The effect of different sterilization techniques on material characteristics of a biodegradable nanocomposite polymer for use in tissue engineering purposes and its in-vitro and in-vivo biocompatibility

Lara Yildirimer[1], Alexander M. Seifalian[1,2]
[1]Centre for Nanotechnology & Regenerative Medicine, Division of Surgery & Interventional Science, University College London, London, United Kingdom, [2]Royal Free Hampstead NHS Trust Hospital, London, United Kingdom.

Statement of Purpose: An oft-underestimated aspect in the development of biodegradable materials for tissue regeneration applications is the ability to ensure sterility. Conventional lab-based sterilization methods frequently damage hydrolytically unstable materials leading to a collapse of the porous internal architecture, unpredictable changes in mechanical and surface properties and altered rates of biodegradation. In our laboratories, we have developed a novel bio-degradable polymer platform for the regeneration of lost skin tissues based on a polyhedral oligomeric silsesquioxane (POSS) incorporated into a poly(□-caprolactone-urea) urethane (PCL) backbone. We investigated the effectiveness of different sterilization methods for our POSS-PCL platform and compared changes in mechanical and surface characteristics induced by each method. Cell viability and DNA content analysis of adult human dermal fibroblasts (aHDF) were carried out. Samples were implanted subcutaneously into rats to investigate biocompatibility, immunogenicity and the rate of vascularization.

Methods: Polymer platforms were fabricated by solvent evaporation or coagulation/phase inversion methods. Sterilization techniques included autoclaving, exposure to microwaves (750 Watt), soaking in an antibiotic mixture or exposure to 70% ethanol. Non-sterilized samples served as controls. Sterility was assessed on cast and coagulated samples which were immersed in normal cell culture medium at 37°C for 7 days. Sterile medium alone served as the negative control. All experiments were conducted in triplicates. Post-sterilization, changes in scaffold characteristics were determined macroscopically (thickness, weight, contact angle) and microscopically (scanning electron microscopy (SEM), atomic force microscopy (AFM)). Mechanical parameters were measured using an Instron-5565 tensile tester (Instron Ltd., Bucks, UK). For in vitro cytocompatibility and proliferation evaluation, aHDF were cultured on porous platforms or on Integra® dermal regeneration scaffolds (Integra Life Sciences, Plainsboro, NJ) for 7 days. Cells were stained with propidium iodide nucleic acid stain (PI) and viewed under a confocal laser scanning microscope. Total DNA content was measured using a dsDNA quantification kit (FluoReporter Blue; Invitrogen Ltd, UK). In vivo subcutaneous implantations were carried out on the backs of male Sprague-Dawley rats. Porous POSS-PCL platforms (n=4) were inserted for 4, 8, or 12 weeks. At each time point, implant vascularization was assessed using oxy-genation and laser Doppler flowmetry (VisiSens, PreSens, Regensburg, Germany). Inflammatory changes in reaction to degradation products were evaluated using H&E staining on explanted POSS-PCL platforms and organs.

Results: After 7 days of incubation, all non-sterilized samples showed signs of infection while media alone remained clear. Autoclaving was excluded despite effective sterilization due to extensive polymer shrinkage. Antibiotic and microwave treatment resulted in unreliable sterilization and were rejected. Soaking in antibiotics further resulted in a significant loss in platform thickness (Fig.1, $p<0.05$). Ethanol effectively sterilized samples and was thus used for all in vitro and in vivo experiments. SEM images showed near-total destruction of platform porosity sterilized with each method bar ethanol treatment (Fig.1).

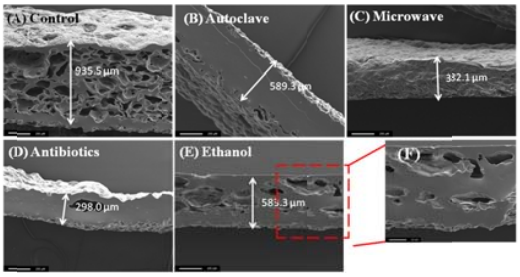

Figure 1.

AFM analysis demonstrated surface flattening effects following sterilization – most notably with antibiotic soaking (Fig.2). Mechanical parameters of sterilized

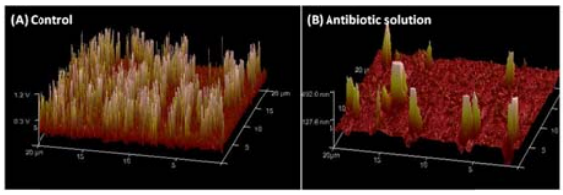

Figure 2.

POSS-PCL platforms did not differ significantly from controls ($p>0.05$). Surface wettability increased with each sterilization method ($p<0.05$). In vitro studies showed enhanced cell attachment, proliferation and transverse migration on POSS-PCL platforms compared to Integra® upon PI staining. Total DNA analyses corroborated these qualitative findings. In vivo exposure of POSS-PCL platforms demonstrated excellent biocompatibility and vascular in-growth into. Histological analyses of explanted organs exhibited no inflammatory reaction towards degradation products.

Conclusions: This study demonstrates sterilization-induced changes in material properties of our novel biodegradable polymer platform. In vitro and in vivo results showed promising biocompatibility profiles which are fundamentally important for clinical translation of any implantable devices. For future studies, the skin regeneration potential of this novel biomaterial shall be evaluated and optimized.