# Tissue Engineering

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## Pulsed Electric Field (PEF) treatment as a sterilisation method for collagen based biomatrices

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**Objectives** To investigate the effect of a Pulsed Electric Field (PEF) as a novel sterilisation method for collagen based biomatrices, testing the resultant collagen structure and function with physical and biological assays. PEF is a non-thermal inactivation method that has been utilized as a sterilisation method for the food industry. There is a need for a compatible method of sterilisation in tissue engineering because current methods of sterilising biomatrices, in particular gamma-irradiation (the most commonly used sterilisation method), have been shown to denature the structure of the collagen molecule.

**Methods** PEF treatment was carried out using a static chamber. For sterilization tests, type I collagen gels contaminated with *E. coli* (at $10^3$ CFU/ml) were subjected to PEF treatment at an electric field strength typically of 45 kV/cm, over a range of pulse numbers. Samples from the gel were enumerated for *E. coli* using nutrient agar, and three replications of the experiment were performed. For collagen structural tests, type I collagen solution was treated with PEF at electric field strengths between 30 kV/cm and 60 kV/cm for 100 pulses. These samples were compared with heat-treated collagen (1 hour at 90 °C in a water bath) and non-treated collagen solution (control) using SDS-PAGE to determine the effect of the treatments on the structure of the collagen monomers. Immortalised osteoblasts (FFC cell line) were cultured on either polystyrene dishes or hydroxyapatite discs coated with either PEF treated collagen, heat-treated collagen or non-treated collagen to determine the effect of PEF on the ability of the collagen to function as a biomaterial. The cells were assessed for viability, function and morphology in the following ways; protein content to assess growth rate, alkaline phosphatase activity to assess function, carboxyfluorescein diacetate/ethidium bromide staining followed by confocal laser scanning microscopy (CLSM) to assess viability and cell morphology, and phalloidin-FITC staining followed by CLSM to detect the actin cytoskeleton.

**Results** Inactivation of *E. coli* contaminated collagen gels was achieved through PEF treatment, with greater inactivation observed with increasing pulse numbers. A 3 $\log_{10}$ reduction in *E. coli* CFU count was obtained after 100 pulses. SDS-PAGE analysis revealed that heat treatment of the collagen solution denatured collagen monomers resulting in fragmentation. PEF treated collagen solution showed no obvious signs of fragmentation occurring, and was comparable to that of the non-treated control collagen. PEF treatment had no marked effect on the viability, growth rate, functional activity or morphology of the cells cultured on either the collagen films on polystyrene dishes or the collagen coated HA discs.

**Conclusions** PEF treatment can successfully inactivate *E. coli* seeded in a collagen gel at field strengths of approximately 45 kV/cm. The structure of the collagen monomers suffers no gross damage as a result of the PEF treatment, nor does the treatment affect the ability of the collagen to function as a biomaterial. The potential for development of the treatment into a safe compatible sterilisation method for tissue engineering matrices has therefore been established.

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## Production of silica-loaded scaffolds using supercritical methods for bone tissue engineering

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**Objectives** 1) To produce polymeric scaffolds loaded with silica using supercritical carbon dioxide as a foaming agent and porogen and 2) to assess the effects of process parameters and silica content on scaffold characteristics. The architecture of tissue engineering scaffolds is important as it influences mechanical properties and the ability to promote guided cell growth and proliferation. In the case of bone tissue engineering, it is desirable for scaffolds to encourage the deposition of the main mineral component of bone (hydroxyapatite; HA) on their surfaces. The incorporation of silica particles in the scaffold may provide nucleation sites for HA, enhancing the rate of mineralisation.

**Methods** Poly (lactic acid) (PLA) was dissolved in dichloromethane to form a viscous gel to which varying amounts of silica particles were added (0–60% w/w). The mixture was then cast as a thin layer onto a PTFE-coated surface and the solvent allowed to evaporate. Pieces of the resultant strip were placed in a cylindrical mould (diameter = 1 cm, length = 2 cm) that was then positioned within a stainless steel pressure vessel. $CO_2$ was introduced into the vessel and the temperature and pressure raised to 160 °C and 160 bar, respectively. At these conditions, the polymer was observed to soften sufficiently to allow the individual strips within the mould to form a single homogeneous mass. After leaving the polymer under pressure for 2 h, the $CO_2$ was evacuated from the vessel at a flow rate of 2.25 litres/minute, which induced foaming in the polymer. After cooling, the scaffolds were removed for analysis. The effect of depressurisation rate on the microstructure of pure PLA scaffolds was also evaluated by varying the evacuation flow rate from 0.2–2.25 litres/minute. The overall void fraction of the scaffolds was determined using geometric measurements and helium pycnometry. Pore size distribution was assessed by mercury intrusion porosimetry. X-ray micro tomography was employed to study the pore structures of the scaffolds.

**Results** Compared with scaffolds made from polymer alone, the incorporation of silica in amounts up to 40% w/w did not have a statistically significant effect on the overall void fraction. However, at silica contents above this, there was an increase in void fraction ($n = 8$, one-way analysis of variance/Dunnett, level of significance = 0.05 for 40% w/w and 0.01 for 50% and 60% w/w). As the proportion of silica in the scaffold was increased, the modal pore size of the scaffolds decreased; from around 0.8 mm in the absence of silica to 0.2 mm when 50% w/w was incorporated into the scaffold. This suggests that silica particles act as sites for pore nucleation. For scaffolds made from pure PLA, increasing the depressurisation rate was found to decrease the overall void fraction, presumably as pore growth was inhibited at higher depressurisation rates.

**Conclusions** Here we have demonstrated for the first time that it is possible to control the pore diameter of a scaffold by the addition of silica particles to a biodegradable polymer. Future work will seek to establish a relationship between silica loading and the mechanical properties exhibited by the scaffold, and its propensity for nucleating HA.