Ionizing Radiation Technologies for Vaccine Development - A Mini Review

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Given the current pandemic the world is struggling with, there is an urgent need to continually improve vaccine technologies. Ionizing radiation technology has a long history in the development of vaccines, dating back to the mid-20th century. Ionizing radiation technology is a highly versatile technology that has a variety of commercial applications around the world. This brief review summarizes the core technology, the overall effects of ionizing radiation on bacterial cells and reviews vaccine development efforts using ionizing technologies, namely gamma radiation, electron beam, and X-rays.

Keywords: ionizing radiation, vaccines, electron beam, gamma irradiation, killed vaccines

INTRODUCTION

Vaccination is a cornerstone of public health measures. It promotes human and animal health as well as prevents the spread of communicable diseases in humans and animals. Over one hundred vaccines are currently licensed for human use in the United States (1). Despite this, many infectious diseases, such as Covid-19, HIV, Influenza, Malaria, and Tuberculosis continue to cause severe illness and death globally. In the feed and livestock animal industries, the use of antibiotic growth promoters has been substantially reduced due to fears of multi-drug resistant bacteria, (2–5). However, with the ban of antimicrobial usage, therapeutic usage of antimicrobials increased in Denmark by 33.6% (6) and mortality in weaning pigs increased by 1.5% (2). The resurgence of previously controlled infections and diseases have led to the intensive investigation and commercialization of multiple methods to control and improve animal health, with vaccinations being the most common (3, 7–9).

Current vaccine technologies have their advantages and disadvantages. Live vaccines often elicit strong immune responses, but a balance between attenuation, safety, and protection must be struck. Vaccination with attenuated strains has often been successful, although this option is not suitable for some diseases (10–13). A disadvantage of attenuated vaccines is the fear of regained virulence. Inactivated, or killed vaccines are inactivated using chemicals such as formalin, diethylpyrocarbonate and ß-propiolactone. Although there are reduced safety risks associated with chemically inactivated vaccines, they often exhibit reduced immunogenicity due to damaged antigenic epitopes. Toxoids, recombinant vaccines, as well as subunit vaccines are typically considered safe because attenuation is induced by deletions preventing the strain from overgrowing and causing disease (14). The disadvantage of sub-unit vaccines is that only a
singular antigen or at times multiple antigens are presented, generally limiting the cross-protective ability of such vaccines.

Given increased urbanization, climate change and close interaction of animals and humans, there is a continuous need to evaluate vaccine technologies to deal with epidemics, pandemics, and rapidly emerging infectious virus variants. The vaccine technologies should be robust and capable of dealing with multiple pathogens, their possible variants and host species (15). Ionizing radiation technology has benefited society for over 65 years. Legacy nuclear technologies based on radioactive isotopes such as cobalt-60 and cesium-137 have resulted in significant benefits to human and animal health and agriculture. Besides radioactive isotope-based ionizing radiation technology, electron beam (eBeam) and X-ray technologies have grown rapidly in the last decade and are now being widely used for a variety of commercial applications. The overall objective of this brief review is to summarize the history and advances of using ionizing radiation technology for developing vaccines against infectious diseases.

**PRINCIPLES OF IONIZING RADIATION**

Ionizing radiation is defined as energy capable of removing electrons from atoms and, thereby, causing ionization. The three main ionizing radiation technologies are gamma radiation technology (based on photons), electron beam (eBeam) technology (based on electrons), and X-rays (based on photons) (16). Gamma rays are electromagnetic radiation composed of photons emitted from the nucleus of a radioactive isotope. In most commercial settings, the isotope source is cobalt-60. In some instances, gamma rays are produced from cesium-137 as well. Electron beam (eBeam) technology is based on highly energetic electrons that are produced from regular electricity using industrial equipment called “eBeam accelerators”. X-rays are also electromagnetic radiation composed of photons. However, they are generated using energetic electrons from accelerators which are allowed to strike an extremely dense metal such as tantalum or tungsten resulting in the formation of X-ray photons. Cobalt-60 is a radioactive isotope and, therefore, it is of serious security concerns. Also, due to increasing cobalt-60 costs, its stringent safe-guarding requirements, and ultimate disposal needs and costs, this legacy technology is quickly becoming commercially unsustainable. Commercially, gamma radiation technology is being quickly replaced with accelerator-based technologies, namely eBeam and X-ray technologies (16, 17). From a commercial perspective, eBeam technology is an attractive technology because of its relatively overall lower costs and relative ease of adoption. One of the key attractive features of eBeam and X-ray technologies is that they are switch-on/switch-off technologies meaning that they can be switched off when not in use. This is in direct contrast to radioactive isotopes such as cobalt-60 where the emission of gamma ray photons cannot be switched off.

Today, eBeam and X-ray technologies are commercial off the shelf technologies with a diverse array of energy and beam power configurations. In commercial settings, eBeam irradiation is generated using accelerators. In these accelerators, electrons generated from commercial electricity are accelerated to approximately 99.999% of the speed of light resulting in electron energies up to 10 MeV (Mega electron volts) (18). These highly energetic electrons are then focused and pulsed uniformly over a material, solid or liquid (16, 18). When the electrons interact with a molecule leading to its ionization, the ejected electron becomes energized, going on to interact with and ionize an adjacent molecule. This chain reaction continues until the energy has fully dissipated (18). High energy eBeam technology is also currently used in the food and medical device industry for its ability to either pasteurize products or achieve complete sterility. In the food industry, this technology is regularly used for phytosanitary treatment, shelf-life elongation, pathogen inactivation, and occasionally terminal sterilization (16, 17). In the medical device industry, this technology is used to sterilize single-use medical devices and laboratory consumables (19).

**EFFECT OF IONIZING RADIATION EXPOSURE ON MICROORGANISMS**

Ionizing radiation inactivates microorganisms through direct and indirect methods. Direct damage is caused as a result of interactions between energetic electrons or photons and the molecules within an organism, while indirect damage is caused as a result of interactions with products of water radiolysis (18, 20, 21). When an energized electron from an accelerator (or a gamma photon emitted from a radioactive isotope) interacts with a material, molecules are ionized, ejecting electrons from the outermost valence shells. These ejected electrons in turn cause a cascade of similar ionization events on adjoining atoms until all its energy is fully dissipated. In microorganisms, DNA is the largest molecule, therefore, resulting in it being the primary target of direct ionization events. The ionization of DNA results in the cleavage of the phosphodiester bonds along the DNA backbone. While single-stranded breaks are repairable, extensive double stranded breaks are much harder for an organism to repair and overcome. Due to excessive shearing of the nucleic acid, the microorganism is ultimately inactivated (21). The other major target of ionizing radiation in a microorganism is its cellular water content, leading to the production of radiolytic species. Radiolysis of water generates a diverse array of highly reactive, but short lived free radical species such as hydroxyl radicals, hydrogen peroxide, hydrogen, hydrated electrons, and hydrated protons. The summary equation for water radiolysis is presented below (Equation 1) with the quantity of each product per 100 eV of energy absorbed shown in parenthesis.

\[
e^- + H_2O \rightarrow ^\cdot OH(2.7) + e_{aq}(2.7) + ^\cdot H(0.55) + H_2(0.55) + H_2O_2(0.71) + H_2O^\cdot(2.7) \quad \text{Equation 1}
\]
The damage to the cellular components often results indirectly from the interaction of these reactive species as opposed to the direct incident electrons. Hydroxyl radicals (•OH) are extremely short lived. However, during their short time, they can cause significant damage to molecules in their immediate surroundings (22). Superoxide radicals (O$_2^-$) are also generated by the radiolysis of water, and it is hypothesized that these molecules accumulate within a microbial cell causing severe damage to proteins such as enzymes with exposed iron-sulfur clusters (23, 24). Additionally, methionine and cysteine have been shown to be especially susceptible to ionizing radiation (25). Superoxide radicals also react with endogenous nitric oxide within a cell, forming reactive nitrogen species (RNS) such as peroxynitrite anion (ONOO$^-$), nitrogen dioxide (NO$^2$), and dinitrogen trioxide (N$_2$O$_3$), which cause further damage to the DNA and are the primary agents of damage to proteins within bacterial cells (26). This protein damage can have significant effects on the microorganism’s ability to function. Taken together, direct and indirect mechanisms of damage lead to the inactivation of microbial cells due to the high number of single and double strand breaks (21). Assuming a hypothetical genome size of 3.5 million base pairs, a dose of 1 kGy would cause approximately 200 single stranded breaks and 14 double stranded breaks, per copy of a bacteria’s genome (18, 27). This extent of DNA damage is irreparable in most microorganisms, resulting in their inactivation due to the inability of the DNA to replicate, thereby, resulting in the microbial population being unable to reproduce. This damage done to microorganisms is extremely rapid. Direct damage due to chemical bonds cleavage is estimated to occur within $10^{-14}$ – $10^{-12}$ seconds of exposure. Within one picosecond ($10^{-12}$ s), superoxide and hydrogen peroxide radicals are formed. By about 1 millisecond after exposure, the reactions of most reactive species are hypothesized to be complete (25, 28).

While microbial cells cease to multiply due to damage to their nucleic acids, multiple studies have demonstrated that their cellular membrane remains intact even after exposure to ionizing radiation. It needs to be pointed out the how microbial cells respond to ionization radiation can be extremely varied depending on the microorganism in question and the ionizing radiation dose applied to the cells. Studies conducted in our laboratory demonstrate that eBeam exposure even at lethal doses does not compromise the bacterial cellular membrane as observed using microscopy (29–33). Similarly, gamma irradiation has also been shown to cause no damage to bacterial cell membranes at lethal doses (34–36). Furthermore, there is now significant evidence that in cells treated with lethal doses of ionizing radiation, there is residual metabolic activity after treatment (33, 35, 37–41). For example, in *Escherichia coli* K-12 metabolic activity of *E. coli* was sustained for up to nine days following treatment, as demonstrated using AlamarBlue™ as well as ATP assays (33). Other studies have demonstrated that gamma radiation also does not significantly hinder cellular functions. Gamma irradiated cells maintained oxidative function and the ability to continue nucleic acid and protein synthesis (35, 38). Furthermore, metabolic activity persists, despite several double stranded breaks of the cell’s genome. Researchers hypothesize that there are portions of genomes which are still intact, enough to sustain cellular functions (35, 39, 42). Bacterial cells exposed to eBeam exposure exhibit similar features. Studies examining the metabolomic state of inactivated *E. coli* and *Salmonella Typhimurium* have shown that immediately after treatment, cells are metabolically active with metabolomic fluxes continuing even 24 hours after eBeam treatment (43). Nevertheless, the ability of microbial cells to continue their metabolic activity even after physical damage to their nucleic acids is a scientific conundrum that is worthy of deeper investigation. Taken together, this state in microbial cells where the cells cannot multiply yet remain metabolically active can be termed as a Metabolically Active, yet Non-Culturable (MAyNC) state. In vaccinology, the term that is often used especially with irradiated malarial sporozoites is “Metabolically active, non-replicating”. This state has potential broad applications in vaccine development. MAyNC cells are inactivated, but maintain cell membrane integrity, and therefore, function as a killed vaccine. The biological significance of residual metabolic activity on the potency of the vaccine is yet to be completely understood. Because ionizing radiation maintains membrane integrity, MAyNC cells may be specifically well-suited for vaccines against pathogens that require immune recognition of multiple antigenic epitopes. Furthermore, due to the growing availability of eBeam and X-ray technologies which can be installed inline to the manufacturing process, the ability to generate MAyNC cells of varying potency can be extremely valuable for vaccine development.

## HISTORY OF VACCINES USING IONIZING RADIATION

The use of ionizing radiation as a method to attenuate or inactive microorganisms for the use as vaccines is not novel, with reports of gamma and x-ray-inactivated vaccine research dating back to the mid-20th century (44–50). The advantage of ionizing-radiation vaccines, or radio-vaccines, is that because they are inactivated, they are able to retain immunogenicity even when stored at non-refrigerated conditions potentially eliminating the need for cold-chain to preserve vaccine potency (31, 51, 52). The ability to store vaccines at ambient or refrigerated storage (as compared to frozen storage) can translate to significantly lower overall costs for vaccine transportation and distribution. The ability to distribute vaccines without the need for cold chain distribution also increases vaccine access in remote areas (53, 54). Importantly, eBeam and X-ray technologies are scalable, with the capability to inactivate large quantities of preparations (55).

Due to the vast commercial capabilities, numerous patents related to “radio-vaccines” have already been filed (Table 1). Interest in radio- vaccines has increased significantly recently, with investigations into the creation of vaccines for bacterial, viral, and protozoan diseases (Table 2). While many of the researched vaccine candidates are based on gamma-irradiation, there is significantly less research conducted on eBeam or X-ray.
inactivated vaccines. This limited amount of information could be attributed to the relatively recent commercial availability of eBeam and X-ray technologies. Among all the research conducted on radio-vaccines, the most progress has been on *Plasmodium* sporozoites attenuated with irradiation to protect against malaria. First examined in 1967 using x-ray irradiation, this idea has evolved considerably over the last 50+ years to its current iteration in phase 2 clinical trials using gamma-attenuated sporozoites (75, 76, 93–97). Studies using gamma-irradiated *Listeria monocytogenes* have demonstrated that unlike other inactivation methods such as heat or formalin, irradiation better maintained antigenic properties and stimulated robust T cell responses (59).

### IMMUNE RESPONSES TO RADIO-VACCINES

In multiple studies investigating the immune response to gamma-irradiated *Brucella* spp., investigators found that gamma-irradiated cells were metabolically active and inactivated cells were able to induce a significant cellular immune response and were protective when challenged (34–37, 56, 98). Furthermore, gamma-irradiated cells have even exhibited an ability to act as an adjuvant, increasing the immune response to co-administered antigens (71). A significant amount of research has been conducted on the development of a gamma-inactivated influenza vaccine, demonstrating that this vaccine is effective in eliciting a strong antigen-specific antibody response as well as protecting mice from challenge with heterologous influenza virus (27, 80, 83).

Electron beam (eBeam) technology has been investigated as a method to generate vaccine-like immunomodulators against *Salmonella* Typhimurium using a mice model (31). This concept has been expanded to demonstrating the immunomodulatory and protective effects of eBeam-inactivated *Salmonella* Enteriditis and Typhimurium in chickens and *Rhodococcus equi* in neonatal fawns (29–32, 40, 41, 64, 67). This concept is now been expanded to include the use of low energy eBeam as an inactivation technology for vaccine development with considerable success (73, 77, 82).

### ROLE OF ADJUVANTS

For a vaccine formulation to be effective upon challenge, it must be able to induce a prolonged and protective immune response. Live attenuated vaccines that retain their ability to replicate with a host, naturally eliciting a strong CD8+ and CD4+ T cell response, as well as a strong humoral response, while inactivated vaccines often require the assistance of an adjuvant to help the vaccine elicit a stronger immune response in the host. An adjuvant is technically defined as a component that is added to vaccine to enhance an immune response, and typically provides the benefits of increased antibody titers and an increased speed, breadth, and duration of an immune response. Because radio-vaccines are unable to replicate within a host, it has been proposed that their immunogenic potential

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**TABLE 1 | A selection of patents relating to radio-vaccines.**

| Patent #                | Country     | Year   | Status* | Title                                                                                     |
|------------------------|-------------|--------|---------|-------------------------------------------------------------------------------------------|
| US3657415A             | USA         | 1969   | Expired | Canine hookworm vaccines                                                                  |
| DE3853854T             | Germany     | 1988   | Expired | Vaccine against group b *Neisseria meningitidis*, gammaglobulin and transfer factor         |
| AU706213B2             | Australia   | 1996   | Ceased  | Method for obtaining a vaccine with wide protective range against group b *Neisseria meningitidis*, the resulting vaccine, gammaglobulin and transfer factor |
| AU6320001A             | Australia   | 2001   | Published | Gamma irradiation of protein-based pharmaceutical products                              |
| KR20090034517A         | South Korea | 2001   | Granted | Vaccination of inactivated chikungunya virus strain                                       |
| US20060147460A1        | USA         | 2002   | Granted | Anticancer vaccine and diagnostic methods and reagents                                    |
| KR101173871B1          | South Korea | 2004   | Granted | Modified free-living microbes vaccine compositions and methods of use thereof             |
| US20050175630A1        | USA         | 2004   | Abandoned | Immunogenic compositions and methods of use thereof                                       |
| US817531981A           | USA         | 2009   | Granted | High energy electron beam irradiation for the production of immunomodulators in poultry   |
| CA2733356C             | Canada      | 2009   | Granted | Influenza vaccines                                                                      |
| US8282924B2            | USA         | 2010   | Granted | Toxoplasma gondii vaccines and uses thereof                                               |
| US2013012045A1         | USA         | 2010   | Abandoned | Cross-protective influenza vaccine                                                       |
| US2015020942A1         | USA         | 2011   | Abandoned | Inactivated varicella zoster virus vaccines, methods of production, and uses thereof     |
| JP2014520117A          | Japan       | 2012   | Granted | Vaccine composition comprising inactivated chikungunya virus strain                      |
| AU2012211043B2         | Australia   | 2012   | Published | Combination vaccines                                                                   |
| US10080795B2           | USA         | 2013   | Granted | Method for inactivating viruses using electron beams                                     |
| WO2014155297A2         | WIPO        | 2014   | Published | Systems and methods for viral inactivation of vaccine compositions by treatment with carbohydrates and radiation |
| WO2014165961A1         | WIPO        | 2014   | Published | Methods and compositions for inducing an immune response                                |
| DE10201522406B3        | Germany     | 2015   | Granted | Irradiation of biological media in transported foil bags                                   |
| KR20180036987A         | South Korea | 2016   | Published | Vaccine composition                                                                      |
| DE102016216573A1       | Germany     | 2016   | Published | Inactivation of pathogens in biological media                                             |
| WO2018167149A1         | WIPO        | 2018   | Ceased  | Method for irradiating mammalian cells with electron beams and/or x-rays                  |
| WO2019191586A2         | Canada      | 2019   | Published | Irradiation-inactivated poliovirus, compositions including the same, and methods of preparation |
| WO2020060942A1         | WIPO        | 2020   | Published | Method for inactivating biologically active components in a liquid                       |

*Status as of November, 2020; © World Intellectual Property Organization.
| Type of Pathogen | Pathogen | Inactivation Method | Inactivation Dose | Model | Notes | Source |
|------------------|----------|---------------------|------------------|-------|-------|--------|
| **Bacteria**     | Brucella abortus | Gamma | 4 kGy | Mice | Irradiated strains induced less of an immune response than live strains | (56) |
| **Bacteria**     | Brucella abortus | Gamma | 3 kGy | Mouse | Antigen specific Th1 response | (34) |
| **Bacteria**     | Brucella abortus | Gamma | 2.5 kGy | Mice | Stimulated IFN-gamma and Th1 cells | (57) |
| **Bacteria**     | Brucella abortus, B. melitensis, and B. suis | Gamma | 3.5 kGy | Mice | Protective upon challenge | (58) |
| **Bacteria**     | Brucella melitensis | Gamma | 3.5 kGy | Mice | Protective upon challenge | (56) |
| **Bacteria**     | Brucella melitensis | Gamma | 3 kGy | Mouse | Cytotoxic T cell response and protective against challenge | (35) |
| **Bacteria**     | Listeria monocytogenes | Gamma | 6 kGy | Mouse | Induced protective T cell responses | (59) |
| **Bacteria**     | Mannheimia haemolytica | Gamma | 2-20 kGy | Rabbit | Protection upon challenge | (60) |
| **Bacteria**     | Orientia tsutsugamushi | Gamma | 2 kGy | Mice | Partially protective upon challenge | (61) |
| **Bacteria**     | Pasteurella tatarica | Gamma | 3 kGy | Mice | Protective upon challenge | (62) |
| **Bacteria**     | Pasteurella tatarica | Gamma | 10 kGy | Mice | Partially protective upon challenge | (63) |
| **Bacteria**     | Rhodococcus equi | Gamma | 4-5 kGy | Mice | Produced cell-mediated and upper respiratory mucosal immune response | (33) |
| **Bacteria**     | Rhodococcus equi | Gamma | 5 kGy | Horse | Not protective upon challenge | (64) |
| **Bacteria**     | Rodentibacter pneumotrophicus | Gamma | 20 kGy | Mice | Protective upon challenge and reduced colonization | (65) |
| **Bacteria**     | Salmonella Enteritidis | Gamma | 2.5 kGy | Chicken | Protective upon challenge and reduced colonization | (66) |
| **Bacteria**     | Salmonella Typhimurium | Gamma | 2.5 kGy | Chicken | Heterophil-mediated innate immune response | (67) |
| **Bacteria**     | Salmonella Typhimurium | Gamma | 2.5 kGy | Chicken | Heterophil-mediated innate immune response | (67) |
| **Bacteria**     | Salmonella Typhimurium | Gamma | 7 kGy | Mouse | Stimulated innate immune markers and reduced colonization | (31) |
| **Bacteria**     | Salmonella Typhimurium | Gamma | 10-50 kGy | Chicken | Protective upon challenge | (68) |
| **Bacteria**     | Shigella dysenteriae | Gamma | Not reported | Rabbits | Bacteria that were treated for a longer time were non-toxic and protective upon challenge | (44) |
| **Bacteria**     | Staphylococcus aureus | Gamma | 2.5-9.9 kGy | Mouse | Induced specific antibody production, but not protective upon challenge | (69) |
| **Bacteria**     | Staphylococcus aureus | Gamma | 25-40 kGy | Mice | Induced B and T cell-dependent protection against challenge | (70) |
| **Bacteria**     | Streptococcus pneumoniae | Gamma | 12 kGy | Mice | Protective upon challenge mediated by B-cells and innate IL-17 response | (71) |
| **Bacteria**     | Streptococcus pneumoniae | Gamma | 25 kGy | Rabbit and Mice | Immunogenic and protective upon challenge | (72) |
| **Protozoa**     | Eimeria tenella | Gamma | 0.1-0.5 kGy | Chicken | Partially protective upon challenge | (73) |
| **Protozoa**     | Eimeria tenella | Gamma | 0.2 kGy | Chicken | Protective upon challenge | (74) |
| **Protozoa**     | Eimeria tenella | Gamma | 0.02-0.15 kGy | Mouse | Protective upon challenge | (75) |
| **Protozoa**     | Plasmodium berghei | Gamma | 0.12-0.15 kGy | Human | Long-lasting protective immunity | (76) |
| **Protozoa**     | Plasmodium falciparum | Gamma | 0.005-0.2 kGy | Mosquito | Sporozoites from irradiated oocysts were non-infective | (49) |
| **Protozoa**     | Plasmodium gallinaceum | Gamma | 20 kGy | Mosquito | Reduction in viral load upon challenge | (77) |
| **Viruses**      | Human Respiratory syncytial virus (HRSV) | Gamma | 12.6 kGy | Mouse | Induced cytotoxic T cells and protective upon challenge | (78) |
| **Viruses**      | Influenza A virus | Gamma | 10-40 kGy | Mice | Cross-reactive and cross-protective cytotoxic T cell responses | (80) |
| **Viruses**      | Influenza A virus | Gamma | 10 kGy | Mice | Protective upon challenge; freeze-drying did not affect cross-protective immunity | (81) |
| **Viruses**      | Influenza A virus | Gamma | 50 kGy | Mice | Inactivated vaccine induced complete protection | (82) |
| **Viruses**      | Influenza A virus | Gamma | 30 kGy | Mice | Elicited a protective immune response | (83) |
| **Viruses**      | Influenza A virus | Gamma | 25-40 kGy | Nonhuman primate | Elicited seroconversion | (51) |
| **Viruses**      | Influenza A virus | Gamma | 10 kGy | Mouse | Protective upon heterotypic challenge | (83) |
| **Viruses**      | Middle Eastern Respiratory Virus (MERs) | Gamma | 50 kGy | Mice | Caused lung immunopathology upon challenge | (84) |
| **Viruses**      | Polio Virus | Gamma | 45 kGy | Mice | Protective upon challenge | (65) |
| **Viruses**      | Polio Virus | Gamma | 30 kGy | Mice | Induced a specific neutralizing-antibody response | (66) |
| **Viruses**      | Polio Virus | Gamma | 50 kGy | Mice | Adjuvanted vaccine elicited T and B cell responses | (52) |
| **Viruses**      | Polio Virus | Gamma | 25 kGy | Mice | Humoral and cellular immune response, induced neutralizing antibodies | (87) |
| **Viruses**      | Polio Virus | Gamma | 0-15 kGy | Rabbit | Inactivated virus was immunogenic | (48) |
| **Viruses**      | Venezuelan Equine Encephalitis Vaccine | Gamma | 80-100 kGy | Guinea Pig | Protective upon challenge | (69) |

(Continued)
TABLE 2 | Continued

| Type of Pathogen | Pathogen                        | Inactivation Method | Inactivation Dose | Model          | Notes                                                                 | Source |
|------------------|---------------------------------|--------------------|-------------------|----------------|----------------------------------------------------------------------|--------|
| Virus            | Venezuelan Equine Encephalitis  | Gamma              | 50 kGy            | Mice           | Protective against subcutaneous challenge and partially protective against aerosol challenge | (99)   |
| Virus            | White Spot Syndrome Virus       | Electron Beam      | 13 kGy            | Shrimp         | Protective upon challenge                                            | (90)   |
| Virus            | Zaire ebola virus               | Gamma              | 100 kGy           | Nonhuman primate | Not protective upon challenge                                      | (91)   |
| Virus            | Zaire ebola virus               | Gamma              | 60 kGy            | Nonhuman primate | Not protective upon challenge                                      | (92)   |

...has to be enhanced by the addition of an adjuvant. There are several reports about coupling radio-vaccines with experimental and commercially available adjuvants. Bayer et al. tested four different adjuvants in combination with Respiratory syncytial virus inactivated with low energy electron beam: Alhydrogel (alum based), MF59 (squalene based), QuilA (saponin based), and Poly IC : LC (synthetic double-stranded RNA based) (77). In their study, strong immune responses and significant reductions in viral loads were detected after immunization and subsequent challenge, although the poly IC : LC adjuvanted vaccine elicited lower titers of neutralizing antibodies than the other adjuvanted vaccines tested (77). Substantial humoral and cellular responses were observed when a gamma-inactivated polio vaccine candidate was combined with an alum adjuvant and when a gamma-irradiated HIN1 vaccine was co-administered with a plasmid encoding mouse interleukin-28B (99, 100). Gamma-inactivated SARS-CoV-2 also benefited from the addition of a GM-CSF adjuvant in order to induce a T cell response (52).

CONCLUSIONS

Though ionizing radiation has been researched as a vaccine technology for nearly a century, only recently have vaccines utilizing ionizing radiation reached commercial development. The general lack of interest in radio-vaccines could be attributed to advances in cloning technologies, mRNA vaccines and gene editing technologies. The recent availability of small footprint, low energy eBeam and X-ray equipment could, however, spur the development of radio-vaccines once again. Commercialization of eBeam and X-ray technologies for the medical device, food, and other industrial applications has led to a decrease in overall technology costs and an increase in technology availability (101). This review highlights the potential of ionizing radiation as a vaccine technology suitable against several pathogens causing diseases in various hosts species. This has been most recently demonstrated in the rapid development of vaccine candidates in response to the COVID-19 pandemic, caused by the virus SARS-CoV-2. Radio-vaccines have even been investigated as a response to previous outbreaks of SARS and MERS, and it was hypothesized that ionizing radiation could be used to rapidly produce a vaccine for SARS-CoV-2 (84, 102–104). Gamma-inactivated SARS-CoV-2 combined with GM-CSF as an adjuvant has demonstrated ability to induce neutralizing antibodies as well as a strong T and B cell response (87, 105).

AUTHOR CONTRIBUTIONS

Major portions of this manuscript have been previously included in a doctoral dissertation by SB (106). SP was involved in writing and editing the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported funds from the USDA-NIFA program administered by Texas A&M AgriLife Research H-87080 as well as funds through contracts from the Pacific Northwest National Laboratories (PNNL).

ACKNOWLEDGMENTS

This work was prepared as part of the activities of the IAEA Collaborating Center for Electron Beam Technology.

REFERENCES

1. FDA. Vaccines Licensed for Use in the United States (2021). FDA. Available at: https://www.fda.gov/vaccines-blood-biologics/vaccines/vaccines-licensed-use-united-states (Accessed September 19, 2021).
2. Wierup M. The Swedish Experience of the 1986 Year Ban of Antimicrobial Growth Promoters, With Special Reference to Animal Health, Disease Prevention, Productivity, and Usage of Antimicrobials. Microb Drug Resist (2001) 7:183–90. doi: 10.1089/10766290152045066
3. Van Immerseel F, Rood JI, Moore RJ, Titball RW. Rethinking Our Understanding of the Pathogenesis of Necrotic Enteritis in Chickens. Trends Microbiol (2009) 17:32–6. doi: 10.1016/j.tim.2008.09.005
4. Maron DF, Smith TJ, Nachman KE. Restrictions on Antimicrobial Use in Food Animal Production: An International Regulatory and Economic Survey. Global Health (2013) 9:48. doi: 10.1186/1744-8603-9-48
5. McEwen SA, Angulo FJ, Collignon PJ, Conly J. Potential Unintended Consequences Associated With Restrictions on Antimicrobial Use in Food-Producing Animals (2017). World Health Organization. Available at: https://www.ncbi.nlm.nih.gov/books/NBK487949/ (Accessed January 20, 2020).
6. DANMAP. DANMAP – Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria From Food Animals, Food and Humans in Denmark (2010). Available at: http://danmap.org/reports/older.
1. Tahergorabi R, Matak KE, Jaczynski J. Application of Electron Beam to IIA and GIPA. Electronic Irradiation of Foods: An Introduction to the Technology

2. doi: 10.1093/af/vfy001

3. Thompson DR, Parreira VR, Kulkarni RR, Prescott JF. Live Attenuated Vaccine Technologies: Essential Components of an Adequate Response to Emerging Viral Diseases.

4. Bhatia SS, Pillai SD. A Comparative Analysis of the Metabolomic Response in Neonatal Foals. Immunogenicity of an Electron Beam Inactivated Rhodococcus Equi Vaccine in Neonatal Foals. PloS One (2014) 9:e105367. doi: 10.1371/journal.pone.0105367

5. Praveen C. Electron Beam as a Next Generation Vaccine Platform: Microbiological and Immunological Characterization of an Electron Beam Based Vaccine Against Salmonella Typhimurium. [Ph.D. Dissertation]. College Station (TX): Texas A&M University (2014).

6. Bordin AI, Pillai SD, Brake C, Bagley KB, Bourquin JR, Coleman M, et al. Immunogenicity of an Electron Beam Inactivated Rhodococcus Equi Vaccine. Front Immunol (2020) 11:583077. doi: 10.3389/fimmu.2020.583077

7. Spreng S, Dietrich G, Weidinger G. Rational Design of Salmonella-Based Vaccine Strategies. Methods (2006) 38:133–43. doi: 10.1016/j.ymeth.2005.09.012

8. doi: 10.1001/jama.2018.0345

9. Graham BS, Mascola JR, Fauci AS. Novel Vaccine Technologies: Essential Components of an Adequate Response to Emerging Viral Diseases. JAMA (2018) 319:1341–2. doi: 10.1001/jama.2018.0345

10. Pillai SD, Shayanfar S. Electron Beam Technology and Other Irradiation Technology Applications in the Food Industry, in: Applications of Radiation Chemistry in the Fields of Industry, Biotechnology and Environment Topics in Current Chemistry Collections (2017). Springer International Publishing (Accessed July 1, 2018).

11. Bhatia SS, Pillai SD. Escherichia Coli Cells Exposed to Lethal Doses of Electron Beam Irradiation Retain Their Ability to Propagate Bacteriophages and Are Metabolically Active. Front Microbiol (2018) 9:2138. doi: 10.3389/fmicb.2018.02138

12. Sanakayaka N, Sokolovska A, Gulani J, HogenEsch H, Sriranganathan N, Boyle JM, et al. Induction of Antigen-Specific Th1-Type Immune Responses by Gamma-Irradiated Recombinant Brucella abortus BRS1. Clin Diag Lab Immunol (2005) 12:1429–36. doi: 10.1128/CDLI.1.12.1429-1436.2005

13. Bhatia SS, Pillai SD. Electron Irradiation of Foods: An Introduction to the Technology. New York: Springer (2005).

14. Pillar SD, Bhatia SS. Electron Beam Technology: A Platform for Safe, Fresh, and Chemical-Free Food, in: Food Safety Magazine – Applications of Radiation to Food Irradiation – (2018) 319:1431–6. doi: 10.1093/af/vfy001

15. Hieke A-SC, Pillai SD. Escherichia Coli Cells Exposed to Lethal Doses of Electron Beam Irradiation Retain Their Ability to Propagate Bacteriophages and Are Metabolically Active. Front Microbiol (2018) 9:2138. doi: 10.3389/fmicb.2018.02138

16. Sanakayaka N, Sokolovska A, Gulani J, HogenEsch H, Sriranganathan N, Boyle JM, et al. Induction of Antigen-Specific Th1-Type Immune Responses by Gamma-Irradiated Recombinant Brucella abortus BRS1. Clin Diag Lab Immunol (2005) 12:1429–36. doi: 10.1128/CDLI.1.12.1429-1436.2005

17. Magnani DM, Harms JS, Durward MA, Splitter GA. Nondividing But Metabolically Active Gamma-Irradiated Brucella Melitensis Is Protecting Against Virulent B. Melitensis Challenge in Mice. Infect Immun (2009) 77:5181–9. doi: 10.1128/IAI.00231-09

18. Moustafa D, Garg VK, Jain N, Sriranganathan N, Vemulapalli R. Immunization of Mice With Gamma-Irradiated Brucella Neotomae and its Recombinant Strains Induces Protection Against Virulent B. Abortus, B. Melitensis and B. Suis Challenge. Vaccine (2011) 29:784–94. doi: 10.1016/j.vaccine.2010.11.018

19. Ahn TH, Nishihara H, Carpenter CM, Taplin GV. Respiration of Gamma Irradiated Brucella abortus and Mycobacterium Tuberculosis. Proc Soc Exp Biol Med (1962) 111:771–3. doi: 10.3181/00379727-111-27917

20. Hiramoto RM, Galisteo AJ, do Nascimento N. Sterilised Toxoplasma Gondii Tachyzoites Maintain Metabolic Functions and Mammalian Cell Invasion, Eliciting Cellular Immunity and Cytokine Response Similar to Natural Infection in Mice. F Vaccine (2002) 20:2072–81. doi: 10.1016/S1567-1359(02)00055-0

21. Sekanella-Fandos S, Nogueira-Ortega E, Olivares F, Luquin M, Julián E. Killed But Metabolically Active Mycobacterium Bovis Bacillus Calmette-Guérin Retains the Antitumor Ability of Live Bacillus Calmette-Guérin. J Urol (2014) 191:1422–8. doi: 10.1016/j.juro.2013.12.002

22. Jesudhasan PR, Bhatia SS, Sivakumar KK, Praveen C, Genovese KJ, He HL, et al. Controlling the Colonization of Clostridium Perfringens in Broiler Chickens by an Electron-Beam-Killed Vaccine. Animals (2021) 11:671. doi: 10.3390/ani11030671

23. Praveen C, Bhatia SS, Alainz RC, Droleskey RE, Cohen JD, Jesudhasan PR, et al. Assessment of Microbiological Correlates and Immunostimulatory Potential of Electron Beam Inactivated Metabolically Active Yet Non Culturable (MAYNC) Salmonella Typhimurium. PloS One (2011) 6:e243417. doi: 10.1371/journal.pone.0243417

24. Trampuz A. Effect of Gamma Irradiation on Viability and DNA of Staphylococcus Epidermidis and Escherichia Coli. J Med Microbiol (2006) 55:1271–5. doi: 10.1099/jmm.0.46488-0

25. Bhatia SS, Pillai SD. A Comparative Analysis of the Metabolomic Response of Electron Beam Inactivated E. Coli O26:H11 and Salmonella Typhimurium ATCC 13311. Front Microbiol (2019) 10:694. doi: 10.3389/ fmicb.2019.00694

26. Moore HN, Kersten H. Preliminary Note on the Preparation of Non-Toxic Shiga Dysentery Vaccines by Irradiation With Soft X-Rays. J Bacteriol (1936) 31:581–4. doi: 10.1128/jb.31.6.581-584.1936
45. Jordan RT, Kempe LL. Inactivation of Some Animal Viruses With Gamma Radiation From Cobalt-60. *Proc Soc Exp Biol Med* (1956) 95:212–5. doi: 10.3813/0037-5292.95.2.212215

46. Tumanyan MA, Dupleshcheva AP, Sedova TS. Influence of Massive Doses of γ-Gamma-Rays on Immunological Properties of Bacteria of Intestinal Group. *Zhur Mikrobiof Epidemiol i Immunobiol* (1958) 43:10.

47. Carpenter CM. Preliminary Report on Vaccines Prepared From Gamma-Irradiated Mycobacterium Tuberculosis and Brucella Suis, in: *American Review of Tuberculosis and Pulmonary Diseases* (1959). Available at: https://www.atsjournals.org/doi/pdf/10.1146/arp15997.3374 (Accessed January 23, 2020).

48. Kaplan C. The Antigenicity of γ-Irradiated Vaccinia Virus. *Epidemic Epidemic* (1960) 58:391–8. doi: 10.1007/BF02217240003855

49. Ward RA, Bell LH, Schneider RL. Effects of X-Irradiation on the Development of Malarial Parasites in Mosquitoes. *Exap Paroet* (1960) 10:324–32. doi: 10.1007/BF0114-4894(60)09007-9

50. Kalenina EF, Abidov AZ. The Effect of Gamma Rays of Co-60 on Smallpox Virus. *Vestnik Mikrobiol Epidemiol i Immunobiol* (1960) 4:32–40.

51. Jordan RT, Kempe LL. Inactivation of Some Animal Viruses With Gamma Radiation From Cobalt-60. *Proc Soc Exp Biol Med* (1956) 95:212–5. doi: 10.3813/0037-5292.95.2.212215

52. Orr MT, Kramer RM, Barnes LV, Dowling QM, Desbien AL, Beebe EA, et al. Preliminary Report of Preclinical Ef

53. Oliveira SC, Zhu Y, Splitter GA. Recombinant L7/L12 Ribosomal Protein -Irradiated Vaccinia Virus. *Proc Vaccinology* (2020) 10.12786. doi: 10.1038/s41598-020-69347-7

54. Fertey J, Thoma M, Beckmann J, Bayer L, Finkensieper J, Reißhauer S, et al. Surendran N, Hiltbold EM, Heid B, Sriranganathan N, Boyle SM, Tumanyan MA, Duplishcheva AP, Sedova TS, et al. Influence of Massive Doses of γ-Gamma-Rays on Immunological Properties of Bacteria of Intestinal Group. *Zhur Mikrobiof Epidemiol i Immunobiol* (1958) 43:10.

55. Jordan RT, Kempe LL. Inactivation of Some Animal Viruses With Gamma Radiation From Cobalt-60. *Proc Soc Exp Biol Med* (1956) 95:212–5. doi: 10.3813/0037-5292.95.2.212215

56. Surendran N, Hiltbold EM, Heid B, Sriranganathan N, Boyle SM, Tumanyan MA, Duplishcheva AP, Sedova TS, et al. Influence of Massive Doses of γ-Gamma-Rays on Immunological Properties of Bacteria of Intestinal Group. *Zhur Mikrobiof Epidemiol i Immunobiol* (1958) 43:10.
81. David SC, Lau J, Singleton EV, Babb R, Davies J, Hirst TR, et al. The Effect of Gamma-Irradiation on the Immunogenicity of Whole-Infected Influenza A Virus Vaccine. Vaccine (2017) 35:1077–9. doi: 10.1016/j.vaccine.2016.12.044

82. Ferrey J, Bayer L, Grunwald T, Pohl A, Beckmann I, Gotzmann G, et al. Pathogens Inactivated by Low-Energy-Electron Irradiation Maintain Antigenic Properties and Induce Protective Immune Responses. Viruses (2016) 8:319–33. doi: 10.3390/v8100319

83. Alsharif M, Furuya Y, Bowden TR, Lobigs M, Koskinen A, Regner M, et al. Intranasal Flu Vaccine Protective Against Seasonal and H5N1 Avian Influenza Infections. PLoS One (2014) 4:e5336. doi: 10.1371/journal.pone.0055366

84. Agrawal AS, Tso X, Algaissi A, Garron T, Narayanan K, Peng B-H, et al. Immunization With Inactivated Middle East Respiratory Syndrome Coronavirus Vaccine Leads to Lung Immunopathology on Challenge With Live Virus. Hum Vaccin Immunother (2016) 12:2351–6. doi: 10.1080/21645515.2016.1177688

85. Tobin GJ, Tobin JK, Gaidamakovka EK, Wiggins TJ, Bushnell RV, Lee W-M, et al. A Novel Gamma Radiation-Inactivated Sabin-Based Polio Vaccine. PLoS One (2020) 15:e0228006. doi: 10.1371/journal.pone.0228006

86. Shahrudin S, Chen C, David SC, Singleton EV, Davies J, Kirkwood CD, et al. Gamma-Irradiated Rotavirus: A Possible Whole Virus Inactivated Vaccine. PLoS One (2013) 8:e598182. doi: 10.1371/journal.pone.0198182

87. Turan RD, Tastan C, Kancagi DD, Yurtsever B, Karakus GS, Ozer S, et al. Gamma-Irradiated SARS-CoV-2 Vaccine Candidate, OZG-38.61.3, Confers Protective Cellular and Mucosal Immune Responses Following Nasal Administration of a Whole Gamma-Irradiated Influenza A (Subtype H1N1) Vaccine Adjuvanted With Interleukin-28B in a Mouse Model. Arch Virology (2021) 166:545–57. doi: 10.1007/s00705-020-09400-3

88. Reitman M, Tribble HR, Green L. Gamma-Irradiated Venezuelan Equine Encephalitis Vaccines. Appl Microbiol (1970) 19:763–7. doi: 10.1128/ amis.19.3.763-767.1970

89. Martin SS, Bakken RR, Lind CM, Garcia P, Jenkins E, Glass PJ, et al. Comparison of the Immunological Responses and Efficacy of Gamma Irradiated V3526 Vaccine Formulations Against Subcutaneous and Aerosol Challenge With Venezuelan Equine Encephalitis Virus Subtype IAB. Vaccine (2010) 28:1031–40. doi: 10.1016/j.vaccine.2009.10.126

90. Motamedi-Sedeh F, Ashar纳斯ab M, Heidari Malea M, Tahami SM. Protection of Lipopolysaccharide Vannamei Against White Spot Syndrome Virus by Gamma-Irradiated Inactivated Vaccine and Prebiotic Immunogen. Radiat Phys Chem (2017) 130:421–5. doi: 10.1016/j.radphyschem.2016.09.020

91. Marzi A, Halfmann P, Hill-Batsoni L, Feldmann F, Shupert WL, Neumann G, et al. An Ebola Whole-Virus Vaccine Is Protective in Nonhuman Primates. Science (2015) 348:439–42. doi: 10.1126/science.aaa4919

92. Geisinger TW, Pushko P, Anderson K, Smith J, Davis KJ, Jahrling PB. Evaluation in Nonhuman Primates of Vaccines Against Ebola Virus. Emerg Infect Dis (2002) 8:503–7. doi: 10.3201/eid0805.010284

93. Clyde DF. Immunity to Falciparum and Vivax Malaria Induced by Irradiated Sporozoites: A Review of the University of Maryland Studie-75. Bull World Health Organ (1990) 68 Suppl9–12.

94. Rieckmann KH. Human Immunization With Attenuated Sporozoites. Bull World Health Organ (1990) 68 Suppl13–6.

95. Luke TC, Hoffman SL. Rationale and Plans for Developing a Non-Replicating, Metabolically Active, Radiation-Attenuated Plasmodium Falciparum Sporozoite Vaccine. J Exp Biol (2003) 206:3803–8. doi: 10.1242/jeb.006244

96. Seder RA, Chang L-J, Enama ME, Zephir KL, Sarwar UN, Gordon IJ, et al. Protection Against Malaria by Intravenous Immunization With a Nonreplicating Sporozoite Vaccine. Science (2013) 341:1359–65. doi: 10.1126/science.1241800

97. Arévalo-Herrera M, Vásquez-Jiménez JM, Lopez-Perez M, Vallejo AF, Amado-Garavito AB, Cepsedes N, et al. Protective Efficacy of Plasmidium Vivax Radiation-Attenuated Sporozoites in Colombian Volunteers: A Randomized Controlled Trial. PloS Negl Trop Dis (2016) 10(10):e0005070. doi: 10.1371/journal.pntd.0005070

98. Velumalapalli R, Cravero S, Calvert CL, Toth TE, Siranganganthan N, Boyle SM, et al. Characterization of Specific Immune Responses of Mice Inoculated With Recombinant Vaccinia Virus Expressing an 18-Kilodalton Outer Membrane Protein of Brucella Abortus. Clin Diagn Lab Immunol (2000) 7:114–8. doi: 10.1128/CDLI.7.1.114-118.2000

99. Alamutui MM, Ravanshad M, Motamedi-Sedeh F, Nabizadeh A, Ahmadi E, Hossienei SM. Immune Response of Gamma-Irradiated Inactivated Bivalent Polio Vaccine Prepared Plus Trehalose as a Protein Stabilizer in a Mouse Model. INT (2021) 64:140–6. doi: 10.1159/000515392

100. Sabbaghi A, Zargar M, Zolfaghari MR, Motamedi-Sedeh F, Ghaemi A. Protective Cellular and Mucosal Immune Responses Following Nasal Administration of a Whole Gamma-Irradiated Influenza A (Subtype H1N1) Vaccine Adjuvanted With Interleukin-28B in a Mouse Model. Arch Virology (2021) 166:545–57. doi: 10.1007/s00705-020-09400-3

101. Pillai SD, Pillai ET. Agriculture: Electron Beam Irradiation Technology Applications in the Food Industry. In: E Greenspan, editor. Encyclopedia of Nuclear Energy. Oxford: Elsevier (2021). p. 313–29. doi: 10.1016/B978-0-12-819725-7.00141-0

102. Beniac DR, deVarannes SL, Andonov A, He R, Booth TF. Conformational Reorganization of the SARS Coronavirus Spike Following Receptor Binding: Implications for Membrane Fusion. PloS One (2007) 2:e1082. doi: 10.1371/journal.pone.0001082

103. Durante M, Schulze K, Incerti S, Francis Z, Zein S, Guzman CA. Virus Irradiation and COVID-19 Disease. Front Phys (2020) 8:565861. doi: 10.3389/fphy.2020.565861

104. Mullbacher A, Pardo J, Furuya Y. SARS-CoV-2 Vaccines: Inactivation by Gamma Irradiation for T and B Cell Immunity. Pathogens (2020) 9:928. doi: 10.3390/pathogens9100928

105. Sir Karakus G, Tastan C, Dilek Kancagi D, Yurtsever B, Tumentemur G, Demir S, et al. Preclinical Efficacy and Safety Analysis of Gamma-Irradiated Inactivated SARS-CoV-2 Vaccine Candidates. Sci Rep (2021) 11:5804. doi: 10.1038/s41598-021-83930-6

106. Bhatia SS. Investigations Into Metabolically Active Yet Non-Culturable (MayNC) Clostridium Perfringens to Control Necrotic Enteritis in Broiler Chickens (2021). Available at: https://oaktrust.library.tamu.edu/handle/1969.1969.1:13018 (Accessed November 9, 2021).

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