Microneedles from Fish Scale Biopolymer

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ABSTRACT: This article reports on microneedles produced from biopolymer films extracted from fish scales of tilapia (*Oreochromiss* sp.) using micromolding technique. Evaluation of the properties of polypeptide films prepared from the fish scales gave refractive index (1.34), protein concentration (78%), ash content (1.6%) at (22%) moisture content. The microneedles successfully inserted into artificial skin models and imaging using digital camera showed microneedles remained intact when inserted and when removed from the skin model. Microneedles also successfully inserted into porcine skin and were shown to dissolve gradually at 0 s, 60 s, 120 s, and 180 s after insertion. Microneedles containing methylene blue as model drug were also produced and successfully pierced porcine skin. 3D finite element (FEM) simulations were performed using the measured mechanical properties of the biopolymer films (Young's modulus 0.23 N/mm^2 and tensile strength 1.8105 N/mm^2) to evaluate the stress distribution on various dimensions of the fish scale derived microneedles and hence, their ability to withstand force necessary to pierce the skin without fracture. Results from mechanical analysis using FEM showed that microneedles with tip radius between 10 and 100 μm could withstand up to 0.12 N of force per microneedle without fracture, which is indicated when the stress at the tip of the microneedle exceeds the ultimate stress of the material of fabrication. Using skin insertion tests and finite element simulations, this study provides evidence that microneedles fabricated from fish scale biopolymer can effectively pierce and degrade into skin and therefore are good candidate for transdermal applications. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40377.

INTRODUCTION

Microneedles have emerged as one of the most effective means of enhancing transdermal penetration of compounds such as proteins, vaccines and therapeutics into the body in a minimally invasive manner.[1–3] Microneedles have been fabricated from materials such as silicone, metal and polymer.[4–9] Biocompatible metals are rather expensive and require more costly processes for microneedle production. Conversely, silicone microneedles have been reported to be relatively brittle.[10] while polymer microneedles are a cheaper option that can use reproducible production methods such as lithography, hot embossing and micromolding.[11–15] Polymers such as Poly glycolic acid,[16] Polycarbonate,[12] carboxymethyl, cellulose,[17] and poly(vinyl methylether-*co*-maleic acid)[11,18] have been used in the fabrication of microneedles in previous studies. Polymer microneedles can be fabricated as solid microneedles which pierce the surface of the skin to create micro conduits through which the compounds to be delivered may permeate the viable epidermis. They can also be designed as biodegradable microneedle structures which when inserted into the skin would dissolve in the moist environment of the viable epidermis to release active ingredients incorporated within the microneedle structure.[3,7,18] This study focuses on the latter, that is, biodegradable polymer microneedles.

More recently, the focus in the area of microneedle has been to develop biodegradable and non-toxic polymer microneedles which can be prepared to have sufficiently strong microstructures that can penetrate the skin and deliver active ingredients into the lower layers of the skin and into the body.[7] New developments in this area include microneedles produced from rapidly dissolving silk fibrions[19] and trans retinoic acid loaded microneedles for treatment of seborrheic keratosis.[20] Natural polymers are a preferred choice in contrast to synthetic polymers due to their inexpensive, biodegradable, non toxic nature, and availability.[21] They have been widely used in cosmetics and pharmaceuticals and especially in transdermal drug delivery for controlled release of agents into the body via the skin.

In order for the microneedles to be readily available and affordable, there is a need to explore a wide range of low cost

materials for fabrication of biodegradable microneedles. It is also an area of immense interest in all industries to convert wastes to useful end products. This minimizes the cost of waste processing and adds further value to available natural resource.

To this effect, the aim of this study is to explore for the first time, the production of microneedles from biopolymer extracted from fish scales and confirm their ability to penetrate into the skin. Extraction of biopolymer from fish scale can be performed using methods such as enzymatic, acid, and thermal hydrolysis.[22–24] The extract is usually a mixture of polypeptides of varying molecular weight, which can be separated by gel chromatography and ultra filtration membrane refining.[25] Biopolymer extracted from fish scale using thermal hydrolysis dissolves in water has good film forming ability[23] and is therefore a good candidate for micromolding. Fish scales are chosen in particular as they are underutilized natural resource that exists abundantly. Fish scales are the organized outermost structure present on the skin surface of fishes such as tilapia, lizard, and grass carp.[22,24] Furthermore, the scales are source of naturally existing protein polymer mainly collagen type 1. Microstructure analysis of fish scales shows collagen fibrils arranged in an orthogonal plywood structure within a hydroxyapatite matrix.[26,27] It is also known that when exposed to water at a temperature of around 80°C, the collagen from the fish scales is hydrolyzed and extracted as collagen polypeptides,[23] a more workable form suitable for micromolding. Recently, protein polymers from fish scales in particular collagen and gelatin have gained more interest as an alternative to that from mammalian source due to the fact that unlike that from mammals, fish scale peptides require a shorter period and less complicated process to extract, eliminate the risk of transferring diseases such as mad cow disease, and has some more favorable physical properties such as higher gel strength and gel viscosity.[24] Compared to mammalian hydrolyzed collagen, that of fish scale dissolves better at room temperature with better film-forming capability.[28]

Delivery of protein biopolymers such as collagen and gelatin to the skin has been shown to have some bioactive properties such as improving skin elasticity, bone strength, antimicrobial activity, mineral binding capacity, lipid lowering effect, and immunomodulatory activity.[29] *In vitro* studies on mice have shown that hydrolyzed collagen can act against the skin damage induced by UV radiation.[30,31] Protein biopolymers in the form of gelatin sponges have also been used for controlling bleeding and healing of surgery wounds.[32,33] Studies on a group of 63 female Taiwanese subjects aged between 23 and 60 years have also shown that polypeptides extracted from tilapia fish scales improve skin elasticity and moisture content when applied topically.[25] In the said studies, despite the penetration of the peptides into the lower layers of the skin, the stratum corneum was found to be a limiting barrier such that the delivery of polypeptides from fish scales to lower layer of the epidermis would prove more effective, creating microneedles from polypeptides of fish scales therefore has great potential in such applications by further enhancing the penetration of polypeptides into the skin for more effectiveness.

In this article, we report on our work to produce microneedles from polypeptides extracted from fish scales through thermal hydrolysis and evaluate their ability to insert and dissolve into the skin. The microneedles presented are biocompatible microneedles which can either be used to deliver polypeptides to the skin or formulated to incorporate active ingredients such that on insertion into skin will dissolve to release the active ingredients into the skin. For microneedles to be effective in transdermal application either as drug delivery devices or as cosmetic devices, it is important that they are strong enough to penetrate into the skin. The aim of this article is to explore the fabrication of novel fish scale biopolymer microneedles and establish their ability to insert and degrade in the skin, thereby determining whether the fish scale biopolymer extracted using thermal hydrolysis is an applicable material for microneedle fabrication in terms of mechanical strength.

MATERIALS AND METHODS

Fish scales of tilapia (*Oreochromis spp.*) were obtained from a local fish trader in Ejigbo, Lagos state in Nigeria. The tilapia fish were matured fish of about 1 kg average weight sourced from the high sea, stored in cold rooms, and then transported to the retailers. Pig ears were obtained from a butcher at the Odo Eran meat market in Lagos state Nigeria. The pigs were slaughtered in the morning, the ears cut off and transported to the laboratory where they were shaved and washed with water. The cartilage and subcutaneous tissue were removed and the skin was washed and stored in the freezer at −20°C until needed for insertion test. Silicone elastomer (Polycraft T4 translucent silicone) was obtained from MBfibreglass, UK and capillary tubes (Pyrex, Stuart, 0.2 mm I.D) were obtained from Finlab, Lagos, Nigeria.

Production of Fish Scale Biopolymer Films

Fish scale biopolymer was extracted according to method reported by Wangtueai and Noomhorm[23] with slight modifications whereby a centrifuge is used for separation rather than vacuum filtering. The fish scales were washed repeatedly to remove all residue, skin, and dirt after which they were kept in the freezer at −20°C until when needed. One hundred grams of thawed fish scale was weighed and treated with 0.6% (w/v) sodium hydroxide concentration to remove non collagen residues from the fish scales. Sodium hydroxide pretreatment was performed at normal room temperature for 1 h duration while swirling every 15 min in a conical flask.

Following pre-treatment, the scales were washed several times using tap water after which the sodium hydroxide was completely removed. The scales were then transferred into the stainless steel pressure vessel of 4.5 L capacity (PC-70001, Binatone, Nigeria) containing 200 mL of water. The vessel was closed and heated at 80°C (±3°C) for 8 h at a pressure of 80 kPa. At these conditions, collagen is hydrolyzed and dissolves in the water.[24,34] Following extraction, the liquid was separated from the solid using a 2 mm aperture sieve and further separation using a centrifuge (R-8C 3/05 Laboratory centrifuge, REMI Motors, Lagos, Nigeria) at 3500 rpm for 20 min. Customized silicone plates were prepared using silicone elastomer and activator (Polycraft T4 Silastic, MBFG, Newtownabbey, UK) in the ratio 10 : 1 by weight. The supernatant was transferred unto the customized silicone plate to oven dry at 150°C for 1 h at atmospheric pressure allowing the water to evaporate. The films resulting were
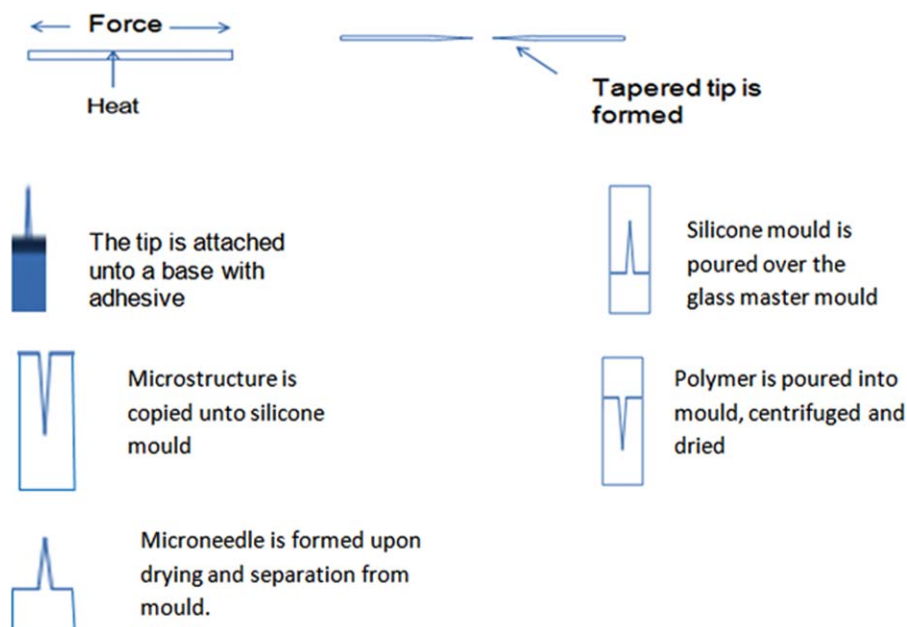
Figure 1. Schematics of microneedle fabrication. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

then left to air dry on the silicone plate at room temperature for 24 h after which dry films had been formed. The films were removed from the silicone plates manually and stored at room temperature (30°C) until next use.

Fabrication of Microneedles

Microneedles were fabricated using established micromolding techniques[11,13,16] to fabricate single microneedles from tips of pulled capillary tubes. Glass capillary tubes were pulled on a Bunsen burner to form sharp tips. The tips were characterized using digital imaging to view tips formed. The formed tips were then separated from the rest of the capillary tube by breaking off with tweezers and attached to flat bases of the fish scale polymer film which was slightly wetted to allow adhesion. Silicon elastomer mixed in the ratio 10 : 1 (weight of silicone to activator) was poured over the glass microneedle master mold and allowed to set for 24 h after which the silicone mold was separated from the glass master mold by hand. Sample of the films were cut with scissors and mixed with a few drops of water to form a thick paste which was then poured unto the silicone mold. The mold filled was then placed in a centrifuge tube and spun at 3500 rpm for 20 min after which it was allowed to air dry at atmospheric temperature and pressure for 24 h. This was followed by manually separating the microneedle formed from the silicone mold. This is illustrated in Figure 1. Microneedles containing methylene blue as model drug were also fabricated by mixing the wet mold with methylene blue prior to micromolding. Methylene blue as well as being a dye can be applied as a drug for treatment of idiopathic pruritus ani through intradermal injection and is therefore a good candidate for delivery using microneedle.[35]

Moisture Content Analysis

The moisture content of the fish scale polymer film was measured using a moisture content analyzer (MS-70, AND, Eng-

land). One gram sample of the film was placed on a steel base unto the sample port of the moisture analyzer, the sample was heated at 180°C (temperature analysis on the moisture analyzer indicates this as the optimal temperature for the material) until all moisture content is removed from the sample. The value of the moisture content was then read. The moisture analysis was performed at the same temperature for all film samples used in this study and each sample was determined in triplicate with average value recorded.

Yield

The yield (Y) was calculated by expressing the mass of the film (M_{film}) as a percentage of the mass of fish scales (M_{scales}) used as feedstock. This gives the % mass of films attainable for every gram of fish scale used.

$$Y(\%) = \frac{M_{film}}{M_{scales}} \times 100. \qquad (1)$$

Refractive Index

The extent to which the polymer produced from fish scales deflected light was measured using a refractometer (60/95 ABBE Type, Bellingham + Stanley Limited, England). Film (0.1 g) is dissolved in 5 mL of water warmed to 40°C and shaken constantly for 1 h until the films were evenly dissolved in the water. The sample port of the refractometer was wiped using cotton wool prior to pouring in the sample and recording the readings of the refractive index.

Protein Concentration

In this study, we use the Lowry test and the nanodrop spectrophotometer (2000/200C, Thermo Scientific, UK) to confirm the extraction of the biopolymer from the fish scales.[36] The Lowry test is used to determine protein concentration based on the presence of peptide bonds which exist between the amino

acids.[36–38] Number of previous studies have also used the Lowry method to test for collagen peptides.[39–41]

Ash Content

The ash content of the extracted fish scale biopolymer is obtained using the ASTM D 2974-87 method C.[42] Briefly a porcelain dish was preheated in an oven at 180°C for 1 h and allowed to cool after which the weight was recorded. The dried biopolymer obtained from moisture analysis was added to the crucible and weighed. The porcelain dish is then placed in a furnace and heated to 440°C until the sample had completely turned to ash, this took approximately 5 h. The sample is then removed from the furnace and weighed to obtain the percent ash content.

Mechanical Strength Test

To obtain the mechanical properties of the films which include tensile strength, young modulus, elongation at break, and yield strength, the Testometric force analyzer (Testometric, England) was used. The films were cut into equal rectangular pieces of 19 mm wide. The thickness of the films was obtained by taking average of the thickness of the films at six different points along the length using a micrometer. The films were placed between the two grips of the force analyzer and set at an initial distance of 40 mm between the two grips thus representing the length over which the tensile stress is exerted. At a set speed of 30 mm/min the film was pulled in opposite direction by the two grips and a graph of force against displacement was obtained using the software attached to the force analyzer. The surrounding temperature ranged between 25 and 30°C over the period of testing.

Insertion Test

Silicone elastomer (Polycraft T4 Silastic, MBFG, UK) prepared from 10 : 1 weight ratio of silicone base:activator was used as an artificial skin model. Materials such as silicone have previously been used to mimic the skin in other reported studies.[43,44] These are used in cases where the skin mechanical properties need to be modeled in a more controlled manner and for more visibility. Single fabricated fish scale microneedles formulated with 0.1% (w/v) brilliant red die (Drimarene X-2B, Sandoz) were inserted into the prepared silicone mold under thumb pressure and imaged from the side view. The microneedle was then removed manually at an estimated speed of 5 mm/s and examined for any sign of fracture. The aim of this experiment was to confirm the physical strength of the microneedles and as the elastomer is moisture free, the microneedle does not dissolve in the artificial skin sample.

Insertion into Porcine Skin

To test the ability of microneedles to pierce real skin, porcine skin was used as model for human skin. Skin insertion experiments were performed according to method used by Yang et al.[45] Porcine skin was removed from the freezer and allowed to thaw completely at room temperature. The prepared skin was then cut into 3 cm^2 area and placed on a ceramic base. The skin was stretched and held in place with the thumb and index finger, microneedles was inserted into the skin and removed immediately after insertion. The points of insertion were stained with methylene blue for 20 min after which excess methylene blue was wipe off with clean tissue and the insertion points

were visualized using digital camera (Nikon, Coolpix L110). To further show the ability of the fish scale microneedles to dissolve in the skin, four identical microneedles made with the same mold were inserted and removed 0 s, 60 s, 120 s, and 180 s after insertion into the skin and images of each microneedle taken immediately after removal from skin.

Finite Element Analysis

Microneedles can be fabricated into various dimensions and the drug delivery rate has been shown to be significantly affected by the geometry of the microneedles such as tip radius.[46,47] Therefore, the fish scale polymer microneedles of various tip sizes must be able to withstand force beyond the force required to penetrate the skin without fracture. Fracture is generally initiated when the stress on the material exceeds the break strength of the material and the maximum stress exists at the tip of the microneedle.[4] To predict the force which the fabricated fish scale biopolymer microneedles of various tip radius can withstand, we simulate the stress distribution on the microneedles using the finite element method. 3D images of the prepared microneedles were drafted in AutoCad (Autodesk, 2009). The analysis was performed using the linear solver independent of time. The images were exported to AutoFEM (AutoFEM Lite®, AutoFEM 2013) and solved using the finite element solver which is inbuilt in the software. The software allows 3D simulations of models which have been drafted using AutoCAD to be meshed and solved for mechanical analysis using the FEM. The properties of the material, young modulus and yield stress obtained from experiment, were incorporated into AutoFEM, the boundary conditions were defined such that a force was applied at the base of the microneedle and the tips position were fixed. The applied force was varied between 0.03 and 0.12 N per microneedle and the maximum stress at microneedle tips ranging between 10 and 100 μm were recorded. The range for applied force is based on insertion force values reported in literature[4,8,11] and is equivalent to force which can be delivered using thumb pressure. The equivalent stress was calculated from the components of stress as follows:

$$\sigma_{eq} = \frac{1}{\sqrt{2}} \sqrt{(\sigma_x - \sigma_y)^2 + (\sigma_y - \sigma_2)^2 + (\sigma_z - \sigma_x)^2 + 6(\tau_{xy}^2 + \tau_{yz}^2 + \tau_{xz}^2)},$$

(2)

where σ_x is the normal stress in the direction of the OX-axis of the global coordinate system; σ_y is the normal stress in the direction of the OY-axis of the global coordinate system; σ_z is the normal stress in the direction of the OZ-axis of the global coordinate system; τ_{xy} is the shear stress acting in the direction of the OY-axis of the global coordinate system on a plane with the normal vector parallel to the OX-axis; τ_{xz} is the shear stress acting in the direction of the OZ-axis of the global coordinate system on a plane with the normal vector parallel to the OX-axis; τ_{yz} is the shear stress acting in the direction of the OZ-axis of the global coordinate system on a plane with the normal vector parallel to the OY-axis.

RESULTS AND DISCUSSION

Values for the chemical properties of the films; moisture content, protein concentration, and ash content alongside refractive

Table I. Properties of Produced Films

Property	Value
Moisture content (% wet basis)	22.16 ± 0.51
Protein concentration (% wet basis)	78.20 ± 0.53
Ash content (% wet basis)	0.10 ± 0.19
Refractive index (−)	1.34 ± 0.00
Yield (%, wet basis)	6.40 ± 1.02

± Standard deviation.

index and yield, are summarized in Table I. Tensile strength, young modulus, and elongation at break of the fish scale biopolymer films were compared with literature values of two other polymers reported in literature. The polymers compared are (1) a mixture of mammalian biopolymer in the form of hydrolyzed collagen and glycerine (Gel:Gly), this has been studied for use as transdermal patches[48] and (2) polymethylvinylethercomaleicanhydride crosslinked with polyethyleneglycol (PMVE/MA:PEG), this polymer has been used in microneedle fabrication.[49,50]

Refractive Index
The refractive index is a basic property of polymers which relates to their ability to bend light, it also correlates with other properties of the polymer such as electromagnetic and chemical properties.[51] Refractive index measured for the fish scale polymer films is within the typical values for polymers where refractive index varies between 1.3 and 1.7. A value of >1.5 indicates a high refractive index polymer while values <1.5 indicate a low refractive index polymer. The average value for the refractive index of fish scales is 1.34 with a standard deviation of 0.00089. The fish scale polymer can therefore be classified as a low density polymer.

Moisture Content
The fish scale polymers retained a level of moisture within their structures. To measure the moisture content, the films can retain at equilibrium with the surrounding environment, the moisture content was measured again after 4 weeks and 3 months, the results are shown in Figure 2. The films maintained moisture content of 18.08% over the duration of 4 weeks. After another 3 months, the moisture contents of the films were analyzed again and found to be 14.27%. Statistical analysis using the Student t test gave P value of 0.00135 between 24 h and 1 month and P value of 0.00018 between 4 weeks and 3 months thus showing a reduced level of significance of time with moisture content at 3 months. This can be explained as water molecules binding with the polymers to give them self plasticizing properties such that a level of moisture is maintained in the films at equilibrium. The change in moisture content after 3 months can be attributed to change in seasonal weather condition hence change in atmospheric conditions thereby shifting the equilibrium. Previous studies that involved extraction of polymer from fish scales reported moisture content values of about 10.5%.[23] The difference in moisture content value could be as a result of different conditions and drying methods used.

In this study, the films were air dried while Wangtueai and Noomhorm[23] used vacuum drying.

Yield
The yield for the thermal extraction process was between 6.40% expressed as percentage of polymer to fish scales on wet basis. Similar studies reported in literature obtained yield values of between 10 and 11% under optimized conditions for hydrolyzed collagen extracted from lizard fish scales.[23]

Protein Concentration
Protein concentration of the polymer films was obtained by Lowry test[41] using BSA as standard. Protein concentration of 78.2% was obtained for fish scale biopolymer films extracted in this study. The protein concentration confirms the extraction of proteins from the fish scales and based on the microstructure of fish scales reported in literature[26,27] and the method of extraction used, the polymer can be said to be hydrolyzed collagen. Although further analysis on the structure of the polymer extracted is not included in this work, we can confirm extraction of protein polymer from fish scales and study its use for microneedle fabrication.

Ash Content
In this study, the ash content obtained for biopolymer of tilapia fish scales is 1.60%. This value is below the maximum ash content recommended for edible hydrolyzed collagen which is between 2 and 2.6%.[52,53] Ash contents of gelatin from tiger toothed croaker and pink perch bones were 2.7% and 2.8%, respectively, while that recorded for the skin was 0.30% and 1.88%. For lizard fish scales ash content of about 2.33% was recorded.[23]

Mechanical Properties of Fish Scale Polymer Films
The mechanical analysis of the films was performed for the purpose of obtaining the mechanical properties of the biopolymer produced to be coupled with FEM analysis to test the ability of fish scale microneedles of varying dimensions to withstand force required for the microneedles to insert into the skin.

Table II shows results of mechanical properties of the films of fish scale biopolymer (FSBP) compared with literature values of mechanical properties of mammalian biopolymer (hydrolyzed collagen) films plasticized with glycerine (GEL:Gly) and films of poly(methylvinylether/maleic anhydride) crosslinked with
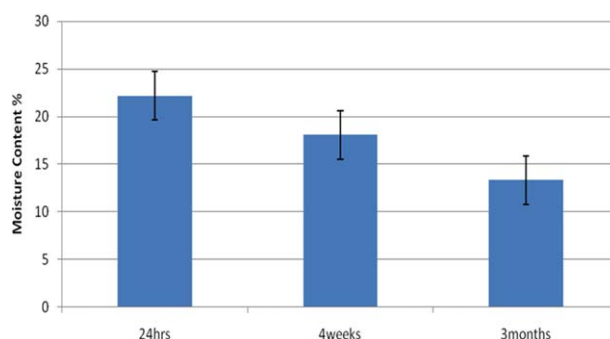
Figure 2. Moisture content of films 24 h, 4 weeks, and 3 months after air drying. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. Mechanical Properties of the Fish Scale Biopolymer Films Compared with Other Polymer Films

	Young modulus (N/mm^2)	Ultimate stress (N/mm^2)	Elongation at break (%)
FSBP	0.2324 ± 0.028	1.8105 ± 0.33	393.45 ± 41.04
Gel:Gly 20 : 5[a]	1.452	1.040	71.66
PMVE/MA:PEG 2 : 1[b]	21.19 ± 6.89	31.60 ± 7.89	311.99 ± 116.12

[a] Values taken from Chai et al.[25]
[b] Values taken from Singh et al.[50]
± Standard deviation.

polyethyleneglycol plasticizer (PMVE/MA:PEG). These two polymers have been used for transdermal drug delivery applications in other studies. PMVE/MA:PEG films in particular have been used for microneedle fabrication.[49] Compared to previous studies which reported hydrolyzed collagen films from mammalian source containing no plasticizer to be brittle and dry with no flexibility,[48] the fish scale polymer films from this study are strong and flexible. Films of hydrolyzed collagen and glycerine (GEL:Gly) in the ratio 20 : 5 polymer had elongation of 71.65% while that of fish scale polymer obtained in this study was up to five times higher at 393.45% with a standard deviation (SD) of 227.54. Similarly, the tensile stress and young modulus were also much higher. The films of 15% PMMVE/MA crosslinked with PEG 10,000 in the ratio 2 : 1 showed tensile strength and young modulus which were 91 times and 17 times higher than that of fish scale polymer ($P = 0.0092$, $P = 0.064$, respectively). However, the fish scale polymer had similar (1.26 times higher) elongation at break than the PMVE/MA films compared ($P = 0.66$). Values for films of PMVE/MA without plasticizer which have been used for microneedle fabrication were not determined.[11,50] From these results, it is observed that values for the mechanical properties of fish scale polymers obtained here vary from that of polymer films which have been studied for microneedle fabrication. However, these results are not conclusive, further analysis is discussed in subsequent sections to determine the suitability of the polypeptides obtained from fish scales for microneedle fabrication.

Microneedle Fabrication

Microfabrication of microneedles using pulled glass capillary tubes and silicone micromolding produced tapered microneedles of about 900 μm in length as shown in Figures 3 and 4. The fish scale polymer formed into microneedles geometries replicating the structure of the glass microneedles used as master molds. Figure 3(a) shows microneedles fabricated with plain fish scale polymer while Figure 3(b) shows fish scale microneedles containing 1% (w/v) methylene blue. This results show that fish scale polymers can be used to produce microstructures suitable for microneedle application using reproducible micromolding techniques. It also shows that the fish scale polymer microneedles can incorporate model drug such as methylene blue and by visual inspection we see that methylene blue was evenly spread across the length of the microneedles. The structures obtained are comparable with silk fibrion microneedles containing methylene blue which were produced by You et al.[19] In this study, the glass capillary tubes were pulled by hand over a Bunsen burner to obtain

microneedle at the tips as described in the method. However, for mass production, sharp tips can be reproducibly produced using micropipette puller as reported in previous studies. For example, Wang et al.[9] have used glass microneedles pulled using automated micropipette puller for microinjection and as master molds for fabricating polymer microneedles. The group obtained similar structures with tips of about 100 μm.

Insertion into Artificial Skin Model

To analyze the strength of the microneedles produced, we insert into a skin phantom made from silicone. Silicone has similar flexibility as the skin with a young modulus of ~0.916 MPa[54] and it has been widely used to model the skin as it has similar physical properties to the skin in terms of toughness and flexibility,[44] and can therefore be used as a model to test the ability of microneedles fabricated in this study to insert into skin without failure. Figure 4 shows microneedle while inserted and following removal from artificial skin model, microneedles remained intact while inserted into the skin model and upon removal without fracture. The results show that fish scale microneedles possess sufficient strength to successfully insert into skin model without breakage or fracture.

Insertion into Porcine Skin

Figure 5 shows points where different microneedles were inserted into porcine skin followed by methylene blue staining. Both plain microneedles and microneedles containing methylene blue successfully pierced the skin. To further show the ability of the fish scale microneedles to degrade in the skin four different microneedles were inserted and removed 0 s, 60 s, 120 s, and 180 s after insertion into the skin. Figure 6 shows images of microneedles gradually dissolving into the skin. The
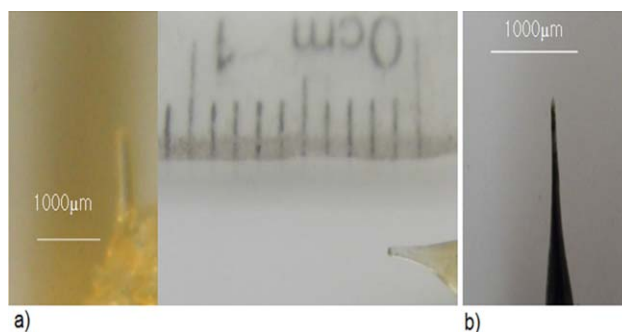

Figure 3. Digital image of microneedles fabricated from fish scale. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
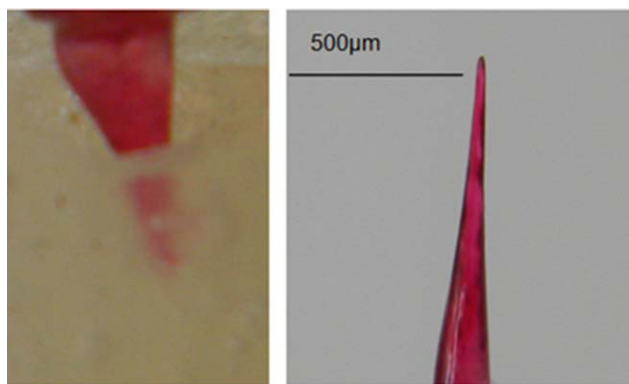
Figure 4. Digital image of microneedles inserted into artificial skin model and after removal from skin model. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
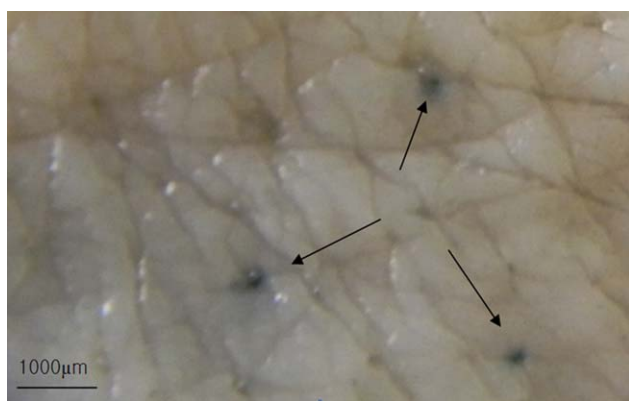

Figure 5. Porcine skin stained with methylene blue with arrows showing microneedle insertion points. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

microneedle tips dissolved completely after 180 s insertion time [Figure 6(d)]. These microneedles take longer to dissolve compared to the silk fibrion microneedles which completely dissolve in 60 s in porcine skin.[19]

FEM Modeling
To further study the effect of applied load on the microneedles, we model microneedles with dimensions as reported in litera-ture.[47,55] The microneedles modeled were 600 μm long with a base radius of 150 μm and tip radius ranging between 10 and 100 μm. Polymer microneedles of similar dimensions have been previously shown to withstand up to 0.03 N force per micro-needle which was necessary for insertion into porcine skin mod-els.[55] Therefore, the microneedles fabricated in this study must be able to withstand such force to be fit to penetrate into the skin. The stress at tip for force ranging between 0.03 and 0.12 N is modeled as shown in Figure 7. The results in Figure 8 show that the single fish scale microneedles with tip radius ranging between 10 and 100 μm can withstand up to 0.12 N force without fracture since for all the simulations, the stress at tip of the microneedles did not exceed the ultimate stress of the fish scale polymer material. The stress was most concentrated at the tip of the microneedles in accordance with other reports[4,56] where it has been reported that microneedles were more likely to fracture at the tip. Finite element modeling has been previ-ously applied in similar manner in biomedical research in for example bone fracture prediction[57] and also predicting optimal drug release from microneedle structures,[46,58] insertion force optimization of microneedles.[8] The results from these simula-tions provide evidence that fish scale polymer microneedles can be fabricated into a range of dimensions and maintain sufficient mechanical strength to pierce the skin.

From the results of the experiment and FEM analysis it seems that despite having much lower tensile strength compared to PMVE/MA formulation which has also been used in micronee-dle fabrication,[49,50,55] the fish scale polymer microneedles can successfully pierce into skin as shown in the insertion test for artificial and porcine skin. This is understandable as the micro-needles fabricated with PMVE/MA have indeed been shown to withstand up to 0.71 N of force per microneedle, forces much higher than the force required to pierce into porcine skin, which is about 0.03 N per microneedle.[55]

This study introduces microneedles from fish scale biopolymer which demonstrate sufficient mechanical strength to pierce skin models without additional polymer incorporated thereby decreasing cost and complexity of production. There have been some previous studies reporting the fabrication of microneedles from biodegradable polymer. However, this is the first time pro-duction of microneedles from polymer extracted from fish
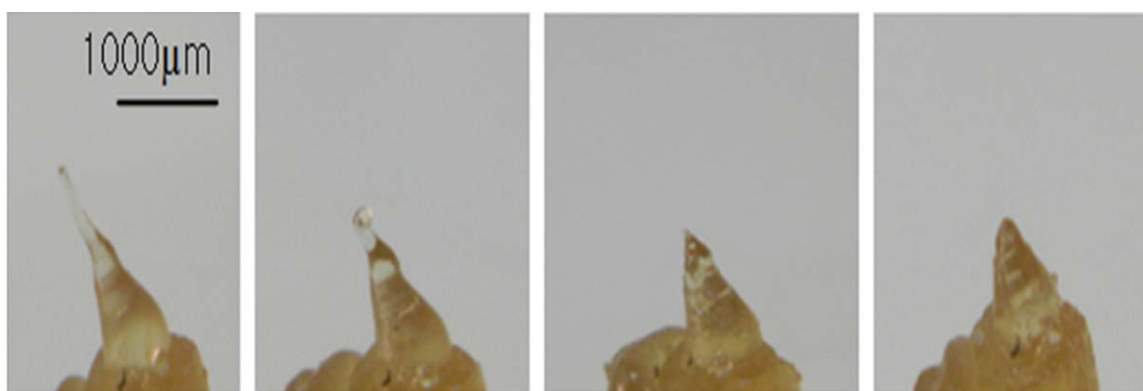

Figure 6. Microneedles removed from porcine skin after (a) 0 s, (b) 60 s, (c) 120 s, and (d) 180 s. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
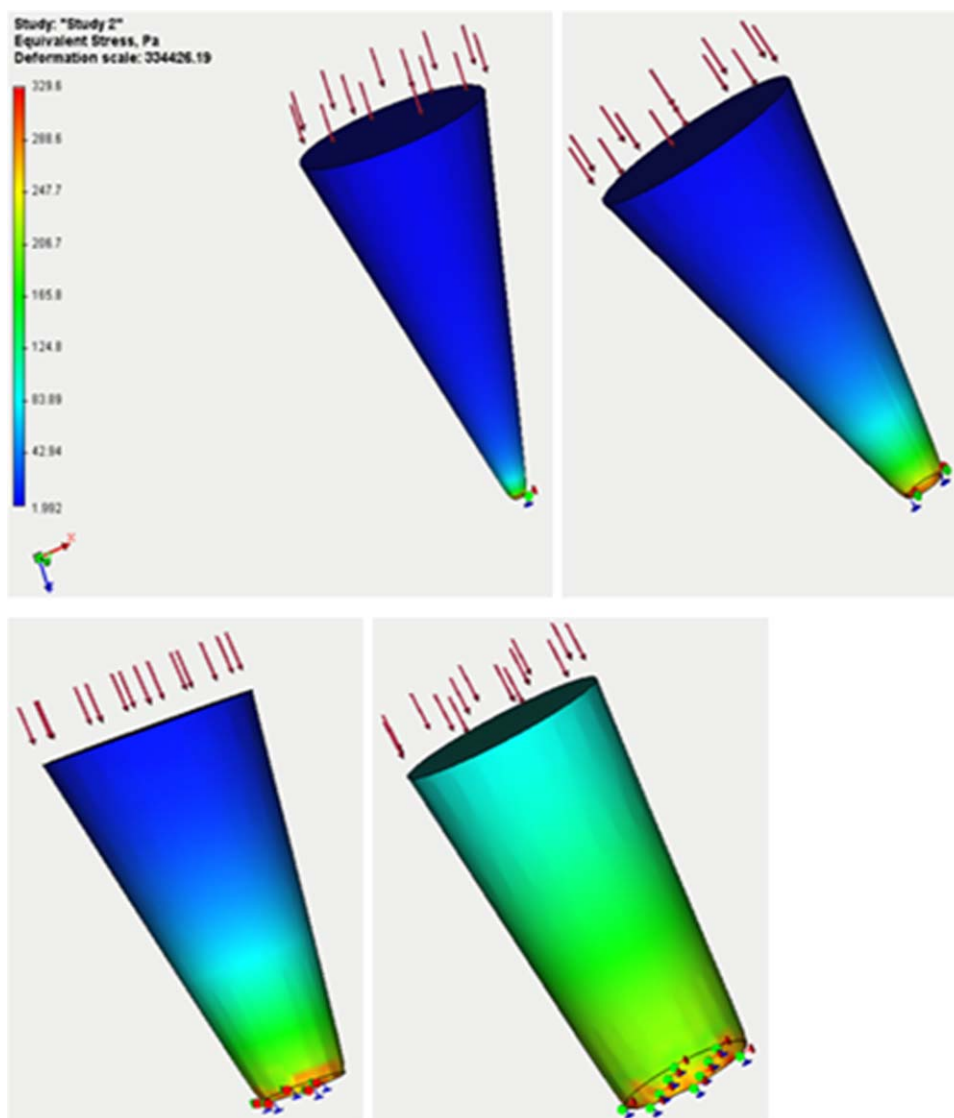
Figure 7. Microneedles with tip radius of 10 μm with 0.06 N evenly distributed force applied. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

scales, an aquatic waste material, is being presented. The microneedles produced in this study demonstrate sufficient strength to penetrate skin model without need for additional component such as copolymer or plasticizer unlike the microneedles fabricated from the other polymers incorporating additional component such as poly lactic acid (PLA), poly glycolic acid (PGA), PVP, and PVA[7,59–62] to achieve sufficient mechanical strength. The fish scale biopolymer is a natural protein which is potentially biocompatible and is therefore an ideal candidate for microneedle production.

CONCLUSION

This study presents biodegradable polymer microneedles fabricated from biopolymer extracted from fish scales. Microneedles were successfully produced from the fish scale polymer films using reproducible micromolding techniques. The microneedles fabricated were shown to have sufficient mechanical strength to

pierce into artificial skin model without fracture and they were further shown to pierce and dissolve into porcine skin without use of additional plasticizer or copolymer. This study explores a
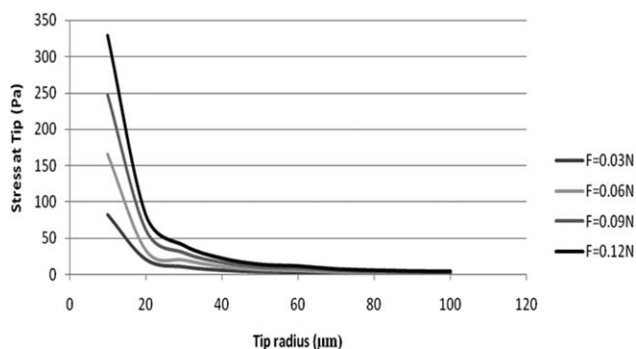

Figure 8. Stress at tip of microneedles when force of 0.03–0.12 N is applied.

cheaply available raw material for the production of biodegradable polymer microneedles. In addition to their use as delivery systems for drugs, these microneedles have great potential for cosmetic application and general health benefits even in their plain form due to the intrinsic bioactivities of the fish scale biopolymer used as material of fabrication.

ACKNOWLEDGMENTS

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