Short Communication

Measurements and models of the toughness of adipose tissue

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Abstract

The measured toughness J_C of adipose and dermal porcine tissues are 4.1 kJ m^{-2} and 17 kJ m^{-2}, respectively, via a trouser tear test. A simplified micromechanical model for toughness is developed for adipose tissue. The analysis shows that the toughness of adipose tissue is determined by the collagen network that surrounds the adipocytes. The volume fraction of the interlobular septa is sufficiently low for it to make a negligible contribution to the macroscopic toughness.

Keywords: adipose tissue, foams, micromechanics, fracture toughness, trouser tear test

1 Introduction

Subcutaneous adipose tissue is a soft connective tissue that resides under the dermal layer of skin (Goldberg and Rabinovitch 1983). The perforation response of a soft tissue is dependent upon its toughness in addition to its stress-strain response, as discussed by Shergold and Fleck (2004). Dermal tissue is made of bundles of collagen fibres, and these fibres possess similar anatomical properties to those of the interlobular septa found within adipose tissue. The toughness of rodent dermis is between 13.2 kJ m^{-2} and 26.9 kJ m^{-2} (Purslow 1983). In order to make predictions for the tear resistance and penetration force of adipose tissue, it is necessary to measure its toughness.

In the current study, the mixed mode toughness of adipose is measured using a trouser tear test as described by Rivlin and Thomas (1953). Purslow (1983) recommended the use of a trouser tear test for soft solids because it is easy to set-up, does not require a large quantity of test material and generates slow and controlled crack propagation. The crack tip zone of adipose tissue is examined by scanning electron microscopy (SEM) and a micromechanical model of toughness is presented. For comparison purposes, a limited set of toughness measurements are also reported for porcine dermal tissue.

1.1 Review of the histology and mechanical properties of adipose tissue

Adipose tissue is comprised of lipid-filled cells called *adipocytes*. Each adipocyte has a diameter of approximately 80 µm and comprises a single lipid vacuole and a nucleus within a phospholipid bilayer (Sheldon 1965; Bjorntorp and Martinsson 1966). Mature adipocytes are arranged within ill-defined lobules of diameter about 1 mm and are enclosed within an extra cellular matrix constructed from two distinct collagen-based structures, reminiscent of closed-cell and open cell-foams (see Fig. 1): *reinforced basement membrane,* a woven filamentous collagenous structure that surrounds each adipocyte (Abrahamson 1986; Comley and Fleck 2009b), and *interlobular septa,* a predominantly type I collagen fibre network (Urmacher 1997). The intervening space is filled with *ground substance*. 60-80 mass % of the adipose tissue is lipid, 5-30 mass % is water and the remaining 2-3 mass % is composed of proteins (Greenwood and Johnson 1983).

At low strain rates adipose tissue is a compliant viscoelastic material with a Young's modulus $E^* \approx$ 1 kPa (Miller-Young *et al.* 2002; Nightingale *et al.* 2003; Gefen and Haberman 2007; Comley and Fleck 2009). At low strain amplitude (< 1 x 10^{-3}) and frequencies up to 100 Hz the compressive storage modulus E' equals 1.8 kPa and the compressive loss modulus E'' equals 0.4 kPa (Patel *et al.* 2005; Geerligs *et al.* 2008; Comley and Fleck 2009).

The toughness J_C of a non-linear elastic material is determined by the rate of change in potential energy of the cracked body with crack length. Gutierrez-Lemini (2002) shows that the methods developed for calculating J-integral for an elastic body can also be applied to viscoelastic solids since J_C is not dependent upon the heredity of the solid. However, only indirect measurements have been reported for the toughness of adipose tissue. For example, in an investigation into stab wounds, O'Callaghan (1999) measured a force of 2 N to penetrate adipose tissue with a sharp instrumented knife.

2 Experimental method

Samples of fresh porcine adipose tissue from the jowl and dermal tissue from the foreleg were obtained[2] and stored in Phosphate Buffered Saline (PBS) prior to testing (maximum 4 hours). Eleven rectangular samples of porcine adipose tissue, of thickness B = 5 mm, length L = 30 mm and width W = 20 mm, were partially slit lengthwise to form two legs with a ligament length of approximately D = *L/3*, as sketched in Fig. 2. Seven similar samples were prepared for dermis of dimension L = 30 mm, W = 20 mm and B = 3 mm. The legs of each specimen were gripped in a

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[2] Tissue samples obtained from Dalehead Foods, Linton, Cambs, UK

screw-driven tensile test machine, see Fig. 2. The grips were pulled apart at a constant displacement rate $\dot{u}$ in the range 0.01 mms^{-1} to 10 mm s^{-1} and the force F versus displacement u was recorded.

3 Results and discussion

The force versus displacement curves for the trouser tear tests of (a) adipose and (b) dermal tissue are shown in Fig. 3 for selected values of $\dot{u}$. In each case the load F increased with displacement until a plateau value $\overline{F}$ was achieved. Subsequent oscillation of F about this mean value was observed. Crack propagation of two samples for each tissue type was monitored using a video camera. This revealed that crack growth initiated when the load achieved the plateau value, and thereafter, the crack growth rate $\dot{a}$ was constant. Thus, it was adequate to deduce $\dot{a}$ from the total crack extension. For steady crack growth at an average fracture force $\overline{F}$, the critical toughness J_C is given by (Rivlin and Thomas 1953; Purslow 1989; Chin-Purcell and Lewis 1996)

$$J_C = \frac{2\overline{F}}{B} \tag{1}$$

The toughness J_C is plotted as a function of crack velocity $\dot{a}$ in Fig. 4 for both tissues. The average toughness is J_C = 4.1 kJ m^{-2} ± 1.2 kJ m^{-2} (1 s.d.) for porcine adipose tissue and J_C = 17 kJ m^{-2} ± 4 kJ m^{-2} (1 s.d.) for porcine dermis, independent of crack velocity.

The above results share some of the features previously observed by Purslow (1989) for trouser tear tests on rat dermis. Purslow observed a fluctuating load history, but found that the toughness increased with crack velocity. The toughness measured here for the dermis is of a similar magnitude to the values reported by Purslow (1983).

3.1 A micromechanical model for toughness

In order to determine the micromechanism of crack advance observations were made of the crack tip. A test at $\dot{u}$ = 0.1 mm s^{-1} was interrupted after a crack extension of 3.8 mm. The test specimen was clamped by cotton thread binding to a glass slide in the deformed configuration, 7 mm from the base of each leg, see Fig. 5a (stress concentrations caused by the cotton clamping were sufficiently far from the crack tip as to have no affect the on its collagen structure). Having clamped the specimen to the glass splint it was removed from the test machine and fixed according to the procedure described by Comley and Fleck (2009b). After fixing the glass slide was removed and the crack tip zone was examined in a SEM.

Scanning electron micrographs of the fracture surface at the crack front are shown in Fig. 5b. Two distinct features of the collagen network are observed: the reinforced basement membrane has collapsed and septa fibres are exposed at the crack tip. The adipocytes have ruptured allowing the lipid to leak out, resulting in the collapse and breakdown of the woven collagen networks. Fractured septa fibres are loose and wavy whereas the intact fibres span the crack wake.

A micromechanical model of the toughness of adipose tissue is now presented. It will be shown that the main contribution to toughness is from the reinforced basement membrane. The contribution of the septa fibres to the macroscopic toughness is deemed to be negligible by the following argument. Assume that the septa fibres resemble an open cell foam of volume fraction $\bar{\rho}_s$ made from elastic-brittle cell walls with cell length *l*, effective modulus E_s^* and fracture strength σ_f. The fracture toughness K_{IC} of the foam is given by (Gibson and Ashby 1997)

$$K_{IC} = 0.65\bar{\rho}_s^{3/2}\sigma_f\sqrt{\pi l} \tag{2}$$

The toughness of the septa follows immediately as

$$J_C = K_{IC}{}^2 / E_s{}^* \qquad (3)$$

Data for the tensile strength of individual septa fibres are not available. However, the tensile strength of the dermis provides a suitable estimate for the tensile strength of the septa fibres, $\sigma_f \approx$ 10 MPa (Ankersen *et al.* 1999). Upon taking $\bar{\rho}_s = 3 \times 10^{-4}$ and l = 1 mm the fracture toughness of the septa network is estimated to be K_{IC} = 1.89 N $m^{-3/2}$ via Eq. (2). Recalling that the estimated effective modulus of the septa network is E_s^* = 90 Pa, the toughness of the septa is J_C = 40 mJ m^{-2}, via Eq. (3). This is substantially lower than the measured toughness of adipose tissue, J_C = 4.1 kJ m^{-2}, and suggests that another source of toughness exists within adipose tissue: the reinforced basement membrane.

In order to estimate the toughness contribution from the reinforced basement membrane, recall that it resembles a closed cell foam in morphology. Now, the toughness of a closed cell foam J_C scales with the toughness of the wall material J_{Cm}, according to the rule-of-mixtures formula $J_C = \bar{\rho}_m J_{Cm}$, where $\bar{\rho}_m$ is the volume fraction of reinforced basement membrane. From the current study the toughness of adipose tissue is J_C = 4.1 kJ m^{-2} and $\bar{\rho}_m = 0.1$. Consequently, the toughness of the reinforced basement membrane is estimated to be J_{Cm} = 40 kJ m^{-2}. No independent measurements are available to confirm or refute this inferred value, but it is plausible it is of similar magnitude to that of dermal tissue.

4 Concluding remarks

The current study has made use of microstructural observations and macroscopic measurements of toughness in order to develop micromechanical models for the toughness of porcine adipose tissue. The models suggest that the toughness of adipose tissue derives from the reinforced basement membrane, with a negligible contribution from the interlobular septa fibres.

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Conflict of interest

All authors deny having any financial and personal relationships with other people or organizations that could inappropriately influence our work.

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Figure Captions

Fig. 1: A sketch of a lobule of adipose tissue (Comley and Fleck 2009).

Fig. 2: Sketch of (a) the dimensions of a tissue sample and (b) the orientation of a sample during a trouser tear test.

Fig. 3: Results from trouser tear tests at selected displacement rates for (a) porcine adipose tissue and (b) porcine dermis.

Fig. 4: Toughness J_C versus crack growth rate $\dot{a}$ for adipose and dermal tissue. The average values of J_C are shown by solid lines.

Fig. 5: (a) the method by which a specimen was fastened to a glass slide immediately following a trouser tear test. (b) Scanning electron micrographs of the crack tip region in adipose tissue fractured during a trouser tear test.

Figure 1

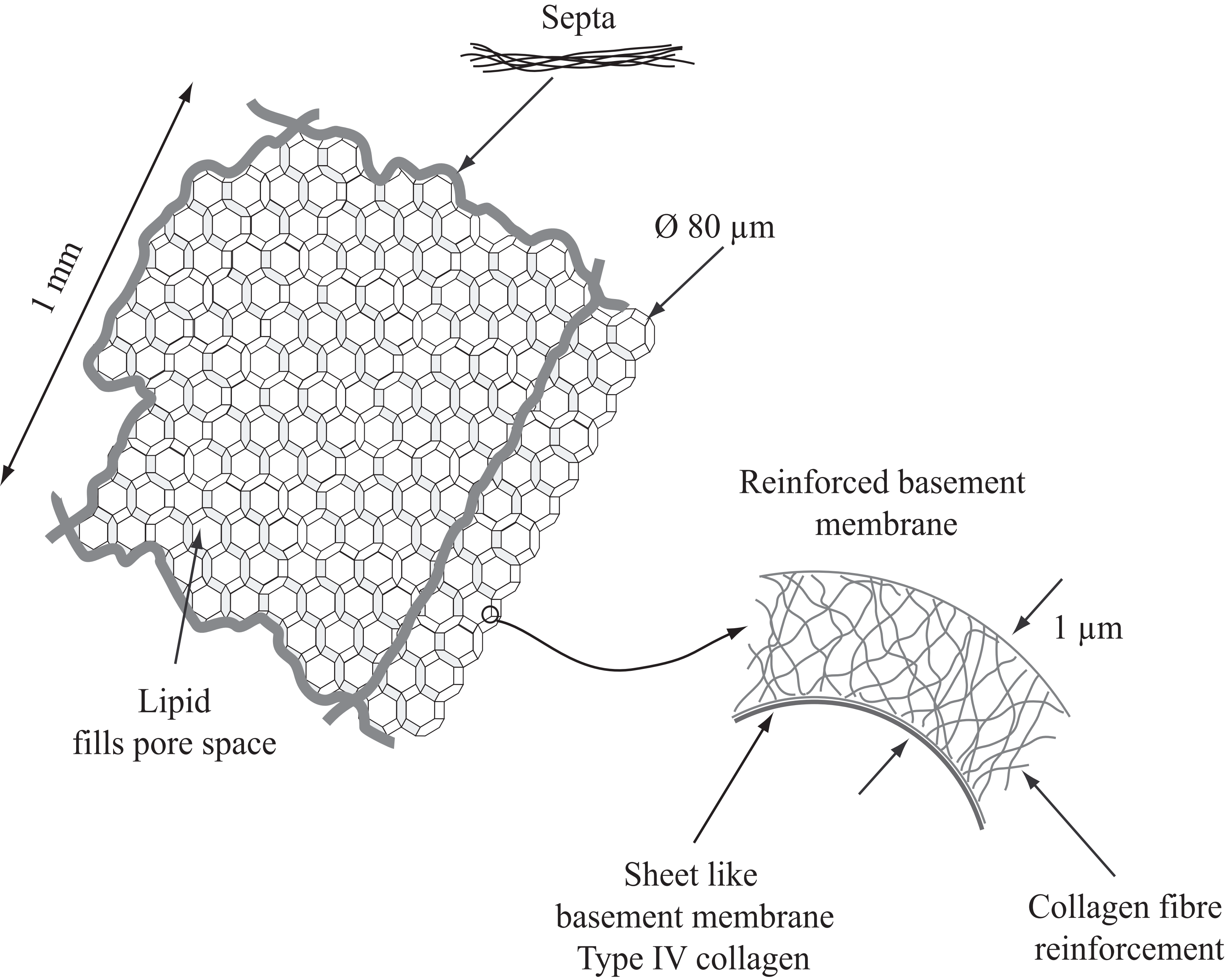

Septa
1 mm
Ø 80 µm
Reinforced basement membrane
1 µm
Lipid fills pore space
Sheet like basement membrane Type IV collagen
Collagen fibre reinforcement

Figure 2

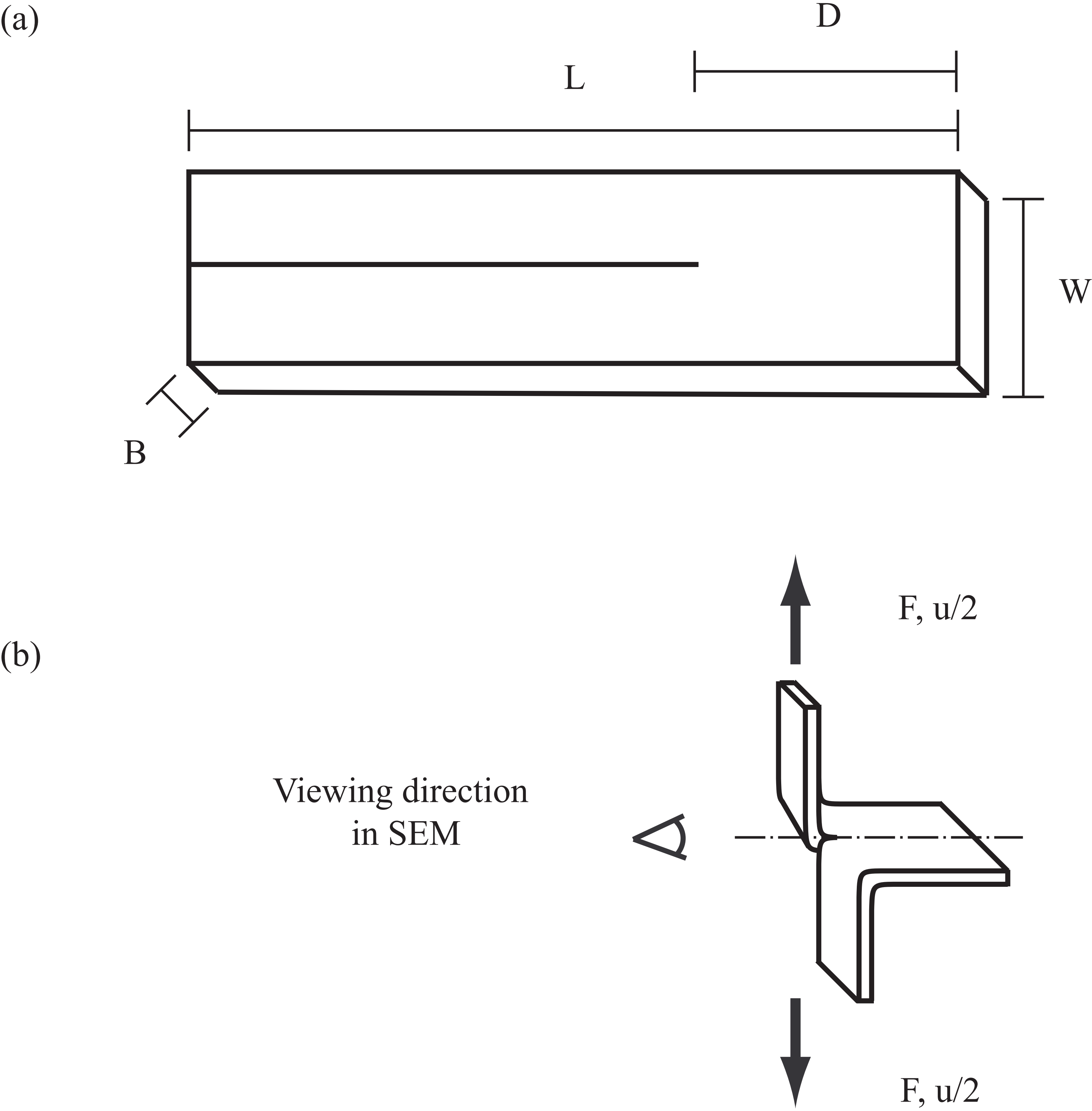


Figure 3

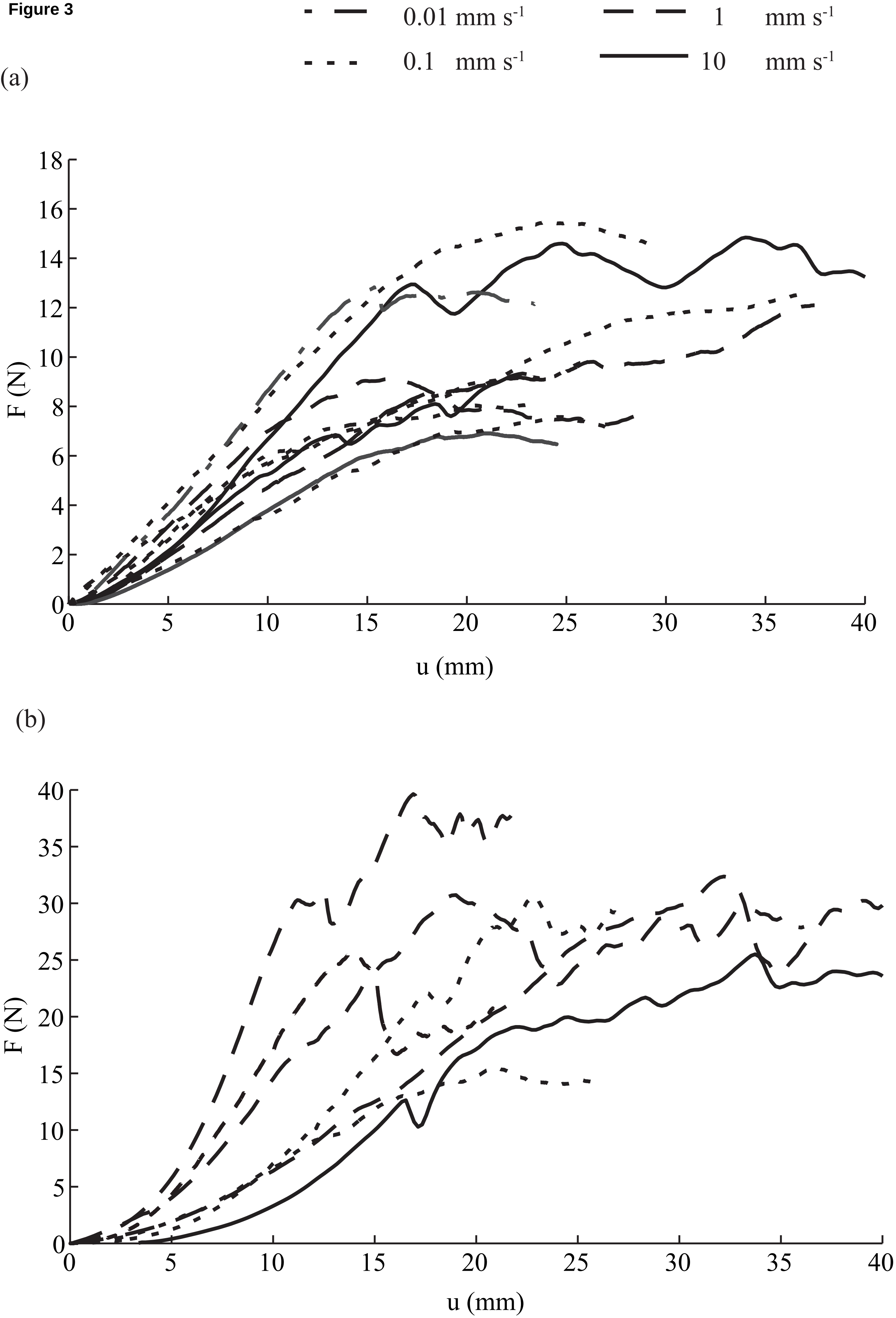

0.01 mm s-1
1 mm s-1
0.1 mm s-1
10 mm s-1
(a)
F (N)
0
2
4
6
8
10
12
14
16
18
u (mm)
0
5
10
15
20
25
30
35
40
(b)
F (N)
0
5
10
15
20
25
30
35
40
u (mm)
0
5
10
15
20
25
30
35
40

Figure 4

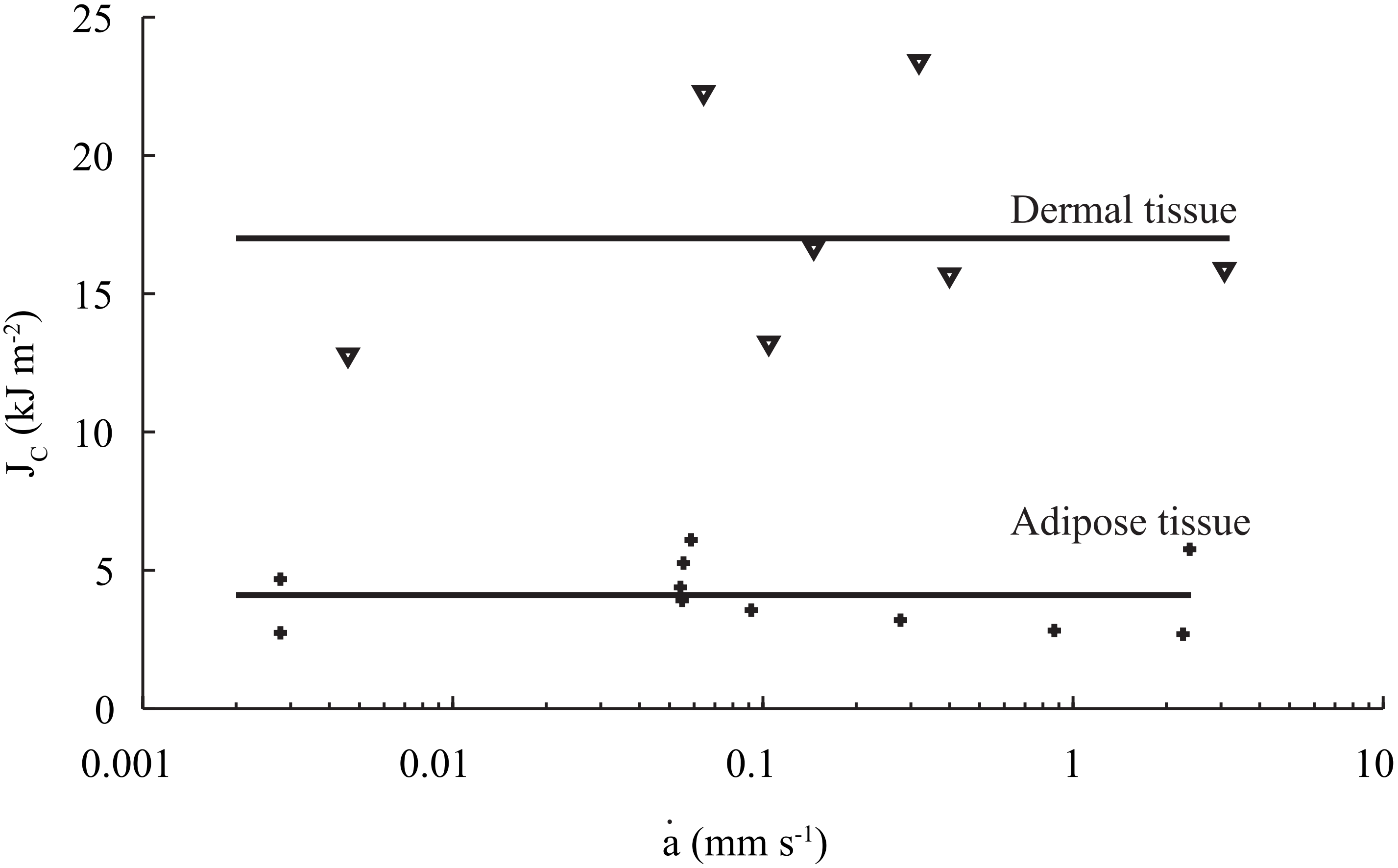
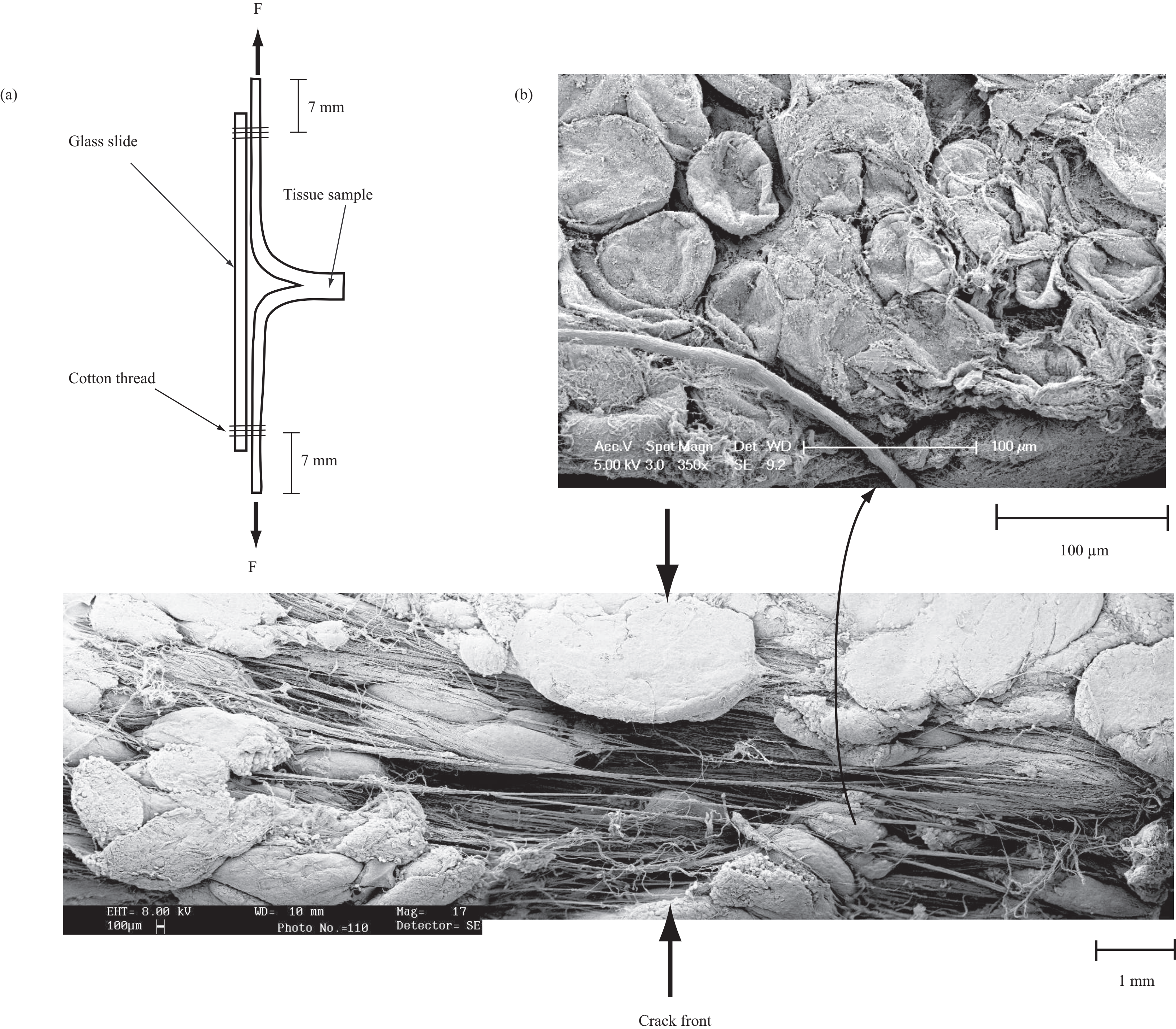

25
20
15
10
5
0
J_C (kJ m^{-2})
Dermal tissue
Adipose tissue
0.001
0.01
0.1
1
10
$\dot{a}$ (mm s^{-1})

Figure 5


(a)
F
7 mm
Glass slide
Tissue sample
Cotton thread
7 mm
F
(b)
Acc.V Spot Magn Det WD
5.00 kV 3.0 350x SE 9.2
100 µm
100 µm
EHT= 8.00 kV WD= 10 mm Mag= 17
100µm Photo No.=110 Detector= SE
1 mm
Crack front