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New application of Pulsed Electric Fields technology to decellularize *Ecklonia radiata* as a sustainable cellulose matrix for tissue engineering

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The development of tissue constructs relies heavily on scaffolding materials that support cell growth. These scaffolds must exhibit appropriate physical, mechanical, and biochemical properties essential for effective tissue repair. Decellularization techniques allow for the extraction of native extracellular matrix (ECM) from animal tissues, yielding biocompatible scaffolds for tissue engineering applications. Additionally, decellularized plant materials offer cellulose-based matrices that mimic ECM, presenting a sustainable, ethical, and cost-effective alternative. However, conventional decellularization processes predominantly use chemicals, raising concerns about their environmental impact.

Pulsed Electric Field (PEF) technology involves the application of high-voltage electric energy to biological materials in short pulses lasting from microseconds to milliseconds. This treatment alters the permeability of cell membranes, leading to the formation of pores, a process known as “electroporation”. Depending on the treatment, these pores can be reversible or irreversible, enabling modifications to the microstructure of biological materials, including seaweed. The efficiency of PEF treatment is influenced by the design of PEF generator including treatment chamber and the intrinsic properties of the biological materials such as composition and electric conductivity. PEF technology offers numerous advantages, including improving mass and heat transfer, and optimizing the efficiency of downstream processes like extraction and drying with minimal energy consumption. Hence, PEF is regarded as an environmentally friendly and sustainable technology. In recent years, PEF has gained attention for its potential applications in bioengineering and development of biomedical devices, particularly in decellularizing plant materials like seaweed to create sustainable cellulose-based bioscaffolds for tissue engineering. By inducing electroporation and facilitating the removal of cellular components, PEF pretreatment is hypothesized to reduce the reliance on chemical usage in plant decellularization processes. Therefore, the aim of this research was to study the potentials to use PEF technology as an eco-friendly alternative to chemical-based protocols for producing bioscaffolds from the brown seaweed *Ecklonia radiata*.

Wild-grown, intact *Ecklonia radiata* laminae were obtained from an authorized seaweed harvester in New Zealand. The laminae were immersed in cold (4°C) deionized water and placed in a PEF batch chamber consisting of two parallel stainless steel electrodes with an 8 cm gap. PEF treatment was performed at an electric field strength of 0.8–2 kV/cm and a specific energy range of 5–50 kJ/kg, with a constant frequency of 100 Hz and a pulse width of 7 microseconds. PEF treatment, with or without chemical post-treatment using a 1% Triton X-100 and 0.1% bleach solution, was applied to decellularize intact *E. radiata* laminae.

The results showed that PEF-treated scaffolds preserved the tri-layered structure. Histological analysis and scanning electron microscopy (SEM) revealed that the resulting scaffolds closely resembled the native structure, consisting of an inner layer of cellulose fibres surrounded by two porous layers, each 8–10 pores thick. PEF-treated scaffolds exhibited larger pores than those observed in chemically treated scaffolds. PEF pre-treatment significantly accelerated the removal of DNA and pigment content from *E. radiata* at a much faster rate compared to chemical treatment alone. The scaffolds remained stable under culture conditions and supported the growth of human BJ/5Ta dermal fibroblasts for seven days. SEM and confocal microscopy demonstrated dense cell populations distributed throughout the inner fibrous layer of the scaffold. PEF-treated scaffolds with reduced chemical intervention exhibited biocompatibility with human cells. These findings suggest that PEF technology provides a promising, sustainable approach for producing cellulose-based bioscaffolds suitable for tissue engineering applications.