Characterisation of the effects of ultraviolet radiation on host-microbiota interactions in skin

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List of Abbreviations

%	Percent
°C	Degree Celsius
12-LOX	12-lipoxygenase
6-4PPs	6-4 photoproducts
6-HAP	6-N-hydroxyaminopurine
AD	Atopic dermatitis
AJs	Adherens junctions
AMPs	Antimicrobial peptides
BCA	Bicinchinonic acid
BSA	Bovine serum albumin
CA-MRSA	Community-acquired methicillin-resistant *Staphylococcus aureus*
CDs	Corneodesmosomes
CFU	Colony forming units
cIAP	Cellular inhibitor of apoptosis protein
Cldn	Claudins
CPDs	Cyclobutane pyrimidine dimers
DAMP	Damaged associated molecular pattern
DAPI	4',6-diamidino-2-phenylindole
DCs	Dendritic cells
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
ETs	Extracellular traps
E value	Expect value
FFAs	Free fatty acids
GAS	Group A streptococcus
GPX4	Glutathione peroxidase 4
GSDMD	Gasdermin D
hBD	Human β-defensin
ICR	International commission on radiation
IL	Interleukin
JAMs	Junctional adhesion molecules

kDa	Kilodalton
KHGs	Keratohyalin granules
KLK	Kallikreins
LCs	Langerhans cells
LGs	Lamellar granules
LTA	Lipoteichoic acid
LUBAC	Linear ubiquitin chain assembly complex
MED	Minimal erythemal dose
MLKL	Mixed lineage kinase domain-like
n	Number of biological replicates
NADPH	Nicotinamide adenine dinucleotide phosphate
NCTC	National collection of type cultures
NHEKs	Normal human epidermal keratinocytes
NMFs	Natural moisturizing factors
ns	Not significant
Ocln	Occludin
OD	Optical density
PAMPs	Pathogen-associated molecular patterns
PARPs	Poly (ADP-ribose) polymerase proteins
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PITs	Pore-induced intracellular traps
PLE	Polymorphic light eruption
PRRs	Pattern recognition receptors
PS	Phospholipid phosphatidylserine
PSMs	Phenol-soluble modulins
RIPK	Receptor-interacting protein kinase
ROS	Reactive oxygen species
qPCR	Quantitative polymerase chain reaction
rRNA	Ribosomal ribonucleic acid
SDS PAGE	Sodium dodecyl sulphate poly acrylamide gel electrophoresis
SB	Stratum basale
SC	Stratum corneum

SEM	Standard error of the mean
SG	Stratum granulosum
SL	Stratum lucidum
SR	Solar radiation
SS	Stratum spinosum
ssDNA	Single stranded DNA
SSR	Simulated solar radiation
TAE	Tris-acetate-EDTA buffer
Th2	T helper 2 cell
TJ/TJs	Tight junction/ Tight junctions
TLR	Toll-like receptor
TNF	Tumor necrosis factor
TRADD	Tumor necrosis factor receptor type-associated death domain
TRAF	Tumor necrosis factor receptor-associated factor
Trp	Tryptophan
TSB-T	Tris buffered saline tween 20
UVA	Ultra-violet A
UVB	Ultra-violet B
UVC	Ultra-violet C
UVR	Ultraviolet radiation
x*g*	Times gravity
ZO	Zonula occluden

Abstract

The elegant structure of the skin renders it an appropriate habitat which harbours numerous commensal microorganisms. These live mutualistically on and in skin and are collectively defined as the skin microbiota. Ultraviolet radiation (UVR) is an important environmental factor that influences the skin and skin microbiota potentially throughout life. Whilst the effect of UVR on skin has been studied for decades, its effect on the skin microorganisms is a new topic in dermatology and microbiology. This thesis aims to investigate the effects of UVR on skin commensal bacteria and how the UVR-irradiated organisms might influence the skin response to UVR.

The sensitivity of eight skin resident bacteria (*Staphylococcus. epidermidis*-three strains, *Staphylococcus hominis*, *Staphylococcus capitis*, *Cutibacterium acnes*, *Micrococcus luteus* and *Corynebacterium jeikeium*) to a single dose of simulated solar radiation (SSR) was investigated. Data indicate that *C. jeikeium* and *M. luteus* are relatively resistant to SSR while *S. capitis* and *C. acnes* are more sensitive, among the microbes tested. The mechanisms behind the different SSR sensitivity between species were explored by comparing expression of the RecA protein pre/post SSR treatment. However, a clear conclusion of how RecA regulates the survival of skin bacteria upon SSR exposure cannot be drawn based on current data.

The presence of an individual strain of *S. epidermidis* reduces the viability of keratinocytes following a single dose of SSR because irradiated *S. epidermidis* can release substance(s) that induce(s) keratinocyte necrosis. The active factor(s) is(are) susceptible to heat and trypsin, indicating that it(they) could be proteinaceous. This hypothesis was investigated using fractionation, from which data suggests that the molecular weights of protein(s) that induce(s) necrosis is(are) between 50-100kDa.

Based on these results, a clinical study was designed to investigate the effect of skin microbiota on the minimal erythemal dose response in healthy humans. This study was not completed due to the pandemic.

In conclusion, the data in this doctoral thesis broaden the knowledge of the effects of UVR on skin commensals and may lead to a better understanding of the relationship between the skin microbiota and skin health.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

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Chapter 1 Review of the literature

1.1 Introduction

Skin, the largest organ in humans, covers the outermost layer of body and serves as a biological barrier to separate the outside world and inner body. The most important function of skin is the barrier function which protects our body from exogenous assaults, such as chemical toxins, pathogens and ultraviolet irradiation (UVR). At the same time the skin prevents the loss of water and solutes from within the body (Baroni et al. 2012). The surface of skin is not sterile and recent research has demonstrated that it is colonized by an abundance of microorganisms, forming a unique skin microbiome. For the most part, the skin microbiome has a positive effect on the skin and plays a significant role in both barrier function and skin metabolism. However, in some situations the skin microbiome can contribute to pathology (Grice and Segre 2011).

During the whole life span of a human, cutaneous cells and skin microorganisms are exposed to UVR from sunlight as well as other sources. The effect of UVR on skin cells has been studied for many decades, revealing both positive and negative effects on human skin. On the one hand, chronic UVR is associated with the pathogenesis of erythema (Robert 2015), photoaging (Fisher et al. 1997), wrinkle formation (Kambayashi et al., 2003), and skin cancer (Gruijl 1999). On the other hand, UVR severs as a primary therapy for many skin diseases, ranging from psoriasis to vitiligo (Robert 2015). However, the effect of UVR on the microbiome has been overlooked. As the skin microorganisms play vital roles in maintaining dermal health, it is valuable to investigate the impact of UVR on skin microorganisms, and how in turn, this may influence the interaction with skin cells. This may lead to a better understanding of photo-

aggravated diseases as well as lay the foundation of a novel microbiology-related therapy in the future. The first part of this literature review will introduce the background knowledge including the structure and barrier function of skin, skin microbiome, UV radiation, and the interaction between them.

1.2 Skin structure and its barrier functions

1.2.1 Skin structure

Skin, from the outermost to the innermost layer, consists of three structural components: the epidermis, the dermis, and the subcutaneous tissue (figure.1.1) (Bäsler et al. 2016). Each of these layers has a crucial function for maintaining skin health.

1.2.1.1 Subcutaneous tissue

The subcutaneous tissue (subcutis, hypodermis) is mainly composed of adipose tissue and interstitial tissue (figure.1.1) (Thomsen et al. 2014). As fat cells are ‘soft’ high-energy tissues, the subcutis plays a vital effect in protecting the body from external insults by providing ‘cushioning’ against trauma. Additionally, the subcutaneous layer acts as a reservoir for energy (Arda et al. 2014). Also in the subcutaneous layer, there are many blood capillary and nerves fibres (figure.1.1), informing an integrated network which permits the prompt absorption of things such as injected medicines (Thomsen et al. 2014).

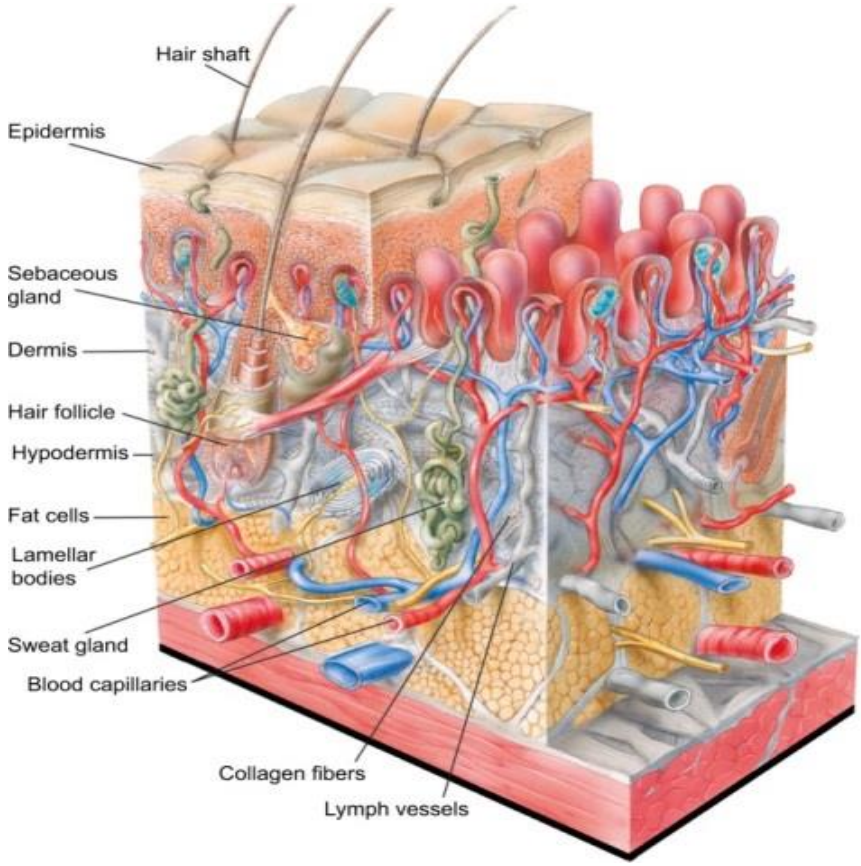

Figure 1.1 The structure of human skin (adapted from 'Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines: a review of the literature.' by Aldag et al., 2016, *Clinical, cosmetic and investigational dermatology*, 9, pp.412)

1.2.1.2 Dermis

The dermis is subdivided into two further layers: the papillary dermis and the reticular dermis. Both contains collagen fibres which maintain the structure of skin (figure.1.1) (Zuber et al., 2015), elastin which provides the elasticity for skin, and the extrafibrillar matrix (Bäsler et al. 2016). Furthermore, glands (hair follicles, sweat glands, sebaceous glands) are observed in this layer (figure.1.1). Glands have various functions in the skin but also they individually influence the distribution of skin commensal bacteria via secreting sebum and sweat (Grice and Segre, 2011). Additionally, some immune cells, such as mast cell and lymphocytes (T and B

cells), alsoexist in the dermis (Lai-cheong 2017). Furthermore, the lymphatic vessels and blood vessels (figure.1.1), which transfuse water and nutrients to organs as well as transport out the metabolic waste constantly to maintain homeostasis and metabolism, are essential components of the dermis (Bäsler et al. 2016).

1.2.1.3 Epidermis

The epidermis is the outermost layer of skin. It is a dynamic, stratified, squamous, keratinized epithelium. The keratinocyte is the dominating cell type of this layer (accounts for 95%). However, the epidermis also contains Merkel cells (for sensory perception), melanocytes (form melanin to protect form UVR), and immune Langerhans cells (LCs) that are indispensable for the sensory perception and barrier function of skin (Arda et al. 2014; Lai-cheong 2017).

Keratinocytes continually migrate from the base of the epidermis to the skin surface. During their migration, their phenotype changes dramatically as they differentiate. This produces 4 characteristic layers of the epidermis which are from inside to out: the stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG), and stratum corneum (SC) (figure.1.2). In some anatomical locations where especially thick skin is required (e.g. palms and soles), there is another layer named stratum lucidum (SL) existing between SG and SC. The different layers of the epidermis will now be discussed in more detail in the following sections.

Stratum basale

The stratum basale (SB), the innermost layer of epidermis, mainly consists of a single layer of undifferentiated cuboidal keratinocytes and is separated from the dermis by a basement

membrane. In order to maintaining the renewal of epidermis, a fraction of basal keratinocytes differentiate continually from SB to SC, while the rest cells remain in an inactive state unless enhanced proliferation is required (Eckert 1989; Baroni et al. 2012). Basal cells adhere to the basement membrane via hemidesmosomes and focal adhesions, and to the adjacent cells via the adherens junctions (AJs) and desmosomes (Moreci and Lechler 2020). Desmosomes are the 'bridge' of the neighboring keratinocytes and bind the adjacent cells tightly (figure 1.2) (Garrod and Chidgey 2008). Structural proteins keratin 5 and keratin 14 are produced by the keratinocytes in the SB. Once released, they assemble and form filaments which connect with desmosomes for the cells' adhesion (Matsui and Amagai 2015).

Melanocytes are also located in the SB. They determine skin color and protect skin from UVR via synthesizing melanosomes, which absorb and scatter detrimental UV rays to protect the DNA of cells (Lai-cheong 2017; Arda et al. 2014). Additionally, a few Merkel cells involved in sensory perception intersperse in the SB as well. They are sensitive to light touch resulting from association with afferent nerve endings and keratinocytes (Arda et al. 2014).

Stratum spinosum

The stratum spinosum (Malpighian layer) is the interlayer between SB and SG. It is composed mainly of several layers of polyhedral-shaped keratinocytes. As keratinocytes leave the SB, they change dramatically in cell shape and express keratin 1 and keratin 10 instead of keratin 5 and keratin 14 (Matsui and Amagai 2015). Once expressed, the keratin 1 and keratin 10 interact with each other and form the tonofibrils (Brod 1960) which are linked to the

desmosomes. As mentioned before, these intercellular junctions can bond the extensions of the spinous keratinocytes together tightly to form the interconnectional net of keratinocytes (Allen and Potten 1975). In addition, the immune-related Langerhans cells are also distributed in the SS (Misery and Dezutter-dambuyant 1995). These cells intersperse among the spinous keratinocytes and are essential for the immune responses of skin, as they participate in the process of antigen presentation (Arda et al. 2014).

Stratum granulosum

As the keratinocytes of SS differentiate into SG keratinocytes, they gradually become flattened (Mackenzie, 1975) and begin to generate lamellar granules (LGs) and keratohyalin granules (KHGs) in their cytoplasm (figure.1.2) (Kubo et al. 2012). KHGs include keratin 1, keratin 10 and keratin-binding proteins. In human skin, the keratin-binding proteins are mainly composed of profilaggrin which can be further degraded and form the filaggrin monomer in the outer layer of the SG (Kubo, Nagao, & Amagai, 2012). The filaggrin is involved in the cell compaction of SC, and its further hydrolysis products - natural moisturizing factors (NMFs), are important for maintaining the skin hydration and reducing the pH of skin surface (Sandilands et al. 2009).

Tight junctions (TJs), the protein complexes between cells, are formed in the intercellular space between SG cells (figure.1.2) (Furuse et al. 2002). They seal the intercellular space between the SG cells and are important components of the skin barrier functions. Moreover, various enzymes and lipids (such as glucosylceramide, cholesterol and phospholipids) are released by the LGs via exocytosis from the SG cells (figure.1.2). These lipids will eventually fill the

intercellular space of the SC and are vital for the formation of the SC barrier (Lee et al. 2006; Kubo et al. 2012). In the outermost layer of the SG, keratinocytes lose the intercellular TJs and initiate the final stage of differentiation which is called cornification (Kubo et al. 2012).

Stratum corneum

The stratum corneum is composed of 10-20 layers of corneocytes (terminally differentiated keratinocytes) embedded in extracellular lipid matirx (Haftek 2015). It is often described as the 'bricks and mortar' model, in which corneocytes are the bricks and the extracellular lipids are the mortar (figure.1.2) (Nemes and Steinert 1999). During the cornification, filaggrin assembles the keratin filaments into bunches, leading to the collapse of keratinocytes (Candi et al. 2005). At the same time, the SG keratinocytes lost their nuclei, organelles and cell membranes, eventually becoming a flat polygonal shape (Kubo et al. 2012). These terminally differentiated keratinocytes are named corneocytes and are encapsued in the cornified envelopes, which are the potein complexes assembled by transglutaminases inside the plasma membrane of the corneocytes and serve as scaffolds for the lipids attachement (Candi et al. 2005; Matsui and Amagai 2015). In addition, the intercellular desmosomes become corneodesmosomes (CDs) in the SC (Matsui and Amagai 2015). The CDs are important not only for the formation of the intact cell layers of SC but also the connection of SC with SG (Lee et al. 2006).

At the uppermost layer of SC, corneocytes continuously slough off to complete the final step of differentiation. Named desquamation, this active process is essential for the renewal of skin and vital for the elimination of pathogens (Simon et al. 2001; Matsui and Amagai 2015). The

mechanism of desquamation is associated with the degradation of CDs, which is caused by the activation of specific serine proteases (kallikreins) (Milstone 2004; Komatsu et al. 2005). Secreted together with their inhibitor from LBs of SG keratinocytes, kallikreins (KLK5/KLK7) are activated in the weakly acidic environment of the upper SC. Thses activated KLK5/KLK7 then cleave the adhesion components of CDs which leads to the final desquamation (figure 1.2) (Matsui and Amagai 2015).

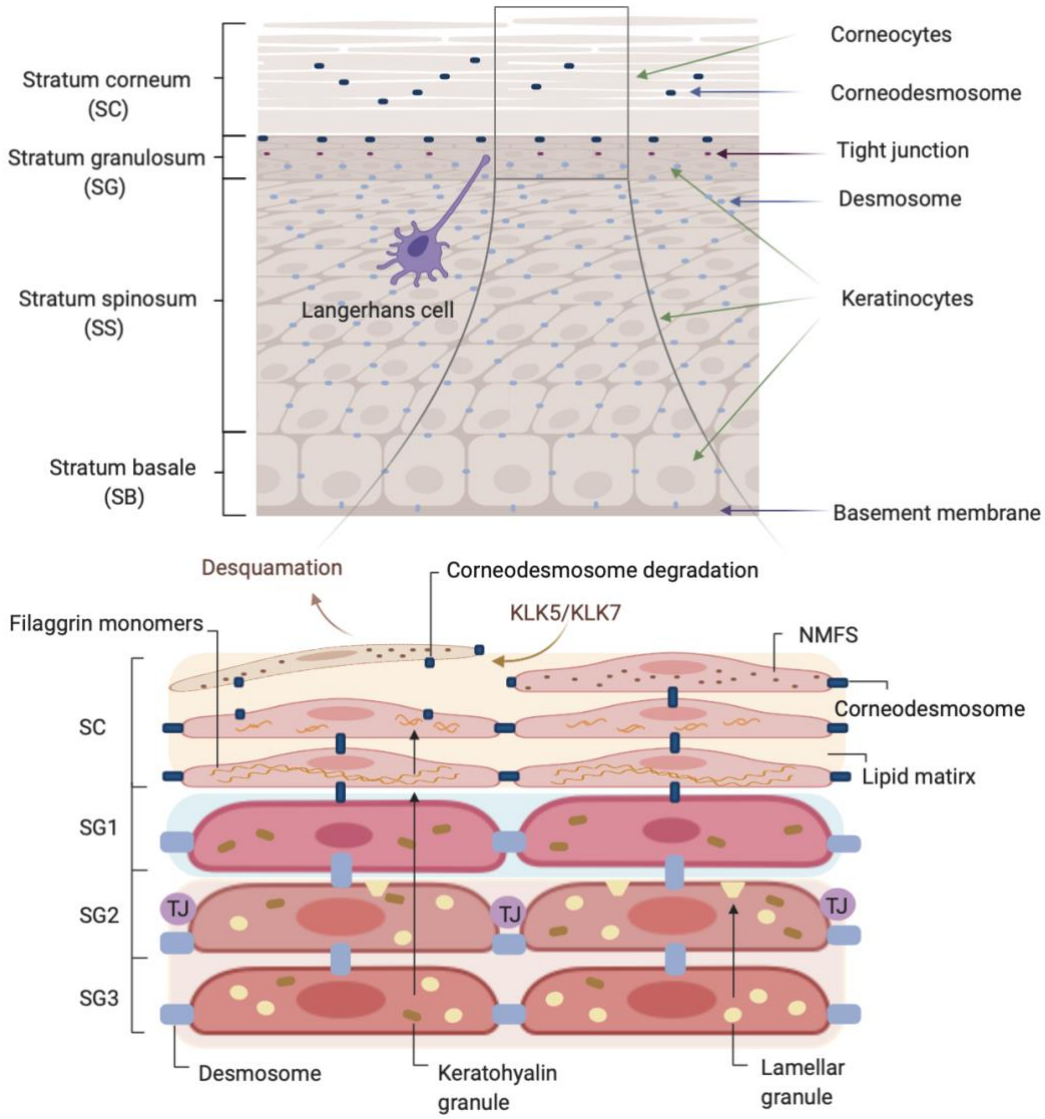

Figure 1.2 The structure of human epidermis and the differentiation of keratinocytes (drawn by Wen Duan)

The main cells of epidermis are keratinocytes which continually differentiate from the SB to the SC, forming the four layers of epidermis: SB, SS, SG, and SC. The important structure TJs are located in the SG2 and seal the intercellular space of SG2 to prevent the loss of water and ions. Keratinocytes are connected by desmosomes in the SB, SS and SG while the corneodesmosomes join the adjacent corneocytes in the SC. The corneocytes and lipid matrix in the SC compose the SC barrier function. Named the 'bricks and mortar' model, the bricks refer to the corneocytes which are the final differentiated keratinocytes while the mortar refers to the lipids released by the lamellar granules from the apical membrane of SG. The corneodesmosomes of the outermost corneocytes are degraded by the activated kallikreins, resulting in the shedding of the outermost corneocytes. This progress is named desquamation which is the last step of keratinocyte differentiation.

1.2.2 The skin barrier functions

Recently it has been suggested that the skin barrier can be considered to be composed of four entities. They are the structural barrier, the chemical barrier, the immune barrier and the microbial barrier (Eyerich et al. 2018). They cooperate with each other closely to maintain the health of the skin. This section will introduce these barriers with the microbial barrier being described in greater detail (as it is central to this thesis) later in section 1.3.2.

1.2.2.1 Structural barrier- the SC and tight junction

It is widely accepted that the SC is an air-liquid barrier between skin and outside environment as the first-line barrier of epidermis (Baroni et al. 2012). SC limits the loss of water, salt and other soluble molecules to prevent the dry skin (Madison 2003). This structure can also inhibit the permeation of detrimental substances from the outside world into the body (Smeden and Bouwstra 2016). Furthermore, potential pathogens on the uppermost layer of SC are continuely removed through desquamation of SC to prevent infections (Matsui and Amagai 2015).

Another essential component of the structural barrier is the tight junction (TJ), a liquid-liquid

interface barrier in the SG. The major function of TJs is to seal intercellular spaces between SG cells and limit movement via the paracellular diffusion pathway. TJs selectively restrict the movement of molecules, water and ions, and separate the adjacent tissues into individual environments (Tsukita et al. 2001; Anderson and Itallie 2009). The critical role of TJ to an epidermal barrier was demonstrated in 2002 when a mouse, deficient in claudin-1 (a major TJ protein) was produced. This mouse died within 24 hours of birth from excessive transepidermal water loss (Furuse et al. 2002).

TJs are composed of transmembrane proteins and cytoplasmic plaque proteins. The former proteins include claudins (Cldn), occludin (Ocln), and junctional adhesion molecules (JAMs). The latter proteins include the zonula occludens (ZO) proteins, MUPP-1 and cingulin (Bäsler et al. 2016). Although all of these proteins are involved in the formation of TJ structures, only a few of them (e.g. Ocln, cingulin) are expressed specifically in SG (Bäsler et al. 2016). For example, Cldn-4, ZO-1, and ZO-2 are found in SS while Cldn-1, Cldn-7 and JAM-A exist in all living cell layers (Brandner 2009). This may suggest that these TJ proteins have multiple functions in maintaining epidermal metabolism (Kirschner et al., 2011).

1.2.2.2 Chemical barrier

Chemical barrier function is executed by three components: the antimicrobial barrier, the acidic pH of skin surface and the NMFs (Eyerich et al. 2018).

The antimicrobial barrier relys on various molecules, such as antimicrobial peptides (AMPs), free fatty acids, glucosylceramides, sphingosine, and hydrolytic products of ceramide (Lee et

al. 2006). Among these elements, AMPs are the dominating molecules for the antimicrobial defense (Lee et al. 2006). They are produced by lamellar granules and immune cells (Braff et al. 2005) and are categorised into three main groups, including defensins, cathelicidins, and dermcidin (Korting et al., 2012). They can eliminate pathogens via activating adaptive immunity. Additionally, they can directly kill pathogens (Baroni et al. 2012). AMPs are demonstrated to have the ability to kill bacteria, enveloped viruses, and fungi (Reddy et al. 2004). This ability is performed by contact of the AMP with the cytoplasmic membrane of the pathogen. This can occur because the surfaces of AMPs are cationic while most bacteria are anionic. When pathogenic microorganisms appear, AMPs attach to their cytoplasmic membranes by electrostatics and insert into their membrane bilayers to break the integrity of membrane, which finally results in the lysis of microbial cells (Nguyen et al. 2011). Furthermore, they may permeate into the cytoplasm and disrupt vital cellular processes, such as the synthesis of DNA, RNA, and protein; protein folding; enzymatic activity; and synthesis of cell-wall (Brogden 2005).

Besides activating immune response and eliminating pathogens, AMPs also can enhance skin barrier via providing several positive effects for TJs. Recently, some researchers showed that the LL-37 enhances the protein expression of Cldn-1, 3, 4, 7, 9, 14 and Ocln. The same study showed that reduced penetration of 4 kDa FITC-dextran, a paracellular marker, is observed showing enhancement of TJ function (Akiyama et al. 2014). Additionally, another study suggests that the antimicrobial protein S100A7/psoriasin increases the expression of claudins and occludin (Hattori et al. 2014).

Since AMPs are an important component of skin barrier, various skin diseases have been

related to their abnormal expression. Reduced expression of AMPs was detected in burns and chronic wounds compared with normal skin (Korting et al. 2012). By contrast, up-regulated AMPs has been associated with various cutaneous diseases. It is reported that nearly all AMPs, especially LL-37, human β-defensin 2(hBD-2) and hBD-3, increase significantly in patients with psoriasis (Nomura et al. 2003; Frohm et al. 1997); for patients with rosacea, the chronic inflammatory reaction of skin may be related to the high expression level of cathelicidins LL-37 (Yamasaki et al. 2007).

The acidic pH of skin surface, which fluctuates from pH 4.5 to pH 5.5, is also crucial for the skin barrier functions (Parra and Paye 2003). First of all, as mentioned before, the acidic pH is essential for the initiation of the desquamation which removes the detrimental microorgaisms regulally. Additionally, the acid mantle provides a suitable environment for the growth of commensal microflora but inhibits the colonization by pathogenic microorganisms (Lambers et al. 2006). In addition, various important enzymes which produce ceramide molecules and free fatty acids, both of which are vital components of the intercellular lipids of the SC, are more energetic in a weakly acidic environment (Lee et al. 2006), demonstrating the weakly acidic pH is important for the formation of the permeability barrier of skin.

Another essential component of the chemical barrier is the natural moisturizing factor (NMF) which helps retain the moisture of the SC. As the permeability barrier, the protective functions of the SC can only be performed in a hydrated condition. The NMF can bind the water from the outside environment to prevent the desiccation of the epidermal surface and thereby support the protective function of the SC (Harding and Rawlings 2000).

1.2.2.3 Immune barrier

The immune barrier consists of the skins' own innate immune system and the adaptive immune system. Innate immunity is a nonspecific and rapid reaction against microorganisms, whereas the adaptive immunity is the response to a special pathogen (Gallo and Nizet 2003). Many components of skin (e.g., lipids, cytokines, and chemokines) participate in the innate immune response, initiating leukocytes to eliminate diverse harmful microorganisms (Niyonsaba et al., 2009).

Human innate immune system depends on various molecules which lead to a rapid response to a potential infection to miantain the physiological health of skin. For instance, the pattern recognition receptors (PRRs) are one of those important molecules. PRRs combine and recognize pathogen-associated molecular patterns (PAMPs) to facilitate the innate immune response. These receptors include lectins and Toll-like receptors (TLRs) (Jouault et al. 2009). Lectins discern yeasts or bacteria by collaborating with TLRs or other signaling receptors. From recent researches, it is suggested that TLRs could be divided into at least 11 different types that respond to different microbial elements. Particularly, TLR2 and TLR4 are crucial for the skin immune system since they recognize a wide spectrum of microorganisms. TLR2 identifies peptidoglycan and lipoteichoic acid of gram-positive bacteria, whereas the TLR4 recognizes the PAMPs which are composed of lipopolysaccharides of gram-negative bacteria (Takeda and Akira 2017). After the PAMPs are recognized by TLRs, transcription factors launch signaling cascades, leading to the generation of various immune molecules, such as cytokines, chemokines, and AMPs (Baroni et al. 2012).

In some skin infections, innate immunity cannot eliminate antigens completely, then the remaining antigens are processed by various professional antigen-presenting cells (dendritic cells (DCs)) to initiate adaptive immunity immediately. A diverse subset of immature DCs are located in skin. LCs are the sole DCs of epidermis, with more subsets existing in the dermis (Merad et al. 2008). It is suggested that LCs have the ability to induce antigen-specific Th2 responses and maintain peripheral tolerance (Malissen et al. 2014). As mentioned above, they are mainly located in the SS. In the inactivated state, their dendrites are placed in intercellular space of the cells below TJs. Once activated by danger signals (e.g. thymic stromal lymphopoietin, tumor necrosis factor (TNF)-α, and Interleukin-1β (IL-1β)) (Kubo et al. 2012), their dendrites are extended through TJs into SG, rapidly taking up extra-TJ antigens and presenting them to adjacent lymph nodes where the adaptive immune T cells exist. Then T cells recognise the antigens and launch their immune activities (Igyártó and Kaplan 2013). Notably, TJs are not broken by dendrites of LCs. When dendrites cross TJs, the TJs are formed between adjacent keratinocytes and extended dendrites, keeping the integrity of TJs (Kubo et al. 2009).

In conclusion, skin is a complex organ which comprises various different kinds of cells. The differentiation of keratinocytes is crucial for the formation of skin structure and barrier functions. The delicate defence system of skin not only protects the inner tissue and organs against outside assaults but also prevents the loss of water and ions in order to maintain normal physiological activities. The four components of the skin barrier complement and reinforce each

other, maintaining skin health together. The final component of the skin barrier, the microbiome, will now be discussed.

1.3 Skin microbiome

The surface of skin is colonized by numerous microorganisms, such as bacteria, fungi, viruses, archaea and mites, forming a unique and dynamic microbial environment on human skin. These microorganisms are named the skin microbiota, and the composition of their genes is defined as the skin microbiome. (Hannigan and Grice 2013; Grice and Segre 2011; Byrd et al. 2018). The skin microbiota varies from person to person, however it is largely stable for each individual over years. If change occurs, new similar species could replace the previous ones to maintain the stability of overall microbiome (Oh et al. 2016).

Based on genomic approaches, most skin bacteria belong to one of four principal phyla: Actinobacteria, Firmicutes, Bacteroidetes and Proteobacteria (Grice et al. 2009). They depend on the function of bacterial adherence to colonize on the skin surface (Roth and James 1988) and make critical contributions to maintain normal skin barrier functions and skin metabolism. Therefore, it is not surprising that changes to the composition of the skin microbiome are associated with various cutaneous diseases (Rosenthal et al. 2011).

1.3.1 Factors influencing skin microorganisms

Both endogenous and exogenous factors can influence the distribution and quantity of skin microbiota. As a result, commensal microbes vary between individuals, and even between different sites of body (Grice and Segre 2011; Rosenthal et al. 2011).

1.3.1.1 Endogenous factors

Temperature and humidity are different on various areas of body thereby modulating the distribution of bacteria. Therefore, skin microbiota varies on different sites of body (figure.1.3). For example, partially occluded areas, such as the fossa axillaries, perineum and toe web, are comparatively higher in temperature and humidity, and are colonized principally by microorganisms which prefer a humid environment (e.g., gram-negative bacteria or coryneforms) (Roth and James 1988). On the contrary, some skin sites have a fluctuating surface temperature and are relatively dry, for example, arms and legs. In these sites, the composition of commensal microorganisms is more complex (Grice and Segre 2011; Hannigan and Grice 2013), and the dominating types of bacteria colonizing on these sites are β-Proteobacteria and Flavobacteriales (Grice et al. 2009).

Skin invaginations, such as sweat glands and sebaceous glands, affect the distribution of skin microorganisms as well. Sweat glands constantly secrete water, salt, and electrolytes to construct a cool, weakly acidic milieu on skin surface, that restricts the species of skin organisms that can live there (Sanford and Gallo 2013). Sweat glands also influence skin microbiota via expressing AMPs (Murakami et al. 2002), as described before, which are important components for the antimicrobial barrier. Sebaceous glands can secrete sebum which contains abundant lipids. Face, chest and back have more sebaceous glands than other body sites, thus they are chiefly colonized by lipophilic microorganisms, such as *Cutibacterium* spp. and *Staphylococcus* spp. (Grice and Segre 2011; Roth and James 1988).

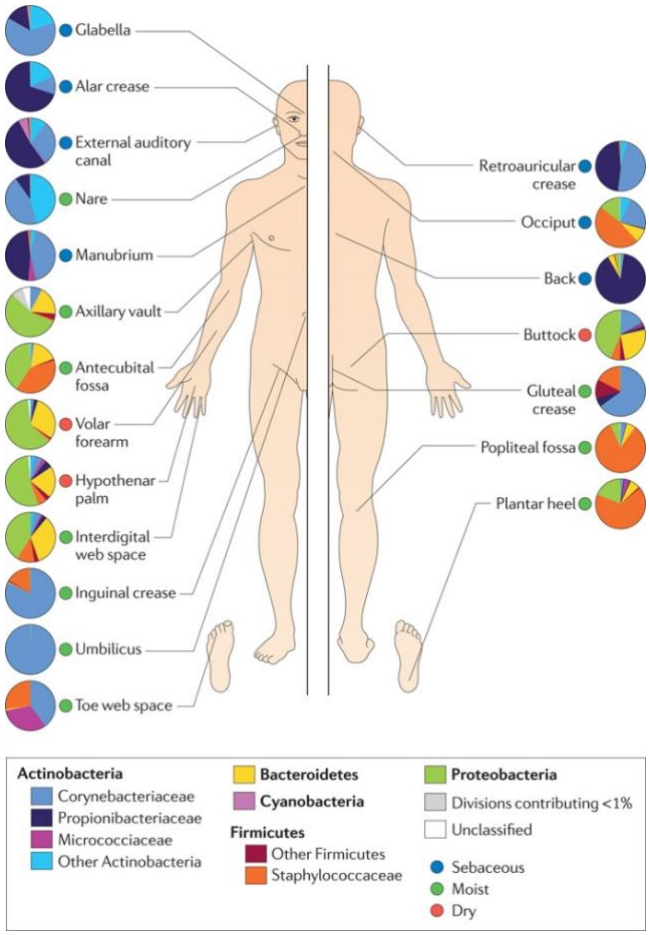

Figure 1.3 The distribution of microorganisms on human body (adapted from 'The skin microbiome' by Grice, E.A. and Segre, J.A. (2011). *Nature Reviews Microbiology*, 9(4), pp.248)

Host factors also affect the composition of the skin microbiome. Firstly, the skin microbiome varies gradually with age. For example, Bacteroidetes and Firmicutes phyla are richer on the skin of younger women while the Proteobacteria and Actinobacteria phyla are richer on that of older women (Kim et al. 2019). Gender also influences the composition of the skin microbial community. In general, males have higher numbers of secretory appendages, resulting in a greater numbers of organisms and biotypes (Marples 1982; Noble and Pitcher 1978). Moreover, the skin microbiome is different between ethnic groups as well. For instance, the microbiome of the human axillary in east Asians has higher total bacterial abundance and the proportion of

Proteobacteria is greater than in Caucasians and Hispanics (Li et al. 2019).

1.3.1.2 Exogenous factors

Various exogenous factors, such as the use of drugs and hand washing, are associated with variations in human skin microbiome. For example, topical antibiotics can quickly alter the skin bacterial communities with the reduction of colonization of normal commensal bacteria such as *Staphylococcus* spp. (SanMiguel et al. 2017); the structure of palm microbiome changed after washing (Fierer et al. 2008). In addition, UVR exposure also affects the skin community. This is supported by the *in vitro* experiment which illustrated that UVR could inhibit the growth of several skin bacteria (Faergemann and Larkö, 1987). Recently, a study also showed the compostion of human skin flora was changed upon UVA and UVB exposure: the Cyanobacteria increased while the Lactobacillaceae and Pseudomonadaceae reduced following UVR treatment (Burns et al. 2019). However, whether this UVR-induced change in skin microbiota is active or passive for skin health is still unclear.

1.3.2 The positive effect of skin microorganisms for skin health- serving as the skin microbial barrier

1.3.2.1 Protecting against infection

Various research has demonstrated that the skin commensal bacteria can prevent potential infection caused by pathogens in both indirect and direct ways (Sanford and Gallo 2013). For example, skin microbes compete with invasive pathogens for the limited space and nutrients of skin surface, greatly reducing the potential colonization of harmful microorganisms (Bäsler et

al. 2016). It has also been demonstrated that various substances produced by skin commensals directly inhibit the growth of pathogens (Harder et al. 2013; Spinola et al. 2016).

Staphylococcus epidermidis (*S. epidermidis*) is one commensal bacterium which is abundant on skin's surface. It has been showed that *S. epidermidis* can produce various bacteriocins to enhance antibiotic function (Jannuzzi et al. 2007). Some researchers showed that a subset of *S. epidermidis* can secrete the serine protease Esp to prevent biofilm formation as well as nasal colonization by the pathogenic *Staphylococcus. aureus* (Iwase et al. 2010). Furthermore, other scholars have demonstrated that *S. epidermidis* can produce phenol-soluble modulins (PSMs) gamma and delta, which are bacteriocins similar to LL-37. These bacteriocins can work with each other as well as host-derived LL-37 to inhibit the growth of *S. aureus* by disrupting the lipidic biofilm of pathogens (Cogen et al. 2010).

The free fatty acids (FFAs) released by skin commensal bacteria can also eliminate pathogens (Spinola et al. 2016). It has been shown that *C. acnes* can inhibit the growth of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) via releasing short-chain fatty acids (SCFAs) produced by fermentating glycerol (Muya Shu et al. 2013). As CA-MRSA is an important pathogen associated with skin infections (Cohen 2007) and is resistant to a wide range of antibiotics (Muya Shu et al. 2013), this finding presents novel microbial treatments for MRSA-related diseases. Another example showing the antibiotic function of microbial FFAs was illustrated through an *in vitro* experiment, which showed that the growth of the pathogen *Streptococcus pneumoniae* (*S. pneumoniae*) was limited in the presence of *Corynebacterium accolens (C. accolens),* a commensal bacterium colonizing the nostril. It was domenstrated that

C. accolens can hydrolyze the skin surface triacylglycerols (e.g. triolein) and then release oleic acid, a FFA which eventually eliminates the *S. pneumoniae* (Bomar et al. 2016).

Various studies have shown that skin commensal organisms can also reinforce both the innate and adaptive immunity to eliminate pathogens (Sanford and Gallo 2013; Hannigan and Grice 2013). Based on an *in vitro* study, it was shown that *S. epidermidis* may influence keratinocytes to secret more AMPs (hBD 2 and hBD 3) to block the growth of group A streptococcus (GAS) and *S. aureus* (Lai et al. 2010). Another study also demonstrated that commensal staphylococcus can increase the quantity of AMPs (hBD-3 and RNase7) produced by keratinocytes to enhance innate immunity (Wanke et al. 2011). Along with keratinocytes, skin commensal bacteria also influence mast cells to intensify the immune response. It has been shown that *S. epidermidis* can produce lipoteichoic acid (LTA) to activate TLR2. The activation of TLR2 can stimulate mast cells to elevate cathelicidin, resulting in a better resistance against vaccinia virus. Meanwhile, more mast cells are also recruited in those skin areas with viral infection, leading to a stronger immune response (Wang, Macleod, & Nardo, 2012).

Additionally, a recent study illustrated the regulatory effect of skin microbiota on encoding the expression of innate immune-related genes. Compared to the germ-free mice, the expression of various genes which modulate the innate immune activations were upregulated in the specific pathogen free mice, such as the PRRs genes, the interferon regulatory factors genes, the complement cascade genes and the AMPs formation genes. These data give strong evidence at a genomic level that the skin microbiota positively affects the skin innate immunity (Meisel et al. 2018).

The effect of skin microbes on enhancing adaptive immunity is suggested by an experiment with germ-free mice. These mice have an insufficient immune response to *Leishmania.* Whereas, after *S. epidermidis* colonized on the skin of those germ-free mice, the level of dermal T cells could be recovered, resulting in rescuing the protective immunity (Naik et al. 2012).

1.3.2.2 Influencing on the skin structural barrier positively

The positive effects of skin commensal microorganisms are also embodied in strengthening the TJs. This was supported by an *in vitro* experiment which showed the production of important TJ proteins, occludin and ZO-1, was improved by the colonization by *S. epidermidis* (Ohnemus et al. 2008). Another study illustrated that *S. epidermidis* can produce LTA to activate TLR2 (Wang, Macleod, & Nardo, 2012) which can subsequently strengthen TJ barrier (Yuki et al. 2011), suggesting that *S. epidermidis* has the ability to enhance the TJ barrier indirectly.

Recent research has suggested that the skin microbiota is involved in the formation of the epidermal permeability barrier of skin. Germ free mice showed much reduced expression of genes involved in the epidermal differentiation complex compared to expression in specific pathogen free mice. These genes included some involved in the formation of filaggrin and the cornified envelope, both of which are essential components for the construction of the SC. These data demonstrate the crucial role of skin microbiota in enhancing skin barrier function at least in the mouse (Meisel et al. 2018).

1.3.2.3 Alleviating inflammation

The skin microbiota can negatively regulate the immune system to suppress the inflammatory

response (Spinola et al. 2016). A study has shown that *S. epidermidis* could reduce the skin inflammation after injury by releasing LTA to suppress the TLR 3 signaling (Lai et al. 2009). This suppression was induced by the activation of the TNF receptor-associated factor-1 (TRAF1, a negative regulator of the TLR3 signaling). This study demonstrated the unique role of skin commensal bacteria in promoting wound healing and proposed a potential treatment for delayed wound healing caused by excessive inflammation. The same anti-inflammatory effect of skin microbiota was demonstrated again in a recent study which used the atopic dermatitis (AD)-like mouse model (Yu et al. 2019). It was demonstrated the skin microbiota could metabolize tryptophan (Trp, an essential amino acid) and produce Indole-3-aldehyde (IAId) to relieve the inflammation of AD-like dermatitis mice by down-regulating the inflammatory $CD4^+$ T cells and Gr11 cells.

1.3.3 Skin microbiota- the relationship with skin disorders

Because of the indispensable role of skin microbiota, it is important to keep it healthy and balanced. The dysbiosis of skin bacterial communities has been associated with various skin disorders, such as AD, psoriasis, acne vulgaris and rosacea (Mańkowska-wierzbicka et al. 2015). The associations of skin disorders and the disruptions of skin microbiota are listed in table 1.1. However, it is important to note that in most cases it is not known whether this change to the microbiota is a cause or a epiphenomenon of disease. Particularly, in some special conditions, the harmless commensal organisms can convert into pathogenic bacteria and lead to diseases (Sanford and Gallo 2013).

Table 1.1 The associations between cutaneous diseases and skin microorganisms'

disruptions

Disease	Changes of skin microbiota	References
Atopic dermatitis	*S. aureus* ↑ *S. epidermidis* ↑ Overall microbial diversity ↓	Kong et al. 2012
Psoriasis	Firmicutes ↑ Actinobacteria ↓	Gao et al., 2008
	Corynebacterium ↑ Propionibacterium ↑ Staphylococcus ↑ Streptococcus ↑	Alekseyenko et al. 2013
Acne vulgaris	Associated with some strains of *C. acnes*, such as type IA	Fitz-gibbon et al. 2013; Kwon et al. 2013
	Microbial density of follicles ↑	Bek-Thomsen et al. 2008

1.3.3.1 Atopic dermatitis

Atopic dermatitis (AD) is a chronic and relapsing inflammatory cutaneous disease which affects principally children and adolescents (Garnacho-Saucedo et al. 2013). The skin lesions of AD normally present on antecubital fossa and popliteal fossa, the moist sites of human body (Grice et al. 2009). It has been identified that the microbial community of AD patients differs from that of healthy people. In an early study, more than 90% of patients' skin were colonized by *S. aureus* on both lesional and non-lesional areas (Leyden et al. 1974). Analyzed by 16S rRNA gene sequencing, the microbial community structure of AD patients showed an higher percentage of *S. aureus* and *S. epidermidis* but a decreased overall microbial diversity during a flare (table 1.1) (Kong et al. 2012). Therefore, antibiotic therapy is an important method for AD treatment to prevent the secondary infection and relieve the severity of lesions.

1.3.3.2 Psoriasis

Psoriasis is a recurring autoimmune inflammatory disease, presenting as distinctly demarcated chronic erythematous plaques covered by silvery white scales. Unlike AD, its lesions preferentially appear on some dry skin sites, such as elbows, knees, scalp, umbilicus and lumbar area (Schön and Boehncke 2005). Various researchers have tested the difference between bacteria colonizing normal skin and psoriasis lesions. Generally it has been shown that psoriasis lesions have an increase of Firmicutes but a decrease of Actinobacteria compared with the heathy skin (table 1.1) (Gao et al. 2008). Other studies found the proportion of corynebacterium, propionibacterium, staphylococcus and streptococcus were relatively elevated on lesions compared with healthy skin (table 1.1) (Alekseyenko et al. 2013). However, although these studies demonstrated different bacteria colonization between healthy and lesional skin, it is still unclear whether those changes are linked to the pathogenesis of the disease, or whether they are a consequence of psoriasis.

1.3.3.3 Acne vulgaris

Acne vulgaris is one of the most common cutaneous disorders occurring during adolescence. It is characterized by non-inflammatory comedone and inflammatory papules, pustules, nodules and cysts in pilosebaceous units (Dawson and Dellavalle 2013). Its pathogenesis has been studied for a century and the skin commensal *C. acnes* has been shown to be directly involved in the pathogenesis of the disease. First of all, the quantity of *C. acnes* parallels the increased secretions of sebaceous glands during puberty, producing more lipases, proteases

and hyaluronidases which damage the tissue of pilosebaceous follicles (Dessinioti and Katsambas 2010). At the same time, *C. acnes* causes inflammatory reactions by activating the classical and alternative complement pathways (Webster et al. 1979; Scott et al. 1979) as well as inducing the production of proinflammatory cytokines (Jeremy et al. 2003; Kim, 2005).

With the development of molecular typing methods, the strains of *C. acnes* correlated with acne vulgaris have been identified. It was found although the relative abundance of *C. acnes* had no apparent difference between patients and healthy individuals. However the specific strains of *C. acnes* found on the skin were dramatically different, and some strains were more related with acne (table 1.1) (Fitz-gibbon et al. 2013). This was supported by another study which showed the percentage of type IA *C. acnes* was higher in acne patients compared with the healthy control (table 1.1) (Kwon et al. 2013). In addition, the pathogenesis of acne was related to the dysbiosis of microbiome on follicles. It was demonstrated the pilosebaceous units of healthy individuals were only colonized by *C. acnes,* whereas follicles afflicted with acne were colonized by not only *C. acnes* but also other bacteria (table 1.1) (Bek-Thomsen et al. 2008).

As mentioned previously, the skin and its microbiome are unique in that are regularly exposed to UVR from sunlight. In the next section, the effects of UVR on skin and its microbiome will be discussed.

1.4 Ultraviolet radiation and its influence on skin

With a wavelength ranging from 100 to 400nm, UVR is a component of electromagnetic spectrum located between visible light and x-rays (figue 1.4). Based on the different biological effects, the International Commission on Radiation (ICR) subdivides UVR into three types which are UVA, UVB, and UVC. The spectrum range is 315 to 400nm for UVA, 280 to 315nm for UVB and 100 to 280nm for UVC (table 1.2). However, some literature suggests that the boundary between UVB and UVA is 320nm while 290nm is the boundary between UVB and UVC (Robert 2015; Hawk et al. 2010).

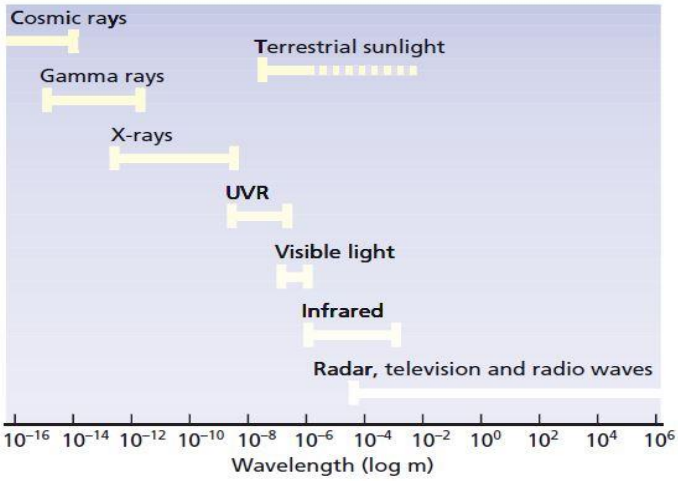

Figure 1.4 The electromagnetic spectrum (adapted from *Rook's Textbook of Dermatology* (pp.29.2) by Hawk et al. (2010), Blackwell Science Ltd.)

It is well known that sun plays a significant role in producing natural UVR. Although it continuously emits a huge amount of UVR, only a small part can reach the Earth's surface as the atmosphere has considerable ability in absorbing the biologically detrimental bands of UVR. As a result, those electromagnetic waves with a wavelength longer than 290nm are resisted and only UVA (96.5%) and UVB (3.5%) can reach the surface of the Earth. At the same time,

the scattering of clouds, oxygen, nitrogen, and some pollutional gases also contributes remarkably to attenuate the energy of UVR (Diffey 2002; Robert 2015). Moreover, in addition to the natural sources, UVR can also be produced by various artificial sources, such as gas discharge lights and arc lamps (Hawk et al. 2010).

Table 1.2 The classification of UVR from ICR (Robert 2015)

Subtype of UV	Waveband(nm)
UVA	315-400
UVB	280-315
UVC	100-280

1.4.1 The beneficial effects of UVR

As one of the most common environmental elements, the biological effects of UVR have been studied for a long time. Although UVR has several harmful influences on skin health, its beneficial effects are also important for humans.

UVR is the main source which induces the synthesis of vitamin D_3 in skin (Trummer et al. 2016). Vitamin D_3 is a vital element in not only maintaining skeletal health, but also decreasing the risk of several chronic diseases, such as autoimmune diseases, cancers as well as heart and circulation problems (Holick 2010). In response to UVB, Epidermal 7-dehydrocholesterol has been shown to form pre-vitamin D_3, which is unstable and immediately converts to vitamin D_3 in a temperature-dependent way. Then vitamin D_3 is transported by vitamin D-binding proteins from the intracellular spaces to the extracellular spaces, participating the circulation and metabolism of body (Holick 2006; Hawk et al. 2010). Since only a small part of vitamin D_3 can be supplemented orally (Greinert et al. 2015), exposing skin regularly in sunlight is important

for maintaining sufficient levels of vitamin D.

UVB can induce the generation of AMPs to enhance the skin barrier. Based on the results of several studies, UVR can directly induce keratinocytes to release various AMPs, such as defensins, RNase 7, and psoriasin (Gläser et al. 2009; Seong et al. 2001).

UVR can serve as an effective therapy for various chronic skin diseases. For example, it is used for the treatment of psoriasis. Although the exact nosogenesis of psoriasis is still unclear, it is suggested to be associated with activated T cells (Wrone-smith and Nickoloff 1996). As UVB has the biological property of immunosuppression, it could ease the symptom of psoriasis by inducing the apoptosis and reducing the activity of disease-modulating T cells (Krueger et al. 1995; Ozawa et al. 1999; Schmitt and Ullrich 2000). Another representative example of UVR phototherapy is the management of vitiligo which is a gene-related autoimmune cutaneous condition (Ezzedine et al. 2015). The potential mechanism of UVR phototherapy in treating vitiligo could be associated with the UVR-induced immunosuppression and redistribution of the melanocytes located in the hair follicles (Juzeniene and Moan 2012).

UVR exposure also has many other benefits for human health, such as protecting the circulatory system (Halliday and Byrne 2014), improving mood and reducing pain (Juzeniene and Moan 2012). However, as the prolonged UVR exposure is detrimental for human skin tissues and cells, the balance between exposure and protection from UVR should be considered carefully.

1.4.2 The detrimental biological effects of UVR

Although UVR has several beneficial effects on human health, its harmful aspects also have been demonstrated by many studies. It is clear that UVR has several negative effects on skin such as damaging DNA (Greinert et al. 2015), immunosuppression (Ullrich and Byrne 2012) and compromising skin barrier function (Biniek et al. 2012). These are discussed in more detail below.

1.4.2.1 DNA damage

DNA damage could be the fundamental pathogenesis of most UVR-related skin conditions and disorders (Robert 2015; Vink and Roza 2001). UVR is reported to cause DNA damage in both direct and indirect ways. Generally, DNA can rapidly convert a great proportion of UVR photons to uninjurious heat energy (Robert 2015). However, there is still a small fraction of photons which cannot be converted. They are absorbed by the double bonds of DNA, then the original double bonds immediately break and new covalent bonds (four-membered ring) are formed between the adjacent pyrimidine bases. These new covalent bonds are known as cyclobutane pyrimidine dimers (CPDs) (figue1.5) (Goodsell, 2001; Ravanat et al. 2001; Vink & Roza, 2001). Fortunately, most of those genetic lesions can be detected and repaired promptly by the process of nucleotide excision repair which is part of the skin's protective mechanism against the development of tumors. In addition, if the DNA damage exceeds a threshold, the keratinocytes would initiate apoptosis (this will be discussed in detail in the next part 1.4.2.2) which could remove the UVR-induced genetic mutations to prevent further cascade effects (Lee

et al. 2013).

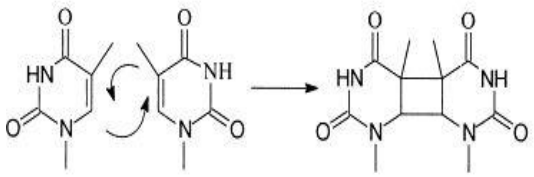

Figure 1.5 The formation of CPD (adapted from **'**Direct and indirect effects of UV radiation on DNA and its components' by Ravanat et al. (2001). *Journal of Photochemistry and Photobiology B: Biology*, 63(1–3), pp.89)

UVR can also injure DNA in an indirect mechanism. In some situations, UVR is absorbed by some chromophores which cannot rapidly convert photons to harmless heat and hence have a relatively long excited state (Cantrell et al. 2001). Bimolecular reactions occur between those chromophores and other molecules to generate free radicals and reactive oxygen species, both of which can break the integrity of DNA and cause damage via oxidative stress (Ribeiro et al. 1992). Meanwhile, as the free radicals can reach the unexposed areas of skin, indirect DNA damage could induce melanoma in those skin areas (Robert 2015). Interestingly, damage to DNA can occur at levels of UVR which do not induce erythema and DNA damage is also the inducer of tanning highly suggestive of the idea that there is no so such thing as 'safe tan'.

1.4.2.2 Immunosuppression

UVR can suppress the immune response in different ways (Ullrich and Byrne 2012): UVB induces immunosuppression via DNA damage, while UVA induces immunosuppression by oxidative stress (Halliday et al. 2012). The first target of UVR is the DNA of keratinocytes. After UVR exposure, those keratinocytes with damaged DNA can express and upregulate various

cytokines (Nishigori et al. 1996) which induces the permeation of suppressor macrophages and neutrophils (Cooper et al. 1993). Immunosuppression caused by these cytokines can occur at both exposed and unexposed sites of body (Vink and Roza 2001). At the exposed skin, these cytokines build a specific environment which could reduce the activity of antigen presenting cells; at the unexposed skin, the number of T helper 2 cell (Th2)-like suppressor cells could be elevated by those cytokines to suppress the immune response systemically (Schwarz 1999).

Additionally, UVR also targets the DNA of several immune cells, such as skin located antigen presenting cells and dendritic cells. This is supported by an animal experiment which showed that the antigen presenting ability of skin dendritic cells with UVR-induced DNA damage is inefficient, and those dendritic cells could participate in inducing suppressor T lymphocytes (Vink et al. 1996). Furthermore, UVR could not only lead to the movement of Langerhans cells from skin cells into the draining lymph nodes (Noonan et al. 1984; Toews, Bergstresser, & Streilein, 1980), but also affects mast cells which play an important role in suppressing immune response (Hart et al. 2001).

1.4.2.3 Compromise of skin barrier functions

Another detrimental effect of UVR is to weaken the skin barrier function. First of all, many researchers have demonstrated that UVR can affect the morphology of skin (Biniek et al. 2012). Pearse et al. showed that both UVA and UVB can elevate the average epidermal thickness (Pearse et al. 1987); Meguro et al. demonstrated that UVR could affect the permeability function of skin, increasing the transepidermal water loss (Meguro et al. 1999). Furthermore, UVR can

also break the mechanical barrier function of epidermis. For example, Biniek et al. indicated that, following the UVR exposure, although the SC stiffness was still constant, the intercellular strength, strain, and lipid cohesion of SC diminished significantly, all of which could be associated with various cutaneous disorders, such as inflammation and infection (Biniek et al. 2012).

1.4.3 The physiological response of skin to UVR

1.4.3.1 Inflammation

Inflammatory responses, such as erythema, swelling, fever, and pain, are suggested to be the most obvious warning of UVR related DNA damage (Hawk et al. 2010). These common reactions can present in hours following UV irradiation (Harrison and Young 2002) although individual susceptibility varies depending on the difference of skin thickness and pigmentation (Vink and Roza 2001). It has been identified that UVR probably induces epidermal keratinocytes to release various cytokines (Barker et al. 1991), which subsequently diffuse to the dermis and lead to dermal haemangiectasis and spongiosis, resulting in skin inflammation (Takashima and Bergstresser 1996; Hawk et al. 2010).

1.4.3.2 Tanning

Tanning is a process in which melanocytes manufacture melanin to absorb both UVR photons and UV-generated free radicals, preventing skin DNA and other important cellular components from reacting with UVR, thus it is considered as the main defensive method to protect skin cells from further UVR-induced damage (Gilchrest and Eller 1999). Depending on the wavelength of

UVR which causes melanogenesis, tanning can be divided into two different types, including the early and the delayed pigmentation (Greinert et al. 2015). The former occurs quickly following UVA exposure, causing photo-oxidative darkening and redistribution of melanin which could provide protections for the genetic sites of SB cells. The latter bases on the mechanism of transporting melanin from melanocytes to neighboring keratinocytes, therefore it initiates gradually from hours to days and can exist for a relatively long time (Hawk et al. 2010; Orazio et al. 2013). Specially, the delayed tanning could reduce the sunburning sensitivity of white skin (Young and Sheehan 2001) while the immediate tanning was ineffective on decreasing the sunburning sensitivity (Hawk et al. 2010).

1.4.3.3 Hyperplasia

UVB exposure accelerates the synthesis of DNA and proteins as well as the speed of cell mitosis, resulting in thickening of epidermis and dermis. This process is known as skin hyperplasia. Commonly in Caucasians who have less melanocyte, it could be an additional method working together with tanning to protect skin against UVR. However the stimulus for hyperplasia is still unclear, and more work is needed in the future for a better understating of its mechanism (Hawk et al. 2010).

1.4.3.4 Premature skin aging

Chronic UVR exposure results in the premature skin aging of skin which is characterized by laxity, wrinkling, dryness, uneven pigmentation and brown spots (Robert 2015; Hawk et al. 2010). These features could be associated with the reduction of collagens, which mainly

support the epidermis and maintain the elasticity of skin (Fisher et al. 1997). Research has demonstrated the UVR could either degrade the existing collagens or limit the generation of new ones, all of which results in the reduction of collagens (Fisher et al. 2002). These data provide compelling evidence that premature skin aging is correlated with UVR exposure.

1.4.4 Keratinocte response to high dose UVR

Apoptosis is a process of programmed cell death, which is characterised by the cell shrinkage and the collapse of the nucleus (Cohen et al. 1992). It is associated with several normal physiological phenomena (e.g. the turnover of old tissue) but also with various pathological conditions, such as skin cancers and immune-related diseases (Assefa et al. 2005). It has been demonstrated in multiple studies that UVR can induce keratinocyte apoptosis and this occurs when the damage caused by UVR is too great to repair. Apoptotic keratinocyte can be seen in skin sections as characeristic sunburn cells (figure 1.6).

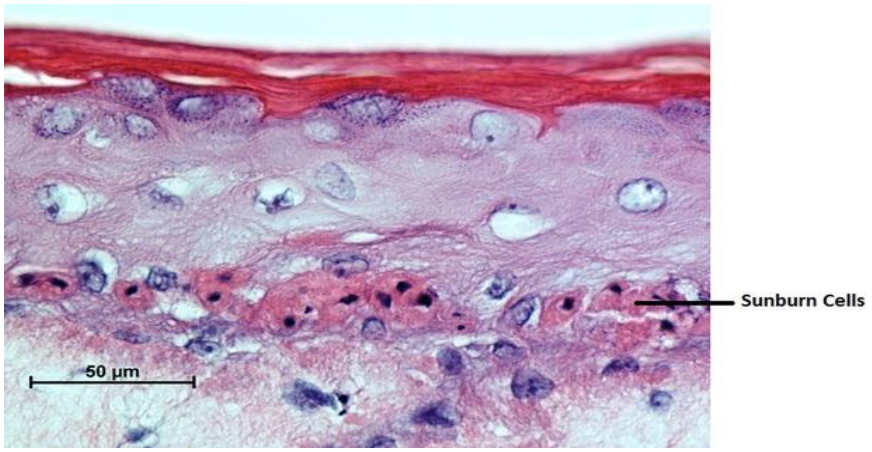

Figure 1.6 Sunburn cells (adapted from 'DNA photoprotection conveyed by sunscreen' by Dehaven, C. et al. (2014). Journal of Cosmetic Dermatology, 13(2), pp.101)

Human skin was exposed to 200 mJ/cm^2 dose of UVR. Sunburn cells in skin sections were measured 24 hours post UVR treatment using haematoxylin and eosin stain. Typical sunburn cells (indicated with the black line) are characterised by pyknotic nuclei and condensed chromatin.

In addition to apoptosis, a high dose of UVR is also associated with the induction of programmed necrosis, including pyroptosis and necroptosis (Tang et al. 2021). The most representative feature of pyroptosis is the formation of gasdermin D (GSDMD) pores in the plasma membrane (Shi et al. 2017). NLRP1 and NLRP3 in the inflammasome complex can detect UVR stimuli and then consequently active caspase-1. The activated caspase-1 can not only promote the maturity of IL-1β and IL-18 but also cleave GSDMD to produce the gasdermin-N domain, which can further result in the formation of GSDMD pore in the cell membrane. The GSDMD pores then discharge the generated inflammatory cytokines (IL-1β and IL-18), leading to cell swelling and lysis due to the alteration of osmotic pressure (figure 1.7) (Tang et al. 2021; Shi et al. 2017; Kim et al. 2019; Fink and Cookson 2005).

Necroptosis is mainly featured by swelling of organelles and damaged cell membrane (Liu et al. 2019). Briefly, the induction of necroptosis is mediated by receptor-interacting protein kinase (RIPK) 1 and RIPK3. TNF binds tumour necrosis factor receptor in the cell membrane and then consequently recruit TNF receptor-associated death domain (TRADD), RIPK1, TNF receptor-associated factor 2/5 (TRAF2/5), cellular inhibitor of apoptosis protein 1/2 (cIAP1/2) and linear ubiquitin chain assembly complex (LUBAC) to form complex I. In the absence of caspase-8, RIPK1 is deubiquitinated and then interacts with RIPK3, inducing the assembly of the necrosome. The activated RIPK3 in the necrosome can further phosphorylate mixed lineage kinase domain-like (MLKL) protein, which eventually causes necroptosis via breaking the integrity of the cell membrane (Dhuriya and Sharma 2018; Kim et al. 2019; Kaczmarek et al. 2013; Liu et al. 2019).

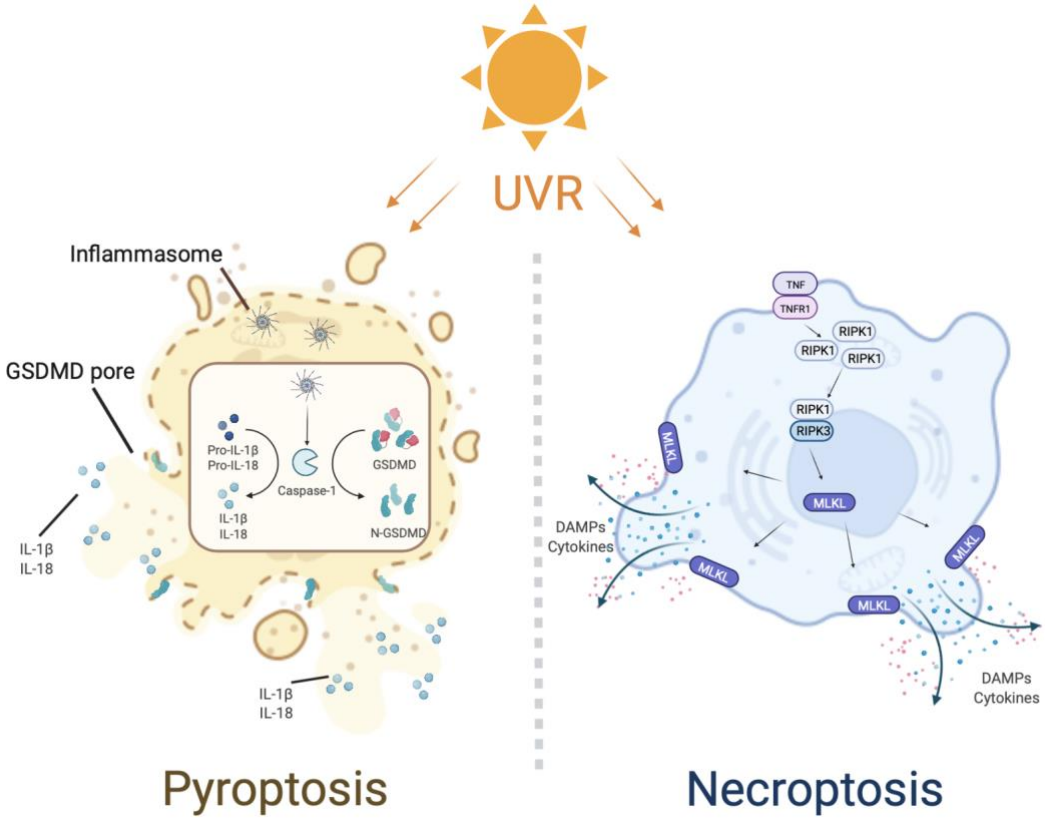

Figure 1.7 The induction of pyroptosis and necroptosis post UVR exposure (drawn by Wen Duan)

High doses of UVR can induce pyroptosis and necroptosis. The initiation of pyroptosis involves the ability of the NLRP1 and NLRP3 inflammasome to detect UVR exposure and then active caspase-1. The activated caspase-1 can either mature IL-1β and IL-18 or cleave GSDMD to form gasdermin-N, which can induce cell membrane perforation. Inflammatory substances then can release into extracellular space via GSDMD pores. The induction of necroptosis is associated with the binding of TNF with TNFR1 in the cell membrane. In the absence or inhibition of caspase-8, RIPK1 and RIPK3 accumulate and form the RIPK1-RIPK3 necrosome complex. RIPK3 in the necrosome then recruit and phosphorylate MLKL, leading to cell necroptosis. IL: Interleukin; GSDMD: gasdermin D; DAMP: damaged associated molecular pattern; TNF: tumour necrosis factor; TNFR: tumour necrosis factor receptor; RIPK: receptor-interacting protein kinase; MLKL: mixed lineage kinase domain-like protein.

1.4.5 Pathophysiological responses of skin to UVR exposure

There are a number of skin disorders that can be induced or aggravated by exposure to UVR. These are collectively known as the photodermatoses. For example, polymorphic light eruption

(PLE), which is characterized by erythematous papules, vesicles or plaques which appear several hours after UVR exposure. PLE is a type II delayed hypersensitivity reaction against an unknown allergen that is produced by UVR (Hawk et al. 2010). It was demonstrated that patients with PLE fail to immunosuppress following exposure to UVR and it is thought that it is the recognition and presentation of the cutaneous antigen in PLE patients, which leads to the clinical symptoms (Lembo and Raimondo 2018). However, the pathogenesis of PLE has not yet been fully characterized. One hypothesis is that the photo allergen, may be produced by the skin microbiota (Patra and Wolf 2016).

1.4.6 UVR and the skin microbiota

The effect of UVR on skin microbiota and the downstream reactions is still a new area in skin microbiology. Recently, as the importance of skin microbiota on maintaining skin health is increasing, this subject has begun to attract more attentions. A samll clinical study exhibited both UVA and UVB exposure can change the composition of male skin microbiome, resulting in a growth in the Cyanobacteria (Burns et al. 2019). As the metabolites of Cyanobacteria are associated with the development of some skin symptoms (Stewart et al. 2006), this finding could lead to a better understanding of the pathogenesis of UVR-induced skin disorders. Another study showed that a strain of *S. epidermidis* could protect against skin cancer after UV exposure by producing 6-N-hydroxyaminopurine (6-HAP). 6-HAP is a chemical molecule that suppresses the growth of skin tumours (Nakatsuji et al. 2018). With the colonization of this strain, the growth of UV-induced skin tumours on mouse skin was limited compared to the control group which were colonized with the strain of *S. epidermidis* deficient in 6-HAP

production. This was in line with another study which illustrated that the skin commensal bacteria can protect skin against melanoma in response to UVB exposure via affacting the survival of melanocytes (Wang et al. 2018). This *in vitro* study showed that the the supernatant of *C.acnes* reduced the survival of melanocytes following the UVB exposure by inducing cell apoptosis. As the irradiated melanocytes containing DNA damage are involved in the fomation of melanoma, this *C. acnes*-induced apoptosis is beneficial for inhibiting the development of UVR-induced skin cancer. Moreover, skin microbiome is also involved in restricting the UVR-induced immunosuppression (Patra et al. 2019). A recent study exhibited that skin microbiota can up-regulate the pro-inflammatory molecules in mice, which could inhibit the UVR-induced immonosuppression. As the UVR-induced immune suppression has been shown to positively correlated with skin cancer (Narayanan et al. 2010), this finding reveals the potential role of skin microbiota in cancer prevention.

1.4.7 The SOS response: an important strategy for bacterial DNA repair

Skin microbiota uniquely, is exposed to UVR potentially on a daily basis. Therefore it is surprising that little is known about how skin bacteria protect themselves from the negative effects of UVR. To date, much of the knowledge regarding bacterial response to UVR has come studies of model bacteria such as *E. coli*. In these studies, damaged DNA endangers bacterial survival following UVR exposure as this could induce cell death via unsuccessful DNA transcription and replication (Sinha and Häder 2002). To maintain cell viability, bacteria have evolved several pathways to repair the UVR-induced DNA lesions (Rastogi et al. 2010; Sinha and Häder 2002). One of the most important mechanisms is named the SOS response, which

is multi-regulated by various proteins and genes to address the DNA lesions by initiating several DNA repair pathways, including the nucleotide excision repair, the recombinational repair and the translesion synthesis (Baharoglu and Mazel 2014).

RecA and LexA are the most crucial proteins for mediating the SOS response (Little and Mount 1982). RecA is a 38 kDa protein that can launch the SOS response in response to the accumulation of damaged DNA (Patel et al. 2010). Post UVR exposure, RecA monomers assembly on the DNA lesions to form filaments which catalyze the proteolysis of the SOS repressor protein LexA, leading to the expression of various genes which participate in addressing DNA damages (Baharoglu and Mazel 2014; Sinha and Häder 2002; Patel et al. 2010; Maslowska et al. 2019). The expression order of these SOS genes is associated with their operators' binding affinity to LexA protein. With the decrease of LexA, this repressor dissociates firstly from the operators of genes that have the weakest LexA binding affinity, e.g., *recA*. In this way, the SOS-mediated genes are sequentially activated for launching DNA repair (Little and Mount 1982; Patel et al. 2010).

1.5 Conclusion and hypothesis

As the outermost and largest organ of human, skin plays a crucial role in protecting body from various challenges. The skin surface is colonised by microorganisms which constitute a unique and diverse skin microbiome which is involved in maintaining homeostasis as well as preventing against skin diseases. Both skin cells and skin microbiome are directly exposed to UVR regularly. As a common environmental element, the influence of UVR on human skin has

been studied for many decades, showing both the positive and negative effects. By contrast, the effects of UVR on skin commensal bacteria have been overlooked.

Aims and hypothesis: members of the skin microbiota may have differential and specific responses to UVR. The response of the microbiota may, in turn, change the physiological response of keratinocytes to UVR.

The aim of this project is to characterize the interaction of skin microbiota and keratinocytes in the context of UVR exposure. The specific objectives of the project are:

1. *In vitro*, to test the sensitivity of individual skin commensal bacteria to simulated solar radiation (SSR);

2. To study the response of normal primary human epidermal keratinocytes (NHEKs) to SSR in the presence of single strains of skin commensal bacteria, using keratinocyte viability as the read out.

3. To investigate the molecules within bacteria producing any effects shown in 2 above.

4. To investigate the Influence of the skin microbiome on the sunburn response in healthy humans *in vivo*.

Chapter 2 Materials and methods

2.1 Materials

All reagents used for skin cell culture were obtained from Promocell (Heidelberg, Germany). Disposable plastics associated with cell culture were obtained from Corning (NY, USA). Reagents and equipment used for biochemical analyses were purchased from Invitrogen by Thermo Fisher Scientific (UK). Unless otherwise stated, all other reagents were obtained from the Sigma Aldrich Chemical Company (Dorset, UK).

2.2 Preparation of normal human primary epidermal keratinocytes (NHEKs)

Proliferating NHEKs in a T25 flask were purchased from Promocell and cultured in a CO_2 incubator (Sanyo, Japan) at 37°C, 5% CO_2 with 8ml of prepared keratinocyte medium (500 ml of Keratinocyte Growth Medium 2 containing 60µl of proprietary $CaCl_2$ solution and 12.3 ml of 'SupplementMix' solution). Once the cells reached 80% confluency, they were passaged as follows: the medium was aspirated and replaced by 3 ml of 0.04% trypsin/0.3% EDTA solution and the flask was placed in the CO_2 incubator at 37°C for 5-7 minutes for cell detachment. Once most of the cells were detached, the trypsin was neutralised using 3 ml of 'Trypsin neutralising solution' (0.05% trypsin inhibitor containing 0.1%/BSA). The solution was then centrifuged at 112 x g for 3 minutes and the cell pellet was resuspended in 2 ml of fresh keratinocyte growth medium. After that, the cell suspension was equally seeded in two T75 cell culture flasks (around $3*10^5$ cells were seeded into one flask), which contained 13ml of fresh keratinocytes growth medium. The cells were maintained at 37°C, 5% CO_2 and their medium were replaced every two days until they exceeded the 80% confluency.

2.3 Preparation of bacteria

2.3.1 Bacterial strains

Staphylococcus epidermidis (NCTC 11047, NCTC 6513 and NCTC 10519), *Micrococcus luteus* (NCTC 2665) and *Corynebacterium jeikeium* (NCTC 11913) were purchased from the National Collection of Type Cultures (NCTC, Public Health England, UK); *Staphylococcus hominis* (ATCC 27844) and *Staphylococcus capitis* (ATCC 27840) were obtained from German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ, Germany); *Cutibacterium acnes*, isolated from the skin microbiota of a healthy volunteer, was obtained from the laboratory of Prof. Andrew Mcbain, University of Manchester.

To preserve the bacterial strains, 500µl of bacterial liquid cultures (described in section 2.2.2) were mixed with 500µl of 50% glycerol (a mixture of 100% glycerol (Acros organics, UK) with an equal volume of sterile distilled water) and stored in the -80°C ultralow temperature freezer (Nuaire, USA) before subculture.

2.3.2 Bacteria identification

2.3.2.1 Extraction of bacterial genomic DNA

3-5 colonies of each bacterium were picked from freshly cultured agar plates and suspended in 50µl of nuclease-free water. These bacterial suspensions were then heated at 100°C for 10 minutes. All samples then were centrifuged at 13000 x g for 10 minutes at 4°C before their supernatants were transferred to sterile Eppendorf tubes for further usage.

2.3.2.2 Bacterial DNA amplification using polymerase chain reaction (PCR)

To prepare samples for PCR, 5µl of each extracted bacterial DNA was mixed with 45µl of PCR master mix (containing 25µl of MyTaq ™ red mix (Bioline, UK), 16µl of nuclease-free water, 2µl of universal primers 27F (5µM) (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5µM) (5'-GGT TAC CTT GTT ACG ACT T-3')) in a PCR tube. All PCR tubes were then placed in a TGradient thermocycler (Biometra, Goettingen, Germany) programmed to run the following cycle: the first denaturation step was set at 94 °C for 3 minutes, followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 56°C for 30 seconds and extension at 72 °C for 1 minute. The final extension step was conducted at 72 °C for 7 minutes. All PCR products were maintained at 4°C until further use.

The purity of the PCR products was then assessed by performing agarose gel electrophoresis: 4µl of each amplified DNA sample was mixed with 1µl of BlueJuice™ gel loading buffer (Thermo Fisher Scientific (UK). These mixtures were then loaded into separate lanes of a 1% agarose gel containing the GelRed™ (Biotium, Middlesex, UK) stain. A 1Kb DNA ladder was also loaded to allow estimation of the size of any PCR products. The PCR products were electrophoretically separated in 1x tris-acetate EDTA (TAE) buffer (pH 8.3, generated by diluting the 50x TAE buffer (Merck, Gillingham, UK)) at 70 volts for 50 minutes before the DNA bands were visualised on an ultraviolet transilluminator (Sigma, UK).

2.3.2.3 DNA purification and Sanger sequencing

The PCR products in each sample were purified using the QIAquick PCR Purification Kit (Qiagen, West Sussex, UK) according to the manufacturer's protocol. The quantification of each

purified DNA sample was measured by using a Nanodrop spectrophotometer. 20-50ng of each purified DNA was then mixed with 4 picomoles of forward primer in a total volume of 10µl of ultrapure water. All prepared samples were sent to the Genomic Technologies Core Facility of the University of Manchester for the 16S rRNA gene sequencing using Sanger sequencing. Each bacterial sample was identified by analysing the generated FASTA files using the BLAST (Basic Local Alignment Search Tool) database (https://blast.ncbi.nlm.nih.gov).

2.3.3 Bacterial culture

Each bacterial stock was streaked onto an agar plate using a sterile loop and incubated for 24-96 hours at 37°C. For generating the liquid culture, a single colony of each bacterium was picked from the agar plate and then seeded into 10ml of sterile broth medium. All bacteria cultures were maintained at 37°C and cultured aerobically in a static Memmert incubator while the *C. acnes* culture was incubated anaerobically (10% CO_2 +10% hydrogen+ 80% nitrogen) at 37°C in a Mark 3 Anaerobic Work Station (Don Whitley Scientific, Shipley, UK). The details of each bacterium's culture medium and incubation time for plate culture and liquid culture are listed in table 2.1. All culture media used were sterilised in advance at a 15 Psi pressure, 121°C for 15 minutes.

Table 2.1 The culture medium and incubation time of each bacterium for plate and liquid culture

Bacterium	Plate culture		Liquid culture	
	Culture medium	Incubation time	Culture medium	Incubation time
S. epidermidis (NCTC 11047)	Sterile trypticase soy agar (TSA) (Oxoid, UK)	24 hours	Sterile trypticase soy broth (TSB) (Oxoid, UK)	Overnight
S. hominis		24 hours		Overnight
S. capitis		24 hours		Overnight
M. luteus		48 hours		48 hours
S. epidermidis (NCTC 6513)	Sterile brain heart infusion (Oxoid, UK) with Tween 80 agar	24 hours	Sterile brain heart infusion (Oxoid, UK) with Tween 80 broth	Overnight
S. epidermidis (NCTC 10519)		24 hours		Overnight
C. jeikeium		48 hours		48 hours
C. acnes	Sterile Wilkins-Chalgren agar (Oxoid, UK)	96 hours	Sterile Wilkins-Chalgren broth (WCB) (Oxoid, UK)	96 hours

2.3.4 Generation of bacterial growth curves

To generate the bacterial growth curves of each bacterial strain, 10 ml of liquid bacterial culture was prepared using the methods mentioned in section 2.3.3 and then 200µl of 1:100 dilution of each bacterial suspension was pipetted into one well of a 96-well plate in triplicate. For *C. acnes*, each well was covered by 1 drop of mineral oil to maintain an anaerobic environment. These 96 well plates were then incubated in a Powerwave XS plate reader (Biotek, Bedfordshire, UK) at 37°C for 24 hours (48 hours for *M. luteus* and *C. jeikeium*; 96 hours for *C. acnes*). The optical density (OD) of each well containing bacterial suspension was measured at 600 nm every 1 hour (every 2 hours for *C. acnes*) and all data generated from the plate reader were analysed

by using the Gen5 Software program (Biotek, Bedfordshire, UK) and GraphPad Prism 7 software (obtained from http://www.graphpad.com) to plot bacterial growth curves. This experiment was repeated thrice for each bacterium.

2.3.5 Generation of bacterial standard curves

To construct bacterial standard curves, an initial bacterial suspension of each organism was created by inoculating a single colony in 10 ml of sterile broth medium (the broth medium of each bacterium was listed in table 2.1) with a loop. The absorbance of this liquid culture was measured at 0, 3, 6, 24 hours (0, 6, 24, 48 hours for *M. luteus* and *C. jeikeium*; 0, 48, 72, 96 for *C. acnes*) after inoculation by a spectrophotometer at 600 nm. At the same time, the corresponding bacterial viable cells counts were made by performing serial dilution and plate counting as follows: 1 ml of bacterial suspension was transferred into a tube containing 9 ml of sterile broth medium to generate a 1:10 dilution and then 1 ml of this dilution was pipetted into another 9ml of fresh broth to create a 1:100 dilution (10^{-2} dilution). Similarly, 10^{-3} to 10^{-9} dilutions were generated by repeating the same procedure. For each dilution, 30μl of bacteria suspension was spread on an agar plate (the agar medium of each bacterium was listed in table 2.1) and then incubated at 37°C in triplicate. Plates were incubated for 24 hours (2 days for *M. luteus* and *C. jeikeium*; 4 days for *C. acnes*) and those with the colony number between 30 and 300 were counted. The colony-forming unit (CFU) of the original bacterial suspension was estimated by using the formula:

$$CFU/ml = \frac{counted\ colony\ number}{volume\ transfered\ to\ plate} \times dilution\ factor$$

The standard curve of each bacterium was then created by using GraphPad Prism 7 software.

2.4 Developing the NHEKs monolayer model colonised with a single commensal skin bacterium

2.4.1 Preparation of NHEKs monolayer in 12-well plates

To prepare the 12-well NHEKs monolayer plates, NHEKs in the T75 cell culture flask with a cell confluency >80% were harvested using the same methods mentioned in section 2.2. The cell pellet was then resuspended in 3 ml of fresh keratinocyte media and the cell concentration was calculated by employing a haemacytometer and adjusted in medium to $5*10^4$ cells/ml. 1ml of this was then seeded in 12-well plates (one well contained 1ml of cell suspension) and incubated at 37°C, 5% CO_2 for further experiments.

2.4.2 Preparation of bacterial suspensions

Stationary phase bacterial liquid culture was prepared and diluted gradually in the corresponding broth medium until a density of 1×10^7 CFU/ml was achieved (This was generated by adjusting the OD_{600} of each bacterial suspension to an appropriate value and the value of each bacterium is listed in table 2.2). 5ml of each bacterial dilution was then centrifuged at 1790 x g for 10 minutes employing a Universal 320 Hehich Zentriugen centrifuge. The harvested pelleted bacteria were washed twice with sterile phosphate buffered saline (PBS, 0.01 M PBS, pH= 7.4) and eventually resuspended in 5ml of fresh keratinocyte medium. The bacterial suspension was then further diluted with keratinocyte medium to obtain a concentration of 1×10^6 CFU/ml (10^7 CFU/ml for all three strains of *S. epidermidis* and 10^4

CFU/ml for *M. luteus*) bacterial dilution, which would be used in the subsequent experiments.

Table 2.2 The OD600 value of each bacterium for constructing a 1×10^7 CFU/ml bacterial suspension

Organisms	OD_{600} value
S. epidermidis (NCTC 11047)	0.047
S. epidermidis (NCTC 6513)	0.157
S. epidermidis (NCTC 10519)	0.162
S. hominis	0.116
S. capitis	0.061
M. luteus	0.105
C. jeikeium	0.059
C. acnes	0.137

2.4.3 Preparation of NHEKs monolayer colonised with a single strain of commensal skin bacterium

NHEKs monolayer was seeded in 12-well plates and incubated appropriately until the cells reached 100% confluency. The media was then replaced by 1 ml of bacterial suspension prepared as described in section '2.4.2' and the plate was then incubated at 37°C, 5% CO_2 for 10, 30, 60 and 90 minutes (while 30, 60 and 90 and 120 minutes for *S. hominis*) respectively. Following the incubation, the infected NHEKs of each well were washed 3 times in PBS to remove the unattached bacterial cells. The treated NHEKs were then harvested by incubating with 0.15 ml of 0.04% keratinocyte trypsin (V_1) at 37°C, 5% CO_2 for 8 minutes. 0.15 ml of trypsin inhibitor (V_2) was mixed with trypsin for inhibiting further trypsinisation following the incubation. The number of bacterial cells attaching to HNEKs (N_B) was measured by conducting serial dilution and viable counting. The bacterial density (D_B) on NHEKs monolayer was calculated

by using the equation below:

$$D_B = \frac{N_B \times (V_1 + V_2)}{The\ aera\ of\ each\ well}$$

This experiment was performed in triplicate. The corresponding time for generating a 10^5 CFU/cm^2 (10^2 CFU/ cm^2 for *M. luteus*) bacterial density (Kloos and Musselwhite 1975) on NHEKs monolayer was used in further experiments.

2.5 Investigating the bacterial sensitivity to solar simulated radiation (SSR)

2.5.1 Calculating the UVR exposure time

A 9600 solar simulator (Applied Photophysics, UK) equipped with a WG320 filter was used to generate the SSR composed of 4.1% UVB and 95.9% UVA. The solar simulator unit was warmed for 30-45 minutes until its energy was stable. Samples were placed on a platform that was approximately 10 cm below the UVR source. The UVR irradiance reaching the samples was monitored using a UVX radiometer (UVP, Jena, Germany) before each exposure treatment. The required dose (in this case 10 or 20 J/cm^2) is a factor of the irradiance and the time of exposure (T_E). This was calculated as follows:

$$T_E(seconds) = \frac{dose}{Irradiance \times Calibration\ factor}$$

Where the calibration factor was 2.88 for the 9600 solar simulator with a WG320 filter.

2.5.2 Treatment of cells with solar simulated radiation

5ml of 1 × 10^7 CFU/ml stationary phase bacterial broths were prepared as described in section

2.4.2 and spun down at 1790 x g for 10 minutes. The bacterial pellets were washed twice with sterile PBS and eventually resuspended in 5ml of PBS. 1 ml of bacterial suspension was then exposed to 10 and 20 J/cm^2 doses of SSR separately and the rest of them were kept in the dark at 4 degrees. After the exposure procedure, the viable bacterial cells number in each sample was counted by performing the plate count. To compare the bacterial sensitivity to SSR, the D_{37} value was then calculated via using the following equation:

$$D_{37} = \frac{37\% - r}{c}$$

Where the *c* and *r* were the slope and the intercept of the equation of the bacterial SSR-survival curves respectively (Appendix 1).

2.6 Immunoblotting of proteins isolated from bacteria

2.6.1 SSR treatment and protein extraction

Bacterial cells in 80ml of stationary phase culture with an of OD_{600} value of 1 were harvested by centrifugation and resuspended in 2ml of PBS. 1ml of this bacterial suspension was then treated with 10 J/cm^2 SSR with continuous stirring (Maclean et al. 2009; Murdoch et al. 2011) while the rest 1ml sample was kept in the dark. Following the UVR exposure, the irradiated bacterial cells were collected and maintained in 40ml of fresh broth media at 37°C. After 1hour's cultivation, these bacteria cells were harvested and resuspended in 1 ml of sterile PBS until future treatments.

Bacterial proteins were extracted by sonication. Before sonication, bacterial cells were pre-

treated with 4µl of 50mg/ml lysozyme solutions and 5µl of protease inhibitor cocktail (P8465, Merck, UK) and incubated at 4°C for 10 minutes. All samples were placed on the ice during the whole sonication process to preserve proteins. Sonication was conducted at 100% amplitude for all samples using a Sonoplus ultrasonic homogeniser (HD2000.2, Bandelin, Berlin) until the protein concentration in bacterial lysates exceeded 1µg/µl (the sonication time for each bacterium is listed in table 2.3). All bacterial lysates were then centrifuged at 13,000 x g for 10 minutes at 4°C and the extracts were filtered by employing the 0.22µm Millex-GV syringe filters. All cell-free lysates were stored in 1.5ml of Eppendorf tubes and maintained on ice to preserve proteins.

Table 2.3 Sonication times (minutes) for the selected skin commensal bacteria

Organisms	Sonication time (minutes)
S. hominis	14
C. jeikeium	13
S. epidermidis (NCTC 11047)	12
S. epidermidis (NCTC 10519)	12
S. epidermidis (NCTC 10519)	12
S. capitis	10
C. acnes	6
M. luteus	1

2.6.2 Quantification of the protein concentration in bacterial lysates

The protein concentration of each bacterial lysate was measured by performing the Bicinchinonic acid (BCA) assay using the Pierce™ BCA protein assay kit. 25µl of bacterial lysates or purified bovine serum albumin (0, 0.025, 0.125, 0.5, 0.75, 1, 1.5, 2 mg/ml) were then pipetted into a well of 96-well plate containing 200µl of working reagents (prepared according

with the manufacturer's instruction) in triplicate. The plate was incubated at 37°C for 30minutes and the OD of each well was then measured at 562nm using a plate reader. A standard curve (figure 2.1) which shows the relationship between the absorbance and the protein concentration was then generated using Office Excel. After that, the protein concentration of bacterial lysates was calculated by employing the equation of the standard curve.

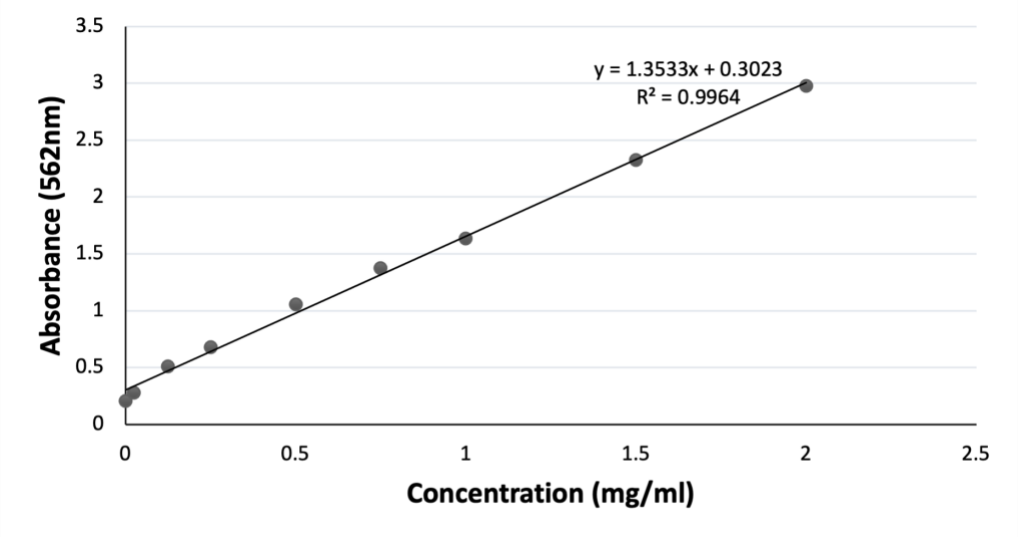

Figure 2.1 The standard curve of BCA assay for protein quantitation (drawn by Wen Duan)

2.6.3 Protein separation and western blot analysis

Bacterial proteins were separated by performing the sodium dodecyl sulphate poly acrylamide gel electrophoresis (SDS PAGE) as follow: 15μg of bacterial proteins was mixed with 6μl of 4X NuPAGE™ LDS Sample Buffer (pH 8.4) and made up to a total volume of 24μl with deionised water in a 0.5 ml Eppendorf tube. All samples were then heated at 100°C for 5 minutes and centrifuged at 1000 x g for 1minute. 24μl of each prepared sample was loaded in a NuPAGE™ 10% Bis-Tris Protein Gel (1.0mm, 10 well) together with 5μl of Spectra™ Multicolor Broad Range Protein Ladder. The gel was then run at 150V constantly in 1X NuPAGE™ MOPS SDS Running Buffer (50 mM MOPS, 50 mM Tris Base, 0.1%SDS, 1mM EDTA, pH 7.7) for 1 hour.

Separated proteins were transferred onto a nitrocellulose membrane by using the Trans-Blot Turbo Transfer Kit (Bio-rad, UK) according to the manufacturer's instructions. To demonstrate the successful transfer and the equality of protein contents, the membrane was then stained by 10 ml of 0.1% (w/v) Ponceau S solution in a $10cm^2$ square petri dish for 5 minutes. During the staining, the 1x tris buffered saline tween 20 (TBS-T, 25 mM Tris, 0.15M NaCl, pH 7.6, 0.5% Tween 20) buffer was produced by mixing 50ml of 20x Pierce™ 20xTBS buffer with 5ml of Tween 20 in 950ml of deionised water. After that, the membrane was washed twice by deionised water and finally blocked in 15ml of 5% skimmed milk (w/v) in TBS-T buffer (blocking buffer) at 4°C overnight.

The membrane was then washed 3 times by TBS-T buffer and placed in 15ml of blocking buffer containing anti-RecA antibody (ab63797, Abcam, Cambridge, UK) at the appropriate concentration (shown in table 2.4) following incubation 1 hour at room temperature with shaking, the membrane was then washed thrice in TBS-T buffer for 10 minutes before it was incubated with 15ml of TBS-T buffer containing 3µl of the goat anti-rabbit horseradish peroxidase-conjugated secondary antibody at room temperature for 1 hour with sharking. After rinsing the membrane with TBS-T for 5 minutes twice, the protein of interest was detected and visualised using the SuperSignal™ West Pico PLUS Chemiluminescent Substrate in a ChemiDoc XRS+ System (Bio-rad, UK). All generated files were then analysed with Image Lab (obtained from http://www.bio-rad.com) for getting optimised figures.

Table 2.4 The optimised concentration (μg/ml) of the anti-RecA antibody for each skin bacterium

Organisms	Optimised concentration (μg/ml)
S. epidermidis (NCTC 11047)	333
C. jeikeium	333
S. epidermidis (NCTC 6513)	200
S. epidermidis (NCTC 10519)	200
S. hominis	200
S. capitis	200
M. luteus	200
C. acnes	133

2.7 Determination of the effects of commensal skin bacteria on the viability of NHEKs in response to SSR

2.7.1 Determination of NHEKs viability in response to SSR

HNEKs were seeded into 12-well plates and incubated until they exceeded 80% confluency. To avoid the influence of scattering, NHEKs of the irradiated group were only cultured in the corner wells. The medium in a NHEKs well was replaced by 1 ml of PBS before the SSR treatment. The cells of the ‘irradiated’ group were immediately exposed to SSR (10 and 20 J/cm^2 doses, respectively) by using the method described in '2.5.1' while the cells of the ‘non-irradiated’ group were covered by aluminium foil. Following irradiation, the PBS in each well was immediately replaced with 1 ml of keratinocyte medium and the NHEKs of both groups were maintained at 37°C in a 5% CO_2 incubator. The cell viability was checked at 0 and 24 hours following UVR exposure by using the method detailed in section 2.7.2.

2.7.2 Measurement of NHEKs viability using Trypan blue exclusion

The medium of confluent NHEK in 12 well plates was replaced by 150µl of 0.04% keratinocyte trypsin, and all cells were kept at 37°C with 5% CO_2 for 5-7 minutes until most cells were detached. 150µl of 0.05% trypsin inhibitor was then pipetted into each cell well to inhibit further tryptic activity. 20µl cells suspension was then stained with 20µl 0.4% trypan blue solution for 10 seconds before the cell viability was measured. The viable and dead cells were then counted using a haemacytometer under a light microscope. The blue cells were assigned as dead while the transparent cells were assigned as viable. The cell viability (V_C) was calculated as follow:

$$V_C = \frac{viable\ cells\ number}{total\ cells\ number} * 100\%$$

2.7.3 Comparation of the viability between the sterile and infected NHEKs in response to SSR

NHEKs monolayer colonised with single commensal skin bacterium was prepared using the methods mentioned in '2.4.3'. The sterile NHEKs and the NHEKs infected with 10^5 CFU/cm^2 (10^2 CFU/ cm^2 for *M. luteus*) single commensal skin bacterium were treated with 10 and 20 J/cm^2 doses of SSR individually by employing the same methods mentioned in '2.5.1'. The cell viability of the keratinocytes was measured after 24 hours of SSR exposure.

2.8 Preparation of bacterial cell-free supernatants

Bacteria were grown as described in section 2.4.2. A 5 ml suspension of 10^6 CFU/ml (10^3 CFU/ml for *M. luteus*) was then prepared in PBS. 1ml of the bacterial suspension was then

exposed to a dose of 10 or 20 J/cm^2 SSR, while the rest of the suspension was maintained in the dark. To collect the cell-free supernatants, both irradiated and non-irradiated bacterial suspensions were centrifuged at 1790 x g for 10 minutes. Their supernatants were then filtered using a 0.22μm Millex-GV syringe filters (Millipore, USA). 30μl of filtered supernatants were then spread on agar dishes and the dishes were cultivated at 37°C overnight (2 days for *M. luteus* and *C. jeikeium*; 4 days for *C. acnes*) to verify that there were no viable bacteria present in the supernatants.

2.8.1 Treatment of NHEKs with bacterial cell-free supernatants

The medium of confluent NHEKs in 12 well plates was replaced with 900μl of fresh keratinocyte media. Next, 100μl of the cell-free supernatants of non-irradiated bacterium and irradiated bacterium (exposed to 10 and 20 J/cm^2) were added to the wells. To eliminate the effects of PBS, one well of NHEKs were co-cultured with 100μl of sterile PBS. These NHEKs were then maintained at 37°C with 5% CO_2 and their cell viability was measured (as described in section 2.7.2) following 24 hours of incubation.

2.9 Measurement of apoptosis using flow cytometry

Cell-free supernatants of 10^6 CFU/ml *S. epidermidis* (NCTC 11047) treated by a dose of 0, 10 or 20 J/cm^2 SSR were produced as described in section 2.8. Confluent NHEKs in 12 well plates were then treated with these supernatants individually and harvested into 1.5 ml Eppendorf tubes as described in section 2.8.1. Before the measurement of NHEKs apoptosis, all cells were stained using Annexin V (eBioscience™ Annexin V Apoptosis Detection Kit APC, Thermo

Fisher Scientific, UK) and 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI) (Thermo Fisher Scientific, UK) as follow: all harvested NHEKs were centrifuged at 400 x g for 5 minutes, and the cell pellet in each tube was washed once using 200µl of 1X Annexin V binding buffer (Thermo Fisher Scientific, UK). To conjugate cells with Annexin V, the cells were spun down at 400 x g for another 5 minutes and resuspended in 200µl of Annexin V binding buffer containing 2µl of Annexin V. All tubes were maintained on ice in the dark. After 20 minutes, Annexin V was removed from cell tubes by centrifuging again at 400 x g for 5 minutes. The cells were then re-suspended in 200µl of ice-cold 1X Annexin V binding buffer before 10µl of 1µg/ml DAPI was pipetted into each sample. The cell apoptosis was measured by using a BD Fortessa X20 flow cytometer (Core Technology Facility, the University of Manchester). All raw data produced from the flow cytometer was analysed by FlowJo software (FLOWJO, LLC). The viable cells, the necrotic cells and the apoptotic cells were classified according to the cell staining results showed in table 2.5.

Table 2.5 The relationships between cell status and staining results

Cell status	Cell staining result
Viable cell	Annexin V (-) + DAPI (-)
Early apoptotic cell	Annexin V (+) + DAPI (-)
Late apoptotic cell	Annexin V (+) + DAPI (+)
Necrotic cell	Annexin V (-) + DAPI (+)

2.10 Protein denaturation of cell-free supernatants of the irradiated *S. epidermidis* (NCTC 11047)

Cell-free supernatant of *S. epidermidis* (NCTC 11047) exposed to the 20J/cm^2 dose of SSR

was prepared as described in section 2.8.

2.10.1 Heat treatment

The prepared supernatant was heated at 100°C for 10 minutes and maintained at room temperature for the cooldown. Confluent NHEKs in 12 well plates were then co-cultured with 100µl of the heated supernatant for 24 hours before the cell viability was measure using the same method of section 2.7.2.

2.10.2 Trypsin treatment

The protein concentration of the prepared supernatant was measured using the same method of section 2.6.2. Protein content in 500µl of the supernatant was calculated and then digested with trypsin solution in the ratio 1:20 (w: w, trypsin: protein) at 37°C. After overnight incubation, trypsin in the supernatant was fully neutralized using a 10X volume of trypsin inhibitor. Confluent NHEKs in 12 well plates were then treated with 100µl of the denatured supernatant and the cell viability was measured after 24 hours using the method described in section 2.7.2.

2.11 Fractionation of cell-free supernatants

5ml of cell-free supernatants of irradiated *S. epidermidis* (NCTC 11047, 10^6 CFU/ml, treated by 20 J/cm^2 does of SSR) was prepared using the same method described in section 2.8 and then maintained on ice. This supernatant was then separated into several fractions by molecular size via utilising the technique below: 4ml capacity Centrifugal Filter Units (Amicon, Darmstadt, Germany) with a filter size of 3, 30, 50 and 100KDa were rinsed using 1ml of sterile PBS and

then 1ml of irradiated cell-free supernatant was added into individual centrifugal units. All units were centrifuged at 1790 x g for 30 minutes at room temperature. The retained and the flow-through fractions in each cut-off unit were collected individually into Eppendorf tubes, and the volume of each was adjusted to 1ml with sterile PBS. 100µl of supernatant fractions were used in subsequent experiments as described in section 2.8.1.

2.12 Statistical analysis

Statistical analysis was implemented employing the GraphPad Prism 7 software. Unpaired student's t-test was utilised for statistical significance analysis between two groups. Results were presented as the mean ± standard error of the mean (SEM) resulting from three individual experiments (n=3), and $P < 0.05$ was regarded as statistical significance. Each experiment was conducted in triplicate and repeated at least three times.

Chapter 3 Construction an *in vitro* model of normal human primary epidermal keratinocytes (NHEKs) colonised with a single skin commensal bacterium

Skin microbiota comprises numerous beneficial microorganisms, including bacteria, fungi, parasites and viruses (Sanford and Gallo 2013). Among all of these microbes, the commensal bacteria dominate in terms of abundance. Although the bacterial density on human skin is variable depending on skin sites, the most predominant skin resident bacterial genera on healthy individuals are propionibacterium, staphylococcus and corynebacterium (Belkaid and Segre 2014; Byrd et al. 2018; Sanford and Gallo 2013; Chen and Tsao 2013). Hence, for this study, the most representative skin commensal bacteria from these genera were selected. These were *Staphylococcus. epidermidis* (*S. epidermidis*), *Staphylococcus hominis* (*S. hominis*) and *Staphylococcus capitis* (*S. capitis*)) (Liu et al. 2020), one of the most dominant propionibacterium species (*Cutibacterium acnes* (*C. acnes*)) and one of the culturable corynebacterium species (*Corynebacterium jeikeium* (*C. jeikeium*)). Notably, as *S. epidermidis* is one of the most common resident skin organisms (Kloos and Musselwhite 1975), plenty of subspecies have been reported previously (Oh et al. 2014). Therefore, three strains of this bacterium (NCTC 11047, NCTC 6513 and NCTC 10519) were chosen. In addition, *Micrococcus luteus* (*M. luteus*), which is also widely found on the skin and has been used in UVR-related studies (Patra et al. 2016; Mahler et al. 1971), was also employed in the current project.

This chapter aims to:

1) verify the identity of all skin commensal bacteria used in this study.

2) generate the growth curves for individual bacteria to identify the time required for organisms to enter the stationary phase.

3) produce standard curves for all selected bacteria to study the relationship between the viable bacterial cell count and the bacterial absorbance.

4) construct an *in vitro* model of NHEKs monolayers colonised by an individual skin commensal bacterium with a similar density as found on human skin.

3.1 Bacterial identification

Several approaches have been developed for bacteria identification, including phenotypic, biochemical, and genotypic methods (Franco-Duarte et al. 2019). While the first two techniques depend on bacterial culture, the genotypic methods that enable the identification of unknown microbes without cultivation have been applied as standard approaches for bacterial identification since the late 20th century (Höfling et al. 1997). 16S ribosomal RNA (16S rRNA) metagenomic sequencing is most commonly used among all genotypic approaches (Weisburg et al. 1991). The 16S rRNA gene exists in all bacteria and archaea (Grice and Segre 2011). It contains several highly conserved regions which are ideal for PCR primer design and some hypervariable regions used for microbial identification. Nowadays, one standard method for microbial identification is to amplify a fraction of the 16S rRNA gene from a microbial sample, and then this amplified product is sequenced to get the final data which contains the critical information of specific taxa (Salipante et al. 2014). Since the 16S rRNA amplicon sequencing only targets bacteria, the potential contaminations from other organisms can be avoided

effectively (Salipante et al. 2014).

S. epidermidis (NCTC 11047), *S. epidermidis* (NCTC 6513), *S. epidermidis* (NCTC 10519), *S. hominis, S. capitis, M. luteus* and *C. jeikeium* were purchased from either National Collection of Type Culture or German Collection of Microorganisms and Cell Cultures. As the bacterial identification of these bacteria was assured by the supplier, no further identification was conducted. For the *C. acnes,* which was isolated from a health volunteer in a previous study, the 16S rRNA gene sequencing was performed. DNA of *C. acnes* was extracted, amplified, purified (as described in section '2.3.2') before the amplicons were sequenced with using Sanger sequencing. Generated data was analysed by BLAST. The identity of greater than 99.5% sequence similarity was used as the criterion for species identification (Janda and Abbott 2007). The results are shown in table 3.1.

Table 3.1 Bacterial identification using 16S rRNA gene sequencing

Bacterial strain	Identities	E value*	Accession no.	Total no. of bases
Cutibacterium acnes strain JCM 6425	99.7	0	NR_113028.1	690

*Expect (E) value: the number of hits could be observed incidentally when searching a database.

3.2 Bacterial growth curves

Bacteria divide exponentially if the available nutrition is sufficient for their growth. The growth rate then gradually reduces with the consumption of nourishing substances and the accumulation of wastes, and eventually reaches a balance with the death rate. This period in bacterial growth is named the stationary phase (Maier 2000). Stationary-phase microorganisms

have been commonly used for microbiology studies as bacterial protein production and the induction of some specific proteins is often maximum in this phase (Ou et al. 2004; Kolter et al. 1993). Bacterial adhesion in particular is better in the stationary phase than that of other phases. For example, it has been shown that a 58 kDa surface protein PA25957, which is involved in bacterial adhesion by interacting with proteins in the extracellular matrix of the host, is synthesized by *C. acnes* in the stationary phase (Grange et al. 2017).

To study the time of each selected microbe entering the stationary phase, growth curves were constructed to illuminate the relationship between the time in culture and the growth phase (figure 3.1). 200µl of diluted bacterial broth cultures (prepared as described in section 2.3.4) were seeded in 96-well plates and cultured as described in section 2.3.4. The OD of each bacterial suspension was recorded throughout a period of 24 to 96 hours. The results in figure 3.1 demonstrate that *S. epidermidis* (NCTC 6513) entered the stationary phase after 8 hours of incubation, followed by the *S. epidermidis* (NCTC 11047) which needed 11 hours' incubation. The growth time for *S. epidermidis* (NCTC 10519) and *S. hominis* to reach the stationary phase was 12 hours which was shorter than that of *S. capitis* (15 hours). The inoculation time for the remaining three bacteria to reach the stationary phase was longer: *M. luteus* and *C. jeikeium* needed 40 hours and 42 hours respectively, while the anaerobic species *C. acnes* took the longest time, which was 76 hours. Notably, the stationary phase of *S. epidermidis* (NCTC 6513) was only sustained for 8 hours, and this bacterium entered the death phase after 16 hours of incubation.

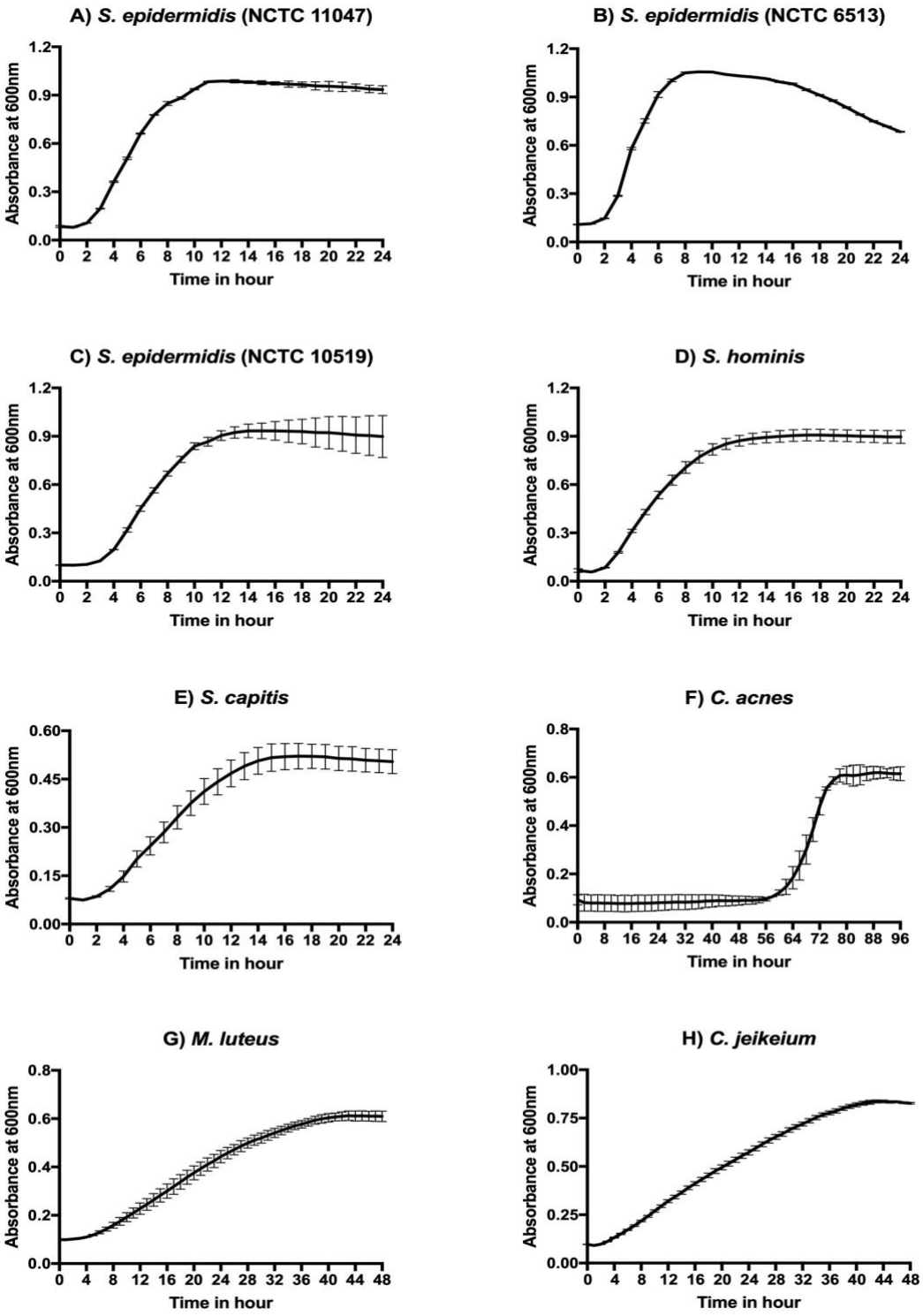

Figure 3.1 The growth curve of selected skin commensal bacteria

The growth curves of A) *S. epidermidis* (NCTC 11047), B) *S. epidermidis* (NCTC 6513), C) *S. epidermidis* (NCTC 10519), D) *S. hominis*, E) *S. capitis*, F) *C. acnes, G) M. luteus, H) C. jeikeium*. Selected skin commensal bacteria entered the stationary phase after 8-76 hours' incubation. Data are presented as mean ± standard deviation (SD), (n=3).

3.3 Bacterial standard curves

To investigate the correspondence between the bacterial absorbance and the viable cell number of a bacterial liquid culture, bacterial standard curves were plotted using the method described in section '2.3.5'. The graphs of absorbance against viable count are shown in figure 3.2. According to these results, the absorbance (measured at 600 nm) of a bacterial suspension with a density of $1*10^7$ CFU/ml should approximately be 0.047 for *S. epidermidis* (NCTC 11047), 0.042 for *S. epidermidis* (NCTC 6513), 0.074 for *S. epidermidis* (NCTC 10519), 0.116 for *S. hominis*, 0.061 for *S. capitis*, 0.137 for *C. acnes*, 0.105 for *M. luteus* and 0.059 for *C. jeikeium,* respectively.

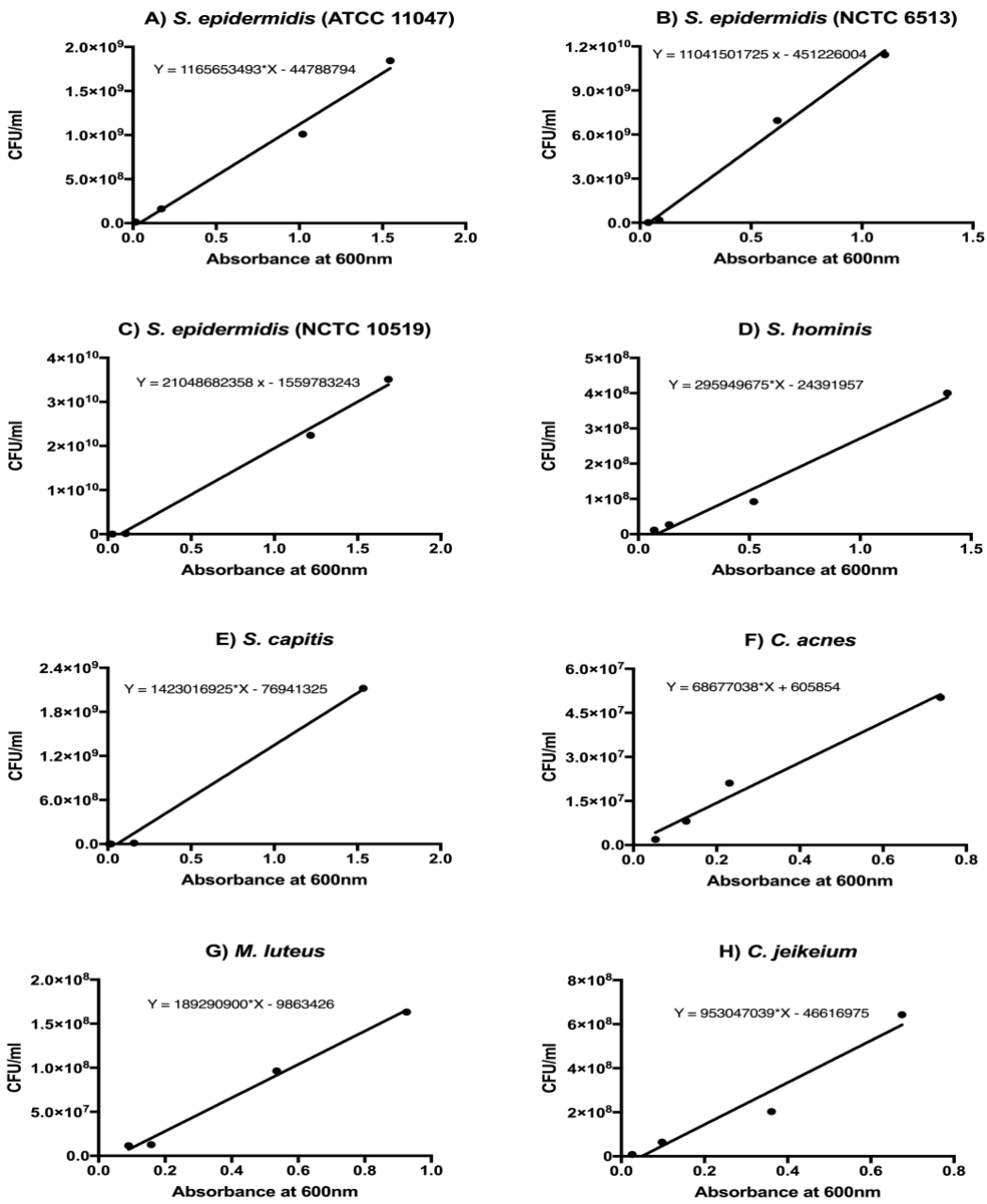

Figure 3.2 The bacterial standard curve of selected skin commensal bacterium

The standard curves (optical density (OD) versus colony forming unit (CFU)) of A) *S. epidermidis* (NCTC 11047), B) *S. epidermidis* (NCTC 6513), C) *S. epidermidis* (NCTC 10519), D) *S. hominis*, E) *S. capitis*, F) *C. acnes, G) M. luteus*, and *H) C. jeikeium*. To create the $1*10^7$ CFU/ml bacterial culture, the OD_{600} of bacterial suspension should be adjusted to 0.047 for *S. epidermidis* (NCTC 11047), 0.042 for *S. epidermidis* (NCTC 6513), 0.074 for *S. epidermidis* (NCTC 10519), 0.116 for *S. hominis*, 0.061 for *S. capitis*, 0.137 for *C. acnes*, 0.105 for *M. luteus* and 0.059 for *C. jeikeium*, (n=3).

3.4 Developing a NHEK monolayer model colonised with a single strain of commensal skin bacterium

To investigate the effects of a skin commensal bacterium on NHEKs response to solar ultraviolet radiation, an *in vitro* model of NHEKs monolayer colonized by a single skin commensal bacterium with a similar bacterial density as found on human skin needed to be constructed. Many studies show that the density of different species of bacteria on human skin is variable, and even for the same bacterium, the density is depend in the particular skin sites (Sanford and Gallo 2013; Byrd et al. 2018). According to a previous study that investigated the distribution of some skin commensal microbes on human skin, the density of $1*10^5$ CFU/cm^2 was chosen as an objective density to build an *in vitro* model of NHEKs colonized with most of the selected skin commensal bacteria (Kloos and Musselwhite 1975). However, as *M. luteus* only takes a small portion in the composition of the skin bacterial community, a $1*10^2$ CFU/cm^2 density was used in the construction of the bacterial NHEKs model (Kloos and Musselwhite 1975; Grice and Segre 2011).

Bacterial suspensions prepared in keratinocyte medium were co-incubated with NHEKs at 37°C in a humidified atmosphere of 5% CO_2 as described in section '2.4'. The number of the adherent bacterial cell was measured at 10 minutes, 30 minutes of incubation and then every 30 minutes (30, 60, 90 and 120 minutes of incubation for *S. hominis*) until the bacterial density on NHEKs reached the required number. To generate a $1*10^5$ CFU/cm^2 bacterial density on NHEKs monolayer, NHEKs were initially co-incubated with $1*10^6$ CFU/ml of *C. acnes, C. jeikeium* and each selected staphylococcus species. The objective density was informed

following an incubation of 30, 100, 25, 75 and 75 minutes for *S. epidermidis* (NCTC 11047), *S. hominis, S. capitis, C. acnes* and *C. jeikeium,* respectively (figure 3.3). However, for two strains of *S. epidermidis* (NCTA 6513 and NCTC 10519), the bacterial density on NHEKs monolayer was still lower than $1*10^5$ CFU/cm^2 after an adhesion up to 2 hours (figure 3.4). To reduce the adhesion time, the initial concentration of these two microbes had been increased to 100 times higher ($1*10^7$ CFU/ml) than the target density. Using this concentration allowed the target density to be achieved following an incubation of 40 and 35 minutes for NCTC 6513 and 10519 respectively (figure 3.3). A similar observation was observed in *M. luteus*, which needed more than 2 hours to develop a $1*10^2$ CFU/cm^2 on NHEKs when its initial concentration was set at 10-times higher than the target density (figure 3.4). Hence, a concentration ($1*10^4$ CFU/ml) of 100 times higher than the objective density was eventually used for the bacterial adhesion of *M. luteus*, and the bacterial density $1*10^2$ CFU/cm^2 was generated on NHEK monolayer after 70 minutes' adherence (figure 3.3).

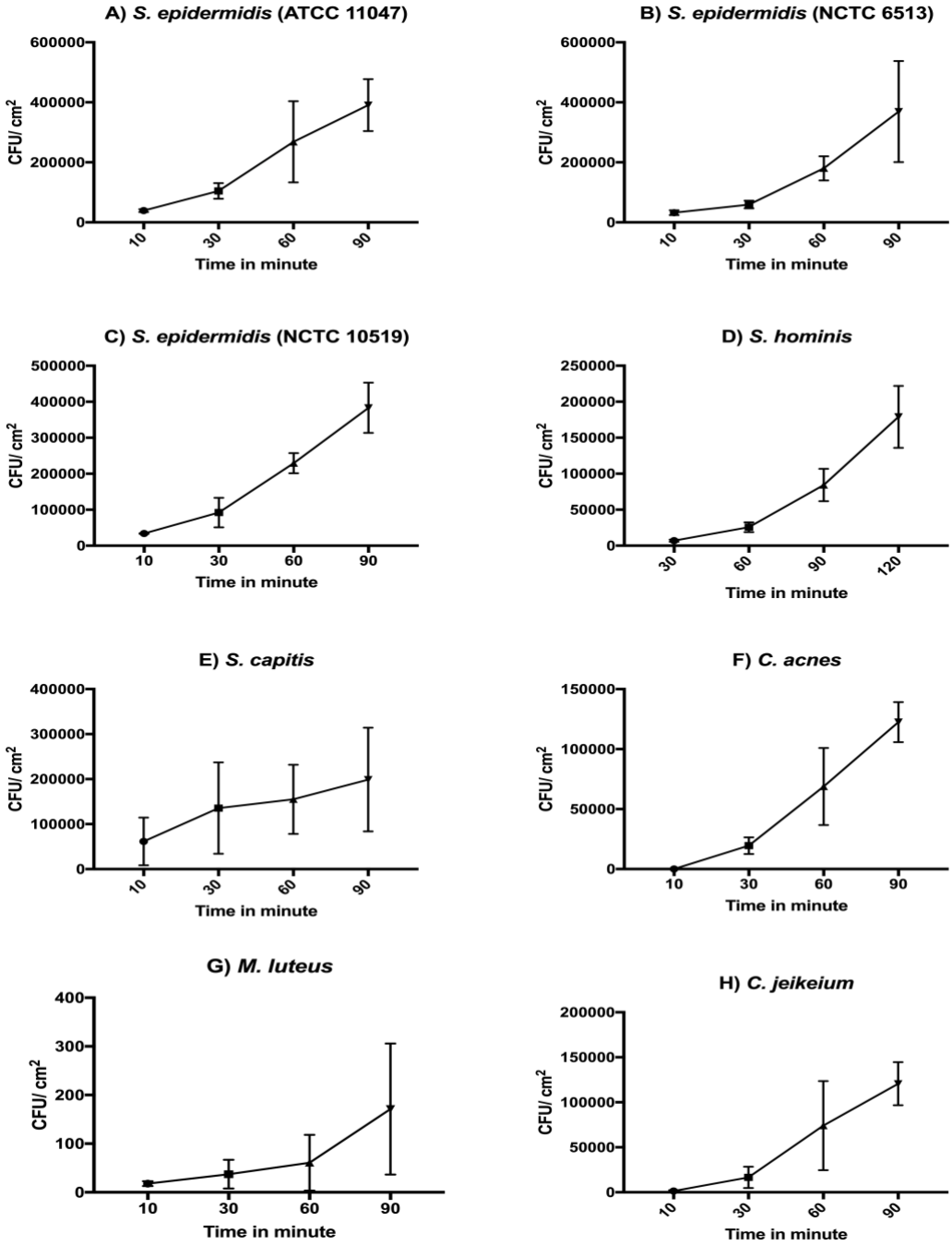

Figure 3.3 The time-dependent increase in attached bacterial cell number on NHEKs

NHEKs were individually co-incubated with bacterial suspensions containing a single strain of bacterium. The concentration of 10^6 CFU/ml was used for A) *S. epidermidis* (NCTC 11047), D) *S. hominis,* E) *S. capitis,* F) *C. acnes* and H) *C. jeikeium*; the concentration of 10^7 CFU/ml was used for B) *S. epidermidis* (NCTC 6513) and C) *S. epidermidis* (NCTC 10519); the concentration of 10^4 CFU/ml was used for G) *M. luteus*. The adherent bacterial cell number was measured at the indicated time points by performing plate count. To generate a $1*10^5$ CFU/cm^2 bacterial density on HNEKs, a co-incubation of 30, 40, 35, 100, 25, 75 and 75 minutes was needed for *S. epidermidis* (NCTC

11047), *S. epidermidis* (NCTC 6513), *S. epidermidis* (NCTC 10519), *S. hominis*, *S. capitis*, *C. acnes* and *C. jeikeium*, separately. For *M. luteus*, 70-minutes of colonization was essential to inform a $1*10^2$ CFU/cm^2 bacterial density on HNEKs. Data are presented as mean ± standard error of the mean (SEM), (n=3).

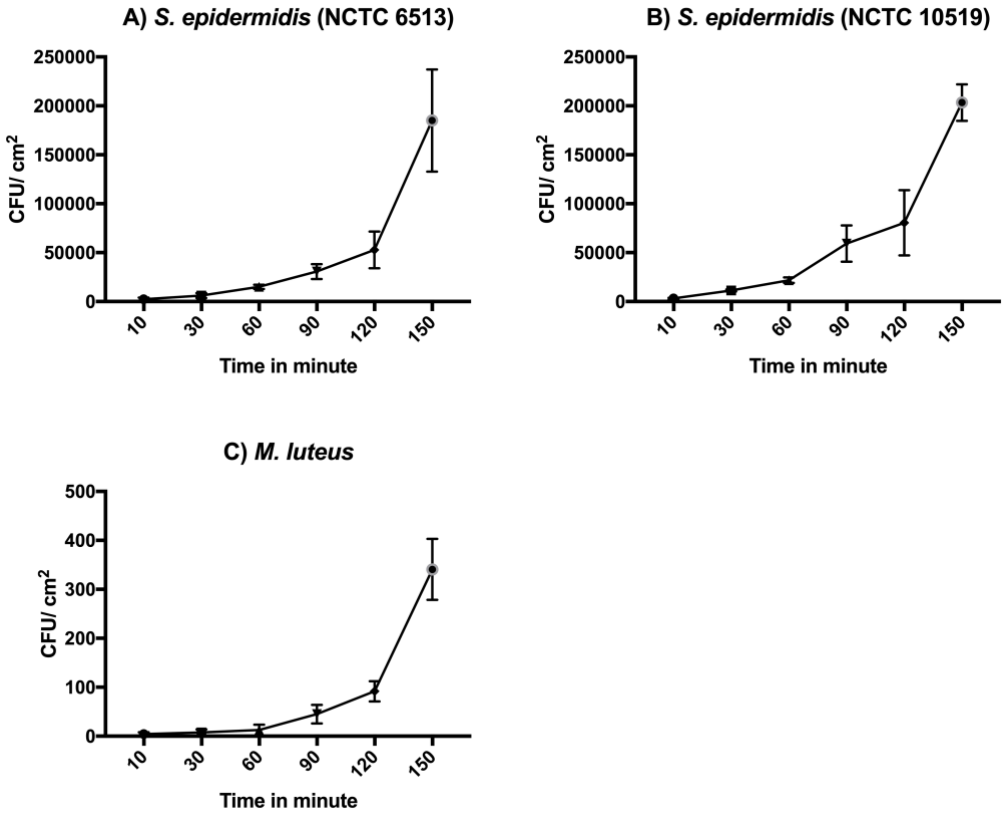

Figure 3.4 The time-dependent increase in attached bacterial cell number on NHEKs

NHEKs were co-incubated with 10^6 CFU/ml A) *S. epidermidis* (NCTC 6513) and B) *S. epidermidis* (NCTC 10519), and 10^3 CFU/ml C) *M. luteus*. The attached bacterial cell number was measured at 10, 30, 60, 90, 120 and 150 minutes of incubation. To develop a $1*10^5$ CFU/cm^2 bacterial density ($1*10^2$ CFU/cm^2 for *M. luteus*) on HNEKs, an adhesion of 130, 125 and 125 minutes was required for *S. epidermidis* (NCTC 6513), B) *S. epidermidis* (NCTC 10519) and C) *M. luteus* respectively. Data are presented as mean ± standard error of the mean (SEM), (n=3).

3.5 Discussion

This chapter aimed to investigate the growth characters of the selected skin commensal members and construct an *in vitro* model of keratinocytes colonized by a single strain of

bacterium.

At the beginning of this project, one of the selected bacteria strains, which was isolated from the health volunteer in a previous study, was confirmed by conducting 16S rRNA gene sequencing (table 3.1). The same method has been widely used in skin microbiota research to identify novel bacteria and investigate the composition of skin bacterial communities on different skin sites (Dekio et al. 2005; Grice et al. 2008; Grice et al. 2009). However, there are limitations of employing the 16S rRNA sequencing-based method for bacterial identification. One major weakness is that if the difference between two bacterial species is so slight that they almost share the same 16S rDNA sequence, this molecular method would lead to a failure in their identification. As a result, the 16S rDNA-based method is inappropriate for the identification of subspecies (Woo et al. 2008; Boyer et al. 2001). However, for the purpose of the present study, 16S rRNA fulfilled the requirements. The data in table 3.1 show a 99.7% homology of the isolated *C. acnes* to an organism identified as *C. acnes* strain JCM 6425. Thus, the conclusion is that the isolated bacterium is *C. acnes* strain JCM 6425.

Next, the growth characteristics of all organisms were established. Figure 3.1 showed that all tested staphylococcus species entered the stationary phase with overnight incubation, which was consistent with the previously reported results (Dodds et al. 2020; Zhu et al. 2010; Liu et al. 2020). *C. acnes* took the longest time (76 hours) to enter that phase, which was ever longer than that in other works (Balgir et al. 2016; Lood and Collin 2011). The more time-consuming growth of *C. acnes* exhibited herein could be associated with the difference in the usage of bacterial strains and growth medium. Figure 3.1 also presented that *M. luteus* needed 40 hours

to enter the stationary phase, however this bacterium only took 13 hours to enter that phase presented in another study (Chandrasekar et al. 2015). As 30 degrees was used in the study of Chandrasekar, the quicker growth of *M. luteus* could be associated with the cultivation at a lower temperature. Notably, the better growth in 30 degrees of this organism also could account for why it represents a small proportion of the composition of the skin microbiota community, as the temperature on human skin surface could not be an optimum condition for its growth.

Figure 3.1 also demonstrated that while all selected skin microbes stayed in the stationary phase until the end of the testing, *S. epidermidis* (NCTC 6513) only maintained in this phase for 8 hours before it entered the death phase. Future works are required to explore the mechanisms behind this phenomenon. Nonetheless, some potential hypotheses could be related to either the production of toxic molecules or the deficient expression of the genes and proteins, which are essential for preserving bacteria survival under stress (Jaishankar and Srivastava 2017).

Plotting standard curves enabled the viable bacterial cell number to be estimated according to the bacterial absorbance. The difference in OD_{600} of each organism for generating a $1*10^7$ CFU/ml bacterial suspension was illuminated in figure 3.2, showing the OD_{600} of *S. epidermidis* (NCTC 6513) was lowest among all tested bacteria while that of *C. acnes* was the highest. This variation in absorbance could be induced by the difference in the cell size of each microbe. At the same absorbance, the viable cell number of *C. acnes* was higher than other selected organisms, suggesting that the cell size of *C. acnes* was relatively larger than other tested species. This result is consistent with the data in the literature, which showed the cell diameters

of staphylococcus species were 0.5 to 1.4μm while the cell size of *C. acnes* was relatively bulkier, which is 3-5μm in length and 0.4-0.7μm in width (Mayslich et al. 2021; Schleifer and Kloos 1975; Schleifer and Kloos 1975).

The time-dependent increase in attached bacterial cell number was shown in figure 3.3 and 3.4, indicating the adhesion of selected skin commensal bacteria to NHEKs was different. When NHEKs were infected with $1*10^6$ CFU/ml bacterial suspension, *S. capitis* only needed 25 minutes to reach the required density (figure 3.3), while *S. epidermidis* (NCTA 6513) and *S. epidermidis* (NCTC 10519) required more than 2 hours to achieve the goal (figure 3.4). Although the mechanisms of skin commensal bacteria attachment to NHEKs are still poorly understood, a recent study has shed light on this question. It has been demonstrated the bacterial adhesion of *S. epidermidis* could be associated with a surface protein SdrF, which facilitated this bacterium's attachment to keratinocytes and keratin (Trivedi et al. 2017). As keratins are one of the most abundant structural proteins on the skin epithelium (Fuchs 1995), the SdrF-mediated keratin binding could be one pathway for host staphylococcus species colonization. Another pathway of *S. epidermidis* colonizing keratinocytes could be correlated with the binding of bacterial surface proteins to keratinocytes extracellular matrix (ECM) components, including fibronectin and collagen (Foster 2020; Alitalo et al. 1982). However, as the strains used in studying the ECM-binding pathway were pathogenetic, further verification is required to investigate the suitability of this pathway in explaining the adherence of host strains of *S. epidermidis*. Furthermore, figure 3.3 and 3.4 also exhibited the difference in adherence to NHEKs between strains of *S. epidermidis*. Although the reasons for this variation are uncleared,

one hypothesis could be related to the different extent of fibronectin binding between these *S. epidermidis* strains (Switalski et al. 1983).

Figure 3.3 also presented that *C. jeikeium* took a longer time (75 minutes) to reach the required density than the *S. epidermidis* (NCTC 11047) and *S. capitis*. The relatively weak adhesion of *C. jeikeium* could be due to the composition of its cell wall, as it has been proven that several substances, such as mannose and galactose, which negatively affected the bacterial adherence to epithelial cells, are included in the cell wall of this bacterium (Romero-Steiner et al. 1990; Leydcn et al. 1987). The adhesion of *C. acnes* and corynebacterium species to keratinocytes has been reported previously, showing corynebacterium species attached better than *C. acnes* to keratinocytes (Romero-Steiner et al. 1990). The same result was not observed from our data, which illuminated that the adhesion of these two bacteria to NHEKs was similar (figure 3.3). As the strains used herein are different from those employed in Romero-Steiner's research, this inconsistency could be associated with the varying strain usage.

In conclusion, the data of this chapter has confirmed the sequence of *C. acnes* used in the following experiments, measured the time for bacteria entering the stationary phase, and explored the bacterial adhesion time for constructing the infected NHEKs model. This model can now be used to assess the effects of bacteria on keratinocytes response to a dose of UVR.

Chapter 4 Investigations into the effect of simulated solar radiation on the viability of skin bacteria and the interactions of irradiated bacteria with human primary epidermal keratinocytes

Solar radiation (SR) is the primary UVR source that bacteria and eukaryotic cells come into contact with. SR can be deleterious to both primarily by causing damage to the DNA and protein structures of cells (Ravanat et al. 2001; Neves-Petersen et al. 2012). The bacterial UVR sensitivity of some organisms has been investigated by previous researchers (Gascón et al. 1995). However, somewhat surprisingly, little is known about the sensitivity of skin commensal bacteria to UVR exposure. Recently, an *in vivo* pilot study demonstrated that both UVA and UVB can differentially change the composition of skin microbiota, suggesting the sensitivity of skin commensal bacteria to UVR is different (Burns et al. 2019). However, this study relied on 16S sequencing and was not able to show the difference in UVR sensitivity between bacterial species.

Furthermore, how the microbiome impacts upon skin physiology following exposure to UVR has been investigated to date in only a single study and this was in mice (Patra et al. 2019). The possibility exists that skin and its microbiota may engage in 'crosstalk' following a dose of UVR and bacteria may modify the response of keratinocytes.

The work described in this chapter aims to investigate:

1) the sensitivity of selected single organisms from the skin microbiota to simulated solar radiation (SSR).

2) how the presence of a single bacterium might change the response of primary human keratinocytes to a single dose of SSR.

4.1 Differential sensitivity of selected skin commensal bacteria to SSR

To investigate the sensitivity of bacteria to SSR, a bacterial suspension containing $1*10^7$ viable cells from a single organism was prepared as described in section '2.4.2'. This suspension was then exposed to 0, 10 and 20 J/cm^2 SSR. The cells were immediately plated onto agar plates post SSR treatment and the viable cell number was counted at times when the diameter of colony reached 1-2mm (the times for counting the bacterial colony number of each organism were outlined in table 4.1). The data is presented in figure 4.1.

Table 4.1 The times for counting bacterial colony number post SSR treatment

Organism	Time (hours)
S. epidermidis (NCTC 11047)	24
S. epidermidis (NCTC 6513)	24
S. epidermidis (NCTC 10519)	24
S. hominis	24
S. capitis	24
M. luteus	48
C. jeikeium	48
C. acnes	96

There was no significant reduction in the CFU of two strains of *S. epidermidis* (NCTC 11047 and 6513), *C. jeikeium* and *M. luteus* following exposure to 10 J/cm^2 doses of SSR. $7*10^6$, $6*10^6$, $9.7*10^6$ and $9.8*10^6$ cells were viable respectively (figure 4.1 A, B, G and H). However, a dose of 10 J/cm^2 SSR exposure significantly decreased the number of viable cells of *S.*

epidermidis (NCTC 10519) to $6*10^6$ (*P*= 0.04), *S. hominis* to $4*10^6$ (*P*= 0.0006) and *S. capitis* and *C. acnes* to $1*10^6$ (*P*= 0.005 and 0.002 respectively) (figure 4.1 C-D-E-F).

The higher dose of $20J/cm^2$ resulted in a significant reduction in bacterial CFU for most tested bacteria (figure 4.1A-G). However, for the *M. luteus* the decrease in CFU was not significant and $6*10^6$ cells were still viable (figure 4.1 H). In response to this UVR dose, viable cell number reduced to $1*10^6$ for *S. epidermidis* (NCTC 11047) (*P*= 0.001), $2*10^6$ for *S. epidermidis* (NCTC 6513), *S. epidermidis* (NCTC 10519) and *S. hominis* (*P*= 0.006, 0.003 and 0.0002 respectively), $7*10^4$ for *S. capitis* (*P*= 0.002), $3*10^5$ for *C. acnes* (*P*= 0.001) and $4*10^6$ for *C. jeikeium* (*P*= 0.02) (figure 4.1 A-G).

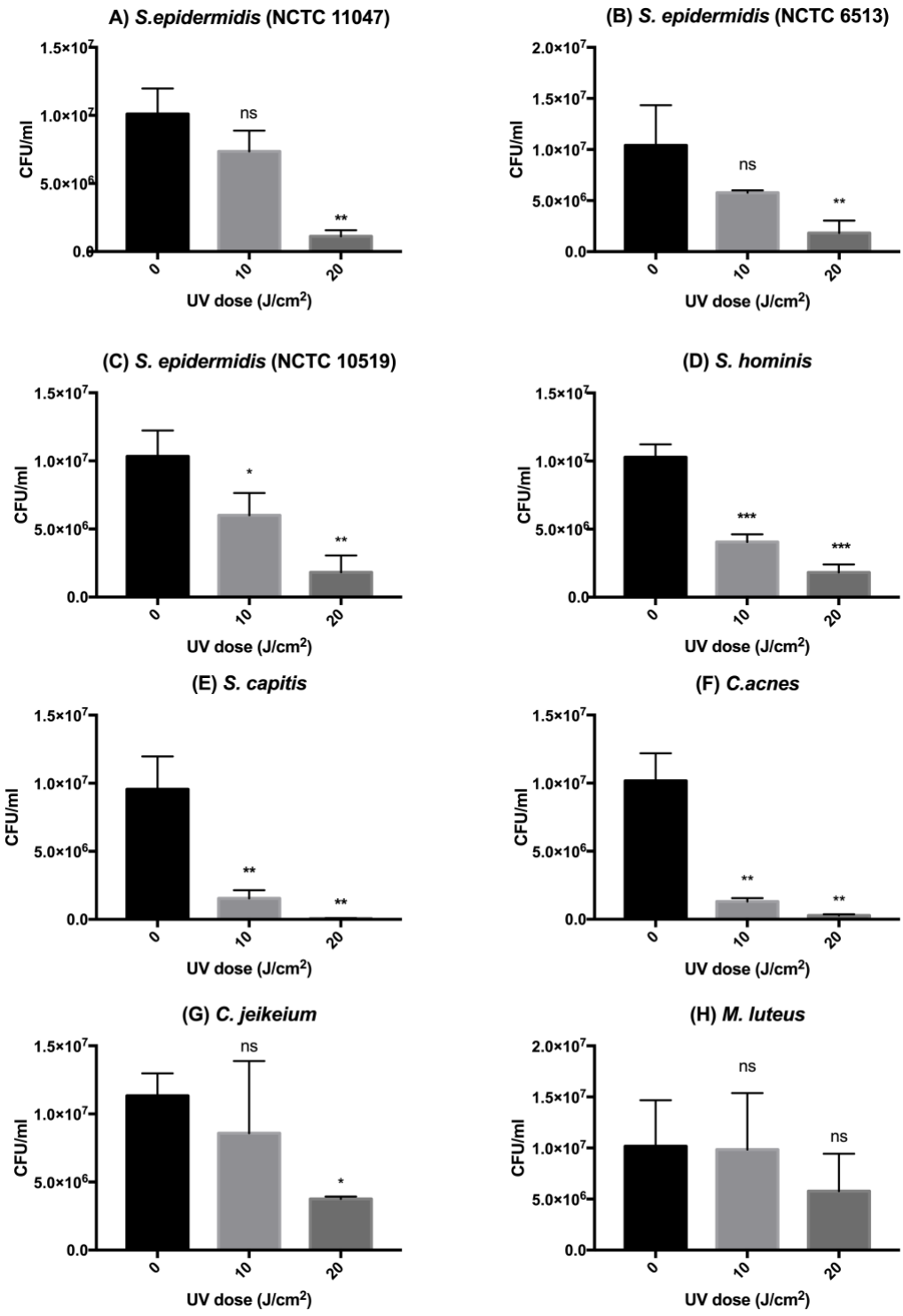

Figure 4.1 The reduction in bacterial viable cells induced by SSR treatment

$1*10^7$ CFU/ml bacterial suspensions were irradiated by 10 and 20 J/cm^2 doses of SSR. For measuring the viable cell number post SSR treatments, viable count was performed at times when the diameter of bacterial colony reached 1-2mm. The reduction in bacterial viable cell number post SSR treatment was shown in A-H. Data are presented as mean ± standard error of the mean (SEM). * P<0.05, ** P<0.01, *** P<0.001, (ns) non-significant. n=3.

To compare the SSR-sensitivity of the selected skin bacteria, the D_{37} value, which is defined as a UVR dose which inactivates 63% viable cells in the original bacterial suspension (Arrange et al. 1993; Gascón et al. 1995), was then calculated by using the slopes and the intercepts (listed in Appendix 1) of the bacterial SSR-survival curves (figure 4.2 A). The statistical difference of the D_{37} value of each organism was then analyzed by using the student -T tested and shown in figure 4.2 B. Among all tested microbes, the D_{37} value of *M. luteus* was the highest (30.8 J/cm^2), which was almost three-fold higher than that of *S. capitis* (10.4 J/cm^2, *P*=0.01) and *C. acnes* (10.4 J/cm^2, *P*=0.01), and two-fold higher than that of *S. hominis* (13.8 J/cm^2, *P*=0.03) and the three strains of *S. epidermidis* (15.6 J/cm^2 for NCTC 11047 and 10519, *P*=0.04; 15.8 J/cm^2 for NCTC 6513, *P*=0.02), suggesting that *M. luteus* is the most resistant bacterium to SSR exposure. The D_{37} value of *C. jeikeium* (19.5 J/cm^2) was lower than that of *M. luteus*, but still significantly higher than that of *S. hominis* (*P*=0.04), *S. capitis* (*P*=0.009) and *C. acnes* (*P*=0.009). On the other hand, the *S. capitis* and *C. acnes* were the most sensitive organisms to SSR exposure among all tested since they had the lowest D_{37} value (10.4 J/cm^2), which were significantly smaller than that of not only *M. luteus* and *C. jeikeium*, but also three strains of *S. epidermidis* (NCTC 11047, *P*=0.001, NCTC 6513, *P*=0.02, and NCTC 10519, *P*=0.03) and *S. hominis* (*P*=0.002).

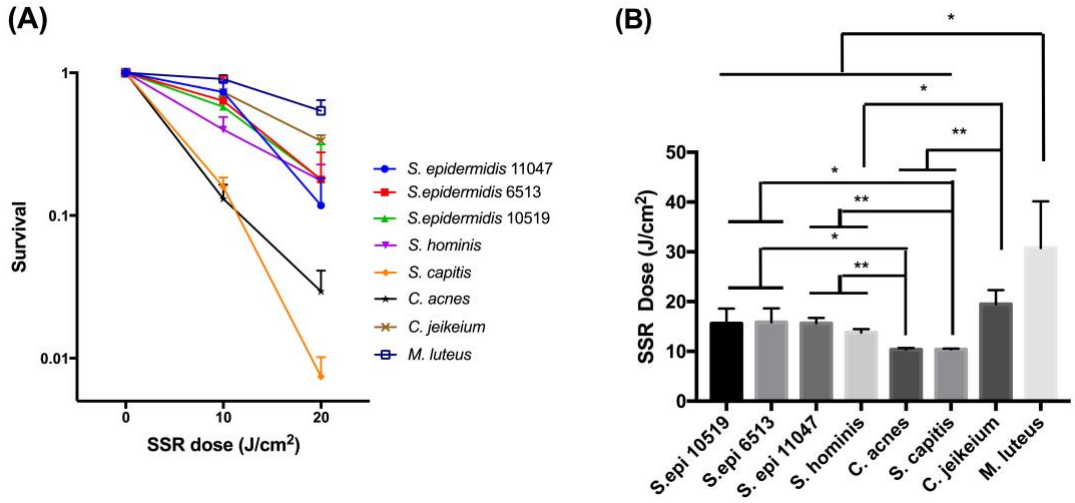

Figure 4.2 The bacterial SSR-survival curves and the D37 values of the selected skin bacteria

(A) The survival curves of (●) *S. epidermidis* (NCTC 11047), (■) *S. epidermidis* (NCTC 6513), (▲) *S. epidermidis* (NCTC 10519), (▼) *S. hominis*, (◆) *S. capitis*, (★) *C. acnes,* (□) *M. luteus*, and (×) *C. jeikeium* post 10 and 20 J/cm^2 doses of SSR treatment. (B) The D_{37} value of each bacterium was then calculated from the SSR-survival curve. Data are presented as mean ± standard error of the mean (SEM). * P<0.05, ** P<0.01. n=3.

4.2 The variation of RecA protein in each selected bacterium upon SSR treatment

The critical capacity of RecA protein in regulating bacterial UVR sensitivity has been demonstrated in a previous study which showed that an *Escherichia coli* (*E. coli*) mutant lacking the expression of the RecA protein was more sensitive to UVR than the *recA*$^+$ strain (Chau et al. 2008). Therefore, differential RecA expression could account for the differences in SSR sensitivity between skin commensal bacteria. To investigate this, immunoblotting was used as a measure of RecA expression.

Bacterial suspensions were exposed to 10J/cm^2 SSR and then cultured in the dark for 1 hour

before extraction of bacterial proteins and analysis by immunoblotting as described in section 2.6. Ponceau S solution was used to stain the membranes to establish equal loading and recombinant RecA from *E.coli* was also loaded to validate the antibody (Gomes et al. 2012) (figure 4.3 A). In each sample, the RecA protein appeared as a single band of 42kDa in agreement with the literature (figure 4.3 B). In the sample form *M. luteus*, the protein appeared be slightly higher molecular weight than the recombinant RecA control (figure 4.3 B).

The intensity of the RecA band in irradiated samples was quantified using Image Lab and expressed as a percentage of the band intensity of the unirradiated control for each bacterial species. This data is shown in figure 4.3 C. While the RecA of *M. luteus* increased by three-fold (P=0.002) post the SSR treatment, the 10J/cm^2 SSR reduced the RecA content in the rest of skin bacteria tested. The RecA expression of *S. epidermidis* (NCTC 11047, P <0.0001) and *S. epidermidis* (NCTC 6513, P=0.0003) reduced most, for which 71% and 67% RecA decreased in response to 10J/cm^2 SSR, respectively, followed by *S. capitis* (P=0.005) and *S. epidermidis* (NCTC 10519, P=0.0002), for which around 50% RecA dropped post SSR exposure. The SSR-induced RecA reduction of *S. hominis* (P=0.04) and *C. acnes* (P=0.02) was the smallest, for which only 29% and 27% RecA decreased respectively following the 10J/cm^2 doses SSR treatment.

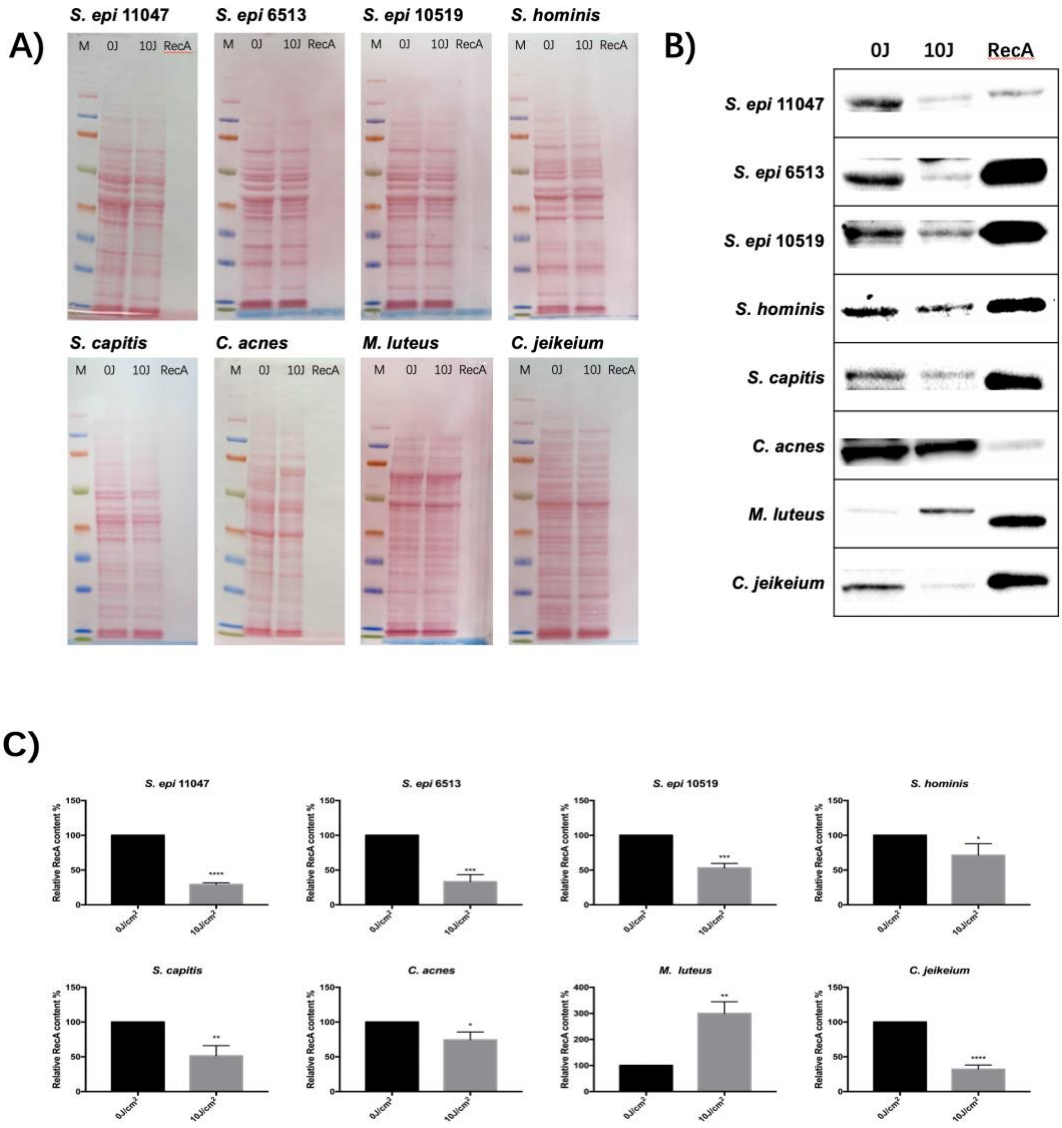

Figure 4.3 Western blot analysis of the RecA protein content of each bacterium pre/post 10J/ cm^2 SSR exposure

Proteins were extracted from the irradiated (treated by 10J/cm^2 SSR) and non-irradiated bacterial samples using sonication. 15μg bacterial proteins were separated on 10% SDS-PAGE gels and then transferred to nitrocellulose membranes. To establish equal loading, bands on the nitrocellulose membranes were stained with Ponceau S solution (A). RecA protein contained in each sample was combined with anti-RecA antibody and analyzed by western blot (B). The intensity of RecA band in irradiated sample was quantified via using Image Lab and presented as a percentage of the RecA band intensity of the unirradiated sample (C). M: marker; 0J: non-irradiated bacterial samples; 10J: bacterial samples treated by 10J/cm^2 SSR; RecA: recombinant *E. coli* RecA protein (used as the positive control). Data are expressed as mean ± standard error of the mean (SEM), (n=3). * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001.

4.3 The viability of NHEKs with/without the colonization of single strains of skin commensal bacterium in response to SSR

UV radiation has been shown to decrease the viability of keratinocytes in numerous studies (Sun et al. 2016; Moravcová et al. 2013; Im et al. 2016) . This reduction in cell viability can be observed after several hours to days of incubation following UVR treatment depending on the dose (Moravcová et al. 2013; Sun et al. 2016). In this laboratory, the gold standard is to measure viability 24hours post exposure and this, as well as the dose response of keratinocyte viability following SSR is well established (Alhasaniah 2020). Therefore, NHEKs were exposed to 10 and 20 J/cm^2 doses of SSR individually, and the cell viability was then measured by conducting trypan blue exclusion assay at 0 and 24 hours following SSR treatment (as described in section '2.7.1'and '2.7.2'). The data are presented in figure 4.4. While 82.3% NHKEs were viable before SSR treatment, the cell viability reduced to 73.8% (P=0.04) and 34.2% (P<0.0001) in response to 10 and 20 J/cm^2 SSR, respectively when cells were harvested after 24 hours post irradiation.

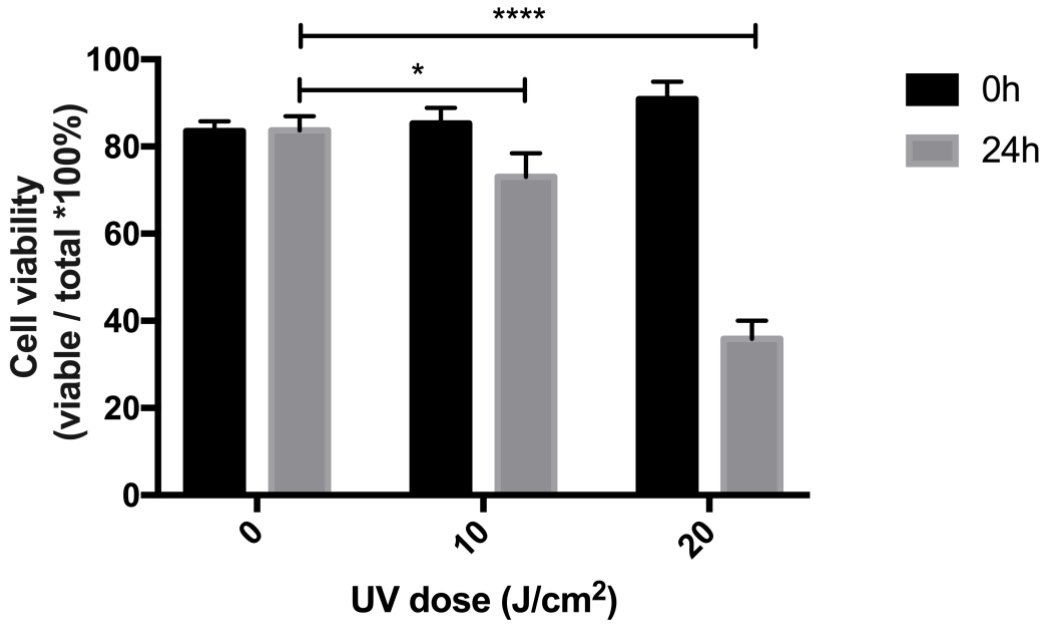

Figure 4.4 Dose-dependent decline in the viability of NHEKs induced by SSR

NHEKs were treated with either 10 or 20J/cm^2 doses of SSR. The number of viable cells was determined by performing a trypan blue exclusion assay at 0 and 24 hours after UVR treatment. When cells were harvested 24 hours after UVR exposure, statistical difference could be observed at both 10 and 20 J/cm^2 doses. Data are presented as mean ± standard error of the mean (SEM), (n=3). * P<0.05, **** P<0.0001.

4.3.1 The comparison of cell viability between NHEKs and NHEKs colonized with a single strain of skin bacterium in response to SSR

Based on the successful construction of the *in vitro* model of NHEKs colonized with a single strain skin commensal bacterium (section 3.4) and the confirmation of a time for harvesting NHEKs after SSR treatment, the effect of the individual skin organism on NHEKs viability in response to SSR was investigated in the following experiment:

Both NHEKs and NHEKs infected with a single bacterial species (prepared as in section 3.4) were exposed to 0, 10 or 20 J/cm^2 doses of SSR. After 24 hours post irradiation, the cell viability

of each group was measured by performing the trypan blue exclusion assay (described in section 2.7.3). As illustrated in figure 4.5 and 4.6, SSR exposure decreased the cell viability of both infected and non-infected NHEKs. For NHEK infected with *S. hominis*, *S. capitis, C. acnes*, *M. luteus* or *C. jeikeium,* the presence of the bacterium did not change the response of the keratinocytes to SSR (figure 4.5 A-E). However, in NHEKs infected with *S. epidermidis* strain 11047, the viability of the keratinocytes was significantly reduced in the presence of the bacterium when exposed to either 10 or 20J/cm^2 SSR (*P*=0.0006 and 0.0012 respectively, figure 4.6 A). Two further strains of *S. epidermidis* (NCTC 6513 and 10519), also produced similar data (figure 4.6 B and C), which showed with the colonization of either these organisms, the viability of keratinocyte decreased more following 10J/cm^2 SSR exposure (*P*=0.03 and 0.002 respectively).

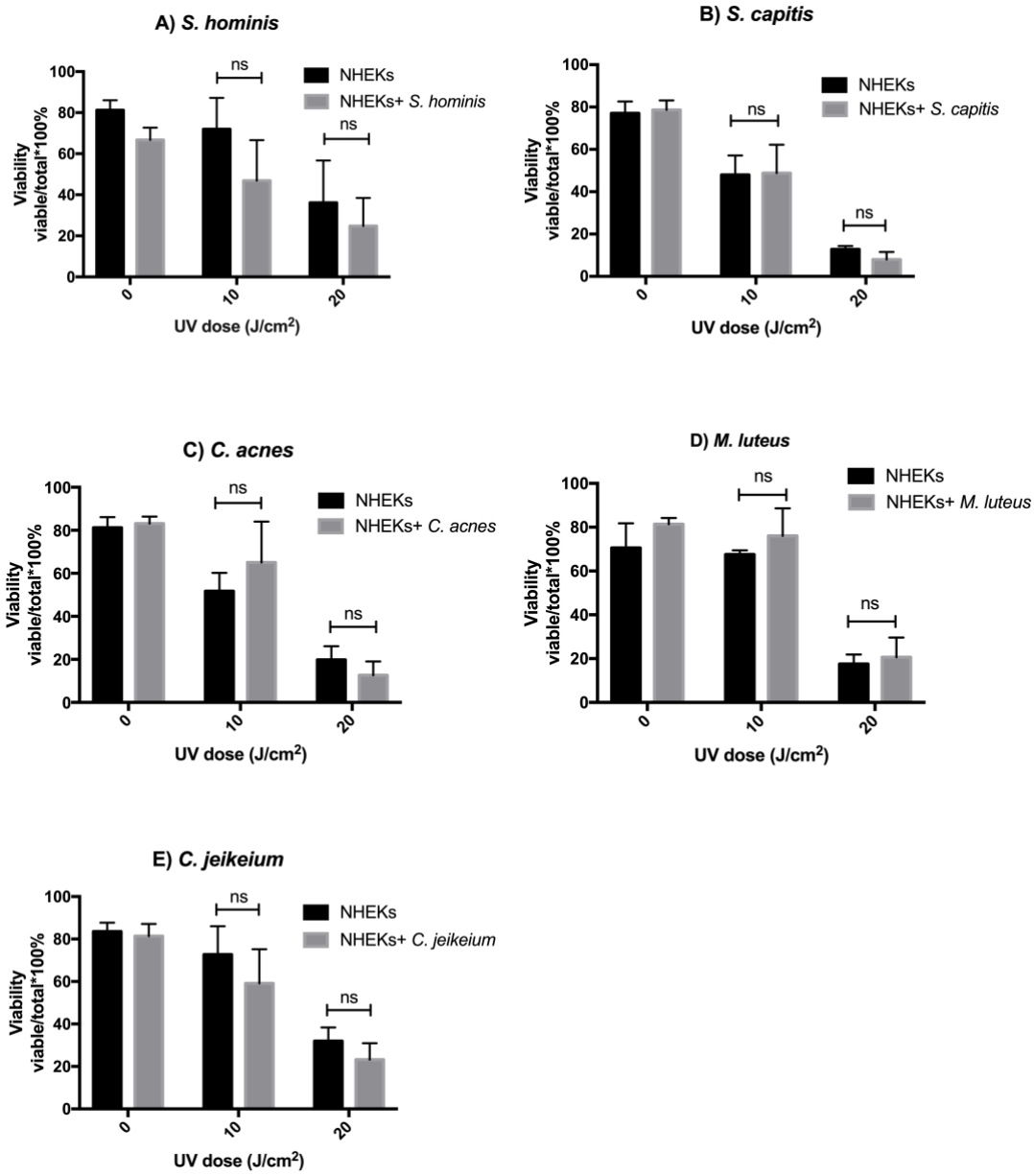

Figure 4.5 The comparison of cell viability between NHEKs and NHEKs colonized with a single strain of skin bacterium in response to SSR

NHEKs with/without the colonization of a single strain of skin commensal bacterium was exposed to 0, 10 and 20 J/cm^2 doses of SSR separately. Trypan blue exclusion assay was then employed to determine the cell viability after 24 hours of irradiation. The adhesion with *S. hominis*, *S. capitis*, *C. acnes*, *M. luteus* or *C. jeikeium* did not significantly influenced the NHEKs viability in response to 10 and 20 J/cm^2 doses SSR exposure. Data are presented as mean ± standard error of the mean (SEM), (n=3). (ns) non-significant.

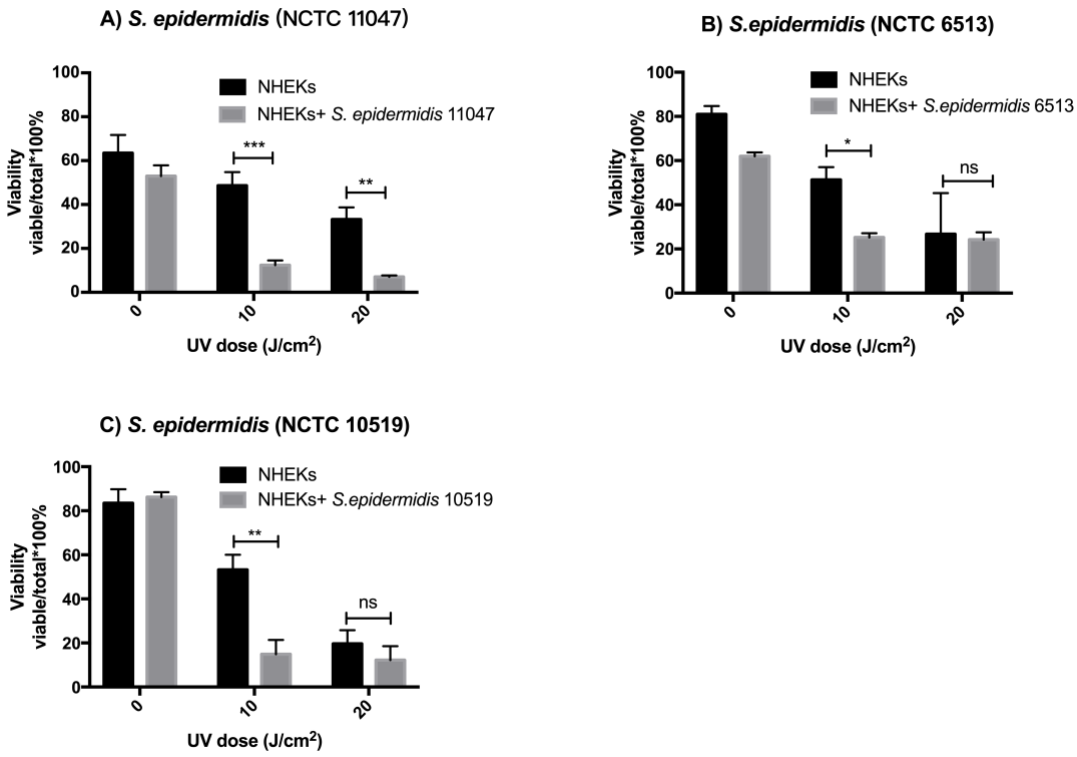

Figure 4.6 The comparison of cell viability between NHEKs and NHEKs colonized with a single strain of *S. epidermidis* in response to SSR

NHEKs with/without the colonization of a single strain of *S. epidermidis* was exposed to 0, 10 and 20 J/cm^2 doses of SSR separately. Trypan blue exclusion assay was then employed to determine the cell viability after 24 hours of irradiation. All three strains of *S. epidermidis* significantly reduced the NHEKs viability following 10 J/cm^2 doses of SSR. A significant reduction in NHEKs viability also presented with the colonization of *S. epidermidis* (NCTC 11047) after the exposure of 20 J/cm^2 doses of SSR. Data are presented as mean ± standard error of the mean (SEM), (n=3). * P<0.05, ** P<0.01, *** P<0.001, (ns) non-significant.

4.4 The variation in NHEKs viability in response to the cell-free supernatants of irradiated bacteria

According to the data of section 4.3, the viability of NHEKs colonized with a single strain of *S. epidermidis* was decreased compared with the sterile NHEKs upon exposure to SSR. This raised the idea that irradiated, but not unirradiated *S. epidermidis* released substances that

could be toxic to NHEKs. To test this hypothesis, cell-free supernatants of irradiated *S. epidermidis* (prepared as in section 2.8) were added to NHEKs, and the cell viability was measured after 24 hours of incubation. As shown in figure 4.7, the viable NHEK number decreased after the 24-hours treatment with the cell-free supernatants of three strains of irradiated *S. epidermidis* compared to the untreated cells. The viability of NHEKs dropped by 20% (P=0.0002), 17% (P=0.04) and 17% (P=0.02) upon co-incubation with the supernatant of strain NCTC 11047, 6513 and 10519 respectively treated by 20J/cm^2 SSR (figure 4.7 A-C). No significant reduction was observed in NHEKs viability in response to the supernatant of strain NCTC 6513 irradiated with 10J/cm^2 SSR (figure 4.7 B). However, a decrease of 14% (P=0.002) and 12% (P=0.01) was observed when NHEKs were incubated with the supernatant of strains NCTC 11047 and 10519 previously irradiated with 10 J/cm^2 SSR (figure 4.7 A and C). In contrast, treatment with the supernatants from other irradiated bacteria did not result in any change to the viability of NHEKs (figure 4.7 D-H).

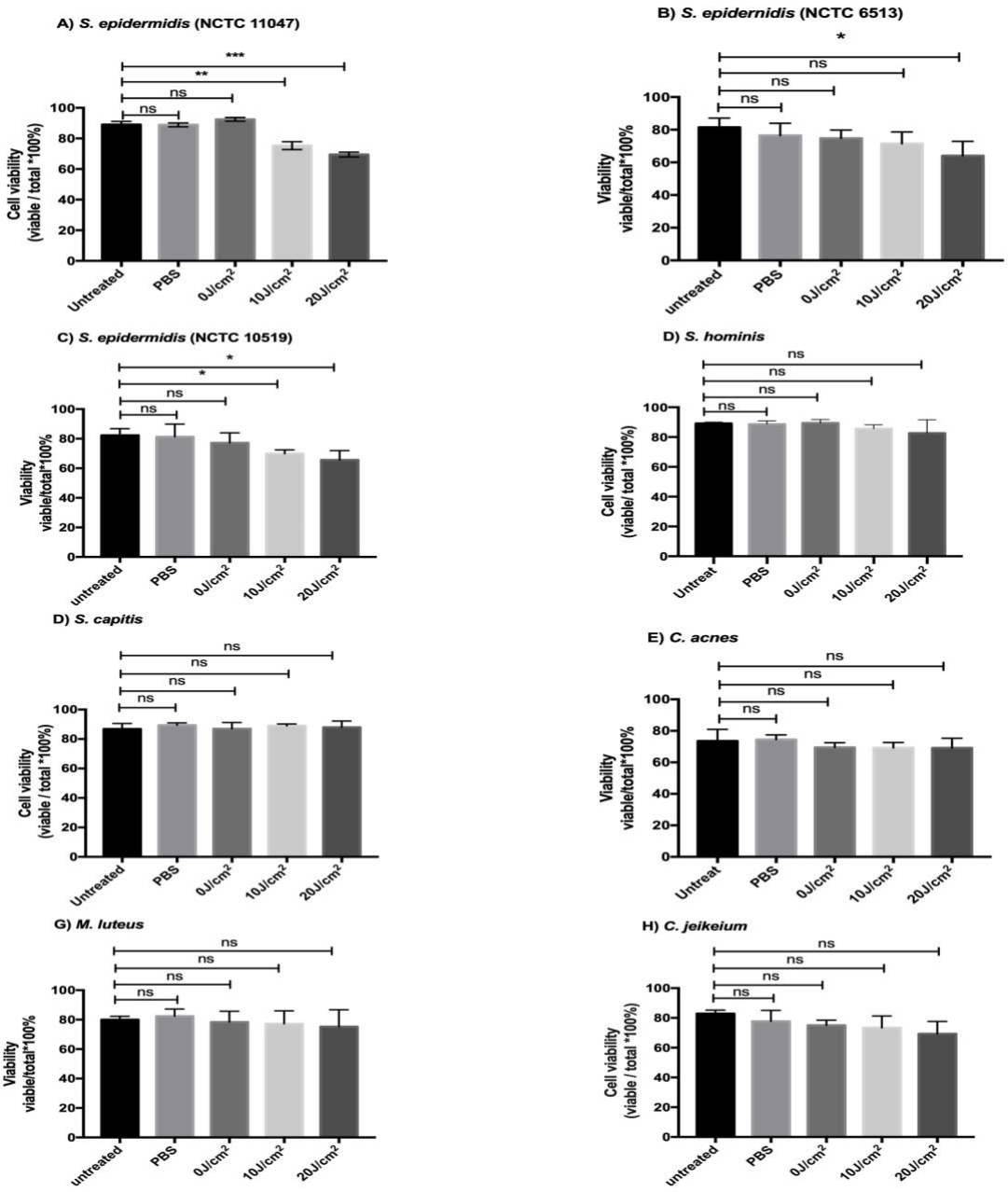

Figure 4.7 The decline of NHEK viability in response to cell-free supernatant of irradiated bacteria

NHEKs were co-incubated with the cell-free supernatant of bacteria irradiated by either 10 or 20 J/cm^2 doses of SSR for 24 hours. The cell viability was then established using trypan blue exclusion assay. NHEKs viability was reduced at 24-hours treatment in the presence of the supernatants of three strains of irradiated *S. epidermidis* (A-C). No statistically significant difference was observed in cell viability after the co-incubation with the cell-free supernatant of other irradiated bacterium (D-H). Data are presented as mean ± standard error of the mean (SEM), (n=3). * $P<$ 0.05, ** $P < 0.01$, *** $P < 0.001$, (ns) non-significant.

4.5 The cell-free supernatant of irradiated *S. epidermidis* (NCTC 11047) promotes keratinocyte necrosis

As presented in section 4.4, the cell-free supernatants form irradiated *S. epidermidis* reduced the viability of NHEK. Since apoptosis is one of the main mechanisms whereby UVR induces NHEKs death, in the following experiment, the idea that *S. epidermidis* could be promoting apoptosis was investigated. As the cell-free supernatant of irradiated *S. epidermidis* (NCTC 11047) had the largest effect on NHEKs viability among the three tested strains (figure 4.7), this was used in further experiments.

The supernatant of *S. epidermidis* (NCTC 11047) treated with either 10 or 20 J/cm^2 SSR (prepared as outlined in 2.8) was used to treat NHEKs for 24 hours. The NHEKs were then harvested and stained with the Annexin V (binds to the phospholipid phosphatidylserine (PS)) and DAPI (binds to DNA) for apoptosis analysis using flow cytometry (as described in section 2.9) (Wallberg et al. 2016).

During apoptosis, due to the translocation of PS from the inner to the outer leaflet of the plasma membrane, the apoptotic cells are bound to Annexin V. As the cell membrane is intact in the early apoptosis, DNA cannot be exposed to DAPI. However, at later stages of apoptosis, with the formation of apoptotic bodies, the integrity of cell membrane can be lost. DAPI then passes thought cell membrane and becomes bound to DNA (Fink and Cookson 2005). As a result, early apoptotic cells are only positive to Annexin V while late apoptotic cells are positive to Annexin V and DAPI. Cell membrane ruptures at the stage of necrosis, leading to the release

of DNA into the extracellular environment (Fink and Cookson 2005; Raffray and Cohen 1997). As the released DNA from necrotic cells are largely intact (Kaczmarek et al. 2013), they can then bind to DAPI in the extracellular environment, and thus, necrotic cells are only positive to DAPI. For the viable cells, as their cell membranes are intact and no PS translocation can be observed, they are negative to both markers. The data in figure 4.8 indicates that the supernatant of irradiated *S. epidermidis* (NCTC 11047) significantly increased the percentage of necrotic NHEKs after 24-hours co-incubation while the percentages of the early and late apoptotic NHEKs were not dramatically affected. The necrotic NHEK% increased by 5% and 10% respectively post the incubation with the supernatant of *S. epidermidis* (NCTC 11047) pre-treated with either 10 or 20 J/cm^2 SSR compared to the control.

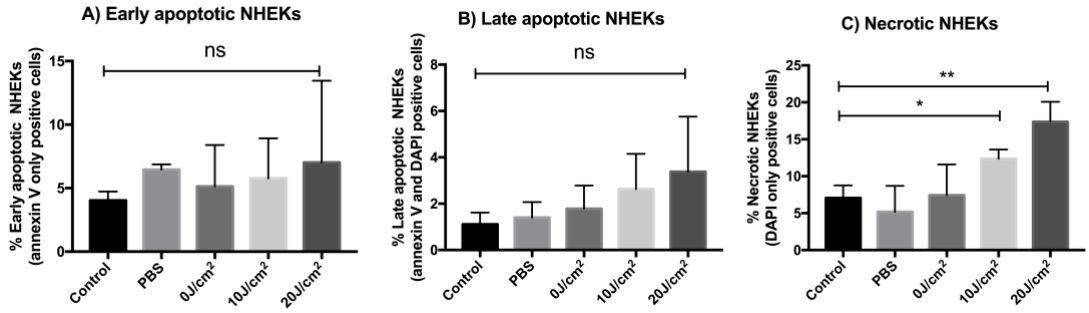

Figure 4.8 The apoptosis of NHEKs after the co-incubation with the cell-free supernatant of irradiated *S. epidermidis* (NCTC 11047)

NHEKs were treated with the cell-free supernatant of irradiated *S. epidermidis* (NCTC 11047) for 24 hours. The cells were then strained with Annexin V and DAPI for apoptosis analysis using flow cytometry. The %apoptotic NHEKs (A and B) were not affected, while the %necrotic NHEKs (C) were significantly increased with the co-incubation of either 10 or 20 J/cm^2 supernatants. Data are presented as mean ± standard error of the mean (SEM), (n=3). * $P < 0.05$, ** $P < 0.01$, (ns) non-significant.

4.6 Characterising the factor from *S. epidermidis* that promotes NHEK necrosis

To investigate the nature of the factor released by irradiated *S. epidermidis* (NCTC 11047), the cell-free supernatant of *S. epidermidis* (NCTC 11047) exposed to 20J/cm^2 doses of SSR (prepared as described in section 2.8) was heated at 100 degrees for 10 minutes or treated with trypsin for 3 hours to denature proteins. 100µl of this protein-denatured supernatant was then co-incubated with NHEKs for 24hours before the cell viability was measured by conducting trypan blue exclusion assay (as described in section 2.10). Figure 4.9 illustrated that while the supernatant of irradiated *S. epidermidis* significantly reduced the NHEK viability, this effect disappeared when it was pre-treated by heat or trypsin. This suggests that the factor promoting NHEK necrosis is not stable to heat or protease treatment, suggestive of the idea that the factor may be a protein.

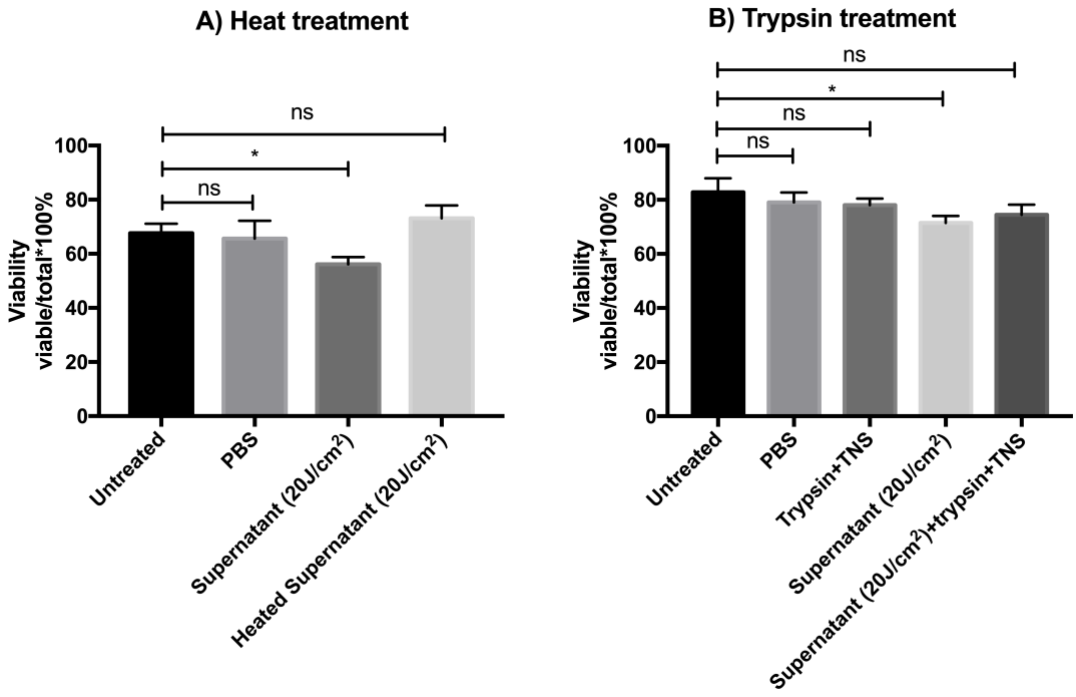

Figure 4.9 The toxic effect of the cell-free supernatant of irradiated *S. epidermidis* (NCTC 11047) after the heat and the trypsin treatment

The supernatant of the irradiated *S. epidermidis* (NCTC 11047) (treated with 20 J/cm^2 SSR) was generated and pre-treated by either 100-degrees heating (A) or trypsin (B). NHEKs were then individually co-incubated with these treated supernatants for 24 hours before the cell viability was measured. While the irradiated supernatant significantly decreased the NHEKs viability compared with the untreated group, neither the heated nor the trypsin-treated supernatant significantly affected the NHEKs viability. Data are presented as mean ± standard error of the mean (SEM), (n=3), * $p < 0.05$, (ns) non-significant.

4.7 Fractionation of the cell free supernatant of irradiated *S. epidermidis* (NCTC 11047) using molecular weight 'cut-off' protein columns

In an attempt to further characterise the protein factor from *S. epidermidis*, the supernatant of *S. epidermidis* (NCTC 11047) treated by 20J/cm^2 SSR (prepared as in section 2.8) was separated into fractions using molecular weight cut-off protein columns (3kDa, 30kDa, 50kDa and 100kDa columns were employed). Each fraction was then co-incubated with NHEKs for 24

hours before the NHEKs viability was measured (as described in section 2.11). In the data of figure 4.10 shows that NHEKs viability reduced significantly after co-incubation with fractions containing proteins with a molecular weight of larger than 3kDa, 30kDa, 50kDa but smaller than 100kDa, illustrating that the weight(s) of the protein(s) was between 50kDa and 100kDa.

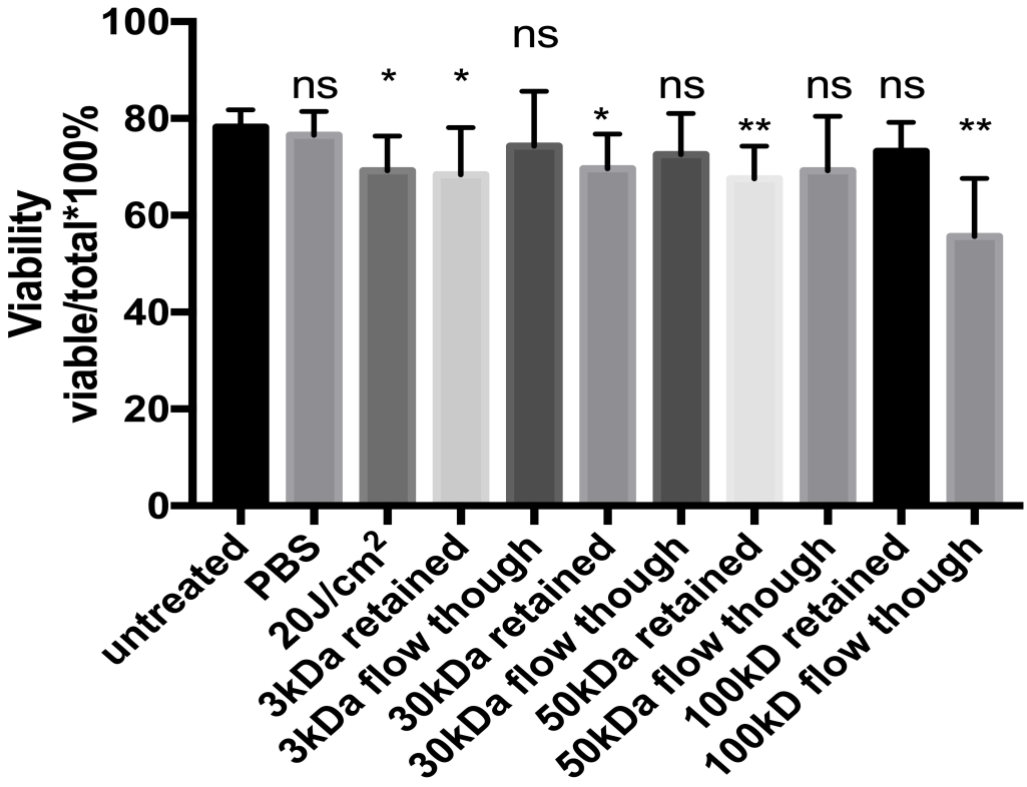

Figure 4.10 The viability of NHEKs in response to the fractions of supernatant of irradiated *S. epidermidis* (NCTC 11047)

The molecules contained in the cell-free supernatant of irradiated *S. epidermidis* were separated according to their molecular weights. Each fraction was then co-incubated with NHEKs for 24 hours, and the cell viability was measured by performing the trypan blue exclusion assay. The fractions containing the substances with a molecular weight of > 3kDa, 30kDa, 50kDa, and < 100kDa decreased the NHEKs viability following the 24-hours incubation. Data are presented as mean ± standard error of the mean (SEM), (n=3), * $p< 0.05$, ** $p< 0.01$, (ns) non-significant.

4.8 Discussion

Little can be found from the literature about how solar radiation affects skin commensal bacteria, and how in turn, the irradiated microbes influence skin physiology. In this chapter, by measuring the viability of individual skin bacteria in response to SSR exposure, it has been shown that the SSR-sensitivity of selected skin bacteria is different. Survival following UVR will depend on:

1) An organism's ability to filter out UVR.

2) An organism's ability to repair any damaged induced by UVR.

It is possible that some skin organisms are innately more able to shield themselves from UVR (Mayser and Pape 1998; Mayser et al. 2002), or can repair more efficiently than others (Nakayama et al. 1992). This may explain in part, be associated with anatomical location. For example, *S. capitis* and *C. acnes* appear to be highly susceptible to SSR and their viability are reduced much more than that of the other tested organisms in response to both 10 and 20 J/cm^2 doses of SSR. This observation could be correlated with the distribution of these two bacteria on human skin, as both of them are mostly isolated from the skin areas that usually experience low UVR exposure. For instance, *S. capitis* is mainly found on the head (and protected by hair), and *C. acnes* generally colonizes inside the sebaceous glands (Kloos and Musselwhite 1975; Fournière et al. 2020). The high UVR sensitivity of *C. acnes* could be associated with the porphyrin content of this bacterium, which can lead to cell death by affecting cell structure under UVR stress (Wang et al. 2012; Kou et al. 2017).

In contrast, *M. luteus* and *C. jeikeium* appear to be highly resistant to a dose of SSR because

more of these cells remain viable (figure 4.1). Although, the mechanisms for the high UVR resistance of *C. jeikeium* are not clear, some researchers have suggested that the high resistance of *M. luteus* to UVR could be correlated with the high production of the UVR-endonuclease by this organism. It has been demonstrated that the UVR-induced DNA damage, pyrimidine dimers, can be repaired by the UVR-endonuclease (Sinha and Häder 2002). Hence, with the production of this DNA repair enzyme, the damaged DNA could be repaired effectively post SSR exposure, leading to an increase survival rate for *M. luteus* (Carrier and Setlow 1970; Mahler et al. 1971; Nakayama et al. 1992). Additionally, the yellow carotenoid pigments in *M. luteus* could also protect this microbe against UVR, as it has been shown previously that these pigments act as a natural sunscreen to filter out UVR and increase survival of the organism (Mohana et al. 2013).

However, these explanations described above do not entirely explain the differences in the UVR sensitivity for all selected skin commensal bacteria. It has been reported that UVR-induced DNA damage is involved in bacterial death (Chau et al. 2008), and the RecA protein is an important regulator for bacterial DNA repair upon UVR exposure (Baharoglu and Mazel 2014; Chau et al. 2008; Patel et al. 2010). Thus, in this study it was investigated whether this protein plays a role in differential SSR-sensitivity of skin bacteria. Iinitially, it was hypothesized that the RecA protein content in each bacterium was negatively correlated to the bacterial SSR sensitivity. The approach to test this hypothesis would be to directly compare the RecA levels within each bacterium. However, proved difficult to find a ‘housekeeping protein’, which was constantly expressed in all selected bacteria to normalise protein levels. Therefore, instead of

comparing the RecA content between each bacterium, its variation in each organism pre/post SSR treatment was investigated, as presented in figure 4.3. After SSR treatment, the RecA increased threefold in *M. luteus* (figure 4.3 C). Therefore, in addition to the mechanisms mentioned in the previous paragraphs, the increase of RecA in *M. luteus* following the UVR stress could be another explanation for the high SSR tolerance of this organism (figure 4.1and 4.2).

For the rest of the selected organisms, a reduction was observed in RecA content after the SSR treatment (figure 4.3 C). Generally, the RecA decreased more in the organisms which were less sensitive to SSR exposure (*C. jeikeium* and the strains of *S. epidermidis*) than the UVR-susceptible species (*S. hominis* and *C. acnes*), whereas this trend was not followed in *S. capitis* (figure 4.3 C). The decrease in bacterial RecA content following UVR exposure has rarely been reported previously. In most studies the UVR-induced changes to RecA expression were investigated using *E. coli* as a model organism (Sommer et al. 1998; Salles and Paoletti 1983). Future studies are needed to investigate whether the RecA protein at least in part, is involved in regulating the skin bacterial sensitivity to SSR.

To investigate the role of skin commensal bacteria in the response of keratinocytes to SSR, an *in vitro* model of NHEKs colonized with an individual skin commensal bacterium has been established in chapter 3. Before treating this model with SSR, a time for cell harvest after SSR exposure was determined. No reduction was observed when cell viability was measured immediately after UV irradiation, while significant differences were shown between non-irradiated and irradiated groups when the cell was harvested after 24 hours of irradiation (figure

4.4). About 70% of NHEK cells remained viable in response to the 10 J/cm^2 solar UVR dose, which decreased to approximately 35% treated with 20 J/cm^2 solar UVR dose. This finding is consistent with the data of Moravcová et al. (2013) and Sun et al. (2016), which demonstrated the significant decrease in keratinocyte viability when the cells were harvested 24 hours post irradiation.

In the irradiated groups, while the presence of most tested bacteria did not dramatically affect the NHEKs viability in response to SSR treatment compared with the non-infected NHEKs, colonizing with strains of *S. epidermidis* reduced the cell viability significantly in response to 10J/cm^2 SSR exposure (figure 4.5). Crucially, *S. epidermidis* did not affect the viability of keratinocytes in the absence of UV irradiation. This suggests that irradiated, but not unirradiated *S. epidermidis*, has a direct effect on the viability of the keratinocytes. Furthermore, the supernatant of irradiated, but not unirradiated *S. epidermidis*, also decreased the number of keratinocytes which were viable following 24 hours incubation. This suggests that irradiation induces the release of molecule(s) from *S. epidermidis*, that affects keratinocyte viability. This could be a general effect of *S. epidermidis* because three different strains of this organism produced similar data. The toxic substance(s) was not stable to heat and trypsin treatment (figure 4.9), suggesting the nature of the detrimental components contained in the supernatants of the irradiated *S. epidermidis* could be protein, probably with a weight between 50kDa and 100kDa. In work not included in this thesis, the supernatant from irradiated *S. epidermidis* was subjected to SDS-PAGE to visualize protein(s) contained. The intention was to perform within gel digestion and identification of the protein by mass spectrometry proteomics. However, the

concentration of the protein(s) must be low because it could not be detected by SDS-PAGE. Work is continuing to try to identify the protein(s).

Our data demonstrated that the increase in keratinocyte loss following SSR induced by *S. epidermidis* was due to the generation of necrotic cells (figure 4.8). Unlike apoptotic cells that are normally considered as non-inflammatory (Kulms and Schwarz 2000a), necrotic cells can induce inflammation by releasing damage-associated molecular patterns, such as the high mobility group 1 (HMGB1) protein, IL-1α, uric acid, etc. (Scaffidi et al. 2002; Westman et al. 2020; Kaczmarek et al. 2013). As % necrotic cells were increased with the presence of *S. epidermidis* upon SSR treatment, the colonization with *S. epidermidis* could aggravate skin inflammation upon UVR exposure if the necrotic cells generated by this organism could not be removed efficiently. However, it is still hard to conclude whether the colonization with *S. epidermidis* is negative or positive in terms of skin health upon UVR stress, as the UVR-protective effects of this organism were also reported previously. For instance, the supernatant from the overnight culture of *S. epidermidis* (ATCC 12228) reduced the damaged DNA of melanocytes post UVB treatment (Wang et al. 2018), the 6-N-hydroxyaminopurine (6-HAP) produced by *S. epidermidis* (MO34) prevented the formation of the UV-induced melanoma in mice (Nakatsuji et al. 2018), and lipoteichoic acid (LTA) released by *S. epidermidis* reduced the generation of the inflammatory cytokine TNF-α from keratinocytes following UVB exposure (Lai et al. 2009). Therefore, more work is still nesssary in the future for determinng the role of *S. epidermidis* on skin health in response to UVR exposure.

In conclusion, the sensitivity of skin commensal bacteria to SSR was different. Among all tested

organisms, while *S. capitis* and *C. acnes* was the most sensitive bacteria to SSR, *C. jeikeium* and *M. luteus* were the most SSR-tolerant species. Bacterial RecA content changed upon SSR exposure. However, the details about how this protein is involves in regulating the sensitivity of skin bacteria to SSR is unclear. The presence of *S. epidermidis* was detrimental to the NHEKs survival in response to SSR exposure. This negative effect of the irradiated *S. epidermidis* (NCTC 11047) on NHEKs viability was probably caused by the release of toxic proteins, which induced the necrosis of NHEKs. These previously unknown data broaden the knowledge of the effect of skin microbiota on epithelial cells upon SSR exposure.

Chapter 5 A proposed study to investigate the influence of the skin microbiome on the sunburn response in healthy humans *in vivo*

5.1 Introduction

In the previous chapters, the data suggested that specific bacteria from skins microbiota can impact the response of keratinocytes to a single dose of SSR. Additionally, a previous study by Patra et al (Patra et al. 2019) used germ-free mice to show that the presence of a microbiome modifies the response to UVR. The work described in this chapter attempts to address this in humans. The hypothesis here is: the absence of a microbiome will alter the response of human skin *in vivo* to a single dose of UVR.

Previous work in the laboratory has shown that it is possible to reduce the microbiome from a healthy human volunteer using ethanol and that levels then remain low for up to 6 hours (figure 5.1). Therefore, reducing the microbiome and irradiation during this 6 hours time frame could provide insight into whether and how the microbiome influences the skins' response to UVR. To achieve this, a minimal erythemal dose (MED) study was designed. The MED is the minimal dose of UVR which causes a mild erythema to the skin (Harrison and Young 2002; Heckman et al. 2013). It will be measured by exposing a specified skin region to serial doses of UVR. The MED is usually measured 24 hours following UVR exposure using a spectrophotometer, as the peak of erythema occurs approximately 8-24 post UVR treatment (Brenner and Hearing 2008).

Unfortunately, because of the covid pandemic, no work could be undertaken in human volunteers. Therefore, this chapter describes only the study design and the process of applying for ethical consent.

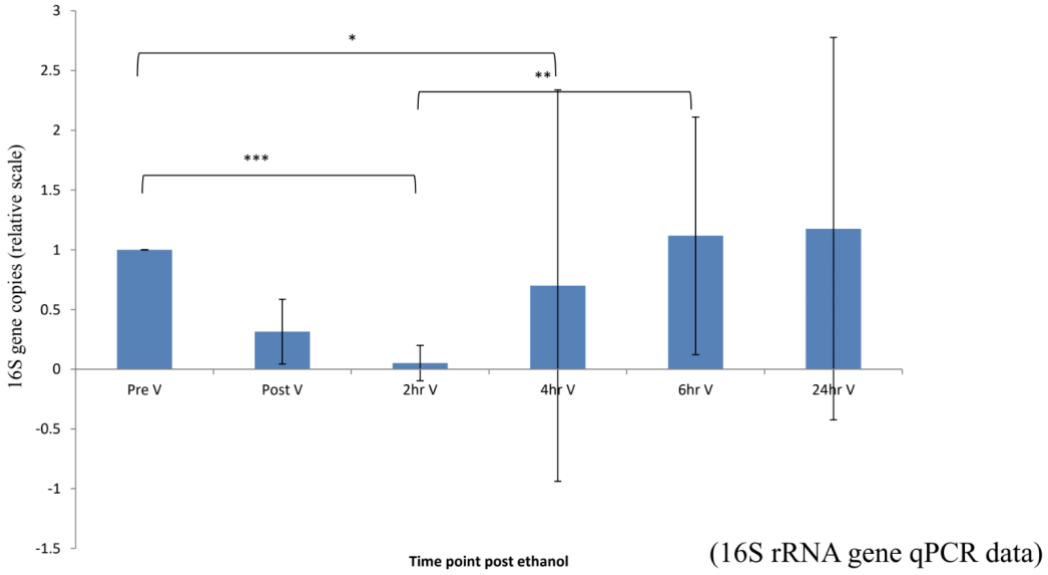

Figure 5.1 The bacterial abundance of volar forearm post 70% ethanol cleaning

The volar forearm skin was wiped with 70% ethanol solution 20 times and the skin microbiome was swabbed at several time points pre and post cleaning. The DNA of the collected skin microbiome samples were extracted and analysed using quantitative PCR (qPCR) (data provided by Professor Andrew McBain).

5.2 Study design and considerations

5.2.1 Study design and ethics approval

This project was reviewed and approved by The University of Manchester Research Ethics Committee (reference 2020-8539-13844, the approved application form is attached in Appendix 2). All procedures involving the participation of volunteers are to be conducted at the Photobiology Unit, Salford Royal Hospital, UK.

5.2.2 The recruitment of participants

Up to 10 participants will be recruited using either a database containing the contact details of potential participants in the Photobiology Unit, Salford Royal Hospital, or via advertisements (figure 5.2) placed in notice boards of The University of Manchester. Statistical power would be iterated from pilot studies. The potential participants will be selected using the inclusion/exclusion criteria (table 5.1). The skin phototype of the potential candidates will be assessed using the form as shown in table 5.2. Participants with the Fitzpatrick phototype I-III (Fitzpatrick 1988) will be invited for further experiments. Suitable volunteers will be given the participant information sheet (Appendix 3) to learn the experimental process and sign the consent form (table 5.3) before conducting all clinical procedures.

Advertisement

Version 2: 12th March 2020

MANCHESTER 1824
The University of Manchester

HEALTHY VOLUNTEERS ARE REQUIRED FOR A STUDY EXAMINING SUNLIGHT EFFECTS ON SKIN

We are performing research to study whether reduction of the bacteria naturally found on the skin increases the skin redness level following exposure to ultraviolet radiation (UVR). This study will be conducted at Salford Royal NHS Foundation Trust on two consecutive days. One side of your mid-back skin will be cleaned and then small areas of both the cleaned and control sides will be exposed to UVR and resulting skin redness will be assessed. Skin swabs will be taken before and after the cleaning to screen the reduction of skin bacteria.

Reimbursement will be provided for travel costs, and the time and inconvenience involved.

We are looking for people who fit the following criteria:

- Aged 18-65 years of any genders
- White Caucasian
- Have NO history of photosensitivity disorders (unusual sensitivity to sunlight) or sensitivity to alcohol
- Have NO history of existing skin disorders
- Having NOT used any photoactive, anti-inflammatory medications or antibiotics in the past 3 months
- Having NOT used/taken any sunbathing/ sunbed/ sunny holiday in the past 3 months

If you are interested in or would like to receive further information, please contact:

Joanne Osman, Research Nurse Tel: 0161 2060457
Email: joanne.osman@manchester.ac.uk
Or
Wen Duan, PhD Researcher Tel: 0161 2755297
Email: wen.duan@postgrad.manchester.ac.uk

University of Manchester Research Ethics Committee reference: 2020-8539-13844

Figure 5.2 The advertisement for participants recruitment (produced by Wen Duan)

Table 5.1 The inclusion and exclusion criteria for participants selection

Inclusion criterions	Exclusion criterions
➓ Healthy, ambulant human adults ➓ Male and female ➓ Aged 18-65 years* ➓ White Caucasian (Fitzpatrick phototype skin types I-III)	➓ History of photosensitivity disorder or skin cancer ➓ Skin sensitivity to ethanol ➓ Taking antibiotics in the past 3 months ➓ Currently taking photoactive or anti-inflammatory therapy ➓ Sunbathing/ sunny holiday/ sunbed used in past 3 months ➓ Unable to comply with the requirements of the study

* Evidence has been shown previously that the composition of skin flora is different between children and adults (Somerville 1969). Only adults (18-65 years old) were recruited herein to reduce the discrepancy caused by the difference of skin microbiota between ages.

Table 5.2 The skin phototype assessment form

Score	0	1	2	3	4
What is the colour of your eyes	Light blue or grey	Blue or green	Hazel or light brown	Dark brown	Brownish black
What is the natural colour of your hair?	Sandy Red	Blond	Chestnut or Light brown	Dark brown	Black
What is the colour of your skin (non-exposed areas)?	Reddish	Very pale	Pale with beige tint	Light Brown	Dark brown
Do you have freckles on unexposed areas?	None	Incidental	Few	Several	Many
What happens when you expose your skin to the sun for more than an hour?	Painful redness, blistering, peeling	Blistering followed by peeling	Burns sometimes followed by peeling	Rarely burns	Never had burns
To what degree do you turn brown?	Hardly or not at all	Light colour tan	Reasonable tan	Tan very easy	Turn dark brown quickly
Do you turn brown within several hours after sun exposure?	Never	Seldom	Sometimes	Often	Always
How does your face react to sun exposure?	Very sensitive	Sensitive	Normal	Resistant	Very resistant
When did you last expose the area to be treated to the sun, artificial sunlamp or tanning cream?	>3 months ago	2-3 months ago	1-2 months ago	<a month ago	<2 weeks ago
Did you expose the area to be treated to the sun?	Never	Hardly ever	Sometimes	Often	Always
Total score	0-7		Fitzpatrick skin phototype I		
	8-16		Fitzpatrick skin phototype II		
	17-25		Fitzpatrick skin phototype III		
	26-30		Fitzpatrick skin phototype IV		
	Over 30		Fitzpatrick skin phototype V- VI		

Table 5.3 The consent form

	Activities	**Initials**
1	I confirm that I have read the attached information sheet (Version 1, Date 10/01/2020) for the above study and have had the opportunity to consider the information and ask questions and have these answered satisfactorily.	
2	I understand that my participation in the study is voluntary and that I am free to withdraw at any time without giving a reason and without detriment to myself. I understand that it will not be possible to remove my data from the project once it has been anonymised and forms part of the data set. I agree to take part on this basis.	
3	I agree to receive ultraviolet radiation (UVR) exposure and 70% ethanol cleaning as described in the information sheet.	
4	I agree to have the standard sunburn threshold testing for the research purpose as explained to me.	
5	I agree to have skin bacteria samples taken for the research purpose as explained to me.	
6	I agree to refrain from having a shower or sauna 6 hours before my participation in the study.	
7	I agree to refrain from exposure my back skin to other UVR lights before the MED assessment.	
8	I understand that data collected during the study may be looked at by individuals from The University of Manchester, Salford Royal Foundation Trust or regulatory authorities, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my data.	
9	I agree that any data collected may be published in anonymous form in academic books, reports or journals.	
10	I agree to take part in this study.	
11	(Optional) I agree that the researchers may contact me in future about other research projects.	
12	(Optional) I agree that the researchers may retain my contact details in order to provide me with a summary of the findings for this study.	

5.2.3 UVR exposure

The back of the torso was selected as the anatomical location to be exposed to UVR. This was chosen because it represents a microbiota-rich site in terms of abundance and species (Grice et al. 2008). The participants would be asked not to apply topicals of any kind to the back skin after their last shower. They would also be asked to refrain from shower or saunas for at least 6 hours before the study.

The right side of the mid-back skin of each volunteer will be wiped with 70% ethanol solution 20 times to reduce the microbiome before UVR exposure. The skin microbiome samples will be collected immediately pre and post wiping using skin swabs to verify the reduction of bacterial abundance. The bacterial DNA in each sample will be then extracted employing the DNeasy PowerSoil Pro Kit (QIAGEN, Netherland) and then the ethanol-induced decrease in bacterial abundance will be analysed using qPCR.

The cleaned and uncleaned skin areas will be separately exposed to a series of 10 doses UVR (7-80 mJ/cm^2, which is roughly equivalent to 11 mins 48 secs -1 hour 58 mins in Manchester summer sun) (figure 5.3). The UVR will be produced by the Waldmann UV 236B unit containing Waldmann CF-L 36 W/UV6 lamps (Waldmann GmbH, Villinge-Schwenningen, Germany). For each UVR dose, a 1*1cm diameter skin area will be exposed. All participants will be asked to protect the exposed skin areas from any UVR sources until the MED has been assessed.

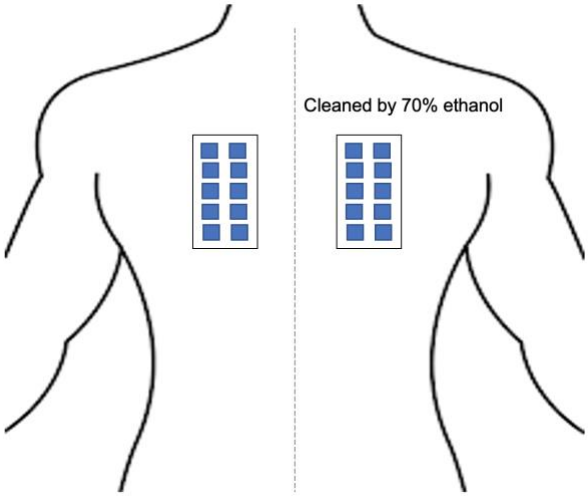

Figure 5.3 A diagrammatic drawing of the template for the MED test (drawn by Wen Duan)

The right side of mid-back skin was cleaned with 70% ethanol before UVR exposure. 10 x 1 cm^2 diameter patches were exposed to 7-80 mJ/cm^2 UVR respectively on the ethanol-cleaned and control sides of mid-back skin immediately after cleaning.

5.2.4 The assessment for the minimal erythema dose (MED)

The MED will be assessed 6 and 24 hours following UVR treatment using a handheld spectrophotometer ((Konica Minolta CM600d, NJ, USA). The redness (*a**) of the central area of each exposed patch and an adjacent unexposed area of the mid-back skin will be measured in triplicate. A 2.5-point difference in the *a** value between the unirradiated and irradiated skin indicates potential burning (Heckman et al. 2013). Any UVR doses that induce an *a** value of 2.5 points higher than that of the exposed skin is considered to have generated an erythema and the lowest one is defined as the MED. A figure showing the typical skin erythema response of MED testing post 24 hours of UVR exposure is presented in figure 5.4.

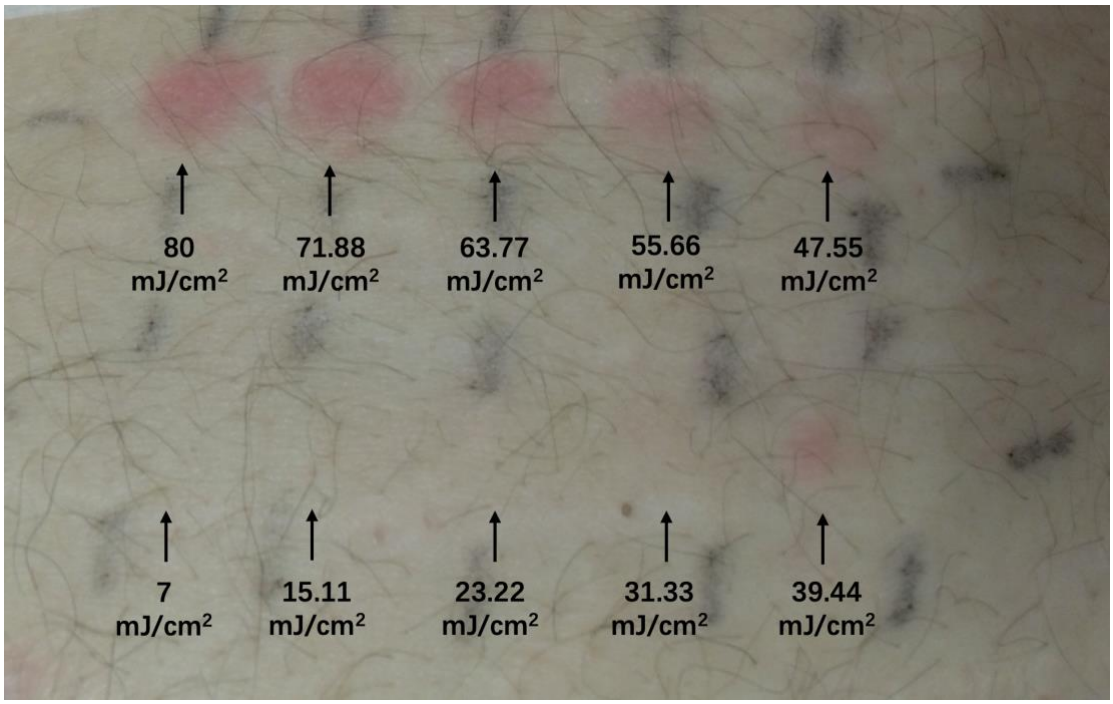

Figure 5.4 The typical skin erythema response of MED testing (provided by Dr. Mark Farrar)

10*1cm^2 area of the dorsal forearm of a healthy adult was exposed to 7-80 mJ/cm^2 doses of UVR individually. The MED was assessed after 24 hours of UVR exposure.

5.2.5 Statistics

Statistical analysis will be implemented employing the GraphPad Prism 7 (GraphPad Software, https://www.graphpad.com). MED between the skin areas with/without the colonisation of skin microbiota will be analysed using the unpaired student-t test. The *P* value < 0.05 is assigned to be statistically significant.

5.3 Discussion

The data generated from this proposed study can broaden the knowledge about the role of the normal skin microbiota in skin response to UVR stress. Recently, Patra and his colleagues have demonstrated the presence of skin microbiota can reduce the UVR-induced

immunosuppression in mice. Specifically, the colonisation of skin microbiota results in a pro-inflammatory condition in mice skin while the absence of skin microbiota leads to an anti-inflammatory milieu following the UVR exposure. The pro-inflammatory environment upon UVR in the presence of the microbiome was associated with the gene expression of cytokines involved in the induction of inflammation (Patra et al. 2019). Based on these data, we hypothesised that the colonization of skin by the microbiome would enhance the skin inflammation upon UVR exposure. In other words, an aggravated skin erythema would be observed in the uncleaned skin area following the treatment of a same dose of UVR. In addtion, as the skin erythema is proportionate to the cutaneous inflammation following UVR stress (Lisi Hruza and Pentland 1993), instead of using a single UVR dose, the usage of a series doses of UVR in this project would reflect the gradual variation of inflammation level in response to the different doses of UVR.

The primary deficiency of this pilot is that it will be difficult to exclude the effects from the re-established skin microbiota. It has been demonstrated that a healthy microbiome is relatively stable and can recover from a perturbation rapidly (Lloyd-Price et al. 2016; Oh et al. 2016). This is supported by our previous work (data is not published), which presented the re-establishment of skin microbiota post ethanol cleaning (figure 5.1). The forearm skin of healthy adults was wiped with 70% ethanol solution and the skin bacterial abundance was compared pre/post the perturbation. The data was analysed using quantitative PCR, showing the skin microbiota re-established in 6 hours following cleaning (figure 5.1). Although the return speed of skin microbiota on back skin has not been reported in the literature, a previous study has

quantified *C. acnes*, which is one of the most predominant bacteria on sebaceous skin regions (Grice et al. 2009). This study showed that *C. acnes* has fully returned in 6 hours after cleaning (Sfriso and Claypool 2020). Thus, it appears that the 6 hours window may be generally true across skin but remains to be firmly established.

Another deficiency is that the skin microbiota could not be removed completely using ethanol solution based on the data in figure 5.1. Thus, swabbing with ethanol reduces but does not entirely remove bacteria. It is possible that the surviving bacteria could interact with skin cells upon UVR exposure and then influence the formation of skin erythema. These are the problems faced when working with human systems *in vivo* instead of germ-free mouse models.

The proposed study was intended to be a pilot for a much more detailed study which has subsequently received funding from the BBSRC. In this study, the skin response to solar UVR will be compared with/without the presence of skin microbiota via comparing not only the erythema degree but also the skin DNA damage, keratinocyte apoptosis, proliferation, and inflammation in skin biopsies taken from irradiated sites with and without microbiome perturbation with ethanol. Specifically, the skin microbiota of the right side of the upper back skin will be wiped with 70% ethanol and then both sides of the upper back skin will be treated with a series of 10 doses of UVR (7-80 mJ/cm^2). 24 hours later, the MED will be assessed and then an unexposed region of the right side of the upper back will be cleaned with 70% ethanol again. The cleaned and the contralateral skin will be treated with an 80 mJ/cm^2 dose of UVR before 2 pieces of 5*5 mm^2 skin biopsies will be collected from the irradiated areas immediately (figure 5.5, marked with blue arrows). Another 4 punch specimens will also be collected: two

pieces from the unexposed skin areas (figure 5.5, marked with black arrows) and two pieces from the MED template area exposed to the 80 mJ/cm^2 dose of UVR (figure 5.5, marked with red arrows). These skin biopsies will be then subjected to immunostaining to compare the markers of skin DNA damages (cyclobutane pyrimidine dimers (CPDs)) (Shih et al. 2018), apoptosis (TUNEL assay, caspase 3) (Kyrylkova et al. 2012; Qin et al. 2002), inflammation (C-C Motif Chemokine Ligand 3 and interleukin 6) and proliferation (Ki67) (Ryser et al. 2014).

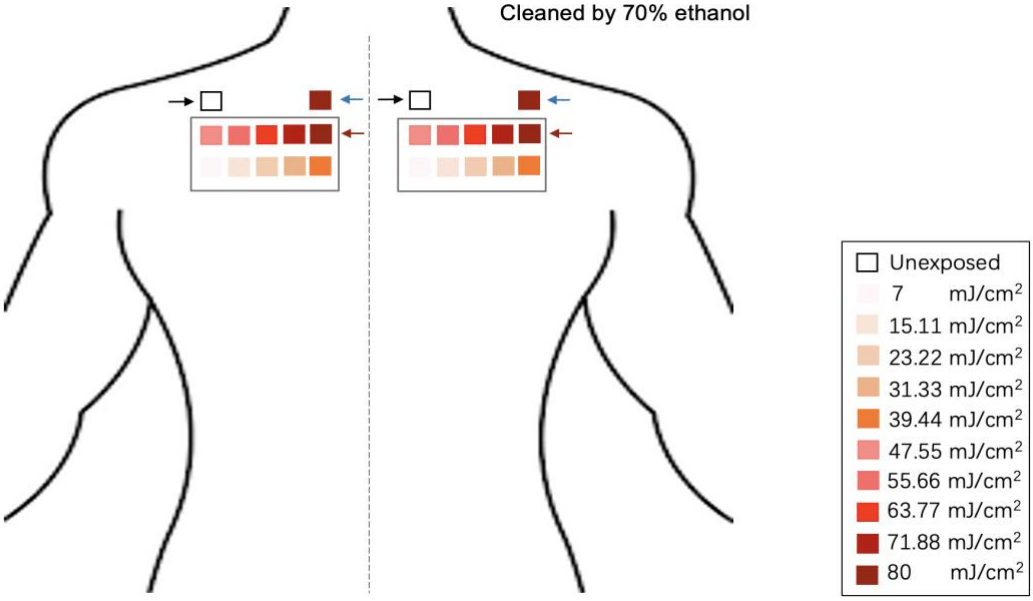

Figure 5.5 A diagrammatic drawing of the UVR treatment and the collection of skin biopsy (drawn by Wen Duan)

The right side of the upper back skin will be wiped using 70% ethanol and then both the clean and the uncleaned skin will be exposed to a series of 10 doses of UVR (7-80 mJ/cm^2). 24 hours later, an unexposed area of the right side of the upper back skin will be wiped again and UVR treatment (80 mJ/cm^2) will be conducted again on the clean and contralateral skin area. 5 mm^2 skin biopsies will be then collected from the regions labelled with arrows.

In summary, although future work is needed to address these questions described above, as one of the few *in vivo* studies that investigated the effects of skin microbiota on the skin upon environmental pressure, the successful conduction of this project could shed light on the role

of healthy skin microbiota on protecting skin from UVR stress.

Chapter 6 General discussion and future work

6.1 Main findings of this thesis

This work aimed to investigate the effects of UVR on host-microbiota interactions in a reductionist model of skin. To mimic the UVR present in the natural environment, simulated solar radiation (SSR) was used as the UVR source. Initially, the sensitivity of selected skin commensal bacteria to SSR was investigated, followed by a study into whether the presence of the commensal bacteria modulate NHEKs responses upon SSR exposure. The main findings of these studies are:

1) An *in vitro* model of NHEK monolayer colonised with a single strain of commensal skin bacterium are constructed, showing the adhesion of selected skin commensal bacteria to NHEKs is different. The adhesion of *S. capitis* is the greatest among all selected organisms while the adhesion of *M. luteus* and two strains of *S. epidermidis* (NCTC 10519 and 6513) are the lowest. The variation in the adherence to NHEKs is even presented between strains of *S. epidermidis.*
2) The SSR sensitivity of skin commensal bacteria is different. Among all tested species, *M. luteus* and *C. jeikeium* are the most tolerant to SSR exposure while *S. capitis* and *C. acnes* are the most SSR-sensitive organisms. To learn more about the possible mechanisms behind the different bacterial UVR sensitivity, the RecA protein content of each organism was then compared. RecA content of *M. luteus* increased following SSR exposure while an opposite trend was observed in the rest of the selected microbes. As the RecA variation post SSR exposure is different between bacteria, future studies are needed to investigate

whether the RecA protein regulates bacterial SSR sensitivity.

3) The colonization of NHEKs monolayers with a single strain of *S. hominis*, *S. capitis*, *C. acnes*, *M. luteus* and *C. jeikeium* did not influence NHEKs survival in response to SSR exposure. In contrast, the presence of *S. epidermidis* decreased NHEKs viability upon SSR treatment. This occurred with three different strains of *S. epidermidis* and may be a general phenomenon of this bacterium.

4) This effect of irradiated *S. epidermidis* on NHEKs could be due to the release of the keratinocyte-detrimental molecule(s) because the cell-free supernatant from irradiated *S. epidermidis* also reduced the viability of NHEKs by a mechanism which appeared to be necrosis.

5) The substance(s) released from the irradiated *S. epidermidis* was (were) sensitive to heat and trypsin treatment, indicating that the nature of this (these) molecule(s) could be protein. This hypothesis was supported by the fractionation data which showed the molecular weight of the factor produced from the irradiated *S. epidermidis* were between 50-100kDa.

6) A clinical study that aims to investigate the effect of skin microbiota on the minimal erythemal dose response in healthy humans is designed. Although it could not be conducted due to the influence of the COVID-19 pandemic, the ethics approval has been conferred by the ethics committee of the University of Manchester.

6.2 The mechanisms of bacterial survival upon UVR exposure

Among the many DNA repair systems in bacteria which could account for bacterial survival following SSR, RecA was selected as the subject of investigation as the RecA-associated DNA

repair pathways (SOS system and recombinational repair) are one of the most important mechanisms for bacterial DNA repair (Patel et al. 2010). However, as the expression of RecA varies between species following exposure to SSR, a clear conclusion cannot be drawn based on current data. Experiments focusing on the gene expression of RecA and other SOS regulators is needed to be conducted in the future to study the SOS response in skin commensals.

The RecA-regulated pathways are not the only strategy that bacteria use to repair DNA damage following UVR. Other systems such as photoreactivation, base excision repair, nucleotide excision repair, mutagenic repair, etc., are also vital mechanisms of bacterial DNA repair (Sinha and Häder 2002). These could be investigated in the future for a better understanding of how skin resident organisms survive UVR exposure. The key regulators of other bacterial DNA repair systems which could be used are listed in table 6.1 (Sinha and Häder 2002; Goosen and Moolenaar 2008). The biomarkers of photoreactivation and base excision repair may not be present in all skin organisms, and some of them are even species-unique (Goosen and Moolenaar 2008). Therefore, it could be challenging to directly investigate the effect of these two pathways on rescuing skin bacteria from UVR exposure. However, since no data is currently available as to what systems skin commensal bacteria utilize in order to protect themselves from UVR, this would be valuable addition to the literature.

Table 6.1 The biomarkers in different bacterial DNA repair systems

DNA repair systems	Biomarkers	Repair mechanisms
Photoreactivation	Photolyase: Phr protein Cry1 protein and Cry2 protein	Reverse CPDs and 6-4PPs
Base excision repair	DNA glycosylases	Remove the damaged bases
Nucleotide excision repair	UvrA, UvrB and UvrC	Remove CPDs and 6-4PPs
Recombinational repair	RecA	Repair ssDNA
SOS system	RecA, LexA	Upregulate the expression of genes that repair DNA damage

CPDs: cyclobutane pyrimidine dimers; 6-4PPs: 6-4 photoproducts; ssDNA: single stranded DNA

In addition to the above mechanisms by which microbes can increase survival rate upon UVR, some organisms could be intrinsically less sensitive to UVR depending on their chromophore content. Chromophores, are molecules that absorb UVR energy and then undergo chemical processes leading to biological responses (Young 1997). UVR chromophores are generally considered as photosensitisers due to their capacity to produce reactive oxygen species or other detrimental photoproducts by which biological damage can be induced (Wondrak et al. 2006). It has been demonstrated that various molecules in human skin can act as UVR chromophores. However, to the best of my knowledge, only limited bacterial UVR chromophores are reported. One of these molecules is porphyrin, which has been demonstrated in some skin resident bacteria (e.g. *C. acnes* and *Corynebacterium minutissimum*) (Shu et al. 2013). The presence of this substance in *C. acnes* could be one intrinsic factor for the high UVR sensitivity of this organism shown in this thesis.

Whether the low UVR tolerance in some skin organisms could be because of high chromophore content needs to be verified. Previous scholars have demonstrated that some amino acids,

including cysteine, histidine, phenylalanine, tryptophan, and tyrosine, can be regarded as UVR chromophores as they show the capacity to absorb solar UVR (Thurstan et al. 2012). Therefore, the UVR sensitivity could be positively correlated with the content of the photosensitive proteins, which contain a high percentage of the UVR absorbing amino acids. Hibbert and colleagues have bioinformatically analysed the UVR chromophore amino acids content of 244 human proteins and successfully identified the UVR chromophore rich proteins of the skin (Hibbert et al. 2015). A similar analysis could also be conducted for skin commensals: essential proteins (e.g., generally distributed in microbes) in skin commensals could be sequenced to identify the UVR chromophore rich proteins in bacteria. The content of these UVR-absorbing proteins could be then compared between organisms to investigate whether some skin resident organisms are intrinsically more UVR susceptible than the others.

6.3 The effects of the protein(s) produced by *S. epidermidis* following UVR exposure on the skin health

Few studies have focused on the interactions between skin and skin microbiota upon UVR stress. Our data have demonstrated that the presence of *S. epidermidis* reduces NHEKs viability upon UVR exposure probably via releasing of protein(s) from the bacterium that induced keratinocyte necrosis. The identification of the protein(s) released from irradiated *S. epidermidis* was begun, but not completed in this project. Specifically, the concentration of this(these) protein(s) was(were) too low for further identification. In future work, methods of increasing protein concentration could be applied, for example, protein precipitation (Jiang et al. 2004). The concentrated protein(s) then could be identified by mass spectrometry. The

success in identifying the detrimental protein(s) produced by the irradiated *S. epidermidis* could lead to a new explanation for the pathogenesis of UVR-related skin disorders, as this organism is widely distributed on the skin.

Keratinocyte apoptosis is the best characterized mechanism of cell death following UVR exposure and occurs in cells that are too severely damaged to repair. It is a part of the anti-tumor mechanism of the skin (Kulms and Schwarz 2000b). However, the data from the present study suggests that the reduction in NHEKs viability induced by irradiated *S. epidermidis* was due to necrosis which is generally considered a consequence of extreme stress (Kaczmarek et al. 2013). It has been reported previously that necrosis of keratinocytes can be induced by the viral infection (He et al. 2014) and high doses of UVB (Mammone et al. 2000). However, little knowledge could be found from literature about the keratinocyte necrosis caused by the products of bacteria. Necrosis especially in response to UVR is now recognized to be a regulated process in some instances and is subdivided into necroptosis and pyroptosis (Tang et al. 2021) ('Accidental' necrosis is also observed depending on the pattern of cell death). Each type of programmed necrosis is characterized by its own biomarkers which are outlined in table 6.2.

Table 6.2 The death regulatory biomarkers in different necrosis systems

necrosis systems	Death regulator biomarkers	References
Pyroptosis	Inflammatory caspase-1 or caspase -11	Shi et al. 2015 Kayagaki et al. 2015
Necroptosis	RIPK1 and RIPK3;	Dhuriya and Sharma 2018
Parthanatos	PARPs	Fatokun et al. 2014
Ferroptosis	GPX4	Yang et al. 2014
Oxytosis	12-LOX	Lewerenz et al. 2018
ETosis/NETosis	NADPH oxidase	Fuchs et al. 2007

RIPK: receptor-interacting protein kinase; PARPs: poly (ADP-ribose) polymerase proteins; GPX4: glutathione peroxidase 4; 12-LOX: 12-lipoxygenase; NADPH: nicotinamide adenine dinucleotide phosphate.

Although the effects of the irradiated *S. epidermidis*-induced necrosis on skin health are not clear now, this cell death could be beneficial in maintaining skin health (figure 6.1). It has been thoroughly documented that UVR is one of the most prevalent inducements associated with the generation of skin cancer. Skin cancer could be induced if the severely impaired cells escape apoptosis post UVR exposure (Armstrong and Kricker 2001). In this circumstance, eliminating malignant cells via necrosis could also act as a substitute pathway that protects skin against tumours. Moreover, as programmed necrosis can induce inflammation, the irradiated *S. epidermidis*-induced necrosis could also be crucial in defending against pathogens (Westman et al. 2020; Kim et al. 2019; Kaczmarek et al. 2013). This could be important as exposure to UVR induces immunosuppression in skin (Schwarz 2010). The antimicrobial function of programmed necrosis has been shown in several subtypes, including necroptosis, pyroptosis and ETosis. Necroptosis is crucial in anti-viral defence as one of the essential adaptor proteins of this necrosis RIPK3 can upgrade cytokine gene expression to kill viruses

(Nailwal and Chan 2019). Pyroptosis can either deconstruct the replication niche of invading microorganisms or render the pathogens susceptible to elimination by phagocytes (Jorgensen and Miao 2015). Additionally, microbes also can be trapped by pore-induced intracellular traps (PITs) during pyroptosis and then cleared by neutrophils (Jorgensen et al. 2016). The pathogen defence mechanism of ETosis is conceptually similar to PITs in pyroptosis. During ETosis, the dying cell releases extracellular traps (ETs), which consist of DNA combined with antimicrobial proteins, to 'grab' detrimental organisms and then inactivate pathogens (Guimarães-Costa et al. 2012). Furthermore, as UVR-induced immunosuppression is associated with the construction of skin malignant tumours (Katiyar 2007), the necrosis-mediated inflammation could also act to inhibit the development of skin cancer.

However, this necrosis-mediated inflammation could be a double-edged sword for human health, as programmed necrosis is also associated with the induction of several diseases (Kim et al. 2019). For instance, overactivated pyroptosis would lead to massive cell death, which is the pathogenesis of multi-organ failure, sepsis and shock (Jorgensen and Miao 2015). Moreover, if programmed necrosis cannot be effectively controlled, the continuously generated DAMPs would lead to autoimmune and chronic inflammatory disorders (Kim et al. 2019) (figure 6.1).

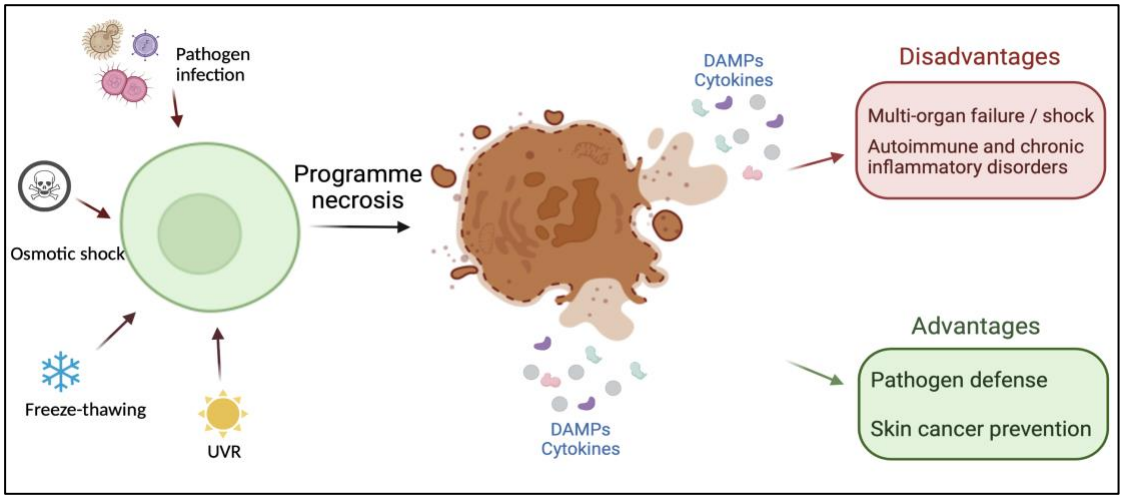

Figure 6.1 The induction of programmed necrosis and its effects on health (drawn by Wen Duan)

At present, it is unknown whether the effects of irradiated *S. epidermidis* on keratinocyte monolayers also occur in human skin. Firstly, unlike the HNEKs monolayer, the skin structure is more complicated. The skin barriers can protect skin from outside hazards via effectively blocking most harmful molecules (Bäsler et al. 2016). As it has been demonstrated that the tight junctions can prevent the entrance of substances of molecular weights >30 kDa (Yokouchi et al. 2015). Hence, the effect of the proteins(s) generated by *S. epidermidis* post UVR exposure on the skin could be restricted via tight junctions. Moreover, the environment on the skin surface where skin microbes are colonised is different from that in the model built herein: keratinocytes are terminally differentiated on the skin surface; the humidity is lower on the skin than in our model (Eckert 1989; Arda et al. 2014). Thus, it will be valuable to investigate whether the colonisation of *S. epidermidis* could influence the response of organ cultured human skin to UVR exposure. In our laboratory, the method of treating organ cultured human skin with bacterial suspensions has been well established. To answer the question raised above, a system of human skin tissue colonised with *S. epidermidis* could be exposed to SSR and then

the UVR-induced DNA lesions (e.g., CPDs) in skin sections could be compared between the samples with/without the colonisation of bacteria.

Additionally, although the NHEKs-bacterium model constructed herein is an effective method to screen the impact of a single skin organism on NHEKs in response to SSR, this model is oversimplified and does not reflect the effects of the entire skin microbiota on the skin upon UVR exposure. In the *in vivo* situation, the skin harbours numerous organisms. As the UVR protective property of skin commensal bacteria has been shown previously in several studies (Hofer et al. 2011; Hug et al. 1999; Wang et al. 2018; Nakatsuji et al. 2018), and thus, the detrimental effect from irradiated *S. epidermidis* may also be diminished/eliminated by the UVR-protective skin organisms. In future work, attention should be paid to identifying the potential for key UVR protective molecules produced by skin commensals. These could be used as novel therapies for UVR-associated skin conditions and sustainable and safe components for development of formulations of sun cream.

6.4 Conclusion

This project focuses on studying the effect of solar UVR on skin microbiota, and how in turn, the irradiated skin bacteria could influence skin health. The skin commensal organisms exhibited different UVR sensitivity. The presence of *S. epidermidis* affected the response of keratinocytes to solar UVR exposure due to the production of the protein(s) which appeared to induce necrosis. This work leads to a better understanding of the relationship between irradiated skin microbiota and skin health and paves the way for future research in this area.

List of References

Akiyama, T. et al. (2014). The human cathelicidin LL-37 host defense peptide upregulates tight junction-related proteins and increases human epidermal keratinocyte barrier function. *Journal of Innate Immunity*, 6(6), pp.739–753.

Aldag, C., Teixeira, D.N. and Leventhal, P.S. (2016). Skin rejuvenation using cosmetic products containing growth factors, cytokines, and matrikines : a review of the literature. *Clinical, Cosmetic and Investigational Dermatology*, 9, pp.411–419.

Alekseyenko, A. V et al. (2013). Community differentiation of the cutaneous microbiota in psoriasis. *microbiome*, 1(31), pp.1–17.

Alhasaniah, A. (2020). *Characterisation of claudin-1 interaction with ultraviolet radiation in human epidermal keratinocytes*.

Alitalo, K. et al. (1982). Extracellular matrix proteins of human epidermal keratinocytes and feeder 3T3 cells. *Journal of Cell Biology*, 94, pp.497–505.

Allen, T.D. and Potten, C.S. (1975). Desmosomal form, fate, and function in mammalian epidermis. *Journal of Ultrastruture Research*, 51(1), pp.94–105.

Anderson, J.M. and Itallie, C.M. Van. (2009). Physiology and function of the tight junction. *cold sping harbor perspectives in biology*, pp.1–16.

Arda, O., Göksügür, N. and Tüzün, Y. (2014). Basic histological structure and functions of facial skin. *Clinics in Dermatology*, 32, pp.3–13.

Armstrong, B.K. and Kricker, A. (2001). The epidemiology of UV induced skin cancer. *Journal of Photochemistry and Photobiology B: Biology*, 63(1–3), pp.8–18.

Arrange, A.A. et al. (1993). Bacterial sensitivity to UV light as a model for ionizing radiation resistance. *Journal of Microbiological Methods*, 18(2), pp.127–136.

Assefa, Z. et al. (2005). Ultraviolet radiation-induced apoptosis in keratinocytes: on the role of cytosolic factors. *Biochimica et Biophysica Acta - Reviews on Cancer*, 1755(2), pp.90–106.

Baharoglu, Z. and Mazel, D. (2014). SOS, the formidable strategy of bacteria against aggressions. *FEMS Microbiology Reviews*, 38(6), pp.1126–1145.

Balgir, P.P., Dhiman, S.R. and Kaur, P. (2016). In silico prediction and qPCR validation of novel sRNAs in Propionibacterium acnes KPA171202. *Journal of Genetic Engineering and Biotechnology*, 14, pp.169–176.

Barker, J.N.W.N. et al. (1991). Keratinocytes as initiators of inflammation. *The Lancet*, 337(8735), pp.211–214.

Baroni, A. et al. (2012). Structure and function of the epidermis related to barrier properties. *Clinics in Dermatology*, 30(3), pp.257–262.

Bäsler, K. et al. (2016). The role of tight junctions in skin barrier function and dermal absorption. *Journal of Controlled Release*, 242, pp.105–118.

Bek-Thomsen, M., Lomholt, H.B. and Kilian, M. (2008). Acne is not associated with yet-uncultured bacteria. *Journal of Clinical microbiology*, 46(10), pp.3355–3360.

Belkaid, Y. and Segre, J.A. (2014). Dialogue between skin microbiota and immunity. *Science*, 346(6212), pp.954–959.

Biniek, K., Levi, K. and Dauskardt, R.H. (2012). Solar UV radiation reduces the barrier function of human skin. *PNAS*, 109(42), pp.17111–17116.

Bomar, L. et al. (2016). Corynebacterium accolens releases antipneumococcal free fatty acids from human nostril and skin surface triacylglycerols. *mBio*, 7(1), pp.1–13.

Boyer, S.L., Flechtner, V.R. and Johansen, J.R. (2001). Is the 16S-23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and population genetics? A case study in Cyanobacteria. *Molecular biology and evolution*, 18(6), pp.1057–1069.

Braff, M.H., Nardo, A. Di and Gallo, R.L. (2005). Keratinocytes store the antimicrobial peptide cathelicidin in lamellar bodies. *Journal of Investigative Dermatology*, 124(2), pp.394–400.

Brandner, J.M. (2009). Tight junctions and tight junction proteins in mammalian epidermis. *European Journal of Pharmaceutics and Biopharmaceutics*, 72(2), pp.289–294.

Brenner, M. and Hearing, V.J. (2008). The protective role of melanin against UV damage in human skin. *Photochemistry and Photobiology*, 84(3), pp.539–549.

Brod, I. (1960). The Ultrostructure of the tonofibrils in the kerotinizotion process of normal humon epidermis. *Journal of Ultrastructure Research*, 4(3–4), pp.264–297.

Brogden, K.A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology*, 3, pp.238–250.

Burns, E.M. et al. (2019). Ultraviolet radiation, both UVA and UVB, influences the composition of the skin microbiome. *Experimental Dermatology*, 28(2), pp.136–141.

Byrd, A.L., Belkaid, Y. and Segre, J.A. (2018). The human skin microbiome. *Nature Reviews Microbiology*, 16(3), pp.143–155.

Candi, E., Schmidt, R. and Melino, G. (2005). The cornified envelope: a model of cell death in the skin. *Nature Reviews Molecular Cell Biology*, 6(4), pp.328–340.

Cantrell, A., Mcgarvey, D.J. and Truscott, T.G. (2001). Chapter 26 - Photochemical and photophysical properties of sunscreens. In Giacomoni, P.U. (editor). *Comprehensive Series in Photosciences*, Elsevier B.V., pp. 497-519.

Carrier, W.L. and Setlow, R.B. (1970). Endonuclease from Micrococcus luteus which has activity toward ultraviolet-irradiated deoxyribonucleic acid: purification and properties. *Journal of Bacteriology*, 102(1), pp.178–186.

Chandrasekar, V., Knabel, S.J. and Anantheswaran, R.C. (2015). Modeling development of inhibition zones in an agar diffusion bioassay. *Food Science and Nutrition*, 3(5), pp.394–403.

Chau, A. et al. (2008). Role of RecA in the protection of DNA damage by UV-A in Escherichia coli. *Journal of Experimental Microbiology and Immunology*, 12, pp.39–44.

Chen, Y.E. and Tsao, H. (2013). The skin microbiome: current perspectives and future challenges. *Journal of the American Academy of Dermatology*, 69(1), pp.143–55.

Cogen, A.L. et al. (2010). Selective antimicrobial action is provided by phenol-soluble modulins

derived from Staphylococcus epidermidis , a normal resident of the Skin. *Journal of Investigative Dermatology*, 130(1), pp.192–200.

Cohen, J.J. et al. (1992). Apoptosis and programmed cell death. *Annual Reviews Immunology*, 10, pp.267–293.

Cohen, P.R. (2007). Community-acquired methicillin-resistant Staphylococcus aureus skin infections: A review of epidemiology, clinical features, management, and prevention. *International Journal of Dermatology*, 46(2), pp1-11.

Cooper, K.D. et al. (1993). Neutrophils, differentiated macrophages, and monocyte/macrophage antigen presenting cells Infiltrate murine epidermis after UV injury. *Journal of Investigative Dermatology*, 101(2), pp.155–163.

Dawson, A.L. and Dellavalle, R.P. (2013). Acne vulgaris. *BMJ*, 8(346), pp.1–7.

Dehaven, C. et al. (2014). DNA photoprotection conveyed by sunscreen. *Journal of Cosmetic Dermatology*, 13(2), pp.99–102.

Dekio, I. et al. (2005). Detection of potentially novel bacterial components of the human skin microbiota using culture-independent molecular profiling. *Journal of Medical Microbiology*, 54, pp.1231–1238.

Dessinioti, C. and Katsambas, A.D. (2010). The role of Propionibacterium acnes in acne pathogenesis : facts and controversies. *Clinics in Dermatology*, 28(1), pp.2–7.

Dhuriya, Y.K. and Sharma, D. (2018). Necroptosis: a regulated inflammatory mode of cell death. *Journal of Neuroinflammation*, 15(199).

Diffey, B.L. (2002). What is light ? *Photodermatology Photoimmunology and Photomedicine*, 18(2), pp.68–74.

Dodds, D. et al. (2020). Controlling the growth of the skin commensal Staphylococcus epidermidis using D-Alanine auxotrophy. *mSphere*, 5(3), pp.1–13.

Eckert, R.L. (1989). Structure, function, and differentiation of the keratinocyte. *Physiological Reviews*, 69(4), pp.1316–1341.

Eyerich, S. et al. (2018). Cutaneous barriers and skin Immunity: differentiating a connected network. *Trends in Immunology*, 39(4), pp.315–327.

Ezzedine, K. et al. (2015). Vitiligo. *The Lancet*, 386(9988), pp.74–84.

Faergemann, J. and Larkö, O. (1987). The effect of UV-light on human skin microorganisms. *Acta dermato-venereologica*, 67(1), pp. 69–72.

Fatokun, A.A., Dawson, V.L. and Dawson, T.M. (2014). Parthanatos: Mitochondrial-linked mechanisms and therapeutic opportunities. *British Journal of Pharmacology*, 171(8), pp.2000–2016.

Fierer, N. et al. (2008). The influence of sex , handedness , and washing on the diversity of hand surface bacteria. *Proceedings of the National Academy of Science*, 105(46), pp.17994–17999.

Fink, S.L. and Cookson, B.T. (2005). Apoptosis, pyroptosis, and necrosis: Mechanistic description of dead and dying eukaryotic cells. *Infection and Immunity*, 73(4), pp.1907–1916.

Fisher, G.J. et al. (2002). Mechanisms of photoaging and chronological skin aging. *Archives of Dermatology*, 138(11), pp.1462–1470.

Fisher, G.J. et al. (1997). Pathophysiology of premature skin aging induced by ultraviolet light. *The New England Lournal of Medicine*, 337(20), pp.1419–1429.

Fitz-gibbon, S. et al. (2013). Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *Journal of Investigative Dermatology*, 133(9), pp.2152–2160.

Fitzpatrick, T.B. (1988). The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatology*, 124(6), pp.869–871.

Foster, T.J. (2020). Surface proteins of Staphylococcus epidermidis. *Frontiers in Microbiology*, 11(1–10).

Fournière, M. et al. (2020). Staphylococcus epidermidis and Cutibacterium acnes: Two major sentinels of skin microbiota and the influence of cosmetics. *Microorganisms*, 8(11), pp.1–31.

Franco-Duarte, R. et al. (2019). Advances in chemical and biological methods to identify microorganisms—from past to present. *Microorganisms*, 7(130), pp.1–32.

Frohm, M. et al. (1997). The Expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *The Journal of Biological Chemistry*, 272(24), pp.15258–15263.

Fuchs, E. (1995). Keratins and the skin. *Annual Review of Cell and Developmental Biology*, 11, pp.123–153.

Fuchs, T.A. et al. (2007). Novel cell death program leads to neutrophil extracellular traps. *Journal of Cell Biology*, 176(2), pp.231–241.

Furuse, M. et al. (2002). Claudin-based tight junctions are crucial for the mammalian epidermal barrier : a lesson from claudin-1 – deficient mice. *The Journal of Cell Biology*, 156(6), pp.1099–1111.

Gallo, R.L. and Nizet, V. (2003). Endogenous production of antimicrobial peptides in innate immunity and human disease. *Current Allergy and Asthma Report*, 3, pp.402–409.

Gao, Z. et al. (2008). Substantial alterations of the cutaneous bacterial biota in psoriatic lesions. *PLoS ONE*, 3(7), pp.1–9.

Garnacho-Saucedo, G., Salido-Vallejo, R. and Moreno-Giménez, J.C. (2013). Atopic dermatitis : Update and proposed management algorithm. *Actas Dermo-Sifiliograficas*, 104(1), pp.4–16.

Garrod, D. and Chidgey, M. (2008). Desmosome structure, composition and function. *Biochimica et Biophysica Acta - Biomembranes*, 1778(3), pp.572–587.

Gascón, J. et al. (1995). Sensitivity of selected bacterial species to UV radiation. *Current Microbiology*, 30(3), pp.177–182.

Gilchrest, B.A. and Eller, M.S. (1999). DNA photodamage stimulates melanogenesis and other photoprotective responses. *Journal of Investigative Dermatology Symposium Proceedings*, 4(1), pp.35–40.

Gläser, R. et al. (2009). UV-B radiation induces the expression of antimicrobial peptides in human keratinocytes in vitro and in vivo. *Journal of Allergy and Clinical Immunology*, 123(5), pp.1117–1123.

Gomes, A. V. et al. (2012). Upregulation of proteasome activity in muscle RING finger 1-null mice following denervation. *The FASEB Journal*, 26(7), pp.2986–2999.

Goodsell, D.S. (2001). The molecular perspective: ultraviolet light and pyrimidine dimers. *Stem Cells*, 19, pp.348–349.

Goosen, N. and Moolenaar, G.F. (2008). Repair of UV damage in bacteria. *DNA Repair*, 7(3), pp.353–379.

Grange, P.A. et al. (2017). Characterization of a Propionibacterium acnes surface protein as a fibrinogen-binding protein. *Scientific Reports*, 7(6428), pp.1–14.

Greinert, R. et al. (2015). European code against cancer 4th edition: Ultraviolet radiation and cancer. *Cancer epidemiology*, 39 Suppl 1, pp.S75–S83.

Grice, E.A. et al. (2008). A diversity profile of the human skin microbiota. *Genome Research*, 18, pp.1043–1050.

Grice, E.A. et al. (2009). Topographical and Temporal Diversity of the Human Skin Microbiome. *Science*. 324(5931), pp.1190–1192.

Grice, E.A. and Segre, J.A. (2011). The skin microbiome. *Nature Reviews Microbiology*, 9(4), pp.244–253.

Gruijl, F.R. De. (1999). Skin cancer and solar UV radiation. *European Journal of Cancer*, 35(14), pp.2003–2009.

Guimarães-Costa, A.B. et al. (2012). ETosis: A microbicidal mechanism beyond cell death. *Journal of Parasitology Research*, 2012(929743).

Haftek, M. (2015). Epidermal barrier disorders and corneodesmosome defects. *Cell and Tissue Research*, 360(3), pp.483–490.

Halliday, G.M. et al. (2012). The suppressive effects of ultraviolet radiation on immunity in the skin and internal organs : Implications for autoimmunity. *Journal of Dermatological Science*, 66(3), pp.176–182.

Halliday, G.M. and Byrne, S.N. (2014). An unexpected role : UVA-Induced release of nitric oxide from skin may have unexpected health benefits. *Journal of Investigative Dermatology*, 134(7), pp.1791–1794.

Hannigan, G.D. and Grice, E.A. (2013). Microbial ecology of the skin in the era of metagenomics and molecular microbiology. *Cold Spring Harb Perspect Medicine*, 3(12), pp.1–15.

Harder, J., Schröder, J.M. and Gläser, R. (2013). The skin surface as antimicrobial barrier: Present concepts and future outlooks. *Experimental Dermatology*, 22(1), pp.1–5.

Harding, C. and Rawlings, A. (2000). Effects of natural moisturizing factor and lactic acid isomers on skin function. *Dry Skin and Moisturizers: Chemistry and Function (2nd ed).* CRC Press, pp. 187–209.

Harrison, G.I. and Young, A.R. (2002). Ultraviolet radiation-induced erythema in human skin. *Methods*, 28(1), pp.14–19.

Hart, P.H., Grimbaldeston, M.A. and Finlay-jones, J.J. (2001). Sunlight, immunosuppression and skin cancer: role of histamine and mast cells. *Clinical and Experimental Pharmacology and Pthsiology*, 28(1–2), pp.1–8.

Hattori, F. et al. (2014). The antimicrobial protein S100A7 / psoriasin enhances the expression of keratinocyte differentiation markers and strengthens the skin' s tight junction barrier. *British Journal of Dermatology*, 171(4), pp.742–753.

Hawk, J. L. M., Young, A. R. and Ferguson, J. (2010). Cutaneous photobiology. *Rook's Textbook of Dermatology*, 1, pp.1-24.

He, Y. et al. (2014). Vaccinia virus induces rapid necrosis in keratinocytes by a STAT3-dependent mechanism. *PLoS ONE*, 9(11), p.e113690.

Heckman, C.J. et al. (2013). Minimal erythema dose (MED) testing. *Journal of visualized experiments : JoVE*, (75), pp.1–5.

Hibbert, S.A. et al. (2015). A potential role for endogenous proteins as sacrificial sunscreens and antioxidants in human tissues. *Redox Biology*, 5, pp.101–113.

Hofer, A. et al. (2011). Topical liposomal DNA-repair enzymes in polymorphic light eruption. *Photochemical and Photobiological Sciences*, 10(7), pp.1118–1128.

Höfling, J.F. et al. (1997). New strategies on molecular biology applied to microbial systematics. *Rev. Inst. Med. Trop. Sao Paulo*, 39(6), pp.345–352.

Holick, M.F. (2006). Resurrection of vitamin D deficiency and rickets. *Journal of Clinical Investigation*, 116(8), pp.2062–2072.

Holick, M.F. (2010). Vitamin D status: measurement, interpretation, and clinical application. *Annals of Epidemiology*, 19(2), pp.7–78.

Hug, D.H., Dunkerson, D.D. and Hunter, J.K. (1999). The degradation of L-histidine and trans- and cis-urocanic acid by bacteria from skin and the role of bacterial cis-urocanic acid isomerase. *Journal of Photochemistry and Photobiology B: Biology*, 50(1), pp.66–73.

Mackenzie, I.C. (1975). Ordered structure of the epidermis. *The Journal of Investigative Dermatology*, 65(1), pp.45–51.

Igyártó, B.Z. and Kaplan, D.H. (2013). Antigen presentation by Langerhans cells. *Current Opinion in Immunology*, 25, pp.115–119.

Im, A.-R. et al. (2016). Protective effect of fermented Cyclopia intermedia against UVB-induced damage in HaCaT human keratinocytes. *BMC Complementary and Alternative Medicine*, 16(261), pp.1–10.

Iwase, T. et al. (2010). Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization. *Nature*, 465(7296), pp.346–349.

Jaishankar, J. and Srivastava, P. (2017). Molecular basis of stationary phase survival and applications. *Frontiers in Microbiology*, 8, pp.1–12.

Janda, J.M. and Abbott, S.L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *Journal of Clinical Microbiology*, 45(9),

pp.2761–2764.

Jannuzzi, D. et al. (2007). Activity of staphylococcal bacteriocins against Staphylococcus aureus and Streptococcus agalactiae involved in bovine mastitis. *Research in Microbiology*, 158(7), pp.625–630.

Jeremy, A.H.T. et al. (2003). Inflammatory events are involved in acne lesion initiation. *Journal of Investigative Dermatology*, 121(1), pp.20–27.

Jiang, L., He, L. and Fountoulakis, M. (2004). Comparison of protein precipitation methods for sample preparation prior to proteomic analysis. *Journal of Chromatography A*, 1023(2), pp.317–320.

Jorgensen, I. et al. (2016). Pyroptosis triggers pore-induced intracellular traps (PITs) that capture bacteria and lead to their clearance by efferocytosis. *Journal of Experimental Medicine*, 213(10), pp.2113–2128.

Jorgensen, I. and Miao, E.A. (2015). Pyroptotic cell death defends against intracellular pathogens. *Immunol Review*, 265(1), pp.130–142.

Jouault, T. et al. (2009). Host responses to a versatile commensal : PAMPs and PRRs interplay leading to tolerance or infection by Candida albicans. *Cellular Microbiology*, 11(7), pp.1007–1015.

JunSeo, S. et al. (2001). Expressions of β-defensins in human keratinocyte cell lines. *Journal of Dermatological Science*, 27(3), pp.183–191.

Juzeniene, A. and Moan, J. (2012). Beneficial effects of UV radiation other than via vitamin D production. *Dermato-Endocrinology*, 4(2), pp.109–117.

Kaczmarek, A., Vandenabeele, P. and Krysko, D. V. (2013). Necroptosis: The release of damage-associated molecular patterns and its physiological relevance. *Immunity*, 38(2), pp.209–223.

Kambayashi, H. et al. (2003). Involvement of changes in stratum corneum keratin in wrinkle formation by chronic ultraviolet irradiation in hairless mice. *Experimental dermatology*, 12 Suppl 2(10), pp.22–27.

Katiyar, S.K. (2007). UV-induced immune suppression and photocarcinogenesis: Chemoprevention by dietary botanical agents. *Cancer Letters*, 255(1), pp.1–11.

Kayagaki, N. et al. (2015). Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. *Nature*, 526, pp.666–671.

Kim, E.H., Wong, S.W. and Martinez, J. (2019). Programmed necrosis and disease: We interrupt your regular programming to bring you necroinflammation. *Cell Death and Differentiation*, 26(1), pp.25–40.

Kim, H.J. et al. (2019). Segregation of age-related skin microbiome characteristics by functionality. *Scientific Reports*, 9(16748), pp.1–11.

Kim, J. (2005). Review of the innate immune response in acne vulgaris: Activation of Toll-Like Receptor 2 in Acne Triggers Inflammatory Cytokine Responses. *Dermatology*, 211(3), pp.193–198.

Kirschner, N. et al. (2011). Tight junctions and differentiation – a chicken or the egg question ? *Experimental Dermatology*, 21(3), pp.171–175.

Kloos, W.E. and Musselwhite, M.S. (1975). Distribution and persistence of staphylococcus and micrococcus species and other aerobic bacteria on human skin. *Applied microbiology*, 30(3), pp.381–5.

Kolter, R., Siegele, D.A. and Tormo, A. (1993). The stationary phase of the bacterial life cycle. *Annual Review of Microbiology*, 47, pp.855–874.

Komatsu, N. et al. (2005). Quantification of human tissue kallikreins in the stratum corneum : Dependence on age and gender. *Journal of Investigative Dermatology*, 125(6), pp.1182–1189.

Kong, H.H. et al. (2012). Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Research*, 22(5), pp.850–859.

Korting, H.C. et al. (2012). Antimicrobial peptides and skin : A paradigm of translational medicine. *Skin Pharmacol and Physiology*, 25(6), pp.323–334.

Kou, J., Dou, D. and Yang, L. (2017). Porphyrin photosensitizers in photodynamic therapy and its applications. *Oncotarget*, 8(46), pp.81591–81603.

Krueger, B.J.G. et al. (1995). Successful ultraviolet B treatment of psoriasis is accompanied by a reversal of keratinocyte pathology and by selective depletion of intraepidermal T cells. *The Journal of Experimental Medicine*, 182(6), pp.2057–2068.

Kubo, A. et al. (2009). External antigen uptake by Langerhans cells with reorganization of epidermal tight junction barriers. *The Journal of Experimental Medicine*, 206(13), pp.2937–2946.

Kubo, A., Nagao, K. and Amagai, M. (2012). Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *The Journal of Clinical Investigation*, 122(2), pp.440–447.

Kulms, D. and Schwarz, T. (2000). Molecular mechanisms of UV-induced apoptosis. *Photodermatology Photoimmunology and Photomedicine*, 16(7), pp.195–201.

Kwon, H.H. et al. (2013). Analysis of distribution patterns of Propionibacterium acnes phylotypes and Peptostreptococcus species from acne lesions. *British Journal of Dermatology*, 169(5), pp.1152–1155.

Kyrylkova, K. et al. (2012). Detection of apoptosis by TUNEL assay. *Odontogenesis: Methods and Protocols*. Totowa, NJ: Humana Press, pp. 41–47.

Lai-cheong, J.E. (2017). Structure and function of skin , hair and nails key points. *Medicine*, 45(6), pp.347–351.

Lai, Y. et al. (2010). Activation of TLR2 by a small molecule produced by Staphylococcus epidermidis increases antimicrobial defense against bacterial skin infections. *Journal of Investigative Dermatology*, 130(9), pp.2211–2221.

Lai, Y. et al. (2009). Commensal bacteria regulate TLR3-dependent inflammation following skin injury. *Nature Medicine*, 15(12), pp.1377–1382.

Lambers, H. et al. (2006). Natural skin surface pH is on average below 5 , which is beneficial for its resident flora. *International Journal of Cosmetic Science*, 28(5), pp.359–370.

Lee, C.H. et al. (2013). Molecular mechanisms of UV-induced apoptosis and its effects on skin residential cells: The implication in UV-based phototherapy. *International Journal of Molecular Sciences*, 14(3), pp.6414–6435.

Lee, S.H., Jeong, S.K. and Ahn, S.K. (2006). An update of the defensive barrier function of skin. *Yonsei Medical Journal*, 47(3), pp.293–306.

Lembo, S. and Raimondo, A. (2018). Polymorphic light eruption: What's new in pathogenesis and management. *Frontiers in Medicine*, 5.252.

Lewerenz, J. et al. (2018). Oxytosis/ferroptosis-(Re-) emerging roles for oxidative stress-dependent non-apoptotic cell death in diseases of the central nervous system. *Frontiers in Neuroscience*, 12:214.

Leydcn, J.J. et al. (1987). Skin Microflora. *Journal of Investigative Dermatology*, 88(3), pp.65–72.

Leyden, J.J., Marples, R.R. and Kligman, A.M. (1974). Staphylococcus aureus in the lesions of atopic dermatitis. *British Journal of Dermatology*, 90(5), pp.525–530.

Li, M. et al. (2019). The influence of age, gender and race/ethnicity on the composition of the human axillary microbiome. *International Journal of Cosmetic Science*, 41(4), pp.371–377.

Lisi Hruza, L. and Pentland, A.P. (1993). Mechanisms of UV-induced inflammation. *Journal of Investigative Dermatology*, 100(1), pp.S35–S41.

Little, J.W. and Mount, D.W. (1982). The SOS regulatory system of Escherichia coli. *Cell*, 29(1), pp.11–22.

Liu, Y. et al. (2019). RIP1/RIP3-regulated necroptosis as a target for multifaceted disease therapy (Review). *International Journal of Molecular Medicine*, 44(3), pp.771–786.

Liu, Yao et al. (2020). Skin microbiota analysis-inspired development of novel anti-infectives. *Microbiome*, 8(85), pp.1–19.

Lloyd-Price, J., Abu-Ali, G. and Huttenhower, C. (2016). The healthy human microbiome. *Genome Medicine*, 8(1), pp.1–11.

Lood, R. and Collin, M. (2011). Characterization and genome sequencing of two Propionibacterium acnes phages displaying pseudolysogeny. *BMC Genomics*, 12(198), pp.1–14.

Maclean, M. et al. (2009). Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array. *Applied and Environmental Microbiology*, 75(7), pp.1932–1937.

Madison, K.C. (2003). Barrier Function of the Skin: "La Raison d'Être" of the Epidermis. *Journal of Investigative Dermatology*, 121(2), pp.231–241.

Mahler, I., Kushner, S.R. and Grossman, L. (1971). In vivo role of the UV-endonuclease from Micrococcus luteus in the repair of DNA. *Nature New Biology*, 234, pp.47–50.

Maier, R.M. (2000). Chapter 3 - Bacterial Growth. *Environmental Microbiology (Second Edition)*. pp. 37–54.

Malissen, B., Tamoutounour, S. and Henri, S. (2014). The origins and functions of dendritic cells and macrophages in the skin. *Nature Reviews Immunology*, 14(6), pp.417–428.

Mammone, T. et al. (2000). Successful separation of apoptosis and necrosis pathways in HaCaT keratinocyte cells induced by UVB irradiation. *Cell Biology and Toxicology*, 16(5), pp.293–302.

Mańkowska-wierzbicka, D. et al. (2015). The microbiome and dermatological diseases. *The microbiome and dermatological diseases*, 69, pp.978–985.

Marples, R.R. (1982). Sex , constancy , and skin bacteria. *Archives of Dermatological Research*, 272(3–4), pp.317–320.

Maslowska, K.H., Makiela-Dzbenska, K. and Fijalkowska, I.J. (2019). The SOS system: A complex and tightly regulated response to DNA damage. *Environmental and Molecular Mutagenesis*, 60(4), pp.368–384.

Matsui, T. and Amagai, M. (2015). Dissecting the formation, structure and barrier function of the stratum corneum. *International Immunology*, 27(6), pp.269–280.

Mayser, P. et al. (2002). Pityriacitrin - an ultraviolet-absorbing indole alkaloid from the yeast Malassezia furfur. *Archives of dermatological research*, 294(3), pp.131–134.

Mayser, P. and Pape, B. (1998). Decreased susceptibility of Malassezia furfur to UV light by synthesis of tryptophane derivatives. *Antonie Van Leeuwenhoek*, 73(4), pp.315–319.

Mayslich, C., Grange, P.A. and Dupin, N. (2021). Cutibacterium acnes as an opportunistic pathogen: An update of its virulence-associated factors. *Microorganisms*, 9(303), pp.1–21.

Meguro, S. et al. (1999). Stratum corneum lipid abnormalities in UVB-irradiated skin. *Photochemistry and photobiology*, 69(3), pp.317–321.

Meisel, J.S. et al. (2018). Commensal microbiota modulate gene expression in the skin. *Microbiome*, 6(20), pp.1–15.

Merad, M., Ginhoux, F. and Collin, M. (2008). Origin , homeostasis and function of Langerhans cells and other langerin- expressing dendritic cells. *Nature Reviews Immunology*, 8(12), pp.935–947.

Milstone, L.M. (2004). Epidermal desquamation. *Journal of Dermatological Science*, 36(3), pp.131–140.

Misery, L. and Dezutter-dambuyant, C. (1995). Precursors of Langerhans cells. *Journal of the European Academy of Dermatology and Venereology*, 5(2), pp.124–131.

Mohana, D.C., Thippeswamy, S. and Abhishek, R.U. (2013). Antioxidant, antibacterial, and ultraviolet-protective properties of carotenoids isolated from Micrococcus spp. *Radiation Protection and Environment*, 36(4), pp.168–174.

Moravcová, M. et al. (2013). Modulation of keratin 1, 10 and involucrin expression as part of the complex response of the human keratinocyte cell line HaCaT to ultraviolet radiation. *Interdisciplinary Toxicology*, 6(4), pp.203–208.

Moreci, R.S. and Lechler, T. (2020). Epidermal structure and differentiation. *Current Biology*, 30(4), pp.R135–R158.

Murakami, M. et al. (2002). Cathelicidin anti-microbial peptide expression in sweat: An innate defense system for the skin. *Journal of Investigative Dermatology*, 119(5), pp.1090–1095.

Murdoch, L.E. et al. (2011). Bactericidal effects of 405nm light exposure demonstrated by inactivation of escherichia, salmonella, Shigella, Listeria, and mycobacterium species in liquid suspensions and on exposed surfaces. *The Scientific World Journal*, 2012, pp.1–8.

Naik, S. et al. (2012). Compartmentalized control of skin immunity by resident commensals.

Science, 337(6098), pp.1115–1120.

Nailwal, H. and Chan, F.K.M. (2019). Necroptosis in anti-viral inflammation. *Cell Death and Differentiation*, 26, pp.4–13.

Nakatsuji, T. et al. (2018). A commensal strain of Staphylococcus epidermidis protects against skin neoplasia. *Science Advances*, 4(2).

Nakayama, H., Shiota, S. and Umezu, K. (1992). UV endonuclease-mediated enhancement of UV survival in Micrococcus luteus: Evidence revealed by deficiency in the Uvr homolog. *Mutation Research-DNA Repair*, 273(1), pp.43–48.

Narayanan, D.L., Saladi, R.N. and Fox, J.L. (2010). Ultraviolet radiation and skin cancer. *International Journal of Dermatology*, 49(9), pp.978–986.

Nemes, Z. and Steinert, P.M. (1999). Bricks and mortar of the epidermal barrier. *Experimental and Molecular Medicine*, 31(1), pp.5–19.

Neves-Petersen, M.T., Gajula, G.P. and Petersen, S.B. (2012). UV light effects on proteins: From photochemistry to nanomedicine. *Molecular Photochemistry - Various Aspects*. pp. 125–158.

Nguyen, L.T., Haney, E.F. and Vogel, H.J. (2011). The expanding scope of antimicrobial peptide structures and their modes of action. *Trends in Biotechnology*, 29(9), pp.464–472.

Nishigori, C. et al. (1996). Evidence that DNA damage triggers interleukin 10 cytokine production in UV-irradiated murine keratinocytes. *Proceedings of the National Academy of Science*, 93(19), pp.10354–10359.

Niyonsaba F. et al. (2009). Multifunctional antimicrobial proteins and peptides: Natural activators of immune systems. *Current Pharmaceutical Design*, 15(21), pp.2393-2413.

Noble, C. and Pitcher, D.G. (1978). Microbial ecology of the human skin. *Advances in Microbial Ecology*. pp. 245–289.

Nomura, I. et al. (2003). Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis : A gene microarray analysis. *Journal of Allergy and Clinical Immunology*, 112(6), pp.1195–1202.

Noonan, F.P. et al. (1984). Mechanism of systemic immune suppression by UV irradiation in vivo . II . The UV effects on number and morphology of epidermal Langerhans cells and the UV-induced suppression of contact hypersensitivity have different wavelength dependencies. *The Journal of Immunology*, 132(5), pp.2408–2416.

Oh, J. et al. (2014). Biogeography and individuality shape function in the human skin metagenome. *Nature*, 514(7520), pp.59-64.

Oh, J. et al. (2016). Temporal stability of the human skin microbiome. *Cell*, 165(4), pp.854–866.

Ohnemus, U. et al. (2008). Regulation of epidermal tight-junctions (TJ) during infection with exfoliative toxin-negative staphylococcus strains. *Journal of Investigative Dermatology*, 128(4), pp.906–916.

Orazio, J.D. et al. (2013). UV radiation and the skin. *International Journal of Molecular Sciences*, 14(6), pp.12222–12248.

Ou, J. et al. (2004). Stationary phase protein overproduction is a fundamental capability of

Escherichia coli. *Biochemical and Biophysical Research Communications*, 314, pp.174–180.

Ozawa, M. et al. (1999). 312-nanometer Ultraviolet B light (narrow-band UVB) induces apoptosis of T Cells within psoriatic lesions. *The Journal of Experimental Medicine*, 189(4), pp.711–718.

Parra, J.L. and Paye, M. (2003). EEMCO guidance for the in vivo assessment of skin surface pH. *Skin Pharmacology and Applied Skin Physiology*, 16, pp.188–202.

Patel, M. et al. (2010). A new model for SOS-induced mutagenesis: How RecA protein activates DNA polymerase V. *Critical Reviews in Biochemistry and Molecular Biology*, 45(3), pp.171–184.

Patra, V.K. et al. (2019). Skin microbiome modulates the effect of ultraviolet radiation on cellular response and immune function. *iScience*, 15, pp.211–222.

Patra, V.K., Byrne, S.N. and Wolf, P. (2016). The skin microbiome: Is it affected by UV-induced immune suppression? *Frontiers in Microbiology*, 7:1235.

Patra, V.K. and Wolf, P. (2016). Microbial elements as the initial triggers in the pathogenesis of polymorphic light eruption? *Experimental Dermatology*, 25(12), pp.999–1001.

Pearse, A.D., Gaskell, S.A. and Marks, R. (1987). Epidermal changes in human skin following irradiation with either UVB or UVA. *Journal of Investigative Dermatology*, 88(1), pp.83–87.

Qin, J.Z. et al. (2002). Regulation of apoptosis by p53 in UV-irradiated human epidermis, psoriatic plaques and senescent keratinocytes. *Oncogene*, 21(19), pp.2991–3002.

Raffray, M. and Cohen, G.M. (1997). Apoptosis and necrosis in toxicology: A continuum or distinct modes of cell death? *Pharmacology and Therapeutics*, 75(3), pp.153–177.

Rastogi, R.P. et al. (2010). Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. *Journal of Nucleic Acids*, 2010:592980.

Ravanat, J., Douki, T. and Cadet, J. (2001). Direct and indirect effects of UV radiation on DNA and its components. *Journal of Photochemistry and Photobiology B: Biology*, 63(1–3), pp.88–102.

Reddy, K.V.R., Yedery, R.D. and Aranha, C. (2004). Antimicrobial peptides : premises and promises. *International Journal of Antimicrobial Agents*, 24, pp.536–547.

Ribeiro, D.T. et al. (1992). Singlet oxygen induced DNA damage and mutagenicity in a single-stranded SV40-based shuttle vector. *Photochemistry and photobiology*, 55(1), pp.39–45.

Robert, D. (2015). Ultraviolet radiation therapy and UVR dose models. *The International Journal of Medical Physica Research and Practice*, 42(1), pp.440–455.

Romero-Steiner, S., Witek, T. and Balish, E. (1990). Adherence of skin bacteria to human epithelial cells. *Journal of Clinical Microbiology*, 28(1), pp.27–31.

Rosenthal, M. et al. (2011). Skin microbiota microbial community structure and its potential association with health and disease. *Infection, Genetics and Evolution*, 11(5), pp.839–848.

Roth, R.R. and James, W.D. (1988). Microbial ecology of the skin. *Annual Reviews Microbiology*, 42, pp.441–464.

Ryser, S. et al. (2014). UVB-induced skin inflammation and cutaneous tissue injury is dependent on the MHC class I-like protein, CD1d. *Journal of Investigative Dermatology*, 134(1), pp.192–202.

Salipante, S.J. et al. (2014). Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. *Applied and Environmental Microbiology*, 80(24), pp.7583–7591.

Salles, B. and Paoletti, C. (1983). Control of UV induction of recA protein. *Proceedings of the National Academy of Sciences of the United States of America*, 80(1), pp.65–69.

Sandilands, A. et al. (2009). Filaggrin in the frontline: Role in skin barrier function and disease. *Journal of Cell Science*, 122(9), pp.1285–1294.

Sanford, J.A. and Gallo, R.L. (2013). Functions of the skin microbiota in health and disease. *Seminars in Immunology*, 25(5), pp.370–377.

SanMiguel, A.J. et al. (2017). Topical antimicrobial treatments can elicit shifts to resident skin bacterial communities and reduce colonization by Staphylococcus aureus competitors. *Antimicrobial agents and chemotherapy*, 61(9):e00774-17.

Scaffidi, P., Misteli, T. and Bianchi, M.E. (2002). Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature*, 418, pp.191–195.

Schleifer, K H and Kloos, W.E. (1975). Isolation and characterization of staphylococci from human skin. I. Amended descriptions of Staphylococcus epidermidis and Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus. *International Journal of Systematic Bacteriology*, 25(1), pp.50–61.

Schleifer, K. H. and Kloos, W.E. (1975). Isolation and characterization of staphylococci from human skin II. descriptions of four new species: Staphylococcus warneri, Staphylococcus capitis, Staphylococcus horminis, and Staphylococcus simulans. *International Journal of Systematic Bacteriology*, 25(1), pp.62–79.

Schmitt, D.A. and Ullrich, S.E. (2000). Exposure to ultraviolet radiation causes dendritic cells/ macrophages to secrete immune-suppressive IL-12p40 homodimers. *The Journal of Immunology*, 165(6), pp.3162–3167.

Schön, M.P. and Boehncke, W.-H. (2005). Psoriasis. *The New England Lournal of Medicine*, 352(18), pp.1899–1912.

Schwarz, T. (2010). The Dark and the sunny sides of UVR-induced immunosuppression : Photoimmunology revisited. *Journal of Investigative Dermatology*, 130(1), pp.49–54.

Schwarz, T. (1999). Ultraviolet radiation-induced tolerance. *Allergy*, 54(12), pp.1252–1261.

Scott, D.G., Cunliffet, W.J. and Gowland, G. (1979). Activation of complement — a mechanism for the inflammation in acne. *British Journal of Dermatology*, 101(3), pp.315–320.

Sfriso, R. and Claypool, J. (2020). Microbial reference frames reveal distinct shifts in the skin microbiota after cleansing. *Microorganisms*, 8(11), pp.1–16.

Shi, J. et al. (2015). Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*, 526(7575), pp.660–665.

Shi, J., Gao, W. and Shao, F. (2017). Pyroptosis: Gasdermin-mediated programmed necrotic

cell death. *Trends in Biochemical Sciences*, 42(4), pp.245–254.

Shih, B.B. et al. (2018). Fractional sunburn threshold UVR doses generate equivalent vitamin D and DNA damage in skin types I–VI but with epidermal DNA damage gradient correlated to skin darkness. *Journal of Investigative Dermatology*, 138(10), pp.2244–2252.

Shu, Muya et al. (2013). Fermentation of Propionibacterium acnes, a commensal bacterium in the human skin microbiome, as skin probiotics against methicillin-resistant Staphylococcus aureus. *PLoS ONE*, 8(2), pp.1–11.

Shu, M. et al. (2013). Porphyrin metabolisms in human skin commensal Propionibacterium acnes bacteria: Potential application to monitor human radiation risk. *Current Medicinal Chemistry*, 20(4), pp.562–568.

Simon, M. et al. (2001). Refined characterization of corneodesmosin proteolysis during terminal differentiation of human epidermis and its relationship to desquamation. *The Journal of Biological Chemistry*, 276(23), pp.20292–20299.

Sinha, R.P. and Häder, D.P. (2002). UV-induced DNA damage and repair: A review. *Photochemical and Photobiological Sciences*, 1(4), pp.225–236.

Smeden, J. van and Bouwstra, J.A. (2016). Stratum corneum lipids: Their role for the skin barrier function in healthy subjects and atopic dermatitis patients. In *Current Problems in Dermatology*. pp. 8–26.

Somerville, D.A. (1969). The normal flora of the skin in different age groups. *British Journal of Dermatology*, 81(4), pp.248–258.

Sommer, S. et al. (1998). Specific RecA amino acid changes affect RecA-UmuD'C interaction. *Molecular Microbiology*, 28(2), pp.281–291.

Spinola, J.J. van R., Dbeibo, L. and Spinola, S.M. (2016). *Skin Stress Response Pathways*. G. T. Wondrak, ed. Switzerland: Springer Nature.

Stewart, I. et al. (2006). Cutaneous hypersensitivity reactions to freshwater Cyanobacteria - Human volunteer studies. *BMC Dermatology*, 6(6), pp.1–9.

Sun, X. et al. (2016). Distinctive molecular responses to ultraviolet radiation between keratinocytes and melanocytes. *Experimental dermatology*, 25(9), pp.708–713.

Switalski, L.M. et al. (1983). Binding of fibronectin to staphylococcus strains. *Infection and Immunity*, 42(2), pp.628–633.

Takashima, A. and Bergstresser, P.R. (1996). Impact of UVB radiation on the epidermal cytokine network. *Photochemistry and photobiology*, 63(4), pp.397–400.

Takeda, K. and Akira, S. (2017). Toll-like receptors in innate immunity. *International Immunology*, 17(1), pp.1–14.

Tang, Z. et al. (2021). Research progress of keratinocyte-programmed cell death in UV-induced Skin photodamage. *Photodermatology Photoimmunology and Photomedicine*, 37(5), pp.442–448.

Thomsen, M. et al. (2014). Model study of the pressure build-up during subcutaneous injection. *PLoS ONE*, 9(8), pp.1–7.

Thurstan, S.A. et al. (2012). Chemical consequences of cutaneous photoageing. *Chemistry*

Central Journal, 6(34).

Toews, G.B., Bergstresser, P.R. and Streilein, J.W. (1980). Epidermal Langerhans cell density determines whether contact hypersensitivity or unresponsiveness follows skin painting with DNFB. *The Journal of Immunology*, 124(1), pp.445–453.

Trivedi, S. et al. (2017). The Surface protein SdrF mediates Staphylococcus epidermidis adherence to keratin. *Journal of Infectious Diseases*, 215, pp.1846–1854.

Trummer, C. et al. (2016). Beneficial effects of UV-radiation : Vitamin D and beyond. *International Journal of Environmental Research and Public Health*, 13(10), pp.1–16.

Tsukita, S., Furuse, M. and Itoh, M. (2001). Multifunctional strands in tight junctions. *Nature Reviews Molecular Cell Biology*, 2(April), pp.285–293.

Ullrich, S.E. and Byrne, S.N. (2012). The immunologic revolution : Photoimmunology. *Journal of Investigative Dermatology*, 132(3 Pt 2), pp.896–905.

Vink, A.A. et al. (1996). Localization of DNA damage and Its role in altered antigen-presenting cell function in ultraviolet-irradiated mice. *The Journal of Experimental Medicine*, 183(4), pp.1491–1500.

Vink, A.A. and Roza, L. (2001). Biological consequences of cyclobutane pyrimidine dimers. *Journal of Photochemistry and Photobiology B: Biology*, 65(2–3), pp.101–104.

Wallberg, F., Tenev, T. and Meier, P. (2016). Analysis of apoptosis and necroptosis by fluorescence-activated cell sorting. *Cold Spring Harbor Protocols*, 2016(4), pp.347–352.

Wang, Y. et al. (2012). The response of human skin commensal bacteria as a reflection of UV radiation: UV-B decreases porphyrin production. *PLoS ONE*, 7(10), pp.e47798.

Wang, Z. et al. (2018). Skin commensal bacteria Staphylococcus epidermidis promote survival of melanocytes bearing UVB-induced DNA damage, while bacteria Propionibacterium acnes inhibit survival of melanocytes by increasing apoptosis. *Photodermatology Photoimmunology and Photomedicine*, 34(6), pp.405–414.

Wang, Z., Macleod, D. and Nardo, A. Di. (2012). Commensal-bacteria LTA increases skin mast cell antimicrobial activity against Vaccinia viruses. *journal of Immunology*, 189(4), pp.1551–1558.

Wanke, I. et al. (2011). Skin commensals amplify the innate immune response to pathogens by activation of distinct signaling pathways. *Journal of Investigative Dermatology*, 131(2), pp.382–390.

Webster, G.F., Leyden, J.J. and Nilsson, U.R. (1979). Complement activation in acne vulgaris: Consumption of complement by comedones. *Infection and Immunity*, 26(1), pp.183–186.

Weisburg, W.G. et al. (1991). 16S Ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology*, 173(2), pp.697–703.

Westman, J., Grinstein, S. and Marques, P.E. (2020). Phagocytosis of necrotic debris at sites of injury and inflammation. *Frontiers in Immunology*, 10:3030.

Wondrak, G.T., Jacobson, M.K. and Jacobson, E.L. (2006). Endogenous UVA-photosensitizers: Mediators of skin photodamage and novel targets for skin photoprotection. *Photochemical and Photobiological Sciences*, 5(2), pp.215–237.

Woo, P.C.Y. et al. (2008). Then and now: Use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories. *Clinical Microbiology and Infection*, 14(10), pp.908–934.

Wrone-smith, T. and Nickoloff, B.J. (1996). Dermal injection of immunocytes induces psoriasis. *The Journal of Clinical Investigation*, 98(8), pp.1878–1887.

Yamasaki, K. et al. (2007). Increased serine protease activity and cathelicidin promotes skin inflammation in rosacea. *Nature Medicine*, 13(8), pp.975–980.

Yang, W.S. et al. (2014). Regulation of ferroptotic cancer cell death by GPX4. *Cell*, 156(1–2), pp.317–331.

Yokouchi, M. et al. (2015). Epidermal tight junction barrier function is altered by skin inflammation, but not by filaggrin-deficient stratum corneum. *Journal of Dermatological Science*, 77(1), pp.28–36.

Young, A.R. (1997). Chromophores in human skin. *Physics in Medicine and Biology*, 42(5), pp.789–802.

Young, A.R. and Sheehan, J.M. (2001). Chapter18 - UV-induced pigmentation in human skin. In Giacomoni, P.U. (editor). *Comprehensive Series in Photosciences*, Elsevier B.V., pp. 357-375.

Yu, J. et al. (2019). A tryptophan metabolite of the skin microbiota attenuates inflammation in patients with atopic dermatitis through the aryl hydrocarbon receptor. *Journal of Allergy and Clinical Immunology*, 143(6), pp.2108–2119.

Yuki, T. et al. (2011). Activation of TLR2 enhances tight junction barrier in epidermal keratinocytes. *The Journal of Immunology*, 187(6), pp.3230–3237.

Zhu, T. et al. (2010). Impact of the Staphylococcus epidermidis LytSR two-component regulatory system on murein hydrolase activity, pyruvate utilization and global transcriptional profile. *BMC Microbiology*, 10(287), pp.1–16.

Zuber, M. et al. (2015). Collagen based polyurethanes — A review of recent advances and perspective. *International Journal of Biological Macromolecules*, 80, pp.366–374.

Appendix

Appendix 1 Slopes and intercepts of the bacterial SSR-survival curves

Organism	Slope(*c*)	Intercept(*r*)
C. acnes	-0.05±0.0006[a]	0.872
S. capitis	-0.05±0.0001	0.885
S. epidermidis (NCTC 10519)	-0.04±0.007	0.996
S. epidermidis (NCTC 6513)	-0.04±0.005	1.016
S. epidermidis (NCTC 11047)	-0.04±0.003[a]	1.058
S. hominis	-0.04±0.003	0.937
C. jeikeium	-0.03±0.002	1.022
M. luteus	-0.02±0.005	1.045

[a] Mean ± SD

Appendix 2 The application form for the clinical project

Ethics Application

Research

Please be mindful that each application, submitted via the University's Ethical Review Manager (ERM), costs the University **£750** due to the number of people required to process, review and approve your application.

Please respect this fact and ensure that you carefully follow the guidance provided and help bubble text in order to complete your application appropriately (and choose the correct route of ethical review). Please **DO NOT** use the ERM system for 'test' submissions. Misuse of the ERM system is a waste of numerous resources which could otherwise be dedicated to research, teaching and social responsibility activities.

You are logged into the Ethical Review Manager (ERM), the system provided by Infonetica Ltd that will process the application on behalf of The University of Manchester. Your contact details will be stored by Infonetica Ltd and used by the University for the purpose of managing your application for ethics review. The University will use your details for that purpose only. The information will be retained, archived and deleted in line with the agreed retention policy. Your details will not be passed to any other third party organisations.

The University, in compliance with the Data Protection Act 2018 (DPA) and the General Data Protection Regulation (GDPR), has a **Data Protection Policy** and **Research Privacy Notice** and any information you provide on this form and associated documents will be protected in accordance with these policies. However, it will be assumed that you have not included any sensitive personal information and you should not, therefore, include a *curriculum vitae* or identifiable information about your racial or ethnic origin, political opinions, religious or similar beliefs, trade union membership, physical or mental health, sexual life, commission of offenses and/or criminal proceedings. Should you feel it essential to include such details in your application please contact the Research Governance, Ethics and Integrity team (research.ethics@manchester.ac.uk).

Please also note this system will send all correspondence related to your ethics application to your University of Manchester email account.

Please do not proceed unless you are content to comply with this.

A0. Data Protection Statement

☑ I confirm that I have read the above information with regard to data protection and will comply with the requirements as described.

Reference #: 2020-8539-13844

A1. Does your study meet the definition of 'research' using human participants or have you been advised to seek ethical approval for your study (either via the Ethics Decision Tool or other guidance)?

Please visit the help bubble (blue circle with the white letter 'i') to the right of this question for a link to the Ethics Decision tool and supplementary information on the types of projects which may or may not require ethical review.

- (•) Yes

You **must read** the information in the help bubble before answering this question. If you cannot answer yes **do not complete the rest of this form, log out of the ERM system** and if you have any queries contact your Ethics Signatory

You should only be submitting this form if you can answer yes to this question.

A02 HRA Approval

A2. Does your study include a component which would require approval by the Health Research Authority (HRA)?

Please visit the Help Bubble in the upper right hand corner for details as to what types of research require NHS REC and HRA approval.

Please choose the option which is most relevant for your study. If you have 2 components (i.e. one using healthy volunteers and one using NHS patients), please speak with a member of the FBMH Research Governance team who will advise on the most appropriate avenue for review.

- (•) Yes: it includes a component that requires review by BOTH the HRA and the University Research Ethics Committee or a Division/School based Committee (e.g. it is being carried out in the NHS but is exempt from NHS REC review)
- ( ) No: it only requires review by the University Research Ethics Committee (UREC) or a Division/School based Committee

A03 - 05 Decision Tree

A3. I confirm that this research project is being conducted by a:

- (•) Student
- ( ) Member of Staff
- ( ) Member of Eurolens Research, Optometry Staff

IMPORTANT: Your answer to **Question A4** will lead you to the correct application form for ethical review and it is important that you answer this question carefully.

Please ensure you read the guidance notes carefully **BEFORE** answering this question and for student projects, discuss the details with your supervisor.

The guidance notes can be found in the Help Bubble (small blue circle with the white letter 'i') to the right of Question A4.

Answering this question incorrectly will result in **SIGNIFICANT** delays to the review process and will result in you needing to **re-apply** for ethical review.

A4. Please select how you will be applying for ethical review:

Please ensure you read the criteria as described in the help bubble carefully before deciding which route of ethical review to select.

****Division/School review is only available for the 10 Schools/Divisions/Departments listed in the help bubble to the right of this question. If your School/Division/Department is not listed you must apply for Proportionate or full UREC review****

- ○ Division/School Review
- ○ Proportionate University Research Ethics Committee (UREC) Review
- ◉ Full University Research Ethics Committee (UREC) Review

STOP!

Are you doing a **student** project?

If so, are you sure that your project could not be ethically reviewed by a **Division/School** panel instead of the UREC?

Incorrectly applying for UREC review rather than Division/School review can result in you needing to re-apply for ethical review and will result in significant delays. In addition, it wastes a considerable amount of UREC resources.

For details on whether your project qualifies for Division/School review please see the help bubble in Question A4.

Please take care when selecting your Division/School/Department/PSS Directorate/Cultural Institution from the drop-down list below.

Mistakes will result in your application being sent to the wrong ethics signatory for review.

Please note, the selection made below should reflect where you are based at the University.

A5 UREC Review (full or Proportionate): Please select which Division/School/Department/Centre/area of PSS/Cultural Institution you are based in:

Division of Musculoskeletal & Dermatological Sciences

Reference #: 2020-8539-13844

A5.1 As you are applying for full UREC review, you will be required to attend a Committee meeting to discuss your project (and provide confirmation of this via email). To assist us with scheduling your application to a meeting which would work best with your current commitments, please indicate your preferences below:

IMPORTANT: Every effort will be made to schedule your application according to your selections below. Please note that this may result in the need to wait longer than 3 weeks to attend the meeting day of your choice. If you indicate that you have no preference and wish to be allocated to the first available Committee meeting, it is imperative that you indicate in **Question A5.2** any dates/times that you are unable to attend.

In **exceptional** circumstances, the Committee will accept attendance via telephone. However please note that although the Committee aims to run to time, delays are possible and therefore if you choose this option you will need to ensure you are available for a 1 hour time slot on the given day. Details of this will be communicated to you in the formal invitation letter sent via the ERM system. If you need to have this option arranged, please indicate this in **Question A5.2** and provide details as to why you are unable to attend the meeting in person.

When you receive the formal invitation via the ERM system **please forward it to research.ethics@manchester.ac.uk and confirm your attendance within the time frame indicated on the PDF letter**.

Failure to do this will result in your application being allocated to an alternative Committee meeting.

- ☐ UREC 1: Thursdays from 9am-12pm
- ☑ UREC 2: Mondays from 11am-2pm
- ☑ UREC 3: Wednesdays from 12pm-3pm
- ☑ UREC 5: Mondays from 12pm-3pm
- ☐ I have no preference and wish to be allocated to the first available Committee meeting.

A5.2 Please list below any upcoming dates/times that you would be unavailable to attend the Committee meeting:

Please note: The scheduling (and re-arranging) of appointments for UREC meetings is a time consuming process which can result in a substantial increase in the workload of our office. We ask you to please respect this fact and make every effort to provide us with as many details as possible regarding your availability so we may minimise the time spent in arranging your appointment. If you **do not** indicate any potential scheduling conflicts, we will allocate your application to the first available Committee meeting based upon your preferences above. Should you contact our office to rearrange this appointment, we will only be able to offer a limited number of alternatives.

26.12.2019 - 03.02.2020, I would be very appreciated if the committee meeting of this application could be arranged as early as possible.

B02 Students

B2. Contact information for the individual completing this form:

Title	First Name	Surname
Miss	Wen	Duan

Email: wen.duan@postgrad.manchester.ac.uk

Reference #: 2020-8539-13844

B2.1 Please confirm one of the following:

- (•) I am the student investigator of this project.
- ( ) I am the supervisor of this project.

B2.2 Please provide the full contact details of your primary supervisor:

This **MUST** be a University of Manchester member of staff with a UoM email address. Please note, the primary supervisor is also the data custodian for your research project. If you have more than one supervisor, please use the '**Add Another**' button below to add the contact details of your additional supervisor(s).

If when using the Search function you cannot locate your supervisor, please ensure they have logged into the ERM at least once. Once they have done this, their details will be stored for future use.

Title	First Name	Surname
Prof	Catherine	O'Neill

Email: catherine.o'neill@manchester.ac.uk

B2.2 Please provide the full contact details of your primary supervisor:

This **MUST** be a University of Manchester member of staff with a UoM email address. Please note, the primary supervisor is also the data custodian for your research project. If you have more than one supervisor, please use the '**Add Another**' button below to add the contact details of your additional supervisor(s).

If when using the Search function you cannot locate your supervisor, please ensure they have logged into the ERM at least once. Once they have done this, their details will be stored for future use.

Title	First Name	Surname
Prof	Andrew	Mcbain

Email: andrew.mcbain@manchester.ac.uk

B2.2 Please provide the full contact details of your primary supervisor:

This **MUST** be a University of Manchester member of staff with a UoM email address. Please note, the primary supervisor is also the data custodian for your research project. If you have more than one supervisor, please use the '**Add Another**' button below to add the contact details of your additional supervisor(s).

If when using the Search function you cannot locate your supervisor, please ensure they have logged into the ERM at least once. Once they have done this, their details will be stored for future use.

Title	First Name	Surname
Prof	Rachel	Watson

Email: Rachel.Watson@manchester.ac.uk

B2.3 Are there any additional collaborators on this project?

Please note: Collaborators are defined as individuals who will assist in either the data collection or data analysis of the project and can be members of staff or students.
Please include any external collaborators from other institutions or organisations. They will **NOT** be involved in any of the electronic correspondence for this project.

- (•) Yes
- ( ) No

B2.4 Will any collaborators be external to this University?

If your study involves an external collaboration please ensure you read the Guidance on External Collaborations

- ( ) Yes
- (•) No

B2.9 Please use the box below to type the title and full name(s) of ALL co-investigators, researchers or collaborators (as well as their role in the project). If individuals are external to this University, please indicate the institution or organisation which they are affiliated with.

To add the name of more than one individual, click the '**Add Another**' button below.

Prof. Lesley Rhodes Professor of Experimental Dermatology, University of Manchester Honorary Consultant Dermatologist Photobiology Unit Salford Royal Foundation Trust: responsible for the clinical aspect of this study.

B2.9 Please use the box below to type the title and full name(s) of ALL co-investigators, researchers or collaborators (as well as their role in the project). If individuals are external to this University, please indicate the institution or organisation which they are affiliated with.

To add the name of more than one individual, click the '**Add Another**' button below.

Mrs Joanne Osman (RN Adult) Research Nurse, University of Manchester conduct the clinical procedures and ultraviolet radiation (UVR) exposures.

B2.12 Please confirm the degree being studied for by the student investigator:

- (•) Postgraduate Research (PGR) (e.g. PhD degree)
- ( ) Postgraduate Taught (PGT) (e.g. masters degree)
- ( ) Undergraduate (UG)
- ( ) Postgraduate Taught + Undergraduate (the study will be conducted by BOTH an UG and PGT student; note: this is rare)

B2.13 IMPORTANT: BEFORE CONTINUING:

Look on the left hand side of the screen for the '**share**' button. Push this button, enter the appropriate email address and be sure to tick all the relevant boxes in the pop up window.

- [x] I confirm that I have pushed the share button on the left hand side of the screen and 'shared' this form with my supervisor.

C01: Compliance & Monitoring

Reference #: 2020-8539-13844

Please note: Everyone is required to complete the compliance & monitoring questions below, whether you are completing a Proportionate University Research Ethics Committee (UREC), full UREC or Division/School template application.

IMPORTANT NOTE: If you will be travelling abroad for your research, and in particular to what is considered to be a risky or dangerous area of the world, you must ensure that you have completed the appropriate Division/School based **risk assessment**, had this **approved** by appropriate individuals within your Division/School and **checked** with the University's Insurance office **regarding travel insurance**. The ERM system **WILL NOT** inform the University's Insurance office of your travel plans automatically (unless you are performing clinical activity) and it is therefore the responsibility of all members of staff and supervisors to contact the Insurance office **prior** to obtaining ethical approval. Please note that specific areas of the world will require additional approvals and this should be taken into consideration when planning a timeline for seeking ethical approval.

C1. Will your research involve any of the following:

Before answering this question please ensure you click on the help bubble to read the guidance information which includes definitions of each of the terms below. Tick all that apply.

- ☐ the use of invasive techniques on participants
- ☑ the use or collection of human tissue
- ☑ the physical testing of participants
- ☐ the use of psychological intervention (please DO NOT tick this option if you are only administering standard psychological tests/questionnaires)
- ☐ the ingestion or inhalation of any substance by participants
- ☐ the use of a medical device or a potential medical device
- ☐ None of the above

C1.1 Will this research be conducted outside of the UK?

- ○ Yes
- ◉ No

C1.2 Will this research involve participants under the age of 5?

- ○ Yes
- ◉ No

C02 Medical Intervention

This study may be reviewed by the UoM Clinical Trial team. If further information is required, the protocol for your study will be requested. Any advice given by the Clinical Trial team is supplementary to the review by your Ethics Signatory (who takes responsibility for your study on behalf of your Division/School) and the ethical issues raised by the UREC.

Reference #: 2020-8539-13844

C2.1 When conducting the proposed methodology, will you be using any piece of equipment that will have direct contact with your potential participants or their samples?

Please open the guidance in the **help bubble** for additional information on this question.

- (•) Yes
- ( ) No

C2.2 Please confirm if an internal risk assessment has been performed for the piece(s) of equipment that you will be using, which has been reviewed by a School Safety Advisor within your faculty and signed off by your line manager:

- (•) Yes
- ( ) No

C2.3 Please confirm that all standard operating procedures (SOPs) are up to date and have been read by all members of your research team for the pieces of equipment that you will be using:

- (•) Yes
- ( ) No

C2.4 Please confirm that all required training has been documented and completed by all members of your research team for the pieces of equipment that you will be using:

- (•) Yes
- ( ) No

C2.5 Will you be administering a drug or other substance to participants?

- ( ) Yes
- (•) No

C2.8 Please describe the procedures to be undertaken

Please include details of any invasive procedures, and any samples or measurements to be taken and/or any psychological tests etc. What is the experience of those administering the procedures?

1. Baseline assessments: Fitzpatrick skin type.
2. Skin microbiota swabs taken from the left mid-back skin.
3. Area of right side of middle back cleaned using 70% ethanol.
4.Skin swabs repeated from same area of back;
5. Both the cleaned area and control area of mid-back will be exposed to a dose serious of 8-80 mJ/cm^2 TL12 broadband UVB. Small (1 cm^2) areas of mid-back skin will be exposed to varying doses of UVR (The exposure progress will be controlled by the research nurse within the Photobiology Unit. Lamp of UVB equipment will be precleaned thoroughly with ethanol, operator wears appropriate Personal Protective Equipment (PPE)
6. 6 and 24 hours later: UVR-erythema (redness) dose response quantified respectively. Non-invasive erythema assessment will be performed.
The research nurse is trained and competent to perform the UVR exposure in accordance with SRFT protocol and completing annual medical devices competences as per SRFT policy.

C2.9 Will any procedures which are normally undertaken be withheld?

- ○ Yes
- ◉ No

C2.11 Will the research participants' General Practitioner be informed that they are taking part in the study?

- ○ Yes
- ◉ No

C2.12 Please explain why not.

Only healthy adults will be recruited to this study, and we do not expect to cause any injuries to all volunteers.

C2.14 What are the criteria that would cause the researcher to electively stop the research project prematurely?

The study may be prematurely stopped for a specific participant or for all included participants if new available data show a potential risk for the participants. A specific participant can withdraw for the following reasons:
1. The participant is violating the protocol.
2. For safety reasons;
In all these cases every attempt will be made to have the participant return for a final visit in order to ensure their safety and wellbeing.

C03 Medical Devices/Testing

C3.1 Which of the following describes the medical device or potential medical device that you will be using?

- ○ CE marked
- ◉ Non CE marked

C3.4 Please provide the following information:

- Who is manufacturing the device or where is it being sourced from?
- Why are you using the device and for what purpose?
- What will you do with the data at the end of the study?

The device that will be used to apply the UV was made in house by Clinical Engineering at SRFT and undergoes annual planned preventative maintenance. There is visual and inclusive of function and clinical safety. In addition, calibration is performed by the Optical and UV radiation specialist Dr Donald Allan Head of Clinical Engineering prior to the study start. The equipment is managed on the In House Quality system.
This device forms part of the standard equipment for performing a minimum erythema dose (MED) test
All raw data will be stored on UoM personal computers and backed up in the university's research data storage systems. However, primary data (paper-based consent forms) will be preserved within the division of Musculoskeletal & Dermatological Sciences, University of Manchester. The data generated form this project will be included as verbatim in the researcher's dissertation and may be published in anonymous form in academic books, reports or journals in the future.

C3.5 Please declare the following:

☑ I confirm that I have attached a completed and signed risk assessment or management plan for this study.

C3.6 Have all members of the research team (including supervisors if applicable) undergone GCP training?

◉ Yes
○ No

C05 Human Tissue

IMPORTANT: ALL STUDIES INVOLVING HUMAN TISSUE MUST HAVE ETHICAL APPROVAL.

PLEASE NOTE: IF YOUR STUDY REQUIRES NHS REC APPROVAL YOU DO NOT NEED TO SUBMIT AN APPLICATION VIA ERM. PLEASE REFER TO THE HRA WEBSITE FOR ALL NHS REC APPLICATIONS.

All staff storing human tissue for research must register their activity with the Research Governance, Ethics and Integrity Team to ensure coverage under the University's HTA Licence for Research. **Email**:HTALicence.Research@manchester.ac.uk

Please note that if you will be using human tissue for your research it is important that you **read the guidance on our website**.

AGAIN: ALL STUDIES INVOLVING HUMAN TISSUE MUST HAVE ETHICAL APPROVAL.

C5 Will your study involve the storage of human tissue samples considered HTA relevant material?

Please see the help bubble in the top right hand corner of this page for guidance on storage.

○ Yes
◉ No

C5.4 Will your study involve the analysis of DNA?

○ Yes
◉ No

C5.5 Please provide details of **how many donors** will be providing human tissue samples:

Number of Donors	Description of Donors
10	Healthy males and females, aged 18-65 years,

Reference #: 2020-8539-13844

C5.6 Please provide details of **how many and what type of human tissue samples** will be obtained/used. If you have more than one type of sample please use the 'Add Another' button to add a new row.

Example:

20 Blood samples

10 Urine samples

Number of Samples	Description of Samples
40	Skin microbiota samples of each volunteer will be collected before and after ultraviolet radiation from both sides of mid-back skin.

C5.7 Please provide details of what will happen to the samples:

For full UREC studies, if you intend to retain the samples for future use please ensure this is made clear in the participant information sheet and that permission is explicitly requested for this in the consent form. Similarly, explicit consent is required if you will be exporting samples overseas.

The skin swab (sample of bacteria present on skin) will be subjected to bacterial DNA extraction procedures, these DNA extracts will be analyzed using next generation sequencing methods and quantitative PCR which will be specific for bacterial DNA only.
All skin swabs got from these volunteers will be processed within 7 days of collection to remove any eukaryotic cells of human origin.

C5.10 If you are performing research using human tissue you MUST confirm the following conditions:

- ☑ I confirm I have completed the MRC online 'Research and human tissue legislation-updated' training
- ☑ I confirm I have registered my study with the Research Governance, Ethics and Integrity team
- ☑ I understand if I am not directly obtaining consent from the participants I must still provide copies of template consent forms and information sheets or a letter from the supplier
- ☑ I understand that if I am importing or exporting samples I will need to instigate a material transfer agreement (MTA) via the Contracts Team

D01 - 02 General Project Information: Resubmission and titles

D1. Is this a re-submission of a project that has previously received an unfavourable ethical opinion?

Please note: this **does not** include applications where revisions have been requested.

- ○ Yes
- ◉ No

D2. Short title of your research project (200 character max)

Influence of the skin microbiome on the sunburn response in healthy humans *in vivo*.

Reference #: 2020-8539-13844

D2.1 Formal title of your research project (if different to short title)

Does reduction of the skin microbiome exacerbate the UV-erythemal response in healthy humans *in vivo*?

D03 Dates of Data Collection/DMP/Data Collection

D3. Will you be collecting data during the course of the research project?

Please note, data refers to any information being gathered about a person or organisation. This information can include specifics such as thoughts, beliefs or characteristics and can be in different formats such as written notes, questionnaires, observations, audio recordings, films, photographs, social media postings or bodily samples.

- (•) Yes
- ( ) No

D3.1 Proposed start date of data collection

Please ensure this date is **far enough in the future to allow for the ethical review process to take place**. The Committee will be **unable to grant approval** to applications which feature a start date **that is in the past**.

01/06/2020

D3.2 Proposed end date of data collection

31/12/2020

D3.3 Please attach a copy of your Data Management Plan:

You **must** use the University's DMP Online system for the creation of your plan and more information can be found in the help bubble.

Please note: if you are not collecting any data for this project, please read the guidance information in the help bubble for additional instructions.

Documents

Type	Document Name	File Name	Version Date	Version	Size
Data Management Plan	Does_reduction_of_the_skin_microbiome_exacerbate_the_UV-erythemal_response_in_healthy_humans_in_vivo_	Does_reduction_of_the_skin_microbiome_exacerbate_the_UV-erythemal_response_in_healthy_humans_in_vivo_.pdf	18/11/2019	1	54.0 KB

D04 Data Protection Training

Reference #: 2020-8539-13844

All staff and students at the University of Manchester are responsible for ensuring they are familiar with the data protection policies and processes and follow these when conducting their research projects. Under the new General Data Protection Regulations (GDPR) the University is required to provide assurances and safeguards to all research participants that their data will be treated confidentially and will be protected as set out to the relevant data protection legislation. To support this, please complete the relevant question below to confirm that you have undertaken the required Data Protection Training or discussed the University's requirements and expectations with your supervisor.

D4. Please tick **each statement** below to indicate that you **understand** and **will adhere to** data protection regulations and The University of Manchester's data protection policies.

For more information, please see the **University's Records Retention Schedule** and **SOP for Recording of Research Participants**.

- ☑ I will ensure that paper data (e.g. consent forms) are stored in a locked cabinet that only the research team has access to.
- ☑ I confirm that all electronic data will be stored on University servers such as my P drive or on the research drive of my supervisor or University approved cloud services e.g. Dropbox for Business.
- ☑ I will NOT use external hard drives, USB sticks or any other portable device to store personal identifiable data as they are subject to loss or theft.
- ☑ I will NOT use personal devices for the recording of audio, video or photographs. (Please refer to the SOP for Recording of Research Participants for more information).
- ☑ I understand that if I need to use a portable device to record and transfer data, this device should be University of Manchester owned and encrypted, the data transferred to a secure server as soon as possible and must be deleted from the portable device following the transfer. (If an encrypted device is not available you will need to make specific arrangements with respect to securing data as soon as possible and this must be detailed in your ethics application).
- ☑ I will NOT store data on cloud based services other than Dropbox for Business approved by the University.
- ☑ I will ensure that all data are anonymised/pseudonymised as soon as possible to protect the confidentiality of my participants.
- ☑ I will only collect the personal information that is required to answer my research question and as approved by the ethics committee.
- ☑ I understand that personal information should be deleted as soon as it is no longer required. If keeping the contact details of participants to contact them about future research or to share findings of my project, I will store these in a separate password protected file or database held on University servers or approved cloud services.
- ☑ I understand that all data should be stored in accordance with the University's Records Retention schedule and must be kept for the period as specified in my data management plan or approved ethics application.
- ☑ I understand that my supervisor MUST be listed as the data custodian for my project and I must ensure that I transfer custody of all paper and electronic data to them before I leave the University.
- ☑ I understand that I SHOULD use encrypted devices when analysing my study data if not accessing the data directly from my P drive or other secure University server.
- ☑ I understand that I MUST ensure that when I am transcribing or analysing data that it is done in a way in which other people are NOT able to see any personal data on my devices.
- ☑ I understand that if I wish to share study data with other researchers or retain the data for use in future studies that I MUST ensure this is explicitly mentioned in the participant information sheet and consent form.
- ☑ I understand that ONLY University of Manchester or study specific email addresses/phone numbers can be used by researchers for their research projects.

E01 Details of Project

Important: You are now completing the **University Research Ethics Committee (UREC)** application form.

Reference #: 2020-8539-13844

E1. What is the principal research question?

Please limit responses to less than 100 words.

Does reduction of the skin microbiome exacerbate the UV-erythemal response in healthy humans in vivo?

E02

E2. What is the academic justification for the research?

Must be in lay language comprehensible to a lay person.

The surface of skin is colonized with an abundance of microorganisms, forming a unique skin microbiome. They play a significant role in maintaining normal skin metabolism, however in some situations they cause some skin diseases as well. During the whole life span of a human, skin cells and skin microorganisms are exposed to an external environment, including to ultraviolet radiation (UVR) in sunlight. The effects of UVR on skin cells has been well studied but there are few studies focusing on the effect of UVR on the skin microbiome. Since the skin microorganisms are important for our skin health, it is valuable to survey the impact of UVR on them, and how in turn, this may influence our skin cells.
Specially, researchers have showed the microorganisms normally found on skin have different UV sensitivity; some species, which are predominantly found on the outermost layer of skin, could be absorbing UV radiation high up in there. Hence, we hypothesize that reducing the skin microbiome will increase UV effects in human skin, such as UV-induced erythema. The data of this research could help us elucidate the role of skin normal microbiota better, and also could lead us a better understanding of some UV-related skin disorders.

E03 Data collection measures

E3. Please indicate if the research involves any of the following:

Tick all that apply

- ☐ Interviews
- ☐ Focus Groups
- ☐ Paper based survey/questionnaires
- ☐ Electronic or online surveys/questionnaires
- ☐ Standard, copyrighted psychology questionnaires/tests
- ☐ Field observation (including participant observation)
- ☐ Child/infant behaviour observation
- ☐ Assessment (such as those used in Education research)
- ☐ Recordings (audio, video, photographs etc)
- ☐ Use of pre-existing media (photographs, video etc from an existing archive/database)
- ☐ Other qualitative methods (e.g. discourse analysis, interaction analysis, conversation analysis)
- ☐ Other on-line or electronic methods (e.g. netography, on-line research, textual analysis of digital sources)
- ☑ The analysis of lab based/human tissue samples
- ☐ Any other method not listed above

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E3.1 Please attach either a copy of the data collection tools you plan to use (e.g., questionnaires) or a very brief protocol describing the procedure (stimuli, responses, conditions manipulated, etc.)

If performing a study with more than one data collection tool, please ensure you include documents for each (i.e. interview topic guides, focus group schedules, copies of all questionnaires and surveys, etc).

IMPORTANT: If you are administering standard, **copyrighted** psychology questionnaires/tests to participants you **MUST** provide a description of the questionnaire/test to the Committee using the approved description form. Please ensure you use a separate form for each test and label each document with the name of the corresponding test before attaching to this question in the application form.

Documents

Type	Document Name	File Name	Version Date	Version	Size
Default	Brief protocol	Brief protocol.docx	12/03/2020	2	17.1 KB

Reference #: 2020-8539-13844

E4. Give a brief summary of the design and methodology of the planned research.

It should be clear exactly what will happen to the research participant, how many times and in what order. Describe any involvement of research participants, participant groups or communities in the design of the research. Please structure your answers into a **bulleted list and be as clear and concise as possible**. Please **do not** copy and paste from your research protocol.

Must be in lay language comprehensible to a lay person.

Potential participants will be provided with information about the study including the participant information sheet (PIS) and the consent form and given at least 24 hours to consider participation. They will be asked to attend the first appointment where the inclusion/exclusion criteria will be assessed (recorded in the Case Report Form) and informed consent will be taken. Before starting the study, the history of photosensitivity will be double checked with each participant in case of the happening of any adverse events (such as photosensitive dermatitis, ethanol and/or UV allergy). And then their skin type will be evaluated according to their tolerance to sun exposure. The study centre is based in an acute general hospital, fully equipped for management of any unforeseen circumstances.

To address our research question, we propose a study investigating does reduction of the skin microbiome exacerbate the UV-erythemal response in healthy humans. One side of mid-back skin of healthy volunteers will be cleaned with 70% ethanol before UV radiation. The skin microbiota sample will be collected immediately before and immediately after the cleaning. And then both of the cleaned area and the control area of the mid back will be exposed to a series of UV light. UVR will be administered topically using a hand held device to give a dose series of 8-80 mJ/cm2 (which is roughly equivalent to 11 mins 48 secs -1 hour 58 mins in Manchester summer sun) ten doses in total as is our standard practice to establish the erythemal threshold of the participant. For each dose, a 1 cm diameter area will be exposed to UVR. At 6 and 24 hours later, the erythemal response of participant will be assessed. Participants will be told not to have a showering, sauna or sunbathing between visits.

All skin swabs obtained from these volunteers will be subjected to bacterial DNA extraction procedures within 7 days of collection. No identifiable human materials will be retained. These DNA extracted will be analyzed using next generation sequencing methods and quantitative PCR which will be specific for bacterial DNA only. The bacterial DNA will be analyzed to determine the types of bacteria present and quantity.

The visit schedule will be as follows:

Visit 1.
- Informed consent procedures
- Assessment of inclusion/exclusion criteria and UV-related skin type
- Skin swab taken from both sides of the mid-back skin
- Area of right side of the mid-back skin cleaned with 70% ethanol
- Skin swab repeated from the cleaned and control skin area
- The cleaned area and the control area of the mid-back exposed to a series of UV light
- Return 6 hours later for assessments of UVR-erythema (redness) dose response

Visit 2. (24h after Visit 1)
- UVR-erythema (redness) dose response measured again to determine minimum erythemal dose.

E05-06

E5. What do you consider to be the main ethical issues which may arise with the proposed study?

Please provide this in a bulleted or numbered list.

The main ethical issues raised by this project relate to confidentiality, consent and skin redness. We do not expect to find anything wrong with participants' skin as only healthy adults without any skin diseases will be invited to take part in this study. We do not expect to cause any infection for participants' skin as no invasive procedure will be conducted during the study. Initial UVR testing to determine the minimal erythemal dose (sunburn threshold) will result in transient redness which may become tanned depending upon the skin type of participant, this will resolve over time.

E6. What steps will be taken to address the issues raised in the question above?

Please provide this in a bulleted or numbered list.

Confidentiality: This issue is raised in relation to the collection of personal data combined skin health assessment. Collection of identifiable data such as the participants name and contact details is required to ensure we can contact the participant with any pertinent study information however, we will be anonymizing the data and creating an unique key in which will link the anonymized data to the participant, this will ensure that the data remain anonymous to the researchers but will allow it to be traced back if we need to contact the participant or if they wish their data to be removed from the study.

Consent: We will seek informed consent from all the participants. Those individuals who are interested in participating in the study will be given a copy of the participant information sheet and consent form at least 24 hours before consent is taken. At their first sampling appointment the potential participants will be asked if they understood the information given about the study and given the opportunity to ask any questions. Information about the study will be explained (again) by the researchers, and the potential participant will be invited to give their consent for the procedures and samples. If they agree they will be asked to sign the consent form. The researcher will be sufficiently qualified and adequately trained in obtaining consent. Participant samples, consent forms and sample information sheet will be labelled with a unique study number this allows the information to remain anonymous to the receiver/processor of the samples, but also allows for the samples to be traced from collection to disposal.

Skin redness: we do not expect to cause any injure to participants since all UVR exposure will be performed at a low dose level. The redness will resolve over time. The study centre specializes in photodermatology and is located within in an acute general hospital, fully equipped for management of any unforeseen circumstance.

E07

E7. What qualifications/experience do the researchers have relevant to the conduct of this research?

Example: training or experience in relation to consent, methodology, data protection, etc

The research team has completed all modules of good clinical practice training. Additionally, the supervisor team has extensive experience in designing clinical studies, complying with relevant regulations including data protection, confidentiality and data analysis to meet study objectives.
The research procedures will be performed in the Photobiology Unit, Salford Royal NHS Foundation Trust under the supervision of Professor Lesley Rhodes who has several years' experience of performing studies with human volunteers.
Consent will be obtained by the Research Nurse who has vast experience of the informed consent process having been trained in a contract research organization and having an MA Healthcare Ethics and Law , University of Manchester)..

E08

E8. Has this, or a similar application, previously been considered by a Research Ethics Committee in the UK, the European Union or the European Economic Area?

- ○ Yes
- ◉ No

E09

Reference #: 2020-8539-13844

E9. How has the scientific quality of the research been assessed?

Tick all that apply.

- [x] Internal review (e.g. involving colleagues or academic
- [ ] supervisor) Review within a multi-centre research group
- [ ] Independent external review
- [ ] Review within a commercial company
- [ ] None external to the investigator
- [ ] Other (e.g. in relation to methodological guidelines)

E9.1 Describe the review process and outcome.

If the review has been undertaken but not seen by the researcher, give details of the body which has undertaken the review.

This research has been fully discussed with all supervisors and collaborators who have extensive knowledge in this field.

E10

E10. Is statistical sampling relevant to this research?

- ○ Yes
- ◉ No

E10.5 If you are not using statistical sampling how was the number of participants decided upon?

This is a small-scale pilot study to determine the effects of UVR on skin microbiota and the different skin response with/ without the existence of skin microbiota after UV irradiation. N=10 participants are sufficient for generating pilot data, whilst recognizing this is a small-scale study with limited time & funding for sample processing and data analysis.

E11

E11. Describe the methods of analysis (statistical or other appropriate methods, e.g. for qualitative research) by which the data will be evaluated to meet the study objectives.

The skin swabs (samples of bacteria naturally present on skin) will be subjected to bacterial DNA extraction and then the qPCR will be carried out on the extracted DNA. Next generation sequencing will be used to determine the types and quantity of bacteria present.
The data derived from the molecular analysis of the samples will be assessed by a comparative analysis software. Counts per bacterial genus will be normalized, assessed by principal components analysis and differential analysis assessed by appropriate linear modelling and false discovery rate correction. This will give us data on the types of bacteria present, and the changes that haveoccurred between sampling time points.
Student t test will be used to analyse the difference of UVR-erythema (redness) dose response between the active group and control group.
The student applicant of this project Miss Wen Duan will be carrying out the sample processing and data analysis as part of her PhD.

E12 Details of Participants

E12. What is the maximum number of participants you plan to recruit (including, if relevant, the potential for dropout)?

An estimate will be required by the University's Insurance Office. Please make every effort to provide this.

If there is more than one group please state how many participants will be recruited for **each group** in a separate line by clicking the 'Add Another' button.

Additionally, if you have more than one group you must also add a line to show the overall total number of participants

For international studies, say how many participants will be recruited in **each country and in total**.

If you are unable to provide an accurate estimate due to extenuating circumstances, please enter a value of 1 and provide a justification in the text box for **E12.1** below.

Number of Participants	Description of Participants
10	Healthy male and female adults.

E12.1 If you are unable to provide an accurate estimate of participants above, please provide a justification below:

Please note: This option is usually **only applicable** to those conducting global surveys, large scale questionnaires or large scale social media data projects. When possible, please try to provide a rough ballpark estimate.

NA

E13

E13. Age range of participants

18-65 years (The composition of skin microbiota is different between children, adults and older people. This study only focuses on the skin microbiota ofadult.)

E14-15

E14. Please list and justify the inclusion criteria for participants.

Participants to be included in the study must:
• Aged 18-65 years of any gender without any skin disorders;
• White Caucasian (sun-reactive skin types I-III): inclusion of a mixed population would increase heterogeneity and thus the number of subjects required;
• Be willing and physically able to carry out all study procedures;
• Be willing and competent (verbally and cognitively) to give written informed consent.

E15. Please list and justify the exclusion criteria for participants.

• Diagnosis or history of photosensitivity;
• Sensitivity to alcohol-based skin preparation;
• A history of existing skin disorders/diseases, such as eczema, xerosis (dry skin), psoriasis, skin lesions etc;
• History of skin cancer;
• Sunbathing or sunny holiday with exposure to planned irradiation site;
• Usage of photoactive, anti-inflammatory medication or antimicrobial medications;
• Participated in any other study in the last 3 months;
• Unable to comply with the requirements of the study.

E16

E16. Will the participants be from any of the following groups?

Tick all that apply

- [x] Adult healthy volunteers (i.e. not under medical care for a condition which is directly relevant to the application)
- [ ] Children under 16 years
- [ ] Adults with learning difficulties
- [ ] Adults who have a terminal illness
- [ ] Adults with mental illness (particularly if detained under mental health legislation)
- [ ] Adults with dementia
- [ ] Adults in care homes
- [ ] Adults or children in emergency situations
- [ ] Prisoners
- [ ] Young offenders
- [ ] Those who could be considered to have a particularly dependent relationship with the researcher (i.e. students taught or examined by the researcher)
- [ ] Other vulnerable groups
- [ ] None of the above

E17

E17. How will the potential participants be identified, approached and recruited?

Please structure your answer into a bulleted list and include specific details regarding how you will identify, approach and recruit participants.

We will recruit participants using a database of previous participants who have consented to their contact details being stored and to being contacted to be informed about future research studies. Potential participants will be invited to participate and sent the Participant Information Sheet if they request more information.
Additionally, participants will be recruit through advertisements placed in notice boards of The University of Manchester, community locations (e.g. libraries, shops), and online (University and Trust websites)
Volunteers will be invited to contact the research staffs to obtain more details of the study prior to recruitment and will only be enrolled onto the study following their written informed consent.

E17.1 Will you be using any of the following to recruit your participants?

- Advertisements
- Emails
- Letters
- Social media postings
- Other forms of recruitment text (e.g. newspaper articles, etc)

Important note: DO NOT include monetary amounts on any advertisement*.

*See help bubble for further information

(•) Yes
( ) No

E17.2 Please attach copies of all emails/letters/text/advertisements that will be used to approach and recruit your potential participants.

Documents

Type	Document Name	File Name	Version Date	Version	Size
Advertisement	Advertisement-2	Advertisement-2.docx	12/03/2020	2	41.9 KB

E18

E18. What is the expected total duration of participation in the study for each participant?

For ethnographic research focusing on one or more groups rather than individual participants, indicate the approximate period of time over which research will focus on each group.

We estimate the total duration of participation for each subject will be up to 2 days.

E19

E19. What is the potential benefit to research participants?

The participant will help researchers to further our knowledge of the role of human skin microbiota on protecting skin against UV light.

E20

Reference #: 2020-8539-13844

E20. Will individual research participants receive reimbursement of expenses or any other incentives or benefits for taking part in this research?

- (•) Yes
- ( ) No

E20.1 Please indicate how much on what basis this has been decided and when participants will be informed of this:

Participants who complete the study will receive £30 for the time, inconvenience and cost (travel) of participation. If a participant is withdrawn by the researcher, they will receive the pro-rata payment (£10 for the visit1 and £20 for the visit2). If they decide not to turn up to the second visit, then they will not receive payment.

E21 Details of Funding

E21. Has funding (internal or external) for the research been secured?

- (•) Yes
- ( ) No

E21.1 Please provide details of the following items:

- Organization
- UK contact at the funding organization
- Amount (£)
- Duration in months
- RMS Reference (if applicable)

The University of Manchester provides a funding of £5000 per year for 36 month, and the internal lab funds are also available for this project (None of the following items are relevant with the internal lab funds as it comes from the income of relevant work of Prof. Catherine O'neill).

E21.2 Please clarify the institution who will be the research governance sponsor for this project:

Please note this is usually The University of Manchester

The University of Manchester

E22 - 23 Details of Consenting Procedures

E22 Please attach a copy of your GDPR compliant participant information sheet(s)/script/summary of information page:

WARNING: Your application will be returned to you and incur substantial delays unless you use the new GDPR compliant templates. Please see the help bubble attached to this question for additional guidance.

For secondary data analysis studies only, please upload a copy of the permission letter from the data controller or external organisation in support of the project.

Documents

Type	Document Name	File Name	Version Date	Version	Size
Participant Information Sheet	participant information sheet (version2)	participant information sheet (version2).docx	12/03/2020	2	54.1 KB

E23 Will informed consent be directly or indirectly obtained from the research participants?

- (•) Yes
- ( ) No

E23.1 How will consent be obtained or verified?

- (•) Written consent
- ( ) Verbally
- ( ) Implied (with the return/submission of a completed questionnaire/survey)
- ( ) A combination of the methods listed above

E23.2 Please provide details of how consent will be obtained.

Please include details relating to **ALL** the methods of data collection/generation that involve participants including:

- how information will be given to participants
- the experience of those who will be taking informed consent
- steps to provide information to participants before the study takes place (e.g. participant information sheet, videos, interactive material)
- extra steps taken to assure the protection of vulnerable participants, including any arrangements to be made for obtaining consent from a legal representative

The trained member of the research team will explain the study to potential research participants. They will also be provided with participants information sheet detailing the conduct of the study. They will have as much time as they need to make their decision within the scope of the recruitment period. They can take the patient information sheet home to discuss the study with their family, friends
and / or general practitioner. The investigator/researcher will answer any questions the potential participant may have before the potential participant can make an informed decision.

E23.3 Please attach a copy of your GDPR compliant consent form(s)/script(s):

WARNING: Your application will be returned to you and incur substantial delays unless you use the new GDPR compliant templates. Please see the help bubble attached to this question for additional guidance.

For secondary data analysis studies only, please provide proof that the analysis you wish to perform falls within the original consent of data subjects.

Documents

Type	Document Name	File Name	Version Date	Version	Size
Consent Form	Consent form (version2)	Consent form (version2).docx	12/03/2020	2	35.5 KB

E24

E24. How long will the participant have to decide whether to take part in the research?

If less than 24 hours please justify

As long as necessary within the scope of the recruitment period and a minimum of 24 hours.

E25

E25. Please clarify any arrangements that have been/will be made in relation to the following:

- participants who might not adequately understand verbal explanations or written information given in English
- participants who have special communication needs (e.g. use of interpreters, etc)
- the use of translated documents (i.e. participant information sheets, consent forms, etc)
- providing a verification of translated documents

Ensure you read the information in the help bubble for specific requirements and expectations in relation to translated documents.

Only those who can read and understand English language will be recruited.

E26 Risks and Safeguards

E26. Please state where the research/data collection will take place including the city and country (even if this is the UK).

For insurance purposes it is **imperative that you include the city and country** where data collection will take place. If performing an online survey with respondents in a variety of countries please state 'global'.

If involving multiple methods, please indicate where the data generation for **EACH** will take place.

Photobiology Unit, Barnes Building, Salford Royal NHS Foundation Trust, Salford, Greater Manchester, UK.

E27 - 30

E27. For your research participants, please consider the potential for the following to occur:

- Adverse events
- Risks
- Hazards
- Pain or distress
- Inconvenience or changes to lifestyle
- Discussion of topics or issues that might be sensitive, embarrassing or upsetting
- Confidential information will be revealed due to their participation in the study (e.g. by participating in your study their colleagues will find out they suffer with their mental health)

We do not expect there to be any disadvantage from taking part. Sunburn threshold testing will result in a transient redness of the skin, but it will resolve over time.
All participants meet the criteria: have no history of sensitivity to alcohol, therefore we do not expect any adverse events from irradiation from alcohol. Only a small volume of ethanol will be used on the skin of participant, the safety manager has confirmed that the 70% ethanol is seen as safe and effective if it's in a small volume.
We do not expect to cause any pain or hazards to any participants by using swabs. All swabs used will be under guarantee during whole study. This method is safe and has been widely used in sampling the microorganisms of the skin.
There will be no irritation from gloves all the gloves are latex free in keeping with NHS policy.

E28. What precautions have been taken to minimise or mitigate the risks identified above?

Participants with history of photosensitivity disorder and sensitivity to 70% ethanol will not be included in this research.

E28.1 If applicable, please attach a copy of your distress protocol or debrief sheet:

E29. Will any benefit or assistance such as medical care, social services or educational activities, which the participant would normally have access to, be withheld as part of the research?

- ○ Yes
- ◉ No

E30. If you are distributing questionnaires or conducting interviews or focus groups, is it possible that criminal or other disclosures requiring action could take place during the study (e.g. in the application of screening tests for drugs)?

- ○ Yes
- ○ No
- ◉ Not applicable as not distributing questionnaires or conducting interviews or focus groups

E31 - 32

E31. For the researchers themselves, please consider the potential for the following to occur:

- Adverse events
- Risks
- Hazards
- Pain or distress
- Inconvenience

None have been identified. Safe handling of UV lamps will be ensured through compliance with Photobiology Unit protocols which adhere to University of Manchester and NHS Trust guidelines.
All researchers will not work alone.

E32. What precautions have been taken to minimise or mitigate the risks identified above?

Please note, if applicable you should also include details as to how you will handle any possible disclosures and ensure this information is present in both the PIS and consent form.

No working alone and as above; working with safety protocols. Effective and timely communication before during and after the study with the Salford Royal Hospital Optical and UV Radiation Specialist Dr Donald Allan.

E32.1 Will your research involve you working away from the University of Manchester premises/facilities, working alone or working outside of normal working hours?

- ○ Yes
- ◉ No

E33

E33. I will report any adverse events (as described in the help bubble) to the Committee.

- ☑ I confirm the above declaration.

You **MUST** tick the box above in order to submit this form.

E34 Conflicts of Interest

Reference #: 2020-8539-13844

E34. Please confirm if any of the following apply:

- ☐ Individual researchers will receive any personal payment over and above normal salary or reimbursement of expenses for undertaking this research.
- ☐ The principal researcher or any other investigator/collaborator has direct personal involvement (e.g. financial, share-holding, personal relationships, etc) in the organisation sponsoring or funding the research, or any other reasons, that may give rise to a possible conflict of interest
- ☐ The host organisation or the researcher's department(s) or institution(s) will receive any payment of benefits in excess of the costs of undertaking the research
- ☑ None of the above

E35 Reporting Arrangements

E35. How is it intended the results of the study will be reported and disseminated?

- ☑ Peer reviewed academic journals
- ☐ Book or contribution to a book
- ☐ Other published outlets (e.g. ESRC, Cochrane Review or NIHR published funder report)
- ☑ Thesis/dissertation
- ☑ Conference presentation
- ☑ Internal report
- ☐ Other (e.g. videos, interactive website)

E36 - 37

E36. How will the results of research be made available to research participants and communities from which they are drawn?

- ☐ Presentation to participants or relevant community groups
- ☑ Written feedback to research participants
- ☐ Other (e.g. videos, interactive website)
- ☐ No feedback will be given

E36.1 Please provide details of the feedback:

Copies of publications arising from this work and a lay summary will be provided to participants upon request.
A lay summary will also be provided to participants upon request in the absence of publication.

E37. Please confirm if any of the following apply:

- ☐ Dissemination will allow identification of individual participants
- ☐ Dissemination will involve publication of extended direct quotations (in which participants can be directly or indirectly identified from the context) from participants
- ☐ Dissemination will involve distribution of audio-visual media in which identified participants play leading roles
- ☑ None of the above

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E38-39 Additional Attachments and Final Declaration

Please use this section to attach any additional documentation that you have not attached previously in this form. If you do not need to attach any additional supporting documentation, please tick the box at the bottom of the page.

Please do not attach a copy of your research protocol as this is not required by the Ethics Committees.

The supporting documents that you may have already been required to attach are:

- Interview guide
- Focus group topic guide
- Questionnaire(s)
- Statistical review
- Advertisements/e-mails/recruitment text
- Consent form(s)
- Participant information sheet(s)
- Distress protocol/debrief sheet
- Lone worker policy/procedure

Examples of documentation that you may wish to attach include, but are not limited to:

- Translated documents
- Verification of translated documents
- Letters from gatekeepers
- Letters of permission from research sites
- Social media recruitment text
- Confidentiality agreements
- Ethical approval from partnering institutions
- Local insurance arrangements
- Completed risk assessment forms

E38. Additional supporting documentation

Documents

Type	Document Name	File Name	Version Date	Version	Size
Additional docs	gcp-certificate	gcp-certificate.pdf	18/11/2019	1	92.5 KB
Additional docs	risk assessment form	risk assessment form.docx	10/01/2020	1	46.8 KB
Additional docs	Case Report Form	Case Report Form.pdf	12/03/2020	1	353.5 KB
Additional docs	Advert_Poster template	Advert_Poster template.docx	12/03/2020	2.0	41.7 KB
Additional docs	Revision form	Revision form.docx	12/03/2020	1	230.0 KB

☑ I confirm that all required supporting documentation for this project is appended.

E39. In order for your application to proceed to review, please confirm the following:

- To the best of my knowledge the information that I have provided here is accurate and I understand that any deliberate attempts to withhold necessary information or mislead the UREC will result in my project being given an unfavourable decision.

☑ I confirm the above declaration.

You **MUST** tick the box above in order to submit this form.

Reference #: 2020-8539-13844

Required Signatures

Final Declarations

1. The information in this form is accurate to the best of my knowledge and belief and I take full responsibility for it.
2. I agree to abide by the ethical principles underlying the Policy on the Ethical Involvement of Human Participants in Research and the University's Code of Good Research Conduct.
3. If the research is approved I agree to adhere to the terms of the full application as approved and any conditions set out by the review body in giving approval.
4. I agree to notify the review body of any amendments to the terms of the approved application (both minor and major), and to seek a favorable opinion from that review body via the formal process before implementing the amendment.
5. I agree to submit annual progress reports setting out the progress of the research as well as end of study reports, as required by the review body for all UREC proposals.
6. I understand that research records/data may be subject to inspe ction by the review body for audit purposes. In addition, I understand that research records/data for those studies that use human tissue, medical devices or pharmaceutical products may be subject to inspection by regulatory authorities for audit purposes.
7. I understand that the information contained in this application, any supporting documentation and all correspondence with the review body or its operational managers relating to the application
   Will be held by the University until at least 5 years after the end of the study or at least 10 years for those studies involving medical data.
   May be disclosed to the operational managers of the review body in order to check that the application has been processed correctly or to investigate any complaint
   May be seen by auditors appointed to undertake accreditation of the University (where applicable)
   Will be subject to the provisions of the Freedom of Information Act and may be disclosed in response to request made under the Act except where statutory exemptions apply
   May be sent by email to members of the review body
8. I understand that information relating to this research, including the contact details on this application, will be held by Infonetica Ltd, and that this will be managed according to the principles established in the Data Protection Act 2018.
9. I confirm that I have not included any sensitive personal information including a curriculum vitae or identifiable information about my racial or ethnic origin, political opinions, religious or similar beliefs, trade union membership, physical or mental health, sexual life, commission of offenses and/or criminal proceedings.

IMPORTANT: Please ensure you request the signatures of the PI or supervisor (if required).

The system now features an automatic submission function which will automatically submit your application (usually within 60 seconds) after all required signatures are obtained as described below.

If you are signing an application, please ensure you remain signed into the ERM system until the screen refreshes and you receive email confirmation that a) your signature has been accepted and b) your application has been successfully submitted.

If you do not receive an email confirmation within 1 hour of signing the form, please perform the following:

1. Open the application and double check the form status as it should be listed as submitted, resubmitted or sent to. If the status is one of these, please email your Ethics Signatory or School Administrator to double check that they have received your application.
2. If the form status is listed as 'changes requested', 'not submitted' or 'returned' then please double check:
   a. That an appropriate signature has been obtained in Section S (it should say for example: Mr Smith has signed on 5/7/2019 at 13.15pm)
   b. That no additional blank signature boxes are listed in Section S
   c. That the application is not pending a mandatory update (listed in a red bar at the top of the screen)
   d. If you have performed all of these checks and the application has still not automatically submitted, please email research.ethics@manchester.ac.uk and provide your project reference number, title and a screenshot confirming these criteria and a member of the team will be able to assist you.

WARNING: Once you have signed the form, it will be locked and if you wish to make further changes you must 'unlock' the form, which will break any signatures already obtained.

For staff projects, if you are NOT the PI, you must obtain their signature (using the request button below).

For student projects, if you are NOT the supervisor, you must obtain their signature (using the request button below).

For student projects, if you ARE the supervisor please ensure you sign the form.

Signature of the Primary Supervisor

To sign this form please look on the left hand side of your screen for an action button called Sign that has a picture of a pencil on it. Please push this button and this button only to sign the form.

Please note that if you are the student requesting your supervisor's signature that by pressing this request button you are confirming that the application is complete, accurate to the best of your knowledge and ready to be signed off by your supervisor for further processing by relevant Division/School/UREC colleagues.

Signed: This form was signed by Prof Catherine O'Neill (catherine.o'neill@manchester.ac.uk) on 03/04/2020 15:45

Reference #: 2020-8539-13844

Appendix 3 Participant information sheets

Influence of the Skin Microbiome on the Sunburn Response in Healthy Humans *in vivo*?

Participant Information Sheet (PIS)

You are being invited to take part in a research study which will survey whether the reduction of the skin microbiome increases the sunburn response in healthy people. This research is part of the PhD project of Miss Wen Duan. Before you decide whether to take part, it is important for you to understand why the research is being conducted and what it will involve. Please take time to read the following information before deciding whether to take part and discuss it with others if you wish. Please ask if there is anything that is not clear or if you would like more information. Thank you for taking the time to read this.

About the research

- **Who will conduct the research?**

This research will be conducted by Wen Duan, PhD Researcher and Mrs Joanne Osman, Research Nurse experienced in Photobiology research, in the Division of Musculoskeletal & Dermatological Sciences, School of Biological Sciences, Faculty of Biology, Medicine and Health, the University of Manchester.

- **What is the purpose of the research?**

The surface of skin is colonized with an abundance of microorganisms, forming a unique skin microbiome. They play a significant role in maintaining normal skin metabolism, however in some situations they cause some skin diseases as well. During the whole life span of a human, skin cells and skin microorganisms are exposed to an external environment, including to ultraviolet radiation (UVR) in sunlight. The effects of UVR on skin cells has been well studied but there are few studies focusing on the effect of UVR on the skin microbiome. Since the skin

microorganisms are important for our skin health, it is valuable to survey the impact of UVR on them, and how in turn, this may influence our skin cells.

Specially, researchers have showed the microorganisms normally found on skin have different UVR sensitivity; some species, which are predominantly found on the outermost layer of skin, could be absorbing UV radiation high up in there. Hence, we hypothesize that reducing the skin microbiome will increase UV effects in human skin, such as UVR-induced erythema. The data of this research could help us elucidate the role of skin normal microbiota better, and also could lead us a better understanding of some UV-related skin disorders.

- **Why have I been chosen?**

This research will involve up to 10 healthy white Caucasian adults who are between 18-65 years old and have no history of sensitivity to UVR or ethanol solution. We are inviting you to take part in this research as you accord with the recruitment criteria.

- **Will the outcomes of the research be published?**

If the results are published in the future, you will be able to request a summary of this from the study team. You will not be identified in any publication of these results.

- **Who has reviewed the research project?**

This project has been reviewed by The University of Manchester Research Ethics Committee 2.

<u>What would my involvement be?</u>

- **What would I be asked to do if I took part?**

This study involves 2 visits to the Photobiology Unit, located within Barnes Building, Salford Royal Hospital, Stott Lane, Salford M6 8HD. The whole study will be finished in 2 days. If you are interested in taking part, some of your personal information (age, the history of skin disorders and the brief skin reaction to sun exposure) will be asked via the telephone in order to helping us estimate your eligibility.

Visit 1

If you appear suitable, you will be invited to attend an initial visit in which your skin phototype will be assessed. You will have the opportunity to ask questions and have them answered, and you are happy to proceed informed consent will be taken. The first part of the visit can take up to 90 minutes.

The right side of your mid-back skin will be cleaned with 70% ethanol solution. The skin microbiota sample will be collected using a simple skin swab immediately before and immediately after the ethanol cleansing. Both the cleansed area and an equivalent area of the left back will be exposed to a UVR lamp. A series of 10 UVR doses (which is roughly equivalent to 11 mins 48 secs -1 hour 58 mins in Manchester summer sun) will be given as is our standard practice to establish your sunburn threshold. For each dose, a 1 cm^2 area will be exposed to the UVR; this will be ten 1 cm sites in total to each side of your back.

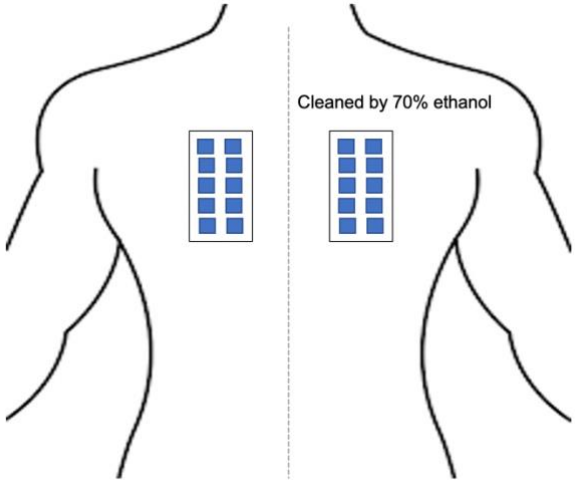

Figure 1 A diagrammatic drawing of the template for standard sunburn assessment test.

Ten x 1 cm^2 area of mid-back skin will be exposed to UVR (both on the ethanol-cleaned and control side).

Six hours later, you will be required to return to the Photobiology Unit and your sunburn response will be assessed and measured.

Visit 2

You need to return at the same time point 24 hours later after the UVR was applied when the sunburn response will be measured a second time.

All skin swabs will be used for bacterial DNA extraction procedures within 7 days of collection. No identifiable human materials will be retained. The bacterial DNA will be analysed to determine the bacterial abundance pre/post the ethanol cleaning .

- **What are the possible disadvantages or risks of taking part?**

You will be informed not to expect individual benefit from participation in the study. Initial UVR testing to determine the minimal erythemal dose (sunburn threshold) will result in transient redness which may become tanned depending upon your skin type. It will resolve over time.

- **Will I be compensated for taking part?**

Volunteers completing the study will receive reimbursement of £30 upon completion of their participation. Reimbursement is for travel costs, which is made at a flat rate, and for the time and inconvenience of taking part. If you do not complete the study, reimbursement will be pro-rata based on the number of visits made and samples provided: If you are withdrawn by the researcher, you will receive a pro-rata payment (£10 for the visit1). If you decide not to turn up to the second visit, then you will not receive payment.

- **What happens if I do not want to take part or if I change my mind?**

You are entirely voluntary in this study; it is up to you to decide whether or not to take part. If you do decide to take part, you will be given this information sheet to keep and will have the opportunity to ask questions. You will then be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time without giving a reason and without detriment to yourself. However, it will not be possible to remove your data from the project once it has been anonymised as we will not be able to identify your specific data. This does not affect your data protection rights. If you decide not to take part, you do not need to do anything further.

<u>Data Protection and Confidentiality</u>

- **What information will you collect about me?**

In order to participate in this research project, we will need to collect information that could identify you, called “personal identifiable information”. Specifically, we will need to collect (These will be recorded in the Case Report Form (CRF)):

1. Name
2. Gender
3. Date of birth
4. Age

- **Under what legal basis are you collecting this information?**

We are collecting and storing this personal identifiable information in accordance with data protection law which protect your rights. These state that we must have a legal basis (specific reason) for collecting your data. For this study, the specific reason is that it is “a public interest task” and “a process necessary for research purposes”.

- **What are my rights in relation to the information you will collect about me?**

You have a number of rights under data protection law regarding your personal information. For example, you can request a copy of the information we hold about you.

If you would like to know more about your different rights or the way we use your personal information to ensure we follow the law, please consult our Privacy Notice for Research.

The full URL of the privacy notice can be found from: http://documents.manchester.ac.uk/display.aspx?DocID=37095

- **Will my participation in the study be confidential and my personal identifiable information be protected?**

In accordance with data protection law, The University of Manchester is the Data Controller for this project. This means that we are responsible for making sure your personal information is kept secure, confidential and used only in the way you have been told it will be used. All researchers are trained with this in mind, and your data will be looked after in the following way:

Only the study team at The University of Manchester will have access to your personal information, but they will anonymise it as soon as possible. Your name and any other identifying information will be removed and replaced with a random ID number. Only the research team will have access to the key that links this ID number to your personal information. Your consent form and contact details will be retained in the University of Manchester for 5 years following

the end of this study. All records will be held in locked offices and secure servers at the University of Manchester.

Please also note that individuals from The University of Manchester or regulatory authorities may need to look at the data collected for this study to make sure the project is being carried out as planned. This may involve looking at identifiable data. All individuals involved in auditing and monitoring the study will have a strict duty of confidentiality to you as a research participant.

What if I have a complaint?

Contact details for complaints

If you have a complaint that you wish to direct to members of the research team, please contact:

Prof. Catherine O'Neill.

Tel: 0161 275 5760

E-mail: catherine.o'neill@manchester.ac.uk

If you wish to make a formal complaint to someone independent of the research team or if you are not satisfied with the response you have gained from the researchers in the first instance then please contact:

The Research Governance and Integrity Officer, Research Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL, email: research.complaints@manchester.ac.uk or telephone: 0161 275 2674.

If you wish to contact us about your data protection rights, email dataprotection@manchester.ac.uk or write to The Information Governance Office, Christie Building, The University of Manchester, Oxford Road, Manchester, M13 9PL and we will guide you through the process of exercising your rights.

You also have a right to complain to the Information Commissioner's Office about complaints relating to your personal identifiable information Tel: 0303 123 1113

The full URL of the ICO's complaints procedure can be found from: https://ico.org.uk/

Contact Details

If you have any queries about the study or if you are interested in taking part then please contact the researcher(s)**:**

Joanne Osman, Research Nurse Tel: 0161 2060457

E-mail: joanne.osman@manchester.ac.uk

OR

Wen Duan, PhD Researcher Tel: 0161 2755297

E-mail: wen.duan@postgrad.manchester.ac.uk