Electronic Supplementary Material (ESI) for ChemComm.
This journal is © The Royal Society of Chemistry 2019

Supporting Information

A bioinspired, photostable UV-filter that protects mammalian cells against UV-induced cellular damage

Camille A. Martin,[a] Mahboobeh Rezaeeyazdi,[b] Thibault Colombani,[b] Sean R. Dinneen,[a] Amrita Kumar,[a] Sidi A. Bencherif,[b,c,d] and Leila F. Deravi*[a]

[a]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, United States
[b]Department of Chemical Engineering, Northeastern University, Boston, MA 02115, United States
[c]Department of Bioengineering, Northeastern University, Boston, MA 02115, United States
[d]John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02128, United States

*Corresponding author: l.deravi@northeastern.edu

Materials and Methods

Potassium ferricyanide (99% for analysis) and hydrochloric acid (ACS Reagent 37% solution) were purchased by Acros Organic. 2,2-Diphenyl-1-picrylhydrazyl (free radical 95% powder) and hydroxyurea (98%) were purchased from Alfa Aesar. Sylgard 184 silicone elastomer curing agent and Sylgard 184 silicone elastomer base were both purchased from Dow Corning Corporation. Methanol (certified ACS), ethanol (anhydrous histological grade), 2-propanol (certified ACS Plus), penicillin and streptomycin were purchased from Fisher Scientific. Avobenzone (pharmaceutical secondary standard certified reference material), oxybenzone (pharmaceutical secondary standard certified reference material), Dulbecco's Modified Eagles Medium (DMEM), fetal bovine serum (FBS), paraformaldehyde, 3-hydroxy-DL-kynurenine, 4',6-diamidino-2-phenylindole (DAPI), potassium phosphate monobasic powder and sodium phosphate dibasic heptahydrate (ACS Reagent 98-102.0 %) were purchased from Sigma Aldrich. Oasis weak ion exchange sample extraction cartridges (1 cc -30 mg) were purchased from Waters (Mildford, MA, USA). ViaQuant™ fixable dead cell assay, ActinGreen™ and CellQuant™ AlamarBlue cell viability assays were purchased from Genecopoeia. A cellular UV-Induced DNA Damage ELISA Kit (6-4PP) was purchased from Cell Biolabs, Inc.

A Molecular Devices Spectra Max M5 spectrophotometer was used for all spectrophotometric analyses. Zeiss Axio Observer Z1 fluorescence microscope was used for imaging ViaQuantTM fixable dead cell assay. Ultraviolet (UV) radiation intensity was measured using Sper Scientific 850009 UV-A/B light meter. Oriel LCS-100TM Small Area Sol1A 94011A was used in all irradiation experiments.

Xa synthesis

0.1 mmol potassium ferricyanide dissolved in deionized water was added to 18.9 μmol of 3-hydroxykynuerine dissolved in 0.2 mmol of phosphate buffer pH 7.4. The reaction mixture was

agitated for 30 minutes prior to purification using weak anionic exchange columns. Successful Xa synthesis was verified by Ultraviolet-Visible Spectrophotometry (UV-Vis spec), Ultra Performance Liquid Chromatography- Mass Spectrophotometry(UPLC-MS) and 1-D Proton Nuclear Magnetic Resonance Spectroscopy (^{1}H NMR) as previously reported.[1] Synthetic Xa was confirmed with UV-Vis spec λ_{max} 430 nm. UPLC-MS confirmed the structure of Xa, m/z $[M+1]_{theoretical}$ 424.0775, $m/z_{observed}$ 424.07223. ^{1}H NMR data where chemical shift (δ ppm), multiplicity (s = singlet, d=doublet, t=triplet, q=quartet, p=pentent, m=multiplet), coupling constant (Hz), and integration (H). ^{1}H NMR (500 MHz, DMSO) analysis of synthetic Xa: δ 8.38 (d, J= 3.85 Hz, 3H), 8.05 (t, J=4.47 Hz, 1H), 7.83 (d, J=4.01 Hz, 2H), 7.70 (s, 1H), 6.67 (s, 1H), 4.45 (d, J=4.96 Hz, 1H), 3.90 ppm (m, J=5.42 Hz, 2H).

Preparation of Xa-coated polymethyldisiloxane (PDMS) films

PDMS substrates were generated by combining Sylgard 184 silicone elastomer curing agent with Sylgard 184 silicone elastomer base (1:5) directly in a Costar 96-well lid (Corning Ref 3931). The mixture was hand stirred for 10 mins and then incubated at 60°C for 4 hours. After 4 hours the lid was cooled to room temperature and the PDMS substrate was then divided into segments. Soluble Xa was drop casted onto the surface of individual imprinted wells (0.32 cm^2) on the PDMS substrate.

Calculating Xa's sun protection factor (SPF)

Xa's SPF was calculated both in solution and as a film. Solutions were prepared containing 0.03-1.00 mM Xa dissolved in phosphate buffer pH 7.4. Xa-coated PDMS films composed of 0.30 - 5.00 mg/cm^2 Xa were prepared as previously described. The solutions and films were analyzed by UV-Vis spec from 290-400 nm. This data was applied to the Sayre *et. al.* derived Mansur equation [2] according to Equation 1:

$$SPF = CF \sum_{290}^{320} EE\ (\lambda)\ I\ (\lambda)\ Abs(\lambda) \quad (1)$$

Where EE (λ) represents the erythemal effect spectrum; I (λ) is solar intensity spectrum; Abs(λ) absorbance of UV-filter; CF is the correction factor (=10). The values for the normalized product function $[EE(\lambda)\ x\ I\ (\lambda)]$ used in the calculation of SPF can be found in Supplemental Table 1. The calculated SPF values were rounded to the nearest whole number.

The critical wavelength was determined by applying this data to Equation 2. [3]

$$\int_{290}^{\lambda_c} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda \quad (2)$$

Where A(λ) represents the absorbance of UV-absorbing compound and λ_c is the critical wavelength.

Measuring the photostability of Xa-coated PDMS films

PDMS films containing 5.00 mg/cm^2 Xa were prepared as previously described These substrates were placed on top of a 96 well plate positioned 23.5 cm from the light source and irradiated with a solar radiation of 820 W/m^2 (UV-near IR). This is equivalent to the global irradiance at solar noon for 4 hours on a clear day in Phoenix, AZ.[4] Each film was analyzed by UV-Vis spec (290 –

700 nm) every 30 mins over the course of 4 hours. The photostability was determined by applying this data to Equations 3 and 4.

$$AUC = \int_{290}^{400} A(\lambda) d\lambda \quad (3)$$

$$AUC\ Index\ (AUCI) = \frac{AUC_t}{AUC_{t=0}} \quad (4)$$

Where AUC is the area under the curve derived from the UV-absorbance spectra from 290 – 400 nm calculated using Equation 3; $AUC_{t=0}$ is the area under the curve determined prior to irradiation; AUC_t is the area under the curve at a specific time point (e.g. 30, 60, and 90 mins); AUC Index (AUCI) is the quotient of AUC after irradiation and AUC before irradiation calculated from Equation 4. A compound or material is considered to be photo-stable if AUCI > 0.8. [5]

Cytocompatibility of Xa

The cytocompatibility of Xa was evaluated with a murine fibroblast cell line (NIH 3T3, CRL-1658, ATCC, Rockville, MD, USA) using CellQuant™ AlamarBlue cell viability assay and ViaQuant™ fixable dead cell assay. Cells were seeded in 96 well plates at 5k viable cells/ well in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 μg/ mL penicillin and 100 μg/ mL streptomycin at 37℃ in a humidified 5% CO_2/95% air-containing atmosphere. After 24 hours the media was then replaced with 100 μL of media containing Xa (0.03-1.00 mM) and incubated for another 24 hours. For the CellQuant™ AlamarBlue cell viability assay, 10 μL of the AlamarBlue reagent (10% of total volume) was added to the cell media for 4 hours following the 24-hour incubation; absorbance was then measured at 570 and 600 nm. The cell viability was calculated as described by the manufacturer. For cell viability analysis using the ViaQuant™ fixable dead cell assay, after 24-hour incubation of cells with Xa (0.03 -1.00 mM), cells were treated with far-red (excitation λ: 633 nm, emission λ: 665 nm) fixable dead cell stain according to the manufacturer's instructions. The cells were then fixed with 4% formaldehyde solution for 30 minutes at room temperature and washed with phosphate buffered-saline (PBS, Sigma-Aldrich, pH 7.4). Prior to microscopy, cells were stained with 4',6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich) (excitation λ: 255 nm, emission λ: 450 nm) according to the manufacturer's protocol. Cells were imaged by fluorescence microscopy (Zeiss Axio Observer Z1). For each sample, 5 representative images were analyzed. Cell viability was determined by calculating the ratio of viable cells to the total number of cells in each image using the Cell Profiler software. Confocal microscopy was used to quantitatively and qualitatively assess the viability and attachment of cells. Following cell seeding with 3T3 fibroblasts, 5k per well and culture for 24 hours, cells were rinsed in PBS and incubated for 15 minutes at 37 °C with a fixable far-red dead (red) cell staining (ViaQuant™) according to manufacturer's instructions. The cells were then rinsed with PBS three times prior to fixation and pemeabilization with 0.1% Triton. Cells were stained with with DAPI (blue) and Alexa Fluor 488-phalloidin (green) according to manufacturer's protocols and imaged with both a fluorescence microscope (Zeiss, Carl Zeiss Microscopy) and a confocal microscope Leica TCS SP5 X WLL Confocal Microscope (Buffalo Grove, IL).

Monitoring the presence of UV-induced cellular damage upon solar irradiation

A total of 4k mouse fibroblasts were seeded in a 96 well plate with DMEM supplemented with 10% fetal bovine serum, 100 μg/ mL penicillin and 100 μg/ mL streptomycin at 37℃ in a humidified 5% CO_2/95% air-containing atmosphere. After 24 hours cell culture media was then removed and replaced with PBS supplemented with calcium and magnesium. Deionized water containing 10 mM hydroxyurea was added to each well and incubated at room temperature for 30 mins prior to irradiation. PDMS substrates with and without Xa were placed on top of each well prior to irradiation with 4.5 kJ/m^2 UV-B, measured using a UV-A/B radiometer, which is equivalent to approximately 11 minimal erythemal doses for skin type III according to the Fitzpatrick skin type classification scale.[6, 7] 1 MED is equivalent to 15-30 minutes of solar noon exposure in northern latitudes (20 °to 40°) therefore this dose represents approximately 4 hours of solar noon radiation felt by a person in North America who typically experiences mild burning. [6] The negative control plate was not exposed to irradiation. The presence of UV-induced photoproducts was evaluated using a cellular UV-Induced DNA Damage ELISA Kit to measure the formation of 6-4PP in intact cells, 30 minutes after irradiation as instructed by the manufacturer.

Evaluating the radical scavenging capacity of Xa using the 2,2-diphenyl-1-picrylhydrazyl (DPPH•) assay

Solutions containing 0.03-1.00 mM of Xa or ascorbic acid were prepared in 100 mM phosphate buffer pH 7.4 and water respectively. A total of 200 μM of DPPH• was prepared in methanol. Next, 20 μL of Xa or ascorbic acid was added to a well containing 100 uL of DPPH•. The 96 well plate was incubated at 37℃ for 30 minutes. DPPH• absorbance was measured at 517 nm. The antiradical activities of Xa and ascorbic acid were determined by calculating the inhibition of DPPH• using Equation 5.

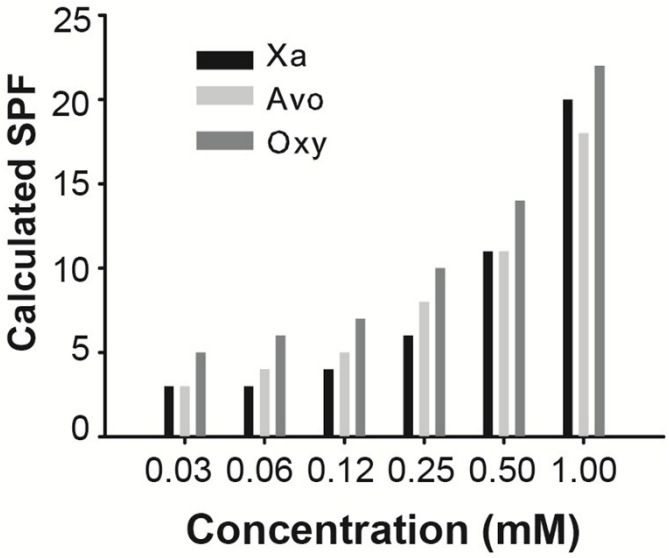
$$\%\, DPPH\cdot_{remaining} = \left(\frac{[DPPH\cdot]_t}{[DPPH\cdot]_{t=0}}\right) \times 100\% \quad (5)$$

Statistical analysis

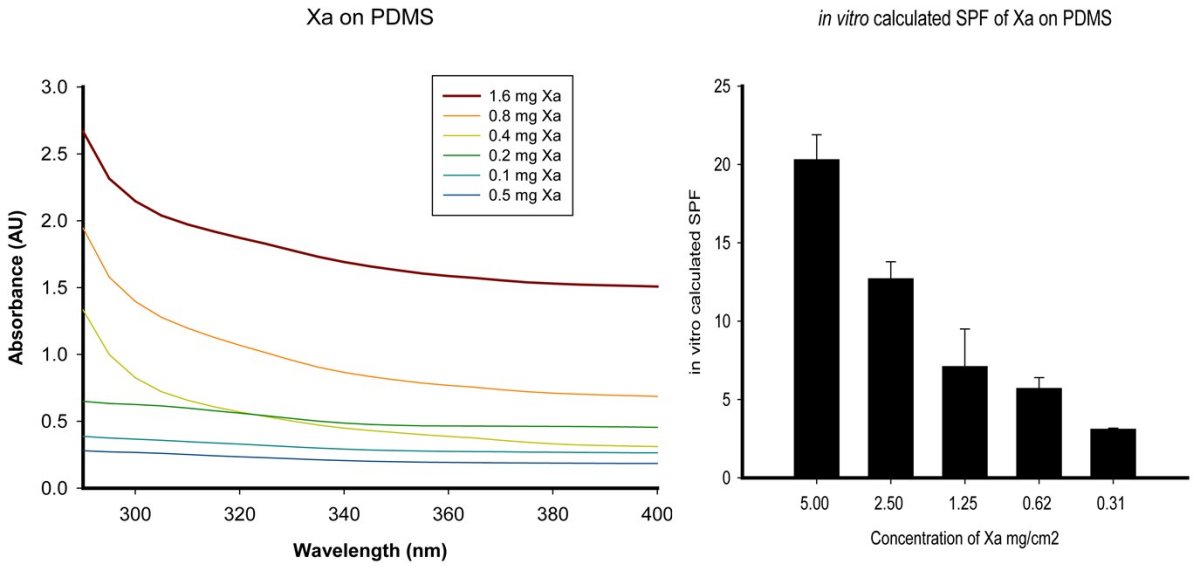
All values in the present study were expressed as mean ± SD. Statistical analyses were performed using the Student's two tail t-test. Differences were considered significant at $p<0.05$.

Supplemental Table 1. Normalized product function used in the calculation of SPF[2]

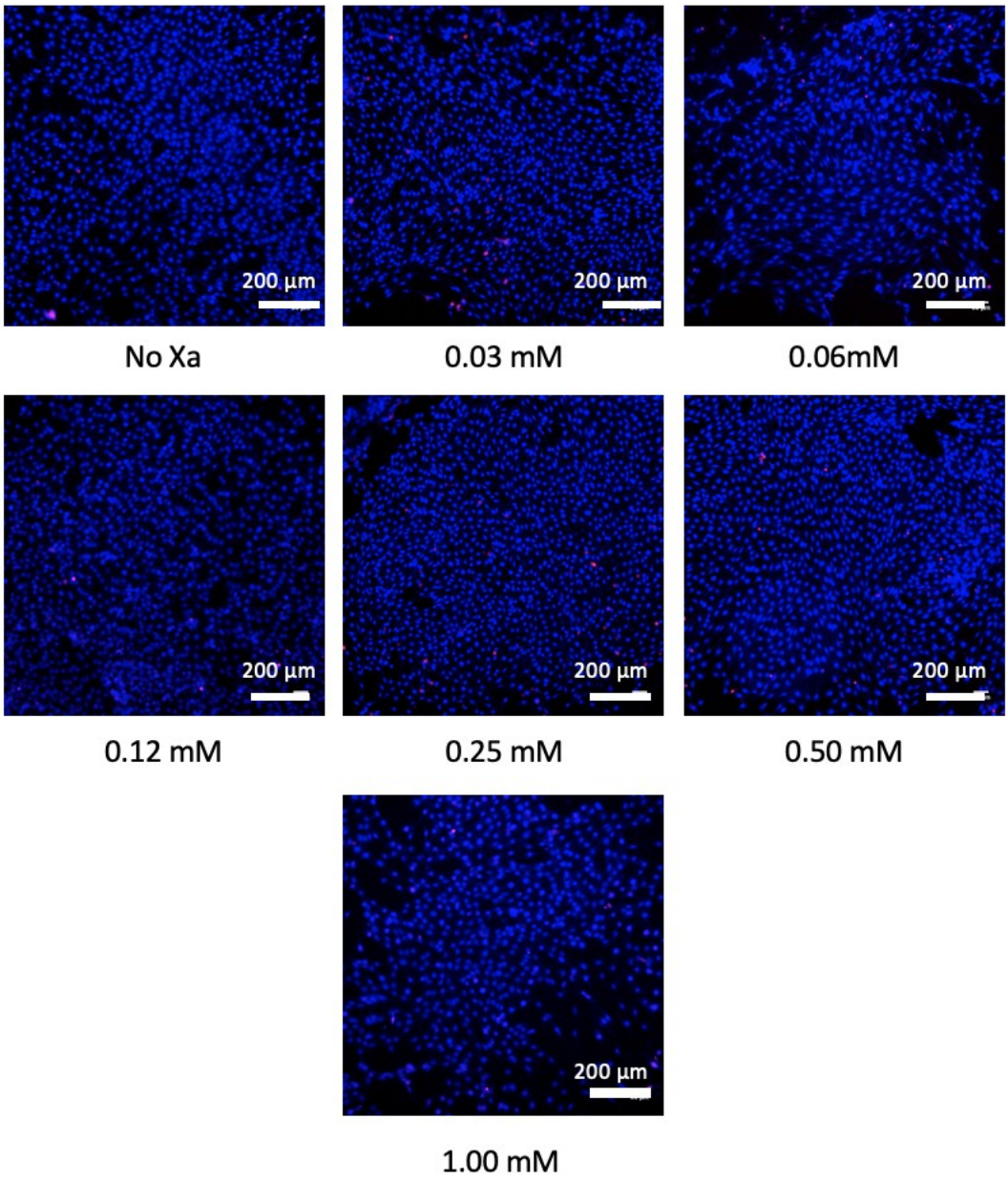
Wavelength (nm)	**EE x I (normalized)**
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1



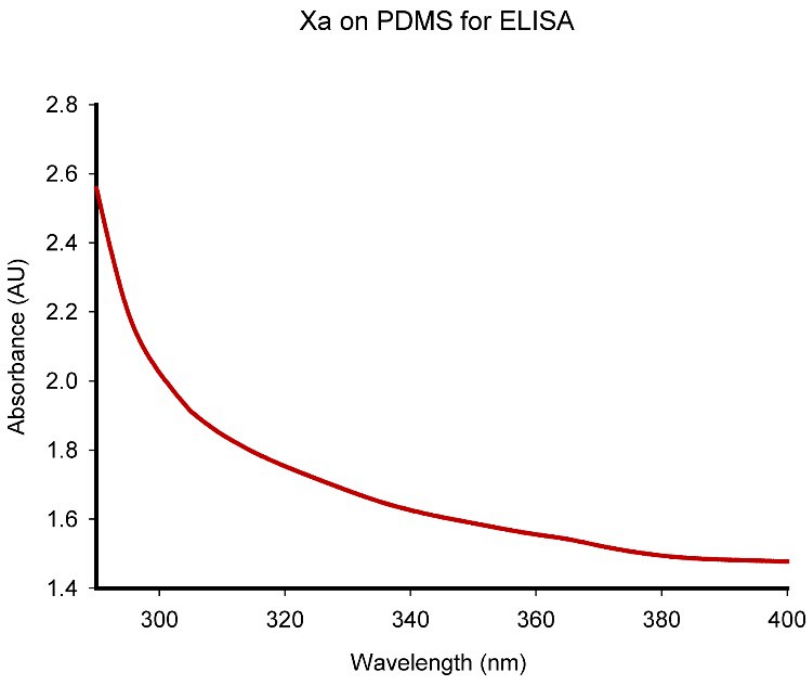
Supplemental Figure 1. The concentration dependent SPF of Xa (in PBS) as calculated from the Sayre derived Mansur equation compared to avobenzone (in isopropanol) and oxybenzone (in ethanol). Three unique solvents were selected in this study to maximize solubility of each material.



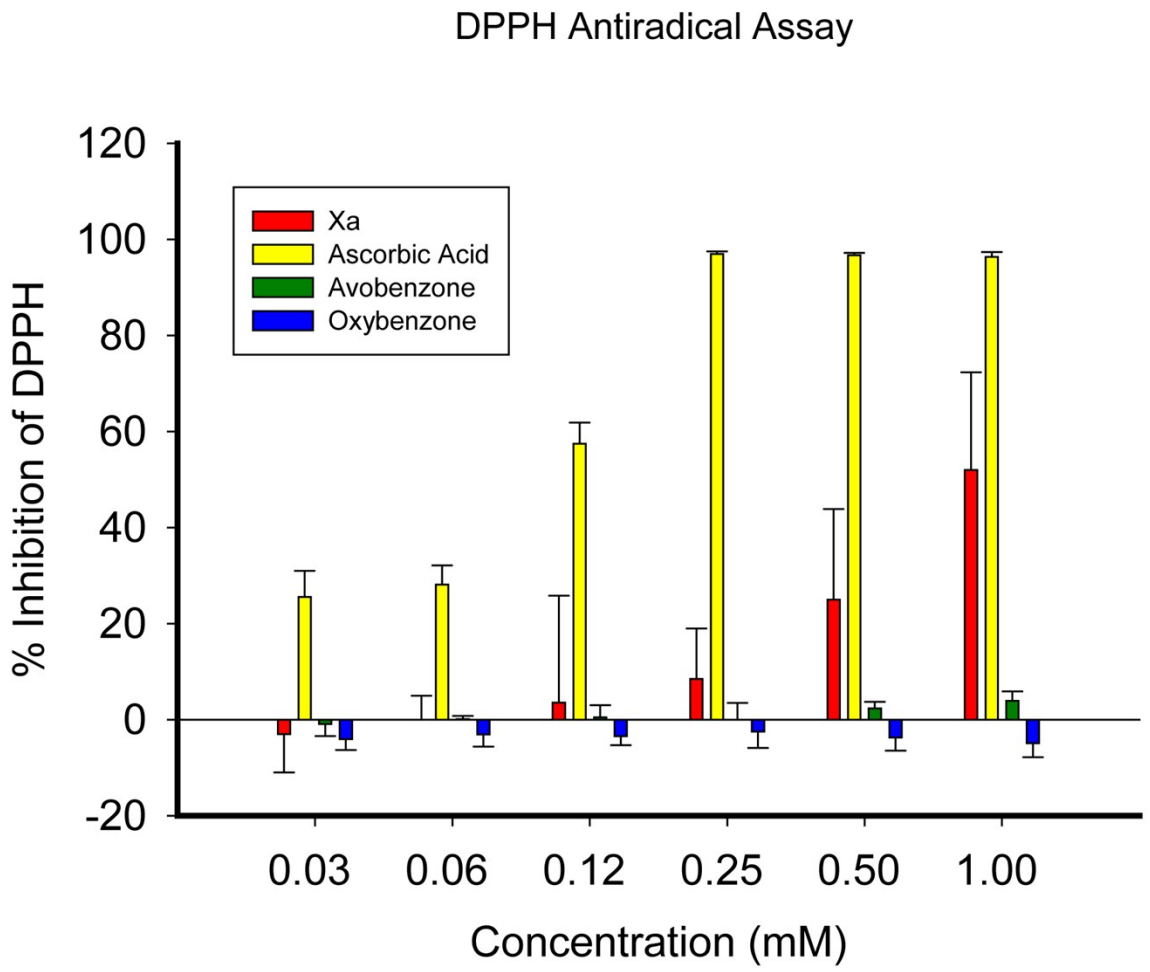
Supplemental Figure 2. A) UV-absorbance of Xa-coated PDMS films. The data represents the average absorbance values for three films composed of 0.31-5.00 mg/cm^2 Xa. B) Calculated SPF of Xa-coated PDMS films. Values represent mean and SD (n= 3).



Supplemental Figure 3. Fluorescence microscopy images of fibroblasts treated with Xa at various concentrations (0.03 -1.00 mM). After 24-hour incubation with Xa, cells were fixed and stained with DAPI and far-red dyes prior to imaging by Zeiss Axio Observer Z1 fluorescence microscope. Each picture is representative of 5 images per condition (n = 3).



Supplemental Figure 4. UV-absorbance of Xa-coated PDMS films used for ELISA assessments. The data represents the average absorbance values for three films composed of 5.00 mg/cm^2 Xa representing an SPF of 19 ± 4. Values represent mean and SD (n= 3).



Supplemental Figure 5. The DPPH assay was used to monitor the antioxidant activity of avobenzone, oxybenzone, ascorbic acid and Xa. Values represent mean and SD (n= 3). The antiradical activity of Xa was statistically similar to oxybenzone and avobenzone at concentrations 0.03-0.12 mM of each UV-absorbing compound, $p>0.05$. At 0.25-1.00 mM the antiradical activity of Xa was statistically different when compared to oxybenzone and avobenzone, $p<0.05$.

References

1. A. Kumar, T. L. Williams, C. A. Martin, A. Figueroa-Navedo and L. F. Deravi, *ACS Applied Materials & Interfaces*, 2018, **10**, 43177-43183.
2. R. M. Sayre, P. P. Agin, G. J. Levee and E. Marlowe, *Photochemistry and Photobiology*, 1979, **29**, 559-566.
3. U. S. F. D. Administration, *Journal*, 2018.
4. Newport, *Journal*, 2018.
5. J. Hojerova, Medovcikova, A. and M. Mikula, *International Journal of Pharmaceutics*, 2011, **408**, 27-38.
6. T. B. Fitzpatrick, *Arch Dermatol*, 1988, **124**, 869-871.
7. B. L. Diffey, *Photodermatol Photoimmunol Photomed*, 2002, **18**, 68-74.