

EFFECT OF DIFFERENT HYDROPHILIC POLYURETHANES ON CELL AND BACTERIAL ADHESION

L.G.Harris, K.Gorna, S.Gogolewski & R.G.Richards

AO Research Institute, Davos, Switzerland.

INTRODUCTION: Studying morphology and adhesion of cells can give an indication to the cytocompatibility of a surface and its suitability for possible further application for orthopaedic implants. In most anatomical areas, soft tissue adherence to implanted material is regarded as one sign of material compatibility and is also important for the prevention of infection. Cells adhere to substrates using special adhesive sites known as focal adhesions. *In vitro* cell adhesion has been used to predict 'implant surface-soft tissue' compatibility¹. The morphology and adhesion of hTERT human fibroblast cells, and the adhesion of *Staphylococcus aureus* (SA) and *S. epidermidis* (SE) were studied on experimental polyurethanes (PUs) with different hydrophobic:hydrophilic (pho:phi) content ratios to determine their cytocompatibility.

METHODS: The surfaces studied were 3 different PUs with different pho:phi content ratios (100%, 70:30 and 30:70), PVC, both sides of Thermanox (controls) and poly(L-DL-lactide) 70/30% (control; results not shown). hTERT fibroblasts were cultured in DMEM with 10% FCS at 37°C. Approximately 20,000 cells were seeded onto each surface for 48h before fixation or immunogold labelling. SA and SE were cultured on the surfaces in brain heart infusion broth (BHI) for 1h at 37°C prior to fixation or fluorescent labelling. For scanning electron microscopy (SEM) study, hTERT, SA and SE were fixed with 2.5% buffered glutaraldehyde for 5 min, post-stained with 1% buffered osmium tetroxide for 1h, dehydrated, critical point dried, coated with Au/Pd, and visualised with an SEM using a backscattered electron detector². Immunogold labelling of vinculin was carried out as previously described, Richards *et al.*³. To quantify the amount of SA and SE adherence on the different surfaces, cultured bacteria were stained with fluorescent redox dye, 5-cyano,2-ditolyl tetrazolium chloride (CTC)⁴ for 1h, and visualised with a Zeiss Axioplan 2 Epifluorescence microscope. The density of live bacteria adhering to the surface observed in each image were counted using KS400 software. All results were statistically analysed to determine whether the adherence of cells and bacteria on the different surfaces varied significantly.

RESULTS: hTERT cells showed the greatest degree of cell spreading and total cell area on the 70:30 and 30:70 pho:phi surfaces, and on both sides of the control Thermanox (Fig. 1).

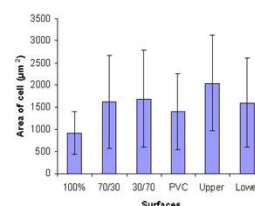


Fig. 1: Graph showing the results of average cell area on each surface.

Less cell spreading was observed on the 100% hydrophobic surface (Fig. 1), most cells were observed in a round state in comparison to flat cells on the 70:30 pho:phi surface (Fig. 2).

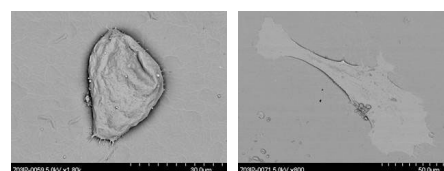


Fig. 2: SEM images of a cell on 100 % hydrophobic surface and on 70:30 hydrophobic/hydrophilic surface.

The adhesion of SA and SE varied depending on the surface, with results from the fluorescence labelling suggesting less SE adhesion than SA, whilst such differences were not observed with the SEM (Fig. 3).

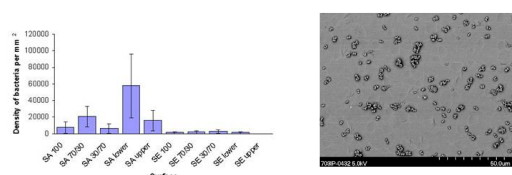


Fig. 3: Graph showing the average density of bacteria on each surface (SA – *S. aureus*; SE – *S. epidermidis*) and an SEM image of SA on the 100% hydrophobic surface.

DISCUSSION & CONCLUSIONS: Initial results have shown that hTERT cells adhere less to the 100% hydrophobic surface in comparison to the 70:30 and 30:70 pho:phi surfaces (Fig. 2). Results so far suggest, neither SA nor SE have a preference to any of the surfaces tested (Fig. 3).

REFERENCES: ¹Richards RG, Owen GR, Ap Gwynn I (1997) Cells Mat 7,15-30; ²Richards RG, ap Gwynn I (1995) J Microsc 177:43-52; ³Richards RG, Stiffanic M, Owen GR, Riehle M, Ap Gwynn I, Curtis AS (2001) Cell Biol Int 25:1237-49; ⁴An YH, Friedman RJ, Draughn RA, Smith EA, Nicholson J, John JF (1995) J Microbiol Methods 24:29-40.