Protocol Title: Singlet oxygen quantification after skin exposure to ultraviolet A (UVA) light

1) Protocol title: Singlet oxygen quantification after skin exposure to ultraviolet A (UVA) light

Protocol version date: May 11, 2017

2) Objectives

The objective of this study is quantification of singlet oxygen species in the skin after exposure to UVA light

3) Background

Ultraviolet (UV) radiation can lead to skin damage and cancer. UVA irradiation is 10 to 100 times more abundant in natural sunlight than UVB, thus human skin is exposed to more UVA irradiation daily.(1-4) UVA irradiation is not completely filtered by clothing and it penetrates deeper into the dermis than UVB, potentially causing more damage.(3-5)

It is believed that skin cancer, photo aging, and skin immunomodulation are mediated by the reactive oxygen species (ROS) that are generated in response to UV radiation.(6) Singlet oxygen ($^1O_2$) is one type of ROS.(6)

The aim of this study is to quantify the level of singlet oxygen generated in the skin after exposure to UVA radiation. Previous studies have used low dose UVA1 irradiation of 20 J/cm^2, which mimics exposure to strong sunlight of approximately two hours.(4) This study will use UVA doses equivalent to or less than what humans are exposed to in daily life. Additionally, the aim is to quantify singlet oxygen produced in individuals of various skin types before and after application of sunscreens containing zinc oxide and avobenzone (SPF 30). To our knowledge, there is no method for quantifying singlet oxygen in human skin after exposure to UVA light. This is a novel method that may help us understand further the protective effects of various skin types, as well as sunscreens.

4) Inclusion and Exclusion Criteria

Inclusion criteria:

- Subjects aged 18 years and older

Exclusion criteria:

- Those who are currently smoking or have smoked within the past 3 years.
- Aspirin use
- Multivitamins and supplements that contain vitamin E
- Adults unable to consent

- Individuals who are not yet adults (infants, children, teenagers)
- Pregnant women
- Prisoners

5) Study Timelines

The study will be conducted over the course of one year.
The duration of each subject's participation is approximately one week.

6) Study Endpoints

Primary study endpoints include:

- To quantify singlet oxygen generated by the skin after exposure to three different UVA radiation doses (each equivalent to or less than UVA dose encountered in daily life)

Secondary study endpoints:

- Difference in singlet oxygen generate between individuals of different skin types
- Difference in singlet oxygen generation with two different sunscreens (zinc oxide and avobenzone, each SPF 30) vs control
- Change in appearance of skin pigmentation

Protocol Title: Singlet oxygen quantification after skin exposure to ultraviolet A (UVA) light

7) Procedures Involved

Subjects meeting the inclusion criteria without any of the exclusion criteria will be enrolled into this study. Up to 17 subjects will be recruited into this study.

Group A: N=7 Fitzpatrick skin type I-II
Group B: N=5 Fitzpatrick skin type III-IV
Group C: N=5 Fitzpatrick skin type V-VI

All subjects will undergo the same procedures, as follows:

Visit 1:

- Informed consent
- Review medical history and concomitant medications
- Fitzpatrick skin type classification
- Three spots measuring 2cm^2 each will be marked on subject's left dorsal forearm and right dorsal forearm
- Each spot will be assigned numbers 1, 2, or 3.
- Each spot will be exposed to corresponding UVA dose.
- UVA exposure at each site and singlet oxygen measurements will be recorded
- Any one spot will be exposed to UVA for up to 5 minutes. The exposure at any one skin site will range from 0.33 J/cm^2, 8.25 J/cm^2, and 16.5 J/cm^2. The exposures used in this study are below those normally used in the literature.(4)
- Subject will be compensated $10 (gift card) at completion of the visit

Visit 2: May occur on the same day as Visit 1 up to 1 month after visit 1

- Three spots measuring 2cm^2 each will be marked on subject's left dorsal forearm and right dorsal forearm
- Each spot will be assigned numbers 1, 2, or 3.
    - First spot: control (no sunscreen)
    - Second spot: 2mg/cm^2 sunscreen with zinc oxide (SPF 30) will be applied
    - Third spot: 2mg/cm^2 sunscreen with avobenzone (SPF 30) will be applied
- Only one UVA dose will be used for all exposures. The dose will be chosen based on dose response during Visit 1 (either 8.25 J/cm^2 or 16.5 J/cm^2)
- Any one spot will be exposed for up to 5 minutes

- Subject will be compensated $15 (gift card) at completion of the visit
- This completes subject's involvement in the study

Subject compensation is outlined as follows:

Visit 1: $10
Visit 2: $15

Protocol activity	VISIT 1	VISIT 2
Informed Consent	X	
Medical History	X	
Review concomitant Medication	X	
Mark 3 areas measuring 2cm^2 each on right and left dorsal forearm	X	X
Expose each of the 3 marked skin areas to one UVA dose (0.33 J/cm^2, 8.25 J/cm^2, and 16.5 J/cm^2)	X	
Determine best UVA dose for subject based on dose response data	X	
Weigh sunscreen 2mg/cm^2		X
Apply zinc oxide containing sunscreen to one marked spot on each arm		X
Apply avobenzone containing sunscreen to one marked spot on each arm		X
Mark one spot as "control" (no sunscreen)		X
Expose each of the 3 marked skin areas to one UVA dose (as determined during visit 1)		X

8) Data and/or Specimen Management and Confidentiality

All data will be coded for the safety of the study subjects. Research data will only be available to the listed research personnel. The research site will maintain appropriate medical and research records for this study in compliance with ICH E6,

Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of subjects.

All of the study data will be coded and stored within a locked cabinet within a locked room with two levels of keyed entry required to gain access. Furthermore, data files will either be stored in password-protected files within a locked room that will require two levels of keyed entry for access.

Precaution will be taken to maintain the privacy of the participants. These include the following: All subjects will be assigned a subject ID number after signing the consent form. This will be their only form of identification throughout the remaining of the study period. Each subject's name, age, and medical record number will be recorded. The consent forms will be kept in a binder. The subjects will be entered into the data analysis sheets as subject ID numbers and a separate password-protected file will contain the key for these codes. All of the files will be saved on password-protected computers within locked rooms. All study related material will be kept in a locked cabinet within a locked room at UC Davis Department of Dermatology. Only the research team personnel will have access to study-related materials. Participant information will not be disclosed to third party individuals except for those authorized to oversee the research project.

Data that are already collected will not be destroyed but will be stored in a codified format that will not allow linkage to identifying information.

The singlet oxygen data are immediately stored after capture into a password-protected computer that will be stored in a locked room within the Institute for Regenerative Cures (IRC) at UC Davis. All data stored in binders will be transported to the UC Davis Clinical Trials unit (CTU).

Each of the subjects' signed consents will be filed in a locked cabinet within a locked room. All of the subjects' data will be entered into the data analysis sheets as codes and a separate password protected file will contain the key for these codes. All of the files will be saved on computers that will be within locked rooms. The research team will follow the UC Davis Institutional Policy for data security to ensure that all subjects and collected data are protected.

9) Data and/or Specimen Banking

All patient information will be only accessible to authorized research personnel, and otherwise personalized information will be locked in a cabinet and password protected electronic drive at the UC Davis Department of Dermatology (IRC and CTU). After the study has been completed and analyzed, all patient information will be discarded. The code keys will be stored in a secure file in a password-protected computer in a locked room and this code key will be deleted upon completion of the analysis.

10) Provisions to Monitor the Data to Ensure the Safety of Subjects

The records and study materials will be maintained in a locked cabinet. Subjects will be asked about their health and any adverse events that may have occurred. Adverse effects will be related to the treating physician. Noted and serious adverse effects will be relayed to the IRB within 24 hours of the occurrence.

11) Withdrawal of Subjects

The subjects may withdraw consent at any time and they will be reassured that their clinical care will not be compromised if a subject were to withdraw. Subjects will be withdrawn from the study if they develop adverse side effects. The investigator can remove the subject from the study. Possible reasons for removal include:

- Failure to follow protocol
- Hypersensitivity to sunscreen

12) Risks to subjects

Subjects will be counseled that the physical risks from sunscreen application may include skin redness or irritation. The physical risks from UVA light may include mild redness or tanning of the spot exposed to UVA light. Subjects will be advised to inform the research team if they experience any side effects.

Although provisions are put in place to protect identifiable data, we cannot guarantee absolute protection of the data collected from each subject. Accordingly, the subjects will be informed of this risk.

13) Potential Benefits to Subjects

There are no direct benefits to the subjects.

This study will help us add to the general scientific knowledge that may benefit individuals in the future.

14) Sharing of Results with Subjects

Any peer reviewed manuscripts that result from the study will be made available to the subjects on request.

15) Provisions to Protect the Privacy Interests of Subjects

Steps will be taken to protect subjects' privacy interests. Subjects will only interact with approved study personnel who have successfully completed human subjects training. This includes those involved in the study visits, consent, and in any study related procedures. We will do our best to make sure that the personal information in the subject's record is kept private. However, no study can guarantee 100% protection of private information despite all of our measures to protect each subject's privacy. Subjects will be made aware of this during the consent process.

16) Compensation for Research-Related Injury

If a subject is injured as a result of being in this study, the University of California will provide necessary medical treatment. Depending on the circumstances, the costs of the treatment may be covered by University or the study sponsor or may be billed to the subjects' insurance company just like any other medical costs. The University and the study sponsor do not normally provide any other form of compensation for injury. For more information about compensation, call the IRB administration at (916) 703-9151 or email at IRBAdmin@ucdmc.ucdavis.edu.

17) Economic Burden to Subjects

Standard of care and other routine costs will be billed to the patient or the patient's insurance carrier, Medicare, or Medi-Cal where appropriate. Only the costs of research and experimental procedures will be paid by the sponsor/department. Subjects may incur a cost in relation to travel needed to get to the UC Davis Dermatology Department for each visit.

18) Drugs or Devices

☒ I confirm that all investigational drugs will be received by the IDS pharmacy, which will store, handle, and administer those drugs so that they will be used only on subjects and be used only by authorized investigators.

☒ I confirm that all investigational devices will be labeled in accordance with FDA regulations and stored and dispensed in such a manner that they will be used only on subjects and be used only by authorized investigators.

Device used in the study:

SINGLET OXYGEN MONITOR FOR UVA-SKIN STUDIES

The UVA-produced Singlet Oxygen monitor detects the weak, near infrared luminescence from the a-X system in molecular oxygen. It uses an ultra-sensitive photomultiplier tube (PMT) coupled to a photon counting system to monitor this weak emission produced by the UVA source that is also part of the system. This non-imaging, point sensor is an ultra-sensitive portable and automated real-time detection system for singlet O_2. The current system includes (1) a fiber –coupled CW UVA source module, (2) PMT detector module, and (3) data acquisition with photon counter board.

A block diagram of the major components of the singlet oxygen sensor is shown in Figure 5.

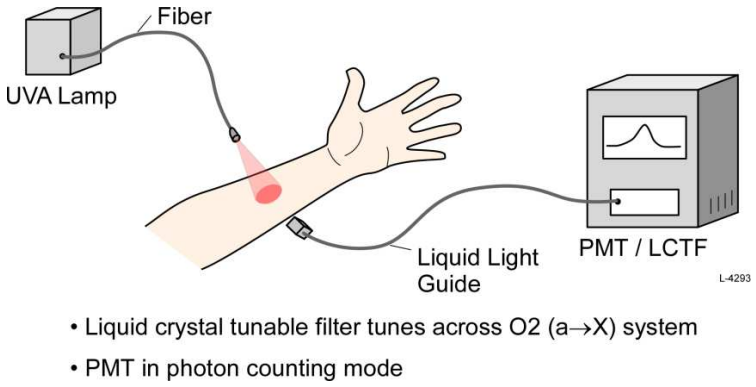

Figure 1. Block diagram of the singlet oxygen monitor.

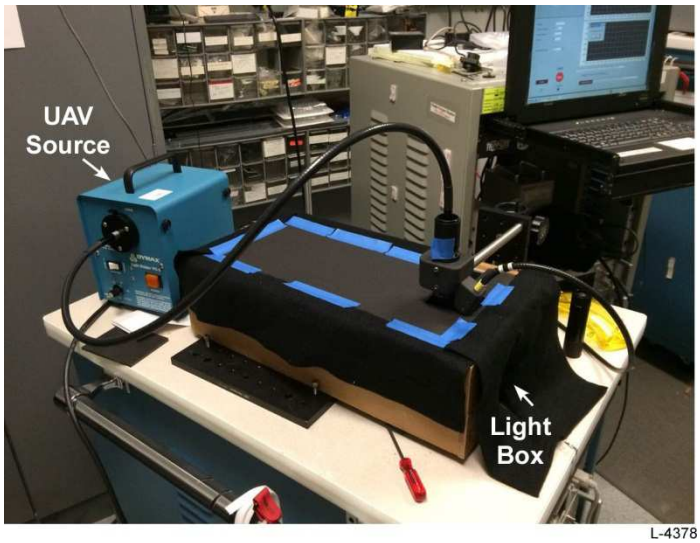

Figure 2. Fiber coupled UVA light source and light box. Subjects insert their arms through the right side of the light box. The equipment rack including the PC screen is shown in the background.

References:

1. Tewari A, Grage MM, Harrison GI, Sarkany R, Young AR. UVA1 is skin deep: molecular and clinical implications. Photochem Photobiol Sci. 2013;12(1):95-103.
2. Tuchinda C, Srivannaboon S, Lim HW. Photoprotection by window glass, automobile glass, and sunglasses. J Am Acad Dermatol. 2006;54(5):845-54.
3. Menter JM, Hatch KL. Clothing as solar radiation protection. Curr Probl Dermatol. 2003;31:50-63.
4. Wang F, Smith NR, Tran BA, Kang S, Voorhees JJ, Fisher GJ. Dermal damage promoted by repeated low-level UV-A1 exposure despite tanning response in human skin. JAMA Dermatol. 2014;150(4):401-6.
5. Sklar LR, Almutawa F, Lim HW, Hamzavi I. Effects of ultraviolet radiation, visible light, and infrared radiation on erythema and pigmentation: a review. Photochem Photobiol Sci. 2013;12(1):54-64.
6. Hanson KM, Clegg RM. Observation and quantification of ultraviolet-induced reactive oxygen species in ex vivo human skin. Photochem Photobiol. 2002;76(1):57-63.