Biomaterials Degradation and Bioabsorbability: Biomedical Potentials of Marine Enzymes

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Abstract

Biomaterials are most commonly recognized as scaffolds potentially able to perform useful functions such as (i) promoting cell attachment, survival, proliferation, and differentiation while possessing minimum toxicity in the original and biodegraded/bioabsorbed forms; (ii) allowing the transport or delivery of gases, nutrients, and growth factors; and (iii) offering sufficient structural support while being degradable/absorbable at appropriate rates for tissue regeneration.

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Biodegradable/Bioabsorbable materials intended to be used as implantable drug eluting scaffolds must fulfill several requirements in order to be considered for clinical integration. They must not elicit abnormal responses in local tissues and should neither produce local nor systemic toxic or carcinogenic side effects. First and foremost, biodegradable/bioabsorbable platforms should serve their intended scaffolding and cell-signaling functions while degrading/absorbing into nontoxic metabolites. Breakdown of artificially manufactured scaffolds requires rigorous toxicological evaluation of each constituent component. Particularly when ambitious strategies involving the use of composite materials with integrated trophic factors are concerned, the importance of material biocompatibility evaluation rises significantly. The desired notion of effecting synergistic actions of GF (growth factor) and other incorporated component requires careful consideration of factor concentrations and release mechanisms in order to avoid potentially harmful overdosing. It therefore remains a priority to conduct systematic and rigorous toxicological studies – both in vitro and in vivo – to (1) eliminate grossly ineffective or toxic delivery platforms in order to (2) narrow down on potentially suitable candidate technologies as well as (3) ascertain any dose or time-dependencies which may influence the materials' suitabilities.

Actually, the performance of many biomaterials depends largely on their degradation/absorbability behavior since the degradation/absorbability process may affect a range of events, such as cell growth, tissue regeneration, drug release, host response, and material function.

Biodegradable/Bioabsorbable medical materials are materials with the ability of functioning for a temporary period and subsequently degrade/absorb in physiological conditions, under a controlled mechanism, into products easily eliminated in the body's metabolic pathways.

The demands for biomaterials with above-mentioned characteristics (controlled, predictable degradation/absorbability kinetics) included a wide range of biomedical applications (such as resorbable surgical sutures, matrices for the controlled release of drugs, and scaffolds for tissue engineering) are becoming more and more crucial and urgent.

Therefore, aim to provide promising potentials of marine enzymes for biomedical materials degradation/absorbability, the relevant potential marine enzymes such as amylases, esterases, cellulases, and laccases are reviewed. It indicates that strategies developed to obtain biomaterials with a controlled degradation/absorbability rate should be based on molecular design principles, such as the introduction of hydrolysable bonds into polymer backbones, copolymerization and blending techniques, crosslinking and surface modification methods, and inclusion of certain additives into polymeric matrices (e.g., excipients, drugs, salts).

Meanwhile, controlled degradation/absorbability of biomedical materials by potential marine enzymes will have several advantages considering the high specificity of enzymes for their substrates and also because enzyme activity can be regulated by environmental conditions (e.g., pH, temperature, the presence of certain substances, like metal ions). In addition, the degradation/absorbability kinetics can be adjusted by the amount of encapsulated enzyme into the matrix.

Introduction

Requirements of Biomaterials

Biomaterials are most commonly recognized as scaffolds potentially able to perform useful functions such as (i) promoting cell attachment, survival, proliferation, and differentiation while possessing minimum toxicity in the original and biodegraded forms; (ii) allowing the transport or delivery of gases, nutrients, and growth factors; and (iii) offering sufficient structural support while being degradable at appropriate rates for tissue regeneration.

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Challenges to Biomaterials

It assumes that the best scaffold for the tissue engineering would be the extracellular matrix (ECM) of the target tissue in its native conformation. Therefore, decellularized organs that retain the ECM [1] present the most common natural scaffold architecture used today, having been incorporated in materials used in heart [2], lung [3], liver [4], bone [5], and blood vessels [6]. At the same time, decellularized organs have a number of shortcomings that have limited their use in biomaterial applications, including long processing times (increasing the costs of production), limitations on sourcing tissues, and potential immunogenicity. Also, decellularization typically involves exposure to nonphysiological chemical and biological agents, such as detergents, enzymes, and physical forces, which cause disruption of the associated ECM, potentially stripping the natural scaffold of its inherent bioactivity [1]. Less expensive bioactive materials can be constructed by modifying traditional "bioinert" materials to mimic physicochemical properties of natural materials [7]. Natural ECM materials, such as collagen and fibrin gels, or recombinant peptides [8, 9] or proteins that mimic natural ECM materials [10, 11] have been used in this way. Hybrid approaches that combine the best qualities of

synthetic materials with biologically active peptides are also the subject of investigation by a number of groups [12–17].

Each of these approaches has their own advantages and limitations. Modification of bioinert materials allows for finer control over material properties; however, recapitulating every physicochemical property of a natural material is nearly impossible. Natural materials such as collagen gels are attractive because of their inherent bioactivity, but the complexity and heterogeneity of these materials can cause unpredictable cellular responses. Furthermore, these natural materials can lack the mechanical strength required for certain applications. Peptides or protein fragments that mimic natural ECM materials can form materials by themselves or can be incorporated into other scaffolds to impart biological activity [8, 9, 18–20]. Biological responses to peptides or protein fragments tend to be more predictable than responses to natural ECM material, but such reductionist approaches often cannot achieve the complexity in interactions and stimuli required to achieve a desired response [21]. The use of selection has the potential to overcome the limitations of these current approaches by specifically identifying material components and scaffolds that meet a set of desired criteria.

Biomaterial Degradation Products and Toxicity

Biodegradable materials intended to be used as implantable drug eluting scaffolds must fulfill several requirements in order to be considered for clinical integration. They must not elicit abnormal responses in local tissues and should neither produce local nor systemic toxic or carcinogenic side effects. First and foremost, biodegradable platforms should serve their intended scaffolding and cell-signaling functions while degrading into nontoxic metabolites [22, 23]. Breakdown of artificially manufactured scaffolds requires rigorous toxicological evaluation of each constituent component. Particularly when ambitious strategies involving the use of composite materials with integrated trophic factors are concerned, the importance of material biocompatibility evaluation rises significantly. The desired notion of effecting synergistic actions of GF (growth factor) and other incorporated component requires careful consideration of factor concentrations and release mechanisms in order to avoid potentially harmful overdosing. It therefore remains a priority to conduct systematic and rigorous toxicological studies – both in vitro and in vivo – to (1) eliminate grossly ineffective or toxic delivery platforms in order to (2) narrow down on potentially suitable candidate technologies as well as (3) ascertain any dose or time-dependencies which may influence the materials' suitabilities.

Currently, there is discord between those postulating the use of naturally derived materials, synthetic materials, or a combination of both − not least due to a lack of consistent proof of one's superiority above the other. Naturally derived biomaterials such as collagen or hyaluronic acid are frequently selected for their benefits including similarity to native ECM, thus favoring natural cell–cell and cell–matrix interactions. Numerous studies have demonstrated both the in vitro and in vivo beneficial potential of natural scaffolds in wound healing [24–26]. Using a genetically diabetic mouse

model, the biocompatibility, and wound-healing properties of a fibrin-based scaffold incorporating both VEGF and bFGF were demonstrated [27]. Degradation of the fibrin scaffold over a 7-day period ensured timely release of GFs and accelerated wound healing compared to wounds not exposed to GFs. As previously mentioned, degradation rates of biological scaffolds may be fine-tuned using crosslinking agents [28, 29], but several crosslinking agents such as glutaraldehyde have demonstrated cytotoxicities [30]. Substitution with other, more biocompatible crosslinking agents is beginning to show tentative success and has to be researched further in terms of their long-term stability and biocompatibility [31–34]. For example, several studies successfully used the natural and noncytotoxic genipin as a crosslinking agent to mechanically stabilize collagen-based scaffolds and demonstrated good cytocompatibility [35–38]. In addition, several studies have demonstrated the cytocompatibility and nontoxicity of scaffolds based on natural biopolymers such as collagen and chitosan [39–43]. However, high purification costs, relative unavailability, and unsuitability for large-scale processing as well as batch to batch variations and an immunogenic potential of naturally derived materials, however, have encouraged scientists to search for realistically applicable synthetic alternatives. Synthetic materials are thought to overcome the aforementioned disadvantages of natural materials and provide additional key benefits including the ability to tailor mechanical properties and degradation kinetics to suit individual applications. Synthetic materials may be fabricated into various shapes with desired pore morphological features conducive and individually tailorable for tissue ingrowth.

The majority of synthetic biodegradable materials studied for tissue engineering applications belong to the polyester family which include polyglycolides, polylactides, and their various co-polymers [44]. They have long been used in a number of clinical applications ranging from biodegradable sutures [45] to orthopedic plates and fixture devices [46] and are also intensively studied as scaffold materials for cell transplantation and tissue regeneration [47]. Polyesters mainly degrade in two stages; at first the amorphous region degrades via hydrolytic chain scission of ester bonds. The second stage involves mainly the degradation of the crystalline areas of the material. Degradation of polyesters releases lactic acid and glycolic acid which are resorbable even at high concentrations. However, concerns regarding their toxicity have been raised; several studies have highlighted the formation of local aseptic sinuses and osteolytic changes in conjunction with intermittent joint swellings in animals and patients treated with rods, screws, and other fixation devices made of degradable PGA or PLA implants [48–50]. Since infections were excluded, it was deemed likely that the observed adverse effects were related to the biodegradation of the PLA–PGA implants. Solazzo et al. studied the effects of PLA/PGA composite plates on osteoblastic differentiation and proliferation of mesenchymal stem cells (MSCs) [51]. Initial results demonstrated strong influences on MSCs by enhancing proliferation, differentiation, and matrix deposition, but long-term effects of degradation products were not studied. Previous studies, on the other hand, already demonstrated long-term dramatic reductions of biocompatibility, likely due to degradation products causing bone resorption in and around the implant site [52]. Many in vitro studies previously determined that both PLA and PGA produced

cytotoxic degradation products but did not consider the buffering effects of physiological solutions present in vivo. On the other hand, systemic distribution may result in potentially harmful degradation products becoming trapped in end-organs such as the liver, kidney, and spleen where they pose an inflammatory risk [53, 54]. Implantation of larger PLA–PGA materials as well as placement in anatomical region without access to sufficient quantities of body fluids may overwhelm the body's capacity to provide adequate buffering. These issues were successfully addressed by incorporating basic salts within PLA–PGA implants which offset the pH decrease observed in the vicinity of degrading implants [55].

Other synthetic materials frequently used in tissue engineering applications include polycarbonates which possess hydrolysable carbonate and ester bonds [56]. Theoretically, the hydrolysis of carbonate groups yields two alcohols and CO_2, thus eliminating the problem of the acid burst seen in polyesters [44]. In practice, however, controversies as to the true chemical reactions occurring during degradation remain with macrophage-induced oxidative and enzymatic break-down being frequently quoted [53, 57–59]. Varying the structure of the pendant R groups allows for tuning of mechanical properties, degradation rates, and subsequent cellular responses to the material.

Polyurethanes (PU) are a major class of synthetic elastomers which are usually used in the manufacture of nondegradable medical implants such as cardiac pacemakers and vascular grafts [60–63]. Biodegradable versions of PU, obtained by incorporating degradable chemical linkages into the backbone, are of increasing interest due to excellent mechanical properties, versatile degradation kinetics, and a compatibility profile which is tunable according to the intended use. However, a major problem has been the toxicity of degradation products, especially those from diisocyanate components such as 4,4′-methylenediphenyldiisocyanate (MDI) and toluene diisocyanate (TDI) [64]. Due to the advantages of precise control over material characteristics, it is possible to design nontoxic degradable PUs using diisocyanates such as lysine diisocyanate (LDI) (2,6-diisocyanatohexanoate) and other aliphatic diisocyanates like hexamethylene diisocyanate (HDI).

Despite excellent and controllable physical properties of synthetic biomaterials, the apparent lack of innate cell recognition entities has led to the fabrication of composite bio-artificial scaffolds which aim to harness the distinct benefits of both material classes while simultaneously compensating for each other's weaknesses. A good idea in theory, biosynthetic composite scaffolds however retain their individual drawbacks which, in the case of synthetic materials, are more predictable and therefore more easily rectifiable. For instance, natural biomaterials pose an innate risk of eliciting an immunological graft rejection which could be detrimental in cases of vital placements of grafts, for example, vascular interpositional implants [65]. With ever advancing material processing techniques, however, synthetic scaffold designs incorporating artificial cell-recognition entities may replace immunogenic cell surface epitopes with synthetic mimics which enhances biocompatibility of synthetic materials [66]. Additionally, synthetic materials may be designed with chemical functional groups which can enhance cell attachment, and proliferation and induce tissue ingrowth.

The apparent difficulty to precisely control fine-tuning of biomaterial degradation has initiated the recent paradigm shift to gradually replace conventional top-down fabrication methods with nature-inspired bottom-up assemblies. Traditionally, top-down approaches involving direct cell seeding into premade porous scaffolds are limited by slow vascularization, slow diffusion, and low cell density as well as nonuniform cell distribution. Bottom-up fabricated scaffolds, on the other hand, benefit from controllable modular assemblies of cell-laden components, thus potentially eliminating the shortcomings of the traditional approach [67]. Such sophistication of material fabrication and construction techniques allows today's scientists to reach beyond conventional natural materials in order to synthesize tomorrow's "designer material." Biodegradable materials have undergone extensive research and represent a popular platform for tissue engineering bone [68], skin [69], cardiovascular tissues [60, 70–72], and nerves [73–76] among many other organs and tissues. Conceptually, degradation is defined as a molecular change due to chemical chain scission within a polymer matrix. The subsequent breakdown into smaller material components and potential release of encapsulated cells or cell-signaling agents have opened medically exploitable avenues, transforming the area of regenerative medicine into a dynamic and self-propagating branch of modern medicine. The aim to synthesize ever more refined scaffolding structures in order to create micro- and nanoenvironments resembling those found in natural tissues now represents an ever growing niche in the materials sciences. Despite enormous efforts, current, as yet insurmountable challenges include precise biomaterial degradation within predetermined spatial and temporal confines in an effort to release bio-signaling agents in such orchestrated fashion as to fully regenerate functioning tissues. It thus appears almost anticlimactic to be asked to step out of the artificially over-constructed spiral of evermore convoluted scaffold fabrication techniques and consider the benefits of controllable bottom-up scaffold fabrication methods.

Marine Enzymes

The oceans provide an almost untapped reservoir of novel enzymes, which might have a potential as biocatalysts for academic research and for industrial processes. With regard to the broad variety of environmental conditions, interesting enzyme with characteristic traits can be isolated. Especially enzymes from extremophiles, which are classified as thermophilic or psychrophilic, are of particular interest for industrial processes in terms of mass transfer or energy savings. Additionally, enzymes that are adapted towards high salt concentrations may be beneficial for industrial biotechnology applications, because the catalytic reactions can be performed in nondiluted solutions. Up to now only a minor amount of this treasure has found an application in the laboratory and industry. However, regarding the ongoing progress and developments in molecular biology, it seems that the portfolio of enzymes that can be applied for the production of bulk and fine chemicals will be broadened in the near future.

The oceans provide a broad variety of marine microorganisms, whose products such as enzymes and bioactive metabolites are of interest for applications in the food and pharmaceutical industries, as well as for the processing of renewable resources to provide raw material, for example, for the production of biofuels. Considering the size of the marine habitats, which range from tropical to cold polar regions and from coastal to hydrothermal deep-sea zones, they harbor a pool of (micro)organisms that are able to produce enzymes with interesting traits like salt and barotolerance and/or adaptations to high or low temperatures, which are sometimes superior in comparison to their counterparts produced by terrestrial microorganisms.

Today, natural product research focuses on the enzyme systems that are responsible for the production of bioactive secondary metabolites, such as polyketide synthases [77, 78] which catalyzes the linkage of acyl-coenzyme A subunits via the generation of cyclic structures (e.g., bryostatin 1). Aside from the natural product research, some (novel) marine enzymes, catalyzing a multitude of processes relevant for biocatalytic processes in industrial biotechnology, show desired properties like high salt tolerance, hyperthermostability, barophilicity, and cold adaptivity as mentioned before. Their performance results from the adaptation of their host organisms during the evolutional development to the environmental surroundings. However, the rising demand for environmentally friendly and beneficial economical manufacturing of bulk and fine chemicals requires the exploitation of these enzymes for the specific and selective production of value-added products like chiral amines, alcohols, halogenated amino acids, as well as the enzymes themselves in the case of, for example, thermostable proteases or polymerases. Thus, marine microorganisms may contribute to expanding the number of (even more) stable and selective biocatalysts for industrial biotransformations. One major concern with establishing these enzymes in industrial-scale applications is their sustainable access. With respect to the difficulties of enzyme expression or cultivation of the natural expression system when transferred from its originally habitat to laboratory conditions, the responsible gene or even the gene cluster must be heterologously expressed. This approach requires identification, isolation, characterization, and cloning of the responsible genes inadequate host organisms (considering the codon usage of marine microorganisms), as well as optimization of enzyme stability and enzyme expression. In addition to microorganisms such as bacteria or fungi, higher organisms such as fish, prawns, crabs, snakes, plants, and algae can also be used for tapping of marine enzymes [79]. In particular, the latter one represents a mentionable source for a number of halogenating enzymes.

Examples of Marine Enzymes

Polysaccharide-Degrading Enzymes

Polysaccharolytic enzymes have well-known applications in the food processing, detergent, paper, and textile industries. Furthermore, they have potential applications in the biofuel industry and in waste management. Cellulase, xylanase, and amylase are used in baking, brewing, and the production of natural sweeteners. Xylanases are also

useful in the prebleaching of paper pulp and amylases are reported to be components of several laundry detergents. Carrageenolytic enzymes are used for liquefaction of carrageenan for applications in the food, pharmaceutical, and cosmetic industries. In addition to food, pharmaceutical, and cosmetic applications, agarases have potential applications in biotechnological research. Cellulolytic and lignolytic enzymes are beneficial in the production of biofuels, since degradation of polysaccharide biomasses is the major challenge in the development of plant-derived biofuels. Plant biomasses often contain complex polysaccharides that need to be converted into simple fermentable sugars for bioethanol production. Cellulose, xylan, and lignocellulose are major groups of polysaccharides that are abundant in nature and they are major components of almost all biowaste and some industrial waste. Degradation of these materials is essential for pollution control. Various polysaccharolytic enzymes may be used for their degradation and conversion to value-added products.

Amylases

Amylase is an enzyme that catalyzes starch hydrolysis and is broadly used for the production of simple sugars. Versatile amylases have been reported from marine organisms.

Cellulases and Lignocellulases

Cellulolytic enzymes were reported from a range of marine microorganisms, including bacteria and fungi.

Chitinases

Chitin is a major component of fungal cell walls, the exoskeleton of arthropods including insects and crustaceans (mollusks, crabs, lobsters, and shrimps), and the internal shell of cephalopods (squids and octopuses). Most of these organisms live in the habitat of the marine ecosystem, and it stands to reason that the chitin hydrolytic enzyme (chitinase) is abundant in the marine environment. Chitinolytic activity was reported in several marine bacteria (pathogens) that attack living arthropods or degrades the chitin-rich biomass derived from their exoskeleton. Chitinase is used for the production of single cell proteins for animal and aquaculture feed, for isolation of fungal protoplasts, and for the preparation of chitooligosaccharides. Chitinases are not widely used at commercial scale due to their high cost, but they have interesting potential applications in pest control and preparation of medicines. Chitinase genes can be used to develop chitinase-producing transgenic plants that are resistant to insects and fungi. The cell walls of some human pathogens including fungi, protozoa, and helminths are composed of chitin, which could be hydrolyzed by microbial chitinases and thus, chitinase may be used for the treatment of these microbial infections.

Agarases

Agarase is an important enzyme distributed in a wide range of marine organisms. This enzyme has potential application in the food, pharmaceutical, and cosmetics industries. It can also be used in microbiology and molecular biology research.

Proteases

Proteolytic enzymes account for above 60% of the global enzyme market [80], and there is no doubt that proteases are one of the most widely studied marine enzymes. They are vastly used in the food processing, detergent, leather, and pharmaceutical industries and in biotechnological research. They have potential applications in bioremediation and waste management. The increased demand for industrial proteases over its supply from plant and animal sources led to an increased interest in microbial proteases; 70% of industrial proteases come from microbial sources. Alkaline and neutral proteases are obtained mainly from bacterial sources, mostly from different species of Bacillus, while fungi are a well-known source of acid proteases. Marine microbial proteases gained attention for their extremophilic properties and stability in the presence of a broad range of chemicals. They possess almost all the characteristics desired for various biotechnological applications of proteases.

Halogenating Enzymes

Halogenated molecules are widely distributed in the environment and their existence is not only related to human industrial processes but also to natural biotic as well as abiotic processes. To date almost 5000 organohalogens are known. Most of them contain chlorine or bromine rather than fluorine or iodine [81]. With respect to the relatively high concentration of bromide found in the marine environment, brominated compounds are found quite often and occur in a larger number than in freshwater samples [82, 83].

In the last decade, there has been a continuous effort to learn more about the still largely unexplored realm of marine enzymes. Tables 1 and 2 show functions and applications of enzymes isolated from various marine animal sources and various marine plant sources, respectively [84].

In the past decade, lots of efforts were made to find an enzyme which can degrade biomaterials such as PEC under mild physiological condition, for example, PBS buffer, pH 7.4. Unfortunately, no degradation had been observed by many enzymes such as lipases, esterases, lysozyme, chymotrypsin, trypsin, papain, pepsin, collagenase and pronase, serum, and whole blood [85]. However, cholesterol esterase (CE), which exists in liver, pancreas, lactating mammary glands, endosome, and lysosome and is overexpressed in macrophages induced by inflammation, is an important hydrolytical enzyme to catalyze the hydrolysis of sterol esters into their component sterols and fatty acids.

Table 1 Functions and applications of enzymes isolated from various marine animal sources [84]

Enzyme Class	Enzyme	Source	Biotechnological applications/ metabolic functions
Hydrolase	Protease	Marine sponge *Spheciospongia vesparia*	Degrade casein, hide powder azure, synthetic substrates
	Cathepsins	Marine sponge *Geodia cydonium*	Major digestive protease in sponges
	Protease	Marine crab, *Scylla serrata*	Collagenolytic metalloprotease closely resembles metalloproteases of vertebrates
	Amylase	*Sparusaurata, Scophthalmus maximus,* and *Sebastes mentella*	Digestive enzymes in marine fishes
	Choline-sterases	Bivalve *Mytilus edulis, Mytilus galloprovincialis, Corbicula fluminea*	Biomarker for aquatic pollution
	AMP-deaminase	Teleost sea scorpion, *Scorpaena porcus*	Purine nucleotide metabolism
	Na, K-ATPase	Spiny lobster *Palinurus elephas*	Generation of osmolyte gradient
	Hyaluronidase	Venom of the stonesh, *Synanceja horrida*	Only marine hyaluronidase
	ATPN-glycosidase	Marine sponge *Axinella polypoides*	Conversion of adenosine-5-triphosphate into adenine and ribose-5-triphosphate
Trans-ferase	α-N-acetylgalactos aminidase	Sea squirt	Structural analyses of the carbohydrate epitopes
	Citratesynthase, Pyruvatekinase	Northern krill, *Meganyctiphanes norvegica*	Metabolic key enzymes, adaptive properties under different thermal conditions
	Transglutaminase	Red sea bream liver	Transfer of amine groups
	Glutathione S transferase	Sea bass (*Dicentrarchus labrax*) liver cytosol	Novel glutathione S transferases belonging to θ and α classes
	cAMP-dependent protein kinase	Marine periwinkle, *Littorina littorea*	In reversible protein phosphorylation, aerobic-anaerobic transitions
Oxido-reductase	Dehalogenating peroxidase	Terebellid polychaete, *Amphitrite ornata*	Removal of anthropogenic or biogenic haloaromatic compounds
	Catalase, Superoxide dismutase	Marine mussel, *Mytilusedulis L.*	Antioxidant enzyme, Potential use in toxicological studies
	Mono oxygenase	Sea bass (*Dicentrarchus labrax*)	Biomarkers of polycyclic aromatic hydrocarbon exposure

Table 1 (continued)

Enzyme Class	Enzyme	Source	Biotechnological applications/ metabolic functions
	Phenoloxidase	Marine mussel *Perna viridis*	Oxidation of phenolic substrates
	20 β-hydroxysteroid dehydrogenase	Japanese eel	Key steroidogenic enzyme
Lyase	Phospholipases A2	Sea snake *Hydrophis cyanocinctus* venom	Novel purification method
Other	Glucose metabolism enzymes	Marine borer *Bankia setacea*	Metabolic enzyme

It is well known that bioabsorbability of the scaffold material is desired to enable improved restoration of targeted tissue. The development of bioabsorbable tissue scaffold materials is a topic of intense research. Such a material is sought for many medical applications, ranging from wound healing to skin, bone, cartilage, nerve, and other tissue regeneration. Synthetic polymers have been extensively examined and implemented as tissue scaffold materials, but many exhibit undesirable characteristics including nonbioabsorbability and low biocompatibility. Although naturally derived polymers are being implemented as alternative materials due to their high biocompatibility, some are not bioabsorbable orelicit undesirable side effects including infection and immune response [86].

Hu et al. [87] investigated a bioabsorbable bacterial cellulose (BBC) incorporating cellulose enzymes. Enzyme sources and characteristics of cellulases obtained from Sigma and e directly used without further purification are shown in Table 3. Results show that compared with pieces containing cellulase A or C, samples incorporating cellulase B in the CA-SC (citric acid-sodium citrate) buffer exhibited the slowest rate of degradation. All BBC pieces tested in the presence of simulated tissue padding exhibited faster degradation performance, suggesting that limited diffusion of cellulases from the top surface of the sample resulted in a higher cellulase concentration in the samples. For a CA-SC buffer pH of 3.0, 50% of the samples incorporating cellulase B in PBS (phosphate-buffered saline) solution were visible after 5 days. The use of aluminum-made simulated tissue padding appeared to affect the degradation. Denser fragments of cellulose were visible on the surface of the simulated tissue padding for days 4–7 in the case of the PBS buffer (Fig. 1d–e), suggesting that the tissue padding may hold the cellulose materials together during the degradation process. These denser fragments may provide additional mechanical integrity for cell growth. Sample pieces incorporating cellulase B and buffer ingredients with pH 3.0 exhibited the best mechanical integrity over 5 days. It has been demonstrated that cellulase B has a relatively long lifetime as well as a relatively low activity, which implies that cellulase B might slowly degrade cellulosic materials over a longer period of time. However, most sample pieces observed in degradations lost more than 50% of their integrity after only 1 day, possibly due to the high

Table 2 Functions and applications of enzymes isolated from various marine plant sources [84]

Enzyme class	Enzyme	Source	Biotechnological applications/ metabolic functions
Oxidoreductase	Iodoper-oxidase	Marine diatom cultures	Iodine incorporating enzyme
	Vanadium-bromoper-oxidase	*Lithophyllum yessoense*	Potential substitute for catalase
	Bromoper-oxidase	Marine algae *Ascophyllum nodosum* and *Corallina officinalis*	Regio-specific bromoper-oxidative oxidation of 1,3-di-*tert*-butylindole
	Nitrate reductase	Eelgrass, *Zostera marina* L.	Key enzyme in nitrate assimilation Metabolic enzyme
	Hydrogenase	Marine green alga, *Chlorococcum littorale*	New source
	Luciferase	Marine dinoflagellates, *Lingulodinium polyedrum* and *Pyrocystis lunula*	Enzymatic oxidation of luciferin
	Xanthine oxidoreductases	*Gonyaulax polyedra*	Role in circadian control
Hydrolase	Protease	Marine green alga, *Codium divaricatum*	Fibrinolytic enzyme
	Cholinesterase	*Gracilaria corticata* (Rhodophyta)	Metabolic enzyme
	ATPase	Zostera marina	Salt-tolerant metabolic enzyme
	Urease	*Aureococcus anophagefferens, Prorocentrum minimum*, and *Thalassiosira weissflogii*	Conversion of urea to ammonia
Lyase	Carbonic anhydrase	Marine diatom *Phaeodactylum tricornutum*	Hydration of CO_2 and the dehydration of bicarbonate
	Myrcene synthase	Marine red alga *Ochtodes secundiramea*	Produces myrcene from geranyl diphosphate
Ligase	Glutamine synthetase	Emiliania huxleyi	Activity related with light and nitrogen availability
Transferase	Ribulose-1,5-bisphosphatecarboxylase/ oxygenase	Marine phytoplankton	Calvin cycle enzyme, Metabolic enzyme
Isomerase	Polyenoic fatty acid isomerase	Marine alga *Ptilota filicina* J	Conversion of arachidonic acid

Table 3 Characteristics of commercial cellulosic degrading enzymes

Enzyme	Activity and activity conditions	Sources
A (powder)	Cellulase from Trichoderma viride, pH 5.0, 37 °C, >5000 U[a]/g solid	Sigma C0615
B (powder)	Cellulase from Trichoderma reesei, pH 5.0, 37 °C, >1000 U/g solid	Sigma C8546
C (powder)	Cellulase from Trichoderma viride, pH 5.0, 37 °C, >3000 U/g solid	Sigma C1794

[a]U in this table is defined as the activity of the enzyme that can liberate 1 lmol of glucose in 1 h at the indicated pH value and temperature in a 2-h incubation time

cellulase loading. In terms of cell growth, the BBC samples need to retain at least 80% of their integrity until day 2 as human skin fibroblast requires a substrate to attach to and quickly proliferate on after injuries occur. Also, the rapid degradation rate of sample pieces could be controlled by using lower loadings of cellulase B once the relatively optimal pH microenvironment for cellulases B was created, as cellulase B exhibited a low and stable activity. The evaluation of different degradations during a period of 7 days is described in Table 4.

Cholesterol Esterase

Cholesterol esterase (CE) is a characteristic hydrolytic activity in MDM (monocyte derived macrophages) and has been shown to increase two to fourfold as monocytes differentiate into macrophages. The CE in MDM has been shown to be identical to that of pancreatic CE. The latter enzyme has been shown to degrade both polyester and polyetherurethanes [88].

For many studies involving homo-chain polymers, the rate of biomaterial degradation becomes slower as the concentration of enzyme is reduced. However, in the
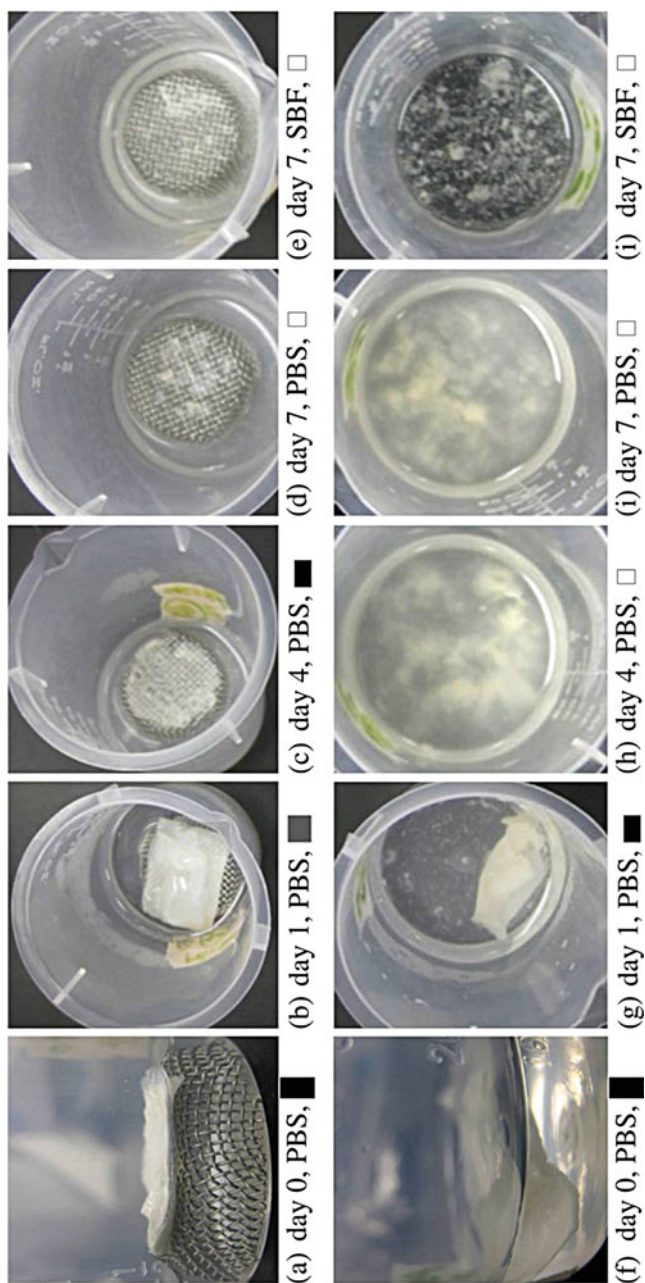
Fig. 1 Macroscopic pictures of degradation of BBC pieces incorporating cellulase B and buffer ingredients with pH 4.0 in PBS and SBF. (**a–e**) Degradation in the presence of simulated tissue padding; (**f–i**) degradation without simulated tissue padding. Degradation levels: none (◻), slight (◼), moderate (◼), extensive (◼), nearly complete (◼), and complete (◻)

Table 4 Description of degrees of degradation of BBC pieces in PBS and SBF

Tab	Tab2
Key: none (■; no degradation), slight (■; very little but visible degradation), moderate (■; at least ~50% of sample was no longer visible), extensive (■; ~90% of sample disappeared and it had decomposed from a single large whole to small patches), nearly complete (■; ~100% of sample vanished but many smaller particles appeared and the solution looked cloudy), and complete (□; no visible particles presented and the solution looked nearly transparent)	Tab2
[a]M1: Method 1, sample pieces positioned at the air–solution interface by a simulated tissue padding	
[b]M2: Method 2, sample pieces submerged in the solution	

case of copolymers, the enzyme dose-response studies are not as simple as that of the homo-chain polymers [89].

Tang et al. [90] investigated the influence of esterase activity (80–400 units/ml) on the biodegradation of polycarbonateurethanes (PCNUs) by cholesterol esterase (CE), with a particular focus on studying the influence of different hard segment structures (physical and chemical) and their contribution to sensitizing the polymer towards enzyme catalyzed hydrolysis.

The polycarbonate-polyurethanes were synthesized using a two-step polymerization. The diisocyanates included MDI, HMDI, or HDI, all obtained from Aldrich, Milwaukee, WI, USA. The soft segment was poly(1,6-hexyl 1,2-ehtyl carbonate) diol (PCN, 1000, received in kind from Corvita Corporation, Miami, FL,USA). 1,4-Butanediol (BD, Aldrich, Milwaukee, WI, USA) was used as the chain extender.

[14]C-labeled HDI or ^{14}C-labeled BD was used in the synthesis, to incorporate a tracer which allowed for the rapid detection of released products following degradation by enzyme. While the ^{14}C-HDI was used to study the relation between hard segment size and the enzyme dose, the ^{14}C-BD label was used as the common component when studying the relation between hard segment types and enzyme concentration.

A semi-batch system was used in the in vitro biodegradation studies. The PCNUs were coated onto small hollow glass tubes (3 mm OD, 2 mm ID) using a 10% w/v polymer-DMAC (dimethylacetamide) solution. Following final curing, the tubing was sectioned and placed into sterile 15 ml vacutainer® tubes (Becton Dickison and Co., Franklin Lakes, NJ). A total polymer surface area of approximately 36 cm^2 was exposed to the incubation solutions. All sample tubes were prepared and handled in a laminar flow hood, employing sterile techniques including sterilized pipette tips and solution filters.

The coated tubes were inserted into vacutainers with 5 ml of either 0.05 M sodium phosphate buffer solution (pH 7.0) or buffer containing CE at concentrations of 16, 80, 160, or 400 units/ml. One unit of CE was defined as the amount of enzyme required to generate 1 nmol/min of p-nitrophenol from the hydrolysis of p-nitrophenyl acetate at a pH of 7.0 and temperature of 25 °C, as determined by spectrophotometric assay at 410 nm. During the degradation period, 1 ml aliquots

were removed from the polymer incubation solutions once a week for 10 weeks and counted in a liquid scintillation counter for radioactivity. The amount of enzyme activity lost every day was calculated based on an enzyme half-life experiment, and fresh aliquots of enzyme were added every day in order to maintain the CE activity. Similar volumes of buffer solution were also added to the control vacutainers in order to maintain a similar volume to surface area ratio for all samples. Each reaction condition was run in triplicate. Bacterial cultures were run on samples at the conclusion of the tests in order to validate that sterility was maintained throughout the experiment.

Results show that polymers with higher hard segment content were shown to be more resistant to the hydrolytic degradation induced by CE. The degradation was highly dependent on enzyme dose. Furthermore, the dose response itself was considered to be a direct function of the surface chemistry and structure of the polymer.

For confirmation, Jahangir et al. [91] studied the influence of fibrinogen (Fg) as a simple model of protein adsorption to determine what effect, if any, that Fg preadsorption would have on modified and nonmodified PEUs and to study its influence on the biodegradation process catalyzed by CE. It states that the preadsorption of Fg onto the modified and nonmodified surfaces provided a temporary protective effect against the hydrolytic function of CE. However, this effect was dissipated by day 70 and both fibrinogenated and non-fibrinogenated groups had the same degradation level by the 126th day. It was observed that the initial gap between the fibrinogenated and nonfibrinogenated groups in the base polymer was significant and that this difference was much smaller for the PPO212L (the most resistant surface to the release of radiolabeled degradation products was PPO212L) containing material as compared to the other polymer surfaces. However, more significant was that preadsorption of Fg did not appear to alter the ability of select SMMs (surface modifying macro-molecules) to provide a more biostable surface.

However, Christenson et al. [92] examined the effect of cholesterol esterase (CE) on the degradation of commercial poly(ether urethane) (PEU) and poly(carbonate urethane) (PCU). Unstrained PEU and PCU films were incubated in 400 U/mL CE solution, a concentration that is significantly greater than the estimated physiological level, for 36 days at 37 °C. PEU and PCU have the same hard segment and similar hard to soft segment ratios.

Cholesterol esterase isolated from bovine pancreas was purchased from Sigma Aldrich (Catalog number C5921, St. Louis, MO). Spectrophotometric assays of enzyme activity were conducted using a *p*-nitrophenol acetate substrate. Solutions were incubated at 37 °C and pH of 7.0 for up to 60 min and read at 410 nm. One unit of CE was defined as the concentration of enzyme required to generate 1 nmol/min of *p*-nitrophenol from the hydrolysis of *p*-nitrophenol acetate. An enzyme concen-tration of 400 units/mL was chosen for this study based on corollary studies of model poly(carbonate urethanes). Unstrained polyurethane films (1.5 cm × 1.5 cm) were incubated in 5 mL of 0.5 M phosphate buffer solution (pH 7.0) or buffer containing
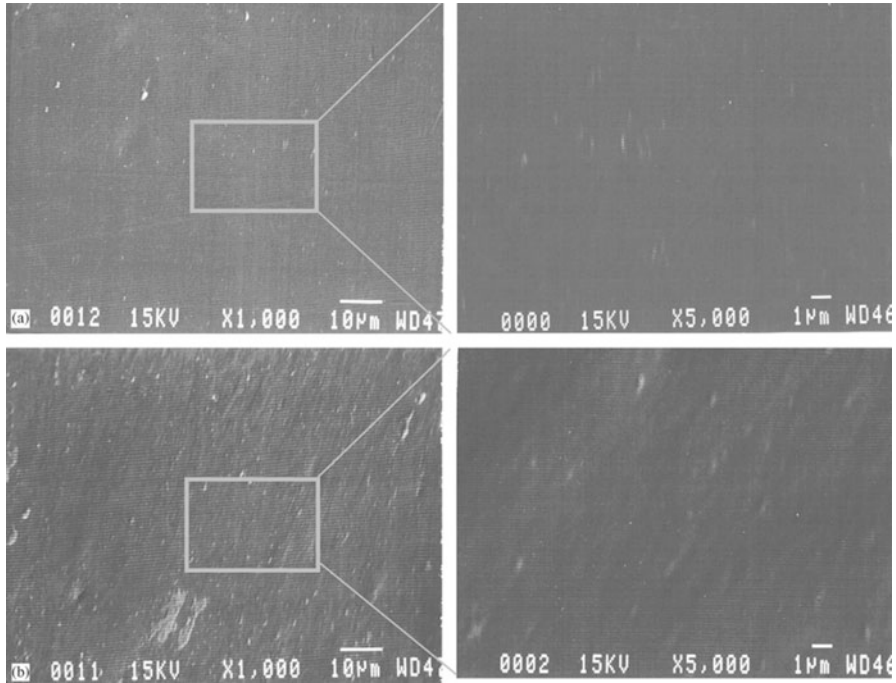
Fig. 2 SEM of polyurethane films treated 36 days in 400 U/mL cholesterol esterase solution: (**a**) PEU and (**b**) PCU

cholesterol esterase for a period of 36 days. Solutions were sterile filtered and a 0.2% w/w sodium azide was used to control microbial contamination. A 1 mL aliquot of fresh CE solution (2000 units/mL) was added to the incubation solutions every day to maintain CE activity. Films were removed every 12 days to examine chemical and physical degradation.

The SEM images of PEU and PCU specimens after 36-day incubation in cholesterol esterase are shown in Fig. 2. Polyurethane film surfaces were smooth and relatively free of surface defects. Previously, pitting of the surface was attributed to extraction of low-molecular-weight degradation products that resulted from chain scission. It was concluded that the extent of degradation with CE was insufficient to cause noticeable surface damage.

It appears that any action of CE was confined to the immediate surface, and the magnitude of the effect was too small to account for the changes observed on implanted films. Degradation processes initiated by CE did not penetrate into the bulk and cause deterioration of bulk properties as was observed with oxidation. It was concluded that in comparison to oxidation, hydrolytic enzymatic degradation of PEU and PCU in vivo is negligible.

Finer et al. [93] also took CE and PCE as suitable models for studying esterase-catalyzed composite resin degradation associated with the oral cavity to determine if

there is a mutual influence between the different esterases with respect to the biodegradation of resin composite.

CE (Cat. #70-1081-01, Lot #9750, Genzyme, Cambridge, MA) and PCE (Cat. #C-5386, Sigma, St. Louis, MO) were prepared by dissolving the enzymes at the required concentrations in phosphate buffered saline (D-PBS, 21600-010, Gibco, Grand Island, NY). All solutions were sterile-filtered using a 0.22-mm filter (Millex®-GP – 0.22 mm Filter unit, Cat. #SLGPR25LS, Millipore, Bedford, MA). The prepared CE and PCE solutions used for replenishing enzyme activity in the biodegradation experiments were stored at −80 °C.

Photopolymerized model composite resin samples (containing 60% by weight fraction of silanated barium glass filler) based on bisGMA/TEGDMA (bis) or urethane-modified bisGMA/TEGDMA/bisEMA (ubis)monomers were incubated in buffer, CE, and/or PCE solutions (pH = 7.0, 37 °C) for 8 and 16 days.

Results indicate that an even greater degradation effect, than the sum of the individual effects by each enzyme measured alone, can be achieved. It also should
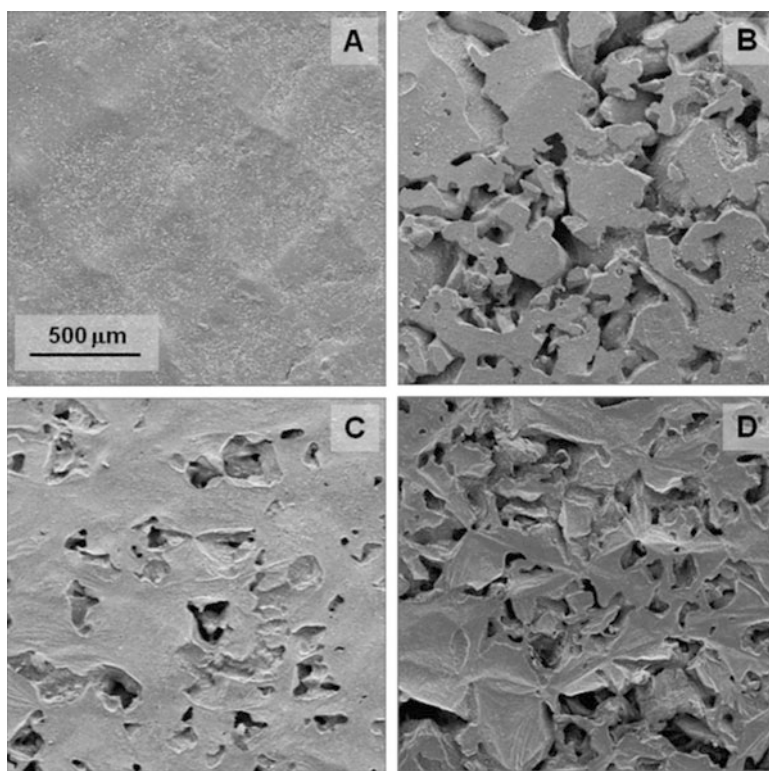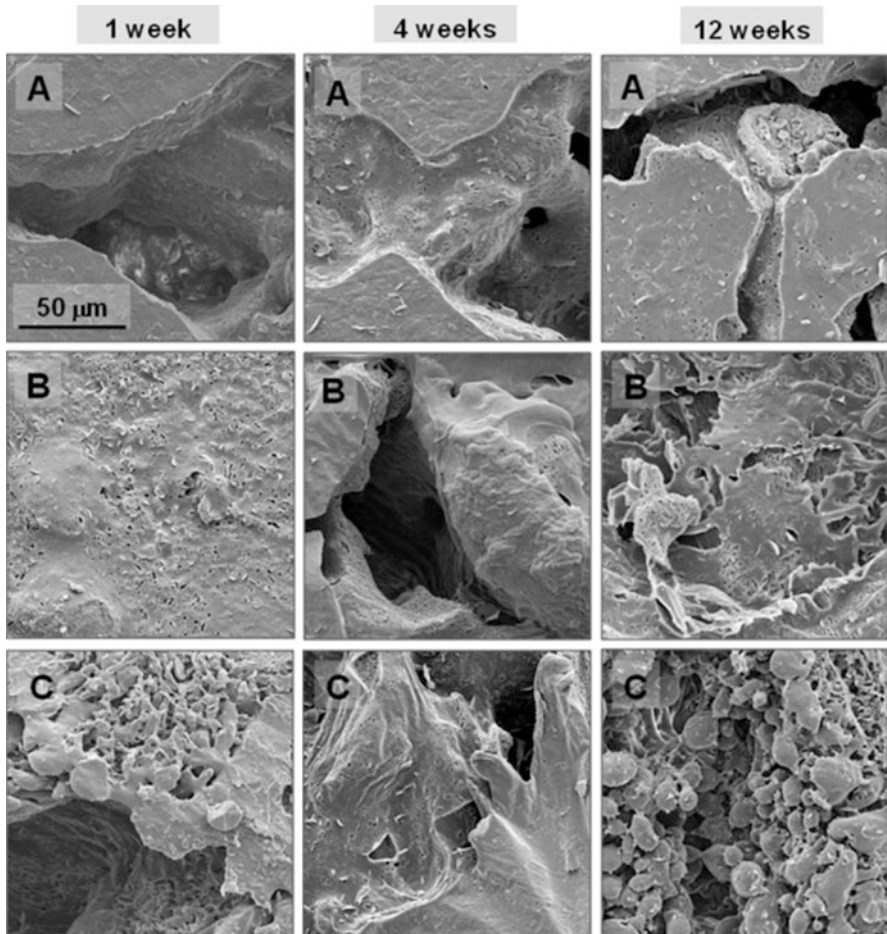

Fig. 3 SEM micrographs of the surface of SPCL disks before (**a**) and after 4 weeks of degradation in PBS (pH 7.4) at 37 °C. Control (**b**), with 0.5% (**c**) and 5% (**d**) encapsulated α-amylase

be noted that the in vivo system also contains many other cofactors, which can inhibit or facilitate the action of enzymes.

The demands for biomaterials with controlled, predictable degradation kinetics includes a wide range of biomedical applications, such as resorbable surgical sutures, matrices for the controlled release of drugs, and scaffolds for tissue engineering. In fact, the performance of many biomaterials depends largely on their degradation behavior since the degradation process may affect a range of events, such as cell growth, tissue regeneration, drug release, host response, and material function.

Biodegradable polymers are materials with the ability of functioning for a temporary period and subsequently degrade in physiological conditions, under a controlled mechanism, into products easily eliminated in the body's metabolic pathways.

Fig. 4 SEM micrographs of the surface of SPCL disks showing the sample morphology in more detail (micrographs at higher magnifications) after degradation for 1, 4, and 12 weeks in PBS (pH 7.4) at 37 °C. Control (**a**), with 0.5% (**b**), and 5% (**c**) encapsulated α-amylase

However, materials to be used in some applications, such as hard tissue replacement, must combine adequate mechanical properties with controlled biodegradability. The material should degrade while maintaining a specified minimum mechanical strength to support the formation of new tissue. It may be difficult to achieve the desired combination of degradation and physical properties in a single material.

Controlled degradation by enzymatic means presents several advantages considering the high specificity of enzymes for their substrates and also because enzyme activity can be regulated by environmental conditions (e.g., pH, temperature, the presence of certain substances, like metal ions). In addition, the degradation kinetics can be adjusted by the amount of encapsulated enzyme into the matrix. Azevedo et al. [94] proposed the enzyme encapsulation technology to incorporate hydrolytic enzymes into the polymeric matrices with controlled degradation. In their work, they investigated the potential of the
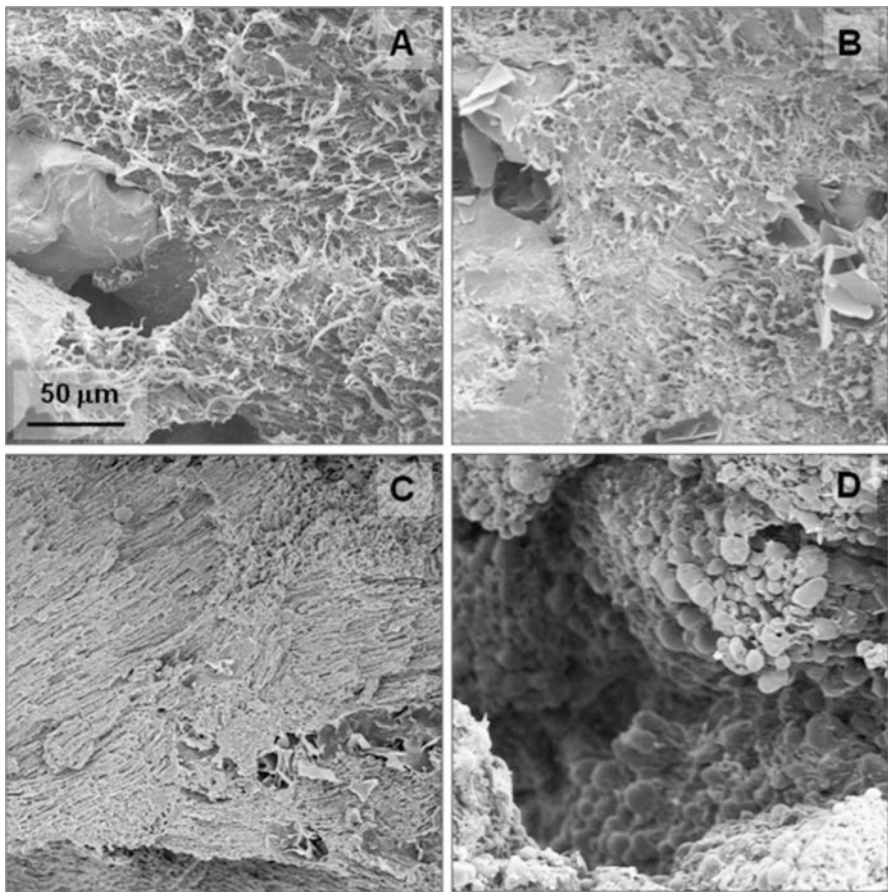
Fig. 5 SEM micrographs of the cross-section (cryogenic fractures) of SPCL disks before (**a**) and after degradation for 12 weeks in PBS (pH 7.4) at 37 °C. Control (**b**), with 0.5% (**c**), and 5% (**d**) encapsulated α-amylase

novel approach to control the degradation kinetics of a starch-based biomaterial by encapsulating a starch-degrading enzyme into the starch matrix using a thermo-mechanical process – a thermostable α-amylase was encapsulated in a starch matrix (processed by compression molding) to tailor the degradation rate.

Preparation of starch-based matrices: the encapsulation method is described as follows:

Prior to use, the enzyme was first lyophilized to obtain the enzyme in powder form. Then the enzyme was mixed with the polymer powder (previously milled in a high speed milling equipment) at different weight percentages (0.5% and 5%, relative to the polymer mass) and processed by compression molding (P = 4 kg cm^{-2}, T = 90 °C, 20 min) in a hydraulic press to prepare disks of about 0.25 g (diameter = 1 cm, thickness = 2 mm). A control, without enzyme, was also prepared. The disks were stored in closed containers at room temperature (away from excess of heat and moisture) until further use.

The effect of enzyme degradation, in both the encapsulated and the free form, on the surface morphology of SPCL disks can be observed in the SEM micrographs shown in Figs. 3, 4, 5, 6. Although the incubation in PBS also causes some erosion on the material surface (Figs. 3 and 4a), the incubation of the disks with the encapsulatedα-amylase leads to a highly porous structure. The degradation effect of the free enzyme (Fig. 6) is not as clear as the one observed with the encapsulated enzyme, which may be related with the starch distribution in the
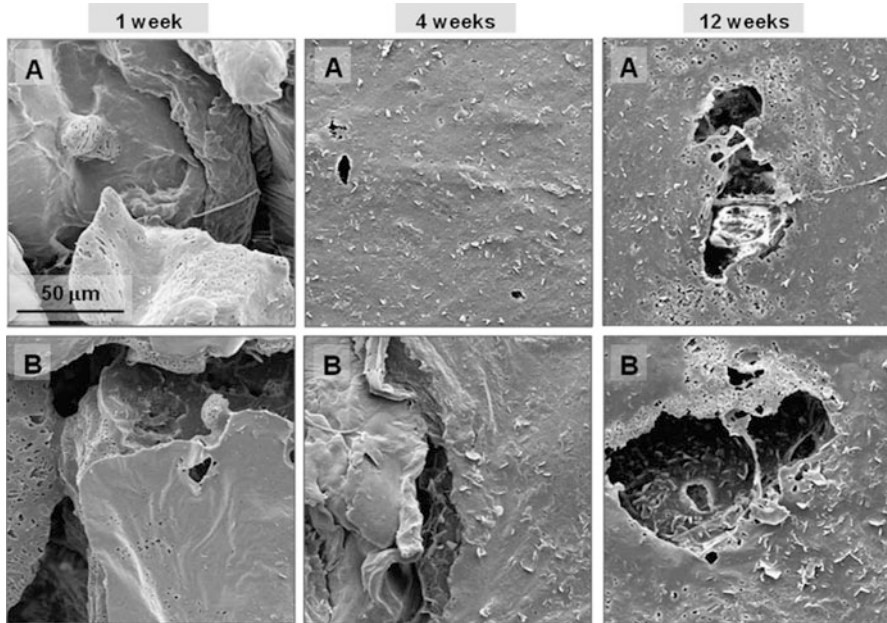
Fig. 6 SEM micrographs of the surface of SPCL disks after degradation for 1, 4, and 12 weeks in PBS containing 0.5% (**a**) and 5% (**b**) a-amylase free in solution

blend. Enzyme hydrolytic activity induces changes in the physicochemical properties of the matrix and, as a result of degradation, the matrix collapses, as can be observed in Fig. 5d.

Results show that by using a thermostable enzyme it is possible to retain its activity during the encapsulation process, and the degradation of the starch matrix can be controlled by encapsulating the proper amount of α-amylase.

Conclusions and Future Perspective

Despite excellent and controllable physical properties of synthetic biomaterials, the apparent lack of innate cell recognition entities has led to the fabrication of composite bio-artificial scaffolds which aim to harness the distinct benefits of both material classes while simultaneously compensating for each other's weaknesses. A good idea in theory, biosynthetic composite scaffolds however retain their individual drawbacks which, in the case of synthetic materials, are more predictable and therefore more easily rectifiable. For instance, natural biomaterials pose an innate risk of eliciting an immunological graft rejection which could be detrimental in cases of vital placements of grafts, for example, vascular interpositional implants. With ever advancing material processing techniques, however, synthetic scaffold designs incorporating artificial cell-recognition entities may replace immunogenic cell surface epitopes with synthetic mimics which enhances biocompatibility of synthetic materials. Additionally, synthetic materials may be designed with chemical functional groups which can enhance cell attachment and proliferation and induce tissue ingrowth.

Biomaterials scientists currently pursue three main avenues for the optimization of tissue engineering scaffold materials: purely natural scaffolds, synthetic alternatives, or biosynthetic composites. It demonstrates that rather than attempting an overcomplicated top down approach by aiming to modify natural materials with inherent material properties which may or may not be suitable, scientists would be better advised to adhere to the bottom-up fabrication of synthetic materials, which may be strictly controlled throughout the fabrication process. Developing nature-inspired materials by copying natural material structures, and using man-made materials for greater control and flexibility during the manufacturing process would ideally enable utilization of the best concepts of both worlds.

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