Investigation into the Effectiveness of Sterilization Procedures for Nanofibrous Scaffolds

Jessica M. Gluck[1], Martin W. King[1], Bhupender S. Gupta[1], Lisa Samuelson[2], David A. Gerber[2]
[1]College of Textiles, North Carolina State University, Raleigh, NC 27695-8301
[2]Department of Surgery, College of Medicine, University of North Carolina – Chapel Hill, NC 27599

Statement of Purpose: When developing implantable biomaterials, the procedure for sterilization is an essential step that has to be addressed. However, when working with nanofibers and nanoparticles, the validity and effectiveness of accepted sterilization techniques cannot be assumed because of the significantly greater surface area and increased fragility of nanomaterials. This study examined the relative effectiveness of 4 different sterilization techniques for polycaprolactone (PCL) nanofibrous webs prepared for tissue engineering applications. The sterilization techniques[1] investigated were i) gamma radiation[2], ii) UV light, iii) UV light with 70% isopropanol, and iv) ethylene oxide. Because of its expected deleterious effect on resorbable materials such as PCL, autoclaving was not included.

Materials and Methods: A 10% by weight solution of polycaprolactone (Mw 65,000) (Sigma-Aldrich), with a melting point of 60°C, density of 1.145 g/cm^3, was made up in a 3:1 chloroform:methanol solvent mixture at room temperature. The electrospinning experiment employed the use of a vertical parallel plate electrospinning system. The top plate was grounded, and the bottom plate was attached to the positive power supply of 48 kV, hence serving as the collection plate for the spun nanofiber web. A meltblown polypropylene nonwoven fabric was used as the collection medium.

Each sample was cut into circles 35mm in diameter, placed into 35mm culture dishes and attached to the bottom of the culture dish with silicone type A medical adhesive (Dow Corning). Each sample was then sterilized by a different method. The methods were i) gamma radiated by a 220 Gamma Cell source with a dose of 2.5 Mrads over a period of 12.6 days. ii) The next samples were exposed to UV light for 30 minutes on each side of their surface. iii) Each sample was exposed to the same conditions of UV light and then were soaked in 70% isopropanol for a period of 12 – 16 hours. iv) The last group of samples was sterilized in ethylene oxide by soaking in ethylene oxide and leaving for 15 minutes before being vacuumed off.

Each set of sterilized samples was then put in media and incubated 37°C and 5% CO_2. The media used was the standard media for hepatocyte culture. The cell line used was from the American Type Culture Collection, designated AML12 (ATCC Catalog No. CRL-2254). The media used did not contain any antibiotic agents. The samples were evaluated for micro-organisms after one week with no media change or significant movement.

Results/Discussion: After a week in media each sample was evaluated for continuing sterility. Valid sterilization was determined by image analysis of the samples viewed at 10x magnification and by viewing the state of the liquid media. If no visible mold or bacteria developed and the media was still in a clear state, then the sample was deemed effectively sterilized.

Figure 1: Radiated samples after 2 weeks in media, yellow bar represents 35mm.

As seen in Figure 1, some samples have a white appearance, which may appear like mold. However, under 10x magnification there was no apparent mold or bacteria. (Figure 2.)

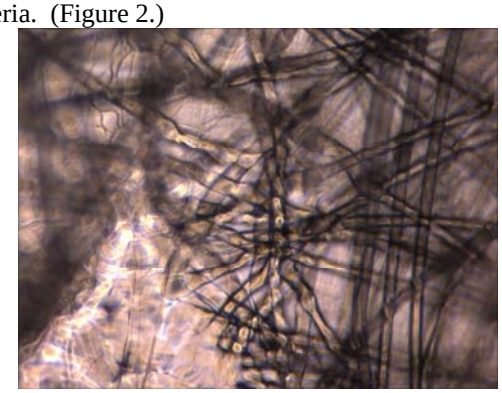

Figure 2: UV light samples after 2 weeks in media, at 10x magnification.

Conclusions: The results from the study show that exposing both sides of the sample to UV light for a minimum of 30 minutes is an effective method of sterilizing nanofibrous webs. However, after a period of one week the UV light samples began to show signs of contamination. By using the standard media with an antibiotic agent, the samples would not be subject to contamination. For long term cultures, gamma radiation is the most efficient sterilization method. Even after seven days of incubation and 14 days in ambient conditions, the samples showed no signs of contamination. Further research is continuing to determine if the sterilization procedures cause any changes in the mechanical properties of the nanofiber webs.

References:

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