

**PREPARATION AND CHARACTERIZATION OF COLLAGEN
GRAFTED POLY(DIMETHYL SILOXANE) MODIFIED FILMS
FOR SKIN SUBSTITUTE APPLICATIONS**

BY

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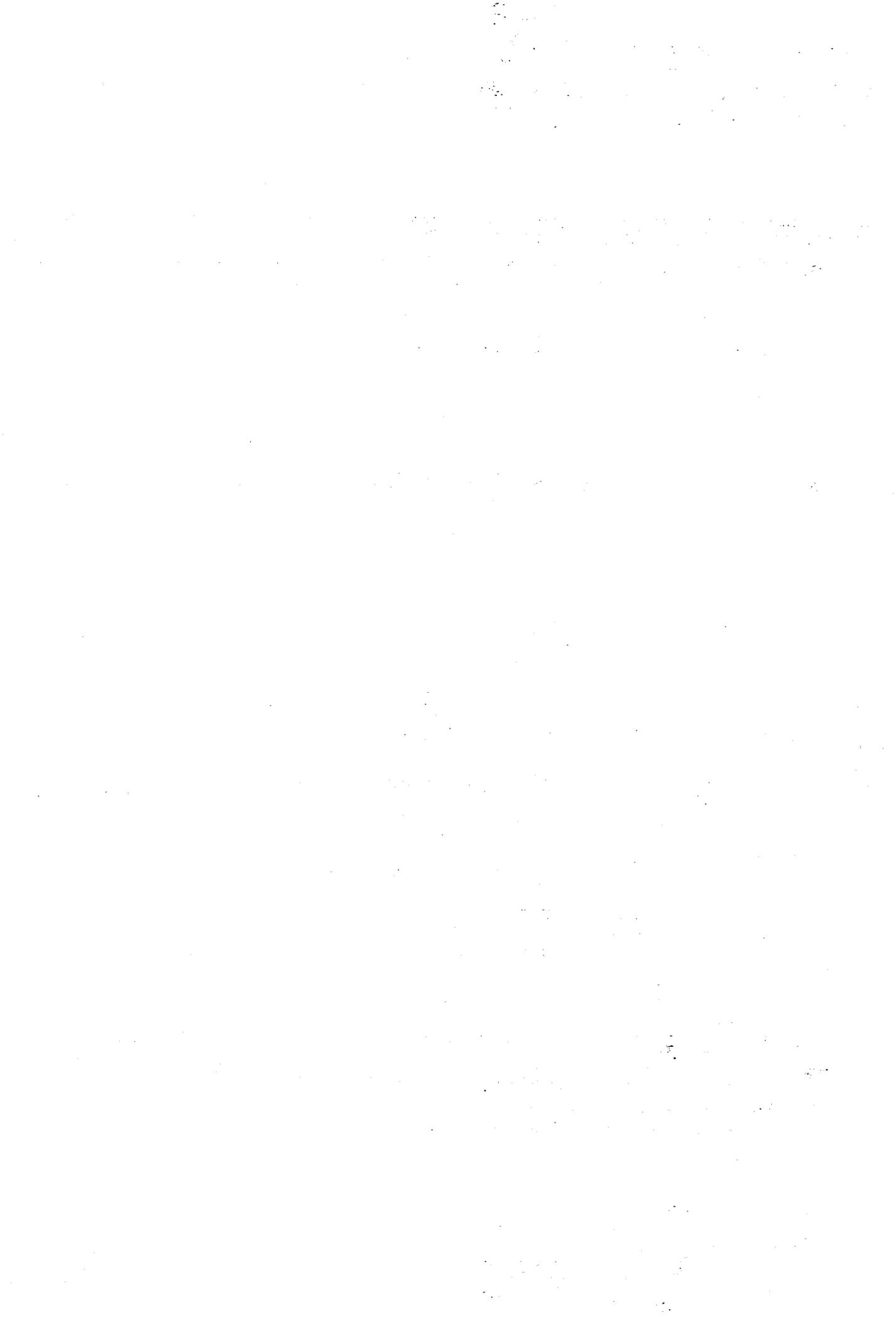


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LIST OF ABBREVIATION

AAC	Acrylic acid
AAM	Acrylamide
AIBN	Azoisobutyronitrile
AN	Acrylonitrile
ANOVA	Analysis of variance
ASTM	American society for testing and material
ATR-IR	Attenuated total reflectance infrared
CDI	Carbonyldiimidazole
COL	Collagen
DMEM	Dulbecco's Modified Eagle's Medium
DMTA	Dynamic mechanical thermal analysis
DOE	Design of experiments
DSC	Differential scanning calorimetry
ECM	Extracellular matrix
EGDMA	Ethyleneglycol dimethacrylate
EGF	Epidermal growth factor
EMA	Ethylene methacrylate
EPR	Ethylene propylene rubber
ESCA	Electron spectroscopy for chemical analysis
FTIR	Fourier transform infrared
F-value	Fisher's F value
GAGs	Glycosaminoglycans
GMA	Glycidyl methacrylate
IPN	Interpenetrating polymer network

L929	Mouse fibroblast cell line
NIPA	N-isopropyl acrylamide
NVP	N-vinyl pyrrolidone
NWF	Nonwoven fabric
OXT	Oxytetracycline
PAA	Poly(acrylic acid)
PBS	Phosphate buffer saline
PCL	Poly(caprolactone)
PDMS	Poly(dimethyl siloxane)
PEG	Poly(ethylene glycol)
PGA	Poly(glycolic acid)
PHEMA	Poly(hydroxyethyl methacrylate)
PLA	Poly(lactic acid)
PMMA	Poly(methyl methacrylate)
PRESS	Prediction error sum of squares
PVA	Poly(vinyl alcohol)
P-value	Probability value
RF	Radio frequency
RSM	Response surface methodology
SD	Standard deviations
SEM	Scanning electron microscopy
UHMWPE	Ultrahigh molecular weight polyethylene
UV	Ultra violet
WSC	Water soluble carbodiimide
XPS	X-ray photoelectron spectroscopy

LIST OF SYMBOLS

eV	Electronvolt for binding energy
g	Gram
h	Hour
Hz	Hertz
IU/ml	Unit per millimetre
kPa	Kilo Pascal
M	Molar
min	Minute
MPa	Mega Pascal
nm	Nanometer
tan δ	Loss factor
T _g	Glass transition temperature
v/v	Volume/ Volume
w/w	Weight/ Weight
wt%	Weight percentage
μm	Micron meter
γ	Interfacial surface free energy
θ	Contact angle
°	Degree
°C	Degree Centigrade
E'	Storage modulus
w _o	Weight of dry sample
w _w	Weight of swollen sample

**PENYEDIAAN DAN PENCIRIAN FILEM KOLAGEN TERCANGKUK POLI
(DIMETIL SILOKSANA) TERUBAHSUAI UNTUK APLIKASI PENGGANTI
KULIT**

ABSTRAK

Poli(dimetil siloxana) (PDMS) filem yang berpusat diubah dengan menggunakan hidrogel dan kolagen untuk menghasilkan pelbagai jenis pengganti kulit. PHEMA (poli(hidroksietil metakrilat)) dan PAA (poli(asid akrilik)) digunakan melalui kaedah jujukan IPN (interpenetrating polymer networks) dan kaedah campuran bagi menghasilkan suatu kumpulan fungsi yang sesuai untuk menempatkan kolagen pada lapisan luar PDMS, dalam usaha meningkatkan kebioserasian mereka. Kolagen (jenis I) dipautkan secara kovalen pada saput yang telah diubah suai dengan menggunakan kopel agen untuk mengaktifkan kumpulan hidroksil pada PHEMA dan karboksil pada PAA. Kolagen bertindak balas dengan kumpulan teraktif untuk membentuk lapisan protein yang terpaut secara kovalen. Sifat permukaan saput yang tidak diubah suai dan yang diubah suai dapat dilihat melalui ATR-FTIR (pantulan jumlah perosotan - spektroskopi inframerah jelmaan Fourier), XPS (spektroskopi fotoelektron sinar X) dan SEM (mikroskopi imbasan electron). DMTA (analisis haba mekanikal dinamik), ujian regangan dan ukuran kekuatan koyak digunakan untuk menyelidik sifat fizikal dan mekanikal sampel yang diubah suai. Ukuran jerapan air dan sudut sentuh air dijalankan pada sampel untuk menilai kebolehbasahan permukaan. Berdasarkan teknik pencirian yang digunakan, didapati bahawa sampel daripada kedua-dua teknik (IPN dan kaedah campuran) lebih hidrofilik dibandingkan dengan kandungan hidrogel. Cantuman kolagen pada permukaan terbukti melalui ATR-FTIR dan XPS. Mikrograf SEM bagi keratan

rentas menunjukkan wujudnya dua sistem fasa pada rangkaian kedua dalam kaedah IPN yang bertambah dengan meningkatnya gabungan PHEMA dan PAA. Sifat mekanikal PDMS yang diubah suai dengan PHEMA tidak berubah sehingga 30% berat daripada kandungan PHEMA, dibandingkan dengan sampel tulen. Walau bagaimanapun, kesan pengukuhan diperhatikan pada sampel PDMS/PAA IPN sehingga hampir 25% berat PAA. Keputusan DMTA menunjukkan terdapat dua T_g s dalam sistem IPN, dan sampel ini adalah system polimer fasa 2. Sampel campuran juga merupakan sistem dwikomponen. Sebaliknya, di dalam PDMS dan hidrogel, ia adalah fasa yang selanjat dan terserak. Penilaian kebioserasian bagi PDMS yang tidak diubah suai dan diubah suai diteliti dalam penilaian in vitro melalui pengkulturan sel fibroblas (L929) pada permukaan mereka. Sampel PDMS yang diubah suai lebih serasi dengan sel fibroblas dibandingkan dengan sampel tulen. Ia juga tidak menunjukkan sebarang kesitotoksikan. Permukaan berkalogen secara signifikan menunjukkan rekatan dan pertumbuhansel dan pertumbuhan berbanding dengan sampel IPN dan sampel campuran, yang kekurangan kolagen.

**PREPARATION AND CHARACTERIZATION OF COLLAGEN GRAFTED
POLY(DIMETHYL SILOXANE) MODIFIED FILMS FOR SKIN
SUBSTITUTE APPLICATIONS**

ABSTRACT

Poly(dimethyl siloxane) (PDMS) based films were modified using hydrogels and collagen to produce different types of skin substitute. Poly(hydroxyethyl methacrylate) (PHEMA) and poly(acrylic acid)(PAA) were used via sequential method of interpenetrating polymer networks (IPNs) and blending methods to create suitable functional groups to immobilize collagen in outer layer of PDMS in order to enhance their biocompatibility. Collagen (type I) was covalently linked onto the modified films via coupling agent to activate the hydroxyl groups of PHEMA and the carboxylic groups of PAA. Collagen reacted with the activated groups to obtain covalently linked protein layers. The surface properties of unmodified and modified films were characterised by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM). Dynamic mechanical thermal analysis (DMTA), tensile testing and tear strength measurement were used to investigate the physical and mechanical properties of the modified samples. Water adsorption and water contact angle measurements were performed on the samples in order to evaluate their surface wettability. The aforementioned characterisation techniques indicated that both IPNs and blends samples exhibited more hydrophilicity according to hydrogels content. Grafting of collagen on the surfaces were confirmed using ATR-FTIR and XPS. The SEM micrographs of the cross section demonstrated the appearance of two-phase system that area phases of the second network in IPN method increased with

increasing incorporation of PHEMA and PAA. The mechanical properties of modified PDMS with PHEMA did not change considerably up to 30 wt% of PHEMA content in comparison to pure samples. However, a reinforcing effect was observed at PDMS/PAA IPNs samples up to approximately 25 wt% PAA. The DMTA results indicated that there are two T_g s in the IPN systems and these samples are two-phase polymeric systems. The blends samples also are a bicomponent system, wherein the PDMS and hydrogels are the continuous and dispersed phases, respectively. The biocompatibility evaluations of unmodified and modified PDMS were investigated in in-vitro evaluation by culturing fibroblast cells (L929) on their surfaces. The modified PDMS samples were more compatible with fibroblast cells than the pure samples and did not show any cytotoxicity. The collagen grafted surfaces showed significant cell adhesion and growth in comparison with IPNs and blends samples which were lack of collagen.

CHAPTER 1

INTRODUCTION

1.1 Polymeric biomaterials

The development of polymeric biomaterials can be considered as an evolutionary process. Reports on the applications of natural polymers as biomaterials date back thousands of years (Barbucci, 2002). However, the application of synthetic polymers to medicine is more or less a recent phenomenon. The use of polymeric biomaterials as we know them today started in the 1940s during the Second World War (Castner and Ratner, 2002). Recent advances in polymeric biomaterials have been focused towards solving problems of patients who have suffered tissue, organ loss and defects (Hu et al., 2003).

Many natural and synthetic or their hybrid matrices have been developed to cover wound sites, replace lost tissue functions and support cell growth. For example, aliphatic polyesters such as poly(lactic acid) are versatile biomaterials due to their biodegradability and biocompatibility (Moon et al., 2000; Moon et al., 2001; Shinoda et al., 2003). Synthetic matrices have many advantages because their molecular design, mechanical and physical properties can be controlled and they can be manufactured on any scale. However, the usage of synthetic biomaterial is still limited because of poor cell attachment/growth, adsorption of proteins and induction of thrombogenesis on the surface. Furthermore, some of the synthetic polymers are difficult to modify due to the lack of sufficient reactive functional groups. On the other hand, natural substrates such as proteins have been extensively used because of excellent biocompatibility and bioactivity (Lee et al., 2001).

Many attempts for establishing novel biomedical applications have been studied by modification or combination of natural polymers including proteins e.g. collagen (Wang et al., 2003; Wang et al., 2006), cellulose (Ishihara et al., 1992), alginate (Knill et al., 2004; Lopez et al., 1997), chitin, and chitosan (Mori et al., 1997). The use of biomaterials to interface with living systems, such as fluids, cells, and tissues of the body, has played an increasingly important role in medical applications. In particular, synthetic and natural polymers, metals, ceramics, composites, and tissue-derived materials have been applied in medicine and pharmaceuticals. These materials can be used in the permanent replacement of defective organs and tissues, temporary support of defective or normal organs, storage and purification of blood, and also control of drug delivery.

The minimum requirements for biomaterials are: non-toxicity, functionality, sterilisability, and biocompatibility. Among these requirements, biocompatibility is essential issue for biomedical applications (Ratner et al., 2004). Polymeric biomaterials are relatively easy to manufacture into products with various shapes, at reasonable cost, and with desirable mechanical and physical properties. However, one of the major factors limiting the use of these materials is their biocompatibility. A challenge is thus to enhance their biocompatibility, at least at the interface with host tissues and fluids. Depending on the intended medical application, all biomaterials are evaluated in terms of biocompatibility. In particular, the design of biocompatible synthetic surfaces that are able to control the interaction between a living system and an implanted material remains a major theme for biomedical applications. The effects of the chemical structure and the surface properties of polymer biomaterials that influence their biocompatibility include: i) the interfacial free energy, ii) balance between the hydrophilicity and the hydrophobicity on the

surface, iii) the chemical structure and functional groups, iv) the type and the density of surface charges, v) the molecular weight of the polymer, vi) conformational flexibility of the polymer, and (vii) surface topography and roughness (Xiong and Bokor, 2004).

1.2 Biocompatibility

The ability to replace or augment damaged organs, blood vessels, tissues, totally or in part, has improved both the quality and the length of life of many people. The decline in surgical risk during recent decades has encouraged the development of more complex procedures for prosthetic implantation. The availability and suitability of traditional natural (autogenous, homogenous) prosthetic element is severely limited, as a result, strong interest has been focused on the use of synthetic materials which would provide an asymptomatic, long term function within the body or in contact with body fluid.

A biomaterial is defined as a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body (Williams, 1999). The study of biomaterials involves investigations into their relevant characteristics, i.e., their mechanical, thermal, electrical and especially surface properties, for the surface is in contact with living tissues. Thus, the study of these surfaces is crucial to determine their biological behaviour and to evaluate their hydrophobic or hydrophilic character (Ratner et al., 2004). Biomaterials are used to manufacture prostheses, implants, and surgical instruments. They can be natural such as collagen, cellulose or synthetic such as metals, ceramics, polymers, and others. Employed in plastic and reconstructive surgery, used to make the tools needed to

examine the human body, and expected to improve the deficiency of an organ, biomaterials must be biologically compatible with the organism. Many biomaterials have been used due to their bioinertness or bioactivity (Park et al., 1995; Hench, 1998; Galletti, 1995) depending on the specific aim, but also on availability. At present, research on biomaterials science is combining biomaterials, biotechnology and molecular biology, in order to have biomaterials with a specific biological functionality (Wang et al., 2006; Aguilar et al., 2002; Patrick Jr et al., 1998; Piskin, 1997).

The essential prerequisite to qualify a material as a biomaterial is that it should be biocompatible. Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application (Williams, 1999). The criteria for determining the biocompatibility of a material depend on its end use application. Consequently, a wide range of materials encompassing all the classical materials such as metals, ceramics, glasses, and polymers have been investigated as biomaterials. Among these, polymers form a versatile class of biomaterials that have been extensively investigated for medical and related applications. This can be attributed to the inherent flexibility in synthesising or modifying polymers matching the physical and mechanical properties of various tissues or organs of the body.

One of the first attempts was the use of the biostable synthetic polymer poly(methyl methacrylate) (PMMA) as an artificial corneal substitute. Encouraged by initial successes, surgeons started using a variety of polymers for different applications such as blood contacting devices, hip joint replacements, and as intraocular lenses (Castner and Ratner, 2002). Even though the application of these polymeric materials significantly improved the advancement of modern health care, the long-term biocompatibility of many of these materials remained a serious

concern. During the latter half of the twentieth century, material scientists began attempts to engineer novel polymeric materials or modify existing polymers which could exhibit biocompatibility and adequate mechanical properties suitable for specific biomedical applications. In addition, recent advances in biotechnology and pharmaceutical science opened novel frontiers in biomedical fields that demanded materials with bioactivity, biocompatibility, and in many cases transient existence.

1.3 Skin template

Human skin is an organ comprised of two horizontal tissues, the dermis overlaid with the epidermis. It covers the human body of an adult over a surface of 1.7 m² (Guerret et al., 2003). The major property of the epidermis, which is mainly composed of keratinocytes and melanocytes, is to protect the body from external insults. It is the first line of defence against infection, and it provides a barrier to pathogens in the environment. The underlying tissue, the dermis, has many fewer cellular components and consists of a dense arrangement of extracellular matrix components that provide a template for cells, vessels, and bioactive molecules. Other important roles of the skin include regulating body temperature and removal of body waste (Silver, 1994).

The epidermis is composed of several layers called strata, and like all epithelial tissues, is a vascular. The main cell types of the epidermis are keratinocytes. These cells produce the fibrous protein keratin that provides the structural toughness of the epidermis. The outermost layer, the stratum corneum, is 20 to 30 cell layers thick. It accounts for approximately three-quarters of the epidermal thickness. It is the abundance of keratin in the stratum corneum that protects the deeper viable cells

from biological, chemical and physical injury (Marcu et al., 2000).

The dermis is strong, elastic and well supplied with blood vessels and nerves. Fibroblasts are the main cell type of the dermis and are responsible for maintenance of the dermal extracellular matrix (ECM). Collagen (types I and III) and elastin are the proteins in the dermis; collagen provides toughness and binds water to keep the skin hydrated and elastin provides elasticity (Marcu et al., 2000). The dermis provides essential structural support and nutrients to the epidermis. Subcutaneous tissue lies underneath the dermis. It consists of loose connective tissue and adipose tissue. The adipose tissue serves as insulation and as an energy reserve. The subcutaneous layer also connects the dermis to the surface muscles.

The mechanical properties of the skin are important to its function. Properties such as tear resistance, shear strength and tensile strength are all important for the proper functioning for the skin as a barrier. Tensile strength has been used extensively as a way to compare the mechanical properties of soft tissue and biomaterials (Berthod et al., 1994; Tomihata and Ikada, 1997; Thacharodi and Rao, 1993). The tensile properties of full thickness skin have been studied by Yamada and Evans (1970). It was found that the tensile properties for the skin as a barrier vary with age, body location and case of gender. The average ultimate tensile strength of skin was reported as 6.3- 7 MPa, elongation at break was 100%, and the elastic modulus was 35 MPa. These mechanical properties demonstrate that skin is a highly extensible material. Fibroblasts are connective tissue cells that play an integral part in dermal maintenance and the wound repair process. Fibroblasts located in the dermis are mainly responsible for the synthesis and secretion of ECM components such as collagen (Singer and Clark, 1999).

Collagen plays a major role in the architecture of the skin; it represents 40-50%

of the dry weight of the skin, the bulk of which is made up of types I and III collagen. Recently, the importance of dermal fibroblasts for the maintenance of phenotype for keratinocytes was reported (Ghalbzouri et al., 2002). In their study, a decellularised dermis was seeded with keratinocytes in the absence and presence of dermal fibroblasts. Immuno histochemical techniques showed that the absence of fibroblasts, the epidermis formed consisted of only two to three viable cell layers with a very thin stratum corneum, however, in the presence of fibroblasts, keratinocyte proliferation and migration was stimulated and epidermal morphology markedly improved. The stimulatory effect of fibroblasts showed a biphasic response: keratinocyte proliferation increased the initial phase but decreased later stages of cell culture.

1.4 Problem statement

Different modification techniques provide good approaches to control the interactions between living systems and implanted materials by modifying the surface characteristics. The study of the interactions of biologically active species with materials is possible through the preparation of structures consisting of materials, cells and proteins that promote a specific biological response after implantation (Ratner et al., 2004; Hasirci et al., 1998).

For many years, poly(dimethyl siloxane) (PDMS, commonly called silicone rubber) has been investigated as a biomaterial for the production of medical devices, such as artificial heart, breast implants, ophthalmologic devices, artificial noses, ears, and chins in maxillofacial reconstruction, and artificial skin (Yannas and Burke, 1980; Lindeman, 1989; Bolz and Schaldach, 1993; Madou and Tierney, 1993). The

stability, toxicity, hydrophobicity, tissue response, and oxygen permeability of this material have been reported in many articles. Although silicone rubber has excellent bioinertness, softness, and stability, serious problems arise when silicone devices are implanted for a long time (Cifková et al., 1990; Khorasani et al., 1996; Vladkova, 2004). Because the body recognizes as foreign hydrophobic biomaterials such as silicone rubber, they stimulate inflammation and fibrosis, the latter process generating a fibrous capsule that isolates the biomaterial. Hydrogels (Okada and Ikada, 1993) and collagen (Kinoshita et al., 1993) coatings have been reported to reduce fibrosis around biomaterials implanted in animals. This approach for enhancing the surface properties of PDMS and their hydrophilicity and biocompatibility is through the incorporating of hydrogels and collagen.

PDMS is modified in order to enhance its hydrophilicity and wettability by poly(hydroxyethyl methacrylate) (PHEMA) and poly(acrylic acid) (PAA) hydrogels via interpenetrating polymer networks (IPNs) and blending methods to introduce a suitable functional group for coupling of collagen in outer layer of PDMS to enhance their biocompatibility. Several approaches based on surface and bulk modifications have been attempted for improving the surface properties of PDMS using hydrogels by laser, plasma, corona discharge and particulate composites methods. Interpenetrating polymerisation is the only way of combining cross-linked polymers. This technique can be used to combine two or more polymers into a mixture in which phase separation is not as extensive as it would be otherwise. These particular methods are advantageous because these are relatively simple, cost effective, and less time-consuming in comparison to other chemical and physical processes.

1.5 Research objectives

In general, the most common polymers used in the medical field are hydrophobic. For this reason, the incorporation of functional groups onto polymer surfaces to improve their hydrophilicity, without changing the mechanical behavior of the material, is widely studied. Different modifications of biomaterials surfaces are normally used to increase the biocompatibility and the adhesion between different biomaterials.

The aim of this work was the modification of PDMS films by hydrogel polymers of PHEMA and PAA via IPN and blending methods to create suitable functional groups for collagen grafting in outer layer of silicone to enhance their biocompatibility. Thus, incorporating of hydrogels and collagen onto PDMS surface may give the silicon substrates with new and interesting properties for applications in silicon-based implantable biomaterials. It is hypothesized that grafted collagen can modulate the foreign body response *in vivo* and lead to improved healing in the area surrounding an implanted material.

The specific aims of this work were to:

- (1) To introduce specific functional groups onto PDMS for biomolecules binding and hydrophilicity improvement of PDMS using hydrogels,
- (2) To improve biocompatibility of PDMS using collagen,
- (3) To investigate and suggest the optimum conditions of hydrogels content in PDMS/ hydrogel IPNs,
- (4) To produce different PDMS based films using hydrogels and collagen as skin substitute,

(5) To evaluate the biocompatibility of different collagen grafted modified PDMS films by in vitro assay to determine their cytotoxicity and investigation of cell behavior.

1.6 Outline of thesis structure

This thesis is organized into five chapters:

Chapter 1 commences with some basic information on the definition of biomaterials and skin composition followed by a brief introduction on the historical overview of uses polymers in the skin substitutes. Issues were of concern, which generated the ideas and inputs for this research work, are also elaborated upon. The primary objectives and the general flow of the research program are also outlined.

Chapter 2 relates some background on engineering polymeric biomaterials for biomedical applications and classification of skin substitutes and burn dressing. Explications on the methods of the surface and bulk modification of PDMS with special focus on the interpenetrating polymer network (IPN) method and applications of hydrogels and biopolymers are also provided. Subsequently, a literature review was done on various published works on silicone, hydrogels and collagen based biomaterials for biomedical applications particularly those that are closely related to this work.

Chapter 3 details the experimental procedures employed in this research. Descriptions of lab equipments used as well as any other processing techniques utilised in generating any data that were used and presented in the research are reported.

Chapter 4 is actually the results and discussion chapter according to the research objectives. The first part of this chapter describes the modification of PDMS via interpenetrating polymer networks by PHEMA and PAA hydrogels and subsequently their collagen grafted experiments. In addition, this part investigates the optimum condition of hydrogel content in silicon/hydrogel IPN. The second part of this chapter introduces the results and discussion of their blending method.

Finally, in Chapter 5, presents some concluding remarks on the present work as well as some recommendations for future studies.

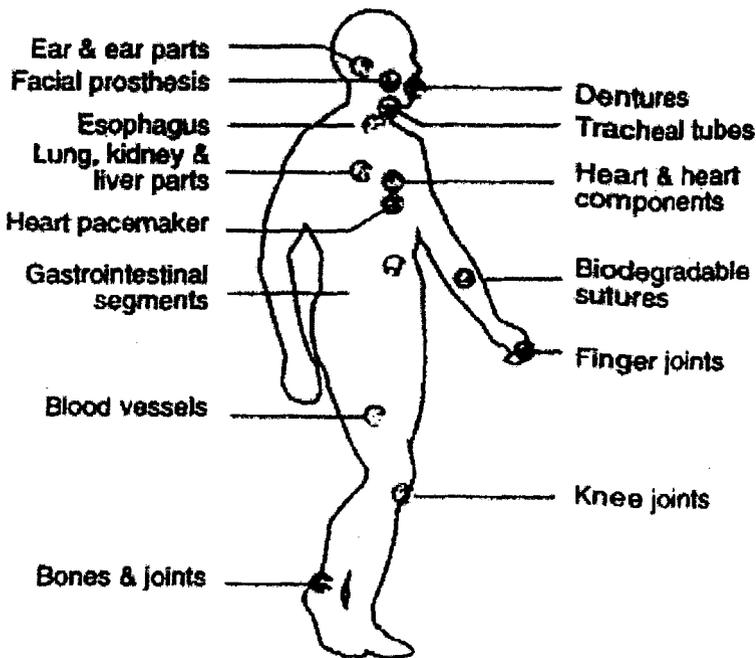
CHAPTER 2

LITERATURE REVIEW

2.1 Biomedical applications of polymers

Polymers have physical properties that most closely resemble those of soft tissues and therefore this class of materials is used extensively to replace the functions of soft tissues including skin, tendons, cartilage, vessel walls, breast and bladder. A number of synthetic polymers find applications as biomaterials. They include polyolefins, polyamides, polyurethanes, polyacrylates, polysulfones, polyethers, and silicone rubbers (Fig. 2.1). Some of these materials are also used as sutures, tissue adhesives, shunts, catheters, and space fillers. Synthetic biomaterials have been evaluated and used for a wide range of medical applications. The ultimate aim in medicine, besides prevention, is the healing of diseases and repairing damage after injuries. The task of engineers, material scientists and physicists is to provide an optimal system for these applications (Ratner et. al., 2004).

From a practical point of view, medical applications of polymers are divided into three broad categories: 1) extracorporeal uses include catheters, tubing, and fluid lines; dialysis membranes, artificial kidney; ocular devices; wound dressings and artificial skin, 2) permanently implanted devices include sensory devices; cardiovascular devices; orthopaedic devices; dental devices, and 3) temporary implants include degradable sutures; implantable drug delivery systems; polymeric scaffolds for cell or tissue transplants; temporary vascular grafts and arterial stents; temporary small bone fixation devices.



Ear & ear parts: acrylic, polyethylene, silicone, poly(vinyl chloride) (PVC)

Dentures: acrylic, ultrahigh molecular weight polyethylene (UHMWPE), epoxy

Facial prosthesis: acrylic, PVC, polyurethane (PUR)

Tracheal tubes: acrylic, silicone, nylon

Heart & heart components: polyester, silicone, PVC

Heart pacemaker: polyethylene, polyacetal

Lung, kidney & liver parts: polyester, polyaldehyde, PVC

Esophagus segments: polyethylene, polypropylene (PP), PVC

Blood vessels: PVC, polyester

Biodegradable sutures: PUR

Gastrointestinal segments: silicones, PVC, nylon

Finger joints: silicone, UHMWPE

Bones & joints: acrylic, nylon, silicone, PUR, PP, UHMWPE

Knee joints: polyethylene

Figure 2.1 Types of polymers used in common biomedical applications (Ratner et al., 2004)

Polymers can be used either as bioinert or bioactive materials, depending on the application. The medical devices that find either intra- or extracorporeal application hold a wide spectrum of synthetic materials such as polyethylene, polypropylene, polyvinylchloride, polyester, polystyrene, polyurethane, silicone, polysulphone, polyamide, polytetrafluoroethylene and their derivatives, that among them silicone is a widely used material due to its good biocompatibility and mechanical properties. Although these products have excellent physical properties, they were nonetheless developed primarily for industrial use and only later found their way into biomedicine. Thus, all these synthetic materials display more or less the same disadvantage that is incompatibility with blood and tissues. Through contact with blood, this incompatibility can irritate a pathophysiological response from the organism, similar to that of traumatic shock. This extensive contact causes a massive activation of the cellular defence systems against the supposed attacker, and with that the human body boosts the various cascade reactions into motion. To avoid this drawback for example heparin-coating was recognized as an improvement on hemocompatibility of the materials used in biomedical applications.

2.2 Classification of skin substitutes and wound dressing

Skin is the largest organ of the human body and functions to protect the body from the external environment by maintaining temperature and haemostasis, as well as by performing immune surveillance and sensory detection. Skin consists of two main layers, an outer epidermis composed of stratified squamous epithelium and an inner dermis composed of dense connective tissue and fibroblasts. Significant skin loss due to injury or illness leading to damage of dermal or sub dermal tissues cannot heal properly and can lead to serious consequences (Sheridan and Tompkins, 1999).

Rapid healing of skin wounds is an important objective. The best way to heal a skin wound is to close it as fast as possible after injury according to surgical standards. However, this method is limited and often does not prove sufficient when the defect area is very large or the recipient microenvironment is poor. Such is the case in extensive skin burns, hard to heal chronic wounds and congenital skin disorders such as epidermolysis bullosa (EB). Over the years, advances in the field of tissue engineering have provided alternative methods to treat hard to heal skin wounds. These currently available products revolve around the in vitro fabrication of biomaterials that imitate natural skin anatomy and/or delivery of growth factors that encourage repair. However, these treatment strategies do not result in optimal healing, in part, due to an inability to fully restore the structure of the dermis. The best way to heal a wound is to close it surgically following injury. However, primary closure is not always possible in large-surface and deep wounds. In such cases it is necessary to replace as much of the missing tissue as possible (Ruszczak, 2003).

Several types of wound dressings are commercially available to support wound healing processes. Sponges, hydrogels, woven and non-woven dressings derived from natural and abundant polymers have been developed for practical use. Historically, wound closure has been achieved via autologous skin grafting. However this method is not always feasible. In such cases, human allogenic skin can be used as a temporary matrix to manage the wound until autologous skin grafting is possible (Ruszczak and Schwartz, 2000). Alternatively, cadaver allograft matrices can be used to achieve temporary wound closure. Such matrices are prepared by removing the epidermal portion of the skin, leaving only the collagen rich dermal matrix. The dermal matrix is made non-viable before use in order to reduce its antigenicity and prevent host rejection. Autologous cultured epidermal sheets are then grafted on top

to enhance healing. Several banks are currently in operation in the world, providing cadaver skin to burn victims. Similarly, temporary wound coverage can be achieved via xenogenic dermal matrices, with porcine skin being the most common. However, sterility and immunogenic rejection is of greater concern with this treatment modality (Ruszczak, 2003).

With the advancement of tissue engineering in the past decade, an array of products have been introduced for the treatment of hard to heal skin wounds and large defects, a number of which are currently available commercially. These tissue engineered skin substitutes are divided into those which utilize synthetic or biological based materials and those that incorporate skin derived cells.

Acellular matrices can be subdivided into single layer and bilayer models. Single layer models include such products as CollatampFascie[®], a bovine/equine collagen membrane and Promogran[®], a spongy collagen matrix containing oxidized regenerated cellulose. Both these products are often used in conjunction with an epidermal allograft. CollatampFascie is used as a wound dressing or implant for healing of partial and full thickness chronic and acute wounds. Promogran has shown success in the treatment of chronic wounds. Namely, randomized clinical trials performed by Vin et al. (2002) and Veves et al. (2002) found that patients with venous and diabetic leg ulcers that were treated with Promogran experienced accelerate healing as compared with those treated with moistened gauze. It is hypothesized that the highly oxidized regenerated cellulose concentration in this product acts as a substrate, reducing their activity and thus improving healing (Lobmann et al., 2005; Cullen et al., 2002).

The bilayer models include such products as Integra[®] (Integra Life Science Corp) and Biobrane[®] (Bertek Pharmaceuticals). Integra is a bilayer membrane

composed of a dermal portion that consists of a porous lattice of fibers of cross-linked bovine collagen and glycosaminoglycans (GAGs) and an epidermal layer consisting of a synthetic polysiloxane polymer (silicone) (Pham et al., 2007). The dermal layer functions as a biodegradable scaffold that allows for organized host dermal regeneration. The epidermal portion is later removed and replaced by an epidermal autograft. Integra is currently approved in for treatment of partial and full thickness burns.

Biobrane, currently in use, is synthetic dressing composed of a nylon mesh coated with polypeptides and bonded to a silicone membrane. Biobrane acts as a temporary covering of partial thickness dermal burns or meshed autografts and is trimmed away as the wound heals. In comparison to allografts, Biobrane demonstrated increased wound healing rates and pain reduction (Pham et al., 2007).

The initial intent of designing biosynthetic skins was to provide a dermal substitute. However, the dermis is a structurally complex tissue, consisting of a variety of cell types, ECM molecules, skin appendages, blood vessels and nerves, none of which are supplied by these synthetic matrices. Thus these early tissue engineered matrices serve simply as bridging devices or imperfect templates for host tissue repair. As such, none of these products have been very successful in promoting and accelerating healing in chronic wound, large full thickness defects, and deep burns (Ruszczak, 2003). More recently these early biosynthetic skin substitutes as well as newer matrix designs have been used as delivery systems for skin derived keratinocytes and fibroblasts. These cells are thought to interact with the implant matrix and the wound bed, producing growth factors and ECM component necessary for healing. There are currently several such products commercially available,

namely, TranCyte[®] (Smith &Nephew), Dermagraft[®] (Advanced Tissue Sciences) and Apligraf[®] (Organogenesis).

TranCyte contains a silicone polymer membrane, seeded with newborn human keratinocytes, on a nylon mesh coated with porcine dermal collagen. This product has been approved for the treatment of full and partial thickness defects and burns. TranCyte is used only as a temporary wound covering and is removed once autografting is possible. Compared to traditional wound management methods, TranCyte was shown to decrease healing time and scar formation.

Dermagraft, currently available in the world, is produced by seeding dermal fibroblasts from human foreskin onto a bioabsorbable polyglactin mesh. The fibroblasts proliferate to fill the scaffold and secrete human collagen, matrix proteins, growth factors and cytokines to produce a neodermis containing living cells. Dermagraft has been designed to overcome the molecular deficiencies associated with chronic wounds by including exogenous growth factors into the polyglactin mesh. Dermagraft has been shown to be effective in promoting colonization of the wound bed, angiogenesis, fibroblast proliferation and matrix deposition and re-epithelialization. In clinical trials, Dermagraft was effective in the healing and wound closure of venous ulcers and diabetic foot ulcers (Gentzkow et al., 1996).

Apligraf, currently available in the world is commercialized form of living skin equivalent (Ruszczak and Schwartz, 2000). The construct contains two layers. The dermal layer is composed of a purified bovine type I collagen matrix mixed with human infant foreskin dermal fibroblasts which cause gel contractions and formation of a dense collagen lattice. The dermal portion is then seeded with human keratinocytes forming an epidermis-like structure. Apligraf has shown success in the treatment of patients with EB, reducing wound healing time and improving quality of

life (Falabella et al., 2000). Apligraf has also been found to act as a natural substrate for proteases. As such, it is thought to be able to counteract the imbalance between matrix production and degradation in chronic wounds and therefore support wound re-epithelialization (Lobmann et al., 2005).

Depending on the patient situation, optimal therapies need to be found for promoting wound closure and dermal regeneration. In the case of severely burned patients, skin has to be replaced rapidly and permanent coverage with split-thickness or cultured epithelial auto graft is required. However, patients may not have sufficient donor sites to cover their wounds if large areas are involved. In the case of chronic wounds attributed to an imbalance of healing factors, infection, reduced collagen deposition, and other cofactors, advanced therapies for treatment are needed. For all of these situations, skin substitutes have been shown to have moderate success and improvements on these substitutes holds great promise for wound healing strategies. Pressure ulcers, venous ulcers and burns are types of wounds that are most often treated with a dressing. Wound dressings are used to protect the site of injury from further insult, contamination and infections that may impede healing. Also, the benefit of a physiologically moist environment has been established (Bello and Phillips, 2000).

Today, there are many wound dressings available on the markets that address different kinds of wounds, treatments and phases in the wound healing process. Example, Comfeel® by Coloplast and Tegaderm® by 3M, hypercolloid and polyurethane dressings respectively are among the most appropriate for minimal to mild exudation (Bello and Phillips, 2000). Also, alginate dressings followed by hypercolloid dressings have shown best results for pressure ulcers. Dressings are fabricated from both synthetic and natural materials. They stated that there has been

no evidence that any dressing type enhances the healing rates of chronic diabetic ulcers.

Over the past two decades extraordinary advances in cellular and molecular biology have led to a greatly improved understanding of the basic biological processes involved in wound repair and tissue regeneration. Ultimately, these great strides in basic knowledge will likely lead to advancements in wound care resulting in accelerated rates of ulcer and normal wound repair, scars of greater strength, and prevention of fibrosis. In addition, this information may translate into better design of artificial organs and tissue substitutes since the exposed surfaces of these materials should be designed so that they integrate completely and continuously with the surrounding tissues.

Some tissue-engineered skin substitutes that are currently available are AHoDerm[®], Integra[®], Apligraf[®] (Graftskin) and Dermagraft[®]. Many of these products have had considerable success in trials, but have not overcome the main wound healing obstruction of tissue regeneration without wound contraction. Thus, tissue engineering skin products have not replaced skin grafting as the method of choice for clinical wound repair. Tissue-engineered skin refers to skin products made up from cells and extracellular matrix alone or in combination with growth factors. A variety of materials have been investigated as matrices including autologous, allogenic, and xenogenic tissues or synthetic and natural polymeric materials for skin tissue engineering. Three different approaches are currently in use to create artificial skin: to recreate the epidermis, to recreate the dermis, and to recreate both the dermis and epidermis using a bilayer graft. Thin layers of keratinocytes as such or cells cultured on polyurethanes (Epicel) and hyaluronic ester membranes (LASERSKIN) have been developed as epithelial replacements. Dermal substitutes make use of

biodegradable polymers such as collagen-glycosaminoglycan matrix covered with a silastic membrane as a barrier layer (Integra) as well as PLAGA (Vicryl) matrices seeded with human fibroblast cells (Dermagraft). A full-thickness graft has been developed using bovine collagen I matrices seeded with fibroblasts and keratinocytes called human skin equivalents (Apligraf). Multicenter clinical trials have revealed the accelerated healing capacity of chronic nonhealing venous stasis ulcers by the tissue-engineered graft. Figure 2.2 depicts some famous biological skin substitutes with their specifications.

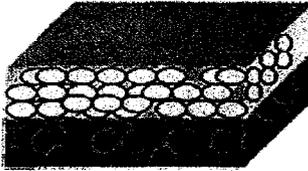
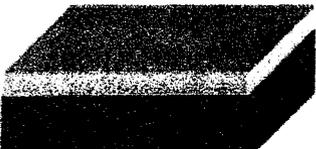
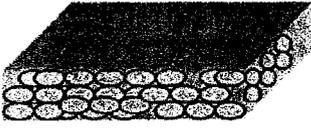
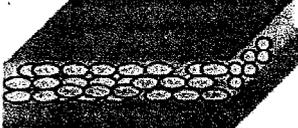
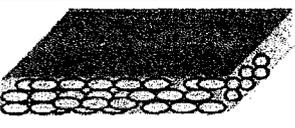
Trade Name	Schematic Representation	Layers
Biobrane™ (Dow Hickam/Bertek Pharmaceuticals, Sugar Land, TX).		<ol style="list-style-type: none"> 1. Silicone 2. Nylon Mesh 3. Collagen
Transcyte® (Advanced Tissue Sciences, Inc. La Jolla, California, USA)		<ol style="list-style-type: none"> 1. Silicone 2. Nylon Mesh 3. Collagen seeded with neonatal fibroblasts
Apligraf® (Organogenesis Inc, Canton, MA and Novartis Pharmaceuticals Corporation, East Hanover, NJ)		<ol style="list-style-type: none"> 1. Neonatal keratinocytes 2. Collagen seeded with neonatal fibroblasts
Dermagraft® (Advanced Tissue Sciences, Inc. La Jolla, California, USA)		<ol style="list-style-type: none"> 1. Polyglycolic acid (Dexon™) or polyglactin-910 (Vicryl™) seeded with neonatal fibroblasts
Integra® (Integra Life Science Corporation, Plainsboro, New Jersey)		<ol style="list-style-type: none"> 1. Silicone 2. Collagen and glycosaminoglycan
Alloderm® (LifeCell, Woodlands, Texas.)		<ol style="list-style-type: none"> 1. Acellular de-epithelialised cadaver dermis
Epicel™ (Genzyme tissue repair corporation, Cambridge, MA.)		<ol style="list-style-type: none"> 1. Cultured autologous keratinocytes
Laserskin™ (Fidia Advanced Biopolymers, Italy) also marketed as Vivoderm™ by ER Squibb & sons Inc,		<ol style="list-style-type: none"> 1. Cultured autologous keratinocytes 2. Hyaluronic acid with laser perforations
Cadaveric allograft (from not for profit skin banks)		<ul style="list-style-type: none"> • cryopreserved in order to retain viability • lyophilised • glycerolised

Figure 2.2 Famous skin substitutes in the world (Jones et al., 2002).

2.3 The criteria for an ideal skin substitute

Ideally a graft material should promote adequate wetting and draping, thus eliminating dead space, exhibit good adherence to the wound, prevent bacterial invasion, and control fluid loss. Further, the material should have adequate mechanical properties, promote endogenous cell infiltration, promote angiogenesis, and degrade with time. The use of biologically active matrices is now well accepted as accelerating wound healing and improving skin reconstruction. It is believed that cells encapsulated within the dermal matrix are able to produce ECM components that contribute to healing. In addition, these cells may interact with pre-existing host cell populations in the adjacent tissue resulting in cell migration into the wound bed.

Despite these successes, there remains a need to stimulate the healing of acute and chronic wounds to a level that is presently not possible with standard methods of care or application of biosynthetic skins. As discussed above, current products demonstrate large variability in success rates depending in the wound type and patient history. In addition, even the best products currently available on the market have success rates below 60% for the treatment of hard to heal wounds. This limited success is mainly due to the inability of the skin substitutes to modulate an adequate matrix-wound cell interaction and integration and to promote wound vascularization necessary for graft take. The ideal skin substitute is inexpensive, long shelf life, used off the shelf, non-antigenic, durable, flexible, prevents water loss, barrier to bacteria, conforms to irregular wound surfaces and drapes well, easy to secure, applied in one operation, and does not become hypertrophic (Sheridan and Tompkins, 1999).

2.4 Applications of silicone based biomaterials

Silicone is the generic name for entirely synthetic polymers containing a repeating Si-O backbone. The organic groups attached to the silicon atom via silicon-carbon bonds define the class of silicone. The most common example is poly(dimethyl siloxane) (PDMS) as shown in Fig. 2.3.

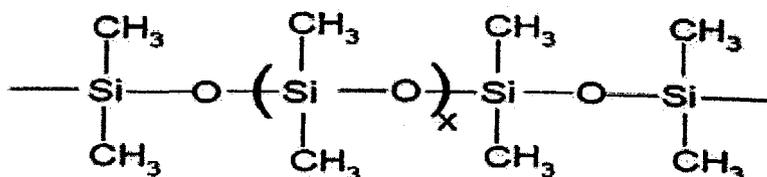


Figure 2.3 The structure of poly(dimethyl siloxane) (PDMS)

Poly(dimethyl siloxane) (PDMS) based elastomers have been used in a wide range of biomedical applications, as a result of their physiological inertness, good blood compatibility, low toxicity, good thermal and oxidative stability, low modulus, anti adhesive properties and many desirable properties including low surface energies, high flexibility and low glass transition temperature. Since the end of 1950, silicone elastomer has been widely used for medical devices such as breast prosthesis, shunt valve for hydrocephalus, drainage implants in glaucoma, artificial skin, maxillofacial reconstruction, denture liners, pace maker lead insulator, contact lenses and catheter, membrane for oxygenator, heart valve and vascular grafts and numerous studies have been conducted on toxicity stability tissue response and oxygen permeability (Walter, 1968; Khorasani et al., 2005).