Published in final edited form as:

Photochem Photobiol. 2012 ; 88(4): 1027–1032. doi:10.1111/j.1751-1097.2012.01163.x.

Nucleotide excision repair is reduced in oral epithelial tissues compared to skin†

David Mitchell^{1,*}, Lakshmi Paniker¹, and Dianne Godar²

¹Department of Carcinogenesis, University of Texas MD Anderson Cancer Center, Smithville, Texas, USA

²US Food and Drug Administration, Center for Devices and Radiological Health, Silver Spring, MD USA

Abstract

Ultraviolet radiation (UVR) exposure to internal tissues for diagnostic, therapeutic and cosmetic procedures has increased dramatically over the past decade. The greatest increase in UVR exposure of internal tissues occurs in the cosmetic industry where it is combined with oxidizing agents for teeth whitening, often in conjunction with indoor tanning. To address potential carcinogenic risks of these procedures, we analyzed the formation and repair of the DNA photoproducts associated with the signature mutations of UVR. Radioimmunoassay was used to quantify the induction and repair of cyclobutane pyrimidine dimers and pyrimidine(6-4)pyrimidone photoproducts in DNA purified from three reconstructed tissues, EpiDerm™, EpiGingival™, and EpiOral™ (MatTek Corp.). We observed comparable levels of DNA damage in all tissues immediately after UVR exposure. In contrast, repair was significantly reduced in both oral tissues compared to EpiDerm™. Our data suggest that UVR exposure of oral tissues can result in accumulation of DNA damage and increase the risk for carcinoma and melanoma of the mouth. Because NER is a broad-spectrum defense against DNA damage caused by a variety of agents in addition to UVR, our data suggest that the relatively low NER efficiency observed in oral tissues may have wide-ranging consequences in this highly exposed environment.

INTRODUCTION

Oral squamous cell carcinoma (SCC) affects ~40,000 Americans, occurs primarily in 2–3% of men and women over the age of 50 and results in ~8,000 deaths (see <http://www.oralcancerfoundation.org/facts/index.htm>). The overall 5-yr relative survival rate estimated from all sites and stages combined is 61%. Because early stage, curable lesions are rarely symptomatic, preventing fatal disease requires early detection and screening. The major risk factors for oral SCC are excessive smoking combined with moderate alcohol use, which together raise the risk ~100-fold in women and ~38-fold in men. In addition, oral SCC has been linked to chronic irritation, such as dental caries or the chewing of tobacco or betel quid, human papillomavirus and exposure to ultraviolet radiation (UVR).

UVR exposures to the oral cavity can occur in a variety of different ways, besides natural exposure to sunlight such as artificial exposure during diagnostic, therapeutic, dental or cosmetic procedures. One of the most promising tools for early diagnosis of neoplasms in oral tissues derives from the seminal work of Alfano who used ultraviolet B (UVB; 290–320

†The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the Department of Health and Human Services.

*Corresponding author: dmitchel@mdanderson.org, 512-237-9474-T; 512-237-2437-F; dmitchel@mdanderson.org.

nm) fluorospectroscopy to detect fluorophore biomarkers such as NAD(P)H, tryptophan and tyrosine (1). The principal that UVB-based fluorescence techniques could discriminate between normal and malignant cells (31) opened the door to *ex vivo* tissue studies with the potential for early detection of cancers of the breast (2, 36), bladder (3, 37), head and neck (17), prostate (1), ovary (13) and oral cavity (22, 23). Along with UVB-based diagnostic procedures, therapeutic applications of UVR have also been developed; for example: treatment of refractory oral chronic graft-versus-host disease (GVHD) following allogeneic stem cell transplantation (10, 32) and rhinophototherapy (RPT) that exposes the nasopharyngeal cavity to UVC/UVB radiation (240–320 nm) to treat allergic rhinitis and oral lichen planus (11, 18, 35).

Dental procedures that utilize UVR include UV photography to monitor plaques during direct bonding procedures (15), UVB excimer laser radiation delivered through optical fibers to detect and ablate residual organic tissues in root canals (30) and other techniques that use ultraviolet LED illumination to remove composite resins (6). However, UVR exposures of oral tissues during diagnostic, therapeutic and dental procedures pale by comparison to those associated with various 'light-activated' or 'light-accelerated' teeth whitening procedures. The basic teeth whitening procedure employs either a hydrogen gel or carbamide peroxide gel combined with UVR to theoretically accelerate the bleaching process via the induction of reactive oxygen species (ROS). Products such as Zoom!® and the Twilight Teeth Platinum Accelerated Whitening System® are commercially available and are used in combination with natural sunlight or sunlamps used for indoor tanning. Recent work from Bruzell and colleagues (19) examined the efficacy and risks of UV-assisted tooth bleaching and found little, if any, enhanced effect by the UVR source, regardless of the chemical product used.

Because the oral cavity is exposed to UVR, it is important to understand the responses of different tissues in the mouth to the same type of DNA damage responsible for the UVB signature mutations associated with SCCs in human skin. Because we know a great deal about the UVR response of skin, but not other potentially exposed internal tissues, comparative repair data can be used to estimate the risks of SCC in response to UVR exposure of the respiratory epithelium as well as other tissues. We know that mutations associated with specific types of DNA photodamage can cause carcinoma (25, 26) and that nucleotide excision repair (NER) removes almost all of this damage and reduces its tumorigenic potential. Indeed, individuals who lack NER, such as patients with the autosomal recessive disorder *Xeroderma pigmentosum*, are orders of magnitude more likely to develop sunlight-induced skin cancers compared to the normal population (7). Using this rationale, we (dlm) previously tested the ability of EpiAir™, a 3-D reconstructed respiratory epithelium tissue model, and EpiDerm™, a similarly reconstructed epidermis, to repair CPDs and (6-4)PPs induced by UVR (25). We found that both tissue models have similar repair rates for the different photoproducts comparable to that observed in human *in vivo*; that is, the UVR response of human respiratory epithelium is comparable to that of human skin. Using a similar approach in the current study, we examined the ability of the major oral tissue types to repair the photoproducts associated with SCC and melanoma. To this end, we quantified NER of CPDs and (6-4)PPs in EpiGingival™ and EpiOral™ 3D-reconstructed models of human gingival and buccal tissues, respectively, and compared their repair capacities to EpiDerm™ 3D-reconstituted model of human skin tissue.

MATERIALS AND METHODS

Tissue culture

The tissues were obtained from MatTek Corporation (Ashland, MA). The EpiDerm™ tissue consisted of normal, human-derived epidermal keratinocytes cultured to form a

multilayered, highly differentiated 3-D model of human epidermis; the tissue possesses all the cell layers and the ultrastructural characteristics of human epidermis. The EpiGingival™ tissue consists of normal, human-derived gingival epithelial cells cultured to form a multilayered, highly differentiated 3-D model of the human gingival; the tissue possesses an organized basal layer and multiple cornified layers analogous to native human gingival tissue. The EpiOral™ tissue consists of normal, human-derived buccal epithelial cells cultured to form a multilayered, highly differentiated 3-D model of the human buccal; the tissue possesses an organized basal layer and multiple non-cornified layers analogous to native human buccal tissue. Tissues were transferred to 6-well culture plates, fed appropriate serum-free, specially prepared phenol red-, antibiotic- and antifungazone-free media supplied by MatTek Corporation (Ashland, MA), and grown overnight at 37°C with 5% CO₂ and humidity prior to exposure. Each experiment was performed in triplicate, except the time course that was performed in duplicate.

UVR irradiation

Tissue samples were irradiated in phenol-free, antibiotic-free, antifungazone-free, serum-free media from above through the plastic lids of 6 well plates placed 58 cm from a bank of eight FS40 lamps (FSX24T12/UVB/HO) installed in a Panosol II Holder (National Biological Corp., Twinsburg, OH) equipped with a cellulose acetate plastic filter (Kodacel, Kodak) to cut-off wavelengths shorter than 290 nm. FS40 sunlamps emit predominantly in the UVB waveband region (290–320 nm) with some radiation in the UVA waveband region (320–400 nm). Tissue samples were either unirradiated (sham-exposed) or irradiated in an incubator at 37°C for multiples of 20 minutes to yield unweighted UVR doses of 2,315 J/m² that are equivalent to CIE erythemally-weighted UVR doses of 132 J/m²; doses were all within 3%. Tissue samples were flash frozen in liquid nitrogen at T=0 or cultured for extended periods (T=24, 48 and 72 hr) using fresh media every 24 h until flash frozen.

DNA isolation and damage analysis

DNA was extracted from MatTek tissues using standard techniques (Gentra Systems Inc, Minneapolis MN, USA). DNA concentrations and purity were determined by reading the absorbance at 230, 260, 280, and 320 nm. DNA damage was quantified using radioimmunoassay (RIA). RIA is a competitive binding assay between radiolabeled DNA and sample DNA for antisera raised against UV-irradiated DNA. DNA damage frequencies in samples used for the standard curve were determined using HPLC-tandem mass spectrometry (Thierry Douki, CEA, Grenoble). These details, as well as those concerning the specificities of the RIAs and standards used for quantification, are described in Mitchell (26, 27).

Statistical Analyses

Two-sample independent t-tests using the R Statistical Package version. 2.7.1 (<http://www.r-project.org/>) were used to determine significant differences between data. Linear regressions exponential decay curves and associated values (Tables) were generated using SigmaPlot version 10 (Systat, San Jose, CA).

RESULTS

We conducted a comparative analysis of 3-D reconstructed epidermal (EpiDerm™), gingival (EpiGingival™), and oral (EpiOral™) tissues to better understand the similarities and differences with regard to their biological responses to UVR. In our initial studies, we measured DNA damage immediately and 24 h after a UVB dose response. Two types of DNA damage were quantified; CPD (Fig. 1) and (6-4)PP (Fig. 2). Fig. 1 shows that comparable amounts of CPDs were initially formed in all three tissue types. The induction

rate for (6-4)PPs was much lower relative to CPDs and increased the variation in the data (Fig. 2). We calculated the slopes and coefficients of determination (r^2) for each curve from the regression analyses of the dose response curves (Tables I and II). The ratio of those slopes was used to determine the 24 h repair rates. Thus, an estimate of the NER capacity in each tissue type was averaged through a range of doses.

We also exposed the MatTek tissues to the highest dose of UVR (750 J/m²) and measured the amount of CPDs and (6-4)PPs at different times post-irradiation, including 0, 24, 48 and 72 h (Fig. 3). The data from the 0 and 24 h dose response curves shown in Figs. 1 and 2 and the single dose repair kinetics shown in Fig. 3 agree well with one another. It is evident that significant differences exist between tissue types. In Fig 3, Panel A, most of the CPDs are removed from the EpiDerm™ tissues by 24 h (~60%) with much slower repair observed at 48 and 72 h, consistent with the biphasic kinetics of NER (14). In contrast, only ~20% of the CPDs are repaired by the EpiGingival™ tissue by 24 h increasing to 40% by 72 h. The EpiOral™ tissue displayed negligible removal of CPDs by 24 h and only 10–20% of these lesions by 72 h after UVR exposure. Consistent with the CPD analysis, (6-4)PPs showed much reduced NER in both the EpiGingival™ and EpiOral™ tissues.

As shown in Figure 3, Panel B (6-4)PP removal in EpiDerm™ was significantly faster and more extensive than CPD repair, consistent with published patterns of the relative repair of these two lesions. Indeed, in a previous study using EpiDerm™ (23), we used a shorter time-course to measure NER and found 40%, 60% and 70% repair at 3, 6 and 12 h post-irradiation, respectively, with no additional repair between 12 and 24 h. In contrast, we see much reduced repair of the (6-4)PP in both the EpiGingival™ and EpiOral™ tissue constructs compared to EpiDerm™. The major observation is that both oral tissues are “repair defective” compared to EpiDerm™ and human skin (23).

DISCUSSION

We analyzed the ability of the diverse tissues in the mouth to repair the high levels of pre-mutagenic damage potentially induced by oral diagnostic, therapeutic, or cosmetic UVR exposures and found that NER was significantly reduced in these tissues relative to skin. Our data suggest that the predominant lesions induced by UVR, as well as the bulky chemical adducts associated with other routes of environmental exposure (e.g., tobacco, air pollution), may persist for prolonged periods of time, significantly increase the probability for mutation induction and cell transformation in oral tissues and increase the risks for oral cancers

Although other bodily locations besides the skin are exposed to UVR (e.g., nasal and mucosal tissues), limited data exist concerning the capacity of these tissues to repair DNA damage. Studies performed on human bronchial fibroblasts and epithelial cells showed similar DNA repair rates as human skin fibroblasts, suggesting that DNA repair mechanisms may be equally efficient in these cell types (12). In addition, a recent pilot study showed that human nasal epithelial cells in vivo are capable of efficiently repairing UV-induced DNA damage in allergic rhinitis patients receiving intranasal phototherapy (19). On the other hand, considerable heterogeneity has been observed in other human cell types, often dependent on the stage of development, with non-proliferating and terminally-differentiated cells (e.g., neurons and astrocytes) showing lower NER rates relative to cells from self-regenerating organs such as the skin (28).

The goal of the current study was to compare the NER capacity of epithelial gingival and oral tissues with skin tissue after exposure to UVR by measuring the induction and loss over time of the two major UVR photoproducts, CPDs and (6-4)PPs. Because there is an

extensive knowledge base available for assessing risks of UVR exposure to skin, reconstructed 3-D human skin models can be used as benchmarks to evaluate the risks associated with UVR exposure of test tissues (*e.g.*, oral) based on quantifiable DNA damage and repair metrics. The deficient NER observed in EpiGingival™ and EpiOral™ reconstructed tissues relative to EpiDerm™ was unexpected and suggests that exposure of the oral cavity to UVR, as well as other environmental agents, may be considerably more dangerous than previously believed based on DNA damage response data derived from human and mouse skin experiments.

The efficiencies of CPD and (6-4)PP repair in the two different oral tissues are reversed: CPDs are repaired slower in EpiOral™ compared to EpiGingival™ tissue; (6-4)PP repair is slower in the EpiGingival™ compared to the EpiOral™ tissue. The mechanism underlying these differences is problematic since NER recognizes and repairs both lesions, albeit with different affinities. It is possible that the tissues differ in their ability to differentially recognize and access CPDs and (6-4)PPs. For example, the UV DNA damage-binding protein (UV-DDB), corresponding to the XPE component of the NER complex, is specifically targeted to CPDs and its absence does not affect (6-4)PP repair (21, 29). Conversely, the DNA damage recognition heterodimer XPC-hHR23B has a high affinity for the (6-4)PP and rarely binds the CPD in *in vitro* systems (33, 34). Hence, reduced UV-DDB activity in EpiOral™ and XPC binding in EpiGingival™ tissues could explain our data. In addition, accessory functions that facilitate NER could also affect differential repair of CPDs and (6-4)PPs. For instance, knockdown of Brg1, the ATPase subunit of the chromatin remodeling complex SWI/SNF, abrogates the excision of CPDs but not (6-4)PPs (38). Presumably, chromatin relaxation by SWI/SNF facilitates CPD repair more than it does (6-4)PP repair, further supporting the idea that the highly distortive (6-4)PP is more readily recognized by NER proteins and less dependent on chromatin remodeling for recognition. Since these lesions have different molecular and biological consequences, the differences in their repair in different tissue types could have different pathological consequences.

The molecular mechanisms underlying the etiology of UVB-induced SCC in mice and humans are fairly well understood. Transition mutations arising at the 3' base of specific T-C dipyrimidine sites have been found in ~50% of the p53 tumor suppressor genes in human skin carcinomas (39). Along with CC→TT tandem double mutations, C→T transitions are considered the 'signature' mutations of UVB irradiation of DNA (5, 39) and, although the mutagenic events responsible for these UVB-induced transitions can be heterogeneous and are not fully understood, it is well-established that CPDs and (6-4)PPs are ultimately responsible. Research suggests that the accumulation of these mutations in oncogenes and tumor suppressor genes of chronically-exposed skin confers a selective advantage on cells with a dysfunctional p53 gene and that reduced apoptosis and increased clonal expansion of these damaged cells leads to formation of mutant p53 clusters, actinic keratoses and, ultimately, carcinomas (4, 5, 16, 39). This model is not limited to UVR. For example, p53 mutations at hotspots of benzo[a]pyrene diol epoxide adduct formation in the p53 gene of cultured lung cells directly link tobacco with lung cancer (9).

Skin cancer is significantly mitigated by NER; the predominant pathway used by mammalian cells to remove the DNA damage responsible for bulky adducts, including UVB signature mutations (8). Unrepaired DNA lesions lead to ~1000-fold greater frequency of skin tumors in *Xeroderma pigmentosum* patients compared to the normal population (20). Hence, the severely reduced rate of NER measured in the gingival and oral mucosa tissue models shown here strongly suggests that the mouth is considerably more vulnerable to the deleterious effects of UVR than either the skin or respiratory epithelium (25). Indeed, because NER is a broad spectrum repair mechanism, its relatively low capacity in oral tissues may increase the risks to other environmental insults in addition to UVR (*e.g.*, DNA

adducts associated with cigarette smoke and smokeless tobacco). Given the clear differences in DNA repair in epithelial tissues in the mouth compared to skin, it is evident that exposure of the oral cavity to UVR should be minimized with regard to time, area of exposure, and accumulated exposures. Furthermore, the data discourages using UVR for cosmetic teeth whitening due to the potential for significantly increasing people's risks for oral cancer.

In summary, our results are consistent with previous EpiDerm™ studies (24), which show that the extent of (6-4) PP repair is significantly greater than CPD repair at 24 h. Furthermore, compared to EpiDerm™, both oral tissues manifest a significant reduction in the ability to repair both CPDs and (6-4)PPs. We conclude that both artificial of the oral tissue constructs examined here are deficient in NER compared to similar epidermal tissue constructs as well as human skin *in vivo* (25).

Acknowledgments

This work was supported by the National Cancer Institute [grant nr. CA113671], the National Institute of Environmental Health Sciences [Center grant nr. ES007784] and the Food and Drug Administration's Critical Path Initiative. The authors would like to thank Robert James for dosimetry of the UVB source and Joshua Pfefer for helpful scientific discussions.

Abbreviations

3-D	3-dimensional
(6-4) PPs	(6-4) photoproducts
CPDs	cyclobutane pyrimidine dimers
UVA	(320–400 nm)
UVB	290–320 nm
UVR	ultraviolet radiation (290–400 nm)

References

1. Alfano RR, Das BB, Cleary J, Prudente R, Celmer EJ. Light sheds light on cancer--distinguishing malignant tumors from benign tissues and tumors. *Bull N Y Acad Med.* 1991; 67:143–50. [PubMed: 2049567]
2. Alimova A, Katz A, Sriramoju V, Budansky Y, Bykov AA, Zeylikovich R, Alfano RR. Hybrid phosphorescence and fluorescence native spectroscopy for breast cancer detection. *J Biomed Opt.* 2007; 12:014004. [PubMed: 17343479]
3. Anidjar M, Ettori D, Cussenot O, Meria P, Desgrandchamps F, Cortesse A, Teillac P, Le Duc A, Avrillier S. Laser induced autofluorescence diagnosis of bladder tumors: dependence on the excitation wavelength. *J Urol.* 1996; 156:1590–6. [PubMed: 8863545]
4. Berg RJ, van Kranen HJ, Rebel HG, de Vries A, van Vloten WA, Van Kreijl CF, van der Leun JC, de Gruijl FR. Early p53 alterations in mouse skin carcinogenesis by UVB radiation: immunohistochemical detection of mutant p53 protein in clusters of preneoplastic epidermal cells. *Proc Natl Acad Sci U S A.* 1996; 93:274–278. [PubMed: 8552621]
5. Brash DE, Rudolph JA, Simon JA, Lin A, McKenna GJ, Baden HP, Halperin AJ, Ponten J. A role for sunlight in skin cancer: UV-induced p53 mutations in squamous cell carcinoma. *Proc Natl Acad Sci U S A.* 1991; 88:10124–10128. [PubMed: 1946433]
6. Bush MA, Hermanson AS, Yetto RJ, Wieczkowski G Jr. The use of ultraviolet LED illumination for composite resin removal: an in vitro study. *Gen Dent.* 2010; 58:e214–8. [PubMed: 20829156]
7. Cleaver, J.; Mitchell, D. Ultraviolet radiation carcinogenesis. In: Hong, W.; Bast, R.J.; Hait, W.; Kufe, D.; Pollock, R.; Weichselbaum, R.; Holland, J.; Frei, E.I., editors. *Cancer Medicine*. McGraw-Hill; UK: 2009. p. 262-269.

8. de Laat WL, Jaspers NG, Hoeijmakers JH. Molecular mechanism of nucleotide excision repair. *Genes Dev.* 1999; 13:768–85. [PubMed: 10197977]
9. Denissenko MF, Pao A, Tang M, Pfeifer GP. Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science.* 1996; 274:430–2. [PubMed: 8832894]
10. Enk CD, Elad S, Vexler A, Kapelushnik J, Gorodetsky R, Kirschbaum M. Chronic graft-versus-host disease treated with UVB phototherapy. *Bone Marrow Transplant.* 1998; 22:1179–83. [PubMed: 9894721]
11. Farkas A, Kemeny L. Applications of the 308-nm Excimer Laser in Dermatology. *Laser Physics.* 2006; 16:876–83.
12. Fornace AJ Jr, Lechner JF, Grafstrom RC, Harris CC. DNA repair in human bronchial epithelial cells. *Carcinogenesis.* 1982; 3:1373–7. [PubMed: 7151252]
13. George R, Chandrasekaran A, Brewer MA, Hatch KD, Utzinger U. Clinical research device for ovarian cancer detection by optical spectroscopy in the ultraviolet C-visible. *J Biomed Opt.* 2010; 15:057009. [PubMed: 21054125]
14. Greinert R, Boguhn O, Harder D, Breitbart EW, Mitchell DL, Volkmer B. The dose dependence of cyclobutane dimer induction and repair in UVB-irradiated human keratinocytes. *Photochem Photobiol.* 2000; 72:701–708. [PubMed: 11107858]
15. Gwinnett J, Ceen RF. An ultraviolet photographic technique for monitoring plaque during direct bonding procedures. *American J of Orthodontics.* 1978; 73:2178–186.
16. Jonason AS, Kunala S, Price GJ, Restifo RJ, Spinelli HM, Persing JA, Leffell DJ, Tarone RE, Brash DE. Frequent clones of p53-mutated keratinocytes in normal human skin [see comments]. *Proc Natl Acad Sci U S A.* 1996; 93:14025–14029. [PubMed: 8943054]
17. Katz A, Savage HE, Schantz SP, McCormick SA, Alfano RR. Noninvasive native fluorescence imaging of head and neck tumors. *Technol Cancer Res Treat.* 2002; 1:9–15. [PubMed: 12614172]
18. Kemeny L, Koreck A. Ultraviolet light phototherapy for allergic rhinitis. *J Photochem Photobiol B.* 2007; 87:58–65. [PubMed: 17329119]
19. Koreck A, Szechenyi A, Morocz M, Cimpean A, Bella Z, Garaczi E, Raica M, Olariu TR, Rasko I, Kemeny L. Effects of intranasal phototherapy on nasal mucosa in patients with allergic rhinitis. *J Photochem Photobiol B.* 2007; 89:163–9. [PubMed: 17997323]
20. Kraemer KH, Lee MM, Andrews AD, Lambert WC. The role of sunlight and DNA repair in melanoma and nonmelanoma skin cancer. The xeroderma pigmentosum paradigm. *Arch Dermatol.* 1994; 130:1018–21. [PubMed: 8053698]
21. Li J, Wang QE, Zhu Q, El-Mahdy MA, Wani G, Praetorius-Ibba M, Wani AA. DNA damage binding protein component DDB1 participates in nucleotide excision repair through DDB2 DNA-binding and cullin 4A ubiquitin ligase activity. *Cancer research.* 2006; 66:8590–7. [PubMed: 16951172]
22. McGee S, Mardirossian V, Elackattu A, Mirkovic J, Pistey R, Gallagher, Kabani S, Yu CC, Wang Z, Badizadegan K, Grillone G, Feld MS. Anatomy-based algorithms for detecting oral cancer using reflectance and fluorescence spectroscopy. *Ann Otol Rhinol Laryngol.* 2009; 118:817–26. [PubMed: 19999369]
23. McGee S, Mirkovic J, Mardirossian V, Elackattu A, Yu CC, Kabani S, Gallagher G, Pistey R, Galindo L, Badizadegan K, Wang Z, Dasari R, Feld MS, Grillone G. Model-based spectroscopic analysis of the oral cavity: impact of anatomy. *J Biomed Opt.* 2008; 13:064034. [PubMed: 19123680]
24. Mitchell D, Paniker L, Sanchez G, Bella Z, Garaczi E, Szell M, Hamid Q, Kemeny L, Koreck A. Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cell Mol Med.* 2008
25. Mitchell D, Paniker L, Sanchez G, Bella Z, Garaczi E, Szell M, Hamid Q, Kemeny L, Koreck A. Molecular response of nasal mucosa to therapeutic exposure to broad-band ultraviolet radiation. *J Cell Mol Med.* 2010; 14:313–22. [PubMed: 18671762]
26. Mitchell, DL. Radioimmunoassay of DNA damaged by ultraviolet light. *Technologies for Detection of DNA.* In: Pfeifer, G., editor. *Damage and Mutations.* Plenum Publishing Corp; New York: 1996. p. 73-85.

27. Mitchell DL. Quantification of photoproducts in mammalian cell DNA using radioimmunoassay. *Methods Mol Biol.* 2006; 314:239–249. [PubMed: 16673886]
28. Mitchell DL, Hartman PS. The regulation of DNA repair during development. *Bioessays.* 1990; 12:74–79. [PubMed: 2188652]
29. Oh KS, Imoto K, Emmert S, Tamura D, DiGiovanna JJ, Kraemer KH. Nucleotide excision repair proteins rapidly accumulate but fail to persist in human XP-E (DDB2 mutant) cells. *Photochemistry and photobiology.* 2011; 87:729–33. [PubMed: 21388382]
30. Pini R, Salimbeni R, Vannini M, Barone R, Clauser C. Laser dentistry: a new application of excimer laser in root canal therapy. *Lasers Surg Med.* 1989; 9:352–7. [PubMed: 2761330]
31. Pradhan A, Pal P, Durocher G, Villeneuve L, Balassy A, Babai F, Gaboury L, Blanchard L. Steady state and time-resolved fluorescence properties of metastatic and non-metastatic malignant cells from different species. *J Photochem Photobiol B.* 1995; 31:101–12. [PubMed: 8583278]
32. Schubert MM, Correa ME. Oral graft-versus-host disease. *Dent Clin North Am.* 2008; 52:79–109. viii–ix. [PubMed: 18154866]
33. Sugawara K, Ng JM, Masutani C, Iwai S, van der Spek PJ, Eker AP, Hanaoka F, Bootsma D, Hoeijmakers JH. Xeroderma pigmentosum group C protein complex is the initiator of global genome nucleotide excision repair. *Mol Cell.* 1998; 2:223–32. [PubMed: 9734359]
34. Sugawara K, Okamoto T, Shimizu Y, Masutani C, Iwai S, Hanaoka F. A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes Dev.* 2001; 15:507–21. [PubMed: 11238373]
35. Trehan M, Taylor CR. Low-dose excimer 308-nm laser for the treatment of oral lichen planus. *Arch Dermatol.* 2004; 140:415–20. [PubMed: 15096369]
36. Yang Y, Katz A, Celmer EJ, Zurawska-Szczepaniak M, Alfano RR. Fundamental differences of excitation spectrum between malignant and benign breast tissues. *Photochem Photobiol.* 1997; 66:518–22. [PubMed: 9337625]
37. Zaak D, Stepp H, Baumgartner R, Schneede P, Waidelich R, Frimberger D, Hartmann A, Kunchel R, Hofstetter A, Hohla A. Ultraviolet-excited (308 nm) autofluorescence for bladder cancer detection. *Urology.* 2002; 60:1029–33. [PubMed: 12475664]
38. Zhao Q, Wang QE, Ray A, Wani G, Han C, Milum K, Wani AA. Modulation of nucleotide excision repair by mammalian SWI/SNF chromatin-remodeling complex. *J Biol Chem.* 2009; 284:30424–32. [PubMed: 19740755]
39. Ziegler A, Leffell DJ, Kunala S, Sharma HW, Gailani M, Simon JA, Halperin AJ, Baden HP, Shapiro PE, Bale AE. Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. *Proc Natl Acad Sci USA.* 1993; 90:4216–4220. [PubMed: 8483937]

Figure 1.

Dose responses for CPDs formation by UVR in 3-D reconstructed MatTek tissues. CPDs were quantified in purified DNA from EpiDerm™ (Panel A), EpiGingival™ (Panel B) and EpiOral™ (Panel C) immediately after UVR irradiation (●) and 24 h later (○). Regression lines were generated using SigmaPlot and slopes were calculated for each curve to yield the repair rates shown in Table I.

Figure 2.

Dose responses for 6-4PPs formation by UVR in 3-D reconstructed MatTek tissues. 6-4PPs were quantified in purified DNA from EpiDerm™ (Panel A), EpiGingival™ (Panel B) and EpiOral™ (Panel C) immediately after UVR irradiation (●) and 24 h later (○). Regression lines were generated using SigmaPlot and slopes were calculated for each curve to yield the repair rates shown in Table II.

Figure 3.

Differential nucleotide excision repair kinetics in artificial skin and oral tissues. The relative amounts of CPDs (Panel A) and (6-4)PPs (Panel B) at 24, 48 and 72 h are shown normalized to the measured values of photoproducts immediately after UV (T_0). Tissues include EpiDerm™ (●), EpiGingival™ (■) and EpiOral™ (▲). The 3-parameter exponential decay curves were generated using SigmaPlot 10.0 and the equation $y = y_0 + ae^{-bx}$.

Table I

Regression analyses and relative 24 h repair rates of CPDs in MatTek 3-D reconstructed tissues. The slopes of the lines shown in Figures 1 and 2 are shown as the number of lesions induced (0 h) or remaining (24 h) in 10^6 bases per initial unit dose of UVR (J/m^2). R^2 is the coefficients of determination. The percentage repair of CPDs at 24 h is determined as $1 - (24 \text{ h slope}/0 \text{ h slope})$.

	Time (h)	R^2	CPDs/mb/J/m ²	Repair (%)
EpiDerm	0	0.9357	0.73	
	24	0.9540	0.30	59
EpiGingival	0	0.9590	0.69	
	24	0.9322	0.50	28
EpiOral	0	0.9613	0.41	
	24	0.9343	0.47	0

Table II

Regression analyses and relative 24 h repair rates of 6-4PPs in MatTek 3-D reconstructed tissues. See Table I.

Tissue	Time (h)	R ²	(6-4)PPs/mb/J/m ²	Repair (%)
EpiDerm	0	0.9650	0.019	100
	24	nd	nd	
EpiGingival	0	0.8332	0.034	0
	24	0.7627	0.035	
EpiOral	0	0.7411	0.025	44
	24	0.8739	0.014	