A Comparison of Sterilization Methods for Dehydrated AC and UC

Taylor J. Sabol, MS, CTBS - BioStem Technologies; Grace S. Tran , BA, CTBS - BioStem Technologies; Christian A. Van Vurst, CTBS - BioStem Technologies; Magnolia West, n/a - BioStem Technologies; Wendy W. Weston, PhD, CTBS - BioStem Technologies

Background: Sterilization of tissue is a crucial part of ensuring that the allograft is safe for the recipient. Sterility Assurance Level (SAL) is the probability that a single unit that has been subjected to sterilization nevertheless remains nonsterile. A very effective sterilization process has a very low SAL, hence, medical device manufacturers design their sterilization processes for an extremely low SAL (10−6). Gamma irradiation (γ-IR) is used by many allograft processers to achieve an SAL of 10−6, but this method has no dose flexibility. Gamma irradiation at the industry standard of 25 kGy has been shown to affect tensile strength, elongation, and water absorption of collagen membranes. E-beam irradiation is very similar to γ-IR in being an ionizing energy. The difference is that e-beam utilizes higher doses with less time of exposure and lower penetration. By limiting the time of exposure, the effect of the sterilization process on tissue structure and endogenous factors should be reduced.

Methods: Amnion/chorion (AC) and Umbilical cord (UC) were processed for dehydrated product. The packaged samples were sent out for E-beam sterilization at VDmax of 0, 10, 20, 40, 60 and 80 kGy and γ-IR. Following sterilization, 10mm punches were taken from each lot and dose. For absorption capacity, punches were photographed, weighed, and rehydrated in DPBS. Punches were removed from the liquid, blotted, weighed and re-photographed. Absorption was calculated as % weight difference between dry and wet conditions. For molecular assays, punches were incubated with DPBS for 72 hours. The supernatant was used for collagen 1A1, hyaluronic acid, IL-1ra and HGF assays. All assays were compared Student's t-test with significance set at $p< 0.05$.

Results: In UC, 80 kGy e-beam had significantly less IL-1ra than 10 kGy, although there did not appear to be a consistent trend in IL-1ra or HGF eluted from the membranes, regardless of type or magnitude of irradiation. HA did not show significant differences between the sterilization treatments, but there is an obvious downward trend in elution from both AC and UC with increasing doses. There was a downtrend in UC collagen 1A1 with increasing doses, but differences were not significant. Absorption results showed a clear downtrend in absorption capacity as irradiation dose increased, with γ-IR results being close to or equal to higher e-beam doses.

Conclusion: Based on these results, e-beam sterilization at increasing doses and γ-IR have differing effects on absorption capacity and growth factor availability. Hence, ideal sterilization technique should be based on the desired product and application (e.g. softer, absorptive membrane with available growth factors vs. stiffer membrane with less/no factors). Based on these results, clinical studies are warranted to elucidate the functional differences in a healing environment.

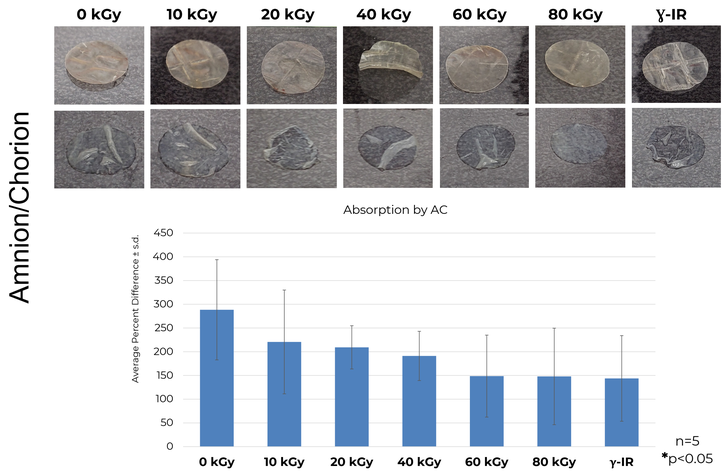
A Comparison of Sterilization Methods for Dehydrated AC and UC

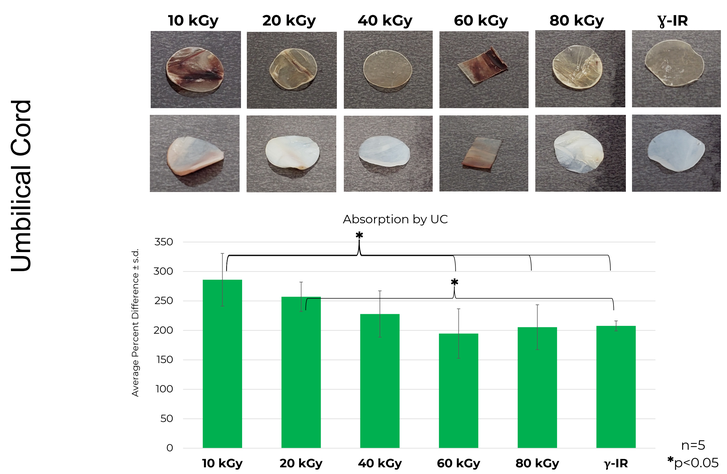
Taylor J Sabol, MS[1,2] , Grace S Tran, BA[1,2], Christian A Van Vurst[1,2], Magnolia West, BS[1,2], Wendy W Weston, PhD[1,2]

[1] BioStem Technologies, Inc., [2] CTBS

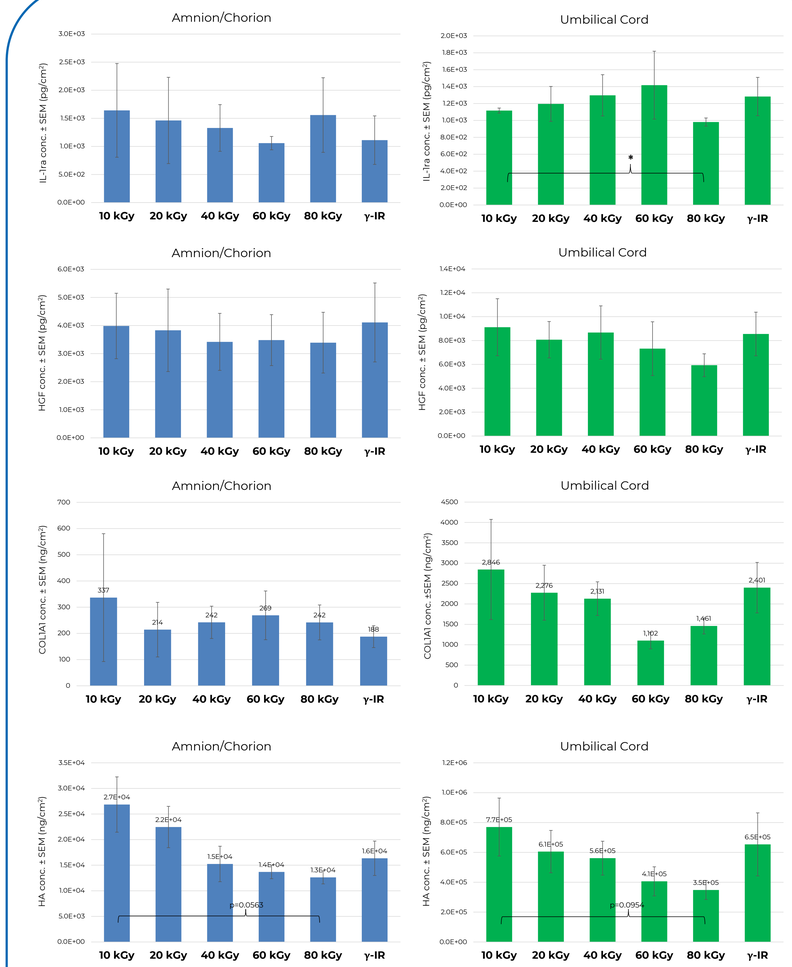
Sterilization of tissue is a crucial part of ensuring that the allograft is safe for the recipient. Medical device manufacturers design their sterilization processes for an extremely low SAL (10^{-6}) which is a 1 in 1,000,000 chance of a non-sterile unit. Gamma irradiation (γ-IR) at 25 kGy is used by many allograft processers to achieve an SAL of 10^{-6}, but this method has no dose flexibility[i] and has been shown to affect tensile strength, elongation, and water absorption of collagen membranes[ii]. Some tissue banks have resorted to lower doses of γ-IR to keep biomechanical and other properties of tissues intact[iii]. E-beam irradiation is very similar to gamma radiation sterilization in being an ionizing energy. The difference is that e-beam utilizes higher doses with less time of exposure and lower penetration. By limiting the time of exposure, the effect of the sterilization process on tissue structure and endogenous factors are reduced. The function of placental allografts as barriers and promoters of healing relies on their ability to absorb eluate, conform to the wound shape and provide factors that promote wound healing. With this in mind, we hypothesized that e-beam sterilization at increasing doses and γ-IR would have differing effects on absorption capacity and growth factor availability. We tested this hypothesis on punches collected from amnion/chorion (AC) and umbilical cord (UC) following sterilization by e-beam irradiation at increasing kGy, as well as gamma-irradiation.

Results





Absorption results showed a clear downtrend in absorption capacity as irradiation dose increased, with γ-IR results being close to or equal to higher e-beam doses. Due to the donor variability, significance was not reached by AC. Significant differences were seen in UC between the lower and higher doses (including γ-IR).



The concentration of **A)** IL-1ra**, B)** HGF, **C)** COL1A1, **D)** HA in the elute of membranes treated with 0 kGy, 10kGy, 20kGy, 40kGy, 60kGy, 80kGy and gamma irradiation (γ-IR). N=5

For all assays, variability between donors was substantial, as expected. HGF did not show significant differences between the sterilization treatments. In UC, 80 kGy e-beam had significantly less IL-1ra than 10 kGy. Interestingly, there did not appear to be a consistent trend in IL-1ra or HGF eluted from the membranes, regardless of type or magnitude of irradiation. Although HA did not show significant differences between the sterilization treatments, there is an obvious downward trend in elution from both amnion/chorion and umbilical cord with increasing doses. There was a downtrend in UC collagen 1A1 with increasing doses, but differences were not significant.

Methods

Five lots of Amnion/chorion and five lots of Umbilical cord were BioRETain® processed. The packaged samples were sent out for E-beam sterilization (Steritek) at VDmax (± 7%) of 0, 10, 20, 40, 60 and 80 kGy and γ-IR (Steris) at 25 kGy (± 7%). Following sterilization, multiple 10mm punches were taken from each lot and dose.

- For absorption capacity: Punches were photographed for visual representation, weighed, and placed in 1.5 ml tubes with 1 mL of $DPBS^{Ca\text{-}Mg\text{-}}$ for 24 hours at 37 °C, 150 rpm. Punches were then removed from the liquid, blotted on a towel for 3 seconds and weighed. The “wet” punches were photographed. Absorption was measured by % weight difference between dry and wet conditions.
- For growth factor and structural component assays: Punches were placed in 1.5 mL tubes with 0.5 mL of $DPBS^{Ca\text{-}Mg\text{-}}$ for 72 hours at 37 °C, 150 rpm. The supernatant was clarified by centrifugation, aliquoted and stored at -20°C until use. ELISA’s for collagen 1A1 and hyaluronic acid were performed. IL-1ra and HGF were assayed by BioPlex cytokine assay. Results were generated against 5-parameter logistic curves.

All assays were compared by a 2-tailed, unequal variance, Student’s t-test with significance set at $p<0.05$.

Discussion

Although IL-1ra and HGF were inconclusive, Collagen 1A1 and Hyaluronic acid show the most favorable elution at 10 kGy of electron beam irradiation. Interestingly, microbiological studies have previously shown that 15 kGy is sufficient for e-beam sterilization techniques. Of the sterilized samples, 10 kGy ± 0.7 e-beam sterilization had the highest absorption. Based on these results, e-beam sterilization at increasing doses and γ-IR have differing effects on absorption capacity and growth factor availability. Hence, sterilization technique should be considered based on the desired product and application (e.g. softer, absorptive membrane with available growth factors vs. harder membrane with less/no factors). Based on these results, clinical studies are warranted to elucidate the functional differences in a healing environment.

i. Silindir Gunay M, Ozer Y. Sterilization methods and the comparison of E-Beam sterilization with gamma radiation sterilization. FABAD J Pharm Sci. 2009;34:43-53.
ii. Komara et al. The Effect of Gamma-Ray Irradiation on the Physical, Mechanical, and Morphological Characteristics of PVA-Collagen-Chitosan as a Guided Tissue Regeneration (GTR) Membrane Material. European Journal of Dentistry. 2022. DOI: https://doi.org/ 10.1055/s-0042-1753451.
iii. Singh R, Singh D, Singh A. Radiation sterilization of tissue allografts: A review. World J Radiol 2016; 8(4): 355-369. PMID: 27158422 DOI: 10.4329/wjr.v8.i4.355.