Potential Applications of Human Artificial Skin and Electronic Skin (e-skin): A review

Asdani Seifullah Dolbashid, BSc
Researcher, Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.
Centre for Innovation in Medical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.

Mas Sahidayana Mokhtar*, PhD
Senior Lecturer, Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.
Centre for Innovation in Medical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.

Farina Muhamad, PhD
Senior Lecturer, Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.

Fatimah Ibrahim, PhD
Professor, Department of Biomedical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.
Centre for Innovation in Medical Engineering, Faculty of Engineering, University of Malaya, 50603, Kuala Lumpur, Malaysia.

*Corresponding Author
mas_dayana@um.edu.my, +60129336621

There is an ever increasing need to develop artificial skin that can fully mimic the human skin that it replaces. Skin substitute has been commercialized and used in cosmetics and wound healing treatment, with mixed results obtained. Apart from artificial skin, electronic skin (e-skin) is also widely researched because it can be customized into wearable devices. E-skin is commonly characterized by its flexibility and ability to accommodate a large range of sensors in ultrathin films. This paper reviews current development and technology applied to artificial skin and e-skin. First, the basic layers of normal human skin are introduced. Then current development of artificial skin in cosmetics and grafting applications are mentioned in the next section. Latest technology in the fabrication of e-skin and some of its characteristics in different applications are also discussed. Animal ban for cosmetics testing and its positive effects on the development of alternatives to animal testing in experiments are also explained in this paper. Lastly, the current challenges in skin research and recommendations for future application of artificial skin as well as electronic skin are presented.

1. Introduction

Skin, or the integumentary system, is our body largest organ. Skin serves as an external barrier that protects unwanted substances from entering the body. Skin consists of three different layers; epidermis, dermis, and hypodermis. These layers and their appendages provide various functions, such as organ and tissue protector, body temperature regulation, and UV protection. Artificial skin aims to mimic the human skin for potential use in various fields. Skin models are being used to study and measure chemical and physiological changes in the human skin. For instance, few skin models are used to predict drug permeability and specific environment of the human skin. There are also artificial skin model that are created specifically to mimic certain skin disease condition; enabling researchers to study the disease further. The most common use of artificial skin is for grafting and a few brands, such as Integra™, is widely used to aid in treatment of burn patients in hospitals.

The development of skin substitutes arises from the problem of inadequate skin surface to cover extensive/extreme damage in patients and the painful procedure patients have to endure during autografts (1). Skin substitutes can be derived from humans (allograft), animal (xenograft), or by using membrane made from natural and/or synthetic polymers (2). Natural polymers have excellent biocompatibility while synthetic polymers have great flexibility and its properties could be tailored according to the specific needs. For example, the poor mechanical properties of collagen could be compensated by adding polyurethane to strengthen the structure of the scaffold (3). The limited use of autografts and allografts and the ever increasing need for wound healing treatment have prompted the development of skin substitutes to treat various skin defects. Mechanical strength is needed as the scaffold also act as a platform for cell functionalization and
differentiation (4,5). To date, there is no artificial skin that could completely replace the actual human skin. However, with the recent progress showing, we are in the right track of producing a reliable and robust artificial skin for general usage.

Electronic skin (e-skin) employs the use of sensors to detect changes in environment such as pressure, strain, temperature, and humidity. Technological advancement in the development of e-skin means it has potential use in wearable individual-centered health monitoring, sensitive tactile information acquiring, minimally invasive surgery, and prosthetics (6). The thinness and flexibility of e-skin are some factors that make e-skin desirable and convenient to use. Nonetheless, the challenge lies in creating a tactile-sensing simulation with high resolution, high sensitivity, and rapid response similar to the human skin characteristics (7). This is important as information need to be delivered the fastest way possible in order to provide precise real-time data of the subject.

2. Skin layers

![Normal skin structure](image)

Figure 1. Normal skin structure. Two main layers of skin are shown here; epidermis and dermis. Use with permission from (8).

Human skin serves as a protection barrier to external environmental elements. The skin or integumentary system as it is called; measures approximately 1.8m² and is composed of many folds, invaginations and specialized niches that support a multitude of microorganisms (9). It consists of epidermal and dermal layers connected by a complex vascular nervous network (10). The skin consists of three different layers; namely epidermis, dermis, and hypodermis. The skin is in a way unique, that it is viscoelastic; it possesses both elasticity of solids and viscosity of fluids at the same time (11). Skin is also home to “normal” bacteria in which a majority of them come from these four phyla; actinobacteria, bacteroidetes, firmicutes, and proteobacteria (12).

2.1 Epidermis

Epidermis is subdivided into several layers of strata, starting from stratum basale at the bottom to stratum corneum at the top. These layers vary from each other as they are on different stages of cells maturation. This is reflected in their movement from stratum basale to stratum corneum, where the cells are finally matured (13). Epidermis primarily serves as a barrier and is comprised mainly of keratinocytes (14). The barriers can be divided into physical, chemical, biochemical, and immunological barriers. The physical barrier consists mainly
of stratum corneum (15,16). The chemical and biochemical barriers are composed of lipids, acids, hydrolytic enzymes, antimicrobial peptides, and macrophages (15). The epidermis is thinner than dermis, at only 10-100µm as compared to the dermis (400-2000µm) thickness.

The epidermis possesses dermal projections called appendages (14). These dermal projections, such as the hair follicles, supply a major pool of new keratinocyte from stem cells located within its bulge. Under normal conditions proliferation of the cells occurs only in the basal layer, while differentiation occurs when basal keratinocytes withdraw from the cell cycle and are detached from the basal membrane (17).

2.2 Dermis

The dermis is derived from mesoderm, which forms a stromal layer below the epidermis (18). It is divided into two regions; which are upper papillary dermis and lower reticular dermis (19). Situated in the middle of the three layers, dermis consists mainly of fibroblasts. The fibroblasts secrete components of the complex extracellular matrix (18). Dermis is largely made up of collagen fibers that are synthesized by fibroblasts (19). Most of the collagen fibers found in the dermis are Type I and III, which provide the dermis with tensile strength and mechanical resistance to the skin (19). The dermis has extracellular matrix (ECM) which provides strength to the skin (14,20).

2.3 Hypodermis

Hypodermis is located in the innermost region of the human skin. This region includes components such as connective tissue, fat, and large blood vessels. Thickness of subcutaneous layer is different from one person to another (21). The hypodermis provides mechanical shock protection, insulates the body against external heat and cold and involves actively in general energy metabolism and storage (15).

3. Artificial Skin

Artificial skin has found its use in various applications such as skin grafting, disease skin model and cosmetic testing. One have to consider these characteristics when developing artificial skin; immunologically compatible, has little or no antigenicity, and does not transmit disease (22). Natural and synthetic polymers are used to produce extracellular matrix of artificial skin with mixed results obtained.

3.1 Artificial Skin: Cosmetics and Pharmacokinetics

With the animal ban in place since the EU regulation in 2013, the development of artificial skin for cosmetics testing was rapid due to the strict enforcement from regulators. While acknowledging that not every test is compatible for artificial skin testing, a lot of research and efforts are underway to overcome these limitations. Limitations of commercially available skin substitutes include reduced vascularization, scarring, failure to integrate, poor mechanical integrity and immune rejection (8,23). In this section, we will look at few artificial skin applications in cosmetics and pharmacokinetics.

HPLC/UPLC- spectrophotometry technique was used to study in vitro skin irritation/corrosion of 50 common chemicals used in cosmetics on EpiSkin™ and the result obtained showed that the technique is capable of reproducing endpoint detection system in the MTT-reduction assay (24). Epiderm™ used in the study of in vitro genotoxicity by using reconstructed skin micronucleus (RSMN) assay demonstrates good consistency and reproducibility; allowing the assay alongside the artificial skin to be considered as the potential alternative for cosmetics related genotoxicity testing in future (25). Hu and colleagues have previously confirmed that in a study involving the comparison of EpiDerm™ and human skin with regard to the expression of 139 genes related to xenobiotic metabolism, 87% of the genes were found to have consistent expression in both EpiDerm™ and human skin (26). Another artificial skin, SkinEthic was also studied for its xenobiotic metabolism and it was found to exhibit similar xenobiotic activity with normal human skin in terms of its monooxygenase, esterase, and transferase activities (27). This further proves the reliability of EpiDerm™ in accessing ingredients used in cosmetics setting. It is also important to note that the European Centre for the Validation of Alternative Methods (ECVAM) has validated the use of EpiSkin™ and EpiDerm™ to replace the usual in vivo rabbit skin irritation test (28). In another study; film thickness, surface topography, contact angle, adhesion, frictional and mechanical properties of artificial skin and rat skin were compared when topical cream was applied, with similar degree of results obtained for the tested parameters (29).
A microfluidic skin created with simple bilayer membrane design, was shown to have the ability to produce constant sweat density mimicking the human skin texture and pore size; enabling rapid and repeatable test for skin wearable device (30). The efficient bilayer design helps in providing pore density similar to sweat pores in skin and uniform flow from all pores at low flow rates associated with sweating (30). A simple gravity approach similar to an intravenous drip bag is used as a pumping mechanism to supply constant fluid at a very low flow rate for a continuous perspiration stimulation. Recently for example, many of such devices were developed to enhance user experience while using a diagnostic device.

Figure 2. An artificial skin developed in the laboratory using methyl acrylates. This artificial skin utilized UV curing in polymerizing the acrylates.

Other than treatment for burns and chronic wounds, skin substitutes were utilized as bioreactors in therapeutics delivery and also as replacement models of the human skin for toxicological testing, speeding up drug development and replacing animal-based tests in many industries (31). Artificial skin models were also tested to investigate the potential benefits of botanical extract in protecting and repairing photogaged skin, with positive result obtained in those particular studies (32–34).

In drug development, in vitro skin models are most commonly used to test for systemically acting drugs application and efficacy testing for drugs at site of action (35). In observing transdermal absorption of a topical drug, flurbiprofen in inflamed skin; an artificial skin model made up of silicon membrane was used together with in vivo experiments to study the complex mechanism of inflamed skin and reduce animal use (36). The use of software that can replicate and predict transdermal skin permeation such as SilicoTM (Xeme, Finland) could accelerate future development of skin models and eliminate the need to use animal for testing (37).

Other applications of artificial skin in cosmetics include the use of skin models to measure skin irritation. Furthermore, a few tested skin models were recognized as the standard test for monitoring skin irritation in humans. In a study performed to evaluate zinc oxide irritation, a substance which is increasingly used in cosmetics, found that zinc oxide does not induce skin irritation and is considered safe for use (38). Another study reported a 3D human epidermis skin model, EpiSkin was validated as in vitro model for skin corrosion test (39).

A majority of available artificial skin models are based on normal human skin and very few are able to mimic the diseased/compromised skin (40). More research needs to be done in order to produce reliable artificial skin with the ability to mimic skin disease condition. 3D skin equivalents made from iPSCs derived keratinocytes and fibroblasts, were shown to be capable of mimicking skin disease condition, paving their way for potential use in skin reconstruction, such as postsurgical skin treatment and wound healing. Recently, a bilayer artificial skin model composed of a very soft under-layer, simulating the dermis and hypodermis and a stiffer hydrophilic top layer simulating the epidermis which have dry and moist friction behavior that are highly similar to the human skin was created (41). Skin model were used to study dielectric properties of human skin layers. This development is very important as it will allow non-invasive monitoring of physiological change in skin layers based on database obtained from the study (42).

In addition to the commercially available skin models, artificial skin membrane and skin-mimicking assay are being developed as multi modal approaches in understanding the human skin. A Parallel Artificial Membrane Permeability Assay (PAMPA) that contain components similar to the real human stratum corneum was created by Sinkó and coworkers to measure skin penetration in drug delivery process as well as its potential usage in cosmetics testing (43). An artificial membrane, Strat-M™ (Merck Millipore, USA) has been used
to measure skin permeation (44). Certain aspects such as cell’s 3D behavior, water contact, biological, topographical, and biomechanical cues needs to be considered when ECM is being developed (45).

Apart from collagen and chitosan; bacterial cellulose has emerged as a promising candidate for skin tissue repair because of its biocompatibility, conformability, elasticity, transparency, ability to maintain a moist environment in a wound and ability to absorb exudate during inflammatory phase, give bacterial cellulose advantages in managing wound healing treatment and cosmetics application (46).

3.2 Artificial skin: Grafting

Dermal substitutes are created to mimic the extracellular matrix (ECM) of skin and have the following criteria; ability to restore skin anatomy and physiological function, biocompatible, ability to receive influx of cells that will function as dermal cells, and resistance to shear forces (47). Artificial skin used for wound treatment is commonly made from collagen or fibronectin that are impregnated with human fibroblast and/or keratinocytes to accelerate wound healing process (48). When it comes to using artificial skin in the treatment of burn patient; the size and severity of the burn would largely affect the treatment result (49). Elastin-based scaffold was proposed as an alternative to collagen-based scaffold for dermal substitute application since it has the potential to significantly improve the outcomes of severe burn injury treatment by reducing wound contraction and severe scarring and shortening wound healing period (50).

The synthetic acellular skin substitutes that are most commonly used in burn injuries treatment and chronic wounds are BiobraneTM, IntegraTM, AlloDermTM, and TransCyteTM while DermagraftTM, ApligrafTM and OrCelTM are the most commonly used natural skin substitutes with allogeneic cells (23). There are already quite a number of literatures that were discussing the use of various commercially available artificial skin as skin substitute (10,23,51–53). In this paper we only mention few of these commercially available skin substitutes relevant applications because we found that it is impossible to provide a comprehensive review of all skin substitutes found in the literature.

A study done by Weigert and coworkers using Integra as skin reconstruction material found that the skin substitute was durable, functional and capable of giving aesthetic coverage in most of the applied skin reconstruction when dealing with severe hand wounds with exposed tendons and/or bone and/or joint in patients follow up ranging from 10-37 months (54). Furthermore, artificial dermis (Integra) was also used as an alternative to flap surgery in the treatment of tendon-exposed wounds, with an overall wound closure rate of 82% obtained, making artificial dermis a viable alternative to the conventional flap surgery treatment which is commonly associated with donor site morbidity and the bulkiness of treated tendons (55). Integra is used in a two-stage procedure in treating patient undergoing radial forearm free flap reconstruction (RFFF) instead of one-stage FTSG. Patients who have undergone Integra grafting followed by STSG placement demonstrated favorable functional outcomes when compared with prior reports of patients who were undergoing primary STSG or FTSG, as well as having superior aesthetic outcomes on the graft site (56).

The post-surgery efficiency (up to 6 months) of Integra dermal regeneration template single layer and split thickness (IDRT-SL) skin treatment for deep facial surgical wounds in elderly multimorbid patients was observed by Koenen and coworkers; and they concluded that the IDRT-SL treatment was a reliable and rapid method for the deep wounds closure in elderly patients with complicated comorbidity (57). A technique that combines cutaneous skin grafting with IDRT to treat skin avulsion injuries demonstrated reduced postoperative immobilization, minimized donor site morbidity and provides good functional and aesthetic result in the twelve-month post-surgery assessment (58).

The use of Integra dermal regeneration template was also applied in degloving injuries, where 90% of tested patients showed excellent post-surgery cosmetic and post-surgery functional results (59). In a study to compare the efficiency of three different methods to cover excised burn wounds; Integra, split thickness skin graft (STSG) and a viscose cellulose sponge Cellonex; fair equally in clinical appearance, histological and immunohistochemical findings (60). Integra was also used to improve postburn scars in patients with contractures and/or hypertrophic scars from previous treatment; with improved function and aesthetics of skin reported after one year of surgery (61). A study done by Hur and colleagues found that scars size in graft patients reduced after Matriderm in the split thickness autograft treatment of 40 patients with acute burn wounds or scar reconstruction (62). Elastin, one of the ingredients in Mariderm, helps regulate collagen contraction, interrupt differentiation of fibroblasts, and reduces scars formation (62,63). Integra was used in the treatment of giant congenital melanocytic naevi (GCMN) in children, a rare condition where a large area of the body is covered by dark pigmentation or moles which cause the patient to have a higher risk of melanoma by 2.3%-10% (64–69). The affected skin area was removed down to the subcutaneous fat and Integra was then attached to the exposed area. Results after follow up which range from six months to four years showed the high take rate of Integra (95%-100%) and excellent functional and cosmetic outcome; suggesting the use of Integra as a viable alternative in the treatment of GCMN (64).
Apart from Integra other skin substitutes are also widely used; which will be mentioned below. Matriderm was found to aid in tissue regeneration and wound closure of patients with post-traumatic wounds when compared with patients treated with only autologous skin graft (70). Faster re-epithelialization was observed during treatment which means Matriderm significantly improve functional and aesthetic outcomes of skin grafting (70). Another study used Matriderm along with unmeshed skin graft to treat full-thickness skin defects in the hand and wrist area, demonstrated high biocompatibility of Matriderm (96% take rate), no limitation of hand function (excellent pliability) and minimal scarring. Thus making Matriderm a viable alternative for skin defect treatment in upper extremity regions (71). A study done over 24-week period in patients who underwent skin reconstruction with either two artificial dermal substitutes (Integra and Hyalomatrix) found better cell regulation, regenerated extracellular matrix. Neoangiogenesis was observed with Hyalomatrix application while more elastic regenerated and overall better physical, mechanical, and optical properties were observed with Integra application (72). Matriderm, another dermal substitute brand, showed stability and minimum complications as well as restoring skin elasticity and skin barrier in a study involving patients with full thickness skin defects who underwent autologous split thickness skin graft with Matriderm (73).

One-stage artificial dermis and skin grafting method was also tested, instead of the conventional two-stage procedure. Increased dermis maturity was shown together with increased fibroblast number and blood supply, demonstrating the technique efficacy (74). In a clinical trial involving 10 patients with severe burns, Matriderm was found to have a significant increase in the objective skin elasticity parameters when Matriderm was applied in one-step procedure (synchronous) instead of two-stage procedure during the full-thickness wound treatment (75). In a study done by Bertolli and colleagues, Matriderm was suggested as the alternative to skin grafting in aiding the margin assessment of patient with dermatofibrosarcoma protuberans (DFSP), a locally invasive neoplasia (76). Recent development has seen the invention of VitriBand, a cell-free bandage made up of silicone-coated film that would be vital in emergency treatment of skin related injuries. The bandage is easy to handle, supplies ECM components to the wounded tissue, and does not cause pain during removal (77). In another study, Alloderm was suggested as one of the alternative to conventional split-thickness skin graft (STSG) as it formed thicker coverage of the affected site apart from having minimal donor site morbidity and superior cosmetic results as compared to STSG alone (78). Researchers also developed their own version of skin substitutes to be used in wound treatment. Yamashita and coworkers proposed a two-step technique which used artificial dermis grafting to treat patients with Hidradenitis suppurativa disease (79). By using a bilayer artificial skin in venous leg ulcer treatment, it was observed that patients showed a better healing chance from the treatment as compared to patients that were treated with only dressing and compression (80). An autologous fibroblast-seeded artificial dermis, created without any animal derived supplement, has shown a comparable healing result with commercially available skin substitutes in treating diabetic foot ulcers (81). Integra was used along with mesenchymal stem cells (MSCs) in a study done on a murine model to treat full thickness burn; which neovascularization was observed in the murine model suggesting the immunoregulatory properties of MSCs may be useful in treating skin wounds (82). MSCs was also used in combination with collagen-glycosaminoglycan (GAG) scaffold to treat deep partial thickness burn wounds on porcine model showed better healing and keratinization, less wound contraction and more vascularization when compared with burn wounds treated with collagen-GAG scaffold only (83). A study utilizing vacuum-assisted closure (VAC) system to treat patients with complex tissue defects by using a combination of STSG and either one of the two brands of artificial dermis (Pelnac and Teruderm) found a lesser waiting period (an average of 7.64 days instead of 2-3 weeks recommended by the company), minimum risk of infection and simpler wound care were observed during treatment (84).

While overcoming inadequate uninjured donor sites and severe scarring in split-thickness skin grafting which is considered as today’s gold standard (85), skin substitutes have to face its own shortcomings such as lack of important structures and cell types (e.g. sebaceous gland, Langerhans cells) and also limited/specific applications in wound treatment system (86,87). Artificial skin focused mainly on mimicking the exact function of the human skin whereas a majority of electronic skin (e-skin) produced attempt to measure human activities based on tactile capabilities of the e-skin. Today with rapid technological advance, it is expected that the gap between artificial human skin and e-skin will be closer in the upcoming years. In the next section few of the main applications of e-skin will be briefly mentioned in order to provide a better understanding of e-skin.

4. Electronic skin (e-skin)

Touch sensing has undergone rapid development since its introduction in robotic field in 1970s, where the earliest stage of development was mainly focused on internal touch sensors until the introduction of tactile sensing/external touch sensing in 1980s (88–90). The rapid advancements of electronic skin capabilities to match the thickness, elastic moduli, and stiffness of the human skin means there is a great potential for e-skin to directly measure human activities (91,92). Ultrathin electronic skin that could be directly applied to the skin would increase the mechanics and robustness of the device (93). Highly desirable characteristics of e-skin includes; biocompatibility, biodegradability, self-healing, temperature sensitivity, and self-powering (94). In developing flexible ultrathin material, researchers must
not overlook the importance of stretchability and self-healing ability of the e-skin as these are vital in creating e-skin with better characteristics of the actual human skin (95). Plastics films are very promising platforms to develop e-skin particularly because they have good mechanical strength, cheap and could be produced in large sheets (96).

In this review paper, we mentioned a few applications of e-skin in various problem-solving approaches to understand the mechanism underlying e-skin and its potential usage better.

Kim and coworkers demonstrated an ultrathin single crystalline silicon nanoribbon (SiNR) which was integrated with mechanical, temperature, and humidity sensors that in turn produce high sensitivity, wide detection ranges and good mechanical durability prosthetic systems (97). An ultrathin e-skin with resistive tactile sensors was developed by using a 1 µm thick polyethylene naphthalate (PEN) foil as a substrate (98).

Zhao and coworkers demonstrated a wearable e-skin composed of PET-based Ag serpentine-shaped electrodes and dielectric layer that can sense contact, pressure, and strain with fast response and shows insignificant resistance changes under stretching, compressing and torsions (99). A compressible silicone foam was utilized as the dielectric and stretchable thin-metal films to develop e-skin with human-like pressure sensitivity around the finger (100), which shows that the development of hand prosthesis with integrated sensing capabilities is just around the corner.

To resolve issues relating to signal interference by mechanical deformation or other stimuli; flexible field-effect transistor (FET) sensor platform was used in designing e-skin that could detect pressure and temperature simultaneously (101). A monolayer capped nano particles (MNCP) e-skin could be produced with large area deposition techniques and multiple sensors, and in addition the sensitivity of the sensor could be tailored by adjusting the substrate thickness (102). A combination of piezoelectric nanogenerators and coplanar-gate graphene transistors was used to develop an e-skin with strain sensor that operates at extremely low voltages, representing a significant development in e-skin studies (103).

A facile galvanic replacement reaction was introduced to obtain greater oxidation resistance on the surface of copper nanowires that serves as conductive fillers for e-skin, which in turn produce high performance e-skin that could be further applied into voice recognition, wrist pulses monitoring and spatial distribution of pressure detection (104). Self-healing composite use conductive fillers in e-skin to mimic the pressure sensitivity and mechanical self-healing of the human skin; which in turn enable the e-skin to have increased initial conductivity efficiency and enhanced mechanical properties (105). The combination between microstructured polydimethylsiloxane (PDMS) dielectric and a thin polymer semiconductor resulted in a flexible plastic pressure-sensitive transistor of e-skin, and improves sensitivity and response time (106).

Figure 3. a) Electronic skin with active-matrix strain sensor array. The inset shows an optical microscopy image of the patterned graphene on a PET substrate. Use with permission from (103). b) User-interactive electronic skin. Organic light-emitting diodes (OLEDs) are turned on locally when the surface is touched, with intensity of light corresponds to the magnitude of applied pressure. Use with permission from (107).

The use of multimodal sensing of organic field-effect transistor (OFET) to mimic multiple layers and constituents of skin resulted in high pressure sensitivity and short mechanical relaxation time of the e-skin (108). Wang and coworkers introduced the first user interactive e-skin that could provide instant response to the magnitude of applied pressure by looking at the intensity of organic light-emitting diodes (OLEDs) that were turned on (107).
E-skin consisting of nanomaterials, such as graphene nanosheets was also developed, producing highly conductive and compressible all-graphene thin film sensor that could sense temperature variation, recognize human touch, and enable human touch locating and pressure level measuring under zero working voltage (109). A low cost graphene-polyurethane piezoresistive sensor was fabricated based on fractured microstructure design which produce high pressure sensitivity, long cycling life, and ability to be produce in large scale fabrication (110). A flexible and high sensitivity e-skin was made by using graphene that could measure human motion detection, acoustic signal acquisition and spatially resolved monitoring of external stress distribution (111). Graphene and polymers were integrated into thin films to produce e-skin that is able to mimic the mechanical self-healing and pressure sensitivity of the human skin without requiring any external power supply (112). There is also a CNT-composite-based elastomer film with interlocked microdome arrays and have giant tunneling piezoresistance which resulted into high sensitivity e-skin to pressure and rapid response/relaxation times that is minimally affected by temperature variation (113).

MoS$_2$ semiconductors was proposed to replace nanomaterials and hybrid composites based sensors as MoS$_2$ possesses great mechanical and optical transmittance, high gauge factor, and tunable band gap over the other sensing materials (114). In pharmacokinetics, a novel electronic skin patch was tested on rats to study the transdermal iontophoresis of donepezil, an oral medication for the treatment of Alzheimer’s disease (115).

In e-skin development, a lot of emphasis is given in using the most flexible and thinnest film possible to create the device. However, sensor fabrication methods must also be taken seriously as it has major impacts in the outcome of e-skin. A technique to fabricate the foldable sensors called screen printing, is shown to be practical and cost effective as it allows large amount of sensors (64 sensors) to be fabricated in one flow without sacrificing much on its piezoelectric properties, thus allowing the sensors to be used in low cost e-skin applications (116).

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Table 1. Characteristics of e-skin in different type of applications
5. Animal use in experiments

Humans always put themselves as the superior of all kinds, being the most complex animals that we are. The concept of sentience in animal is argued by both philosophers and scientists. Rene Descartes, the Enlightenment philosopher stated that animals are just like machines that can move and make sounds but have no feelings (123). As time goes by, there are very significant changes in our attitudes towards animal welfare. The sustainability of a system or procedure must take into account the resource availability, consequences, and morality of action (124). Rationale for involving animals and the appropriateness of the numbers and species used are among the guideline criteria in animal testing (125). In a study done in 2014, they found that consumers in both developed and developing countries are concerned on issues surrounding ethical and animal welfare when it comes to buying cosmetics products (126).

Based on the 2003’s European Directive 86/609/EEC and the 7th Amendment to the Cosmetics Directive 76/768/EEC; animal testing for cosmetics ingredients was banned since 2009 except for repeated dose toxicity tests until 2013 (127,128). There is also a report that details the lack of alternative method for animal testing in five main areas of cosmetics testing, which are toxicokinetics, skin sensitization, repeated dose toxicity, carcinogenicity, and reproductive toxicity (129). An estimated five billion cosmetics items are sold per year in the EU by 2000 companies (130).

The introduction of 3Rs (Refinement, Reduction, and Replacement) has met with some success. The ban put in place really helps in transforming artificial skin development in a way that larger amounts of capital are poured to search for an alternative to animal testing. The critics said that human safety may be jeopardized as the result of rushing implementation of the ban without fully understanding the complexity and dynamics of all fields involved (131). Skin testing such as 4-h patch test was done in humans where the risks are fairly low and the safety of the test when performed in humans is well documented (132). Outside the EU region, there are ethical committees as well to oversee the handling and ethical use of animals in research. Some of these committees are Institutional Animal Ethics Committees (Canada), Institutional Animal Care and Use Committee (USA), and Ethics Committee (Australia) (133).

In skin grafting, commercial products that are readily available in the market do not have to go through the same regulation as artificial skin intended for cosmetics testing. Different from artificial skin for cosmetics testing, artificial skin for grafting have various characteristics and caters to different types of grafting; most importantly it is directly applied on the patient himself. For e-skin development, most of the e-skin developed involved the monitoring of tactile sensing in the device. The parameters tested, such as strain and pressure sensitivity of the skin, are usually based readily available data. This eliminates the need for e-skin to be tested with animal. While the law regarding the use of animal for e-skin testing is unclear because the field is still considered relatively new, it is expected that the same law applied to human artificial skin will also apply to e-skin in future, because the mimicking of the actual human skin is getting better and more accurate.

6. Current challenges and limitations

There are many challenges faced by researchers in developing skin substitutes. Apart from regulations and ethics, there are many other issues that have to be considered when developing skin substitutes. Although e-skin is not subjected to the same regulations for artificial skin, there are still many challenges that lie ahead in developing an ideal e-skin for use.

The complexity of human skin proves to be a continuous challenge for researchers. The commercially available artificial skin is still far from providing a complete and feasible solution for patient in need of grafting. Aspects concerning the vascularity and ECM of the skin have to be improved to cater for different patient needs. The combination of biologically active and automated tissue printing technique provides assured quality and quantity of the skin substitute produced (85). However, there lies one main obstacle which is the cost to accelerate the use of artificial skin in grafting. For a patient, high cost of artificial skin means only some will get the treatment while for a manufacturer, smaller manufacturing scale means less distribution of cost, thus the higher costs incurred. There are still works to be done in enhancing skin model so that it is more responsive to particular conditions such as traumatic injury and wound damage (134).

Materials such as graphene, CNT, and nanowires exhibit excellent capabilities to be developed as e-skin sensors (135). However the high cost of these materials proves to be a hurdle when developing the sensors. The rapid development of multifunctional e-skin is much needed news for the industries as different sensors can be inserted in just one thin layer of e-skin, catering to different applications (7). Other than multifunctional sensors, researchers also have to consider the integration with transistors to develop active matrix and signal amplification better (136). While acknowledging flexibility and stretchability as the most desired aspects of e-skin, real-time monitoring will enable tactile information to be used in the system control loop, thus providing more control to users (137).

7. Recommendations and potential applications
Skin research is a field that still has a lot of improvements to be made. The combination of natural and synthetic material can be useful in fabricating artificial skin that has adjustable characteristics and customizable applications. Recent development of artificial blood from hematopoietic stem cells should be greatly welcomed by artificial skin researchers as it opens up many possibilities ahead in this field of research. Artificial skin that is able to mimic the vascularity of the human blood vessels and surrounding ECM will enable it to be used on patient with amputated limbs, thus eliminating the need for obtaining transplant from other recipient.

In skin grafting, the requirement for the injured area should be considered before beginning the treatment. For example, STSG may require different type of skin substitute from FTSG as different layers of skin are involved in the injury. The use of suitable skin substitute can shorten the recovery time, allow for better vascularization, and better overall aesthetic outcomes on the treated area. Functionality aspects such as the pliability of treated hand injury should be monitored closely to increase the quality of life of patient who underwent the treatment.

Ethical aspects of handling animal should always be followed, even in region where the use of animals for cosmetics testing is not banned yet. There should be greater emphasis on ethics in dealing with animal in experiment as well. The 3Rs concept (replace, reduce, refine) is a good guideline to follow when dealing with animal in experiment. With the increasing awareness of animal rights, we can expect tougher regulations and better handling of animal use for experiment.

For e-skin, while it does not fall under the same regulations as the artificial skin; the tactile sensing needs to be more adaptive and responsive to surrounding environment. The flexibility and thinness of the sensor film provide e-skin with brighter prospect for it to be used as the main diagnostic tool in accessing health conditions in the future. Sensor sensitivity can be optimized so that it will mimic the sensitivity of the specific limb exactly and enabling it to be used on the artificial limb. This will provide an alternative to more expensive and complex robotic limbs in the market.

Real-time monitoring is vital in measuring health conditions of the patient as it will give an insight of what happening in the body at the specific time and this would allow for faster response and better treatment of the patient. Embedded e-skin with long lasting power or capability to be charged wirelessly will provide real-time monitoring and enable health practitioners to give a better diagnosis to the patients. Embedded e-skin is also very useful in helping us understanding certain health conditions better, as certain rare health condition or disease are not fully understood yet by us. Furthermore, the use of a new type of material, such as graphene, can provide the e-skin with longer durability and practicality to be mass-produced thus lowering the cost of the e-skin. Real-time disease monitoring, ultra-sensitive disease detection, and interactive health monitoring are some of the potential that e-skin hold for us in future.

Conclusion

The development of artificial skin evolved greatly since its first introduction in the 1980s. Artificial skin enables researchers to gain valuable insights into various biochemical and physiological reactions happening within the skin. The combination of natural and synthetic materials in developing artificial skin has tremendously improved the overall quality of artificial skin or skin model produced. However, we are still looking for artificial skin models that have the following characteristics; fully mimicking the human skin, fast healing, cost effective, fully compatible and feasible to be applied in current settings of a majority of hospital today.

The current skin substitute used for grafting are usually combined with autologous skin to produce better cosmetics and functional results. With increased reliability and functionality of skin substitutes, it is hoped that no more autologous skin will be used for skin grafting in future. Therefore, scarring will be minimized and better aesthetics of the recovered skin would be obtained after the treatment. Efforts are being taken to reduce the recovery time and degree of pain in patient who undergo skin grafting. Skin substitutes used as replacement for the conventional autografting needs to be more reliable and consistent in producing results; while still remain affordable for general public. More research needs to be done in order to accelerate the development of skin substitute, such as by having larger pool of patients who will undergo the wound healing treatment. Recent development is very positive as the healing time, functionality of the skin post-surgery and severity of scarring are greatly improved.

E-skin represents our advancement and futuristic way in tackling the human skin mimicking problem. Different sorts of materials used to make the e-skin, which caters a multitude of functions, provides them with flexibility and adaptability in solving the problem that lies ahead in future. E-skin made with new type of material such as graphene represents ongoing innovations to improve the overall qualities of e-skin. The race to use thinner and more flexible material would ultimately bring greater adaptability to the devices created. High sensitivity sensors improve the tactile sensing of e-skin, giving a more ‘human feel’ to the device. Flexible ultrathin material paired with ultra-sensitive sensors embedded within skin allows real-time monitoring of the subject, thus improving the quality of results obtained by e-skin. With the current rate of developments in place, we are very optimistic that artificial skin capable of fully mimicking the human skin would be in market in the upcoming years.

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a) Figure 1. Normal skin structure.
b) This figure shows the two main layers; epidermis and dermis with other basic components of the human skin structure.
Figure 2. An artificial skin developed in the laboratory using methyl acrylates. This artificial skin utilized UV curing to polymerize the acrylates.

Figure 3. a) Electronic skin with active-matrix strain sensor array. The inset shows an optical microscopy image of the patterned graphene on a PET substrate b) User-interactive electronic skin. Organic light-emitting diodes (OLEDs) are turned on locally when the surface is touched, with intensity of light corresponds to the magnitude of applied pressure.
<table>
<thead>
<tr>
<th>Application(s) of electronic skin</th>
<th>Material</th>
<th>Fabrication technique</th>
<th>Advantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voice recognition and real time wrist pulse detection</td>
<td>PDMS and Single-walled carbon nanotubes (SWNTs)</td>
<td>Combination of micro-patterned PDMS and SNWTs</td>
<td>Fast response time, great stability and repeatability</td>
<td>(117)</td>
</tr>
<tr>
<td>Optical and pressure sensor</td>
<td>PDMS</td>
<td>Mechano-optical transduction</td>
<td>Peak sensitivity suitable for touch interfaces</td>
<td>(118)</td>
</tr>
<tr>
<td>Real time radial artery waveforms monitoring</td>
<td>Copper 7,7,8,8-tetracyano-p-quinodimethane (CuTCNQ)</td>
<td>Conventional double layer structure</td>
<td>Very high sensitivity and stability; fast response time; low detection limit; working voltage and power consumption.</td>
<td>(119)</td>
</tr>
<tr>
<td>Multidirectional force-sensing testing</td>
<td>PDMS and Multi-walled carbon nanotubes (MWNTs)</td>
<td>Thermal curing</td>
<td>Ability to distinguish different mechanical stimuli and ability to identify intensities and directions of air flows and vibrations</td>
<td>(120)</td>
</tr>
<tr>
<td>Optical pressure sensor</td>
<td>PDMS</td>
<td>Embossed gratings</td>
<td>High sensitivity and tolerance to bending</td>
<td>(121)</td>
</tr>
<tr>
<td>Detection of extremely small stimuli such as minute vibration and sound stimuli</td>
<td>PDMS and ZnO nanowires</td>
<td>Thermal curing</td>
<td>High sensitivity and able to detect high frequency vibration (250 Hz)</td>
<td>(122)</td>
</tr>
</tbody>
</table>

a) Table 1. Characteristics of e-skin in different type of applications
b) This table shows different applications of e-skin with various fabrication techniques applied and also advantages of each one of them.