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Electric Field Induced Super-cooling System for Long Term Dry-aged Beef Loin

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Abstract This study investigates the utilization of an electric-field-induced super cooling system in long-term dry aging of beef loin. Analyzed quality properties of dryaged beef loin applied with electric field refrigeration (EFR) versus commercial refrigeration (CR). Quality properties was including aging loss, pH, water holding capacity (WHC), cooking loss, color, warner-bractzler shear force (WBSF), total plate count (TPC), and thiobarbituric acid reactive substances (TBARS). Aging loss of wk 1 EFR was significantly lower than CR ($p<0.05$). pH of EFR was slow change tendency compared CR. WHC of both aging methods were higher with increase in aging duration. Cooking loss of wk 1, 2, 4, and 10 EFR were significantly lower than CR ($p<0.05$). Lightness and redness of EFR was slow change tendency compared CR. However, yellowness of EFR was increased until wk 2, 3, and significantly decreased at wk 10 $(p<0.05)$, but vellowness of CR was decreased until wk 3 and significantly increased with an increasing aging weeks ($p<0.05$). Both aging methods of WBSF was decreased with increase in aging weeks; however, wk 10 of CR was significantly lower than EFR (p <0.05). TPC after wk 3 EFR groups were significantly lower than CR groups (p <0.05), and TBARS of EFR groups were significantly lower than CR $(p<0.05)$. The present results show that application of the EFR system for dry aging beef loin can extends its shelf life and induce changes of several aging properties in similar to commercial aging.

Keywords dry aging, beef loin, electric field refrigeration, aging properties

Introduction

Recently, aged beef-produced through the aging of beef loin for a specific period-has raised consumers' interest (Boleman et al., 1997). Aged meat is produced through wet aging or dry aging, both of which lead to a more tenderized texture of meat compared to that of fresh meat. This texture originates from meat proteases that induce myofibrillar fragmentation during the aging process following the animal's slaughter (Carlson et al., 2017; Quali et al., 2013; Smith et al., 2008).

The dry-aging of beef exposes the unpacked meat to air in a dry-aging refrigerator for 28–35 days (Perry 2012; Smith et al., 2008). During this process, the surface of the

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beef dries, and microorganisms proliferate. Therefore, long-term aging that lasts six or more weeks is prohibited considering food safety (Dashdorj et al., 2016; Laster et al., 2008; Smith et al., 2008;). Nevertheless, the unique flavor and palatability of dry-aged beef compared to those of fresh beef or wet-aged beef (Campbell et al., 2001) introduce the need for a stable dryaging technology that minimizes changes in the quality properties of the beef during the aging process.

The main causes of meat degradation are microbial metabolites (toxic substances) due to microbial growth, and the production of peroxides and aldehydes due to lipid oxidation, which reduce the values of meat concerning hygienic safety (Frankel, 1984). A low-temperature environment that inhibits microbial growth, lipid oxidation, and protein deterioration prevents such meat contamination (Dashdorj et al., 2016; Gill and Newton, 1978). In general, refrigeration occurs at 0℃– 15℃ and freezing at −18℃ or below. However, the microbial inhibitory capability of refrigeration is not as effective over long periods of storage. In addition, frozen meat, despite the possible long-term storage, may undergo quality deterioration due to freezing and thawing, such as a decrease in its water holding capacity, and drip or texture loss.

An electric field super-cooling system (refrigerator) induces a super-cooled state of the meat from −3℃ to −1℃. The system operates by maintaining the vibration of water molecules and preventing them from freezing with the use of a steady electric-field current, simultaneously circulating a cooling air current with an air blower (Iwasaka et al., 2011). Microbial contamination and lipid oxidation of meat are relatively suppressed between −3℃ and −1℃, in contrast with those at 4℃ (Stonehouse and Evans, 2015), so long-term storage of meat in an unfrozen state is possible.

A soft texture and unique flavor are critical factors in the quality of dry-aged beef. During the dry-aging process, cathepsin and calpain that mediate autoproteolysis, induce myofibrillar fragmentation and, consequently, soft texture (Spanier et al., 1997). Thus, the aging of beef increases the levels of several monosaccharides and free amino acids that influence the flavor and palatability, whereby flavor potentiators are produced and enhance the flavor of meat (Mullen et al., 2000). The activities of such proteases are retained even in an electric field (Bhat et al., 2019b) or in a super-cooled state between −3℃ and −1℃ (Bahuaud et al., 2008), which suggests a potential application of an electric field super-cooling system to the production of dry-aged beef.

The present study utilized an electric field super-cooling system in the long-term dry-aging of beef loin. After dry-aging different groups of beef loin under commercial dry-aging conditions for the same period, the quality properties of the beef were analyzed to determine the suitability of the electric field super-cooling system for the production of dry-aged beef.

Materials and Methods

Preparation of dry-aged beef loin

Beef loin (*Musculus longissimus dorsi*; Refrigerated for 24 h after slaughter; I home meat, Seoul, Korea) was used for dry aging. Seven samples of beef loin were subjected to dry aging with an electric field refrigeration system (EFR; air velocity, 5±2 m/s; temperature, –1℃; ARD-090RM-F, Mars, Fukushima, Japan) at different aging durations of 0 (fresh meat), 1, 2, 3, 4, 7, and 10 wk. For comparison, beef loin (Refrigerated for 24 h after slaughter; I home meat) was dry aged in a commercial refrigerator (CR; air velocity, 5±3 m/s, temperature, 4℃; CA-H17DZ, LG, Seoul, Korea). After aging, the sample surfaces were cut and thermally processed in a 70℃ chamber (10.10ESI/SK, Alto Shaam, Menomonee Falls, WI, USA) for 120 min, followed by cooling at 10℃ for 1 h.

Aging loss

The fresh beef loin (initial weight) aging processed at EFR and CR for different aging durations (1, 2, 3, 4, 7, and 10 wk).

After aging, aging beef loin were weighed (aging weight) and percentage aging loss was determined using the following formula.

$$
ext{Dry aging loss } (\%) = \frac{\text{Again } weight (g)}{\text{Initial weight } (g)} \times 100
$$

pH

For measuring pH, each 4 g of a sample was mixed with 16 mL distilled water. After sample preparation, in an ultra-turrax homogenizer (HMZ-20DN, Poolim Tech, Seoul, Korea) at 10,923×g for 1 min. The pH was measured using a pH meter (Model S220, Mettler-Toledo, Schwerzenbach, Switzerland).

Water holding capacity (WHC)

WHC of samples was measured by filter paper press method (Grau and Hamm, 1953) with slight modification. Except fresh meat sample, the dry aging meat of dried surfaced were trimmed off and each 300 mg sample was placed at filter paper (Whatman No. 2, GE Healthcare, Chicago, IL, USA) and using a filter-press device and compressed for 3 min. The WHC calculated using the following methods by measuring the meat area and the total area.

WHC (%) =
$$
\frac{\text{Meat area (mm}^2)}{\text{Total area (mm}^2)} \times 100
$$

Cooking loss

Uncooked samples (weighed before cooking) were heat-processed in a chamber (70℃ for 120 min), such that the core temperature approached 70±1℃. After cooling at 10℃ for 1 h, cooked samples were weighed (after cooking) and percentage cooking loss was determined using the following formula.

$$
Looking loss (\%) = \frac{Weight before cooking - Weight after cooking (g)}{Weight before cooking (g)} \times 100
$$

Color

The inner surface of the uncooked samples was measured using a colorimeter (CR-10, Minolta, Tokyo, Japan) for lightness (L^*) , redness (a*), yellowness (b*) A white standard plate with a CIE L^* of +97.83, a* of -0.43, and b* of +1.98 was used for reference.

Warner-Bractzler shear force (WBSF)

The shear force of each sample was assessed by cutting samples into $1.3 \times 3.0 \times 1.3$ cm³ blocks and analyzed using a V-blade attached to a Texture Analyzer (test speed, 21.0 mm/s; head speed, 21.0 mm/s; distance, 22.0 mm; force, 5.6 N; TA 1, Ametek, Largo, FL, USA). Measured values are expressed in Newton (N).

Total plate count (TPC)

Microbial growth during aging was evaluated via a total viable count. Ninety milliliters of a peptone solution was added

into 10 g samples and homogenized using a bag mixer, and serially diluted samples were plated onto plate count agar (Difco potato agar, Becton, Dickinson and Company, Franklin Lakes, NJ, USA), followed by incubation at 36℃ for 24 h. TPC was determined from the mean of three assessments and expressed as Log CFU/g values.

Thiobarbituric acid reactive substances (TBARS)

TBARS levels in samples were measured using the method of Tarladgis et al. (1960). Upon the formation of malondialdehyde and 2-thiobarbituric acid resulting from lipid peroxidation, and the absorbance of samples was measured at 538 nm, using a multi-mode microplate reader (Spectra Max iD3, Molecular Devices, San Jose, CA, USA). The TBARS value was expressed as mg malondialdehyde/kg sample.

Statistical analysis

Experimental were assessed after a minimum of three repeated trials. Statistical analysis of variance were performed on all variables measured using the General Linear Model (GLM) procedure of the SAS software program (SAS version 9.3 for window: SAS Institute, Cary, NC, USA), ANOVA and Duncan's multiple range were performed for verifying significances of differences ($p<0.05$). And the results are presented as mean \pm SD values.

Results and Discussion

Aging loss

During the aging process for dry-aged beef, the moisture content of the meat gets evaporated upon exposure to air, leading to moisture loss. There is also trimming loss by trimming the crust that has formed on the surface of the meat by dehydration and microbial growth following aging (Parrish et al., 1991). The aging loss-the weight of final dry-aged beef loin after trimming over the weight of fresh meat prior to aging is given in Table 1; both EFR and CR aging processes led to an increase in aging loss with a prolonged aging period. The difference in aging loss between aging methods was not significant across all groups except wk 1 of aging. At wk 1, the EFR aging group displayed a significantly lower value than the CR aging group (p<0.05). Similarly, Laster et al. (2008) reported an increase in trimming loss with an increasing aging period when beef loin (ribeye) was dry-aged for 14–35 days. Smith et al. (2008) also reported a decrease in the final yield of dry-aged beef loin (short loin) when increasing the dry-aging period, while considering various conditions such as surface contraction, crust, and fat removal.

All values are mean±SD.

 A –E Means on the same row with different numbers are significantly different (p<0.05).

a,b Means on the same column with different numbers are significantly different (p <0.05).

EFR, electric field refrigeration; CR, commercial refrigeration.

pH, WHC, and cooking loss

Compared to non-aged beef, the beef in the early aging groups (EFR: wk 2, 3; CR: wk 1) displayed a steep increase in pH $(p<0.05)$, followed by a slight decrease (Table 2). The EFR aging groups displayed a significant increase in pH up to 6.72 until wk 2 (p<0.05), while a significant decrease was observed in the groups at wk 3, 4 and wk 7, 10 of aging (p<0.05). The CR aging groups showed a significant increase in pH to 6.75 at wk 1 ($p \le 0.05$), followed by a significant decrease with the increasing aging period ($p<0.05$). The CR groups showed significantly higher pH values at wk 1 ($p<0.05$), whereas at all subsequent weeks, significantly higher values were observed for the EFR groups ($p<0.05$). This behavior indicated a slower pH change in the EFR aging groups than in the CR groups. Juárez et al. (2009) reported that upon pork loin aging, the aging group at day 14 had higher pH than the group at day 2, and Iida et al. (2016) reported significantly lower pH values for dryaged beef after aging for 11, 50, or 60 days, compared to those of beef aging for 4 days, when the *Longissimus thoracis* muscle of Japanese black cattle was dry-aged for 60 days. In addition, when pork loin was aged under EFR (Park et al., 2019), the increase in pH continued until wk 3, 4 of aging before a decrease, which corroborated the results in this study.

Among the quality properties of dry-aged beef is WHC. This property changes because the meat undergoes autoproteolysis upon dry aging, and during that period, the Z line in the meat is cleaved by proteases, such as cathepsin and calpain, which increases the surface area for moisture retention in the meat (Huff-Lonergan et al., 1996), while water evaporates and moisture concentrates in the crust (Dashdorj et al., 2016). In this study, both EFR and CR aging groups exhibited an increase in WHC with the increasing aging period (Table 2); nonetheless, the difference in WHC between aging methods across all groups was insignificant. Campbell et al. (2001) reported that the juiciness was higher in the 14- or 21-day dry-aged group than that in the 7-day group or the control group that was not dry-aged. This observation led to the conclusion that the high WHC of aged beef influenced sensory characteristics compared to that of non-aged beef.

The cooking loss with respect to the aging method and period is given in Table 2. All EFR aging groups exhibited significantly lower cooking loss values than non-aging groups $(p<0.05)$, while an increased cooking loss was detected following wk 2 of aging. All CR aging groups exhibited significantly lower values than non-aging groups (p<0.05), while a significantly higher value was measured for wk 4, 10 of aging than that of wk 3 ($p<0.05$). The EFR aging groups allowed significantly lower cooking loss at wk 1, 2, 4, and 10 ($p<0.05$) than the CR groups. The change in cooking loss according to the aging period has been previously investigated: Ellis et al. (1997) reported a lower cooking loss of pork in the longer aging

All values are mean±SD.

 A –G Means on the same row with different numbers are significantly different (p<0.05).

a,b Means on the same column with different numbers are significantly different ($p<0.05$).

EFR, electric field refrigeration; CR, commercial refrigeration; WHC, water holding capacity.

period group (16 days) than that in the shorter one; Choe and Kim (2017) reported on dry-aged beef loin where the cooking yield was higher after 14 days of dry-aging than that in the non-aged beef loin; Iida et al. (2016) reported on the dry-aging of beef (Tajima Japanese black cattle), where the cooking loss was significantly lower after 11, 20, and 30 days of aging, compared to 4 days of aging, while the 40- and 50-day aging groups did not differ significantly from the 4-day aging group, a trend similar to the one observed in this study. Regarding the effect of the electric field treatment on cooking loss, Bhat et al. (2019a) reported that when *biceps femoris* was used in beef aging under pulsed electric field (PEF) treatment, the cooking loss did not differ between PEF and non-PEF groups. Based on that, an appropriate electric field does not impart any damage to the meat.

Color

During the same aging period, different aging methods induced color variations (Table 3). Lightness measurements showed that the lightness of the EFR groups did not vary relative to the aging period, whereas the CR group at wk 10 showed significantly lower lightness than the groups at other aging weeks ($p<0.05$). Within the same aging period, the CR groups at wk 1 and wk 10 showed significantly higher values than the EFR ones ($p<0.05$). The EFR groups had a redness increase tendency with an increasing aging period, reporting a significantly higher value of 9.46 at wk 7 than at wk 0-2 ($p<0.05$). In contrast, the CR groups exhibited a redness increase of up to 9.40 until wk 4. Yellowness in the EFR groups increased until wk 2, 3, followed by a significant decrease at wk 10 ($p<0.05$), whereas in the CR groups, it decreased until wk 3 to the significantly lower value of 3.20 than that in the non-aging groups $(p<0.05)$. This process was followed by a significant increase after wk 4 until wk 10 ($p<0.05$). During the same aging period, the yellowness difference between aging methods was as follows: the EFR groups showed significantly lower values than the CR groups at wk 1 ($p<0.05$) but significantly higher values in all subsequent aging weeks (p<0.05). In line with our results, several previous studies have reported a decrease in lightness and an increase in redness and yellowness with a prolonged aging period upon beef loin aging (Choe and Kim, 2017; Wicklund et al., 2005). Park et al. (2019) aged pork loin under EFR and reported that lightness decreased with increasing aging period, while yellowness increased until wk 2 of aging and then decreased, a result similar to that of this study. However, redness also decreased after wk 2, which partially contradicted the result in this study. Choe and Kim (2017) attributed such color change to the decrease in the moisture content during dry-aging, which relatively increased the content

Table 3. Instrumental color of beef loin with different dry aging methods and periods

Trait	Dry aging methods	Dry aging time (wk)								
		θ		2	3	$\overline{4}$		10		
L^*	EFR	37.10 ± 2.33	38.06 ± 0.29 ^a	37.63 ± 0.68	37.70 ± 2.34	37.87 ± 0.86	37.40 ± 0.80	37.40 ± 0.43 ^a		
	CR	37.10 ± 2.33 ^A	35.84 ± 1.50 ^{Ab}	$36.42\pm0.80^{\rm A}$	35.80 ± 0.97 ^A	35.60 ± 1.47 ^A	36.16 ± 1.93 ^A	31.84 ± 0.33 ^{Bb}		
a^*	EFR	$7.17 \pm 0.50^{\circ}$	7.00 ± 0.16 ^{Cb}	$7.94 \pm 0.21^{\rm BC}$	8.50 ± 0.34 ^{AB}	8.82 ± 1.78 ^{AB}	9.46 ± 0.57 ^A	9.37 ± 0.25 ^A		
	CR	$7.17\pm0.50B$	8.10 ± 0.10^{ABa}	8.29 ± 0.46 ^{AB}	8.50 ± 0.10 ^{AB}	$9.40 \pm 1.91^{\text{A}}$	8.48 ± 0.98 ^{AB}	8.17 ± 0.35 ^{AB}		
b^*	EFR	5.00 ± 0.64 ^{BC}	$3.83 \pm 0.12^{\text{CDb}}$	6.86 ± 1.38 ^{Aa}	6.88 ± 1.63 ^{Aa}	6.63 ± 0.99 ^{ABa}	6.52 ± 1.51 ^{ABa}	3.02 ± 0.04^{Da}		
	CR	5.00 ± 0.64 ^{AB}	4.76 ± 0.36 ^{BCa}	4.62 ± 0.26 ^{BCb}	3.20 ± 0.35^{Db}	4.20 ± 0.66 ^{Cb}	4.62 ± 0.72 ^{BCb}	5.52 ± 0.19 ^{Ab}		

All values are mean±SD.

 $A-D$ Means on the same row with different numbers are significantly different (p<0.05).

a,b Means on the same column with different numbers are significantly different ($p<0.05$).

EFR, electric field refrigeration; CR, commercial refrigeration.

of the meat pigment hemoglobin in aged beef compared to that in non-aged beef.

Waner-Bratzler shear force (WBSF)

Rigor after slaughter is followed by the aging of meat. During this process, Ca^{2+} is released from cell organelles and activates proteases that ultimately destroy the Z line of the muscle (Bhat et al., 2018), whereby the meat texture is tenderized and acquires its characteristic soft texture. The WBSF of both EFR and CR aging groups displayed a tendency to decrease with increasing aging period (Table 4). The WBSF in the EFR groups exhibited a significant difference at wk 4 compared to that at wk 0 (p<0.05), while that in the CR groups showed a significantly lower value at wk 10 than at wk 0 (p<0.05). The difference between aging methods within the same aging week was not significant at wk 0-7, whereas at wk 10 of aging, the CR group exhibited a significantly lower WBSF than the EFR group $(p<0.05)$. Such a decrease in shear force is one of the most distinguishing characteristics of aged beef. Campbell et al. (2001) reported that the group with a long aging period (21 days) showed lower shear force than the groups with relatively short aging periods (7 days and 14 days). Choe and Kim (2017) reported on beef loin that the 14-day dry-aged group had a lower shear force than the non-aged beef. Smith et al. (2008) reported that the shear force of beef loin (short loin) tended to decrease upon aging.

Total plate count (TPC), Thiobarbituric acid reactive substances (TBARS)

The exposure of dry-aged beef to air through dry-aging results in the evaporation of moisture from the meat surface, which lowers its water activity; this promotes the proliferation of yeasts because they require low water activity (Ahnström et al., 2006). In addition, various microorganisms grow on the surface of dry-aged beef during the aging period. Among them is *Thamnidium,* which enhances the aging flavor, taste, and tenderness. However, the growth of other microorganisms, such as *Rhizopus*, *Mucor genera*, and *Penicillium* is undesired, since they harm the human body (PrimeSafe, 2017). TPC of beef loin exhibited an increasing tendency over the increasing dry-aging period across all EFR and CR aging groups (Table 5). The TPC per aging period between aging methods did not exhibit a significant difference at wk 1, 2, while at wk 3-10, the EFR groups showed significantly lower values than the CR groups ($p<0.05$). The meat degrades when TPC exceeds 7 Log CFU/g (Lambert et al., 1991). The TPCs of the aging groups at wk 7, 10 were 8.86 Log CFU/g and 9.14 Log CFU/g, respectively, which exceed the meat degradation criteria. Low temperatures can inhibit microbial growth on meat to some extent (Gill and Newton, 1978), so the relatively lower temperature used in EFR aging compared to that in CR aging could have the same effect. Lins et al. (2017) also reported that, concerning the refrigeration (4℃) of ground beef, the group treated under a pulsed magnetic field showed lower counts of total aerobic bacteria than the untreated group. This result may be due to the effect of pulsed magnetic field on microbial growth and migration via DNA molecular changes, which inhibits the proliferation of the

All values are mean±SD.

 $A-D$ Means on the same row with different numbers are significantly different ($p<0.05$).

WBSF, warner-bractzler shear force; EFR, electric field refrigeration; CR, commercial refrigeration.

Trait	Dry aging methods	Dry aging time (wk)								
		θ				4		10		
TPC	EFR	$2.18\pm0.14E$			2.68 ± 0.12^{DE} 3.42 ± 0.43^{CE} 3.89 ± 0.97^{BCb}	4.45 ± 0.48 BCb	4.72 ± 0.92 ^{Bb}	6.85 ± 0.06 ^{Ab}		
(Log CFU/g)	CR.	2.18 ± 0.14^{D}	3.17 ± 0.34^{D}	$4.29 \pm 1.29^{\circ}$	5.88 ± 0.06 ^{Ba}	6.91 \pm 0.19 ^{Ba}	8.86 ± 0.05 ^{Aa} 9.14 \pm 0.61 ^{Aa}			
TBARS	EFR	$0.21 \pm 0.05^{\rm D}$		0.20 ± 0.01^{Db} 0.22 ± 0.02^{Db} $0.23\pm.02^{Db}$		0.34 ± 0.01 ^{Cb}	$0.49 \pm 0.01^\mathrm{Bb}$	0.72 ± 0.01 ^{Ab}		
(mg MDA/kg meat)	CR.	$0.21 \pm 0.05^{\circ}$	0.21 ± 0.03 ^{EFa} 0.25 ± 0.01 ^{Ea}		0.30 ± 0.02^{Da}	1.02 ± 0.01 ^{Ca}	1.24 ± 0.02 ^{Ba}	1.64 ± 0.01 ^{Aa}		

Table 5. TPC and TBARS levels of beef loin with different dry aging methods and periods

All values are mean±SD.

 $A-F$ Means on the same row with different numbers are significantly different (p<0.05).

a,b Means on the same column with different numbers are significantly different ($p<0.05$).

TPC, total plate count; TBARS, thiobarbituric acid reactive substances; EFR, electric field refrigeration; CR, commercial refrigeration.

microorganisms that grow on meat (Barbosa-Cánovas et al., 1998; Hofmann 1985).

The levels of TBARS is an important indicator of meat degradation (Frankel, 1984; Gray et al., 1996). In this study, the amount of TBARS increased with the aging period, similar to TPC (Table 5). EFR led to significantly lower TBARS values than CR (p<0.05). Lins et al. (2017) reported that TBARS did not differ significantly when an electric field was used for the refrigeration (4℃) of ground beef, which contradicted the respective result in this study; however, lipid oxidation is generally promoted at higher temperatures, and as TBARS is influenced by temperature (Jakobsen and Bertlsen, 2000), the TBARS measured at a general aging temperature (4℃) was higher than that at an electric-field dry-aging temperature (−1℃). In conclusion, the CR aging group at wk 4 closely reached the criteria for meat spoilage; therefore, dry-aging by CR is not suitable for long-term dry-aging (more than 4 weeks) concerning hygienic safety. Conversely, EFR is suitable for long-term dry-aging because it prevents spoilage by keeping water molecule unfrozen below the freezing point (0℃).

Conclusion

EFR system application for beef loin dry aging was similar change tendency in aging loss, WHC and WBSF compared with commercial aging method. In case of cooking loss, several aging weeks $(1, 2, 4,$ and (10 wk) of EFR were significantly lower than CR (p<0.05). TPC and TBARS change stability related of hygienic safety were excellent of using EFR system. Therefore, EFR system appropriate for beef loin dry aging, also capable for long-term dry aging.

Conflicts of Interest

The authors declare no potential conflict of interest.

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Author Contributions

Conceptualization: Kim HY. Data curation: Kim HY. Formal analysis: Park SY. Methodology: Kim HY. Software: Park SY. Validation: Kim HY. Investigation: Park SY. Writing - original draft: Park SY. Writing - review & editing: Park SY, Kim HY.

Ethics Approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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