JOURNAL OF MATERIALS SCIENCE. MATERIALS IN MEDICINE 5 (1994) 500 502

Quantification of fibroblast adhesion to biomaterials using a fluid mechanics approach

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Adhesion of cells to the surface of a biomaterial is one of the major factors mediating its biocompatibility. Despite the importance of this parameter, reliable means for its quantification still need to be developed. In the research described here, the jet impingement method has been investigated as to its ability to resolve differences in fibroblast adhesion. A wide spectrum of materials has been studied. To visualize the cell layer on opaque surfaces, a fluorescent staining method has been developed. The measurements show statistically significant differences between material classes, with the highest degree of adhesion about twice that of the lowest degree of adhesion. The greatest adhesion was observed with metallic materials which exhibit a shear stress to erode cells from the material surface of about 5.65×10^{-3} N cm^{-2}. The tensile stress of adhesion (about 2.15×10^{-2} N cm^{-2}) was similar for most materials. The interpretation of the results of jet impingement testing and their relation to *in vivo* cell and soft tissue adherence to biomaterials merits further investigation.

1. Introduction

Cellular interactions with implants are of primary importance to the biomaterials community, since they mediate a great many aspects of biocompatibility. For example, optimal adhesion of soft tissues and cells to biomaterials would minimize infection at implant sites by eliminating dead spaces between the implant and the tissue plane where bacteria can flourish. Adhesion can be influenced by biomaterial surface factors such as charge, energy, and topography. These characteristics can be altered by various materials processing methods, so, in principle, a surface can be designed with a desired degree of adherence. A basic requirement for such efforts, however, is a reliable means for quantification of the degree of adhesion which exists between cells and surfaces. In past research, we have developed *in vivo* methods for measuring the interfacial strength of tissue adhesives and of soft tissue/biomaterial interfaces using peel test methods [1]. In that study it was found that large differences in adhesive strength existed between electropolished 316L stainless steel and plasma-sprayed commercially pure Ti.

For purposes of screening new materials, it is desirable to have *in vitro* methods for measurement of cellular adherence. Development of such methods has been the focus of previous investigation [2] in which a fluid mechanics method, jet impingement, was used to quantify the adhesion of fibroblasts to 316L stainless steel samples of varying roughness. The purpose of the present investigation was to extend jet impingement bioadhesion measurements to additional materials of biomedical interest.

2. Materials and methods

The materials used in this investigation were three metals: Ti–6Al–4V, 316L stainless steel, and Haynes 25 (a cobalt-based alloy very similar in composition to the ASTM F-90 material); graphite; tissue culture polystyrene; and glass. The metals had a roughness characteristic of a 120 grit finish. The nonmetallic materials were used in the as-received condition. The control materials, polystyrene and glass had the smoothest finish. These materials were cleaned by rinsing in deionized water, scrubbing in a detergent solution, and then were thoroughly rinsed with tap water, deionized water, and finally distilled water. The nonpolymeric materials were sterilized by autoclaving at 123 °C. The polystyrene was prepackaged in sterile and disposable form. All test specimens were in the form of circular discs (135 mm × 3 mm), expect for the polystyrene and glass, which were petri dishes.

The specimens were either placed in glass petri dishes, or (for the control materials) used as is, and on their surfaces 3T3 embryonic mouse fibroblasts (BALB/c clone A31, ATCC CCL 163, American Type Culture Collection, Rockville, MD) were grown as a monolayer to near confluence ($6–8 \times 10^4$ cells cm^{-2}). The cells were cultured in Dulbecco's Modified Eagle

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Medium, supplemented with 10% bovine calf serum (Hyclone Laboratories, Logan, Utah) and an antibiotic solution used at a concentration of 10 ml per litre of medium. The antibiotic consisted of 5000 U ml^{-1} of penicillin, 5 mg ml^{-1} streptomycin, and 10 mg ml^{-1} neomycin. The cell cultures were incubated at 37 °C in an atmosphere of 5% CO_2 at 95% humidity.

When the cells were ready for testing, the culture dish was transferred to a constant temperature bath. The fluid used to create the jet impinging on the cell layer was phosphate buffered physiological saline solution at 37 °C. It was delivered by a Masterflex Model 7021-20 peristaltic pump through an extensive tubing system which damped the pulsations and made the flow steadier. The fluid was directed by a three way value through a nozzle which had an internal diameter of 0.838 mm. The flow rate was such that the Reynolds number of the flow was 1750. The cells were subjected to the flow field for 30 s. This is the same period as that of Deshpande *et al.* [3] who used this method to study canine cardiac endothelium. The nozzle tip was at a distance above the specimen of four times the nozzle diameter, as recommended by Deshpande *et al.* [3].

The flow creates a lesion in the cell layer in the areas where the normal and shear stresses are of sufficient magnitude to disrupt its integrity. The stresses applied by the fluid jet have been computed by Deshpande and Vaishnav [4]. The stress levels tend to decay assymptotically with radial distance from the axis of the jet nozzle. By measuring the size of the lesion and using theoretical stress versus radial distance calculations [4], the stresses required to erode off the intact cell layer can be determined.

Special staining techniques were needed to visualize the lesions on opaque surfaces. The stain was 5% fluoroscein diacetate in acetone added to a phosphate-buffered saline solution (at a concentration of 4 mg ml^{-1}). This is a modification of the technique described by Schmalz and Netuschil [5]. The cells were illuminated with ultraviolet light to cause the stain to fluoresce, and the lesions were then photographed. The area of the lesion on enlarged photographs was measured using a magnetic contact digitizing tablet (Kontron MOP system). The lesion is not necessarily a perfect circle, but the area measurement allows an effective diameter to be computed.

3. Results

Table I shows the means shear and tensile stresses, τ and σ respectively, at the border of the lesion for the various materials tested. These are measures of the apparent strength of adhesion. The shear stresses required to erode the cells fall into three groups. Highest are the metals (at about 5.65×10^{-3} N cm^{-2}), followed by graphite and polystyrene, with glass having the lowest τ value. Student *t*-test comparisons show the following differences to be statistically significant at *p* values of 0.025 or better:

Ti–6Al–4V, Haynes 25 > graphite,

polystyrene > glass

TABLE I Apparent adhesive strengths of fibroblasts to various materials

Material	No. of Lesions Tested	τ^a (N $cm^{-2} \times 10^{-5}$)	τ^b (N $cm^{-2} \times 10^{-5}$)
Ti–6–4	8	605 ± 75	1911 ± 431
Haynes 25	9	561 ± 80	2161 ± 229
316L	3	531 ± 48	2243 ± 117
Graphite	4	480 ± 37	2264 ± 81
Polystyrene	4	445 ± 31	2202 ± 82
Glass	8	321 ± 23	1410 ± 210

[a] shear stress at border of lesion
[b] tensile stress at border of lesion

For 316L, the following differences are significant at $p = 0.05$ or less:

316L > polystyrene, glass

The normal stresses were about 2.15×10^{-2} N cm^{-2}, approximately three to five times that for the shear stresses at the lesion border. They were quite similar between materials (expect for glass which was lower). Only the following difference was statistically significant at $p < 0.01$:

all materials > glass

At a $p = 0.05$ value, graphite and 316L were larger than Ti–6Al–4V.

4. Discussion

The results of the jet impingement tests shown in Table I clearly indicate that the jet impingement method is capable of resolving apparent differences in fibroblast adhension to various biomaterials. The ranking of the strengths given in Table I suggests a relationship between surface energy and adhesive shear stress. As would be expected on this basis, the cells were most adherent to the metallic materials.

On the other hand various questions regarding the interpretation of these tests still remain. The first is whether the failure in the integrity of the cell layer results from cohesive failure of the fibroblasts themselves, parting at the cell/biofilm interface, or separation at the biofilm/material interface. This aspect is currently under investigation via electron microscopy.

A second question, which possibly is related to the first one, is the degree to which jet impingement tests correlated with *in vivo* soft tissue adherence. In the testing here, a titanium alloy showed a degree of apparent adherence equivalent to a stainless steel, yet peel testing *in vivo* [1] showed that subcutaneous soft tissue was much more adherent to plasma-sprayed titanium than to electropolished stainless steel, as previously pointed out. Although this discrepancy could be related to a difference in surface roughness, a recent study of 316L stainless steel showed equivalent jet impingement results for a variety of surfaces ranging from a milled finish to a 1 μm diamond polish [6]. One interpretation of these various studies is that jet

impingement measures the degree of adherence of cells to biomaterials up to a certain point, i.e. that where the cell/biofilm interfacial strength exceeds the cohesive strength of the cells themselves. But where this limit is exceeded, as could be the case for all the metals tested here, then *in vivo* soft tissue adherence might be governed by two effects: cellular and intercellular matrix interactions with the surface. Further experiments are needed to clarify this situation.

5. Conclusions

Jet impingement is a method which is able to resolve differences in apparent fibroblast adhesion to biomaterials. Metals show the greatest degree of cell adhesion. while glass displays the least. At present there are some uncertainties as to how to interprete such measurements. Whether the adhesive strengths obtained with this technique are well correlated with *in vivo* soft tissue adherence, or whether above a threshold stress level this is primarily a test of cell biocompatibility with the surface remains to be determined.

Acknowledgements

Financial support from the Swiss National Science Foundation (Grant 32-31017.91) and an NIH BRSG grant from Tulane University is gratefully acknowledged.

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