

Combined Treatment of Biomatrices with Nisin and Pulsed Electric Fields as a Potential Decontamination Method?

S. Griffiths^{1,2}, M. Maclean², S.J. MacGregor², J.G. Anderson², M.H Grant¹

¹ [Bioengineering Unit](#), ² [The Robertson Trust Laboratory for Electronic Sterilisation Technologies \(ROLEST\)](#), University of Strathclyde, Wolfson Building, 106 Rottenrow, Glasgow, UK.

INTRODUCTION: Pulsed electric field (PEF) treatment has been shown to achieve bacterial inactivation in collagen gels whilst retaining the ability of the collagen to function as a biomaterial [1, 2]. Nisin, an antimicrobial peptide, has been used widely as a food preservative and has shown bactericidal action against a number of Gram-positive bacteria [3]. The potential of nisin to increase the efficacy of PEF disinfection of collagen gels to be used for tissue engineering applications was investigated.

METHODS: Collagen gels, produced using type I collagen, were seeded with *Staphylococcus epidermidis* at concentrations of approximately 10³ CFU/ml. Firstly, seeded collagen gels were subjected to PEF treatment (45 kV/cm, 100 pulses, each of 1µs duration) using a static test chamber. Next, collagen gels, seeded with *S. epidermidis*, were produced containing either 500 or 3000 IU/ml of nisin and used to test the effect of nisin with and without PEF treatment on *S. epidermidis*. The surviving bacteria were enumerated as CFU counts after plating samples onto Brain Heart Infusion agar and incubating at 37 °C for 18-24 h. All treatments were repeated in triplicate and the level of inactivation determined.

The viability of mammalian cells cultured on collagen gels containing nisin was also assessed. Collagen gels were produced containing a range of nisin concentrations (0-3000 IU/ml) and then seeded (10⁴ cells/cm²) with 3T3 cells. After 3 days of culturing their viability was assessed by carboxyfluorescein diacetate/ethidium bromide staining and observed under a Carl Zeiss AxioImager fluorescence microscope.

RESULTS: Treatment with nisin alone at 500 IU/ml caused no significant reduction in the *S. epidermidis* cell population. At 3000 IU/ml a similar level of inactivation to PEF treatment alone was achieved (see Table 1). The greatest microbial inactivation was achieved with a combined treatment of nisin and PEF. These combined treatments achieved inactivation values greater than 3 log₁₀ CFU/ml.

No change to 3T3 cell viability or morphology was

observed when cultured on collagen gel containing nisin (see Figure 1).

*Table 1. Inactivation of S. epidermidis in collagen gel after exposure to nisin or PEF treatment alone and in combination. Results are mean ± SD, n=3. * P<0.05, compared with treatment with PEF or nisin alone, ANOVA with Dunnett's comparison.*

| Collagen gel treatment | Inactivation (Log ₁₀ CFU/ml) |
|-----------------------------------|---|
| PEF alone | 0.66 ± 0.09 |
| Nisin level at 500 IU/ml: | |
| Nisin alone | 0.01 ± 0.03 |
| PEF + nisin | 3.40 ± 0.23 * |
| Nisin level at 3000 IU/ml: | |
| Nisin alone | 0.61 ± 0.21 |
| PEF + nisin | 3.29 ± 0.08 * |

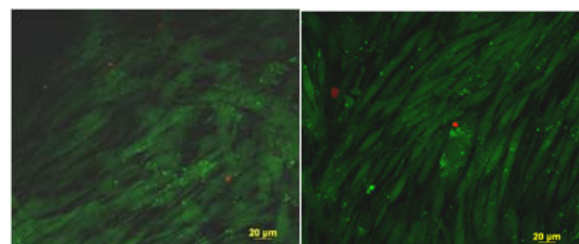


Fig. 1: 3T3 cells cultured for 3 days on native collagen gel (left) and on collagen gel containing 3000 IU/ml of nisin (right), stained with Ethidium bromide and CFDA

DISCUSSION & CONCLUSIONS: The incorporation of nisin into collagen gel greatly enhanced the lethal effects of PEF treatment. Further development may offer a safe compatible decontamination method for tissue engineering matrices.

REFERENCES: ¹ S. Smith, S. Griffiths, S.J. Macgregor, et al. (2008) *J Biomed Mater Res A*. DOI 10.1002/jbm.a.32150. ² S. Griffiths, S. Smith, S.J. Macgregor, et al. (2008). *J Appl Microbiol*. 105(4): p. 963-969. ³ C.K. Bower, J.E. Parker, A.Z. Higgins, et al. (2002). *Colloids and Surfaces B: Biointerfaces*. 25: p. 81-90.

ACKNOWLEDGEMENTS: EPSRC studentship, Doctoral Training Centre in Medical Devices- S.G.