Mass spectrometry in bioresorbable polymer development, degradation and drug release tracking

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Mass spectrometry in bioresorbable polymer development, degradation and drug release tracking

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Abstract

A detailed characterization of polymeric matrices and appropriate degradation monitoring techniques are required to sustain the development of new materials as well as to enlarge the applications of the old ones. In fact, polymer analysis is essential for the clarification of the intrinsic relationship between structure and properties that ascertains the industrial applications in diverse fields. In bioresorbable and biodegradable polymers, the role of analytical methods is dual since it is pointed both at the polymeric matrices and degradation tracking. The structural architectures, the mechanical and morphological properties, as well as the degradation rate are of outstanding importance for the peculiar application. In some cases, the complexity of the polymer structure, the processes of decomposition or the low concentration of the degradation products need the concurrent use of different analytical techniques, which complement each other, to give detailed information of the reactions taking place. Several analytical methods are used in bioresorbable polymer development and degradation tracking. Among them, mass spectrometry (MS) plays an essential role and it is used to refine polymer syntheses, for its high sensitivity, to highlight degradation mechanism by detecting compounds present in trace amount, or tracking the degradation product profile as well as to study drug release. In fact, elucidation of reaction mechanisms and polymer structure, attesting the purity and detecting defects as well as residual catalysts, in biodegradable and bioresorbable polymers requires sensitive analytical characterization methods that are essential in providing an assurance of safety, efficacy and quality. This review aims to provide an overview of the MS strategies used to support research and development of resorbable polymers as well as to investigate the degradation mechanism. It is focused on the most significant studies concerning synthetic bioresorbable matrices (polylactide, polyglycolide and their copolymers, polyhydroxybutyrate, etc.), published in the last ten years.

Keywords: bioresorbable polymers, structural characterization, polymer degradation, mass spectrometry, biomedical applications, drug release

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I. Introduction

Synthetic polymers, with an extensive variety of mechanical properties, performance, durability and cost, are widely employed in the daily requests of contemporary society, ranging from packaging to electronic devices, buildings, medical purposes, etc. In the last decade, the attention and worldwide consumption of biodegradable have undoubtedly increased, conditioned also by legislative choices. At present, several different kinds of biodegradable polymers have been developed and introduced into the market with an estimation of a rising share in the next years. Bioresorbable polymers belong to a class of biodegradable materials that can be easily absorbed by the body. They will be named "bioresorbable polymers" throughout the text. Their bioresorption can occur hydrolytically or enzymatically. Their physical properties, such as flexibility, strength, and adaptable degradation rates, make them suitable for various end use application such as orthopedic, drug delivery, and cardiology (**Figure 1**). The market of bioresorbable polymers is related to natural and synthetic polymers, among which poly(lactide) (PLA) and its derivatives, poly(glycolide) (PGA), poly(lactide-*co*-glycolide) (PLGA), poly(ε-caprolactone) (PCL), poly(ethylene glycol) (PEG), proteins, polysaccharides, polydioxanone (PDS), etc. (**Figure 2**). Additionally, PLA is the most widely employed as poly(L-lactide) (PLLA), poly(D-lactide) (PDLA), and poly(DL-lactide) (PDLLA).[1]

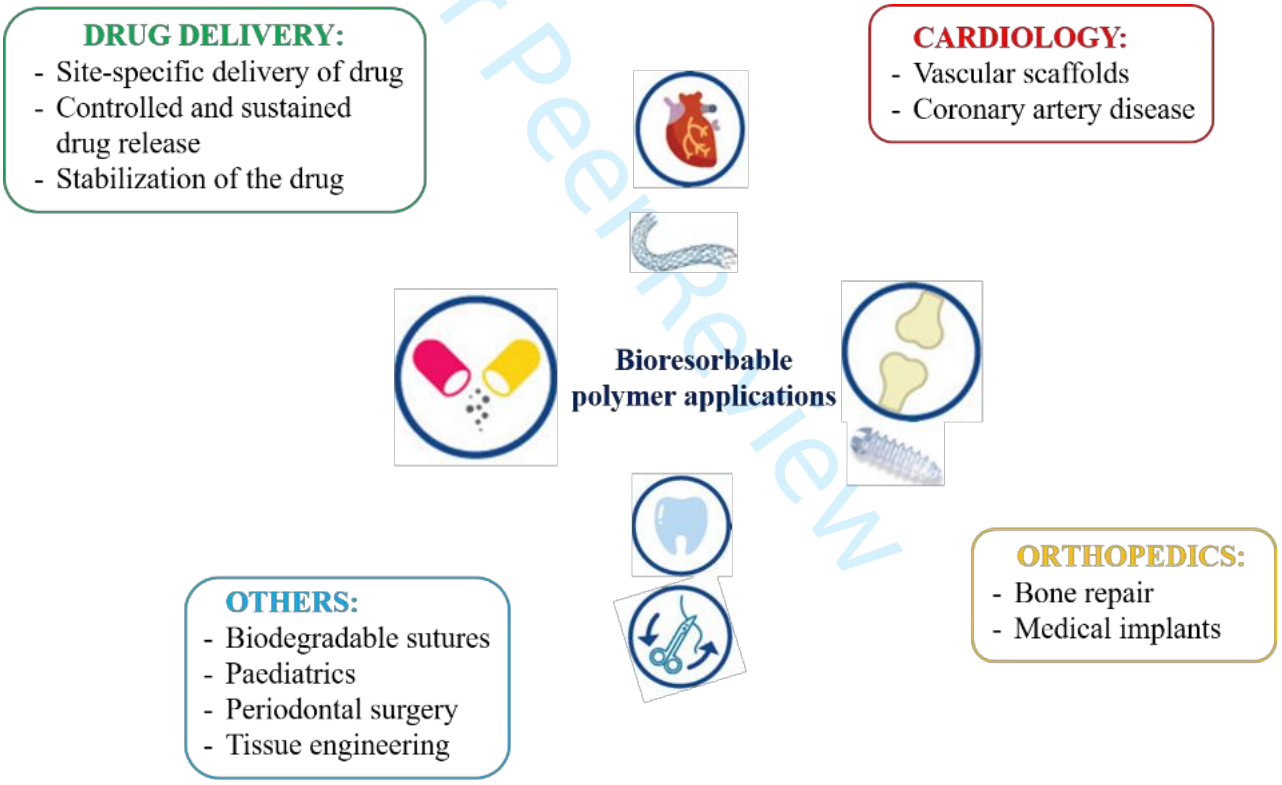

Figure 1. General bioresorbable polymer applications.

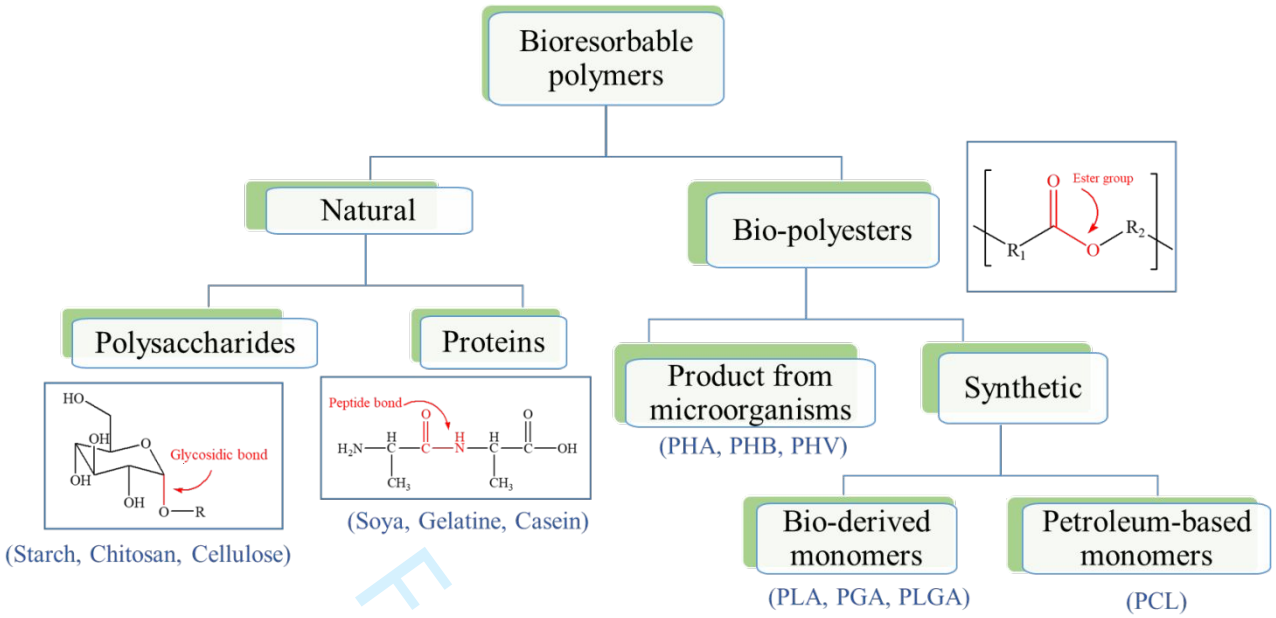

Figure 2. Classification of bioresorbable polymers.

Although there are several alternative systems on the market, an outstanding economical effort is still focused in refining thermo-mechanical performance and tailoring structural properties in order to further extend the range of applications. Improving the properties of polymers and polymer-based materials involves a good understanding of characterization features. Therefore, the structural analysis of bioresorbable polymers is of remarkable importance for the innate relationship between structure and properties that influences the industrial applications in different fields.[2]

Polymer analysis can be related to many different points moving from average molar mass determination to detailed characterization of chemical structures or compositions. It is fundamental for quality control of polymeric products as well as in troubleshooting of a polymer industrialized process. Presently, there is a high demand for developing specialty materials in many innovative biomedical applications, mainly based on bioresorbable polymeric materials. Both the chemical and physical characteristics of the polymers can have a large influence on the rheological and mechanical behavior as well as on biodegradation kinetics or how cells will interact with the material. Noteworthy, progress in organic synthesis and characterization methods have yielded synthetic bioresorbable polymers with well-defined, three-dimensional structures and with the prospective to mimic biomacromolecules. These well-structured polymers include block copolymers, branched, dendritic, graft and star-shaped polymers.[3] Additionally, reliable design of the polymer synthetic methods allows tailoring of the mechanical, physicochemical and degradation properties or drug release kinetics of the resulting materials. In some cases, because of the structural and compositional complexity of synthetic polymers, the detailed characterization of a newly developed polymeric material is a challenging task, principally when it is prepared from innovative polymer chemistry, catalysis, or formulation process. Analytical approaches and methods, for both characterization and degradation features, have a central role for the development of bioresorbable polymer systems and proving the suitability aimed at a specific application.[4-6] Mass spectrometry (MS) has played an increasingly important role in polymer analysis, thanks also to the improvement of instrumental techniques and the introduction of up-to-date configurations. In recent times, several reviews,[7-11] books,[12-14] and book chapters[3] on modern MS in polymer chemistry have been published. Undoubtedly, MS methods, for their high sensitivity, selectivity, and rapidity, provide the opportunity to look at the finest structural details even in complex polymer samples. Modern MS techniques for their high sensitivity provide a valuable support for the whole characterization of bioresorbable polymeric materials. In fact, sensitive analytical methods are required in order to checking the purity and identifying defects in this kind of polymers, also in trace

amounts, essential information in providing an assurance of safety, efficiency and quality. Most of the polymers used for biomedical applications are copolyesters. Because the composition is regularly tuned to optimize crystallinity, degradation rate, and mechanical properties, its accurate knowledge is essential. Experimentally, the composition is typically determined by proton nuclear magnetic resonance (^{1}H NMR) spectroscopy that is not always able to provide the finest details. Amongst the MS techniques, matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) has successfully changed the practice of polymer characterization. Compared to other MS techniques, MALDI MS provides several distinctive features that make it a powerful tool for analyzing a wide range of polymers. In fact, MALDI-TOF MS is a high sensitivity technique that offers detailed structural information about the macromolecules contained in a polymer sample without previous sample treatment or time-consuming separation. Information on the nature of monomers and end groups can be obtained from the accurate mass measurement of the individual oligomers. As a consequence, MALDI MS has become a routinely used tool for polymer characterization and has been applied also in the structural characterization of biodegradable and bioresorbable polymers.[9,15-20] In MALDI analysis, the selection of the matrix and the sample preparation procedures held an essential role. Recently, a second generation ionic liquid matrices (ILM II) were tested in the spectral analysis of biodegradable and bioresorbable polymers (PLLA, PCL, PEG, block and random copolymers) with the aim of understanding the physical parameters, which bother or help the MALDI-TOF analysis of this class of polymers.[21-23]

Nevertheless, the differentiation between a pure polymer and its side products is a difficult issue and single stage-MS is not always decisive. Tandem mass spectrometry (MS/MS), especially in homopolymers analysis, can be very advantageous for the detection of shortcomings, being also able to highlight the nature of the end groups as well as structural data for copolymers. The understanding of polymer-fragmentation mechanisms is propedeutic and consequently of primary importance for the analysis of MS/MS data, as shown in a number of experimental and theoretical studies.[15,24] Furthermore, MS/MS can also be used to distinguish isobaric and isomeric species, and highlight differences within a mixture of linear and cyclic systems. However, in some cases, a previous separation by hyphenated techniques is required to fully differentiate macromolecular architectural differences. The separation of polymer mixtures due to differences in polarity or hydrophobicity by liquid chromatography (LC) prior to ionization can provide complementary information and simplify the MS/MS data, above all in the analysis of mixtures. In a similar way, ion mobility (IM) MS can provide additionally gas-phase separation, based on measurements of the collisional cross-section (CCS) of ions, before and/or after fragmentation and it has proved to be helpful in the elucidation of the detailed three-dimensional structure of synthetic bioresorbable polymers.[25] Sodium cationized PLA and PEG were also selected as calibrants with reference CCS to define a calibration procedure traveling wave ion mobility spectrometry (TWIMS).[26]

The final performance of a material in many traditional and modern applications not only depends on its bulk properties but also is heavily connected with its surface microstructure and interfacial behavior. Several recent papers appeared in the literature reviewing a variety of important issues in the surface sciences of biodegradable and bioresorbable materials, mainly involved in drug-delivery. Surface analysis of biodegradable polymers provided information about the chemical structure of the polymer and surface-active additives as well as surface contamination, which can prejudice the surface properties in many processes. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) is frequently used in surface chemistry characterization of bioresorbable polymers and in monitoring the changes induced by degradation processes.[27] TOF-SIMS has been recently used to understand physiochemical surface interactions between degradable biopolymers and biological environments.[28-33] SIMS can work in the static mode (SSIMS) or dynamic mode (DSIMS). The former mode gives hints about molecular composition whereas the dynamic one provides elemental and isotopic information.[12,13]

Interestingly, inductively coupled plasma mass spectrometry (ICP-MS) was recently employed in bioresorbable and biodegradable polymer analysis to check metals due to residual catalyst,[34] correlate their contents with polymer degradation,[35] or for drug delivery studies.[36,37]
Furthermore, modern soft ionization MS techniques have been used to point out the detection of primary thermal, thermo and photo-oxidative decomposition products, providing detailed information on the relationships between polymer end-chain structures and degradation processes.[9,12,13] MS techniques have been also applied to follow the hydrolytic and enzymatic degradation of polymeric materials; among them electrospray ionization mass spectrometry (ESI MS) has been more commonly used because of the advantage of being readily interfaced with solution - based separation techniques such as high-performance liquid chromatography (HPLC).[9]
Overall, inspection of the literature reveals more and more interest on bioresorbable polymers and a progressive trend in the application of MS, in the characterization as well as in the degradation features, and the relates drug release researches. Thus, the current review will be focused on the most significant – in our opinion – characterization, degradation and drug release studies on bioresorbable polymers by MS methods. The selected papers were published between the beginning of 2009 and september 2019. This review provides an overview of the MS analytical tools used to study bioresorbable polymers and the kind of information that can be obtained. Each MS method presents advantages and disadvantages in being applied in the characterization, degradation or release tracking studies. The choice of the MS technique is of crucial importance to succeed in the reliability and utility of the data obtained. We hope that this review will be helpful for this purpose, further extending the fields of application of MS in the development of bioresorbable polymers.

II. Characterization of bioresorbable polymers by MS

The identification of molecular parameters and structures is the first step in the chemical analysis of a polymeric material. Undoubtedly, NMR and Fourier transform infrared spectroscopy (FTIR), combined or by themselves, represent the major and more frequently analytical tools adopted to follow and verify each step of the synthesis of polymers, also in studies concerning bioresorbable matrices.[38-49] Size exclusion chromatography (SEC) and MS techniques are currently applied to determine the molar mass (MM) and check the structural changes induced by degradation source in the polymer samples.[9] Several MS methods are adopted for the structural characterization of biodegradable polymers.[3,9,19,50] Checking the structure, purity and defects in this kind of polymers requires good analytical characterization techniques above all for trace amounts detection and it is crucial as a guarantee of safety, efficiency and quality. Accordingly, modern MS techniques is a valuable support for the characterization and development of bioresorbable polymeric materials. In fact, MS techniques for their high sensitivity, selectivity, and quickness provide the opportunity to investigate the finest structural details yet in complex polymer samples. In particular, MALDI MS has been confirmed a powerful tool for analyzing a wide range of polymers, including bioresorbable ones, providing detailed structural information about the individual molecules contained in a sample with the advantage of no prior sample treatment or time-consuming separation. In several cases, the synergic combination of information from more than one analytical technique, more regularly NMR and MALDI or FTIR, provides a better understanding of the polymer architecture and the suitability for the designed application.[18,51,52] Bioresorbable polymer studies by MS mainly concern the structural investigation of synthetic samples (identification of end groups, functionalization, presence of contaminants, etc.). However, several relevant features have been investigated by different MS techniques (**Figure 3**). In fact, MS has been applied to confirm the functionalization of polymer-drug coniugates systems, ascertain the polymerization mechanism, analyse the surface and establish the distribution of bioactive substances or drugs in polymeric matrices. Several studies have been supported by tandem MS/MS analysis being propedeutic and, in some case, essential to define the precise macromolecular structures (end groups, architectures, and sequences). All these features are crucial in the development of polymeric systems oriented to the biomedical field market where the purity and the real structural corrispondence are a warranty of healthy. **Table 1** summarizes the bioresorbable polymers analysed, the MS, the traditional methods used and the kind of information acquired by MS, in the papers discussed in the present review.

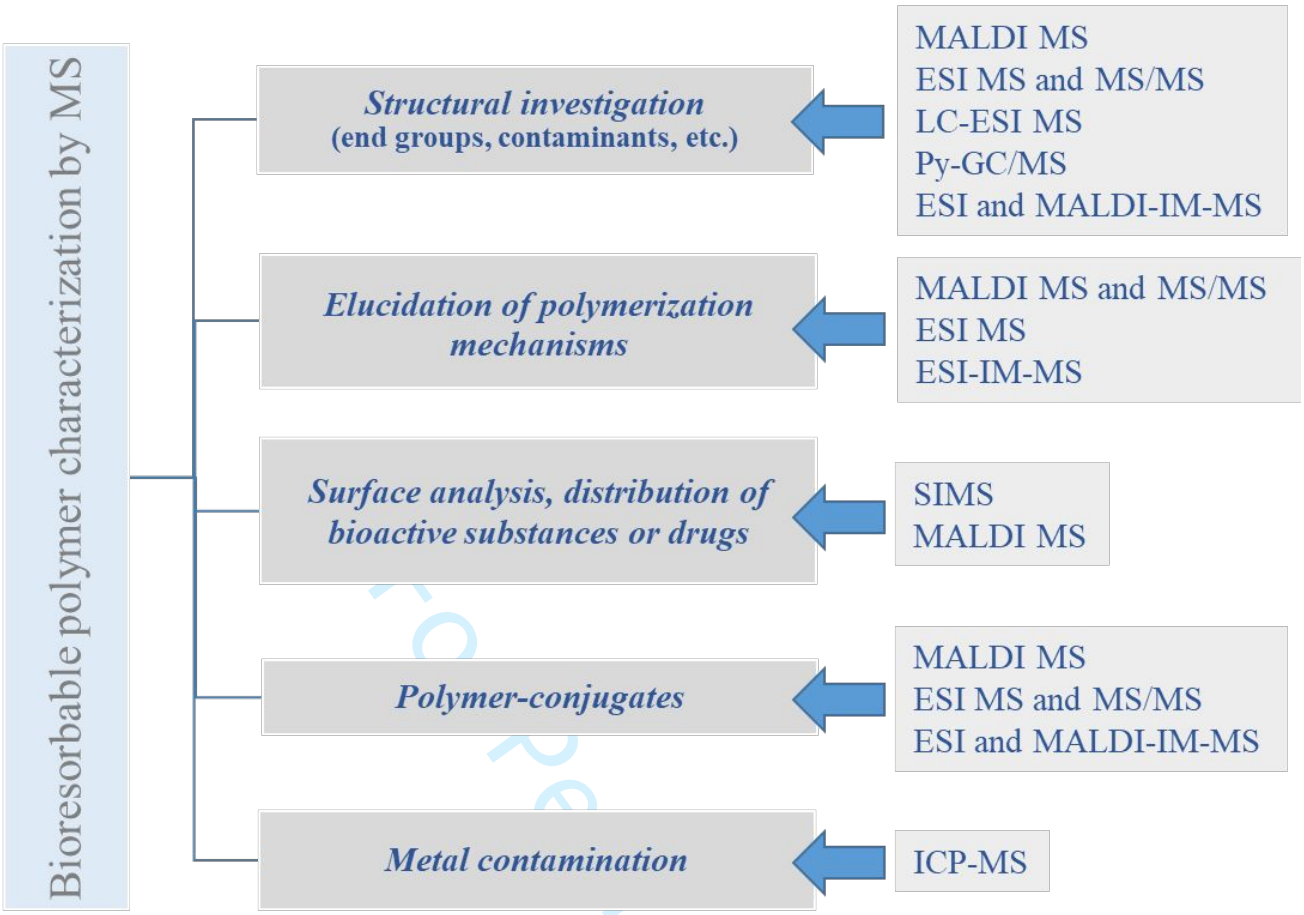

Figure 3. Overview of the MS techniques used in the characterization of bioresorbable polymer.

Structural investigation

The chemical analysis, i.e. the identification of molecular parameters and structures, is the first step in the development of any type of polymeric material. Modern MS, mainly MALDI-TOF, can provide fundamental information about synthetic polymers: the mass of the repeating units, end group pattern, monomer composition, and, in some cases, average molar masses. MALDI-TOF spectra can be helpful to ascertain a variety of polymer structures, distinguishing among linear, cyclic, and branched chains, copolymers and homopolymers, supplying information on star polymers with diverse numbers of arms. Understanding of the structure of chain end groups, detecting species in trace amounts, can be crucial in polymer analysis and can confirm the success of the synthetic pathway or the introduction of chemical modifications in the polymer structure by further functionalizations as well as on the occurrence of side reactions.[53] In the last decade, a variety of biodegradable and bioresorbable polymers has been characterized by MS. Polyesters, especially poly(α-hydroxy acids), PGA, PLA and their copolymers PLGA (**Table 2**), are the principal bioresorbable polymers used in biomedical applications.[54] Among them, PLA has received significant attention due to its excellent biocompatibility and biodegradability. The major applications include resorbable sutures, implants, drug delivery systems (DDS), tissue engineering and orthopedic devices. Data concerning the synthesis (polycondensation, ring opening polymerization, chain extension), physical-chemical (thermophysical, solubility, miscibility, stereocomplexes), mechanical properties, degradation behavior, applications in medicine and pharmacy of poly(α-hydroxyacids) and PLA have been widely reported and reviewed in the literature.[55-62] MS, mainly MALDI MS, has been used in the last decade to confirm the successful preparation of PLA, PGA and PLGA copolymers.[51,52,63-65] and drug-polymer conjugate,[66,67] or check the stucture of commercial polymer samples.[34] Dria et al. described the synthesis and characterization of a series of multi-armed resorcinarene- and calixarene-core PLA star

polymers. These macrocyclic-core, four- and eight-armed star PLAs were prepared by tin(II)-catalyzed ring opening polymerizations (ROP) of L-lactide (LLA) and racemic DL-lactide (DLLA) using hydroxyl-functionalized calixarene and resorcinarene initiators. The resulting polymers had narrow dispersity (D) and molar masses close to those targeted based upon monomer/initiator ratios, as determined by SEC, NMR spectroscopy, and MALDI-TOF MS. MS analysis of selected ''lower'' molar mass samples (Mn < 20 kDa) confirmed the incorporation of the initiator within the star PLAs. The Mn and D values of the star PLAs determined by MALDI MS were slightly lower than those measured by NMR spectroscopy and SEC. The detection of adjacent peaks with $\Delta m/z$ of 72 and the presence of the half numbers of monomer units within the polymer chains in acquired spectra suggested the occurrence of trans-esterification reaction during star polymer production.[52]

Hetero-telechelic, low molar mass PLAs were prepared by the zinc-catalyzed ROP of LLA or D-lactide (DLA) using functional initiators and subsequent reaction with termination reagents, yielding –OH, –COOH, $–NH_2$ and –SH as functional chain ends. Structural characterization was performed by molar mass analysis, NMR spectroscopy and MALDI-TOF MS in the linear mode using [2-(4-hydroxyphenylazo)benzoic acid] (HABA) as the matrix. In the MS spectra, litium or sodium adduct ions related to oligomers having molar masses M = nM(LLA) + M(initiator) were detected as the main series of signals, confirming the success of the synthesis. Afterwards, the influence of the functional end-groups on the thermal behavior of PLAs, both as single enantiomer polymer chains and as their corresponding stereocomplexes, was investigated. Again, MS highlighted that, under the synthetic conditions applied for these materials, inter-molecular ester-exchange reactions occurred to a lower extent.[64] Grignard et al. reported on the successful metal- and solvent-free synthesis of homoPLAs and PLA-based di and triblock copolymers in supercritical carbon dioxide ($scCO_2$). ^1H NMR, Raman spectra, SEC, and MALDI MS were used for the characterization of the samples. MALDI MS highlighted limited transesterification reactions and confirmed the end groups of the PDLLA chains expected from the synthetic pathway. Whatever the amine cocatalyst, MALDI-TOF spectra showed one main distribution corresponding to PDLLA chains (cationized by Na^+ or K^+) bearing benzyl alcohol end group, each peak separated by 144 mass units (**Figure 4**). A limited transesterification was evidenced by the presence of a second minor distribution separated from the major one by 72 mass units. Furthermore, MALDI-TOF spectra were characterized by a less abundant third population of PDLLA chains due to a low chain initiation by water residues.[68]

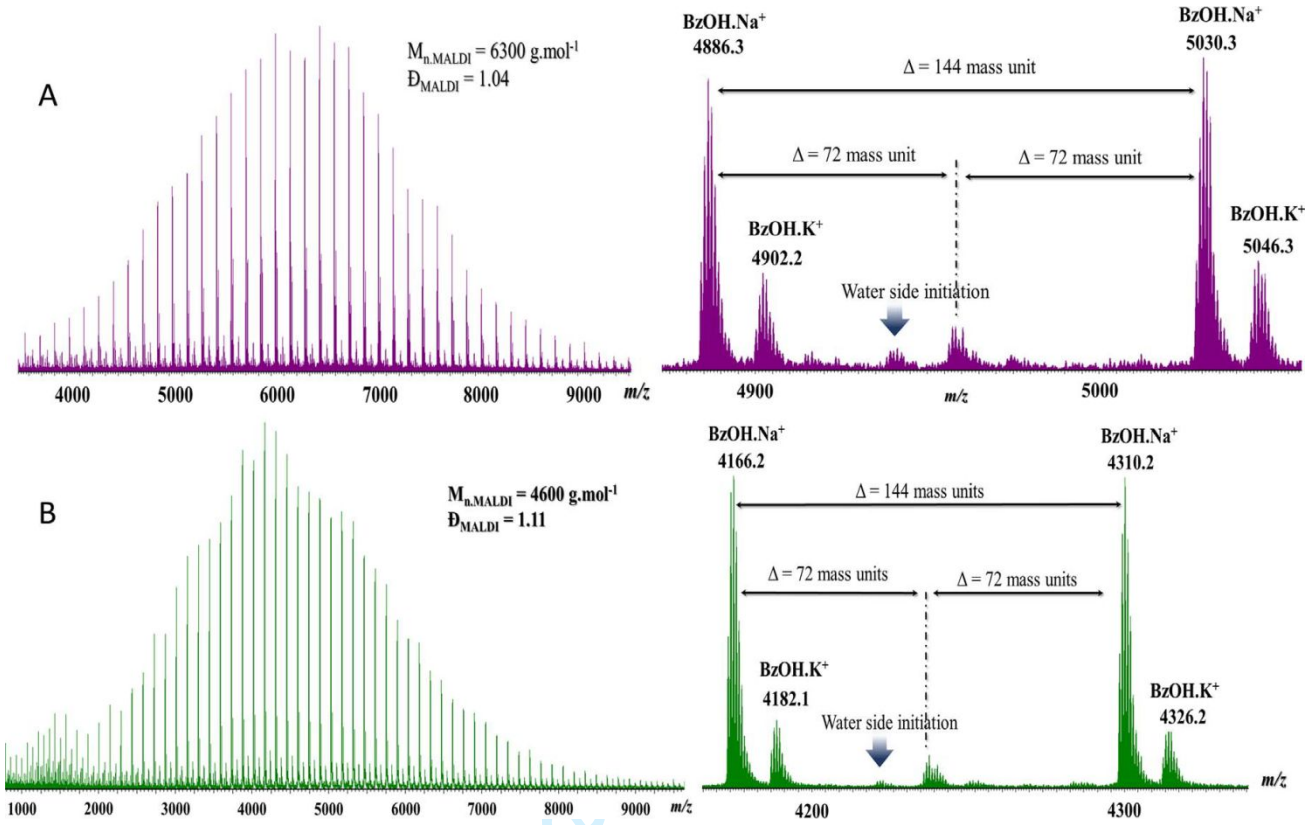

Figure 4. MALDI mass spectrum recorded for P(D,L-lactide) synthesized using (A) TU/PMDETA and (B) TU/DBU as catalytic systems and benzyl alcohol as an initiator. **Reprinted with permission from Grignard et al.,[68] copyright (2017) Elsevier**.

Liénard et al. prepared cyclo-PLAs (Mn ≈ 4 000 g/mol, purity range 93-99.9 %) by the optimization of the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction applied on α-azide-ω-alkyne linear PLA precursors. The structure of the PLA precursors was established by ^{1}H NMR and MALDI-TOF. The success of cyclization high efficiency was confirmed by traditional methods (^{1}H NMR and SEC) and was also attested by IM-MS, able to discriminate linear from cyclic polymer ions.[69]
Very recently, Rizzarelli et al. both by pyrolysis-gas chromatography mass spectrometry (Py-GC/MS) and NMR analyses showed the presence of 1-dodecanol end groups in a commercial medical-grade PLA (PURASORB® PL 10). 1-dodecanol end groups were also confirmed by ESI MS carried out on the PLA oligomers. Moreover, ICP-MS highlighted the presence of metals, among which Sn, Fe and Cu that can influence polymer degradation.[34] High-resolution ESI MS (HR ESI MS) of poly(tartronic-*co*-glycolic acid) confirmed the existence of a polymer/oligomer mixture but the interpretation of mass spectra was complicated, in particular in the higher mass ranges, because of the multiply charged ions due to the pendent carboxyl groups.[70] Recently, Chen et al. prepared pyrene-labeled polymer (PEG-PLA-pyrene) by coupling carboxyl pyrene with a PEG-PLA copolymer. MALDI-TOF MS was used to measure the detailed molar mass of the fluorescent pyrene-labeled PEG-PLA block copolymer. The peaks in the PEG-PLA spectra were separated by 44 and 72 mass units, which corresponded to the molecular weight of the oxyethylene units (44.03 g/mol) and lactyl units (72.06 g/mol), respectively. The molar mass of PEG-PLA ranged from 1,500 to 2,800 g/mol. After coupling with 1-pyrenebutyric acid, the molar mass distribution of the resulting polymer shifted to 1,800-3,200 g/mol, which indicated the chain extension of the pyrene moiety onto PEG-PLA.[71]
Hydrogels present growing interest for applications as controlled drug-delivery carriers and tissue engineering scaffolds because of their excellent biocompatibility related to the presence of large amounts of water. Among the various hydrogel systems, injectable and bioresorbable hydrogels appear

to be the most promising. In particular, the synthetic hydrogels possess a tunable chemical composition and three-dimensional physical structure that enable to control mechanical properties, biodegradation, and biocompatibility. In the literature, there are several studies addressed to the synthesis, characterization, citocompatibility and degradation of hydrogels based on biodegradable moieties.[3] Bencherif et al. synthesized a series of resorbable hydrogels by crosslinking polymerization of PEG-*co*-PGA macromonomers containing three types of end-group functionalities: diacrylates, dimethacrylates, and urethane dimethacrylates. ^{1}H-NMR and MALDI-TOF were used to follow and verify each step of the synthesis. These techniques together provided comprehensive information regarding the degree of acrylate, methacrylate, and isocyanatoethyl methacrylate conversions, molecular mass, and product purity. By varying the chemistry of the cross-linker group, the hydrophobicity of a single core polymer consisting of PGA could be fine-tuned, leading to significant variations in the mechanical, swelling and degradation properties of the gels.[67]

Poly(ε-caprolactone) (PCL) belongs to the first generation of synthetic biodegradable polyesters tested as resorbable materials, particularly in DDS. Different types of PCL block and random copolymers were synthesized and characterized with the support of diverse MS tecniques.[33,65,72-76] New copolymers of ε-caprolactone (CL) with three hydroxy-fatty acids, 12-hydroxy stearic acid, 16-hydroxyhexadecanoic acid and ricinoleic acid, were prepared by catalytic polyesterification. The syntheses were carried out in solvent-free systems and in organic solvents as well, using tin(II) 2-ethylhexanoate as catalyst, at different temperatures and molar ratios of the comonomers. The cyclic and linear chemical structures of polymeric products were confirmed by FT-IR, NMR and MALDI-TOF MS analysis. Notheworthy, the synthesis parameters were optimized and the CL/hydroxy acid molar ratio was set as 5:1 thanks to the MS results.[73] The average molar mass (Mn) and D of a series of novel triblock copolymers (PBCL-*b*-PEG-*b*-PBCL) composed of PEG and PCL-bearing benzyl carboxylate on the α-carbon of CL (PBCL) were estimated by ^{1}H NMR and MALDI-TOF. The debenzylation of the synthesized (PBCL-*b*-PEG-*b*-PBCL) copolymer was carried out to achieve copolymers with various degrees of free α-carboxyl to α-benzyle-carboxylate groups on the hydrophobic block. Incomplete reduction of PBCL led to the formation of poly(α-carboxyl-*co*-benzyl caboxylate-ε-caprolactone) (PCCL) in the lateral blocks at 27 %, 50 % and 75 % carboxyl group substitution. 2,5-dihydroxybenzoic acid (DHB) matrix with NaCl as a cationization agent was selected for the MALDI analysis. The peaks corresponding to each block series observed in the MALDI mass spectra of triblock copolymers were compared with that of PEG. In the PBCL-*b*-PEG-*b*-PBCL spectra, peaks related to the hydrophobic block containing BCL and CCL units showed peak-to-peak differences of 248 and 158 corresponding to the mass of BCL and CCL repetitive units, respectively. The spectra of not completely debenzylated copolymers showed a higher D; thus, for these copolymer samples Mn and D values, obtained by the ^{1}H NMR and MALDI-TOF methods, were not comparable.[76]

Polyhydroxyalkanoates (PHAs) are commercially-valuable biocompatible and biodegradable polymers with many potential medical, pharmaceutical and other industrial applications. They can be microbial or synthetically produced.[77] The analysis of PHAs monomeric composition is especially challenging due to the broad chemical diversity of PHA monomers and has been supported by MS methodologies.[78,79] Ge et al. proposed an on-line liquid chromatography-ESI mass spectrometry (LC-ESI MS) based strategy to elucidate the structures of unknown PHA monomers and determine the monomeric composition of seven bacterial PHA monomers after hydrolysis by the standard addition method.[78] Among bacterial PHAs, poly(3-hydroxybutyrate) (PHB) is the most extensively studied biodegradable thermoplastic polymer. It is a fully biodegradable and biocompatible but useful application of PHB has been often limited by its brittleness and narrow processing window (thermal decomposition temperature near to the melting point). Bio- or chemosynthesis of (R)-3-hydroxybutyric acid (HB) copolymers, and blending with other polymers have been adopted to overcome the above drawbacks and obtain new useful materials based on PHB. Impallomeni et al. synthesized copolymers containing HB, 1,4-butanediol (B), and adipic acid (A) by microwave-assisted transesterification of

biodegradable PHB and poly(1,4-butyleneadipate) (PBA) in solution at different reaction times, composition of the starting mixture, and amount of 4-toluenesulfonic acid, used as a catalyst. A detailed characterization of the copolyesters by diverse analytical methods was carried out. MALDI-TOF mass spectra were acquired in reflection mode using the trans-3-indoleacrylic acid matrix, allowing the microstructure and end-groups determination.[80] Chemical modifications, to introduce functional groups, add valuable features to PHAs that can not be easily achieved by bioconversion processes.[81] Kwiecień et al. described a highly selective method for controlling the degradation of PHA, via a reduction reaction by lithium borohydride, potentially useful in further synthesis of tailor-made biodegradable materials. PHA oligodiols derived from a poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) biopolyester [poly(3HB-*co*-4HB)] and from synthetic atactic PHB were prepared. The structural characterization of the PHA oligodiols was carried out by NMR, ESI MS and MS/MS, which confirmed that the obtained oligomers were terminated by two hydroxyl end groups.[82]

Elucidation of polymerization mechanisms

Both MALDI and ESI MS are frequently used in the elucidation of polymerization reaction mechanisms.[10,12] Recently, MALDI MS analyses in reflectron mode have been performed to obtain information about the structure and the mechanism of reaction in the synthesis of PLA, PGA and random PLGA by ROP, using sodium hydride as the environmentally friendly and nontoxic initiator. The nominal mass of the repetitive unit in PLA is 144 g/mol (**Table 2**). A broadened distribution was revealed with a series of peaks spaced by 72 mass units, instead of 144 mass units, confirming that significant transesterification reactions occurred. The presence of alcohol-functionalized end groups, observed by NMR and MALDI, supported the hypothesis of anionic ROP mechanism operating by cleavage of acyl-oxygen bond of the cyclic di-esters. Furthermore, MALDI showed the presence of carboxyl end groups, reasonably derived by the anionic initiation by cleavage of the alkyl-oxygen bond of the monomers.[63] Multidimensional MS methodologies, interfacing MALDI and ESI with TOF mass analysis, tandem MS (MS/MS) fragmentation and/or ion mobility MS (IM-MS), have been employed to elucidate the structural details (composition, end groups, chain sequence and isomeric purity) of the copolyesters poly(propylene maleate) (PPM) and poly(propylene fumarate) (PPF). In particular, MALDI mass spectra showed one major and two minor distributions having different end groups (EGs) and having the composition $[R_n + EG_s + Na]^+$ in which R is the propylene maleate/fumarate copolyester repeat unit ($C_7H_8O_4$, 156 g/mol) (**Figure 5**). The detection of C_2H_5O-chain end was related to $Mg(OC_2H_5)_2$ catalyst used in the synthesis, which evidently behaved also as initiator of the polymerization; conversely, the –H end group was introduced upon termination with aqueous hydrochloric acid. The minor products (symbols B and C in **Figure 5**) highlighted the incorporation of one or two additional propylene oxide comonomers, respectively (see structures in Figure 4); these byproducts pointed out that some oligomerization of propylene oxide may occur during copolymerization. The ESI-MS/MS and MALDI-MS/MS helped to establish the copolymer connectivity and confirmed the end group natures (**Figure 6**). Additionally, IM-MS was used to differentiate the isomeric PPM and PPF samples and evaluate the extent and efficiency of PPM to PPF (i.e. all-cis to all-trans polymer chains) isomerization (**Figure 7**).[20]

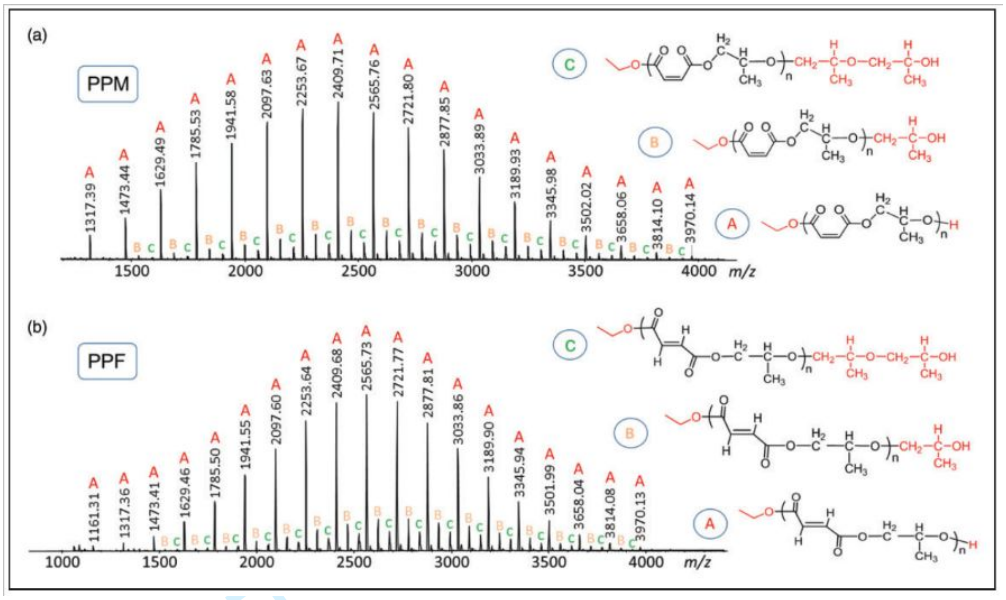

Figure 5. MALDI mass spectra of **(a)** poly(propylene maleate) (PPM) and **(b)** poly(propylene fumarate) (PPF). All ions are sodiated species with the composition $[R_n + EG_s + Na]^+$, where R and EG_s designate the PPM/PPF repeat unit ($C_7H_8O_4$, 156 Da) and the corresponding end groups (in red color), respectively. **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

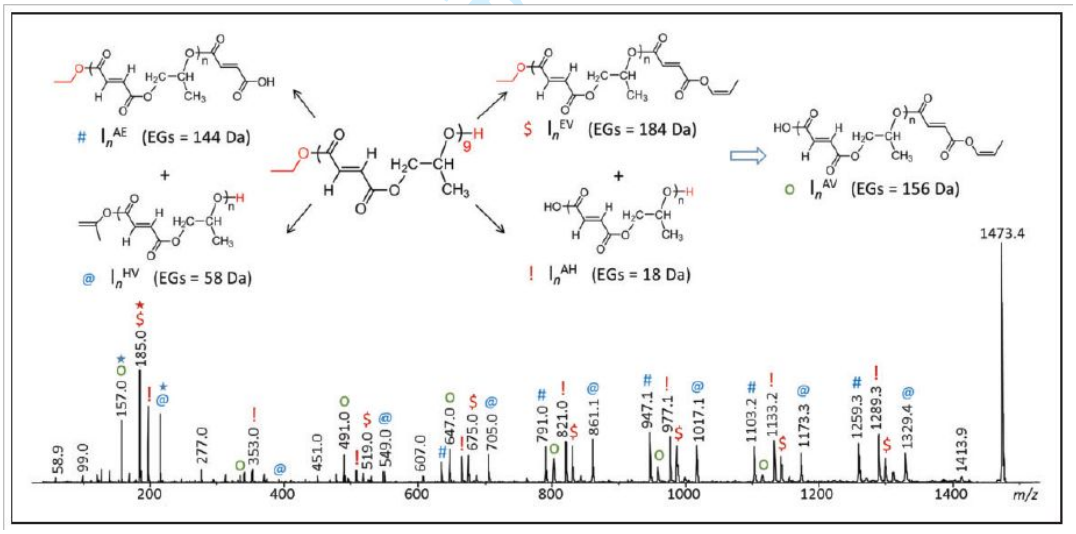

Figure 6. MALDI-MS/MS spectrum of the $[M + Na]^+$ ion from the PPF 9-mer with CH_3CH_2O– and –H end groups (*m/z* 1473.4). The scheme on the top shows the fragment ions arising from 1,5-hydrogen rearrangement over ester groups facing the CH_3CH_2O- ($, !) or -H (#, @) chain end. Consecutive dissociation of these fragments (⇒) leads to internal fragments (o). The Na^+ ion has been omitted for brevity. An asterisk above the fragment notation (*) indicates fragments ionized by H^+ (Na^+ is eliminated with the neutral fragment). **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

IM-MS analysis separated PPM and PPF ions according to their drift time through the IM region (IM dimension), which depends on the charge and collision cross-section (CCS or Ω) of the ions, and by their *m/z*, which is determined by the composition and charge (MS dimension) of the ions. **Figure 6a** shows the result of such 2D analysis for PPF ionized by ESI. The ions are separated based on their

charge state (+1 to +3) into unique 2D locations with specific drift times and *m/z* ratios. The singly charged species are the most intense; the mass spectrum extracted from their mobility region (+1 region in **Figure 7a**) is depicted in **Figure 7b** and clearly showed that ESI conditions partly degraded the polyesters, probably because of electrochemically produced acid at the ESI electrode. Nevertheless, a significant portion of the PPF with the end groups introduced during polymerization (i.e. CH_3CH_2O– and –H) survives intact to permit IM-MS analysis of the corresponding $[M + Na]^+$ ions, which had the composition $[R_n + C_2H_6O + Na]^+$. By the ESI-IM-MS drift time distributions (IM-MS chromatograms) and comparing the measured and calculated collision cross-sections (Ω) of singly sodiated PPF and PPM oligomers a quantitative assessment of the influence of cis vs. trans double bond geometry on the resulting macromolecular architecture was obtained.[20]

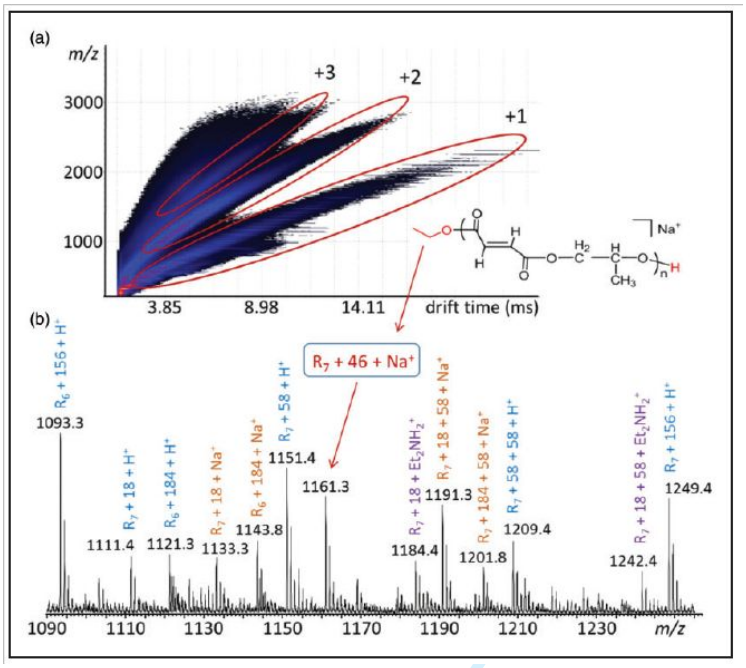

Figure 7. (a) 2-D ESI-IM-MS plot (*m/z* vs. drift time) of PPF; the mobility regions of singly, doubly and triply charged ions are encased in ovals. **(b)** Mass spectrum extracted from the region of singly charged ions, containing several ion distributions which include intact PPF ions with CH_3CH_3O– and –H end groups (46-Da end group mass) and degradation products with various end group masses (noted after the number of repeat units; see **Figure 5** for plausible structures). Charge is provided by addition of H^+, Na^+ or $(C_2H_5)_2NH_2^+$ (from residual PPM to PPF isomerization reagent). PPM leads to very similar ESI-IM-MS characteristics, except for the absence of $(C_2H_5)_2NH_2^+$ adducts. **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

Surface analysis and MS studies on distribution of bioactive substances or drugs in bioresorbable polymeric matrices

Biodegradable and bioresorbable polymers are widely used in medical applications to provide scaffolding for cell growth and proliferation as well as drug release.[83] Consequently, the analysis of polymer surfaces can be relevant since biological events occur when the surface come in contact with biological media. SIMS is a powerful tool that can be fruitfully used to get detailed information on the polymeric surface.[84] TOF-SIMS was recently employed for monitoring the selective protein adsorption on mixed polymer brushes composed of poly(ethylene oxide) (PEO), a protein-repellent polymer, and poly(acrylic acid) (PAA), a weak polyacid whose conformation changes according to the pH and ionic strength of the surrounding medium. A mixture of lysozyme (Lyz), human serum albumin (HSA), and human fibrinogen (Fb) was used to demonstrate the success of this strategy.[29]

Electrospinning (ES) of polymer solutions generates non-woven webs of nanofibres. The fibre diameter ranges between 10 nm and 1 μm depending on the operating conditions. Surface functionalisation can be performed by the use of suitable additives. Detailed characterisation of the molecular composition at the fibre surface is a key issue. Van Royen et al. prepared biodegradable nanowebs with potential antibacterial activity by ES of solutions containing PCL and a functionalising additive with PCL segments and hexyldimethylammonium groups (PCLhexaq). SSIMS has been applied to characterize the surface functionalisation of electrospun nanofibres. In particular, the method yielded both qualitative and quantitative information on the molecular composition of the outer monolayer of individual nanofibres. Detailed interpretation of the positive ion mass spectra allowed complete diagnostic information on each of the surface components to be obtained in spite of the fact that the PCL fibre matrix and the PCLhexaq additive are structurally quite close to each other and in spite of the low additive concentration (0.16-1.4 % w/w relative to PCL). Imaging of structural ions highlighted the homogeneous distribution of PCLhexaq over the individual fibre surface. Additionally, quantifying the surface concentration of PCLhexaq relative to that of PCL revealed electric field-driven surface enrichment of the additive during ES. Finally, the SSIMS analysis of fibres exposed for increasing periods to an aqueous solution showed that the additive surface concentration decreased to 56 % in about 72 h, almost linearly with time at a rate of 0.6 % h^{-1}.[33] Bege et al. investigated a reproducible spray-coating process for stents coated with poly(ethylene carbonate) (PEC) and Paclitaxel, a natural product with antitumor activity. They clearly showed that TOF-SIMS analysis is a useful method to verify the order of the coating layers and the study paved the way to examine drug eluting stents with a chemically sensitive technique.[28]

Dynamic SIMS can provide deph profiles and bulk analysis, useful to determine the distribution of drug or bioactive substance or three-dimensional imaging of surface modifications in scaffold pores.[30,85] Burns et al. formulated two poly(α-hydroxy esters), PLA and PLGA 80/20, with a surfactant stabilizer (Aerosol-OT, AOT) to encapsulate the protein keratinocyte growth factor (KGF) for its controlled release. KGF is involved in a number of crucial biologic processes, most notably epithelial growth and repair. The membranes were analyzed by TOF-SIMS to determine the distribution of KGF and AOT within the film. They used an instrument equipped with a C_{60} polyatomic ion source that can be used as a sputtering gun during depth profile analysis. Depth profiling was used to determine the relative AOT and KGF peak intensity in comparison to that of PLA, before and after the soaking procedure in phosphate buffer solution (PBS). The depth profile analysis revealed that both the PLLA and PLGA membranes had a high-surface AOT ion signal at the zero-time point. After a soaking/washing procedure, the intensity of the AOT peak at the surface decreased substantially in both the PLLA and PLGA membranes (**Figure 8a, c**). This decrease in AOT at the surface allowed cells to adhere to the polymer membranes. Conversely, the KGF present in each of the polymer membranes was almost unaffected by the soaking period (**Figure 8b, d**).[30]

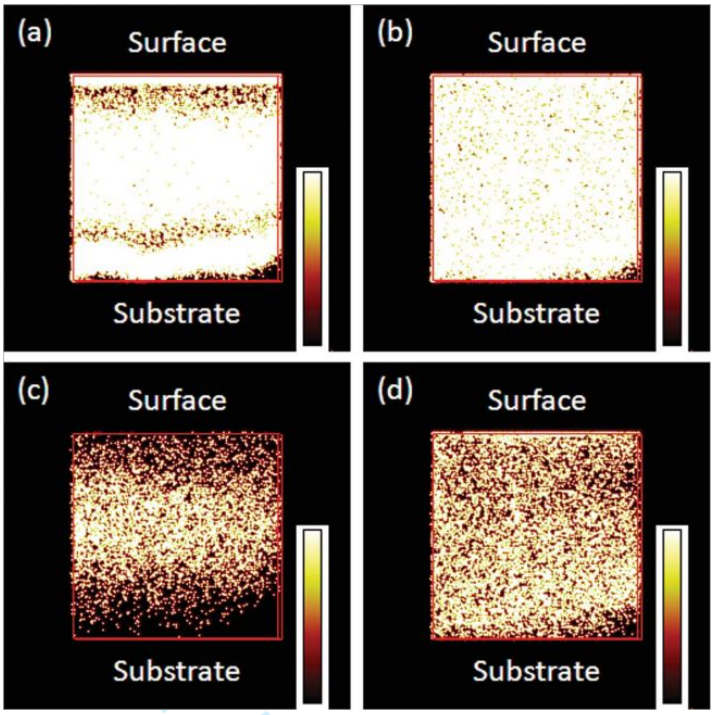

Figure 8. (a) and **(b)** PLLA/AOT/KGF at 0 h **(c)** and **(d)** PLLA/AOT/KGF at 24 h soak time. The depth of each film was measured by profilometry to be ~500 nm for both the 0- and 24-h time point. The entire depth of the membrane was sputtered using C_{60}^{+++} until the substrate (Si) was reached for each profile. 3D reconstruction using Ion-ToF software of TOF-SIMS depth profiling data. **Figure 8 (a,c)** represent the distribution of AOT at the surface of a PLLA/AOT/KGF membrane at the 0 h and 24 h soak time. **Figure 8 (b,d)** represent the distribution of KGF at the surface of a PLLA/AOT/KGF membrane at the 0 h and 24 h soak time. The zero-time point has a high-ion signal of AOT at the surface and a depletion zone where little AOT is present. The surface layer of AOT above the depletion zone is removed after the soaking procedure in PBS solution. The distribution of KGF is more concentrated at the 0-h time point versus the 24-h time point but is still present through out the surface and bulk of the PLLA polymer membrane. Similar results were obtained from the membranes composed of PLGA/AOT/KGF. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]. **Reprinted with permission from Burns et al.,[30] copyright (2010) John Wiley and Sons**.

SIMS is not the only technique employed in the characterization of the surface of bioresorbable polymer items. Typically, SIMS shows a relatively high noise level. This represents a limitation in imaging devices with low drug concentrations, such as in drug-eluting stents (DESs). To overcome this restriction, a MALDI Qq-ToF method was developed to acquire information on the distribution of chemical species (drug, drug-related degradation products or polymer-related degradation products) on the stent surface (or at different depths of the polymer coating). In particular, the molecular imaging MALDI Qq-TOF method was developed for analyzing the drug (sirolimus) distribution on CYPHERW and NEVO™ DESs. The technique proved to be powerful in studying drug distribution. Pre-selected concentrations of the matrix solution (alpha-cyano-4-hydroxycinnamic acid in methanol) were sprayed with an automated sprayer to ensure an evenly distributed matrix deposition on the samples. The coating matrix, laser energy, laser frequency, spatial resolution (related to rastering speed) and mass spectrometer parameters were optimized to analyze drug distribution in both durable and biodegradable polymer matrices. The developed method allowed low level detection of the target molecule without biological interferences from the blood or tissue and could be further extended.[86]

Tandem MS analysis and fragmentation studies

Tandem MS (MS/MS) is increasingly applied to analyse synthetic polymers since it can provide information on chain-end or in-chain substituents, discriminate isobaric and isomeric species, differentiate linear and cyclic polymer and establish macromolecular connectivities, sequences and architectures.[87,88] In fact, in several cases, single-stage mass data may not be sufficient to unequivocally establish the polymer structure. For confident structural assignments, the fragmentation studies are propaedeutic to understand tandem mass spectra. In fact, knowledge of fragmentation mechanisms of polymer ions provides guidelines on how to get the desired information from the fragment ions detected in MS/MS spectra and deduce the truthful macromolecular architecture.[24] Amongst the tandem MS tools, the collision induced dissociation (CID) approach represents the privileged technique for a deep structural characterization of gas-phase ions.[17] The low-kinetic energy CID behavior of different sodium-cationized PLA oligomers was thoroughly investigated by De Winter et al. Investigation of several end-groups modified PLA revealed that, in addition to the expected end-group specific fragmentation pathways, upon collisional activation, PLA Na^+ systematically underwent end-group specific dissociations. Those dissociations proceeded through favorable six-membered ring transition states (McLafferty-like rearrangement). Consecutive and competitive fragmentations were also highlighted and were due to progressive fragmentations of the oligomer chain starting from both the end-groups. In addition, the collisionally-excited PLA Na^+ competitively suffered from two sequential backbone cleavages leading to sodium-bound dimer and trimer cations that finally caused the loss of a monomeric residue, corresponding to neutral acrylic acid. The experiments, performed on a hybrid Q-ToF instrument, were also supported by a theoretical study.[89] ESI-MS/MS has been extensively used for the end groups, degree of purity and sequence analysis of natural and synthetic biodegradable copolyesters.[9,90-94] The success of the synthetic routes adopted to obtain pesticide[91] and lipoic acid[92] oligo(3-hydroxybutyrate) (OHB) conjugates was verified by NMR and ESI-MS/MS. The ESI-MS/MS experiments, performed on selected Na-adduct of the pesticide- and lipoic acid-OHB conjugates, established that respectively the initiators, (4-chloro-2-methylphenoxy)acetate or (2,4-dichlorophenoxy)acetate, and the lipoic acid were covalently bonded to the OHB chains. Scionti and Wesdemiotis validated the electron transfer dissociation (ETD) as a suitable complementary ESI-MS/MS technique for the characterization of biodegradable polyesters and compared the resulting MS/MS spectra with those generated by the classical collisionally activated dissociation (CAD) method on the same set of precursor ions. The compounds studied included PLA and two copolymers based on ethylene and butylene adipate units. CAD of $[M+2Na]^{2+}$ ions from these polyesters was suggested to proceed via charge-remote 1,5-H rearrangements over the ester groups, leading to cleavages at the (CO)O-alkyl bonds. ETD of the same precursor ions created instead a radical anion at the site of electron attachment, which were broken by radical-induced cleavage of the (CO)O-alkyl bonds and by intramolecular nucleophilic substitution at the (CO)-O bonds. One of the advantages over the classical CAD method is that ETD does not activate consecutive fragmentation reactions in any significant extent, which simplifies spectral interpretation and permits conclusive identification of the correct end groups.[93] Josse et al. developed a tandem MS-based method to determine the degree of purity achieved in the cyclization of a linear PLLA prepared by Cu-catalyzed alkyne-azide cycloaddition. Outstandingly, when the traditional polymer characterization techniques (^{1}H NMR, SEC and single-stage MS) were unable to prove the presence of residual linear polymer, the designed ESI-MS/MS methodology allowed the detection of trace amounts (< 5%) of the starting material, as a result of radically different CID behaviours. The developed technique could be readily adaptable to other isomeric macromolecules displaying different CID characteristics.[94]

MS researches on polymer-conjugates

Both MALDI and ESI MS, in some cases with the support of MS/MS and/or IM-MS, have been used in the characterization of polymer-conjugates, to confirm the effective functionalization and the success of the synthetic approch.[66, 95-107] The transesterification reaction of PHAs has been used as a

strategic and simple tool for the synthesis of delivery systems for selected bioactive compounds, holding carboxyl or hydroxyl functionalities. The structures of transesterification products were established at the molecular level by ESI-MS/MS and ^{1}H NMR.[95-97] The transesterification reaction of bacterial biopolymers with tyrosol was applied as a convenient method for obtaining polymer-conjugates. Tyrosol is a naturally occurring phenolic bioactive compound with a hydroxyl group. Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) (P(3HB-*co*-4HB)) and poly-γ-glutamic acid (γ-PGA) were selected as biodegradable polyester and polyamide. The (trans)esterification reactions were carried out in melt mediated by 4-toluenesulfonic acid monohydrate. The structural characterization by ESI-MS/MS confirmed that the developed method leads to the formation of conjugates in which bioactive compounds are covalently bonded to biopolymer chains. Kwiecien et al. showed that transesterification of P(3HB-*co*-4HB) with tyrosol leads to the (3HB-*co*-4HB) oligomers that contain one bioactive molecule covalently bonded to the oligomer chain, while esterification of γ-PGA with tyrosol results in conjugates with increased amount of biologically active moieties along the oligomer chain.[95] Pignatello et al. described the synthesis and characterization of different series of mono- and bis(carboxy)- and (amino)-PEG (2000 – 5000 Da) amphiphilic conjugates with α-lipoamino moiety (LAA). The structure of the synthesized conjugates was confirmed by ^{1}H-NMR, FTIR and MALDI-TOF.[98-100] PHB found applications in biomedical fields, including surgery and DDS. Because of its insolubility in most solvents, PHB-based DDSs are usually prepared through a limited number of techniques, such as spray-drying and high-pressure homogenization, while simpler approaches, like solvent evaporation methods, are unsuccessful in obtaining micro- and nanoparticles. Applying a solvent-deposition method to synthetic poly(3-hydroxybutyrate-*co*-ε-caprolactone) (P(HB-*co*-CL)) copolymers and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-caprolactone) (P(HB-*co*-HV-*co*-CL)) terpolymers, Pignatello et al. produced colloidal micro- and nanosuspensions. They described the synthesis and a detailed analytical characterization of the terpolymers carried out by SEC for MM, NMR to check sequences and composition, MALDI-TOF and SEC/MALDI-TOF for the end group analysis. They observed that the composition and crystallinity of the tested polymers affected the type and size of micro- or nanoparticles, whereas the MM mainly influenced the probes encapsulation and release.[101] Among various smart materials, thermo-responsive bioresorbable polymers have been studied intensively because of the easiness to handle their temperature changes, such as from room temperature to steady human body temperature.[102-104] Recently, the synthesis, characterization and application of a thermo-responsive polyhydroxyalkanoate-*graft*-poly(N-isopropylacrylamide) (PHA-*g*-PNIPAm) has been reported. The complex structures and MMs of the intermediates and products were confirmed by ^{1}H-NMR, MALDI-TOF MS and SEC.[105]

Bioconjugates are hybrid materials containing biomolecules covalently linked to synthetic polymers among which PEG is the most widely used. PEGylation, i.e. covalent attachment of PEG to other molecules, is primarily applied to therapeutic peptide and protein drugs. PEGylated therapeutic drugs are employed for the treatment of several chronic diseases. These compounds and several similar bioconjugates can not usually be made with high purity and average spectroscopic methods, such as NMR or X-ray diffraction spectroscopy, can not provide an adeguate structural characterization. This drawback has been overcome by MS, supported by separation techniques and MS/MS fragmentation.[24] IM-MS, which interfaces dispersion according to *m/z* (MS dimension) with collision cross section and charge (IM dimension), provides further separation efficiency as well as shape/size selectivity. Building a multidimensional technique, by combining soft ionization methods (i.e. MALDI or ESI) with IM-MS and MS/MS fragmentation, relevant insights into the composition, structure, and architecture of bioconjugates and other complex biomacromolecules has been gained. Sallam et al. highlighted the usefulness of combining MALDI, ESI, MS/MS and IM-MS experiments for the comprehensive characterization of the primary structure and architecture of a polyether dendron conjugated with two different bioactive peptides.[106] Additionally, the MS/MS and IM-MS/MS techniques were applied by Alawiat et al. in the determination of the sequence, derivatization site, and conformation of two alanine-rich peptides (AQK18 and GpAQK18, Gp: Lpropargylglycine) and their

conjugates with PEG. In particular, the sequence and conformation of AQK18 and GpAQK18 polypeptides and their conjugates with PEG were revealed by MALDI MS, ESI MS, MS/MS fragmentation while shape-specific dispersion by IM-MS. MS/MS fragmentation studies by both MALDI and ESI asserted that the PEG chain was attached to the C-terminus of the peptides. Remarkably, the IM-MS experiments showed the existence of random coil and helical conformers in both the peptides and bioconjugates. Moreover, the IM-MS data also suggested that the helical structure was stabilized by PEG attachment at the C-terminus.[107]

MS analyses of polymeric excipients

The development of excipients can strongly influence the progress of potential new drug carriers. They are defined as inactive ingredients, which are mixed with active pharmaceutical constituents to yield a drug product that is ready for a specified target. Excipients may enhance the bioavailability and stability of the drug, as well as enable the construction of drug forms of controlled or localized substance release.[108] Polymeric excipients constitute a very large and varied family of substances. A detailed characterization is required as assurance of safety, efficiency and quality. Modern MS techniques, being able to examine individual components and discriminate components in a mixture, provide a valuable support also for the characterization of polymeric excipients. Hurtado et al. analysed by high resolution MS two polymeric excipients, i.e. Gelucire 44/14 and polysorbate 80, typically used in drug delivery formulations and also as cosmetic and food additives. These excipients are known to improve solubility of poorly water-soluble drugs and, therefore, increase their effective bioavailability. High resolution Fourier transform ion cyclotron resonance MS (FTICR MS) was used to compare two different batches of Gelucire 44/14 and polysorbate 80. Thanks to the high resolving power of FTICR MS, it was possible to discriminate between batches of excipients from differences in the identified components and detect additional constituents respect to those assigned by lower resolution TOF MS. In particular, the improved resolution provided by FTICR MS allowed the identification of four polymeric series differences in the two batches of polysorbate 80 and showed the presence of one compound and three polymeric series differences in the two batches of Gelucire 44/14 (**Figure 9**).[119]

Surfactants are extremely important, versatile excipients. They can be divided into anionic, cationic, and nonionic surfactants. The in-depth characterization of surfactants can be a challenge, since many industrial products are mixtures of variable composition. In the past, mass spectrometric methods, mainly MALDI MS, has been successfully applied to characterize several ethoxylated surfactants, widely used by the pharmaceutical industry.[110] Polysorbates are a distinctive class of nonionic surfactants that include fatty acid ester moieties bonded to PEO chains condensed onto a sorbitan core. They have a star-branched structure and are used to improve the solubility of hydrophobic analytes. Erdem et al. provided an in-depth characterization of polysorbate 85 by two different multidimensional techniques, reverse-phase LC or IM separation, supported by online ESI MS and MS/MS. Interactive LC in reverse-phase mode allowed separating the oligomers of the surfactant according to their hydrophobicity/hydrophilicity balance. Instead, IM dispersed the surfactant oligomers in relation to their charge and collision cross section (i.e. size/shape). By both separation method, an increased number of fatty ester groups and/or absence of the polar sorbitan (or isosorbide) core led to higher retention/drift times, allowing the separation of isobaric species or with superimposed isotope patterns, so that their ester content could be unambiguously identified by MS/MS. Moreover, LC-MS and IM-MS let to detect several byproducts besides the main PEO-sorbitan oleate oligomers. Remarkably, the optimized LC-MS experimental conditions provided the necessary separation resolution for the quantitative determination of the degree of esterification. Even though, IM-MS reduced analysis time and solvent consumption, in such case, LC-MS/MS enabled a more complete analysis.[111]

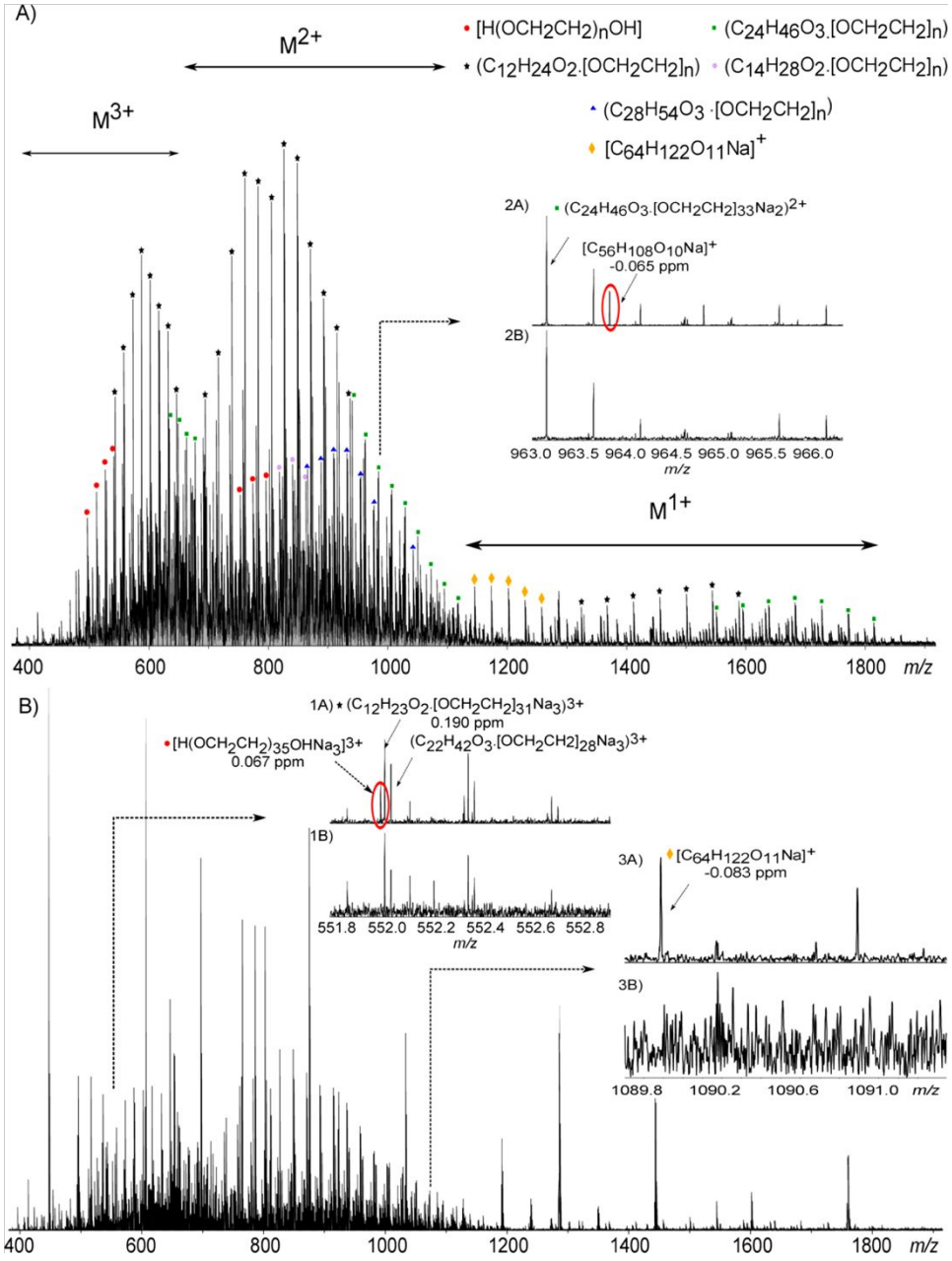

Figure 9. (a) Spectrum of polymeric distribution patterns in Gelucire 44/14. **(b)** Spectrum of polymeric distribution patterns in Gelucire 44/14. Inserts 1A-1B, 2A-2B, and 3A-3B show the differences between the two spectra. **Reprinted with permission from Hurtado et al.,[109] copyright (2012) American Chemical Society**.

III. Bioresorbable polymer degradation studies by MS

Polymer degradation is one of the most significant areas of polymer chemistry being a major factor restraining application of these outstanding and versatile materials. Polymer degradation can involve diverse processes and named as thermal, thermo- or photo-oxidative, chemical, thermo-mechanical, biological, etc. (**Figure 10**). Whatever the cause (heat, light, microorganisms, or chemicals), the deterioration mainly gives rise to degradation products usually with characteristic functional and end groups, which can be identified by different analytical methods (MS, NMR, FTIR) and highly informative of the degradation mechanism. The degradation can be stimulated by more than one environmental factor and it is the consequence of permanent structural changes that are usually undesirable or, in some cases, needed, as in biodegradation or recycling or in light-responsive degradation systems, otherwise stimulated to support structure elucidation, such as in pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) studies.[112]

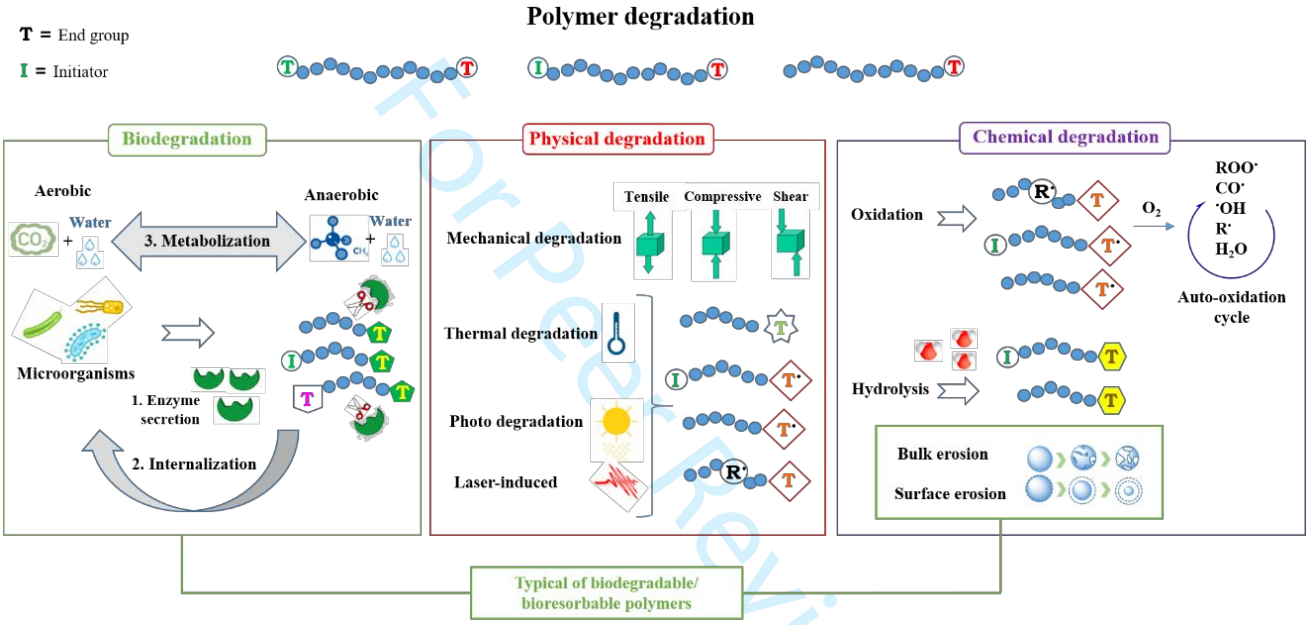

Figure 10. Polymer degradation processes.

The peculiarity of a device made of a bioresorbable polymeric material in comparison with a nonresorbable one is that not only the device itself but also the degradation products must show a biocompatible behavior. Therefore, in the designer of a bioresorbable device it should be helpful to exactly estimate the quantity, size and typology of the products originated from the degradation process and establish whether they are well-matched with the timing in which the phenomenon occurs. To escape a harmful decay of the biocompatibility, great attention must be paid to potential low MM degradation products, that can produce tissue damage. The design of a safe and effective device must consider the entire medical device life cycle and include considerations about processing, manufacturing, sterility and shelf life. All these steps contribute in producing a reliable device, above all when bioresorbable materials are used. MS can provide a valuable support in bioresorbable polymer development providing detailed qualitative and quantitative information on degradation products and on polymer modifications induced by different deterioration causes (**Table 1**, **Figure 11**).

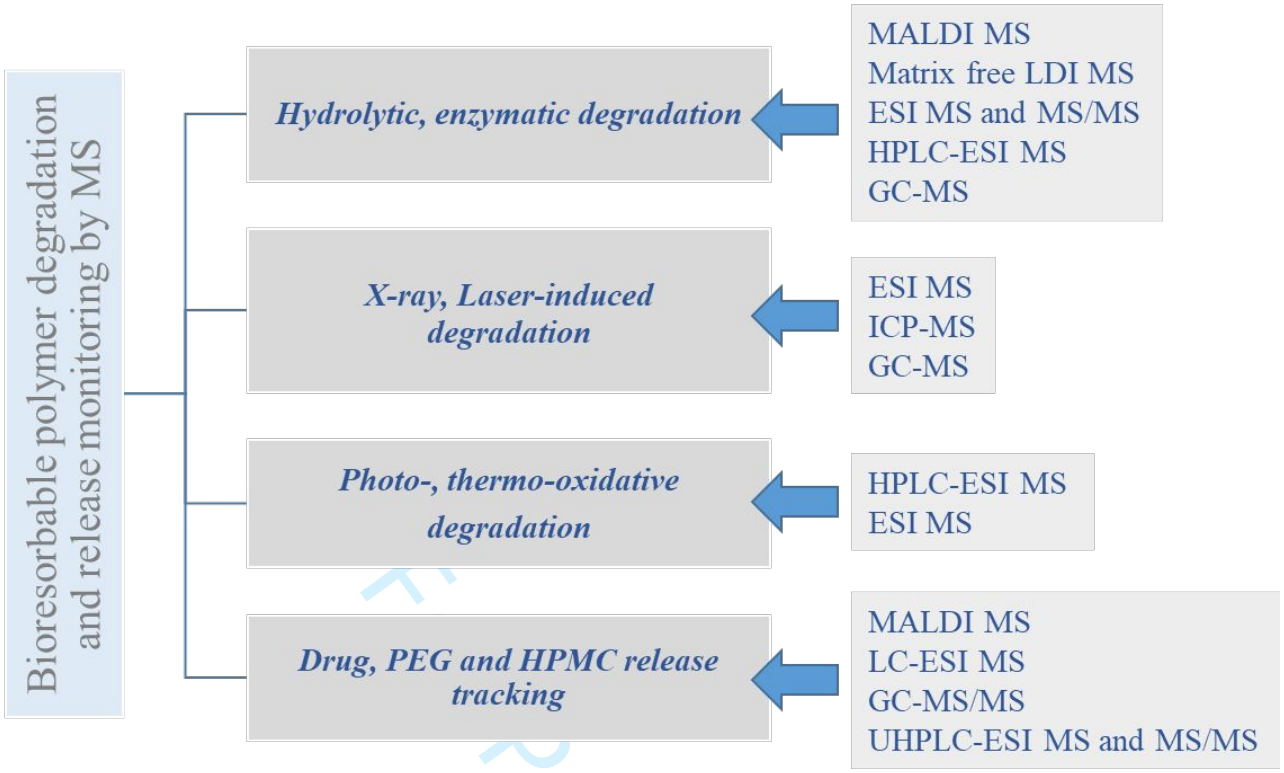

Figure 11. Overview of the MS techniques used in bioresorbable polymer degradation and release tracking.

The deterioration influences the physical, chemical, and mechanical properties. Tracking and controlling degradation requires understanding of many different phenomena, among which the diverse chemical mechanisms underlying structural changes in macromolecules, the influence of additives, the interactions of fillers, etc. Morphological, compositional, crystallinity, mechanical properties, molar mass, and surface wettability changes are checked to acquire information on degradation mechanisms and kinetics of bioresorbable polymers. In fact, the knowledge of degradation mechanisms and rates are crucial to choosing and designing bioresorbable polymers for a specific biomedical application, such as DDSs. Noticeably, degradation rate can be modulating by polymer structure and/or composition. The degradation kinetic of aliphatic polyesters, for example, can be tailored by the introduction of functional groups in their backbone, leading to a modification of their morphology, hydrophilicity and wettability,[113] or by crosslinking,[114] improving also their mechanical properties.[115-117]

The progress of hydrolytic or enzymatic degradation can be studied by a broad range of techniques. MS methods for their high sensitivity, selectivity, and speediness offer the opportunity to explore the structural details in polymer degradation.[9,27,92,118,119] ESI MS represents the most useful technique for characterizing low molar mass polymers possessing different end group structures, with the advantage of being easily interfaced with solution-based separation techniques such as HPLC.[9,75,120] Profiling of product formation by ESI MS and MS/MS has offered new routes to identify damage markers for use in tracking and controlling oxidative damage to polypeptides.[121] An HPLC-MS/MS method has been optimized to track the in vivo degradation of zein porous scaffold.[122] Recently, GC/MS was used to check the chemical structure of the enzymatic degradation products originated from PCL copolymers,[73] and GC tandem MS in drug release to measure the residual prostaglandin E2 (PGE2) content into biodegradable PLGA microspheres.[123]

Due to their good mechanical properties, their biodegradability and biocompatibility aliphatic polyesters derived from cyclic monomers, such as lactones or lactides, are widely used for biomedical

applications such as surgical sutures, DDSs and tissue engineering scaffolds. Both enzymatic and chemical catalyzed hydrolytic degradation of PCL have been extensively investigated, showing that several lipases accelerate the degradation significantly. It has been shown, that degradation preferentially occurs in the amorphous domains. Increasing the amorphous domains of a PCL based material can be used to modify the degradation rate and thus make it fit for new applications. With the support of ESI MS analysis, crosslinking has been shown to reduce the crystallinity implying a higher degradation rate.[114] Additionally, in situ cross-linking of PCL fiber with bis-(ε-caprolactone-4-yl) (BCY) was shown to be a feasible approach to avoid the reduction in molar mass of PCL during melt-spinning. ESI MS was used to evaluate the effect of in situ cross-linking on the degradation profile of melt spun PCL fibers with different amounts of BCY. Remarkable differences in the degradation product profiles with linear, cyclic or BCY-related low molar mass compounds were detected, noticeably proving the influence of cross-linking and processing on the degradation process and water-soluble products.[115]

Interestingly, Vermet et al. functionalized a 100 % PLLA resorbable knit with a polymer of cyclodextrin (polyCD) to provide a reservoir capacity towards antibiotic (ciprofloxacin), to extend locally the release of the antibiotic and obtain an antibacterial efficiency against generally encountered bacteria. Concerning the cyclodextrin finishing process, the main challenge was to define the curing conditions in order not to alter the PLLA material and preserve its bio-resorbability and its mechanical properties. The degradation products of polyCD resulting from the hydrolysis of the polymer were analyzed by HR ESI MS in positive ion mode coupled with LC and supported by MALDI analysis.[124]

A resorbable device for ligation of blood vessels was made of poly(glycolide-*b*-trimethylene carbonate) (poly(GA-*b*-TMC)) triblock copolymer by injection molding. The developed device was tested in vitro to reveal the evolution of degradation products and changes in mechanical properties. A new rapid matrix-free laser desorption ionization-MS (LDI-MS) method was developed for direct screening of degradation products released into the degradation medium. The combination of LDI-MS and ESI MS analyses enabled the comparison of the degradation product patterns in water and buffer medium (**Figure 12**). The detected degradation products ranged from linear TMC monomer to oligomers with up to 10 repeating units with $m/z < 1000$ Da. The most abundant ions in the buffer solution was due to the oligomers with two repeating units of GA and three of TMC while the most abundant one in the water fraction contained one repeating unit of GA and two of TMC. The detected ions were related to oligomers generally with a smaller number of GA than of TMC units, which was in agreement with the ESI MS analysis. Overall, LDI-MS analysis showed a TMC-rich degradation product pattern in both water and buffer solution, indicating preferential degradation of the soft blocks consisting of random GA-TMC units.[125]

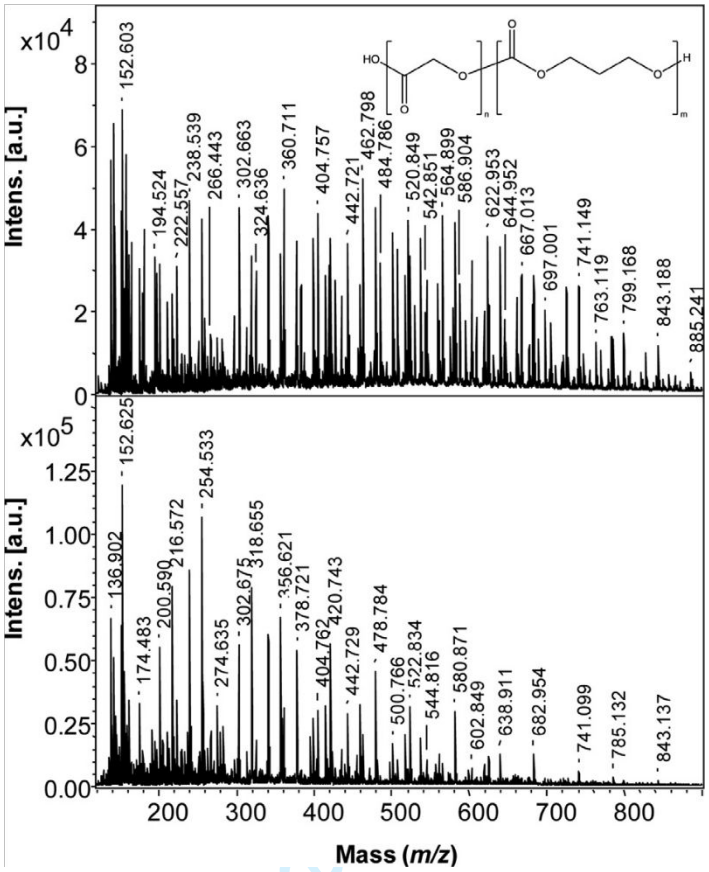

Figure 12. LDI-MS spectrum of poly(GA-*co*-TMC) degradation products in buffer (above) and water (below) after 60 days of hydrolysis. **Reprinted with permission from Aminlashgari et al.,[125] copyright (2013) Elsevier**.

Poly(ester amide)s (PEAs) have emerged in the last years as an important family of biodegradable and bioresorbable synthetic polymers. Currently, several papers in the literature describe the synthesis, characterization, degradation and biomedical applications of biodegradable PEAs.[27,126-133] HPLC-ESI-TOF/MS was successfully used for monitoring the enzyme-mediated degradation of degradable multiblock PEAs based on natural amino acids, such as lysine and leucine, for controlled drug delivery applications. Enzymatic degradation was performed with serine proteases (R-chymotrypsin (R-CT) and proteinase K (PK)). The technique allowed the identification of fully and partially degraded polymer fragments, thus providing information on the polymerization process and on the intrinsic polymer structure. Tracking the release of monomeric and oligomeric products into the enzyme media during the course of enzymatic degradation revealed the preferences of R-CT and PK toward ester and amide bonds.[134]

A MS-based approach to profiling degradation at the amino acid residue level was used to study protein and peptide oxidation, a key feature in the progression of a variety of disease states and in the poor performance of protein-based products. Synthetic peptides containing the photosensitive residues, tryptophan and tyrosine, were selected as models for protein-bound residue photodegradation. ESI-MS/MS was employed to characterise and provide relative quantitative information on the formation of photoproducts localised to specific residues, including the characterisation of low abundance photomodifications. The identification of the degradation products yielded information on the formative mechanisms.[121]

Shape memory polymers (SMPs) are a class of smart polymeric materials that have the ability to keep a temporary shape, and later recover to its "memorized" original (permanent) one, upon an external stimulus (heat, light, electromagnetic induction, solvents). Their properties and shape-memory effects

have been examined by different analytical methods among which MS. Weems et al. determined the degradation rate and product concentrations using LC-MS, of porous and non-porous SMPs intended for implantable vascular medical devices, yielding a previously unexamined degradation mechanism for these biomaterials.[135]

Studies by MALDI are usually limited by the mass range of the degradation products whose signals usually overlap with matrix interfering. However, in the absence of matrix-related background, desorption-ionization MS on porous silicon is able of monitoring low MM components released during degradation. In fact, a MALDI-TOF MS study directly on the DIOS (desorption/ionization on silicon) plate was successfully carried out to identify low MM components released during degradation of diblock copolymer PEG-PLA stabilized emulsions. SEC and MALDI-TOF profiles of PEG-PLA during degradation in distilled deionized water at 37 °C showed that the MM decrease was fast at the early stages. The MePEG2000 spectra were well resolved, and the peaks were separated by 44 mass units, which corresponded to the MM of the EG monomer (oxyethylene units = 44.03 g/mol). After polymerizing with DL-lactide, other peaks separated by 72 mass units appeared (lactyl units = 72.06 g/mol), in agreement with the presence of PLA blocks. After 2 weeks, the peaks corresponded to lactyl units strongly decreased, indicating the loss of PLA component. Beyond 3 months, no signal characteristics of PLA were detected on the MALDI-TOF spectra.[136,137]

Degradation plays a key role in every life phase of a polymer, i.e. during its synthesis, processing, usage and even after it has achieved its scheduled purpose. In the advance of bioresorbable polymers, not only the hydrolytic and/or enzymatic degradation should be taken into account but also deterioration prompted by processing, sterilization methods or manufacturing and MS can be a helpful support.[34,138,139] Polymers in fact may be subjected to quite high temperatures during processing or manufacturing, and during this time, degradation may damage the properties of the neat material. Very recently, Rizzarelli et al. have investigated the laser-induced degradation occurring when ultrashort laser pulses (ULP) were employed to cut extremely thin PLA films prepared by solvent-casting. ULP polymer cutting technology is an interesting manufacturing process for its advantages in potential medical applications. Actually, heat transmission to the region surrounding the cuts is limited, so that the incisions are precise and the effects on the regions around them are small. In this way, the need for post-processing is reduced and ULP cutting becomes interesting for industrial applications. However, degradation induced by ULP may occur and impair the properties of the polymer samples. To investigate this possibility, portions of PLA films, ULP cut and uncut, were analysed by SEC, differential scanning calorimetry (DSC), NMR and FTIR. Furthermore, PLA oligomers were studied by ESI MS. The complementarity of the techniques used in this study allowed highlighting a laser-induced degradation. Both SEC and NMR showed a decrease in the molar mass. FTIR, ESI MS and NMR spectra revealed the presence of olefin end groups originated from a β-H transfer mechanism, induced by heat and/or light (Norrish II mechanism). Additionally, the inspection of the ESI mass spectra highlighted the cleavage of ester bonds related to the Norrish I type mechanism, undetected by the other techniques.[34]

In a very nice paper, Sun et al. described the use of light-degradable aliphatic PTMC nanoparticles as drug carrier for photosensitizer. They synthesised a new sixmembered cyclic carbonate monomer (LrM) with a lightresponsive 4,5-dimethoxy-2-nitrobenzyl pendent group attached via a carbamate linkage, then copolymerized with TMC to afford light-responsive copolycarbonate (LrPC). ESI-TOF/MS was helpful to determine the degradation products of LrM upon UV light irradiation. In fact, ESI mass spectra (**Figure 13**) highlighted that LrM was decomposed into an intermediate 5-(aminomethyl)-5-methyl-1,3-dioxan-2-one (I) (undetected by MS) and 4,5-dimethoxy-2-nitrosobenzaldehyde (II) via a radical redox photoisomerization mechanism. Then the reactive intermediate I evolved into a more thermally stable six-membered cyclic carbamate (III) via intramolecular transcarbamation of the functional amine group with the carbonate group. The intermolecular degradation product IV was observed as well and its detection was related to the

intermolecular ring-opening reaction of I with LrM. In addition, imine V was suggested as a minor degradation product generated from the primary amine I and aldehyde II.[140]

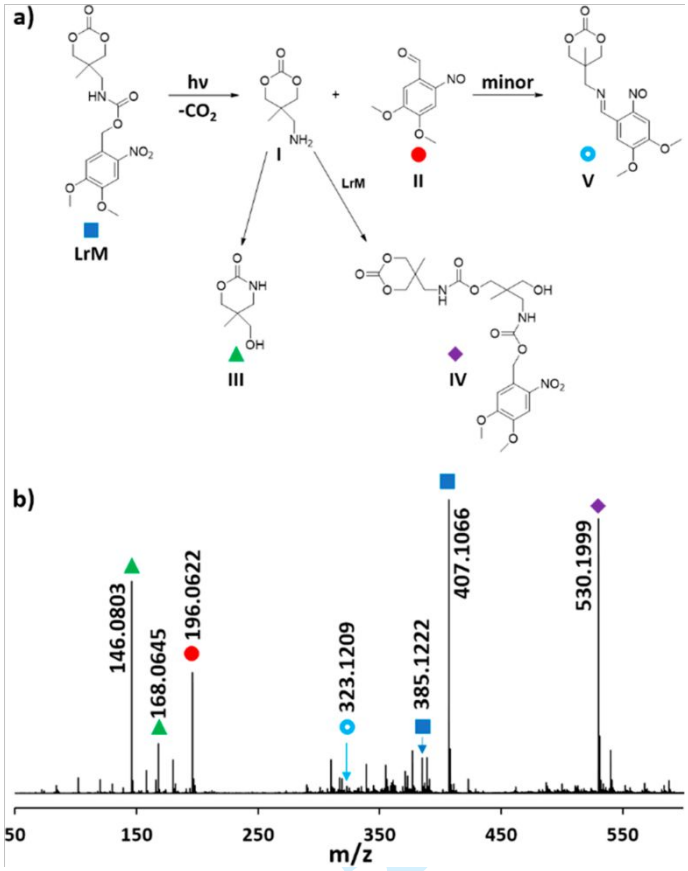

Figure 13. (a) Degradation mechanism of **LrM** upon irradiation. **(b)** ESI-ToF mas spectrum for degradation products of LrM after irradiation (320−480 nm, 0.607 W/cm^2) for 15 min. [**III** + H]$^+$: calcd, 146.0812; found, 146.0803; [**III** + Na]$^+$: calcd, 168.0631; found, 168.0645; [**II** + H]$^+$: calcd, 196.0604; found, 196.0622; [**V** + H]$^+$: calcd, 323.1238; found, 323.1209; [**LrM** + H]$^+$: calcd, 385.1242; found, 385.1222; [**LrM** + Na]$^+$: calcd, 407.1061; found, 407.1066; [**IV** + H]$^+$: calcd, 530.1980; found, 530.1999. **Reprinted with permission from Sun et al.,[140] copyright (2018) American Chemical Society**.

IV. Bioresorbable polymer drug release tracking by MS

Effective performance of any controlled DDS depends on the drug elution kinetics, which in bioresorbable polymer matrices is strictly connected to the degradation behavior. Therefore, understanding of the polymer degradation phenomena is a crucial aspect in the design of controlled DDS. Several papers are addressed to investigate the degradation rate and drug release profile of bioresorbable polymeric matrices by MS (**Table 1, Figure 11**).[92,122,141-147]

Phan et al. determined the release of PEG and HPMC (hydroxypropyl methylcellulose) from a daily disposable hydrogel contact lens material (nelfilcon A; Dailies AquaComfort PLUS; DACP) over 24 hrs by a developed an in vitro eye model (OcuFlow), simulating physiologically relevant tear volume and natural tear flow, air exposure, and mechanical rubbing. The elution of PEG and HPMC from DACP lenses was analyzed using LC-ESI MS. LC-ESI MS experiments with PEG and HPMC showed distinctive peaks representative of the polymer. LC-MS data showed that the release of all wetting agents from the lenses followed a burst release pattern, which occurred within the first 1.5 hrs. Moreover, the release of PEG was greater than that of HPMC.[144] Peer et al. provided new insights on how the surface nanopatterning of biomaterials can functionalize the surface and tailor the release

kinetics of therapeutic agents coated on it for controlled drug elution. They compared the rates of drug release from nanopatterned and flat PLLA surfaces using HPLC-MS. PLLA, frequently used for fabricating drug-eluting coronary stents, was nanopatterned through microtransfer molding and solvent casting. The impact of nanopattern on the release of sirolimus, an immunosuppressant agent, coated on the PLLA surface, was investigated. A significantly lower release rates (25-30 %) from the nanopatterned surfaces than that of the flat surface was detected, counter-intuitive given the nanopattern-induced increase in their surface area. The authors ascribed the decreased drug release rate to the partial wetting of the nanopatterned surface.[143] Tang et al. optimized a mPEG-PLGA-mPEG-based delivery system for long-term controlled release of salmon calcitonin (sCT), the most widely used calcitonin (used for many years in the treatment of metabolic bone diseases, particularly osteoporosis) after single subcutaneous injection. The DDS was prepared by dissolving sCT into polymer solution and in vitro release studied in phosphate buffer saline (PBS, pH 7.4) at 37 °C. MALDI-TOF MS was used to evaluate the stability of released sCT and sCT remaining in gel formulation. Ions due to a degradation product was observed for the sample withdrawn at day 35, even though the peak corresponding to the sCT was still the major peak on the spectra, indicating that most of the peptides retained their chemical integrity.[146]

IV. Conclusions

Bioresorbable synthetic polymers represent an important class of materials in the biomedical field. The design of suitable polymers for diverse biomedical applications and, at the same time, with safe degradation products is one of the main challenges faced by researchers around the world. For this reason, characterization is a prerequisite for understanding their structure-property relationships regardless of the specific biomedical application. Among the analytical approaches and methods, for both characterization and degradation features, those based on mass spectrometry have been extensively used in the last ten years. Overall, MS is more frequently empoyed in the characterization than in the degradation or release studies of bioresorbable polymers (**Figure 14a**). Among the MS techniques, MALDI MS represents the more applied method for the characterization of biorsorbable polymers (**Figure 14b**); it is much less used in degradation monitoring studies due to the possible interference between the matrix signals and low molar mass products resulting from deterioration processes (**Figure 14c**). On the other hand, ESI MS has proved to be a successful method for obtaining structural and quantitative information on water-soluble monomers and oligomers originated from the bioresorption. Moreover, the advantage of being readily interfaced with solution-based separation techniques, such as HPLC for separating complex mixtures, designates ESI MS and MS/MS a relevant and elective analytical tool of screening in degradation and release studies (**Figure 14c**). Recently, identification of residual catalyst in polymers (tin, zirconium, etc.), heavy metals in bioabsorbable implants rather than release of therapeutic metal ions have been checked by ICP-MS. The combination of SIMS and TOF (TOF-SIMS) gives rise to a very sensitive surface analytical technique, well established for many applications. Infact, by providing detailed information about the surface, interfaces of the sample and giving a full three-dimensional analysis, TOF-SIMS allowed monitoring the distribution or absorption of proteins on the surface of resorbable polymers, as well as surface interactions between biomaterials and biological systems. In cases where the characterization of the polymer architecture is complex, for example due to isomeric structures, multidimensional MS techniques provided unequivocal identification. Finally, MS techniques have been also helpful to investigate the degradation rate and drug release profile of bioresorbable DDS, which are closely interconnected.

Undoubtly, several "traditional" methods have proved very successful at studying bioresorbable polymer (i.e. FTIR and NMR spectroscopies). Thus, a legitimate question is: "What does MS provide additionally in bioresorbable polymer investigations?" There are important reasons to use MS in the development of bioresorbable polymers: classical analytical tools, for example, are always averaging methods; i.e. they measure the average properties of a mixture of macromolecules and thus do not examine individual ones. Furthermore, more traditional techniques do not typically yield information on the different types of oligomers and additives that may be present in polymer samples. Unlike NMR, MS can distinguish between cyclic and linear species. Moreover, MS provide the opportunity to explore the finest structural details, required as assurance of safety, efficiency and quality in bioresorbable matrices. MS clearly has great potential to examine individual components originated from degradation in polymeric systems, and this can add much information to support and broaden the "classical" methods. Finally, most "classical" methods do not provide absolute, direct MM values for polymers. Overall, in the last decade several MS methods have been successfully employed in bioresorbable polymer development and degradation tracking for the complexity of the issue. In fact, a growing interest and an increasing number of papers concerning bioresorbable polymers arise from the literature. However, in several cases, more techniques are combined to clarify better the features of bioresorbable polymers, to overcome some drawbacks, such as discrimination of isobaric species, and obtain relevant information for tailored applications. It is desirable that, in the near future, attention

to bioresorbable polymers and advances in analytical techniques will act as an engine to understand and improve the performance of these outstanding materials, promoting greater diffusion. In the meanwhile, we hope that this review will be helpful for the choice of the right MS technique in future studies, supporting the further extension of MS applications in the development of bioresorbable polymers.

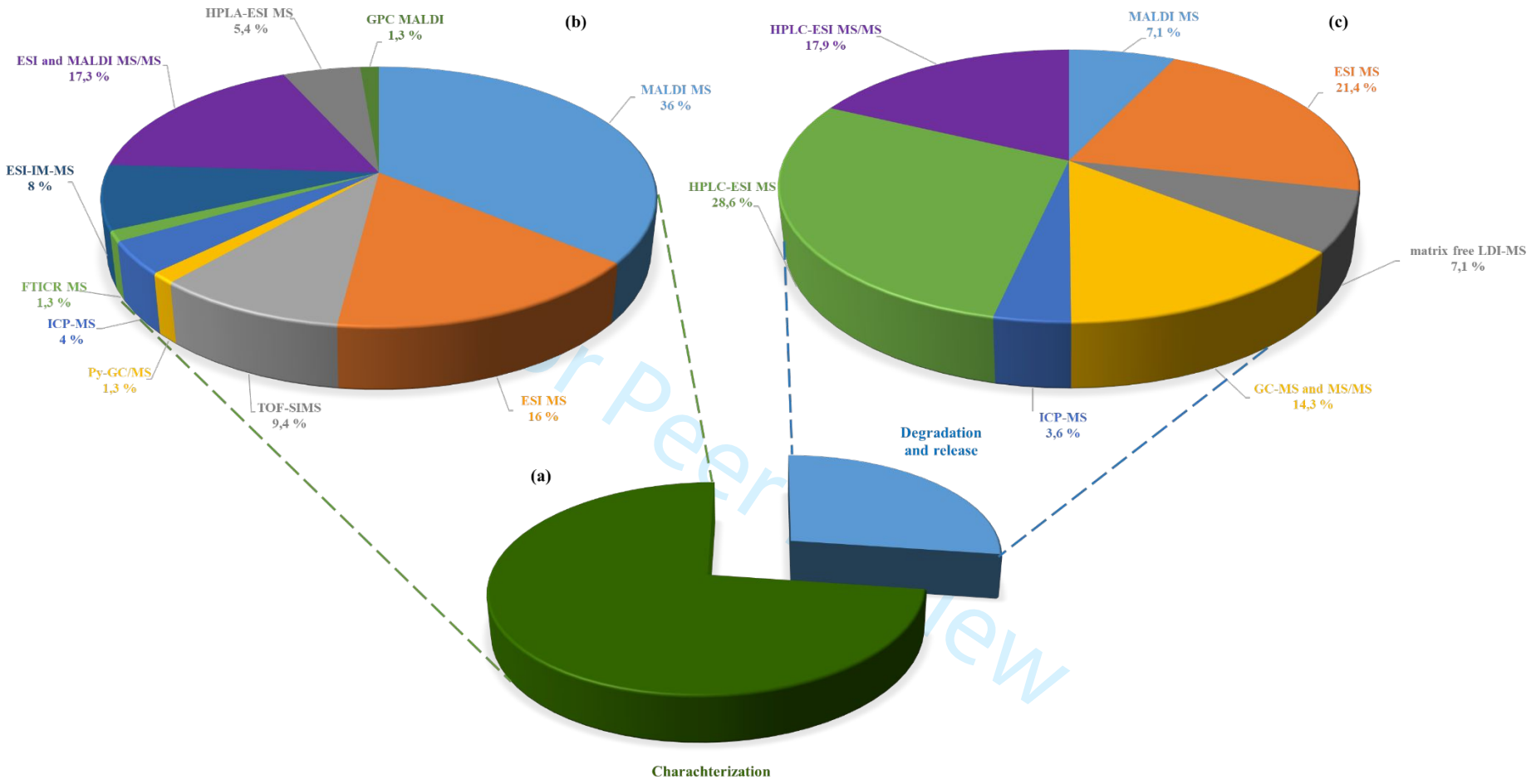

Figure 14. (a) Percentage of the papers (mentioned in this review) that deal with the characterization of bioresorbable polymer, degradation and release tracking by MS methods. Pie chart of MS techniques used in the **(b)** characterization and **(c)** monitoring of degradation and release tracking in the papers mentioned in this review.

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Table 1. Bioresorbable polymers analysis by MS.

Polymers[1]	MS techniques[2]	Other analytical methods[3]	Information acquired by MS	Ref.
mPEG	MALDI-TOF MS and MS/MS	^{1}H NMR; SEC	Nature of end groups and architectural modifications	17
PPM; PPF	ESI MS, ESI-IM-MS and MS/MS; MALDI MS and MS/MS	SEC	Composition, end groups and chain sequence differentiation in the isomeric PPM and PPF	20
α-methyl, ω-hydroxy PLA	MALDI and ESI-TOF; ESI-IM-MS	/	Structural investigation	25
PEC	TOF-SIMS (depth profile)	SEC; SEM; CLSM	Evidence on the order of the coating layers	28
PEO - PAA Brushes	TOF-SIMS	Gel Electrophoresis SDS-PAGE	Protein identification after adsorption	29
PLLA; PLGA	TOF-SIMS (depth profile)	/	Distribution of surfactant stabilizer and protein drug through the polymer membrane	30
Aliphatic poly(ester urethane) urea	TOF-SIMS	SEC; ^{1}H NMR; FTIR; DSC; XPS; CA; SEM; tensile testing	Insight into the outermost surface composition	31
Hydrogel composite Gg-co-poly(AA-ANI)	TOF-SIMS	SEM; FTIR; XRD; TGA	Compositional identification of backbone	32
PCL; PCLhexaq	TOF-SIMS	/	Qualitative and quantitative information on the molecular composition of the outer monolayer of nanofibers	33
PLA	ICP-MS; Py-GC/MS; ESI MS	SEC; TGA; DSC; NMR; FTIR	Occurrence of residual catalyst; identification of end groups and degradation mechanisms	34
PLA	ICP-MS	SEM; DSC; FTIR	Presence of transition metals	35
mPEG-*b*-PCL-*b*-PLL; copolymer-cisPt(IV)	ICP-MS	TEM	Cellular uptake of polymer-di-cisPt(IV) micelles and Pt–DNA adducts measurement	36
PSA-*b*-mPEG; SPIO-NPs	ICP-MS	SEC; ^{1}H NMR; FTIR; TEM; DLS	Iron content in SPIO-PNPs	37
PLys-*b*-PLLA;	MALDI-FTMS	^{1}H NMR; SEC; SEM;	Structural investigation	51

Polymers[1]	MS techniques[2]	Other analytical methods[3]	Information acquired by MS	Ref.
PLys-*b*-PLLA-*b*-PLys		AFM; Dynamic CA and Surface Energy	confirming successful preparation	
Multi-armed resorcinarene- and calixarene-core PLA star polymers	MALDI-TOF	SEC; ^{1}H NMR; UV-Vis; DSC	Evidence for incorporation of the initiator within the star	52
PLA; PGA; PLGAs	MALDI-TOF	^{1}H and ^{13}C NMR; SEC; TGA; DSC; XRD; CA	Identification of end groups and polymerization mechanisms	63
Hetero-telechelic, low-molecular-weight PLAs	MALDI-TOF	^{1}H NMR; DSC	Structure and end groups identification	64
PCL, PGA, PLA, and copolymers	MALDI-TOF	^{1}H and ^{13}C NMR; SEC	Determination of average MM, end groups and evidence of intermolecular transesterification	65
PLGA-alendronic acid conjugate	MALDI-TOF	^{1}H NMR; DSC	Confirmation of the reaction between the copolymer and drug; semi-quantitative estimation	66
Hydrogels based on PEG-GA macromonomers with different photopolymerizable end-groups	MALDI-TOF	^{1}H NMR	Degree of end group conversions, MM, and product purity	67
PEO-*b*-PLA, PLA-*b*-PCL-*b*-PLA, PLA-*b*-PBS-*b*-PLA; PPE-*b*-PLA	MALDI-TOF	^{1}H NMR; SEC; Raman	Absence of transesterification and chain end correspondence	68
α-azide-ω-hydroxy PLLA; α-azide-ω-alkyne PLLA; cyclic PLLA	MALDI-TOF ESI-IM-MS	^{1}H NMR; SEC	End group functionalization reaction; evidence for the cyclization efficiency	69
Poly(TA-*co*-GA)	HR ESI MS	^{1}H and ^{13}C NMR; FTIR; TEM; TGA; DLS	Confirmation of the presence of a polymer/oligomer mixture	70
Pyrene-labeled PEG-PLA conjugate	MALDI-TOF	^{1}H NMR; FTIR; DSC; TGA; absorbance and fluorescence measurements; DLS; SEC	Average MM determination	71
Chain-extended PCL-diol	HR ESI MS	^{1}H and ^{13}C NMR; ATR-FTIR; DSC; TGA; CA	Chemical structure determination	72
CL and hydroxy-fatty acids copolymers	MALDI-TOF GC-MS	^{1}H and ^{13}C NMR; 2D-NMR; ATR-FTIR	Average MM and optimization of synthesis parameters; degradation products characterization	73
5-Z-amino- δ-VA homopolymer and its copolymers with CL	HPLC-ESI MS	^{1}H and ^{13}C NMR; SEC; DSC	Monomer characterization	74
P(CL-*co*-δ-VA)	MALDI-TOF	^{1}H NMR; SEC;	Structural analysis of the copolymers;	75

Polymers[1]	MS techniques[2]	Other analytical methods[3]	Information acquired by MS	Ref.
	HPLC-ESI MS	DSC;CLSM	Degradation products characterization	
PBCL-*b*-PEG-*b*-PBCL	MALDI-TOF	^{1}H NMR; DLS	Determination of the MM and degree of polymerization	76
PHAs	ESI MS and HPLC-ESI MS	HPLC - NMR off line	Qualitaty and quantitaty determination of PHA monomers	78
P(HB-*co*-BA)	MALDI-TOF	^{1}H and ^{13}C NMR; SEC; DSC; WAXS	End-group analysis	80
Oligo(3HB-*co*-4HB)]; oligo(a-PHB)	ESI MS and MS/MS	^{1}H NMR; SEC; elemental analysis	Structural characterization	82
PCL	TOF-SIMS	/	Three-dimensional imaging of surface modifications in scaffold pores	85
PLA Oligomers	ESI MS and MS/MS (CID)	^{1}H NMR	CID behavior and influence of end-groups	89
LA-OHB	ESI MS and MS/MS HPLC-ESI MS	^{1}H NMR; FTIR; SEC	Structural characterization and successful polymerization; monitoring hydrolytic degradation products and α-lipoic acid release	92
Cyclic PLA; α-azide-ω-alkyne-PLLA	MALDI-TOF; ESI MS and MS/MS	^{1}H NMR; SEC	Determination of the architectural purity	94
Tyrosol-P(3HB-*co*-4HB); tyrosol-γ-PGA	ESI MS and MS/MS	^{1}H NMR; SEC	Structural characterization and successful polymerization	95
PEG2000 – Lipoamino acids conjugates	MALDI-TOF	^{1}H and ^{13}C NMR; FTIR; DSC; DLS	Structure confirmation	98,99, 100
P(HB-*co*-CL); P(HB-*co*-HV-*co*-CL)	MALDI-TOF; SEC-MALDI-TOF offline	^{1}H and ^{13}C NMR; UV; DLS	Structure confirmation	101
PHA-*g*-PNIPAm	MALDI-TOF	^{1}H NMR; UV/vis; SEC; TGA; SEM; CA	MM and structural determination	105
Peptides-PEG conjugates	MALDI MS and MS/MS; ESI MS, IM-MS, and MS/MS	CD	Elucidation of alanine-rich polypeptides sequence	106, 107
Polymeric excipients	FTICR MS; MALDI-TOF	/	End-group determination; presence of contaminants in different batches	109
Polysorbate 85	MALDI MS; ESI-IM-MS, LC-MS and MS/MS	/	Detailed compositional and structural characterization; detection of minor components; comparison between two MS methods	111

Polymers[1]	MS techniques[2]	Other analytical methods[3]	Information acquired by MS	Ref.
Cross-linked PCL fibers	ESI MS	^{1}H NMR; SEM	Degradation product profiles and effect of cross-linking and processing conditions	115
PCL-*b*-PEG	ESI MS	WL; SEC; ^{1}H-NMR; DSC; FTIR; XRD; SEM	Determination of the nature of the water soluble degradation products	120
Zein-based biomaterial	HPLC-ESI MS and MS/MS	/	Tracking the in vivo degradation, detection of the changes of amino acids levels in plasma and different organs after the implantation of scaffolds	122
PLGA - prostaglandin E2 (PGE_2) microspheres	GC-MS/MS	SEM; AFM	Measurement of PGE_2 release	123, 147
Cyclodextrin modified PLLA	HR ESI MS	DSC; SEM; tensile testing	Degradation products monitoring	124
Triblock P(GA-*co*-TMC)	matrix-free LDI-MS; ESI MS	DSC; tensile testing	Identification of degradation products, rich in TMC units, and evidence of preferential hydrolysis of amorphous regions	125
Multiblock PEAs based on natural amino acids	LC-ESI MS	NMR; DSC; SEC	Identification and semiquantitative analysis of degradation products	134
Shape memory PUs	LC-ESI MS	^{1}H and ^{13}C NMR; DSC; DMA; SEM; ATR-FTIR	Degradation rate and product concentrations by studying model compounds	135
PEG-*b*-PLA	matrix-free LDI-MS	^{1}H NMR; GPC	Degradation rate and monitoring the release of low molecular weight degradation components	136
PTMC with oligo OEG side groups	MALDI MS	^{1}H-NMR; SEC; FTIR	Analysis of the degradation compounds	137
PCL; PU	GC-MS	SEC; DSC; FTIR; SEM	No release of volatile degradation products	138
PTMC-based copolymer	ESI MS	^{1}H and ^{13}C NMR; UV/vis; FTIR; DSC; SEC; AFM	Degradation products characterization	140
CS-coated-DAUN-PLGA NPs	UHPLC-ESI MS and MS/MS	^{1}H NMR; FTIR; DSC; TEM; DLS	Plasma quantification and pharmacokinetic analysis of DAUN	141
PLGA-*b*-PEG-*b*-PLGA based DDSs	HPLC-APCI-MS/MS	/	Determination of serum LNG concentration	142
PLA	HPLC-ESI MS	SEM; AFM	Drug release monitoring	143

Polymers[1]	MS techniques[2]	Other analytical methods[3]	Information acquired by MS	Ref.
PEG; HPMC	HPLC-ESI MS and MS/MS	/	Detection and quantification of PEG and HPMC release	144
PECA DDSs	UPLC-ESI MS and MS/MS	SEM	Determination of ketorolac concentrations in rat plasma	145
mPEG-*b*-PLGA-*b*-mPEG based sCT DSs	MALDI MS	HPLC-UV; CD	Assessment of chemical stability of released sCT	146

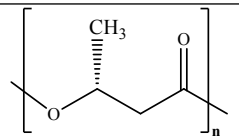
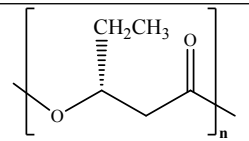
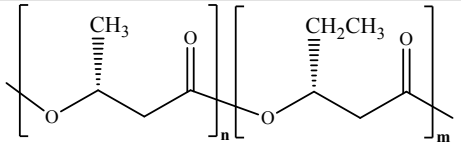
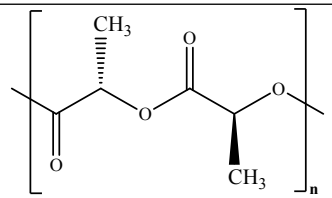
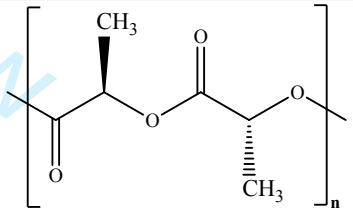
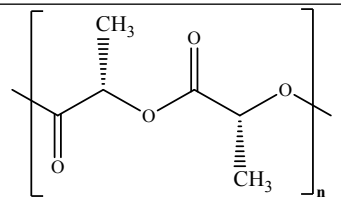
[1] mPEG = substituted methoxy poly(ethylene glycol); PPM = poly(propylene maleate); PPF = poly(propylene fumarate); PLA = poly(lactide); PEC = poly(ethylene carbonate); PEO = poly(ethylene oxide); PAA = poly(acrylic acid); PLLA = poly(L-lactide); PLGA = poly(lactide-*co*-glycolide); Gg-*co*-poly(AA-ANI = gum ghatti-*co*-poly(acrylic acid-aniline); PCL = poly(ε-caprolactone); PCLhexaq = hexyldimethylammonium functionalised PCL; mPEG-*b*-PCL-*b*-PLL = methoxy poly(ethylene glycol)-*b*-poly(caprolactone)-*b*-poly-L-lisina; PSA-*b*-mPEG = poly(sebacic anhydride)-*block*-methyl ether poly(ethylene glycol); SPIO = superparamagnetic iron oxide; NPs = nanoparticles; PLys-*b*-PLLA = poly(L-lysine)-*block*-poly(L-lactide); PLys-*b*-PLLA-*b*-PLys = poly(L-lysine)-*block*-poly(L-lactide)-*block*-dendritic poly(L-lysine); PGA = poly(glycolide); PEG-GA = poly(ethylene glycol-*co*-glycolide); PEO-*b*-PLA = poly(ethylene oxide)-*block*-poly(lactide); PLA-*b*-PCL-*b*-PLA poly(lactide)-*block*-poly(ε-caprolactone)-*block*-poly(lactide); PLA-*b*-PBS-*b*-PLA = poly(lactide)-*block*-poly(butylene succinate)-*block*-poly(lactide); PPE-*b*-PLA = poly(phosphoester)-*block*-poly(lactide); poly(TA-*co*-GA) = poly(tartronic-*co*-glycolic acid); VA = δ-valerolactone; CL = ε-caprolactone; PBCL-*b*-PEG-*b*-PBCL = poly(α-benzyl carboxylate-ε-caprolactone)-*block*-poly(ethylene glycol)-*block*-poly(α-benzyl-carboxylate-ε-caprolactone); PHAs = polyhydroxyalkanoates; P(HB-*co*-BA) = poly(3-hydroxybutyrate-*co*-butyleneadipate); oligo(3HB-*co*-4HB) = oligo(3-hydroxybutyrate-*co*-4-hydroxybutyrate)diols; oligo(a-PHB) = oligo[(R,S)-3-hydroxybutyrate]; LA-OHB = lipoic acid-oligo-(3-hydroxybutyrate) conjugates; tyrosol-P(3HB-*co*-4HB) = tyrosol-poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate); tyrosol-γ-PGA = tyrosol-poly-γ-glutamic acid conjugates; P(HB-*co*-CL) = poly(3-hydroxybutyrate-*co*-ε-caprolactone); P(HB-*co*-HV-*co*-CL) = poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-ε-caprolactone); PHA-*g*-PNIPAm = polyhydroxyalkanoate-*graft*-poly(N-isopropylacrylamide); PCL-*b*-PEG = poly(ε-caprolactone)-*block*-poly(ethylene glycol); triblock P(GA-*co*-TMC) = poly(glycolide-co-trimethylene carbonate) triblock copolymer; PEAs = poly(ester amide)s; PUs = polyurethanes; PEG-*b*-PLA = poly(ethylene glycol)-*block*-polylactide; PTMC = poly(trimethylene carbonate); OEG = oligo ethylene glycol; CS = chitosan; DAUN = daunorubicin hydrochloride; DDSs = drug delivery systems; HPMC = hydroxypropyl methylcellulose; PECA = polyethylcynanoacrylate; sCT = salmon calcitonin; DSs = delivery systems.

[2] MS = mass spectrometry; MALDI = matrix-assisted laser desorption ionization; TOF = time of flight; MS/MS = tandem mass spectrometry; ESI = electrospray ionization; IM = ion mobility; SIMS = secondary ion mass spectrometry; ICP-MS = inductively coupled plasma - mass spectrometry; Py-GC/MS = pyrolysis-gas chromatography/mass spectrometry; MALDI-FTMS = matrix-assisted laser desorption ionization - Fourier transform mass spectrometry; HR-ESI MS = high resolution-ESI MS; GC/MS = gas chromatography/mass spectrometry; HPLC-ESI MS = high-performance liquid chromatography - ESI MS; SEC = size-exclusion chromatography; FTICR MS = Fourier transform ion cyclotron

resonance mass spectrometry; CID = collision-induced dissociation; LDI-MS = laser desorption ionization-mass spectrometry; UHPLC = ultra-high performance liquid chromatography; APCI = atmospheric pressure chemical ionization.

(3) NMR = nuclear magnetic resonance; SEM = scanning electron microscopy; CLSM = confocal laser scanning microscopy; SDS-PAGE = gel electrophoresis with silver staining; FTIR = Fourier transform infrared spectroscopy; DSC = differential scanning calorimetry; XPS = X-ray photoelectron spectroscopy; CA = contact angle; XRD = X-ray diffraction; TGA = thermogravimetry analysis; TEM = transmission electron microscopy; DLS = dynamic light scattering; AFM = atomic force microscopy; ATR-FTIR = attenuated total reflectance-Fourier transformed infrared; WAXS = wide-angle X-ray scattering; CD = circular dichroism; WL = weight loss measurements; DMA = dynamic mechanical analysis.

Table 2. Sources, names, abbreviations and structures of the main bioresorbable polyesters.

Source	Name and Abbreviation		Structure and nominal mass of the repetitive units (g/mol)	
Microorganisms	Poly(R-3-hydroxy butyrate)	PHB		86
	Poly(R-3-hydroxy valerate)	PHV		100
	Poly(R-3-hydroxy butyrate-*co*-R-3-hydroxy valerate)	PHBV		86/100
Biobased monomers	Poly(L-lactide)	PLLA		144
	Poly(D-lactide)	PDLA		144
	Poly(DL-lactide)	PDLLA		144

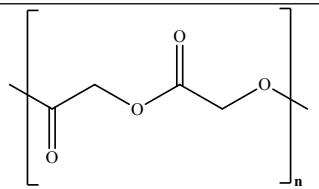
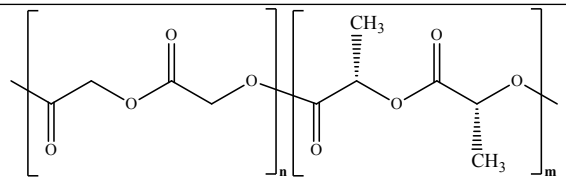
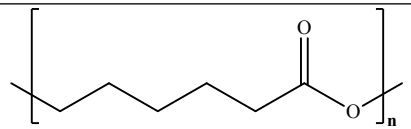
Source	Name and Abbreviation		Structure and nominal mass of the repetitive units (g/mol)	
	Poly(glycolide)	PGA		116
	Poly(lactide-*co*-glycolide)	PLGA		144/116
Petroleum-based monomers	Poly(ε-caprolactone)	PCL		114

Figure captions

Figure 1. General bioresorbable polymer applications.

Figure 2. Classification of bioresorbable polymers.

Figure 3. Overview of the MS techniques used in the characterization of bioresorbable polymer.

Figure 4. MALDI mass spectrum recorded for P(D,L-lactide) synthesized using (A) TU/PMDETA and (B) TU/DBU as catalytic systems and benzyl alcohol as an initiator. **Reprinted with permission from Grignard et al.,[68] copyright (2017) Elsevier**.

Figure 5. MALDI mass spectra of **(a)** poly(propylene maleate) (PPM) and **(b)** poly(propylene fumarate) (PPF). All ions are sodiated species with the composition $[R_n + EG_s + Na]^+$, where R and EG_s designate the PPM/PPF repeat unit ($C_7H_8O_4$, 156 Da) and the corresponding end groups (in red color), respectively. **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

Figure 6. MALDI-MS/MS spectrum of the $[M + Na]^+$ ion from the PPF 9-mer with CH_3CH_2O– and –H end groups (*m/z* 1473.4). The scheme on the top shows the fragment ions arising from 1,5-hydrogen rearrangement over ester groups facing the CH_3CH_2O- ($, !) or -H (#, @) chain end. Consecutive dissociation of these fragments ($\Rightarrow$) leads to internal fragments (o). The Na^+ ion has been omitted for brevity. An asterisk above the fragment notation (*) indicates fragments ionized by H^+ (Na^+ is eliminated with the neutral fragment). **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

Figure 7. (a) 2-D ESI-IM-MS plot (*m/z* vs. drift time) of PPF; the mobility regions of singly, doubly and triply charged ions are encased in ovals. **(b)** Mass spectrum extracted from the region of singly charged ions, containing several ion distributions which include intact PPF ions with CH_3CH_3O– and –H end groups (46-Da end group mass) and degradation products with various end group masses (noted after the number of repeat units; see **Figure 5** for plausible structures). Charge is provided by addition of H^+, Na^+ or $(C_2H_5)_2NH_2^+$ (from residual PPM to PPF isomerization reagent). PPM leads to very similar ESI-IM-MS characteristics, except for the absence of $(C_2H_5)_2NH_2^+$ adducts. **Reprinted with permission from Sallam et al.,[20] copyright (2017) Sage Publications**.

Figure 8. (a) and **(b)** PLLA/AOT/KGF at 0 h **(c)** and **(d)** PLLA/AOT/KGF at 24 h soak time. The depth of each film was measured by profilometry to be ~500 nm for both the 0- and 24-h time point. The entire depth of the membrane was sputtered using C_{60}^{+++} until the substrate (Si) was reached for each profile. 3D reconstruction using Ion-ToF software of TOF-SIMS depth profiling data. **Figure 8 (a,c)** represent the distribution of AOT at the surface of a PLLA/AOT/KGF membrane at the 0 h and 24 h soak time. **Figure 8 (b,d)** represent the distribution of KGF at the surface of a PLLA/AOT/KGF membrane at the 0 h and 24 h soak time. The zero-time point has a high-ion signal of AOT at the surface and a depletion zone where little AOT is present. The surface layer of AOT above the depletion zone is removed after the soaking procedure in PBS solution. The distribution of KGF is more concentrated at the 0-h time point versus the 24-h time point but is still present through out the surface and bulk of the PLLA polymer membrane. Similar results were obtained from the membranes composed of PLGA/AOT/KGF. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]. **Reprinted with permission from Burns et al.,[30] copyright (2010) John Wiley and Sons**.

Figure 9. (a) Spectrum of polymeric distribution patterns in Gelucire 44/14. **(b)** Spectrum of polymeric distribution patterns in Gelucire 44/14. Inserts 1A-1B, 2A-2B, and 3A-3B show the differences between the two spectra. **Reprinted with permission from Hurtado et al.,[109] copyright (2012) American Chemical Society**.

Figure 10. Polymer degradation processes.

Figure 11. Overview of the MS techniques used in bioresorbable polymer degradation and drug release tracking.

Figure 12. LDI-MS spectrum of poly(GA-co-TMC) degradation products in buffer (above) and water (below) after 60 days of hydrolysis. **Reprinted with permission from Aminlashgari et al.,[125] copyright (2013) Elsevier**.

Figure 13. (a) Degradation mechanism of **LrM** upon irradiation. **(b)** ESI-ToF mas spectrum for degradation products of LrM after irradiation (320−480 nm, 0.607 W/cm^2) for 15 min. [**III** + H]$^+$: calcd, 146.0812; found, 146.0803; [**III** + Na]$^+$: calcd, 168.0631; found, 168.0645; [**II** + H]$^+$: calcd, 196.0604; found, 196.0622; [**V** + H]$^+$: calcd, 323.1238; found, 323.1209; [**LrM** + H]$^+$: calcd, 385.1242; found, 385.1222; [**LrM** + Na]$^+$: calcd, 407.1061; found, 407.1066; [**IV** + H]$^+$: calcd, 530.1980; found, 530.1999. **Reprinted with permission from Sun et al.,[140] copyright (2018) American Chemical Society**.

Figure 14. (a) Percentage of the papers (mentioned in this review) that deal with the characterization of bioresorbable polymer, degradation and release tracking by MS methods. Pie chart of MS techniques used in the **(b)** characterization and **(c)** monitoring of degradation and release tracking in the papers mentioned in this review.

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