View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Aston Publications Explorer

Plasma generated poly(allyl alcohol) antifouling coatings for cellular attachment

Lucy M. Watkins,[a] Adam F. Lee,[b*] James W. B. Moir[c] and Karen Wilson[b*]

[a]Department of Chemistry, University of York, York YO10 5DD, UK

[b]European Bioenergy Research Institute, Aston University, Birmingham B4 7ET, UK

[c]Department of Biology, University of York, York, UK

[*]Corresponding authors: Email: k.wilson@aston.ac.uk, a.f.lee@aston.ac.uk; Tel: +44 (0)121 2045456

Abstract

Conformal poly(allyl alcohol) (PAA) coatings were grown on a biomedical grade polyurethane scaffold using pulsed plasma polymerization of the allyl alcohol monomer. The creation of a continuous wave polymer primer layer increases the interfacial adhesion and stability of a subsequent pulsed plasma deposited PAA film. The resulting PAA coatings are strongly hydrophilic and remain unchanged following seven days incubation in biological media. Films prepared through this two-step process promote human dermal fibroblast cell culture, while resisting E. Coli biofilm formation.

Keywords: Plasma; Polyurethane; XPS; Biofilm; Polymerisation; Fibroblast

Introduction

The design and development of medical devices and related biomaterials is a major growth area in biochemistry and tissue engineering [1, 2]. Clinical applications range from ‘active’ gauzes and bandages, to fibres for cell growth and scaffolds for tissue culture and body implants. Almost 80 % of hospital acquired infections are associated with bacterial adhesion at the surfaces of such materials [3], resulting in growth of antibiotic-resistant colonies termed biofilms. Such biofilms are of particular concern during the use of surgical devices e.g. scalpels and joint implants, with many chronic infections arising from accidental transfer of persistent antibiotic-resistant bacteria [4]. There is thus an urgent need for new surface coatings able to combat biofilms through simple application methodologies [5].

Plasma polymerisation (PP) is a versatile vapour phase method for functionalising a wide range of materials including polymers, wood, metal, ceramic or glass [6-8]. PP can achieve profound changes in surface chemical functionality, enabling control over key factors such as wetting or adhesion, without changing the corresponding bulk properties of the solid [9, 10]. There is great freedom in the range of functional monomers amenable for PP including

alcohols [11, 12], amines [13-15], cyano groups [16], carboxylic acids [17], anhydrides [18], epoxides [19], fluorocarbons [20, 21], ferrocenes [22] and aromatic compounds [23]. The resulting polymer films can be tailored only a few atomic layers thick, and are conformal, following the three dimensional contours of (even complex) substrate surfaces. Such properties minimize coating costs, and renders PP ideal for coating fibrous materials such as membranes or gauzes which possess porous architectures. Control over the resulting coating surface functionality also affords the ability to promote (or suppress) cell adhesion [24], and is preferable to gas phase corona or conventional plasma routes which often offer only short-lived benefits [25, 26].

Plasma polymer coatings of acrylic acid [27], isopropyl alcohol [28], allylamine [**Error! Bookmark not defined.**] and isopropylacrylamide [29], as well as co-polymers of allyl alcohol [30], acrylic acid [**Error! Bookmark not defined.**] or allylamine [30, 31] with 1,7-octadiene have been successfully applied for the adhesion and proliferation of various cell types including fibroblasts, corenal and aortic endothelial cells. Hydroxyl functionalised surfaces are particularly interesting coatings for biomaterials, since not only do they exhibit increased cell attachment and proliferation [24, 32], but surfaces exposing polyethylene glycol groups also possess remarkable antifouling properties and resistance towards biofilm formation [33, 34]. Although cell attachment and proliferation has been previously studied over alcohol based plasma polymers, these have focused on substrates such as polystyrene [**Error! Bookmark not defined.**], aluminium foil, glass, and tissue culture plastic [**Error! Bookmark not defined.**]; materials incompatible with the manufacture of durable medical implants. In contrast, polyurethane (PU) is a practical medical substrate [35] which already finds application in catheters, vascular grafts, artificial tendons or as a wound dressings. We previously reported that pulsed PP of allyl alcohol monomer permits the growth of hydrophilic PAA films with high surface hydroxyl densities on medical grade PU (Estane 58201) [36]. However, such PAA coated surfaces have not been investigated in biomedical applications such as cell adhesion for tissue culture or the genesis of antibacterial surfaces. Here we report on the development of an antifouling coating for PU medical devices which both hinders bacteria adhesion and promotes dermal fibroblast cell attachment and proliferation.

Experimental

Plasma polymerisation. Thin films (1.5 mm) of PU (Estane 58201, Noveon) were prepared by compression moulding of beads between sheets of release film (Tygaflor) using a Fontyne Press (Mackey Bowley) at 220 °C and 50 kNm^{-2} pressure. Prior to compression, beads were dried overnight in a vacuum oven at 80 °C to remove excess water. Films were cut into 28 mm discs after pressing, and then sonicated in distilled water for 10 min and finally washed in ethanol before use.

Plasma coatings were generated in an inductively coupled RF pulsed plasma reactor operating at 13.56 MHz, the details of which have been previously reported [**Error! Bookmark not defined.**]. Allyl alcohol (Aldrich >99 %) was subjected to several freeze-thaw cycles prior to use, and dosed into the reactor via a needle valve to achieve a constant pressure of $5x10^{-2}$ Torr. A pulse generator was employed to set different duty-cycles (t_{on} /(t_{on} + t_{off})) which were monitored via an oscilloscope. Samples were prepared at 20 W either by treating for 40 min with a fixed duty cycle of 1 ms/5 ms (pulsed) or by first treating with a continuous wave (CW) plasma for 2 min followed by 38 min of 1 ms/5 ms PP duty cycles (CW-primed+pulsed). This pulsed duty cycle was chosen as previous studies [**Error! Bookmark not defined.**] showed that it afforded excellent retention of surface -OH functionality. Film thicknesses were calculated from deposition rates under the different growth conditions from attenuation of the N 1s XPS signal of the underlying PU substrate, and assuming a layer-by-layer growth mode. Deposition rates of ~2.4 and 3 $nm.min^{-1}$ were obtained for CW and 1 ms/5 ms PP sequences respectively, which were used to adjust treatment times to obtain a common coating thickness of ~120 nm. Treated samples were stored in vacuo in a dessicator prior to XPS and contact angle analysis, which were performed within 3 h of their preparation.

XP spectra were obtained using a Kratos AXIS HSi photoelectron spectrometer with a charge neutralizer and Mg K_α excitation source (1253.6 eV). Energy referencing was employed using the carbon C 1s (C-C/C-H) 285 eV peak and valence band. Survey spectra were acquired for each sample, together with high-resolution C, N and O 1s spectra at pass energies of 160 eV and 20 eV respectively. Peak analysis of XP spectra was performed using CasaXPS Version 2.1.9 software, with all spectra Shirley background-subtracted prior to fitting. A common lineshape was adopted for each element, based on a Gaussian-Lorentzian mix, with FWHM = 1.23 (C 1s), 1.44 (N 1s), 1.76 (O 1s), and percentage Lorentzian convolution = 30 % (C), 30 % (N), 70 % (O) respectively. Elemental compositions were determined using the appropriate instrumental response factors for C (0.318), O (0.736) and N (0.505).

ATR-IR measurements were carried out on a Bruker Equinox 55 Infra-red spectrometer using a silicon ATR crystal (IR Select) 50×20×20 mm aligned to an incident angle of 45°. Advancing contact angles were measured at room temperature via the dynamic sessile drop method using a Krüss DSA 10 MK2 instrument. Deionized water from a Nanopure system (Barnstead, Dubuque, IA, conductivity 1.8 $m\Omega.cm^{-1}$) was used as the test liquid. The tangent method was used to compute contact angles in all cases with an average of 10 readings taken across the surface. Zeta potential measurements were conducted on an Anton Parr KG Electrokinetic Analyzer using a flat cell compartment based on the streaming potential method. Time and pH dependent zeta potential measurements were performed in 1 mmol aqueous KCl supporting electrolyte solution while varying pH with 0.1 M KOH using an Anton Parr KG

remote titration unit at 20 °C.

Cell culture experiments. Cell culture studies utilized 28 mm discs of Estane 58201 (Noveon) which were placed in six well untreated polystyrene culture plates (Falcon®, Becton Dickinson). Samples were sterilized with UV radiation for 2 h (UVasol 450) prior to addition of the cell suspension or media. Cells were seeded onto the centre of the disc using of stainless steel rings (5 mm I.D.) to ensure initial cell confinement at the centre of the scaffold. 500 μl aliquots of cell suspension containing 70,000 cell.cm^3 were pipetted into the well formed within the steel rings. The cell suspension was then made up to a volume of 2 cm^3 with tissue culture media (TCM) (Dulbecco's Modified Eagles Medium+10 vol% Foetal Calf Serum). The rings were carefully removed and an additional 2 cm^3 of TCM added to the wells to ensure coverage of the scaffolds. Plates were placed in an incubator at 37 °C under 5 % CO_2. Every 2 days half of the TCM (2 cm^3) was removed from the six well plates to allow removal of cell debris. 2 cm^3 of fresh media was then carefully added to provide nutrients for the living cells to continue growing.

Samples were then analysed via live/dead, and WST-1 (4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assays at 2, 4 and 7 day time points. The live/dead assays were performed utilising two fluorescent dyes. Target cells (live cells) are stained green by a fluorescent membrane dye 3,3-dioctadecyloxacarbocyanine perchlorate. A second dye, propidium iodide which only penetrates dead cells whose cell walls are not intact, was used to stained these nuclei a fluorescent red. Samples for live/dead assaying had TCM removed and were washed several times with phosphate buffered saline (PBS) after which 2 cm^3 of the live/dead dye was added, sufficent to cover the scaffold. The dye was left in contact with samples for 20 min to allow full interaction with cells and then removed for analysis by confocal microscopy.

Colorimetric WST assays were performed by first removing TCM from samples, then adding 4 cm^3 of fresh TCM along with 400 mm^3 of cell proliferation reagent (WST-1 Roche Diagnostics Ltd). Plates were incubated in 5 % CO_2 at 37 °C for 3 h. Readings were taken by placing 100 mm^3 aliquots of samples into a 96 well TCP plate. Absorbances were measured by UV-Vis spectroscopy against a blank background control using a 450 nm test wavelength and 655 nm reference wavelength. Quantification was performed against a multi-point calibration curve of absorbance for known cell concentrations.

Bacterial adhesion experiments. A culture of *Escherichia Coli* (*E. Coli*, W3110-K12 strain) was grown overnight in a Petri dish, after which bacteria were re-suspended in 500 μl of Luria Bertani (LB) media containing 10 g.L^{-1} tryptone, 5 g.L^{-1}

yeast extract and 5 g.L^{-1} NaCl. Polymer samples were washed with ethanol and distilled water to sterilize the surface prior to bacterial exposure. Samples were then placed into a fulcrum tube containing 5 cm^3 of LB media and 100 mm^3 of E. Coli suspension (10^6 cells.cm^3) and incubated under gentle agitation at 37 °C. Analysis was undertaken via a confocal microscope after one and four days incubation.

Results and Discussion

Strong adhesion between the PU substrate and plasma film is essential for coating longevity, and hence the effect of surface pretreatment on PAA coating stability was first explored. Film degradation was assessed by monitoring breakthough of the N 1s XP signal from the underlying PU substrate following seven days incubation in PBS. Direct deposition of PAA (1 ms/5 ms) onto untreated PU was compared to films deposited onto surfaces pretreated by either argon etching or a CW-primer PAA layer; the latter were expected to enhance covalent interfacial interactions. All films were prepared with similar 120 nm thicknesses as described in the experimental. Resuting surface compositions are shown in **Figure 1**, which revealed that the O:C ratio of all as-prepared films was similar, and higher than that of untreated PU as expected following allyl alcohol functionalization. However, following incubation only the film deposited onto the CW-primed layer remained stable, with nitrogen breakthough evident for PAA films prepared on both untreated and Ar$^+$ etched PU surfaces; the concomitant decrease in O:C ratio with increasing N:C ratio suggests that the latter films underwent fracturing or delamination from the PU substrate.

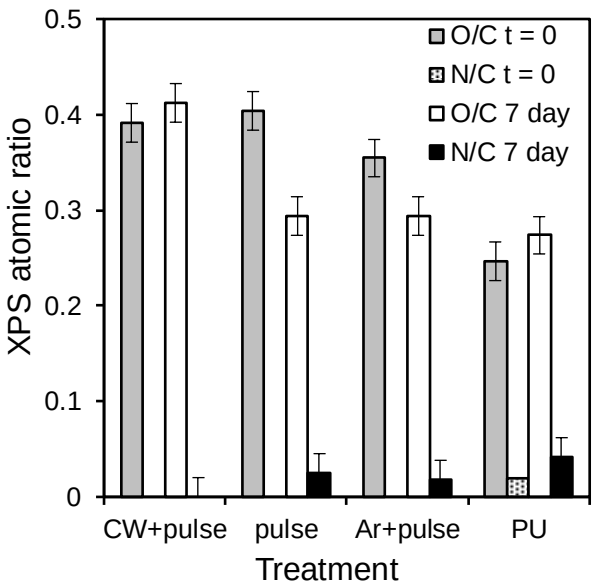

Figure 1. Effect of PP treatments on PAA film stability over PU. O:C and N:C atomic ratios from XPS of PAA coatings on as-prepared and 7 day incubated PU samples. Films prepared with allyl alcohol monomer using either 2 min CW-primed+38 min pulsed, 2 min Ar^+ etch+40 min pulsed, or 40 min pulsed only.

Figure 2 shows the high resolution N and C 1s XP spectra of fresh and PAA-modifed PU before and after PBS incubation. The parent PU exhibits 3 main components in the C 1s spectra arising from the aryl, ether and amide functionalities of the urethane monomer at 285, 286.5 and 288 eV respectively, with a single N 1s component at 400.2 eV attributed to the amide function. Both pulsed PAA treatments, either onto the CW primer layer or directly on the underlying substrate, completely attenuated the amide fingerprint for as-deposited films >100 nm thick (**Figure 2a**). The C 1s spectra of the as-deposited CW-primed substrate is oxygen deficient and exhibits a high CH_x:C-OH(R) ratio of ~5:1 (**Figure 2b**), arising from extensive fragmentation of the allyl alcohol monomer under under CW conditions. However, pulsed plasma treatment of the analogous bare or CW-primed substrates yielded C 1s spectra exhibiting two main components in a 2:1 ratio at 285 and 286.5 eV, consistent with the CH_x and C-OH(R) species expected for PAA. Uniformity of the as-deposited CW-primed+pulsed film on the PU substrate was verified by XPS imaging of C 1s, O 1s and N 1s regions (**Figure S1**) and AFM (**Figure S2**), with the latter also evidencing a decrease in surface roughness relative to the parent PU substrate (**Table S1**).

Film integrity after immersion at 37 °C in PBS, a model biological media, was subsequently assessed by monitoring the N 1s XP signal from the PU surface amide functionality. Results were striking: after a 7 day exposure to PBS solution, the PAA coating prepared by pulsed PP alone had degraded significantly, re-exposing the PU substrate, whereas that grown over the CW-primed surface remained entirely intact (**Figure 2a**). Preservation of the latter CW-primed surface was verified by C 1s XPS (**Figure 2b**) which showed that the CH_x:C-OH(R) ratio remained ~2:1 following incubation, i.e. the two-step PP method increased the long-term stability of the PAA films in biological media. Changes in the C-OH(R) region of the C 1s spectra of the pulsed only PAA coating after PBS incubation are attributed to oxidative degradation of the underlying PU substrate during incubation [37], similar to that observed for untreated PU (**Figure S3** and **Table S2**), highlighting the need for the CW-primer to preserve the subsequent pulsed PAA overlayer.

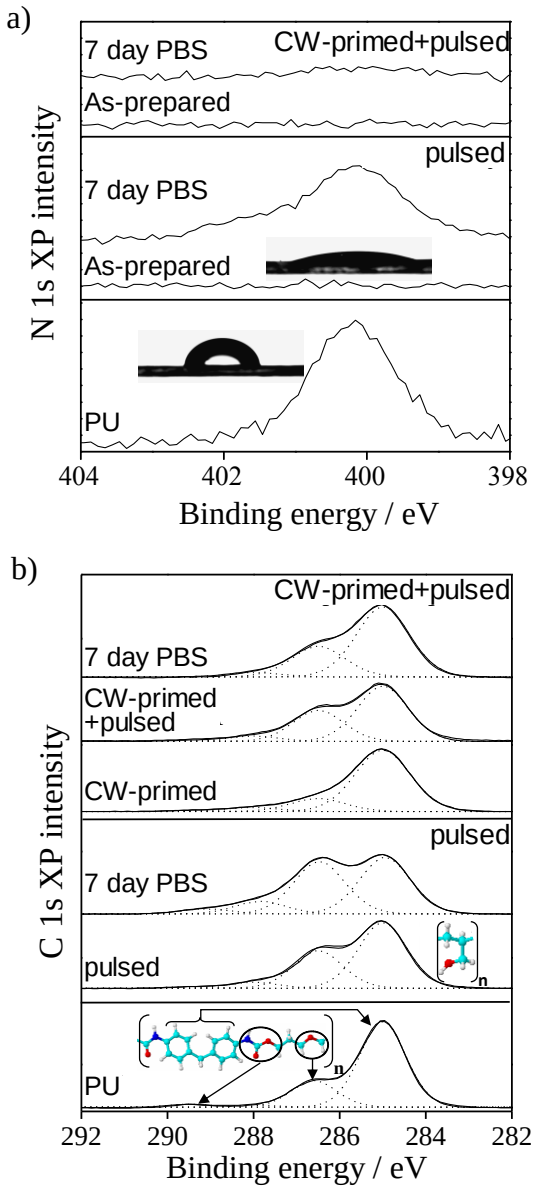

Figure 2. a) N 1s and b) C 1s XP spectra of PU and pulsed plasma PAA treated PU films prepared with and without CW primer layer before and after 7 days PBS incubation.

The atomic compositions of parent and treated PU surfaces are given in **Tables 1** and **2**, alongside the evolving substrate hydrophobicity from contact angle measurement. Pulsed treatments produced the most polar surfaces (low contact angles), reflecting greater incorporation of intact alcohol groups, and this improved hydrophilicity was in all cases retained after PBS exposure. In contrast, degradation of the allyl alcohol monomer during growth of the CW-primer resulted in a poorly hydroxylated surface and corresponding small increase in surface hydrophilicity (evidenced by a slight decrease in the contact angle from 82 ° to 60 °).

Table 1 Surface atomic compositions and hydrophilicity of PU surfaces.

Sample	C / %	O / %	N / %	Contact angle / °
PU substrate	84.3	14.5	1.2	82
CW-primed only	82.5	17.4	0.1	62
Pulsed only	71.2	28.8	0	18
CW-primed+pulsed	73.8	26.2	0	18
Pulsed only+7 days PBS	75.5	22.2	1.8	21
CW-primed+pulsed+7 days PBS	70.8	29.2	0	20

[a]Determined from XPS.

Table 2 Fitted C 1s XP components of PU surfaces.

Sample	C 1s components / %				
	285 eV CH_x	286.5 eV C-OH(R)	288 eV C=O	289.5 eV COOH	CH_x:COH(R)
PU	81.3	13.7	2.2	2.8	5.9
CW primed	77.2	16.3	5.2	1.3	4.7
CW-primed+pulsed	59.6	33.4	5.7	1.3	1.8
CW-primed+pulsed+7 days PBS	66.7	29.2	4.1	0	2.2

Figure 3 shows the ATR-IR spectra of untreated PU, and those following CW-priming and subsequent pulsed PAA treatments. PU exhibits strong bands spanning 1531 to 3318 cm^{-1} arising from the polyether and amide functions. Deposition of a CW-primer attenuates the C-N and aromatic C-C stretches, and broadens the 1700 cm^{-1} C=O region, but had no impact on the hydroxyl region, in agreement with XPS. Subsequent growth of a pulsed PAA overlayer fully attenuated the PU features, accompanied by the emergence of two poorly resolved CH_x stretches at ~2900 cm^{-1} and a strong, broad OH band at 3375 cm^{-1}, confirming the genesis of a hydroxyl terminated surface. The sharp C=O vibration at 1706 cm^{-1} in the CW-primed+pulsed PAA film is also consistent with XPS which suggests that ~5 % of the surface carbon is present as carbonyl functions, likely arising from transformation of allyl alcohol monomers into the resonant CH_2^+-CH_2-HC=O keto-form during plasma activation [**Error! Bookmark not defined.**][38]. These assignments are summarized in **Table 3**.

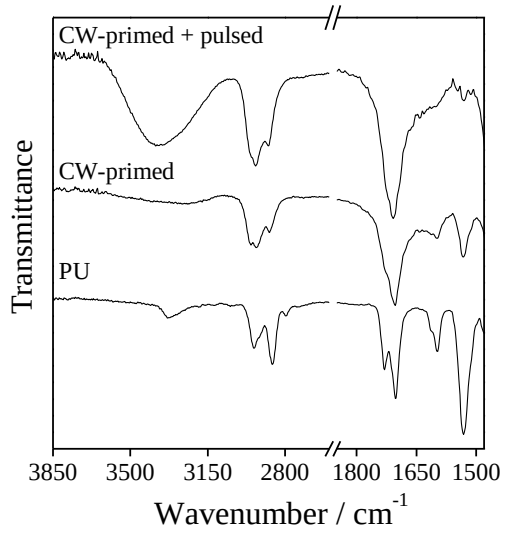

Figure 3. ATR-IR spectra of untreated, CW-primed and CW-primed+pulsed PAA treated PU surfaces.

Table 3 IR assignments for PU surfaces.

Assignment	Untreated PU / cm^{-1}	CW-primed / cm^{-1}	CW-primed+pulsed / cm^{-1}
ν C-N	1531	1531	
ν C-C aromatic	1599	1598	
ν C=O (H bonded amide)	1703	1702	
ν C=O		1706	1706
ν C=O (Free amide)	1732		
νs CH (polyether)	2794		
νas CH (polyether)	2856		
ν CH		2871	2879
ν CH		2927	2931
νas CH (polyether)	2937	2952	
ν NH (H-bonded)	3318		
ν OH		3324 (b)	3376

Figure 4 highlights the inverse relationship between contact angle and surface C-OH:CH_x ratio as a function of PU treatment, and resiliance of the CW-primed+pulsed PAA treated PU surface to PBS incubation.

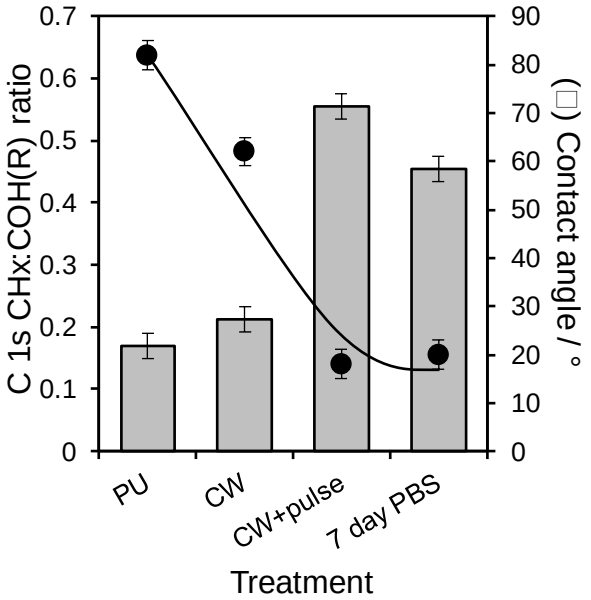

Figure 4. Contact angle and C 1s CH_x:COH ratios of PU and pulsed plasma PAA coated films prepared with and without CW primer layer, and after 7 days PBS incubation.

Bacterial adhesion to surfaces, and subsequent biofilm growth, is facilitated by biospecific (protein-protein, carbohydrate-protein) and non-specific (hydrophobic or electrostatic) surface interactions of pili and flagella [29, 39]. Surfaces terminating in poly(ethylene glycol) chains or hydrophilic hydrogen bonding acceptor groups exhibit antifouling properties [40], and hence the biofilm resistance of our hydroxyl-rich, CW-primed PAA coatings was explored through incubation with *E. coli* (**Figure 5**). Bacterial binding studies were undertaken by suspending PU samples in a fulcrum tube containing Luria Bertani (LB) media and *E. Coli* W3110 suspension under gentle agitation at 37 °C; the number of bacteria in suspension far exceeded that required to saturate the substrate geometric surface area. The CW-primed+pulsed PAA coating exhibited a striking resistance towards *E.Coli* adhesion, even after 4 days continuous incubation, with bacterial densities reduced by 88 % relative to untreated PU (44,743 mm^{-2}). This suppressed adhesion may reflect different surface charges of the bare and treated PU surfaces: zeta potential measurements revealed that $\zeta_{plateau}$ increased from -28±2 mV to -35±2 mV following the CW-primed+pulsed PAA treatment (**Figure S4**), expected to induce stronger repulsive electrostatic interactions with *E. Coli* bacterium (which possess a negative surface charge [41]). AFM studies have also shown that *E.coli* adhesion depends on the structure and length of the carbohydrate chain attached to the lipopolysaccharide coating of the cell, which affects the hydrophobicity of the bacterium surface. *E.coli* adhesion is favoured on high surface energy, hydrophobic [42] substrates with rough surface morphologies [43], consistent with our observation of a weakened bacterial interaction

over the hydrophilic, negatively charged, and smoother two-step PAA coated PU. There are few reports on *E. coli* adhesion over plasma polymerized surfaces, however our results compare favouably with those for plasma deposited triglyme (triethylene glycol dimethyl ether, $C_8H_{18}O_4$), wherein biofilm formation on stainless steel from mixed cultures of *Salmonella typhimurium*, *Staphylococcus epidermidis* and *Pseudomonas fluorescens* was reduced by 94 % after 24 hours [44].

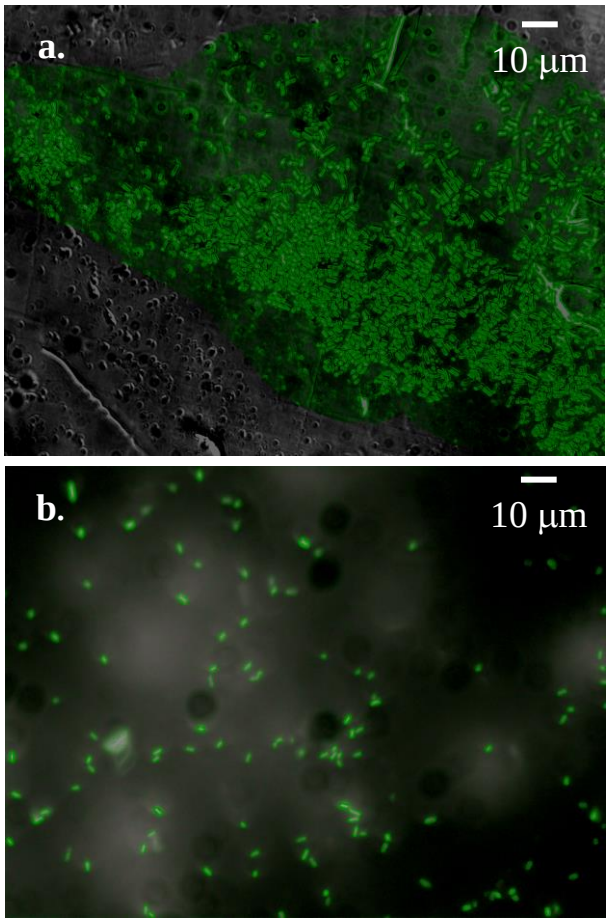

Figure 5. Confocal micrographs of *E. Coli* over a) bare PU and b) CW-primed+pulsed PAA treated PU surfaces.

In contrast to the preceding bacterial adhesion studies, dermal fibroblasts grow well over oxygen-rich plasma polymer surfaces such as isopropyl alcohol [28] and plasma co-polymers of allyl alcohol/1,7-octadiene [30]. We therefore explored the viability of our coatings for Human Dermal Fibroblast (HDF) attachment to determine whether the hydroxyl-rich CW-primed+pulsed PAA coating would improve skin cell adhesion and proliferation over PU. Samples were analyzed for HDF binding over a one week period, via live/dead and quantitative WST assays. **Figure 6** shows micrographs of the live/dead stained samples after 2 and 7 days culturing, which reveal a progressive increase in viable HDF surface coverage over the two-step PAA treated sample. The parent PU exhibited low

fibroblast growth, with cells presenting rounded morphologies, indicative of poor fibroblast attachment. In contrast, the CW-primed+pulsed PAA treated substrate exhibited far higher cell densities, with fibroblasts displaying extended, cross-linked structures indicative of excellent cell proliferation. A WST quantitative assay was also performed to quantify cellular coverages and the proportion of viable cells over untreated and (CW-primed+pulsed PAA treated PU (**Figure 7**). HDF cell densities of 150 and 330 mm^{-2} were measured after 2 and 7 days respectively over the two-step PAA treated sample, representing increases of 17 % and 58 % respectively relative to the parent PU substrate. This enhanced cell attachment compares very favourably with HDF growth over isopropyl alcohol [**Error! Bookmark not defined.**] and acrylic acid [**Error! Bookmark not defined.**] plasma films. These observations support the XPS results in **Figure 2** that evidence a hydroxyl-rich surface is retained even after 7 days incubation in biological media, highlighting the enhanced performance offered by the two-step coating for medical applications. Note that there was no observable cell attachment over PU subject solely to a pulsed PAA treatment, i.e. without the primer interface, which we attribute to the associated film instability/roughness and consequent inhibited cell adhesion and proliferation; human fibroblasts proliferate preferentially over smooth polymer surfaces [45]. The latter observation is in accordance with cell culture studies over poly(L-lysine)/hyaluronan films where swelling of unstable films prevents cellular attachment [46].

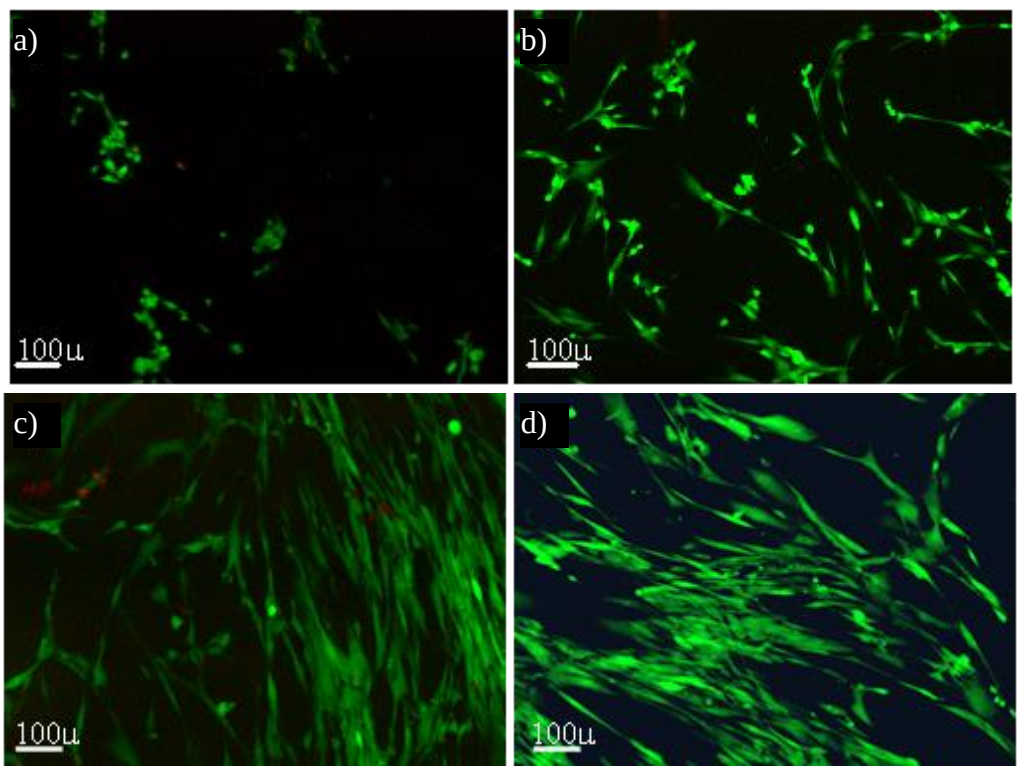

Figure 6. Live/dead stained micrographs of cells on a, c) PU and b, d) CW-primed+pulsed PAA coated PU after 2 and 7 days incubation.

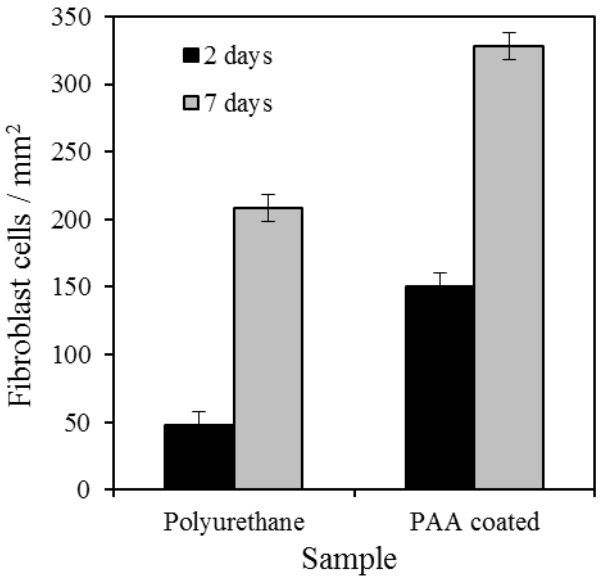

Figure 7. WST assay showing dermal fibroblast cell count on untreated and CW-primed+pulsed PAA coated PU after 2 and 7 days incubation.

Conclusions

Plasma polymerisation offers a simple means to functionalize polyurethane surfaces with coatings which are stable towards extended incubation in biological media. Use of a continuous wave primer layer improves the adhesion and stability of subsequent thin, hydrophilic poly(allyl alcohol) films. Such a two-step PAA coating imparts excellent resistance towards biofilm formation over PU, and improved surface compatibility with human dermal cells, offering diverse biomedical applications. The different performance of the CW+pulsed PAA coated PU substrate towards bacteria versus dermal fibroblasts likely reflects a combination of the enhanced hydroxyl density and film stability/smoothness which favours fibroblast proliferation, and negative surface charge which weakens *E.Coli* attachment.

Acknowledgements

LW thanks Smith & Nephew Research Centre (York, UK) and Dr Jonathan Maughan for studentship support and assistance with fibroblast growth, and Prof Alexander Bismarck (Department of Chemical Engineering, Imperial College London) for access to an electrokinetic analyzer.

Author contributions

LW prepared and characterized the surfaces, and conducted biological tests assisted by JWBM. KW and AFL conceived the study, designed and coordinated the experiments and wrote the manuscript.

Competing financial interests statement

The authors declare that they have no competing interests.

Conflicts of interest

None.

References

[1] L.S. Nair, C.T. Laurencin, Biodegradable polymers as biomaterials, Progress in Polymer Science, 32 (2007) 762-798.

[2] A.J.T. Teo, A. Mishra, I. Park, Y.-J. Kim, W.-T. Park, Y.-J. Yoon, Polymeric Biomaterials for Medical Implants and Devices, ACS Biomaterials Science & Engineering, 2 (2016) 454-472.

[3] J.D. Bryers, B.D. Ratner, Bioinspired implant materials befuddle bacteria, Asm News, 70 (2004) 232-237.

[4] D. Lindsay, A. von Holy, Bacterial biofilms within the clinical setting: what healthcare professionals should know, Journal of Hospital Infection, 64 (2006) 313-325.

[5] E.M. Hetrick, M.H. Schoenfisch, Reducing implant-related infections: active release strategies, Chemical Society Reviews, 35 (2006) 780-789.

[6] P.K. Chu, J.Y. Chen, L.P. Wang, N. Huang, Plasma-surface modification of biomaterials, Materials Science & Engineering R-Reports, 36 (2002) 143-206.

[7] R. Morent, N. De Geyter, J. Verschuren, K. De Clerck, P. Kiekens, C. Leys, Non-thermal plasma treatment of textiles, Surface & Coatings Technology, 202 (2008) 3427-3449.

[8] D. Merche, N. Vandencasteele, F. Reniers, Atmospheric plasmas for thin film deposition: A critical review, Thin Solid Films, 520 (2012) 4219-4236.

[9] H.K. Yasuda, Plasma Polymerization, Academic Press1985.

[10] F.F. Shi, Recent advances in polymer thin films prepared by plasma polymerization Synthesis, structural characterization, properties and applications, Surface and Coatings Technology, 82 (1996) 1-15.

[11] A.P. Ameen, R.D. Short, R.J. Ward, The formation of high surface concentrations of hydroxyl groups in the plasma polymerization of allyl alcohol, Polymer, 35 (1994) 4382-4391.

[12] S. Candan, Radio frequency-induced plasma polymerization of allyl alcohol and 1-propanol, Turkish Journal of Chemistry, 26 (2002) 783-791.

[13] P. Hamerli, T. Weigel, T. Groth, D. Paul, Surface properties of and cell adhesion onto allylamine-plasma-coated polyethylenterephtalat membranes, Biomaterials, 24 (2003) 3989-3999.

[14] A. Harsch, J. Calderon, R.B. Timmons, G.W. Gross, Pulsed plasma deposition of allylamine on polysiloxane: a stable surface for neuronal cell adhesion, Journal of Neuroscience Methods, 98 (2000) 135-144.

[15] J.D. Whittle, R.D. Short, C.W.I. Douglas, J. Davies, Differences in the Aging of Allyl Alcohol, Acrylic Acid, Allylamine, and Octa-1,7-diene Plasma Polymers As Studied by X-ray Photoelectron Spectroscopy, Chemistry of Materials, 12 (2000) 2664-2671.

[16] C. Tarducci, W.C.E. Schofield, J.P.S. Badyal, S.A. Brewer, C. Willis, Monomolecular Functionalization of Pulsed Plasma Deposited Poly(2-hydroxyethyl methacrylate) Surfaces, Chemistry of Materials, 14 (2002) 2541-2545.

[17] J.M. Kelly, R.D. Short, M.R. Alexander, Experimental evidence of a relationship between monomer plasma residence time and carboxyl group retention in acrylic acid plasma polymers, Polymer, 44 (2003) 3173-3176.

[18] M.E. Ryan, A.M. Hynes, J.P.S. Badyal, Pulsed Plasma Polymerization of Maleic Anhydride, Chemistry of Materials, 8 (1996) 37-42.

[19] C. Tarducci, E.J. Kinmond, J.P.S. Badyal, S.A. Brewer, C. Willis, Epoxide-Functionalized Solid Surfaces, Chemistry of Materials, 12 (2000) 1884-1889.

[20] V. Panchalingam, B. Poon, H.H. Huo, C.R. Savage, R.B. Timmons, R.C. Eberhart, Molecular-Surface Tailoring Of Biomaterials Via Pulsed RF Plasma Discharges, Journal of Biomaterials Science-Polymer Edition, 5 (1993) 131-145.

[21] C.I. Butoi, N.M. Mackie, L.J. Gamble, D.G. Castner, J. Barnd, A.M. Miller, E.R. Fisher, Deposition of Highly Ordered CF2-Rich Films Using Continuous Wave and Pulsed Hexafluoropropylene Oxide Plasmas, Chemistry of Materials, 12 (2000) 2014-2024.

[22] L.M. Han, K. Rajeshwar, R.B. Timmons, Film Chemistry Control and Electrochemical Properties of Pulsed Plasma Polymerized Ferrocene and Vinylferrocene, Langmuir, 13 (1997) 5941-5950.

[23] N.M. Mackie, D.G. Castner, E.R. Fisher, Characterization of Pulsed-Plasma-Polymerized Aromatic Films, Langmuir, 14 (1998) 1227-1235.

[24] K.S. Siow, L. Britcher, S. Kumar, H.J. Griesser, Plasma Methods for the Generation of Chemically Reactive Surfaces for Biomolecule Immobilization and Cell Colonization - A Review, Plasma Processes and Polymers, 3 (2006) 392-418.

[25] R. Förch, A.N. Chifen, A. Bousquet, H.L. Khor, M. Jungblut, L.Q. Chu, Z. Zhang, I. Osey-Mensah, E.K. Sinner, W. Knoll, Recent and Expected Roles of Plasma-Polymerized Films for Biomedical Applications, Chemical Vapor Deposition, 13 (2007) 280-294.

[26] D.B. Haddow, S. MacNeil, R.D. Short, A Cell Therapy for Chronic Wounds Based Upon a Plasma Polymer Delivery Surface, Plasma Processes and Polymers, 3 (2006) 419-430.

[27] L. Detomaso, R. Gristina, G.S. Senesi, R. d'Agostino, P. Favia, Stable plasma-deposited acrylic acid surfaces for cell culture applications, Biomaterials, 26 (2005) 3831-3841.

[28] S.A. Mitchell, M.R. Davidson, N. Emmison, R.H. Bradley, Isopropyl alcohol plasma modification of polystyrene surfaces to influence cell attachment behaviour, Surface Science, 561 (2004) 110-120.

[29] H.E. Canavan, X. Cheng, D.J. Graham, B.D. Ratner, D.G. Castner, Surface Characterization of the Extracellular Matrix Remaining after Cell Detachment from a Thermoresponsive Polymer, Langmuir, 21 (2005) 1949-1955.

[30] R.M. France, R.D. Short, E. Duval, F.R. Jones, R.A. Dawson, S. MacNeil, Plasma Copolymerization of Allyl Alcohol/1,7-Octadiene: Surface Characterization and Attachment of Human Keratinocytes, Chemistry of Materials, 10 (1998) 1176-1183.

[31] M. Zelzer, R. Majani, J.W. Bradley, F.R.A.J. Rose, M.C. Davies, M.R. Alexander, Investigation of cell–surface interactions using chemical gradients formed from plasma polymers, Biomaterials, 29 (2008) 172-184.

[32] A.S. Curtis, J.V. Forrester, C. McInnes, F. Lawrie, Adhesion of cells to polystyrene surfaces, The Journal of Cell Biology, 97 (1983) 1500-1506.

[33] D. Cunliffe, C.A. Smart, C. Alexander, E.N. Vulfson, Bacterial adhesion at synthetic surfaces, Applied and Environmental Microbiology, 65 (1999) 4995-5002.

[34] E. Ostuni, R.G. Chapman, M.N. Liang, G. Meluleni, G. Pier, D.E. Ingber, G.M. Whitesides, Self-Assembled Monolayers That Resist the Adsorption of Proteins and the Adhesion of Bacterial and Mammalian Cells, Langmuir, 17 (2001) 6336-6343.

[35] G. Baer, T.S. Wilson, D.L. Matthews, D.J. Maitland, Shape-memory behavior of thermally stimulated polyurethane for medical applications, Journal of Applied Polymer Science, 103 (2007) 3882-3892.

[36] L. Watkins, A. Bismarck, A.F. Lee, D. Wilson, K. Wilson, An XPS study of pulsed plasma polymerised allyl alcohol film growth on polyurethane, Applied Surface Science, 252 (2006) 8203-8211.

[37] P.A. Gunatillake, D.J. Martin, G.F. Meijs, S.J. McCarthy, R. Adhikari, Designing Biostable Polyurethane Elastomers for Biomedical Implants, Australian Journal of Chemistry, 56 (2003) 545-557.

[38] C. Susut, R.B. Timmons, Plasma enhanced chemical vapor depositions to encapsulate crystals in thin polymeric films: a new approach to controlling drug release rates, International Journal of Pharmaceutics, 288 (2005) 253-261.

[39] B. Gottenbos, D.W. Grijpma, H.C. van der Mei, J. Feijen, H.J. Busscher, Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria, Journal of Antimicrobial Chemotherapy, 48 (2001) 7-13.

[40] S. Lowe, N.M. O'Brien-Simpson, L.A. Connal, Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates, Polymer Chemistry, 6 (2015) 198-212.

[41] W.W. Wilson, M.M. Wade, S.C. Holman, F.R. Champlin, Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements, Journal of Microbiological Methods, 43 (2001) 153-164.

[42] Y.-L. Ong, A. Razatos, G. Georgiou, M.M. Sharma, Adhesion Forces between E. coli Bacteria and Biomaterial Surfaces, Langmuir, 15 (1999) 2719-2725.

[43] K.D. Park, Y.S. Kim, D.K. Han, Y.H. Kim, E.H.B. Lee, H. Suh, K.S. Choi, Bacterial adhesion on PEG modified polyurethane surfaces, Biomaterials, 19 (1998) 851-859.

[44] A.R. Denes, E.B. Somers, A.C.L. Wong, F. Denes, 12-crown-4–ether and tri(ethylene glycol) dimethyl–ether plasma-coated stainless steel surfaces and their ability to reduce bacterial biofilm deposition, Journal of Applied Polymer Science, 81 (2001) 3425-3438.

[45] T.P. Kunzler, T. Drobek, M. Schuler, N.D. Spencer, Systematic study of osteoblast and fibroblast response to roughness by means of surface-morphology gradients, Biomaterials, 28 (2007) 2175-2182.

[46] L. Richert, F. Boulmedais, P. Lavalle, J. Mutterer, E. Ferreux, G. Decher, P. Schaaf, J.-C. Voegel, C. Picart, Improvement of Stability and Cell Adhesion Properties of Polyelectrolyte Multilayer Films by Chemical Cross-Linking, Biomacromolecules, 5 (2004) 284-294.