Removing Endotoxin from Metallic Biomaterials with Compressed Carbon Dioxide-Based Mixtures

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Statement of Purpose: Depyrogenation of biomaterials can be difficult due to high variability in molecular weight, thermal stability, and resistance to pH of endotoxin molecules. The most common technique is dry heat, which requires exposure to temperatures of 250 ºC for more than 30 min or 180 ºC for more than 3 h[1]. Other techniques include washing with non-pyrogenic solvents and chemical inactivation using strong acid/base solutions[2]. Obviously, only heat-tolerant and corrosive-resistant materials can withstand these protocols while heat damage and oxidation are also possible. This study evaluated liquid carbon dioxide (CO_2)-based mixtures for the removal of *E. coli* endotoxin from smooth and porous titanium (Ti) surfaces and stainless steel (SS) lumens. This is a low-temperature process using mild conditions that avoids some of the complications mentioned above. Both Ti and SS are selected as the model biomaterials due to their relatively high surface energies which support strong endotoxin adherence[2,3].

Methods: Commercially pure Ti disks, porous-coated Ti coupons (150 µm pore size), and SS tubing (2.159 mm ID) were the substrates. *E. coli* endotoxin (O55:B5) was employed as the bio-contaminant. Bone-dry grade CO_2 along with surfactant Dehypon® Ls-54 and endotoxin-free water were employed as the cleaning fluids. A uniform endotoxin film was coated individually on each substrate. The contaminated materials were then treated with liquid CO_2 and liquid CO_2 + mixtures of water and surfactant in a pressure vessel at 25 C and 27.6 MPa for 2 h providing mechanical stirring. After treatment any residual endotoxin in the substrates was individually recovered by sonication in water. The aqueous solutions were then analyzed with the limulus amebocyte lysate (LAL) assay for endotoxin quantification. Endotoxin levels were compared against negative controls (contaminated/untreated samples) to determine the endotoxin removal level.

Results: Figure 1 shows the percentage endotoxin removal for Ti disks processed with either pure CO_2 or mixtures of CO_2, water and surfactant. For experiments involving CO_2 + Ls-54 and water, no residual endotoxin was detected. The probable mechanism is the formation of a water-in-CO_2 microemulsion. The water in the microemulsion will dissolve the endotoxin in the fluid phase, facilitating its removal. For those experiments employing CO_2 with either Ls-54 or water, 85% and 83% endotoxin removal was obtained, respectively. CO_2 alone will not dissolve endotoxin, hence the low level of removal (17%) with pure CO_2 is probably due to agitation and physical dislodgment. Figures 2 and 3 show the percent of endotoxin removal attained from the SS lumens and the porous-coated coupons, respectively. No residual endotoxin was detected in any lumen treated with the liquid CO_2 microemulsion. These results suggest facile penetration of the CO_2 solvent with no mass transfer limitation under the experimental conditions tested. In the porous-coated coupons no residual endotoxin was detetected after processing with liquid CO_2 microemulsions. This indicates that the water-in-CO_2 microemulsion system completely penetrated the porous layer and detached all the endotoxin

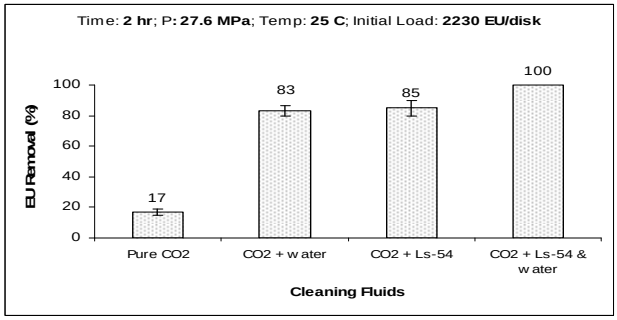

Figure 1. Endotoxin removal from Ti disks using liquid CO_2 and mixtures of water and Ls-54

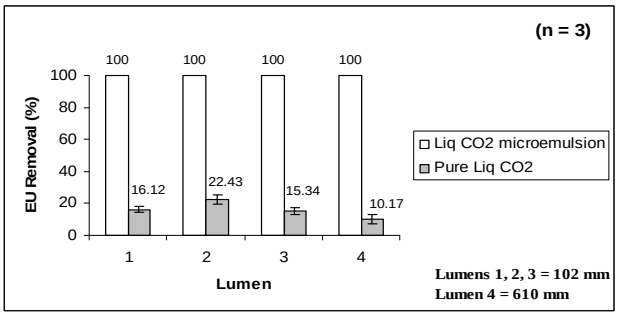

Figure 2. Endotoxin removal from SS lumens

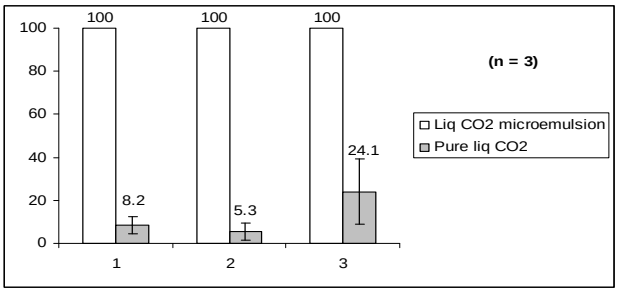

Figure 3. Endotoxin removal from porous Ti coupons

Conclusions: The present study demonstrated that a well agitated water-in-CO_2 microemulsion system at room temperatures and moderate pressures (25 ºC and 27.6 MPa) can effectively remove endotoxin from smooth and porous Ti surfaces and in SS lumens. Significant fractions of endotoxin (83 to 85%) were also removed from the Ti disks when employing binary mixtures of CO_2 with either water or Ls-54. Pure CO_2 did not remove significant amount of endotoxins from all of the tested substrates because the biomolecule is not soluble in CO_2.

References: 1. Tsuji K. Appl Environ Microbiol 1978;36:710-714. **2.** Ragab AA. J Orthop Res 1999;17:803-809. **3.** Nelson SK. J Prosthet Dent 1997;77:76-82.