Correlation between surface properties of polystyrene and polylactide materials and fibroblast and osteoblast cell line behavior: a critical overview of the literature

Rémi G. Tilkin[1,2], Nicolas Régibeau[1,2], Stéphanie D. Lambert[1], Christian Grandfils[2]*

1: Department of Chemical Engineering – Nanomaterials, Catalysis and Electrochemistry (NCE), University of Liège, B-4000 Liège, Belgium

2: Interfaculty Research Center of Biomaterials (CEIB), University of Liège, B-4000 Liège, Belgium

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ABSTRACT

Bone reconstruction remains an important challenge today in several clinical situations, notably regarding the control of the competition occurring during proliferation of osteoblasts and fibroblasts. Polystyrene and polylactide are reference materials in the biomedical field. Therefore, it could be expected from the literature that clear correlations have been already established between the behavior of fibroblasts or osteoblasts and the surface characteristics of these materials. After an extensive analysis of the literature, our critical review has highlighted the need to develop a more in-depth analysis of the surface properties of these materials. Moreover, the large variation noticed in the experimental conditions used for *in vitro* animal cell studies impairs comparison between studies. From our comprehensive review on this topic, we have suggested several parameters that would be valuable to standardize in order to integrate the data from the literature and improve our knowledge on the cell-material interactions.

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1. INTRODUCTION

Worldwide, over 4 million surgical operations involving bone grafting or bone graft substitutes are performed every year.[1] Bone represents the second most grafted organ after blood transfusion.[2] Moreover, the importance of bone repair is expected to increase continuously in the next decades with the population aging and the constant increase of need for healthcare worldwide.[3]

If bone is well-known to have self-regeneration properties capacity, large fracture defects (*i.e.* gaps beyond two and a half times the bone radius, 13 % of tibia fractures [4]) request the assistance of implants in order to restore the original structure and function of the native bone.[5] If the grafting of implants is successful in several clinical situations [6–8], those foreign materials can raise several issues, like infections, alteration of the implant (*e.g.* fracture, wear, corrosion), or implant loosening due to the well-known stress shielding effect.[9–11] An attractive alternative is organ transplantation.[12,13] However, this approach suffers also from important drawbacks, such as a lack of donors, risk of infection transmission, difficult management in case of multiple surgery sites, or worse, rejection by the recipient.[14–16] Tissue engineering has become one of the most promising techniques to maintain, improve, or reconstruct human tissue, even complete human organs.[17–19] This strategy relies mostly on the administration of stem cells that are injected under a free form or combined with scaffolds. Those materials are made from degradable porous three-dimensional matrices which support and control the spatial organization of stem cells.[17,20] During the reconstruction process, scaffolds can be tailored to degrade according to a kinetics synchronized with the neoformation of bone, in order to support the biomechanical solicitations imposed by the body. At the end of this reconstruction process, only repaired tissue remains, thereby avoiding long-term biocompatibility problem of synthetic implants. Various materials have been proposed for the conception of scaffolds. Several are frequently used nowadays, like ceramics (*e.g.* hydroxyapatite), polymers (*e.g.* aliphatic polyesters), and metals (*e.g.* magnesium).[21–24] These materials have to satisfy very strict and specific requirements, notably in term of biocompatibility, porosity, degradability, and mechanical properties. [20,25,26] Besides these bulk properties, surface properties of the implant are also particularly important. [27,28]

During bone reconstruction several cell types colonize the bone defect, notably osteoblasts. Osteoblasts are mostly responsible for bone remodeling.[29–31] These cells have a cuboidal shape *in vivo* while in vitro they adopt an elongated flattened form with extensions as long as 90 μm. With a large Golgi apparatus-these polarized cells are able to secrete large amount of proteins on the bone matrix face [30–32] They also regulate the activity of osteoclasts (*i.e.* cells responsible for bone resorption) via the production of macrophage-colony stimulating factors. After active bone formation, osteoblasts are embedded in the bone matrix and differentiate into osteocytes.[32]

Fibroblasts can also be found at the fracture site during wound healing. [33,34] Fibroblasts are the most abundant cell type in connective tissue.[33] These cells disclose an elongated flattened stellate form with long cytoplasmic extensions extending along the connective tissue fibers (*i.e.* extension from 50 to 70 μm).[35] Fibroblasts contain an oval nucleus and also a large endoplasmic reticulum connected with the Golgi apparatus.[36] In contrast to osteoblasts, the cytoskeleton of fibroblasts is significantly more rigid because composed of contractile bundles of microfilaments aligned in the direction of the cell movement. These bundles are attached to the focal contacts responsible of cell adhesion to the extracellular matrix (ECM). One of the key function of fibroblasts is structural, with the secretion of proteins composing the ECM.[36] Their regulation role is also crucial thanks to their ability to produce several factors responsible for the connective tissue growth or for the degradation of cell mediators such as cytokines and growth factors.[33,36] Finally, fibroblasts are involved in wound healing.[33,34]

Fibroblasts and osteoblasts have different spreading behavior. Compared to fibroblasts, osteoblasts spread so extensively on surface that their distinction from the substrate is difficult without adopting specific cell markers. This adhesion behavior comes from their softer cytoskeleton whose viscoelasticity properties allow them to adapt to very irregular surfaces. This contrast of cytoskeleton rigidity between fibroblasts and osteoblasts can easily explain that the latter ones prefer rough surfaces while fibroblast proliferation is enhanced on smooth surfaces.[37]

A competition between fibroblasts and osteoblasts occurs at the fracture site.[38,39] This competitive growth leads either to the formation of a healthy bone if osteogenesis is favored, or to the development of a fibrous capsule with the production of pathological scar tissue if fibroblasts are dominating. This cell interplay is also present at the surface of implants.[38,40] The presence of an external body at the fracture site can impair the differentiation of osteoblasts by altering the ideal environment requested for osteoblast differentiation and proliferation. In these conditions, the proliferation of fibroblasts will be enhanced with the formation of a fibrous tissue between the implant and the undamaged tissue, preventing the expected integration of the implant within the surrounding healthy bone [40,41]. This process results in an excessive implant mobility and micromotion, ultimately leading to implant failures.[41] Moreover, fibroblasts are known to produce cytokines able to stimulate osteoclast amplification, to repress osteoblast functions, and to activate local inflammation. The combination of these three events are prone to bone resorption and implant loosening. Accordingly the implant should simultaneously induce the promotion of osteoblast differentiation and proliferation while limiting any fibroblast adhesion and proliferation. From this brief description of the *in vivo* bone repairing process, we could anticipate that several efforts have been made at least *in vitro* in order to highlight the optimal surface properties of implants promoting bone cell proliferation while counteracting fibroblast amplification. Accordingly, an in-depth description of the interactions existing between these two cell types and the material surface seems to us essential.

To analyze the literature in this regard, we decided to focus our attention on two well-known polymers typically adopted for animal cell culture, *i.e.* polystyrene (PS) and polylactide (PLA).

Polystyrene represents obviously a key reference to perform animal cell culture *in vitro* since more than 40 years.[42] Atactic polystyrene typically adopted for animal cell culture is an amorphous hard stiff transparent thermoplastic produced by the polymerization of styrene. This material is further processed under 2D objects (*e.g.* plates, flasks) but also 3D configurations (*e.g.* microcarriers, such as Plastic or PlasticPlus). Due to its high hydrophobicity, pure polystyrene cannot be used as such to support cell culture.[43] Hydrophilization of the surface of PS is therefore requested adopting either chemical etching with sulfuric acid, flame treatment, or corona discharge.[42,44] This functionalized polystyrene is typically called Tissue Culture Polystyrene (TCPS). Nevertheless, researchers are still trying to improve its ability to sustain cell adhesion.

Polylactic acid is a rigid thermoplastic produced by the polymerization of lactic acid or more frequently from its dimer, the L-lactide.[45] Although existing under other enantiomeric forms (*i.e.* poly-D-lactide, poly-L-lactide, and poly-D,L-lactide), most of the research dedicated to bone repair has been focused on PLLA due to its superior mechanical behavior. Characterized by a well-established biocompatibility, the L-lactic acid released from its degradation can be eliminated through kidney filtration or recycled into the tricarboxylic acid cycle.[45–47] Macromolecular features of PLA, optimal isomer ratio, molecular weight, architecture, and crystallinity can be finely tuned to adjust its mechanical properties and its degradation rate.[45,46] With a melting temperature around 180°C, PLA can be easily processed using traditional processing technologies, like extrusion, injection molding, compression molding, or blow molding.[45,48] PLA is notably adopted for screws and plates used in bone fixation, for surgical sutures in wound management, but also for drug delivery systems.[18,45–47] More recently, this polymer has been imposed as one of the most popular to produce scaffolds, notably using 3D printing.[49]

In vitro, fibroblasts and osteoblasts can only survive if they can meet and interact with a surface on which they can adhere, spread, and proliferate.[50] Accordingly, the presence or absence of interaction with the surface induces signal transduction to the nucleus that respectively causes cell growth and differentiation or apoptosis and cell death. As detailed below, many investigations focus on the surface modification of substrates to promote *in vitro* cell adhesion and proliferation. In the next two paragraphs, we will summarize the main observations reported on the interaction of osteoblasts and fibroblasts with the PS and PLA. Two-dimensional substrates are considered to highlight the link between surface modification and cell behavior, without taking into account the micro-environmental aspect present in tri-dimensional substrates. To facilitate the integration of our observations, they are be grouped within 4 tables, each presenting the behavior of a given cell type on one given substrate. Each table is divided in 4 sections, depending on the modification approach: (i) unspecific interactions, (ii) specific interactions, (iii) roughness modification, and (iv) combination of these approaches. Two tables comparing osteoblast and fibroblast behavior at the surface of PS and PLA are also presented. In a second step, the main results are discussed in order to highlight the main molecular features determining fibroblast and osteoblast in vitro behavior.

2. CELL BEHAVIOR ON POLYSTYRENE

Surprisingly, only one study compared the adhesion and proliferation of fibroblasts and osteoblasts on polystyrene. Lavenus *et al*. have compared the proliferation of fibroblasts and osteoblasts on TCPS, glass, and titanium substrate (see Table 1).[51] For both cell types, proliferation rate and cell adhesion was significantly higher on TCPS than on glass and titanium. In the case of fibroblasts, the difference between glass and titanium was negligible. 90 % of fibroblasts were attached after 16 days on TCPS while only 60 % were attached on glass and titanium. The ratio between the number of attached cells after 16 days and the number of seeded cells is 8 for TCPS and 4 for glass and titanium. For osteoblasts, 80 % were attached after 8 h on TCPS while 30 % were attached on glass and 20 % on titanium. Regarding osteoblast proliferation, the difference between glass and titanium was only negligible until day 8. The ratio between attached osteoblasts after 16 days and cells seeded is 30 on TCPS, 17 on glass, and 10 on titanium.

Table 1. Comparison of fibroblast and osteoblast behavior on PS

Modification	**Physicochemical characterization**	**Cell behavior**	**Ref.**
None	F: n.a. C: -29 mV H: 64° R (Ra): 5 nm	Ad T 8 h: 90 % (fibroblast) and 60 % (osteoblast) attached Prol T 16 days: 8-fold (fibroblast) and 30-fold (osteoblast) increase in attached cell number compared to seeded cell number.	51

n.a. = not available ;

C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion ; prol = cell proliferation

2.1 Adhesion and proliferation of fibroblasts on polystyrene

2.1.1 Non-specific surface functionalization

The most common and simple strategy that was reported to enhance animal cell adhesion on native polystyrene relied upon the generation of hydrophilic functional groups at the surface of this polymer. Two main techniques were adopted for this purpose: plasma treatment and irradiation. Lee *et al*. used air plasma treatment to generate carbonyl, ammonium, and amide groups at the surface of PS plates.[52] Compared to fibroblasts that did not adhere and kept a round shape on untreated PS, these cells quickly adhere and spread on plasma-treated PS. More living cells were observed on treated PS (no quantitative results). Plasma treatment of PS dishes was also used by Mitchell *et al*. but in the presence of acetone.[53] The authors reported a correlation in cell density observed after 1 or 2 days with the acetone flow rate imposed during plasma exposition. XPS analysis of the functionalized PS allowed these authors to establish a clear dependence between cell proliferation rate and surface oxygen concentration with an optimal around 5 atomic %. Godek *et al*. used different combinations of gas and monomers such as C_3F_8, acrylic acid, N-vinyl-2-pyrrolidone, N-vinylformamaide, allylamine, and hexylamine for plasma deposition on PS dishes.[54] The authors reported that all surfaces except the FC treated one enhanced fibroblast attachment and proliferation, without providing any quantitative data.

Laser irradiation in the UV range was adopted by Pfleging *et al*. using different laser wavelengths under atmospheric conditions to functionalize PS surface.[55,56] This technique produced -COOH groups in the presence of He or O_2 and primary amino groups in the presence of 2-amino-ethanol. A minimal energy delivered per unit area was highlighted in order to promote cell adhesion. Above this wavelength-dependent threshold, the surface roughness increased significantly while polar groups were removed with as consequence an inhibition of cell adhesion.

Different synthetic macromolecules were also grafted on PS surface to enhance fibroblast interaction. After plasma activation under Ar, O_2, or Ar+O_2, Chen *et al*. polymerized N-vinyl-2-pyrrolidone on PS films.[57] The hydrophilicity increase arising from the presence of poly-N-vinyl-2-pyrrolidone was increased in function of the polymer surface density (ranging from 0.7 to 1.3 mg/cm²). These surface modifications also enhanced fibroblast adhesion and proliferation in relationship with the increase in hydrophilicity. In a similar way, after γ-irradiation activation, Biazar *et al*. grafted poly-N-isopropylacrylamide, a temperature-responsive polymer, on PS dishes.[58] According to microscopic observations only, the authors noticed an enhancement in cell adhesion after 48 h of culture. Interestingly enough, thanks to a lower critical solution temperature of poly-N-isopropylacrylamide close to 37°C, the authors succeeded to detach the cells by a decrease of temperature below 32°C, thus without trypsinisation (no quantitative results). Beside these covalent grafting approaches, Hatanaka *et al*. succeeded to coat TCPS multiwells with poly(uridine 5'-p-styrenesulfonate) using only a physical adsorption process.[59] Compared to untreated multiwells, cell adhesion after 2.5 h of culture was increased by a factor 1.2 and 1.5 using 0.01 mg/ml or 50 mg/ml of poly(uridine 5'-p-styrenesulfonate) respectively. Polyelectrolyte complexes made from polyethyleneimine and heparin were deposed on TCPS dishes by Mao et *al*. [60] Within these thin films, acid fibroblasts growth factors was also incorporated. After 10 days of culture, a 2.5 times increase in cell density was counted on film made from five bilayers and loaded with growth factors compared to TCPS control.

2.1.2 Specific functionalization

The physisorption or the chemical grafting of proteins or peptides was also frequently reported in order to promote a specific cell interaction with PS through adhesion peptide sequences.

Adhesion of peptides

Kurihara *et al*. coated PS sheets with homemade recombinant proteins characterized by increasing density of arginine-glycine-aspartic acid (RGD) sequences.[61] Compared to unmodified PS, this protein coating enhanced cell adhesion with a clear dependence of RGD surface density. Indeed, a 5-fold, 12-fold, and 14-fold increase was noticed in the presence of recombinant proteins bearing 2, 21, and 43 RGD sequences respectively. A similar approach was reported by Vashi *et al*..[62] The authors incubated TCPS plates in a solution comprising polypeptides based on resilin (*i.e*. an elastic and resilient protein found in most insects) before proceeding to a UV irradiation to crosslink the polypeptides. Interestingly, the dependence of fibroblast adhesion with peptide surface density passed through a maximum. Indeed, above a concentration of peptide solution ranging from 0.1 or 10 mg/ml, depending on the polypeptide type, fibroblast adhesion was inhibited.

Proteins from extracellular matrix

Alternatively to a physisorption approach, different surface pretreatments was applied on PS to generate functional groups adopted to graft peptides or proteins on the surface. Plasma activation was the most widely used for this purpose. Bax *et al*. used a nitrogen plasma exposure to attach covalently tropoelastin, an ECM protein providing elasticity to tissue, on PS sheets.[63] If no cell spreading could be observed after 90 min of cell culture on PS control, cell spreading was already detectable on plasma activated PS, while high cell spreading could be observed after tropoelastin grafting. The introduction of a spacer between the surface and peptide or protein was proposed to prevent any steric hindrance and facilitate cell - ligand interaction. For example, Sasai *et al*. used vinyl methyl ether maleic acid copolymer as a bridge to attach GRGDS peptides on petri dishes.[64] After 6 days of culture, the cell number was multiplied by 4.5 compared to seeding. Choi *et al*. used allylamine as a linker to immobilize collagen on PS films.[65] After 7 days of culture, no significant difference was observed between allylamine treated PS and untreated PS while the attachment to collagen surface significantly increased the cell proliferation rate.

2.1.3 Surface roughness modification

Modification of topography of PS and the outcomes on cell shape and movement was investigated by Frey *et al*..[66] In this optic, pillars were printed on PS films adopting a molding process (see pillar features given in Table 1). The authors found that this increase in roughness increased the cell migration 1.5 times compared to the native flat PS, but also promoted a dentritic shape to the cells (*i.e*. longer perimeter relative to the area). In a similar way, Crouch *et al*. have assessed the effect of aspect ratio (*i.e*. depth/width) of gratings grafted on TCPS plates on the anisotropy of fibroblasts.[67] An increase of cell alignment was noticed in the presence of gratings with groove-like morphology. This specific roughness elongated up to two times the fibroblasts.

Table 2. Fibroblast behavior on PS

Class of modification	Specific nature of the modification	Physicochemical characterization	Cell behavior	Ref.
Non-specific functionalization – functional groups	Air plasma	C: n.a. H: p = 82° ; m = 16° F: C=O, NH_4, NH R (R_a): p = 12 nm ; m = 11 nm	Ad T 4 h: quick adherance (no quantification) Morph T 4 h: quick spreading (no quantification)	52
	Acetone plasma	C: n.a. H: p = 92° ; m = 70-77° F: C=O, C-OR, O-C=O R: RMS = 3-5 nm	Prol T 2 days: maximum fold increase of cell number (i.e. ratio of the number of cells T 2 days / T 1 day) of up to 14.3 (compared to a value of 2.2 for untreated TCPS)	53
	Plasma activation in presence of C3F8, acrylic acid, N-vinyl-2-pyrrolidinone, N-vinylformamide, allylamine, hexylamine	n.a.	Ad and prol: enhanced (no quantification)	54
	Laser irradiation in UV	C/R: n.a H: p = 82° ; m = 90-100° F: O-C=O, NH_2	Ad: occurs under a threshold for laser energy delivered (no quantification)	55 56
Non-specific functionalization – macromolecules	Grafting of poly-N-vinylpyrrolidone	C/H/R: n.a. H: p = 88° ; m = 36-51° F: C=O, C-N ; grafting yield = 0.7-1.3 mg/cm²	Prol T 6 days: O.D. 0.52-0.70 (highest proliferation for lowest contact angle = highest grafting yield) compared to 0.35 for PS	57
	Grafting of poly-N-isopropylacrylamide	C: n.a. H: p = 94° ; m = 61° F: C=O, N-H R: t = 100 nm	Ad T 2 days: enhancement (no quantification)	58
	Coating of poly(uridine 5'-p-styrenesulfonate)	C/R: n.a. H: p = 80° ; m = 22° F: presence of polymer	Ad T 2.5 h: increase of 1.5 compared to PS	59

	Coating of (growth factor/heparin)/polyethyleimine layers	C/F: n.a. H: p = 70° ; m = 45° R (RMS): p = 1.38 nm ; m = 4.52 nm	Prol T 10 days: 2.5 times number of cells compared to TCPS	60
Specific functionalization – adhesion of peptides	Physi-sorption of homemade recombinant proteins containing 2, 21 or 43 repetitive RGD sequences	C/H/R: n.a. F: amount of ligand (no numerical value)	Ad T 3 h: 5-fold, 12-fold, and 14-fold increase compared to unmodified PS, for ligands with 2, 21, and 43 RGD	61
	Physi-sorption and reticulation of polypeptides based on resilin (*i.e.* an elastic and resilient protein found in most insects)	R: n.a. C: 10% of charged residues H: p = 62° ; m = 17-27° F: presence of polypeptide	Ad T 1 day: inhibition above a critical peptide concentration (no quantification)	62
Specific functionalization – proteins from extracellular matrix	Grafting of tropoelastin	C/H/R: n.a. F: amount of protein (no numerical value)	Morph T 90 min : 83 % of spreading on modified PS, undetectable on PS	63
	GRGDS with a spacer made from a copolymer of vinyl methyl ether maleic acid	n.a.	Prol T 6 days: 4.5-fold increase in cell number compared to seeding	64
	Allylamine plasma treatment and grafting of collagen	C/R: n.a. H: p = 87°, m (allylamine) = 55° ; m (collagen) = 50° F: presence of allylamine (C-N groups) and collagen	Prol T 7 days: rate 1.6 times higher on collagen modified PS, compared to unmodified PS. No signifant difference between allylamine modified PS compared to unmodified PS	65
Roughness	Contact pressing	C/H/F: n.a. R: 1.78 µm in height, 10.30 µm in diameter, 15.76 µm spaced center-to-center	Mob T 2 h: 1.5 times faster on pillar that flat Morph T 2 h: more branched on pillat than flat	66
	Nanoimprint lithography	C/H/F: n.a. R: width = 0.1-10 µm, depth = 0.1-1 µm.	Mob T 1 day: increase in alignement with the aspect ratio (*i.e.* depth/width) up to 97 % Morph T 1 day: increase of elongation with aspect ratio	67
Combination	n.a.	n.a.	n.a.	n.a.

n.a. = not available ;

p = pure substrate ; m = modified substrate ;

t = thickness ; C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion ; prol = cell proliferation ; via = cell viability ; morph = cell morphology ; mob = cell mobility

2.2 Adhesion and proliferation of osteoblasts on polystyrene

Although that several authors tried to promote fibroblast interaction on polystyrene *in vitro*, all the studies identified have discarded a chemical or/and physical routes reported above to graft simple chemical groups to hydrophilize PS.

2.2.1 Non-specific surface functionalization

Only one biomacromolecule, pectine, (and not synthetic one) has been adopted to promote adhesion of osteoblasts on PS, and with results that are not really convincing (see Table 3). Kokkonen *et al*. coated TCPS dishes with pectin modified hairy regions using allylamine plasma deposition as pretreatment [68]. Their observation have highlighted that, even if osteoblasts could adhere and spread, cell proliferation rate was higher on the TCPS control compared to the same substrate coated with pectin. Moreover, their microscopic observations were not supported by quantitative data. More recently, pectin enriched with galactose residues was coated on TCPS plates by Folkert *et al*.[69]. If this coating increased osteoblast proliferation rate and mineralization compared to the unmodified TCPS plates, the differences were not significant statistically.

2.2.2 Specific surface functionalization

Coating or grafting of adhesion peptides or biomacromolecules was adopted to promote osteoblast adhesion on this polymer.

Adhesion of peptides

In order to guarantee a better accessibility to cell binding domains and to prevent any potential conformational change of proteins once in contact with the surface, the introduction of short peptides on PS was attempted. Harbers *et al*. grafted several specific ligands, including peptides containing RGD sequence, heparin binding peptides (*i.e*. peptides containing RRI sequences), and collagen binding peptides (*i.e.* peptides containing DGEA sequences). These moieties were coated at different surface densities via a polyethylene glycol-amine spacer precoated on PS (see Table *3*).[70] This interesting comparison study evidenced the higher potency of RGD to enhance osteoblast adhesion compared to the two others families. Focusing on RGD binding peptides, the authors found that the increase of ligand density resulted in an increase of the adhesion force between the cells and the substrate (*i.e.* a 18-fold increase of the ligand density led to a 30-fold increase of force needed to detach 70 % of the cells from the surface via centrifugal cell adhesion assay). High surface density of RGD also led to a higher cell density and a higher proliferation rate (see Table 3).

Proteins from extracellular matrix

Hanagata *et al*. examined the effect of pre-adsorbed type I collagen structure (*i.e.* feltwork structure from acid solution and fibrils from neutral solution) and density (*i.e.* concentration of impregnation solutions: 9 and 90 µg/mL) on osteoblasts during incubation.[71] Interestingly enough, the collagen conformation showed a marked effect on the proliferation rate, even though no detectable differences were observed regarding the types and levels of integrin produced in initial cell attachment. Indeed, cell confluence was noticed after 3 days for the feltwork structure and only after 9 days for the fibril structure. Mineralization was also delayed by the fibril structure. In contrast, the surface density of collagen had no influence on cell proliferation. Wende *et al*. chemically grafted collagen type I on TCPS pretreated with NH_3 plasma and using glutaraldehyde as cross-linker.[72] As detailed in Table 3, these changes only slightly affected the osteoblast proliferation rate.

The influence of the concentration gradient of fibronectin adsorbed on PS surface of osteoblast behavior can be presumed from the comparison of the two following studies. When this protein was adsorbed without spatial control of its distribution of the surface of PS or TCPS, no significant change in osteoblast adhesion and proliferation rate was noticed by Stephansson *et al*..[73] In contrast, the controlled deposition of fibronectin according to patterning via microcontact printing by Pan *et al*. highlighted that osteoblasts preferentially attached on area printed with this protein.[74]

The physical deposition of osteopontin, *i.e*. a main protein component of the organic bone matrix, was also investigated on TCPS by Liu *et al*. [75]. These authors adsorbed this biomolecule either directly on PS, either on a collagen layer first deposed on PS. This pre-coating of collagen significantly increased the density of cells adhering after 2 h compared to osteopontin directly applied on PS. Accordingly, a better control of the conformation of osteopontin with higher number of accessible RGD sites for cell adhesion can be expected when this protein was interacting with collagen instead of PS.

2.2.3 Surface roughness modification

The effect of topography at different scales on osteoblasts was investigated.

For example, Lenhert *et al*. nanoimprinted regularly spaced grooves of different depths (*i.e*. 50 and 150 nm) with a periodicity of 500 nm onto PS petri dishes, followed by a O_2 plasma treatment.[76] As already described in the literature, the authors observed that 40 % and 60 % of the osteoblasts aligned within 30° of the groove direction for 50 nm and 150 nm grooves respectively. Interestingly enough, the osteoblasts presented an elongated morphology (*i.e*. aspect ratio significantly higher in the parallel direction), and their migration was 1.46 times faster in the parallel direction for the 150 nm-grooves. Their observation also indicated that a minimum depth of 50 nm was required to control osteoblast cell behavior.

In a similar way, but adopting electron beam lithography, Lamers *et al*. evaluated the influence of several nano-topographical parameters of the surface such as depth, width, (an)isotropy, and spacing on osteoblast behavior (see Table 3).[77] Their results also supported the observations reported by Lenhert *et al*., highlighting that anisotropic surfaces induced an elongated shape to osteoblasts that aligned. Moreover, cell motility was highly dependent on the ridge-groove ratio of anisotropic patterns.

More recently, adopting a similar approach, but considering another scale, Sun *et al*. micro-replicated 2-4 µm wide microgrooves onto PS sheets with a distance between groove of 6-11 µm.[78] This surface roughness also imposed cell alignment along the grooves (*i.e*. all cells aligned in a direction within 10 % of the groove direction). Moreover, for thinner grooves, cells were found on top of the ridge while, for larger grooves, they were found inside the grooves. No influence of topography could be observed on cell proliferation or metabolic activity.

2.2.4 Combination of modification of roughness and surface chemistry

Other authors coated inorganic materials on TCPS, modifying both the roughness and the chemical composition of the PS surface. For example, Goldberg *et al*. coated TCPS dishes with a 7 µm-layer of carbonated hydroxyapatite, a component of the bone calcified matrix.[79] This coating enhanced cell attachment, growth, and proliferation of osteoblasts for 21 days. However, surprisingly enough, the number of adherent cells after 4 and 48h was significantly lower on carbonated hydroxyapatite coating compared to the TCPS control (see Table 3). The highly roughened sharp crystal topography of the ceramic attachment points (*i.e.* crystal size of 27 nm) could explain the limited number of cells adhering initially on this surface.

In order to control independently surface topography and wettability of PS films, Yun Yang *et al*. submitted PS films to CF_4 plasma followed by O_2 plasma for different exposure times.[80] According to this two steps approach, CF_4 activation increased both the contact angle and the roughness of PS films. In contrast, O_2 treatment only decreased the contact angle, while keeping the roughness constant. This study showed that the initial adhesion of osteoblasts was determined by the roughness generated by the CF_4 plasma treatment. This treatment significantly increased osteoblasts proliferation compared to untreated PS and TCPS (see Table 3). On the other hand, the cell adhesion and proliferation were enhanced after the O_2 plasma activation giving rise to an optimal surface roughness.

Table 3. Osteoblast behavior on PS

Class of modification	Specific nature of the modification	Physicochemical characterization	Cell behavior	Ref.
Non-specific functionalization – functional groups	n.a.	n.a.	n.a.	n.a.
Non-specific functionalization – macromolecules	Grafting of pectine	n.a.	Prol T 2 days: lower compared to TCPS (no quantification)	68
	Coating of pectine	C/H/R: n.a. F: presence of pectine	Prol T 12h to T 3 days: increase compared to TCPS (not significant)	69
Specific functionalization – adhesion of peptides	Peptides : RGD, RRI, DGEA	n.a.	Results significantly higher for RGD (=> focus on RGD) Ad T 21 h: 3-fold increase of force needed to detach 70 % of the cells, compared to TCPS Prol T 4 days: 2-fold increase in cell density between ligand density of 1 and 5 pmol/cm², plateau over 5.5 pmol/cm² with similar results as TCPS	70
Specific functionalization – proteins from extracellular matrix	Coating of collagen	C/H/F: n.a. R: 2 structures (felwork vs fibrils)	Prol: confluence T 3 days (felwork) or T 9 days (fibrils). No influence of collagen density (no quantification)	71
	Grafting and coating of collagen	C/R: n.a. H: p = 51°, m = 43° F: presence of collagen	Prol T 7 days: cell surface density on covalently bound collagen 1.24 times higher than TCPS, 1.21 times higher than adsorbed collagen	72
	Coating of fibronectin, collagen	n.a.	Ad, morph, prol: no difference between sample	73
	Coating of fibronectin	n.a.	Ad T 4 days: preferably on coated PS compared to PS b (no quantification)	74
	Coating of osteopontin and/or collagen	C/H/R: n.a. F: 0.38 ng/mm²	Ad T 2 h: 1.9-fold increase in cell density for osteopontin + collagen compared to adsorbed osteopontin	75

Roughness	Langmuir–Blodgett lithography	C/H/F: n.a. R (depth): 50-150 nm	Ad T 2 days: no significant difference Morph T 2 days: 40 % (50 nm) and 60 % (150 nm) of cell aligned within 30° of the groove direction; higher elongation in direction of groove Mob T 4 days: 1.46 times faster in the parallel direction	76
	Electron beam lithography	C/H/F: n.a. R (pitch size): 40-1000 nm	Morph T 1 day: highly elongated and aligned on anisotropic surfaces Mob T 1 day: increase on isotropic surface	77
	Micro-replication	C/H/F: n.a. R (width): 2-4 µm	Morph T 1 day: all cells aligned in within 10 % of the groove direction). Prol T 7 days: no influence of topography (no quantification)	78
Combination	Hydroxyapatite	C/H: n.a. F: Ca/P ratio = 1.59 R (R_a): p = 1.76 µm , m = 1.23 µm ; t = 7 µm	Ad T 4 h: 1.7-fold decrease in cell number compared to TCPS Prol T 2 days: 1.9-fold decrease in cell number compared to TCPS	79
	CF_4 and O_2 plasma	C: n.a. H: p = 90°, m(CF_4) = 130°, m(CF_4+O_2) = 20° F: F- and O-containing groups R (R_a): p= 0.9 nm, m(CF_4) = 70 nm, m(CF_4+O_2) = 70 nm	Ad T 6 h for CF_4: 2.6-fold (for CF_4) and 3.7-fold (for CF_4+O_2) increase in absorbance compared to untreated PS and 1.9-fold increase compared to TCPS. Prol T 4.5 days : 4.2-fold (for CF_4+O_2) increase in absorbance compared to TCPS	80

n.a. = not available ;

p = pure substrate ; m = modified substrate ;

t = thickness ; C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion ; prol = cell proliferation ; via = cell viability ; morph = cell morphology ; mob = cell mobility

3. CELL BEHAVIOR ON POLYLACTIDE

As for polystyrene, very few studies comparing fibroblasts and osteoblasts behavior on polylactide were identified in our literature survey. Adopting a plasma deposition technique, Reno et *al*. grafted -COOH and -NH_2 groups on the surface of PLA films using respectively acrylic acid and 1,2-diaminopropane (see Table 4).[81] The expected decrease in contact angle arising from this hydrophilization and the increase in surface charge density have been correlated with a protein adsorption enhancement (*i.e.* 1.9 times and 2.4 times for carboxylic and amino groups respectively). Compared to the unmodified PLA, these surface modifications also promoted osteoblast proliferation rate. After 48 h of culture, osteoblasts showed a proliferation 2.7 and 1.8 higher on acrylic acid or 1,2-diaminopropane modified PLA respectively compared to PLA. However, surprisingly enough, these surface chemistry modifications of PLA did not impact significantly the fibroblast behavior. Fakhry *et al.* highlighted *in vitro* that chitosan films better support the initial attachment and spreading of osteoblasts preferentially over fibroblasts.[82]

Table 4. Comparison of fibroblast and osteoblast behavior on PLA

Modification	Physicochemical characterization	Cell behavior	Ref.
Acrylic acid and 1,2-diaminopropane plasma	C/R: n.a. H: p = 71°, m(acrylic acid) = 50°, m(1,2-diaminopropane) = 67° F: COOH (acrylic acid) , NH_2 (1,2-diaminopropane)	Prol T 2 days: for osteoblasts, 2.7-fold (acrylic acid) and 1.8-fold (1,2-diaminopropane) increase in cell number compared to unmodified PLA ; for fibroblasts, no significant difference	81

n.a. = not available ;

p = pure substrate ; m = modified substrate ;

C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion

3.1 Adhesion and proliferation of fibroblasts on polylactide

3.1.1 Non-specific surface functionalization

Different techniques were proposed to introduce ionizable groups on PLA in order to promote fibroblast adhesion on this neutral polyester. Surface hydrolysis of PLA was realized by Wang *et al*. by immersing PLLA films in NaOH solution [83]. Simply in function on the hydrolysis duration, these authors adjusted the hydrophilicity and roughness of the polyester film. Cell viability after 24 h of fibroblasts was significantly improved after chemical hydrolysis compared to untreated PLA, leading to similar results as TCPS. CO_2 plasma treatment of PLLA films was used by Khorasani *et al*. to incorporate oxygen containing polar groups (see Table 5). However, the resulting gain in hydrophilicity did not significantly change the fibroblast behavior both in terms of adhesion and growth rate.[84] Jacobs *et al*. compared fibroblast reactivity with PLA films exposed under either He, Ar, or dry air plasma [85]. All these treatments increased the surface hydrophilicity of PLA (see Table 5) and enhanced cell adhesion and spreading, showing already an elongated shape after 1 day of culture. Yet, no significant difference was noticed in terms of cell proliferation after 7 days of culture. The influence of the nature of gases on cell attachment and proliferation was negligible. Moreover, cell viability was 2 times lower on all PLA samples compared to the TCPS control. Anhydrous NH_3 was also used as discharging gas by Yang *et al*..[86] After 4 days of culture, the cell density measured on the modified PDLLA was 1.9 times higher compared to unmodified PDLLA.

Grafting of tris(2-aminoethyl)amine onto PLA films to build branched architectures containing amine functionalities was reported by Janorkar *et al*..[87] This chemical approach decreased the contact angle of PLA film but accentuated their roughness. Although only qualitative, these changes slightly promoted fibroblast adhesion and viability (see Table *5*).

Polysaccharides (*e.g*. cellulose and chitosan) were frequently adopted to promote adhesion and proliferation of fibroblasts on polylactide. A direct coating approach was used by Hossain *et al*. to adsorb a blend of cellulose-polyvinyl acetate on the surface PLA fibers.[88] The authors reported an improved cell adhesion compared to the PLA control, without supporting their findings by quantitative results. In the other studies, either the surface was activated before adsorption of the polysaccharide adsorption, either bi-functional groups were used to graft these biomacromolecules. Plasma treatment was the most popular technique to activate PLA surface, although alternative approaches were also reported such as chemical treatment or photo-oxidation under UV light exposure. Adopting activated PLLA films under Ar plasma before chitosan coating, Ding *et al*. showed that fibroblasts hardly spread on chitosan PLA (*i.e*. round cells), while their proliferation rate was similar to PLA control and glass petri dish.[89]

Photooxidation was adopted by Zhao *et al*. in order to graft methacrylic acid on PLA surface as a first step.[90] The negative charges arising from this activation facilitated the adsorption of chitosan by ionic interaction realized in a second phase. After 7 days of culture, an increase in cell viability was found on chitosan samples compared to unmodified PLLA (see Table 5). Photooxidation under UV light exposure was also used by Zhu *et al*. in order to immobilize chitosan previously functionalized with 4-azidobenzoic acid, a photosensitive hetero-bifunctional crosslinking reagent.[91] Heparin was additionally coated on chitosan to form a polyelectrolyte complex at the surface of the PLA film. This double macromolecular layer increased 3.5 times the fibroblast adhesion estimated 24 h after cell seeding compared to the untreated PLA. Moreover, phenotype change were reported with fibroblasts having a spindle form on modified PLA compared to a round shape on unmodified PLA.

3.1.2 Specific surface functionalization

Adhesion of peptides

Following Ar plasma with acrylic acid, Jung *et al*. immobilized GRDG and GRGD peptides at a density of 145 and 138 pmol/cm² respectively on PLLA films.[92] These bio-functionalized PLLA films were more hydrophilic and promoted fibroblast adhesion and spreading after 48 h, in particular for GRGD modified substrates (see Table 5).

Proteins from extracellular matrix

Yang *et al*. also attempted to enhance fibroblast adhesion on PDLLA combining O_2 or NH_3 plasma treatment and anchorage of collagen with or without post-collagen coating.[93]

Aminolysis or hydrolysis of PLA was used by Xu *et al*. to graft gelatin on PDLLA films using 1,6-hexanediamine as cross-linker.[94] If their microscopic observations were supporting a fibroblast adhesion enhancement in the presence of gelatin grafted on polyester surface, these authors did not provide any quantitative data. Cai *et al*. used the same chemical approach to anchor collagen type I or fibronectin.[95] Already after 2 h, an increase in cell adhesion was noticed in the presence of these extracellular matrix proteins. Interestingly, the spreading of fibroblasts on fibronectin modified PLA was twice more rapid and extensive compared to collagen modified and unmodified PLA (see details in Table 5). PLA hydrolysis was also used by Mateos-Timoneda *et al*. in order to graft collagen type I and to evaluate the fibroblast response.[96] After already 4 h of culture, these authors reported a 2-fold increase in adhesion efficiency compared to PLA control but also PLA with adsorbed collagen. Interestingly, they also demonstrated that fibroblasts had a 10-fold higher affinity for TCPS compared to PLA. After PLA film hydrolysis, Nagai *et al*. covalently bonded fibronectin using a water-soluble carbodiimide. [97] As noticed by the former authors, the presence of fibronectin raised also fibroblast adhesion after 1.5 h of culture. Moreover, fibroblasts adhering on PLA disclosed a similar morphology compared to TCPS.

Table 5. Fibroblast behavior on PLA

Class of modification	Specific nature of the modification	Physicochemical characterization	Cell behavior	Ref.
Non-specific functionalization – functional groups	Hydrolysis treatment	C/F: n.a. H: p = 80°, m = 53-68° R (R_a): p = 18.7 nm, m = 43-145 nm	Via T 1 day : 2-fold increase in absorbance compared to unmodified PLA, similar to TCPS	83
	CO2 plasma	C/R: n.a. H: p = 86°, m = 45-50° F: O-containing groups	Ad T 2 days: no significant difference between treated and untreated PLA (no quantification)	84
	He, Ar, air plasma	C/R: n.a. H: p = 73°, m = 50-57° F: COOH, C-O, C-N	Negligible influence of gas nature Ad T 1 day: 1.6-fold increase in living cell number compared to unmodified PLA, 50 % of TCPS Prol T 7 days: no significant difference in living cell number compared to unmodified PLA, 50 % of TCPS	85
	NH3 plasma	C/R: n.a. H: p = 78°, m = 22° F: OH, C-O-C, NH_2	Prol T4 days: 1.8-fold increase in cell number compared to unmodified PDLLA	86
	Grafting of tris(2-aminoethyl)amine	C: n.a. H: p = 82°, m = 61° F: CN, NH_2 R (RMS): p = 0.3 nm, m = 4.9 nm	Morph T 3 days: 1.5-fold increase in cell coverage compared to unmodified PLA Via T 5 days: less dead cells compared to unmodified PLA (no quantification)	87
Non-specific functionalization – macromolecules	Coating of a blend of cellulose-polyvinyl acetate	C/H/F: n.a. R (R_a): p = 0.2 µm, m = 2.8-3 µm	Ad T 2 days: increase compared to the PLA control (no quantification)	88
	Ar plasma activation followed by chitosan coating	C: n.a. H: p = 70°, m = 47° F: OH, C-O-C, NH_2 R: t < 6-7 nm	Morph T 2 days: small spreading and round shape (no quantification) Prol until 5 days: similar rate as PLLA and glass petri dish	89

	Photooxidation of PLA before grafting methacrylic acid, followed by ionic adsorption of chitosan	C: n.a. H: p = 98°, m(grafted) = 83°, m(grafted+adsorbed) = 75° F: presence of chitosan R: p = smooth, m(grafted) 200-300 nm, m(grafted+adsorbed) = 200-800 nm	Via T 7 days: 1.8-fold (grafted and anchored) and 1.6-fold (grafted) increase compared to unmodified PLLA	90
	Grafting of chitosan and heparin	C/R: n.a. H: p = 65°, m = 40° F: presence of heparin and chitosan	Ad T 1 day: 2.0-fold (chitosan) and 3.5-fold (chitosan+heparin) increase in cell number compared to untreated PLA, respectively 40 and 70 % of TCPS. Morph T 12 h: spindle shapes compared to round and spherical shapes on unmodified PLA.	91
Specific interactions – adhesion of peptides	Ar plasma with acrylic acid, followed by grafting of GRDG or GRGD peptides	C/R: n.a. H: p = 74°, m = 50° F: density = 145 (GRDG) and 138 (GRGD) pmol/cm²	Ad T 2 days: increase compared to unmodified PLLA, in particular for GRGD (no quantification). Morph T 2 days: higher spreading compared to unmodified PLLA, in particular for GRGD (no quantification)	92
Specific interactions – proteins from extracellular matrix	O_2 or NH_3 plasma treatment and anchorage of collagen with or without collagen coating	C/R: n.a. H: p = 78°, m(coated) = 62°, m(grafted) = 54° F: presence of collagen	Prol T 4 days: without PBS washing, 2.5-fold increase in cell density for both compared to untreated PDLLA samples. After PBS washing, 1.4-fold decrease in cell number for coated collagen but no difference for grafted collagen.	93
	Grafting of gelatin	C/R: n.a. H: p = 76°, m = 45° F: presence of gelatin	Prol T 2 days: increase in adherent cell number compared to unmodified PLA (no quantification)	94
	Grafting of collagen or fibronectin	C/H/F: n.a. R: p = 1.2 nm, m(collagen) = 1.5 nm, m(fibronectin) = 4.1 nm	Ad T 2 h: 2.8-fold (collagen) and 3.4-fold (fibronectin) in adhering cell number compared to unmodified PLA Morph T 8 h: 4-fold (collagen) and 8-fold increase in cell surface area compared to unmodified PLA	95

	Hydrolysis treatment and grafting of collagen	C: n.a. H: p = 80°, m (hydrolysis) = 34° F: density = 0.2-1.3 (covalent) and 0.02 µg/cm² (adsorbed) R (R_a): p = 79 nm, m(hydrolysis) > 2 µm	Ad T 4 h: 2-fold increase in cell number on covalent collagen compared to hydrolysis, physisorbed collagen, and unmodified PLA, 10-fold smaller than TCPS Prol T 7 days: 2.1-fold increase in absorbtion on covalent collagen compared to physisorbed collagen	[96]
	Grafting of fibronectin	C/H/R: n.a. F: presence of fibronectin	Ad T 1.5 h: 7-fold (covalent) and 3.4-fold (adsorbed) increase compared to unmodified PLA, 2.9-fold (covalent) and 1.4-fold (adsorbed) compared to TCPS	[97]
Roughness	n.a.	n.a.	n.a.	n.a.
Combination	n.a.	n.a.	n.a.	n.a.

n.a. = not available ;

p = pure substrate ; m = modified substrate ;

t = thickness ; C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion ; prol = cell proliferation ; via = cell viability ; morph = cell morphology ; mob = cell mobility

3.2 Adhesion and proliferation of osteoblasts on polylactide

3.2.1 Non-specific surface functionalization

The influence of chiral properties of PLA was studied by Yi *et al.*, comparing protein adsorption and osteoblast behavior cultured on films composed of either PLLA, PDLA, PDLLA, or stereocomplexes made from a blend of PLLA and PDLA.[98] Protein adsorption was higher on PLA films compared to TCPS, with a maximum adsorption of fibrinogen on PLLA (*i.e.* 3.4-fold increase) and of albumin on stereocomplexes (*i.e.* 18-fold increase). Cell proliferation rate was lower on PLA samples than TCPS after 14 days of culture. Differences in cell amplification between PLA samples appeared from day 4 to day 14 days of culture, proliferation rate being significantly higher on PLLA, PDLLA, and stereocomplex compared to the amorphous PDLA. Taking into account that no statistic differences were observed in wettability and average roughness for all PLA films, the authors hypothesized that the stereoform should be mainly responsible of the differences in protein adsorption and cell behavior.

The influence of functional groups grafted PLA surface on protein adsorption efficiency and osteoblast adhesion was investigated by Alves *et al*. after O_2 plasma treatment of PDLLA film. [99] This activation enhanced protein adsorption (*i.e*. up to 15 % increase for fibronectin) on plasma treated PDLLA compared to untreated PDLLA. After 1 day of culture, in the absence of previously adsorbed proteins, the attachment of osteoblasts did not significantly increase (lower than TCPS). After adsorption of fibronectin, a 2.8-fold increase in osteoblast attachment was noticed, with similar behavior compared to TCPS. Surprisingly enough, after 7 days of culture, the higher cell surface density was observed for untreated PDLLA.

Macromolecules

The coating of PDLLA film with derivatives of chitosan, N-butyl or N-cetyl chitosan or o-carboxymethyl chitosan, was explored by Cai *et al*. to improve interaction with osteoblasts *in vitro*.[100,101] N-cetyl chitosan did not change cell adhesion while cell proliferation was enhanced in the presence of the N-butyl chitosan after 7 days of culture. A higher benefit was observed with o-carboxymethyl chitosan, which increased both cell adhesion and cell proliferation.

Cai *et al*. adopted also a layer-by-layer approach relying on polystyrene sulfonate–chitosan polylelectrolyte complexes to coat PDLLA film with chitosan.[102] In the presence of 5 bilayers the cell density was enhanced compared to non modified PDLLA, becoming similar to TPCS.

Instead of adding positive charges to the polyester surface with chitosan, the same research group adopted poly(aspartic acid) with molar mass of 5 or 12 kDa to add negative charges on the surface of PDLLA films.[103] As detailed in Table 6, this change in surface charge also enhanced osteoblast adhesion and proliferation rate in the same magnitude range as observed with chitosan.

An original approach was also proposed by Cai *et al*. with the adsorption or grafting of silk fibroin on PLA.[104] Those modifications have also enhanced cell adhesion, proliferation rate, and viability (see details in Table 6).

3.2.2 Specific surface functionalization

Adhesion of peptides or other specific ligands

After loading baicalin (*i.e*. a flavonoid with anti-inflammatory activity) within PDLLA films Liu *et al*. have noticed a significant enhancement of osteoblasts adhesion and proliferation rate.[105]

Proteins from extracellular matrix

As for polystyrene, several authors immobilized typical proteins of the extracellular matrix on polylactide to promote osteoblast adhesion. Más *et al*. grafted collagen type I on PDLLA membranes using polyacrylic acid as cross-linker.[106] Osteoblast density after 21 days of culture was increased. As outline in Table 6, the morphological (higher spreading) and metabolic behavior (collagen production) of osteoblasts were also enhanced after this surface functionalization. Zhu *et al*. built 15 nm-thick multilayers made from polyethyleneimine and gelatin on the surface of PDLLA films.[107] Compared to PDLLA control, a 1.5-fold increase in cell proliferation was noticed by these authors, close to the cell multiplication rate observed on TCPS. This surface change also promoted osteoblasts spreading and protein synthesis like observed on TCPS, in contrast to pure PDLLA.

3.2.3 Combination of modification of roughness and surface chemistry

Inorganic materials were applied on PLA by Hirata *et al*. to improved cell attachment on PLLA sheets using a coating of -COOH functionalized multiwalled carbon nanotubes.[108] Qualitatively, more cells were observed on coated PLLA than untreated PLLA after 2 h of culture. Moreover, after 2 h of culture, the osteoblasts kept their round shape with not extended filopodia on untreated PLLA while filopodia were well stretched on coated PLLA.

Table 6. Osteoblast behavior on PLA

Class of modification	Specific nature of the modification	Physicochemical characterization	Cell behavior	Ref.
Non-specific functionalization – functional groups	Change in chirality of PLA. Osteoblast behavior cultured on films composed of either PLLA, PDLA, PDLLA, or stereocomplexes made from a blend of PLLA and PDLA	C/F: n.a. H:PLLA = 71°, PDLA = 76°, PDLLA = 73°, mix = 78° R (R_a): 3.8 (PLLA, PDLA, PDLLA) and 5.9 (mix) nm ; (Rz): 31.6 (PLLA), 33.3 (PDLA, PDLLA), and 34.5 (mix) nm	Prol T 14 days: up to 1.6 times lower on PLA compared to TCPS, 1.2 times higher on PLLA, PDLLA, and mix than PDLA.	98
	O_2 plasma	C: n.a. H: p = 75°, m = 59° F: COOH, increase in O content R: t = top few atomic layers	Ad T 1 day: in the absence of proteins, no difference compared to unmodified PDLLA ; with preadsorbed fibronectin, 2.8-fold increase compared to untreated PDLLA and similar to TCPS. Prol T 7 days: in the absence of proteins, 1.3-fold increase ; in the presence of preadsorbed proteins, up to 2-fold decrease.	99
Non-specific functionalization – macromolecules	Grafting of n-butyl and n-cetyl chitosan	C/R: n.a. H: p = 69°, m(butyl) = 43°, m(cetyl) = 51° F: NH_2, COHN ; presence of alkyled chitosan	Ad T 8 h: no significant difference compared to unmodified PDLLA. Prol T 7 days: 1.6-fold increase in cell density compared to unmodified PDLLA (butyl), no difference (cetyl) Via T 7 days: 1.2-fold (butyl) and 1.3-fold (cetyl) increase in MTT absorbance compared to unmodified PDLLA.	100
	Grafting of o-carboxymethyl chitosan	C/R: n.a. H: p = 69°, m = 44° F: NH_2, COHN ; presence of alkyled chitosan	Ad T 8 h: 1.4-fold increase in cell density. Prol T 7 days: 1.4-fold increase in cell density. Via T 7 days: 1.2-fold increase in MTT.	101
	Coating of polystyrene sulfonate/chitosan layers	C/R: n.a. H: p = 83°, m = 74° F: NH_2, COHN ; presence of chitosan	Prol T 7 days: 1.3-fold increase in cell density compared to untreated PDLLA, similar to TCPS. Via T 7 days: 1.2-fold increase in MTT absorbance compared to unmodified PDLLA, similar to TCPS.	102

	Physical entrapment of polyaspartic acid of Mw of 5 or 12 kDa	C/R: n.a. H: p = 69°, m = 59° F: presence of polyaspartic acid	Ad T 8 h: 1.3-fold increase in cell density Prol and Via T 7 days: 1.3-fold increase in cell density for 12 kDa (not significant for 5 kDa).	[103]
	Grafting and physical entrapment of fibroin	C/R: n.a. H: p = 69°, m = 49° F: NH_2, COHN ; presence of fibroin	Ad T 8 h: 1.5-fold (entrapped protein) and 1.3-fold (immobilized protein) increase in cell density compared to unmodified PDLLA. Prol T 7 days: 1.5-fold (entrapped protein) and 1.4-fold (immobilized protein) increase in cell density compared to unmodified PDLLA. Via T 7 days: 2-fold (entrapped protein) and 1.4-fold (immobilized protein) increase in MTT absorbance compared to unmodified PDLLA.	[104]
Specific interactions – adhesion of peptides	Physical entrapment of baicalin	C/R: n.a. H: p = 69°, m = 50° F: surface coverage = 72 %	Ad T 8 h: 1.4-fold increase in cell density Prol T 7 days: 1.4-fold increase in cell density Via T 7 days: 1.1-fold increase in MTT	[105]
Specific interactions – proteins from extracellular matrix	Grafting of collagen	C: n.a. H: p = 82°, m = 77° F: presence of collagen R (R_a): p = 7 nm, m = 31 nm	Prol T 21 days: 1.3-fold increase in cell number compared to untreated PDLLA. Morph T 12 days: spreading on modified PDLLA but not on PDLLA (no quantification).	[106]
	Coating of polyethyleneimine/gelatin layers	H/F: n.a. C: p = 0 mV, m = -19 mV R: t = 15 nm	Via T 5 days: 1.5-fold increase compared to the control PDLLA, similar to TCPS. Morph T 14 days: spreading on modified PDLLA and TCPS, but round sphere morphology on PDLLA.	[107]
Roughness	n.a.	n.a.	n.a.	n.a.
Combination	Coating of COOH functionalized carbon nanotubes	C/F/R: n.a. H: p = 64°, m = 30°	Ad T 2 h: spreading on modified PLLA, but not on modified PLLA (no quantification). Prol T 3 days: increase in cell number compared to untreated PLLA (no quantification).	[108]

n.a. = not available ;

p = pure substrate ; m = modified substrate ;

t = thickness ; C = charge ; H = hydrophobicity (contact angle given in °) ; F = functional groups ; R = roughness ;

ad = cell adhesion ; prol = cell proliferation ; via = cell viability ; morph = cell morphology ; mob = cell mobility

4. DISCUSSION

4.1 Limitations in the comparison of data reported in bibliography

Before starting to discuss the different strategies identified in the literature to control fibroblast and osteoblast behavior *in vitro*, our bibliography survey allowed us to drawn some limitations arising when trying to compare the different studies. Indeed, one of the main conclusions met in our literature review came from the difficulty of comparison of studies reported in the literature due to the lack of standardization in methodologies adopted for material characterization and for *in vitro* cell behavior analysis.

If several parameters were considered for material analysis, such as hydrophobicity, elemental composition, and functional group composition, some were frequently lacking. As example, surface parameters such as roughness and surface charge were frequently forgotten, even though they are well known to influence cell behavior. Other parameters were also typically missing like bulk characterization of materials, *e.g.* purity, solvent or other chemical residues arising from material processing, macromolecular features (including mean molecular weight and polydispersity, tacticity).

A second weakness noticed in our review arose from the lack of standardization in surface analyses, which were performed following different experimental conditions. It is also worth to mention the different modes adopted to present experimental data did not facilitate data comparison between studies. For example, surface roughness was presented either in terms of amplitude parameters (*e.g.* arithmetic average height R_a, root mean square average RMS, height of pitches, vertical z-range R_z) or spacing parameters (*e.g.* spacing between pitches, grooves width).

The same limitations was observed for cell behavior evaluation both in terms of technique standardization to monitor cell adhesion, proliferation, viability, and morphology, but also in data analysis. For instance, when the total number of cells were counted, the cell viability was rarely determined, although the ratio between total cell number and living cells is known to be critical to assess surface biocompatibility. Large variability in *in vitro* testing was also noticed. Seeding conditions (*e.g*. initial number of cells per surface area unit), cell type (*e.g*. osteoblasts vs preosteoblasts), cell conditioning, passage number, and duration of cell culture were all experimental parameters that significantly differed from one study to another. Knowing that all these variables are well-known to impact animal cell behavior, comparison between studies was not straightforward. As for material characterization, the results of cell adhesion and proliferation were expressed in variable ways. For example, cell number were given per well, surface area unit or in some cases simply given in optical density, thus without converting this raw data in cell number.

The introduction of reference materials as control was unfortunately frequently forgotten, although being systematically imposed in certified laboratories. For example, the inclusion of TCPS, as positive control, was only given in a limited number of studies focused on PLA samples.[83,85,91,96–99,106,107,109]

Last but not least and as mentioned several times in this review, results were frequently expressed in a qualitative way without providing any quantitative data, therefore limiting the establishment of correlations between surface features and cell behavior.

4.2 General overview of the strategies adopted to control cell behavior

Due to the complexity of the control of the dynamic and complex interplay occurring between cells and surfaces, it is important to stress that no general principle has been found to predict and control the effect of specific surface modifications on animal cell attachment and spreading.[110]

Nevertheless, general trends were highlighted in the literature, facilitating the optimization of substrate selection in the frame of the development of a new biotechnology process. To increase cell affinity on a given substrate, a wide variety of surface parameters were tuned, notably the topography, the nature of the surface functional groups, the surface hydrophobicity, the surface charge, and the presence of specific interactions.

The reader has to keep in mind that these parameters are linked. Modifying the roughness of the surface can induce changes in the surface hydrophilicity by the presence of roughness itself or the chemical modification occurring during surface treatment.[37,111] Inversely, changes in the surface chemical properties can influence the surface topography. Therefore, attention has to be paid to these parameters in order to compare correctly literature data. Moreover, cell origin has to be taken into account.[111] Results reported in the literature are referring in majority to cell lines from different origin. Yet, different cell types present different morphologies and metabolisms that result in differences in term of cell response to the same surface modifications.[50,112] In particular, primary cell lines are usually replaced by immortalized cells derived from tumors because of their high proliferation rate and their ability to be indefinitely cultured.[27,111] However, these immortalized cell lines present phenotypes slightly different that those of primary lines, leading to potential differences regarding cell behavior.

Our literature survey identified two main categories of strategies to improve and control fibroblast and osteoblast behavior on modified PS and PLA surface. Either functional non specific groups were anchored to these polymers in order to increase at least their hydrophilicity and their surface charge density. Either ligands recognizing specific cell integrins were attached to these polymeric surfaces. For these two approaches, the authors anchored on the polymer surface either low molecular weight moieties, either macromolecules from synthetic or natural origin. A limited number of studies also combined these two strategies. In spite that the literature clearly established the needs to promote osteoblast amplification while counteracting fibroblast proliferation to promote bone reconstruction, very few studies directly compared the adhesion and proliferation of these two cell types on PLA. Moreover, we did not identified publication studying the competition of these cells via co-culture. In the limited number of studies reported, the authors cultured separately fibroblasts and osteoblasts and reported that osteoblast proliferation could significantly be enhanced by surface modifications compared to fibroblasts. [51,81]

Surprisingly, studies comparing the adhesion of fibroblasts and osteoblasts on PS versus PLA could not be found in the literature. TCPS was nevertheless used as a reference material in studies focusing on cell behavior on PLA. For example, different authors have observed that osteoblasts could proliferate at a similar rate on PLA and TCPS after surface modification of the polyester (*i.e*. plasma treatment or immobilization of chitosan or gelatin).[99,106,107,109] In contrast, for fibroblasts, the same types of modification led to a lower cell adhesion and proliferation compared to TCPS [85,87,91,96] while only a very limited number of studies found similar or improved results on modified PLA and control TCPS.[83,97]

4.3 Detailed overview of the main surface parameters adjusted in order to control fibroblast and osteoblast behavior

4.3.1 Modification thickness

When dealing with surface modifications, a special attention should be paid on the thickness of the surface that was altered. Function of the methodology applied, this thickness could range from a sub-nanometer scale (such as those given after plasma treatments) to more than 100 nm, for example in the case of multiple layer polymer coatings. Following these latter changes, the native surface should be totally covered with substantial deviation of its original properties, including roughness and rheological characteristic. Therefore, when evaluating these deeply modified samples, it should be anticipated that their biological performances should be mostly under the control of the new interphase generated and not by the underlying substrate. The reader should nevertheless keep in mind that cells could also be influenced by other factors than the surfaces, including through a degradation of the material core, or larger scale roughness, but also in function of the macroscopic mechanical properties of the material. Interestingly, only 6 studies from our bibliography survey estimated the thickness of their surface modifications. [58,76,79,89,99,107] In their study, Lenhert *et al.* found that a minimum depth of 50 nm was required to control osteoblast cell behavior.[76]

4.3.2 Surface roughness

A first parameter influencing cell adhesion is surface topography. Topography should be considered at different scales compared to the cell size.[27] Irregularity of the surface at a macroscopic scale (*i.e.* > 50 µm, such as a curved surface) prevent a homogeneous distribution of cells on a given substrate after seeding, although this roughness range could appear smooth and flat at the cell scale level (*i.e.* < 50 µm). We can also readily anticipate that a roughness in a range of 1 to 50 µm could interfere with cell behavior, in particular in terms of spreading, migration, and multiplication. At higher resolution, the roughness could also impact cell behavior, taking into account that animal cells do not adhere continuously on a substrate but through focal points that are distant at a submicrometer scale. Regarding PS and PLA, two different scales were considered: nanometric [60,80,83,87,95,96,106] and micrometric scales [88,90,96]. It could be observed that the surface modifications led to an increase in roughness, accompanied by a decrease in water contact angle. Moreover, the effect on roughness of physical treatment (*e.g.* hydrolysis treatment, plasma treatment) was higher than macromolecule grafting and coating. For example, hydrolysis treatment could induce a micrometric roughness from a nanometrically rough substrate.[96] This increase in roughness was reported to enhance osteoblast and fibroblast adhesion, proliferation, and viability. Moreover, well-spread cells could be observed on modified substrates. However, the degree of improvement of cell behavior caused by the roughness modifications did not seem to be dependent on the magnitude of these roughness modifications.

A specific attention was also paid to the possible influence of an anisotropic roughness. When cells were cultured on anisotropic surfaces, containing for example grooves or pillars in the micron domain range and orientated in a specific direction, cells showed a capacity to align themselves and to elongate in this specific direction.[66,67,76–78,80,113] This cell orientation process, identified as contact guidance, compresses the cytoskeleton but also deforms the nucleus shape.[111] As a consequence of nucleus alteration, the expression of genes is also affected, which in turn greatly influences the proliferation and differentiation of animal cells. No consensus has yet been found regarding the minimal depth and width of grooves needed to induce cell orientation.[27] Nevertheless, results found by Lenhert *et al.* supported that a minimum depth of 50 nm was required to control osteoblast cell behavior.[76]

4.3.3 Impact of the hydrophilicity, chemical functional groups, and electrokinetic potential of the surface

With very poor aqueous wetting ability by nature, the **hydrophilic increase** of PS and PLA was typically reported to enhance fibroblast and osteoblast adhesion, a general trend also noticed for other animal cell types.[27,28,114,115] Even though hydrophobic surfaces adsorb a larger amount of proteins than hydrophilic surfaces, these proteins undergo conformational changes that reduced their interaction with cell integrins. [27,50,116] A compromise must therefore be found between the amount of proteins adsorbed and their conformational changes. This optimal has been quantified, *i.e.* water contact angle should ideally ranges between 50° and 70°.[115] On the opposite, lower (*i.e.* below 30°) or higher (*i.e.* above 90°) contact angles results in poor cell adhesion. More specifically for PS and PLA, our bibliography survey highlighted that the majority of modifications leading to cell interaction were corresponding to water contact angles ranging between 40° and 60° for osteoblasts [72,99–101,103,105,117] and between 40° and 70° for fibroblasts.[53,57,91–94,58,60,65,83–85,87,89] However, some articles also reported an increase in osteoblast and fibroblasts adhesion and proliferation for substrates with water contact angles over 90° (up to 120°) and below 30° (down to 15°).[52,55,56,59,80,86,96,108]

The influence of **well-defined chemical functional groups** grafted on the surface was also well documented in the literature to improve cell behavior.[27] More specifically, it was shown that surfaces containing oxygen (like -COOH groups) or nitrogen (like $-NH_2$ groups) promoted cell adhesion, proliferation, and viability. These groups included -COOH, and $-NH_2$ for osteoblasts [80,99–101,109,117] and -C=O, -COOH, -OH, -C–N, $-NH_4$, $-NH_2$ for fibroblasts. [52,53,55–58,84–86,89] Keeping in mind that several of these studies did not characterize deeply enough their changes in surface chemistry, we could not conclude that these differences in chemical affinity were really specific either to osteoblasts or fibroblasts. Mitchell *et al*. also established a clear dependence between the cell proliferation rate and the surface oxygen concentration with an optimal around 5 atomic % (measured via XPS).[53] The influence of this atom on cell behavior could be explained by its expected contribution both in terms of hydrophilicity increase and influence on electrokinetic potential, which in turn are known to affect protein adsorption.

Surface charge can greatly influence protein adsorption and cell adhesion. Although hydrophobic interactions are driving protein adsorption, ionic interactions are also significantly contributing to the thermodynamic balance controlling protein adsorption on interphase.[50,116,118] Several studies demonstrated that the introduction of positive or negative charges on the surface of biomaterials increased cell adhesion, proliferation, and spreading.[27,39,116,118,119] This cell interaction enhancement was correlated to the amount of adsorbed proteins via electrostatic interactions.[119] Already in 1979, Levine *et al*. reported that an optimal charge density was required to avoid the toxicity and to promote cell adhesion and proliferation.[120] This optimal value corresponds to the so called discrete range of surface charges, which is within the range of 2-10 charges/nm^2 for negatively charged surfaces.[121] At lower surface potential, the density of cells attached to the surface remained limited and, after some days of culture, cells finally desorbed. For higher electrokinetic potential, cell toxicity was observed as a result of an inhibition of transmembrane protein movements resulting from multiple adhering bonds.[122,123] The comparison of adhesion and spreading behavior of osteoblasts and fibroblasts in function of the sign and amplitude of the electrokinetic of a substrate remains nevertheless controversial. For example, Schneider *et al*. reported that cell adhesion was significantly increased on positively charged hydrogels compared to negative or neutral surfaces, with a two-fold enhancement for osteoblasts compared to fibroblasts.[39] In our bibliography survey, only 2 studies quantified the surface charge.[51,107] Both studies found that negatively charged surface promoted osteoblasts and in a smaller way fibroblasts proliferation. On the hand, TCPS surface with a charge of -29 mV led to a 30-fold (for osteoblast) or a 8-fold (for fibroblast) increase in attached cell number after 16 days of culture compared to seeded cell number.[51] On the other hand, PLA modified with gelatin bearing a charge of -19 mV increased 1.5 times osteoblast viability compared to the uncharged control PDLLA. Moreover, cells were spread on modified PDLLA, but round on unmodified PDLLA. However, due to the lack of information regarding the determination of surface charge, no clear trends could be extracted from our bibliography survey.

4.3.4 Adhesive peptides and proteins from the extracellular matrix

The grafting of specific peptidic domains on the surface also received considerable attention to improve cell behavior. As reported above, two main approaches were adopted to promote a selective interaction between cell integrin and the surface, *i.e.* grafting of short peptide sequences (*e.g.* such as the so-called universal RGD-sequence) or immobilization of ECM proteins (*e.g.* fibronectin, collagen, gelatin).[124] Shorter peptides are more attractive due to their stability (no risk of unfolding during or after adsorption, higher stability regarding storage and sterilization, but also regarding safety aspects if *in vivo* applications are considered). RGD coated surfaces already demonstrated their performance to increase *in vitro* cell spreading, proliferation, and differentiation but also *in vivo* bone formation.[125] In our bibliography survey, the specific comparison of osteoblast and fibroblast behavior on PS or PLA confirmed the higher potency of RGD sequence on other peptides such as RRI, DGEA, RDG. [61,64,70,92] Cell behavior of this bio-functionalized materials was also affected by the surface density of peptides, of its accessibility in function of the distance from the surface, and of its structure.[124,126–129] A minimum surface density of peptides is known to be required to promote cell spreading.[130] This critical concentration depends on the cell type. Regarding osteoblasts, Chollet *et al*. showed that a minimal RGDC density of 1 pmol/mm² (*i.e*. a spacing of 2 nm if a coverage of 80 % is assumed) improved cell response on polyethylene terephthalate surfaces.[128] To enhance fibroblasts cell spreading on glass substrates, Massia *et al*. reported that the minimal amount of RGD peptides was 1 fmol/cm² (*i.e*. 440 nm spacing), while at least 10 fmol/cm² (*i.e*. 140 nm spacing) was required to observe focal contact formation.[131] Unfortunately, no quantitative conclusions could be drawn for our literature survey focused on PS – PLA and fibroblasts or osteoblasts. Only the study realized by Kurihara *et al*. established a relationship between peptide surface density and fibroblast adhesion, with an increase in cell adhesion with the peptide surface density.[61]

Comparable conclusions were drawn from the different studies reporting a biofunctionalization with ECM proteins. Several adhesive proteins were grafted at the surface of PS and PLA: collagen (mainly) [65,71–73,75,93,106], fibronectin [73,74,97], gelatin [94,107], osteopontin [75], and tropoelastin [63]. All these biological macromolecules were reported to enhance cell behavior via specific interaction. However, an increase in hydrophilicity or / and in roughness was also observed after protein grafting. The structure of the proteins was also important. The higher enhancement of proliferation osteoblasts by felwork type I collagen over fibril type I collagen reported by Hanagata *et al*. could be explain by the fact that conformation state of these macromolecules should affect cell recognition.[71] In a similar way, Liu *et al*. showed that a precoating of collagen raised the accessibility of osteopontin (*i.e*. higher number of accessible RGD sites for cell adhesion), compared to osteopontin directly adsorbed on TCPS. Regarding protein density, no significant effect of collagen density was noticed on osteoblast proliferation rate by Hanagata *et al*.. [71]

4.3.5 Chemical and biological stability of the surface modifications

Stability of the surface modification was another aspect often neglected by authors, although the new engineered surfaces could be profoundly affect afterwards. On the one hand, surface stability should be verified after storage and sterilization in order to verify the **chemical stability**. On the other hand, the incubation of the materials in the cell culture medium can also profoundly alter the new surface. Indeed, any protein adsorption contributes at inducing **biological modification of the surface**. Whatever their origins, these surface evolutions are of course important to control in order to correlate actual surface properties with cell behavior.

As example of chemical modifications, the plasma-modified substrates tended to recover their original hydrophobic state over time at normal atmosphere due to a reorientation of the neo-formed chemical groups towards the bulk but also due to their reactivity with CO_2 or H_2O of the atmosphere.[85] A right material conditioning (*i.e.* temperature, atmosphere, packaging nature) should therefore be optimized and fully described in material and method sections.[86] Sterilization processes, including mild ones typically adopted for laboratory purposes (*i.e.* UV radiation or ethanol treatment) can also greatly impact the surface of these polymers.[99] This step must therefore be carefully taken into consideration. Chemical stability of the materials should also be also verified during the period of cell incubation. Indeed migration of low molecular weight compounds/residues from the core towards the surface cannot be neglected and could influence cell behavior. For PLA substrates, the by-products released for ester hydrolysis, lactic monomers but also oligomers, have been reported to influence cell behavior. Notably, Yi *et al*. found that D-monomer seemed to be an inhibitor of cell proliferation.[98]

4.4 Protein adsorption

As already mentioned, in most of the cases, cells never directly interact with the native surface but rather with proteins, exogenous or secreted ones by cells, which can unspecifically adsorb to most of the surfaces.[27] Cells recognize specific amino-acid sequences such as the so-called universal RGD sequence present on these proteins through specific receptors like integrins. It can be therefore understood that cell behavior is influenced by biomaterials surface properties through protein adsorption. The surface properties greatly influence the conformation and composition of the adsorbed protein layer.[52] These observations allowed to explain why hydrophobic surfaces, with higher ability to induce protein conformational changes, are less bioadhesive than hydrophilic substrates.[50,81] A compromise must therefore be optimized between the amount of protein adsorbed per surface unit of material and their possible conformational changes. This important parameter is however most of the time overlooked. Reno *et al*. found that PLA presenting -COOH (29.5 µg/mL) or -NH_2 groups (37.2 µg/mL) was able to adsorb a higher serum protein concentration compared to TCPS (19.5 µg/mL) and untreated PLA (15.2 µg/mL).[81] This gain in hydrophilicity led to a significant increase of osteoblast proliferation while no significant difference could be observed for fibroblasts. Regarding osteoblasts, the higher amount of adsorbed protein on PLA with -NH_2 groups compared to PLA with -COOH groups did not change the cell proliferation rate. In view to better understand these variations in cell behavior, more in-depth studies should be realized in order to identify precisely the nature and amount of proteins adsorbing to a given surface and if possible to monitor their eventual conformational changes. Accordingly, Yi *et al*. compared the relative surface density of bovine of serum albumin and fibrinogen after interaction with PLA or TCPS surfaces.[98] They found that both protein adsorption were higher on PLA compared to TCPS, while cell proliferation was significantly lower on PLA samples than TCPS.

Monitoring the adsorption of bovine serum albumin and fibronectin on plasma treated PLA substrates, Alves *et al*. highlighted an up to 1.5-fold increase in adsorption of these two proteins after surface activation.

This knowledge in protein adsorption events is well-recognized to control bone repair. In particular, the proteins involved in osteoblast adhesion (*e.g*. extracellular matrix proteins, cytoskeletal proteins, integrins, cadherins) have been well described in a former review published by Anselme.[118] During osteoblast/material interactions, their expression (qu'entends-tu par "expression"… qui a publié cette phrase ?) are modified according to the surface characteristics of materials. Their involvement in osteoblastic response to mechanical stimulation highlights the significance of taking them into consideration during development of future biomaterials.

The elucidation of the interfacial kinetics of cell adhesion on surfaces can also contribute to the rational engineering of surface modification of biomaterials with as final purpose their biointegration.[132]

5. CONCLUSIONS AND PERSPECTIVES

This critical review focused on the interaction of osteoblasts and fibroblasts on modified PS and PLA surfaces. Our bibliography survey highlighted the need of a more in-depth analysis of the surface properties of these materials and their evolution during animal cell culture, including the need to standardize analytical and biological assays. Indeed, all the results identified in our bibliography survey did not facilitate the understanding of the interplay existing between animal cells.[110] No clear correlation could be established between the behavior of fibroblasts or osteoblasts and surface characteristics of the two reference materials that we have selected PS and PLLA, even if several general tendencies could be drawn.

In line with our observations, we would advise for the future to edit specific guidelines and standard protocols in order to improve the comprehension of the interactions between surface and cells.

Regarding material characterization, a list of critical parameters would be interesting to establish in order to report their in-depth characterization. Regarding bulk properties, the molecular weight, glass transition temperature, crystallinity percentage, melting temperature, proportion of enantiomers (in the case of PDLLA), and tacticity should always be evaluated. Concerning surface parameters, roughness profiles (*e.g.* R_a, R_t, R_z) at nanometric and micrometric scales, surface energy (via contact angle analysis), elementary composition (via XPS), electrokinetic potential, and functional group composition (via FTIR/Raman spectra) would be helpful to report. For *in vitro* evaluations, several cell behaviors should be assessed: cell adhesion (*i.e.* percentage of adherent cells and surface cell density after seeding), cell proliferation rate, cell viability, and cell morphology (*i.e.* spreading area in μm^2 for cell and profile). In order to facilitate comparison, these *in vitro* studies should respect standard incubation times such as for cell adhesion: 1 h, 4 h, and 24 h ; for cell proliferation rate and cell viability: 24 h, 2 d, 4 d, and at least 7 d. Free access to standardized protocols for the determination of cell behavior would be helpful in order to promote the adoption of the same experimental variables for *in vitro* evaluation. Common cell types should also be used such as L929 cell line for fibroblasts, as advised in ISO 10993 and human osteoblast-like cells (MG63) for osteoblasts. Cell adhesion and proliferation should be evaluated by manual or automatic cell counting, with a preference for automatic counting to improve counting statistics. Cell viability should be determined by MTT or MTS assays, which are also reported in ISO norms. Additionally, new tools coming from molecular biology would be interesting to implement including proteomics, molecular assays for matrix production, reverse transcriptase polymerase chain reaction (RT-PCR), and subcellular imaging techniques accessible with confocal microscopy. All these new techniques should contribute to better probe animal cell function and their signaling status. From these standardized and more comprehensive view of material-cell interaction, further developments should be facilitated with potential applications for the design of new *in vitro* models and implants.

AUTHOR INFORMATION

Corresponding Author

*Christian Grandfils, c.grandfils@uliege.be, Allée du Six Aout 11, B-4000 Liège, Belgium

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ABBREVIATIONS

MSC, mesenchymal stem cells; ECM, extracellular matrix; RGD, arginine-glycine-aspartic acid sequence; PS, polystyrene; PLA, polylactide; TCPS, tissue culture polystyrene; PDLA, poly-D-lactide; PLLA, poly-L-lactide; PDLLA, poly-D,L-lactide; WSC, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide; XPS, X-ray photoelectron spectroscopy; FTIR, Fourier transform Infrared; MTT, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide.

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