# Sterilisation of PLGA flat sheet and hollow fibre tissue engineering scaffolds

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**INTRODUCTION:** The design and production of scaffolds for tissue engineering is becoming increasingly sophisticated. Scaffold properties such as mechanical strength, architecture and degradation rate are known to have a significant influence on the ultimate success of the engineered tissue. Post-production scaffolds require sterilisation prior to use in cell culture. Typical sterilisation techniques, such as autoclaving, can have a significant impact on the properties of the scaffold. In this study the effects of four sterilisation methods (ethanol, UV, peracetic acid and antibiotics) are investigated on two different scaffold architectures (flat sheets and hollow fibres); success of sterilisation and effect on the scaffold architecture and pore size are investigated.

**METHODS:** Polymer scaffolds were fabricated by solvent exchange from a 20%w/w solution of 50:50 poly(D,L-lactic-co-glycolic) acid (PLGA) (Medisorb, Alkermes) in 1-methyl-2-pyrrolidinone (NMP) (Arcos Organics). Flat sheets were cast on glass sheets and immersed in a water bath; hollow fibres were extruded through a spinneret nozzle, solvent exchange occurred via water in the lumen and an external water bath.

Flat sheet scaffolds were prepared by placing the polymer in 13mm diameter cells (Minucells and Minutissue), hollow fibres were cut into 20mm lengths. Samples were sterilised with either: 70% ethanol (Fisher Scientific) [1], UV irradiation [2], Peracetic acid solution: (0.1% peracetic acid (Sigma-Aldrich), 4% ethanol [3] or Antibiotic antimycotic solution (Sigma-Aldrich) for varying durations.

Sterilisation effectiveness was determined following incubation in cell culture medium (10%FCS, 1% NEAA, 1%SP, 88%DMEM, Sigma Aldrich) for up to 48h. Effects on the structure were determined though SEM. Changes in pore size were measured through gas permeation experiments.

**RESULTS:** All sterilisation methods tested showed no signs of infection during culture when sterilised for typical durations as quoted in literature [1-3]. It was also found that all sterilisation techniques resulted in some degree of damage to the polymer structure; increased treatment duration lead to increased damage (Fig. 1). Polymer structures were seen to exhibit larger pores, tears associated with increased fragility of structure and folding and wrinkling of the surface.

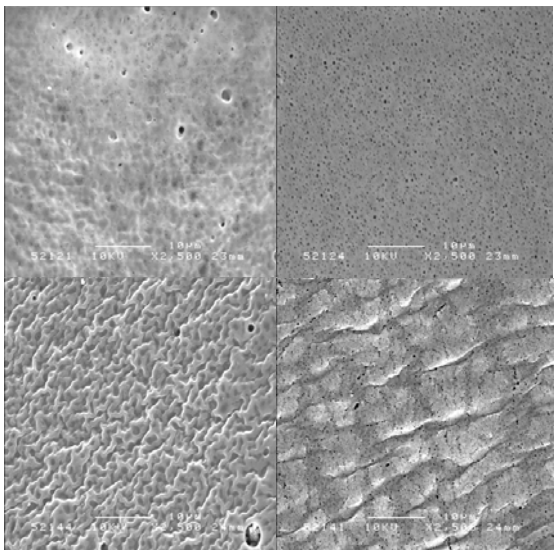

*Fig. 1: Effect of antibiotic sterilisation on flat sheet polymer morphology: control bottom (top left) control top (top right) sterilised bottom (bottom left) sterilised top (bottom right).*

**DISCUSSION & CONCLUSIONS:** All sterilisation techniques were associated with changes in the scaffold topography in terms of pore size and surface roughness. These changes may be beneficial; increasing the ability of cells to penetrate and adhere to the scaffold. However, they may also have detrimental effects on the mechanical integrity and degradation rates of the scaffold *in vivo*. The use of peracetic acid and antibiotic treatments for typical durations were found to have the most significant changes on the scaffold structure. The required ethanol treatment of 30mins led to a small increase in pore diameter, which may potentially enhance nutrient transfer through the scaffold.

**REFERENCES:** [1] J.M. Karp, M.S. Shoichet, and J.E. Davies, (2003) *J. Biomed. Mater. Res.* **64A**:388-96. [2] C. Fischbach, J. Tessmar, A. Lucke, E. Schnell, G. Schmeer, T. Blunk, and A. Gopferich, (2001) *Surf. Sci.* **491**:333-45. [3] P.S. McFetridge, (2002) *PhD* University of Bath.