PolySciTech Division of
AKINA, INC.
3495 Kent Avenue
West Lafayette, IN 47906
Tel: (765) 464-0501; Fax: (765) 464-0820 www.polyscitech.com

PLGA-PEG-PLGA Sterilization Protocol

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Whitepaper Authors
Chloe Stewart, Postgraduate Research Student, School of Pharmacy, University of Nottingham
Anna Piccinini, Assistant Professor of Inflammation Biology, Faculty of Science, University of Nottingham
John Garner, General Manager, Akina, Inc.

Polymer Product

Cat# AK097 Poly(lactide-co-glycolide)-b-Poly(ethylene glycol)-b-Poly(lactide-co-glycolide) (nominal: (Mw ~1700-1500-1700 Da) LA:GA 15:1)

Background

Utilization of PLGA-PEG-PLGA thermogels in biological testing and applications requires careful sterilization of the material. These thermogels present a unique challenge to sterilization, however due to their physical structure, biodegradability, and interactions with water.

Methods/Observations

Submicron Filtration Diluting the PLGA-PEG-PLGA into deionized water under cold conditions yielded a solution however this solution was too viscous to successfully filter through 0.22 µm or 0.45 µm filter under the conditions tested at either 1% or 20% w/v.

Dry Heat Sterilization 1 hour at 180 °C caused the polymer to discolor to a brownish color, likely due to polymer/PEG degradation under non-specific oxidation conditions.

Autoclave Sterilization Autoclave sterilization was conducted as described in the protocol below. The material successfully formed a thermoresponsive gel afterwards and was utilized in research. The full results of the research are described in article: Stewart, Chloe L., Andrew L. Hook, Mischa Zelzer, Maria Marlow, and Anna M. Piccinini. "PLGA-PEG-PLGA hydrogels induce cytotoxicity in conventional in vitro assays." *Cell Biochemistry and Function* 42, no. 5 (2024): e4097. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/cbf.4097

Autoclave Protocol

Perform the following steps under aseptic conditions in a laminar flow hood operated per manufacturer's instructions. As relevant, utilize disinfecting spray (70% ethanol or 3% bleach solution) and UVC illumination to sanitize work surfaces prior to initiating operations.

1. Place a 20 mL glass scintillation vial (without lid) and a clean metal spatula into an oven at 180 °C overnight to remove any lipopolysaccharide (LPS) contamination/endotoxins.
2. Using the sterilized spatula, weigh an appropriate amount of polymer into the vial (normally 200-500 mg) and record the combined weight of the vial and polymer.
3. Cover the top of the vial with fresh/clean aluminum foil and loosely tape down with autoclave tape.
4. Place the vial into a glass beaker to make sure it doesn't fall over in the autoclave.

5. If also using magnetic stirring fleas to dissolve the polymer, thoroughly wash the fleas with soap and water and place into a fresh 20 mL glass vial covered with foil and tape as above.
6. Using a benchtop Prestige Medical Classic Media 12L autoclave (or equivalent device), empty any remaining water and refill with fresh MilliQ water.
7. Place the beakers containing the polymer and flea vials into the autoclave basket and run a standard autoclave cycle (121 ºC for 28 minutes).
8. Whilst the polymer is autoclaving, the vial caps (polyethylene) can be cleaned with 70% ethanol and left to dry under the clean air flow of a laminar flow hood.
9. After autoclaving, the polymer will have spread to form a thin layer across the bottom of the glass vial and some water droplets will remain in the vial.
10. Dry the outside of the vial, remove the aluminum covering and reweigh the polymer vial to calculate the amount of water inside.
11. Once weighed, close the vial with the clean cap.
12. Deduct the amount of water remaining in the vial from the amount of water that is needed to make the polymer into a 20% w/v solution.

Conclusion

Autoclaving the polymer following the protocol described provides for suitable endpoint sterilization that does not drastically alter the polymer functionality and obviates the problems encountered with either dry heat sterilization (polymer oxidation) or filtration (viscosity).

References

[1] Stewart, Chloe L., Andrew L. Hook, Mischa Zelzer, Maria Marlow, and Anna M. Piccinini. "PLGA-PEG-PLGA hydrogels induce cytotoxicity in conventional in vitro assays." *Cell Biochemistry and Function* 42, no. 5 (2024): e4097. https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/abs/10.1002/cbf.4097