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Cell Adhesion on Natural Rubber Latex Films: Influence of Surface Properties

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Adhesion, proliferation and spreading of cells on polymeric surfaces are regulated by the various surface properties of substrates, and cell responses are often indicative of their biocompatibility nature. Three differently prevulcanised natural rubber latexes i.e. *irradiated (IR), peroxide (PX) and sulphur (SPV) vulcanised latexes and one non-vulcanised high ammoniated latex (HA) were examined for their wettability, surface microstructure and surface chemical composition. Influence of these surface properties on the cell activities of L929 fibroblasts was studied. Scanning electron microscopy showed that IR and PX supported greater cell activity than HA while SPV was inferior. Cell activity was enhanced on the rougher, less hydrophobic and 'cleaner'* (i.e. *surfaces with fewer chemicals) IR and PX surfaces. HA and SPV surfaces showed additional presence of a myriad of chemical elements not detected in IR or PX. SPV was particularly detrimental to cells, likely due to the distinct presence of zinc-related chemicals on its surface. It is apparent that the prevulcanisation process has imparted greater surface microstructure, and hydrophilicity to IR and PX surfaces, the probable reason for these two latex materials to be more superior for adhesion, proliferation and spreading of cells.*

Key words: surface properties; biocompatibility; prevulcanised natural rubber latexes; wettability; surface microstructure; hydrophilicity

Soft tissue attachment to implants is important to reduce infection[1], provide proper and tight apposition of tissue to prosthesis[2], and inhibit thrombus formation[3]. Upon implantation, the implant surface is first conditioned by tissue fluids[4]. which produced a layer of adsorbed macromolecules and water. This adsorbed layer would then influence the behaviour of cells when they come in contact with the implant surface. Cellular interactions at tissue (or blood): implant interface regulate the onset of inflammatory responses[5], resulting in the production of a myriad of mediators[6]. These might sometimes be followed by fibrous capsule formation around the implant[7], which could proceed over a long period and ultimately

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affect the implant functions[8] Surface-cell interaction is a complex phenomenon and no single physical or chemical property is sufficient to predict its nature over a broad range of polymers It is not surprising that processes like adhesion, spreading and proliferation of cells on polymeric surfaces are influenced by various surface properties of the materials This study aims to relate the influence of the surface properties of natural rubber latex (NRL) films on such cell activities Although at present NRL is yet to be used in implant devices, these data could be useful in other medical applications because the cell responses might be indicative of the biocompatibility nature of the NRL medical devices NRL is renowned for its tensile strength and elasticity, which are superior to most other polymeric materials[9] NRL being a natural polymer is also much cheaper than the synthetic polymeric materials Study into this areas can help to create more biocompatible and safer NRL products, widens its greater applications in sophisticated medical devices, possibly as a competitive implant biomaterial with excellent physical properties

MATERIALS AND METHOD

Natural Rubber Latex

All NRL materials described in *Table 1* were obtained from the Malaysian Rubber Board, Kuala Lumpur, Malaysia and the Tun Abdul Razak Research Centre, Hertford, United Kingdom

Cells

The L929 cell line was selected for use in this study because it is a well established cell line, and relatively easy to culture and maintain L929 cells are fibroblastic mouse connective tissue cells of the parental strain L sub-clone derived from normal subcutaneous areolar and adipose tissue of C34/An mouse These cells were supplied by the European Collection of Animal Cell Cultures (ECACC), Salisbury, United Kingdom Cells were cultured at $5 \times 10^3 - 2 \times 10^4$ cells per cm^2 in Dulbecco medium (DMEM) supplemented with penicillin 100 IU mL^{-1}, streptomycin 100 µg mL^{-1}, 2-mercaptoethanol 10 µM and fetal bovine serum (FBS) 10% Cells were maintained in humidified atmosphere with 5% CO_2 at 37°C Upon confluence cells were detached from the culture flask surface by incubating in 0 25% Trypsin-Gibco Solution A (Gibco BRL Co Ltd , UK) for 5–10 min Cells were washed twice with supplemented DMEM medium to remove the trypsin residues

Preparation of Latex Cast Films

Latex was first sieved through muslin cloth to remove coagulum and debris Air bubbles

TABLE 1 NRL FROM DIFFERENT VULCANISATION SYSTEMS

Sample code	Description of the latex
HA	NRL preserved with 0 7% ammonia non vulcanised
IR	NRL prevulcanised using the gamma irradiation system
PX	NRL prevulcanised using the peroxide system
SPV	NRL prevulcanised using the sulphur system

were gently scooped from the surface of latex. 5 mL of the latex was poured and spread evenly onto a 94 mm diameter glass petri dish. The latex was left to dry overnight at ambient temperature to form solid films.

Exposure of Cells to Latex Cast Films

Test piece measuring 1.0 cm × 2.0 cm was cleaned with distilled water (20 mL) and ethanol 70% (5 mL) in an ultrasonic bath for 1 h and 5 min, respectively. Samples were attached onto the bottom of a 6-well culture dish (*Nunc*, Denmark) by sticking the longer ends with sterile skin closure adhesive tapes (*BDF leukostrip*, NHS Supplies, UK), giving a final exposed area of approximately 1.0 cm × 1.0 cm. This adhesive tape has been found to have no adverse reaction towards the cells in a separate experiment. Test samples were equilibrated in 2 mL of complete DMEM culture medium for 1 h at 37°C and 5% CO_2 humidified atmosphere. After the equilibrating period, the medium was removed and replaced with 1 mL of L929 cell suspension (density at 6–8 × 10^4 cells mL^{-1}). 2 mL of culture medium was added to prevent the wells from drying out. Cells were maintained at conditions described before. Tissue culture polystyrene (TCPS) wells and borosilicate glass slides were used as controls.

Scanning Electron Microscopy (SEM)

When cells were fully confluent, the NRL samples were detached from wells, rinsed twice with phosphate buffer saline (PBS, 0.1 M, pH 7.2), and fixed with glutaraldehyde (2.5%, 15 min). Fixed samples were again rinsed twice in PBS and dehydrated sequentially in 20%, 40%, 60%, 80%, and absolute ethanol, with 20-min dehydration period at each interval. Dehydrated samples were critical-point-dried, and spur coated with gold.

Water Contact Angles

Water contact angles were measured using the sessile drop method[10]. Measurements were determined on both sides of the latex films namely, the surface exposed to air (upper surface) and the surface that was in direct contact with the glass petri dish (lower surface) during the film drying process.

Atomic Force Microscopy (AFM)

Topographic images of latex films were collected under 'contact' mode[11]. Measurements were made with Nanoscope® III Multimode Scanning Probe Microscope (Digital Instruments, Santa Barbara, California, USA.) using a 200 μm long silicon nitride cantilever and a D scanner, which allows a maximum 12 μm *x,y* scanning range. Measurement was carried out in an air-supported unit to ensure a vibration-free environment.

X-ray Photoelectron Spectroscopy (XPS)

Silicone wafers (1.0 cm × 1.0 cm) were cleaned with absolute methanol in an ultrasonic bath (10 min) and dried under nitrogen gas flow. 50 μL of liquid latex was deposited onto the wafers and allowed to evaporate overnight. Surface chemical composition of the dried samples was analysed using *Scienta 300* X-ray photoelectron spectrometer.

Data Analysis

Where applicable, inferential statistics by ANOVA (analysis of variance) was carried

out followed by the *posthoc* Tukey B test at 0.05 significance interval. All statistical analysis was carried out using the SPPS software (version 6.0 for Windows).

RESULTS

Wettability of Latex Cast Films

Both the upper and lower film surfaces of the four NRL samples showed relatively similar wettability ranking (*Table 2*). In all cases, HA was significantly most hydrophobic, followed by PX (Tukey B test at 0.05 significance level). There was no significant difference between IR and SPV (*Table 3*). The upper surfaces of all latex films showed no significant difference in their receding angles while with the lower surfaces. HA film gave significantly higher value ($P < 0.001$, ANOVA).

All four latex films showed distinct hysteresis ranging from 87° – 67.5° (*Table 2*). For both the upper and lower film surfaces, HA showed greatest hysteresis, followed by PX, IR and SPV. During the water contact measurement, a distinct water stain mark was seen on SPV surface. This stain mark was moderate on IR and PX and negligible on HA film surfaces. This observation concurs with the water contact angle values because wettability of a material is not only characterised by the spreading behaviour of water on its surface, but by the nature of the material itself for example, its ability to absorb water or flexibility of molecular segments existing on the material surface[12].

Surface Topography of Latex Films

HA film surface was reasonably flat and smooth except for several holes and pits measuring approximately 0.1 μm – 0.5 μm in diameter (*Figure 1a*). IR (*Figure 1b*) and PX (*Figure 1c*) surfaces have fewer holes but distinct features resembling particulates of

TABLE 2[a]. ADVANCING (θ_A) AND RECEDING (θ_R) WATER CONTACT ANGLES OF NRL CAST FILMS MEASURED BY THE SESSILE DROP METHOD[10]

NRL film	Advancing angle, θ_A (Degree)		Receding angle, θ_R (Degree)		Hysteresis[b] (Degree)	
	Upper surface	Lower surface	Upper surface	Lower surface	Upper surface	Lower surface
HA	101.2 (1.8)	98.6 (0.5)	14.2 (1.1)	22.0 (0.9)	87.0	76.6
IR	80.1 (0.9)	85.5 (1.0)	17.3 (0.7)	15.0 (1.0)	69.8	70.5
PX	89.8 (1.5)	91.6 (0.4)	17.5 (1.2)	18.4 (0.9)	72.3	73.2
SPV	82.2 (1.3)	84.1 (1.0)	14.7 (1.0)	16.6 (1.1)	67.5	67.5

[a]Data are expressed as means (n = 6) and values in parentheses represent the standard error of mean (SE).
[b]Calculated as $\theta_A - \theta_R$

various sizes protruded from these surfaces, giving them a rougher appearance than HA film. Particulates on PX surface were in random clusters, smaller and comparable in size while those on IR surface were bigger and distributed more uniformly across the film surface. SPV surface appeared rougher than HA, the pits being relatively bigger (1.0 μm – 1.5 μm in diameter), and more uniform in size (*Figure 1d*). These pits were located closely together and appeared only on a certain region of the film surface.

Contrary to the AFM micrographs, calculation of the root mean square of roughness (RMS) showed that HA has a higher RMS value than PX (RMS = 46.6 nm and 19.5 nm, respectively), suggesting the former surface to be rougher (*Table 4*). This higher RMS value was largely due to the surface features of HA, which showed a wide difference between the highest and lowest peak heights (height difference = 194.1 nm). Measurement on areas devoid of holes and pits gave a markedly lower value (RMS = 9.2 nm). The surface features of PX showed a much lower height difference (approximately 67.7 nm), indicating the presence of small-scale roughness. SPV gave the highest RMS value (RMS = 66.3 nm), suggesting it to be the roughest surface. The RMS value of the IR (RMS = 42.3 nm) was slightly lower than HA.

Surface Chemical Composition of Latex Films

Except for SPV, there was generally no distinct difference among the other latex samples, although binding energies for some peaks differed slightly (*Table 5*). The elemental ratio (calculated against carbon) showed that the oxygen content of SPV and HA (O_{1s}:C_{1s} ratio of 0.84 and 0.74, respectively) was almost 2-fold greater than PX and IR (O_{1s}:C_{1s} ratio of 0.48 and 0.37 respectively) (*Table 6*). All samples showed comparable N_{1s}:C_{1s} ratios within the range of 0.08 – 0.12, indicating relatively similar nitrogen content. The potassium content of IR and PX was similar ($K_{2p3/2}$:C_{1s} ratios of 0.04), the values being intermediate of SPV and HA. SPV ($K_{2p3/2}$:C_{1s} ratio of 0.02) and HA ($K_{2p3/2}$:C_{1s} ratio of 0.06) showed the lowest and highest potassium content, respectively.

TABLE 3. COMPARISON OF THE SIGNIFICANT DIFFERENCE BETWEEN WATER CONTACT ANGLES ON THE UPPER AND LOWER SURFACES OF NRL CAST FILMS[a]

NRL film	Advancing angle, θ_A			Receding angle, θ_R		
	Upper surface of NRL cast Film					
	HA	IR	PX	HA	IR	PX
IR	S			N		
PX	S	S		N	N	
SPV	S	N	S	N	N	N
	Lower surface of NRL cast Film					
IR	S			S		
PX	S	S		S	N	
SPV	S	N	S	S	N	N

[a]Tukey-B test at 0.05 significance level; S = significant and N = not significant

TABLE 4. COMPARISON OF THE ROUGHNESS OF NRL CAST FILM SURFACES[a]

NRL film	Roughness measurement (nm) Mean roughness	RMS (root mean square)	[b]Height difference
HA	35.7 (7.5*)	46.7 (9.2*)	194.1
IR	34.3	42.3	168.2
PX	15.4	19.5	67.7
SPV	48.4	66.3	439.6 (265.6#)

[a]Measurements were based on an approximately 6 μm × 6 μm surface area of the AFM images
[b]Height difference describes the difference between the lowest and highest peak heights associated with the surface features
*Represent measurements on an area devoid of holes and pits
#Difference between the average and lowest peak heights of the surface features on SPV film surface

Trace amounts of sulphur were detected on HA ($S_{2p3/2}$:C_{1s} ratio of 0.02), IR ($S_{2p3/2}$:C_{1s} ratios of 0.01) and PX ($S_{2p3/2}$:C_{1s} ratio of 0.01) but not on SPV surfaces, although the latter was prepared from the sulphur prevulcanised latex. This could be due to the high noise to signal ratio at the 0-300 eV regions of the SPV spectrum possibly 'masking' the S_{2p} signal, which otherwise usually occurs at approximately 165 eV[13]. Phosphorus was detected on SPV ($P_{2p3/2}$:C_{1s} ratio 0.05) and HA ($P_{2p3/2}$:C_{1s} ratio of 0.02) but not on IR or PX film surfaces. SPV showed a distinct presence of zinc element (Zn_{auger}:C_{1s} ratio of 0.51), which was not observed on other NRL samples.

Adhesion, Spreading and Proliferation of Fibroblasts on Latex Films

In general, cell proliferation at areas surrounding the perimeter of the latex samples occurred in a more uniform layer while more cell clusters or patches were observed at areas towards the center of the samples. Compared to HA (*Figure 2a*), IR and PX (*Figures 2b,* and *2c*) showed a greater number of adherent cells with most cells displaying the full-spread spindle morphology characteristic of fibroblasts. Adherent cells on HA were sparse and more rounded in shape. A small number of cells were found on SPV (*Figure 2d*) and majority of the cells were round with numerous blebs and ruffles and no extension of the characteristic pseudopodia. All cell activities (*i.e.* adhesion, spreading, and proliferation) were comparatively lower than the TCPS or glass controls.

DISCUSSION

Morphology and Density of Adherent Cells

Upon contact with surfaces, cells generally start to spread[14], and the constant motion of filopodia and lamellopodia during the cytoplasmic spreading gives rise to ruffles. In the event of compatible surface, cell attachment would be followed by spreading, characterised by the ceasing of ruffles and blebs, and extension of cytoplasm with

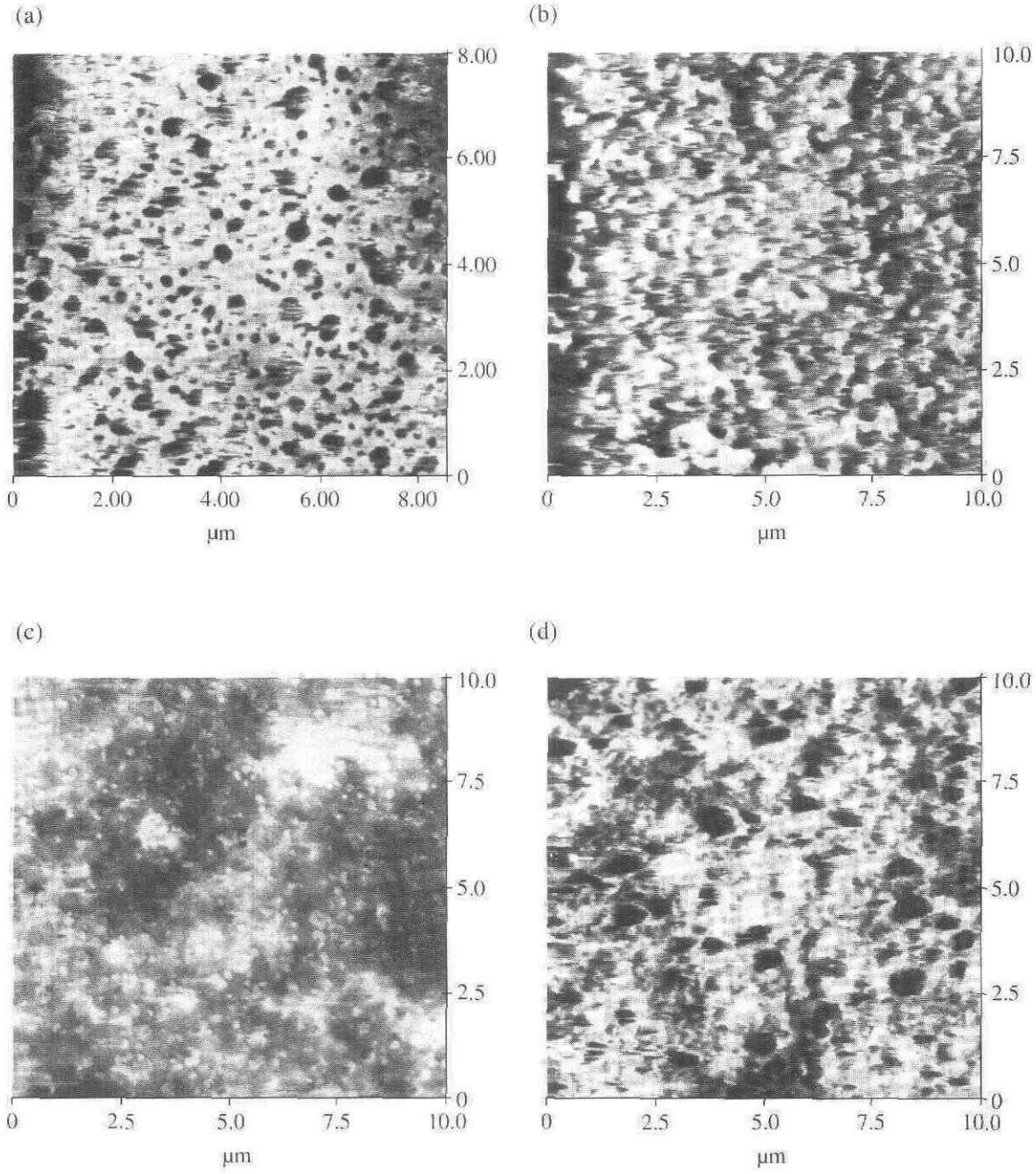

Figure 1. Surface topography of (a) HA, (b) IR, (c) PX and (d) SPV latex films analysed by contact mode AFM. Measurements were made on an area of approximately 10 μm × 10 μm.

TABLE 5. SUB-SHELL BINDING ENERGIES OF ELEMENTS[13] DETECTED ON NRL FILMS[a]

Element	Sub-shell		Binding energies (eV)			
	Photoelectron lines	Auger lines	HA	IR	PX	SPV
Zn		$L_3M_{23}M_{45}$ ^1P				586.4
Zn		$L_3M_{23}M_{45}$ ^3P				577.4
Zn		$L_2M_{23}M_{45}$ ^1P				562.0
O	1s		531.0	529.8	530.0	530.6
Zn		$L_3M_{45}M_{45}$				499.0
Zn		$L_2M_{45}M_{45}$				475.0
N	1s		398.6	397.8	397.8	398.6
K	2s		376.2	375.4		
K	$2P_{1/2}$		294.2	293.4	293.0	294.6
K	$2P_{3/2}$		290.8	290.4	290.4	291.4
C	1s		283.0	282.0	282.4	283.4
S	2s		231.0		230.6	
P	2s		189.4			190.4
S	$2p_{1/2}$, $2p_{3/2}$		166.6	166.2	166.0	
Zn	3s					139.2
P	$2p_{1/2}$, $2p_{3/2}$		131.4			132.6
Zn	$3p_{3/2}$		87.0			88.0
O	2S		25.0	23.6	24.2	24.2
K	$3p_{1/2}$		14.8	14.8	15.0	16.6
Zn	$3d_{5/2}$					9.4

[a]Measurements were made at 45° take-off angle. Elements are represented as follows: zinc (Zn); oxygen (O); nitrogen (N); potassium (K), carbon (C); sulphur (S) and phosphorus (P)

TABLE 6. ELEMENTAL RATIOS OF HA, IR, PX AND SPV SAMPLES CALCULATED AGAINST THEIR RESPECTIVE C_{1s} PEAK[a]

NRL film	Element to carbon ratio (based on integrated area under curve)					
	O_{1s}:C_{1s}	N_{1s}:C_{1s}	$K_{2p3/2}$:C_{1s}	Zn_{auger}:C_{1s}	$S_{2p3/2}$:C_{1s}	$P_{2p3/2}$:C_{1s}
HA	0.74	0.08	0.06	nd	0.02	0.02
IR	0.37	0.08	0.04	nd	0.01	nd
PX	0.48	0.12	0.04	nd	0.01	nd
SPV	0.84	0.10	0.02	0.51	nd	0.05

[a]Results were not corrected against relative cross-sectional factors. For the Zn_{auger} peak, the photoelectron emission related to the $L_3M_{45}M_{45}$, was used for calculation.
nd = non-detactable

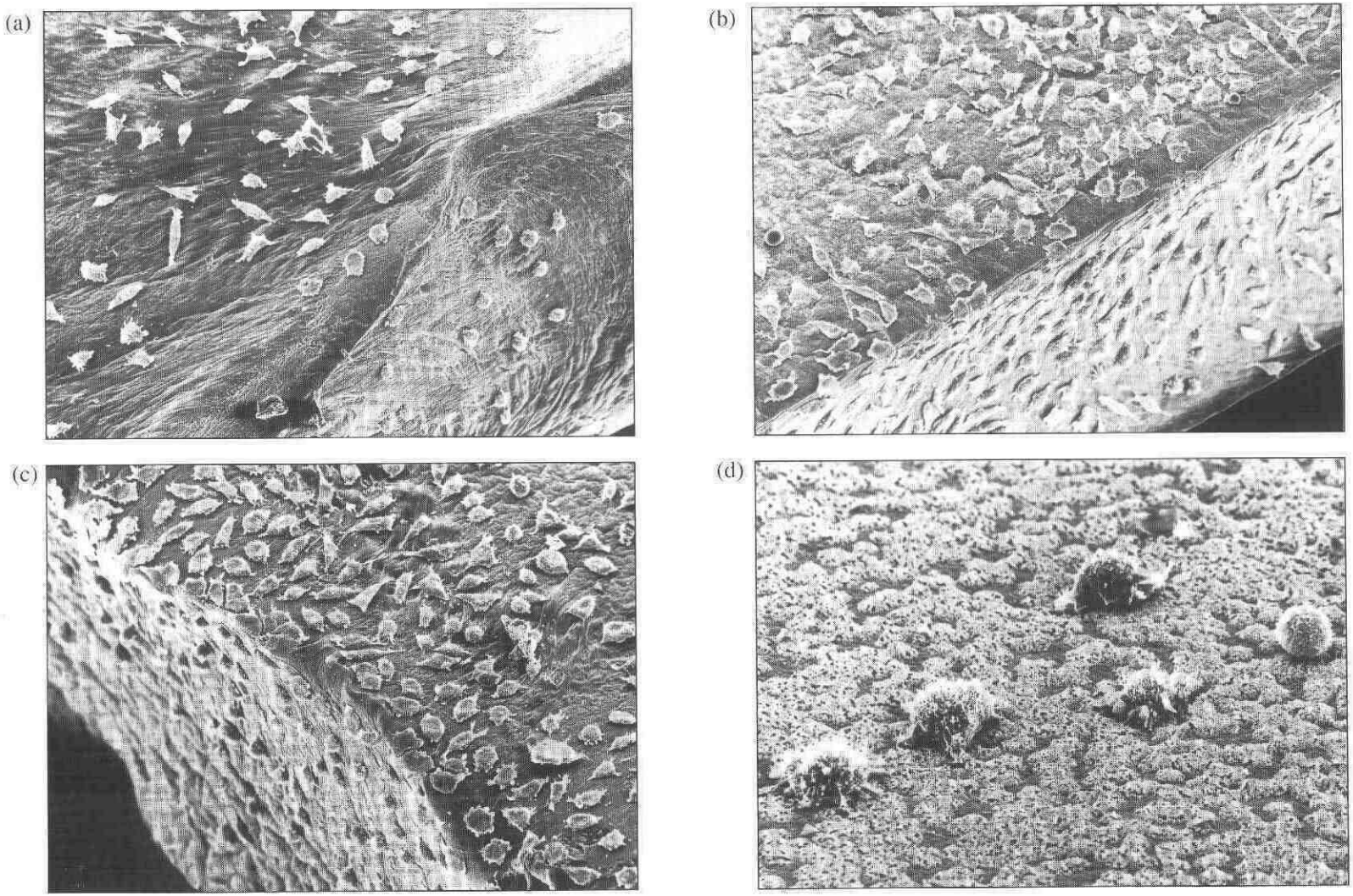

Figure 2. Adhesion and proliferation of L929 fibroblasts on (a) HA, (b) IR, (c) PX and (d) SPV film surfaces. SEM micrographs were taken at 1600 × (a, b and c) and 3200 × (d) magnifications, respectively.

concomitant flattening of the central mass[15] With fibroblasts, the cytoplasmic spreading occurs initially in a radial symmetrical fashion and upon establishment of a distinct leading edge, cells become polarised, resulting in diminished ruffling and appearance of typical spindle-shape morphology mass[16] This cell morphology is characteristic of those seen on IR and PX film surfaces and to a lesser extent on HA surface This indicates that these latex surfaces possess certain degrees of cell compatibility that enables establishment of a stable adherent stage and considerable cell spreading activity Compared to HA sample, IR and PX surfaces showed substantially higher number of adherent fibroblasts indicating that the cells were able to spread and develop firm cell-substratum adhesion The rounded fibroblasts on HA surface (and to a greater degree on SPV surface) indicate poor cell adhesion where the cell microtubules were impeded from extending across the film surface and eventually failed to initiate any pseudopodia extension characteristic of cell spreading[1] The ruffled cells on SPV surface also shows an active motion of cells that nevertheless, failed to attach firmly to the substratum surface and the lamellopodia were eventually pulled back by the cortical tension producing ruffles[16]

Unlike the uniform cell monolayer observed with TCPS or glass controls, fibroblast proliferation on HA, IR, PX and SPV were found to exist generally in patches or clusters with some areas having markedly higher cell density than the others These clusters resembles pattern associated with the dynamic adsorption and desorption of adhesion proteins to polymer substrates[17] suggesting that some degrees of protein interaction have occurred between these latex surfaces and the proteins present in the culture medium It is also possible that these latex samples possess irregular surface properties, causing the initial cell adhesion to be greater at certain sites These sites then act as central points for cell proliferation, giving rise to cell patches or clusters Cells that are adhered closely together are also known to cause progressive cell flattening that leads to cell proliferation[18] Another possible reason for the clustered cells is the non-uniform initial cell distribution across the test samples, resulting in cells settling as aggregates onto the sample surfaces Subsequent secretion of growth and proliferation factors or other extracellular matrix proteins[5,19] by these aggregated cells might then develop an environment more favourable to growth and proliferation of cells compared to that by individual cells Also, unlike TCPS and glass, it is possible that certain degrees of leaching of impurities from the latex samples have occurred, and the local effects on cells adjacent to these sites eventually caused variation in cell spreading[20]

Effects of Surface Chemical Composition

Studies have shown that cell adhesion to biomedical polymers is dependent on the surface chemistry of the materials[3,21] HA, IR, PX and SPV surfaces displayed varying surface chemical compositions, and most elements are attributed to the non-rubber substances in the NRL[9] The ability of these latex samples to support cell activity could be attributed to some of these elements For example the naturally occurring latex proteins in NRL could provide the surface nitrogen-containing groups, which were reported to promote attachment and/or proliferation of cells[22] Likewise surface oxygen on the latex films could support cell adhesion by facilitating cell interaction *via* hydrogen bonding between groups on cell membranes and polymers[23] Other chemical functional groups could

also provide interaction points for cells *via* short-range interaction between ionizable groups on the heterogeneous cell membrane and the chemical groups, triggering cell adhesion[23]

In general IR and PX showed relatively similar cell activity which could be attributed to their remarkably similar elemental ratios particularly the O_{1s} C_{1s} and $K_{2p3/2}$ C_{1s} ratios IR and PX also showed better cell adhesion and spreading, probably because of the fewer non-hydrocarbon elements present on these surfaces thus creating a 'cleaner and conducive environment for cell activity The specific presence of chlorine and phosphorus on HA surface and the myriad of other elements, especially zinc on SPV surface could be detrimental to cells This could be one reason for poor cell adhesion on these film surfaces Zinc element on SPV surface was undoubtedly from the zinc accelerators used in compounding and cells have been shown to react negatively to zinc accelerators especially those from the dithiocarbamate group[24] Phosphorus was only detected on HA and SPV film surfaces, both of which showed distinctly poorer fibroblast adhesion than IR or PX samples The negative effect of phosphorus is uncertain since it is present in cell culture medium as part of the nutrient formulation Although phosphorus is also present in the phospholipid membranes of rubber particles[9] the selective detection of phosphorus on HA and SPV film surfaces suggests that this element might be related to other substances existing specifically in these two latex samples

Effect of Surface Wettability on Cell Adhesion

Adhesion spreading and proliferation of cells on polymeric surfaces are dependent on the overall nature of the material, particularly the hydrophobic/hydrophilic balance of polymeric surfaces[25] rather than whether the material is entirely hydrophilic or hydrophobic[26,27] Cell adhesion is increased on wettable surfaces[18], especially the moderately hydrophilic surfaces because of the preferential adsorption of adhesion proteins from the cell culture media[22,28] This explains the superior cell adhesion on the TCPS control compared to any latex samples because of its moderately wettable surface Unexpected ability of some highly hydrophilic or hydrophobic materials to support cell adhesion is usually caused by modulation of the protein behaviour towards the polymer surfaces particularly protein adsorption efficiency and possible desorption[18] This could explain the ability of HA IR and PX to support some degrees of cell adhesion despite their relatively strong hydrophobic nature Likewise, the highly hydrophilic glass control used in this study supported excellent cell adhesion because of its unique ability to absorb adhesion proteins from cell culture media[18] In general cell adhesion is more inhibited on the hydrophobic latex surfaces because adhesion, which increases contact areas of cells is thermodynamically favoured with increasing surface energy of materials[29]

Although all latex materials were clearly hydrophobic (illustrated by their high θ_A) IR and PX by comparison, were more hydrophilic than HA surface Study has shown that cell adhesion is favoured with increasing hydrophilicity[14] and in serum containing media, cell adhesion decreased with increasingly hydrophobic substrates[30] due to the reduced spontaneous serum protein adsorption on the more hydrophobic substrates This may explain the better cell adhesion on IR and PX surfaces because of their greater hydrophilicity compared to HA sample The increased hydrophilicity of IR and PX was likely attributed to the added compounding chemicals[9], which affected their

wettability[23]. Also, unlike the non-vulcanised HA latex, the pre-vulcanisation process of IR and PX latexes might have liberated bound proteins from the rubber particles. Higher content of free proteins, especially on the film surface could enhance the polarity[9] and consequently increase wettability. Study has also shown that poorly hydrophilic polar surfaces promote good cell adhesion[25], which possibly explains the better cell adhesion on IR and PX surfaces since they were comparatively closer to the 'poorly hydrophilic' category than HA due to their distinctly lower θ_A values. SPV although more hydrophilic than HA, did not support appreciable cell activity because of its inherent surface toxicity. Interestingly, all NRL samples displayed large contact angle hysteresis, which is likely caused by the adaptation of polymer surface to the polar environment of water[31].

Although several studies concurred that surfaces of certain wettability (especially moderately wettable) promote cell adhesion[18,25] there is no distinct wettability criteria that can clearly classify a material of being definitely cell adhesive or non-adhesive, particularly over a broad selection of biomaterials. This reflects the difficulties in relating the effects of hydrophilicity-hydrophobicity on cell behaviour. In general, this study indicates that cell adhesion improves with increasing wettability of the latex film surface only when other factors such as cytotoxicity (as with SPV sample) are absent or less prominent.

Effects of Surface Topography on Cell Adhesion

Except for SPV, cell adhesion on the other three latex samples increased with increasing surface roughness. This is contrary to some studies that showed cell adhesion to be lower on rougher surfaces[1,32]. Yet, rough surfaces have been reported to promote better cell adhesion although the surface topography did not appear to affect subsequent cell spreading[33]. It is therefore obvious that cell behaviour on polymeric surfaces does not depend only on the surface roughness, but on the types of cell used[1] and the surface microstructures[32,34]. Unlike the surface treated materials used in other studies[1,32,34], it is possible that the extent of roughness of the untreated latex films in this study was not enough to manipulate specific cell behaviour. Nevertheless, the increased roughness on IR and PX has managed to provide adequate contact points for the anchorage of fibroblasts, resulting in greater cell adhesion compared to the smoother HA surface. SPV despite having the roughest surface, showed poor cell adhesion because of its cytotoxicity caused by the added compounding chemicals.

NRL is also a highly structured material renowned for its stereo regularity because of the lack of isomerism in its polyisoprene chains[9]. Cells are known to be sensitive to microtopography[34] and surface regularity affects the behavioural characteristic of cells[32]. It has been suggested that structured polymers support cell adhesion while amorphous materials inhibit similar cell activity[25]. IR and PX in particular, showed certain degree of regularity and relatively organised surface microstructures compared to HA sample and this could substantiate the better cell adhesion on the former two samples.

Protein Adsorption and Cell Adhesion

FBS which is present in the cell culture medium used in this study, contains numerous glycoproteins, two of which, fibronectin (Fn)

and vibronectin (Vn) are essential for cell attachment[35] These proteins have been found to promote adhesion of anchorage-dependent cells such as fibroblasts and epithelial cells onto synthetic surfaces *via* receptor-ligand interaction leading to further cell activation[35,36] On the contrary, albumin (Ab), which is a predominant component in FBS, impairs cell adhesion[37] Therefore, cell adhesion is dependent on the net outcome exerted by the stimulatory (Fn and Vn) and the non-adhesive (Ab) components in FBS

Although direct pre-adsorption of latex samples with these serum proteins was not carried out in this study, it should be noted that all samples were equilibrated for one hour in complete culture medium containing FBS prior to the addition of cells It is possible that some degree of protein adsorption onto the latex film surfaces have occurred during this period While Vn adsorbs strongly on both hydrophilic and hydrophobic surfaces Fn is reported to adsorb more preferentially on hydrophilic[36] and rougher surfaces[38] This could explain the enhanced proliferation spreading, and flattening of cells[35] on IR and PX compared to HA as their relatively rougher and more hydrophilic surfaces would have triggered greater Fn absorption Cell activity on HA, and to a lesser degree SPV surface could be aided by the absorption of Vn onto their surfaces Adsorption of Vn is expected to be similar among the latex materials because of its non-dependence on wettability

Generally, cell activity on all four NRL samples was inferior to the TCPS or glass controls One probable reason for this is that NRL is a substance of natural origin with innate latex proteins[9] It is possible that these latex proteins could deter cell activity by promoting Ab and/or reducing Fn and Vn adsorption from the culture medium Likewise these innate proteins might interfere with the conformation of absorbed serum proteins, and subsequently hinder the serum protein binding activity with cell surface receptors[16] Rubber particles are also enveloped within a phospholipid membrane[9] Study has shown that a stable phospholipid layer could impair the interaction between serum proteins and cells[39] consequently reducing protein adsorption that could promote cell adhesion TCPS and glass have superior cell adhesion because they possess excellent ability to absorb adhesion proteins, particularly Vn[35] due to the specific influence of their high surface energies on the amount and conformation of absorbed proteins[40] Therefore it is not unexpected that the TCPS and glass controls used in this study displayed excellent cell activity compared to the latex film surfaces

CONCLUSION

Generally, this study shows that cell activities upon contact with NRL film surface are dependent on various surface properties Among the more influential factor is the surface chemical composition, which could predispose varying degrees of surface toxicity This was illustrated in SPV sample, which showed a distinct presence of zinc element on its film surface, likely attributed to zinc-related chemicals used in its compounding formulation SPV also showed specific presence of phosphorus, calcium, and copper elements These together with the zinc element could be the reason for its surface toxicity, consequently impairing its ability to promote cell adhesion The inability of SPV sample to support good cell adhesion could also be due to the leaching of cytotoxic compounds from this sample This study also indicates that 'cleaner' surfaces (*i e* surfaces with fewer chemicals) for example, IR and PX could promote better

cell adhesion than surfaces with a myriad of chemical elements This was evident in HA which despite being non-cytotoxic in a separate experiment nevertheless failed to support cell adhesion comparable to IR or PX, possibly because of the specific presence of chlorine and phosphorus on its film surface Surface topography and wettability appear to be the next two factors that regulate cell activity on these latex film surfaces, probably through their effects on the adsorption and desoprtion of adhesion proteins from the culture medium However, it could not be ascertained which surface property is more influential in this matter Findings suggest that rougher and less hydrophobic surfaces (*i e* IR and PX samples) are more likely to promote better cell adhesion, spreading, and proliferation compare to smoother or more hydrophobic surfaces (*i e* HA sample) It is noteworthy to mention that all surface properties characterised in this study were carried out using films prepared from the same batch of latexes Some surface properties may differ between batches possibly affecting the cell activity trend reported in this study Nevertheless it should be noted that all four latexes are distinctly different in their compounding formulations and vulcanisation processes[9] Taking this factor into consideration, the cell activity trend observed in this study is unlikely to differ substantially where comparison between these four latexes are concerned

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