EP3HTMed: Relative Biological Effectiveness of Alpha Radiation in Cultured Porcine Aortic Cells

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Application

The authors of this paper sought to design an experiment to assess the RBE of alpha radiation from an external polonium-210 source using cultured porcine aortic endothelial cells as a surrogate for human tissues. As polonium-210 ingestion is associated with diets high in meat, especially caribou, this work seeks to provide insight into the risks associated with this activity. Further, this work sought to develop an improved method of RBE determination using an external radiation source; other common methods require adding radioactive salts to the cell culture medium itself. To perform these important experiments, a test apparatus was created using autoclaving resistant Master Bond EP3HTMed, a one-part epoxy that meets USP Class VI and ISO 10993-5 criteria for use as a biocompatible epoxy.

Key Parameters and Requirements

In the construction of their test assembly, shown in **Figure 1**, the experimenters needed an adhesive to bond the glass support rings to the thin mylar sheets. Measuring 2.5 µm, the thin mylar film enabled transmission of the easily attenuated alpha particles emitted from the polonium-210 source to the test cells. In addition to forming a strong, water-tight bond between the glass and the mylar film, the adhesive must also possess the ability to resist autoclave sterilization as well as being biocompatible and non-cytotoxic. As the experiment primarily was assessing the continued viability of the porcine endothelial cells, it is critical that the test apparatus itself does not contribute to cellular harm. After assembly of the test apparatus and prior to introducing the cellular tissues, it is critical that the apparatus be sterilized. Autoclaving, exposure to high temperatures and pressures, is an effective and common means to sterilize and destroy any microbiological contaminants that may interfere with the scientific experiments. The experimenters constructed the apparatus shown in the below figure by bonding the glass support rings to the mylar film; curing of the epoxy was done at 150°C for 10 minutes. The dishes were then cut out from the excess mylar sheet, checked for watertightness, and were then autoclaved.

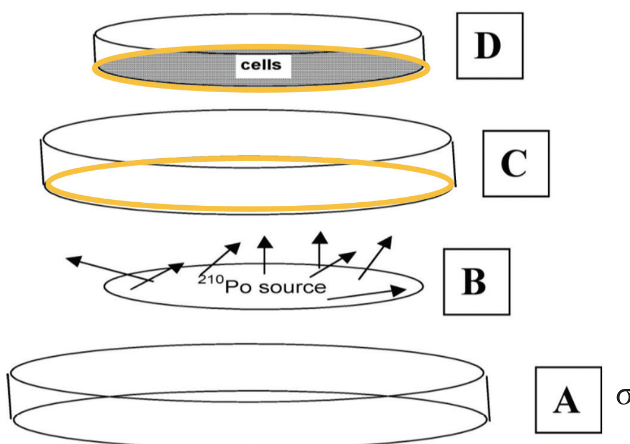

Figure 1. 60 mm plastic culture dish (A), Po-210 radiation source, (B), larger mylar dish (C), and smaller mylar dish containing test cells (D). The Master Bond EP3HTMed, shown in gold, was used to bond the glass support rings to the thin mylar sheet on the bottom of dishes C and D.[1]

Master Bond EP3HTMed is a one-part, thermal curing epoxy that meets USP Class VI and ISO 10993-5 for biocompatibility. These are rigorous protocols that ensure that any plastic, rubber, adhesive or sealant used in the construction of implantable medical devices possesses no cytotoxicity. During certification, various extracts from the material to be certified are introduced to cell cultures or directly to test animals; the test specimens are then assessed to ensure that the extracts do not impart any toxicity. For this application, an adhesive compliant with USP Class VI and ISO 10993-5 ensures that the adhesive itself will not interfere with the experimenter's assessments of cell viability during the experiment. Master Bond EP3HTMed also possesses exceptional resistance to autoclaving—**Figure 2** presents the autoclave resistance of EP3HTMed when compared to a standard epoxy. A lower weight change indicates a greater resistance to the harsh temperatures and pressures experienced during repeated autoclave cycles.

In addition to meeting biocompatibility requirements and possessing autoclave resistance, Master Bond EP3HTMed is a versatile one-part, thermal curing epoxy that requires no measuring or mixing prior to use. It bonds well to a variety of substrates including glass, metals, ceramics, and most plastics. It has a wide service temperature range of -60 to +400°F (-51 to +204°C), and it possesses superb strength and modulus.

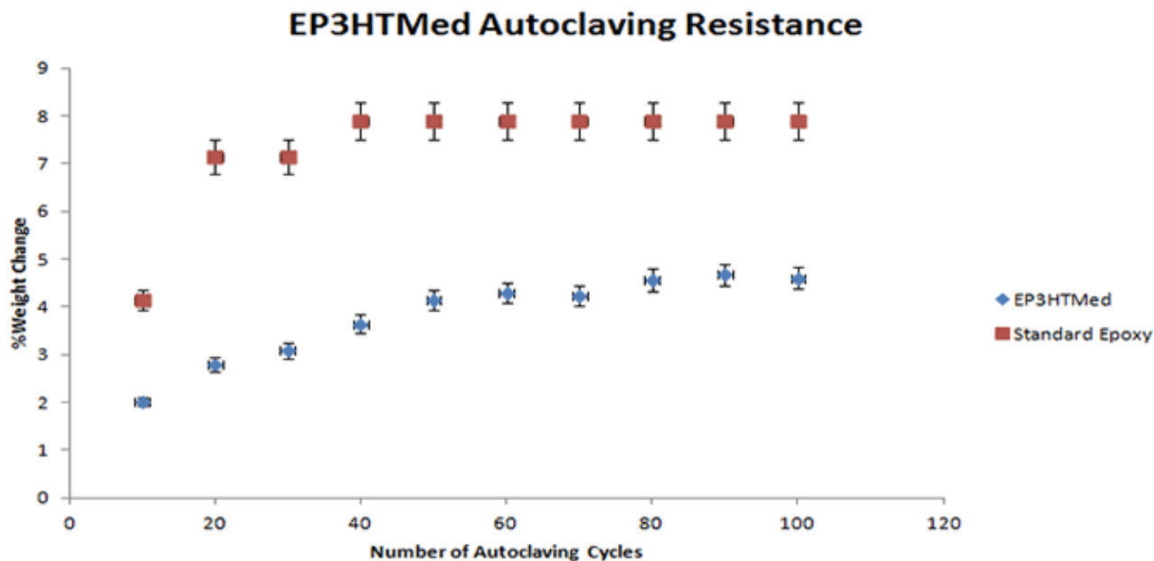

Figure 2. Autoclave resistance of Master Bond EP3HTMed compared with a standard epoxy over 100 autoclave cycles. A lower weight change indicates greater resistance to the harsh conditions of the autoclave.

Results

The experimenters determined the relative biological effectiveness (RBE) of the porcine aortic endothelial cells using the device constructed in **Figure 1**; this methodology employed an external radiation source.[1] Another common methodology in this field of research uses an internal radiation source—in this method, polonium-210 citrate is added to the cell culture and diluted. Their work demonstrated that the external radiation source method provides a much more consistent dose response when compared with the internal radiation source method. The construction of the test apparatus, using Master Bond EP3HTMed, then allows for a more accurate and reproducible means to assess RBE.

Endpoint	Slope (m) ± SE*	n	R^2	p**	Porcine RBE ± SE	Bovine RBE ± SE
Cell viability: Exponential fit where $y = e^{-mx}$						
x-ray	0.007 ± 0.001	7	0.75	0.0008		
external alpha	0.149 ± 0.021	5	0.78	0.002	21.2 ± 4.5	13.1 ± 2.5
Live Cells: Exponential fit where $y = e^{-mx}$						
x-ray	0.038 ± 0.007	7	0.79	0.001		
external alpha	0.49 ± 0.05	5	0.87	0.0008	12.9 ± 2.7	9.7 ± 0.6
LDH: Linear fit where $y = mx + 100$						
x-ray	14.2 ± 0.5	7	0.98	0.0000002		
external alpha	75.7 ± 5.6	5	0.96	0.0002	5.3 ± 0.4	11.1 ± 3.0
Clonogenic Survival Fraction: Exponential fit where $y = e^{-mx}$						
x-ray	0.65 ± 0.06	8	0.89	0.00001		
external alpha	1.03 ± 0.03	6	0.99	0.0000001	1.6 ± 0.1	14.0 ± 1.0***

Figure 3. Experimental results of the relative biological effectiveness (RBE) determined from four different endpoints on cultured porcine aortic endothelial cells. The porcine RBE values were determined from an external polonium-210 alpha emitter source. The Bovine RBE values, determined from an internal radiation source, are cited from a previous work.[2]

From the data presented in **Figure 3**, it can be shown that the RBE values determined in porcine tissues using the external radiation method follow a logical progression and indicate a more consistent dose response when compared with the bovine RBE values measured by the internal radiation method.[1] The RBE values were determined by dividing the determined slope of the external alpha radiation series by the slope of the reference radiation (x-rays) series. The dose response curves were constructed by irradiating samples over a range of dosages; the irradiated cell monolayers were then assessed through various standard methods to determine changes to cell viability, number of live cells, lactate dehydrogenase (LDH) release, and clonogenic survival fraction.

The experimenters concluded that the external radiation source method is a simple and superior method for determining RBE values. The dosimetry is simplified relative to the internal method; when using the internal method, radioactive polonium-210 citrate must be added to the culture medium itself which means that additional radiation dose is being delivered to the cells during colony formation. The external method simplifies dosimetry, as no dose is being provided to the cells unless it is fixtured in the test apparatus allowing for greater control of the dose and determination of the exposure time. Using Master Bond EP3HTMed, the experimenters were able to construct a test apparatus that allowed for a more reliable and simple method to determine RBE values and to assess the extent of cell damage associated with alpha irradiation from a polonium-210 source.

References

[1] Thomas, P., Tracy, B., Ping, T, et al. International Journal of Radiation Biology. Relative biological effectiveness (RBE) of alpha radiation in cultured porcine aortic endothelial cells. Volume 83; 2007, pp. 171-179.

[2] Thomas, P., Tracy, B., Ping, T., et al. International Journal of Radiation Biology. Relative biological effectiveness (RBE) of polonium-210 alpha particles vs. x-rays on lethality in bovine endothelial cells. Volume 79; 2003, pp. 107-118.