

A Comparison of Sterilization Methods for Dehydrated AC and UC

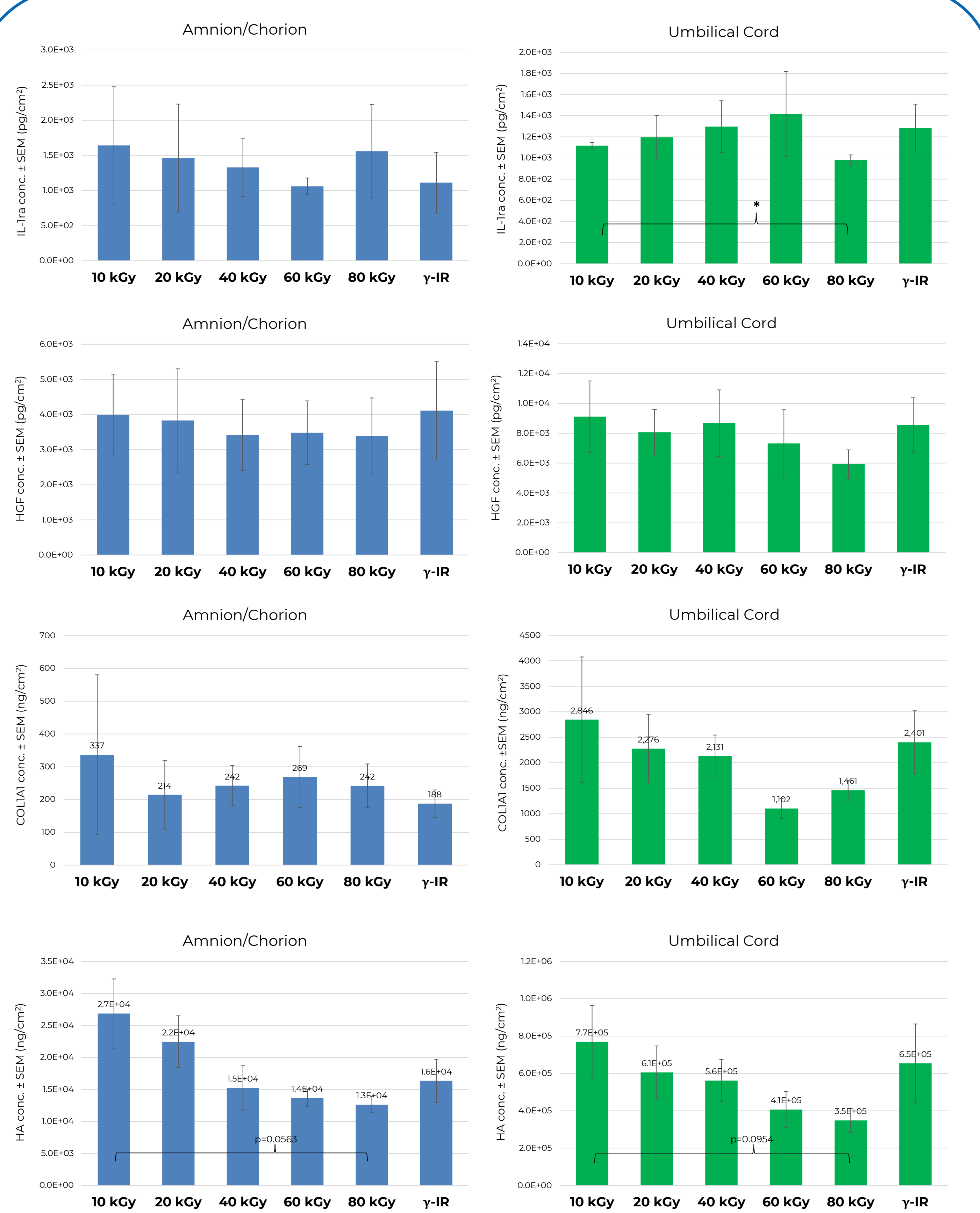
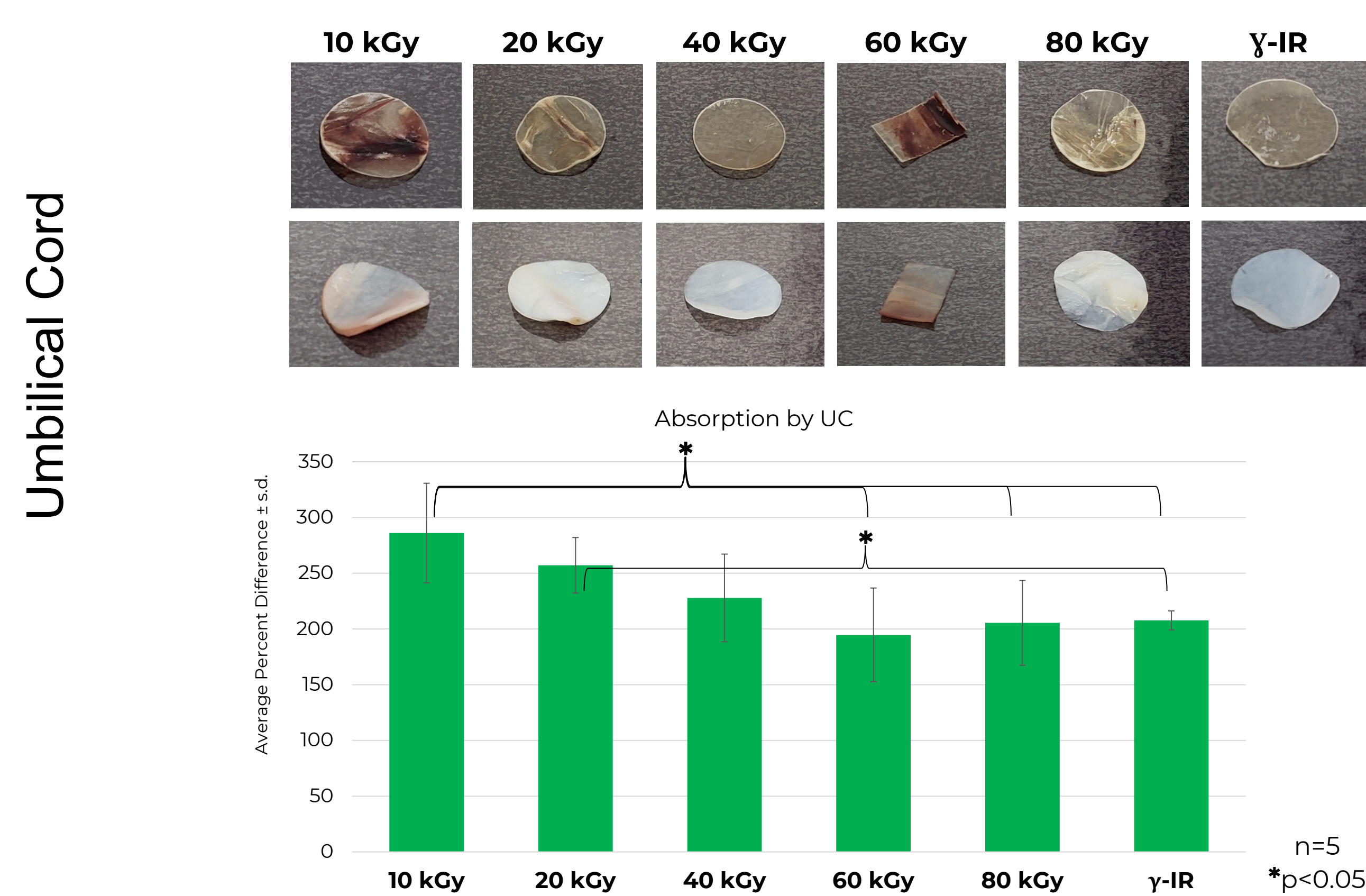
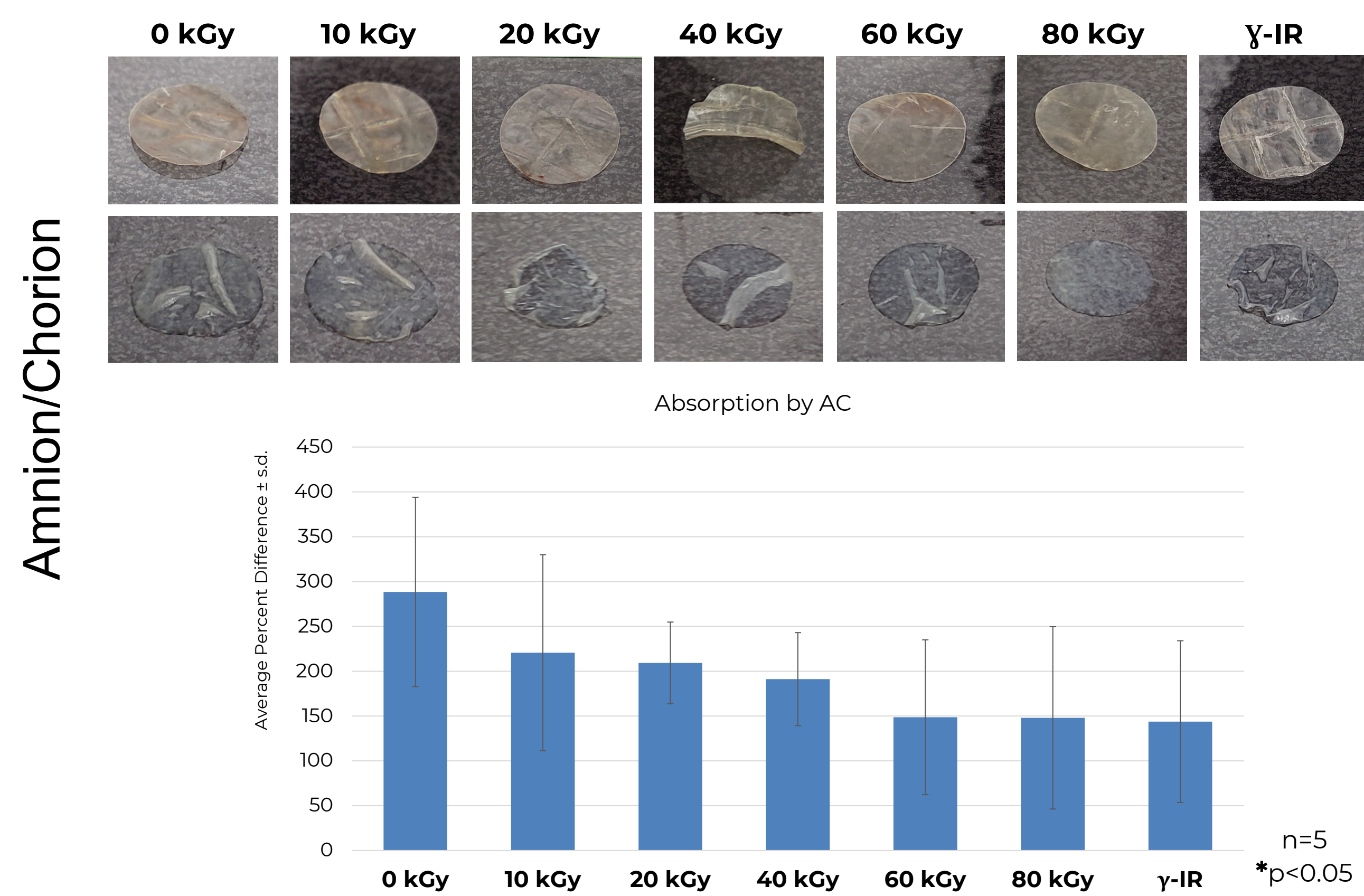
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Sterilization of tissue is a crucial part of ensuring that the allograft is safe for the recipient. Medical device manufacturers design their sterilization processes for an extremely low SAL (10^{-6}) which is a 1 in 1,000,000 chance of a non-sterile unit. Gamma irradiation (γ -IR) at 25 kGy is used by many allograft processors to achieve an SAL of 10^{-6} , but this method has no dose flexibilityⁱ and has been shown to affect tensile strength, elongation, and water absorption of collagen membranesⁱⁱ. Some tissue banks have resorted to lower doses of γ -IR to keep biomechanical and other properties of tissues intactⁱⁱⁱ. 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



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 ($\pm 7\%$) of 0, 10, 20, 40, 60 and 80 kGy and γ -IR (Steris) at 25 kGy ($\pm 7\%$). 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-Mg} 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-Mg} 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.



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