Development of Artificial Human Skin Substitute for Medical Research

Khushboo Danish
Department of Biomedical Engineering
Mehran University of Engineering
& Technology Jamshoro Pakistan
17bm18@students.muet.edu.pk

Dr. Ahsan Ahmed Ursani
Department of Biomedical Engineering
Mehran University of Engineering
& Technology Jamshoro Pakistan
ahsan.ursani@faculty.muet.edu.pk

Moatter Waqar
Department of Biomedical Engineering
Mehran University of Engineering
& Technology Jamshoro Pakistan
17bm09@students.muet.edu.pk

Dr.Noor Ahmed Sanbhal
Department of Textile Engineering
Mehran University of Engineering
& Technology Jamshoro Pakistan
noor.sanbhal@faculty.muet.edu.pk

Abstract

Skin substitutes are necessary for the accountability of dreadful incidents in life. They are important for clinical use, particularly in the administration of intense wounds Moreover, Diabetes is a common disease, affecting 422 million people worldwide and causing 1.6 million deaths. requiring patients to administer doses orally or via insulin pen markers. Research aims to develop advanced drugs that can perform activity for the entire day, but this is difficult due to insulin's high molecular weight and long uptake time. Artificial skin substitutes are being developed to reduce animal rights violations in laboratories.. There are numerous skins substitutes available economically, each with specific purposes and skin qualities, for both dry and clammy skin conditions, with mechanical properties within the range of human skin. An artificial skin substitute based on polyurethane material is proposed, with a secondary goal of transdermal drug delivery based on substrate. Using various measurement and evaluation techniques, the surface, mechanical, and behavioral properties of the designed model would be focused to meet the actual skin model. The designed model would be the result of altered substrate ratios, soluble drugs, and materials. In any case, a significant disadvantage of these materials is that they are hydrophobic and incapable of absorbing adequate dampness to successfully mimic skin erosion conducts under changing ecological conditions. As a result, a water-permeable material capable of imitating human skin may be required.

Keywords : Skin . Substitute. Polyurethane. Drug Permeability.

Introduction

Skin is the most important organ in the body, accounting for 16% of body weight [1-2]. The barrier provides a physical barrier to the environment and protects against microorganisms, toxic agents, and ultraviolet radiation. Skin, depending on donor age and dehydration level, is nonlinear, anisotropic, heterogeneous, viscoelastic, and incompressible[3]. The epidermis, dermis, and subcutaneous layer are the three basic layers of the skin. Even though it is structurally continuous throughout the body, skin thickness varies with age, anatomical site, and cell keratinization[4]. It is also made up of pigment-producing melanocytes, sensory Merkel cells, and specialized dendritic Langerhans cells, which play an important role in the skin's immune defense system[3]. In vivo and ex vivo tests with human skin require artificial and recreated skin models[5]. The artificial skin models extend from straightforward homogeneous polymer materials, such as poly (dimethoxy silane) or silicone films through to lipid-based parallel counterfeit membrane-permeability test (PAMPA) or phospholipid vesicle-based permeation-assay membranes, with the last-mentioned fabric outlined to imitate the SC[6]. Artificial skin models are highly reproducible due to them.

standardized development[4]. This artificial skin should mimic some of the most important features of natural skin. It should protect the wound by creating a barrier between it and the outside world. It should also regulate water dissipation as well as protein and electrolyte loss[7]. Other important properties include limiting excessive heat loss, reducing pain, allowing early mobilization, facilitating quick wound healing, and improving the corrective appearance of the scar[8]. The primary use is to treat skin damage, wounds, and burn patients. Alternatively, artificial skin is now being used on some platforms to treat patients with skin diseases such as diabetics, foot ulcers, and so on. In addition, laboratories have created in vitro skin models for use in cosmetics and medical research[9]. This is a more cost-effective and ethical method of testing surface reactions to topical solution treatments. In addition to tactile recognition, artificial skin devices must also communicate with the nervous system[10]. This will be accomplished through electrical stimulation of extremely precise silicon-based cathode[11].

To develop a reproducible model of skin by a simple in vitro method, that could be used as mechanical skin substitutes, with silicone elastomers and polyurethanes being the most used materials[12]. Lines of water-permeable material capable of acting flawlessly on human skin may be required to recreate human skin[13]. The materials used in this experimental work for the development of artificial human skin model are Polyurethane ,DMF and Levofloxacin 500mg. Polyurethanes have been utilized for almost 50 years in biomedical applications, especially as the blood-reaching material in cardiovascular gadgets[3].

Polyurethanes have been widely used in a variety of biomedical applications due to their excellent physical properties and biocompatibility, including aortic grafts, heart valves, pacing leads covers, cardiac catheters, and controlled sedate release devices[1]. Polyurethanes were used for controlled delivery of various pharmaceutical specialists such as caffeine, rifampicin, and nystatin. Many of these systems are based on the physical interaction of a drug and polymers[14]. Depending on the chemical composition, versatile properties, hydrophilicity, debasement rates, and porosity, the polyurethanes and polyurethane acrylates can be utilized as adhesion barriers, frameworks for the repair and recovery of different tissues, strong tissue defect fillers an d fluid injectable materials which cement after infusion[15]. Moreover, synthetic polyurethane-based hydrogels have been developing intrigued as biomaterial and they have amazing properties such as biodegradable, non-toxic and versatile within the later a long time[16].

N, N-Dimethylformamide is broadly utilized as a natural dissolvable in the research centers and in the synthetic business[17-18].It is used as solvent in the proposed model due to its

aprotic nature of the solvent, wide liquid range and low volatility[19]. It is frequently used for chemical reactions and other applications, which require a high solvency power[20]. Levofloxacin has been classified among the fluoroquinolones against infections[3]. It is effectively used in patients with respiratory problems, skin diseases and urinary bladder problems[21]. In patients with simple disorders of the skin and delicate tissues[22].

It is not required that an artificial skin model keep the wound sterile, but a strong interaction between the skin substitute and the host should prevent local and systemic disease. Providing a climate to hasten injury healing and improving the corrective appearance of the scar are all benefits of early preparation.

Experimental

Materials

Polyurethane & DMF (Dimethylformamide - (CH3)2NC (O) H) were employed in this experiment for the development of an artificial human skin model. Polyurethane in the form of chips was sourced through Sigma Aldrich in the United States and DMF was provided by Nanomaterials research lab in Dept. of textile engineering, MUET . The Levofloxacin drug in Fig 1 used in drug permeability test was purchased from local market in Hyderabad, Pakistan.

Fig 1 Levofloxacin tablet

Methods

Polyurethane and DMF Solution

Based on the volatile nature and dissolving characteristics, a list of solvents for polyurethane were considered, with DMF being the most suitable. Based on the calculations, the concentration of Polyurethane and DMF was determined & weighed using a digital balance. The stock solution was adjusted to approximate the thickness of the developed model, which was needed to be 2mm thickness approximated to the human skin. The mixture of PU and DMF was magnetically stirred for 1-2 hours at room temperature until uniformly mixed.

$$\text{Amount of PU(g)} = \left(\frac{\%\text{ of PU}}{100}\right) \text{x stock solution.}$$

$$\text{Amount of DMF} = \text{Stock Solution} - \text{Amount of PU}$$

Layer formation in Petri Dish

The mixture was poured into a glass petri dish and covered with aluminium foil to avoid dirt particles. The mixture was kept at room temperature overnight and remained undisturbed for 3-4 days to allow the solvent to fully evaporate at room temperature. Once the layer was

prepared, it was dried in the oven for 24 hours at 40°C for best results. This is illustrated in Fig 2 given. A digital vernier calliper was used to measure its area diagonally and thickness multiple times from different dimensions to estimate the mean value.

(a) (b) (c) (d)

Fig 2 (a) Mixture of PU and DMF Poured into Petri dish (b) Solution covered with Aluminium Foil (c) Result of developed model layer after 2 days (d) Final result of layer after complete drying (5-6 days)

Loading of LVFX HCL

The oven-dried sample was then loaded with levofloxacin HCL to determine drug loading, permeability, and release characteristics of developed sample. To load the drug solution, a standard LVFC solution was made by dissolving 0.5g LVFX HCL in 250mL distilled water. The prepared sample was placed into the 50ml of the standard solution and kept for 2 hours. Subsequently, the drug-loaded sample was dried in oven at 50°C for 20 minutes and weighed to determine the drug absorption concentration. The weights of samples 1, 2, and 3 were calculated, and the difference between the weights before and after levofloxacin loading. The results are validated by the illustrations provided in Figures 3 a-f.

The % increase in weight and the & increase in drug uptake of samples were determined using the formula $\%\textbf{Increase in weight } \left(\frac{\mathbf{W2-W1}}{\mathbf{W1}}\right)\mathbf{x100}$ & $\textbf{Drug uptake } (\mathbf{g}) = \mathbf{W3} - \mathbf{W1}$ whereas

W_1 = Weight of sample before loading of drug

W_2= Weight of sample after loading of drug

W_3 = Weight after oven-drying the drug loaded sample

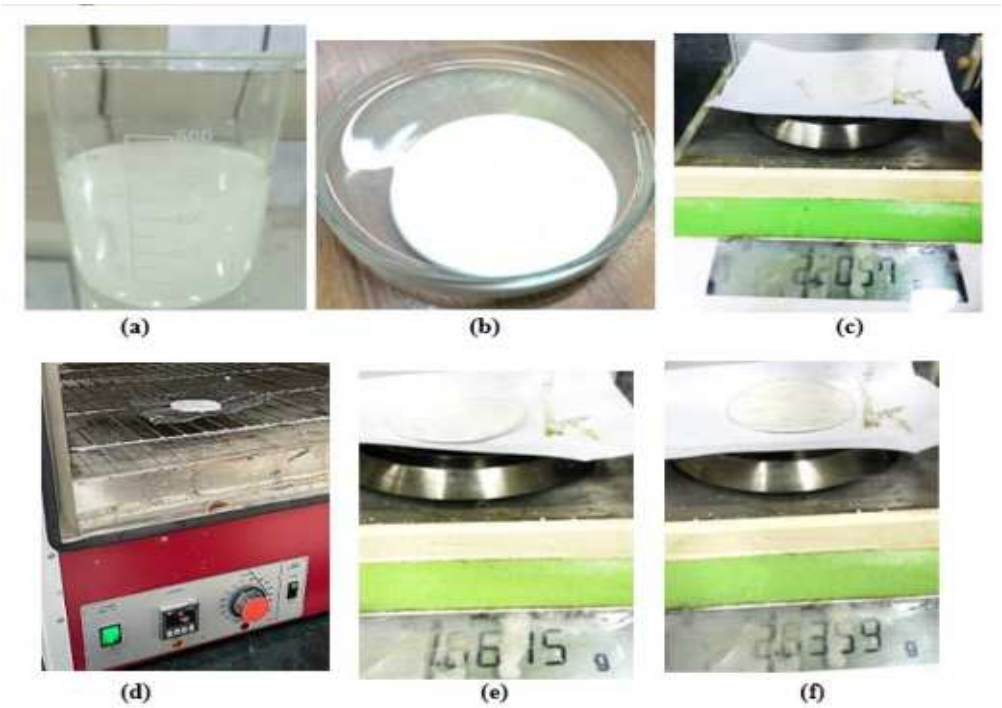

Fig 3 (a) Prepared LVFX Solution (b) Prepared sample 1 immersed in LVFX Solution and kept for 2 hours (c) Weight of drug loaded sample 1 (d) Drug loaded sample 1 in oven (e) Weight of oven-dried sample 1 (f) Weight of Drug loaded Sample 2

Characterization

Drug permeability test

The drug permeability of prepared sample was determined used spray rating tester equipment in Fig 4 offered at MUET, Jamshoro, Sindh's Department of Textile Engineering, which is used to determine the water permeability of textile-based textiles. The sample was packed securely into the conical flask, making sure there was no space for the solution to escape via the corners. 5ml of the standard LVFX Solution was used and poured drop by drop using pippet at the top of the layer with a dropper. The permeability of the drug was measured over time.

Fig 4 Spray Rating Tester M232 - Water repellence (Spray Method)

Surface Morphology

Th texture and distribution of localized molecules at the medium's surface are determined by surface morphology. The pore size of our prepared sample was observed using a digital microscope **LABOMED LX-400 Digital Microscope** given in Fig.5 at 100x before and after the drug loading experiment to observe the surface appearance of our prepared sample.

Fig 5 LABOMED LX-400 Digital Microscope Equipment

Porosity test by weight method

The ability of the medium's absorption rate and the density of the solution are used to determine porosity using the weight technique. It's the proportion of Pore volume to Bulk volume. Pore volume is calculated as the ratio of the weight of the medium to the density of the solution. The overall volume of the occupied medium is referred to as the bulk volume. The calculated 50ml of LVFX and distilled water solution with known density was used to immerse the prepared sample for 2-3 hours approximately. The weight of the sample before and after immersion in the prepared LVFX solution was recorded. Provided the density of solution (LVFX in distilled water) is 2.4 g/cm^3

$$\Phi = \left(\frac{Vp}{Vb}\right) \therefore Vp = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Density of Solution}}$$ Vp = Pore volume Vb = Bulk volume

Porosity test by Area Method

ImageJ software was used to calculate porosity using the area approach. To determine the porosity, two pictures of a sample medium were processed, one before and one after drug loading . The photos were processed using the steps outlined below in Fig 4

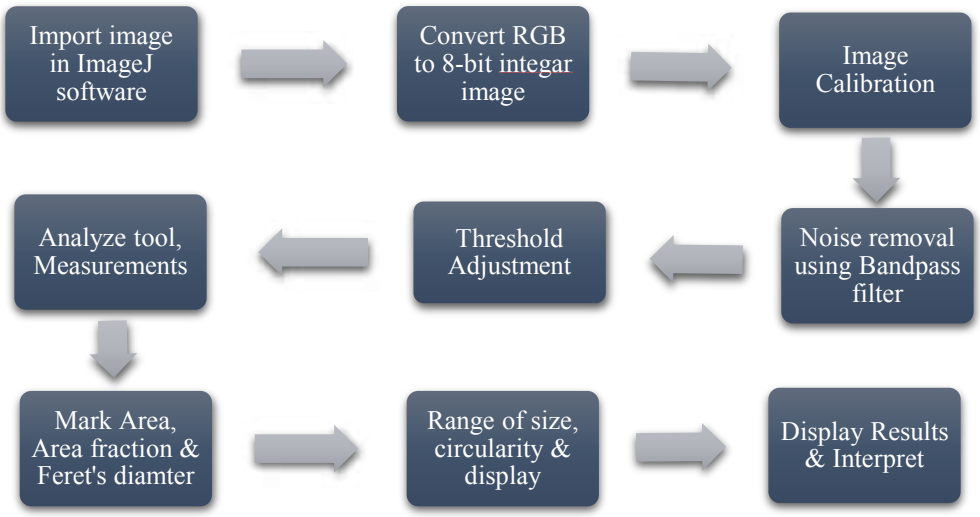

Fig 4. Flow chart representation of determination of porosity using ImageJ software

Results & Discussion

Preparation of Artificial Skin Model

The 8g stock solution was the most suitable among the prepared solutions (30 % PU). The 8g (30% PU) reference sample came close to the specified thickness of 1.80mm. A tabulated data is provided to display results obtained by varying the concentration of Polyurethane in stock solution in given Table.1 Because of its high volatile nature, a rise in the number of pores was noticed as the concentration of DMF as a solvent in the stock solution was increased. As the concentration of PU was increased, the viscosity of the solution decreased, and the time it took to completely dissolve PU in DMF increased. Furthermore, when the concentration of PU rose, the time required for solvent evaporation and full drying of the produced sample at room temperature increased. The thickness of the prepared sample layer obtained at different stock solutions of the sample is shown by the curve in Figure 2. This illustrates an increasing layer thickness curve as the stock solution increases. At 8g stock solution, the best results are obtained.

Table 1 . Thickness of layer formed with respect to stock solution and % of PU

Stock solution	% of PU in solution	Thickness of layer(mm)
2g	15	0.13
2g	20	0.21
3g	20	0.36
3g	25	0.46
5g	30	0.78
8g	30	1.85
8g	35	1.96

Drug Permeability Experiment

The sample was packed securely into the conical flask, making sure there was no space for the solution to escape via the corners. 5ml of the standard LVFX Solution was used and poured drop by drop at the top of the layer with a dropper. The drug went through the prepared layer, and a drop was seen dripping from the filter and onto the cloth, confirming the prepared sample's drug permeability. The time it took for each drop to fall was estimated to be between 20 and 25 minutes. It took more than 3-4 hours to perform the entire experiment. The drug drops were permeable, as shown in Fig 14, and the permeability was measured for 6 hours as the average of three samples.

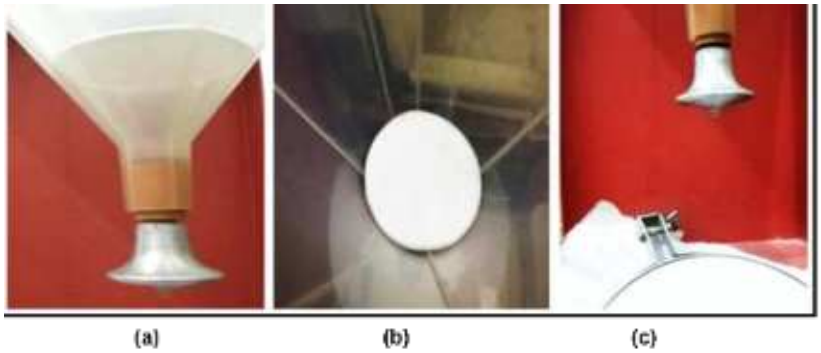

Fig 14 (a) Sample placed in flask of instrument (b) Top View of Sample (c) Falling of drop from filter after passing through sample layer

Drug Uptake Experiment

Table 2 shows that the permeability of the generated sample model is validated by the results of water and drug uptake studies. The performance of the three supplied samples varies depending on their concentration. The drug absorption rate in the sample is the most encouraging outcome. Sample-3 displays the highest drug absorption rates. It demonstrates that rate drug uptake is increasingly linearly in 3 consecutive samples provided.

Table 2 : Observations and Calculations for Water uptake and Drug uptake experiment

	Weight of Sample 1	**Weight of Sample 2**	**Weight of Sample 3**
Before loading LVFX	1.84g	2.35g	2g
After loading LVFC	2.2057g	2.8359g	2.42g
Difference	0.3657g	0.4859g	0.42g
% Increase in weight	19.87 %	20.67%	21%
Weight after oven-dried	1.86g	2.371g	2.022g
Drug Uptake	20mg	21mg	22mg

Surface Morphology

The pore size of the sample before and after drug loading was measured using a microscope, and the results show a difference. The sample before drug loading has a greater pore size, while the sample after drug loading has a smaller pore size, indicating that the loaded drug has occupied the vacant spaces. The results of porosity inside the sample medium obtained using the weight method presented.

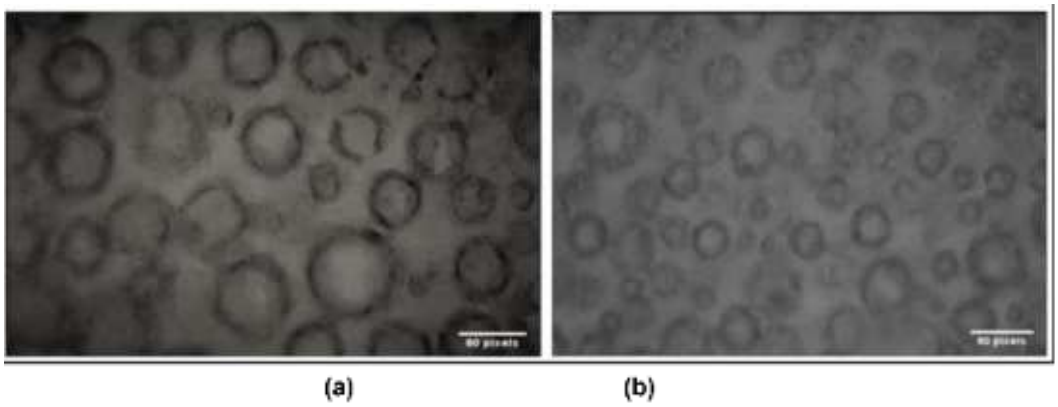

Fig 15 (a) Microscopic Image of Sample before Drug loading (b) Microscopic Image of Sample after Drug loading

5.5 Porosity determination

The weight method was used to determine porosity in the prepared sample. With the provided numerical data in table 3 & mathematical formula, the porosity of 3 different samples was determined. Sample 3 exhibited maximum porosity showing maximum numbers of pore count.

Table 3 Observations and Ccalculations to determine porosity in samples.

	Sample 1	**Sample 2**	**Sample 3**
Dry Weight	1.84g	2g	2.35g
Wet Weight	2.2058g	2.42g	2.8359g
Pore Volume	0.152	0.175	0.2024
Bulk Volume	93.0625	93.0631	93.0644
Porosity (%)	1.6×10^{-3}	1.88×10^{-3}	2.1×10^{-3}

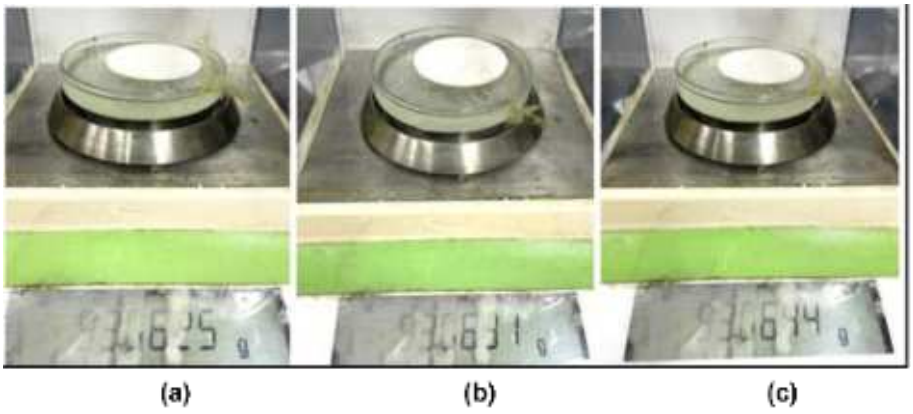

Fig 16 (a) Bulk volume of Sample-1 (b) Bulk volume of Sample 2 (c) Bulk Volume of Sample 3

Area method using ImageJ Software

Before drug loading, the image of the sample medium showed a count (number of pores) of 28 and a surface area (% Area Fraction) of 36.895, however after drug loading, the picture showed a count of 33 and a surface area (% Area Fraction) of 38.081. The sample after drug loading exhibits an increase in count and surface area, indicating that the LVFX drug has occupied the pore spaces in the sample medium.

Table 4 Data of sample (before drug loading) obtained using ImageJ software.

Count	Total Area	Average Size	% Area	Feret	FeretX	FeretY
28	113340	4047.857	36.895	121.677	308.964	226.714

Table 5 Data of sample (after drug loading) obtained using ImageJ software.

Count	Total Area	Average Size	% Area	Feret	FeretX	FeretY
33	97080	2941.818	38.081	76.636	256.485	245.364

Conclusion

An artificial skin model based on polyurethane was prepared in laboratory for medical research purposes. The developed model is aimed to imitate the properties of human skin at research experiments level. The developed model has approximate thickness 1.8±0.2 mm, nearby to human skin thickness of 2mm. The surface morphology was observed using Digital microscopy. The results demonstrated the efficiency of developed artificial skin model in permeation of levofloxacin drug with an uptake of 2mg per 2-hour duration. The surface morphology demonstrated the difference in pore size of the sample medium before and after drug loading experiments validating the successful results obtained. All results have shown that the design experiment carried out for development of artificial skin model is a suitable candidate for human skin substitute in medical research purposes. The developed skin model may be further evaluated for its biocompatibility and cell viability to make sure that prepared skin model is safe and nontoxic. Secondly the skin model is prepared with its limited characterization sources so the work may be continued to develop and characterize artificial skin model for further Laboratory testing of surface morphology as well as in vivo drug permeability.

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