An improved peel test method for measurement of adhesion to biomaterials

K. BUNDY
Biomedical Engineering Department, Tulane University, New Orleans, LA 70118, USA
E-mail: kbundy@mailhost.tcs.tulane.edu

U. SCHLEGEL, B. RAHN, V. GERET, S. PERREN
AO Research Institute, Clavadelerstrasse, CH-7270 Davos, Switzerland

Adhesion of tissues to biomaterials is desirable to prevent bacterial proliferation and for epithelial/transmucosal sealing of transcutaneous appliances, but can be counter-productive elsewhere, e.g. implants contacting tendons or maxillofacial subcutaneous tissue. It is therefore important to gauge adhesion strength of tissues to biomaterials before clinical use. Peel-testing is widely used for industrial product adhesion monitoring, but has rarely been applied biomedically. Here we describe peel-testing instrumentation designed for testing adherence of soft tissues to biomaterials. It offers the advantage that a 90° angle between peel and substrate is maintained, simplifying determination of applied normal forces separating tissue layers from material surfaces. The device is portable and can be brought directly to the specimen removal site. This minimizes time delays between explantation and testing, maintaining the tissue/biomaterial interface in the freshest possible state closely approximating *in vivo* conditions, and so avoids measurement artifacts. So far, the instrument has been used to test adhesion of tape to a biomaterial surface (for determining the device's technical performance), assess strength of tissue adhesives, and measure adhesion of subcutaneous tissue to orthopaedic biomaterials. However, its versatility suggests additional applications for the peel-tester where adhesion of soft tissue to biomaterials is of interest.

1. Introduction

Peel-testing is a well established methodology in industrial applications involving tapes, adhesives and the like, and has been used to a limited extent in the biomaterials field [1, 2]. Various standard methods for conducting peel tests have been described [3–15] including T-peel, cleavage peel, climbing drum and floating roller techniques. Although these tests have many important industrial uses, our main interest in peel-testing involves measurement of soft tissue adherence to biomaterials. For this purpose standard techniques present certain disadvantages. For example, the status of the soft tissue/biomaterial interface may be rather unstable and susceptible to dehydration and other changes, while at the same time the delay between surgical removal of the specimen and the actual conduct of the peel test may be substantial. During sample transport and storage, the tissue/material interface may therefore be unacceptably modified. Thus, for biological samples, it would be beneficial to minimize the delay between explantation and testing. Secondly, the variable angle between the peeling force and the substrate in some of the standard tests presents a complication from the biomechanical point of view; it would be desirable to maintain a constant angle throughout the test. The device described in the present study overcomes both of these disadvantages.

2. Materials and methods

2.1. Peel-tester design, specifications and capabilities

The basic idea behind our peel-tester is that the sample to be tested is held in a holder which moves horizontally at the same velocity V as the tissue is pulled from the specimen vertically, maintaining a 90° angle between the peel and the substrate. The preamplified output of a force transducer serves as the input to a chart recorder, producing a force versus time record. Because of the constant velocity of peeling, this record is equivalent to a force (F) versus displacement (δ) curve. The principle behind our peel-tester is shown in Fig. 1.

The overall dimensions of the peel-testing device are 11 cm × 11 cm × 21 cm, and it weighs only 2.5 kg. Peel test measurements can be made directly on-site and completed within approximately 2 min from the time when the specimen is surgically removed from the animal. A photograph of the actual device is shown in Fig. 2. After removal from the animal, the specimen with its soft tissue covering is placed in a well of appropriate

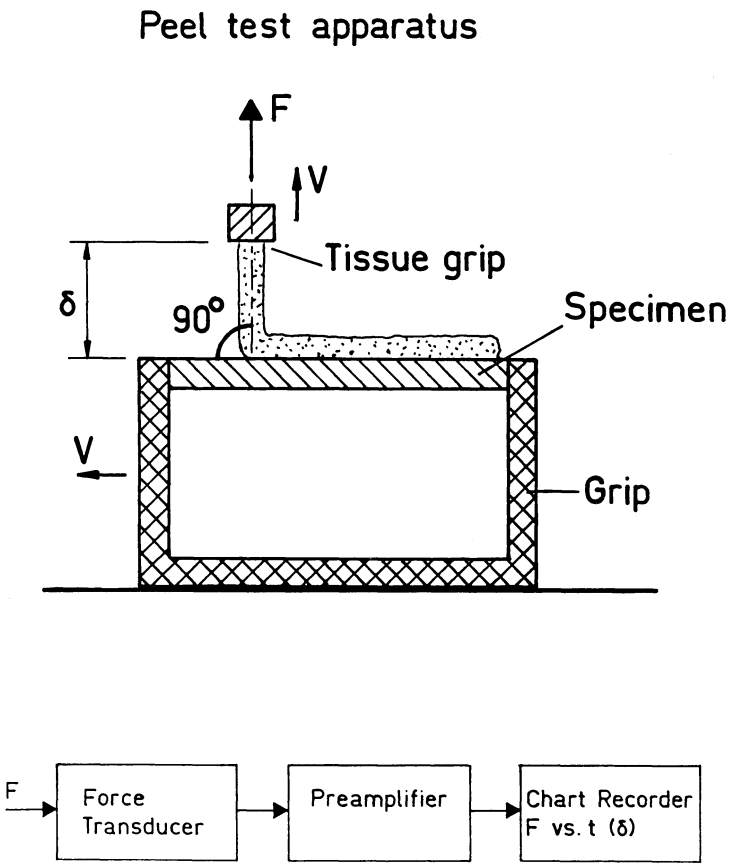

Figure 1 Schematic diagram for peel-tester that maintains a right-angle between specimen and tissue.

size, shown at the extreme right-hand side of Fig. 2, on the base of the device. A rectangular, four-sided knife made from microtome blades can be seen directly above this well. This knife is precisely positioned so that the handle shown in the figure can be used to lower the knife so that it cuts through the soft tissue layer directly at the border of the specimen, nontraumatically trimming off excess tissue. The specimen with its remaining tissue

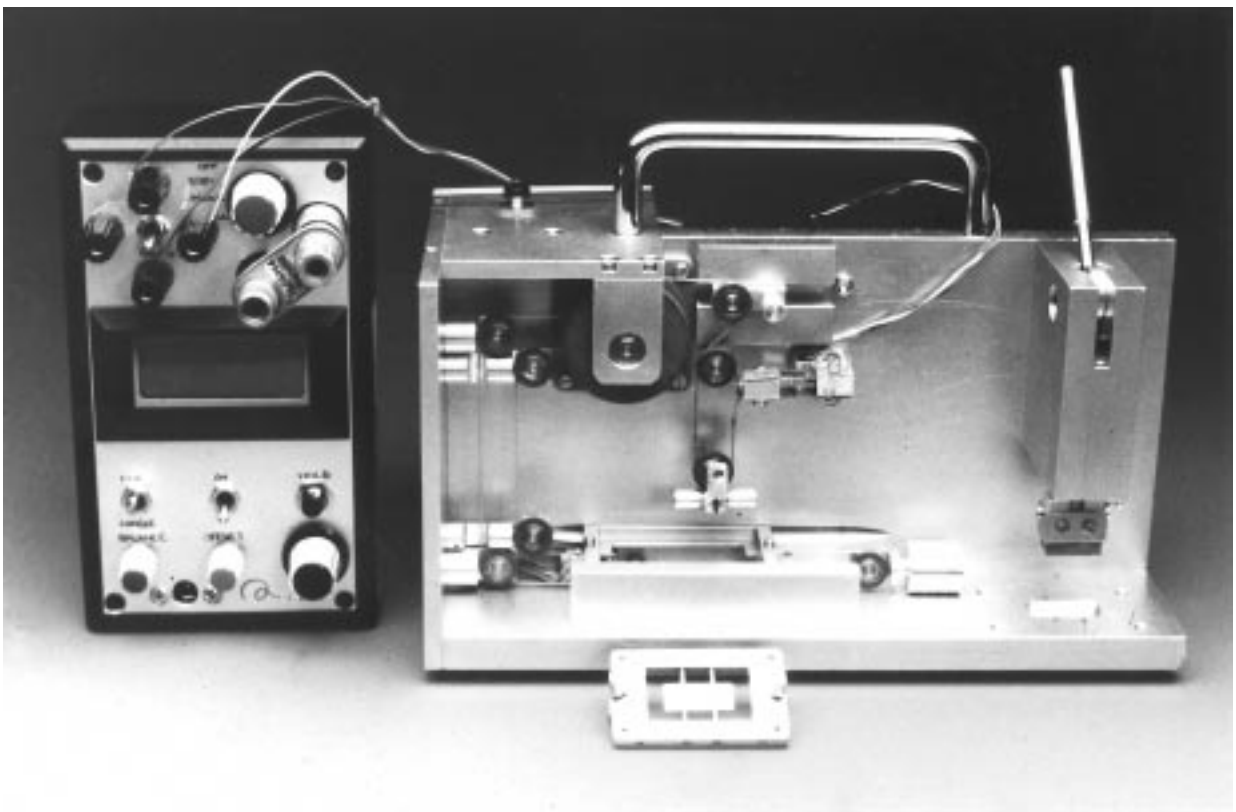

Figure 2 Peel-testing device with specimen holder, tissue trimmer and preamplifier.

covering is then quickly removed from the well and placed in the specimen carrier shown in the foreground of the figure. To show the rectangular test specimen with clarity, a sample without an overlying tissue layer is shown in the figure. The specimen carrier is then placed in a holder (shown in Fig. 2 directly above the carrier), and the tissue is gripped in a clamp.

An actual peel test in progress is presented in Fig. 3. Note the perpendicular orientation of the tissue peel to the substrate. The tissue grip and force transducer (constructed in our laboratory) are clearly shown, as is the gear and cabling system which creates equal horizontal and vertical velocity components. The transducer is a double bending bar with strain gauges in a half bridge. A DC motor with electronic speed control provides the power for the peel-tester. The signal from the force transducer is amplified using a pre-amplifier that was constructed based upon an analog device type 2B30 strain gauge signal conditioner.

The maximum load capacity of the force transducer was 2.94 N, although this can be changed to a greater or lesser value as needed for other applications by selecting a different transducer. The intrinsic system noise, measured by hanging a 20 gm weight from the grip was found to be $\pm$ 0.5 g, which sets the lower limit of load detection sensitivity for the device. For the tests reported here, the peel velocity used was 4.3 mm/sec (to maintain quasistatic conditions and minimize viscoelastic effects associated with the material being peeled), although this too can be changed depending on the application.

2.2. Tests conducted and materials used

Our experiments were conducted in three phases. This testing approach was based on the strategy of first using an easily implemented, reproducible system for testing (electrical insulation tape placed upon an electropolished stainless steel substrate); secondly testing of biologically-based products (tissue adhesives); and thirdly testing of biomaterial/subcutaneous tissue interfaces that were formed *in vivo*. For these tests, the maximum peeling force normalized to the width of the peel was taken to be the interfacial strength.

Three substances were used in the tests with tissue adhesives: N-butyl-2-cyanoacrylate (HISTOACRYL blue, B. Braun Melsungen AG, Melsungen, Germany); a two component fibrin sealant (Tisseel, Immuno AG, Vienna, Austria); and glycerol. Glycerol is not a tissue adhesive *per se*. However, because of its surface tension and viscosity, it forms a weak attachment and was used to verify the lower force detection limit capability of the peel test instrument.

The cyanoacrylate and glycerol were used to attach NMRI mouse skin to an electropolished 316L stainless steel substrate to form the interface for testing. To test the fibrin glue, a piece of mouse skin was attached to a steel substrate with cyanoacrylate, and another piece of mouse skin was attached to the first, so that the subcutaneous sides were in contact, using the fibrin glue to form the interface for peel testing. The manufacturers' instructions were followed in the use of the two tissue adhesives. The skin tested was obtained from the backs of the mice. The side of the skin in contact with the adhesive was the inner (i.e. non-hair) side. Before forming the interfaces, the steel was ultrasonically cleaned in isopropyl alcohol. The cyanoacrylate and fibrin sealant specimens were allowed to set for 10 min prior to testing. This was unnecessary for the glycerol.

For the third phase of the testing using interfaces formed *in vivo*, rectangular specimens of electropolished

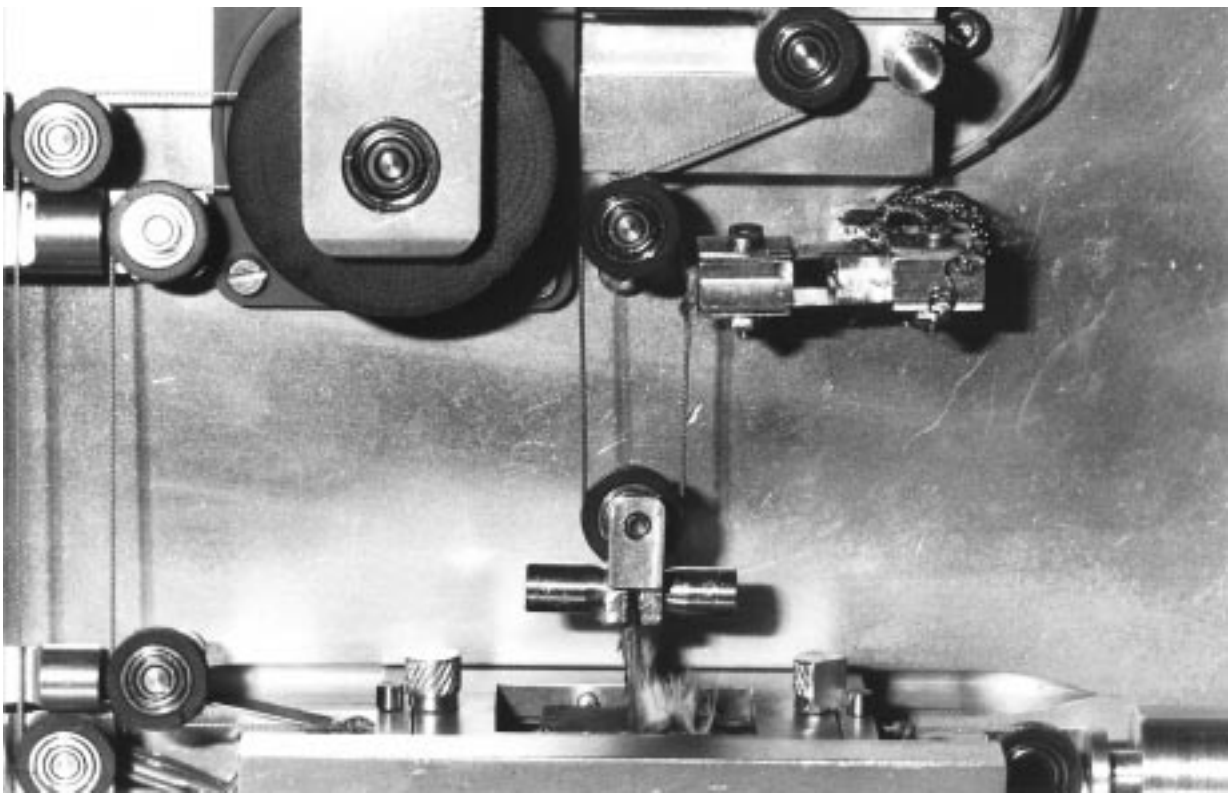

Figure 3 Tissue being pulled at ninety degrees from specimen.

316L stainless steel and plasma-sprayed titanium were implanted in the backs of NMRI female mice, and later explanted and tested regarding their adherence. These two materials were chosen since it was expected that the steel would show little tissue adherence while the titanium material would be highly adherent, thus presenting a wide range of conditions over which we could validate the capability and versatility of our peel tester. Details regarding these studies and their significance with respect to the scientific investigation of tissue adherence will be discussed in a future paper. The present article focuses on the peel-testing method itself.

3. Results

In all of the tests that were conducted, the peel tester was found to be very easy to use. In its operation, it was seen to function very well and reliably in a technical sense. It did not limit in any way the acquisition of the data that we wanted, either by producing artifacts that obscured the measurement, being too slow, or otherwise. As mentioned previously, for the *in vivo* phase of our testing program, we were able to complete the peel test within two min from the time the specimen was removed from the animal.

Fig. 4 shows a typical force versus displacement curve for the subcutaneous implant specimens. In the beginning little load is applied as slack in the system is taken up. A linear region is then noted, the slope of which represents the stiffness of the system (peeled material plus loading mechanism). The load then drops, presumably as the overlying tissue starts to peel from the surface. Secondary peaks can be noted as the peeling continues. Often (as shown in the figure) though not always, the maximum force was associated with the first peak.

For the well characterized system (electrical tape on stainless steel), for six measurements the mean value of the peel strength was 0.250 N/mm. The standard deviation was 3.33×10^{-2} N/mm, 13.3% of the mean value. Fig. 5 presents the results for the peel tests with the tissue adhesives.

n refers to the number of interfaces measured. As would be expected, the interfacial strength ranking from highest to lowest is cyanoacrylate, fibrin glue and glycerol. Here rather large standard deviations were observed.

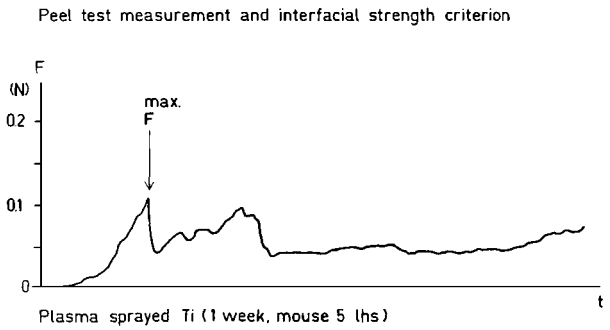

Figure 4 Typical force versus displacement curve exhibiting a maximum, from which peel strength is determined.

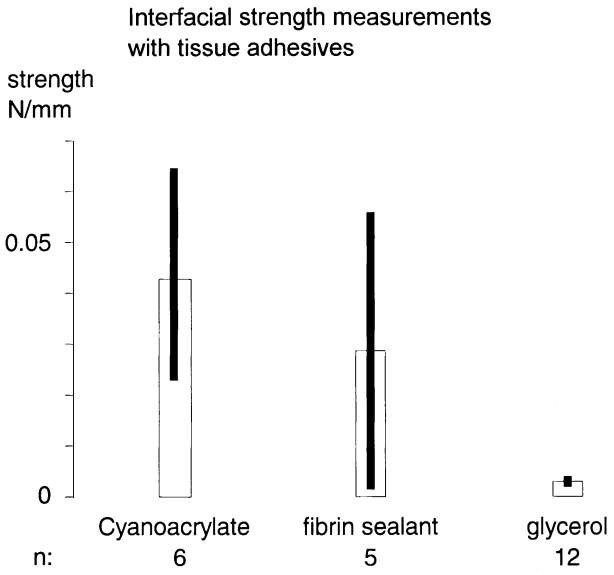

Figure 5 Interfacial strength measurements with tissue adhesives (error bar indicates standard deviation).

Student's *t*-test was used to determine the degree of statistical difference among the peel strength values for these substances. Both of the tissue adhesives had bonds to the substrate stronger than that for glycerol. For the cyanoacrylate and fibrin glue these differences were statistically significant at the 99.5% and 95% confidence levels, respectively. Because of the greater similarity in means and the large standard deviations mentioned above, the confidence level for the difference between the two tissue adhesives was only 80%.

For the tests with the implant materials in contact with subcutaneous tissue, substantial and statistically significant differences were observed in peel strength between the rough plasma-sprayed titanium surface and the smooth electropolished stainless steel. The steel displayed hardly any adherence at all. On the other hand, the plasma-sprayed titanium showed a considerable degree of adherence, and the peel strength value increased over time. Further details regarding the *in vivo* testing have been given elsewhere [16–20] and will be the subject of a future paper.

4. Discussion

In this research we have developed improved methodology for quantifying the degree of adherence of soft tissue to various types of materials and surfaces. Our portable peel-testing device allows a more reliable result to be obtained since it minimizes the time between explantation and testing. It also maintains the angle between the peel and substrate at 90°, which makes it easier to use the force versus displacement curve to find the peel strength of the biomaterial/tissue interface. Our device is capable of measuring over a wide range of adhesive strength. The peel strength data points taken during the three testing phases, for example, ranged from 1.5×10^{-3} N/mm to 0.317 N/mm. The intrinsic range of the force transducer thus allowed pronounced differences in adhesive strength to be resolved. This range can be extended considerably, if necessary, by selection of other force transducers.

The wide standard deviation of the fibrin glue that we observed is consistent with investigations of others who have tested similar products [21, 22]. It is reportedly due to mixing of the components, partial disruption of the clot, rate of elongation, fibrin concentration, etc.

The versatility of this peel tester should make it useful for many other applications. It could be applied to testing of virtually any kind of biomaterial substrate regarding adhesion of soft tissue to its surface. Testing for purposes other than those described in this article, such as measuring the effectiveness of wound dressings, adhesion of tissue to ophthalmologic materials, etc., would require other force transducers, tissue grips and specimen carriers, but these modifications could easily be implemented.

5. Conclusion

A peel-testing apparatus, maintaining a right angle between the peel and the substrate, has been constructed for rapid measurement of adhesion directly at the site of specimen removal. In the research presented here, it has been successfully validated in tests measuring the strength of tissue adhesives and soft tissue adhesion to various orthopaedic materials. The basic design of the peel tester that has been presented in this paper is that for a versatile instrument. We feel that it could have uses in many other applications where adhesion of soft tissues is a matter of interest (besides the ones we have directly examined) such as testing of wound dressings, ophthalmologic materials, etc.

Acknowledgments

Partial support for K. Bundy from the NIH Fogarty Center Senior International Fellowship No. 1 F06 TWO1603–01 ICP(6) is gratefully acknowledged. Immuno AG provided us with the Tisseel fibrin glue.

References

1. J. D. BOBYN, G. J. WILSON, D. C. MACGREGOR, R. M. PILLIAR and G. C. WEATHERLY, *J. Biomed. Mater. Res.* **16** (1982) 571.
2. H. KURZWEG, R. B. RHEIMANN and T. TROCYNSKI, *J. Mater. Sci.: Mater. Med.* **9** (1998) 9.
3. ASTM Standard Test Method D1876–95 Peel Resistance of Adhesives (T-Peel Test).
4. ASTM Standard Test Method C906–95 T-Peel Strength of Hot Applied Sealants.
5. ASTM Standard Test D3167–93 Floating Roller Peel Resistance of Adhesives.
6. ASTM Standard Practice D2918–93 Durability Assessment of Adhesive Joints Stressed in Peel.
7. ASTM Standard Test Method D1781–93 Climbing Drum Peel for Adhesives.
8. ASTM Standard Test Method D3807–93 Strength Properties of Adhesives in Cleavage Peel by Tension Loading (Engineering Plastics-to-Engineering Plastics).
9. ASTM Standard Test Method D903–93 Peel or Stripping Strength of Adhesive Bonds.
10. ASTM Standard Test Method D3330–96 Peel Adhesion of Pressure Sensitive Tape at {I15 Angle}.
11. ISO Standard 813: 1997 Rubber, Vulcanized or Thermoplastic–Determination of Adhesion to a Rigid Substrate-90° Peel Method.
12. ISO Standard 4578: 1997 Adhesives-Determination of Peel Resistance of High-Strength Adhesive Bonds-Floating Roller Method.
13. ISO Standard 8510–1: 1990 Adhesives-Peel Test for a Flexible-Bonded-to-Rigid Test Specimen Assembly-Part 1: 90° Peel.
14. ISO Standard 8510–2: 1990 Adhesives-Peel Test for a Flexible-Bonded-to-Rigid Test Specimen Assembly-Part 2: 180° Peel.
15. ISO Standard 1139: 1993 Adhesive-180 Degree Peel Test for Flexible-to-Flexible Bonded Assemblies (T-Peel Test).
16. K. BUNDY, B. RAHN, U. SCHLEGEL, V. GERET and S. PERREN, in Transactions of 17th Annual Meeting of the Society of Biomaterials, May (1991), Scottsdale, AZ, p. 229.
17. K. BUNDY, B. RAHN, U. SCHLEGEL, V. GERET and S. PERREN, *Biofouling* **4** (1991) 246.
18. K. BUNDY, B. RAHN, U. SCHLEGEL, V. GERET and S. PERREN, in Abstracts of the 28th Annual Technical Meeting of the Society of Enginering Science, November (1991), U. Florida, Gainesville, p. 175.
19. K. BUNDY, B. RAHN, U. SCHLEGEL, S. PERREN and P. CHAN, in Transactions of 4th World Biomaterials Congress, April (1992), Berlin, Germany, p. 348.
20. K. BUNDY, B. RAHN, H. GERBER, U. SCHLEGEL, R. PETER and V. GERET, in Transactions of 39th Annual Meeting of the ORS, February, (1993), San Francisco, CA, p. 513.
21. A. D. M. TORIUMI, *Plastic and Reconstructive Surgery* **89** (1992) 973.
22. B. D. H. SIERA, D. S. FELDMAN, R. SALTZ and S. HUANG, *J. Appl. Biomat.* **3** (1992) 147.

Received 4 August 1998
and accepted 14 October 1999