

## BIOENGINEERING EYELID TARSAUS

**Purpose:** Sourcing a suitable substitute for eyelid tarsus during reconstruction remains an ongoing challenge. We aimed to develop a bioengineered tarsus tissue complex by combining a novel chitosan scaffold with orbital skin fibroblasts.

**Methods:** Cryogelation was used to produce three-dimensional macroporous chitosan hydrogel scaffolds with pore sizes ranging from 10-100 $\mu$ m. The internal architecture and mechanical properties were regulated by controlling various processing parameters. Tensile testing was performed throughout development to ensure optimal biomechanical properties mimicking native tarsus tissue. Orbital skin fibroblasts were cultured using 1-2mm samples of human eyelid skin. Immunohistochemical staining was used to characterise the rate of fibroblast de-differentiation in vitro to identify the optimum fibroblasts for culture onto the chitosan scaffolds.

**Results:** The elastic modulus of the bioengineered chitosan scaffold was found to closely resemble human tarsus tissue. Orbital skin fibroblast culture typically reached confluence within 3 weeks and fibroblasts from the second passage of culture stained strongly for all fibroblast markers. The scaffolds were found to successfully support the growth and proliferation of human orbital skin fibroblasts in vitro as demonstrated by scanning electron and confocal microscopy. Further studies evaluating the safety of this bioengineered tarsus tissue are already underway.

**Conclusion:** Our novel chitosan scaffold has demonstrated both biomechanical compatibility and the ability to support orbital skin fibroblast culture. This study represents the first of its kind in the field of oculoplastic ophthalmology which has tremendous potential to be further developed and applied to clinical practice in order to improve patient outcomes following eyelid reconstruction.