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# The Biomechanics of eyelid tarsus tissue

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ABSTRACT

Reconstruction of the eyelid remains challenging due to the unique properties of the tarsal plate, which is a fibrocartilagenous structure within the eyelid providing structural support and physical form. There are no previous studies investigating the biomechanical properties of tarsus tissue, which is vital to the success of bioengineered tarsal substitutes. We therefore aimed to determine the biomechanical properties of human tarsus tissue, and used a CellScale BioTester 5000 (CellScale, Waterloo, Canada) to perform uniaxial tensile tests on ten samples of healthy eyelid tarsus. All samples were tested 'fresh' within two hours of harvest. A tensile preload of 50 mN was applied for 10 min before the sample was subjected to uniaxial tension under linear ramp displacement control. Maximum strain was 30% of the original tissue length and thirty dynamic cycles were performed at a strain rate of 1%/s using a triangular waveform. Of the samples tested, the mean (SD) width was 5.51 mm (1.45 mm) whilst mean thickness was 1.6 mm (0.51 mm). The mean toe modulus was 0.14 (0.10) MPa, elastic modulus was 1.73 (0.61) MPa, with an extensibility of 15.8 (2.1)%, and phase angle of 6.4° (2.4)°. After adjusting for the initial tissue slack, the maximum strain ranged from 23.8% to 30.0%. At maximum strain, it was observed that the linear region of the stress–strain curve was reached without the sample slipping out of the clamps. Our results establish a benchmark for native tarsus tissue, which can be used when evaluating tissue engineered tarsal substitutes in the future.



## 1. Introduction

The eyelid is supported by a fibrocartilagenous layer named the tarsus. The tarsus provides both structural support and physical form, making it an essential component of the eyelid's function and appearance. In the upper eyelid, the tarsal plate measures approximately 25 mm in length, with maximal central height of 10 mm. Full thickness eyelid defects which cannot be closed directly require reconstruction of both the anterior and posterior lamellae, which include the tarsal plate and palpebral conjunctiva (Fig. 1) (DiFrancesco et al., 2004). Tarsal repair is vital for functional eyelid reconstruction, but presently remains limited by the complexity of tarsus tissue and lack of suitable tarsal substitutes.

Reconstruction of the eyelid is commonly required following large tumour excision, trauma or congenital defects. The former is particularly significant in Australia, where non-melanoma skin cancer represents the most common and expensive cancer nationwide, with the periorbital region involved in approximately 10% of cases (Cook and Bartley, 2001; Fransen et al., 2012). Basal cell carcinoma, and squamous cell carcinoma are the two most common eyelid cancers in Australia, with a mean age at presentation between 61 and 67 (Donaldson et al., 2002; Malhotra et al., 2004a, 2004b). Tarsal substitutes described previously include nasal septal cartilage and mucous membrane, auricular cartilage and skin, buccal mucous membranes, hard palate, preserved sclera, irradiated homologous tarsus, aorta and artificial tarsal plates (DiFrancesco et al., 2004; Ito et al., 2001; Jordan and Anderson, 1997; Jordan et al., 1990; Kamiya and Kitajima, 2003). Issues encountered with each of these substitutes include any, or a combination of: difficulty with harvest, inadequate strength to support the reconstructed eyelid, thickness and rigidity of the material, deformity or shrinkage over time, difficult donor-site healing and local inflammation. Presently there are no reconstruction methods that are completely satisfactory, and a new solution is required to achieve the desired outcomes for patients (Ito et al., 2001, 2007; Kamiya and Kitajima, 2003).

Tissue engineering aims to produce functional substitutes to repair defects, and the use of engineered three-dimensional

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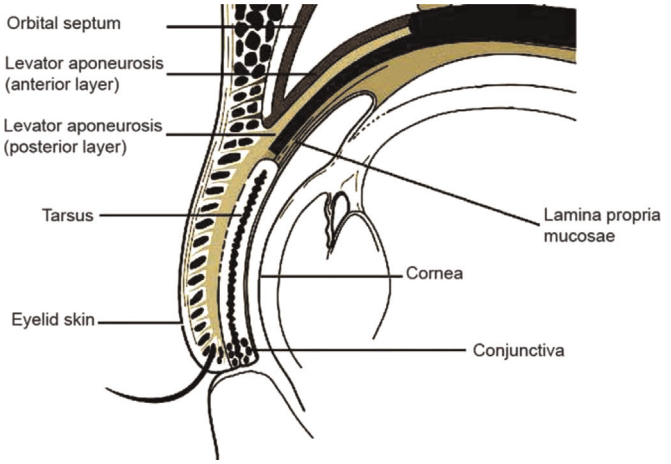


**Fig. 1.** The anatomy of the eyelid.

biomaterial constructs to reconstruct or repair living tissue has been widely investigated over the last two decades (Ladewig et al., 2012; O'Connor and Morrison, 2013; Sun et al., 2011). Most recently, Zhou et al. (2010) developed a synthetic tarsal substitute using microbial poly (3-hydroxybutyrate-co-3-hydroxyhexanoate) with promising results in animal studies. However, the mechanical properties of the bioengineered tarsus were not tested, and, to the best of our knowledge, there are no previous studies investigating the biomechanics of normal tarsus tissue. It has been shown that the behaviour of cells, including their adhesion, migration, proliferation, differentiation and gene expression, is affected by their local physicochemical microenvironment (Discher et al., 2005; Engler et al., 2006). Therefore it is important to understand the mechanics of the tissue to be engineered in its native state in order to design suitable scaffolds for tissue engineering. We aimed to better understand the viscoelastic behaviour of normal human tarsus tissue by undertaking biomechanical testing on fresh samples of human tarsus following surgical removal.

## 2. Materials and methods

Ten samples of healthy tarsus tissue were obtained from ten patients, 7 male and 3 female (median age (range): was 71.5 (63–86)), undergoing various ophthalmic procedures involving the removal of eyelid tissue at the Royal Adelaide Hospital. Examination prior to surgery revealed normal lid laxity in all patients. The removal of healthy eyelid tissue in all cases was in keeping with standard practise, and it would otherwise have been discarded if not used in our study. Ethics approval was obtained from the Royal Adelaide Hospital and the Southern Adelaide Clinical Human Research Ethics Committees and all patients provided informed consent. All tissue samples studied were free from disease, and eyelid laxity assessed clinically prior to the procedure was normal in all cases. The tarsal layer was dissected from the eyelid into approximately 5 mm wide $\times$ 1.5 mm thick samples. All samples were placed in a solution of phosphate buffered saline (PBS) at room temperature and immediately transported to the laboratory for biomechanical testing.

All tarsus samples were tested 'fresh' within 2 h of excision (i.e. without any prior freezing or storage). Upon arrival at the laboratory, each tarsus sample was trimmed into a rectangular shape using a scalpel. Uniaxial tensile tests were performed using a CellScale BioTester 5000 (CellScale, Waterloo, Canada), a micromechanical testing system specially designed for biological materials (Fig. 2). The sample was inserted into a pair of custom-made tissue clamps, fixed to two opposing actuator arms, and, in keeping with the anatomical alignment of the tarsus, the loading axis was aligned in the mediolateral direction. This direction represents the primary direction of tension on the eyelid, as the tarsoligamentous sling between the medial and lateral orbital rims supports the eyelid against gravity, resulting in minimal tension in the vertical direction. This is also evidenced by the horizontal tension lines in the eyelid and the general surgical principle of repairing defects using incisions parallel to relaxed tension lines (Rosser and McCormick, 2008). The clamped samples, oriented horizontally, were lowered into a PBS bath maintained at $37 \pm 1$ °C for the entire duration of the experiment (Fig. 3). Sample length, width and thickness were measured photogrammatically using the overhead charge-coupled device (CCD) camera. The camera was

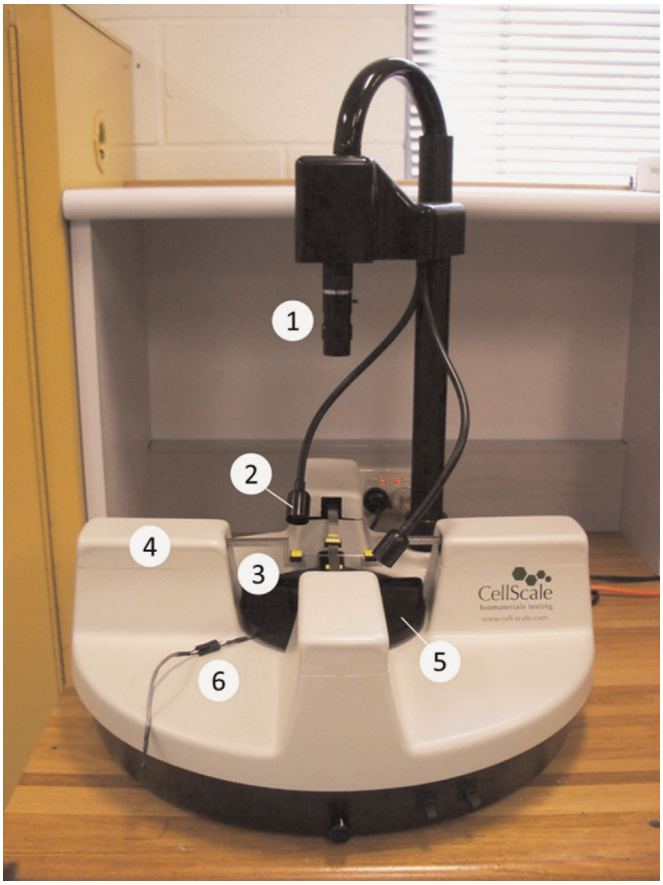


**Fig. 2.** The CellScale BioTester. (1) An overhead charge-coupled device (CCD) camera, which monitors the sample as it is being stretched. (2) Overhead lamps to illuminate the sample. (3) Four actuator arms fitted with custom-made tissue clamps that hold the sample in place. As uniaxial testing was performed in this study, only two of the four actuator arms were required. (4) Load cells for measuring the force on the sample. The setup in this research used 23 N load cells ($\pm$0.1% error), which were housed in protective compartments on the sides of the device. (5) A water bath, which sits on top of a heated platform. (6) A temperature gauge, which is placed in the water bath to automatically monitor and control the temperature of the testing environment.

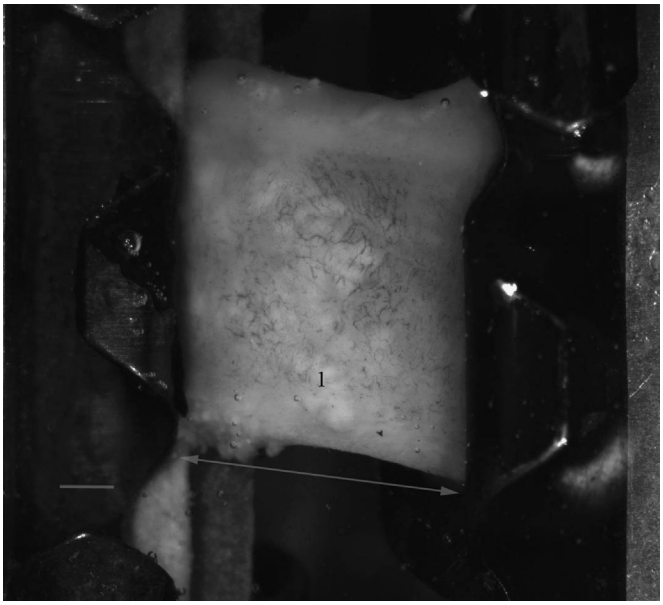


**Fig. 3.** A tarsus sample being tested fresh using the CellScale BioTester. The tarsus sample (1) is clamped in the mediolateral (arrow) direction. Scale bar represents 1 mm.

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