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Evaluation of Radiological Effects on the Aptamers to Remove Ionic Radionuclides in the Liquid Radioactive Waste

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ABSTRACT

Background: Aptamers are currently being used in various fields including medical treatments due to their characteristics of selectively binding to specific molecules. Due to their special characteristics, the aptamers are expected to be used to remove radionuclides from a large amount of liquid radioactive waste generated during the decommissioning of nuclear power plants. The radiological effects on the aptamers should be evaluated to ensure their integrity for the application of a radionuclide removal technique.

Materials and Methods: In this study, Monte Carlo N-Particle transport code version 6 (MCNP6) and Monte Carlo damage simulation (MCDS) codes were employed to evaluate the radiological effects on the aptamers. MCNP6 was used to evaluate the secondary electron spectrum and the absorbed dose in a medium. MCDS was used to calculate the DNA damage by using the secondary electron spectrum and the absorbed dose. Binding experiments were conducted to indirectly verify the results derived by MCNP6 and MCDS calculations.

Results and Discussion: Damage yields of about 5.00×10^{-4} were calculated for 100 bp aptamer due to the radiation dose of 1 Gy. In experiments with radioactive materials, the results that the removal rate of the radioactive ^{60}Co by the aptamer is the same with the non-radioactive ^{59}Co prove the accuracy of the previous DNA damage calculation.

Conclusion: The evaluation results suggest that only very small fraction of significant number of the aptamers will be damaged by the radioactive materials in the liquid radioactive waste.

Keywords: Aptamer, Liquid Radioactive Waste, Decommissioning, Decontamination, Radiological Damage

Original Research

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Introduction

In 1990, Ellington and Szostak [1] discovered RNA molecules that bind specific ligands and termed these molecules 'Aptamer,' from the Latin '*aptus*,' meaning to fit. The aptamers are single-stranded DNA or RNA that, through intramolecular interactions, fold into unique tertiary conformations capable of binding to a target with a high affinity and specificity [2]. According to the high affinity and specificity, the aptamers can bind to the targets, such as metal ions, chemical compounds, proteins, cells, and whole micro-organisms [3].

Due to these target binding characteristics, the aptamers have been studied and uti-

lized in a range of fields. In particular, the aptamers are similar to antibodies but have advantages such as high chemical stability and productivity compared to the antibodies, so they have been studied as alternatives to the antibodies [4, 5]. For example, by using the aptamers that can bind to a specific cell such as a cancer cell, the aptamers are used as a drug delivery agent [6].

Also, the aptamers can be used to develop aptamer-based new drugs, and in 2005, Pfizer developed an neovascular age-related macular degeneration treatment called 'Macugen (pegaptanib sodium; Pfizer, New York, NY, USA),' which was first marketed with approval of the U.S. Food and Drug Administration [7]. In addition, many studies have been conducted to apply the aptamers to diagnostics [8–10], aptamer-based biosensors [11, 12], and imaging systems [13].

Even though the aptamers are applied in so many fields as above described, there is no noticeable movement in the nuclear power field to utilize the target-binding characteristics of the aptamers. Therefore, this study suggested that the aptamers could be applied to the treatment of liquid radioactive waste (hereinafter referred to as "liquid waste") generated in nuclear power plants by developing the aptamers that can bind to specific radioactive materials.

During the reactor operation, a large amount of the liquid waste is generated mainly by the coolant used to transfer thermal energy produced in nuclear fission [14]. Currently, in the Republic of Korea, the liquid waste generated from the nuclear power plants, before recycled or released into the environment, is controlled below the activity concentration limits in the effluent water through the treatment processes applying reverse osmosis, an ion exchange, and an evaporation method.

Liquid waste treatment technologies such as the reverse osmosis, the ion exchange, and the evaporation method cannot completely specify the target radionuclides to be removed from the liquid waste. Accordingly, various studies have been conducted to increase the selectivity of nuclides for the above technologies [15, 16]. On the other hand, since the aptamers can selectively remove the only specific atoms such as cobalt using the target binding characteristics, it is suggested that aptamers can be used as a removal technology of ionic radionuclides in the liquid waste.

In particular, during decommissioning the nuclear power plant, a large amount of the liquid waste is generated through the processes such as decontamination and dismantling of various structures contaminated by radioactive materials generated during the operation. Until now, adsorbents, reverse osmosis, ion exchange, and evaporation methods have been widely studied as removal technologies for radionuclides in liquid waste generated during the decommissioning of nuclear power plants, as well as after the Fukushima Daiichi nuclear accident [17–19]. By developing the aptamers with the binding capability for specific ionic radionuclides in the liquid waste, it could be applied to reducing a large amount of the liquid waste generated from decommissioning.

The aptamers consist of short oligonucleotides, a mixture of a single-strand and a double-strand as a structure analogous to hairpin structure of the single-stranded DNA (ssDNA) [20]. It is widely known that nucleic acids such as DNA can be damaged by radiation. Therefore, the radiological effect of the liquid waste on the aptamers should be evaluated quantitatively. In this paper, the radiological effect on the aptamers in the liquid waste was evaluated for the successful application of aptamers as a removal technology of the ionic radionuclides.

Materials and Methods

So far there has been no reported evaluation of the radiological effects on the aptamers during liquid waste treatment. For this reason, we have conducted an evaluation of the radiological effect on the aptamers that can be received during the liquid waste treatment by applying a radiological damage assessment method for the DNA. DNA is damaged directly by ionizing radiation or indirectly by free radicals produced from interactions of the ionizing radiation with water, which results in strand breaks and base damages [21]. Energy deposition in the cell nucleus has been shown to be related to the radiological effect of the cells, and various studies have been conducted to apply Monte Carlo simulation methods to the assessment of DNA damage caused by the energy deposition [22].

Monte Carlo N-Particle transport code version 6 (MCNP6) [23] and Monte Carlo damage simulation (MCDS) [24–26] were used as the evaluation tools for the DNA damage in this study. MCNP6 was used to evaluate the secondary electron spectrum and the absorbed dose in a medium using photons from gamma-generating nuclides, and MCDS was used to calculate the DNA damage by using the secondary electron spectrum and the absorbed dose as inputs [27].

As the results of the MCNP6 and MCDS calculations, the

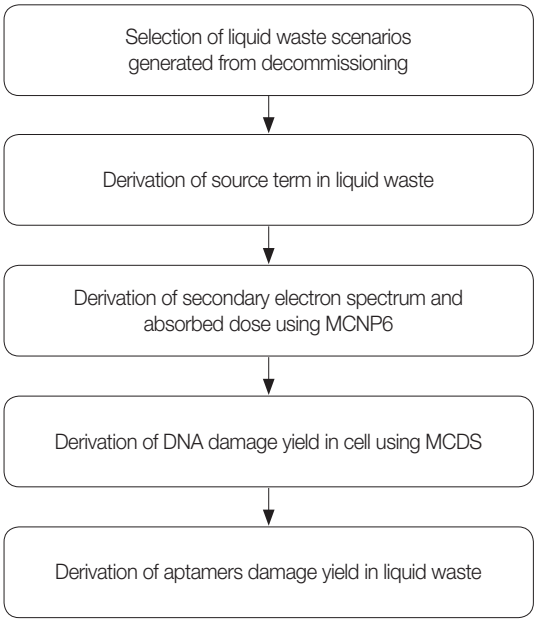

Fig. 1. The evaluation process of radiological effect on the aptamers. MCNP6, Monte Carlo N-Particle transport code version 6; MCDS, Monte Carlo damage simulation.

damage yields of the DNA in a cell by the secondary electrons and the free radicals generated by the photons emitted from the radionuclides were derived. However, the results were not completely corresponding to the damage on the aptamers in the liquid waste, given that those results were derived for the DNA in a cell rather than for the aptamers in the liquid waste. Therefore, to apply those calculated results of the MCNP6 and MCDS for the DNA in cells to the damage on the aptamers in the liquid waste, an additional process was employed to treat and correct the differences between the DNA in a cell and the aptamers in the liquid waste. The overall evaluation process of the radiological effect on the aptamers is shown in Fig. 1.

1. Liquid Waste Generation Scenarios Applied to the Evaluation

1) Scenario 1: Liquid waste generated by underwater cutting of reactor vessel internals

Reactor vessel internals (RVIs) are very large components that should be segmented into small parts for the decommissioning and dismantling of nuclear power plants, but they should be generally segmented underwater remotely because they are high-radioactive components difficult for operators to access [28, 29]. Some of the radioactive materials of the RVIs' residues after underwater cutting remain ionic in the water.

The specific activity of ^{60}Co in a barrel among RVIs was assumed to be about 10^8 Bq/g based on the activation calculation results [30]. Among the radionuclides of the RVIs, the ionic radioactive materials floating in the water after the cutting were evaluated as the following. The RVIs are supposed to be cut by 1 m thickness, of which a thickness of 0.5 cm sinks into the water. At this time, if the RVIs structure is symmetric and RVIs' weight is 100 tons, 500 kg of RVIs sinks into the water. Assuming that only 1% of RVIs' radioactivity exists as an ionic state in water, ^{60}Co source terms in liquid waste were derived as 500 Bq/mL when the size of the pool where the underwater cutting was performed was 10 m × 10 m × 10 m.

2) Scenario 2: Liquid waste generated by a wire saw cutting of bio-concrete

Bio-concrete surrounding the reactor core for the shielding can be radioactivated by the neutrons produced in the core. Since the bio-concrete is very bulky and thick, its radioactivity reduces as the distance from the reactor core increases [31]. Therefore, the bio-concrete should be cut for the decommissioning of a nuclear power plant according to its activity level.

A diamond wire saw is used to cut the bio-concrete, and the water is required for cooling the wire saw and for the dust control during cutting, which produces the secondary liquid waste [32]. According to the reference [31], the main gamma-ray source of the bio-concrete is ^{60}Co and europium (Eu) isotopes (^{152}Eu and ^{154}Eu). The summed specific activity of the ^{60}Co and the Eu isotopes in the bio-concrete is assumed to be about 10^5 Bq/g [30]. The ionic radioactive materials floating in the water by diamond wire saw cutting were evaluated by assuming as the following. The gamma energies of 1.274 MeV (35% yield) from ^{154}Eu and 1.408 MeV (21% yield) from ^{152}Eu are comparable to those from ^{60}Co having two gammas of 1.17 (100% yield) and 1.33 MeV (100% yield). We employed ^{60}Co, which is more conservative than Eu radionuclides considering both the energy and yields of europium and cobalt. In addition, by assuming that the water density is 1 g/mL and the ratio of water supplied for cutting to the cut concrete is 10 to 1, ^{60}Co source terms in liquid waste were derived as 10,000 Bq/mL.

3) Scenario 3: Liquid waste generated by high-pressure water jetting used for surface contamination

The high-pressure water jetting technique is effective in surface decontamination [33]. Secondary liquid waste is gen-

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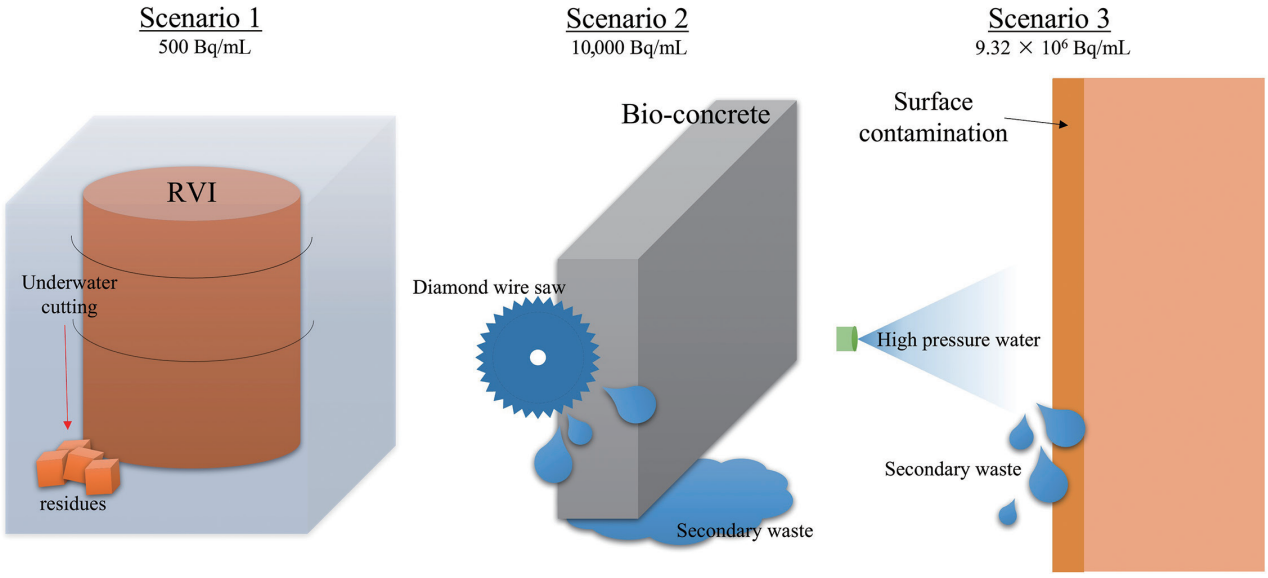

Fig. 2. Summary of the liquid waste generation scenarios. RVI, reactor vessel internal.

erated by this technique of using water for decontamination [34]. According to the radiological site characterization report of the United States [35], the maximum level of surface decontamination is about 5.03×10^6 Bq/cm^2. In addition, since the secondary liquid waste generation rate is about 5.4 L per 1 m^2 of the surface decontaminated by high-pressure water washing [34], the specific activity of about 9.32×10^6 Bq/mL may be contained in the liquid waste. By assuming that all surface contamination sources were ^{60}Co, ^{60}Co source terms in the liquid waste were derived as 9.32×10^6 Bq/mL. All liquid waste generation scenarios described in this section are summarized in Fig. 2.

2. DNA Damage Evaluation Using MCNP6 and MCDS

MCNP6 was used to evaluate the secondary electron spectrum and the absorbed dose in the medium due to the source terms for each liquid waste generation scenario. The evaluation was conducted with an assumption that the aptamers and the ^{60}Co sources were distributed uniformly within the 500 mL column corresponding to the size of a trial product. A cell flux tally (F4), which calculates flux averaged over a cell, of MCNP6 was used to calculate the secondary electron spectrum. Also, an energy deposition tally (F6), which calculates energy deposition averaged over a cell, was used to evaluate the absorbed dose. The photon cross-section library was applied with mcplib84 based on final release of version VI of the Evaluated Nuclear Data File (ENDF/B-VI).

MCDS was used to evaluate the DNA damage by using the secondary electron spectrum and the absorbed dose resulting from the MCNP6 calculations. MCDS results were drawn as single-strand break, double-strand break, and base damage.

3. Binding Experiments between Aptamer-Bead Complex and Ionic Nuclides

Binding experiments between the aptamer-bead complex and ionic nuclides were conducted to indirectly verify the results derived by MCNP6 and MCDS calculations. Through these experiments, we have tried to estimate the possibility and extent of damage that the aptamer itself would receive by the radioactivity of nuclides by comparison assessment for amounts of removed nuclides of both stable isotope ^{59}Co (non-radioactive) and the radioisotope ^{60}Co by the same aptamers.

The cobalt-specific aptamer LoFA-C1 (biotin–5′–GGTAATACGACTCACTATAGGGAGATACCAGCTTATTCAATTTTGCTTGACGAGCCTGTACGTGGTTCCCTCCAGATGGTCGAGATTGCACTTACTATCT–3′) used in the experiments was selected as a specific aptamer for Co^{2+} by systematic evolution of ligands by exponential enrichment (SELEX) and synthesized by IDT (Integrated DNA Technologies, Inc., Coralville, IA, USA). The aptamer-bead complex was prepared by binding the aptamer to the streptavidin beads (Pierce Streptavidin Agarose; Thermo Scientific, Dreieich, Germany). Aptamer amount used in each experiment was 1.2 nmol (number of molecules: 7.2×10^{14}).

A solution of ^{59}Co is prepared from cobalt(II) chloride made

Table 1. Damage Yields of DNA in a Cell by ^{60}Co Radioactivity from Liquid Waste Generation Scenarios

Scenario	Damage yields (bp^{-1})			
	SSB	DSB	BD	Total
Liquid waste generated by underwater cutting of RVI	1.36×10^{-16}	3.10×10^{-15}	7.00×10^{-15}	1.02×10^{-14}
Liquid waste generated by wire saw cutting of bio-concrete	2.73×10^{-15}	6.21×10^{-14}	1.40×10^{-13}	2.05×10^{-13}
Liquid waste generated by high-pressure water jetting used for surface contamination	2.55×10^{-12}	5.79×10^{-11}	1.31×10^{-10}	1.91×10^{-10}

bp, base-pair; SSB, single-strand break; DSB, double-strand break; BD, base damage; RVI, reactor vessel internal.

by Sigma-Aldrich (449776-5G; Burlington, MA, USA). To measure the concentration of the ^{59}Co in the solution after the removal of ^{59}Co by the aptamer-bead complex, plasma-optical emission spectroscopy was used. Also, we prepared a solution of ^{60}Co by diluting ^{60}Co stock solution made by Eckert & Ziegler (Atlanta, GA, USA). To measure the concentration of the ^{60}Co in the solution after the removal of ^{60}Co by the aptamer-bead complex, the radioactivity of the ^{60}Co was measured by the high-purity germanium detector (Mirion Technologies [Canberra] Inc., Meriden, CT, USA).

The removal rates of nuclides (^{59}Co and ^{60}Co) in both solutions were derived by measuring the amounts of nuclides before and after passing through the aptamer-bead complex as the relative ratios, and all experiments were repeated three times independently.

Results and Discussion

1. DNA Damage in a Cell

To evaluate the radiological effects on the aptamers in the liquid waste, DNA damage yields of the irradiated cells were evaluated by using MCNP6 and MCDS. The assumed ^{60}Co radioactivity in the liquid waste was used as an initial radioactive source for the DNA damage evaluation. Table 1 shows the damage yields of DNA in a cell by the ^{60}Co radioactivity in the liquid waste. This result shows that the damage yields of DNA in a cell are directly proportional to the assumed ^{60}Co activity concentrations in the liquid waste streams.

2. Aptamers Damage in a Cell

DNA has a double-strand structure while the aptamer consists of a single-strand. This structural difference can lead to the difference in damage yields with the same radioactivity. To apply the evaluated radiological effect on the DNA in the cell calculated by MCNP6 and MCDS to the radiological effect on the aptamers in the liquid waste, a correction factor with respect to the DNA structure was used based upon the previous research [36], where the damage ratio between ssDNA and double-stranded DNA (dsDNA) was evaluated to be 3.6 with the same irradiation. Therefore, the effect magnitude by a factor of 4 was conservatively multiplied to the DNA damage yields shown in Table 1.

3. Aptamers Damage in Liquid Waste

To correct the evaluated radiological effect on the aptamers in cells to the effect on the aptamers in the liquid waste, additional study was conducted. The most abundant substance in cells is water which accounts for about 70% of the cell's weight, but other substances such as inorganic ions and organic molecules also exist [37]. Therefore, the following evaluation was performed to consider the difference in the water content and in the chemical composition between the cell and the liquid waste.

The difference in the radiological effect due to the different chemical composition between the cell and the liquid waste was evaluated through two simple MCNP6 models. One of the models used a cytoplasm, which is the remainder of the cell except for the cell nucleus and accounts for most of the cell volume, and the other model used liquid waste as a medium. The energy deposition rates were compared between two models, one filled with liquid waste in the medium and the other filled with chemical composition of cytoplasm in the medium, with same geometry and same radioactive source terms.

F6 tally was used to calculate the energy deposition in the medium. Absorbed dose of 9.56×10^{8} and 1.25×10^{9} MeV/g was evaluated for the cytoplasm and the liquid waste, respectively, confirming that about 1.3 times higher energy was deposited in the liquid waste. Since DNA is damaged indirectly by the radicals generated by water radiolysis, the energy deposition in the water is important from the viewpoint of DNA damage. Accordingly, the DNA damage is assumed to be proportional to the amount of energy deposited in the water. It is also supposed that only 70% (6.69×10^{8} MeV/g) of

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Table 2. Damage Yields of the Aptamer (Based on 100 bp) in the Liquid Waste by ^{60}Co Radioactivity from Liquid Waste Generation Scenarios

Scenario	Total damage yields of 100 bp aptamer	
	Aptamer in a cell	Aptamer in the liquid waste
Liquid waste generated by underwater cutting of RVI	4.10×10^{-12}	8.20×10^{-12}
Liquid waste generated by wire saw cutting of bio-concrete	8.19×10^{-11}	1.64×10^{-10}
Liquid waste generated by high-pressure water jetting used for surface contamination	7.65×10^{-8}	1.53×10^{-7}

bp, base-pair; RVI, reactor vessel internal.

Table 3. Absorbed Dose by ^{60}Co Radioactivity from Liquid Waste Generation Scenarios

Scenario	Absorbed dose (Gy)
Liquid waste generated by underwater cutting of RVI	1.64×10^{-8}
Liquid waste generated by wire saw cutting of bio-concrete	3.28×10^{-7}
Liquid waste generated by high-pressure water jetting used for surface contamination	3.06×10^{-4}

RVI, reactor vessel internal.

the energy deposited in the cytoplasm (9.56×10^{8} MeV/g) is deposited in water because water accounts for about 70% of the cell's weight.

Therefore, it was evaluated that the aptamers could be damaged up to twice as much in the liquid waste as in the cell according to the energy deposition in the water, and for this reason, the effect magnitude by a factor of 2 was multiplied to the DNA damage yields shown in Table 1. As a result, the damage yields of the aptamer in liquid waste were derived as shown in Table 2 by applying the effect magnitude from the structural difference between DNA and aptamer as well as the difference in the water contents between a cell and liquid waste. The aptamer damage yields were calculated per 100 base-pair (bp) since the number of bp was 100 for the template DNA used in this work.

4. Aptamers Damage per Absorbed Dose

The damage yields of aptamers for each liquid waste generation scenario can be expressed as a function of absorbed dose, and the absorbed dose evaluated for each liquid waste generation scenario is shown in Table 3. Fig. 3 shows the aptamer damage yields according to the absorbed dose, and it was confirmed that the radiological damage on aptamers increase in proportion to the absorbed dose. As a result, damage yield of about 5.00×10^{-4} occurs to 100 bp aptamer per radiation dose of 1 Gy. These results suggest that only very small fraction of significant number of the aptamers will be damaged by the radioactivity of the liquid waste.

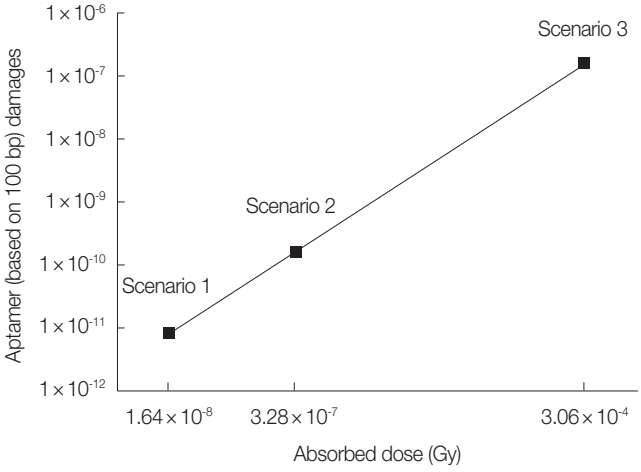

Fig. 3. Aptamer damages with absorbed doses of liquid waste generation scenarios. bp, base-pair.

5. Evaluation Results of Removal Rates of Nuclides (^{59}Co/^{60}Co) by the Binding Experiments

The above derived aptamer damages (Table 2) are resulted by the consideration of the structural difference between DNA and aptamer as well as the difference in the water contents between a cell and liquid waste. Nevertheless, there may be additional differences in the real damages on the aptamers in the liquid waste due to the factors that are difficult to quantify, such as radical scavenging effects in a cell. Since the quantitative difference caused by these additional factors is not known yet, some experiments were conducted to verify the aptamer damages indirectly. As the experiment results show, the ^{59}Co and ^{60}Co were removed by $48.99\pm2.93\%$ and $49.22\pm4.68\%$, respectively, through the aptamer-bead complex.

These results demonstrate that the cobalt-specific aptamer LoFA-C1 could remove the two isotopes of ^{59}Co and ^{60}Co with very similar fraction of about 50% regardless of the presence of radioactivity. From these experimental results, it was evaluated that the damage on the binding characteristics of the aptamers by the radiation from the target nuclides such as ^{60}Co would be negligible.

Conclusion

Radiation damage of aptamers by the radioactive material in the liquid waste was evaluated in this work, so that aptamers could be used as a removal technology of radionuclides. The damage yields were evaluated to be about 5.00×10^{-4} for 100 bp aptamer per radiation dose of 1 Gy from the MCNP6 and MCDS calculation and application of a correction factor, which considers the structural difference between a DNA and aptamer besides the difference in the water contents between a cell and liquid waste. The evaluation results suggest that only very small fraction of a significant number of the aptamers would be damaged by the radioactive materials in the liquid waste. These analytical results were verified indirectly by the experiment that bound the stable isotope ^{59}Co and the radioisotope ^{60}Co to the same aptamer-bead complex, respectively, and then evaluated the nuclide removal fraction for each isotope. Furthermore, in order to practically apply the aptamers to the removal of radionuclides in the liquid waste generated from the operation and decommissioning of nuclear power plants, more study would be required to discover the factors that could damage the aptamers besides the radiological effects. Finally, it is suggested that if there are no additional damage factors except for the radiation in the liquid waste, the aptamers could be considered as a removal technology of radionuclides in the liquid waste.

Conflict of Interest

No potential conflict of interest relevant of RADCORE, Co., Ltd., to this article was reported.

Acknowledgements

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Ethical Statement

According to Bioethics and Safety Act in the Republic of Korea, an approval from the ethics committee is not required for this study.

Author Contribution

Conceptualization: Yun Y, Kim SH, Kim S. Methodology: Lee M, Cha G, Kim D, Jang D, Lee S. Project administration: Kim S. Writing – original draft: Lee M. Writing – review & editing: Yun M, Kim S, Kim SH. Investigation: Lee M, Cha G, Kim D, Jang D, Lee S. Supervision: Kim S. Validation: Kim H. Approval of final manuscript: all authors.

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