STRUCTURAL INTEGRITY OF SHIGA TOXIN AFTER ELECTRON BEAM (EBEAM) INACTIVATION OF SHIGA TOXIN PRODUCING *E. COLI*

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Structural Integrity of Shiga Toxin After Electron Beam (eBeam) Inactivation of Shiga Toxin Producing *E. coli*

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Electron beam (eBeam) technology is an innovative resource used to rid a variety of pollutants including microbial pathogens. This technology utilizes a linear electron accelerator that is capable of generating highly energetic electrons resulting in rapid microbial inactivation. Microbial inactivation is the result of the electrons causing a chaotic ionization event that eventually causes lethal single and double-strand breaks in DNA. Due to this, the cell is incapable of replicating its DNA leading to its inactivation. This technology is widely used in the medical device sterilization industry, in food processing, and is starting to be employed for environmental remediation. Toxin-producing pathogens such as *Listeria spp.*, Shiga-toxin producing *E. coli* and *Clostridium perfringens* are all targets in food processing. Shiga-toxin producing *E. coli* (STEC) are responsible for a large number of food-associated outbreaks as well as fatalities and severe morbidities. While eBeam is capable of inactivating STEC, (i.e., preventing cell multiplication), there is convincing evidence that the cells are still metabolically active. The concept of these cells being Metabolically Active yet Non-Culturable (MAyNC) has been coined for this scenario; do these cells still produce functional toxins? The underlying hypothesis is that the toxins are

structurally degraded and consequently non-functional. Therefore, the primary goal of this research was to analyze the structural integrity of shiga-toxins from MAyNC STEC after exposure to eBeam technology. This was done using in vitro ELISA assay in order to quantify shiga toxin production post-irradiation. This research would allow for a deeper understanding of ionizing radiation–based microbial inactivation.

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NOMENCLATURE

eBeam	Electron Beam
E. coli	*Escherichia coli*
STEC	Shiga-toxin producing *E. coli*
MAyNC	Metabolically Active yet Nonculturable
kGy	KiloGray
MeV	Mega electron-volt
EHEC	Enterohemorrhagic *E. coli*
HC	Hemorrhagic colitis
HUS	Hemolytic uremic syndrome
Stx	Shiga toxin
kDa	kilodalton
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
PBS	Phosphate Buffer Saline

CHAPTER I

INTRODUCTION

Electron Beam (eBeam) Technology

Electron beam (eBeam) technology has the potential to employ a novel approach to sterilization techniques. This technology exploits radiation from wavelengths shorter than 10^{-10} m that are generated by linear accelerators [1]. The location of ultraviolet light on the electromagnetic spectrum creates a generalized division between ionizing and nonionizing radiation. Ionizing radiation consists of wavelengths shorter than 10^{-7} m and include X-ray, eBeam, and gamma radiation (Figure-1). This type of radiation is characterizing by its ability to displace electrons and create ions unlike its counterpart, nonionizing radiation, that does not possess enough energy to do so. In contrast to other sources of radiation, eBeam technology is not produced by radioactive materials, rather it is produced in an on/off style through commercial electricity, making it more cost effective, safe, and environmentally favorable [1].

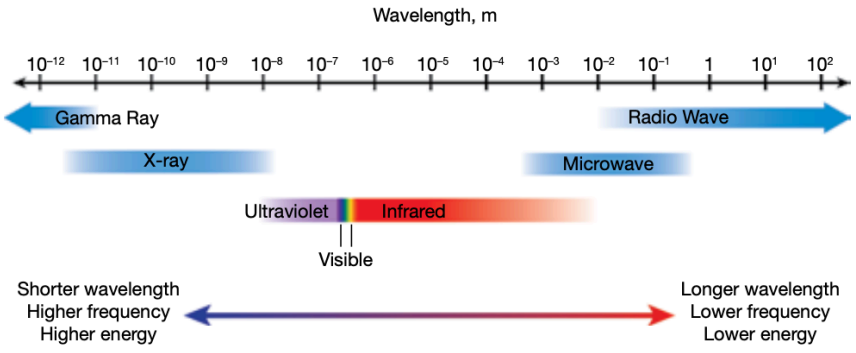


Figure-1: Electromagnetic Spectrum illustrating ionizing and nonionizing radiation [1].

Electrons are propelled from linear accelerators above and below a conveyor system, causing a surge of ionizing events in the target substance (Figure-2). This type of radiation is

tunable, meaning the desired dose for a target substance can be adjusted by changing the speed at which the substance moves under the linear accelerators.

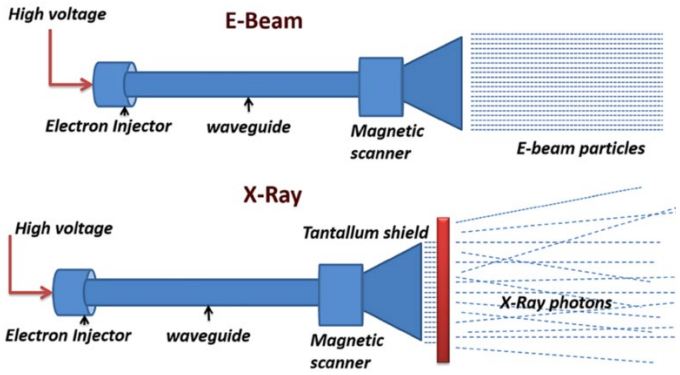


Figure-2: Comparison of eBeam and X-Ray linear accelerators [2]

Minimum and maximum dose points are adjustable by desired product treatment (phytosanitary, pasteurization, or sterilization) with respect to certain regulatory limits [1]. The standard unit for absorbed dose is a kilogray (kGy) and is measured by alanine (L-α-alanine) dosimeters and a Bruker E-scan spectrometer. The energy used for electron beam technology is measured in mega electron-volts (MeV) and is subject to certain regulatory limits set by the US Nuclear Regulatory Commission, including an upper limit of 10 MeV [1]. When a target substance is subjected to eBeam processing, electrons bombard the substance, creating ionizing events that are capable of creating single- and double-stranded breaks in DNA rendering pathogens inactive due to the inability to replicate. Therefore, this technology is especially useful for processing foods and ensuring their sterility for consumers.

Shiga Toxin Producing *E. coli* (STEC)

Shiga toxin producing *Escherichia coli* (STEC) is a type of *E. coli* that are capable of releasing toxins that cause diarrhea in humans [3]. There are various groups of *E. coli* that include enteropathogenic *E. coli* (EPEC) enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC),

enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC) and adherent invasive *E. coli* [4]. Shiga toxin producing *E. coli* (STEC) falls under the category of Enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 associated with hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) [4]. To clarify, all EHEC strains produce shiga toxins (are STEC) but not all STEC are enterohemorrhagic and cause HC or HUS. A distinguishing factor for EHEC, and therefore STEC, is the major virulence factor shiga toxins that are released and associated with severe morbidities of the gastrointestinal tract [5]. Shiga toxins are encoded on bacteriophages which are found inserted in the bacterial chromosome that can be released upon induction of the lytic cycle such as during an SOS response [3].

STEC has two toxins, Shiga toxin 1 (Stx1) and 2 (Stx2), that are structurally expressed on lambdoid bacteriophages [5]. These two toxins can be expressed individually, together, or in the case of Stx2, in multiple forms [5]. Stx2 is the hallmark toxin found in most cases of STEC and was principally investigated in this study. The structure of shiga toxins are that of AB_5 toxins that are comprised of an A subunit and 5 B subunits. This AB_5 structure is similar to several other bacterial pathogens such as *Bordetella pertussis*, *Vibrio cholerae*, *Shigella dysenteriae* (Beddoe, 2010). The A subunit is responsible for catalytic activity and the B subunit binds to specific Gb_3 receptors on the surface of target cells. The structure of AB_5 toxins include an A subunit which when nicked reveal a 28 kDa peptide (A_1) and a 4 kDa peptide (A_2) linked via disulfide bond; the B subunit contains five identical 7.7 kDa peptides [5]. [6]

While the structure of the AB_5 toxins is shared among different pathogens, their specificity, catalytic activity, and intracellular activities are unique [7]. The A subunit in the case of STEC,

acts as an N-glycosidase and removes an adenine base from the 28 S rRNA of the 60S ribosomal subunit in an infected cell, which shuts down protein synthesis in the target cell and subsequently leads to cell death [5]. The B subunit binds the toxin to a glycolipid receptor (Gb_3) which then leads to the toxin being transported inside the cell to the Golgi apparatus and finally to the endoplasmic reticulum. Events such as exposure to UV light, chemical agents, and breaks in DNA encourage STEC to generate bacteriophage progeny upon exposure and ultimately release shiga toxins that, in turn, can cause diarrheal disorders in the host.

Novelty of the Study

This study was designed to observe the effect of irradiating shiga toxin producing *E. coli* at 0 kGy, 1 kGy, 3 kGy, and 7 kGy and analyze toxin production after 12 and 24 hours. Since eBeam processing induces breaks in DNA, it is reasonable to analyze how STEC responds to these breaks as studies have revealed that damage to DNA induces STEC into the lytic cycle, thereby releasing shiga toxins. ELISA assays provided means to quantify Stx production after 12- and 24-hour incubation periods and provide insight to variances in toxin production after these time intervals. This data will provide a clear understanding on how shiga toxin producing *E. coli* reacts metabolically to electron beam processing and if there are changes in toxin production over time.

This study is applicable to ensuring that foods contaminated with STEC are safe for consumers, (i.e. the toxins are non-functional). The foods most commonly associated with STEC outbreaks are fresh produces, beef, and unpasteurized juices. Ionizing radiation has shown promising efficacy in treating these pathogens [7]. Studies have shown lower doses of irradiation, such as 1-2 kGy in beef contaminated with *E. coli*, are sufficient in remediating pathogens while

preserving flavor and color [7]. Thus, eBeam technology is applicable to the food industry and presents as a reliable sterilization technique. In sum, the objective of this study is to analyze the relationship between the plausible induction of the lytic cycle at various dose points in conjuncture to two post-irradiation incubation periods (12 and 24 hours).

CHAPTER II

METHODS

Bacterial Preparation

A stock culture of shiga-toxin producing *E. coli* (STEC) was obtained from the Pillai laboratory culture collection. A glycerol stock culture of *E. coli* O157:H7 was prepared by first making an overnight culture. The overnight culture was made by inoculating liquid trypic soy broth (TSB) medium with the bacteria at 37°C in a shaking incubator for 12 hours. 1 ml of the overnight culture was then aliquoted into a 15 ml conical tube and centrifuged at 4500 rpm for 2 minutes. The resulting supernatant was pipetted out and an additional 1ml of the overnight culture was added to the conical tube. After centrifuging once more and discarding the supernatant in the same matter as before, 1 mL of a 70% TSB and 30% glycerol solution was added and vortexed to resuspend the pellet. The stock culture was then stored in a -80°C freezer. To confirm the presence of *E. coli* in the glycerol stock cultures, MacConkey agar was inoculated with stock culture and observed for lactose fermenting colonies that appear a dark pink color. In addition, a Gram stain was performed on the stock culture to confirm the Gram reaction of the bacterial cells.

A tryptic soy agar (TSA) plate was inoculated with the culture obtained from the glycerol stock and incubated at 37°C for 24 hours. Overnight broth cultures were prepared in triplicate prior to eBeam processing by inoculating TSB media with a single colony from the TSA plate. Once propagated, the overnight cultures were washed three times with Phosphate Buffer Saline (PBS) solution. To wash the overnight cultures, they were each centrifuged at 4500 rpm for 2 minutes leaving a bacterial pellet at the bottom of the conical tube. The supernatant was discarded, and 15

mL of PBS was added then vortexed to resuspend the pellet in solution. This process was repeated three times and then resuspended in PBS to a final volume of 25 mL at the end of the third wash.

Electron Beam Processing

The samples prepared were taken to the National Center for Electron Beam Research in College Station, TX. Prior to eBeam processing, biological replicates were aliquoted into 5 mL Whirlpack bags, heat sealed, and then triple bagged per Texas A&M University's BSL-2 safety protocols. In addition, a sample for the dosimetry (speed check) was made by aliquoting 5 mL of PBS in a Whirlpack bag and triple bagged in the same manner as the samples.

The speed check was required for eBeam processing in order to achieve the targeted eBeam dose. The dose of the speed check was monitored in the same matter as the experimental samples. Alanine pellets were secured to the top and bottom of the Whirlpak bag, the sample was then sent through on a conveyor system and retrieved after eBeam processing. The alanine pellets were then analyzed via a Bruker E-scan spectrometer and the dose received was recorded. Use of the speed check allowed the dosimetrist to alter the speed of the conveyor and ensure that it coincided with the target dose.

Confirmation of Bacterial Inactivation

After eBeam processing, the samples were analyzed to confirm bacterial inactivation. In the biosafety cabinet, the Whirlpack bags were cut open with sterilized scissors. The contents of the bag were carefully pipetted out and transferred to 15 ml conical tubes that were then placed in an incubator at 37°C. At 12 and 24 hours, 1 ml of the samples were pipetted into freezer tubes labeled according to the dose point (0, 1, 3 or 7 kGy), biological replicate (A, B, or C) and hour of

toxin analysis (12 or 24 hour). The freezer tubes were transferred to the -80°C freezer in the Pillai Laboratory at either the 12^{th} or 24^{th} hour after eBeam processing [8]. The control samples were used to make ten-fold serial dilutions by transferring 100µL of the control sample into 900µL PBS dilution blanks. The last four dilutions (10^{-5}, 10^{-6}, 10^{-7},10^{-8}) were plated on TSA plates and incubated at 37°C for 24 hours. Colonies of each dilution were counted to calculate initial concentration of *E. coli* in each replicate. To confirm the inactivation of irradiated samples, TSA plates were inoculated with irradiated samples and incubated at 37°C for 24 hours. Inactivation was confirmed by observing no growth after 24-hour incubation.

ELISA Toxin Analysis

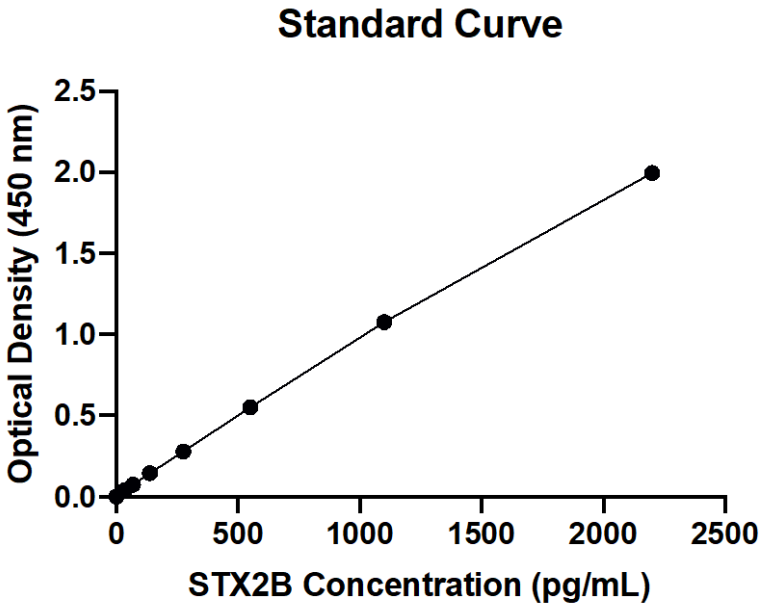
An enterohemorrhagic *E. coli* STX2B ELISA kit from Sino Biological Wayne, PA was used to quantify toxin production after eBeam processing at two different time intervals (12 and 24 hours). The protocol adapted from Sino Biological is as follows: A 96-well microplate was prepared by coating each well with 100µL of the diluted capture antibody and incubated in a 4°C refrigerator for 24 hours. The microplate was then washed three times with 200µL of wash buffer, then blocked with 200 µL blocking buffer, and incubated at room temperature for a minimum of 1 hour. The microplate was then washed again three times with wash buffer and ready for sample addition. A standard curve was made by diluting the standard in sample dilution buffer and adding 100µL to each well of the first two rows of the microplate. For sample addition, the samples were thawed and centrifuged for 2 minutes at 4500 rpm. The supernatant of each sample was added to the subsequent wells of the microplate and the plate was incubated at room temperature for 2 hours. After incubation, 100µL of the detection antibody diluted in antibody dilution buffer was added to each well and incubated at room temperature for 1 hour. The wash step was repeated after the

incubation period then 200µL of the substrate solution was added to each well and incubated for 20 minutes at room temperature. Finally, 50µL of the stop solution was added to each well and the optical density of each well was read with a microplate reader set to 450 nm.

CHAPTER III

RESULTS

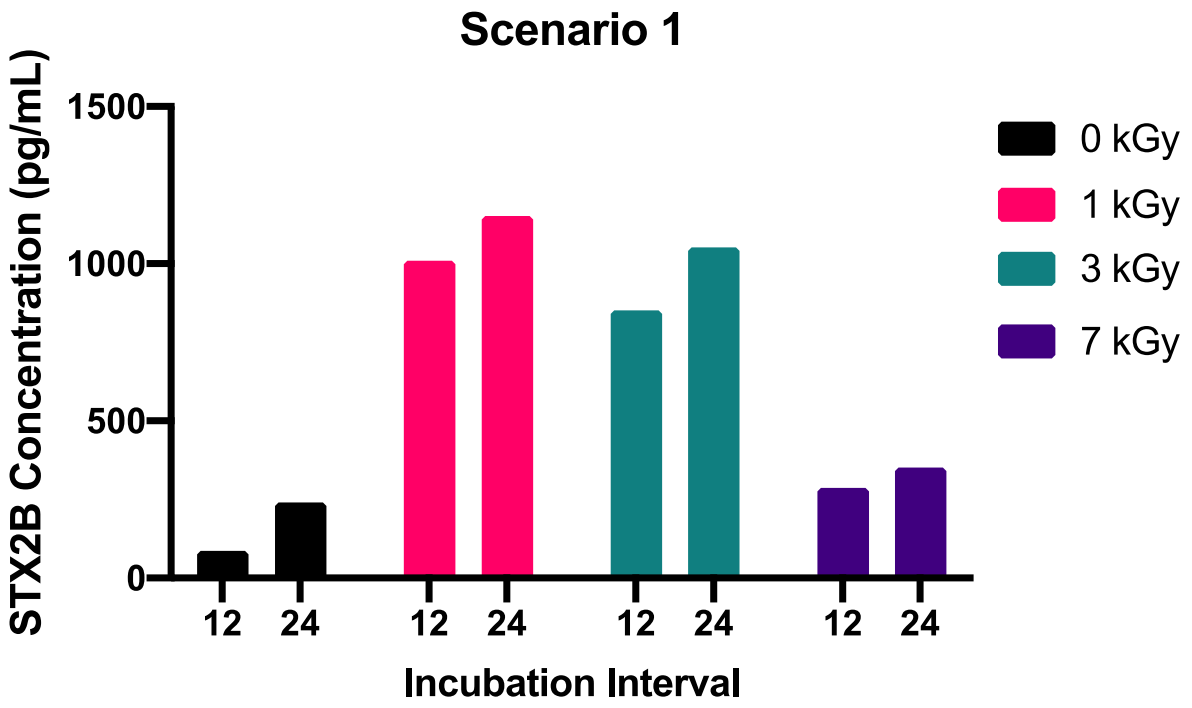
A sample standard curve provided by the ELISA kit used to make theoretical data for discussion is shown in Figure- 4 and includes plausible data for a standard curve adapted from the kit insert.



STX2B Concentration (pg/mL)	Optical Density (450 nm)
0.00	0.000
34.38	0.038
68.75	0.074
137.50	0.145
275.00	0.278
550.00	0.550
1100.00	1.077
2200.00	1.995

Figure-4: Representation of the standard curve provided by the enterohemorrhagic *E. coli* STX2B ELISA kit from Sino Biological Wayne, PA.

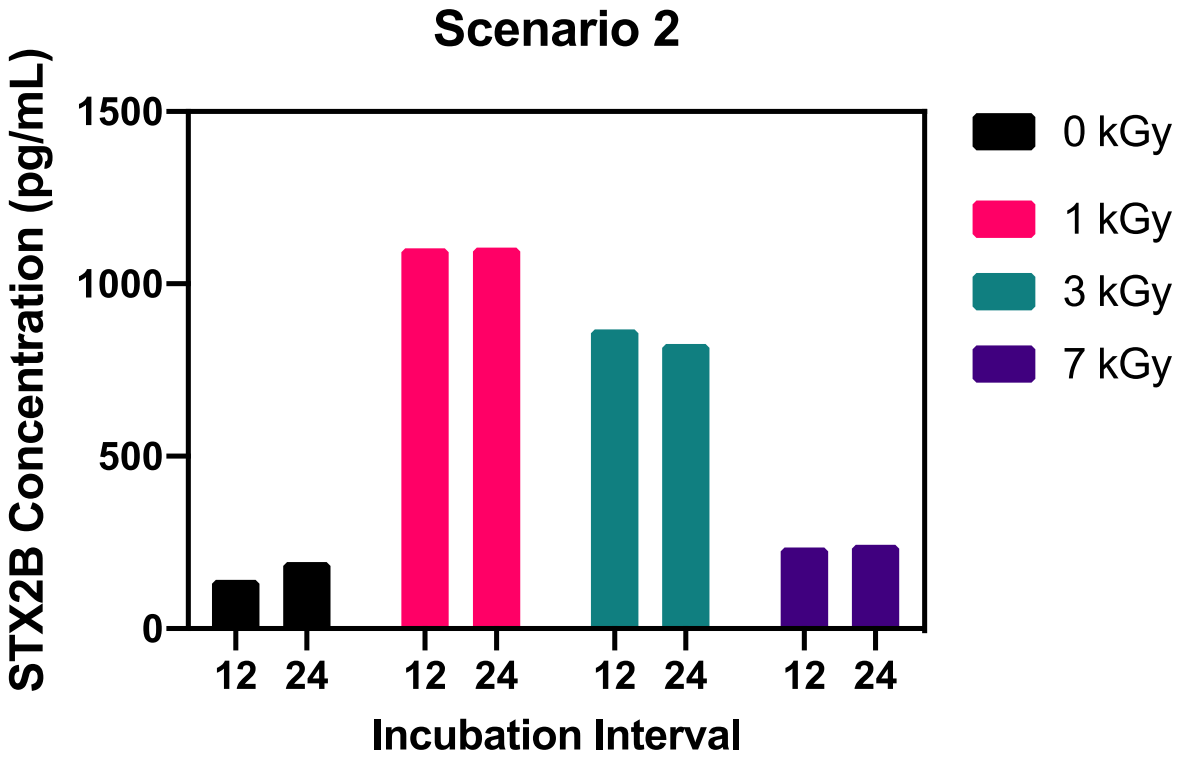
Scenario 1 observed data that shows a correlation between increased STX2B production and incubation periods that approach the 24th hour (Figure-5). A general trend of decreasing STX2B production is seen as follows: 1 kGy, 3 kGy, 7 kGy, and 0 kGy.



		12	24
0 kGy	STX2B Concentration (pg/mL)	173.0000	482.0000
	Optical Density (450 nm)	0.1779	0.4560
1 kGy	STX2B Concentration (pg/mL)	2016	2301
	Optical Density (450 nm)	2	2
3 kGy	STX2B Concentration (pg/mL)	1702	2103
	Optical Density (450 nm)	2	2
7 kGy	STX2B Concentration (pg/mL)	573.00	703.00
	Optical Density (450 nm)	0.54	0.65

Figure-5: Theoretical data illustrating Scenario 1.

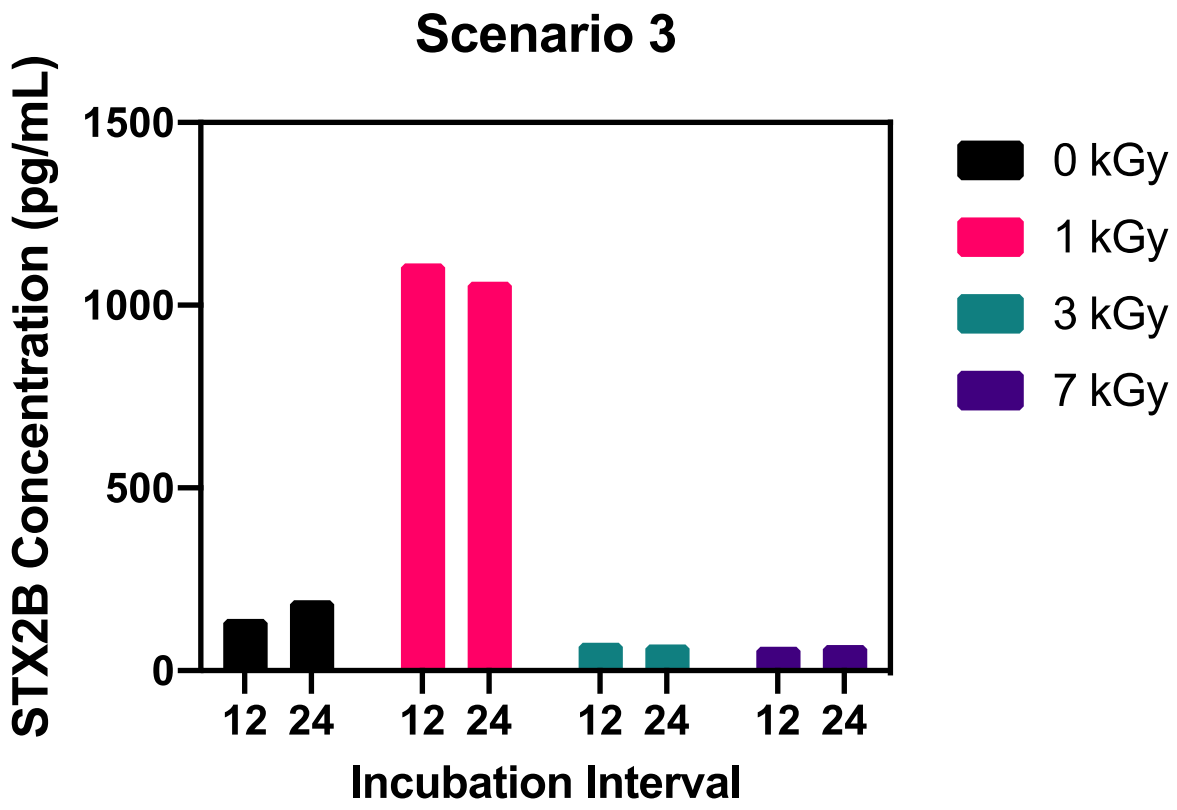
Scenario 2 shows no significant changes in STX2B production after either 12- or 24-hour incubation periods (Figure-6). The general trend of Stx production is shown to have decreased from 1 kGy, 3 kGy, 7 kGy, and 0 kGy, respectively.



		12	24
0 kGy	STX2B Concentration (pg/mL)	284.0000	385.0000
	Optical Density (450 nm)	0.2778	0.3687
1 kGy	STX2B Concentration (pg/mL)	2204	2210
	Optical Density (450 nm)	2	2
3 kGy	STX2B Concentration (pg/mL)	1735	1649
	Optical Density (450 nm)	2	2
7 kGy	STX2B Concentration (pg/mL)	472.00	486.00
	Optical Density (450 nm)	0.45	0.46

Figure-6: Theoretical data illustrating Scenario 2.

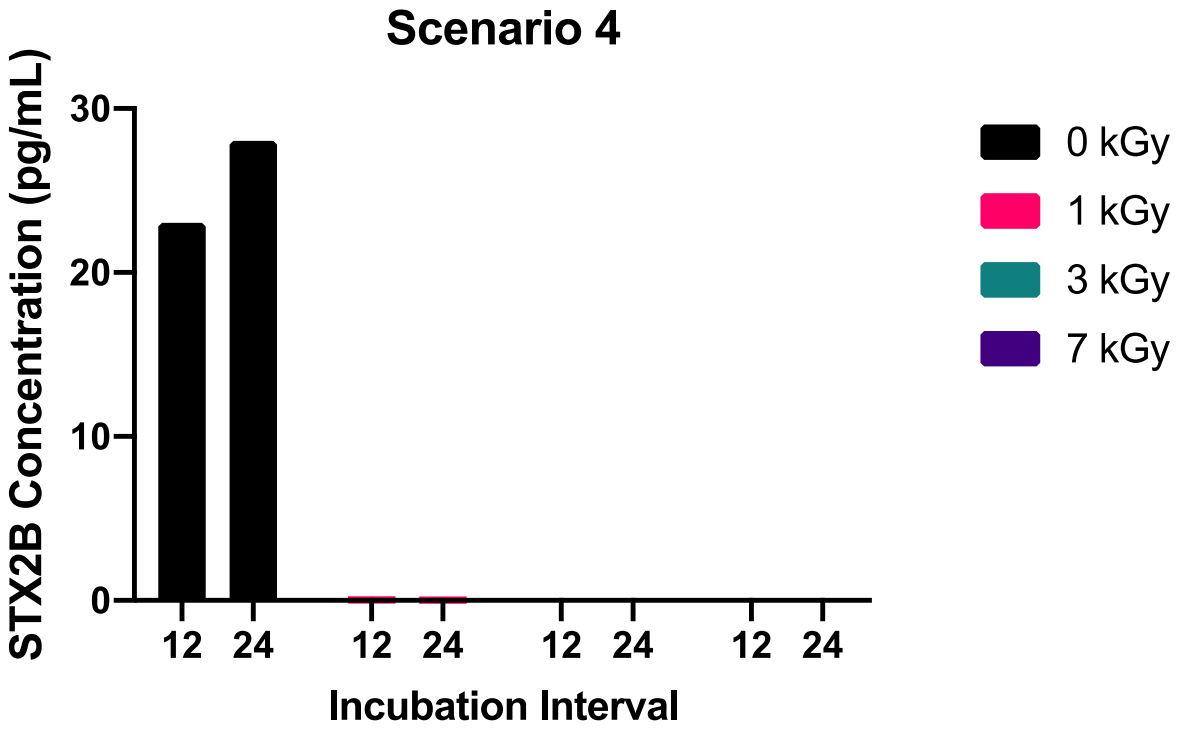
Data from Scenario 3 shown in Figure-7 presents a plausible relationship between a lethal dose (3 kGy) and a significant decrease in STX2B production preceding that dose point. Samples treated at a dose below a lethal level (1 kGy) show a spike in Stx production.



		12	24
0 kGy	STX2B Concentration (pg/mL)	284.0000	385.0000
	Optical Density (450 nm)	0.277800	0.368700
1 kGy	STX2B Concentration (pg/mL)	2226	2128
	Optical Density (450 nm)	2.0256	1.9374
3 kGy	STX2B Concentration (pg/mL)	152.00	142.00
	Optical Density (450 nm)	0.159000	0.150000
7 kGy	STX2B Concentration (pg/mL)	130.00	142.00
	Optical Density (450 nm)	0.139200	0.150000

Figure-7: Theoretical data illustrating Scenario 3.

Theoretical data for Scenario 4 is depicted in Figure-8. In this case, there is a noteworthy absence of STX2B production in eBeam treated cells. The control group (0 kGy) presented a relatively low amount of Stx, but in comparison to the eBeam treated cells, it had the greatest amount of Stx production.



		12	24
0 kGy	STX2B Concentration (pg/mL)	46.0000	56.0000
	Optical Density (450 nm)	0.0636	0.0726
1 kGy	STX2B Concentration (pg/mL)	0.10	0.03
	Optical Density (450 nm)	0.022290	0.022227
3 kGy	STX2B Concentration (pg/mL)	0.003	0.002
	Optical Density (450 nm)	0.0222027	0.0222018
7 kGy	STX2B Concentration (pg/mL)	0.002	0.001
	Optical Density (450 nm)	0.0222018	0.0222009

Figure-8: Theoretical data illustrating Scenario 4.

CHAPTER IV

DISCUSSION

Due to unforeseen circumstances related to COVID-19 during the spring semester of 2020, results of this study were unable to be completed. In addition, the ELISA kit used for this study did not function as anticipated leading to weeks of troubleshooting. Ultimately, research was ceased due to COVID-19 restrictions. In light of this unprecedented obstacle, theoretical results will be explored to justify the significance of this study.

Standard Curve

The enterohemorrhagic *E. coli* STX2B ELISA kit from Sino Biological provided a typical standard curve achieved with the kit standards. This standard curve was utilized to construct theoretical data to illustrate different scenarios that could have been observed from this study.

Scenario 1

The ELISA data in Scenario 1 indicated the greatest concentration of STX2B in the 1 kGy samples incubated for 24 hours (Figure-5). The general trend observed indicated that there is an increase in toxin production after the 24^{th} hour of incubation. The control sample (0 kGy) showed a minimal amount of Stx production likely due to minor damage to DNA and this was observed across Scenarios 1-4 [10]. The 1 kGy sample produced the most Stx followed by 3 kGy, 7 kGy, and 0 kGy, respectively. Studies have determined that 3 kGy is a lethal dose for *E. coli,* thus it is relevant to compare Stx production above and below that limit [11]. Toxin

production at 1 kGy could have been the result of cells not being completely inactivated, and the survivors actively producing STX2B as a stress response to a low eBeam dose [11]. Data for this scenario indicated the cells were capable of remaining intact, metabolically functional, and consequently able to support bacteriophage proliferation [10]. This data also suggested that the most suitable dose for alleviating toxin production by STEC would be 7 kGy. However, the cells were MAyNC and continued to produce functional toxins as the incubation period approached the 24th hour [10]. Broadly speaking, at a dose of 1 kGy, the pathogens were at a peak threshold of metabolic activity because the cells were not completely inactivated and continue to produce toxins post-eBeam processing. This could have confirmed that the double-stranded breaks in DNA caused by eBeam irradiation will induce STEC into the lytic cycle and cause Stx production. It also suggested that a higher dose inhibits the amount of Stx produced by the pathogen. Recent studies have shown the capability of irradiated STEC to mend damaged DNA and be metabolically active [11].

Scenario 1 ultimately proposed that while these pathogens were inactivated, eBeam technology has the capability to stress the cells into toxin production if the cells were not completely inactivated. As the dose increased beyond a lethal dose (3 kGy) the metabolic activity was shown to decrease as a function of cell inactivation [11]. An increase in Stx production as the incubation period is prolonged suggested that survivors are presently releasing Stx, or the cells were MAyNC and still capable of releasing Stx.

Scenario 2

In Scenario 2 The ELISA kit indicated little or no change in toxin production between incubation periods (Figure-6). The greatest amount of STX2B concentration was seen at 1 kGy,

3 kGy, 7 kGy, and 0 kGy, respectively. If the study exhibited these results, it could be concluded that there was no significance in incubation time post-irradiation and therefore, the amount of toxin production was restricted; and the cells were not continuously metabolically active (i.e. continually producing toxins). In comparison to Scenario 1 where the cells were increasingly metabolically functional, Scenario 2 proposed that the cells showed a decline in toxin production as a result of cell death [10]. Ultimately, this data showcased that permanent DNA damage to the cell allowed only a specific margin of metabolic activity because of the cell's inability to repair DNA damage imposed by eBeam processing. In conjuncture, incubation was not relevant to STEC's capability to produce Stx as it was shown to be dependent upon the survival of the cells and the extent of DNA damage.

This data would be significant because it demonstrated that STEC exposed to eBeam processing produced toxins in response to DNA damage but, once the cells exhausted their metabolic capabilities, toxin production was reduced. Additional studies to monitor Stx production beyond the 24th hour would be needed in order to confirm the presence or absence of Stx production. In this case, it was assumed that an optimal dose could have been determined to achieve minimal or no toxin production. Although, confidence that STEC did not increase in metabolic activity with incubation time beyond the 24th hour would have required further studies.

Scenario 3

In this scenario, the ELISA kit showed a negative linear association between STX2B concentration and received dose (Figure- 7). Previous studies have determined that 3 kGy is a lethal dose for *E. coli,* so it is reasonable to assume that after the pathogen was inactivated, the metabolic activity decreased as well [11]. This would suggest that while the cells produced Stx

after DNA damage, pending an optimal dose greater than or equal to 3 kGy, toxin production decreased rapidly with respect to dose. Metabolic activity would therefore depend on the received dose and whether said dose was high enough to inactivate the cells completely. Possible survivors in the 1 kGy dose samples did not receive a lethal dose, thus the data indicated a spike in Stx production. This was likely due to the cells' ability to repair mild DNA damage and remain metabolically active [11]. Incubation periods were not significant in this case and suggested that metabolic activity and toxin production was restricted to a threshold as discussed in Scenario 2.

We can conclude that eBeam processing was a sufficient means of inactivating STEC in addition to the reduction of Stx with respect to an increased dose beyond 3 kGy. This data could establish an understanding that STEC is MAyNC but does not produce a sufficient amount of functional toxins after receiving a lethal dose. Furthermore, this established evidence that STEC was shown to produce Stx as an SOS response, but cell death as a result of a lethal dose decreased metabolic activity [11].

Scenario 4

Theoretical data in Scenario 4 suggested that eBeam processing mitigated STX2B production after a low dose of 1 kGy and thereafter with increasing doses of 3 kGy, and 7 kGy (Figure-8). The single- and double-stranded breaks in DNA imposed on the cells should have prompted STEC into the lytic cycle and resulted in toxin production [1,3]. This gives the impression that subjecting STEC to eBeam irradiation, and subsequent DNA damage, should show Stx production. Although, if this data was observed, there would be evidence that eBeam irradiation is capable of not only inactivating the cells, but the metabolic activity was also

impaired leading to little or no toxin production after any exposure to eBeam irradiation. In recent studies, STEC that caused illness in humans was more sensitive to gamma radiation than STEC that was identified to not cause illness [12]. Although this was seen in gamma radiation, the findings suggest that susceptibility of STEC to gamma radiation could be applied to another source of ionizing radiation (eBeam processing). Survivors in the 1 kGy samples showed a depletion in metabolic capabilities as a result of eBeam processing and prompts the assumption that even with mild DNA damage, the cells were not able to repair the damage and release Stx as an SOS response as seen in Scenario 3.

This data set correlates with the hypothesis that eBeam processing, beyond a low dose point of 1 kGy, renders STEC nonfunctional. This is an ideal situation that would provide confidence that eBeam processing is applicable to diminishing Stx output in addition to disabling STEC beyond repair.

CHAPTER V

CONCLUSION

Shiga toxin producing *E. coli* (STEC) has been extensively researched over the years and the results of this study would have provided a robust interpretation of how these enterohemorrhagic bacteria cells can be eradicated from various products associated with STEC outbreaks. This study is the first step into a large-scale study that would provide insight into how STEC behaves metabolically to certain stressors such as eBeam processing. It is disheartening to be unable to report data with this study due to the unanticipated outbreak of COVID-19, but the relativeness of this study is still significant.

Electron beam technology is an innovative resource that poses as a reliable source of pathogen inactivation in various products. Treatment with eBeam irradiation makes single- and double-stranded breaks in DNA that ultimately halts pathogen replication and results in cell death. In some cases, such as with STEC, these pathogens can be rendered Metabolically Active yet Non-Culturable (MAyNC). It is important to consider the implications of STEC being non-culturable (meaning there is no evidence of cell growth) but that the cells could continue to produce toxins. In conjuncture, STEC releases shiga toxins as an SOS response to DNA damage, therefore, it is reasonable to ask whether or not eBeam processing is inactivating STEC cells but also inducing toxin production. The specific aim of this study would answer this question and provide information applicable to other AB_5 toxins and hopefully lead to further investigation of the relationship between eBeam technology and pathogen inactivation.

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