Effect of Sterilization on the Activity of a Biomimetic Coating containing Polymer and Protein Components

Wade A. Takeguchi[1], Tara Kupumbati[2], and Jennifer A. Neff[1]
[1]Allvivo Vascular, Inc., 20914 Bake Parkway, Suite 112, Lake Forest, CA 92630
[2]Medtronic Heart Valves, 1851 E. Deere Avenue, Santa Ana, CA 92705

Statement of Purpose: Combination products represent an important advance in medical device technology that hold great promise for improving patient care. A novel surface coating comprised of a hemocompatible polymer and protein called factor H has been developed to reduce device related complement activation. This coating has been applied to coronary stents to reduce restenosis. A major challenge of bringing a combination product like this to the market is selecting a method of sterilization that is compatible with all of the device components. Problems associated with some sterilization methods include degradation of polymers, inactivation of drugs or biologics, and alteration of the physical or mechanical properties of device components. This paper describes the effects of different types of sterilization on (1) the activity of functional groups in a polymer surface coating and (2) the activity of factor H.

Methods: End Group Activated Pluronic (EGAP) equipped with a terminal pyridyl disulfide functional (PDS) group was synthesized according to the procedure of Li et al by Allvivo Vascular, Inc. (Lake Forest, CA) (1). Electropolished, 316L stainless steel samples (Phoenix Specialty Manufacturers) were pretreated with CIP 100 (Steris), silanized with octadecyltrimethoxysilane (ODtMOS, Sigma), and coated with EGAP as described by Neff et al (2). Effect of sterilization on PDS activity: To test the effect of sterilization on the retention of the polymer's PDS functional group activity, β-galactosidase (Calbiochem) was activated with N-succinimidyl S-acetylthioacetate (SATA, Pierce), deacetylated, and coupled to EGAP coated substrates that had been sterilized by ethylene oxide (EO), hydrogen peroxide, e-beam or γ-irradiation. The amount of surface bound β-galactosidase was measured using a FluoReporter® *lacZ*/ Galactosidase Quantitation Kit (Molecular Probes). Effect of sterilization on factor H: Factor H (donated by the Nilsson-Ekdahl lab, University of Kalmar, Sweden) was activated with SATA, deacetylated, and coupled to EGAP-coated substrates. After coupling factor H, samples were sterilized via EO, e-beam or γ-irradiation. The activity of factor H was measured using an assay for complement convertase formation as described previously (2). Briefly, samples were incubated in serum containing 10% VBS for 1 hr at 37°C, rinsed with PBS, and incubated with an AMC modified peptide that contains the cleavage site for the C3/C5 convertase. The amount of AMC released upon cleavage of the peptide was measured using a flourimeter.

Results / Discussion:
Over 90% of the functional groups on EGAP remained active for coupling after sterilization by EO, e-beam, and gamma irradiation. However, hydrogen peroxide sterilization resulted in 77% loss of coupling activity. Unmodified Pluronic elicited greater complement activation than a cellulose acetate positive control. Addition of factor H significantly reduced complement activation. The complement reducing activity of factor H was not significantly diminished by EO, e-beam, or γ-irradiation sterilization.

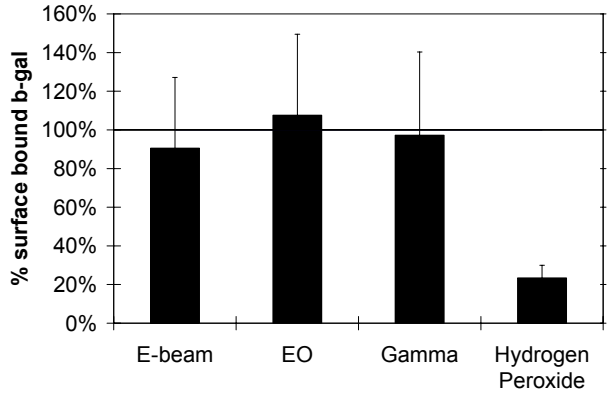

Figure 1: Percent Beta Galactosidase activity obtained on EGAP coated, sterilized samples relative to EGAP coated samples that were not sterilized (line at 100%).

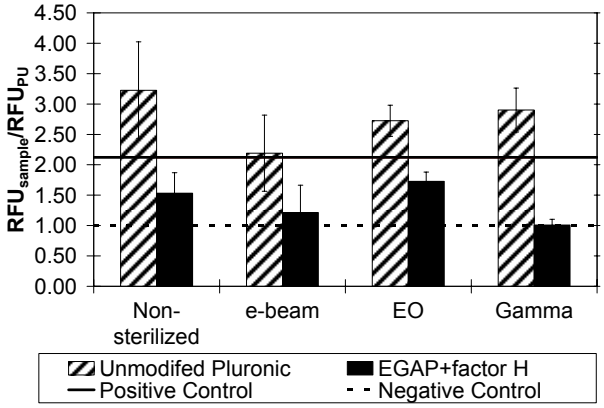

Figure 2: Complement activation levels of unmodified Pluronic-coated and EGAP+factor H -coated samples relative to negative (polyurethane) controls, with positive (cellulose acetate) and negative controls shown.

Conclusions: EO, e-beam, and γ-irradiation sterilization did not cause a significant loss of either the functional group activity of EGAP or the activity of factor H. Hydrogen peroxide sterilization resulted in a significant decrease in EGAP's coupling potential. There was a small trend toward better retention of factor H activity with e-beam and γ-irradiation compared to EO sterilization.

References:

1. Li JT. Bioconjug Chem. 1996;7:592-599.
2. Neff JA. Proc of the ASM MPMD Conf, St. Paul, MN Aug 2004