Is Lotion Sunscreen Better than Spray?

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Experiment 9: Efficacy of Sunscreen

I. Introduction

Section 1. Description of Problem

In the normal epidermis, the layers of cells go, top to bottom, squamous cells, basal cells, and melanocytes. (Doonan, Lecture, Nov 13, 2019) Below the epidermis is the dermis, and here there are neurons and blood vessels. When skin cells receive repeated UV radiation, over time the cells cannot repair themselves fast enough and, with prolonged exposure, will lead to cancer. (Doonan, Lecture, Nov 13, 2019)

There are various types of skin cancer that a person can get. Actinic Keratosis is precancerous and involves having dry, scaly patches of skin. (Doonan, Lecture, Nov 13, 2019) Squamous Cell Carcinoma, the second most common type of skin cancer, affects the squamous cells and goes the least deep into the dermis of the cancers. (Doonan, Lecture, Nov 13, 2019) Basal Cell Carcinoma, the most common type, affects basal cells and goes farther into the dermis, but not as far as the last type, Melanoma.

Melanoma affects about 192,000 people a year and about 7,000 people a year die from it. This type of skin cancer goes deep into and through the dermis and reaches the fatty layer underneath. (Doonan, Lecture, Nov 13, 2019) Not only is it bad for the cancerous growth to reach into the fat layer, but also this cancer is due to mutations in melanocytes, which when mutated grow incredibly quickly. (Doonan, Lecture, Nov 13, 2019)

To avoid melanoma, the major recommendations are to put on sunscreen, cover your arms and legs with clothes, and cover your face with a hat. (Doonan, Lecture, Nov 13, 2019) Though this is good advice, sometimes sunscreen alone is not enough and due to heat, people don't want to cover up in layers of clothing.

Section 2. UV Light and DNA Damage

UV light cause damage to cells through the energy from the light waves transferring to the nucleotide bonds. Specifically, bonds between cytosines (C), thymines (T), and C to T are the ones that get changed. This change is that two adjacent C, T, or C and T will break their bonds with their complementary bases on the opposite DNA strand and will bind to each other instead. This causes a hump in the DNA strands which messes with DNA transcription. (Doonan, Lecture, Nov 13, 2019)

UV light is light in the 200-400 nm range. Within this range, there are 3 main types of UV light, UVA, UVB, and UVC. (Doonan, Lecture, Nov 13, 2019) UVA is 314-400 nm and it is not absorbed by the protective ozone layer around the earth. This isn't a large issue since it causes the least damage of the UV types, due to its lower energy. (Doonan, Lecture, Nov 13, 2019) UVB is from 280-315 nm. It's mostly absorbed by the ozone layer but also is the type of UV that causes the most cancer. (Doonan, Lecture, Nov 13, 2019) UVC is from 100-280 nm and the ozone layer entirely absorbs it, (Doonan, Lecture, Nov 13, 2019) so as long as we get the ozone layer repaired, we should be safe from UVC damage.

Sunscreens prevent UV damage through two main ways, organic and inorganic. The inorganic way involves zinc or titanium oxide and those molecules physically block sun rays

from getting to the skin. (Doonan, Pre-lab Talk, Nov 13, 2019) The organic method involves avobenzone or oxybenzone chemicals that actually absorb the sun rays before they can get to the skin. (Doonan, Pre-Lab Talk, Nov 13, 2019) The efficacy of sunscreen is measured in sun protection factor (SPF) which is measured by the ratio of damaged cells over total cells. There are conflicting results about whether higher amounts of SPF actually make a difference above about 15 SPF. (Doonan, Pre-lab Talk, Nov 13, 2019)

Section 3. Yeast as a Model Organism

A good model organism is one with simple biological processes and are easy to take care of. They should reproduce quickly and be similar in the pathway in question in another organism. (Doonan, Lecture, Nov 13, 2019) The specific yeast used in this experiment is a sugar fungus, *Saccharomyces cerevisiae*. Yeast is a good model organism because it fits the requirements mentioned before. It is simple, replicates quickly, and is in the fungus Kingdom, which is much closer evolutionarily to humans than bacteria is.

Yeast is being used for this experiment of its similarity to humans genetically. Additionally, this experiment includes damaging yeast under UV light. There aren't ethical issues with killing off lots of yeast, but there would be for larger creatures.

Section 4. Description of Project

What question did you seek to answer? Provide the rationale for the question you are asking (this will likely require some research!). Succinctly describe your experimental design. What was your hypothesis?

Our experiment is comparing lotion versus spray sunscreen. We wanted to know if lotion was better than spray sunscreen. The reason behind this is that we've been told before that spray is worse than lotion, and wanted to know if this reason was simply application or if there was a chemical difference. Many people, including the FDA, are not even sure of the safety of spray sunscreen. (UPMC)

To test spray versus lotion, we will apply protect plates of yeast with either spray or lotion sunscreen. Then, we will place them under UV exposure for different amounts of time to look at the efficacy of spray versus lotion over time. We hypothesised that spray would in fact be worse than lotion, and that there would then be more damaged yeast cells over time in the spray samples than in the lotion.

II. Methods

UV Exposure

Our hypothesis was that spray sunscreen would not be as good as lotion sunscreen. We conducted an experiment to test whether spray sunscreen is as good as lotion or not. In this experiment, we exposed *Saccharomyces cerevisiae* (yeast) to UV light while covered in a layer of protection of one of the types of sunscreen. After they were exposed to UV, we incubated the samples for 48 hours at 32^{o}C so that the yeast would recover, commit apoptosis if too damaged, and replicate if not too damaged. (Doonan, Lecture, Nov 20, 2019) After this growth period, we

counted the number of colonies on the plates and compared the numbers from plates that were exposed to UV light to the plates that were not. From comparing the samples exposed to the UV light to the negative controls, we were able to figure out the efficacy of the spray and lotion sunscreens both relative to each other and in general.

The sunscreen was applied onto a sheet of plastic wrap that covered the yeast sample on YEPD plates. Both sunscreens were Coppertone Sport with SPF 50 and accounted for both UVA and UVB light. We had 4 samples of yeast per sunscreen, and each of these were exposed to UV light for different amounts of time. Our time steps were 0, 10, 20, and 30 minutes. Additionally, for each sample, we had a plate with 10^{-4} and 10^{-5} concentration to increase the likelihood that we would have countable results.

Therefore, the independent variables are the type of sunscreen (spray versus lotion) and the amount of exposure. This experiment then has 8 levels of independent variables. The dependent variable is the percentage of the yeast that is still alive after the UV exposure. The controlled variables are the amount of initial yeast, the intensity of UV light, and the company, SPF, and kind (both “sports”) of the two sunscreens. Finally, the positive control is a plate of yeast also exposed to UV light, but without either of the sunscreens applied for protection, for the longest time (30 minutes). (Madigan, Pre-Lab Talk, Nov 20, 2019) The negative controls were the plates with time steps of 0, so they weren’t exposed to UV light. (Madigan, Pre-Lab Talk, Nov 20, 2019)

Nuclear Fragmentation Assay

We completed a Nuclear Fragmentation Assay to understand the detrimental effects of long-term UV light exposure on cells, specifically looking at *Saccharomyces cerevisiae* (yeast) cells. This assay was a way to check if apoptosis had occurred in yeast cultures under UV light. (Doonan, Lecture, Nov 20, 2019) To check for apoptosis, we looked specifically for genomic DNA (gDNA) fragmentation, which is a sign of apoptosis. (Doonan, Lecture, Nov 20, 2019) The whole process included exposing the yeast cells to UV light, extracting the gDNA, and running the gDNA on an agarose gel to see fragmentation.

More specifically, we exposed 4 samples of 5mL of yeast cells on petri dishes to UV light for different amounts of times. These timepoints were 0, 10, 20, and 30 minutes, the 0 minute sample being our control since it was not exposed to UV light. After exposing them to UV light, we incubated the samples for 30 minutes at 32^{o}C to give the cells time to activate apoptosis if needed. (Doonan, Pre-lab Talk, Nov 13, 2019)

To extract the gDNA, we added Releasing Reagents A and B and then a protein removal reagent. After these, we added first isopropanol which pulled down the gDNA and then ethanol to remove any remaining contaminants. (Madigan, Pre-Lab Talk, Nov 20, 2019) We then ran each sample on a 0.8% agarose gel. If the gel contained multiple lines at sizes of 200, 400, 600, and 800 bp, we would know that gDNA fragmentation and so apoptosis had occurred.

III. Data & Results

Figure 1. Nucleosome Fragmentation Assay of gDNA of *S. cerevisiae* at Different Time Steps of UV Exposure on 0.8% agarose gel.

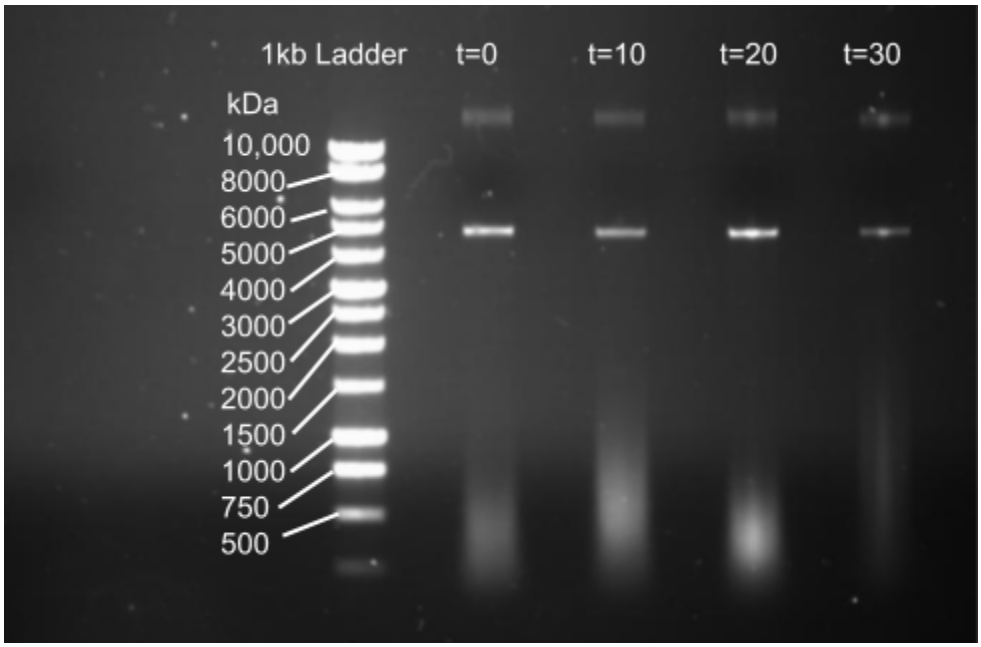


Figure 2. Graph of log molecular weight vs distance traveled of 1kb Ladder on .8% agarose gel.

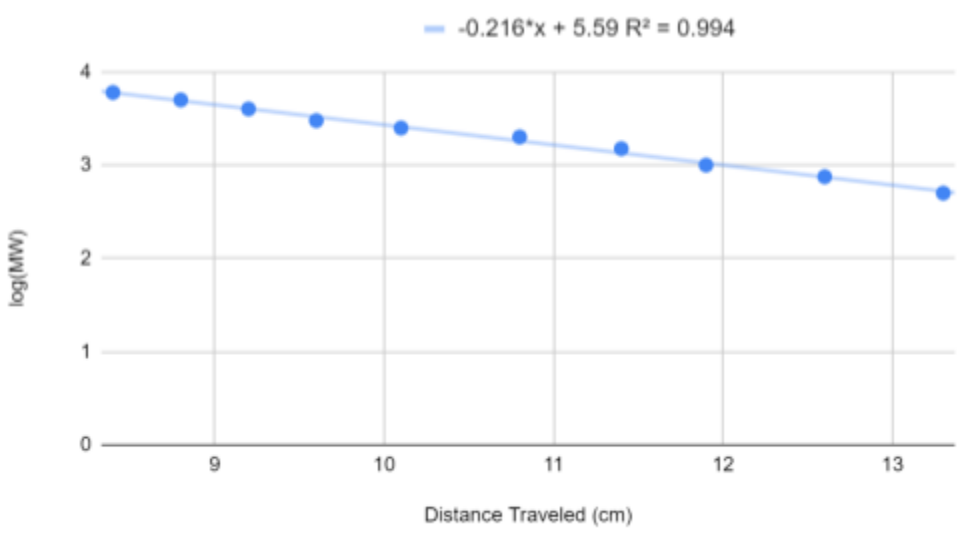


Table 1. Raw Plate Counts and Viable Counts of *S. cerevisiae* for 10^{-4} and 10^{-5} dilutions.

Condition	**10^{-5} dilution**	**10^{-4} dilution**	**Viable Count ($\frac{CFU}{mL}$)**

Spray t=0 min	58	TMTC	5.80×10^7
Spray t=10 min	45	TMTC	4.50×10^7
Spray t=20 min	46	TMTC	4.60×10^7
Spray t=30 min	48	TMTC	4.80×10^7
Lotion t=0 min	64	TMTC	6.40×10^7
Lotion t=10 min	66	TMTC	6.60×10^7
Lotion t=20 min	55	TMTC	5.50×10^7
Lotion t=30 min	53	TMTC	5.30×10^7
Positive Control t=30	N/A	TMTC*	N/A

**TMTC = Too many to count*

Sample calculation:

The general equation is:

$$Viable\ Count = \frac{raw\ count}{volume\ plated} dilution\ factor$$

For each one, the volume plated was .1 mL. The dilution factor was either 10^5 or 10^4. Since only 10^{-5} was countable, 10^5 was the only dilution factor used.

Looking at the Condition spray, t=0 min,

$$Viable\ Count = \frac{58\ cells}{.1\ mL}\ 10^5 = 5.80 \times 10^7 \frac{CFU}{mL}$$

Table 2. Percent Viability of *S. cerevisiae* after UV Exposure for all Conditions.

Condition	% Viability
Spray t=0 min (10^{-5})	100.00%
Spray t=10 min (10^{-5})	77.59%
Spray t=20 min (10^{-5})	79.31%
Spray t=30 min (10^{-5})	82.76%
Lotion t=0 min (10^{-5})	100.00%
Lotion t=10 min (10^{-5})	103.13%
Lotion t=20 min (10^{-5})	85.94%
Lotion t=30 min (10^{-5})	82.81%
Positive Control t=30 (10^{-4})	N/A (TMTC)

Sample calculation:

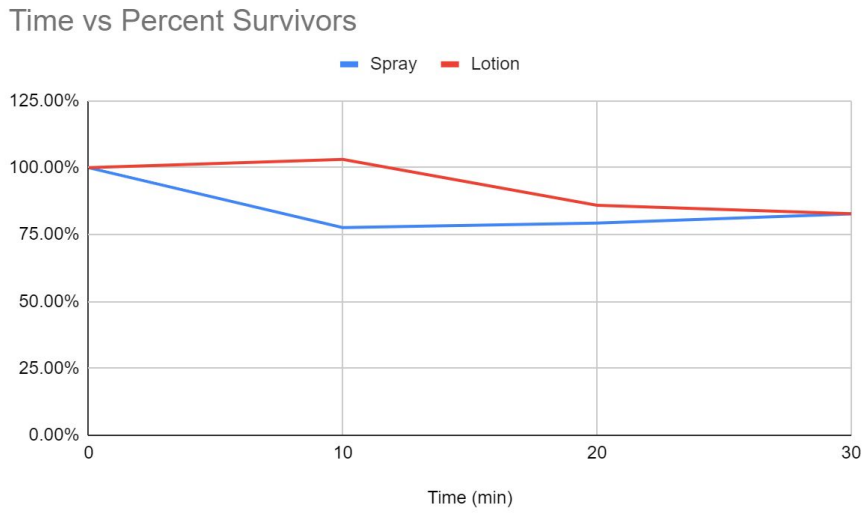
Percent viability was the viable count of the time in question divided by the initial viable count (at t=0 min).

$$\%Viability = viable\ count_{t=n}/viable\ count_{t=0}$$

Looking at t=10 min for spray as an example, viable $count_{t=n}$ is 4.50×10^7 and viable $count_{t=0}$ is 5.80×10^7 :

$$\%Viability = \frac{4.50\times10^7}{5.80\times10^7} = .7759 = 77.59\%$$

Figure 3. Graph of Time vs Percent Survivors for Spray and Lotion after UV Exposure.



IV. Discussion

Nucleosome Fragmentation Assay

1. A Nucleosome Fragmentation Assay indirectly tests for apoptosis through looking at nuclear DNA fragmentation. Nuclear DNA fragmentation is a sign that apoptosis has occurred, since one of the results of apoptosis is DNA fragmentation. Thus, the presence of DNA fragmentation would indicate apoptosis has occurred, but it may have occurred even if there is no evidence of DNA fragmentation. (Doonan, Lecture, Nov 20, 2019)

2. We exposed yeast to UV light for four different amounts of time, 0, 10, 20, and 30 minutes. We chose this because we wanted to see a relationship between time and how much apoptosis has occurred. Since UV light affects yeast cells within a few minutes, (Magidan, Pre-Lab Talk, Nov 20, 2019) we chose earlier time sections, an equal amount of time apart from each other.

3. Our control was a negative control where the yeast exposed to UV light for no amount of time, 0 minutes. This gave us an idea of what impacts the UV light had on a healthy yeast culture.

4. If I did this experiment again, I would likely include a positive control where I induced apoptosis and nuclear DNA fragmentation through a different method. This would give me a reference for what I should expect to see if apoptosis was successfully induced, and was displayed through nuclear DNA fragmentation. This would be important to have in case the results we got seemed to be nuclear DNA fragmentation, but were actually a contaminant. Comparing the contaminant to the DNA that we know is fragmented due to apoptosis would enable us to more confidently establish that apoptosis occurred within the cell.

5. The nucleosome fragmentation assay shown in Figure 1 suggests that there was no nuclear DNA fragmentation at any of the time points, though this does not mean that apoptosis did not occur. The reason that it appears that fragmentation did not occur is that each of the time point lanes look pretty much the same, even the lane that was not exposed to UV radiation (t=0 minutes). Additionally, if there had been nuclear fragmentation, we would have seen much more smearing all throughout the lane. (Doonan, Pre-lab Talk, Dec 4, 2019) As it is, since there is only a bit of smearing at the bottom of the lane and the samples all look about the same, it's likely that the smearing seen on the assay is RNA and not DNA, since the preparatory kit did not have a step to remove RNA from the samples. (Doonan, Lab, Dec 4, 2019)

6. We cannot know if apoptosis occurred or not. If there had been evidence of nuclear DNA fragmentation, it would have been reasonable to conclude that apoptosis had occurred. However, while there was no evidence of nuclear DNA fragmentation, it is important to note that apoptosis may have still occurred, (Doonan, Pre-Lab Talk, Nov 13, 2019) perhaps simply without getting to the nuclear DNA fragmentation step.

7. To determine an equal concentration, we would consider any differences in dilution the samples may have experienced. There were not many times where this may have occurred, since most steps for each of the samples involved the same amount of added liquid and each sample started out with the same concentration. However, changes in concentration may have occurred in the step of resuspending the gDNA in TE buffer after extracting the gDNA from the cells. Here, if there appeared to be contaminants such as salt, simply more buffer was added, ranging from an amount of 50-100μL of extra buffer. (Doonan, Pre-Lab Talk, Dec 4, 2019) Accounting for this dilution difference, one could quickly obtain the concentrations of the gDNA to load onto the gel. However, this would not really be necessary, since the purpose of this experiment was to use nuclear DNA fragmentation as a measure to see if apoptosis had occurred. If the experiment was instead interested in how much apoptosis occurred relatively between the samples, then loading an equal concentration would be more fitting.

8. Since there was no evident nuclear fragmentation, to improve this experiment I would likely change the time steps to be longer. With longer time steps, it would be more likely for more cells to be more damaged and thus begin apoptosis. With more cells undergoing apoptosis, it's more likely for there to be nuclear DNA fragmentation that we would then have been able to see. Since there wasn't any fragmentation at 30 minutes, I would suggest having time steps around and after 30 minutes, in addition to the control at 0 minutes.

However, it is also possible that there was no fragmentation due to there being no apoptosis potentially from some experimental error. It's possible that we left on the lids or that the UV machine wasn't working for some reason. One of the other experimental errors that may have resulted in there being seemingly no nuclear DNA fragmentation was that some of the samples were sitting out for a while before being put in the incubator for their recovery period. This could have led to healthy cells having time to replicate and consume the cells that underwent apoptosis. To check for these potential experimental errors, I would also redo the experiment with the same time steps to verify if there was in fact some experimental error.

Efficacy of Sunscreen

9. We had both positive and negative controls to test the efficacy of the two sunscreens. The positive control was a petri dish without any sunscreen placed under the UV light for the longest time step (30 minutes). This was all that was needed, since after 10 minutes under UV light without protection, yeast cells will be dead. (Magidan, Pre-Lab Talk, Nov 20, 2019) The negative controls that we did were 2 petri dishes exposed to no UV radiation, but still covered in each of the sunscreens. This was to hold as much as possible the same between the control and the variables but to show what would happen to the yeast cells without any exposure to UV radiation.

10. Referring to Figure 3, we see only a slight decrease in percent survivors over the 30 minutes of UV exposure. The sample protected by lotion at t=10 seems to improve, going to 103% survivors, but this is not significantly larger and suggests there was actually no change. At 20 minutes, percent survivors on the lotion protected sample goes down to 86% and stays about the same at 83% for t=30 minutes. The t=10 protected by spray, went down to 78% survivors and then stayed about the same at 79% survivors at t=20. At t=30, however, the percent survivors of the spray protected sample goes up to 83% which suggests again.

These suggest that the percent survivors decreases over time, though not by very much. Though there is some change, the fact that there were both increases and decreases in percent survivors, this suggests there wasn't a significant change over time in percent survivors. Additionally, the positive control at t=30 was too many to count, when it should have 0% survivors. The combination of these factors suggests that there was an issue with the experimental design and not that the slight decrease of percent survivors was significant.

11. Initially, the lotion seems to do better than the spray sunscreen. It has 103% and then 85% survivors whereas the spray sunscreen drops down to 78% and 79% survivors initially. However, at the end, both samples covered by lotion and spray have approximately 83% survivors. This would suggest there's not much difference between spray and lotion sunscreen, and if there is any, it's only a small benefit initially.

Importantly, though, our percent survivors of our positive control, was too many to count when it should have been 0% survivors. Since our positive control did not give the results we expected, this suggests that something went wrong with our experiment. Everyone else in the

lab experienced this issue as well, which suggests that there was something likely wrong with the UV machine. This may have been that it wasn't turned on or that the UV light wasn't working.

12. Our results refute our hypothesis; spray sunscreen seems to be as good as lotion, while we hypothesised that the lotion would be better. While it isn't initially, according to our results, they reach the same after 30 minutes, with only a little variation beforehand.

13. As mentioned before, due to the positive control not having 0% viability, there was likely something wrong in the experiment, so while our results do refute our hypothesis, they are not necessarily significant. Additionally, there are various factors in the experiment itself that are important to note alongside the results. One was that the sunscreen was applied onto a plastic wrap before being wrapped around the plates which the yeast were on. Since plastic wrap is not as absorbent as human skin, the spray would not stay on very well and frequently ran off the edges. While the lotion could be spread out relatively evenly over the yeast samples, it was difficult to completely and as thickly protect the yeast with the spray sunscreen. Additionally, since the UV light from the UV machine should be much stronger than the UV radiation from the sun, the times from this experiment would not line up exactly with the times if the samples had instead been laid out in the sun.

14. With the above limitations and caveats in mind, there are a few recommendations for a sunscreen user that this experiment leads to. One is that spray sunscreen is often hard to get on in the same amounts as lotion. It must be applied more conscientiously than lotion, Additionally, these sunscreens can likely be used for longer than this experiment uses while offering the same level of protection. Those said, it is important also to note that the UV light was likely not functioning for this experiment, so the results would even further not directly pertain to a user.

15. One of the issues that we encountered was that the spray sunscreen did not stay on the plastic wrap very well. However, plastic wrap is often used and seems to be one of the better methods for testing efficacy of sunscreen over a culture on a petri dish. One way to work around the issue of there being too much spray sunscreen to stay on well is to lower the amount of sunscreen that we cover the yeast cultures with. If this becomes an issue with the lotion sunscreen not spreading well enough, we could simply mix it with some water so that it spreads evenly and easily over the plastic wrap, while the spray sunscreen does not fall off, as was done in "Comparison of the photoprotective effects of sunscreens using spectrometric measurements of the survivability of yeast cells exposed to UV radiation". (Mesías et al, 2017)

One way to account for the intensity of UV light not directly replicating the effects of the sun would be to instead place the petri dishes directly in the sunlight outside. This has been done in numerous other experiments looking at the efficacy of sunscreen. (Beck-Winchatz et al, 2014; Kay et al, 2009) One other change that this would require would likely be being exposed to sunlight for longer periods of time than our experiment did since the radiation will not be as intense.

16. I would also look at the effects on living animals, though not under UV radiation directly. As done in “Effects of UVA radiation on an established immune response in humans and sunscreen efficacy,” I would get volunteers if I chose to use humans or simply lab rats or another small model organism to use either spray or lotion sunscreen. They would spend a certain amount of time outside with this protection. To measure the differences in efficacy, I would likely need to use a different factor than cell death or sunburn. One that the study looking at the effects of UVA radiation uses is immune response, since UVA decreases immune response. Since both sunscreens that we used in the experiment are broad band, I could similarly use this measurement. (Moyal, 2002)

17. If I were to do this experiment again, I would use skin tissue samples. They have been used in studies interested in space-like and high altitude environments (Beck-Winchatz et al, 2014) but also specifically in studies interested in efficacy of sunscreen. (Sohn et al, 2015; Caspers et al, 1998) Tissue samples either from humans or from porcine, as Sohn et al use, are evidently more closely related to humans than yeast is. This model organism can be plated and irradiated without much ethical concern, since it is a collection of cells. However, there are some downsides including the fact that it’s replication time is much slower, at about 20 hours (Phillips, 2015) compared to the 45 minutes of yeast. (Doonan, Lecture, Nov 13, 2019) Additionally, among other more technical issues, there are ethical considerations that need to be taken when collecting tissue samples. (Grizzle, 2010) While we potentially could use this in the lab, as skin samples can often be collected from hospitals, (Grizzle, 2010) the issue of replication time might make the experiments more difficult than necessary, when yeast samples often work quite well and are simpler.

18. I would expect to see more fragmentation on the gDNA isolated from the exposed cells. This is because the gDNA fragments under UV light due to an intracellular process that activates DNAses. (Doonan, Lecture, Nov 20, 2019) gDNA on its own would mainly mutate, not fragment, with UV radiation exposure.

19. Another way to cause DNA damage is to use dimethyl sulfate. Dimethyl sulfate affects chromosomes as well as DNA, causing both breaks in DNA and alkylated bases. (IARC, 1999) The breaks caused in DNA could potentially be measured in our lab setting, similarly to the nuclear fragmentation assay. However, this is not necessarily true since there’s not a guarantee that there are many DNA breaks. Impacts of the alkylated bases could either be physiologic changes or cell death. If there are enough alkylated bases, they might result in apoptosis being induced since they would impact transcription, similarly to the nuclear fragmentation experiment, which would then also be measurable in our lab setting.

20. Yeast as a model organism was reviewed in “New insights into cancer-related proteins provided by the yeast model.” This is important since cancer affects many people’s lives and gaining a deeper understanding of the mechanisms of cancer can lead to better treatments. This study was specifically interested in the use of yeast to study various cancer-related

proteins and some of the underlying causes of cancer. Yeast was used by this and previous papers since it is evolutionarily similar to mammalian cells and has similar cellular processes while also being much simpler and easier to care for, replicate, and manipulate. One of the major experiments discussed in this paper was a p53 FASAY assay, where p53 uptake was measured by plating cells without histidine, since p53 was His+ and could then grow on the plates. The conclusion of the review was that past uses of yeast as a model were justified and valid. Additionally, it claimed that yeast will also have future uses, specifically to go hand in hand with the sequencing of the human genome to identify the function of genes. (Pereira et al, 2012)

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