Cell responses to plasma polymers – implications for wound care

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Abstract

Materials used in modern wound dressings have been designed to provide optimum levels of hydration, pH and gas exchange for wound healing. However, materials that provide these properties do not always provide the optimum conditions for cell attachment and growth. Plasma polymerisation is a method by which a thin 'pin-hole' free coating can be deposited on the surface of materials, optimising the surface for cell growth whilst leaving the bulk properties unchanged. Plasma polymerised coatings have found use as surfaces for *in vitro* cell studies and in wound care applications. In this article we will first give a brief introduction to plasma polymerisation. Subsequently the attachment, proliferation and migration of cells involved in wound healing on plasma polymers are reviewed. The attachment of keratinocytes, fibroblasts and endothelial cells to surfaces have been studied in detail. Cell proliferation and, in particular, cell migration have been studied to a lesser extent.

Keywords: wound healing, plasma polymer, cell attachment, keratinocyte, fibroblast.

Introduction to plasma polymerisation

In 1928, Irving Langmuir wrote, "We shall use the name plasma to describe this region containing balanced charges of ions and electrons"[1], and from there the term plasma was born. Plasma is commonly described as the fourth state of matter; it is a natural process seen in lightning and the Northern and Southern lights. Plasmas have been recreated in the laboratory using electric discharge and consist of fragments, ions, radicals and other charged species[2]. Plasma polymerisation is a technique that uses plasmas produced from organic vapours to create thin, functionalised, polymer films upon a substrate. The vapour is excited by energy from radio waves produced at a frequency of 13.56 MHz. This provides energy to excite and fragment the monomer, and deposit a highly cross-linked, homogeneous film upon the substrate surface that leads to surface property changes. Surface chemistry, wettability, adhesion and roughness are among the properties that can be controlled and altered. This technique is attractive compared to traditional polymerisation, because it leads to an increase in efficiency; it only has one step, is solvent-free and it can be used to polymerise a large range of monomers. The coatings produced are thin, nanometre thick, smooth, generally uniform films. It also has an advantage in that films can be deposited onto almost all solid materials and has been shown to have no effect on the original, mechanical properties of the substrate[3]. Thus it is geometry-independent and requires little or no substrate pre-treatment. The main disadvantage of plasma polymerisation is that, because of the fragmentation of the monomer, it can be hard to control chemical functionality. The monomer choice offers control over the functional groups present at the surface. However, by careful control of pressure, power and flow rate, the degree of fragmentation of the monomer molecules can be varied and so changes the density of the functional groups on the surface. Another disadvantage is that high-cost vacuum conditions are generally needed. Plasma polymer films are used in a large range of applications such as barrier coatings[4,5], surfaces for corrosion prevention[6], alcohol electrodes for measuring ethanol content[7], sterilisation of surgical tools[8], ophthalmic lenses[9], biosensors[10] and wound healing[11].

The properties required of a dressing depend on the wound that it is to be used on. For simple, superficial wounds the majority of wound dressings aim to be non-adherent. However, for deeper, more complex wounds

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a dermal replacement is often required. In the case of dermal replacements the wound dressings form a temporary scaffold. This aids the migration of cells within the wound, so increasing the rate of wound healing whilst reducing scaring. Dressings such as Alloderm (Lifecell, USA), Biobrane (Smith and Nephew, UK), Dermagraft (Advanced BioHealing, USA) and Integra (Integralife, USA) are widely used as dermal replacements. Whilst these scaffolds provide an environment rich with biological molecules along which cells can migrate, the newer biodegradable, polymeric scaffolds still need coating with biomolecules to encourage cell migration. The coating of these synthetic scaffolds with plasma polymers can aid the adsorption of biomolecules and therefore produce better dressings and dermal replacements for the healing of wounds.

Effect of surface chemistry on cell attachment

The initial, and therefore perhaps most important, step required for most cells to survive, proliferate and migrate is attachment and so it is essential to provide a surface to which these cells can initially attach. Adherent cells are cultured routinely on tissue culture polystyrene (TCPS), the majority in serum-containing media. However, cells do not possess the necessary integrins to attach directly to TCPS. They, therefore, must attach via a protein layer that has been adsorbed to the surface, normally from the cell culture medium[12-14]. However, as in the case of keratinocytes and corneal epithelial cells, occasionally a more specialised protein surface is required[15]. Due to the complexity and variability of the serum used in cell culture media it is difficult to understand and evaluate which proteins are adsorbed. The proteins that are absorbed also have to be in the correct configuration to interact with the cells. Many cell-adhesion studies have been undertaken to try and obtain a better understanding of the interaction of cells with a range of biomaterials[13,16]. Plasma polymerised surfaces have been used to try and improve the attachment of cells for a range of biomedical applications such as stents used in coronary heart disease[17], catheters[18] and dressings for wound healing[19].

Many studies have looked at keratinocyte behaviour on plasma polymerised surfaces because of their importance in wound care and healing. Keratinocytes form the epidermis and when terminally differentiated provide a waterproof, protective layer. Once breached, it is essential to re-form the barrier function as quickly as possible to prevent infection and dehydration in a wound. It is the stratum corneum that has been shown to be largely responsible for the skin's barrier function[20]. Keratinocytes present a particular problem in that in the lower layer of the epidermis, the stratum basale, they are proliferative, but as they move up through the epidermis they undergo terminal differentiation, forming a layer of dead skin cells on the surface; the stratum corneum. This property means that surfaces that support keratinocyte attachment also need to be able to support keratinocytes in a proliferative, undifferentiated state.

Collagen I has shown to be a preferred substrate for keratinocyte attachment[15] and therefore is typically used as a positive control in studies of keratinocyte attachment to plasma polymerised surfaces. Conversely, hydrocarbon surfaces are an unfavourable surface for keratinocyte attachment[21,22]. This is unsurprising due to their high hydrophobicity.

Plasma polymerised surfaces with acid, alcohol and/or nitrogen functionalities have been found to enhance keratinocyte attachment when compared to a control surface of tissue culture polystyrene. This work has been expanded to produce a range of plasma polymers with a range of functional group concentrations. These polymers are produced by the inclusion of a hydrocarbon monomer, such as 1,7-octadiene, in the monomer feed. These plasma polymers produce bespoke thin films with a range of different surface chemistries, therefore different surface energies, charge and measurable differences in wettability[21,23]. It was found that for plasma polymerised acrylic acid (ppAA) a low carboxylic acid (-COOH) functional group concentration of 2.3% was found to be optimal for keratinocyte attachment. Keratinocyte attachment levels on this 2.3% COOH surface were comparable to keratinocyte attachment on collagen I coated surfaces[21]. For plasma copolymerised (PCP) surfaces of allyl amine, optimal keratinocyte attachment was observed on surfaces with higher functional group concentration. The greatest attachment was seen on the plasma polymer with the greatest N/C ratio, a value of 0.37. In the case of the allyl amine surface the authors were unsure if the increase in attachment was due to an increase in specific nitrogen containing group functionality (amine/amide/imine) or an increase in nitrogen functionality in general[21]. Plasma polymers with hydroxyl (-OH) functionality have been studied by the same group and found that increasing the concentration of hydroxyl groups correlated with an increase in keratinocyte attachment. This correlated nicely with a corresponding increase in the surface energy of the PP film. However, when the surface energy of the PCPs of acrylic acid/1,7-octadiene was analysed, and this theory applied, the opposite trend was observed. Thus the authors concluded that surface chemistry was the principal contributing factor for protein adhesion and, therefore, cell attachment to a substrate, not surface energy[23].

Another cell type of importance when considering cutaneous wound healing are fibroblasts, which synthesise the extracellular matrix proteins of the dermis. The dermis provides the elasticity of the skin but also contains blood vessels and nerves. Fibroblasts need to be cultured in cases where the dermis as well as the epidermis needs to be regenerated, that is, in a burn. A common cell line used to study fibroblast – biomaterial interactions is the murine 3T3 cell line.

Detomaso *et al.*[24] looked at the attachment of 3T3 fibroblasts on two different ppAA surfaces, one with a high surface

concentration of carboxyl groups (16%) and one with a low surface concentration (4%). The surface with low carboxyl group concentration was found to be very stable as opposed to the higher carboxyl surface, whose carboxyl group concentration dropped to 10% after 24 hours soaking in water. The stable, low acid surface had a greater number of cells attached, agreeing with what was seen for keratinocytes[21]. Detomaso *et al.* suggested that the poor ability of the 16% COOH surface to support fibroblast cell attachment may be due to the instability of the film rather than the high concentration of the COOH functional group[24].

When looking at the human fibroblast cell line, 1BR.3N, Mitchell *et al.* looked at attachment on different plasma polymerised isopropyl alcohol (ppIPA) surfaces. They found that, despite differences in hydrophobicity, surface oxygen and surface functional group concentration, all of the surfaces supported cell attachment of approximately 40 cells/mm^2 after 24 hours. They also looked at micro-patterned surfaces and saw that cells attached to the hydrophilic areas preferentially, rather than the untreated hydrophobic areas, again agreeing with keratinocyte behaviour. Once the fibroblasts had reached confluency in the hydrophilic areas, the fibroblasts possessed the ability to grow on the hydrophobic, untreated areas showing adaptive behaviour of fibroblasts[25].

Vascular endothelial cells (EC) form a thin monolayer that lines the entire blood and lymphatic (vascular) system, allowing regular blood flow, preventing platelet adhesion and thrombosis. In coronary heart disease, stents have been used to keep arteries open and since their introduction have saved many lives. Problems associated with the use of these stents are restenosis and thrombosis, the restenosis being minimised by the use of drug eluting stents (DES)[26]. However, thrombosis is still a problem for patients[27] and one cause may be due to the slow formation of the endothelial monolayer on the stent surface. The lack of an EC monolayer means that the essential actions of EC, such as their antithrombogenic behaviour, are not occurring. The slow growth and poor adhesion of endothelial cells to the biomaterial on stents has led to research in designing plasma polymer surfaces specifically for ECs to try to produce new surfaces aimed at reducing thrombosis in patients[17,28].

It has been shown by many that factors such as wettability, surface charge, surface chemistry and topography have an effect on cell attachment to surfaces[29,30]. If evaluating this purely from a point of view of surface chemistry it could be hypothesised that the surface charge will exert the greatest effect due to the size of the interaction forces compared as to if there were just dipole–dipole interactions, for example. However, the wettability of the surface has been shown to affect the attachment of EC by Van Wachem *et al.*[29] and Dekker *et al.*[30]. Moderately hydrophilic surfaces with contact angles between 20 and 45° provided a preferential surface for attachment. Surface charge of the polymer has been shown to have a larger affect, with positively charged surfaces proving preferential over negatively charge surfaces for ECs[17,31]. When looking at plasma polymerised surfaces, Muguruma *et al.*[31] compared an oxygen and an allyl amine plasma treated surface, finding only attachment to the positively charged nitrogen surface. This group found no attachment to the oxygen treated surface; however, this could to be due to the low amounts of carboxyl groups produced. It is more likely the main functionality would be alcohol groups with a few carboxyl groups present[32], as it has been shown that ECs will attach to plasma polymerised acrylic acid (ppAA)[17]. Furthermore, it has been shown that increasing the concentration of carboxyl groups in ppAA surfaces increases EC attachment, which would be expected as the increase in carboxyl groups correlates to an increase in hydrophilicity[33]. The group studied human aortic endothelial cells on plasma polymers of poly(vinylacetic acid), producing polymers with three different concentrations of carboxyl groups on the surface. The surface containing the highest concentration of carboxyl groups, 9%, proved significantly better for EC attachment. Bhattacharyya *et al.* also made films of differing thickness, with the same 9% surface carboxyl concentration, showing that, for initial attachment, a thickness of 100 nm was significantly better than the 25, 50 and 200 nm thick films. However, after seven days, there was no significant difference of cell density between the different film thicknesses. In all cases the carboxyl surfaces proved better for cell growth than TCPS.

Yang *et al.* showed increased EC attachment on a plasma polymerised allyl amine/acrylic acid (ppAam/AA) bipolar film over ppAA, ppAAm and ppAA/Aam films. A bipolar film consists of a stacked film of alternating layers, in this case allyl amine and acrylic acid, and so layers of alternating -COOH/-NH_2 groups. They also showed an increase in EC attachment with increased concentrations of C-NH_3^+ groups[17].

Cell proliferation

Once cell attachment has occurred, cells are able to form colonies and proliferate to increase in number until a confluent layer has been formed. The ability of cells to attach and proliferate upon a surface is of great importance when designing biomedical implants. When developing vascular stents it is important to have a surface that will support the proliferation of endothelial cells, as a confluent layer of endothelial cells is necessary to increase success rates and reduce the risk of restenosis. In wound healing it is desirable to have high cell proliferation to increase cell numbers and close the wound before infection or dehydration. Cell-surface interactions are known to affect cell proliferation but the mechanism by how this happens is poorly understood.

In a study by Haddow *et al.* (1999) keratinocyte attachment on ppAA was found to outperform collagen I. Haddow *et al.*[34] investigated keratinocyte proliferation on a 2% COOH ppAA

surface, an acid functionalised self-assembled monolayer (SAM), and collagen I, a hydrocarbon plasma polymer, TCPS, a methyl-terminated SAM and gold as reference surfaces. They showed that keratinocyte proliferation was similar on the ppAA and the acid-terminated SAM, and that both surfaces supported keratinocyte proliferation at a higher rate than that of collagen I. The authors concluded that the proliferation of keratinocytes is promoted by carboxyl functionality, due to the protein layer adsorbed from the serum[34].

Proliferation of transformed human fibroblasts have been studied on plasma polymerised isopropyl alcohol (ppIPA)[25] and on plasma polymerised acetonitrile (ppAN)[35], the latter also being UV-ozone treated after plasma polymerisation. For ppIPA, fibroblast proliferation was greatest on the film with the highest surface oxygen concentration, hydrophilicity and surface carbonyl and hydroxyl groups[25]. For ppAN, all surfaces supported fibroblast proliferation; the ppAN with no UV-ozone treatment gave similar levels of cells as TCPS. This surface contained less than 3% surface oxygen so the growth of cells was credited to the presence of nitrogen containing functional groups. As the UV-ozone treatment time increased, the concentration of carbonyl groups on the surface increased. Some carboxyl groups were also present in the films UV-ozone treated the longest. These carboxyl containing surfaces had the greatest proliferation and number of cells after 72 hours. The proliferation in the ppAN UV-ozone treated for 15 seconds film was comparable to the ppAN with no UV-ozone treatment, despite the presence of more carbonyl groups in the modified film. These factors contributed to the authors' conclusions that the carboxyl groups are responsible for the increase in fibroblast cell growth[35].

Another group looked at plasma polymerised allyl amine surfaces for studies of human fibroblast cell growth. A positive correlation between amine group surface concentration and metabolic activity of cells was found, the difference being significant after six days of cell culture. All of the ppAam surfaces studied showed improved proliferation over the control PET surface, showing that the surface properties such as increased hydrophilicity and the presence of amine functionalities improve cell growth[36].

Another group trying to find correlations between surface functional group concentration and cell growth looked at bovine aortic endothelial cells (BAEC) and their growth on hydroxyl, carbonyl and carboxyl functionalised surfaces[37]. The surfaces were created by plasma deposition using organic vapours and the surfaces quantified using various reagents for each functional group. No correlation between BAEC growth and carboxyl or hydroxyl functionality was

seen. They found a positive correlation between carbonyl group concentration and BAEC growth. The carbonyl groups were likely to be mainly ketone due to the lack of a reaction with Schiff's base as would be seen if they were aldehyde. In contrast to this, Tidwell *et al.* found the best results for cell growth with carboxyl group terminated self-assembled monolayers (SAMs), with hydroxyl groups performing the worst[38]. The difference in these two results was put down to the low levels of carboxyl groups in the surfaces produced by Ertel *et al.* None of the surfaces produced by Tidwell *et al.*[38] performed as well as TCPS, the authors concluding that multifunctional surfaces may be better for cell growth. Yang *et al.* found increased proliferation on a bipolar film of ppAam/Aac[17].

Cell migration

Whilst there are numerous studies looking at the attachment and proliferation of a variety of cells upon different surface chemistries, the literature is surprisingly sparse upon the effect of surface chemistry upon cell migration. Cell migration is an important process for embryo development, healing of the nervous system and wound healing. Cell migration on solid substrates occurs by an amoeboid movement, in simpler terms known as cell crawling, using actin filaments[39].

Having also looked at attachment and proliferation of endothelial cells, Yang *et al.* also studied migration using a standard scratch assay (illustrated in Figure 1)[17]. In this assay a scratch is gently made using a fine, plastic pipette tip in a confluent layer of cells and watching the cells migrate across the gap. The rate of migration can then be calculated. Yang *et al.* looked at endothelial cell migration on ppAA, ppAAm and a control surface 316L stainless steel, as well as bipolar films of ppAam/AA and ppAA/Aam as described earlier. Images were taken after one day (Figure 1). The ECs migrated faster on the surfaces containing amine groups than the stainless steel control and the ppAA surface. The cells migrated fastest on the ppAam/AA surface, within one day the ECs were covering the complete scratch. This surface had the greatest density of C-NH_3^+ groups and was the most hydrophilic out of the surfaces studies. Yang *et al.* hypothesised that high densities of C-NH_3^+ groups and hydrophilic surfaces were critical for EC migration[17].

Whilst the direct effect of surface chemistry on plasma polymerised surfaces needs to be explored further, the effect of various protein coatings on cell migration has been looked at extensively. The fibronectin cell binding domain RGD (Arginine-Glycine-Aspartic acid) has been bound to various different surfaces to improve cell migration[40, 41]. Additionally, various collagens and other adhesive extracellular matrix proteins such as laminin and fibronectin have also been attached to coatings. Endothelial cells have been shown to have a higher rate of migration on interstitial collagens (types I, III and IV) whilst fibronectin allowed lower rates of migration[42]. Keratinocytes have also been shown to migrate along laminin, vitronectin, fibronectin and collagens I and IV. In a recent review by Siow *et al.*[43] and publications by Whittle *et al.*[44] the effect of surface chemistry upon protein adsorption was studied. It could, therefore, be hypothesised that the preparation of haptotactic gradients of varying surface chemistry could be produced that would result in directed cell migration and potentially increased rates of migration.

Conclusions

All of the above papers report improved cell growth with improved surface functionality, with groups such as carboxyl, hydroxyl and amine. These groups are responsible for increasing the hydrophilicity of the surface, which is shown to correlate with increased cell growth due to hydrophilic surfaces being preferable for protein adsorption. This alone shows the importance of plasma polymerisation as a low-cost and effective technique for the manufacture of biomaterials and in the context of this review, surfaces for wound healing.

Out of all of the three areas discussed above, cell attachment has been investigated the most thoroughly using a wide range of plasma polymerised surfaces and cell types. It has been shown that surfaces containing carboxyl, hydroxyl and nitrogen functional groups support all cell types discussed in this report to some level. It can be hard to compare which surfaces are preferential for each cell type between groups, as often different conditions are used and the data presented in different formats. For comparing keratinocytes, extensive research has been done by the group of Short *et al.* on many different surfaces. It can be concluded from the work of this group that acid surfaces are preferential for keratinocyte growth and proliferation. The effect of surface chemistry on cell migration has not been studied extensively and further research needs to be done in this area due to the importance of cell migration in wound healing.

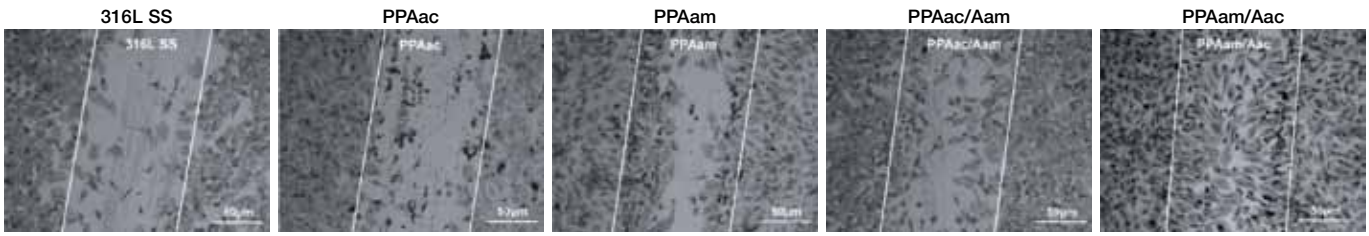

Figure 1. "Migration of HUVECs on 316 SS, PPAac, PPAam, PPAac/Aam and PPAam/Aac multistacked layered bipolar films". Image from[17].

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References

1. Langmuir I. Oscillations in Ionized Gases. Proc Natl Acad Sci U S A 1928; 14(8):627–37.
2. Shen M & Bell Alexis T. A Review of Recent Advances in Plasma Polymerization. Plasma Polymerization: American Chemical Society 1979; 1–33.
3. Colley HE, Mishra G, Scutt AM & McArthur SL. Plasma Polymer Coatings to Support Mesenchymal Stem Cell Adhesion, Growth and Differentiation on Variable Stiffness Silicone Elastomers. Plasma Process Polym 2009; 6(12):831–9.
4. Agres L, Ségui Y, Delsol R & Raynaud P. Oxygen barrier efficiency of hexamethyldisiloxane/oxygen plasma-deposited coating. J Appl Polym Sci 1996; 61(11):2015–22.
5. Zheludkevich ML, Serra R, Grundmeier G, Yang LH & Ferreira MGS. Barrier properties of polyurethane coil coatings treated by microwave plasma polymerization. Surf Coat Technol 2006; 200(12–13):4040–9.
6. Schulz DL, Sailer RA, Braun C *et al.* Trimethylsilane-based pretreatments in a Mg-rich primer corrosion prevention system. Progress in Organic Coatings 2008 Sep; 63(2):149–54.
7. Cokeliler D & Mutlu M. Performance of amperometric alcohol electrodes prepared by plasma polymerization technique. Analytica Chimica Acta 2002; 469(2):217–23.
8. Chu PK, Chen JY, Wang LP & Huang N. Plasma-surface modification of biomaterials. Mater Sci Eng R Rep 2002; 36(5–6):143–206.
9. Wohlrab C & Hofer M. Application of plasma polymerization on ophthalmic lenses – Equipment and Processes. Opt Eng 1995; 34(9):2712–8.
10. Hiratsuka A, Muguruma H, Lee KH & Karube I. Organic plasma process for simple and substrate-independent surface modification of polymeric BioMEMS devices. Biosens Bioelectron 2004; 19(12):1667–72.
11. Haddow DB, Steele DA, Short RD, Dawson RA & MacNeil S. Plasma-polymerized surfaces for culture of human keratinocytes and transfer of cells to an *in vitro* wound-bed model. J Biomed Mater Res A 2003; 64A(1):80–7.
12. Norde W. Protein Adsorption at solid-surfaces – A thermodynamic approach. Pure Appl Chem 1994; 66(3):491–6.
13. Mrksich M. Tailored substrates for studies of attached cell culture. Cell Mol Life Sci 1998; 54(7):653–62.
14. Wahlgren M & Arnebrant T. Protein adsorption to solid-surfaces. Trends Biotechnol. [Review]. 1991; 9(6):201–8.
15. Dawson RA, Goberdhan NJ, Freedlander E & MacNeil S. Influence of extracellular matrix proteins on human keratinocyte attachment, proliferation and transfer to a dermal wound model. Burns. [Article]. 1996; 22(2):93–100.
16. Ziats NP, Miller KM & Anderson JM. *In vitro* and *in vivo* interactions of cells with biomaterials. Biomaterials 1988; 9(1):5–13.
17. Yang Z, Tu Q, Wang J *et al.* Bioactive Plasma-Polymerized Bipolar Films for Enhanced Endothelial Cell Mobility. Macromol Biosci 2011; 11(6):797–805.
18. Onder S, Kazmanli K & Kok FN. Alteration of PTFE Surface to Increase Its Blood Compatibility. J Biomater Sci Polym Ed 2011; 22(11):1443–57.
19. Haddow DB, MacNeil S & Short RD. A cell therapy for chronic wounds based upon a plasma polymer delivery surface. Plasma Process Polym [Article]. 2006; 3(6–7):419–30.
20. Madison KC. Barrier function of the skin: "La Raison d'Etre" of the epidermis. J Invest Dermatol 2003; 121(2):231–41.
21. France RM, Short RD, Dawson RA & MacNeil S. Attachment of human keratinocytes to plasma co-polymers of acrylic acid octa-1,7-diene and allyl amine octa-1,7-diene. J Mater Chem 1998; 8(1):37–42.
22. Haddow DB, Steele DA, Short RD, Dawson RA & MacNeil S. Plasma-polymerized surfaces for culture of human keratinocytes and transfer of cells to an *in vitro* wound-bed model. J Biomed Mater Res A. 2003 Jan 1; 64(1):80–7.
23. France RM, Short RD, Duval E, Jones FR, Dawson RA & MacNeil S. Plasma copolymerization of allyl alcohol 1,7-octadiene: Surface characterization and attachment of human keratinocytes. Chemistry of Materials 1998; 10(4):1176–83.
24. Detomaso L, Gristina R, Senesi GS, d'Agostino R & Favia P. Stable plasma-deposited acrylic acid surfaces for cell culture applications. Biomaterials 2005; 26(18):3831–41.
25. Mitchell SA, Davidson MR, Emmison N & Bradley RH. Isopropyl alcohol plasma modification of polystyrene surfaces to influence cell attachment behaviour. Surface Science 2004; 561(1):110–20.
26. Zilberman M & Eberhart RC. Drug-eluting bioresorbable stents for various applications. Annu Rev Biomed Eng 2006; 153–80.
27. Schulz S, Schuster T, Mehilli J *et al.* Stent thrombosis after drug-eluting stent implantation: incidence, timing, and relation to discontinuation of clopidogrel therapy over a 4-year period. Eur Heart J 2009; 30(22):2714–21.
28. Avci-Adali M, Paul A, Ziemer G & Wendel HP. New strategies for *in vivo* tissue engineering by mimicry of homing factors for self-endothelialisation of blood contacting materials. Biomaterials 2008; 29(29):3936–45.
29. Vanwachem PB, Beugeling T, Feijen J, Bantjes A, Detmers JP & Vanaken WG. Interaction of cultured human endothelial cells with polymeric surfaces of different wettabilities. Biomaterials 1985; 6(6):403–8.
30. Dekker A, Reitsma K, Beugeling T, Bantjes A, Feijen J & Vanaken WG. Adhesion of Endothelial Cells and Adsorption of Serum Proteins on Gas Plasma-Treated Polytetrafluoroethylene. Biomaterials 1991; 12(2):130–8.
31. Muguruma H, Murata N, Kawasaki N, Kuretoko S & Kudo S. Growth of endothelial cells on surfaces modified by a plasma-polymerized coating. IEICE Transactions on Electronics 2007; E90C(9):1844–7.
32. Yoshinari M, Hayakawa T, Matsuzaka K, *et al.* Oxygen plasma surface modification enhances immobilization of simvastatin acid. Biomedical Research – Tokyo 2006 Feb; 27(1):29–36.
33. Bhattacharyya D, Xu H, Deshmukh RR, Timmons RB & Nguyen KT. Surface chemistry and polymer film thickness effects on endothelial cell adhesion and proliferation. J Biomed Mater Res A 2010; 94A(2):640–8.
34. Haddow DB, France RM, Short RD, MacNeil S & Dawson RA. Comparison of proliferation and growth of human keratinocytes on plasma co-polymers of acrylic acid/1,7-octadiene and self assembled monolayers. In: Lee WW, D'Agostino R & Wertheimer MR, eds. Plasma Deposition and Treatment of Polymers 1999; 39–44.
35. Davidson MR, Mitchell SA & Bradley RH. UV-ozone modification of plasma-polymerised acetonitrile films for enhanced cell attachment. Colloids Surf B Biointerfaces 2004; 34(4):213–9.
36. Hamerli P, Weigel T, Groth T & Paul D. Surface properties of and cell adhesion onto allylamine-plasma-coated polyethylenterephtalat membranes. Biomaterials 2003; 24(22):3989–99.
37. Ertel SI, Chilkoti A, Horbett TA & Ratner BD. Endothelial Cell Growth on Oxygen Containing films deposited by Radiofrequency Plasmas – The role of surface Carbonyl Groups. JJ Biomater Sci Polym Ed [Article]. 1991; 3(2):163–83.
38. Tidwell CD, Ertel SI, Ratner BD, Tarasevich BJ, Atre S & Allara DL. Endothelial cell growth and protein adsorption on terminally functionalized, self-assembled monolayers of alkanethiolates on gold. Langmuir 1997; 13(13):3404–13.
39. Arcangeli ABaA. Integrins and Ion Channels in Cell Migration; 2010.
40. Schneider GB, English A, Abraham M, Zaharias R, Stanford C & Keller J. The effect of hydrogel charge density on cell attachment. Biomaterials 2004; 25:3023–8.
41. Cavalcanti-Adam EA, Volberg T, Micoulet A, Kessler H, Geiger B & Spatz JP. Cell Spreading and Focal Adhesion Dynamics Are Regulated by Spacing of Integrin Ligands. Biophys J [DOI: 10.1529/biophysj.106.089730]. 2007; 92(8):2964–74.
42. Madri JA, Pratt BM & Yannariello-Brown J. Matrix-driven cell size change modulates aortic endothelial cell proliferation and sheet migration. Am J Path 1988; 132(1):18–27.
43. Siow KS, Britcher L, Kumar S & Griesser HJ. Plasma methods for the generation of chemically reactive surfaces for biomolecule immobilization and cell colonization – A review. Plasma Process Polym 2006; 3(6–7):392–418.
44. Whittle JD, Bullett NA, Short RD, Ian Douglas CW, Hollander AP & Davies J. Adsorption of vitronectin, collagen and immunoglobulin-G to plasma polymer surfaces by enzyme linked immunosorbent assay (ELISA). J Mater Chem 2002; 12(9):2726–32.