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Presence of Bacterial DNA Fragments and Their Molecular Size after Heat Disinfection

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Objectives : Quality control of dialysates is crucial in hemodialysis treatment. Dialysates contaminated by bacterial DNA fragments (bDNA) have been reported to induce inflammatory responses in dialysis patients. The effectiveness of using an endotoxin retentive filter (ETRF) for removing bDNA fragments is still unclear. In the present study, we attempted to examine the molecular sizes and concentration of bDNA generated by heat disinfection of bacterial suspensions.
Methods : Pseudomonas aeruginosa was cultured and serially diluted by 10-fold from 10^8 to 1 CFU/mL. The bacterial suspensions (1 mL each) were then subjected to heat treatment at 99°C for 15 min. After the heat treatment, the suspensions were cooled on ice water for 1 minute and centrifuged at 12,000 rpm for 10 min. The concentrations of double-stranded DNA (dsDNA) and single-stranded DNA (ssDNA) fragments in the supernatants were measured. The molecular sizes of the dsDNA fragments were evaluated by polyacrylamide gel electrophoresis.
Results : bDNA was not detected in disinfected suspensions containing $\leq 10^5$ CFU/mL. The concentrations of dsDNA and ssDNA (ng/mL) in the disinfected supernatants derived from bacterial suspensions containing initial concentrations of 10^6, 10^7, and 10^8 CFU/mL were 1±3 and 22±6, 330±74, and 640±151, and 966±154 and 3113±486, respectively. The molecular sizes of the dsDNA were mostly less than 25 bp, and less than 10 bp in some cases. Therefore, bDNA fragments might still pass through ETRFs which have a cutoff value of 6,000 Da. These results suggest that if heat water disinfection releases a significant amount of suspended bacteria from the biofilm, it may lead to contamination with a large amount of bDNA.
Conclusions : bDNA fragments were detected even after heat disinfection in bacterial suspensions containing $\geq 10^6$ CFU/mL, and their molecular sizes were such that they could still pass through ETRFs.