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BAKALÁŘSKÁ PRÁCE

Vliv UV záření na kůži studované pomocí ultra-slabé emise fotonů

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Vedoucí bakalářské práce: doc. RNDr. Pavel Pospíšil, Ph.D.

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BACHELOR THESIS

Effects of UV radiation on skin – ultra-weak photon emission study

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Study programme: Biophysics

Supervisor: doc. RNDr. Pavel Pospíšil, Ph.D.

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Abstrakt:

Bakalářská práce byla zaměřena na studium vlivu UV záření na kůži pomocí ultra-slabé emise fotonů. Dopad UV záření na kůži způsobuje tvorbu reaktivních forem kyslíku, které mohou oxidovat biomakromolekuly a tím způsobovat oxidativní poškození organismu. Tvorba elektronově-excitovaných stavů a následná oxidace biomakromolekul je spojena s vyzářením velmi malého množství fotonů, tzv. ultra-slabá emise fotonů. Cílem bakalářské práce bylo studovat vliv UV záření na kůži a vzniklé oxidativní poškození pomocí dvou dimenzionálního zobrazování ultra-slabé emise fotonů. Práce potvrdila teorii, že melanin v kůži může být excitován absorpcí fotonů emitovaných jinými excitovanými molekulami. Práce také ukázala, že zobrazování ultra-slabé emise fotonů je užitečná a kvalitní metoda pro studium oxidativního poškození v lidské kůži.

Klíčová slova: ultra-slabá emise fotonů, reaktivní formy kyslíku, melanin, UV záření, kůže

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Abstract:

The bachelor thesis was focused on the study of ultra-weak photon emission from UV irradiated skin. UV irradiation of the skin leads to a formation of reactive oxygen species, which oxidize biomacromolecules and cause oxidative damage to the organism. Formation of electron-excited states and subsequent oxidation of biomacromolecules is accompanied by an emission of very small amounts of photons, called an ultra-weak photon emission. The aim of this thesis was to study the effect of UV radiation on the skin and resulting oxidative damage using two-dimensional ultra-weak photon emission imaging. The thesis confirmed the theory, that melanin can become excited through absorption of photons emitted by other excited molecules. The thesis also showed that ultra-weak photon emission imaging is a useful method to study oxidative damage in human skin.

Key words: ultra-weak photon emission, reactive oxygen species, melanin, UV radiation, skin

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Prohlašuji, že jsem bakalářskou práci na téma „Vliv UV záření na kůži studované pomocí ultra-slabé emise fotonů“ vypracoval samostatně pod vedením doc. RNDr. Pavla Pospíšila, Ph.D. za použití literatury uvedené v závěru práce.

V Olomouci dne…….. ……

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I declare that I have written the bachelor thesis titled „Effects of UV radiation on skin – ultra-weak photon emission study" individually under the guidance of doc. RNDr. Pavel Pospíšil, Ph.D. using sources and references cited at the end of the thesis.

In Olomouc …….. ……

CONTENT

LIST OF ABBREVIATIONS

CAT	catalase
CCD	charge-coupled device
DMSO	dimethyl sulfoxide
EPR	electron paramagnetic resonance
GPx	glutathione peroxidase
GSH	glutathione
H_2O_2	hydrogen peroxide
$HO^{\bullet}$	hydroxyl radical
$HO_2^{\bullet}$	hydroperoxyl radical
HPLC	high-performance liquid chromatography
$L^{\bullet}$	lipid alkyl radical
$LOO^{\bullet}$	lipid peroxyl radical
LOOH	lipid hydroperoxide
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
$^1O_2$	singlet oxygen
$O_2^{\bullet -}$	superoxide anion radical
$[^3(R=O)^{\bullet}]$	triplet excited carbonyl
$RO^{\bullet}$	alkoxyl radical
$ROO^{\bullet}$	peroxyl radical
ROOOOR	tetroxide
ROOR	dioxetane
ROS	reactive oxygen species
SOD	superoxide dismutase

1. INTRODUCTION

This thesis focuses on the effects of ultraviolet radiation on skin using ultra-weak photon emission. Exposure of the skin to ultraviolet radiation leads to formation of reactive oxygen species.

Reactive oxygen species are highly reactive molecules with one or more oxygen atoms. Reactive oxygen species are produced in biological systems during metabolic processes and play an important role in some biological processes such as cell signalling. Increase in formation of reactive oxygen species causes oxidation of important biomacromolecules such as lipids and proteins. This higher number of reactive oxygen species being formed can be a result of inability of antioxidants, which function as defence of organism against oxidative damage, to scavenge them. They can also be formed in higher numbers because of the exposure to stresses such as heat, physical damage, ultraviolet radiation, and others.

Oxidation of biomacromolecules in amounts not neutralizable by antioxidants leads to a chain reaction, during which an increasing number of biomacromolecules is oxidized and destroyed in the process. Such oxidation in skin leads to skin ageing, inflammation, or in some cases even skin cancer.

Relaxation of excited molecules to grounds state during oxidative damage is accompanied by an emission of a very small number of photons, this process is called ultra-weak photon emission. Ultra-weak photon emission can be divided into two categories, spontaneous, which occurs during metabolic processes in biological systems, and stress induced, which is initiated by external stresses such as ultraviolet radiation. Ultra-weak photon emission imaging uses highly sensitive cameras to study the formation of reactive oxygen species and their damage to organism.

This thesis is divided into three main parts. Theoretical part which focuses on reactive oxygen species, their damage to biomacromolecules and their influence on ultra-weak photon emission. Methodical part focusing on methods and materials used in the measurements and result and discussion part, which focuses on demonstration and discussion of results made during experimental part of the thesis.

2. THEORY

2.1 Ultraviolet radiation

Ultraviolet (UV) radiation is electromagnetic radiation found between x-ray and visible radiation, its wavelength is between 10-400 nm. Excessive exposure of the skin to UV radiation results in weakened skin immune system and leads to changes on cellular level (Clydesdale *et al.* 2001).

Natural cause of human skin being exposed to UV radiation is exposure to sun rays during outdoor activities without the use of protective measures (clothes, umbrellas, …). Intensity of UV radiation on the skin is highly dependent on geographical location (D'Orazio *et al.* 2013). UV radiation is absorbed and reflected by Earth's atmosphere, therefore intensity is lower, if it must pass through thicker atmosphere, resulting in the highest intensity of UV passed through the atmosphere being around the equator, in higher altitudes and in parts of Earth, where clouds are scarce.

In recent years, there has been an increase in skin damage due to exposure to artificial UV light produced by tanning booths. Tanning booths are known to increase risk of damage to the skin and skin cancer. Tanning booths are more frequently visited by children and adolescents, mainly in countries of Northern Europe and in the United States of America (Doré and Chignol 2012).

Ultraviolet radiation induces several cellular and molecular changes when it comes to contact with the skin. When UV penetrates the skin, it causes reactions leading to formation of ROS, which are responsible for oxidation of biomacromolecules.

2.1.1. UV components

2.1.1.1. UVA

UVA is a long-waved component of UV radiation, located between 315-400 nm and has the lowest energy of all UV radiation components. Ozon layer very efficiently absorbs other components of UV radiation, but has only a small effect on UVA, which results in over 90 %

of UV radiation not blocked by the atmosphere being UVA. UVA easily penetrates skin and causes formation of reactive oxygen species (D'Orazio *et al.* 2013).

2.1.1.2. UVB

UVB is located between 315-400 nm. About 97 % of UVB from the Sun is absorbed by ozone in the atmosphere (Turtoi and Borda 2013). UVB penetrates skin less efficiently than UVA because of its wavelength, most of it is absorbed by upper parts of skin. Absorption of UVB by skin results in molecular changes leading to skin cancer, mutations, and sunburn (D'Orazio *et al.* 2013). Absorption of UVB through the eye causes damage to cornea, rods, and cones, which can result in loss of eyesight. It is necessary to use protective goggles when working with UV radiation (van Kuijk 1991).

2.1.1.3. UVC

UVC is a component of UV radiation located between 200-280 nm and is completely absorbed by the ozone layer. UVC irradiation is an extensively used method for sterilization. UVC is absorbed by nucleic acids and nucleus leading to genetic material damage and its destruction or malfunction (Dai *et al.* 2012).

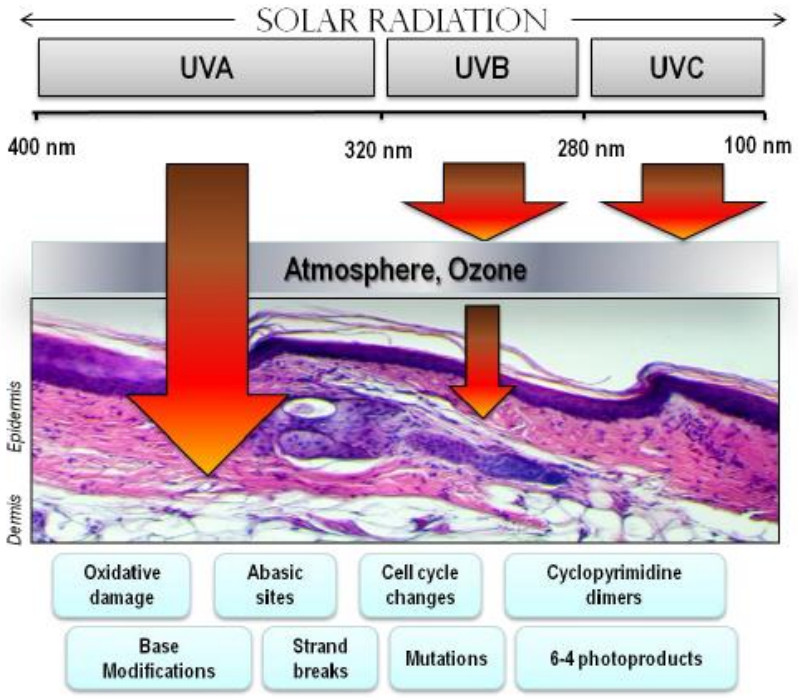

Figure 1: Ultraviolet radiation spectrum, its components and effects on skin (D'Orazio *et al.* 2013).

2.2. Reactive oxygen species

Reactive oxygen species (ROS) are molecules with one or more oxygens. In the human body, they are naturally produced by electron transport in mitochondria, breakdown of fatty acids and amino acids in microbodies and during protein folding in the endoplasmic reticulum. In plants, they are produced during energy transfers and electron transports in chloroplasts. They are extremely reactive and cause oxidation of biomacromolecules.

ROS are separated into radical and non-radical forms. Radical ROS contain unpaired electrons in their electron shells. Radical ROS members are superoxide anion radical ($O_2^{\bullet -}$), hydroperoxyl radical ($HO_2^{\bullet}$), hydroxyl radical ($HO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$) and high energy singlet oxygen ($^1O_2$). Non-radical ROS contain paired electrons, members are hydrogen peroxide (H_2O_2), hypochlorous acid anion, ozone, and low energy $^1O_2$.

2.2.1. Reactive oxygen species – overview

2.2.1.1. Superoxide anion radical

Superoxide anion radical is a two-atom molecule with an unpaired electron in oxygen atom. It is a base, meaning it can accept protons. Accepting a proton by $O_2^{\bullet -}$ leads to formation of $HO_2^{\bullet}$, which is capable of oxidation. Superoxide anion radical can be both oxidant and reductant depending on the other atom or molecule present in the redox reaction. Lifetime of $O_2^{\bullet -}$ is in microseconds (Gechev *et al.* 2006).

Physical ways to create $O_2^{\bullet -}$ are irradiation of water by gamma radiation, called radiolysis, photolysis, process where water is irradiated by ultraviolet light, oxidation of dimethyl sulfoxide and acetonitrile and irradiation of pigments (D) by UV or visible light, called photosensitive reaction type I (Figure 2).

$$D + UV\ radiation \dashrightarrow {}^1D^{\bullet} + {}^3D^{\bullet}$$

$${}^3D^{\bullet} \dashrightarrow D^{\bullet+} + D^{\cdot-}$$

$$D^{\bullet-} + O_2 \dashrightarrow O_2^{\bullet-}$$

$$O_2^{\bullet-} + O_2^{\bullet-} \dashrightarrow H_2O_2$$

Figure 2: Photosensitisation reaction type I.

One of the places, where $O_2^{\bullet-}$ is formed is in mitochondria by reduction of molecular oxygen in complexes I and III. In complex I reduction of molecular oxygen is caused by nicotinamide adenine dinucleotide (NADH), in complex III reduction is caused by coenzyme Q10. In plants, reduction of molecular oxygen takes place in photosystems I and II in chloroplasts. Another organelle, where $O_2^{\bullet-}$ is formed is a plasmatic membrane, where molecular oxygen is reduced by NADPH oxidase. Last organelle where $O_2^{\bullet-}$ is formed are microbodies, reduction here is induced by xanthine oxidase.

2.2.1.2. Hydrogen peroxide

Hydrogen peroxide is a molecule containing two hydrogen atoms and two oxygen atoms. It's a weak acid and can act as both reductant and oxidant. Lifetime of H_2O_2 ranges from milliseconds to seconds. Because of its relatively long lifetime, it can leave and enter cells through cellular walls.

Formation of H_2O_2 by physical means is similar to the formation of $O_2^{\bullet-}$, photolysis, radiolysis, and photosensitisation reaction type I (Figure 2). Chemically, H_2O_2 is formed on cathode in sulfuric acid electrolysis.

In biological systems H_2O_2 is formed by dismutation, which is a redox reaction of two $O_2^{\bullet-}$ leading to formation of H_2O_2 and molecular oxygen (Figure 3). One of the organelles where H_2O_2 is formed is mitochondria by enzymatic dismutation. First enzyme catalysing dismutation is Cu-Zn superoxide dismutase (Cu-Zn SOD), which catalyses dismutation in intermembrane space. Second enzyme is Mn superoxide dismutase (Mn SOD) catalysing dismutase in matrix. In plants, dismutation takes place in chloroplasts. Hydrogen peroxide is also formed in the matrix of plasmatic membrane, in microbodies and in the endoplasmic reticulum.

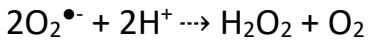

Figure 3: Dismutation of superoxide anion radicals.

2.2.1.3. Hydroxyl radical

Hydroxyl radical is a two-atom molecule with an unpaired electron located on oxygen. It is the most reactive member of all ROS and is extremely dangerous to proteins, lipids and other biomacromolecules, which it attacks immediately after its formation (Schmitt *et al.* 2014). It is oxidant and its lifetime is really low, about one nanosecond. Because of its low lifetime, it is incapable of diffusion out of cells.

Physical way of forming $HO^{\bullet}$ is through photolysis. Chemically, it is created by a Fenton reaction or a reduction of H_2O_2 by $O_2^{\bullet-}$, called Haber-Weiss reaction. In biological systems $HO^{\bullet}$ is formed by a Fenton reaction (Schmitt *et al.* 2014) of free transition metal with H_2O_2 or by Fenton reaction of metal found in metalloproteins with bound H_2O_2 (Figure 4).

Figure 4: Fenton reaction in biological system with bound hydrogen peroxide.

2.2.1.4. Singlet oxygen

Singlet oxygen is a molecular oxygen in singlet excited state. It exists in two forms, high energy $^1O_2$ has a small lifetime and quickly transfers to a low energy excited state. It contains unpaired electron and therefore is a member of radical ROS. Low energy $^1O_2$ is more stable than high energy $^1O_2$ and contains no unpaired electrons. Lifetime of $^1O_2$ is dependent on its current form. Gas form has a lifetime of over 70 minutes, liquid form's lifetime is dependent on the solvent in the solution. Lifetime of $^1O_2$ in water solution is approximately 3,1 μs (Egorov 1989).

In plants, $^1O_2$ is formed in leaves, during intersystem crossing of triplet excited chlorophylls to singlet excited state in the thylakoid membrane. Formation of $^1O_2$ in the human body is linked with energy transfers and intersystem crossings of skin and eye pigments.

2.2.2. Formation of reactive oxygen species

2.2.2.1. Formation of ROS by redox reactions

Redox reaction is a chemical reaction during which electrons are transferred. If an atom or molecule loses electron, reaction is called oxidation, if it gains electron, it is called reduction. First redox reaction resulting in formation of ROS is a reduction of molecular oxygen (Figure 5).

$$O_2 \dashrightarrow O_2^{\bullet -} \dashrightarrow H_2O_2 \dashrightarrow HO^{\bullet} \dashrightarrow H_2O$$

Figure 5: Reduction of molecular oxygen through ROS leading to formation of water.

One-electron reduction of molecular oxygen leads to the formation of $O_2^{\bullet -}$. Consequent reduction of $O_2^{\bullet -}$ and acceptance of proton leads to the formation of H_2O_2, this reaction can be catalysed by superoxide reductase among others. Second to last reaction is reduction of H_2O_2 and formation of $HO^{\bullet}$, for example Fenton reaction. Lastly, by reduction of $HO^{\bullet}$, water is formed. Reduction of $HO^{\bullet}$ in the organism is part of the redox reaction, where the oxidized component is a biomacromolecule. This redox reaction creates organic radical and water.

Second redox reaction causing formation of ROS is an oxidation of water, this reaction occurs as a part of light reactions in photosynthesis (Figure 6).

$$H_2O \dashrightarrow HO^{\bullet} \dashrightarrow H_2O_2 \dashrightarrow O_2^{\bullet -} \dashrightarrow O_2$$

Figure 6: Oxidation of water through ROS leading to formation of molecular oxygen.

Oxidation of water would form $HO^{\bullet}$, however, this reaction is thermodynamically impossible in biological systems. Oxidation of $HO^{\bullet}$ leads to the formation of H_2O_2, this reaction is possible thermodynamically, but is impossible kinetically. By losing an electron, H_2O_2 transforms into $O_2^{\bullet -}$ and consequent oxidation creates molecular oxygen.

2.2.2.2. Formation of ROS by energy transfer

ROS can be formed not only by a redox reaction, but also by energy transfer reactions. One of these reactions is called type II reaction, it's a reaction, where energy is transferred from pigment on molecular oxygen forming $^1O_2$. Another energy transfer reaction forming ROS is breakdown of peroxides.

Dioxetane (ROOR) is a peroxide formed by recombination of two ROO$^\bullet$, recombination of two RO$^\bullet$ or by cyclization of ROO$^\bullet$. Dioxetane breaks down into triplet excited carbonyl ([^{3}(R=O)$^\bullet$]), alcohol, and molecular oxygen. Triplet excited carbonyl then transfers its energy on molecular oxygen which becomes $^1O_2$ (Figure 7).

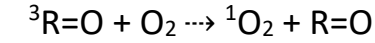

Figure 7: Energy transfer from ROOR to molecular oxygen leading to the formation of $^1O_2$.

Tetroxide (ROOOOR) is peroxide formed by a recombination of two ROO$^\bullet$ or two RO$^\bullet$. Tetroxide breakdown is similar to a breakdown of ROOR, [^{3}(R=O)$^\bullet$] is formed, it transfers its energy to molecular oxygen and $^1O_2$ is formed. Tetroxide can breakdown without any intermediates such as [^{3}(R=O)$^\bullet$]. During direct breakdown, ROOOOR breaks down into $^1O_2$, carbonyl, and alcohol (Figure 8).

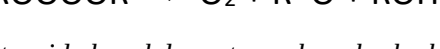

Figure 8: Tetroxide breakdown to carbonyl, alcohol, and $^1O_2$.

2.3. Formation of ROS in skin by UV radiation

Skin is the largest organ of human body and acts as a barrier against physical and chemical stresses (Albrecht *et al.* 2019). Skin irradiation by UV radiation leads to the formation of ROS. During excessive formation of ROS in the skin, the organism tries to scavenge them using antioxidants. When the amount of ROS in the skin is higher than the antioxidants are capable of scavenging, they start to attack biomacromolecules. These attacks cause oxidative

damage to skin, which manifests as skin ageing, inflammation or, due to genetic damage, even skin cancer.

UV irradiation of skin causes an increase in formation of ROS. These primary radicals are extremely reactive and interact with biomacromolecules such as lipids, proteins, or nucleic acids. This interaction causes damage to biomacromolecules resulting in them becoming highly reactive organic radicals (Albrecht *et al.* 2019). A model which replicated human skin was exposed to UVB radiation and spatial formation of ROS and formation of ROS in time was observed (Hakozaki *et al.* 2007). Authors detected formation of $^1O_2$ and $O_2^{\bullet-}$ by a chemiluminescent probe. They consider human equivalent skin model with a combination of used imaging techniques as good method for observation of ROS in skin.

2.3.1. Skin

Epidermis is the upper part of skin and is in direct contact with UV radiation, which the human body is exposed to. Epidermis is mostly composed of cells known as keratinocytes, these cells are tightly connected and act as a protective barrier of the body. Beside their protective function, keratinocytes also accumulate pigment melanin, which blocks UV radiation impacting skin and prevents its entrance deeper into the body (D'Orazio *et al.* 2013).

For practical reasons, human skin is not widely used in experiments, different types of animal skins are usually used. Choosing correct animal skin is influenced by many factors such as availability, price, and anatomical and functional similarities with human skin (Prasad *et al.* 2018). It was discovered that pig skin shares many similarities with human skin in morphology, composition, and its reaction to stimuli (Vardaxis *et al.* 1997). Due to these observations, low price and relatively easy means of acquisition, pig skin is an ideal substitute for human skin in studies.

2.3.1.1. Melanin

Melanin is a pigment, which is formed in special organelle called melanosome. These organelles are located in melanocytes, which form only a very small part of epidermal cells

(Brenner and Hearing, 2008). Melanin formed in melanocytes is transferred to keratinocytes, where it accumulates.

Melanin exists in many forms, most important forms are eumelanin, darker pigment found more in people with darker skin colour, and pheomelanin, lighter pigment (D'Orazio *et al.* 2013). Eumelanin is more effective at blocking UV radiation than pheomelanin (Vincensi *et al.* 1998), resulting in people with more eumelanin having darker skin and their skin being much less susceptible to damage from UV irradiation. Data exists that support the hypothesis that pheomelanin helps oxidative damage by forming free radicals in melanocytes even without UV irradiation (Mitra *et al.* 2012).

Electron paramagnetic resonance (EPR) spectroscopy was used to observe effects of UVA on non-pigmented, light-pigmented, and dark-pigmented melanocytes (Kassouf *et al.* 2018). Using spin traps, radicals were detected in all types of cells, however, the biggest number of radicals was detected in light-pigmented cells. Authors concluded that the melanin layer in skin reduces UVA damage and in lightly pigmented skin they observed that excess amount of UVA damages primarily basal cells.

2.3.2. Photosensitisation reaction type II

Reactive oxygen species in human skin form primarily by type II reaction. During this reaction, pigments in skin, mostly melanin, absorb excitation energy from UV radiation and becomes excited to singlet excited state. Afterwards, singlet excited pigments undergo intersystem transfer and become triplet excited pigment. These triplet excited pigments transfer their excitation energy to molecular oxygen, which is normally in triplet energy state, leading to the formation of $^1O_2$. This energy transfer is possible, because energy of pigments in triplet excited state is higher than singlet energy of molecular oxygen. Singlet oxygen after excitation exists in higher energy singlet state and lower energy singlet states. High energy singlet states are highly unstable and very quickly relax into lower energy singlet states, therefore lower energy singlet state of oxygen is commonly referred to as $^1O_2$.

2.4. Lipid peroxidation in skin

During excessive formation of ROS in the skin caused by stresses such as UV radiation and chemical damage, antioxidant systems in skin are unable to scavenge all formed ROS and ROS start to oxidise biomacromolecules. Lipid peroxidation is a well-known process of cell damaging in different organisms and is used as an indicator of the damage caused by ROS to an organism (Prasad *et al.* 2018).

2.4.1. Mechanism of lipid peroxidation

Lipid peroxidation is a complex mechanism separated into three steps (Yin *et al.* 2011). First step in lipid peroxidation is called initiation, it's a process, during which lipid alkyl radical ($L^{\bullet}$) is formed by an abstraction of hydrogen from lipid by ROS. Most common reactive species responsible for lipid peroxidation are $^1O_2$, $HO^{\bullet}$, ozone and nitrogen dioxide radical (Niki 2014).

Second step in lipid peroxidation is called a propagation. In this step, $L^{\bullet}$ interacts with molecular oxygen, resulting in a formation of lipid peroxyl radical ($LOO^{\bullet}$). Lipid peroxyl radicals are dominant radicals in the chain reaction of lipid peroxidation because the speed of their reactions is much slower than those of other radicals. Next part of propagation is an attack on organic molecules, such as lipids and proteins, by $LOO^{\bullet}$. Lipid peroxyl radical subtracts hydrogen from them and forms lipid hydroperoxide (LOOH). Attacked biomacromolecule becomes new organic radical, that once again interacts with molecular oxygen and attacks other lipids and proteins, leading to chain reaction.

Last step in lipid peroxidation is a termination. The termination occurs when chain reaction of damaging lipids and creating new radicals stops. This can be achieved by two means. Termination occurs if reactive species are scavenged by antioxidants or by interaction of two radicals. Entire process of lipid peroxidation can be seen on (Figure 9), with the addition of the fact, that termination can also be caused by antioxidants neutralizing $LOO^{\bullet}$.

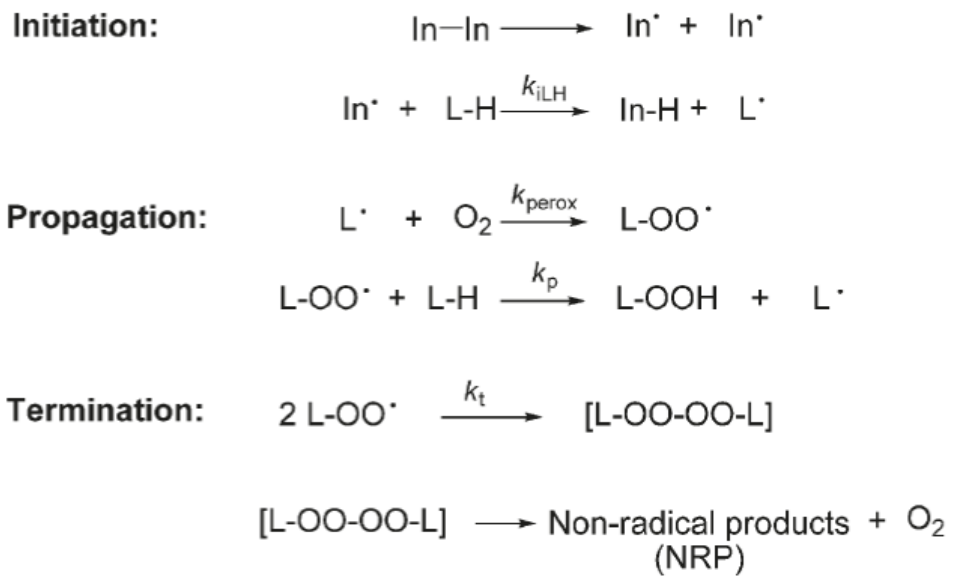

Figure 9: Mechanism of lipid peroxidation. Figure taken from (Yin et al. 2011).

2.4.2. Lipid peroxidation caused by different types of stress

Oxidative damage and lipid peroxidation in skin caused by ozone was measured on hairless mice (Thiele *et al.* 1997). Mice were exposed to ozone to study the effects of ozone on lipid peroxidation in their skin. The results show that ozone reacts directly with lipids in the skin and induces lipid peroxidation. Authors suggest that exposure to ozone found in urban smog could lead to lipid peroxidation in human skin and cause damage to the skin.

Lipid vesicles prepared from extracted human skin were exposed to UV radiation and lipid peroxidation was studied using HPTLC and EPR spectroscopy (Lasch *et al.* 1997). The results show that UV irradiation can split hydroperoxides generating $HO^{\bullet}$ causing hydrogen abstraction from lipid leaving lipid radicals. Authors also conclude that sterols are effective ROS scavengers because they compete with other susceptible lipids in human skin for ROS.

2.5. Protein oxidation in skin

During excessive production of ROS in the skin, biomacromolecules can be oxidized, besides lipids, their peroxidation is described in the previous chapter, proteins can also undergo oxidation.

In the skin, oxidation of proteins by ROS due to UV radiation or visible light is frequent. Higher possibility of oxidation is caused by an abundance of proteins in human skin and body. Other reasons for this higher oxidation is the fact that proteins have chromophores in their structure, they bind chromophores from outside easily and they have fast reaction speeds with other excited biomacromolecules (Davies 2003).

2.5.1. Mechanism of the protein oxidation

Protein oxidation due to an irradiation can happen by two means. First option is a direct oxidation. Direct oxidation occurs, when chromophores inside protein absorb UV radiation, become excited and create ROS. Second option is an indirect oxidation, which happens, when protein is attacked by $^1O_2$ formed outside, for example by energy transfer from other proteins, lipids or pigments (Davies and Truscott 2001).

Direct oxidation occurs after chromophores inside protein absorb UV radiation, examples of the chromophores are tryptophan, cysteine, histidine, and others. After absorption of UV radiation, these chromophores become excited, transfer their energy to molecular oxygen and create $^1O_2$.

Both options lead to a ROS interacting with a protein, during this contact, multiple reactions are possible, examples are hydrogen abstraction from protein, oxidation, addition, and fragmentation (Davies 2016). These reactions lead to formation of protein peroxyl radical, which then attacks biomacromolecules. Mechanism of protein oxidation is similar to the mechanism of lipid peroxidation.

2.6. Antioxidants

Reactive oxygen species have both positive and negative effects on the human body (Valko *et al.* 2006). Positive effects occur when concentration of reactive oxygen and nitrogen species is regulated. Some of these effects are defence against infectious agent and signalization function of cells (Valko *et al.* 2007). If free radicals are formed at an excessive amount, negative effects start to show. This situation occurs when there is an abundance of ROS due to various stresses, when effectivity and number of antioxidants is low, or by combination of both. The

balance between antioxidants and reactive forms, and therefore between negative and positive effects, is extremely important for living organisms. This balance is maintained by a process called redox regulation. This process maintains redox homeostasis and protects the organism (Dröge 2002).

Human body has multiple defences against damage caused by ROS, such as preventive protection, repairing damaged cells and antioxidants.

2.6.1. Functions of antioxidants

Antioxidants are separated into two categories, enzymatic, such as superoxid dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT), and nonenzymatic, vitamins C and E, glutathione (GSH) and carotenoids (Valko *et al.* 2007).

Superoxid dismutase is important antioxidant responsible for a catalysis of dismutation, this reaction transforms $O_2^{\bullet -}$ to molecular oxygen and less reactive H_2O_2. Hydrogen peroxide is then neutralized by CAT or GPx (Fridovich 1995). In the human body, there are three forms of SOD, Cu-Zn SOD in cytosols, Mn-SOD in mitochondria and extracellular SOD.

Mitochondrial Mn-SOD is biologically very important, as shown by many studies, where deactivation, elimination, or other forms of destruction of Mn-SOD were studied, these led to mutations and illnesses in study subjects (Matés *et al.* 1999). Cytosol Cu-Zn SOD was tested on calves, which received higher amounts of zinc and copper, these cows had better immune reactions and bigger SOD activity compared to other cows (Prasad and Kundu 1995).

Neutralization of H_2O_2 in cells is catalysed by CAT and GPx. Catalase neutralises H_2O_2 formed inside cells by breaking them down to water and molecular oxygen. One study gave rats liposomes containing CAT a SOD directly into veins, and then exposed them to 100 % oxygen atmosphere, these rats had higher survival rates than rats without additional liposomes (Turens *et al.* 1984). Main source of the protection against small amounts of oxidative damage is GPx, which reacts effectively with lipid and other hydroperoxides and neutralises them (Matés *et al.* 1999).

2.7. Ultra-weak photon emission

Ultra-weak photon emission, also called autoluminescence, biophoton emission or weak chemiluminescence, is an emission of photons from plants, animals, and humans. It is caused by oxidative chemical processes, during which excited molecules such as [^{3}(R=O) $^\bullet$] are formed. Excited molecules then relax to their ground state, this transition is accompanied by emission of a small number of photons (Cifra and Pospíšil 2014).

When other molecules are close to the excited molecules, they can become excited through energy transfer. These molecules can for example be pigments, resulting in excited pigments, or molecular oxygen, resulting in the formation of $^1O_2$. Relaxation of molecules excited through energy transfer to ground state is also accompanied by ultra-weak photon emission. Ultra-weak photons are therefore emitted both from molecules excited during metabolic processes and from molecules excited via energy transfer.

Detection and imaging of ultra-weak photon emission are utilized in many fields (Cifra and Pospíšil 2014). In agriculture ultra-weak photon emission imaging is used to study reactions of plants to diseases and stresses such as pathogens (Mansfield 2005), drought (Ohya *et al*. 2002) and salinity related stress (Ohya *et al*. 2000). Ultra-weak photon emission can be also used in food quality control and in many medical fields, such as dermatology (Khabiri *et al* 2008) or neurology (Tang and Dai 2014).

Ultra-weak photon emission isn't a part of thermal emission from the dark body (Cifra and Pospíšil 2014). Even though number of photons produced by ultra-weak photon emission is very low, it is still much higher than thermal radiation generated by black body with temperature of 300 K. It was also found (Kobayashi *et al.* 2009), that when imaging human body with CCD camera, ultra-weak photon emission and thermal emission are located in different parts of body.

2.7.1. Spontaneous and stress induced ultra-weak photon emission

Ultra-weak photon emission can be divided into two categories. Spontaneous ultra-weak photon emission is linked with metabolic oxidative processes, where it is created as a secondary product of these processes without any external influence of stresses or stimuli.

Stress induced ultra-weak photon emission is linked with stress induced oxidative processes (Cifra and Pospíšil 2015). Stresses which lead to creation of ultra-weak photon emission are UV and visible radiation, excessive heat, chemical damage, viral and bacterial factors, and others. These stresses induce higher formation of ROS and subsequent higher emission of ultra-weak photons. Intensity of spontaneous emission ranges in tenths photons in second per centimetre squared. Stress induced emission has intensity several orders higher, it can be as much as thousands in second per centimetre squared (Cifra and Pospíšil 2014). These intensities are undetectable by human the eye, special cameras are needed to observe ultra-weak photon emission.

2.7.2. Mechanism of ultra-weak photon emission in skin induced by UV radiation

During oxidative processes in skin, lipid and protein oxidation occurs, these processes are described in detail in their own chapters. Formed peroxyl radicals of lipid and proteins go through various chemical reactions, which lead to a formation of [^{3}(R=O) $^\bullet$]. Triplet excited carbonyl can interact with molecular oxygen creating $^1O_2$. Triplet excited carbonyls in skin can also excite pigments in skin, mainly melanin. Formation of $^1O_2$ and pigments and their intersystem transitions are described in specific chapters.

Studies found spectral ranges of transitions linked with ultra-weak photon emission (Cifra and Pospíšil 2015). Transition from triplet excited carbonyl to its ground state is accompanied by emission ranging in 350-550 nm. Monomol emission is in the infrared part of the spectrum and collision of two $^1O_2$ leads to double emission on wavelengths 653 and 703 nm. Excitation of pigments by triplet excited carbonyls leads, in a case of singlet excited pigment, to emission in 550-750 nm and in a case of triplet excited pigment to emission in 750-1000 nm. Transitions of excited states and their respective wavelengths are shown in Figure 10.

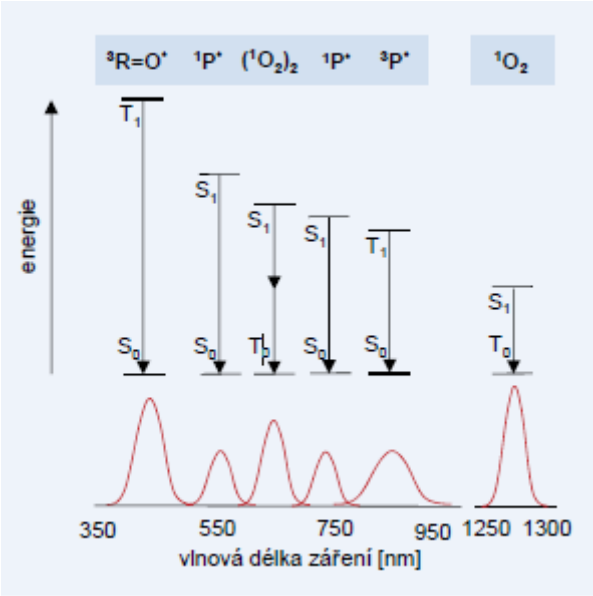

Figure 10: Transitions of electronically excited molecules. From left: triplet excited carbonyl, singlet excited pigment, singlet oxygen (dimol emission), triplet excited pigment and singlet oxygen (monomol emission). Figure taken from (Cifra. and Pospíšil 2015).

2.7.3. Ultra-weak photon emission imaging

2.7.3.1. One-dimensional ultra-weak photon imaging

One-dimensional ultra-weak photon emission imaging is done using photomultiplier tubes, which count number of photons captured. One-dimensional ultra-weak photon emission imaging gives an information about the number of ultra-weak photons emitted and captured but does not give any information about the location they were emitted from. The effects of temperature and oxygen concentration on ultra-weak photon emission from human hand were studied using photomultiplier tubes (Nakamura and Hiramasu 2005). It was observed, that decrease in both temperature and oxygen concentration lowers the ultra-weak photons emitted from the human hand.

2.7.3.2. Two-dimensional ultra-weak photon imaging

Two-dimensional ultra-weak photon emission imaging is, because of a low number of photons created, difficult. Normal cameras are uncappable of imaging such small intensities, it is necessary to use special cameras. These cameras are called charge-coupled device (CCD) cameras, they are cooled using either liquid nitrogen or Peltier effect (Prasad and Pospíšil 2013).

To get a quality picture using a CCD camera, it is necessary to measure for at least 15 minutes, ideally 30 minutes. This relatively high measuring time is caused by a readout of a CCD chip, which adds to every image created by CCD chip (Cifra and Pospíšil 2014). It is also better to use binning mode, that is a mode, which makes adjacent pixels act as one pixel, increasing quality of signal but decreasing resolution of the picture. It is also important to choose correct objective lens to increase a quality of images.

Two-dimensional ultra-weak photon emission imaging is more complicated than one-dimensional but offers additional information and localization of oxidative damage in sample. Combination of both methods gives the information of the amount of photons emitted, the emissions evolution in time and the place from which ultra-weak photons were emitted.

Two-dimensional ultra-weak photon emission imaging from dorsal and palmar sides of the human hand as the reaction to UVA and visible radiation was done using CCD camera (Prasad and Pospíšil 2012). Authors found out that oxidative damage in the skin is generated when exposed to both types of radiation, but a higher amount of ROS is formed when irradiated by UV radiation. They also found out that ultra-weak photon emission is higher in the palmar side of hand than in dorsal, meaning that the palmar side underwent higher oxidative damage. Ultra-weak photon emission imaging can be used to study effects of various stresses and is not limited to UV induced ultra-weak photon emission. Fenton reagent was applied on a pig skin in different concentrations. It was found that changes in ultra-weak photon emission corresponded with different oxidative damage (Prasad *et al.* 2018). Authors pointed out that to use ultra-weak photon emission imaging in dermatological or clinical research, technological advancements in sensitivity of cameras are necessary.

3. AIM OF THE THESIS

The aim of this thesis was to study the effects of UV radiation on the skin using two-dimensional imaging of ultra-weak photon emission by CCD camera. Additionally, to study properties and effects of UV radiation on melanin in the skin to provide better understanding of its excitation and function in oxidative damage to skin. This thesis was also focused on demonstrating usability of ultra-weak photon detection and imaging as a quality method to study damage to the skin caused not only by UV radiation but also all kinds of stresses.

4. MATERIAL AND METHODS

4.1. Skin samples

The skin used for measurements in this thesis was collected from pig ear which has many anatomical and physiological similarities with human skin. Pork ears used in experiments were collected from nearby slaughterhouse the same day as the measurements were made to provide the best possible approximation of human skin.

Process of separation of the skin from the ear was made in the same steps for each measurement. First, a mask from black paper, which was tested to ensure that it doesn't emit light if kept in a dark room, was made to ensure that each piece of skin would be the same size. Then a slightly bigger part than the mask was cut from the ear and the inner part of the skin was separated using a scalpel in such a way that only skin was left with no meat from inside the ear and that the skin wasn't penetrated.

Separated inner skin from pork ear was then washed in a 0,9 % solution of NaCl, also known as saline solution, to clean the skin from contamination and after that in distilled water. After separation and cleaning, skin was placed in a petri dish. Paper mask was put over the skin and secured using black adhesive tape, which was also tested to ensure that it doesn't emit light in a dark room.

4.2. Light exposure

Petri dish with skin and mask was exposed to UV radiation for various times based on the type of measurement done, these times are specified in the results and discussion section. Distance from the tip of the light cable to the surface of the skin was kept at 10 cm. The source of UV radiation used was LIGHTNINGCURE Spotlight source LC8 (Hamamatsu Photonics K.K., Shizuoka, Japan), the spectral characteristic of used UV lamp is shown in Figure 11. The spectral characteristic shows, that UV radiation used to expose the skin consisted mainly of UVA and UVB part of UV radiation, with UVA being the most dominant. However, the spectroradiometer used for measurement could only measure from 300 nm upwards. It is possible that UVB part in spectrum is higher but can't be seen in the part of the spectrum shown

on measured characteristic. The irradiance on the surface of the skin was 449,26 $W \cdot m^{-2}$. The spectral characteristic of the lamp was measured using LI-1800 Portable Spectroradiometer (LI-COR, Lincoln, Nebraska, USA).

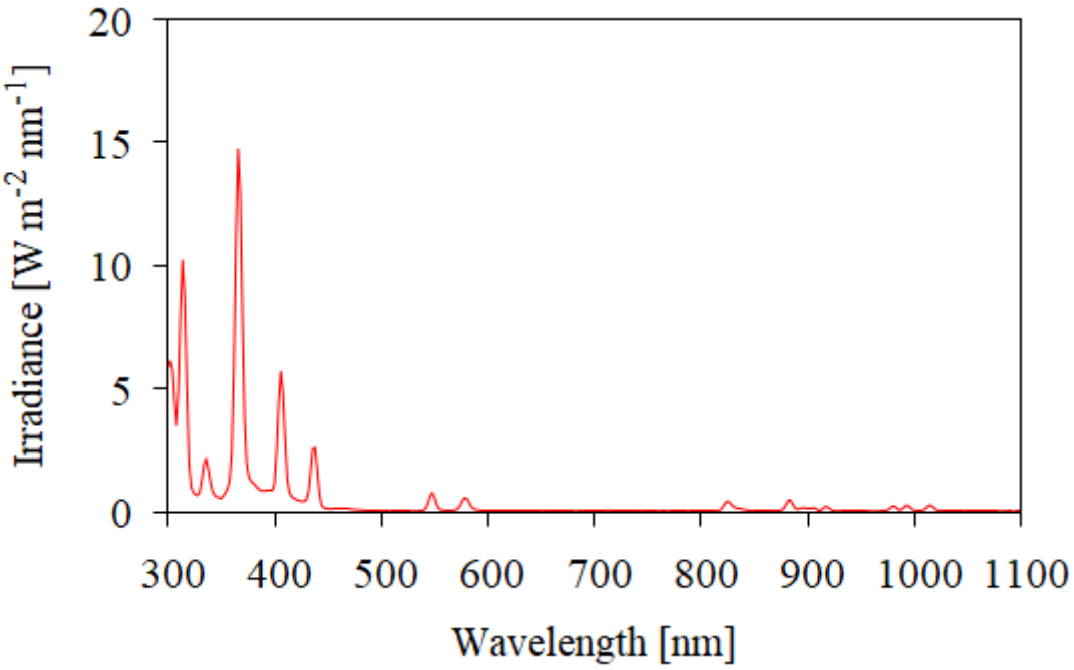

Figure 11: Spectral characteristic of UV lamp used for measurements.

Exposure to UV source generated an excessive amount of heat on the skin, temperature on the surface of skin was around 60 ºC, to prevent heat damage, petri dish with skin was irradiated while being placed in a beaker inside a box filled with ice (Figure 12). While in the box, temperature on the surface of the skin during measurement was 20 ºC.

Figure 12: Box filled with ice containing a beaker for petri dish with sample.

To protect the author from the damage caused by UV radiation, security measures were made, author was wearing special protective goggles to cover eyes, petri dish was placed under turned off light cable, source was turned on and author left the room and came back only after a certain amount of time to turn off the source.

The entire process from preparation and cleaning of the skin to irradiation was performed in a semi-dark room, with only a very limited amount of light present to prevent irradiation and exposure of skin samples to visible light. After irradiation, the skin sample was dark-adapted for 5 minutes in an outer dark room to avoid delayed luminescence during measurement.

4.3. Ultra-weak photon emission

After irradiation and dark-adaptation, petri dish with sample was placed in front of CCD camera in an inner dark room with black painted walls and no source of light, the door to both inner and outer room were closed for the entirety of measurement.

Two-dimensional ultra-weak photon emission imaging was accomplished using highly sensitive CCD camera VersArray 1300B (Princeton Instruments, Trenton, NJ, USA) with spectral sensitivity in the range of 200 to 1000 nm. Objective lens of 50 mm focal distance (F

mount Nikkor 50-mm, f:1.2, Nikon) was used to enhance light collecting efficiency. The CCD unit contained a Dewar bottle filled with liquid nitrogen and camera was cooled down to -102 ºC to reduce photons emitted by the heat of the camera. The temperature was locked by the software for the entirety of measurement.

The software used to control the temperature and the measurements was WinView 32 (Princeton Instruments, Trenton, NJ, USA). Parameters set for all measurements were 100 kHz scan rate, gain 2, image format 1340 x 1300 pixels and accumulation time of 30 min. The distance between the surface of the skin and detector during measurement was 35 cm.

Before measuring the first sample of the day, data correction was made by measuring the background signal for 30 minutes and using the software to subtract this background signal from each measurement. The background subtraction was kept the same for each sample of the day, to ensure no difference in signal caused by different subtraction.

Images from CCD camera were modified using software Andor Solis (Oxford Instruments, Tubney Woods, Abingdon, UK). These modifications include changing the range of intensity and colour for clearer image of sample, export of data to images and others.

4.4. Addition of melanin on samples

Some measurements were made with addition of melanin after the exposure of the sample to UV radiation. Melanin used in this thesis was in a solid form, which was combined with DMSO to create a 7 mM and 20 mM stock solutions. These stock solutions were then diluted into different concentrations for specific measurements. These concentrations are specified in results and discussion sections. The volume of melanin solution used in the measurements was 30 µl.

Melanin was in some measurements added straight on the surface of the skin using pipette and then spread to cover the entire surface which was exposed to UV radiation. In the other type of measurements, melanin was not added directly on the skin but was separated to study effects without direct contact. Melanin was in this case put between two cover slips connected using black adhesive tape, these cover slips were then taped to a mask on the sample.

4.5. The usage of filters

Measurements were made using edge filters to separate emissions of different wavelengths. Long-pass edge filter allowing photons with wavelength longer than 500 nm was used, its transmission characteristic was measured and is shown in Figure 13. Two short-pass edge filters had to be used simultaneously to ensure, that no photon with wavelength higher than 500 nm were able to pass through. Their combined transmission characteristic is shown in Figure 14.

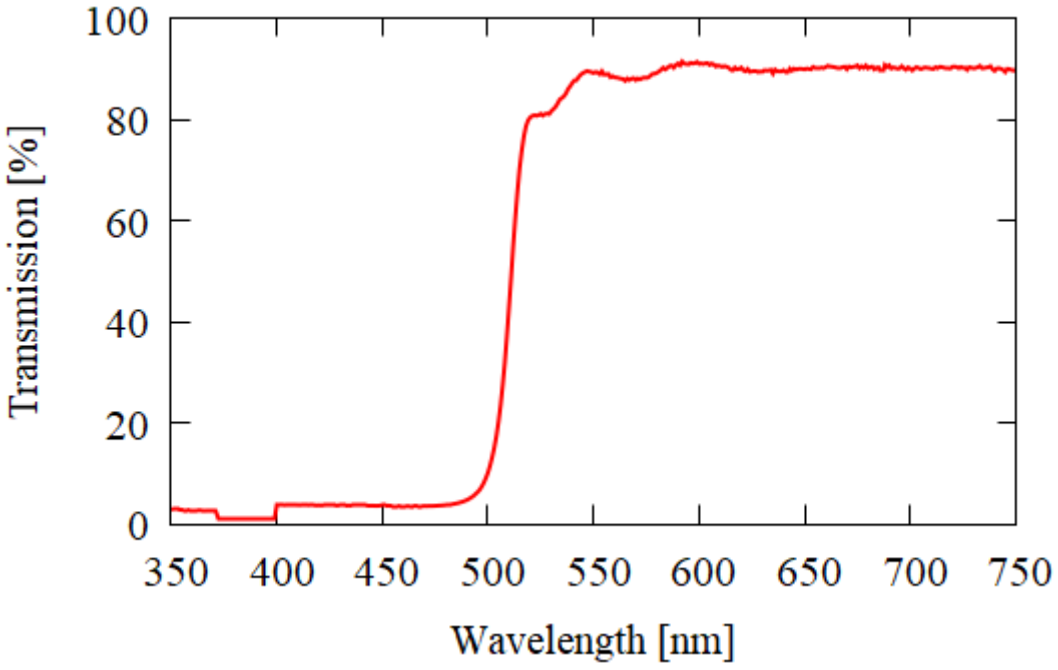

Figure 13: Transmission characteristic of long-pass edge filter used in measurements.

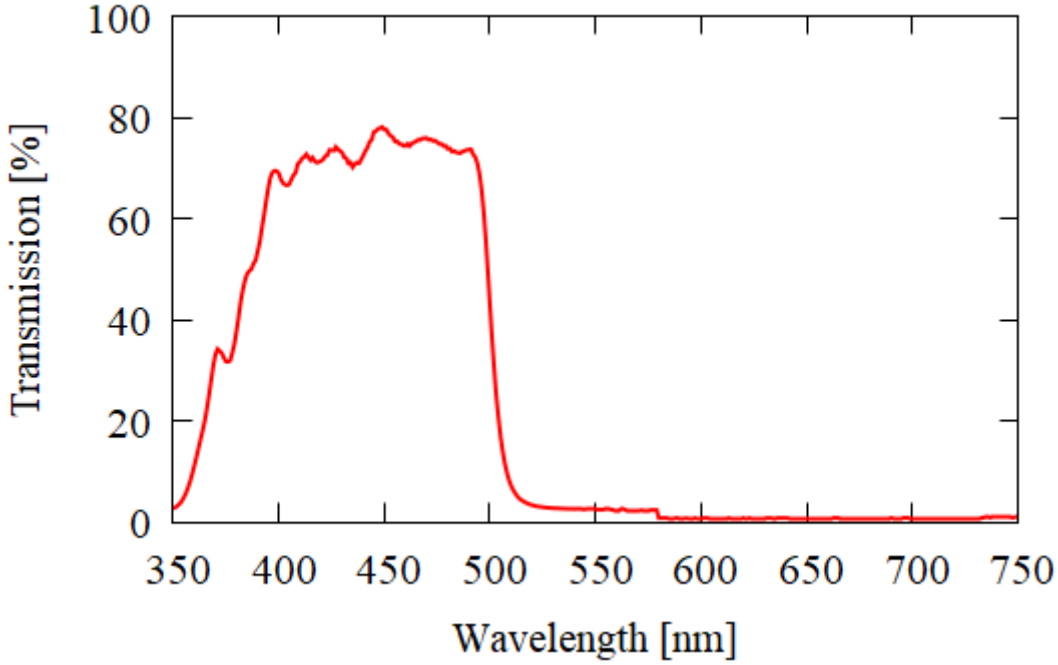

Figure 14: Transmission characteristic of two combined short-pass edge filters used in measurements.

5. RESULTS

5.1. Spontaneous and UV induced ultra-weak photon emission from pig ear

Two-dimensional imaging of ultra-weak photon emission was first measured on the entire pig ear with most of the ear covered in a mask made from black paper. Only a small part of the ear, not covered by a mask, was exposed to UV radiation for 5 minutes. The photograph and corresponding image of spontaneous and UV induced ultra-weak photon emission are shown in Figures 15a and 15b, respectively. The Figures 15a-b show the difference between UV induced ultra-weak photon emission and spontaneous ultra-weak photon emission.

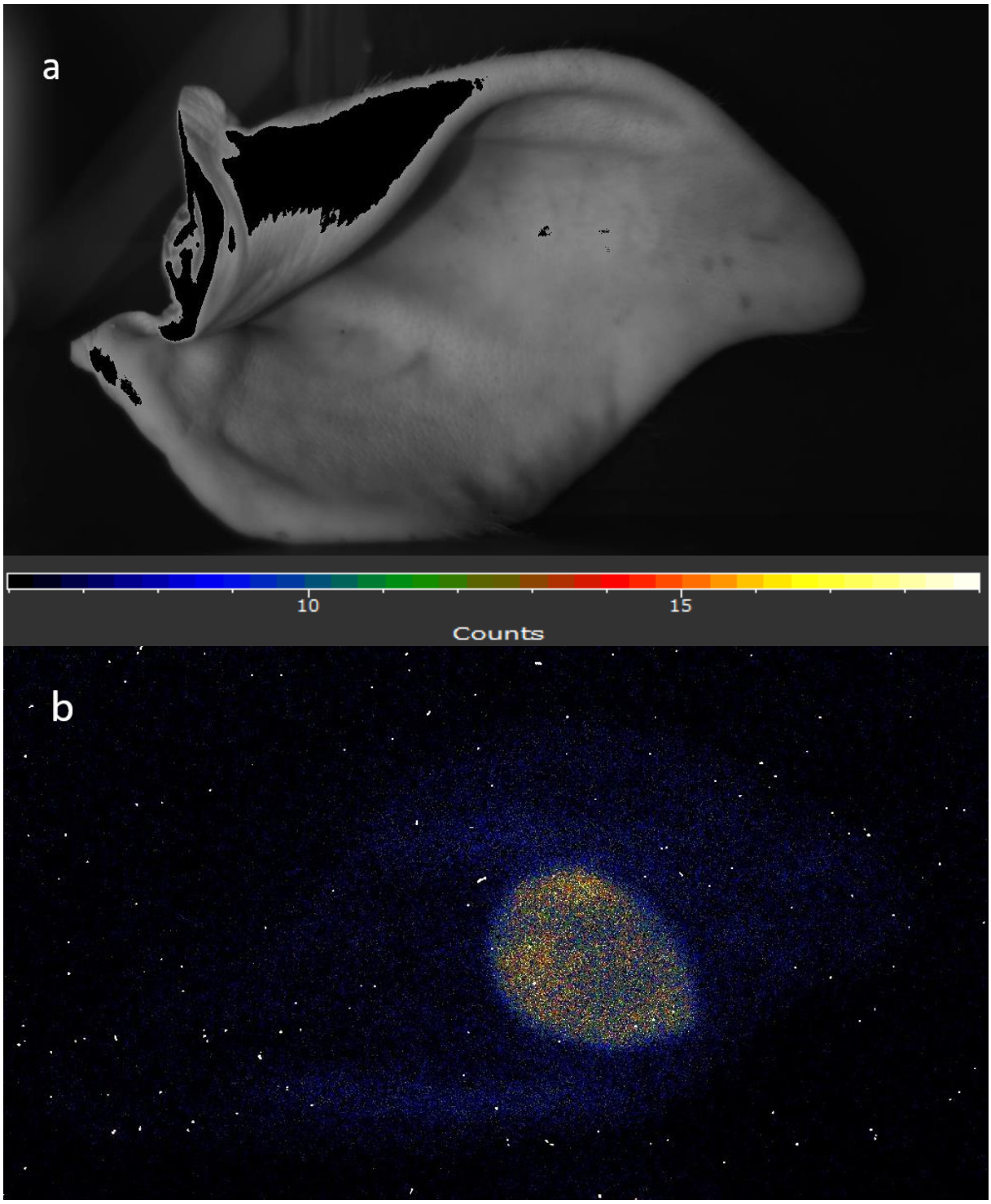

Figure 15: Photograph of the pig ear used in measurements of spontaneous and UV induced ultra-weak photon emission (a). Two-dimensional imaging of spontaneous and UV induced ultra-weak photon emission on pig ear (b).

5.2. Ultra-weak photon emission imaging from pig ear induced by UV using filters

UV induced ultra-weak photon emission in the pig ear is mostly caused by the relaxation of [^{3}(R=O)$^\bullet$] and excited melanin molecules formed during the oxidation of biomacromolecules. To study the involvement of both molecules in ultra-weak photon emission separately, edge filters were used on the objective lens of the camera. The pig ears were exposed to UV radiation for 3 minutes using the mask the same way as in the previous measurement. First ear was exposed to UV radiation and measured without any filter on the camera. The second ear was measured with 500 nm long pass edge filter. The ultra-weak photons emitted by the relaxation of [^{3}(R=O)$^\bullet$] have shorter wavelength than 500 nm resulting in no photons caused by the relaxation of [^{3}(R=O)$^\bullet$] to be captured. The last ear was measured with two short pass edge filters that allowed only photons with wavelength shorter than 500 nm to pass through, as the photons emitted by the relaxation melanin have longer wavelength than 500 nm, no photons caused by relaxation of melanin were captured. The images showing full ultra-weak photon emission, ultra-weak emission of photons with wavelength 500 nm or longer and ultra-weak emission of photons with wavelength 500 nm or shorter are shown in Figures 16a-c, respectively.

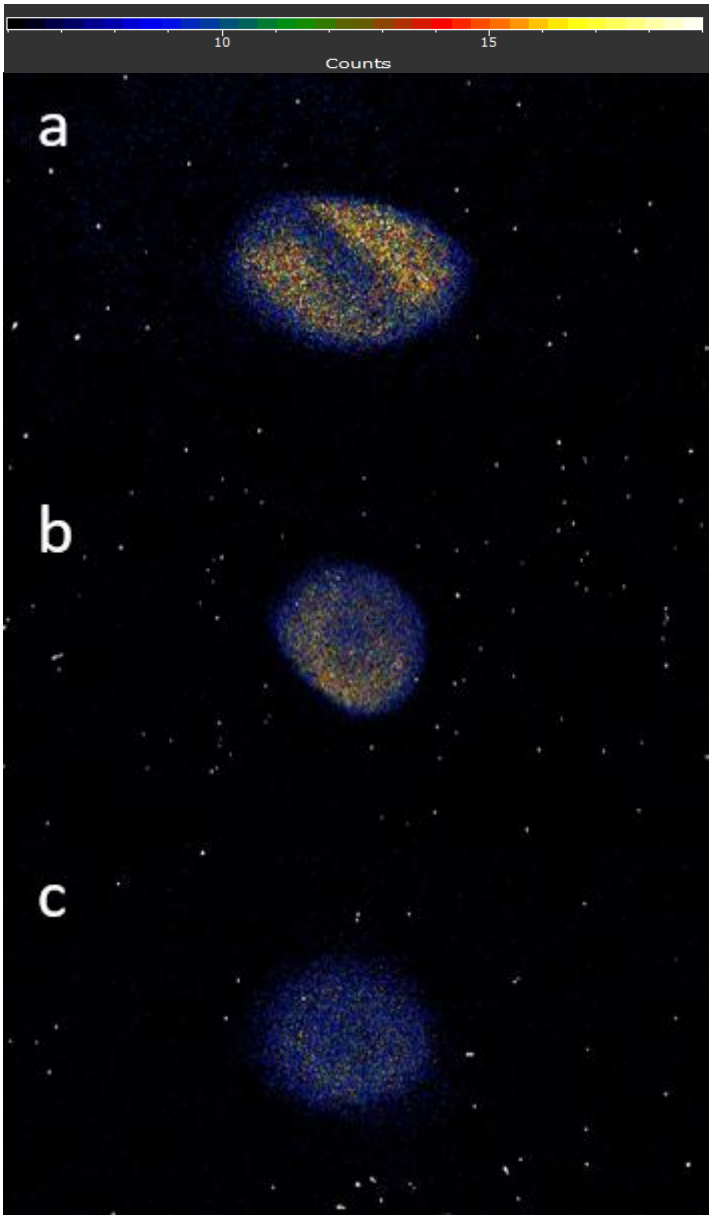

Figure 16: Two-dimensional imaging of ultra-weak photon emission from pig ear after 3 min UV radiation exposure with no filter (a), with long pass edge filter (b) and with two combined short pass edge filters (c).

5.3. Effects of different UV radiation exposure times on ultra-weak photon emission

To demonstrate the effects of longer UV exposure times on the pig skin, the skin was irradiated for increasing time periods. Measurements were made for exposure times 0, 1, 3, 5, 7 and 10 minutes. Two-dimensional images of UV induced ultra-weak photon emission are shown in Figures 17a-f. Two-dimensional images captured by CCD camera demonstrate an increase in ultra-weak photon emission caused by higher oxidative damage from UV irradiation of the skin.

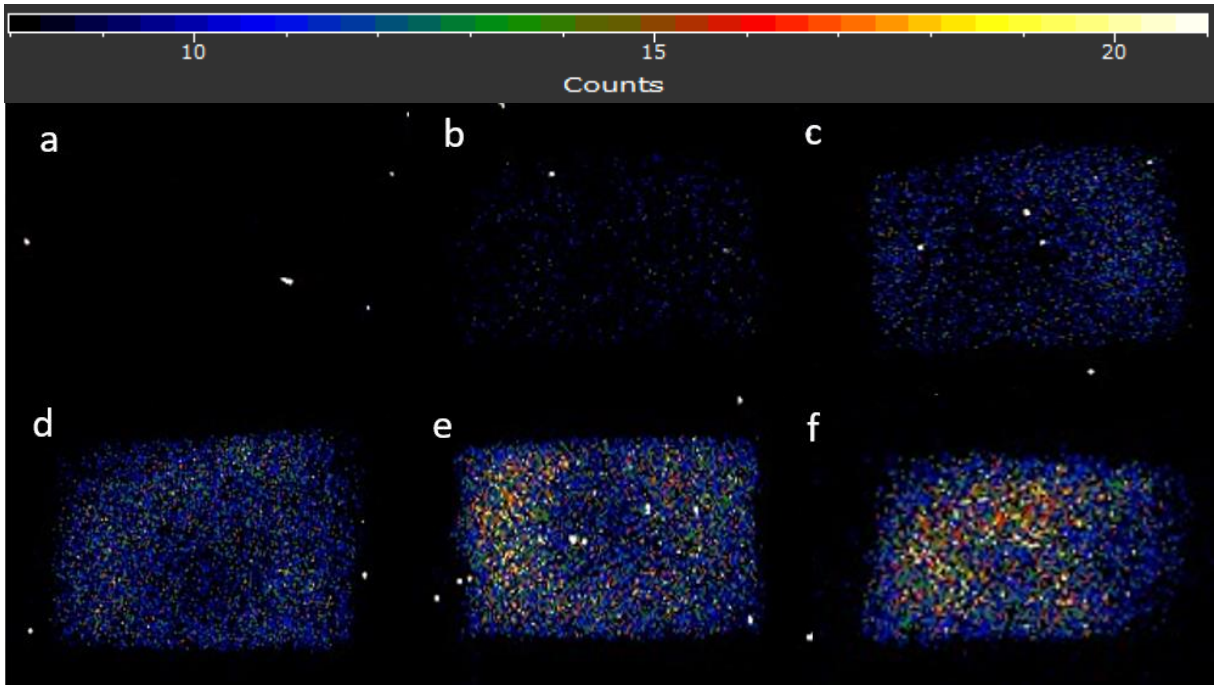

Figure 17: Two-dimensional imaging of ultra-weak photon emission from pig skin after being exposed to UV radiation for 0 (a), 1 (b), 3 (c), 5 (d), 7 (e) and 10 (f) minutes.

5.4. Effects of different melanin concentrations on ultra-weak photon emission

Melanin was applied on the surface of the skin after the irradiation in different concentrations to demonstrate increase in ultra-weak photon emission with higher melanin concentrations. Exposure time to UV radiation was 3 min. Two-dimensional images with concentrations 0 mM, 1 mM, 4 mM, and 16 mM of melanin added on the skin are shown in Figures 18a-d, respectively. The results show that increase in ultra-weak photon emission corresponds with increased melanin concentration.

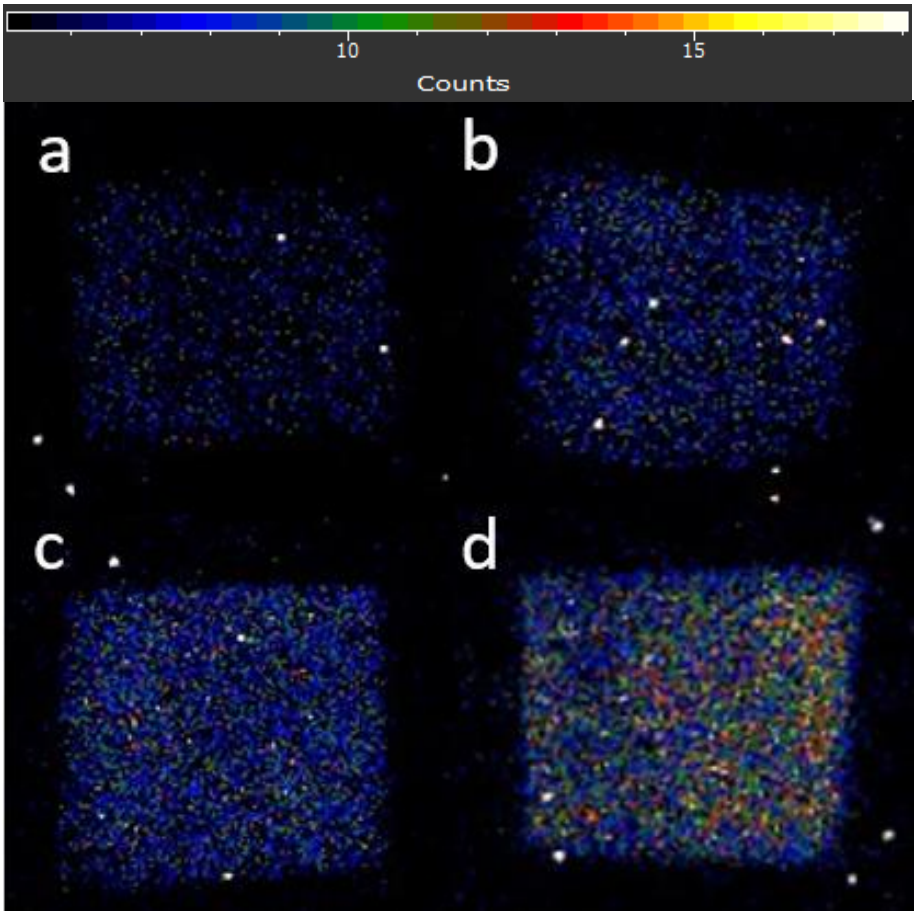

Figure 18: Two-dimensional images of ultra-weak photon emission from skin after 3 min UV irradiation with melanin added after the exposure in concentrations 0 mM (a), 1 mM (b), 4 mM (c) and 16 mM (d).

5.5. Effects of addition of melanin with separation from skin on ultra-weak photon emission

Melanin was placed between two cover slips and placed on the skin, ensuring no direct contact to demonstrate the excitation of melanin through absorption of photons emitted by excited molecules during their relaxation. Exposure time was 3 min, and the concentration of melanin between cover slips was 1, 4 and 16 mM. Two-dimensional images of control measurement with solvent and of the skin with cover slips containing melanin in concentrations 1, 4 and 16 mM are shown in Figures 19a-d, respectively. The Figures 19a-d show increase in ultra-weak photon emission which corresponds with increased melanin concentration even when melanin was separated from the skin.

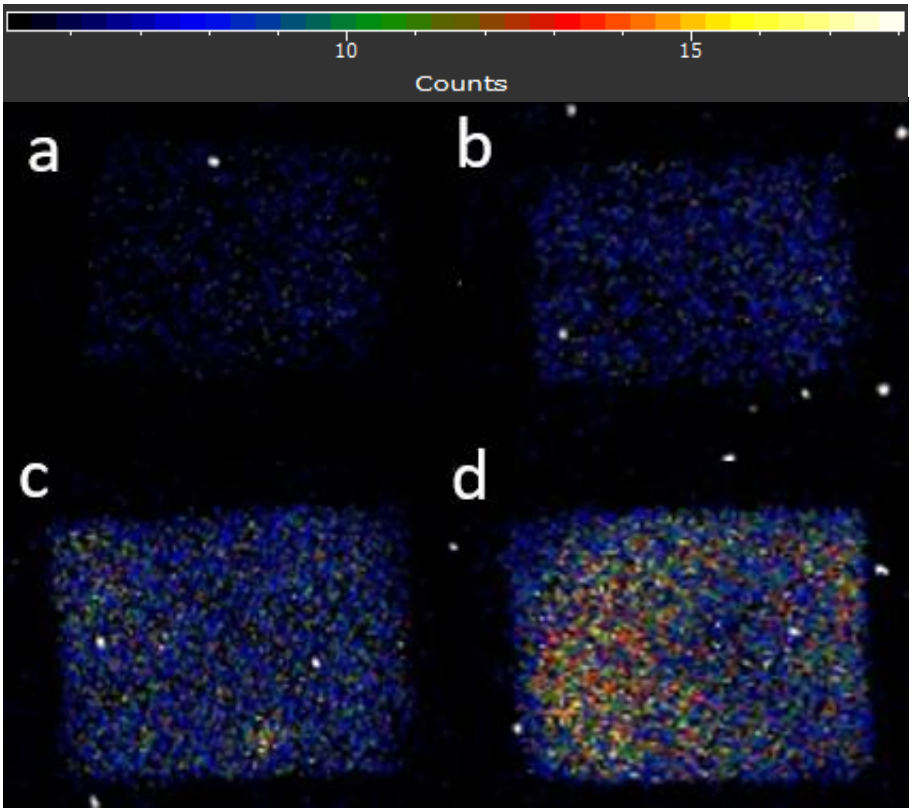

Figure 19: Two-dimensional images of ultra-weak photon emission after 3 min irradiation with UV. Control measurement with solvent between cover slips (a) and measurements with melanin in concentrations 1 mM (b), 4 mM(c) and 16 mM (d) between cover slips.

6. DISSCUSION

Reactive oxygen species are the subject of many studies because their overabundance causes several medical problems not only in the skin, but in the entire organism. In the skin, melanin is one of the main defences against ROS formed by UV radiation and is one of the main subjects of this thesis.

The first measurement performed on pig ear demonstrated the difference between spontaneous and UV induced oxidative damage and subsequent ultra-weak photon emission. Spontaneous emission is caused by oxidative metabolic processes inside the skin, even when the sample is not alive some metabolic processes are still occurring inside if captured in matter of hours after its death. The irradiation of the skin causes increased oxidative damage caused by formed ROS, resulting in increased ultra-weak photon emission. Results correspond with study made on a human hand exposed to UV radiation (Prasad and Pospíšil 2012). In this study, authors show among other results spontaneous ultra-weak photon emission and UV induced emission from human hand, both of these emissions are higher than emission shown in this thesis from pig ear, due to the human hand being alive in their measurements with functioning oxidative metabolic processes.

The ultra-weak photon emission in skin after the exposure to UV radiation is thought to be mainly caused by the relaxation of [^{3}(R=O) $^\bullet$] and excited melanin. To study their ultra-weak photon emissions separately filters must be used. When no filter is added, the resulting ultra-weak photon emission is a caused by a relaxation of many molecules, mainly [^{3}(R=O) $^\bullet$] and excited melanin. To study the influence of only excited carbonyl triplets on ultra-weak photon emission, long-pass 500 nm edge filter must be used. Because photons emitted during the relaxation of [^{3}(R=O) $^\bullet$] have wavelength shorter than 500 nm (Cifra and Pospíšil 2015), no emission from [^{3}(R=O) $^\bullet$] is going to be captured. Separating the emission caused by the relaxation of excited melanin requires the use of 500 nm and 600 nm short-pass edge filters in combination. The combination of these two filters ensures, that no photon with wavelength longer than 500 nm is going to be captured, however, combining filters decreases the transmission. The relaxation of excited melanin is accompanied by photon with wavelength longer than 500 nm, resulting in no photons caused by relaxation of melanin to be captured when short-pass edge filters are used. The results indicate that the ultra-weak photon emission

is mainly caused by the relaxation of melanin. However, because of the decrease of transmission caused by the combination of two short pass edge filters and by the quantum characteristic of the camera, the difference measured is not precise and serves only as a demonstration and not exact measurement.

During the exposure of the skin to UV radiation, ROS are formed. ROS formation and subsequent oxidative damage and ultra-weak photon emission is affected by the exposure time in such a way, that higher exposure times lead to higher ultra-weak photon emissions. This increase can be attributed to higher number of molecules being excited by UV radiation and their subsequent oxidation of biomacromolecules. Two-dimensional ultra-weak photon emission images captured in this thesis show the increase in ultra-weak photon emission with increasing exposure times.

UV irradiation of the skin causes various pigments, mainly melanin, to get to their excited state. When these pigments relax to their ground state, they emit an ultra-weak photon, which can be detected using a CCD camera. Pigments get this energy either through absorption of UV photons from the source, or through energy transfer from other excited molecules such as triplet excited carbonyls. This energy transfer from molecule to molecule however can occur only, when the distance between the receiving molecule and the donating molecule is extremely small. When melanin is added on the surface of the sample after the irradiation of the sample, added melanin cannot absorb any photons from the source of the UV radiation and has to accept the energy to become excited either through energy transfer from excited molecules or through absorption of photons emitted by these excited molecules during their relaxation. Two-dimensional images captured show an increase in ultra-weak photon emission clearly corresponding with increased concentration of melanin.

Excitation of melanin is also theorized to be possible through absorption of photons, but not from the UV source, but from the excited molecules in the skin. These molecules become excited after their irradiation, then they relax to their ground state and emit a photon, this photon can be absorbed by another molecule, such as melanin, to become excited. To eliminate the energy transfer option of excitation and focus solely on the absorption of photons, melanin has to be separated from the skin sample, the addition of some form of barrier, such as cover slip increases the distance between the excited molecules and melanin to such numbers, that energy transfer is not possible at all. Two-dimensional ultra-weak photon emission images captured

during this thesis show, that increase in ultra-weak photon emission with melanin separated is similar to increase in emission, when melanin isn’t separated. These images support the theory that it is possible for melanin to become excited by three different methods. First, by photoexcitation caused by photons emitted by the source of UV radiation. Next option is excitation by energy transfer from molecules excited by UV radiation. The last possibility of excitation is the emission of photons from excited molecules in the skin and their absorptions by melanin.

7. CONCLUSION

This bachelor thesis focused on the effects of UV radiation on the skin and pigments present in the skin, which was studied by two-dimensional ultra-weak photon emission imaging. This thesis also focused on excitation options for melanin in the skin and tried to demonstrate two-dimensional ultra-weak photon emission imaging as a viable and useful method to study effects of stress on skin and oxidative damage.

The difference between spontaneous ultra-weak photon emission caused by metabolic processes and induced ultra-weak photon emission caused by stresses was discussed and demonstrated on pig ears with UV radiation source. The proportion of the ultra-weak photon emission caused by the relaxation of excited melanin compared to the relaxation of $[^3(R=O)^\bullet]$ was also demonstrated on the pig ear using edge filters.

Measurements of different UV radiation exposure times on the pig skin sample were performed to demonstrate changes in ultra-weak photon emission. An increase in ultra-weak photon emission corresponding with longer UV radiation exposure times was measured and two-dimensional images were taken using CCD camera

To study energy transfer from UV excited molecules to melanin, melanin was added on the surface of the skin after the irradiation. Increase in oxidative damage and subsequent ultra-weak photon emission with increasing melanin concentrations was demonstrated.

The theory that melanin can become excited not only through energy transfer from already excited molecules or through absorption of photons emitted by UV radiation source, but also through absorption of photons emitted by these excited molecules was shown and confirmed. Increase in ultra-weak photon emission can be seen in two-dimensional images and supports the theory that melanin can become excited by photons emitted from molecules in the skin.

The work done in this thesis could be in future expanded upon for example by studying the products of the irradiation with high-pressure liquid chromatography or by one-dimensional imaging of ultra-weak photon emission with photomultiplier tubes.

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