Poly(butylene succinate)-based polyesters for biomedical applications: a review

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In Memory of our Beloved Colleague and Friend Dr. Lara Finelli

Abstract

This contribution aims to provide an overview on the use of poly(butylene succinate) (PBS) and its copolymers in biomedicine, with particular attention to emerging topics, such as controlled drug release and tissue engineering.

Indeed, PBS is a well-known aliphatic polyester, given its interesting thermo-mechanical properties and the proven biodegradability, combined with acceptable raw material and production costs. Moreover, the reactants employed in the synthesis can be obtained also from renewable resources, making PBS a fully bio-based and sustainable polymer. Although its commercialization is mainly devoted to biodegradable packaging, the use of PBS in the biomedical field has recently attracted considerable attention.

The synthetic strategies adopted and the solid-state properties, together with data on the biodegradation rate and biocompatibility of this class of polyesters are here reported and compared. Finally, the envisioned applications have been described.

keywords: poly(butylene succinate); biomedical applications; PBS-based copolymers; solid-state properties; biodegradability; biocompatibility.

abbreviations: 3HB, 3-Hydroxybutylene; 3H-T, [3H]-Thymidine; 5Cl8HQ, 5-Chloro-8-Quinolinol; ε_b, Elongation at break; σ_b, Stress at break; σ_y, Stress at yield; χ_c, Degree of Crystallinity; ALP, Alkaline Phosphatase; a. st., after sterilization; BACs, Bovine Articular Chondrocytes; Balb/C, Mouse Splenocytes; BD, 1,4-Butanediol; BMC9, Bone Marrow–derived Mesenchymal progenitor Cell; BMSCs, Bone Marrow Stem Cells; BPF, Bovine Plasma Fibrinogen; BSA, Bovine Serum Albumin; b. st. = before sterilization; C2C12, Mouse Skeletal Myoblasts; C57B/6, Mouse Splenocytes; C, Chitosan; CMC, Carboxymethylchitosan; CSF, Chestnut Shell Fiber; DAPI, 4',6-Diamidino-2-Phenylindole; DMS, Dimethyl Succinate; DTXL, Docetaxel; DOX, Doxorubicin; E, Elastic Modulus; ECM, Extracellular Matrix; ELISA, Enzyme-Linked Immunoadsorbent Assay; f, film; FA, Fluorapatite; FDA, Food and Drug Administration; FDA/EB, Fluorescein Diacetate/Ethidium Bromide; FITC, Fluorescein Isothiocyanate; Fn, Fibronectin; GAG, Glycosaminoglycan; H9c2, Rat Cardiac Cells; H&E, Hematoxylin and Eosin; HA, Hydroxyapatite; hASCs, human Adipose Stem Cells; hBMSCs, human Bone Marrow Mesenchymal Stem Cells; HFN, Human plasma Fibronectin; hFOB 1.19, human Osteoblasts; hMSCs, human Mesenchymal Stem Cells; HPBS, Hydrolyzed PBS; HPLC, High Performance Liquid Cromatography; HS-68, normal Human Foreskin Fibroblasts; HSA, Human Serum Albumin; K5N8Q, 5-Nitro-8-hydroxyquinoline; L929, Mouse Fibroblasts; LDH, Lactate Dehydrogenase; LDPE, Low Density Polyethylene; MA, Maleic Anhydride; MC3T3-E1, Mouse Calvaria pre-osteoblastic Cells; MCF-7, human Breast Cancer Cells; MEM, Minimum Essential Medium; MG-63, human Osteosarcoma; M_n, Number Molecular Weight; MSCs, Mesenchymal Stem Cells; MTS, 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2(4-sulfofenyl)-2H-tetrazolium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NIH 3T3, Mouse Fibroblasts; n.r., not reported; PBS, Poly(Butylene Succinate); PBSUI, Poly(Butylene Succinate) Urethane Ionenes; PBSxGyGz^I, Poly(Butylene Succinate-*co*-2-trimethylammonium Chloride Glutarate); PBxGluxyS, Poly(Butylene-*co*-2,4,:3,5-di-O-methylene-D-Glucitol Succinate); PBxManxyS, Poly(Butylene-*co*-2,4,:3,5-di-O-methylene-D-Mannitol Succinate); PBSxSSy, Poly(Butylene Succinate-*co*-butylene

Sulfonated Succinate); PC, Phosphorylcholine; PCL, Poly(ε-caprolactone); PCR, Polymerase Chain Reaction; PDI, Polydispersity Index; P(DLBS), Poly(ω-pentadecalactone-*co*-butylene succinate); PEG, Poly(Ethylene Glycol); PET, Poly(Ethylene Terephthalate); PGA, Poly(Glycolic Acid); PGS, Poly(Glycerol Sebacate); phys. cond., physiological conditions; PHA, Polyhydroxyalkanoates; PHB, Poly(3-hydroxybutyrate); PHBV, Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate); PIII, Plasma Immersion Ion Implantation; PLA, Poly(Lactic Acid); PLLA, Poly(L-Lactic Acid); PLGA, Poly(Lactic-*co*-Glycolic Acid); PMLA, Poly(Malic Acid); PP, Polypropylene; PTES, Poly(Triethylene Succinate); PTX, Paclitaxel; PVC, Poly(Vinyl Chloride); PZL, Poly(Z-L-Lysine); ROP, Ring Opening Polymerization; RT, Room Temperature; s, scaffold; SA, Succinic Acid; SaOs-2, human Osteosarcoma; SEM, Scanning Electron Microscope; SMCS, Sheep Marrow Stem Cells; TBT, Titanium(IV) Butoxide; TCP, Tricalcium Phosphate; TCPS, Tissue-Culture Polystyrene plate; TCSF, crosslinked CSF; T_g, glass transition Temperature; T_m, melting Temperature; TTCP, Tetracalcium Phosphate; VSA, Vinyl Sulfonic Acid; WCA, Water Contact Angle.

Contents

1. Introduction

Technological challenges more and more require multidisciplinary contributions to be addressed. Researchers from different scientific areas need to cooperate and to join their skills to solve the unmet issues affecting modern society. In this respect, medicine probably represents one of the most topical examples. Especially in the case of regenerative medicine or nanomedicine, matched efforts of biologists, engineers, physicists and chemists are extremely important.

By using a combination of cells, gene-based methods and biomaterials, regenerative medicine aims to replace damaged human tissues restoring the complete function and structure of the organ. [1,2] On the other hand, one of the forefront aspects of nanomedicine, which basically means the application of nanotechnology to medicine, [3] is the controlled delivery of pharmaceuticals, therapeutic and diagnostic agents to specific targets with the aid of engineered constructs. [4] These last ones, also called nanocarriers, include ceramic, polymeric as well as metal particles; they can be effectively used to target cancer cells with high specificity and affinity. [5]

In this framework, biomaterials acquire a prominent importance, being involved in both the above mentioned medicine fields. Various definitions of biomaterial are available, probably the most updated being: “A biomaterial is a substance that has been engineered to take a form which, alone or as part of a complex system, is used to direct, by control of interactions with components of living systems, the course of any therapeutic or diagnostic procedure, in human or veterinary medicine”. [6] Polymers are the most extensively studied biomaterials because, by the combination of different monomers, a broad spectrum of new materials with completely diverse physical-mechanical behaviour can be easily prepared. To be used in contact with human cells and body, polymeric materials must satisfy very strict requirements and, above all, they have to be biocompatible. Biocompatibility has been recently defined as “the ability of a biomaterial to perform its desired function […] without eliciting any undesirable local or systemic effects in the recipient”. [7]

In addition, when intended for temporary use, biodegradability is another advantageous property of biomaterials, because the use of surgery to remove the carrier/implant can be avoided since it undergoes degradation and subsequent excretion (or reabsorption) when its useful function has ended. It is worth mentioning that not only the biomaterial, but the degradation products as well have to result as nontoxic for the host. [8]

Among polymeric biomaterials, aliphatic polyesters have been widely employed in biomedicine, [9-12] as they combine interesting properties with easy synthetic strategies and acceptable raw materials and production costs. [13] The abundance of monomers employed in their synthesis allows to prepare a wide spectrum of polymers possessing specific characteristics for the intended application. Moreover, different synthetic pathways can be adopted, such as polycondensation, ring opening polymerisation (ROP) and in some cases, bacterial and enzymatic syntheses are available as well. [14]

Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(ε-caprolactone) (PCL) and their copolymers are the most researched and investigated aliphatic polyesters for biomedical purposes; this is due to their proven biodegradability, biocompatibility and bioresorbability. [15-17]

The use of poly(glycolic acid) and poly(lactic acid) in medicine dates back to the late ‘60s, when bioresorbable sutures based on these materials obtained the Food and Drug Administration (FDA) approval in 1969 and 1971, respectively. [18] In the following years the first commercial product, a PGA-based absorbable suture named DEXON®, was marketed. [19] Since then, many items have been developed, comprising screws, suture anchors, and articles for fracture fixation and meniscus repair. [19] More recently, also polyesters containing dioxanone (1981) and caprolactone (1997) have been approved by FDA for medical purposes. [19]

Polyhydroxyalkanoates (PHA) are a class of polyesters produced via microbial fermentation. They serve as carbon and energy storage for bacteria and are deposited as insoluble spherical inclusions in the cell cytoplasm. [20] The most known PHA is poly(3-hydroxybutyrate) (PHB), which is

synthesized from 3-hydroxybutyrate (3HB). However, different bacteria use hydroxy fatty acids of varying chain length to produce a range of PHAs. [20]

Due to properties such as biocompatibility, biodegradability, and production from renewable resources, PHAs have been extensively explored in recent years for uses in medicine. [21]

PHAs have been employed to develop various devices, such as sutures, cardiovascular patches and orthopaedic pins, tissue engineered nerve, tendon and articular cartilage, repair patches, slings, adhesion barriers, stents, wound dressings and carriers for controlled drug release [21,22,23]

Although less important, it is worth mentioning also poly(malic acid) (PMLA), a water soluble, bioresorbable and biocompatible polymer.[24] PMLA can be obtained from natural and/or bacterial resources, but also synthetically by polycondensation and ring-opening polymerization.[25]

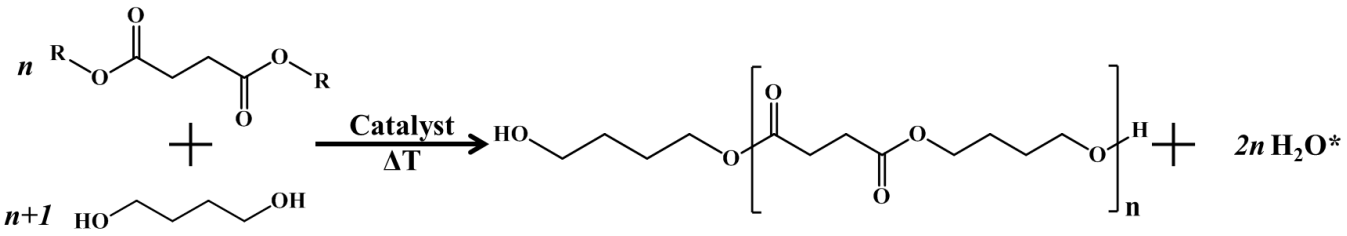
In the last years, another member of the aliphatic polyester class has received increasing attention as regards its possible applications in biomedicine: this is poly(butylene succinate) (PBS). This trend is confirmed by the growing number of articles available in the literature reporting the employment of PBS and PBS-based copolymers in tissue engineering and controlled drug delivery.

The aim of this contribution is therefore to collect, analyse and compare the results obtained by the scientific community on the use of PBS and its copolymers for biomedical applications.

2. Poly(butylene succinate)

Since the very early work of Carothers and his group in the early ‘30s, [26] many efforts have been directed to the realisation of industrially relevant aliphatic polyesters. Among other successful cases, PBS is commercially available since 1993. [27] It is produced under the tradename Bionolle™ by Showa-Denko K.K. [27] and by Mitsubishi Chemical Corporation under the tradename GS Pla™. [28] Its main uses regard environmental purposes, such as mulching films, compostable bags, nonwoven sheets & textiles, catering products and foams. [27, 28]

The monomers employed in the PBS synthesis are succinic acid (SA) and 1,4-butanediol (BD) (Figure 1), which are commonly obtained from fossil resources and are readily available on the market.



R: H or CH_3
* or CH_3OH if dimethylsuccinate is employed

Figure 1. Reaction scheme showing PBS polymerization.

Interestingly, both SA and BD can be obtained not only from oil, but through fermentation as well. In the last years, various microorganisms have been screened and tested for the production of succinic acid via biotechnological processes, with good yields. [29] The so-obtained SA can then be converted into 1,4-butanediol through hydrogenation. [30] This would lead to a complete bio-based PBS. Various companies such as Succinity® (a joint venture between BASF and Purac), Reverdia, BioAmber and Myriant are operating in the production of biosuccinic acid at industrial scale.

PBS is commonly synthesized by polycondensation in two steps: in the first one, esterification (or transesterification in the case of dimethylsuccinate, DMS) reactions with removal of water (or methanol) occur, while in the second, conducted at higher temperature and under reduced pressure to remove BD (generally an excess of glycol of about 10-20% is used), high molecular weight PBS is obtained. [31]

Different catalysts are traditionally employed for the synthesis of PBS, one of the most common being titanium(IV) butoxide (TBT). Different other types of catalysts, such as organometallic or metal-oxide compounds, have been tested and the results obtained have been fully discussed by Jacquel *et al*. [32]

Recently, the possibility to obtain PBS by using enzymatic catalysis, in particular with a lipase from *Candida antarctica*, has been explored. [33-35] Two different ways have been tested: direct polycondensation or ROP of cyclic oligomers (firstly obtained by lipase catalysed condensation of DMS and BD). In all cases the ester has been employed, as the use of SA led to unsuccessful polymerizations. Indeed, it seems that the ability of lipases to catalyse the direct polycondensation of a α,ω-linear aliphatic diacid and a diol is strongly related to the monomer chain length; the higher the number of carbon atoms, the better the result. [33] The positive results achieved have demonstrated the availability of a greener route to the PBS synthesis. However, the issues related to enzyme leaching and inactivation and the use of solvents to avoid polymer precipitation and consequent limitation of the molecular weight growth, hampered the diffusion of this technique.

The success of PBS as thermoplastic materials is closely linked to its properties, which are reported in Table 1. As a matter of fact, PBS is a semicrystalline polymer with high crystallization ability (χ_c = 35-45%) [36] and its melting temperature is one of the highest among poly(alkylene dicarboxylate)s. [37,38] The glass transition temperature is well below room temperature, therefore PBS possesses a broad workability range which allows its processing through extrusion, injection moulding and thermoforming. [39-41]

Table 1. PBS properties: lab scale synthesized PBS and commercial BIONOLLE™.

PBS	M_n (g/mol)	PDI	T_g (°C)	T_m (°C)	χ_c (%)	E (MPa)	σ_y (%)	σ_b (%)	ε_b (%)
Lab scale without chain extender [31]	51200	2.1	-34	115	41±4	337±27	/	31±2	24±4
Lab scale without chain extender [38]	64400*	n.r.	-18	112	40	n.r.	35±1	29±2	275±35
Lab scale with chain extender [41]	81200	2.5	-30	112	44±4	366±5	/	27±2	303±20
Lab scale with chain extender [42]	$[\eta]$ = 2.72 dLg^{-1}		-37	111	n.r.	372±30	/	45±1	530±10
Bionolle™ [27, 43]	66600	2.2	-32	114	35-45	470-540	31	59-62	660-710
LDPE [44, 45]	n.r.	n.r.	-120	108	39-42	200-300	12	36	400
PP [46]	n.r.	n.r.	5	162	51	855	25	31	500

* M_w (g/mol)

As to the mechanical properties (Table 1), they are strictly dependent on the presence of small amounts of diisocyanates, typically hexamethylene diisocyanate, used as chain extenders. High molecular weight PBS synthesized without chain extenders shows a brittle behaviour, with very short elongation at break (ε_b), [31] while the use of isocyanates significantly improves its elongation, [41] up to values comparable to those of polyolefins (Table 1). [47]

Poly(butylene succinate) has shown proven biodegradability in the environment, both in compost as well as under natural conditions (soil and water). [27] Hydrolytic degradation and biocompatibility of PBS will be discussed more in detail in the following.

3. PBS-based copolymers

Literature analysis shows that the most studied biomedical application of PBS and PBS-based copolymers is tissue engineering, where these polymers have been employed both in the form of films [42,44, 47-72] as well as scaffolds, obtained by salt leaching, [73-80] electrospinning, [81-92] or extrusion. [93,94] In some cases, realization of microspheres [95-97] or nanocarriers [99,100] as drug delivery systems has been also reported.

PBS has been employed as homopolymer with or without the use of isocyanates, [44,49,50,52,54, 55,63,64,76,83,85,89,94,98] as a blend with PLA, [59,91] poly(L-lysine), [69] poly(3-hydroxybotyrate-*co*-valerate), [81] PCL [95] or in copolymeric systems.

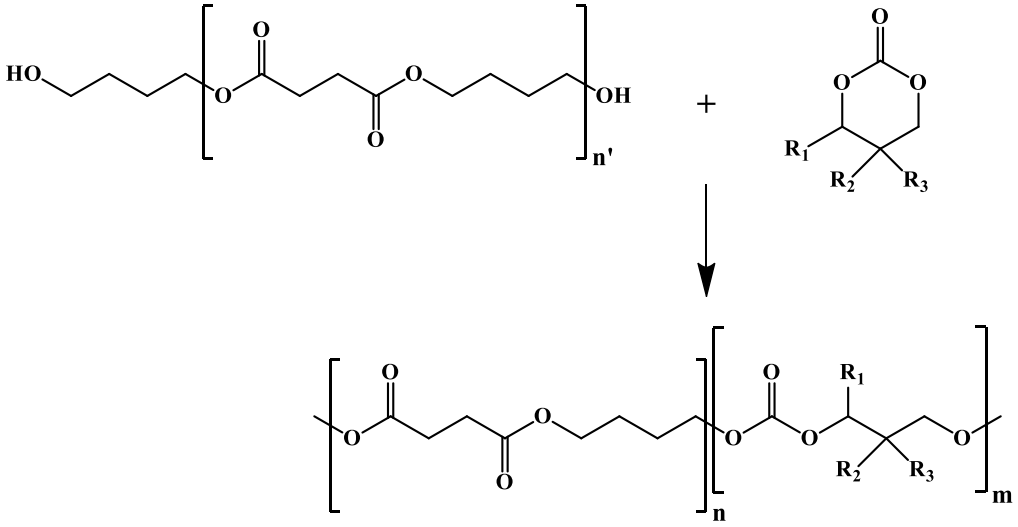
PBS-based copolymers have been prepared with the main purpose of modifying and tuning the PBS properties, such as thermal and mechanical behaviour or biodegradability rate.

The two main synthetic strategies adopted to obtain PBS-based copolymers are copolycondensation and reactive blending. TBT has been the catalyst prevalently employed, but lipase assisted polymerizations (Novozyme 435) have been carried out, too.[96]

Copolycondensation allowed to synthesize random copolymers by means of a two-step catalytic reaction and it has been used in most of the studies. Different comonomeric units have been introduced along the PBS macromolecular chain such as dilinoleic acid, [66,92,99] ω-

pentadecalactone, [96] itaconic acid, [101] cyclic carbonates (Figure 2), [48] and diethanolamine. [42]

Aliphatic/aromatic copolyesters have been also synthesized (i.e. poly(butylene terephthalate/succinate)s). [73] To these last, poly(ethylene glycol) subunits have been also added. [73] On the other hand, by reactive blending, it has been possible to obtain multiblock copolymers with different block lengths by simply varying the mixing time. To this purpose, PBS has been copolymerized with other aliphatic polyesters bearing ether and thioether-linkages, such as poly(butylene thiodiglycolate), [56] poly(thiodiethylene succinate), [57] poly(triethylene succinate), [58] poly(diethylene glycol succinate), [87] and poly(butylene diglycolate). [60]



1. $R_1=R_2=R_3=H$
2. R_1= Me, $R_2=R_3=H$
3. $R_1=H$, $R_2=R_3$=Me
4. $R_1=R_2=H$, $R_3=OCH_2Ph$
5. $R_1=H$, R_2=Et, $R_3=CH_2OCH_2Ph$

Figure 2. Structure of Poly(butylene succinate-*co*-cyclic carbonate)s. [48]

Recently, the introduction of sugar-based subunits, namely 2,4:3,5-di-O-methylene-D-mannitol [103] and 2,4:3,5-di-O-methylene-D-glucitol [104] along the PBS backbone has been studied, leading to the formation of PBxManxyS and PBxGluxyS copolyesters (Figure 3).

Last but not least, PBS ionomers containing dimethyl 5-sodium sulfoisophthalate [49] or sulfonated succinate units [105,106] have been synthesized and characterized.

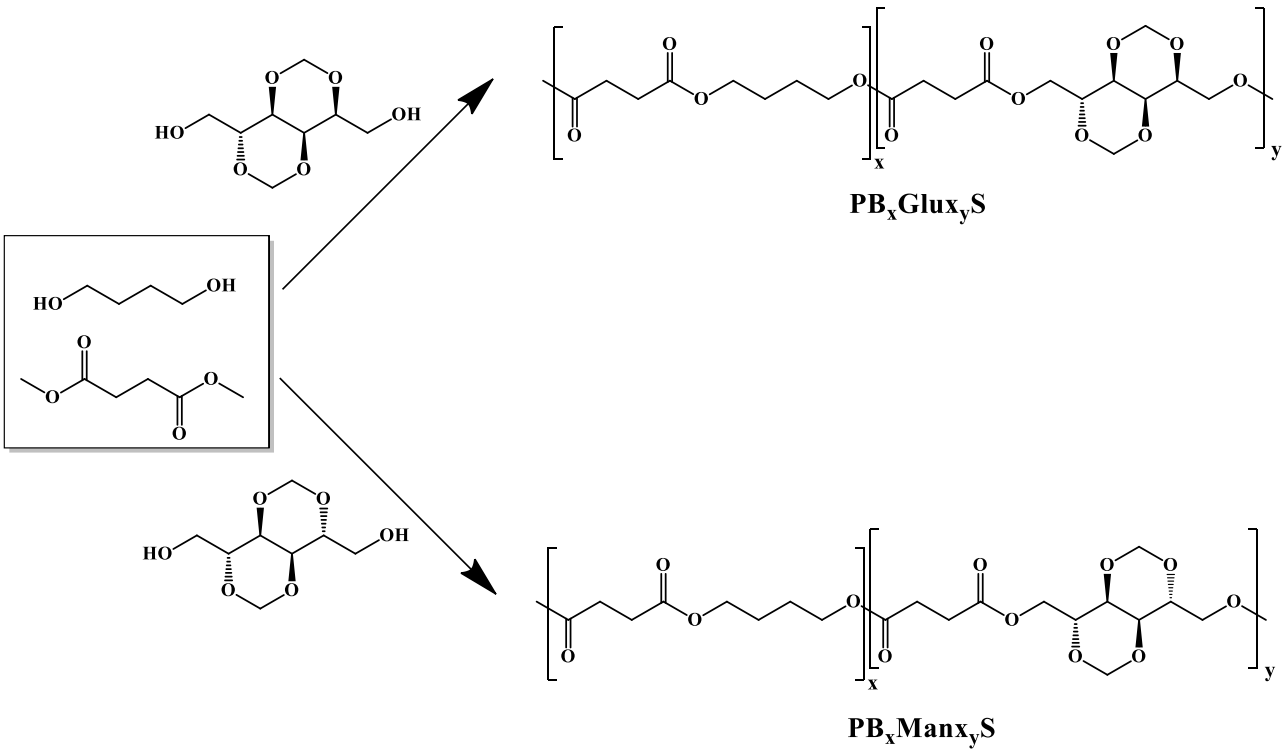


Figure 3. Structure of PBxManxyS and PBxGluxyS copolyesters. [103,104]

Other authors realized PBS-based composites by blending the polyester with organic materials, like chitosan, [51,53,74,75,77-79,80,86,93] collagen, [84] and chestnut fibers (CSF) [72] or inorganic ones such as calcium phosphate, [65,67,68,71] hydroxyapatite (HA), [51,61,84,88,90] fluoroapatite [70] and wollastonite apatite. [82] In one case PBS has been also incorporated in carboxymethylchitosan scaffolds. [97]

As it can be seen, chitosan (C) and hydroxyapatite were the most used compounds; HA is a calcium phosphate ceramic and it is the main mineral constituent of teeth and bones. It displays excellent biocompatibility with hard tissues [107] and it is a bioactive compound, which means that it has a significant ability to promote bone growth. [108] Unfortunately, its poor mechanical properties render it non suitable for load-bearing applications [107,108] and this is the reason why the

realization of composites capable of improving its load capacity without compromising the bioactivity is being investigated. [51,53,74,75,77-79,80,86,93]

On the other hand, chitosan, the second most abundant natural polysaccharide on Earth, finds extensive use in biomedical applications because of its biodegradability and biocompatibility. [109] Again, the poor mechanical properties limit the chitosan employment for particular applications and therefore the study of chitosan-based composites has been promoted. [110]

4. Physical properties

4.1. Thermal properties and wettability

As expected, the properties of PBS-based polymeric systems were significantly affected by the synthetic strategy adopted (copolycondensation, reactive blending, physical blending, etc...) and on the kind of comonomer/copolymer employed. Among other properties, the degree of crystallinity and wettability are very important parameters that can influence both biodegradation rate and biocompatibility of the material. In particular, it is well-known that the higher the degree of crystallinity the lower the hydrolytic and enzymatic degradation rate, since the less organized and packed amorphous domains are more easily attacked. [111-113] Similarly, the higher the hydrophilicity, the higher the hydrolysis rate, due to a greater water adsorption. On the other hand, enzymatic degradation is faster for a balanced hydrophobicity–hydrophilicity ratio, as the interaction of enzymes with the polymer surface is controlled by this parameter. [114]

Different studies have been also carried out to analyse how surface wettability can affect the cell adhesion and proliferation, thus the biocompatibility. [115,116] It is in fact well established that surface chemistry, wettability and roughness are three of the most important factors influencing biological reactions at biomaterial surfaces.[116] Results demonstrated that adhesion is favoured on hydrophilic substrates.[115] As regards the surface roughness, it has been observed that the cells better spread on low-rough substrates, confirming that the cell attachment and proliferation are enhanced by a certain surface topography.[116] This is because, for substrates displaying a suitable

roughness, the cells would modify their shape accordingly to the surface topography, increasing the contact area and, consequently, the interfacial force. [116] This would result in higher cell attachment and proliferation.

As a confirmation of the above mentioned findings, some studies carried out on PBS-based substrates have demonstrated that surface modifications that generate a certain degree of surface roughness pronouncedly promote osteoconductive property of scaffold materials. [65,71,90]

In this framework, Table 2 reports the crystallinity and water contact angle (WCA) data for the PBS-based polymers together with the mechanical properties (that will be discussed in chapter 4.2.).

Table 2. Degree of crystallinity, wettability and mechanical properties of PBS-based systems.

Polymeric material	χ_c (%)	WCA (°)	E (MPa)	σ_b (MPa)	ε_b (%)	form
Poly(butylene succinate-*co*-dimethyl 5-sulfoisophtalate sodium salt)s (PBS-*co*-BSi) [49]	n.r.	50-76	n.r	n.r	n.r.	f
Poly(butylene succinate) [50]	n.r.	59	500	33	126	f
Poly(butylene succinate)-*blend*-chitosan/hydroxyapatite (PBS/C/HA) [51]	58-78	n.r.	610-1950	12-39	1-264	f
Poly(butylene succinate) [44]	n.r.	25-50*	n.r.	n.r.	n.r.	f
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [53]	n.r.	78-107	n.r.	n.r.	n.r.	f
Poly(butylene succinate-*block*-butylene thiodiglycolate) (PBS-*b*-PBTDG) [56]	20-41	n.r.	61-337	7-31	24-713	f
Poly(butylene succinate-*block*-thiodiethylene succinate) (PBS-*b*-PTDGS) [57]	14-38	73-93	72-326	4-31	24-699	f
Poly(butylene succinate)-*block*-poly(triethylene succinate) (PBS-*b*-PTES) [58]	27-54	25-96	47-490	6-34	20-700	f
Poly(butylene succinate-*block*-butylene diglycolate) (PBS-*b*-PBDG) [60]	21-41	n.r.	83-337	14-31	17-883	f
Poly(butylene succinate)-hydroxyapatite (PBS/HA) [61]	n.r.	n.r.	280-560*	10-52*	n.r.	f
Poly(butylene succinate)-*end capped*-phosphorylcholine (PBS-PC)[63]	n.r.	15-66	n.r.	n.r.	n.r.	f
Poly(butylene succinate)-silica nanocomposites (PBS/Si); poly(butylene succinate)-strontium hydroxyapatite nanocomposites (PBS/SrHA) [64]	37-39	62-64 b.st. 63-75 a.st.	470-640	10-12	n.r.	f
Poly(butylene succinate)-β-tricalcium phosphate (PBS/TCP); poly(butylene succinate)-hydroxyapatite (PBS/HA)[65]	n.r.	60-93	n.r.	n.r.	n.r.	f
Poly(glycerol sebacate)-*blend*-poly(butylene succinate-*block*-dilinoleate) (PGS/P(BS-DLA)) [66]	n.r.	24-79	10-54	2-8	20-29	f
Poly(butylene succinate)-β-tricalcium phosphate (PBS/TCP); poly(butylene succinate)-hydroxyapatite (PBS/HA) [67]	n.r.	71-94	n.r.	n.r.	n.r.	f

Poly(butylene succinate)-*blend*-polylactic acid-*graft*-tetracalcium phosphate (PBS/PLA-*g*-TCP) [68]	n.r.	n.r.	542-1287	21-35	4-109	f
Poly(butylene succinate)-*blend*-poly(Z-L-lysine) (PBS/PZL) [69]	52-64	n.r.	82-480	3-19	6-63	f
Poly(butylene succinate)-fluorapatite (PBS/FA) [70]	n.r.	0-67	391-734	n.r.	n.r.	f
Poly(butylene succinate)-*blend*-chestnut shell fiber (PBS/CSF); **(Poly(butylene succinate)-*graft*-maleic anhydride) - *blend*-chestnut shell fiber (PBS-*g*-MA/CSF)** [72]	n.r.	n.r.	750-1250*	27-40*	155-250*	f
			1250-1600*	39-49*	200-249*	f
Poly(butylene succinate) urethane ionenes (PBSUI) [42]	n.r.	63-77	243-372	39-45	516-543	f
Poly(butylene terephthalate)-*co*-poly(butylene succinate)-*block*-poly(ethylene glycol) (P(BSBT)-*b*-PEG) [73]	5-23	n.r.	7-35	10-23	567-1438	f
Poly(butylene succinate) (PBS) [76]	n.r.	n.r.	0.16-0.72	0.02-006	n.r.	s
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [83]	n.r.	n.r.	176-296	6-7	8-11	f
Poly(butylene succinate) (PBS) [85]	n.r.	49-70	617	3	47	f
		79-114	43	1	122	s
Poly(butylene succinate-*block*-diethylene glycol succinate) (PBS-*b*-PDGS) [87]	49-59	86-96	96-88	6-34	20-24	f
	44-55	n.r.	9-23	2-12	127-135	s
Poly(butylene succinate) [89]	65-71	106	n.r.	n.r.	n.r.	s
Poly(butylene succinate)-hydroxyapatite (PBS/HA) [90]	n.r.	0-110	n.r.	n.r.	n.r.	s
Poly(butylene succinate)-*blend*-polylactic acid (PBS/PLA) [91]	34-53	127-132	70-380	2-10	20-130	s
Poly(glycerol sebacate)-*blend*-poly(butylene succinate-*co*-butylene dilinoleate) (PGS/PBS-DLA) [92]	n.r.	39-49	14-54	2-8	20-29	f
			1-6	2-3	86-148	s
Poly(butylene succinate) [94]	n.r.	61-117	n.r.	n.r.	n.r.	s
Poly(ω-pentadecalactone-*co*-butylene succinate) (P(DLBS)) [96]	47-64	n.r.	n.r.	n.r.	n.r.	
Poly(butylene-*co*-2,4,:3,5-di-*O*-methylene-D-mannitol succinate) (PB_XMan_xS) [103]	17-52	113-117	351-515	20-28	2-19	f
Poly(butylene-*co*-2,4,:3,5-di-*O*-methylene-D-glucitol succinate) (PB_xGlux_yS) [104]	n.r.	n.r.	348-1356	11-40	3-10	f
Poly(butylene succinate-*co*-butylene sulfonated succinate) (PBS_xSS_y) [105]	n.r.	n.r.	403-1050	26-42	3-282	f
Poly(butylene succinate-*co*-2-trimethylammonium chloride glutarate) ($PBS_xG_yG_z^I$); **Poly(butylene succinate)-*blend*-cloisite · w%(poly(butylene succinate-*co*-2-trimethylammonium chloride glutarate) ($PBS/CL·w\%(PBS_xG_yG_z^I)$)** [106]	n.r.	n.r.	149-440	2-35	0.3-282	f
			550-747	39-50	16-144	f

n.r. = not reported
f = film
s = scaffold
b.st. = before sterilization
a.st. = after sterilization
* = extracted from graph

Almeida *et al*., [89] analysed the degree of crystallinity of PBS granules and of extruded fibres: interestingly, the fibres displayed a higher degree of crystallinity (χ_c = 71.4%) as compared to the

raw PBS (χ_c = 65%). The authors attributed this result to the induced orientation of PBS chains during the extrusion process.

The preparation of PBS composites led to different outcomes. One study reported the thermal properties behaviour of PBS blends with HA and chitosan. [51] A decrease in the melting point and a slight increase of the degree of crystallinity were observed and linked to the decrease in the molecular weight due to the processing (shear stress) and to the presence of moisture. Similar findings have been reported by Carrasco *et al*. [117] for PLA: the polymer processing generated a molecular weight decay because of chain scission reactions.

On the contrary, Grigoriadou *et al*. [64] prepared PBS composites containing silica nanotubes or strontium hydroxyapatite nanorods: no decrease in the melting point was detected; as to the degree of crystallinity, the addition of a small amount of nanofiller increased the χ_c because the nanoparticles acted as nucleating agents; on the other hand, for loading concentrations higher than 20 wt% a slight decrease in the χ_c was reported. This has been explained as due to a hindering effect of the nanoparticles, which hampered the macromolecular chains folding into crystalline structures. [64] A similar effect has been shown by Tan *et al*. [69] in PBS physically blended with polylysine: the introduction of this latter in the polyester matrix reduced its crystallization ability and led to the formation of less perfect crystals.

In PBS/PLA blends, a plasticizing effect of PLA by PBS was observed. [91]

As far as the crystallisation behaviour of block and random copolymers is concerned, different situations have been reported depending on the crystallization ability of the two (or more) comonomeric units. Multiblock poly(ether ester) copolymers [73] showed microphase separation, while poly(ω-pentadecalactone-*co*-butylene succinate) copolymers highlighted the coexistence of two crystalline phases only for a specific composition, namely 65 mol% of PBS. [96]

Finally, the degree of crystallinity and the melting temperature of heteroatom-containing multiblock copolymers was influenced by the block length of the crystallisable co-unit (BS): the shorter the

length, the lower both the χ_c and T_m because of the formation of less and less perfect crystals due to increased difficulty of PBS chain folding. [56-58,60]

Many authors analysed the wettability of PBS and its copolymers and composites. The specimen morphology has a great importance for polymer hydrophilicity: as a matter of fact, Sutthiphong *et al.* [85] evidenced greater contact angles for electrospun PBS fibres with respect to the film because of the increased surface roughness. Similar findings have been highlighted by Tallawi *et al*. [66] on poly(glycerol sebacate) blended with poly(butylene succinate/dilinoleate) copolymers: smoother films surfaces displayed higher wettability compared to rougher surfaces.

Alkaline hydrolysis permitted to increase the polymer surface roughness, due to surface erosion, thus to tailor the material biocompatibility. [65,71,90]

Plasma treatment is another well-known process to modify the wettability of a polymer, and consequently favours the cell attachment, spreading and proliferation. [118] Through this technique new polar functional groups can be inserted at the specimen surface and therefore the wettability can be significantly enhanced. PBS films have been subjected to H_2O and NH_3 plasma immersion ion implantation: as a result OH and NH_2 groups were created and the surface water contact angle decreased from 50° to about 25°. [44]

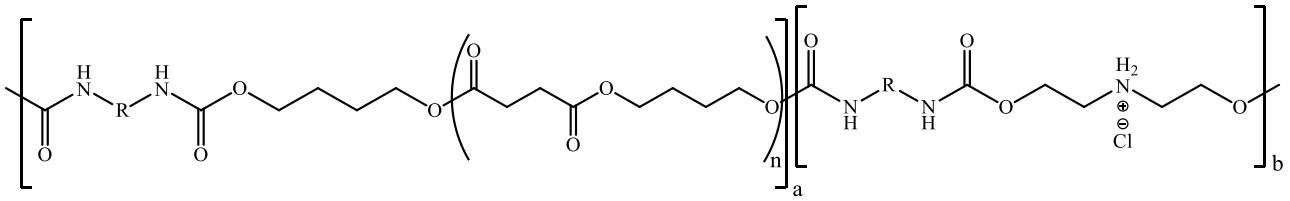
PBS scaffolds produced through weft knitting (meaning that the wales are perpendicular to the course of the yarn) were subjected to different treatments to modify the surface roughness and hydrophilicity. [94] The plasma/vinyl sulfonic acid (VSA) treatment increased the hydrophobicity of the substrate, while UV/O_3 treatment and NaOH etching significantly enhanced the wettability behaviour. The water contact angle decreased from 106° to 94° and 61°, respectively. [94]

As to PBS composites, the presence of chitosan enhanced the surface hydrophilicity because of the presence of a large number of polar groups; plasma etching of these composites showed a further effect in this direction due to the removal of a thin film layer which revealed the inner core richer in chitosan. [53] HA and tricalcium phosphate (TCP) [65,67] increased the surface roughness, and

therefore the wettability of PBS films as well as nanofluoroapatite, [70] while the addition of silica nanotubes or strontium hydroxyapatite nanorods had no significant effect. [64]

Hydrophilic treatments, which involve surface structure reorganization, have been also conducted on phosphorylcholine end-capped poly(butylene succinate) to increase the PBS wettability by immersing the polymer specimens in water and varying temperature and time of treatment. [63]

Also the incorporation of urethane ionic groups increased the PBS surface hydrophilicity (Figure 4). [42]



R: hexamethylene

Figure 4. Structure of poly(butylene succinate) urethane ionene containing secondary amine cation.[42]

The presence of an increasing amount of P(BS60DLA40) in blends with poly(glycerol sebacate) (PGS) enhanced the wettability of the electrospun fibres; no significant differences were observed when compared to the corresponding films. [92]

Finally, the introduction of polar groups (even to a small extent: 3-5%) [49] or of ether [58,88] and thioether [57] linkages (and therefore of electronegative oxygen and sulphur atoms) along the PBS backbone enhanced its surface hydrophilicity. If the block length did not impact the water contact angle when sulphur atoms were added, [57] a significant increase of the wettability was observed with the increase of block length in PBS-based copolymers containing PEG-like sequences. Moreover, a decrease of the contact angle with time was highlighted. [58] Because of the exclusion of water absorption in the time scale explored, the authors explained this behaviour on the basis of a quick chain spatial reorganization that brought the more hydrophilic PEG-like units to the interface with water during WCA measurements. [58]

4.2. Mechanical properties

The mechanical properties are of crucial interest to evaluate the applicability of new synthesized polymers and composites as biomaterials, as similarity between the properties of the polymeric matrix with a specific tissue can not only favour cell proliferation, [119] but also induce stem cells differentiation. [120,121] The mechanical data about the PBS-based systems for biomedical applications are collected in Table 2, which reports the range of elastic modulus (E), the elongation at break (ε_b) and the stress at break (σ_b) as reported by the various authors.

As far as multiblock copolymeric systems containing ether and thioether linkages are concerned, the mechanical properties of the PBS homopolymer were modified as follows: a reduction of E and σ_b and an increase of ε_b were observed with the decreasing of the block length. [56-58,60] This was mainly due to the reduced degree of crystallinity of the synthesized copolymers. In addition, a comparison between oxygen and sulphur containing copolyesters of similar degree of crystallinity and block length has been performed: thioether linkages caused a decrease in the rigidity as compared to ether linkages (E decrease of 25% and ε_b increase of 20% were observed). This result has been correlated by the authors to two different factors: 1) the high chain flexibility of sulphur containing copolymers, due to the presence of longer C-S bonds with respect to C-O ones and 2) the weaker interchain interactions because of the lower electronegativity of sulphur atoms with respect to oxygen ones. [56]

The blending of poly(glycerol sebacate) with poly(butylene succinate/dilinoleate) (PBS-DLA) multiblock copolymer led to a reduction of the elastic modulus and of the stress at break; no yield was observed in all cases. [66] It is worth pointing out that the PBS-DLA showed a thermoplastic elastomeric behaviour. [66] Elestrospun fibres of the same blends displayed higher strains as compared to the corresponding films. [92] Young's modulus increased with the increasing of the PBS-DLA content. [92]

The introduction of PEG and PBS segments in PET, significantly contributed to modify its mechanical properties: as a matter of fact copolymers containing higher mol% of soft segment (PBS

and PEG) with respect to the hard segment (PET) displayed a decrease in the elastic modulus and in the tensile strength and an increase in the elongation at break, because of the reduced χ_c. [73]

Blending of PBS with polylysine led to a worsening of the mechanical properties, especially for polylysine amounts higher than 20%; this has been correlated to the phase separation between the two domains. [69]

The addition of organic or inorganic fillers had a significant impact on the PBS properties. In general, elastic modulus and elongation to break respectively increased and decreased with the increasing filler content as due to the composite enhanced stiffness, [83] while no general trend can be observed for tensile strength.

Guo *et al.*, evaluated the mechanical properties of poly(butylene succinate)/hydroxyapatite (PBS/HA) nanocomposites at RT and at -30°C. [61] With the increasing of HA content, the tensile strength decreased, while the tensile and flexural modulus increased (up to respectively 66% and 69% higher for 20% HA as compared to neat PBS). This was probably due to the presence of inorganic nanofillers which displayed higher modulus, while the lower tensile strength has been attributed to the decrease of ε_b, once again due to the HA particles which modified the original PBS structure. [61] On the other hand, the impact strength of the composites, was significantly worsened by the HA particles, whose aggregation acted as defects in the polymer matrix. [61] Properties evaluated at -30°C showed a higher tensile strength, of about 55%, and lower impact strength when compared to the value measured at room temperature. The decrease in the impact strength has been ascribed to the restricted mobility of the polymer chains. [61]

The addition of HA to PBS blended with chitosan, caused an increase of the elastic modulus and a decrease both of the tensile strength and of the elongation at break. [51] Silica-nanotubes and strontium hydroxyapatite nanorods raised the PBS elastic modulus, while no significant effect was recorded for the tensile strength; this last has been linked by the authors to the lack of interaction between inorganic nanoparticles and polymer matrix. [64]

When chitosan nanofibers have been used to reinforce PBS matrix, no negative effect has been observed in the tensile strength meaning that no critical size defects were present in the material structure; tensile modulus was significantly enhanced and elongation at break decreased. [83]

Niu and co-workers [70] observed an increase in the compressive strength and elastic modulus of the nanofluoroapatite/PBS composites up to a critical value (40 wt%) above which the mechanical properties dropped. The presence of a critical concentration for the enhancement of the mechanical properties of PBS nanocomposites, was confirmed by the studies of Fan *et al.* [68] conducted on PBS grafted tetracalcium phosphate (TTCP). As a matter of fact, the tensile strength increased up to a concentration of TTCP equal to 10%, then decreased because of the particles agglomeration and formation of micropores. [68]

Mechanical properties of PBS/Chestnut Shell Fiber (CSF) composites have been analysed by Wu and co-workers. [72] Tensile strength and tensile modulus at failure of the PBS/CSF composites decreased with increasing CSF content, because of the poor dispersion of the CSF in the PBS matrix. On the other hand, PBS-g-MA/TCSF composites (i.e. maleic anhydride-grafted poly(butylene succinate) and crosslinked CSF) displayed enhanced tensile strength and tensile modulus at failure with respect to neat PBS as due to an enhanced dispersion of TCSF in the PBS-g-MA matrix because of the formation of branched or crosslinked polymeric chains. [72] Elongation at break decreased in all cases with respect to PBS. [72]

Salt leaching technique has been used to prepare scaffolds from chain-extended PBS. [76] Compression and tensile tests revealed a strict relationship between porosity and mechanical properties. With the increasing of the pore size, i.e. the salt amount used to prepare the scaffolds, the mechanical properties decreased. [76]

Scaffolds of chitosan/PBS blends prepared by salt leaching exhibited a compressive modulus of 1.7 MPa. [75] Similar findings have been displayed in another PBS/C system, the compressive modulus of PBS and PBS containing 25% and 50% of chitosan being 16 MPa, 9.0 MPa and 22.8 MPa, respectively. [80]

Mechanical properties of electrospun scaffolds from PBS/PLA blends have been analysed. Two different rotating speeds of the collecting plate were used for the preparation of the mats: 600 and 1900 rpm, since the higher rotational speed is known to increase fibers' alignment. [91] It has been in fact reported in the literature that the higher the fibers' alignment, the better the mechanical strength; a reduced failure strain can be instead observed. [122] Similar behaviour has been observed for the PBS/PLA blends, even if in this case also different parameters have to be taken into account, since polyester miscibility, blend morphology and crystallinity also played a role. [91]

Mechanical behaviour of PBS fibres obtained via weft knitting was compared to that of silk fibres of similar linear density. [94] PBS fibres displayed a lower specific strength to rupture, but a higher elongation (120%) as compared to silk ones (30%). [94]

Lastly, the mechanical properties of films and scaffold obtained from the same polymers have been compared: [85,89] both studies reported that scaffolds were much softer than the films (E and σ_b ten to twenty times and two to three times lower for scaffolds, respectively), while the elongation at break considerably increased in the case of scaffolds (about four times). The authors explained these results on the basis of the porous structure of the electrospun fibres and of the lower degree of crystallinity of the scaffolds. [89]

5. Hydrolytic and enzymatic degradation

The hydrolytic and enzymatic degradation studies conducted on PBS and PBS-based systems are reported in Table 3, that contains the degradation conditions, the time scale explored and the weight loss range.

Table 3. Hydrolytic and enzymatic degradation studies of PBS-based systems.

Polymeric material	Degradation conditions	Time (d)	Weight loss (%)
Poly(butylene succinate-*co*-cyclic carbonate)s (PBS-*co*-CC) [48]	Hydrolytic: phys. cond.	40	0
	Enzymatic: lipase Novozyme 435	25	40-70

	Enzymatic: lipase porcine pancreas	20	40-90
Poly(butylene succinate-*co*-dimethyl 5-sulfoisophtalate sodium salt)s (PBS-*co*-BSi) [49]	Hydrolytic: phys. cond.	80	3-5*
Poly(butylene succinate) (PBS) [50]	Hydrolytic: phys. cond.	105	65
Poly(butylene succinate)-*block*-poly(triethylene succinate) (PBS-*b*-PTES) [58]	Hydrolytic: phys. cond.	225	0-35
Poly(butylene succinate)-silica nanocomposites (PBS/Si); poly(butylene succinate)-strontium hydroxyapatite nanocomposites (PBS/SrHA) [64]	Enzymatic: *R. delemar* and *P. cepacia* lipases	30	2-16*
Poly(glycerol sebacate)-*blend*-poly(butylene succinate-*co*-dilinoleate) (PGS/P(BS-DLA)) [66]	Hydrolytic: phys. cond.	28	3-13
Poly(butylene succinate)-*blend*-poly(Z-L-lysine) (PBS/PZL) [69]	Enzymatic: *P. cepacia* lipase	28	8-26*
Poly(butylene succinate) urethane ionenes (PBSUI) [42]	Hydrolytic: phys. cond.	4	5-35
Poly(butylene terephthalate)-*co*-poly(butylene succinate)-*block*-poly(ethylene glycol) (P(BSBT)-*b*-PEG) [73]	Hydrolytic: phys. cond.	63	3-33*
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [83]	Hydrolytic: phys. cond.	30	1-7
Poly(butylene succinate-*block*-diethylene glycol succinate) (PBS-*b*-PDGS) [87]	Hydrolytic: phys. cond.	235	0-20
Poly(butylene succinate) [89]	Hydrolytic: phys. cond.	30	2-3*
	Enzymatic: *P. cepacia* lipase	30	4*
	Enzymatic: protease XIV from *S. griseus*	30	5*
Poly(butylene-*co*-2,4,:3,5-di-*O*-methylene-D-mannitol succinate) (PB_XMan_XS) [103]	Hydrolytic: phys. cond.; pH 2.0, 37°C.	56	10-15
	Enzymatic: porcine pancreas lipase	56	24-29
Poly(butylene-*co*-2,4,:3,5-di-*O*-methylene-D-glucitol succinate) (PB_xGlux_yS) [104]	Hydrolytic: phys. cond.; pH 2.0, 37°C.	40	10-24*
	Enzymatic: porcine pancreas lipase	40	15-25*
Poly(butylene succinate-*co*-butylene sulfonated succinate) (PBS_xSS_y) [105]	Hydrolytic: phys. cond.	56	1-25*
	Hydrolytic: pH 4.0, 37°C.	56	5-35*
	Hydrolytic: pH 10, 37°C.	56	15-60*

phys. cond. = physiological conditions (37°C, pH=7.4)
* = extracted from graph

Polymers used for temporary biomedical applications have to satisfy an important prerequisite, i.e. biodegradability. In this particular field the study is preferably conducted under physiological conditions (phys. cond., i.e. 37°C, pH 7.4, phosphate-buffered saline). As it is well known, *in vitro* hydrolysis of aliphatic polyesters is a bulk phenomenon (Figure 5) that proceeds in two stages and it is affected by different factors, e.g. chemical structure, hydrophilic–hydrophobic balance, molecular weight and molecular weight distribution, solid-state morphology and degree of crystallinity. [111,112] In the first step a random chain scission, occurs and a decrease in the molecular weight

and an increase in the degree of crystallinity (due to the faster crystallization of chain fragments) can be usually observed. [123] When the molecular weight reaches a critical value of about 13.000 Da, the second stage, accompanied by mass loss, starts. [125] To analyse the degradation profile, molecular weight changes and weight losses as a function of incubation time are therefore the easiest and most extensively used techniques. Many authors reported that PBS homopolymer hydrolytically degrade very slowly under physiological conditions, and its weight remains relatively constant for several weeks: [49,50,58,87,89] only a decrease in the molecular weight could be detected. [87] This result has been imputed to the high crystallinity and hydrophobicity of this polymer. [57]

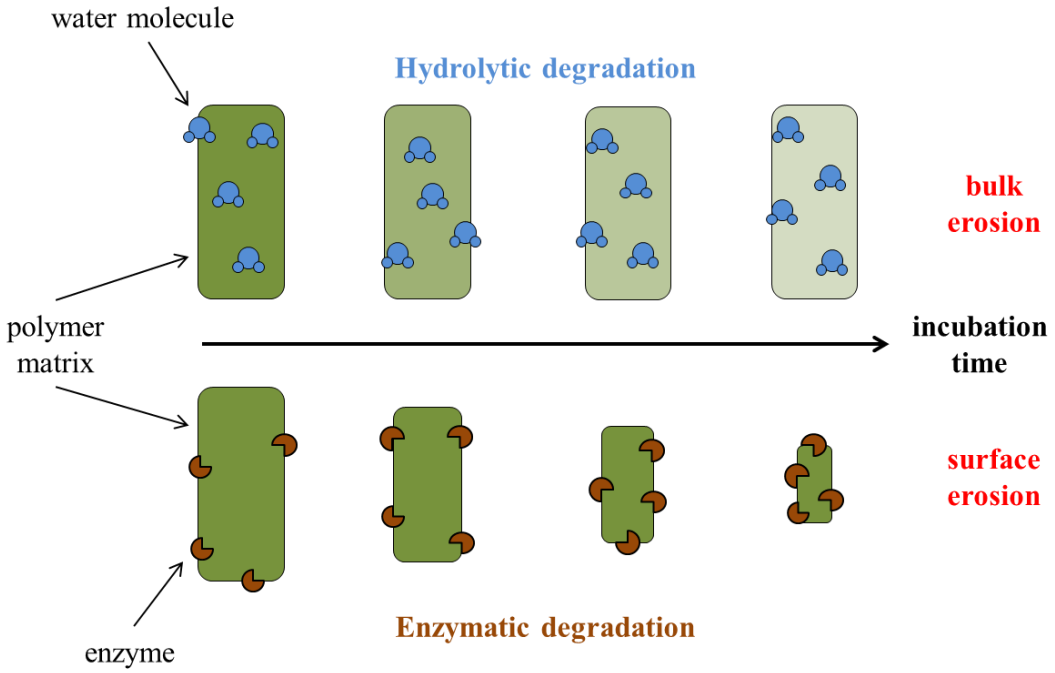


Figure 5. Hydrolytic degradation and enzymatic degradation process.

On the other hand, copolymerisation of PBS with hydrophilic sequences had a notable effect on its hydrolysis rate. [57,74,87] Wang *et al.* [73] reported that the longer the PEG segment, the higher the weight losses, because of a greater water adsorption. Similarly, PBS-based copolymers containing PEG-like sequences, showed a faster hydrolytic degradation rate with the increasing of block length, due to the preferential cleavage of the ester bonds on those chain segments, which,

due to their hydrophilic nature, were easily solubilised in water. [57] Also the introduction of ionic groups increased the PBS degradation rate. [49] The introduction of urethane ionenes increased the PBS degradation rate because of the increased hydrophilicity and reduced degree of crystallinity with the increase amount of urethane groups. [42]

By a comparison between copolymeric films and scaffolds it can be evicted that the film underwent faster degradation. [87] The reason seems to be linked to the carboxylic end-groups produced during hydrolysis, which could display an autocatalytic effect on the degradation kinetic. In polymer films, the diffusion of the acidic polymeric fragments is much lower with respect to the scaffolds due to size and morphology reasons. Therefore, they accumulate in the sample core, catalysing the hydrolysis of the macromolecular chains. [124]

Jager *et al.* [100] observed fast degradation of nanoparticles made of poly(butylene succinate-*co*-dilinoleate): after three weeks of incubation they collapsed and a substantial decrease in the molecular weight was observed. This result has been strictly connected by the authors to the structure of the particles, as they were soft and with a density of 0.37 g/mL, meaning that they contained a high water amount which could increase the hydrolysis rate.

In the case of PBS/C blends, it has been reported that the presence of chitosan enhanced the composite erosion because of an increased water uptake, due to swelling and opening of the outer layer of the composite. This effect was even more evident in the case of PBS/C blends reinforced with chitosan nanofibres, because of the higher hydrophilicity of these last. [84]

Last but not least, various enzymatic degradation studies have been conducted on PBS-based polymeric systems in the presence of *Candida antartica* lipase (Novozyme 435) or porcine pancreas lipase, [48] *Pseudomonas cepacia* lipase, [70] a mixture of *Rizopus delemar* and *Pseudomonas Cepacia* lipases, [64] *Aspergillus oryzae* lipase, lysozyme or a mixture of the two. [93]

Lipases are in fact well-known enzymes capable of catalysing the hydrolysis of aliphatic polyesters. [114] Degradation kinetic depends on the same factors mentioned above in the case of simple hydrolysis, i.e. chemical structure, hydrophilic–hydrophobic balance, molecular weight, solid-state

morphology and degree of crystallinity, but differently from it, enzymatic hydrolysis is a surface eroding process (Figure 5). Therefore, molecular weight of the substrate does not significantly changes, as the low molecular weight products are easily solubilized in the aqueous medium. [114]

In all the analysed cases, copolymerisation [48] or blending [70] favoured the enzymatic biodegradation.

Yang *et al.* [48] reported the degradation of PBS copolymerized with poly(cyclic carbonate)s in the form of thin films. In the time scale explored (25 days) PBS did not lose any weight up to an enzyme concentration of about 1.3 mg/mL, while copolymers appreciably degraded; as expected, the lower the degree of crystallinity, the molecular weight and the melting point of the samples under study, the higher the degradation rate. On the other hand, no significant degradation was observed for all samples if hydrolytic tests in buffer were performed (1000 h of incubation, 37°C, pH 7.4).

Also the blending with poly(lysine) [69] enhanced the PBS degradation rate, due to a reduced degree of crystallinity.

In scaffolds from PBS/C blends, the presence of lysozyme, known to degrade chitosan, did not significantly influence the biodegradation behaviour with respect to the control (hydrolysis). [93] Higher weight losses were observed when lipase was supplemented. The contemporary addition of lipase and lysozyme fastened the degradation: as a matter of fact after 6 weeks of incubation all the scaffolds lost their structural integrity, as evidenced by SEM analysis. [93]

Lastly, silica-nanotubes and strontium hydroxyapatite nanorods significantly contributed to the enzymatic degradation behaviour of PBS composites, not only because of the presence of hydroxyl groups on the surface of both nanoparticles, capable of catalysing the process, but of the formation of holes on the polymer, due to the removal of the nanoparticles. [64] This formed a porous structure which could facilitate the lipase diffusion in the polymer matrix and accelerate the degradation. [64]

6. Biocompatibility evaluation

The study of polymer cytotoxicity is of primary importance to assess their potential as substitutes of organic tissues or as drug carriers. Most of the studies have been conducted *in vitro* by employing different kinds of animal (27 reports) or human cells (17 reports) (Figure 6). In few cases *in vivo* trials were performed, too.

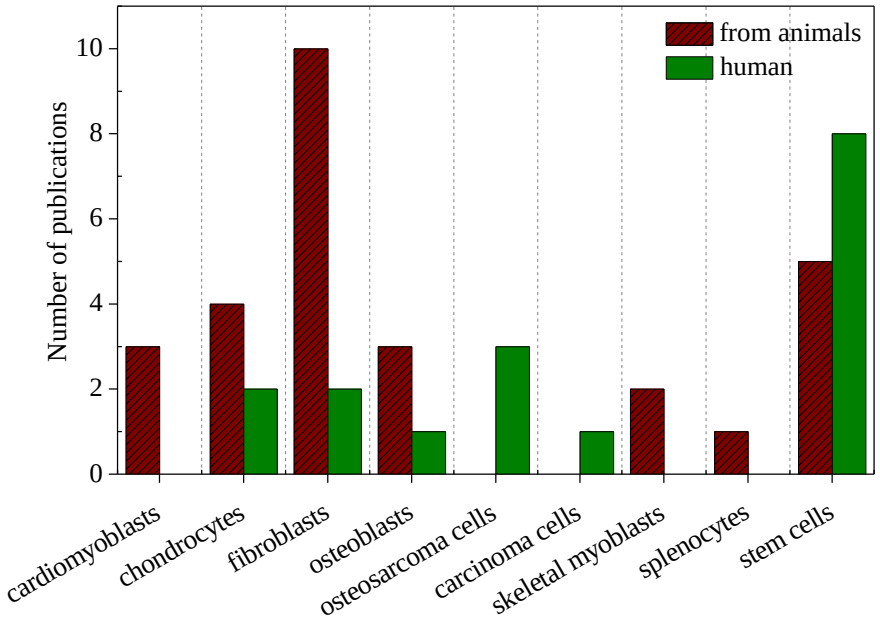


Figure 6. Number of published reports with respect to the cell type employed for in vitro cytotoxicity studies on PBS and PBS-based copolymers.

6.1. In vitro cytotoxicity tests with animal cells

Table 4 reports the different cell lines tested and the characterization techniques adopted to assess the biocompatibility of the PBS-based copolymers.

Table 4. *In vitro* biocompatibility tests with animal cells.

Polymeric material	Cell line tested	Adopted methods of analysis
Poly(butylene succinate-*co*-cyclic carbonate)s (PBS-*co*-CC) [48]	Mouse fibroblasts (NIH 3T3)	Optimas 5.2 image analysis system MTT assay
Poly(butylene succinate) (PBS)[50]	Rats osteoblasts	MTT assay ELISA assay ALP assay

Poly(butylene succinate) (PBS) [52]	Rats calvaria osteoblasts	Kit-8 test ALP assay PCR assay
Poly(butylene succinate-*block*-thiodiethylene succinate) (PBS-*b*-PTDGS) [57]	Rat cardiac cells (H9c2)	Alamar Blue assay SEM analysis
Poly(butylene succinate)-*block*-poly(triethylene succinate) (PBS-*b*-PTES) [58]	Mouse fibroblasts (L929)	LDH assay SEM analysis ELISA assay MTT assay
Poly(butylene succinate)-*blend*-poly(lactic acid) (PBS/PLA) [59]	Mouse fibroblasts (L929); bone marrow stem cells (BMSCs)	Kit-8 test LDH assay
Poly(butylene succinate)-*end capped*-phosphorylcholine (PBS-PC) [62]	Mouse fibroblasts (L929)	MTT assay SEM analysis
Poly(glycerol sebacate)-*blend*-poly(butylene succinate-*co*-dilinoleate) (PGS/PBS-DLA))[66]	Mouse skeletal myoblasts (C2C12)	WST-8 test Alexa Fluor1555 Phalloidin assay SEM analysis
Poly(butylene terephthalate)-*co*-poly(butylene succinate)-*block*-poly(ethylene glycol) (P(BSBT)-*b*-PEG) [73]	Mouse fibroblasts (L929); Sheep marrow stem cells (SMSC)	MTT assay Olympus light microscope analysis
Poly(butylene succinate)-*blend*-chitosan/ hydroxyapatite (PBS/C/HA) [74]	Mouse fibroblasts (L929)	MTS assay SEM analysis
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [75]	Mouse fibroblasts (L929); mouse mesenchymal stem cells (BMC9)	MEM assay MTS assay SEM analysis Western blot assay
Poly(butylene succinate) (PBS) [76]	Mouse calvaria (MC3T3-E1); Mouse pre-osteoblastic cells (ATCC CRL-2593)	MTT assay MEM assay
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [77]	Mouse fibroblasts (L929); mouse mesenchymal stem cells (BMC9)	MTS assay SEM analysis Olympus light microscope analysis ALP assay
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [78]	Bovine articular chondrocytes (BAC)	SEM analysis GAG assay Immunolocalization of type I and II collagens
Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)-*blend*-poly(butylene succinate) (PHBV/PBS) [81]	Rabbits mesenchymal stem cells (MSCs)	LDH assay SEM analysis MTT assay
Poly(butylene succinate) (PBS) [85]	Mouse fibroblasts (L929);	MTT assay SEM analysis ALP assay
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [86]	Bovine articular chondrocytes (BAC)	GAG assay Immunolocalization of type I and II collagens
Poly(butylene succinate-*block*-diethylene glycol succinate) (PBS-*b*-PDGS) [87]	Rat cardiac cells (H9c2)	Alamar Blue assay SEM analysis Leitz Diaplan light microscope
Poly(butylene succinate)-hydroxyapatite (PBS/HA) [88]	Porcine chondrocytes	Alamar Blue assay PCR test GAG assay
Poly(butylene succinate) [89]	Mouse fibroblasts (L929)	SEM analysis DNA quantification

Poly(glycerol sebacate)-*blend*-poly(butylene succinate-*co*-dilinoleate) (PGS/P(BSBL)) [92**]**	Mouse skeletal myoblasts (C2C12); Rat cardiomyocytes	WST-8 test SEM analysis Alexa Fluor1555 Phalloidin assay Connexin 43 expression
Poly(butylene succinate)-*blend*-silk (PBS/S) [94]	Mouse fibroblasts (L929)	SEM analysis DNA quantification
Poly(butylene succinate)-carboxymethylchitosan composites (PBS-CMC) [97]	Rat chondrocytes	Alamar Blue assay GAG assay SEM analysis
Poly(butylene succinate-*co*-butylene dilinoleate) (PBS-DLA) [99]	Mouse splenocytes (C57B/6, H-2b, Balb/c, H-2d)	3H-T assay

MTT assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *in vitro* mitochondrial metabolic activity test
ELISA assay = enzyme-linked immunoadsorbent assay, *in vitro* cells viability test
ALP assay = alkaline phosphatase activity assay, *in vitro* cells viability test
Kit-8 test = cell count kit
PCR assay = Quantitative real-time polymerase chain reaction, *in vitro* gene expression test
Alamar Blue assay = quantifying the metabolic activity, *in vitro* cells viability test
LDH assay = lactate dehydrogenase, *in vitro* cells viability test
Alexa Fluor1555 Phalloidin assay = *in vitro* immunofluorescence analysis
MEM assay = minimum essential medium, *in vitro* cells viability test
MTS assay = 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2(4-sulfofenyl)-2H-tetrazolium, *in vitro* cells viability test
Western blot assay = collagen type II protein extraction, *in vitro* cells viability test
GAG assay = dimethylmethylene blue assay for *in vitro* glycosaminoglycan quantification
3H-T assay = [3H]-thymidine incorporation assay, *in vitro* cells proliferation test

6.1.1. Fibroblasts and stem cells

Fibroblasts, i.e. the cells that synthesize extracellular matrix and collagen, are the most commonly used cells for the biocompatibility evaluations. Two different cell lines have been employed: NIH 3T3 [48] and L929. [58,59,62,73-75,77,85,94]

Yang and co-workers [48] synthesized PBS copolymers containing carbonate building blocks. Their biocompatibility was monitored by incubating NIH 3T3. Results showed comparable responses to PLLA films under the same conditions, demonstrating that high compatibility can be obtained by varying the amount of carbonate in the copolymers. [48]

L929 and Bone Marrow Stem Cells (BMSCs) viability was measured on PBS and PBS/PLA blends: similar and higher cell viabilities were found as compared respectively to PLA and PVC. [59] Lactate dehydrogenase (LDH) quantification showed a percentage of dead cells of about 5% for L929 and 6% for BMSCs, similarly to PLA (5 and 7% respectively) and lower than PVC (16 and 25% respectively). [59]

PBS and PBS copolymers containing PEG-like sequences were treated with fibronectin (Fn) to test the ability of these materials to adsorb bioactive proteins. Fn has been shown to regulate cell growth and shape, cytoskeletal organization (i.e. the process of assembly, arrangement or disassembly of cytoskeletal structures), differentiation, migration, and apoptosis (i.e. the process of programmed cell death) of almost all tissue cells. Fibronectin coating increased the cell adhesion by 5–7% with respect to L929 cells cultivated on neat polymeric films. [58]

Bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF) adsorption was tested on chloroethylphosphoryl functionalized poly(butylene succinate) (PBS-Cl) and phosphorylcholine end-capped PBS (PBS-PC). PBS surface adsorbed the highest amount of proteins, while the PBS-Cl and PBS-PC surfaces adsorbed lower amounts. These polymers were also cultured with L929 cells; cell viability was found comparable with phenylic acid, used as control. [62]

The same authors ran hemolysis (i.e. the rupturing of erythrocytes with consequent release of their cytoplasm into the surrounding fluid) test on PBS, PBS-Cl and PBS-PC leaching solutions. [62] Hemolysis testing is a common method to determine the biocompatibility of biomaterials. The leaching solutions from the three polymers were all below the safe value of 5%, meaning that they would not lead to severe hemolysis according to ISO 10993-4: 2002. The lowest values were found for neat PBS. [62]

In another work, [73] poly[(butylene terephthalate)-*co*-poly(butylene succinate)-*block*-poly(ethylene glycol)] multiblock copolymers cytotoxicity was tested towards the growth and morphology of L929 cells and Sheep Marrow Stem Cells (SMSC) cells before and after gamma irradiation sterilization. Both cell lines were able to form a cell monolayer on polymers not treated with gamma irradiation, similarly to negative control. No morphological changes were observed. On the contrary, the sterilization process of gamma irradiation had some effect on the cytotoxicity of the copolymers (growth inhibition of about 12-13%), possibly due to the presence of terephthalic acid, succinic acid and hydroquinone derivatives, produced during the irradiation process. [73]

Some authors [74,75,77] conducted cytotoxicity studies on fluids extracts from PBS-based scaffolds, by using L929 cells. Latex rubber was used as positive control, as it has a strong cytotoxic effect leading to extensive cell death. [74]

Scaffolds from PBS/C blends showed good viability of L929 cells regardless the blend composition. Extracts of scaffolds containing HA, displayed viability of about 80% with respect to negative control. Significant result variability was however observed when replicate experiments were run. This behavior has been explained by the authors as due to the presence of some salt particles used during the scaffold preparation (salt leaching), which could not be removed, especially in the case of small pores (63 -125 μm). [74] Direct contact assays were also carried out on PBS/C blends using a mouse bone marrow–derived mesenchymal progenitor cell line (BMC9). Cells were allowed to grow under chondrogenic differentiating conditions. Results evidenced that BMC9 cells not only adhered and grew on the scaffold surface, but they showed a transition from an initial fibroblast-like morphology to a rounder shape, typical of articular chondrocytes cultivated in 2D. [75] Further evidence of this differentiation was given by the collagen II expression, which also suggests the production of cartilage-like extracellular matrix. [75]

Cell monolayer was already observed after one week of cell seeding, but no pore occlusion was present, indicating the adequacy of the scaffold pore size (250–500 μm). [77] Alkaline phosphatase (ALP) increased in the first two weeks, probably because of early osteogenic differentiation, and then decreased because of the starting of the mineralization process. [77]

Warp knitted PBS scaffolds in the native form or after NaOH, UV and plasma treatment were tested by means of L929 cells and compared to silk fibres. [94] When L929 cells were seeded, many differences were highlighted already after the first hours of incubation, meaning that the substrate surface played a significant role. [94] Round morphology was observed for the cells deposited on the untreated surface as well as on the NaOH and UV/O_3 treated ones, while those seeded on the plasma/VSA treated fibres showed the typical spindle-like fibroblast morphology. This is probably due to the presence of sulfonated moieties that can better mimic the natural extracellular

environment and modulate the cell attachment. [94] At the end of the experiment all the scaffolds supported cell adhesion and proliferation. [94]

Finally, L929 cells were as well used for indirect cytotoxicity determination on chain-extended PBS films and electrospun scaffolds; results were comparable to the negative control (tissue-culture polystyrene plate, TCPS). [85]

In vitro biocompatibility of scaffolds from PBS and PBS blends with poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) was studied by means of mesenchymal stem cells (MSCs) isolated from the bone marrow of rabbits. [81] All blends were able to stimulate less cell adhesion than the control.

6.1.2. Osteoblasts

Osteoblasts, i.e. cells responsible for bone synthesis, isolated from the calvaria of neonatal Sprague–Dawley rats were used to assess biocompatibility of poly(butylene succinate). [50] Alkaline phosphatase (ALP) activity was used as an early marker for osteoblast differentiation. ALP activity of the osteoblasts on PBS substrates significantly increased throughout the cultivation period to values comparable to those observed for TCPS. [50] Similar experiments were conducted on PBS surfaces treated by O_2 or N_2 plasma immersion ion implantation (PIII). [52] Results displayed an improvement of cell viability and ALP activity for plasma treated PBS with respect to the neat polymer. [52] PIII treatment produced also different effects with respect to the osteogenic gene expression. Lastly, PBS surfaces modified with N_2 plasma exhibited antibacterial properties as due to the presence of amino and imino groups. [52]

Cytotoxicity of scaffolds from chain extended PBS was evaluated by means of mouse calvaria-derived, pre-osteoblastic cells (MC3T3-E1). [76] Indirect cytotoxicity tests conducted on scaffolds extraction media and LDH assay showed good biocompatibility to the bone cells. [76] Non-toxicity was also confirmed through direct cytotoxicity studies. MC3T3-E1 cells adhered well on the scaffolds surface, and cytoplasmic expansion of the cells over the surfaces and inside the pores was also observed. [76]

6.1.3. Chondrocytes

Chondrocytes, i.e. cells found in healthy cartilage, were used to assess biocompatibility of carboxymethylchitosan (CMC) scaffolds containing surface hydrolysed poly(butylene succinate) (HPBS) microspheres [97] and of HPBS scaffolds. [88] In the first case, it was found that the number of cells grown on the scaffolds was not influenced by the scaffold composition after 7 days of incubation. For longer incubation times the number of cells decreased. This has been explained on the basis of CMC degradation, which caused the cell detachment from the substrate. [97] For scaffolds containing bigger microspheres and a higher amount of HPBS particles, this effect was less influential since a greater number of proliferated cells was observed. Interestingly, the cells spread on the surface of the microsphere presented a fibroblast-like morphology, while those in the scaffold pores exhibited a more spherical and aggregated structure, typical of chondrocytes. This phenomenon was attributed to the higher surface hydrophilicity of CMC with respect to HPBS. [97] Glycosaminoglycan (GAG) secretion was also monitored The GAG amount increased as a function of incubation time, particularly when chondrocytes were cultured on scaffolds with a higher CMC content and smaller microspheres. [97]

Biocompatibility of HPBS scaffolds was monitored not only under static conditions, but also under dynamic ones. This is because in static culture oxygen and nutrient gradients are formed, which give rise to a decrease of cell proliferation from the surface to the inner part of the scaffold. [88] Results did not display significant differences between the number of chondrocytes. Cartilage-specific gene expression monitored by PCR analysis demonstrated higher expression of collagen II with respect to TCPS. This is because the 3D scaffold better simulate the chondrocyte function. Dynamic culture better induced cartilage-specific gene expression. [88] Finally, much higher amounts of extracellular matrix were secreted by chondrocytes (GAG secretion) under dynamic cultures with respect to static ones, being attributed to better diffusion of oxygen and nutrients through the scaffold pores. [88]

Also PBS/C scaffolds biocompatibility was tested by means of bovine articular chondrocytes cultured under orbital rotation. Results were compared to those obtained on PGA scaffolds under the same conditions. [86] After the cultivation period, the chondrocytes completely penetrated the scaffolds and homogeneously dispersed within the constructs. Similar cell distribution was found in PGA scaffolds, even if some necrotic regions were presents, due to accumulation of acidic products from polymer degradation or to dense cell population combined with scarce nutrient exchange. [86] A constant increase of the proteoglycan content during cell cultivation was observed for both PBS/C and PGA incubated scaffolds, indication of the formation of new cartilage-like tissue. Proteoglycan is indeed a very important extracellular matrix (ECM) molecule, since it is responsible of water binding to cartilage and accounts for cartilage compressive stiffness and elasticity. [86] Expression of collagen I and collagen II was verified as further confirmation that a cartilage tissue was formed. Cells seeded on C/PBS scaffolds displayed a morphology better resembling that of native cartilage with respect to cells seeded on PGA ones. [86]

Pore structure and size is a crucial factor in tissue regeneration, as the cell attachment and proliferation is dependent on optimal pore size and geometry. [78] In addition, the pore size affects the mechanical behaviour of the scaffold, thus influencing the stability of the construct, particularly important in load-bearing areas. [78] In this framework, Alves da Silva et al. studied the biocompatibility of PBS/C scaffolds obtained by compression moulding followed by salt leaching by using bovine articular chondrocytes (BACs). [78] The experiments have been carried out under dynamic (spinner flasks) and static culture conditions.

As a matter of fact, dynamic culture conditions may support higher seeding densities and therefore facilitate cell growth, proliferation and production of extracellular matrix. Moreover, the dynamic culture may lead to more stable constructs and therefore enhance its integration within the tissue at the site of implantation. The results obtained by hematoxylin and eosin (H&E) staining showed more cells covering the surface of the scaffolds with bigger pore size, both for dynamic as well as for static cultures. Consequently, more proteoglycans were observed (Figure 7).

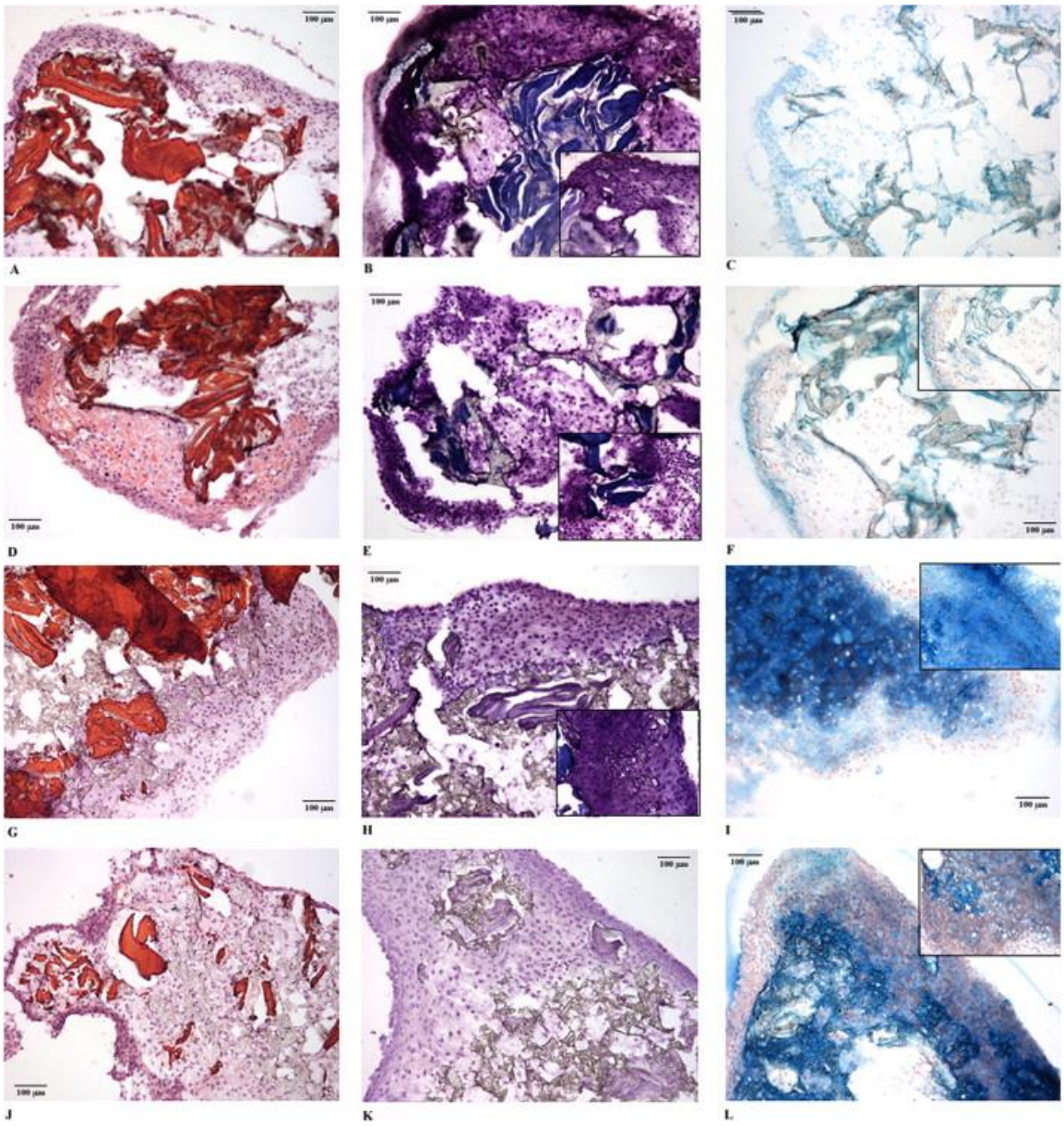


Figure 7. Production of ECM in PBS/C scaffolds at 4 weeks of culture: (A–F) results for static (A–C) and dynamic (D–F) cultures in 60% porosity scaffolds; (G–L) results for static (G–I) and dynamic (J–L) cultures in 80% porosity scaffolds. Cells were able to attach to scaffolds, as shown by H&E staining for (A and D) 60% porosity and (G and J) 80% porosity scaffolds. Cells produced proteoglycans, detected by toluidine blue staining, either in (B and E) 60% porosity or in (H and K) 80% porosity scaffolds. Sulphated proteoglycans were detected by alcian blue staining, again for both (C and F) 60% porosity and (I and L) 80% porosity scaffolds. Two magnifications (10x and 20x) were used in each staining for microscopic observation. Scale bar = 100 μm. Reproduced with permission of Elsevier from ref. [78]

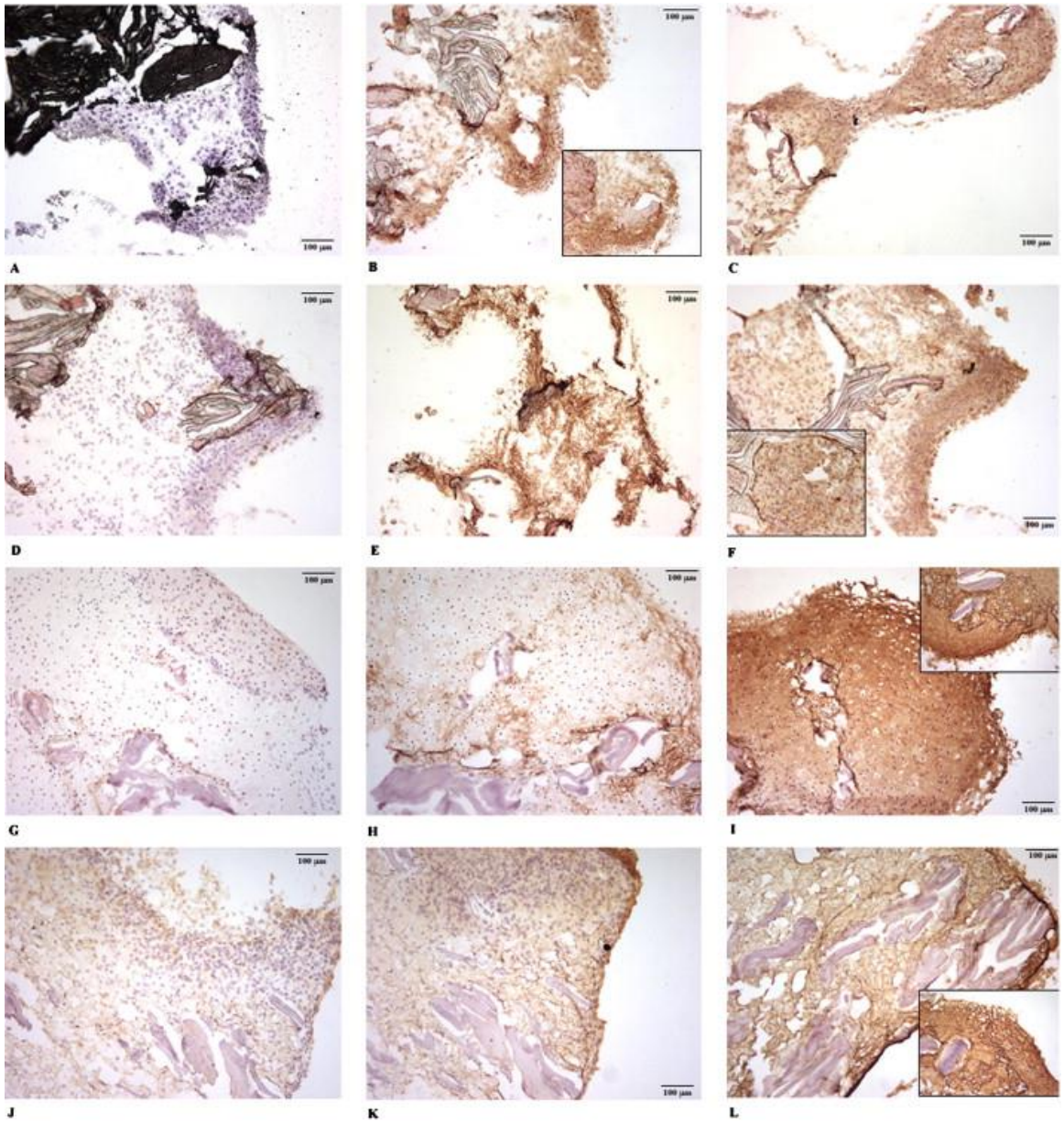


Figure 8. Immunolocalization of collagens in PBS/C scaffolds at 4 weeks of culture: (A–F) results for (A–C) static and (D–F) dynamic cultures in 60% porosity scaffolds; (G–L) results for (G–I) static and (J–L) dynamic cultures in 80% porosity scaffolds. Controls (A, D, G, and J) were performed with normal goat serum. Collagen type I (B–K) and collagen type II (C–L) were detected. Two magnifications (10x and 20x) were used in each staining for microscopic observation. Scale bar = 100 μm. Reproduced with permission of Elsevier from ref. [78]

For scaffolds with bigger pores, collagen II was synthesized in all samples, whereas less collagen type I was present (Figure 8G-L). Cells seeded into scaffolds with smaller pore sizes produced both collagen type I and type II (Figure 8A-F).

Finally, glycosaminoglycan (GAG) production was significantly higher in the smaller pore sized scaffolds with respect to those containing bigger pores. Moreover, stirred cultures significantly enhanced the GAG production in both scaffolds as compared to static ones.

6.1.4. Cardiomyocytes

The cardiomyocytes are the muscle cells that form the cardiac muscle. Embryonic rat cardiac H9c2 cells were used to assess indirect and direct cytotoxicity of PBS-based copolymers containing ether (PBS-*b*-PDGS) [87] or thioether linkages (PBS-*b*-PTDGS). [57] Tests were performed on compression molded films and on electrospun scaffolds for PBS-*b*-PDGS polymers. Indirect cytotoxicity analyses did not evidence any difference between negative control, PBS, and newly synthesized copolymers. Cell proliferation results highlighted a slight increase with the incubation time for all the polymers under investigation both in the form of film and scaffold. [57,87]

Immunohistochemistry studies were conducted to understand whether the scaffolds could induce a modification of the phenotype of H9c2 cells. Results demonstrated that the cells were able to maintain the markers of the cardiac phenotype. [87]

In another study, the cytocompatibility of different blends of poly(butylene succinate-*co*-butylene dilinoleate) (PBS-DLA) copolymers with poly(glycerol sebacate) was monitored with a mouse skeletal myoblast cell line (C2C12). [66] No significant differences in cell adhesion were displayed by the different blends after 3 h of incubation. On the contrary, with the increasing of culturing time, higher cell proliferation on stiffer substrates was observed. As a matter of fact, cells spread all over the substrate and appeared more stretched on stiffer polymer matrices than on softer ones. However, cells exhibited the typical myoblast-like morphology and a correct cytoskeleton organization on all substrates after 72 h. [66] Similar trends were observed in the case of electrospun fibres from the same blends. [92] Scaffolds have been cultured for 5 days with postnatal

cardiomyocytes from Sprague Dawley rats. Samples containing higher amounts of PGS showed better mitochondrial viability. However, it was observed that the cardiomyocytes were able to behave on these substrates similarly than on fibronectin (their natural substrate). [92] Opposite trends were observed for C2C12 cells, where the blend with higher PGS content displayed lower viability. [92] This behavior has been explained on the basis of the different mechanical properties of the substrates, which can diversely affect the attachment and proliferation of the two cell lines. [92]

6.1.5. Splenocytes

With splenocyte is intended any of the different white blood cell types situated in the spleen or purified from splenic tissue. *In vitro* cell proliferation of mice C57B/6 and Balb/c splenocytes was used to assess cytotoxicity of poly(butylene succinate-*co*-butylene dilinoleate) nanoparticles. [98] Interestingly, with the increasing of the nanoparticle concentration a significant increase of the number of viable cells was observed for both C57B/6 and Balb/c type. [98]

Various cell lines have been tested to evaluate the biocompatibility of PBS and PBS-based copolymers. Many studies have focused on scaffolds, being the 3D constructs commonly used for tissue engineering. The results highlighted a good biocompatibility of this polyester with many different cells. Indeed, the biocompatibility of PBS has been often comparable to that of PLA, whose use in biomedicine is well established.

Further improvements of PBS mechanical properties can be achieved through copolymerization or realization of composites. These strategies permit a better coupling of the substrate properties with the cells requirements (i.e. the site of implantation).

The use of animal cells to verify the biocompatibility of PBS, although very useful, represents only the first step toward the evaluation of the applicability of this polymer in biomedicine. Tests ran with human cells would give a better indication in this respect.

6.2. In vitro cytotoxicity tests with human cells

Human cells have been employed to assess cytotoxicity of PBS and PBS-based systems, stem cells being the most extensively used. In Table 5 are contained the cell lines employed for the studies together with the techniques adopted for the biocompatibility determination.

Table 5. *In vitro* biocompatibility tests with human cells.

Polymeric material	Cell line tested	Adopted methods of analysis
Poly(butylene succinate-*co*-dimethyl 5-sulfoisophtalate sodium salt)s (PBS-*co*-BSi) [49]	Human dermal fibroblasts	SEM analysis Confocal microscopy MTT assay FDA/EB assay
Poly(butylene succinate) (PBS) [46]	Human fetal osteoblast (hFOB 1.19)	SEM analysis
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [53]	Human osteosarcoma (SaOs-2)	SEM analysis DNA quantification ALP assay ELISA assay
Poly(butylene succinate) (PBS) [55]	Human adipose stem cells (hASCs)	Alamar Blue assay ELISA assay SEM analysis
Poly(butylene succinate)-silica nanocomposites (PBS/Si); **poly(butylene succinate)-strontium hydroxyapatite nanocomposites (PBS/SrHA)** [64]	Human osteosarcoma (MG-63)	Calcein Am assay Alamar Blue assay SEM analysis
Poly(butylene succinate)-β-tricalcium phosphate (PBS/TCP); **poly(butylene succinate)-hydroxyapatite (PBS/HA)**[65]	Human mesenchymal stem cells (hMSCs)	MTT assay ALP assay Alizarin red S assay
Poly(butylene succinate)-β-tricalcium phosphate (PBS/TCP); **poly(butylene succinate)-hydroxyapatite (PBS/HA)**[67]	Human mesenchymal stem cells (hMSCs)	Nikon Eclipse TS100 light microscope analysis MTT assay ALP assay Alizarin red S assay
Poly(butylene succinate)-fluorapatite (PBS/FA)[70]	Human mesenchymal stem cells (hMSCs)	MTT assay ALP assay SEM analysis
Poly(butylene succinate)-β-tricalcium phosphate (PBS/TCP) [71]	Human mesenchymal stem cells (hMSCs)	MTT assay Fluorescence microscopy SEM analysis
Poly(butylene succinate)-*blend*-chestnut shell fiber (PBS/CSF); (Poly(butylene succinate)-*graft*-maleic anhydride)-*blend*-chestnut shell fiber (PBS-*g*-MA/CSF) [72]	Normal human foreskin fibroblasts (HS-68)	MTT assay Collagen quantification Annexin V-FITC assay
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [79]	Human bone marrow mesenchymal stem cells (hBMSCs)	SEM analysis MTS assay ALP assay
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [80]	Human bone marrow mesenchymal stem cells (hBMSCs)	SEM analysis MTS assay ALP assay PCR test
Poly(butylene succinate)-*coated*-collagen;	Human chondrocytes	Alamar Blue assay

poly(butylene succinate)-*coated*-hydroxyapatite [84]		RT-PCR test
Poly(butylene succinate) (PBS) [85]	Human osteosarcoma (SaOs-2)	MTT assay SEM analysis ALP assay
Poly(butylene succinate)-hydroxyapatite (PBS/HA) [90]	Human chondrocytes	Alamar Blue assay PCR test
Poly(butylene succinate)-*blend*-silk (PBS/S) [94]	Human adipose stem cells (hASCs)	SEM analysis DNA quantification ALP assay Ca^{2+} quantification
Poly(butylene succinate-*co*-butylene itaconate) (PBS-co-BI) [101]	Human breast cancer (MCF-7)	MTT assay

MTT assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *in vitro* mitochondrial metabolic activity test
FDA/EB assay = fluorescein diacetate/ethidium bromide, *in vitro* cells viability test
ELISA assay = enzyme-linked immunoadsorbent assay, *in vitro* cells viability test
ALP assay = alkaline phosphatase activity assay, *in vitro* cells viability test
PCR test= real-time polymerase chain reaction, *in vitro* gene expression test
Alamar Blue assay = quantifying the metabolic activity, *in vitro* cells viability test
Calcein AM assay = *in vitro* cells distribution growth
Alizarin red S assay = *in vitro* calcium containing deposits and mineralized matrix analysis
Nikon Eclipse TS100 light microscope = *in vitro* morphology analysis
MTS assay = 3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2(4-sulfofenyl)-2H-tetrazolium, *in vitro* cells viability test

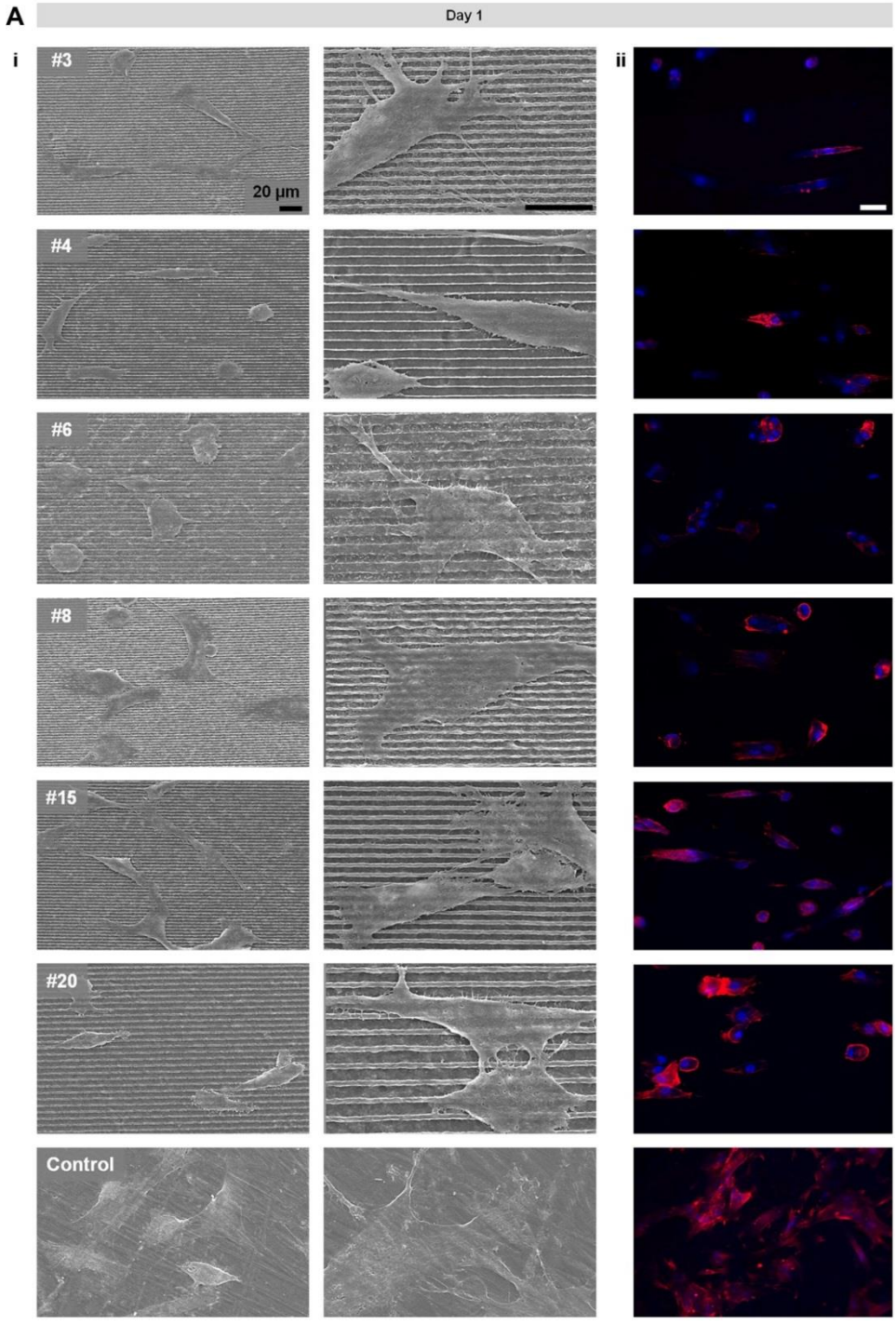
6.2.1. Stem cells

Coutinho *et al*., [53] reported a simple method to control the alignment of human adipose stem cells (hASCs) by micropatterning PBS surface. Micropatterns have been obtained by using a patterned polydimethylsiloxane mould where a PBS solution is casted and covered with a PBS disc. As the solvent evaporates, micropatterns are transferred to the PBS disc used as substrate. [55]

Natural tissues and ECM are composed of micro- and nanoscaled elements, which arrange in specific architectures (for example, fibroblasts and cardiomyocytes in native myocardial tissue align themselves in parallel arrays in order to guarantee the electrical and mechanical properties of the heart). Their replication, while developing an engineered tissue, is of crucial importance to modulate the tissue function and to determine the success of the implant. [55]

Indeed, immediately after implantation, the substrate gets covered by a layer of proteins. Besides the material chemistry, the adsorptive behaviour of these proteins is linked to the surface properties of the substrate (e.g. its micro- and nanostructure). [55]

The hASCs viability was similar for patterned and non-patterned PBS, thus the cell growth was not influenced by the microengineered surfaces. Lower DNA content was observed for patterns, suggesting that hASCs increased their DNA content faster on the non-patterned surfaces. [55]



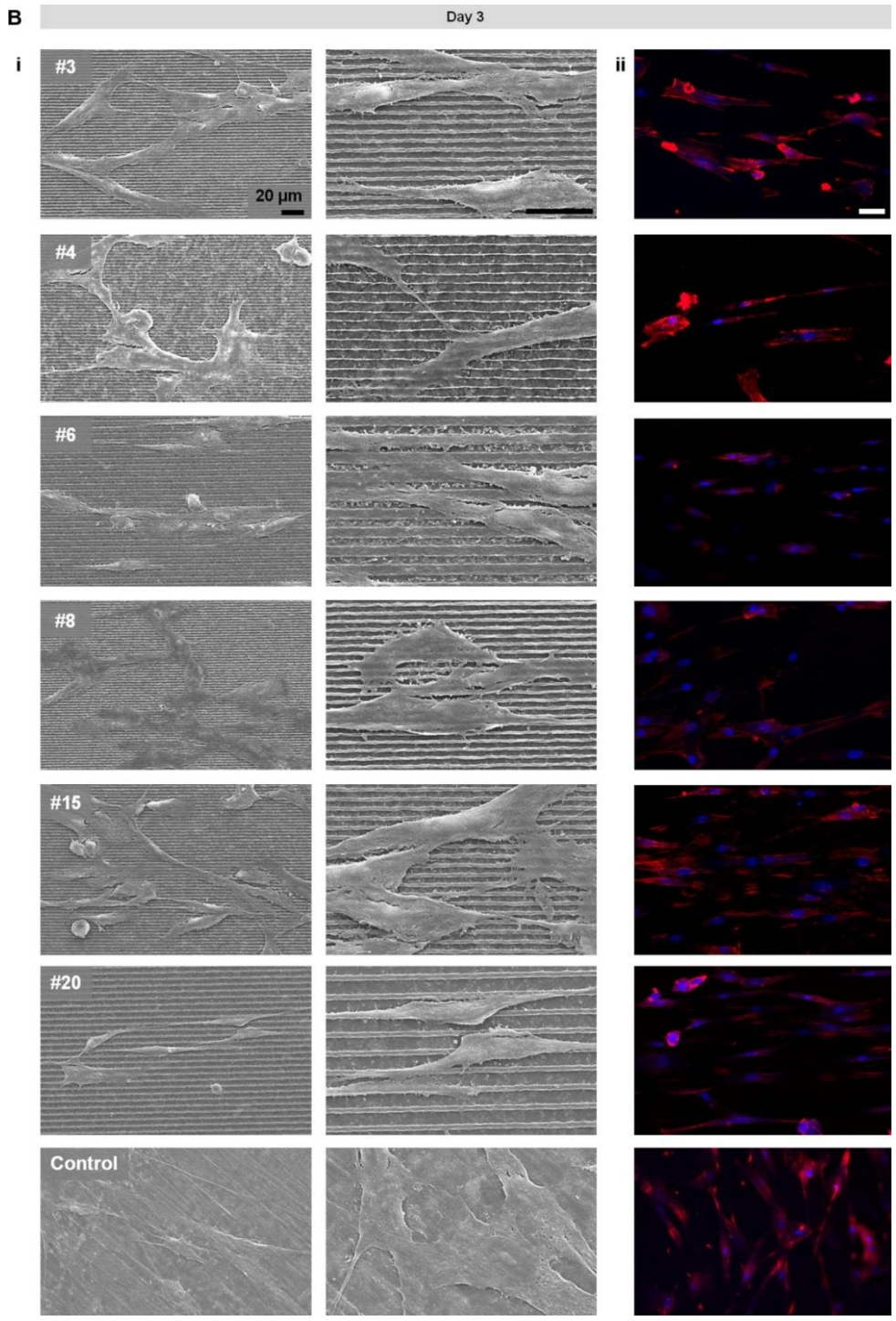


Figure 9. SEM at two magnifications (i) and (ii) of immunostaining of hASCs cultured onto micropatterned PBS surfaces after (A) 1 day and (B) 3 days of culture: cell cytoskeleton stained red with phalloidin and cell nucleus counterstained blue with DAPI.

Reproduced with permission of Elsevier from ref. [55]

However, on the patterned surfaces, cells must rearrange to respond to the substrate, and this could explain the slower cell division. Already after 1 day of culture, hASCs started to orient along the direction of micropatterns, while on non-patterned surfaces, cells formed a uniform layer and were randomly oriented (Figure 9). Results from different micropatterned surfaces also highlighted that hASCs better align along the patterns in those surfaces with groove/ridge width ratio bigger than 1. [55]

Warp knitted PBS scaffolds in the native form or after NaOH, UV and plasma treatment were tested by means of hASCs and compared to silk fibres. [94] hASCs were able to adhere and proliferate on both the PBS and silk fibres. [94]

3-D PBS/C scaffolds obtained by salt leaching have been seeded with bone marrow mesenchymal stem cells (hBMSCs). [79,80]

Practically no cell adhesion was found on neat PBS substrate, while the presence of chitosan promoted the cell adhesion and proliferation of hBMSCs. [80] Higher cell viability was found for higher chitosan content. [80]

After one week, significant cell adhesion was observed, which increased over incubation till a formation of a dense multi-layered cell structure and the production of mineralized extracellular matrix (Ca–P deposits were observed), confirming osteogenic differentiation. [79] Interestingly, alkaline phosphatase activity (ALP) of hBMSCs, did not follow the usual trend. As a matter of fact, ALP activity continued to increase till the end of the experiment, while typically a maximum peak is reached for shorter incubation times. [79]

Real-time quantitative PCR was performed to evaluate the expression of osteogenic related genes. All genes were expressed in the PBS/C blends, evidencing a successful osteogenic differentiation. [80]

With the aim to be used for bone regeneration purposes, cytocompatibility of different PBS-based composites was tested using human mesenchymal stem cells from bone marrow tissue. [65,67] In particular, the two studies analysed films of PBS, PBS/HA and PBS/TCP composites [67] and

surface modified films obtained from the same materials. [65] Better results were obtained on hydrolysed surfaces when compared to non-hydrolysed ones. In particular, higher viability was found on PBS/TCP composites with respect to neat polymer or PBS/HA composite. [65] ALP activity highlighted the same trend. Alzarin Red S staining was also used to monitor the presence of calcium-containing deposits, as indication of osteogenic differentiation. Once again, hBMSCs produced the highest amount of Alzarin Red S positive deposits when cultured on hydrolysed PBS/TCP films. [65] The authors explained these results on the basis of the improved surface roughness and therefore the increased hydrophilicity of the hydrolysed samples, since increased wettability can improve osteogenesis. [65]

Similar findings have been reported in the case of PBS/TCP composites treated with NaOH. [71] This hydrolysis technique increased the surface roughness and surface hydrophilicity. Biocompatibility studies, conducted using hMSCs, showed that the alkaline hydrolysis significantly enhanced cell adhesion and proliferation. [71]

Even if the role of surface roughness is not fully explained, it seems that initial cell attachment occurs through filopodia that directly adhere to the rough surface irregularities, while smooth surfaces suppress the filopodia formation, thus resulting in flattened cells with decreased adhesion capacity. [125]

Niu and co-workers developed nanofluorapatite–poly(butylene succinate) (n-FA–PBS) bioactive composites and tested their antibacterial properties as well as cell attachment and proliferation by means of human mesenchymal stem cells (hMSCs). [70] n-FA–PBS composite showed promising antibacterial properties (*E. coli* adherence was tested) as compared to tissue-culture polystyrene plates and neat PBS, probably due to the presence of n-FA or the release of F ions. Increased cell proliferation and ALP activity were found with respect to neat PBS [70]

6.2.2. Chondrocytes

Human articular chondrocytes were used to analyze biocompatibility of PBS-based polymers and composites when cartilage repair was intended. In particular, scaffolds obtained from surface-

hydrolysed PBS/HA composites [90] of from surface-hydrolysed PBS coated with collagen or HA [84] were studied. The authors reported that surface-hydrolysed PBS scaffolds enhanced cell proliferation as compared to not hydrolysed ones, because of the higher surface hydrophilicity. [90] In addition, among the coated PBS scaffolds, best results were obtained when HA was applied.

6.2.3. Osteoblasts

Human fetal osteoblast cell line (hFOBs 1.19) was employed to assess biocompatibility of PBS films treated with H_2O or NH_3 plasma immersion ion implantation (PIII). [44] The plasma treatments enhanced osteoblast compatibility, as already observed in another report from the same authors, above described. [52] In particular, the samples treated with H_2O exhibited better biocompatibility compared to those treated with NH_3. Similarly, H_2O PIII treated samples induced higher bioactivity than the NH_3 PIII ones. Since the surface roughness and hydrophilicity of the two samples was comparable and higher than the non-treated ones, the observed behavior has been ascribed to the different functional groups which formed on the polymer surface consequently to the treatment, i.e. OH and NH_2 respectively. [44]

6.2.4. Fibroblasts

In another study, [49] random PBS-based copolymers containing 5-sulfoisophthalate sodium salt comonomeric units (PBS-co-BSi) were prepared and their cytotoxicity was tested by means of human dermal fibroblasts and compared to that of PLGA, under the same conditions. Tests conducted on polymer extracts and on polymer films evidenced that the new PBSi copolymers induced a similar cellular metabolic activity to PLGA regardless of the ionic content. Cells cultivated on PBSi more widely spread on the polymer surface and displayed more filopodial growth as compared to those on PBS film. the reason being the higher surface hydrophilicity (WCA of about 50-55°) of the PBSi films, which can favour the adsorption of cell-adhesion proteins such as fibronectin and vitronectin. [49]

Biocompatibility of PBS/CSF composites was analysed by employing human fibroblasts cell line (HS-68). [72] Cell viability was similar for the different CSF compositions and comparable to the

control. In the case of maleic anhydride-grafted PBS, cell viability was slightly lower, probably because of a cytotoxic effect of maleic anhydride. [72] Collagen production was also evaluated, since it is an important component for cell proliferation and tissue formation and imparts mechanical strength to the tissue. Collagen content was higher in the PBS/CSF composites with respect to the neat PBS, while in the PBS-g-MA collagen was produced to a lower extent and did not increase significantly with the incubation time. [72]

6.2.5. Carcinoma and osteosarcoma cells

Gowsika and Nanthini [101] tested the anti-cancer activity of poly(butylene succinate-*co*-butylene itaconate) (PBS-*co*-BI) against MCF-7 cell line from human breast cancer with promising results.

Osteosarcoma cells (i.e. cells from a bone cancerous tumour) were used to analyse the biocompatibility of PBS/C blends treated with mechanical and plasma etching, [53] of PBS nanocomposites containing silica-nanotubes and strontium hydroxyapatite nanorods [64] and of chain-extended PBS scaffolds. [85]

The absorption of two different proteins, human plasma fibronectin (HFN) and human serum albumin (HSA) was tested. [53] The highest absorption level was found for both proteins in the mechanically polished samples because of the highest chitosan content on the surface and the higher surface roughness. However, chitosan presence played an important role as well, since a significant difference was found between HSA adsorption on non-treated PBS/C blend and neat PBS. [53]

Osteosarcoma cells (SaOs-2) were able to attach and proliferate on all the substrates tested, however higher spreading degree and the presence of some extended lamellopodia were found for cells seeded over the PBS/C blend surface. The effect was more evident for etched surfaces, where cells had a more flattened morphology and higher number of adhesion points to the surface. [53] ALP activity was higher for PBS/C blends with respect to PBS, even if highest cell viability was obtained in the case of pristine PBS, probably because of the chitosan presence. Indeed, this last caused a higher protein absorption that could enhance the ALP expression. [53]

In the case of chain extended PBS, extraction media from both scaffolds and films did not present any toxicity to SaOs-2 cells. [85] Direct cytotoxicity experiments highlighted an increase in cell attachment and proliferation with the increasing of culturing time. Highest cell viability was recorded for cells cultured on electrospun fibres, probably because of the higher surface area. [85]
PBS nanocomposites containing silica-nanotubes and strontium hydroxyapatite nanorods have cultured with osteosarcoma MG-63 cells. [64] MG-63 cells were able to attach and proliferate on neat PBS and on SiO_2 containing composites, even to a higher extent than TCPS reference, while no living cells were detected on $Sr_5(PO_4)_3OH$ nanorods containing composites, indicating a cytotoxic effect of these last ones. [64] Incorporation of Si resulted in higher cell viability, since it is known that Si can stimulate MG-63 cell proliferation. [64] On the other hand, the authors explained the unexpected cytotoxic effect of $Sr_5(PO_4)_3OH$ nanorods on the basis of the nanoscaled topography dimension of the Sr inclusion, because strontium is an important trace element in human bone. [64]

PBS-based systems displayed good biocompatibility also with respect to different kinds of human cells, as already observed for animal ones. In addition, human cells provide a more specific indication of the possibility to employ these substrates as substitutes of human tissues. Again, the mechanical properties of PBS have been specifically tuned by the realization of composites and copolymers to adapt its performances, in particular with respect to the mechanical behavior, to the replaced tissue. Various treatments enabled the modification of the PBS surface morphology, by increasing roughness and hydrophilicity.
Last, but not least, most studies reported the osteogenic differentiation of mesenchymal stem cells, indicating that PBS substrates are very promising for bone tissue engineering.

6.3. In vivo studies

The *in viv*o experiments (Table 6) have been conducted through subcutaneous implantation in rats, up to 36 weeks, [59,73] in mice calvaria for 8 weeks, [79] in cranial defect and iliac submuscular region of Wistar rats for 12 weeks, [80,93] and in femoral bone of rabbits for 4 and 8 weeks. [70]

Table 6. Biocompatibility evaluations *in vivo*.

Polymeric material	Place of implantation
Poly(butylene succinate)-*blend*-poly(lactic acid) (PBS/PLA) [59]	subcutaneously in healthy male rats
Poly(butylene succinate)-*blend*-fluorapatite (PBS/FA) [70]	femoral bone of white rabbits
Poly(butylene terephthalate)-*co*-poly(butylene succinate)-*block*-poly(ethylene glycol) (P(BSBT)-*b*-PEG) [73]	subcutaneously in the back of male Wistar rats
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [79]	cranial defect in nude mice
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [80]	cranial defect in Wistar rats
Poly(butylene succinate)-*blend*-chitosan (PBS/C) [93]	subcutaneously in male Wistar Han rats
Poly(butylene succinate-*co*-butylene dilinoleate) (PBS/DLA) (core) and N-(2hydroxypropyl) methacrylamide-based polymers (shell) [100]	nanoparticles intravenously injected in B/6 mice

PBS/PLA blend *in vivo* tissue compatibility was tested in male rats. [59] After 1 week the typical characteristics of acute inflammatory response after surgical procedures were observed on the harvested tissue, which appeared as edematous. Thin layer of elongated fibroblasts enveloped the implant. After one month no eosynophiles and few macrophages were found. The implant was encapsulated with connective tissue, few new blood vessels and immature fibroblasts. The inflammatory response was therefore alleviated. Similar results occurred for PBS, while in the case of PLA the inflammation was still occurring to some extent. After three and six months the situation well resembled that of month 1, with a thickening of the fibrous capsule. In the case of PLA the situation reached a similarity to PBS/PLA and neat PBS. [59]

In vivo biocompatibility of poly[(butylene terephthalate)-*co*-poly(butylene succinate)-block-poly(ethylene glycol)] copolymers (PEG molecular weight 1000 g/mol) and different PBS contents (20–30 mol%) was analyzed by subcutaneous implantation in rats. [73] After 1 week, the copolymer underwent fragmentation and connective tissue grew between the fragments. Copolymer

was surrounded by fibrous tissue containing macrophages. At the end of week 4, the fragmentation increased and more tissue grew within the fragments. On the other hand, inflammatory response decreased, indicating good biocompatibility of the sample. [73]

Athymic nude mice were used to examine the healing of cranial critical size bone defects in response to transplants of constructs based on PBS/C scaffolds in the defects. [79] The constructs consisted of pure scaffolds and scaffolds implanted with human bone marrow mesenchymal stem cells (hBMSCs). At the end of the experiment (8 weeks of implantation), the constructs promoted bone regeneration of the defects; this effect was enhanced in the case of scaffolds cultured with hBMSCs. [79]

In another study, [80] PBS and PBS/C scaffolds of different composition have been implanted in hard tissue (cranial defect) and iliac submuscular regions of Wistar rats. After one month of implantation, host cells diffused throughout the porous structure even if a mild inflammatory response was observed (more pronounced in the submuscular region). [80] PBS/C scaffolds evidenced a higher production of collagen with respect to PBS alone. [80]

After 7 days of subcutaneous implantation, PBS/C scaffolds kept their porous morphology; inflammatory infiltrates were mainly constituted by neutrophils, responsible for the phagocytosis of microorganisms and foreign materials (not possible in the case of implants due to the size difference). [93] After three weeks, the presence of neutrophils was negligible, while blood vessels were formed within the scaffolds. Simultaneously, collagen started to be deposited by fibroblasts. [93] At week 6, the acute inflammatory response evolved into a chronic one (foreign body reaction). [93] At the end of the experiment (week 12), the scaffolds' structure and integrity was still visible. However, cells started to colonize the scaffold interior and an increase in vascularization and the presence of adipocytes were highlighted. [93]

Niu *et al.*, [70] conducted in vivo trials on PBS/ nano-fluorapatite (nFA) composites by inserting samples into the femoral bones of rabbits for 4 and 8 weeks. After 4 weeks of implantation only a small amount of new tissue was formed on the composite surface, while after 8 weeks a higher

amount of new tissue and no gap between the composite and the original bone were observed (Figure 10). Moreover, no fibrous capsule was present, indicating good osteoconductivity of the composite. [70] This phenomenon has been ascribed to the presence of the bioactive nFA that increases the surface activity, making it capable of bonding bone tissue. [70]

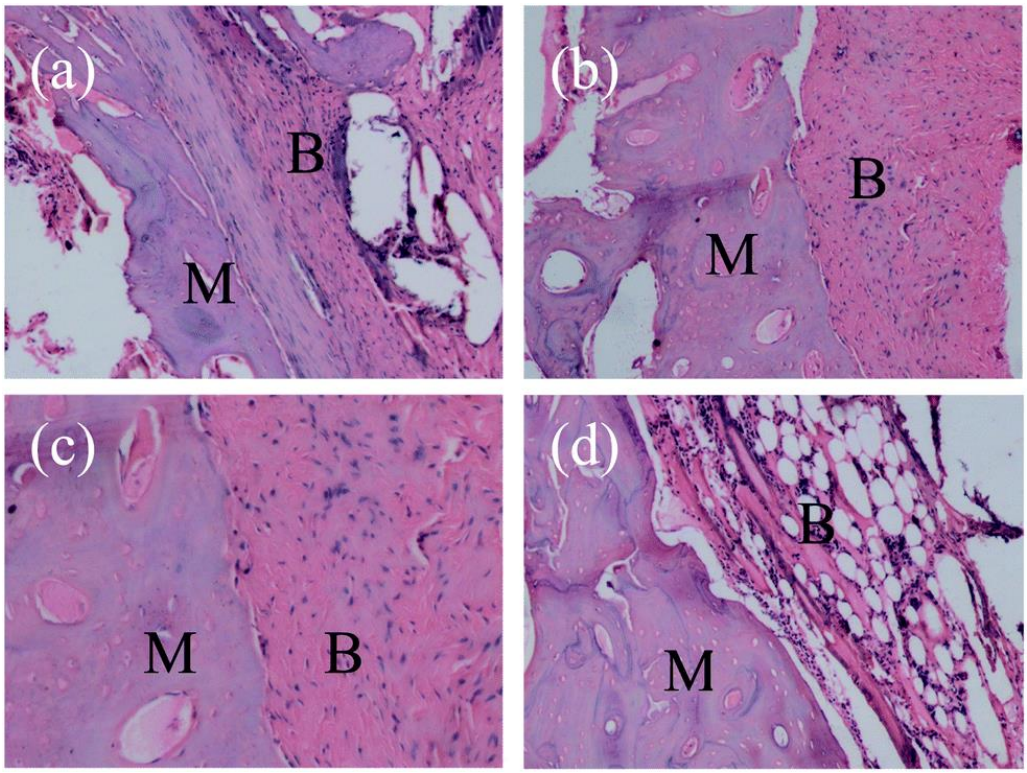


Figure 10. Histological evaluation of (a–c) the PBS/nFA composite and (d) PBS implanted into the femoral bone of rabbits for 4 (a) and 8 (b–d) weeks. a, b, d: 20x magnification; c: 40x magnification. M represents gradually degraded materials and B represents new bone tissue. Reproduced with permission of Royal Society of Chemistry from ref. [70]

Last, but not least Jager *et al*. [100] intravenously injected core shell nanoparticles composed of poly(butylene succinate-*co*-butylene dilinoleate) (core) and N-(2-hydroxypropyl) methacrylamide-based polymers (shell) in mice bearing EL-4 T cell lymphoma. The nanoparticles were loaded with docetaxel (DTXL) and doxorubicin (DOX), two anticancer drugs. This approach permitted to significantly reduce the tumor growth and to extend the survival time of the animals with respect to

the untreated mice and to the delivery of either free DTXL or free DOX at the same dose concentrations.[100]

The results obtained by the studies *in vivo* demonstrated that the PBS-based systems are promising candidates for tissue regeneration applications, especially for bone repair. The response elicited by the host to PBS is similar to that of PLA. [59]

Cell diffusion through the construct and formation of new blood vessels has been observed in all cases, thus indicating a good compatibility between the implant and the existing tissue.

Unfortunately, only few examples of *in vivo* studies regarding PBS and PBS-based systems are present so far in the literature. More research is needed to confirm and validate the possibility to employ this polymer in biomedicine.

7. Biomaterial applications

Thin films, mainly obtained through compression molding and scaffolds have been successfully realised starting from PBS and PBS-based copolymers and composites. The main techniques adopted in the scaffold production are salt leaching, [73-80] electrospinning, [81-92] or extrusion (Figure 11). [93,94]

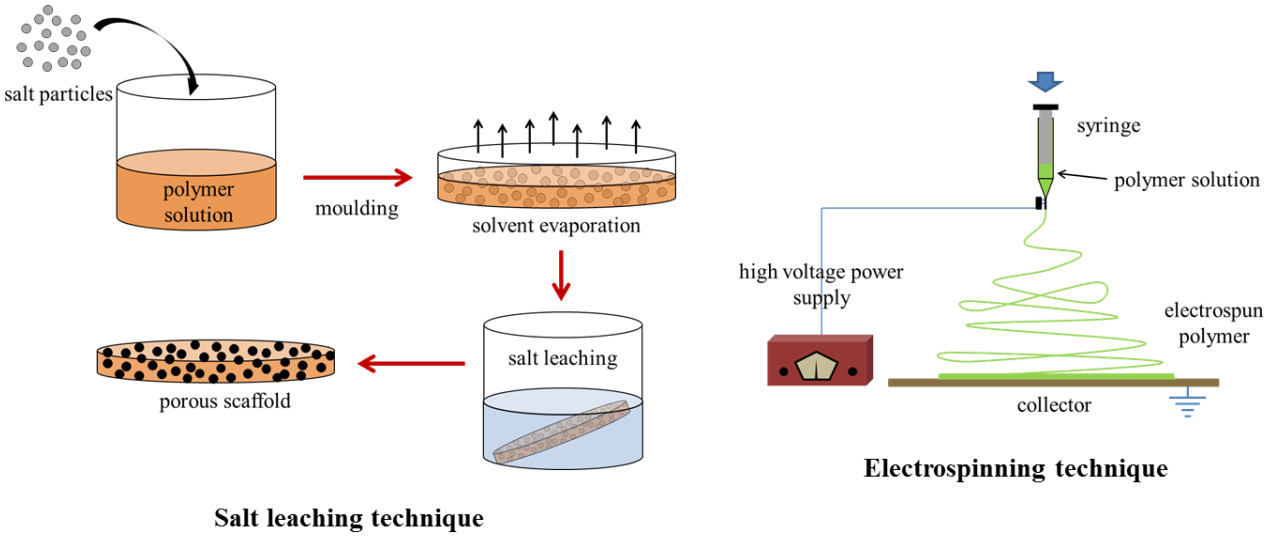


Figure 11. Common techniques employed for the preparation of polymer scaffolds.

Although envisioning a possible application, most studies were focused on the synthesis and characterization of the new developed PBS-based systems, rather than on the final use. However, few reports provided additional data in this respect.

Bone repair has been evaluated by employing PBS/C scaffolds, [75,78,86] surface hydrolysed scaffolds of PBS, [84] PBS/HA composites, [88,90] and PBS microspheres incorporated in carboxymethylchitosan scaffolds. [97]

Extensive research has been also conducted in bone tissue engineering by using PBS [76,85] and plasma treated PBS, [44,52] PBS/C scaffolds, [53,77] surface hydrolysed PBS/TCP composites, [67,71] PBS/FA composites [70] and scaffolds from PBS wollastonite/apatite composites. [82] In addition, it has been observed that PBS/C scaffolds could induce the osteogenic differentiation of stem cells. [79,80]

Finally, PBS-DLA copolymers displayed good potential for cardiac tissue engineering.[66,92]

With the aim of using PBS-based copolymers and composites in drug delivery applications, some authors investigated the release of molecules from films, [58] micro and nanoparticles, [95,96,98-100] or scaffolds [76] realised with the new materials.

Broadly speaking, the drug release through a polymer matrix is governed by two mechanisms, i.e. a diffusion-controlled release (dependent on the diffusion coefficient, matrix porosity and tortuosity) or a triggered pathway initiated by changing the environmental conditions such as pH or temperature. [99] Moreover, since biodegradable polymers are considered, their bulk erosion must be taken into consideration (Figure 12).

In vitro release of ipriflavone (a compound used to inhibit bone resorption, to maintain bone density and to prevent osteoporosis) was studied in chain extended PBS scaffolds prepared by particulate leaching. [76] Two phases could be highlighted from the release profiles: one initial burst phase (possibly due to the dissolution of drug aggregates present on the scaffold surface) and a sustained release which showed a gradual decrease of release rate up to a plateau value. No effect of the porosity and pore size of the scaffolds was detected, since high load efficiency and capacity were

observed. Release rate differences observed during the experiment among the scaffolds were explained on the basis of inter-cellular walls thickness, which can significantly influence the drug diffusion from the specimen. [76]

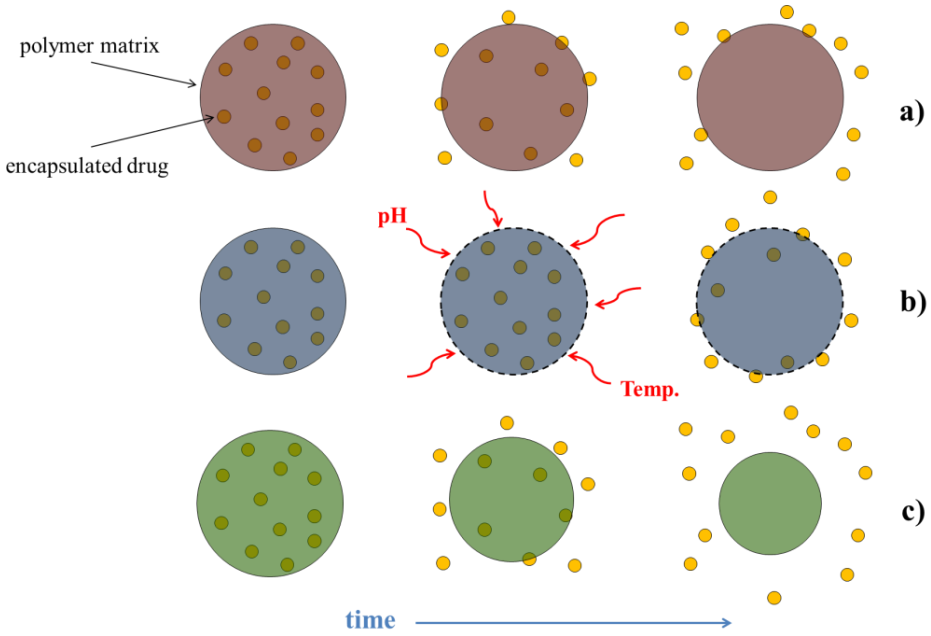


Figure 12. Drug release mechanism. a) diffusion controlled release, b) triggered release, c) erosion controlled release.

Similar results have been found by studying release kinetic of indomethacin, a non-steroidal anti-inflammatory drug used in the treatment of soft tissue problems, from poly(butylene succinate)/poly(caprolactone) (PBS/PCL) microcapsules, [95] and by evaluating the release of Camptothecin (a natural plant alkaloid which has shown a broad spectrum of antitumor activity) from poly(ω-pentadecalactone-*co*-butylene succinate) nanoparticles. [96]

Mohanraj *et al.* prepared PBS microcapsules loaded with Levodopa, an anti-Parkinson's drug. [98] They observed that smooth microspheres exhibited higher encapsulation efficiency with respect to porous ones. Moreover, the drug release was higher in simulated cerebrospinal fluid as compared with phosphate buffer, showing that composition of release medium is an important factor affecting the drug release. [98]

In another study, [58] polymeric films containing fluorescein isothiocyanate (FITC) were prepared by solvent casting. FITC release, used as model molecule, was then monitored by measuring UV absorbance. PBS and multiblock PBS-based copolymers containing ether-linkages (PBS-*b*-PTES) were investigated. All polymers displayed a diffusion driven profile, with a burst release within the first 6 hours, followed by a sustained release. The release profiles significantly differed from one polymer to another, material hydrophilicity and chain mobility playing an important role in this respect. [58] PBS, the most hydrophobic material, showed the slowest release, while the introduction of TES co-monomeric units, increased the polymer hydrophilicity and therefore the FITC release. Another important factor seems to be the crystal phase, since crystal lamellae probably act as a barrier delaying FITC diffusion during the burst phase. However, at longer incubation times, this effect was no longer observed because of a possible switching from diffusion-based to an erosion-based release mechanism as a consequence of polymer hydrolysis. [58]

Jeger *et al*., [99] analysed the release of hydrophobic model drug paclitaxel (PTX) from PBS-DLA nanoparticles; as a comparison PLA and PLGA drug-loaded nanoparticles were also considered. PTX release has been monitored by HPLC and scattering measurements. About 40% of the encapsulated drug was released during the first 12 h of incubation, while only another 10% was released from the nanoparticles after 120 h. Interestingly, the presence of the model drug modified the structure of the nanoparticles, which were able to hold a high amount of water in the drug-free conditions. On the contrary, PTX encapsulation led to a shrinking of the nanoparticles, as water was replaced with the hydrophobic drug. [99] Once again, the release profile was controlled by the diffusion of the PTX and water draining through the polymer matrix. [99] The same authors prepared self-assembly core-shell nanoparticles composed of poly(butylene succinate-*co*-butylene dilinoleate) and N-(2-hydroxypropyl) methacrylamide-based polymers loaded with docetaxel and doxorubicin for the treatment of EL-4 T cell lymphoma. [100]

Another possible application of PBS-based copolymers is as antimicrobial biomaterials. In this respect, some authors investigated the antimicrobial activity against *Staphylococcus aureus* of

PBS/PLA electrospun scaffolds loaded with potassium salt of 5-nitro-8-hydroxyquinoline (K5N8Q) or of 5-chloro-8-quinolinol (5Cl8HQ). [91] Neat PBS/PLA scaffolds did not exhibit any antibacterial effect, while in the drug containing samples well-distinguished zones of inhibition of the bacterial cells growth were detected, indicating that the drugs are capable of retain their activity when incorporated in the electrospun mats. [91]

8. Conclusions

The use of poly(butylene succinate) in biomedicine as an alternative to the well-established products is attracting considerable attention as documented by the growing number of publications on this topic (Figure 13).

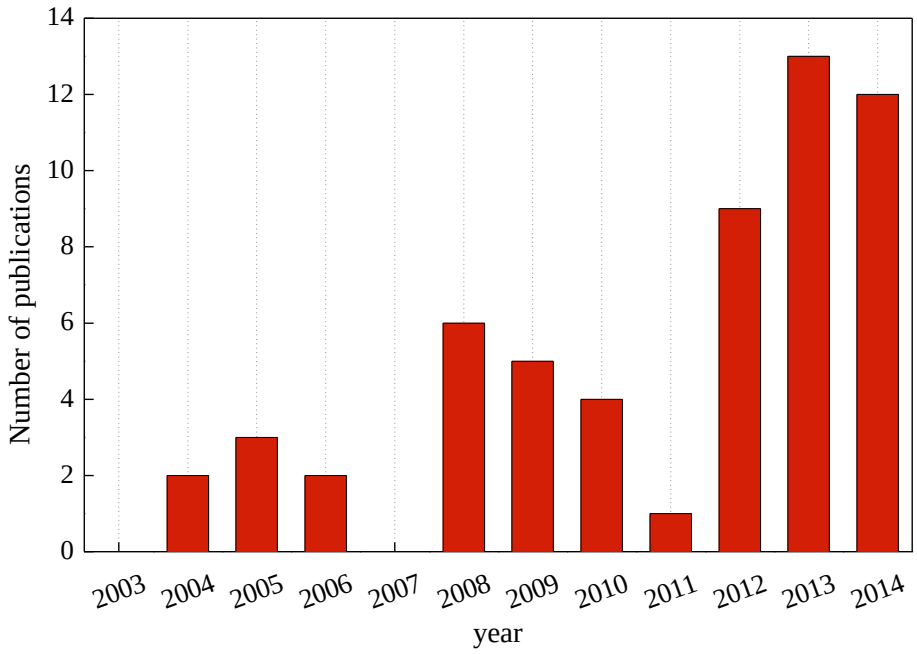


Figure 13. Number of published reports per year on PBS and PBS-based copolymers for biomedical applications.

PLA, PGA, PCL and their copolymers are already present on the market since decades as resorbable sutures. PLGA is the most popular among these polymers because of its long clinical experience, favourable degradation characteristics and possibilities for sustained drug delivery. [11]

Also PHA-based polymers have been considerably studied for the realization of 3D constructs or drug delivery systems, as they display interesting features with respect to bone, nerve, cardiovascular and cartilage tissue engineering and as drug carriers. [21] However, due to their poor mechanical properties and the high costs for their microbial production, PHA exploitation is so far mainly limited to academic research. [123]

On the other hand, PBS possesses interesting physic-mechanical properties and it can be easily synthesized by melt polycondensation at moderate costs. Additional value is given by the possibility to obtain both succinic acid and 1,4-butanediol from renewable resources, that makes PBS a fully bio-based and biodegradable polymer.

It is worth highlighting that to be employed as biomaterials, materials must fulfil strict requirements. Besides biocompatibility, the mechanical properties and the biodegradation rate must be coupled with the site of implantation. [16]

PBS itself displays a quite slow hydrolysis rate and low flexibility that could hamper its uses for certain applications. Therefore, blending and/or copolymerization have been used to tailor the PBS characteristics in order to satisfy many different requirements. Surface wettability, degree of crystallinity, mechanical properties and biodegradation rate have been tuned by acting on the molecular architecture and/or on the nature of comonomeric unit. The addition of organic or inorganic fillers, mainly chitosan or hydroxyapatite, played also a significant role in this respect.

Different techniques, such as salt leaching or electrospinning have been employed to realise scaffolds for tissue engineering. On the other hand, micro- and nanoparticles for controlled drug release applications have been successfully prepared.

Biocompatibility evaluations, conducted under different experimental conditions and with the use of various cell lines, highlighted the good *in vitro* and *in vivo* biocompatibility of the PBS-based copolymers and composites.

With respect to the final application, the studies demonstrated the potentiality of PBS to be employed in different areas of tissue engineering ranging from myocardial, thanks to the introduction of soft co-units, to the bone tissue replacement, through the realisation of composites.

On the contrary, only few reports dealt with the controlled release of model molecules or drugs from thin films, scaffolds or micro- and nanocarriers. A burst release followed by a sustained one, governed by diffusion processes, has been observed in all cases.

Although many promising results have been achieved, further research, such as additional *in vivo* tests and clinical trials need to be carried out before PBS and PBS-based materials can be commercialized for biomedical applications, as to the best of our knowledge, PBS has not received the Food and Drug Administration approval for uses in biomedicine, yet.

References

[1] C. Mason, P. Dunnil, A brief definition of regenerative medicine, Regen. Med. 3 (2008) 1-5.

[2] R. Lanza, R. Langer, J.P. Vacanti, Principles of Tissue Engineering, Third Edition, Elsevier Academic Press, London, UK.

[3] K. Riehemann, S.W. Schneider, T.A. Luger, B. Godin, M. Ferrari, H. Fuchs, Nanomedicine-Challenge and perspectives, Angew. Chem. Int. Edit. 48 (2009) 872 – 897.

[4] S.M. Moghimi, A.C. Hunter, J.C Murray, Nanomedicine: current status and future prospects. FASEB J. 19 (2005) 311-330.

[5] S. Nie, Understanding and overcoming major barriers in cancer nanomedicine, Nanomedicine-UK 5 (2010) 523–528.

[6] D.F. Williams, On the nature of biomaterials, Biomaterials 30 (2009) 5897–5909.

[7] D.F. Williams, On the mechanisms of biocompatibility, Biomaterials 29 (2008) 2941–2953.

[8] B.D. Ulery, L.S. Nair, C.T. Laurencin, Biomedical applications of biodegradable polymers, J. Polym. Sci. Pol. Phys. 49 (2011) 832–864.

[9] H.Y. Cheung, K.T. Lau, T.P. Lu, D. Hui, A critical review on polymer-based bio-engineered materials for scaffold development, Compos. Part B-Eng. 38 (2007) 291–300.

[10] M.I. Sabir, X. Xu, L. Li, A review on biodegradable polymeric materials for bone tissue engineering applications, J. Mater. Sci. 44 (2009) 5713–5724.

[11] H.K. Makadia, S.J. Siegel, Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier, Polymers 3 (2011) 1377-1397.

[12] Y. Li, G.A. Thouas, Q.Z. Chen, Biodegradable soft elastomers: synthesis/properties of materials and fabrication of scaffolds, RSC Adv. 2 (2012) 8229–8242.

[13] V. Tserki, P. Matzinos, E. Pavlidou, D. Vachliotis, C. Panayiotou, Biodegradable aliphatic polyesters. Part I. Properties and biodegradation of poly(butylene succinate-co-butylene adipate), Polym. Degrad. Stabil. 91 (2006) 367-376.

[14] L.S. Nair, C.T. Laurencin, Biodegradable polymers as biomaterials, Prog. Polym. Sci. 32 (2007) 762–798.

[15] J. Panyam, V. Labhasetwar, Biodegradable nanoparticles for drug and gene delivery to cells and tissue, Adv. Drug Deliv. Rev. 55 (2003) 329–347.

[16] M. Vert, Degradable and bioresorbable polymers in surgery and in pharmacology: beliefs and facts, J. Mater. Sci. Mater. Med. 20 (2009) 437–446.

[17] H. Tian, Z. Tang, X. Zhuang, X. Chen, X. Jing, Biodegradable synthetic polymers: Preparation, functionalization and biomedical application, Prog. Polym. Sci. 37 (2012) 237– 280.

[18] J. Kohn, W.J. Welsh, D. Knight, A new approach to the rationale discovery of polymeric biomaterials, Biomaterials 28 (2007) 4171–4177.

[19] J.C. Middleton, A.J. Tipton, Synthetic biodegradable polymers as orthopedic devices, Biomaterials 21 (2000) 2335-2346.

[20] K. Grage, A.C. Jahns, N. Parlane, R. Palanisamy, I.A. Rasiah, J.A. Atwood, B.H.A. Rehm, Bacterial Polyhydroxyalkanoate Granules: Biogenesis, Structure, and Potential Use as Nano-/Micro-Beads in Biotechnological and Biomedical Applications, Biomacromolecules 10 (2009) 660–669.

[21] D.B. Hazer, E. Kılıçay, B. Hazer, Poly(3-hydroxyalkanoate)s: Diversification and biomedical applications. A state of the art review, Mater. Sci. Eng. C 32 (2012) 637–647.

[22] S.P. Valappil, S.K. Misra, A.R. Boccaccini, I. Roy, Biomedical applications of polyhydroxyalkanoates, an overview of animal testing and in vivo responses, Expert Rev. Med. Devic. 3 (2006) 853-868.

[23] C.G. Jaffredo, S.M. Guillaume, Benzyl b-malolactonate polymers: a long story with recent advances, Polym. Chem. 5 (2014) 4168-4194.

[24] M. Vert, Chemical routes to poly(β-malic acid) and potential applications of this water-soluble bioresorbable poly(β-hydroxy alkanoate), Polym. Degrad. Stab. 59 (1998) 169–175.

[25] O. Coulembier, P. Degée, J.L. Hedrick, P. Dubois, From controlled ring-opening polymerization to biodegradable aliphatic polyester: Especially poly(b-malic acid) derivatives, Prog. Polym. Sci. 31 (2006) 723–747.

[26] W.H. Carothers, Polymerization, Chem. Rev. 8 (1931) 353-426.

[27] http://www.showa-denko.com

[28] http://www.m-kagaku.co.jp

[29] I. Bechthold, K. Bretz, S. Kabasci, R. Kopitzky, A. Springer, Succinic acid: a new platform chemical for biobased polymers from renewable resources, Chem. Eng. Technol. 31 (2008) 647–654.

[30] S. Varadarjan, D.J. Miller, Catalytic upgrading of fermentation-derived organic acids, Biotechnol. Progr. 15 (1999) 845-854.

[31] M. Gigli, N. Lotti, M. Gazzano, L. Finelli, A. Munari, Novel eco-friendly random copolyesters of poly(butylene succinate) containing ether-linkages, React. Funct. Polym. 72 (2012) 303–310.

[32] N. Jacquel, F. Freyermouth, F. Fenouillot, A. Rousseau, J.P. Pascault, P. Fuertes, R. Saint-Loup, Synthesis and properties of poly(butylene succinate): efficiency of different transesterification catalysts, J. Polym. Sci. A1 49 (2011) 5301–5312.

[33] H. Azim, A. Dekhterman, Z. Jiang, R.A. Gross, *Candida antarctica* lipase B-catalyzed synthesis of poly(butylene succinate): shorter chain building blocks also work, Biomacromolecules 7 (2006) 3093-3097

[34] S. Sugihara, K. Toshima, S. Matsumura, New strategy for enzymatic synthesis of high-molecular-weight poly(butylene succinate) via cyclic oligomers, Macromol. Rapid Comm. 27 (2006) 203–207.

[35] T. Yagihara, S. Matsumura, Enzymatic synthesis and chemical recycling of novel polyester-type thermoplastic elastomers. Polymers 4 (2012) 1259-1277.

[36] M. Soccio, N. Lotti, L. Finelli, M. Gazzano, A. Munari, Influence of transesterification reactions on the miscibility and thermal properties of poly(butylene/diethylene succinate) copolymers, Eur. Polym. J. 44 (2008) 1722–1732.

[37] E.S. Yoo, S.S. Im, Melting behaviour of poly(butylene succinate) during heating scan by DSC, J. Polym. Sci. Pol. Phys. 37(1999) 1357–1366.

[38] J. Xu, B.H. Guo, Poly(butylene succinate) and its copolymers: research, development and industrialization, Biotechnol. J. 5 (2010) 1149-1163.

[39] T. Miyata, T. Masuko, Crystallization behaviour of poly(tetramethylene succinate), Polymer 39 (1998) 1399-1404.

[40] G.Z. Papageorgiou, D.N. Bikiaris, Crystallization and melting behavior of three biodegradable poly(alkylene succinates). A comparative study, Polymer 46 (2005) 12081–12092.

[41] M. Fabbri, M. Gigli, R. Gamberini, N. Lotti, M. Gazzano, B. Rimini, A. Munari, Hydrolysable PBS-based poly(ester urethane)s thermoplastic elastomers, Polym. Degrad. Stabil. 108 (2014) 223-231.

[42] F. Wu, C.L. Huang, J.B. Zeng, S.L. Li, Y.Z. Wang, Synthesis and characterization of segmented poly(butylene succinate) urethane ionenes containing secondary amine cation. Polymer 55 (2014) 4358-4368.

[43] V. Siracusa, N. Lotti, A. Munari, M. Dalla Rosa, Poly(butylene succinate) and poly(butylene succinate-co-adipate) for food packaging applications: Gas barrier properties after stressed treatments, Polym. Degrad. Stab. 119 (2015) 35-45.

[44] H. Wang, J. Ji, W. Zhang, J. Jiang, Z. Wu, S. Pu, P.K. Chu, Biocompatibility and bioactivity of plasma-treated biodegradable poly(butylene succinate), Acta Biomater. 5 (2009) 279–287.

[45] A.G. Pedroso, D.S. Rosa, Mechanical, thermal and morphological characterization of recycled LDPE/corn starch blends, Carbohyd. Polym. 59 (2005) 1–9.

[46] M.A. Lopez Manchado, L. Valentini, J. Biagiotti, J.M. Kenny, Thermal and mechanical properties of single-walled carbon nanotubes–polypropylene composites prepared by melt processing, Carbon 43 (2005) 1499–1505.

[47] T. Fujimaki, Processability and properties of aliphatic polyesters, 'BIONOLLE', synthesized by polycondensation reaction, Polym. Degrad. Stabil. 59 (1998) 209-214.

[48] J. Yang, W. Tian, Q. Li, Y. Li, A. Cao, Novel biodegradable aliphatic poly(butylene succinate)s bearing functionalizable carbonate building blocks: II. Enzymatic biodegradation and in vitro biocompatibility assay, Biomacromolecules 5 (2004) 2258-2268.

[49] S.I. Han, S.W. Woong, B.S. Kim, S.S. Im, A novel polymeric ionomer as potential biomaterial: crystallization behavior, degradation, and in-vitro cellular interactions, Adv. Funct. Mater. 15 (2005) 367-374.

[50] H. Li, J. Chang, A. Cao, J. Wang, In vitro evaluation of biodegradable poly(butylene succinate) as a novel biomaterial, Macromo. Biosci. 5 (2005) 433–440.

[51] V.M. Correlo, L.F. Boesel, M. Bhattacharya, J.F. Mano, N.M. Neves, R.L. Reis, Hydroxyapatite reinforced chitosan and polyester blends for biomedical applications, Macromol. Mater. Eng. 290 (2005) 1157–1165.

[52] H. Wang, J. Ji, W. Zhang, W. Wang, Y. Zhang, Z. Wu, Y. Zhang, P.K. Chu, Rat calvaria osteoblast behavior and antibacterial properties of O_2 and N_2 plasma-implanted biodegradable poly(butylene succinate), Acta Biomater. 6 (2010) 154–159.

[53] D.F. Coutinho, I.H. Pashkuleva, C.M. Alves, A.P. Marques, N.M. Neves, R.L Reis, The effect of chitosan on the in vitro biological performance of chitosan-poly(butylene succinate) blends, Biomacromolecules 9 (2008) 1139–1145.

[54] J.S. Lim, J.H. Kim, New application of poly(butylene succinate) (PBS) based ionomer as biopolymers: a role of ion group for hydroxyapatite (Hap) crystal formation, J. Mater. Sci. 44 (2009) 6398–6403.

[55] D.F. Coutinho, M.E. Gomes, N.M. Neves, R.L. Reis, Development of micropatterned surfaces of poly(butylene succinate) by micromolding for guided tissue engineering, Acta Biomater. 8 (2012) 1490–1497.

[56] M. Gigli, N. Lotti, M. Gazzano, L. Finelli, A. Munari, Macromelecular design of novel sulfur-containing copolyesters with promising mechanical properties, J. Appl. Polym. Sci. 126 (2012) 686–696.

[57] M. Soccio, N. Lotti, M. Gazzano, M. Govoni, E. Giordano, A. Munari, Molecular architecture and solid-state properties of novel biocompatible PBS-based copolyesters containing sulphur atoms, React. Funct. Polym. 72 (2012) 856-867.

[58] C. Gualandi, M. Soccio, E. Saino, M.L. Focarete, N. Lotti, A. Munari, L. Moroni, L. Visai, Easly synthesized novel biodegradable copolyesters with adjustable properties for biomedical applications, Soft Matter 8 (2012) 5466-5476.

[59] H. Kun, Z. Wei, L. Xuan, Y. Xiubin, Biocompatibility of a novel poly(butylene succinate) and polylactic acid blend, ASAIO J. 58 (2012) 262-267.

[60] M. Gigli, N. Lotti, M. Gazzano, L. Finelli, A. Munari, Synthesis and characterization of novel poly(butylene succinate)-based copolyesters designed as potential candidates for soft tissue engineering, Polym. Eng. Sci. 53 (2013) 491-501.

[61] W. Guo, Y. Zhang, W. Zhang, Mechanical properties and crystallization behavior of hydroxyapatite/poly(butylenes succinate) composites, J. Biomed. Mater. Res. 101A (2013) 2500-2506.

[62] S.P. Zhang, L.L. Wang, S. Yang, Y.K. Gong, Improved Biocompatibility of phosphorylcholine end-capped poly(butylene succinate), Sci. China 56 (2013) 174-180.

[63] N. Hao, Y.B. Wang, S.P. Zhang, S.Q. Shi, K. Nakashima, Y.K. Gong, Surface reconstruction and hemocompatibility improvement of a phosphorylcholine end-capped poly(butylene succinate) coating, J. Biomed. Mater. Res. 102 (2014) 2972-2981.

[64] I. Grigoriadou, N. Nianias, A. Hoppe, Z. Terxopoulou, D. Bikiaris, J. Will, J. Hum, J.A. Roether, R. Detsch, A.R. Boccaccini, Evaluation of silica-nanotubes and strontium hydroxyapatite nanorods ad appropriate nanoadditives for poly(butylene succinate) biodegradable polyester for biomedical applications. Composites 60 (2014) 49–59.

[65] S. Arphavasin, W. Singhatanadgit, P. Ngamviriyavong, W. Janvikul, P. Meesap, S. Patntirapong, Enhanced osteogenic activity of a poly(butylene succinate)/calcium phosphate composite by simple alkaline hydrolysis, Biomed. Mater. 8 (2013) 1-8.

[66] M. Tallawi, R. Rai, M.R. Gleixner, O. Roerick, M. Weyand, J.A. Roether, D.W. Schubert, A. Kozlowska, M. El Fray, B. Merle, M. Goken, K. Aifantis, A.R. Boccacini, Poly(glycerol sebacate)/poly(butylene succinate-dilinoleate) blends ad candidate materials for cardiac tissue engineering, Macromol. Symp. 334 (2013) 57–67.

[67] P. Ngamviriyavong, S. Patntirapong, W. Janvikul, S. Arphavasin, P. Meesap, W. Singhatanadgit, Development of poly(butylene succinate)/calcium phosphate composites for bone engineering, Compos. Interface. 21 (2013) 1-11.

[68] R.R. Fan, L.X. Zhou, D.X. Li, D.M. Zhang, M. Wu, G. Guo, Preparation and characterization of composites based on poly(butylene succinate) and poly(lactic acid) grafted tetracalcium phosphate, J. Macromol. Sci. Phys. 53 (2014) 296-308.

[69] L. Tan, J. Hu, S. Ye, J. Wei, Y. Chen, Crystallization and degradation behaviors of poly(butylene succinate)/poly(Z-L-Lysine) composites, Thermochim. Acta 575 (2014) 279– 284.

[70] Y. Niu, L. Cao, J. Wei, Y. Ma,S. Song, W. Weng, H. Li, C. Liu, J. Su, Development of a bioactive composite of nano fluorapatite and poly(butylene succinate) for bone tissue regeneration, J. Mater. Chem. B 2 (2014) 1174-1181.

[71] S. Patntirapong, W. Singhatanadgit, P. Meesap, T. Theerathanagorn, M. Toso, W. Janvikul, Stem cell adhesion and proliferation on hydrolyzed poly(butylene succinate)/b-tricalcium phosphate composites, J. Biomed. Mater. Res. A 103 (2015) 658-670.

[72] C.S. Wu, Y.C. Hsu, H.T. Liao, F.S. Yen, C.Y. Wang, C.T. Hsu, Characterization and biocompatibility of chestnut shell fiber-based composites with polyester, J. Appl. Polym. Sci. 131 (2014) 40730(1-10).

[73] L.C. Wang, J.W. Chen, H.L. Liu, Z.Q. Chen, Y. Zhang, C.Y. Wang, Z.G. Feng, Synthesis and evaluation of biodegradable segment multiblock poly(ether ester) copolymers for biomaterial applications, Polym. Int. 53 (2004) 2145-2154.

[74] V.M. Correlo, L.F. Boesel, E. Pinho, A.R. Costa-Pinto, M.L. Alves de Silva, M. Bhattacharya, J.F. Mano, N.M. Neves, R.L. Reis, Melt-based compression-molded scaffolds from chitosan-polyester blends and composites: morphology and mechanical properties, J. Biomed. Mater. Res. 91 (2008) 439-504.

[75] J.T. Oliveira, V.M. Correlo, P.C. Sol, A.R. Costa-Pinto, P.B. Malafaya, A.J. Salgrado, M. Bhattacharya, P. Charbord, N.M. Neves, R.L. Reis, Assessment of the suitability of chitosan/polybutylene succinate scaffolds seeded with mouse mesenchymal progenitor cells for a cartilage tissue engineering approach, Tissue Eng. A 14 (2008) 1651-1661.

[76] P. Hariraksapitak, O. Suwatong, P. Pavasant, P. Supaphol, Effectual drug-releasing porous scaffold from 1,6-isocyanatohexane-extended poly(1,4-butylene succinate) for bone tissue regeneration, Polymer 49 (2008) 2678-2685.

[77] A.R. Costa-Pinto, A.J. Salgado, V.M. Correlo, P. Sol, M. Bhattacharya, P. Charbord, R.L. Reis, N.M. Neves, Adhesion, proliferation, and osteogenic differentiation of a mouse mesenchymal stem cell line (BMC9) seeded on novel melt-based chitosan/polyester 3D porous scaffold, Tissue Eng. A 14 (2008) 1049-1057.

[78] M.L. Alves de Silva, A. Crawford, J.M. Mundy, V.M. Correlo, P. Sol, M. Bhattacharya, P.V. Hatton, R.L. Reis, N.M. Neves, Chitosan/polyester-based scaffolds for cartilage tissue engineering: assessment of extracellular matrix formation, Acta Biomater. 6 (2010) 1149-1157.

[79] A.R. Costa-Pinto, V.M. Correlo, P.C. Sol, M. Bhattacharya, S. Srouji, E. Livne, R.L. Reis, N.M. Neves, Chitosan-poly(butylene succinate) scaffolds and human bone marrow stromal cells induce bone repair in a mouse calvaria model, J. Tissue Eng. Regen. Med. 6 (2012) 21-28.

[80] A.R. Costa-Pinto, I. Vargel, V.M. Correlo, P.C. Sol, S. Faria, E. Piskin, R.L. Reis, N.M. Neves, Influence of scaffold composition over in vitro osteogenic differentiation of hBMSCs and in vivo inflammatory response, J. Biomater. Appl. 28 (2014) 1430-1442.

[81] J.X. Tang, Q.Y. Wu, Mesenchimal stem cellular adhesion and cytotoxicity study of random biopolyester scaffolds for tissue engineering, J. Mater. Sci. Mater. Med. 17 (2006) 627-632.

[82] D. Zhan, J. Chang, Y. Zang, Fabrication of fibrous poly(butylene succinate)/wollastonite/apatite composite scaffolds by electrospinning and biometric process, J. Mater. Sci. Mater. Med. 19 (2008) 443-449.

[83] E.D. Pinho, A. Martins, J.V. Araujo, R.L. Reis, N.M. Neves, Degradable particulate composite reinforced with nanofibres for biomedical applications, Acta Biomater. 5 (2009) 1104-1114.

[84] W. Kosorn, B. Thavornyutikarn, B. Phumsirl, P. Uppanan, P. Meesap, W. Janvikul, Hydrolyzed poly(butylene succinate) scaffolds coated with bioactive agent, J. Metals Materials Minerals 20 (2010) 98-99.

[85] S. Sutthiphong, P. Pavasant, P. Supaphol, Electrospun 1,6-diisocyanatehexane-extended poly(1,4-butylene succinate) fiber mats and their potential for use ad bone scaffolds, Polymer 50 (2009) 1548-1558.

[86] J.T. Oliveira, A. Crawford, J.L. Mundy, P.C. Sol, V.M. Correlo, M. Bhattacharya, N.M. Neves, P.V. Hatton, R.L. Reis, Novel melt-processable chitosan-polybutylene succinate fibre scaffolds for cartilage tissue engineering, J. Biomater. Sci. 22 (2011) 773-788.

[87] C. Gualandi, M. Soccio, M. Govoni, S. Valente, N. Lotti, A. Munari, E. Giordano, G. Pasquinelli, M.L. Focarete, Poly(butylene/diethylene glycol succinate) multiblock copolyester as a candidate biomaterial for soft tissue engineering: solid-state properties, degradability, and biocompatibility, J. Bioact. Compat. Pol. 27 (2012) 244-264.

[88] P. Kaewkong, P. Uppanan, B. Thavornyutikarn, W. Kosorn, W. Janvikul, Chondrocyte growth and function on HPBS/HA composite scaffolds: static versus dynamic culture, IPCBEE 43 (2012) 11-14.

[89] L.R. Almeida, A.R. Martins, E.M. Fernandes, M.B. Oliveira, V.M. Correlo, I. Pashkuleva, A.P. Marques, A.S. Ribeiro, N.F. Duraes, C.J. Silva, G. Bonifacio, R.A. Sousa, A.L. Oliveira, R.L. Reis, New biotextiles for tissue engineering: development, characterization and in vitro cellular viability, Acta Biomater, 9 (2013) 8167-8181.

[90] P. Uppanan, P. Meesap, B. Thavornyutikarn, W. Kosorn, W. Janvikul, Study on surface-hydrolyzed poly(butylene succinate)/hydroxyapatite composite scaffolds for cartilage regeneration, Adv. Sci. Lett. 19 (2013) 3070-3072.

[91] N. Stoyanova, D. Paneva, R. Mincheva, A. Toncheva, N. Manolova, P. Dubois, I. Rashkov, Poly(L-lactide) and poly(butylene succinate) immiscible blends: from electrospinning to biologically active materials, Mater. Sci. Eng. C 41 (2014) 119–126.

[92] M. Tallawi, D.C. Zebrowski, R. Rai, J.A. Roether, D.W. Schubert, M. El Fray, F.B. Engel, K.E. Aifantis, A.R. Boccaccini, Poly(glycerol sebacate)/poly(butylene succinate-dilinoleate) (PGS/PBS-DLA) fibrous scaffolds for cardiac tissue engineering, Tissue Eng. Pt. C Meth. 20 (2014) 412-22.

[93] A.R. Costa-Pinto, A.M. Martins, M.J. Castellhano-Carlos, V.M. Correlo, P.C. Sol, A. Longatto-Filho, R.L. Reis, N.M. Neves, In vitro degradation and in vivo biocompatibility of chitosan poly(butylene succinate) fiber mesh scaffolds, J. Bioact. Compat. Pol. 29 (2014) 137-151.

[94] V.P. Ribeiro, A.S. Ribeiro, C.J. Silva, N.F. Durães, G. Bonifácio, V.M. Correlo, A.P. Marques, R.A. Sousa, A.L. Oliveira, R.L. Reis, Evaluation of Novel 3D Architectures Based on Knitting Technologies for Engineering Biological Tissues, Journal of Donghua University 5 (2013) 421-426.

[95] S.J. Park, Y.M. Lee, S.K. Hong, Release behaviors of porous poly(butylene succinate)/poly(ε-caprolactone) microcapsules containing indomethacin, Colloid Surface B 47 (2006) 211-215.

[96] J. Liu, Z. Jiang, S. Zhang, W.M. Saltzman, Poly(ω-pentadecalactone-co-butylene-co-succinate) nanoparticles as biodegradable carriers for camptothecin delivery, Biomaterials 30 (2009) 5707-5719.

[97] P. Meesap, P. Uppanan, B. Thavornyutikarn, W. Kosorn, W. Janvikul, Surface hydrolysed poly(butylene succinate) microspheres incorporated carboxymethylchitosan scaffolds for cartilage tissue engineering, J. Metals Materials Minerals 20 (2010) 107-111.

[98] K. Mohanraj, S. Sethuraman, U.M. Krishnan, Development of poly(butylene succinate) microspheres for delivery of levodopa in the treatment of Parkinson's disease. J. Biomed. Mater. Res. 10 (2013) 840-847.

[99] A. Jager, D. Gromadzki, E. Jager, F.C. Giacomelli, A. Kozlowska, L. Kobera, J. Brus, B. Rihova, M. El Fray, K. Ulbrich, P. Stepanek, Novel "soft" biodegradable nanoparticles prepared from aliphatic based monomers ad a potential drug delivery system, Soft Matter 8 (2012) 4343-4354.

[100] E. Jager, A. Jager, P. Chytril, T. Etrych, B. Rihova, F.C. Giacomelli, P. Stepanek, K. Ulbrich, Combination chemotherapy using core-shell nanoparticles through the self-assembly of HPM-based copolymers and degradable polyester. J. Control. Release 165 (2013) 153-161.

[101] J. Gowsika, R. Nanthini, Synthesis, characterization and cytotoxicity of certain itaconic acid based biodegradable aliphatic copolyesters. J. Chem. Pharm. Res. 6 (2014) 1452-1461.

[102] J. Yang, Q. Hao, X. Liu, C. Ba, A. Cao, Novel Biodegradable Aliphatic Poly(butylene succinate-co-cyclic carbonate)s with Functionalizable Carbonate Building Blocks. 1. Chemical Synthesis and Their Structural and Physical Characterization, Biomacromolecules 5 (2004) 209-218.

[103] C. Lavilla, A. Alla, A. Martínez de Ilarduya, S. Muñoz-Guerra, High Tg Bio-Based Aliphatic Polyesters from Bicyclic D‑Mannitol, Biomacromolecules 14 (2013) 781−793.

[104] E. Zakharova, A. Alla, A. Martinez de Ilarduya, S. Muñoz-Guerra, Bio-based PBS copolyesters derived from a bicyclic D-glucitol, RSC Adv., 5 (2015) 46395-46404.

[105] M. Bautista, A. Martínez de Ilarduya, A. Alla, S. Muñoz-Guerra, Poly(butylene succinate) Ionomers with Enhanced Hydrodegradability, Polymers 7 (2015) 1232-1247.

[106] M. Bautista, A. Martinez de Ilarduya, A. Alla, S. Munoz-Guerra, Poly(butylene succinate) Ionomers and Their Use as Compatibilizers in Nanocomposites, DOI 10.1002/pc.23454, 2015.

[107] W. Suchanek, M. Yoshimura, Processing and properties of hydroxyapatite-based biomaterials for use as hard tissue replacement implants, J. Mater. Res. 13 (1998) 94-117.

[108] A.A. White, S.M. Best, Hydroxyapatite-carbon nanotube composites for biomedical applications: a review, Int. J. Appl. Ceram. Tec. 4 (2007) 1–13.

[109] M. Dasha, F. Chiellini, R.M. Ottenbrite, E. Chiellini, Chitosan - A versatile semi-synthetic polymer in biomedical applications, Prog. Polym. Sci. 36 (2011) 981–1014.

[110] S.C.M. Fernandes, C.S.R. Freire, A.J.D. Silvestre, C.P. Neto, A. Gandini, Novel materials based on chitosan and cellulose, Polym. Int. 60 (2011) 875–882.

[111] M. Gigli, A. Negroni, M. Soccio, G. Zanaroli, N. Lotti, F. Fava, A. Munari, Influence of chemical and architectural modifications on the enzymatic hydrolysis of poly(butylene succinate), Green Chem. 14 (2012) 2885-2893.

[112] M. Gigli, A. Negroni, G. Zanaroli, N. Lotti, F. Fava, A. Munari, Environmentally friendly PBS-based copolyesters containing PEG-like subunit: effect of block length on solid-state properties and enzymatic degradation, React. Funct. Polym. 73 (2013) 764-771.

[113] M. Gigli, A. Negroni, M. Soccio, G. Zanaroli, N. Lotti, F. Fava, A. Munari, Enzymatic hydrolysis studies on novel eco-friendly aliphatic thiocopolyesters, Polym. Degr. Stab., 98 (2013) 934-942.

[114] M. Mochizuki, M. Hirami, Structural effects on the biodegradation of aliphatic polyesters, Polym. Advan. Technol. 8 (1997) 203-209.

[115] Y. Arima, H. Iwata, Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers, Biomaterials 28 (2007) 3074–3082.

[116] A. Ranella, M. Barberoglou, S. Bakogianni, C. Fotakis, E. Stratakis, Tuning cell adhesion by controlling the roughness and wettability of 3D micro/nano silicon structures, Acta Biomaterialia, 6 (2010) 2711–2720.

[117] F. Carrasco, P. Pagès, J. Gámez-Pérez, O.O. Santana, M.L. Maspoch, Processing of poly(lactic acid): characterization of chemical structure, thermal stability and mechanical properties, Polym. Degrad. Stabil. 95 (2010) 116-125.

[118] R. Morent, N. De Geyter, T. Desmet, P. Dubruel, C. Leys, Plasma surface modification of biodegradable polymers: a review. Plasma Process. Polym. 8 (2011) 171–190.

[119] S.C. Baker, G. Rohman, J. Southgate, N.R. Cameron, The relationship between the mechanical properties and cell behavior on PLGA and PCL scaffolds for bladder tissue engineering, Biomaterials 30 (2009) 1321–1328

[120] A.J. Engler, S. Sen, H. Lee Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, Cell 126 (2006) 677–689.

[121] G.C. Reilly, A.J. Engler, Intrinsic extracellular matrix properties regulate stem cell differentiation, J. Biomech. 43 (2010) 55–62.

[122] M.S. Rizvi, P. Kumar, D.S. Katti, A. Pal, Mathematical model of mechanical behavior of micro/nanofibrous materials designed for extracellular matrix substitutes, Acta Biomater. 8 (2012) 4111–4122.

[123] Q. Chen, S. Liang, G.A. Thouas, Elastic biomaterials for tissue engineering, Prog. Polym. Sci. 38 (2013) 584-671.

[124] A. Gopferich, Mechanism of polymer degradation and erosion, Biomaterials 17 (1996) 103-114.

[125] X. Zhu, J. Chen, L. Scheideler, T. Altebaeumer, J. Geis-Gerstorfer, D. Kern, Cellular reactions of osteoblasts to micron- and submicron-scale porous structures of titanium surfaces, Cells Tissues Organs 178 (2004) 13–22.