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# A review of *biomaterial* degradation assessment approaches employed in the biomedical field



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The biological response to biomaterials plays a crucial role in selecting suitable materials for the formulation and development of tissue engineering platforms. Biodegradation is one of the properties that is considered in selecting appropriate biomaterials for biomedical applications. Biodegradation is the process of breaking down large molecules into smaller molecules with/without the aid of catalytic enzymes. The biodegradation process is crucial in the chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) process of biomaterials and small molecules in the body. Degradation of biomaterials can be followed by assessing the physical, mechanical, and chemical attributes of biomaterials. There are several techniques/parameters that can be targeted when studying the degradation of biomaterials, with gravimetric analysis, surface erosion, and morphological changes being the largely employed techniques. However, the techniques present a few limitations, such as technical errors and material solubility being mistaken for degradation, and these techniques can infer but not confirm degradation as they do not provide the chemical composition of fragmenting/fragmented molecules. The American Society for Testing and Materials (ASTM) guidelines provide techniques and parameters for assessing biodegradation. However, the ASTM guidelines for degradation assessment approaches and techniques need to be updated to provide sufficient evidence to draw conclusive decisions regarding the degradation of biomaterials. In this review, the degradation assessment approaches and techniques are critically reviewed about their advantages and disadvantages, and to provide suggestions on how they can still play a role in assessing the degradation of biomaterials. This review could assist researchers employ cost-effective, efficient, and multiple degradation assessment techniques to evaluate and provide sufficient information about the degradation of biomaterials. Suggested future ASTM guidelines for assessing biodegradation should include measuring parameters (such as chemical, mechanical, or physical attributes of biomaterials) in real-time, employing non-invasive, continuous, and automated processes.

Biodegradation is the biological catalytic reaction of reducing complex macromolecules into smaller, less complex molecular structures (by-products)[1]. Biodegradable materials have been widely used in the biomedical field due to their tuneable nature. Biomaterials' tunability includes, but is not limited to chemical, physical, mechanical, and biological functionalisation tailored for a wide range of applications in the biomedical field such as drug delivery, tissue engineering, and wound healing[2–4]. The processing of biomaterials allows for the development of solid and liquid-based formulations. Biomaterials in the biomedical field can be processed via a wide range of approaches to produce solid formulations such as polymeric sutures[2], nanoparticles[5,6], scaffolds[7,8], and fibres[9]. The liquid-based biomaterial formulations include, but are not limited to hydrogels, gels, suspensions, and foam. The differences in biomaterial physical forms suggest that different

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degradation assessment approaches should be employed to assess the biomaterial degradation processes.

The mechanism of degradation, the formed by-products, and the desirable properties of biomaterials are well-documented[10–13]. However, in vitro, and in vivo assessment of biomaterial biodegradation approaches remain a matter of concern given that the approaches employed to evaluate biodegradation present several limitations. Conventional in vitro approaches for assessing the biodegradation of biomaterials include physical, chemical, and mechanical characterisation of biomaterials. Biomaterial degradation can occur via three interconnected processes, and it can be assessed by monitoring these processes namely physical, chemical, and mechanical changes. Biomaterials have characteristic functional groups that can be chemically/enzymatically cleaved during the degradation process. These functional groups include but are not limited to ester, ether, amide, imide, thioester, and anhydride which allows for hydrolytic or enzymatic biomaterial cleavage during the degradation process[10,13,14]. The reaction mechanism for the hydrolysis of polyanhydrides, acetals, polyamides, ketals, polycarbonates, ortho esters, and other related moieties is summarised in Fig. 1 below[13,15,16]. Several moieties that can be hydrolysed during degradation are well-documented[11,13,15]. This process of chemical/biological cleavage of biomaterials can result in surface erosion/weight loss (which is also a physical process)[17,18], molecular weight change (which is both chemical and physical)[19,20], and changes in mechanical properties[19,21]. These physical, chemical, and mechanical changes in biomaterials degradation further support the notion of interconnected degradation processes.

Physical and mechanical degradation assessment approaches infer (not confirm) that degradation is taking place and changes in biomaterial properties (i.e., shape, size, morphology, tensile strength, viscosity, storage modulus, etc) can be graphically represented. However, only the chemical-degradation assessment approach can confirm the degradation of biomaterials by assessing absorptive properties, measuring the molecular weight of molecules, and determining the structures of disintegrated biomaterial or produced by-products. Each of the degradation assessment approaches (physical, mechanical, and chemical) presents a few drawbacks. One of the physical approaches that are highly employed in assessing degradation is observing weight loss (via gravimetric analysis) accompanied by visualisation of scanning electron microscope (SEM) images of the degrading biomaterial over time. The approach is easy to execute in a laboratory setting without any economic constraints, and for inferring degradation. However, there are major issues with employing this technique in a laboratory setting. Including that weight loss can simply be mistaken for degradation, whereas it may be attributed a biomaterial that is dissolving (soluble) in simulated bodily fluid or buffered solution. This concern was mentioned at in a study by Mndlovu et al., which followed the degradation of partially crosslinked chitosan-alginate bioplatforms[4].

Other notable drawbacks with degradation assessment approaches discussed in this review include: (i) the inability to employ the physical degradation assessment approaches such as surface erosion on liquid-based formulations, (ii) the requirement for changing from a liquid- to solid-based formulation to assess morphological changes via SEM, (iii) questionability in reproducing the degradation profile of solid materials via the gravimetric analysis approach, and (iv) high costs associated with employing high throughput analytical techniques (nuclear magnetic resonance (NMR), high-performance liquid chromatography (HPLC), size exclusion chromatography (SEC), mass spectrometry etc) in assessing degradation of biomaterials. These limitations led to the conceptualization of the purpose of this review, which was to evaluate the current approaches in assessing degradation and critically assess their strengths, limitations, and possible future applications in assessing the degradation of biomaterials in the biomedical field. Biodegradation mechanisms, biodegradation calculations, and equations were not included in this review. A general step-by-step process for assessing degradation was covered together with the possible techniques that can be combined to produce reproducible degradation results. Suggestions on updating the current degradation assessment approaches, aligned with the American Society for Testing and Materials (ASTM) guidelines, are provided.

## General biodegradation processes and biodegradation assessment approaches

The properties, applications, advantages, and disadvantages of biodegradable biomaterials are well-documented in literature[3,22–24]. Herein, the focus is on the biodegradation assessment approaches. Biomaterials in the biomedical field are designed to be biocompatible and interact with body tissues. The biocompatibility of biomaterials infers that the body does not react to them severely. However, the by-products of the degraded biomaterials may trigger a severe reaction as they may have a different level of tissue

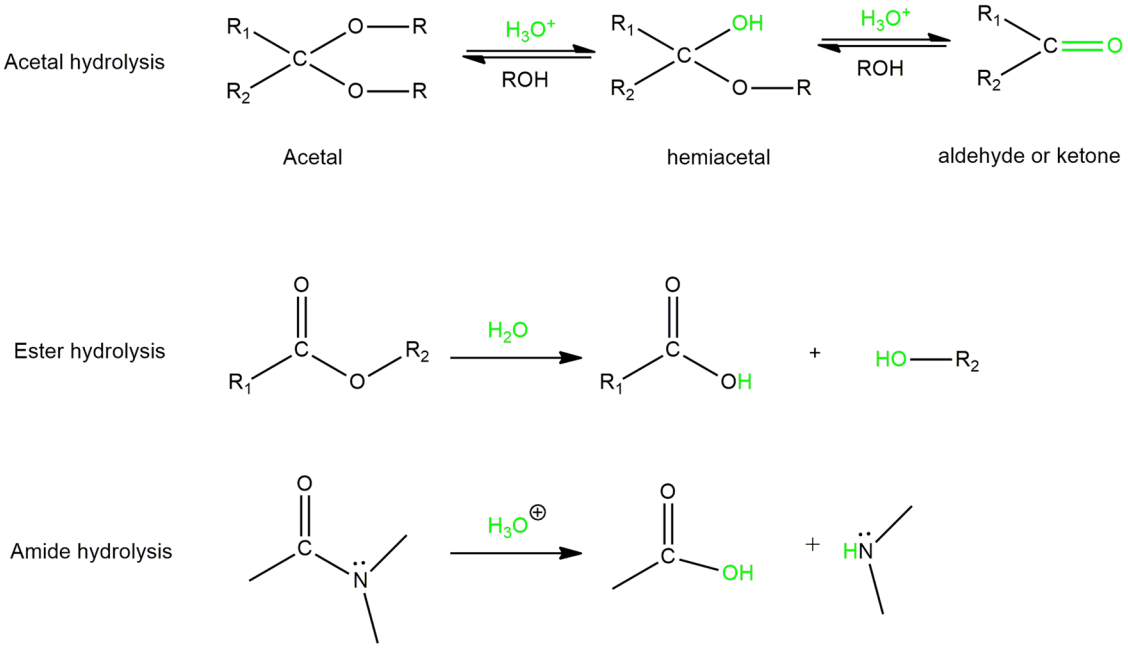


**Fig. 1 | Schematic representation of hydrolytic cleavage of targeted bonds.** The chemical cleavage mechanism of acetals, esters, amides, and related moieties during degradation is highlighted.

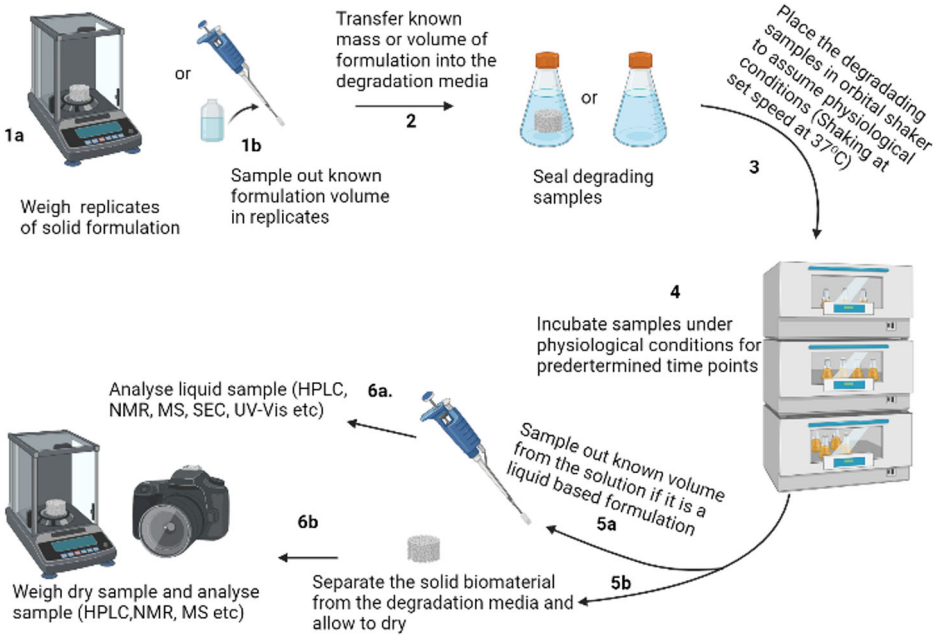


**Fig. 2 | Schematic representation of experimental set-up for assessing degradation of solid and liquid formulations.** Image produced via BioRender.

compatibility compared to the starting material. To this end, the degradation of peptide and polymeric biomaterials was researched, including the mechanism degradation[25,26]. The requirement of degradation of biomaterials is to enable absorption of the by-products, induce therapeutic effects (which may be associated with release of a bioactive from the biomaterial), and undergo elimination from the body.

Desirable in vitro and in vivo biodegradation properties of biomaterials include the following; (a) the material should not induce a sustained inflammatory or toxic response upon implantation in the body, (b) the material should have an acceptable shelf-life, (c) the degradation time of the material should match the healing or regeneration process for tissue engineering, (d) the material should have appropriate mechanical properties for the targeted application and the variation in mechanical properties with degradation should be compatible with the healing or regeneration process, (e) the degradation by-products should be non-toxic, and undergo metabolization and clearance from the body, (f) the material should have appropriate permeability and processability for the intended application[12,27,28].

The experimental approach in constructing an in vitro/in vivo degradation study proceeds as depicted in Fig. 2. The first step allows for pre-degradation assessment of the formulation which involves the analytical techniques that would be used to monitor degradation progress. The second step allows for the immersion of formulation into the degradation media (body fluid, PBS, or enzymatic buffers) to initiate the degradation process. ASTM F1635-11 highlights the degradation testing conditions as indicated above and maintained at a pH of 7.4 or at documented specific pH conditions for the targeted bodily environment, as indicated in the third and fourth steps of Fig. 2. The fifth and sixth steps allow for the processing of degraded samples using various techniques such as gravimetric analysis, surface area changes, and chemical composition qualification and quantification. The ASTM F1635-11 guidelines highlighted that degradation shall be monitored via mass loss (gravimetric analysis), changes in molar mass, and mechanical testing. Furthermore, the guidelines indicated that molar mass shall be evaluated by solution viscosity or SEC, while weight loss shall be measured to a precision of 0.1% of the total sample weight and that the sample should be dried to a constant weight. There are few concerns that the ASTM guidelines does not consider (i) invasiveness of the degradation approaches which can disturb degradation during the sampling period, (ii.) continuity of degradation during the degradation assessment process, and (iii.) real-time assessment of degradation of biomaterials. Herein, several approaches extracted from literature reports were evaluated and critiqued to suggest a cost-effective and reproducible degradation assessment approach.

Physical characterisation approaches include surface morphology assessment via SEM[29], mass and molecular balance transitions after exposure to simulated body fluid[30,31], changes in mechanical properties[27,32], and surface/bulk erosion[33–35] of biomaterials. The chemical characterisation approach for assessing degradation includes the use of specialised equipment such as fourier transform infrared spectroscopy (FTIR)[29,36], NMR[37], and mass spectrometry[38,39] to name a few. The third approach to assessing the biodegradation of biomaterials employs analytical techniques that can quantify the degrading biomaterials or the produced by-products after degradation. The specialised analytical techniques that can quantify biodegrading biomaterials include equipment such as X-ray photoelectron spectroscopy (XPS)[29], ultraviolet–visible spectrophotometry (UV-Vis)[40], high-performance anion-exchange chromatography/pulsed amperometric detection (HPAEC-PAD)[41], and chromatographic techniques[41,42].

Chemical, physical, and mechanical attributes of the formulation can be monitored before degradation, during degradation, and at the endpoint of the degradation process. Chemical composition, texture, colour, rigidity, porosity, surface area, shape, elasticity, viscosity, and electrical charge for solid/liquid formulation are among the properties that can be assessed to qualify and quantify the degradation of biomaterials. When a solid formulation/biomaterial is insoluble in the release media, the gravimetric analysis and surface erosion approach can be carried out effectively. However, if soluble polymeric/biomolecules are used in formulating the biomaterials, the biomaterials would dissolve in the degradation media which renders the gravimetric analysis and surface erosion degradation assessment approach ineffective in accurately monitoring the degradation processes[4,43]. Further techniques such as vacuum drying or lyophilisation/freeze-drying may have to be incorporated after each degradation time point to remove all liquid and measure the residual weight of biomaterial. Biomaterial solubility is one of the leading limitations in employing physical degradation assessment approaches such as gravimetric analysis and surface erosion. The current review focuses on steps 5 and 6 in Fig. 2, examining the approaches employed in assessing the biodegradation of biomaterials and providing insight into the improvements required to ensure that the approaches are reliable and valid for assessing the degradation of biomaterials.

## Factors affecting degradation of biomaterials

### Biomaterial processing

**Biomaterial crosslinking**. Degradation profiles of hydrogels can be affected by the biomaterial composition such as polymer concentration,

**Table 1 | Materials and their impact on degradation and degradation assessment**

| Material type | Materials sub-groups | Polymer examples | Impact on degradation | Refs. |
|---|---|---|---|---|
| Natural | polysaccharides | Alginate, hyaluronic, acid and chitosan | Can increase degradation rate of hydrogels in general. However, the reaction used during biomaterial development can control degradation rate of biomaterial, i.e., strain promoted alkyne–azide cycloadditions reaction can slow down degradation of rate of natural based biomaterials. | 30,50,51 |
| | polypeptides | Collagen, Gelatin, Silk | Can increase degradation rate of biomaterials. biomaterial development reactions can slow down degradation even when natural polymers are utilised. | 50,51,145,146 |
| | Lipids | Triglycerides, fatty acids | Can slow down degradation rate of nano formulations. | 53,147 |
| Synthetic | Polyesters and polylactide | Poly(Glycolic Acid), Poly(L-lactic acid), and PLGA | Varying monomer ratios in synthetic polymers can influence the degradation of biomaterials, i.e., high G/L rations in PLGA-based formulations decrease the rate of degradation. | 19,49,54,148 |
| | aliphatic copolymer | Polycaprolactone (PCL), Polybutylene succinate (PBS) | These polymers slow down degradation rate of biomaterial. | 49,54,149 |
| | Others-relative low degradation | PVA | These materials slow down degradation rate. | 150 |
| Hybrid/composite (Natural and synthetic) | Polyesters with non-degradable polymers | PLA/PU | Degradation rate is increased by adding PLA to PU | 56 |
| | Slow-degrading materials with natural polymers | PVA/HA hydrogels, PCL hydrogels, | degradation rate can be improved through utilising a biodegradable material such as HA. Degradation can also be decreased by blending natural polymers with synthetic polymers. | 56,150,151 |

formulation type (solid/solution), crosslinker functionality, and overall charge of the biomaterial due to functionalisation and/or conjugation. Literature reports have highlighted that the hydrogel degradation profile can be controlled by altering hydrogel composition[44,45]. Crosslinked hydrogels tend to induce resistance to degradation. Ester hydrolysis rate constant is higher for crosslinked gels compared to non-crosslinked hydrogels[45]. Degradation via hydrolysis of esters is inclined to proceed slowly in biomaterials constructed from negatively charged polymers[45].

**Material selection and combinations (natural versus synthetic materials)**. Biomaterial crosslinking has tremendous impact on degradation. Biomaterial processing such as thermomechanical properties (extrusion, compression molding) and polymer selection (natural versus synthetic, hydrophilicity/hydrophobicity, size, charge etc) can also impact degradation kinetics of hydrogels. Biodegradable natural, synthetic, and a hybrid of both polymer types have been extensively reported in literature[46–48]. In this study, the different polymeric types will be discussed briefly on their general impact on degradation of biomaterials (Tables 1 and 2).

Natural polymers are naturally occurring macromolecular compounds. These natural occurring compounds such as polysaccharides, polypeptides, and lipids are characterised by their chain of sugar units attached by a glycosidic bond in polysaccharides whereas a in polypeptides the amino acids connected via a peptide bond form the repeating units chain[46,49]. Natural polymers such as alginate, hyaluronic acid (HA), collagen, and chitosan are readily degradable in their respective in vitro degradation media as well as their in vivo biological processes. The use of natural polymers in the biomaterial development can control the rate of degradation of the biomaterial. Natural polymers such as gelatin, alginate, and HA have been noted to accelerate degradation of various formulations whereas silk fibroin slowed down degradation rate[50]. Natural polymers can increase degradation rate of hydrogels in general. However, the reaction used during biomaterial development can control degradation rate of biomaterial. On contrary to the general impact of natural polymers on degradation of biomaterials, strain promoted alkyne–azide cycloadditions reaction slows down degradation rate of natural based biomaterials[51]. This attest to the effect of certain reactions on the overall degradation of biomaterials. Lipids are susceptible to chemical oxidation which can increase the rate of degradation biomaterials[52]. However, lipids can also protect drugs from hydrolysis reactions which is a crucial property for the overall degradation of formulations[53].

Synthetic polymers are not natural occurring, they are synthesised via polymerisation procedures such as aliphatic polyesters, polylactide, aliphatic copolymer[54]. Synthetic polymeric materials can easily be hydrolysed during the degradation process which makes them suitable for controlling degradation of biomaterials[49]. Degradation rate can be controlled by varying specific monomers of these synthetic polymers i.e. increasing the proportions of L/G in PLGA based biomaterials can decrease the rate of biodegradation[55]. Aliphatic polyester such as PCL have a very slow degradation rate. However, degradation of biomaterials can be improved by utilising specific type of PCL with certain degree of crystallinity and molecular weight[49]. Furthermore, co-polymerisation of PCL with other polyesters can also improve degradation of biomaterials. Relatively slow degrading materials such as PVA can reduce the degradation rate of biomaterials.

Blends or composite of natural with synthetic materials can improve degradation of biomaterials by varying proportions of natural to synthetic materials[56]. PVA and PLC have relatively slow degradation rate compared to synthetic polyesters. Blending PVA with HA has been shown to improve degradation rate of biomaterials[57]. Degradation can be improved by blending certain natural with synthetic polymers. However, degradation can also be decreased by mixing natural with synthetic polymeric materials[56,58].

## Environmental factors

The well-known factors that impact the degradation of biomaterials are temperature, humidity, specific pH conditions, and mechanical loading. A study by Hosseini et al. indicated that humid conditions or wet conditions at high temperatures induced a strong hydrolytic degradation of poly(-ethyleneterephthalate) products[59]. To evaluate the effect of pH on the degradation of biomaterials; poly(lactic acid) (PLA) brushes were incubated in phosphate buffer solutions at different pH conditions at 37 °C[60]. PLA brushes exhibited an increased susceptibility to degradation with increasing pH conditions[60,61]. it was also observed that the PLA brushes degraded rapidly as the temperature was increased[60]. Similar effects of pH on degradation were observed in other reports[61]. However, there are some cases where biomaterials exhibited less pH effect on degradation. A study by Juuti et al. indicated that poly(l-lactide-co-glycolic acid) (PLGA) stents' degradation was not impacted by the different pH conditions[62]. Stress from environment conditions can affect degradation of biomaterials. Mechanical load can cause biomaterials to degrade faster than the expected degradation rate[63]. Micro and macro structural, mechanical, and morphological

**Table 2 | Properties of degradation assessment approaches**

| Degradation assessment approach | Monitored parameters | Advantages | Disadvantages | Quantification/qualification | Equipment/software | Refs. |
|---|---|---|---|---|---|---|
| Physical | Gravimetric analysis | - Cost-effective<br>- Easy to undertake in a lab setting<br>- Can quantitatively infer degradation | - Measurements affected by polymer solubility<br>- Technical weighing error<br>- Cannot qualify degradation<br>- No by-product information<br>- No clear indication of the biodegradation mechanism<br>- Limited applicability in liquid formulation | - Quantification of residual mass | - Weighing balance | 4,83,87,90,91,99 |
| | Surface erosion | - Easy to execute in the laboratory setting<br>- Does not require sophisticated analytical equipment<br>- Macroscopic photographs provide a visible representation of biomaterial degradation | - Solubility affects the size and shape of the formulation<br>- The approach is limited to solid biomaterials | - Quantification of formulation physical size reduction | - Camera<br>- Zeta sizer for particle size | 87,90,91,152 |
| | Morphological assessment | - Can assess both liquid and solid-based formulations i.e., nano/microparticles, scaffolds, fibres, sutures, films, creams, gels, and suspensions<br>- can infer degradation through affected morphological properties | - Cannot quantify degradation<br>- May require flash freezing to preserve the morphology of the formulation<br>- Does not give sufficient information regarding biofragmented molecules and formed by-products | - Observation of surface morphological changes in formulations | - SEM<br>- TEM<br>- Image assessment software i.e., *image J*, MATLAB®, Wolfram Mathematica™ | 87–91,93–95,153,154 |
| Mechanical attributes | Storage modulus, tensile strength, compressive strength and viscosity | - Can infer degradation and testing parameters can be quantified for both liquid and solid-based formulations | - Inability to chemically confirm and quantify degradation<br>- Requires the use of sophisticated equipment rendering it economically challenging | - Quantifying loss of mechanical properties i.e., storage modulus, tensile strength, compressive strength and viscosity | - Rheometer<br>- Viscometer<br>- Rheolution Elastosens™ bio2 (simplifies the characterization of hydrogels during and after gelation) | 99,101–103,155,156 |
| Chemical attributes | Chemical composition | - Provide reproducible results<br>- Provides both quantification of degrading materials and confirms the structure of the formed by-products or dissociation of macromolecules | - High costs associated with equipment and running costs<br>- Series of steps required for sample preparation | - Quantification of disintegration of starting biomaterials and formation of smaller biomolecules or by-products)<br>- Qualitative analysis of specific properties of formed by-products or fragmenting biomolecules | - MRI<br>- NMR<br>- Mass spectrophotometer<br>- Respirometer<br>- pH metre | 45,107,111,132 |
| | Absorption and fluorescent properties | - Accurate measurement of degrading materials via absorptance and fluorescent properties of biomolecules | - Limited to biomolecules with the capability of absorbing visible light or fluorescent properties<br>- May require sophisticated assays<br>- High costs may be expected with equipment and running costs | - Quantification of absorbance or fluorescence | - UV-Vis<br>- Fluorescence Microscopy<br>- Chromatography (HPLC/UPLC) | 115,119,134,143 |

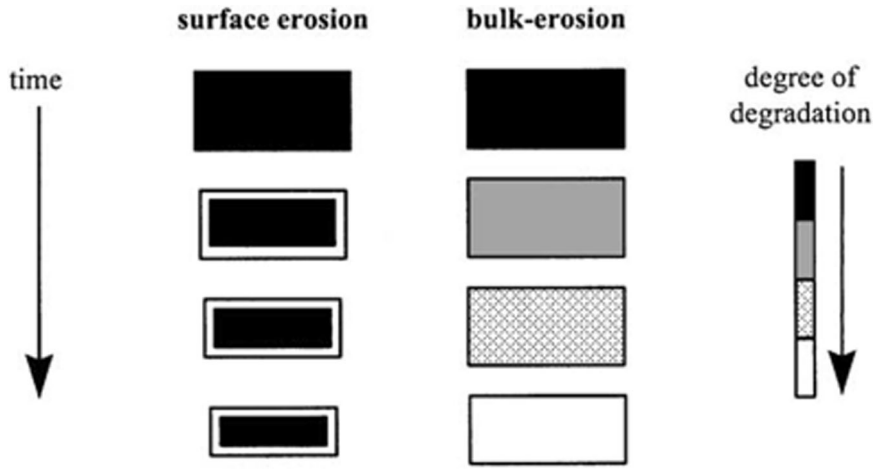


**Fig. 3 | Schematic representation of surface and bulk erosion of polymeric formulations.** Image obtained with permission from Von Burkersoda et al. Von Burkersroda et al.[76].

properties of polyester based biomaterials are susceptible to accelerated degradation rate due to mechanical load exerted to the biomaterials[63].

### Biomaterial form (solid versus liquid)

Solid polymeric biomaterials generally tend to degrade slower than in their liquid form[64]. A study by Wach et al. noted a low rate of viscosity reduction in solid hydroxypropyl cellulose compared to liquid form when irradiated (ɣ-rays)[65]. The decrease in viscosity rate in solid-to-liquid was attributed to the breakdown of polymers to their respective low molecular compounds (by-products) such as oligo/monosaccharides[65]. Similar observations were noted in an unrelated study where the hydrolysis rate constant ($k_{obs}$) values in gels (solid) were roughly 1.8-fold greater in gels than in polymer solutions (liquid)[45]. A study by Nagasawa et al. evaluated the irradiation-induced degradation of alginate biomaterials and observed that degradation was higher in the aqueous alginate than in the solid form[66]. The susceptibility of liquids to degradation was attributed to the presence of water which accelerated the degradation[66]. The increased rate of degradation of liquid-based biomaterials compared to solid formulations could also be explained by the slower rate of polymer matrix hydration in solid formulations compared to liquid forms.

### Biomaterial chemical, physical, and mechanical properties

Biomaterial chemical properties can impact their biodegradation properties. Functionalisation of biomaterials affects the degradation of biomaterials. The effect may be attributed to the changes in hydrophilicity of the biomaterial after modifying/adding certain functional groups on/to the chemical structures of the biomaterial. A study by Kumar et al. noted the effect of methoxypolyethylene glycol (mPEG) derivatives (mPEG (-$OCH_3$ functionality), mPEG-aldehyde (mPEG-CHO) and mPEG-acetic acid (mPEG-COOH)) on the degradation of these chitosan (CHT)-mPEG derivative scaffolds[67]. The mPEG hydrophilicity allowed for ease of water penetration to the scaffold leading to faster degradation. However, scaffolds containing conjugated mPEG chains (CHT-mPEG-CHO and CHT-mPEG (-$OCH_3$ functionality) exhibited slower degradation compared to scaffolds containing free mPEG chains (CHT-mPEG)[67]. CHT-mPEG-CHO scaffold underwent the slowest degradation due to the strongest conjugation of mPEG-CHO with CHT[67]. Strong interactions and conjugations within biomaterials can limit degradation of biomaterials whereas the use of free branched hydrophilic biomolecules can enhance degradation of biomaterials. Presence of certain functional groups, i.e., ester, ether, amide, imide, thioester, and anhydride groups, can have an impact on the degradation of biomaterials. Esters allows for ease of degradation whereas biomolecules with aldehyde and -$OCH_3$ functional groups showed decreased degradation[67]. Degradation does not solely depend on the presence of certain functional groups but also on the interactions between biomolecules. Strong conjugation can lead to slow or limited degradation whereas weak conjugations or presence of free polymeric chains can disrupt the H-bond rendering the chains more mobile, hence decreasing the water holding capacity[67]. Biodegradable biomaterials can be developed from synthetic polymers with hydrolysable functionalities such as polyesters, polyether, polyamide etc[68,69].

The crystallinity of biomaterials is another degradation-impacting factor. A study by Pantani and Sorrentino indicated that crystallinity decreased the PLA degradation rate where crystallinity affected the first stages of water diffusion into the polymer matrix and also significantly affected swelling and biodegradation rate[70]. The study noted that semi-crystalline injection-moulded PLA showed a much slower degradation rate compared to the amorphous injection-moulded PLA[70]. In a different study, it was indicated that highly crystalline polymeric biomaterials have poor solubility kinetics[71]. The studies above highlighted that the crystallinity of the material can affect the degradation process. Therefore, crystallinity can be explored to regulate biomaterial degradation.

The biomaterial viscosity affects the overall degradation of biomaterial. Antti et al. assessed carboxymethylcellulose degradation and noted that degradation is faster in biomaterials with initial higher dynamic viscosity than in biomaterials with initial lower dynamic viscosity[72]. The observation was attributed to the stronger cavitation collapse of viscosity in highly viscous liquids causing faster degradation compared to less viscous solutions with weaker cavitation collapse presenting slower degradation[72]. This means that in viscous solutions, the negative pressure in the rare fraction region of wave function does not easily overcome the natural cohesion forces acting within the liquid thereby creating voids or bubbles which bursts and result in strong collapse of the viscosity in viscous liquids compared to less viscous liquid. In the same study, when comparing biomaterials having a controlled similar dynamic viscosity with varying molecular mass, biomaterials composed of higher molecular mass polymers displayed a higher extent of degradation than the biomaterials composed of polymers of lower molecular mass[72]. Degradation was also reported to be affected by polymeric concentration as high polymeric concentrations displayed faster degradation than biomaterials with low polymer concentrations[72]. The accelerated degradation of highly concentrated polymeric biomaterials was attributed to the presence of more molecules that would undergo degradation in highly concentrated liquids compared to a lower concentration of molecules[72].

Hydrophobicity can also affect the degradation of biomaterials and the effect is mostly associated with reduced biomaterial exposure and interaction with water. Increasing hydrophobicity lead to a decrease in the amount of surrounding water that can facilitate hydrolytic cleavage on the scissile bond of biomaterials[73]. The other effect of degradation is the steric hindrance and bulkiness around the area where hydrolytic cleavage occurs. When steric bulk around esters is increased, the bonds become less susceptible to hydrolysis[73,74]. A study by Zhong et al. investigated a series of hyperbranched poly(α-amino ester)s which showed that the more

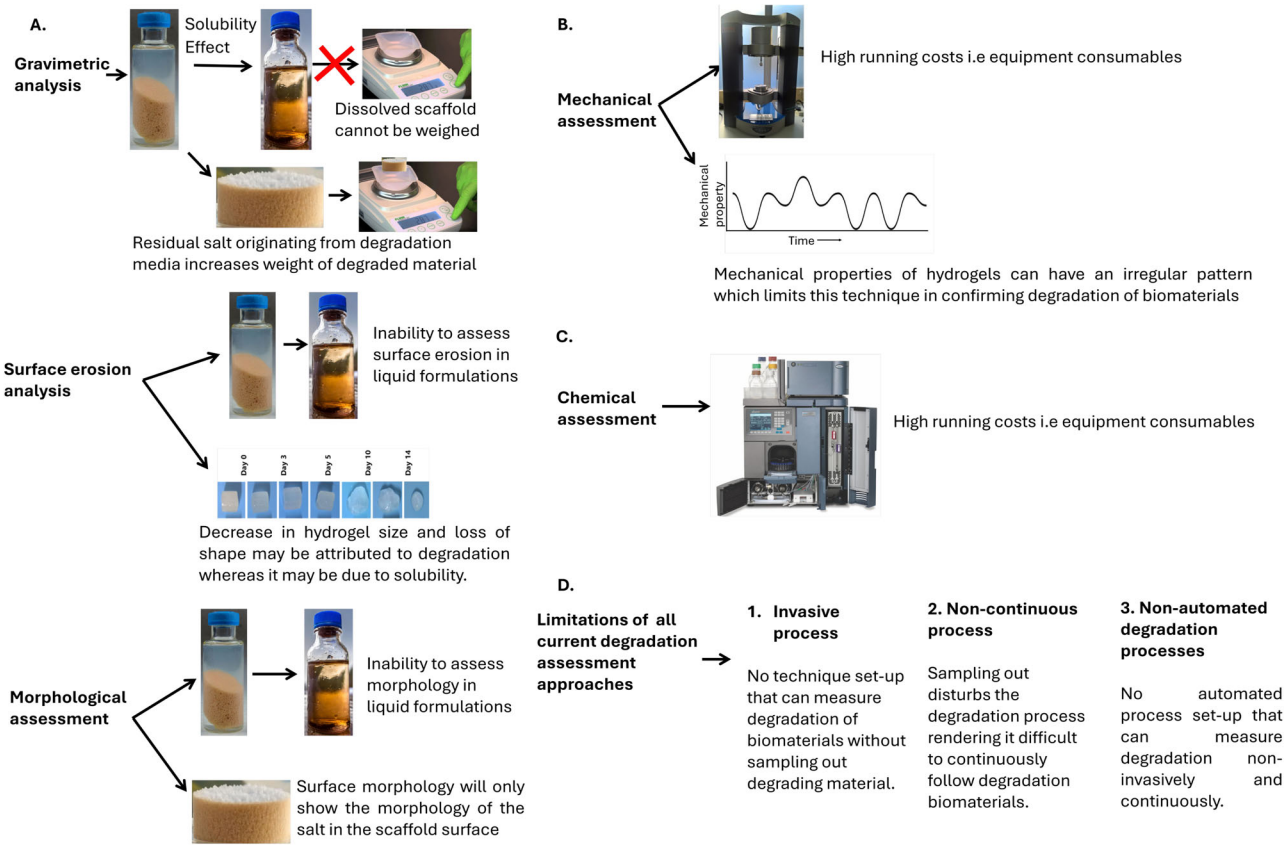


**Fig. 4 | Schematic showing degradation approaches and factors affecting degradation assessment of biomaterials. A** shows the limitations of physical degradation assessment approaches. **B** Shows the limitations of mechanical degradation assessment approaches. **C** shows the limitations of chemical-degradation assessment approaches. **D** explains the three limitations of all current degradation assessment approaches/techniques.

hydrophobic polymers degrade at a slower rate compared to hydrophilic polymers[75].

Peptides and protein-based formulations also tend to be more stable in solid form compared to liquid form. Another contributing factor to faster degradation occurring in liquid formulations compared to solid formulations is the mechanism of degradation. Liquid formulations tend to follow bulk erosion whereas solid formulations may follow either/both bulk and surface erosion. This is due to the degrading media that easily and rapidly interacts with all molecules throughout the liquid suspension thus inducing bulk erosion[76]. The process of bulk and surface erosion of polymeric materials is depicted in Fig. 3 below. In surface erosion, the materials tend to erode from the surface moving towards the centre of the polymeric formulation as time proceeds, whereas in bulk erosion, degradation occurs throughout the formulation matrix instead of starting in one specific location.

### Biomaterial geometries (shape and size) influence in the degradation of biomaterials

Biomaterials in the biomedical field can be designed into various shapes and sizes to suit targeted application. However, certain shapes and sizes are more susceptible to degradation than others. A study by Chen et al. developed biodegradable magnesium vascular stent implant designed by shape optimisation strategy for arterial applications[77]. The stent was developed into a sine-wave shape with several layers between the inner diameter and the outer diameter of the stent[77]. Proximal and distal rings of the stent exhibited high corrosion at the corners of those rings and the overall degradation of the stent was from the surface and moved layer-by-layer to the interior of the stent[77]. Biomaterials with single layers have a higher degradation rate compared to those with multiple layers[78]. Increasing surface area increases the rate of biodegradation[79]. Single layer films, nanospheres and scaffolds have higher degradation rate compared to multilayered counterparts[78,79]. Small spheres degrade faster than larger spheres[80]. The observations above attest to the impact of biomaterial geometry on the overall degradation of biomaterials.

## Degradation assessment approaches employed in the biomedical field

Biodegradation can proceed via two mechanisms which include bio-deterioration and bio-fragmentation. The biodegradation mechanism normally occurs at the surface level whereas bio-fragmentation involves the cleavage of bonds into smaller molecular structures[81]. Bio-deterioration and bio-fragmentation may occur at the same time and can both affect the physical, chemical, and mechanical properties of biomaterials[81]. Exposure to certain temperatures, chemicals/enzymes, and light in the environment, such as degradation media at physiological conditions can initiate and influence biodegradation. Progress in degradation can be assessed using the techniques below to quantify and qualify degradation of biomaterials and by-products formed during the degradation process (Fig. 4).

### Physical degradation assessment approaches

**Gravimetric analysis approach for assessing degradation of biomaterials.** The gravimetric analysis approach focuses on measuring the residual weight of biomaterial after biodegradation. The kinetics of fluid uptake or wettability directly affects weight loss. Fluid-based hydrogels/biomaterials tend to allow the fluid to reach the biomaterial matrix faster than solid biomaterials. Moreover, nanoparticles present a high surface area which allows for fluid to reach the core of the biomaterial faster than in scaffolds/microparticle-based biomaterial formulation. Thus, nanoparticles along with liquid-based formulations tend to display a high degree of degradation compared to microparticles and scaffolds due to varying surface erosion and bulk erosion that exist during the degradation of the formulation[82].

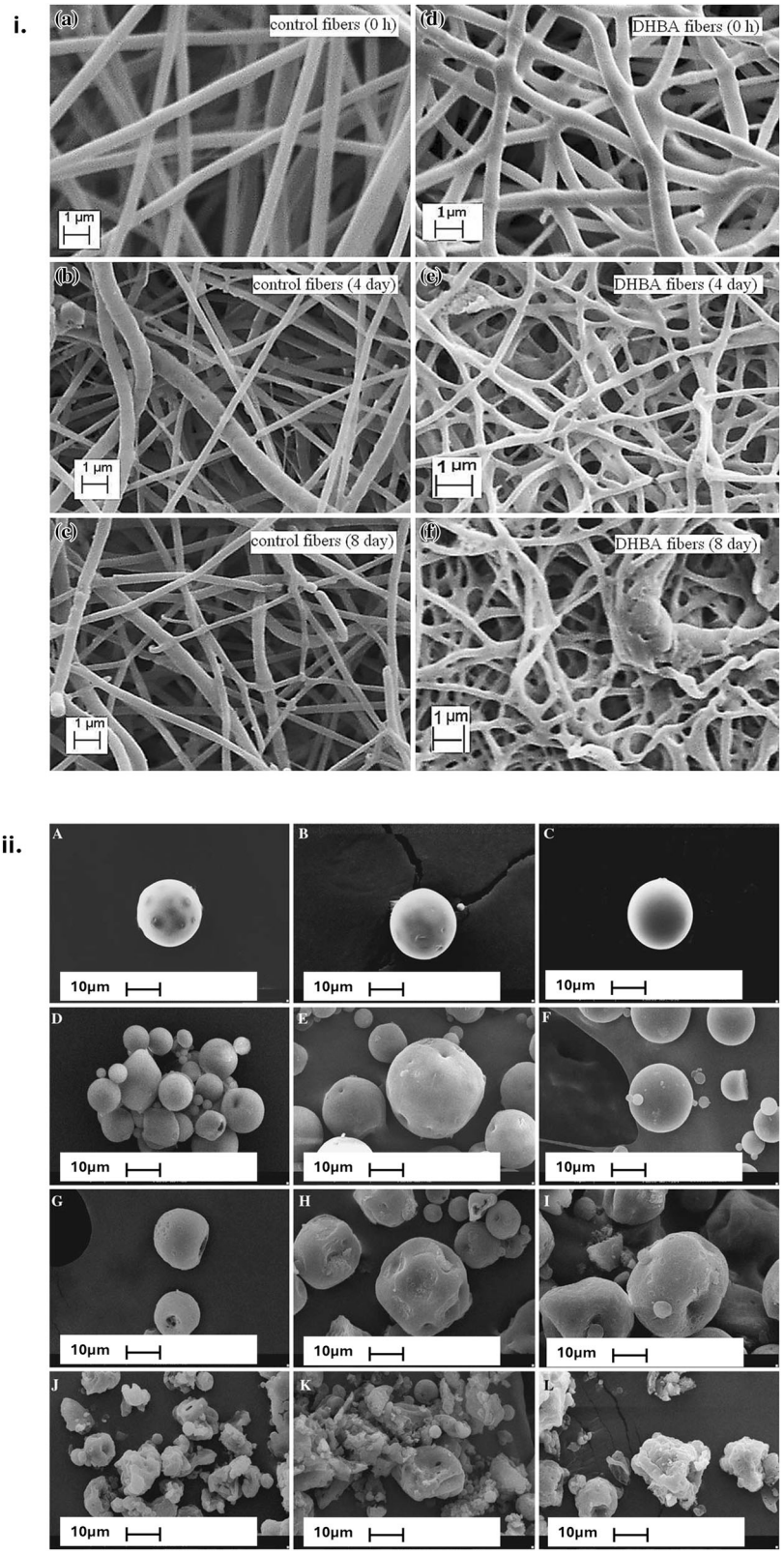


**Fig. 5 | SEM surface morphological changes on biomaterials due to degradation.** Degradation of fibers and microspheres at different time points are provided: (i) images of nanofibers without DHBA (control fibres) and nanofibers containing DHBA (images of the fibres were obtained at different time points, **a**–**c** are control fibres at time 0, 4, 8 h, whereas **d**–**f** are fibres at time 0, 4, and 8 h, respectively. (ii) SEM images of microspheres at time 0 (**A**–**C**) and degrading microspheres after 8 (**D**–**F**), 15 (**G**–**I**) and 21 (**J**–**L**) days of degradation. Microsphere formulations: PLGA10 (**A**, **D**, **G**, **J**), PLGA15 (**B**, **E**, **H**, **K**), PLGA20 (**C**, **F**, **I**, **L**). Images reproduced with permission from Ahire et al.[90], and Biondi et al.[91].

The gravimetric analysis approach is cost-effective and easy to undertake in a laboratory setting. Therefore, this renders it one of the highly explored degradation assessment approaches in the biomedical field. However, the approach presents a few limitations that need to be taken into consideration when assessing the biodegradation of biomaterials. The limitations of the gravimetric analysis approach include; (a) the solubility of polymeric biomaterials which can be incorrectly considered as degradation when biomaterial is exposed to degradation media, (b) high chances of technical error given that solid biomaterials can disintegrate into several smaller pieces which may cause it to be difficult to accurately collect for drying, (c) biomaterials can still hold water into their core thus increasing residual weight, (d) the approach can quantify but cannot qualify

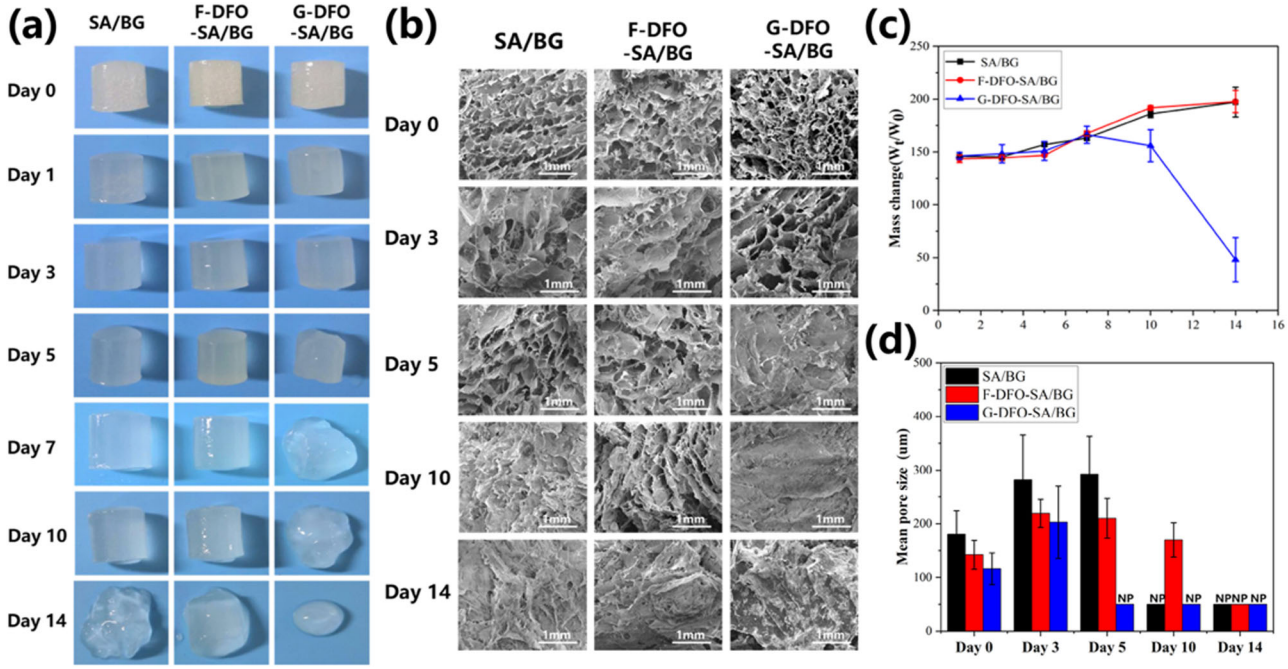


**Fig. 6 | The use of multiple degradation assessment techniques to monitor degradation of hydrogels.** Degradation assessment of SA/BG hydrogel, F-block deferoxamine -SA/BG hydrogel, and G-block Deferoxamine -SA/BG hydrogel. **a** Macroscopic photographs of hydrogels from day 0 to day 14. **b** SEM images of interior structure of hydrogels from 0 to day 14. **c** Quantitative analysis of mass change of hydrogels with degradation time. **d** Statistics of mean pore size measured from the representative SEM images. NP means not porous. Image obtained with permission from Zhang et al.[87].

degradation as it does not include information on the produced by-product, (e) it is limited to solid formulations and has limited applicability in liquid formulation, and (f) liquid-based formulations requires conversion to solid form with the use of freeze-drying technique.

Alginate is a highly employed polymeric biomaterial in the biomedical field. Alginate is highly soluble in water, which could render degradation assessment via gravimetric analysis difficult. Mndlovu et al. indicated that the degradation of alginate is highly affected by its solubility in water[4]. The swelling behaviour of pristine alginate decreased from 619.69% in 8 h to 111.14% after 24 h which resulted in approximately 90% degradation within 3 days[4]. The decrease in swelling showed that the polymer might have been dissolving, and the high degradation rate highlighted one of the drawbacks of the gravimetric analysis technique[4]. The degradation media, such as PBS or enzymatic media, may contain salts which can add to residual weight if not taken into consideration during the experiment and analysis. A washing step needs to be included to remove excess salts on the degrading biomaterial. Alternatively, the collected degradation media (buffer solutions) can be lyophilised and weighed to be used as a control to determine the weight attributed to the salt contents of the buffer solution[83].

Air-drying of biodegraded samples is an easy approach that is widely used to measure the residual weight of biodegraded biomaterials. However, biomaterials can hold fluid in the matrix core which can prolong the air-drying process. Thus, inaccurate measurements may be obtained when using the air-drying approach. A solution to the air-drying issue would be to use other equipment that can quantify moisture content on degraded biomaterial. Alternatively, the freeze-drying technique or vacuum drying oven may be employed. Freeze-drying/lyophilization allows for the complete removal of water from the biomaterials by using a vacuum on frozen biomaterials to change the phase of water from solid (ice) to vapour without passing through a liquid phase[84,85]. The high cost associated with lyophilization limits its application in biodegradation studies and the biomedical field[84–86]. The limitations and solutions provided above can mediate issues with the gravimetric analysis approach as a technique to follow the degradation of biomaterials. However, the gravimetric analysis approach should not be used solely as a degradation assessment technique as the technique only infer degradation, and degradation cannot be fully confirmed by changes in the weight of biomaterials. The gravimetric analysis technique should be coupled with chemical-degradation assessment techniques such as HPLC, Mass spectrometry, SEC, and NMR.

**Surface erosion approach for assessing degradation of biomaterials.** Surface erosion allows for degradation assessment by measuring reduction in the size of solid hydrogel (gel, scaffold, fibres, sutures, tablets etc) with time. Macroscopic photographs are captured during the degradation period and measurements such as surface area and diameter are acquired for quantification of degradation. Macroscopic photographs can be presented according to the degradation time point whereas calculations can be conducted via *Image J* software (Figs. 5 and 6a)[87]. Similar to the gravimetric analysis approach, the surface erosion degradation assessment approach is easy to execute in a laboratory setting, does not require sophisticated analytical equipment, and the macroscopic photographs provide a visible representation of biomaterial degradation.

The limitation of the surface erosion degradation assessment approach is the solubility of polymers that affect the measurement of surface erosion in the sense that soluble polymers would dissolve and lose their size and shape. The surface erosion degradation assessment approach is limited to solid biomaterials. The approach infers degradation by the reduction in size and change in the physical shape of biomaterial but cannot confirm degradation or differentiate between degradation and solubility of biomaterials. The limitations mentioned above render the surface erosion degradation approach questionable to be used alone. Therefore, it is often undertaken along with other degradation assessment approaches such as gravimetric analysis and morphological assessment as depicted in Fig. 5.

**Assessment of biomaterial morphology as a degradation assessment approach.** Morphological assessment of degrading biomaterials provides valuable information concerning the effect of degradation on the porosity and textural properties of biomaterials. SEM and transmission electron microscopy (TEM) are the most used equipment in

assessing the morphological properties of scaffolds. Images acquired through SEM can be processed in *Image J* software which calculates the pore size and distribution on scaffolds. Different biomaterial formulations can be assessed for degradation through this approach, and this includes microparticles[88], scaffolds[87], fibres[84], sutures[89], etc. A study by Ahire et al. followed the degradation of electrospun Poly(D,L-lactide) (PDLLA)/Poly(ethylene oxide) (PEO) nanofibers in phosphate buffered saline (PBS) solution[90]. The SEM images indicated a reduction in nanofiber size and loss of 58% weight in 8 days (Fig. 5i)[90]. Furthermore, functionalisation of the nanofibers with PEO contributed to major degradation observed on 2,3-Dihydroxybenzoic acid (DHBA)-containing nanofibers compared to the nanofibers without DHBA[90]. Another example of transitions in the morphological features of biomedical materials is the transformation in shape of biomaterials. A study by Biondi highlighted the loss of spherical shape in degrading PLGA microspheres (Fig. 5ii)[91]. The loss of the spherical shape of the microspheres was also accompanied by the progressive decrease in molecular weight and loss of mechanical properties of PLGA microspheres[91].

The surface morphological assessment approach presents a few limitations which include, but are not limited to, (i) the approach can infer degradation through observing the changing morphological properties but cannot quantify degradation, (ii) in some instances the approach may require flash freezing to preserve morphology at each degradation time point, (iii) the approach does not give sufficient information with regards to biofragmented molecules and formed by-products, (iv) morphological changes may not be significant in some instances which renders this degradation approach questionable for application in isolation. RGD-Paclitaxel-Curcumin-nanoliposomes were monitored using TEM and zeta sizer for stability over 3 months in a stability chamber. The stability results exhibited a slight increase in particle size and a slight decrease in zeta potential[92]. However, there was no significant change in the physical appearance and particle aggregation of the nanoliposomes[92]. The observation above attests to the limitations associated with the morphological analysis as a degradation assessment approach.

The analysis of degradation via physical assessment approaches presents several limitations as indicated above. None of the physical assessment approaches mentioned above can be employed alone in assessing degradation, instead, they should be coupled with additional degradation approaches such as NMR, MS, FTIR, or HPLC to follow the formed by-products and confirm degradation. Gravimetric analysis, surface erosion, and morphological changes were conducted concurrently to study the effect of degradation of sodium alginate/bio-glass (SA/BG) hydrogel. The surface erosion photographs exhibited the reduction in size and loss of shape which inferred degradation; the SEM images showed changes in the morphology of SA/BG due to degradation, and mass change allowed for quantification of degradation (Fig. 6)[87]. The use of two or more degradation assessment approaches allows for an improved description, representation and characterisation of the degradation of the biomaterials (Fig. 6). Changes in mechanical properties, morphological properties and macroscopic images can infer degradation. However, quantification of degradation cannot be solely based on gravimetric analysis, instead degradation studies may include one or more of the robust analytical techniques involved in chemical-degradation approaches.

**Assessment of biomaterial degradation using in silico tools**. SEM/TEM already employs imaging softwares that can allow for biomaterial degradation assessments. *Image j* is a java-based image processing programme that has been predominately used as an image processing tool to quantify degradation on biomaterials in SEM/TEM images. However, there are other image processing tools that can provide robust degradation assessment and such in silico tools include MATLAB®, which is a multi-paradigm programming language and numeric computing environment software and Mathematica™, which is a software system with built-in libraries for several areas of technical computing. The image processing software's highlighted above can provide information such as pore uniformity and distribution in scaffolds/biomaterials. The pore size, uniformity and distribution can be acquired on images of scaffolds that were allowed to degrade for specific period. The acquired information above can then be used to quantify degradation over time by monitoring the transitions in pore size, uniformity, and distribution in scaffolds. Investigations undertaken by du Toit et al. and Kumar et al. highlighted the application of Mathematica™ on SEM images to assess the pore uniformity and distribution in biomaterials and the obtained results were plotted as histograms[93,94]. Degradation was observed by convolving the SEM image with a low pass filter to blur the image in Mathematica™, followed by quantifying the extent of blur in the image using the custom blurring function, colorQuantize the image to provide approximation of the image that uses only 5 distinct colours, and lastly, plot histograms for each colour channel to discriminate between morphological features in the SEM image[93]. A different study by Omranian employed response surface method (RSM), *Image J* and MATLAB® to validate image processing outcomes[95]. One of the ways to optimally represent the results from the two softwares would be to express pore size transitions/pore distribution/pore uniformity variation as a function of time. This would allow for optimum quantification degradation over time and the graph can be accompanied by the SEM/TEM images.

### Assessment of mechanical properties as a measure of degradation

Biomaterial mechanical properties such as viscosity, rigidity (storage modulus), elasticity, and tensile strength are affected during degradation. Young's moduli/ rigidity/ storage modulus/elastic modulus ($G'$) informs of the amount of energy stored within the structure of materials whereas the loss modulus ($G'$) provides more information about the viscous part of the material or the amount of energy dissipated in the material[96]. Complex viscosity ($\eta^*$) furnishes insights into the viscosity of the material measured in a steady shear test[96]. The elasticity of materials can be inferred when the storage modulus is higher than the loss modulus[96]. Tensile strength represents the maximum stress that a material can withstand during stretching or pulling before it breaks.

Biodegrading materials exposed to degradation media tend to lose their mechanical properties such as the decrease in storage modulus, tensile strength, and viscosity[97,98]. A study by Tibbitt et al. highlighted the degradation of poly(ethylene glycol) diphotodegradable acrylate (PEG-di-PDA) hydrogel via irreversible photo-cleavage of *O*-Nitrobenzyl ether moieties that reside within the PEG-di-PDA monomer in the presence of light (one-photon, $\lambda = 320-436$ nm; two-photon, $\lambda = 740$ nm)[99]. Mechanical properties such as lost elasticity of network strands and an exponential decrease in the shear storage modulus were observed on the degraded biomaterials[99]. The cleavage of the bonds within the biomaterial did not only affect the chemical properties of the biomaterials, but also resulted in surface erosion and mass loss[99].

The observations above correlate with other literature reports on the loss of mechanical properties such as the decrease in the elastic modulus[100], compressive modulus and ultimate stress[101], elongation at break and tensile strength[102] of biomaterials during degradation. The observations above highlight the effective use of two or more degradation assessment approaches to analyse the degradation of biomaterials. The loss of mechanical properties of biomaterials during degradation can be followed using equipment such as the rheometer, texture analyzer, viscometer, and Elastosens™ Bio2, to name a few.

Loss of mechanical properties can infer degradation and the testing parameters above can be quantified over time for both liquid and solid-based formulations. The changes in mechanical properties of biomaterials can be graphically represented as shown in Fig. 6 below. However, this degradation assessment approach also presents a few limitations such as (i) the inability to chemically confirm and quantify degradation, instead, degradation is inferred by changing mechanical properties, (ii) requires the use of sophisticated equipment which renders it economically challenging to employ this approach, (iii) an additional analytical technique may be

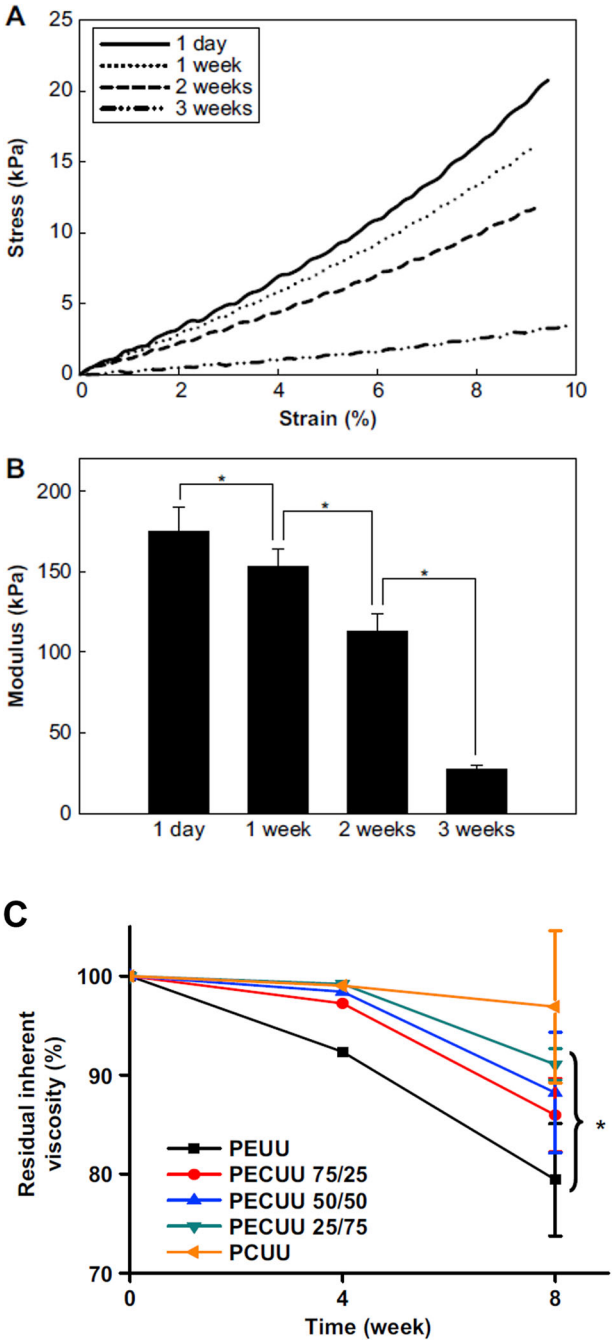


**Fig. 7 | Effect of degradation on mechanical properties.** Three of the various mechanical properties employed in the monitoring of biomaterial degradation are provided. **A** Stress-strain curve, **B** elastic moduli in compression of photo crosslinked MAALG-25 hydrogels during degradation. *$p < 0.05$. **C** residual inherent viscosity of PEUU, PECUUs, and PCUU films after PBS immersion at 37 °C over a period of 8 weeks. Image adapted with permission from Joen et al.[156], and Hong et al.[103].

required to confirm the chemical composition of biomaterials that can support degradation observations. One example of lost mechanical properties during degradation is the decrease in biomaterial viscosity as they degrade (Fig. 7C)[100,103]. A study by Hong et al. highlighted the differences in percentage degradation assessed in different approaches[103]. Synthesised poly(ester carbonate) urethane ureas (PECUUs) exhibited 9% mass loss in PEUU, whereas other PECUUs and PCUU did not show detectable loss of mass over a period of 56 days[103]. In the same time frame, the PEUU had 80% of its original inherent viscosity whereas PCUU displayed a statistically similar viscosity to that recorded at time zero[103]. This study highlights the implementation of the two approaches (MASS difference and mechanical properties) to follow the degradation of biomaterials.

### Chemical properties as a degradation assessment approach

The degradation assessment approaches mentioned above have one common limitation which is the inability to confirm degradation; they infer degradation by gravimetric analysis, surface erosion, morphological changes, and loss of mechanical properties of biomaterials. Chemical composition and formed by-products from biodegraded biomaterials are one of the best degradation assessment approaches that can chemically confirm the degradation of biomaterials. Adding certain functional groups may render biomaterials more hydrophilic or hydrophobic which in turn affects the degradation profile. Ma et al. highlighted that the degradation of graphene family materials is affected by the carbon-to-oxygen ratio (C/O), the lateral size and the number of layers in the 2-D structure[104]. Multiple graphene oxide layers degraded faster than a one-dimensional layer[105]. Material functionalisation such as coating graphene oxide with PEG and BSA (bovine serum albumin) protected the material from enzymatic degradation[105,106]. This degradation assessment approach does not need to be coupled with physical or mechanical degradation assessment approaches; the approach can qualify and quantify degradation. However, the use of high throughput analytical techniques and costly accessories renders this degradation assessment approach economically challenging. Despite the economic challenges associated with this technique, it is of utmost importance in confirming the degradation of biomaterials. The cost challenges can be mediated by the availability of various analytical techniques that can be employed in assessing degradation.

**Assessing degradation of biomaterials via pH monitoring.** Biomaterials tend to have different degradation kinetics in different pH conditions and the pH of the medium changes over time as biomaterials degrade[61,107–109]. The degradation assessment via pH offers few advantages such as indirectly monitoring degradation by measuring the pH of the surrounding degradation media[107] or performing direct degradation by monitoring the molecular weight (via gel permeation chromatography; GPC) of biomaterials at different pH conditions[108]. Degradation of liquid, semi-solid and solid formulations can be followed through this approach. The approach is cost-effective given the low running cost and inexpensive equipment for performing the study. One major drawback with this approach is that degradation cannot be accurately quantified but can be inferred by observing pH changes that occur in the surrounding solution (Fig. 8a). Furthermore, pH can increase and decrease during the degradation period, which renders it difficult to quantify degradation (Fig. 7A)[107]. One of the approaches to mediate the degradation quantification limitation is to employ this approach with another degradation assessment technique, such as GPC, to quantify the molecular weight of degrading biomaterials (Fig. 8b)[108].

**Employing respirometry to assess biomaterial degradation.** Respirometer is an analytical device that can measure the concentration of gases ($O_2$, $CO_2$, $CH_4$, and $H_2S$) under a controlled environment, such as temperature, airflow, and humidity/moisture. The respirometry technique is largely employed in industrial biomass oxygen and carbon dioxide monitoring and the principle behind the technique is well documented[110]. This technique allows for degradation of biomaterials to be followed by measuring the produced $CO_2$. The use of analytical equipment can confirm the degradation of biomaterials given that by-products are measured and represented graphically. One major advantage of this technique is the ability to follow the degradation of liquid, solid and semi-solid-based biomaterials. The other benefit of this technique is the ability to measure the degradation of blank and different samples at the same time. This would allow for simultaneous assessment of relative degradation of crosslinked vs. non-crosslinked biomaterials, functionalised vs non-functionalised biomaterials and bioactive loaded vs plain

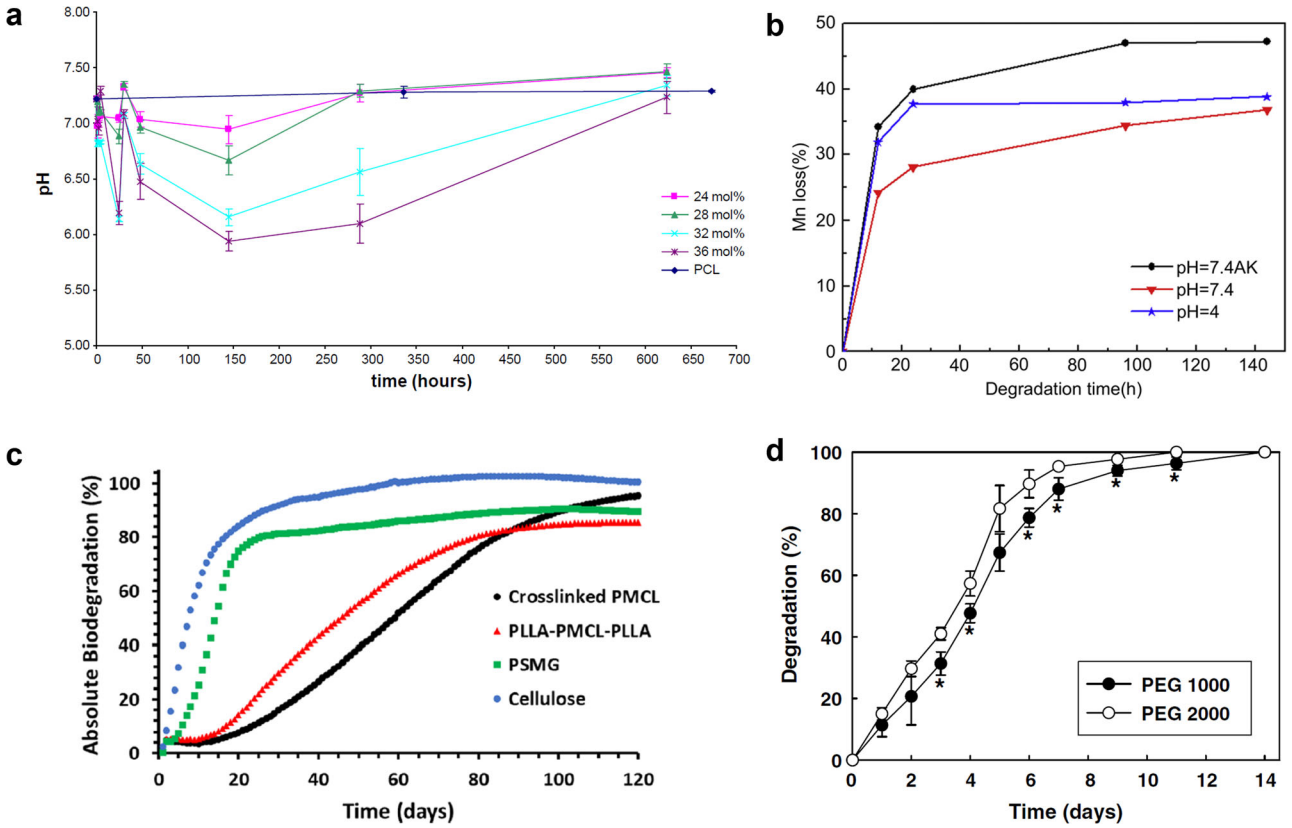


**Fig. 8 | Assessing chemical degradation through monitoring chemical properties of biomaterials.** Various chemical properties monitored during degradation are provide: **a** pH profile of solution where PCL with different calcium oxide (mol%) composition were incubated for degradation. **b** molecular weight loss of poly(ε-caprolactone) ehydrazoneepoly (ethylene glycol) ehydrazoneepoly (ε-caprolactone) macrodiol (PCLH) polyurethane at different pH conditions analysed via gel permeation chromatography. **c** Absolute cumulative biodegradation profiles of polyesters and the positive control cellulose assessed by following $CO_2$ yield via respirometer. **d** Degradation of crosslinked HA hydrogels at a theoretical cross-linking density of 20%. Image adapted with permission from Prabhakar et al.[107], Zhou et al.[108], Reisman et al. [111] and Jeon et al.[115].

biomaterials. This technique allows for small-scale and up-scaled industrial biodegradation assessment. One drawback of the techniques is the running cost and purchasing different sensors for specific gas detection. A study by Reisman et al. employed the respirometry technique to follow the degradation of polyesters (poly(L-lactide), poly-γ-methyl-ε-caprolactone and poly(salicylic methylglycolide) and the technique allowed for accurate long-term (120 days) monitoring of $CO_2$ produced by raw compost (Fig. 8c)[111].

**Employing UV-Vis as a degradation assessment technique**. One of the techniques employed in the chemical composition degradation assessment approaches is the UV-Vis which can measure the concentration of starting biomaterial or follow the formation of by-products. Protein-based biomaterials are easy to follow using UV-Vis or fluorescence. However, some polymeric biomaterials do not possess UV-Vis absorptive properties, which limit this technique from being used to assess degradation. This limitation can be mediated by following the degradation of bioactives/drugs incorporated in biomaterials. An alternative approach to overcoming the limitation above is to use quantification assays[112,113]. A study by Jeon et al. employed the carbazole assay[114] to quantify the degradation of crosslinked HA hydrogels[115]. The study utilised HAase media to facilitate the degradation of the hydrogel. The supernatant fluid of degraded crosslinked HA hydrogels was collected and saturated with benzoic acid followed by quantification of uronic acid content in the samples employing the carbazole assay using D-glucuronic acid lactone as the standard[115]. The study above highlights the measurement of formed by-products (uronic acid) after degradation by using known oligosaccharides of polymeric biomaterials. The mechanism utilised an enzyme (hyaluronidase) to cleave high molecular weight HA into smaller oligosaccharides and measure the formed by-products[116]. The approach used above can be employed with various polymeric biomaterials to study degradation. Understanding the properties of the polymers and the mechanism of degradation would allow for ease of application of the technique above to assess degradation. One of the advantages of using the technique above is the accurate assessment of degradation by analytically quantifying degradation as shown in Fig. 8.

**Degradation assessment via fluorescence**. Fluorescence is another technique that can be employed in quantifying degradation. Similarly, to the UV-Vis approach, the fluorescence technique requires the molecules to fluoresce. Biomolecules tend to lose their fluorescence activity as they degrade[117–119]. This allows for the quantification of the degradation of biomaterials by monitoring the decrease in fluorescence of certain molecules. The major drawback of using this technique is that the technique is rendered inapplicable if the biomolecules used to formulate the biomaterials do not possess any fluorescent properties. This drawback can be mediated by incorporating biomolecules with fluorescent properties, incorporating fluorescent dyes and also using fluorescent kits[120,121].

The advantage of using this technique include accurate quantification of degradation and the ability to graphically display the degradation of biomaterials by showing the decreasing fluorescence over time (Fig. 9). A study by Dong et al. highlighted the application of the imaging technique to quantify and display the degradation of Dox-loaded porphyrin conjugated polyethylene-glycol-ε-caprolactone (PEG-PCL) hydrogel[119]. Degradation was tracked with multispectral fluorescence imaging employing the Maestro CRI in vivo imaging system with dual excitation

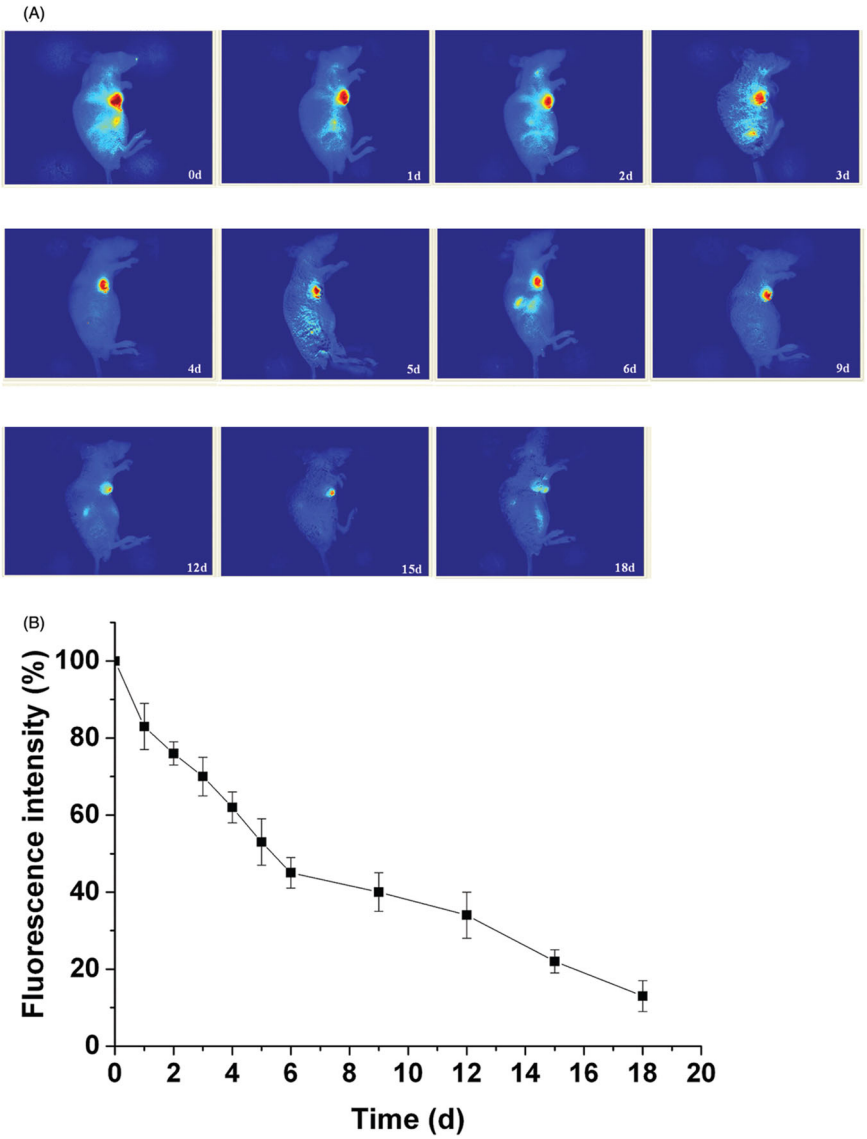


**Fig. 9 | Hydrogel degradation assessment by fluorescence imaging technique. A** The fluorescence signals of the hydrogel were recorded with an excitation wavelength of 595 nm. **B** Quantitative analysis of the hydrogel erosion by fluorescence imaging technique. Image obtained with permission from Dong et al.[119].

wavelengths of 523 and 595 nm at an exposure time of 300 ms[119]. The single signal of the Dox and the hydrogel were separated with green and red by the spectral species (Fig. 9). The bioluminescence imaging was undertaken after intraperitoneal injection with D-luciferin potassium salt solution for 5 min on each of the degradation assessment days[119]. The fluorescence emission signal of the Dox was collected from 560 to 750 nm with an excitation wavelength of 523 nm, while that of the hydrogel was collected from 630 to 800 nm with an excitation wavelength of 595 nm[119]. The drugs, together with the polymeric biomaterials, displayed a decrease in fluorescence as time proceeded (Fig. 9)[119].

**Degradation assessment employing NMR spectroscopy**. NMR spectroscopy is one of the analytical techniques that can be employed in assessing degradation. The principle and pharmaceutical application of NMR is well documented in literature[122–124]. Degradation quantification via $^{1}$H-NMR and C-NMR can be followed by relating the peak area of interest in the degraded sample to a signal from an appropriate internal standard, without the need for a reference standard of the same chemical structure as the degraded sample[125–127]. Alternatively, a reference standard can be used to follow biomaterial disintegration or by-product formation. However, this degradation assessment technique presents a few drawbacks such as high costs associated with the equipment and accessories. Degradation of hydrogels can be quantified followed by observing the hydrolysis rate constant ($k_{obs}$) and half-life ($t_{0.5}$) of esters in hydrogels via $^{1}$H-NMR. A study by Lau et al. followed the hydrolysis of hydrogels and highlighted that the $k_{obs}$ in hydrogels decreased as degradation proceeded[45]. This allows for the quantification of degradation, schematically (chemical structures of starting materials or by-products), and graphical representation of degrading biomaterials.

**Mass spectrometry as a degradation assessment approach**. The mass spectrometer allows for the identification of biomolecules by molecular weight. This is one of the most applicable techniques to measure degradation given that it can detect the molecular weight of fragmented biomolecules. Biodegradation can be accurately followed with this technique. Polymeric chain molecules are made up of monomers that can be fragmented from the polymeric structures and measured via light scattering measurements[128,129]. Literature reports showed the successful application of MS in the identification of degrading products[129–131]. However, the high costs associated with the equipment and the running costs, render the application of this technique in quantifying degrading materials economically challenging. Thus, the technique is mostly used to identify instead of quantifying biomaterials.

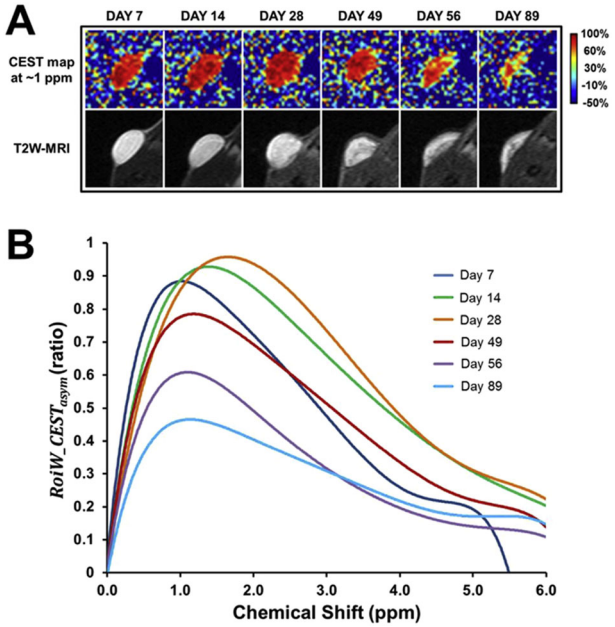


**Fig. 10 | Degradation assessment via MRI.** MRI signals were used to map out and quantify degradation of biomaterials over a certain period: (**A**) CEST maps and corresponding T2W MRI images from a representative sample of hydrogel. **B** Time-course of the RoiW_CESTasym signal for the corresponding images in **A** at the respective time points. Image acquired with permission from Shazeeb et al.[140].

However, it is possible to use the technique for quantitative analysis of the degradation of biomaterials. One of the examples of exploring both quantitative analysis and confirming degradation of degraded hydrogel was a study by Van Hove et al. which observed the molecular weight of degrading hydrogel by mass spectrometer[132]. Fragmenting molecules can be schematically presented along with graphically displaying quantification of formed by-products or diminishing starting materials.

**Gel permeation or SEC as a degradation assessment approach.** Gel permeation or size exclusion chromatography is one of the effective approaches to measure degradation as it can separate molecules based on molecular mass. The technique is widely employed in the analysis of polymer degradation. One of the drawbacks includes the high costs associated with sample preparation and equipment. A study by Hendrickson et al. highlighted a step-by-step sample preparation and analysis of polymer degradation via the SEC technique[133]. In a different study, the effect of differences in polymeric backbone length on degradation was evaluated with GPC[134]. The study indicated that the increase in thiol monomer functionality and decrease in thiol functional group concentration increase the average molecular weight and polydispersity of the distribution of thiol-polyacrylate backbone chains[134]. The changes in molecular weight were measured with GPC/SEC and correlated to the changing weight loss to assess degradation. This attests to the possibility of using two techniques to evaluate the degradation of biomaterials.

**Degradation assessment via magnetic resonance imaging.** Biomaterial degradation can also be followed via the use of magnetic resonance imaging (MRI). The principle behind MRI and its application in the medical field is well documented in literature[135–138]. MRI uses a strong magnetic field and low-energy radiofrequency signals to gather information from certain atomic nuclei (i.e., $^{1}H$, $^{31}P$, etc) to produce images of body/biomaterials[136,138]. The application of the MRI technique provides advantages such as the ability to follow the degradation of both liquid and solid-based biomaterials, and degradation can be presented as both images and quantitative graphs (Fig. 9)[135,139]. The disadvantage of this technique is the high cost associated with it, along with the long sample preparation and analysis. A study by Shazeeb et al. followed in vivo degradation of hyaluronic acid (HA) based hydrogel by employing the T2-weighted (T2W) images technique and chemical exchange saturation transfer (CEST) MRI to acquire images of the hydrogel by monitoring the endogenous signal of the hydrogel[139,140]. The T2-weighted (T2W) images technique allows for the visualisation of hydrogels without using contrast agents while the CEST contrast signal was generated by monitoring the protons in specific molecules in the hydrogel based on their ability to exchange with bulk water protons using specific radiofrequency (RF) pulses[139,140]. The T2W MRI images of the hydrogels were able to provide information about hydrogel volume due to water influx and efflux while the CEST signals of the hydrogel showed a gradual decrease with an influx of water[140]. The two techniques allow for degradation assessment along with the mechanism of degradation such as the decreased CEST signal due to an increase in fluid uptake/swelling (Fig. 10).

**Degradation assessment via UPLC/HPLC.** One of the most applicable techniques to assess degradation is UPLC/HPLC[125,141,142]. The technique allows for degradation assessment by following the formation of by-products or diminishing of starting materials. The technique works by sorting out biomolecules based on retention time. This allows for the most accurate quantification of specific biomolecules. Standards are used to calculate the concentration of formed by-products or diminishing biomaterials. The technique works as both qualitative and quantitative analysis given that biomolecules have specific retention time which can be detected and quantified. Some of the drawbacks of this technique are the high cost associated with the equipment along with the consumables required to assess degradation. A study by Al-Sibani et al. highlighted the use of weight loss, UV-Vis, and HPLC to comparatively quantify the degradation of HA-based hydrogel within 24 h[143]. The observations from the study indicated that UV-Vis and HPLC degradation assessment had similar results, whereas weight loss showed a higher degradation rate which exaggerated the rate of degradation[143]. Degradation was quantified with the three degradation assessment techniques above; however, degradation was measured at a one-time point (24 h). The reason behind quantifying degradation at a one time point instead of several consecutive time points could be due to the high running cost associated with using more than one degradation assessment technique. However, it is possible to use two or more techniques to assess degradation and provide reproducible results. Thus, selecting specific techniques that can yield good quantification and confirming the disintegration of biomaterials plays a vital role in the accurate assessment of biomaterial degradation.

**Zeta sizer and isothermal titration calorimeter to measure degradation.** Employing two complementary approaches could provide an expanded analysis of degradation biomaterials. One of the degradation assessment approaches can be employed in measuring the zeta potential of degrading biomaterials. The use of a zeta sizer can quantify both particle size distribution and surface potential of degrading biomaterials. The use of one piece of equipment to measure two properties makes it an economically attractive technique to measure degradation. The change in particle size can infer degradation which can be supported by a change in the surface charge of suspensions[64]. The technique is not highly used in degradation assessment. However, it can be used along with other cost-effective physical degradation assessment techniques such as gravimetric analysis and morphological assessment. Another useful technique that can be employed in quantifying degradation is isothermal titration calorimetry. The technique was explored in detail in a study where the quantification of microbial degradation of trace pollutants was investigated[144]. One of the limitations of employing this technique could include costs associated with instrument or sample preparations.

## Conclusion and future recommendation

The current review highlighted the different approaches used to assess the degradation of biomaterials in the biomedical field. The review also highlighted over-reliance on physical degradation assessment approaches

(gravimetric analysis, surface erosion, and morphological changes) over mechanical and chemical assessment approaches. The degradation assessment approaches were critically reviewed on how they are carried out, their advantages, disadvantages, and mediation of specific limitations associated with each degradation assessment approach. Examples were given on the way each approach can be carried out, the type of information they give, and the conclusions that can be drawn from each of the degradation assessment approaches.

The physical and mechanical degradation assessment approaches infer degradation, whereas chemical-degradation assessment approaches provide more information on degradation such as molecular weight or correct quantification of formed by-products. The physical and mechanical degradation assessment approaches offer cost-effective degradation assessment of biomaterials whereas chemical-degradation assessment offers high throughput degradation assessment. Suggestions that can be drawn from the review are that physical and mechanical degradation assessment approaches should be coupled with chemical-degradation assessment approaches to provide more information to make conclusive decisions on the degradation of biomaterials. The high cost associated with high throughput analytical techniques renders the chemical-degradation assessment approach economically challenging. However, the abundance of analytical equipment increases choices for selecting a suitable, cost-effective technique that can be coupled with a routine physical/mechanical degradation assessment approach.

The three degradation assessment approaches (physical, mechanical, and chemical) have one limitation, which is the need to sample out at each predetermined time point to assess the degradation of biomaterials. This indicates that the degradation assessment approaches are invasive and are not continuous. Sampling out may disturb the degradation process, and using different vials to assess degradation could result in errors in accurately following the degradation process. Issues associated with the limitations above include (i) fluctuation in degradation percentages at different time points due to the use of separate samples to assess degradation, (ii) disturbing degradation by sampling out, thus preventing continuous degradation assessment of biomaterials, and (iii) inability to assess degradation in real-time. Future degradation assessment approaches should have three key parameters such as (i) continuous, (iii) automated, (iv) and non-invasive degradation assessment processes. The inline and/or continuous parameter refers to the ability of the degradation assessment approach to be carried out without having to sample out during the degradation process. This can be achieved using machine learning to create automated degradation assessment equipment or 3-D imaging software to view the degradation of biomaterials in real time. Automated three-dimensional imaging of degrading biomaterials will resolve the need to sample out at different degradation time points, instead different images can be acquired while the biomaterial is degrading. This approach will make the degradation assessment process continuous and non-invasive. Future degradation assessment equipment could allow for degradation to be carried out over a prolonged period. The ASTM guidelines for assessing the degradation of biomaterials should be updated to meet the three key points (continuous, non-invasive, and automated).

## Code availability

No codes were generated or analysed during the current study.

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## Author contributions

Hillary Mndlovu: writing—original draft, writing—review & editing. Pradeep Kumar: writing—review & editing, methodology, conceptualisation. Lisa C. du Toit: writing—review & editing. Yahya E. Choonara: writing—review & editing, funding acquisition, conceptualisation.

## Competing interests

The authors declare no competing interests.

## Additional information

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