array and evaluate the prepared biodegradable microneedles using *in vitro* techniques.

Materials and Methods: Biodegradable polymeric microneedle arrays were fabricated out of poly lactic-co-glycolic acid (PLGA) using the micromolding technique under aseptic conditions, and the morphology of the microneedles was characterized using light microscopy. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to rule out drug-polymer interactions. Standard procedures were used to analyze the prepared microneedle arrays for *in vitro* drug release and to perform a microneedle insertion test. Enzyme-linked immunosorbent assay was used to quantify rHuKGF.

Results: The PLGA polymer was safe for use in the fabrication of rHuKGF microneedles as there was no interaction between the drug and the polymer. The fabricated rHuKGF microneedle arrays had fully formed microneedles with a height of 600 µm and a base of 300 µm. The drug from the microneedle patch was released *in vitro* within 30 minutes. The strength of the microneedles in the patch was good, as they were able to reach a depth of 381±3.56 µm into parafilm without any structural change or fracture.

Conclusion: Microneedle transdermal patches were successfully prepared for rHuKGF, and their evaluation suggested excellent quality and uniformity of patch characteristics. This can have potential applications in the therapeutic arena, offering advantages in terms of reduced dosing frequency, improved patient compliance, and bioavailability.

Key words: Transdermal drug delivery, microneedles, recombinant human keratinocyte growth factor, micromoulding, poly-lactide-co-glycolide

ÖZ

Amaç: Mikro iğne transdermal yamalar, hem enjeksiyonların hem de yamaların bireysel sınırlamalarının üstesinden gelmek için kullanılan hipodermik iğneler ile transdermal yamaların bir kombinasyonudur. Bu çalışmanın amacı, minimal invaziv, biyobozunur polimerik rekombinant insan keratinosit büyüme faktörü (rHuKGF) mikro iğne dizisini tasarlamak; hazırlanan biyolojik olarak parçalanabilir mikro iğneleri *in vitro* teknikler kullanarak değerlendirmektir.

Gereç ve Yöntemler: Biyolojik olarak parçalanabilen polimerik mikro iğne dizileri, aseptik koşullar altında mikro-kalıplama tekniği kullanılarak poli laktik-ko-glikolik asitten (PLGA) üretildi ve mikro iğnelerin morfolojisi ışık mikroskobu kullanılarak karakterize edildi. İlaç-polimer etkileşimlerini belirlemek için sodyum dodesil sülfat-poliakrilamid jel elektroforezi kullanıldı. Hazırlanan mikro iğne dizilerini analiz etmek; *in vitro* ilaç salımı ve mikro iğne yerleştirme testi gerçekleştirmek için standart prosedürler kullanıldı. RHuKGF'yi miktar tayini için enzime bağlı immünosorbent deneyi kullanıldı.

Bulgular: PLGA polimeri, ilaç ve polimer arasında hiçbir etkileşim olmadığından rHuKGF mikro iğnelerinin imalatında kullanım için güvenlidir. Üretilen rHuKGF mikroiğne dizileri, 600 µm yükseklikte ve 300 µm tabanlı mikroiğnelere sahipti. Mikro iğne yamasından ilaç *in vitro* koşullarda 30 dakika içinde salındı. Herhangi bir yapısal değişiklik veya kırılma olmaksızın 381±3,56 µm'lik bir parafilm derinliğine ulaşabildikleri için yamadaki mikro iğnelerin gücü iyi olarak değerlendirildi.

Sonuç: rHuKGF için mikroiğneli transdermal yamalar, başarıyla hazırlandı ve yamanın mükemmel kalitede ve istenen tekdüzelikte olduğu gösterildi. Hazırlanan mikro iğneli transdermal yamanın, terapötik alanda potansiyel uygulamalara sahip olabileceği ve artmış biyoyararlanım, azaltılmış dozlama sıklığı, iyileştirilmiş hasta uyuncu gibi avantajlar sunabileceği belirlendi.

Anahtar kelimeler: Transdermal ilaç taşıma, mikroiğneler, rekombinant insan keratinosit büyüme faktörü, mikro-kalıplama, poli-laktit-ko-glikolid

***Correspondence:** melbha.starlin@gmail.com, **Phone:** +60122309605 **ORCID-ID:** orcid.org/0000-0002-6496-7080

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INTRODUCTION

Many active drugs cannot be delivered effectively using current drug delivery systems, such as injection and pills.[1] Microneedle transdermal patches consist of a plurality of microprojections, which help to pierce the upper epidermis of the skin far enough to improve the delivery of a broad range of molecules and nanoparticles. Pain-free drug administration is promised, as it is brief enough avoiding the stimulation of nerve fibers.[2] The skin will be restored within one to three days after being treated and no bacterial contamination or long-term irritation occurs. To date, microneedles have been used to deliver drugs of varying molecular weight, bio-therapeutics, vaccines, small molecules, and proteins.[3] They are also used in cosmetology as rollers and pens to facilitate transdermal delivery of peptides and proteins.

Compared with solid microneedles made of silicon or metal, polymeric microneedles have attracted extensive attention because of their excellent biocompatibility, biodegradability, and non-toxic properties.[4] Polymeric microneedles will not leave any sharp biohazard medical waste after use. Polymers with different degradation profiles and swelling properties allow microneedles to be fabricated with different mechanical properties and functions.[5] Poly lactic-co-glycolic acid (PLGA) is one of the most favored Food and Drug Administration (FDA)-approved polymers used in designing biodegradable polymeric microneedles.[6]

Mucositis is one of the main oncological problems caused by high-dose cytotoxic cancer chemotherapy or radiotherapy in patients with hematological malignancies. It is defined as inflammatory or ulcerative lesions on the mucous membranes lining the entire gastrointestinal tract from the mouth to the anus.[7] According to the clinical practice guidelines developed by the Mucositis Study Group of the Multinational Association for Supportive Care in Cancer and the International Society of Oral Oncology, recombinant human keratinocyte growth factor (rHuKGF) is recommended for the prevention and treatment of oral mucositis.[8] The United States FDA also approved the utilization of rHuKGF to treat oral mucositis in patients with hematologic malignancies who are receiving myeloablative radio-chemotherapy with autologous hematopoietic stem cell support.[9]

Endogenous KGF is a 28 kDa protein produced naturally in the body by dermal fibroblasts within the skin, lamina propria cells of the intestines and, most importantly, mesenchymal cells. Its epithelial cell proliferative properties help to maintain epithelial integrity.[10] The specificity of KGF for epithelial cells is due to its exclusive action on KGF receptors, which are present on epithelial cells and absent on cells of hematopoietic origin.[11,12] rHuKGF is a recombinant N-terminal truncated form of human KGF prepared from *Escherichia coli* using recombinant DNA technology. The molecular weight of rHuKGF is only 16.2 kDa, which is smaller than that of endogenous KGF due to the removal of the first 23 N-terminal amino acids with an elimination half-life ($t_{1/2}$) of 4.5 hours.[13] It has similar biological activity to the native protein but higher stability.[14]

Currently, 60 µg/kg/day of rHuKGF is administered intravenously daily for 3 consecutive days before and 3 consecutive days after the patient receives chemotherapy. The drug is commonly dosed in a hospital setting, which means that patients must be hospitalized for a week in order to receive the injection and chemotherapy treatment.[15-17] Because of the inconveniences, pain, and economic burden due to hospital charges, patient compliance will be highly affected, requiring an alternative route of administration. In our previous research, we developed chitosan nanoparticles and β-cyclodextrin-based delivery systems to deliver rHuKGF.[9,10,13] Stability issues due to their complex nature makes proteins difficult drug candidates for transdermal delivery. Therefore, an alternative route, such as transdermal microneedles, is designed to solve the limitations of the current parenteral route.

MATERIALS AND METHODS

Materials

rHuKGF (Sigma-Aldrich, USA), chlorotrimethylsilane, poly (D, L-lactide-co-glycolic acid) in a 75:25 ratio, and polyethylene glycol 400 were purchased from Sigma-Aldrich (M) Sdn. Bhd., Malaysia. A polydimethylsiloxane (PDMS) microneedle mold with 11x11 arrays (Blueacre Technology, Ireland), polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP) [Chemiz (M) Sdn. Bhd., Malaysia], and an ELISA kit ab183362-KGF-FGF-7 (Abcam, USA) were used. Animal studies were not conducted for the prepared microneedles.

Pre-formulation studies

Drug-polymer interaction studies using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

A Bio-Rad brand SDS-PAGE electrophoresis system was used. Denaturing SDS-PAGE was performed by mixing 10 µL of diluted rHuKGF sample (20 ng of protein) with 10 µL of sample loading buffer and heated in boiling water for 5 to 10 minutes. Samples and Thermo Scientific™ Spectra™ Multicolor Broad Range Protein Ladder were then loaded into precast 12% Tris-Glycine 1.0 mm minigels according to Figure 1. Electrophoresis was then performed at room temperature for approximately 45 minutes with a constant voltage of 120 V in running buffer until the dye front reached the end of the 60 mm gel. Subsequently, the gel was removed and washed three times, 5 minutes each, in ultra-pure water. The gel was then stained using Bio-Safe™ Coomassie Stain for an hour.[18] The de-staining process was completed by agitating the gel in 50 mL of distilled water for a minimum of 30 minutes. Lastly, the gel was imaged using a GS800 calibrated densitometer.[19]

Preparation of the backing membrane

The film casting method was used to fabricate films, and Table 1 shows the formula and composition for the different types of formulated patches.[20] Petri dishes were first treated with 0.2 mL of physically mixed liquid paraffin:dichloromethane (1:10) mixture to facilitate the removal of films from the dishes. Polymer solutions were prepared in distilled water at a concentration of 10% by dissolving dried powder samples of

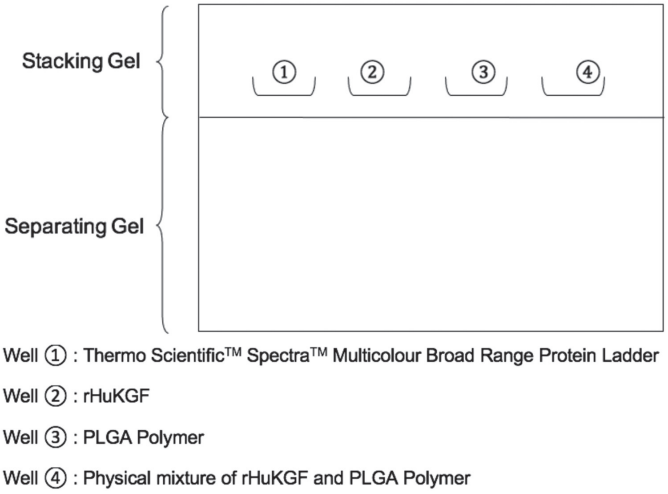

Figure 1. Loading arrangement of marker protein ladder and samples in vertical SDS-PAGE system
rHuKGF: Recombinant human keratinocyte growth factor, PLGA: Poly lactic-co-glycolic acid, SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

Table 1. Composition of backing membrane formulations

Formulation code	Ratio of polymer (10% w/v)	Plasticizer (% w/w of total polymer)	Solvent/patch (mL)
	PVA:PVP	PEG 400	Ultra-pure water
A1	4:6	0	150
A2	1:1	0	150
A3	6:4	0	150
B1	4:6	20	150
B2	4:6	25	150
B3	4:6	30	150

PVA: Polyvinyl alcohol, PVP: Polyvinyl pyrrolidone

PVA and PVP at 100˚ and at room temperature, respectively, followed by vigorous stirring until a clear gel was formed.[21] Varying proportions of PVP and PVA polymer solutions and different percentages of plasticizer were then mixed well to form a final volume of 150 mL. Bubbles were removed by centrifugation. The resultant solutions were poured onto the petri dishes and dried under ambient conditions for 24 hours in a Gelman Sciences microessentials class100 laminar flow work station. The dried cast films were then detached from the petri dish and wrapped in aluminum foil for further use. Evaluation tests were carried out on the next day, and the formulation with the best characteristics was chosen to be used in fabricating microneedle arrays.

Evaluation of the backing membrane

The commercial product Kefentech (ketoprofen plasters) was chosen as the reference.

Physical appearance

All the prepared patches were inspected visually for color and smoothness.

Thickness of the film

The thickness of the films was measured using an electronic digital micrometer screw gauge at three different places.[22] Average and standard deviation (SD) values of the three readings were calculated for each prepared film.

Tensile strength of the film and percentage elongation break test

Three pieces of film strips (4 cm x 2 cm) from each formulation were cut evenly, and the tensile strength and percentage elongation at break were evaluated using a tensiometer. The tensiometer consists of two load cell grips, a lower fixed one and an upper movable one. A film strip was fixed between these cell grips, and the force was gradually applied until the film broke.[23] The tensile strength and percentage elongation at break were read directly from the dial reading.

Preparation of medicated polymeric microneedle arrays

First, the PDMS mold was treated with 0.15 mL of chlorotrimethylsilane and air dried to facilitate the release of replicated PLGA microneedle arrays from the PDMS mold. Different formulations with different concentrations of PLGA polymer solution, as shown in Table 2, were used to fabricate the microneedles.

PLGA was weighed accurately and dissolved fully in acetone. The volume was made up to 1 mL and the solution passed through a 0.22-µm membrane filter to remove contaminants. Subsequently, 1 µL of rHuKGF (5 µg/mL) was pipetted and incorporated into the molten polymer solution. A 300-µL amount of the drug-polymer mixture was then cast into the PDMS microneedle mold using a micropipette. The filled PDMS mold was placed gently in the centrifuge and centrifuged at 2000 rpm for 20 minutes to entrap the drug-polymer mixture into the microneedle array cavity in the PDMS mold. The temperature was fixed at 37˚ during the molding process. The microneedles were then dried for 24 hours under ambient conditions in a controlled air environment in a class 100 laminar flow work station.

After drying of the microneedles, a polymer blend (PVP:PVA) devoid of the drug was cast onto the mold. The best formulation of the polymer blend (PVP:PVA in a 4:6 ratio with 30% plasticizer) that we determined from the previous backing membrane evaluation was used. The polymer solution was poured onto the mold, and the whole device was air-dried at room temperature in a laminar flow hood or freeze-dried overnight. After drying, the replicate PLGA microneedles connected to the polymer blend were released from the PDMS mold. Optical images of the microneedle arrays were obtained using a light microscope.[24,25]

Table 2. Formulation of medicated polymeric microneedles

Formulation code	Polymer (% w/v)	Solvent (mL)
	PLGA (75:25)	Acetone
M1	7	1
M2	9	1
M3	11	1

PLGA: Poly lactic-co-glycolic acid

Evaluation of polymeric microneedles

Morphological characterization of polymeric microneedle arrays

A Zeiss Axio Vert. A1 inverted microscope (Carl Zeiss, Germany) equipped with an HBO 50W mercury vapor lamp and exciter/emitter filter combinations was used for physical characterization of polymeric microneedles. Zen 2012 software (Blue edition) was used for image processing and analysis. Different image sizes were captured and visualized at 5x, 10x, and 20x magnifications with different viewing angles. Observations were carried out from three sides: (A) Top view of the microneedle patch (B) cross section view of the microneedle patch and (C) 70° view of the microneedle patch.[26]

Microneedle insertion test using light microscopy

An eight-layer folded parafilm sheet was used. The thickness of the parafilm sheet was measured at three different places using an electronic digital micrometer screw gauge. The microneedle array was first inserted with 20 N of force into the parafilm sheet for 30 seconds and removed. The parafilm sheets were then unfolded, and the layers containing holes were counted using a light microscope.[27]

In vitro release test in saline

Drug release from microneedles loaded with rHuKGF was determined by using a modified dissolution method. The microneedle array was first pressed against a layer of parafilm in order to expose only the needles. After insertion, the full penetration of microneedles was confirmed by observation under the light microscope. The parafilm with microneedles was then attached to the bottom of a hollow glass tube in which the backing film faced the inside of the tube while the microneedles tips were exposed to the outside. The bottom of the hollow glass tube was immersed in freshly prepared 150 mL pH 6.2 phosphate buffered saline (PBS). The PBS solution was magnetically stirred at 30 rpm and maintained at 37° throughout the test period. Periodically, a 100 µL aliquot of PBS was sampled and immediately replaced with fresh PBS. The concentration of the drug was analyzed using an ELISA kit, and the results were compared with the calibration curve of rHuKGF.

Statistical analysis

The results were expressed as mean ± standard deviation with n=3. Simple regression analysis[26] was performed using GraphPad Prism 7 software.

RESULTS AND DISCUSSION

Drug-polymer interaction studies using SDS-PAGE

Drug-polymer compatibility evaluation was performed using SDS-PAGE for the drug (rHuKGF), PLGA polymer, and their physical mixture (1:1) separately. The results clearly indicate the absence of any chemical interaction between the drug and polymer and thus confirm that rHuKGF is compatible with the PLGA polymer and could be used for the preparation of an rHuKGF-incorporated microneedle transdermal patch. The rHuKGF used in this study was manufactured by Sigma-Aldrich, United States and had a molecular weight of 18.9 kDa which can be proved by Figure 2 as the gel band of rHuKGF located in between the gel band representing 15 kDa and 25 kDa. Figure 2 (A) shows the gel band of the Spectra Multicolor Broad Range Protein Ladder corresponding to its representative molecular weight. The gel band for the PLGA polymer is not prominent, due to the specificity of Coomassie stain, which can only be used to stain and visualize proteins. From the resulting bands, no interaction was observed between the PLGA polymer and rHuKGF, as the band for the drug-polymer physical mixture was located at the same level as that of the drug alone. This means that the gel band shown for the mixture was actually the gel band of rHuKGF, evidencing that the drug had not undergone any structural modification or structural change due to chemical interaction.

Preparation and characterization of the backing membrane

Both PVA and PVP are hydrophilic polymers, and it was found that the thickness of the film increased when the concentration of PVA was increased. Among formulations A1-A3, the thickness of the fabricated films varied from 0.236±0.004 mm to 0.335±0.005 mm as shown in Table 3.

Non-plasticized patches were physically clean, transparent, and had a smooth surface. However, the patches were very fragile, and thus addition of plasticizer was necessary to improve the mechanical properties of the placebo patches.

Formulation A1 with the thinnest film containing a PVA:PVP ratio of 4:6 was chosen as the control formulation. Further

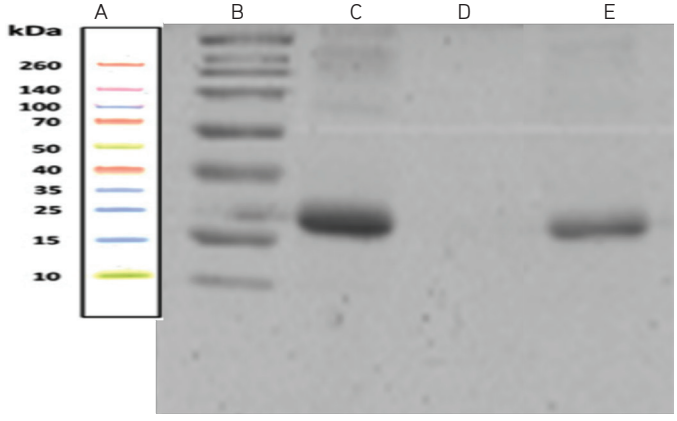

Figure 2. SDS-PAGE gel band of (A) spectra multicolor broad range protein ladder with its molecular weight; (B) spectra broad range protein ladder; (C) rHuKGF; (D) PLGA polymer; and (E) the drug-polymer mixture

SDS-PAGE: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, rHuKGF: Recombinant human keratinocyte growth factor, PLGA: Poly lactic-co-glycolic acid

Table 3. Thickness of backing membrane without plasticizer

Formulation code	Ratio of polymer (10% w/v)	Mean thickness (mm)
	PVA:PVP	
A 1	4:6	0.236±0.0044
A 2	1:1	0.320±0.0131
A 3	6:4	0.335±0.0045

PVA: Polyvinyl alcohol, PVP: Polyvinyl pyrrolidone

fabrication of films (formulation B1-B3) with a fixed PVA:PVP polymer ratio of 4:6 but different concentration of plasticizer (20%, 25%, and 30% w/w of total polymer) was carried out, and the films were evaluated for physical appearance, thickness of the film, tensile strength, and percentage elongation break of the film.

The results for mechanical properties of the films, including tensile strength and percentage elongation at break, are shown in Table 4. The commercial product Kefentech, chosen as the reference, had a tensile strength of 51.448±8.095 mPa and 266.1% ±7.411% elongation at break. As shown in Table 4, film fabricated from Formulation B3, which contained 30% w/w plasticizer, had the best characteristics as it was the thinnest and had better tensile strength and elongation properties. It had a thickness of 0.117±0.004 mm, tensile strength of 61.362±3.376 mPa, and 288.500% ±11.653% elongation at break. Moreover, the results showed that the patches were of uniform thickness as evidenced by the SD value, which was less than 0.01 mm. Therefore, formulation B3 was chosen as the model backing membrane for further fabrication of microneedle transdermal patches.

Upon addition of plasticizer, the flexibility of polymer macromolecules or macromolecular segments increases as a result of loosening of tightened intermolecular forces.[28] This study indicated that lower concentrations of plasticizer were found to give rigid and brittle patches, whereas higher concentrations gave soft patches. Plasticizer at a concentration of 30% w/v was found to give good flexible patches and was easily removed from the glass surface without any brittle fracture. All the films formed were transparent, flexible, non-sticky, and had a smooth surface. This ensures that the films will maintain a smooth and uniform surface when applied to the skin.

The tensile strength and percentage elongation value of the fabricated formulations depicts that the flexibility increased as the concentration of plasticizer was increased. Sufficient mechanical strength and elongation properties of the backing membrane are important to ease the removal of the microneedle transdermal patch from the skin and to help avoiding tearing of the film during removal.

Preparation and evaluation of medicated microneedle patches

PDMS is commonly used to prepare micro-mold micro-devices because it is chemically inert, non-hygroscopic, thermally stable, and mechanically durable.[29] In this study, PDMS micro-mold was used to fabricate microneedles using the micromolding technique. Preparation of the polymer solution is an important step in the fabrication of microneedles. Each batch of microneedle patches was fabricated using a fresh drug-loaded polymer solution and was stirred well to obtain uniform dispersion of the drug in the solution. However, many bubbles were produced in the long process of stirring and might adversely affect the casting process and microneedle shape. The presence of bubbles also might decrease the mechanical strength of the microneedles, which could be a limitation in some situations. Therefore, an optimal centrifugal force and duration of centrifugation were investigated and were fixed at 2000 rpm and 20 minutes, respectively. Bubbles were successfully removed after centrifugation.

From the results shown in Figure 3, it could be concluded that Formulation M2 with a concentration of 9% w/v PLGA polymer solution was the most suitable for the fabrication of complete and fully formed rHuKGF polymeric microneedles.

The morphology of microneedles was determined at 4x and 10x magnification as shown in Figure 4. Figure 4 (D) shows that each needle was 600 µm in height and 300 µm in base width and was arranged in an 11x11 array with 600 µm tip-to-tip spacing. Microneedles of this size can penetrate the outer skin barrier and deliver drugs to the epidermis and superficial dermis, where drugs can diffuse rapidly for local delivery to the skin or systemic distribution via uptake by dermal capillaries.

Incomplete needle formation by 7% w/v and 11% w/v PLGA polymer solutions could be due to the viscosities of the respective solutions. A solution with low viscosity might be easily spun-off from the opening of the cavity of the micro-mold instead of filling the holes of the micro-mold, while high viscosity might result in difficulty filling in the microneedle mold cavity.

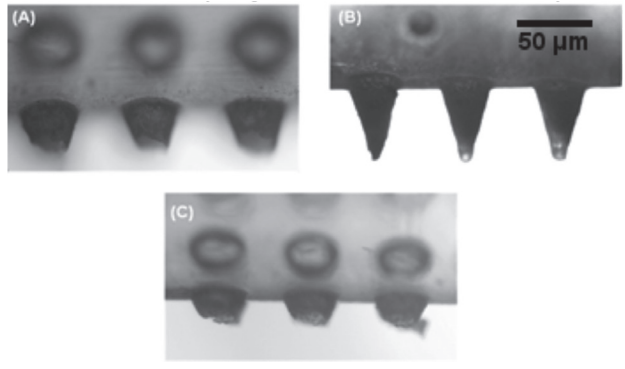

Figure 3. Cross-sectional light microscopy images of a polymeric microneedle array made from (A) 7% w/v PLGA polymer solution; (B) 9% w/v PLGA polymer solution; and (C) 11% w/v PLGA polymer solution
PLGA: Poly lactic-co-glycolic acid

Table 4. Physical characteristics of films fabricated with formulations B1 to B3

Formulation code	Ratio of polymer (10% w/v)	Plasticizer (% w/w of total polymer)	Thickness (mm)	Tensile strength (mPa)	% elongation
	PVA:PVP	PEG 400			
B1	4:6	20	0.140±0.0030	24.800±6.679	244.967±21.170
B2	4:6	25	0.127±0.0015	43.356±6.092	284.767±12.586
B3	4:6	30	0.117±0.0036	61.362±3.376	288.500±11.653

PVA: Polyvinyl alcohol, PVP: Polyvinyl pyrrolidone

Microneedle insertion test using light microscopy

Microneedles should have sufficient mechanical strength to be inserted successfully into the skin without failure during insertion. Results of research carried out by Larrañeta et al.[27] proved that although parafilm presents slightly lower penetration depths than porcine skin, it could still be a promising material to replace biological tissue for insertion studies. The average thickness of a parafilm layer is 127±3.560 µm. The third layer of the parafilm sheet can be reached as shown in Figure 5, but holes did not form in the third layer, which means that the microneedles can correspondingly reach insertion depths between 254±3.560 µm and 381±3.560 µm.

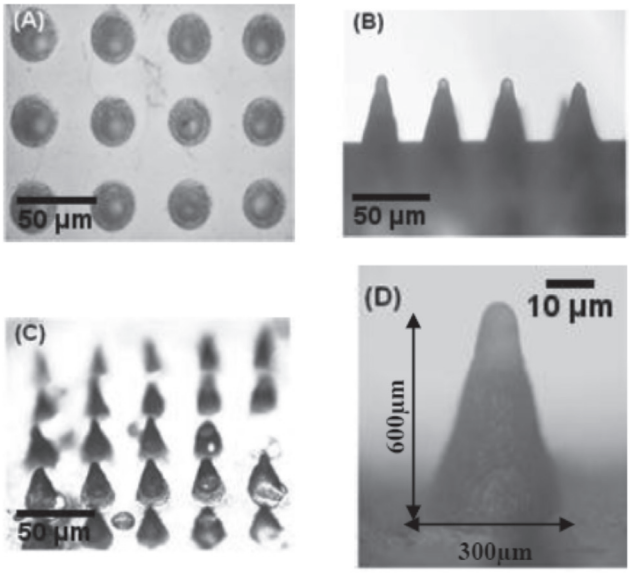

Figure 4. Light microscope images of a section of an 11x11 polymeric microneedle array fabricated using formulation M2: (A) top view at 4x magnification; (B) cross-sectional view at 4x magnification; (C) 70° view at 4x magnification; and (D) cross-sectional view at 10x magnification

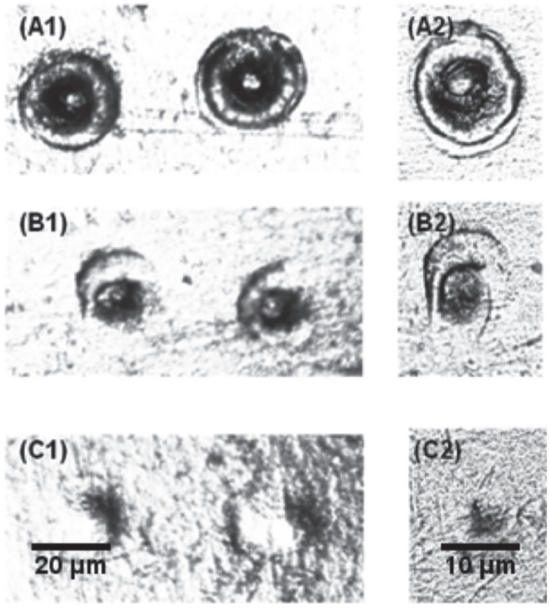

Figure 5. Microscopic observations of holes left on the different layers of a parafilm sheet (A) first layer; (B) second layer; and (C) third layer at (1) 10x magnification and (2) 20x magnification

The average thickness of the stratum corneum and epidermis is between 0.01 and 0.02 mm and 0.1 mm, respectively. Therefore, the results proved that rHuKGF microneedles can successfully overcome the barrier of the stratum corneum and could reach the dermis layer of the skin for drug release. Blood vessels are mostly on the lower part of the dermis and would not be punctured as the needles penetrate only the upper layers of the skin. Besides, the mechanical strength of microneedles was sufficient as it did not undergo structural change or fracture inside the parafilm sheet after insertion test. Thus, the safety of rHuKGF polymeric microneedles is ensured.

In vitro release test

Microneedles loaded with rHuKGF released their contents efficiently within 30 minutes upon incubation in 150 mL PBS solution with pH 6.2. Figure 6 shows that 96.67% of rHuKGF can be successfully released within 15 minutes, and 100% drug release was obtained within 30 minutes. This rapid release might be due to the burst effect of PVP and the solubility of the polymer in the solution. Controlled release was not achieved during the release study, which might have been due to the fabrication methodology.

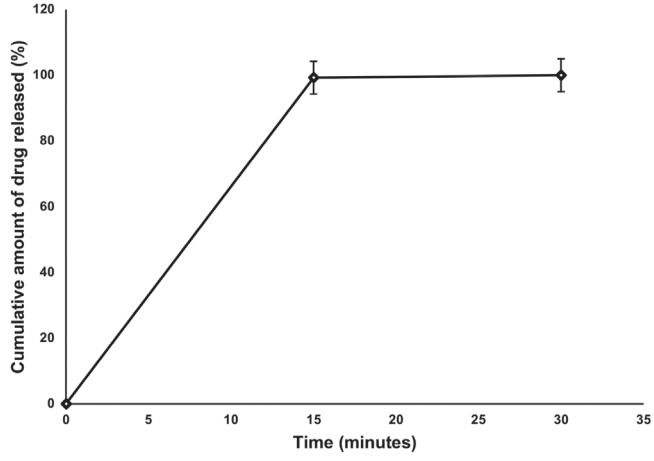

Figure 6. Graph of the cumulative release of rHuKGF into phosphate buffered saline solution over time
rHuKGF: Recombinant human keratinocyte growth factor

As in research performed by Park et al.[30] additional steps were added before direct encapsulation of the drug within microneedles in order to achieve controlled release. The author mentioned that for controlled release, double encapsulation is necessary, in which the drug must first be encapsulated in either carboxymethylcellulose or poly-L-lactide before encapsulation in microneedles. Although controlled release was not achieved, the results could prove that delivery of rHuKGF by using the microneedle technique was successful. Future directions can be focused on the fabrication of controlled release rHuKGF microneedles by using the double encapsulation method in order to solve the frequent administration of drugs before and after chemotherapy.

Theoretically, drug release from microneedles is facilitated either by drug diffusion through the polymer or by degradation

of the polymer. Diffusion is the major pathway of drug release in most controlled release devices and is strongly influenced by the polymer matrix, as the motion of a small molecule is restricted by the three-dimensional network of polymer chains. In diffusion-controlled release, the molecular size and weight play important roles.

However, in the case of dermal application, the surface of the microneedles that pierce the skin will determine how fast small molecules diffuse from the microneedle arrays into the skin. There are several product-related factors that determine the rate of drug delivery. These factors include the solubility and concentration of the drug molecule, the thickness of the back plate, and properties of the microneedle array itself such as length, sharpness, porosity, strength, surface area, and density.[31] However, the rate of drug delivery is also dependent on variables more difficult to control such as the quality of the penetration, the manner of microneedle application and the type of skin. To gain more insight into the usability of polymeric microneedles for drug release into the skin, *in vivo* diffusion studies should be performed.

CONCLUSION

The present study demonstrates that polymeric microneedles for transdermal delivery of rHuKGF can be developed using the PDMS micromolding method. These polymeric microneedle arrays can be fabricated on a large scale at low cost. Polymeric microneedles may provide advantages that overcome the limitations of silicon and metal microneedles. Many polymer materials are inexpensive, mechanically strong, and have been used to fabricate medical devices. PLGA polymer-fabricated microneedles in this research possessed good mechanical strength and could withstand high forces as they were fractured following insertion into a parafilm sheet, which makes these microneedles safe for patients. No interactions were found between rHuKGF and the PLGA polymer, and the drug could be successfully released from microneedles *in vitro*. However, some improvement in the reproduction of small-scale features such as microneedle tips may be possible. Further investigations, such as *in vivo* diffusion studies and fabrication of rHuKGF microneedles for controlled and prolonged release kinetics, can be performed.

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