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Review Article

BIODEGRADABLE POLYMERS: A SMART STRATEGY FOR TODAY'S CRUCIAL NEEDS

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Abstract

In the recent years, bio-based and biodegradable products have raised high interest since sustainable development policies tend to expand with the decreasing reserve of fossil fuel and the growing concern for the environment. Biodegradable polymers (BPs) are designed to degrade upon disposal by the action of living organisms. These polymers bring a significant contribution to the sustainable development in view of the wider range of disposal options with minor environmental impact. The market of these environmentally friendly materials is in rapid expansion, 20–25 % per year. Extraordinary progress has been made in the development of practical processes and products from polymers such as starch, cellulose, and lactic acid. The need to create alternative biodegradable water-soluble polymers for down-the-drain products such as detergents and cosmetics has taken on increasing importance. Biodegradable polymers mainly classified as agropolymers and biodegradable polyesters. Biopolyesters productions are obtained mainly from renewable resources. Consumers, thus far attached little or no added value to the property of biodegradability, forcing industry to compete head-to-head on a cost performance basis with existing familiar products. In addition, no suitable infrastructure for the disposal of biodegradable materials exists as yet. This article intends to present the properties and applications of BPs.

Key Words: Biodegradable, biopolymer, renewable resources, degradation, microorgani

1. Introduction:

Conventional polymers such as polyethylene and polypropylene persist for many years after disposal. Built for the long haul, these polymers seem inappropriate for applications in which plastics are used for short time periods and then disposed. Furthermore, plastics are often soiled by food and other biological substances, making physical recycling of these materials impractical and generally undesirable. In contrast, biodegradable polymers (BPs) disposed in bioactive environments degrade by the enzymatic action of microorganisms such as bacteria, fungi, and algae [1].

Increasing concern exists today about the preservation of our ecological systems. Most of today's synthetic polymers are produced from petrochemicals and are not biodegradable. Persistent polymers generate significant sources of environmental pollution, harming wildlife when they are dispersed in nature. For example, the disposal of non-degradable plastic bags adversely affects sea-life. It is widely accepted that the use of long- lasting polymers in products with a short life-span, such as engineering applications, packaging, catering, surgery, and hygiene, is not adequate. Moreover, incineration of plastic waste presents environmental

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issues as well since it yields toxic emissions (e.g., dioxin). Material incineration is also limited due to the difficulties to find accurate and economically viable outlets.

In addition, plastic recycling shows a negative eco-balance due to the necessity, in nearly all cases, to wash the plastic waste as well as the energy consumption during the recycling process phases (waste grinding and plastic processing). As plastics represent a large part of the waste collection at the local, regional, and national levels, institutions are now aware of the significant savings that compostable or biodegradable materials would generate. For these different reasons, reaching the conditions of conventional plastic replacements by degradable polymers, particularly for short-term applications (packaging, agriculture…), is of major interest to the society as a whole, from the plastic industries to the citizens [2].

A variety of natural, synthetic, and biosynthetic polymers are bio and environmentally degradable. A polymer based on a C-C backbone tends to resist degradation, whereas heteroatom-containing polymer backbones confer biodegradability. Biodegradability can, therefore, be engineered into polymers by the judicious addition of chemical linkages such as anhydride, ester, or amide bonds, among others. The usual mechanism for degradation is by hydrolysis or enzymatic cleavage of the labile heteroatom bonds, resulting in a scission of the polymer backbone. Macro organisms can eat and, sometimes, digest polymers, and also initiate a mechanical, chemical, or enzymatic aging [3].

Biodegradable polymers with hydrolysable chemical bonds are researched extensively for biomedical, pharmaceutical, agricultural, and packaging applications. In order to be used in medical devices and controlled-drug-release applications, the biodegradable polymer must be biocompatible and meet other criteria to be qualified as biomaterial-processable, sterilizable, and capable of controlled stability or degradation in response to biological conditions.[4] The chemical nature of the degradation products, rather than of the polymer itself, often critically influences biocompatibility. Poly (esters) based on polylactide (PLA), polyglycolide (PGA), polycaprolactone (PCL), and their copolymers have been extensively employed as biomaterials [4-7].

Their polymer chains may also be broken down by non-enzymatic processes such as chemical hydrolysis. BPs is often derived from plant processing of atmospheric CO_2. Biodegradation converts them to CO_2, CH_4, water, biomass, humic matter, and other natural substances. BPs are thus naturally recycled by biological processes (Fig. 1).

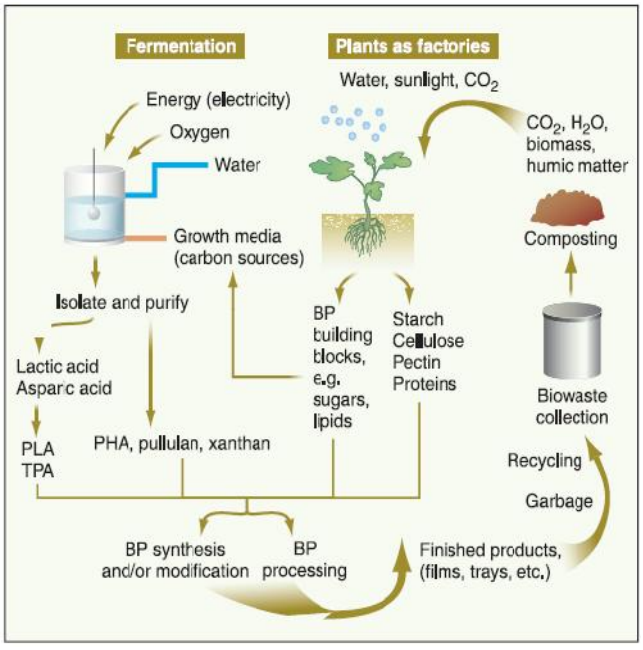

Fig.1. Cyclic process by which agricultural products and fermentative routes can yield biodegradable polymers. Upon disposal in bio-bins and exposure to a bioactive environment, BPs will biodegrade to natural substances such as CO_2, water, humic matter, and biomass. New agricultural crops, using nutrients from compost and fixing CO_2, will produce new polymer building blocks, monomers, and polymers. (Source: SCIENCE VOL 297 AUGUST 2002)

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1.2. Recent Past of Biodegradable Polymers:

The worldwide consumption of biodegradable polymers has increased day-by-day. Target markets for BPs include packaging materials (trash bags, wrappings, loose-fill foam, food containers, film wrapping, laminated paper), disposable nonwovens (engineered fabrics) and hygiene products (diaper back sheets, cotton swabs), consumer goods (fast-food tableware, containers, egg cartons, razor handles, toys), and agricultural tools (mulch films, planters). BP commercialization is, however, hampered by competition with commodity plastics that are inexpensive and familiar to the customer. Also, an infrastructure for the disposal of BPs in bioactive environments must be developed and will require capital investments. Without an extensive network of efficient composting and other bioconversion facilities that, in addition to compost, yield other valuable chemical intermediates, BPs and other bio-disposables (food, yard-waste, non-recycled paper) are intended to be entombed in dry landfill environments designed to retard biodegradation [8-10].

The potential of biodegradable polymers has been recognized for a long time since they could be an interesting way to overcome the limitation of the petrochemical resources in the future. The fossil fuel and gas could be partially replaced by green agricultural resources, which would also participate in the reduction of CO_2 emissions. However, till now, biodegradable polymers have not found extensive applications in industries to largely replace conventional plastic materials, reasons being their high production costs and sometimes their underperformed properties.

1.3. Biodegradable Polymers and Coming Era:

Mounting consumer pressure and legislation such as plastic bag bans and global warming initiatives will increase demand for biodegradable plastics in North America, Europe and Asia by nearly 15% annually until 2015, according to a new report by IHS Chemical. Demand will grow from 269,000 metric tons in 2012 to nearly 525,000 MT in 2017. Biodegradable polymers are a part of the larger overall bio-plastics market. Typically, bio- plastics are either bio-based or biodegradable, although some materials are both. The biodegradable polymers market is still young and very small, but the numbers are off the charts in terms of expected demand growth and potential for these materials in the coming years. Food packaging, dishes and cutlery constitute a major market for the product because these materials can be composted with the food waste without sorting, which is a huge benefit to the waste management effort and to reducing food waste and packaging disposal in landfills. Increasing legislation and consumer pressures are also encouraging retailers and manufacturers to seek out these biodegradable products and materials. These biodegradable polymers offer expanding uses for biomedical applications. Another developing use for these biodegradable polymers is in the shale gas industry, where they are used during hydro-fracking. In 2012, Europe was the dominant market for biodegradable polymers, consuming about 55% of world consumption; North America accounted for 29%; and Asia approximately 16%. The most acceptable disposal method for biodegradable polymers is composting. However, composting requires an infrastructure, including collection systems and composting facilities. Composting has been a growing component of most European countries' municipal solid waste management strategies for some time, and the continent has an established and growing network of facilities, while the U.S. network of composting facilities is smaller, but expanding. North American consumption of biodegradable polymers has grown significantly in recent years, according to the IHS report, primarily due to the following factors—biodegradable polymers have become more cost competitive with petroleum-based products, and there has been growing support at the local, state and federal levels for these products (for example, legislation defining biodegradability, and plastic bag bans). In addition, there has been progress in addressing issues relative to solid waste disposal, such as improving

composting infrastructure. Said Malveda, "A couple of main barriers to these biodegradable polymers are price and performance, which will become less significant as processing technologies improve, more applications for their use are developed, and production increases. Regulations such as plastic bag bans are being enacted in many countries, and this stimulates new research investments for alternative materials and new uses." In Asia, there has been some growth of biodegradable polymers use due to government and industry promoting their use. This also includes plastic bag bans and global warming initiatives. However, Asian consumption of biodegradable polymers has not increased as much as expected. Current market prices of biodegradable polymers continue to be higher than conventional, petroleum-based resins. However, the Chinese market is expected to grow rapidly due to new capacity and government legislation supporting the environment. Future growth will also depend on price reductions. In 2012, the two most important commercial, biodegradable polymers were polylactic acid (PLA) and starch-based polymers, accounting for about 47% and 41%, respectively, of total biodegradable polymers consumption. Starch sources vary worldwide, but include corn, potatoes, cassava and sugar beets. In Europe, starch-based biodegradable polymers are the major type consumed, accounting for 62% of the market, due to Europe's large, starch-based capacity and their use in many applications. This is followed by PLA, with 24% and other biodegradable polymer types with 14%.

As per Markets and Markets, the use of renewable resources such as biomass-and bio-based raw materials such as starch, vegetable crop derivatives in manufacturing of plastics is driving the market for biodegradable plastics. As of 2009, bio-based plastics accounted for about 93% of the overall biodegradable plastics market. The use of biodegradable plastics in various applications such as packaging, domestic goods has enabled the plastic manufacturers to lessen the dependence on petroleum-based plastics. Increasing consumer awareness for sustainable products and manufacturers concern for developing eco-friendly packaging is shifting the trend towards biodegradable plastics from their petrochemical counterparts.

The overall scenario for biodegradable plastics is significantly positive with a critical need to expand production capacities and increase consumer awareness. The increasing demand for renewable and bio-based materials and shift in consumer preference for eco-friendly packaging is driving the market for global biodegradable plastics. The global biodegradable plastics market in terms of volume is expected to grow from 664,000 metric tons in 2010 to 2330,000 metric tons by 2016, at an estimated CAGR of 20.24% from 2011 to 2016.

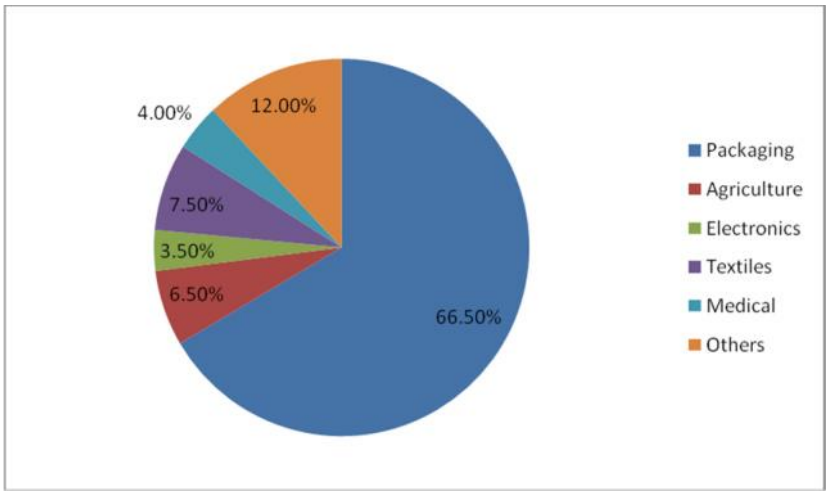

Fig. 2: Global Bio-Based Biodegradable Plastics Market (By Applications, 2016).

Biodegradable plastics offer tremendous potential in various applications including packaging, electronics, transport, textiles, and medical. Amongst all market segments, the

starch-based plastics market commands the largest share in terms of volume, while PLA-based plastics lead the market in terms of value. Packaging forms the largest application market due to increased consumer awareness for sustainable packaging. The packaging application contributed over 50% of the global biodegradable plastics market in 2010. In PLA-based plastics, textiles applications are expected to have the highest CAGR of 23.16% from 2011 to 2016. Europe accounted for the major share for the global biodegradable plastics market estimated to be 40.6% in 2010. This was primarily due to the fact that focus on sustainability is significant in Europe, especially in the European Union. Due to this, Europe is the most regulated market especially when it comes to certifying and commercializing new plastic products. North America forms the second largest market for biodegradable plastics in the world. The market players are focusing in agreement and collaboration in order to share technical expertise in the production of biodegradable polymers, which therefore accounted for the highest share of the total competitive developments in the global biodegradable plastics market from May 2008 to April 2011. Industry participants with the most agreement and collaboration and significant product developments include Cardia Bioplastics Limited, Cereplast, Purac, and Telles. In 2011, Cereplast Inc. has concluded a multi-million dollar distribution agreement with BioWorks Pl for the distribution of Cereplast bioplastic resins in the Poland market. (Source Technical Articles & Reports on Plastic Industry from plastemart.com on 05/11/2013)

In this viewpoint we report on progress, technical and social challenges and environmental benefits of BPs. Some of highly promising biodegradable polymers that are either in development or already marketed.

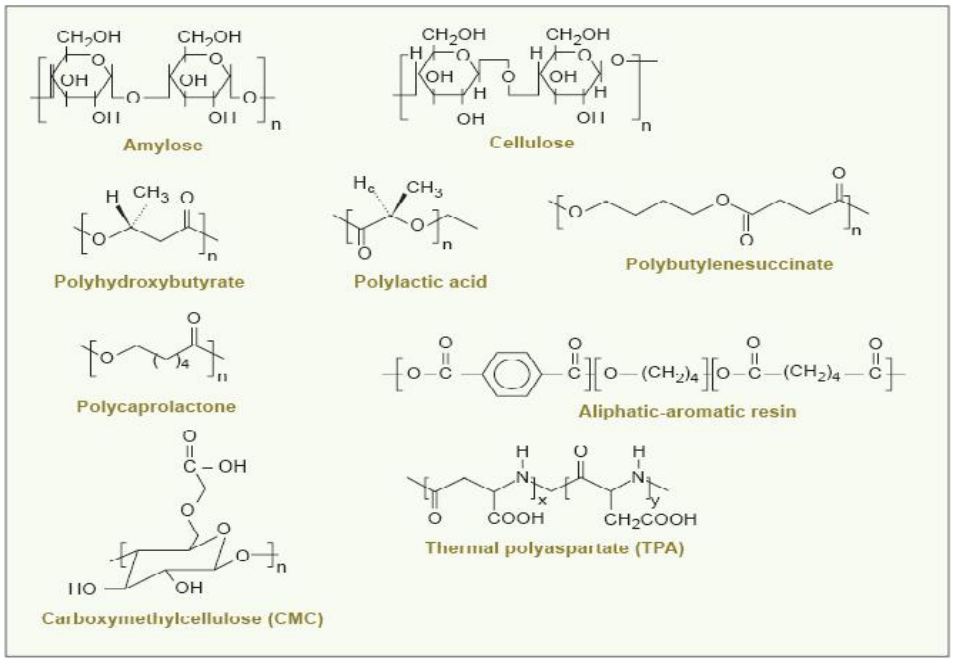

Fig.3. Structures of some selected bio-degradable polymers. (Source: SCIENCE VOL 297 AUGUST 2002)

1.4. Renewability and Sustainable Development:

Renewability is more or less linked to the concept of sustainable development. The UN World Commission on ‘‘Environment and Development in Our Future’’ defines sustainability as the development which meets the needs of the present without compromising the ability of future generations to meet their own needs. The use of annually renewable biomass must be understood in a complete carbon cycle. The carbon cycle is a complex process by which carbon is exchanged between the four main reservoirs of carbon on the planet i.e., the lithosphere (e.g., limestone), the biosphere (plant and animal), the hydrosphere (e.g.

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bicarbonate dissolved in the oceans), and the atmosphere (CO_2). Recent human activities (burning fossil fuel and massive deforestation) lead to an important imbalance in the carbon cycle with a huge and rapid release of CO_2 to the atmosphere, which cannot be fully compensated by the photosynthesis activity and the dissolution in the oceans.

It results in a large accumulation of CO_2 in the atmosphere, which contributes to the global warming. People are now aware that efforts have to be made to re-balance the carbon cycle by reducing the amount of CO_2 production. Part of the carbon cycle re-balancing concept is based on the development and manufacture of products based on renewable and biodegradable resources. By collecting and composting biodegradable plastic wastes, we can generate much-needed carbon-rich compost: humus materials. These valuable soil inputs can go back to the farmland and ‘‘reinitiate’’ the carbon cycle. Then, the plants growth contributes to reducing CO_2 atmospheric accumulation through photosynthesis activity. Besides, composting is an increasing key point to maintain the sustainability of agricultural systems by reducing the consumption of chemical fertilizers [11].

2. Biodegradability and Compostability:

According to ASTM standard D-5488-94d and European norm EN 13432, ‘‘biodegradable’’ means ‘‘capable of undergoing decomposition into carbon dioxide, methane, water, inorganic compounds, and biomass’’. The predominant mechanism is the enzymatic action of microorganisms, which can be measured by standard tests over a specific period of time, reflecting available disposal conditions. There are different media (liquid, inert, or compost medium) to analyze biodegradability. Compostability is material biodegradability using compost medium. Biodegradation is the degradation of an organic material caused by biological activity (biotic degradation), mainly microorganisms’ enzymatic action. The end-products are CO_2, new biomass, and water (in the presence of oxygen, i.e. aerobic conditions) or methane (in the absence of oxygen, i.e., anaerobic conditions), as defined in the European Standard EN 13432-2000 [12].

We must also take into account the amount of mineralization as well as the nature of the residues (commonly called ‘by-products’) left after biodegradation. The accumulation of contaminants with toxic residues can cause plant growth inhibition. The key issue is to determine the environmental toxicity level for these by-products, which is known as eco-toxicity. Some general rules enable the determination of the biodegradability evolution. For example, an increase in parameters such as the hydrophobicity, the macromolecules molecular weights, crystallinity or the size of crystalline domains decreases the biodegradability [13-15].

2.1. Biodegradable Polymers Classifications:

Biodegradable polymers represent a growing field. A vast number of biodegradable polymers (e.g. cellulose, chitin, starch, polyhydroxyalkanoates, polylactide, polycaprolactone, collagen and other polypeptides…) have been synthesized or are formed in natural environment during the growth cycles of organisms. Some microorganisms and enzymes capable of degrading such polymers have been identified [16-19].

Different classifications of various biodegradable polymers have been proposed. Here we propose to classify the biodegradable polymers according to their synthesis process (Fig. 4): (i) polymers from biomass such as agro-polymers from agro-resources (e.g., starch or cellulose), (ii) polymers obtained by microbial production such as the polyhydroxyalkanoates (PHAs), (iii) polymers conventionally and chemically synthesized from monomers obtained from agro-resources, e.g., the polylactic acid (PLA), and (iv) polymers obtained from fossil resources.Only the first three categories (i–iii) are obtained from renewable resources. We can further classify these biodegradable polymers into two main categories: the agro-polymers (category i) and the biodegradable polyesters or biopolyesters (categories ii–iv).

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The purpose of this article is to give a brief overview on representative biodegradable polymers that have already been commercialized or are under investigation for biomedical and ecological applications.

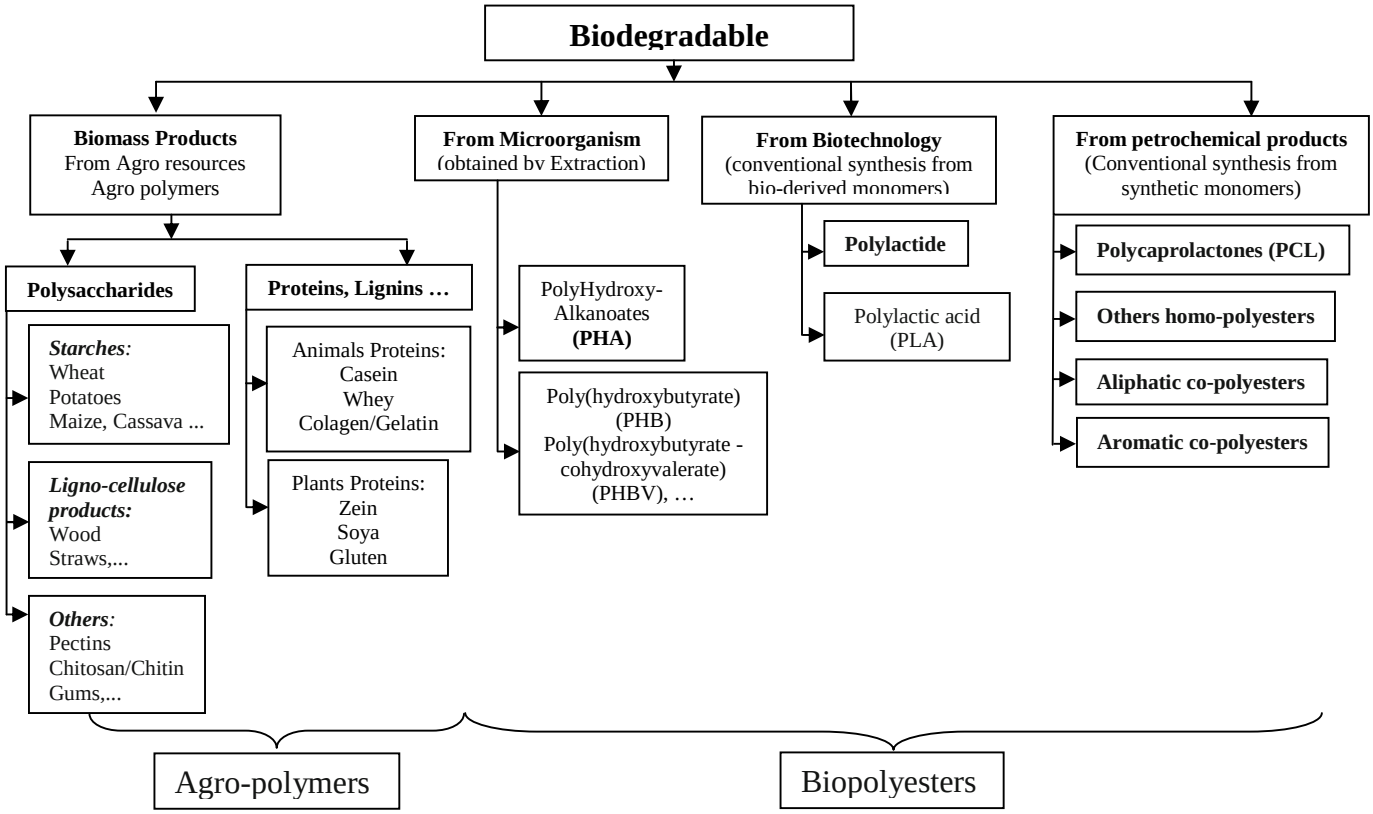


Fig.4: Classification of Biodegradable polymers.
(Source: L. Avérous & E. Pollet (eds.), Environmental Silicate Nano-Biocomposites, Green Energy & Technology, DOI: 10.1007/978-1-4471-4108-2_2, © Springer-Verlag London 2012)

3. Biodegradable polyesters for medical and ecological applications:

Numerous biodegradable polymers have been developed in the last two decades. In terms of application, biodegradable polymers are classified into three groups: medical, ecological, and dual application, while in terms of origin they are divided into two groups: natural and synthetic.

Among the biodegradable polymers, recent developments of aliphatic polyesters, especially polylactides and poly(lactic acid)s, will be mainly described in the last part.

In a strict sense, such polymers that require enzymes of microorganisms for hydrolytic or oxidative degradation are regarded as biodegradable polymers. This definition does not include polylactides in the category of biodegradable polymers, because polylactides are hydrolyzed at a relatively high rate even at room temperature and neutral pH without any help of hydrolytic enzymes if moisture is present. This often gives rise to confusion when we say that polylactides are biodegradable. As will be shown later, polylactides, especially polyglycolide, are readily hydrolyzed in our body to the respective monomers and oligomers that are soluble in aqueous media [20]. Generally, such a polymer that loses its weight over time in the living body is called an absorbable, resorbable, or bioabsorbable polymer as well as a biodegradable polymer, regardless of its degradation mode, in other words, for both enzymatic and non-enzymatic hydrolysis. To avoid this confusion, some people insist that the term “biodegradable” should be used only for such ecological polymers that have been developed aiming at the protection of earth environments from plastic wastes, while the

polymers applied for medical purposes by implanting in the human body should not be called biodegradable but resorbable or absorbable (Fig. 5).

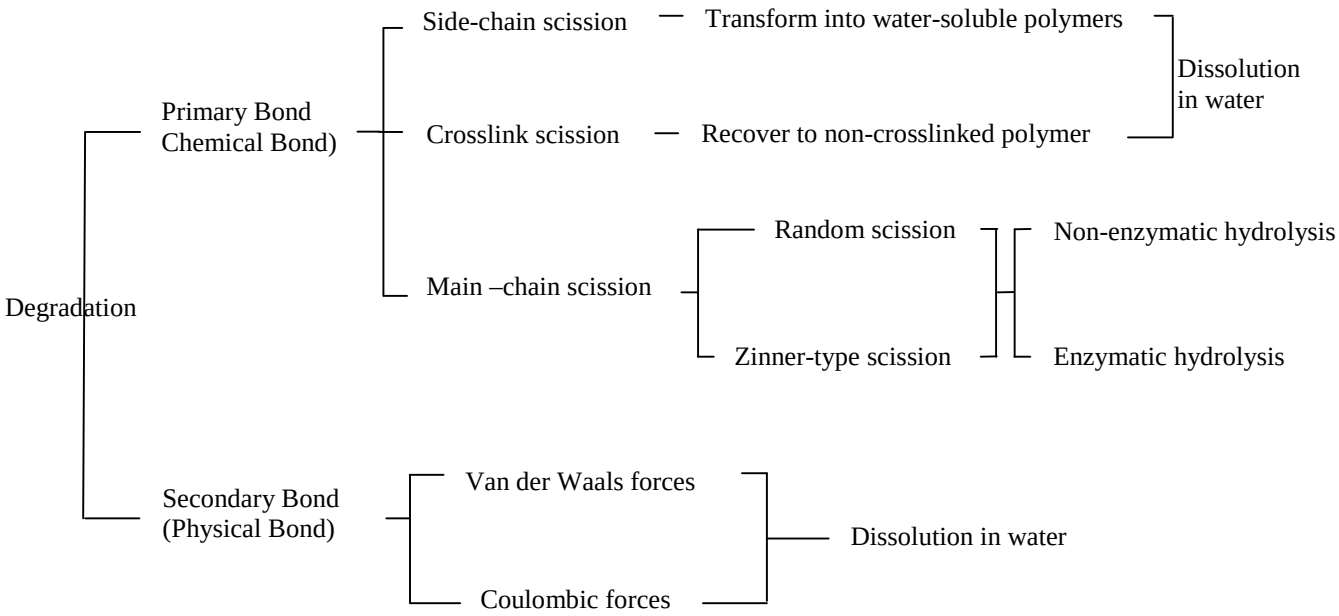
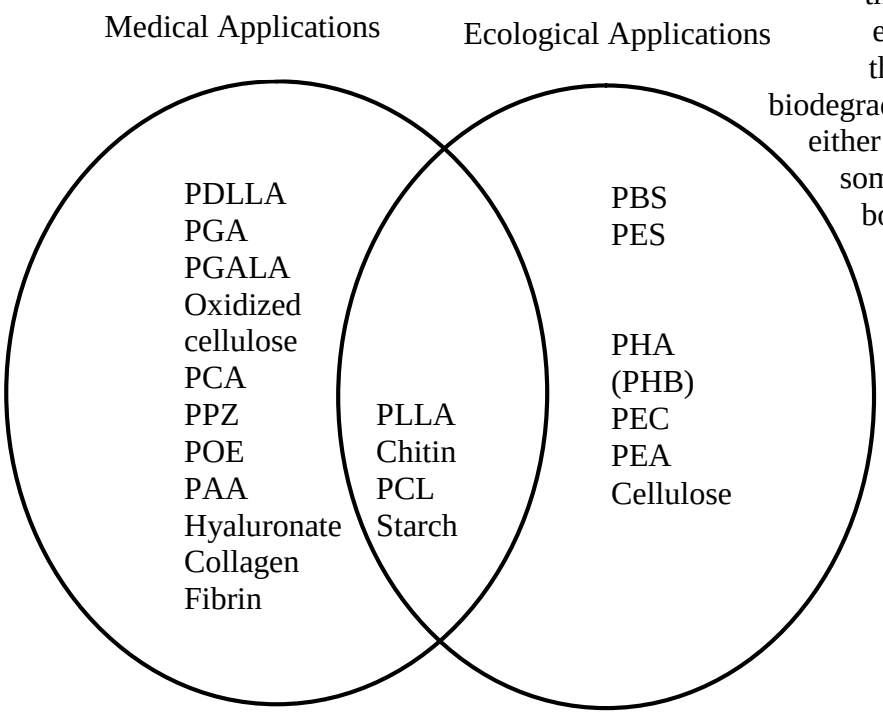


Fig.5: Modes of resorption of polymers

These biodegradable polymers have currently two major applications; one is as biomedical polymers that contribute to the medical care of patients and the other is as ecological polymers that keep the earth environments clean. Most of the currently available biodegradable polymers are used for either of the two purposes, but some of them are applicable for both, as illustrated in Fig. 6.



F***ig.6: Application of biodegradable polymers.*** ***PAA: Poly- (acid anhydride); PBS: Poly(butylene succinate); PCA: Poly(acyanoacrylate); PCL: Poly(e-caprolactone); PDLLA: Poly(DLlactide), Poly(DL-lactic acid); PEA: Poly(ester amide); PEC: Poly(ester carbonate); PES: Poly(ethylene succinate); PGA: Poly(glycolide), Poly(glycolic acid); PGALA: Poly(glycolideco- lactide), Poly(glycolic acid-co-lactic acid); PHA: Poly(hydroxyalkanoate); PHB: Poly(3-hydroxybutyrate); PLLA: Poly(L-lactide), Poly(L-lactic acid); POE: Poly(orthoester).***

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3.1. Biomedical applications:

3.1.1. Biomaterials:

A variety of polymers have been used for medical care including preventive medicine, clinical inspections and surgical treatments of diseases [21-25]. Among the polymers employed for such medical purposes, a specified group of polymers are called ***polymeric biomaterials,*** when they are used in direct contact with living cells of our body. Typical applications of biomaterials in medicine are for disposable products (e. g. syringe, blood bag, and catheter), materials supporting surgical operation (e. g. suture, adhesive, and sealant), prostheses for tissue replacements (e.g. intraocular lens, dental implant, breast implant), and artificial organs for temporary or permanent assist (e.g. artificial kidney, artificial heart and vascular graft).

The biodegradable medical polymers have attracted much attention. There are at least two reasons for this new trend. One is the difficulty in developing such biocompatible materials that do not evoke any significant foreign-body reactions from the living body when receiving man-made biomaterials. At present we can produce biomaterials that are biocompatible if the contact duration of biomaterials with the living body is as short as several hours, days, or weeks. However, the science and technology of biomaterials have not yet reached such a high level that allows us to fabricate biocompatible implants for permanent use. On the contrary, biodegradable polymers do not require such excellent biocompatibility since they do not stay in body for a long term but disappear without leaving any trace of foreign materials [26-27].

The other reason for biodegradable polymers attracting much attention is that nobody will want to carry foreign materials in the body as long-term implants, because one cannot deny a risk of infection eventually caused by the implants.

These biomaterials are quite different from other nonmedical commercial products in many aspects. For instance, neither industrial manufacturing of biomaterials nor sales of medical devices are allowed unless they strict complies regulatory issues. The minimum requirements of biomaterials for such governmental approval include non-toxicity, sterilizability, and effectiveness. Biocompatibility is highly desirable but not indispensable; most of the clinically used biomaterials lack excellent biocompatibility, although many efforts have been devoted to the development of biocompatible materials by biomaterials scientists and engineers. A vast unsolved problem of biomaterials is lack of biocompatibility, especially when they are used permanently as implants in our body.

Minimal requirements for being biomaterials:

1. Non-toxic (Biosafe): Non-pyrogenic, Non-hemolytic, Chronically non-inflammative, Non-allergenic, Non-carcinogenic, Non-teratogenic, etc.
2. Effective: Functionality, Performance, Durability, etc.
3. Sterilizable: Ethylene oxide, c-Irradiation, Electron beams, Autoclave, Dry heating, etc.
4. Biocompatible: Interfacially, Mechanically, and Biologically.

Although biodegradable polymers seem very promising in medical applications, these kinds of polymers currently do not enjoy large clinical uses, because there is a great concern on biodegradable medical polymers. This concern is the toxicity of biodegradation by-products, since the causes of toxicity of biomaterials are mostly due to low-molecular-weight compounds that have leached from the biomaterials into the body of patients. However, biodegradable polymers always release low molecular-weight compounds into the outer environment as a result of degradation. If they can interact with the cell surface or enter into the cell interior, it is possible that the normal condition of the cell is disturbed by such foreign compounds. One can say that an implanted biomaterial induces cyto-toxicity if this disturbance is large enough to bring about an irreversible damage to the cell. Purified polyethylene and silicone are not toxic but also not biocompatible, because thrombus formation and encapsulation by collagenous fibrous tissues take place around their surface when implanted [28].

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3.1.2. Surgical use:

Application of biodegradable polymers to medicine did not start recently and has already a long history. Actual and possible applications of biodegradable polymers in medicine are shown in Table 1 and lists representative synthetic biodegradable polymers currently used or under investigation for medical application are shown in Table 2. As is seen, most of the applications are for surgery. The largest and longest use of biodegradable polymers is for suturing. Collagen fibres obtained from animal intestines have been long used as absorbable suture after chromium treatment [29].

The use of synthetic biodegradable polymers for suture started in USA in the 1970's [26, 30]. Commercial polymers used for this purpose include polyglycolide, which is still the largest in volume production, together with a glycolide-L-lactide (90:10) copolymer. The sutures made from these glycolide polymers are of braid type processed from multi-filaments, but synthetic absorbable sutures of mono-filament type also at present are commercially available.

The biodegradable polymers of the next largest consumption in surgery are for hemostasis, sealing, and adhesion to tissues [31]. Liquid-type products are mostly used for these purposes. Immediately after application of a liquid to a defective tissue where hemostasis, sealing, or adhesion is needed, the liquid sets to a gel and covers the defect to stop bleeding, seal a hole, or adhere two separated tissues. As the gelled material is no longer necessary after healing of the treated tissue, it should be biodegradable and finally absorbed into the body. The biomaterials used to prepare such liquid products include fibrinogen (a serum protein), 2-cyanoacrylates, and a gelatin/resorcinol/formaldehyde mixture.

Regenerated collagen is also used as a hemostatic agent in forms of fibre, powder, and assemblies. Another possible application of biodegradable polymers is the fixation of fractured bones. Currently, metals are widely used for this purpose in orthopaedic and oral surgeries in the form of plates, pins, screws, and wires, but they need removal after re-union of fractured bones by further surgery. It would be very beneficial to patients if these fixation devices can be fabricated using biodegradable polymers because there would be no need for a re-operation.

Table 1: Medical applications of bioabsorbable polymers

Function	Purpose	Examples
Bonding	Suturing	Vascular and intestinal anastomosis
	Fixation	Fractured bone fixation
	Adhesion	Surgical adhesion
Closure	Covering	Wound cover, Local hemostasis
	Occlusion	Vascular embolization
Separation	Isolation	Organ protection
	Contact inhibition	Adhesion prevention
Scaffold	Cellular proliferation	Skin reconstruction, Blood vessel reconstruction
	Tissue guide	Nerve reunion
Capsulation	Controlled drug delivery	Sustained drug release

3.1.3. Pharmaceutical use:

For delivery of drugs to diseased sites in the body in a more effective and less invasive way, a new dosage form technology, called drug delivery systems (DDS), started in the late 1960's in the USA using polymers. The objectives of DDS include sustained release of drugs for a desired duration at an optimal dose, targeting of drugs to diseased sites without affecting healthy sites, controlled release of drugs by external stimuli and simple delivery of drugs mostly through skin and mucous membranes [32].

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Table 2: Representative synthetic biodegradable polymers currently used or under investigation for medical application

Polymers	Structure	M_w/kD	Degradation rate	Medical application
Poly(glycolide)	Crystalline	–	100% in 2–3 months	Suture, Soft issue anaplerosis
Poly(glycolic acid-co-L-lactic acid)	Amorphous	40–100	100% in 50–100 days	Suture, Fracture fixation, Oral implant, Drug delivery microsphere
Poly(L-lactide)	Semicrystalline	100–300	50% in 1–2 years	Fracture fixation, Ligament augmentation
Poly(L-lactic acid-co-e-caprolactone)	Amorphous	100–500	100% in 3–12 months	Suture, Dural substitute
Poly(e-caprolactone)	Semicrystalline	40–80	50% in 4 years	Contraceptive delivery implant,
Poly(p-dioxanone)	Semicrystalline	–	100% in 30 weeks	Suture, Fracture fixation
Poly(orthoester)	Amorphous	100–150	60% in 50 weeks (saline, 378C)	Contraceptive delivery implant

Polymers are very powerful tools for this new approach of delivery system. If a drug is administered through a parenteral route like injection, the polymer used as a drug carrier should be preferably absorbable, because the polymer is no longer required when the drug delivery has been accomplished. Therefore, biodegradable polymers are widely used, especially for the sustained release of drugs through administration by injection or implantation into the body. For this purpose, absorbable nanospheres, microspheres, beads, cylinders and/or discs are widely prepared using biodegradable polymers [33-35].The shape of the most widely used drug carriers is a microsphere, which incorporates drugs and releases them through physical diffusion, followed by resorption of the microsphere material. Such microspheres can be prepared with a solvent-evaporation method using glycolide-lactide copolymers. Naturally occurring biodegradable polymers are also used as drug carriers for a sustained release of drugs. If the drug carrier is soluble in water, the polymer need not to be biodegradable, because this polymer will be excreted from the body, associated with urine or feces although excretion will take a long time if the molecular weight of the polymer is extremely high.

3.1.4. Use for Tissue Engineering:

Tissue engineering is an emerging technology to create biological tissues for replacements of defective or lost tissues using cells and cell growth factors. Scaffolds are also required for tissue construction if the lost part of the tissue is so large that it cannot be cured by conventional drug administration. At present, such largely diseased tissues and organs are replaced either with artificial organs or transplanted organs; but both the therapeutic methods involve some problems like the biocompatibility of clinically used artificial organs is mostly not satisfactory enough to prevent severe foreign-body reactions and to fully perform the objective of the artificial organs aimed for patients. The bio-functionality of current artificial organs is still poor. On the contrary, the bio-functionality of transplanted organs is as excellent as healthy human organs, but the patients with transplanted organs are suffering from side-effects induced by immunosuppressive drugs administered. Another major problem of organ transplantation is shortage of organ donors [25].

The final objective of tissue engineering is to solve these problems by providing biological tissues and organs that are more excellent in both bio-functionality and biocompatibility than the conventional artificial organs.

Biodegradable polymers are required to fabricate scaffolds for cell proliferation and differentiation which result in tissue regeneration or construction. Biodegradable polymers are necessary also for a sustained release of growth factors at the location of tissue regeneration. Generally, scaffolds used in tissue engineering are porous and three-dimensional to encourage infiltration of a large number of cells into the scaffolds. Currently, the polymers used for scaffolding include collagen, glycolide-lactide copolymers, other copolymers of lactide, and crosslinked polysaccharides [36].

3.2. Ecological applications:

3.2.1. Classification of Ecological Plastics:

Biodegradable ecological plastics are defined as polymers that maintain mechanical strength and other material performances similar to conventional non-biodegradable plastics during their practical use but are finally degraded to low-molecular-weight compounds such as H_2O and CO_2 and non-toxic by-products by microorganisms living in the earth environments after their use. Therefore, the most remarkable feature of ecological plastics is their biodegradability [37].

In the pre-primary stage of ecological plastics, natural polymers, especially polysaccharides were promising candidates for biodegradable polymers. They included starch, chitin, cellulose and mucopolysaccharides; but nowadays not much attention is paid to these polysaccharides except the cellulose and its derivatives because of their low processability in molding. Although, chemically substituted, grafted and blended starch and cellulose have been intensively taken in account and studied to improve processability and physical properties. For example, cellulose acetate has been proven to be a thermoplastic and exhibit good barrier properties to grease and oil though chemical substitution of cellulose is well known to slow down its biodegradation, while starch-poly(vinyl alcohol) (PVA) blend has been investigated for replacement of low density polyethylene (LDPE) and polystyrene (PS).

Among the biodegradable polymers that have been most intensively investigated are aliphatic polyesters of both natural and synthetic origins [38-40].

The synthesis of poly(α-hydroxyacid)s such as polyglycolide or poly(glycolic acid) is carried out by direct condensation polymerization of HO-R-COOH or ring-opening polymerization of

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[R-CO-O-R-CO-O] ıtion results in high- molecular-weight polymers while former generally yıelds oılgomers [41]. Poly(hydroxyalkanoate)s (PHA) are biosynthesized by microorganisms such as *Bacillus megaterium* using starch from corn and potato as raw materials, while poly(x-hydroxyalkanoate)s are synthesized by ring-opening polymerization of lactones. Poly(alkylene dicarboxylate) s are generally produced by condensation of prepolymers having hydroxyl or carboxyl terminal groups using chain extenders such as diisocyanate. Direct condensation polymerization between low-molecular-weight HO-R_1-OH and HOOC-R_2-COOH generally produces only low-molecular-weight polymers [42-43].

3.2.2. Processing of plastic wastes:

The other major application of biodegradable polymers is in plastic industries to replace bio-stable plastics for maintaining our earth environments clean. The first choice for processing of plastic wastes is reuse, but only some plastic products can be re-used after adequate processing and many of them are very difficult to recycle. In these cases, wastes are processed by landfill or incineration, but these processes often pollute the environments. If biodegradation by-products do not exert adverse effects on animals and plants on the earth than biodegradable plastics can be regarded as environment-friendly or ecological materials.

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Therefore, much attention has been focused on manufacturing biodegradable plastics which, however, should have several requirements.

- ✓ They are to be low in product cost,
- ✓ Satisfactory in mechanical properties and
- ✓ Not harmful to animals and plants when biodegraded.

The biodegradation kinetics is also an important issue of biodegradable plastics.
Expected applications of biodegradable polymers in plastic industries are listed in Table 3[32]

Table 3: Ecological applications of biodegradable polymers.

Application	Fields	Examples
Industrial applications	Agriculture, Forestry	Mulch films, Temporary replanting pots, Delivery system for fertilizers and pesticides
	Fisheries	Fishing lines and nets, Fishhooks, Fishing gears
	Civil engineering and construction industry	Forms, Vegetation nets and sheets, Water retention sheets
	Outdoor sports	Golf tees, Disposable plates, cups, bags, and cutlery
Composting	Food package	Package, Containers, Wrappings, Bottles, Bags, and Films, Retail bags, Six-pack rings
	Toiletry	Diapers, Feminine hygiene products
	Daily necessities	Refuge bags, Cups

The applications cover a wide range of industries including agriculture, fishery, civil engineering, construction, out- door leisure, food, toiletry, cosmetics, and other consumer products. It is possible that the waste left as a result of outdoor activity and sports will stay for a long time in natural environments, possibly damaging them. On the other hand, when plastics are used indoors as food containers that are difficult to separate from the food remaining after use, the waste can be utilized as compostable if it is biodegradable.

3.2.3. Physical properties of ecological plastics:

Biodegradable polymers can be divided into two groups, that is, polyethylene (**PE**)-like and poly (ethylene terephthalate) (**PET**)-like polymers. The biodegradable polymers with a relatively large number of methylene groups and planar zigzag structure in a molecule are PE-like, including poly(ε-caprolactone) and poly(butylenes succinate) (PBS), while PET-like polymers such as poly(3-hydroxybutyrate) (PHB) and poly(L-lactide) (PLLA) have helix structures and bulky side-chains.
However, the elongation-at-break of PHB and PLLA, tensile testing is much lower than that of PET, resulting in low toughness and poor impact strength. This means that some modifications, for instance, copolymerization, blending, or addition, are essential for a large industrial production of these biodegradable polymers as real ecological plastics [42-43].
Another disadvantage of biodegradable polymers is their low crystallization temperature, which lowers the crystallization rate. This property brings about low processability when fibres are manufactured from these polymers.
Table 4 shows the moisture barrier, oxygen barrier, and mechanical properties of some representative biodegradable polymers. Evidently, physical properties as well as the cost of these polymers depend on their chemical and physical structures.

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Table 4: Moisture barrier, oxygen barrier & mechanical properties of representative biodegradable polymers [39].

Materials	Moisture barrier	Oxygen barrier	Mechanical Properties
Collagen	Poor	Good	Moderate
Gelatin	Poor	Good	NA
High Amylose Starch	Poor	Moderate	Moderate
Methyl Cellulose	Moderate	Moderate	Moderate
Cellulose Acetate	Moderate	Poor	Moderate
Starch/PVA	Poor	Good	Good
P(3HB-4HV)	Good	Good	Moderate
PLA	Moderate	Poor	Good

3.2.4. Biodegradability:

Similar to biodegradation of cellulose and chitin by *cellulase* and *chitinase*, aliphatic polyesters undergo enzymatic degradation. *Esterases* are the enzymes responsible for hydrolytic degradation of aliphatic polyesters [44]. As this enzymatic reaction is of heterogeneous type, hydrolytic enzyme molecules first adsorb on the surface of substrate polymers through the binding site of enzyme molecules. Then, the active site of the enzyme comes into direct contact with the ester bond of the substrate molecule.

Different activities of different hydrolytic enzymes for the same substrate polymer may be due to different binding capacities of the enzymes to the substrate, as there is no large difference in the hydrolytic activity among enzymes. The enzymes excreted from microorganisms may hydrolyze polymers to low-molecular weight compounds which will serve as a source of nutrients to the mother microorganisms [45-47].

An important group of *esterases* for biodegradation of aliphatic polyesters are lipases 40, 48]. These enzymes are known to hydrolyze triacylglycerols (fat) to fatty acid and gycerol. It seems probable that lipase can hydrolyze aliphatic polyesters in contrast with aromatic polyesters, because the flexibility of the main-chain and the hydrophilicity of aliphatic polyesters are so high to allow intimate contact between the polyester chain and the active site of lipases in marked contrast with the rigid main chain and hydrophobicity of aromatic polyesters.

The biodegradability of polyesters is investigated in terms of the hydrophilic/hydrophobic balance of polyester molecules, since their balance seems to be crucial for the enzyme binding to the substrate and the subsequent hydrolytic action of the enzyme. Interestingly, lipases are not able to hydrolyze polyesters having an optically active carbon such as PHB and PLLA [49].

The hydrolysis of PHA is catalyzed by *PHA depolymerise* which has a sequence of -Asn-Ala-Trp-Ala-Gly-Ser-Asn-Ala-Gly-Lys- as the active center [46]. It is reported that PHB is hydrolyzed by *PHA depolymerase* more quickly than a copolymer of 3-hydrolxybutyrate (3HB) and 3-hydroxyvalerate (3HV) [P(3HB-3HV)] but more slowly than the copolymer of 3HB and 4-hydroxyvalerate (4HV) [P(3HB-4HV)] [50]. This difference may be due to bulkiness of the side-chain of PHA which hinders the enzymatic attack on the ester bond of PHA through a steric hindrance effect.

Both *lipases* and *PHA depolymerase* are enzymes of the endo-type which break bonds randomly along the main-chain of the substrate polymer, in contrast to enzymes of the exo-type which attack zipper-like the bonds at the end of the main-chain [51].

The hydrolysis rate of films prepared from copolymers of butylene succinate (BS) and ethylene succinate (ES) by *lipase* from *Phycomyces nitensas* a function of the BS content in the copolymers were studied and recorded by Mochizuki *et al* (1997) and it seems that the

enzymatic hydrolysis of the copolymers greatly depends on the chemical composition. However, the more direct factor influencing the hydrolysis is not the chemical composition but the crystallinity of the copolymer films, since there is a linear correlation between the hydrolysis rate and the crystallinity of the films, where the film crystallinity is plotted against the chemical composition of the films [52].

Table 5: Physical properties of PGA, PLLA, PDLLA, and PCL

Parameter	PGA	PLLA	PDLLA	PCL
T_m/ ˚C	225–230	170–190	–	60
T_m^0 a)/ ˚C	–	200–215	–	71, 79
T_g / ˚C	40	50–60	50–60	–60
ΔH_m (xc = 100%)/(J/g)	180–207	93	–	142
Density/(g/cm^3)	1.50–1.69	1.25–1.29	1.27	1.06–1.13
Solubility parameter (25 ˚C)/(J/cm^3)$^{0.5}$	–8	22.7.	21.1	20
$[a]^D_{25}$ in chloroform	–	–155 l 1	0	0
WVTR[b]/(g/m^2/day)	–	82–172	–	177
σB[c]/(kg/mm^2)	8–100[d]	12–230[d]	4–5[e]	10–80[d]
E[f])/(kg/mm^2)	400–1400[d]	700–1000[d]	150–190[e]	–
ε_B[g]/%	30–40[d]	12–26[d]	5–10[e]	20–120[d]

a) Equilibrium melting temperature. b) Water vapor transmission rate at 25˚C. c) Tensile strength.
d) Oriented fiber. e) Non-oriented film. f) Young's modulus. g) Elongation-at-break.

There is a group of polymers that is used for both medical and ecological applications. Among them are PLLA (poly(L-lactide)) and PCL (Poly(ε-caprolactone)). Both aliphatic polyesters are synthesized by ring opening polymerization. PLLA is degraded non-enzymatically in both earth environments and the human body, while PCL is enzymatically degraded in earth environments, but non-enzymatically in the body [53-56].

There are debates on the future potential of PLLA and PHA. Some researchers think that PHA will dominate PLLA in the future when plants modified with gene technology will become capable of producing PHA on a large scale, while others say that ring-opening polymerization in chemical industries is more controllable and produces a larger amount of polymer than biosynthesis in the outdoor field. It seems too early to give a conclusion on this issue, although it is clear that the most important influential factor is the production cost of these polymers, and this is a complex issue depending on many factors.

4. Biodegradable Polymers for Protein and Peptide Drug Delivery:

Over the past decade developments in the field of biotechnology have led to the cloning, characterization, and commercial availability of many clinically useful proteins and peptides. While the technology exists for the discovery and development of these molecules, several challenges need to be solved with regard to their delivery in convenient, controlled release, and targeted formulations. In contrast to conventional synthetic pharmaceuticals, proteins are large molecular weight polypeptides which are susceptible to proteolysis, chemical modification, and denaturation during storage and administration [57-58].

The most convenient route for the systemic delivery of pharmaceuticals is oral; however, attempts to deliver large molecular weight proteins and peptides orally have not been widely successful. Bioavailability via this route is poor for molecules of molecular mass greater than

several hundred daltons. In addition, proteins are susceptible to hydrolysis and modification at gastric pH levels and can be degraded by proteolytic enzymes in the small intestine [59]. Parenteral delivery of proteins and peptides has been the method of choice for systemic delivery due to ease of administration, the avoidance of biological barriers through which it is difficult for proteins to pass, and the ability to achieve pharmacologic levels of circulating protein over a relatively short period of time. In addition to parenteral administration, interest has increased in the area of local delivery of proteins to mucosal tissues of the gut, sinus, and lungs by both oral and inhalation delivery systems [60-62]. In these applications, proteins must be administered in formulations which protect against proteolysis and target the mucosal tissues. Recently, there has been interest in the use of degradable polymer systems for controlled release of protein vaccines to be administered, either via the parenteral route or targeted to mucosal tissues [63-67].

The use of degradable microspheres that contain protein vaccines can potentially reduce the number of inoculations, reduce the total antigen dose required to achieve immune protection, and enhance the immune response [68-69]. Site-specific delivery of proteins to topical wounds and bony defects through the use of degradable polymer delivery systems has also been reported. The primary interest for degradable polymers in drug delivery has been in controlled release systems [70-72].

Polymers have also been widely investigated for use in protein-polymer conjugates. Degradable polymeric drug delivery systems have several advantages compared to conventional drug therapies. These include improved patient compliance, avoidance of the peaks and valleys of drug plasma levels associated with conventional injections, localized delivery of the drug to a particular body compartment or cell type, thereby lowering the systemic drug level, protection of drugs that are rapidly degraded in the body, and improved drug efficacy. The obvious advantage of biodegradable polymers for drug delivery over non-degradable systems is that they do not have to be removed from the patient.

There are many different types of biodegradable polymers that can potentially be used in the preparation of protein delivery systems. They include both naturally derived and synthetic materials (Table 6). The development of biodegradable polymers for drug delivery has been largely empirical; that is, few polymers have been developed specifically for the purpose of drug delivery.

A case in point is the widespread use of poly(1actic-coglycolic acid) (PLGA) homo- and copolymers for the preparation of degradable microspheres. These polymers were first used in the production of biodegradable sutures and later found to have properties desirable for controlled release devices. The degradation characteristics of PLGA and the elimination of the breakdown products are well documented [73-75].

4.1. Biocompatibility of Polymeric Systems:

Polymers used as drug delivery systems for protein pharmaceuticals need to exhibit “biocompatible” characteristics in terms of both the polymer’s effect on the organism receiving the drug delivery system and the polymer’s effect on the protein to be delivered. The polymer itself, which consists of a repeating monomeric species, may potentially be antigenic [76-77], carcinogenic [78-79], or toxic [80-81] or have some inherent incompatibility with organisms. The shape of an implanted material has been implicated in its biocompatibility as well, smooth surfaces being less irritating and more biocompatible than rough surfaces [82]. A key factor which influences the biocompatibility of an implanted polymer is the presence of low molecular weight extractables, or unreacted residual monomers and polymerization initiators 83].

4.2. Protein Pharmaceuticals and Protein Stability Issues:

Many low molecular weight drugs have been successfully incorporated into degradable polymeric delivery systems and released in an active form. Larger molecular weight proteins,

however, behave quite differently in such systems. Serum albumin is among the most well studied of proteins in the development of drug delivery systems [84-85]. However, the properties of serum albumin do not in general mimic those of specific protein pharmaceuticals. Therefore, the extension of the results achieved with low molecular weight drugs or serum albumin to other types of high molecular weight protein pharmaceuticals is limited at best.

Table 6: Natural, Derived & Synthetic Biodegradable Polymers utilized in Protein drug delivery.

Polymer	Protein delivered and reference
Naturally Derived	
Albumin	Insulin [192], urokinase [193], YIGSR [221], gp120 peptide[*222*], IIF-2 [223], growth hormone [224], SOD [225], CD4 [226]
Alginate	Albumin [85], TGF- β_1 [178], bFGF [179], TNF receptor [180], angiogenesis factor [181], EGF [181], urogastrone [181], NGF [182]
Cellulose derivatives	TGF–β_1 [169-171], aFGF [172]
Collagen	IL-2 [183,184], NGF [185], insulin [186], EGF [187], TGF-β_1 [188]
Gelatin	IFN$_\alpha$ [189, 227], insulin [190], albumin [191], IFN$_\gamma$ [191], GM-CSF [191], SOD [228], IL-lα [229].
Hyaluronic acid	Insulin [177], NGF [176].
Polysaccharides	IFN$_\alpha$ (194), albumin [195], lysozyme [195], immunoglobulin G [195], carbonic anhydrase [195].
Synthetic	
Maleic anhydride-alkyl vinyl ether copolymers	IFN$_\alpha$ [168], HSA [168].
Pluronic polyols	BSA [123], IL-2 [159-160], urease [161], natriuretic factor [162].
Poly(acry1ic acid)	EGF [99].
Poly(cyanoacrylates)	Insulin [134], growth hormone-releasing factor [135-136], calcitonin [137].
Poly(amino acids)	Antibody [216]
Poly(anhydrides)	Insulin [145-146], myoglobin [145,146], lysozyme [147], trypsin [147], heparinase [147], ovalbumin [147], albumin [147], immunoglobulin [147].
Poly(esters): Poly(1actic acid) (PLA)	HSA [87], insulin [110], LHRH [112], albumin [121] , BSA [123,128], bone morphogenetic protein [129].
Poly(1actic-co-glycolic acid) (PLGA)	Carbonic anhydrase [88], IL-2 [96], G-CSF [97], insulin [109], LHRH [111], LHRH analogs *[74,100,113*-116], BSA [117-118, 128], diphtheria toxoid [120], calcitonin [122], cytochrome c [124], myoglobin [124], somatrotropin [124], albumin [124], TGF-β_1 [197].
Poly(ethy1ene glycol)	IL-2 [96, 206, 207, 209], G-CSF [97], BSA [128,167], bone morphogenetic protein [129], immunoglobulin *[210]*.
Poly((hydroxypropy1) methacrylamide)	Transferrin [203], antibodies [200, 201, 203].
Poly(orth0 esters)	LHRH analog [151], insulin [152, 153], lysozyme [154].
Poly(viny1 alcohol)	Cytochrome c [124], myoglobin [124], somatrotropin [124], albumin [124], BSA[163-165].
Poly(vinylpyrro1idone)	Chymotrypsin [166], BSA [167].

Interactions between proteins and polymeric materials appear to be protein and polymer specific. At issue are the following: (i) the protein molecular weight, which is an important parameter with regard to diffusion characteristics, (ii) the isoelectric point (P^I) of the protein (and polymer as well in some cases), which governs chargecharge interactions (protein-polymer and proteinprotein), (iii) the presence of cysteines on the protein which may participate in the formation of intermolecular (i.e., protein-polymer) disulfide bonds, (iv) the primary amino acid sequence of the protein which may be rendered susceptible to chemical modification in association with a polymeric material (e.g., β-elimination, or other modification), (v) the presence or absence of carbohydrates on the protein, which may enhance or prevent interaction with polymeric materials and affect the protein's hydrodynamic volume, (vi) the relative hydrophobicity of a protein which could interact with hydrophobic sites on a polymer, and (vii) the heterogeneity of protein pharmaceuticals, which often exists for proteins produced by recombinant methods. While a certain degree of pre-evaluation is feasible, each type of delivery system needs to be tested independently with each protein of interest in order to evaluate the specific protein-polymer interactions involved with each particular protein-polymer pair. These interactions, as well as the rates of biodegradation of the polymeric system, will ultimately influence the protein release rate and the overall condition of the released protein.

There are several challenges in the development of drug delivery systems with regard to maintaining the integrity and activity of incorporated proteins. First, in the process of preparing drug delivery systems, proteins may be exposed to extreme stresses. Necessary manufacturing steps may include excessive exposure of the protein to heat, shear forces, pH extremes, organic solvents, freezing, and drying, to name a few. Following manufacture or preparation, the drug delivery systems must be stored for some extended period of time prior to administration. While many studies have described the storage stability of lyophilized or liquid formulations of proteins, relatively little information is available on the subject of long term stability of proteins within biodegradable drug delivery systems. Next, when biodegradable polymer drug delivery systems are administered, the incorporated proteins may become hydrated at relatively high concentrations for prolonged periods of time. Proteins in this type of environment are susceptible to denaturation and aggregation [86]. Also, when a polymer begins to degrade following administration, a highly concentrated microenvironment is created from the released protein and polymer breakdown byproducts in andaround the microspheres (such as acidic monomers).

Proteins may be susceptible to aggregation, hydrolytic degradation, and/or chemical modification in such an environment. Finally, proteins may undergo reversible or irreversible adsorption to the polymers used to fabricate degradable delivery systems, which can affect the drug delivery rate and ultimately lead to denaturation, aggregation, and inactivation of the protein. Protein adsorption to polymeric materials has been widely studied in the area of polymeric implants; however, the deleterious effects of protein adsorption are no less significant for protein drug delivery applications. In a specific example, human serum albumin was shown to undergo a multilayer adsorption to PLA nanospheres [87]. Furthermore, some of the albumin was found to be irreversibly adsorbed to this material.

Several approaches have been taken to stabilize proteins and reduce denaturation in polymeric delivery systems. These include the following: (i) the addition of stabilizing additives to prevent protein aggregation or adsorption to the polymer's surface [86, 88-89], (ii) the addition of excipients to increase hydration of the system and enhance both protein diffusion and polymer degradation [90-91] and (iii) the modification of the protein or the polymer with water-soluble polymers to prevent protein aggregation [92] and/or adsorption [93-94].

Protein modification with PEG has been demonstrated with two proteins in PLGA delivery systems: interleukin-2 (IL-2) [95] and granulocyte colony-stimulating factor (G-CSF) [96]. In

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both cases, the unmodified protein exhibited a poor release profile, and much of the protein remained trapped within the polymer after several weeks of incubation in solution. The poor release was attributed to difficulty in resolubilization of the encapsulated protein. The PEG-modified proteins, however, were released much more readily form the systems, probably due to increased protein solubility, decreased aggregation, and decreased protein adsorption to the polymeric surfaces.

4.3. Sterilization:

Sterilization, although issues of sterilization of drug delivery systems are rarely discussed in the literature, many challenging problems in this area need to be solved. Several approaches which are routinely applied to the sterilization of polymers or implantable polymeric devices are ethylene oxide gas, steam, sub-micron filtration in organic solvents, or y-irradiation. These methods, however, are not generally applicable to proteins. Proteins can be denatured by ethylene oxide gas, by exposure to organic solvents, and by temperatures required for steam sterilization (121 ˚C). In addition, proteins may undergo severe aggregation and degradation following exposure to y-irradiation. Conversely, typical sterilization methods for protein pharmaceuticals such as filtration through sub-micron filters in aqueous solution would not be applicable to polymeric drug delivery systems which may be greater than 100 pm in diameter, be water insoluble, or undergo hydrolysis on exposure to water.

Therefore, methods of sterilization of polymeric drug delivery systems must be tailored to each individual product. For parenteral systems, all components must be sterile filtered in solution prior to formation of the delivery system. This involves filtration of both an aqueous protein solution and often an organic polymer solution. Once the individual components are filtered, an aseptic process must be employed during fabrication of the delivery system. The use of clean rooms and validation of an aseptic manufacturing process can add considerable cost to a parenteral controlled release product. The existing marketed peptide delivery products, Lupron Depot (Takada Abbott) and Zoladex (ICI), are manufactured under aseptic conditions. Both of these products release peptide analogs of luteinizing hormone releasing hormone (LHRH) for 1 month. Decapeptyl (Ipsen Biotech), another LHRH delivery system, is terminally sterilized by y-irradiation since the incorporated peptide was found to be resistant to degradation by this treatment.

4.4. In-vitro Vs In-vivo Analysis Comparisons:

Many excellent studies have been published which describe the in vitro release of proteins from degradable delivery systems. These in vitro studies are essential for determining the reproducibility of a system's release kinetics and the integrity of the released protein. In vitro release kinetics, however, often do not mimic in vivo performance of the system. Many polymers, for example, may degrade faster in the body than in a test tube due to the presence of proteolytic enzymes. Makino et al. have reported that the degradation rate of PLA microcapsules in aqueous solution was accelerated by the addition of albumin, y-globulins, and fibrinogen [97]. They showed that these proteins adsorbed to the polymer surface and also increased the polymer solubility. On the other hand, certain polymers can become encapsulated with fibrotic tissue and adsorb proteins from serum or interstitial fluid in vivo, which results in a slower release. In vitro hydration of a polymer may occur more rapidly than in vivo, particularly in hydrogel systems, resulting in a faster in vitro release. In an attempt to more closely mimic in vivo release conditions, in vitro release studies have been performed in more physiological solutions such as serum or fetal calf serum [87]; however, in vivo data must always be included in a complete characterization of a new degradable drug delivery system.

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4.5.Polymers for Controlled Release:

4.5.1. Polymer Degradation Mechanisms:

Schematic representation of different polymer degradation mechanisms are shown in fig.7: (1) Hydrolysis of the polymer backbone may occur via acid, base, or enzymatic mechanisims. The degradation byproducts are of low molecular weight and are generally water soluble, which allows the embedded protein or peptide to be released. (2) Hydrolysis of a cross-linked polymer network is catalyzed via acid, base, or metal ion chelator, or enzymatically. Cross-links may be made with divalent cations (sodium alginate), or with a divalent activated chain such as glutaraldehyde or methylenebis-(acry1amide). Broken cross-links allow protein or peptide release. (3) Hydration of a polymer matrix allows for diffusion of proteins or peptides. In some examples (e.g., esterified hyaluronic acid), solubilization occurs through hydrolysis of a hydrophobic side chain, resulting in a main-chain molecule which is hydrophilic and will solubilise in water.

4.5.2. Delivery System Morphologies and Release:

Mechanisms: Biodegradable protein and peptide delivery systems can be fabricated in a variety of morphologies. These systems can be classified as reservoir or monolithic matrix devices [72, 98]. In a biodegradable reservoir system a core of drug is surrounded by a polymer coating. In a monolithic matrix system, the drug is uniformly distributed throughout the solid polymer. The majority of systems discussed below are of the monolithic matrix system type. Microspheres and nanospheres are one of the most desirable types of parenteral delivery systems since they can be administered by a routine injection with a narrow gauge needle. Larger systems such as cylindrical implants have also been fabricated which require injection through a trochar or surgical implantation. Polymeric gels are another type of system that can be administered by injection but can suffer drawbacks related to local inflammation if organic solvents are used to solubilise the polymer. Gels have also been studied extensively for the topical administration of proteins for applications such as wound healing, in particular, the administration of epidermal growth factor (EGF) for dermal wounds [99].

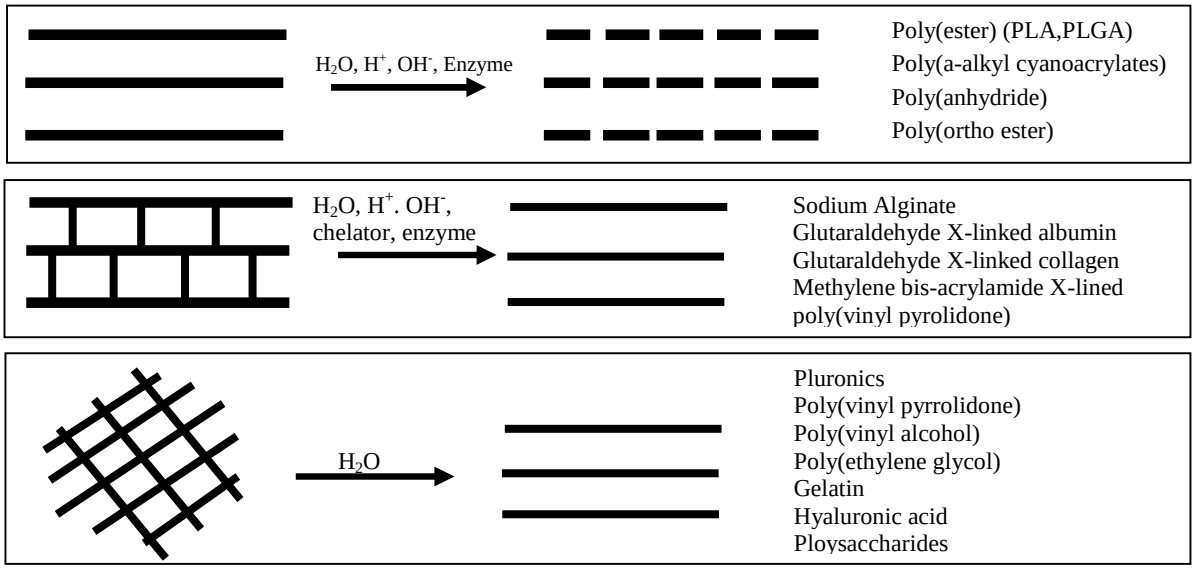

Fig. 7: Schematic representation of different polymer degradation mechanisms.

The release of a protein from a degradable delivery system can be governed by several mechanisms: (i) pure drug diffusion through the polymer matrix (diffusion controlled), (ii) degradation of the polymer (erosion controlled), or (iii) counter-current diffusion of aqueous medium into the polymer (swelling controlled). These classifications are useful for

understanding a given delivery system and in the development of mathematical models to describe in vitro drug release. However, many biodegradable polymer-protein delivery systems are very complex, and the release of the drug is often due to a combination of mechanisms. For example, in a degradable PLGA microsphere system, release of a protein is often initially controlled by desorption of protein from the surface of the PLGA microsphere, followed by diffusion of the protein through porous channels in the polymer matrix which in turn is influenced by the swelling rate of the system. At later times, the polymer begins to degrade, and a combined erosioddiffusion-controlled release mechanism occurs. In addition, the physical state of a polymer can change as it degrades, which can further complicate the release kinetics. Park has reported that water hydration in PLGA microspheres allowed the polymer morphology to change from a glassy to a rubbery state by lowering the glass transition temperature. This in turn led to a faster degradation rate[100].

It would be difficult to describe all of the degradable polymeric protein and peptide delivery systems that have been reported over the past decade. This review will focus on some of the more significant reports in the published literature in an attempt to provide a broader overview of the different types of systems that have been developed.

4.5.3. Bulk Erosion Polymers:

4.5.3.1. Poly(1actic-co-glycolic acid) (PLGA) Copolymers.

The use of PLGA copolymers for the controlled release of proteins and peptides is widely described in the literature [101-104]. These polymers have been used successfully for several decades in biodegradable sutures and more recently as drug delivery microcarriers, and as a result much is known about their biocompatibility and physicochemical characteristics [105-107]. PLGA copolymers are well suited for use in delivery systems since they can be fabricated into a variety of morphologies including films, rods, microspheres, and nanospheres by solvent casting, compression molding, or solvent evaporation techniques. PLGA copolymers are prepared by polycondensation reactions with lactic and glycolic acids [108]. On exposure to water, PLGA undergoes random chain scission by simple hydrolysis of the ester bond linkage (Figure 8). Devices made from PLGA copolymers undergo bulk erosion as compared to surface erosion.

The chemical composition and ratio of monomers used in the polycondensation reaction strongly influence the degradation characteristics of the copolymer, and thus drug release kinetics as well. The degradation rates for PLGA (which range from weeks to months under physiologic conditions) have been shown to be influenced by factors which affect polymer chain packing (i.e. crystallinity) and hydrophilicity. Since PLGA degradation is catalyzed by hydrolysis, a crystalline or hydrophobic polymer composition disfavors dissolution and degradation and slows drug release kinetics.

One of the first studies describing the delivery of a protein from PLGA microcapsules was reported by Chang in 1976 [109]. Insulin was incorporated into the delivery system, and its release rate was varied from 50% in 5 h to 2.5% in 24 h. A more detailed study was reported 10 years later in which both in vitro and in vivo release of insulin was demonstrated from pellets and microspheres made from poly(1actic acid) (PLA) [110]. A pore-release model was used to describe the mechanism of insulin release from both microbeads and pellets.

The desired clinical effect is therefore one of downregulation, and a well-defined delivery pattern is not necessary as long as a sufficient quantity of drug is provided. Since these drugs were originally administered by injection one or more times daily, the development of a once monthly delivery system was desirable from a patient compliance point of view. An initial burst effect of these peptides was not a problem due to their low toxicity. The peptides are also relatively stable compounds that can be incorporated into polymeric devices with minimal loss of bioactivity. Finally, the PLGA polymers used in the devices required minimal toxicological testing.

Zoladex is a cylindrical implant approximately 1 mm in diameter and 3-6 mm in length [111]. The device is made from a 50:50 PLGA copolymer and contains 3.6 mg of drug which is homogeneously dispersed throughout the matrix. After subdermal injection in the abdominal wall, the drug is released over 28 days. Release of the peptide is initially controlled by a dissolutioddiffusion mechanism from polypeptide domains at or near the surface of the device. At later times, the degradation of the polymer leads to the generation of microporosity and enhanced water uptake by the system, which ultimately results in further release of the drug. A second generation product designed to continuously deliver LHRH for 3 months has recently been described [112]. After screening several PLA and PLGA polymers, it was determined that microspheres prepared from PLA with a molecular weight of 15 000 that contained 12% LHRH by weight gave the most desirable release profile.

The absence of water-soluble oligomers (less than 0.1%) in the polymer was important for reducing the initial burst of drug. The system was proven to be pharmacologically active in rats and provided linear sustained release and persistent serum levels of LHRH for over 3 months.

There are a number of additional reports that describe the use of PLGA microcapsules for the delivery of LHRH analogs [74,113-115]. Microspheres containing the analog nafarelin were prepared by a coacervation technique, and the release of peptide was described as triphasic. In the first phase the drug was released by diffusion from the surface of the spheres. The second phase exhibited a slow or negligible release rate. The third phase was characterized by a more significant release of the drug and was attributed to degradation of the PLGA matrix. By selection of the appropriate polymer molecular weight and copolymer composition, the second phase of low peptide release could be minimized. Another PLGA delivery system for a LHRH analog was prepared by a hot-press technique [116].

A water/oil/water emulsion preparation technique that was similar to the process used to manufacture Lupron Depot has been used to incorporate the model proteins bovine serum albumin (BSA) and horseradish peroxidise into PLGA microspheres [117]. More than 90% incorporation efficiency was achieved, and different in vitro release rates were obtained by modifying factors in the preparation procedure such as mixing rate and the volume of inner water and organic phases. A more recent report by Sah et al. describes the modification of BSA release kinetics from PLGA microspheres by the blending of different molecular weight polymers prior to preparation of the delivery system [118]. Zero- or first-order release kinetics could be achieved by using a combination of a high molecular weight PLGA (75:25) and a low molecular weight PLA 2000. The porosity, degree of water uptake, and degradation rate of the microspheres could also be varied by changing the polymer composition.

The incorporation of several protein antigens in PLGA microspheres for vaccine delivery has also been reported for tetanus [119] and diphtheria toxoid [120]. These systems were capable of inducing an immune response in mice that was comparable to conventional multidose injections.

Cylindrical monolithic matrix release devices were made by extruding an albumin suspension in a PLA acetone solution, to form rods [121]. The rods were then coated with pure poly (D, L-lactide) and cut into different lengths. Release of the albumin from the short cylinders (0.5-1 cm) was primarily diffusion controlled. Release of the protein from longer devices (2-4 cm) was controlled by a combination of diffusion and osmotic pressure. The duration of release could range from 200 to 800 h depending of the loading and length of the device. The tendency for some proteins to adsorb to PLGA polymers has been used in the development of a microsphere delivery system for salmon calcitonin [122].

4.5.3.2. PLGA Polymer blends:

One approach that has been used to modify the release of proteins from PLGA and PLA delivery systems is to blend together several different types of polymers. In one study, films

containing bovine serum albumin were prepared from blends of pluronic polyols and poly (L-lactic acid) (Fig 9) [123]. The addition of the nonionic pluronics to the system resulted in films with different phase-separated morphologies and different degrees of hydration. When used as drug releasing matrices, these blends extended protein release and minimized the initial protein burst compared to the pure polymer.

Films based on blends of poly(viny1 alcohol) (PVA) (Fig 9) with PLGA have been prepared with incorporated cytochrome c, myoglobin, somatotropin, or albumin [124].

4.5.3.3. Block Copolymers of PEG, and Lactic and Glycolic Acid:

Copolymers of PEG and PLA have been synthesized for use in delivery systems [125-126]. The net result is a biodegradable polymer with a reduced amount of hydrophobicity that is an inherent property of PLA systems. These copolymer systems can be composed of:

(i) random blocks of the two polymers,
(ii) two blocks in which case the molecules are amphiphilic, or
(iii) triblocks in which hydrophilic microphases are present [127].

Proteins which are incorporated into devices made from these copolymers are less likely to adsorb to the delivery system through hydrophobic interactions. A study by Youxin describes the release of BSA from ABA triblock copolymers consisting of PLA or PLGA A-blocks attached to central PEG B-blocks [128]. The polymers were shown to swell very rapidly due to microphase separation, and degradation occurred over 2-3 weeks. Microspheres containing BSA were prepared from the copolymers. Continuous release was attained when the A-blocks were made from PLPG, while pulsatile release was observed with A-blocks made from PLA. In another study, PLAPEG block copolymers were used as a delivery system for bone morphogenetic protein (BMP) [129]. The copolymer consisted of a PLA segment with a molecular weight of 650 and a PEG segment with a molecular weight of 200. The copolymer containing the BMP was an injectable viscous semiliquid. When implanted under the fasciae of the dorsal muscles of mice, the composites were completely absorbed and replaced by newly induced bone with hematopoietic marrow. The composites induced twice as much bone as composites of BMP and a 650 dalton homopolymer.

The amphiphilic nature of the PLA-PEG two block copolymer systems has also been used to create nanoparticulate carriers using a two-phase oil-in-water emulsion system [130]. Block copolymers of PIA-PEG were used to surface coat PLGA nanospheres [131]. The result was an increase in surface hydrophilicity and decrease in surface charge of the nanospheres. A PEG chain length of 2000 daltons was shown to provide an effective repulsive barrier to albumin adsorption. In vivo clearance studies in a rat model showed that the PIA-PEG-coated PLGA nanospheres had a dramatically increased blood circulation time and decreased hepatic uptake as compared to uncoated PLGA nanospheres.

4.5.3.4. Poly(cyanoacrylates):

Poly(cyanoacry1ates) have gain much of attention to be used as delivery systems for proteins and peptides because they undergo spontaneous polymerization at room temperature in the presence of water, their erosion has been controlled by the length of the monomer chain and the pH [132]. Once formed, the polymer is slowly hydrolyzed, leading to a chain scission and liberation of formaldehyde (Fig 10). While the polymers are not toxic, the formaldehyde released as the degradation byproduct does create a toxicity concern [133]. A nanocapsule delivery system for insulin was prepared by the interfacial emulsion polymerization of alkyl cyanoacrylates [134]. The nanospheres had an average diameter of 220 nm and were capable of sustaining the release of insulin when administered either subcutaneously or orally. Nanospheres made from poly(isohexy1 cyanoacrylate) were shown to deliver growth hormone releasing factor in a rat model for 24 h after subcutaneous injection [135-136]. This was a significant improvement compared to the injection of free drug which was undetectable after 100 min. Release of the protein from the nanoparticles resulted from degradation of the

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polymeric matrix and was not due to passive diffusion of peptide through the polymer. Detailed autoradiography studies and transmission electron microscopy analysis showed that the particles containing radiolabeled polymer remained intact at the site of injection for at least 24 h. Poly(isobuty1 cyanoacrylate) nanoparticles have also been used as a sustained release system for calcitonin [137].

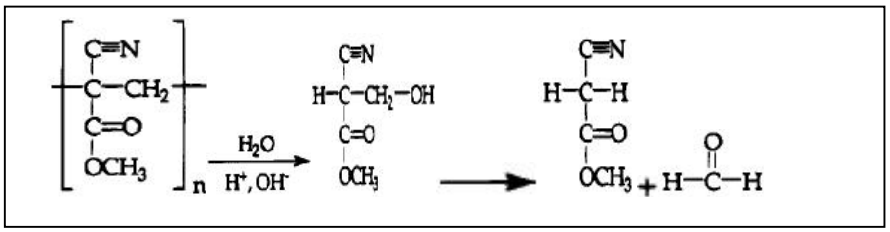

Fig. 8: Chemical formula for poly(lactic-co-glycolic acid) PLGA. The hydrolysis of PLGA is catalyzed by water and is accelerated by the presence of acid or base, the degradation byproducts of PLGA are lactic and glycolic acid.

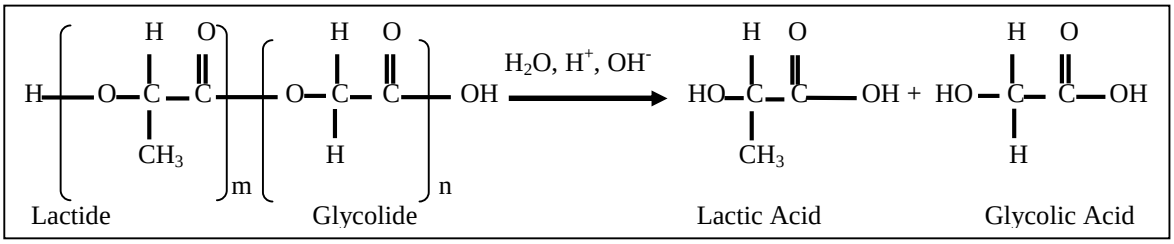

Fig. 9: Chemical Structure of three common hydrophilic polymers used in degradable drug delivery systems.

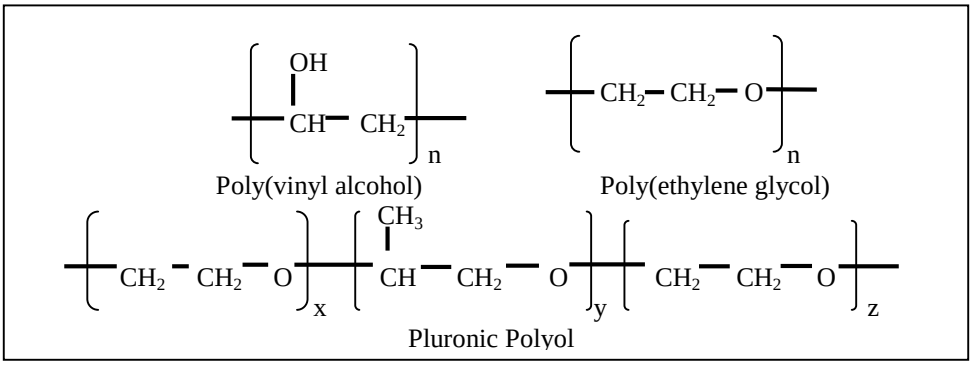

Fig. 10: The hydrolytic degradation of poly(cyanoacrylate).

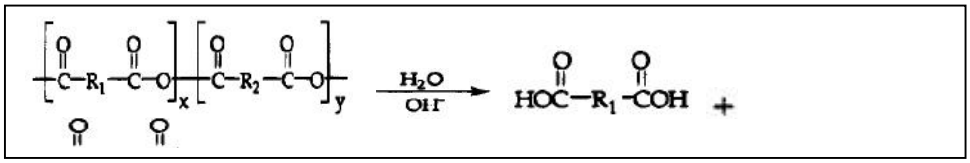

Fig. 11: The hydrolytic degradation of poly(anhydrides).

4.5.4. Surface Erosion Polymers:

4.5.4.1. Poly(anhydrides):

Poly(anhydrides) were developed for drug delivery applications by Langer and collaborators, represent a class of surface eroding polymers [138-140]. Tamada reviews the different types of polyanhydrides with a detailed description of their erosion kinetics [141] Hydrolysis of the anhydride bond is suppressed by acid, which results in an inhibition of bulk erosion by the acidity of the carboxylic acid products of the polymer hydrolysis process [142]. By varying the ratio of the hydrophobic component 1, 3-bis(p-carboxyphenoxy)pro-pane and sebacic acid, degradation rates ranging from days to years can be achieved [143].

Poly(anhydrides) can be fabricated into delivery systems by injection molding or compression molding. Injection molding requires higher than ambient temperatures and can result in a reaction of the amine groups on a drug with the anhydride linkage, thus making this technique difficult to implement with easily denatured proteins [144]. Several proteins have been successfully incorporated into, and released, from poly-(anhydride) delivery systems. The incorporation of insulin and myoglobin has successfully been achieved in poly(anhydride) microspheres using both a hot-melt microencapsulation technique [145] or microencapsulation by solvent removal [146]. A recent report describes the incorporation of several proteins into poly(anhydride) microspheres including lysozyme, trypsin, heparinase, ovalbumin, albumin, and immunoglobulin [147]. The microspheres were prepared by a solvent evaporation technique method using a double emulsion. All proteins were released at a near-constant rate for more than 25 days without any large initial burst, irrespective of the polymer molecular weight and protein loading.

4.5.4.2. Poly(ortho esters):

Poly(ortho esters) are another example of surface-eroding polymers that have been developed for drug delivery systems [148]. Hydrolysis of the ortho ester group is acid-catalyzed [149]. One particular type of poly(ortho ester) is made of 2,2- dialkoxytetrahydrofuran, 1,6-hexanediol, and 1,4-cyclohexanedimethanol. Upon hydrolysis, the acidic byproduct hydroxybutyric acid is released, which causes an increasing erosion rate of the system over time. Basic additives such as $Mg(OH)_2$ can be included in the delivery system to suppress bulk hydrolysis and enhance surface erosion. Conversely, the inclusion of an acidic species such as 9, l0-dihydroxystearic acid can be used to increase the rate of surface erosion [150]. The rate of the poly-(ortho ester) surface erosion can also be controlled by the hydrophobicity of the polymer and the cross-link density. An increase in the cross-linking can also reduce the diffusional release of drug.

Several proteins and peptides have been incorporated into poly(ortho ester) delivery systems including the LHRH analog nafarelin , insulin and lysozyme. In the latter system the polymer was prepared by a trans-esterification reaction between a triol and an alkyl orthoacetate to produce a viscous ointment at room temperature. Protein incorporation into this system was accomplished by simple mixing at room temperature without solvents [151-154].

4.5.5. Hydrogel Systems:

The use of biodegradable hydrogels as delivery systems for proteins is of particular interest due to their biocompatability and their relative inertness toward protein drugs [155-156]. Hydrogels are the only class of polymer that can enable a protein to permeate through the continuum of the carrier. The initial release rate of proteins from biodegradable hydrogels is therefore generally diffusion controlled through the aqueous channels of the gel and is inversely proportional to the molecular weight of the protein. Once polymer degradation occurs, and if protein still remains in the hydrogel, erosion-controlled release may contribute to the system. Several disadvantages must

be considered when using a biodegradable hydrogel system for the release of proteins. Their ability to rapidly swell with water can lead to very fast release rates and polymer degradation rates. In addition, hydrogels can rapidly decrease in mechanical strength upon swelling with water.

Biodegradable hydrogels have been prepared from natural or synthetic polymers [157]. Three examples of polymers commonly used to prepare hydrogels are shown in Fig 9. Formation of the hydrogels can be achieved by both chemical and physical means. Chemically crosslinked gels are prepared by polymerization of monomers by chemical cross-linking of water-soluble polymers. Upon hydrolysis of the cross-links, the polymer becomes water soluble and is eliminated from the body. Hydrogels formed by physical means contain polymers that are associated through extended junction zones. These associations can be created by a simple entanglement of polymer chains or by interactions between hydrophobic or crystalline regions of the polymer or may be ionic in nature. Many water-soluble polymers form hydrogels by simple entanglement, particularly, naturally occurring polymers such as hyaluronic acid. The synthesis of block copolymers or the blending of two different polymers has been used to create physical hydrogels with a wide variation in physical and mechanical properties. Several polysaccharide systems such as alginates and pectin will form hydrogels upon the introduction of counterions.

4.5.5.1. Pluronic Polyols:

Pluronic polyols or polyoxamers are block copolymers of poly(ethy1ene oxide) and poly-(propylene oxide) (Fig 9). One particular polymer, pluronic F127, has been used extensively as a gel forming polymer matrix to deliver proteins. Pluronic F127 consists by weight of approximately 70% ethylene oxide and 30% propylene oxide, with an average molecular weight of 11 500. The polymer exhibits a reversible thermal gelation in aqueous solution at concentrations of 20% or more [158]. Thus, a solution of this polymer is liquid at room temperature, but rapidly gels in the body. Although this polymer is not metabolized by the body, the gels do slowly dissolve over time and the polymer is eventually cleared. As a result of its good biocompatibility and nondenaturing effects on proteins, pluronic F127 gels have been used as delivery systems for several proteins including IL-2 [129-130], urease [131] and rat intestinal natriuretic factor [132]. These systems are easily administered by subcutaneous injection and generally release the protein over a period of 1-2 days.

4.5.5.2. Poly(vinyl alcohol):

Poly(viny1 alcohol) (PVA) is another polymer that can be made into a hydrogel that degrades by solubilization (Fig 9). Bovine serum albumin was incorporated into PVA discs, and release of the drug was studied ***in vitro*** [163]. The initial release of the drug was attributed to diffusion of drug through water-filled pores near the surface of the polymer matrix. As the polymer swelled, structural changes occurred in the polymer and diffusion of the protein occurred through both the hydrated polymer matrix and the water-filled pores.

Physically cross-linked PVA gels have been prepared by a freeze-thawing process which causes structural densification of the hydrogel due to the formation of semicrystalline structures [164-165]. When BSA was incorporated into these gels, the release was essentially complete within **50** h and was controlled by a pure diffusional mechanism.

4.5.5.3. Poly(viny1pyrrolidone):

The earliest studies reporting on the incorporation of chymotrypsin in poly(viny1pyrrolidone) (PVP) gels is use of a chemically cross-linked biodegradable hydrogel as a protein delivery system [166]. The PVP was cross-linked with N, N'-methylenebis(acrylamide). Hydrolysis of the

cross-linking agent resulted in the production of formaldehyde and degradation of the hydrogel. Degradation of this type of hydrogel was very sensitive to the concentration of cross-linking agent. More than 1-2% cross-linking agent produced an essentially noneroding hydrogel. Once the cross-link density fell below a critical value of 1% the hydrogel began to dissolve and the enzyme was released by diffusion from the gel. The cross-link density therefore controlled both the rate of hydrogel solubilization and the release rate of the chymotrypsin. Gels with too high a cross-link density remained insoluble, and only a fraction of the chymotrypsin was released. Gels with too low of a cross-link density, on the other hand, completely dissolved. Because of their high porosity, however, the protein underwent a rapid diffusional release within a time of **2-3** days, and kinetics was difficult to control.

Heller et al. were fabricate a degradable hydrogel system by first preparing a prepolymer of PEG and either fumaric acid, ketomaionic acid, ketoglutaric acid, or diglycolic acid. The prepolymers were then crosslinked to PVP by copolymerization. When BSA was entrapped in microspheres of these gels, the duration of ***in vitro*** zero-order release could be varied between 10 days up to 7 weeks by the choice of ester structure and the amount of vinylpyrrolidone cross-links [167].

4.5.5.4. Maleic Anhydride-Alkyl Vinyl Ether Copolymers:

Maleic anhydride-alkyl vinyl ether copolymers have been used to fabricate polymeric films containing a-interferon (IFNα) [168].These devices were designed as ophthalmic implants.

4.5.5.5. Cellulose:

Methylcellulose gels have been effectively used for the site-specific delivery of several proteins. Beck et al. used **3%** methylcellulose gels to deliver transforming growth factor-P1 (TGF-Pl) both to topical skin wounds [169-170] and to bone defects [171]. In both cases, the protein in the gel showed a significant enhancement in the healing of skin wounds or bone defects when compared to protein that was applied to the site in a saline buffer solution.

A 1% (hydroxyethyl)cellulose gel was used to incorporate acidic fibroblast growth factor (aFGF) for a wound healing formulation [172]. The addition of heparin to the gel in a 3:l ratio with aFGF was found to be necessary for maintaining full biological activity and conformational stability of the growth factor. ***In vitro,*** aFGF was release from the gel over 24 h. Application of the gel to fullthickness wounds in diabetic mice was found to accelerate healing when compared to a phosphate buffer control.

4.5.5.6. Hyaluronic Acid Derivatives:

Hyaluronic acid derivatives are a good example of naturally occurring polymers that have been modified to control the degradation and release rates. Hyaluronic acid is a naturally occurring mucopolysaccharide consisting of residues of D-glucuronic acid and N-acetyl-D-glucosamine in an unbranched chain. The polymer has an average molecular weight of **(5-6)** $x10^6$ and exhibits excellent biocompatibility. Both chemical cross-linking and derivatization of hyaluronic acid have been used to enhance the rheological properties or increase the degradation time [173-175].

This system has also been used as a delivery device for nerve growth factor (NGF) & microspheres prepared from hyaluronic acid esters were used for the nasal delivery of insulin [176-177].

4.5.5.7. Alginate:

Alginate is a linear polysaccharide that is extracted from red-brown seaweed. It contains the repeating units of 1,4-linked a-L-guluronic acid and P-Dmannuronic acid. In the presence of divalent cations such as calcium, sodium alginate spontaneously forms a hydrogel matrix. Cross-linking occurs through the guluronic acid residues. Ionically cross-linked alginate gels have been

used to incorporate several different proteins for controlled release applications including TGF-β_1 [178], basic fibroblast growth factor (bFGF) [179], tumor necrosis factor receptor (TNFR) [180], and angiogenic molecules such as angiogenesis factor, epidermal growth factor (EGF), and urogastrone [181]. Because alginate is an anionic polymer at pH 7.4, proteins with a net positive charge can ionically bond to the polymer and thus exhibit reduced bioactivity upon incorporation into an alginate delivery system. TGF-β_1, with a pI of 9.8, is one example of a protein with a net positive charge at physiologic pH. When incorporated into alginate beads, ^{125}I-labeled TGF- β_1 was not released when the beads were incubated in 0.1 N HCl [178]. The protein did release when the beads were incubated in phosphate-buffered saline, pH 7.4. Furthermore, when the released TGF- β_1 was assayed by ELISA, little binding of the monoclonal antibody to the protein occurred. The addition of poly(acry1ic acid) to the alginate bead was shown to prevent the inactivation of TGF- β_1 by the alginate.

Proteins have been successfully delivered from alginate beads in several ***in vivo*** models. NGF was incorporated into poly(L-lysine)-coated alginate microspheres. When implanted in the cerebral cortex of rats that had received a cortical lesion, the neural degeneration in these animals was decreased when compared to the controls [182]. The alginate system was found to be much more efficient than intravenous delivery at depositing bFGF within the arterial wall.

4.5.5.8. Collagen:

The majority of collagen-based systems are in the form of either implantable devices or injectable gels. Several researchers have demonstrated the release of proteins from collagen matrices. One concern with collagen is its potential for causing an immunogenic response in the patient. Atelocollagen has been used in order to decrease the potential immunogenicity of collagen [183]. This material is collagen that has been subjected to protease treatment to remove the telopeptides.

Fujiwara et al., incorporated **IL-2** into a collagen pellet that was prepared by homogeneously mixing an aqueous solution of atelocollagen with the protein to obtain a uniform gel mixture [184-185]. The gel mixture was then subjected to molding and then drying to produce cylindrical pellets 1 mm in diameter and 10 cm long. The pellets were implanted subcutaneously in mice containing solid tumors and were found to have a significant effect in the inhibition of tumor growth.

A similar collagen system was used to continuously deliver NGF to the hippocampus of gerbils**.** The NGF was colyophilized with human serum albumin prior to mixing with the collagen. The delivery of NGF was able to prevent neuronal cell damage, and NGF concentrations in the hippocampus were shown to remain high in for **5** days as determined by an enzyme immunoassay [186].

Collagen monolithic devices varying in cross-link density, collagen structure, and type of cross-linking agent were fabricated for the controlled release of the model macromolecule inulin [186]. ***In vitro*** release rates were linear with the square root of time, indicating a diffusioncontrolled system. Collagen gels have been reported to effectively deliver epidermal growth factor (EGF) [187] and TGF-β_1 [188] to experimentally induced wounds in a mouse model. In both cases, the growth factors were shown to accelerate wound healing.

4.5.5.9. Gelatin:

A gelatin-based microsphere delivery system containing IFNα was prepared by sonication of an aqueous solution of the drug and gelatin in toluene and chloroform that contained the surfactant span 80 [189]. The gelatin was then cross-linked with glutaraldehyde. ***In vitro*** degradation of this

system was observed with the addition of collagenase and was inversely proportional to the cross-linking density. A potential problem with this system is the use of glutaraldehyde as a nonspecific cross-linking agent which can potentially bond to both the collagen and the interferon, thus inactivating some of the drug.

Gelatin-based films originally designed as a wound dressing material have been used to deliver ^{125}I-labeled insulin [190]. The film adhered to open wounds but was permeable to body fluids. Insulin was released ***in vitro*** for **4** days. Incorporation of collagen into the release solution resulted in a significant increase in the insulin release rate.

Investigators have utilized the process of complex coacervation to prepare microspheres containing albumin, γ-interferon, and granulocyte macrophage colony-stimulating factor (GM-CSF) [191]. The system relies on the spontaneous phase separation process that occurs when oppositely charged polyelectrolytes are mixed in an aqueous medium.

4.5.5.10. Albumin:

Albumin microspheres were developed as an injectable degradable system for the delivery of insulin [192]. Insulin crystals were suspended in a phosphate buffer solution that contained bovine serum albumin. While the suspension was stirred rapidly in a mixture of petroleum ether and corn oil to form a waterin-oil emulsion, cross-linking of the spheres was initiated by the addition of **2.5%** or **5%** glutaraldehyde. Microspheres ranging in diameter from **50** to 1000 pm were obtained. A sustained release of bioactive insulin in rats was obtained over for more than 60 days. Albumin microspheres have also been prepared that contained covalently immobilized urokinase using glutaraldehyde chemistry [193].

4.5.5.11. Starches and Dextrans:

Cross-linked polysaccharide microparticles have been used by several groups as protein delivery systems. In one study, recombinant mouse IFNα was covalently coupled to polyacryl starch microspheres using carbonyldiimidazole chemistry [194]. The bound IFNα was found to activate cultured macrophages for nitrite production and had an anti-leishmania1 effect in mice. Low doses of IFNα, which had no effect in the free form, when bound to microparticles significantly reduced the load of ***Leishmania donovani*** in infected mice. Other biodegradable polysaccharides have been used to deliver the model proteins, albumin, lysozyme, immunoglobulin G, and carbonic anhydrase [195]. The polysaccharides maltodextrin or hydroxyethyl starch was derivatized with acrylic acid glycidyl ester.

The protein polymer solution was then polymerized in a water-in-oil emulsion. Proteins were released from the microspheres over a 12 week period. Polyacryldextran microspheres containing several different proteins were prepared using a similar polysaccharide derivatization system [196]. The heat stability of carbonic anhydrase was improved when incorporated into the microspheres, and degradation was enhanced in the presence of dextranase.

4.5.6. Composite Systems:

The combination of synthetic polymers with natural materials is another approach that has been taken in the development of protein delivery systems. In these systems the polymer can afford mechanical strength while the natural material affords protein stability. As mentioned above, the Lupron Depot microspheres contain PLGA, gelatin, and the drug LHRH. In another study, the protein, TGF-PI, was first absorbed onto demineralized bone matrix (DBM) [197]. This material is a bone derivative that is prepared by demineralizing cadaver bone with HCl and is comprised of more than 90% collagen along with small amounts of lipids, proteins, and proteoglycans. **A** colyophilized preparation of the protein and the DBM was then incorporated into a PLGA matrix

and fabricated into **2** mm thick discs which were designed to stimulate bone growth. In *vitro* release studies demonstrated that the released TGF-β_1, retained between 80% and 90% of its bioactivity.

Duncan and Kopecek have described a unique composite hydrophilic gel comprised of hydroxypropyl methacrylamide copolymers which are cross-linked via a degradable oligopeptide [198]. Fluorescent labeled dextrans of different molecular weights were incorporated into the gels. The rate of release was found to depend mainly on the equilibrium degree of swelling and not on the structure of the cross-links. However, the degradation of the gels by a mixture of lysosomal enzymes or chymotrypsin was dependent on both swelling and crosslink structure (length of the oligopeptide and type of amino acid residues).

In another example of composite systems Saffran and co-workers have described a novel system for oral delivery of insulin and vasopressin to the colon. Their work takes advantage of the fact that certain azopolymers are resistant to degradation by proteolytic enzymes in the stomach; however, on passage to the colon these polymers may be cleared by bacterial reductases.

4.6. Protein-Polymer Conjugates:

4.6.1. Potential Applications and Challenges with Conjugates:

In the preceding sections biodegradable protein drug delivery systems were described where proteins were noncovalently embedded or contained within degradable matrices. Proteins may also be delivered in the form of covalent conjugates with water-soluble, biodegradable polymers. Modifications of proteins via conjugation with polymers have been envisioned and investigated for a variety of purposes. Ringsdorf first described protein-polymer conjugates as a means to create “pharmacologically active polymers” [199]. In his model a biodegradable or biostable polymer chain serves as a backbone carrier for at least three different species. First, the pharmaceutical agent (or protein) is linked to the polymer via stable or degradable linkages. Also conjugated onto the polymer or designed into the monomer repeat units of the polymer itself may be a solubilizer to enhance the water or lipid solubility of the conjugate. Finally, a homing device such as an antibody [200-201], a carbohydrate [202], a receptor binding ligand [203], or simply an electrically charged species [204] may be conjugated onto the polymer backbone to assist in targeting specific tissues or regions of the body.

There are many potential advantages to forming protein-polymer conjugates as drug delivery systems. Among these is the ability to alter the circulation pharmacokinetics of the protein-polymer conjugate. The kidney glomerular membrane serves to clear small circulating molecules (less than 70 kDa) by filtration. Conjugation of low molecular weight proteins with watersoluble polymers effectively increases their hydrodynamic radius, thereby reducing renal clearance. Systematic studies investigating proteins conjugated with noncationic polymers of various molecular weights have clearly demonstrated that circulation half-lives are increasingly prolonged when larger molecular weight polymer chains are conjugated onto these proteins [205-206].

Other potential advantages of forming protein-polymer conjugates include the reduction of antigenicity of the protein [207-208], improvement of protein solubility [209], and a reduction in the susceptibility of a protein to proteolysis [210]. Several protein-polymer conjugates are now entering the clinic for treatment of a variety of disorders [211].

Problems associated with conjugating polymers and proteins often involve inactivation or alteration of protein activity. While convenient conjugation chemistries take advantage of the ε-amino group of lysine or carboxylic acid side chains, proteins which are conjugated with

polymers at these positions often suffer from inactivation or alteration of bioactivity. The extent of inactivation or alteration is protein specific, depending on the position of the conjugation sites relative to the active region of the protein molecule. For many protein-polymer conjugation schemes, a balance between the desirable effects of conjugation and the loss of bioactivity must be established.

Recently, investigators have attempted to avoid the problem of inactivation by the use of site-specific conjugation strategies. A popular technique with antibody conjugation is to utilize the carbohydrate in the hinge region, a site distant from the antigen binding domain, to safely form conjugates [212].

For the purposes of this review we will consider a protein-polymer conjugate to be biodegradable if either (i) the polymer carrier is hydrolytically or enzymatically degradable or

(ii) the chemical linkage between the protein and polymer carrier is hydrolytically or enzymatically degradable. Each of these possibilities is discussed below.

4.6.2. Conjugates where the carrier is Biodegradable:

A clear advantage for biodegradable polymeric carriers of proteins is that the carrier can be metabolized or hydrolyzed and will eventually be eliminated from the body. The importance of this attribute is underscored by the undesirable effects of the accumulation of high molecular weight poly(vinylpyrrolidone), a non-biodegradable polymer, following administration as a plasma expander [213-214]. Biodegradable polymers to be used as carriers for conjugates must be soluble in aqueous solutions. This suggests that either the polymer is inherently hydrophilic or it is of low molecular weight.

One family of polymers which has been widely reported as biodegradable carriers for synthetic pharmaceuticals are polyamino acids: polyb-lysine) [215], poly(L-glutamic acid) [216] and polyb-aspartic acid) [217]. Numerous reports of conjugating peptides to poly(amino acids) suggest that these carriers could also be useful in protein drug delivery applications; however, few reports exist in the literature. In order to be biodegradable, the monomers used in the preparation of these polymers must be of the L configuration, the **D** configuration being nonbiodegradable.

Unlike poly(ethy1ene glycol), poly(amino acids) are negatively charged at physiological pH, and this charge influences their biological behavior. For example, circulation half-lives of poly(amino acids) have been demonstrated to depend on electrical charging as well as molecular weight [218]. Another biological feature of poly(amino acids) which is most likely related to electrical charging is their propensity to serve as adjuvants for conjugated peptides and elicit an immune response [219-220]. This would clearly be an undesirable feature in many protein drug delivery applications.

Pharmacologically inactive proteins have also been utilized as carriers for other pharmacologically active proteins. In this scheme the carrier protein is chemically linked to a protein pharmaceutical. The conjugation of the two proteins together acts to increase circulation halflife and shield the protein pharmaceutical from proteolytic digestion and immunologic detection.

Albumin has been used as a pharmaceutical carrier to enhance circulation half-lives of peptides such as the laminin cell binding peptide YIGSR [221] and SP68-a 21 amino acid peptide from gp120 of the human immunodeficiency virus type ***HIV*-1 [**222]. Albumin conjugated with tumor invasion-inhibiting factor-2 (IIF-2) demonstrated a 40-60-fold reduction in the amount of peptide required to inhibit cancer cell invasion [223] and albumin conjugated with human growth factor

led to a 20-40-fold increase in stability as compared to uncoupled growth hormone [224]. A significant increase in circulation half-life, from 4 min to 6 h following intravenous administration, was achieved when albumin was conjugated with superoxide dismutase [225]. Undesirable heterogeneities which resulted from chemical conjugation techniques have led to the development of a recombinant fusion protein of albumin and CD-4 [226]. In this system a fusion protein may be produced in yeast at large scale without the heterogeneities observed with chemical coupling. Gelatin and succinyl-gelatin have also been investigated as biodegradable protein carriers for the delivery of α-interferon [227] superoxide dismutase [228], IL-lα [229] and TNF [236]. In the case of cytokine delivery (¥Jinterferon, IL-lα, and TNF) gelatin appears to enhance the performance of the conjugated proteins by binding to cells of the immune system and eliciting a mild immune reaction.

In addition to those biodegradable carriers mentioned above, investigators have conjugated proteins with molecules of DNA for use as amplification probes or reporter systems [239]. While these reports have not suggested the use of DNA as a biodegradable protein carrier, the versatile chemical properties and desirable pharmacokinetic properties of DNA [230] suggest that poly(nuc1eic acids) may be investigated for this purpose in the future.

4.6.3. Conjugates where the Protein-Polymer linkage is Biodegradable:

Polymers may be conjugated to proteins with either stable or biodegradable linkers. The purpose of biodegradable linkages may be to release a protein from a polymer in a time controlled fashion, or to release a protein in response to certain physiological conditions. In certain situations a degradable linkage may be necessary to regain the activity of a linked protein. One class of biodegradable linkages are those in which the chemical bond between the protein and polymer degrades hydrolytically. Some of the more common chemical linkages between proteins and polymers include reactions with amino acid side chains: (1) the ε–amino group of lysine and the a-amino groups of proteins (amide, thiourea, alkylamine, and urethane linkages), (2) the thiol group of free cysteine residues (thioether linkage), and **(3)** carboxylic acid groups of aspartic and glutamic acid (amide and alkylamine) [231-233]. Amide linkages generated with succinate esters such as N-hydroxysuccinimide (NHS) have been widely utilized in conjugation chemistries and are well characterized with regard to their hydrolytic instability [234].

Protein-polymer conjugates formed with succinate esters such as succinimidyl succinate have been demonstrated to degrade under physiological conditions, i.e. PBS, pH 7.4, at*37* ℃ [235-236]. Thiol conjugation chemistries are also degradable under physiological reducing conditions and have also been investigated as reversible protein-polymer linkages [237].

Another class of biodegradable linkages are those which are susceptible to enzymatic degradation. Several examples in the literature describe proteins or pharmaceuticals which are linked to polymeric carriers via short polypeptide sequences. The specific sequence and length of the peptide strongly influence the ability of specific enzymes to degrade the linkages [238-242].

However, it should be emphasized that proteins themselves are clearly susceptible to enzymatic cleavage as well. Therefore, protein-polymer conjugates designed with enzymatically degradable linkages should be designed for specific protease action within specific compartments of the body.

4.6.4. Some other examples form literature:

Alcalá-Alcalá *et al* (2013) proposed a biodegradable polymeric system for formulating peptides and proteins. The systems were assembled through the adsorption of biodegradable polymeric

nanoparticles onto porous, biodegradable microspheres by an adsorption/infiltration process with the use of an immersion method. The peptide drug is not involved in the manufacturing of the nanoparticles or in obtaining the microspheres; thus, contact with the organic solvent, interfaces, and shear forces required for the process are prevented during drug loading. Leuprolide acetate was used as the model peptide, and poly(d,l-lactide-co-glycolide) (PLGA) was used as the biodegradable polymer. Leuprolide was adsorbed onto different amounts of PLGA nanoparticles (25 mg/mL, 50 mg/mL, 75 mg/mL, and 100 mg/mL) in a first stage; then, these were infiltrated into porous PLGA microspheres (100 mg) by dipping the structures into a microsphere suspension. In this way, the leuprolide was adsorbed onto both surfaces (i.e. nanoparticles and microspheres). The adsorption efficiency and release rate are dependent on the amount of adsorbed nanoparticles. As expected, a greater adsorption efficiency (~95%) and a slower release rate were seen (~20% of released leuprolide in 12 hours) when a larger amount of nanoparticles was adsorbed (100 mg/mL of nanoparticles). Leuprolide acetate begins to be released immediately when there are no infiltrated nanoparticles, and 90% of the peptide is released in the first 12 hours. In contrast, the systems assembled in this study released less than 44% of the loaded drug during the same period of time. The observed release profiles denoted a Fickian diffusion that fit Higuchi's model ($t_{1/2}$) [243].

Mishra *et al* (2008) in review have summarized various aspects related to the formulation and processing of biodegradable polymerized microparticles/ nanoparticles for delivery of therapeutic proteins and peptides. A brief introduction of biodegradable polymers has been incorporated for reader’s benefit. In addition, biodegradable polymers based carriers designed for vaccine delivery has been incorporated in detail. Functionalized biodegradable carrier(s) for site specific delivery of proteineous matter has also been discussed [244].

Saini et al (2012) study about the rapid advances in synthesis of peptide and protein compounds for therapeutic activity; now-a-days enormous interest in developing microparticles based drug delivery using biodegradable polymers. A key factor in the design of injectable protein delivery systems is the choice of an appropriate biodegradable polymer. Biodegradable polymers, either synthetic or natural, are capable of being cleaved into biocompatible by-products through chemical or enzyme catalyzed hydrolysis. In systemic delivery of proteins; biodegradable microspheres as parenteral depot formulation occupy an important place because of several aspects like protection of sensitive proteins from degradation, prolonged or modified release/pulsatile release patterns. Biodegradable polymer microspheres delivery systems offer a lot of advantages in chemotherapy where they provided localized sustained release and reduced toxicity at the same time, since the drug can be localized by direct injection of drug-loaded-microspheres into tumor tissues, thus minimizing negative effect to the healthy tissues [245].

Li Zhang et al (2009) developed Injectable Biodegradable Polymer Depots For Minimally Invasive Delivery of Peptides and Proteins [246].

Vila *et al* studies about the major research issues in protein delivery include the stabilization of proteins in delivery devices and the design of appropriate protein carriers in order to overcome mucosal barriers. They have attempted to combine both issues through the conception of new biodegradable polymer nanoparticles: (i) poly(ethylene glycol) (PEG)-coated poly(lactic acid) (PLA) nanoparticles, chitosan (CS)-coated poly(lactic acid–glycolic acid (PLGA) nanoparticles and chitosan (CS) nanoparticles. These nanoparticles have been tested for their ability to load proteins, to deliver them in an active form, and to transport them across the nasal and intestinal mucosa. Additionally, the stability of some of these nanoparticles in simulated physiological

fluids has been studied. Results showed that the PEG coating improves the stability of PLA nanoparticles in the gastrointestinal fluids and helps the transport of the encapsulated protein, tetanus toxoids, across the intestinal and nasal mucosa. Furthermore, intranasal administration of these nanoparticles provided high and long-lasting immune responses. On the other hand, the coating of PLGA nanoparticles with the mucoadhesive polymer CS improved the stability of the particles in the presence of lysozyme and enhanced the nasal transport of the encapsulated tetanus toxoids. Moreover, these particles were very efficient in improving the nasal absorption of insulin as well as the local and systemic immune responses to tetanus toxoids by intranasal administration [247].

5. Biodegradable polymers as biomaterials:

6.

During the past two decades significant advances have been made in the development of biodegradable polymeric materials for biomedical applications. Degradable polymeric biomaterials are preferred candidates for developing therapeutic devices such as temporary prostheses, three-dimensional porous structures as scaffolds for tissue engineering and as controlled/sustained release drug delivery vehicles. Each of these applications demands materials with specific physical, chemical, biological, biomechanical and degradation properties to provide efficient therapy. Consequently, a wide range of natural or synthetic polymers capable of undergoing degradation by hydrolytic or enzymatic route are being investigated for biomedical applications.

Both synthetic polymers and biologically derived (or natural) polymers have been extensively investigated as biodegradable polymeric biomaterials. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in the polymer leading to polymer erosion [248]. Depending on the mode of degradation, polymeric biomaterials can be further classified into hydrolytically degradable polymers and enzymatically degradable polymers. Most of the naturally occurring polymers undergo enzymatic degradation.

Natural polymers can be considered as the first biodegradable biomaterials used clinically. The rate of *in vivo* degradation of enzymatically degradable polymers however, varies significantly with the site of implantation depending on the availability and concentration of the enzymes. Chemical modification of these polymers also can significantly affect their rate of degradation. Natural polymers possess several inherent advantages such as bioactivity, the ability to present receptor-binding ligands to cells, susceptibility to cell-triggered proteolytic degradation and natural remodeling. The inherent bioactivity of these natural polymers has its own downsides; these include a strong immunogenic response associated with most of the polymers, the complexities associated with their purification and the possibility of disease transmission.

Synthetic biomaterials on the other hand are generally biologically inert, they have more predictable properties and batch-to-batch uniformity and they have the unique advantage having tailored property profiles for specific applications, devoid of many of the disadvantages of natural polymers. Hydrolytically degradable polymers are generally preferred as implants due to their minimal siteto-site and patient-to-patient variations compared to enzymatically degradable polymers [248]. The successful performance of the first synthetic poly(-glycolic acid) based suture system during the late 1960s led to the design and development of a new array of biodegradable polymers as transient implants for orthopaedic and related medical applications.

6.1. Hydrolytically degradable polymers as biomaterials:
6.2.

Hydrolytically degradable polymers are polymers that have hydrolytically labile chemical bonds in their back bone. The functional groups susceptible to hydrolysis include esters, orthoesters, anhydrides, carbonates, amides, urethanes, ureas, etc [249]. Two general routes are used to develop hydrolytically sensitive polymers for biomedical applications. They are step (condensation) polymerization and addition (chain) polymerization including ring-opening polymerization. Step process is used to prepare a variety of hydrolytically sensitive polymer classes, such as polyanhydrides, poly(ortho esters) and polyurethanes. Ring opening polymerization (ROP) is an extensively investigated polymerization route to develop hydrolytically sensitive polymers, including the poly(α-esters) and polyphosphazenes. In addition, several polymers developed by microbial bioprocess are gaining significant interest as biodegradable polymers.

6.2.1. Poly(~-esters):

Poly(α-ester)s are thermoplastic polymers with hydrolytically labile aliphatic ester linkages in their backbone. The uniqueness of this class of polymers lies in its immense diversity and synthetic versatility. Poly(α-ester)s can be developed from a variety of monomers via ring opening and condensation polymerization routes depending on the monomeric units. Bacterial bioprocess routes can also be used to develop some poly(α-ester)s. Various synthetic routes for developing polyesters have been reviewed by Okada et al [250]. Among the class of poly(α-ester)s, the most extensively investigated polymers are the poly(α-hydroxy acid)s, which include poly(glycolic acid) and the stereoisomeric forms of poly(lactic acid). Several other aliphatic polyesters were developed since then as biodegradable biomaterials and are attracting significant attention as biomaterials due to their good biocompatibility and controllable degradation profiles. Figure 12 shows some of the commercially developed meniscus repair devices based on poly(α-ester)s [251].

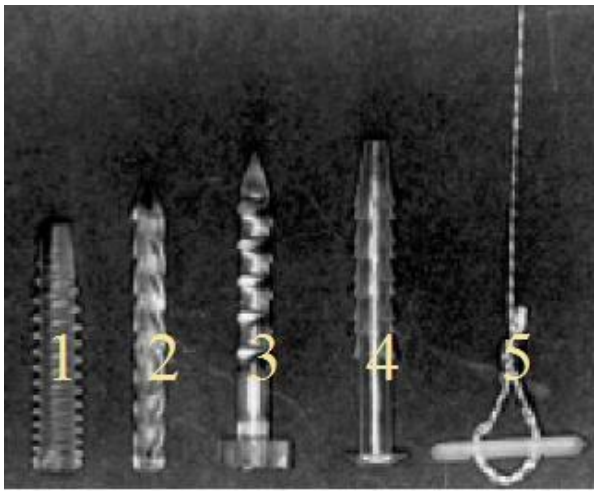

Fig.12: Some commercially developed and used poly ester-based meniscus repair devices.
1. Mitek Meniscal Repair system,
2. Clearfix Screw,
3. Arthrex Dart,
4. Bionx Meniscus Arrow,
5. Linvatec Biostinger suture.
(Source reprinted from Reference [251]).

The most extensively studied monomers for aliphatic polyester synthesis for biomedical applications are lactide, glycolide and caprolactone [252]. Another method for developing polyesters is by bacterial enzymatic poly-esterification [253]. Poly(α-ester)s mainly undergo bulk erosion i.e., the polymeric matrices degrade all over their cross-section and have erosion kinetics that are non-linear and usually characterized by a discontinuity [254]. Both homo-polymers and co-polymers of poly(α-ester)s have been investigated as potential biomaterials for a variety of biomedical applications.

6.2.1.1. Polyglycolide:

Polyglycolide can be considered as one of the first biodegradable synthetic polymer investigated for biomedical applications. Polyglycolide is a highly crystalline polymer (45–55% crystallinity) and therefore exhibits a high tensile modulus with very low solubility in organic solvents. The glass transition temperature of the polymer ranges from 35 to 40 ˚C and the melting point is greater than 200 ˚C. In spite of its low solubility, this polymer has been fabricated into a variety of forms and structures. Extrusion, injection and compression molding as well as particulate leaching and solvent casting, are some of the techniques used to develop polyglycolide-based structures for biomedical applications [255].

Due to its excellent fiber forming ability, polyglycolide was initially investigated for developing resorbable sutures. The first biodegradable synthetic suture called DEXON® that was approved by the US-FDA in 1969 was a polyglycolide.

The polymer is known to lose its strength in 1–2 months when hydrolyzed and losses mass within 6–12 months. In the body, polyglycolides are broken down into glycine which can be excreted in the urine or converted into carbon dioxide and water via the citric acid cycle [256].

6.2.1.2. Polylactides:

U1nlike glycolide, lactide is a chiral molecule and exist in two optically active forms; L-lactide and D-lactide. The polymerization of these monomers leads to the formation of semi-crystalline polymers. The polymerization of racemic (D, L)-lactide and meso-lactide, results in the formation of amorphous polymers. Among these monomers, L-lactide is the naturally occurring isomer. Similar to polyglycolide, poly(L-lactide) (PLLA) is also a crystalline polymer (~37% crystallinity) and the degree of crystallinity depends on the molecular weight and polymer processing parameters. It has a glass transition temperature of 60–65 ˚C and a melting temperature of approximately 175 ˚C [252].

Poly(L-lactide) is a slow-degrading polymer compared to polyglycolide, has good tensile strength, low extension and a high modulus (approx 4.8 GPa) , so that it has been considered an ideal biomaterial for load bearing applications, i.e. orthopaedic fixation devices. Some of the PLLA-based orthopaedic products include: the Phantom Soft Thread Soft Tissue Fixation Screw®, Phantom Suture Anchor®(DePuy), Full Thread Bio Interference Screw® (Arthrex), BioScrew®, Bio-Anchor®, Meniscal Stinger® (Linvatec), and the Clearfix Meniscal Dart® (Innovasive Devices).

PLLA can also form high strength fibres and was FDA approved in 1971 for the development of an improved suture over DEXON®. Due to the high strength of PLLA fibres, it has been investigated as scaffolding material for developing ligament replacement or augmentation devices to replace non-degradable fibres, such as Dacron [257-258].

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Polylactides undergo hydrolytic degradation via the bulk erosion mechanism by the random scission of the ester backbone. It degrades into lactic acid a normal human metabolic by-product, which is broken down into water and carbon dioxide via the citric acid cycle [256]. However, being more hydrophobic than polyglycolide, the degradation rate of PLLA is very low. It has been reported that high molecular weight PLLA can take between 2 and 5.6 years for total resorption in vivo [252 & 259].

6.2.1.3. Poly(lactide-co-glycolide):

Among the co-polyesters investigated, extensive research has been performed in developing a full range of poly(lactide-co-glycolide) polymers (PLGA). Both L- and DL-lactides have been used for co-polymerization. In the composition range of 25–75%, poly(L-lactide-co-glycolide) forms amorphous polymers. The intermediate co-polymers were found to be much more unstable compared to the homopolymers. Thus, 50/50 poly(DL-lactide-co-glycolide) degrades in approximately 1–2 months, 75/25 in 4–5 months and 85/15 in 5–6 months [260]. Different ratios of poly(lactide-co-glycolides) have been commercially developed and are being investigated for a wide range of biomedical applications. PuraSorb® PLG is a semicrystalline bioresorbable co-polymer of L-lactide and glycolide with a monomer ratio of 80L: 20G [261]. A co-polymer containing 90% glycolic acid (GA) and 10% L-lactic acid (LA) was initially used for the development of the multifilament suture Vicryl®. A modified version of the suture, Vicryl Rapid®, is currently on the market, which is an irradiated version of the suture to increase the rate of degradation. PANACRYL® is another commercially developed suture from the co-polymer with a higher LA/GA ratio in order to decrease the rate of degradation.

PLGA has been shown to undergo bulk erosion through hydrolysis of the ester bonds and the rate of degradation depends on a variety of parameters including the LA/GA ratio, molecular weight, and the shape and structure of the matrix. The major popularity of these biocompatible co-polymers can be attributed in part to their approval by the FDA for use in humans, PLGA demonstrates good cell adhesion and proliferation making it a potential candidate for tissue engineering applications. Various studies have been performed so far using micro- and nanofabrication techniques to form three-dimensional scaffolds based on PLGA [262-265]. Figure 13 shows three structures developed from PLAGA using various micro- and nano-fabrication techniques.

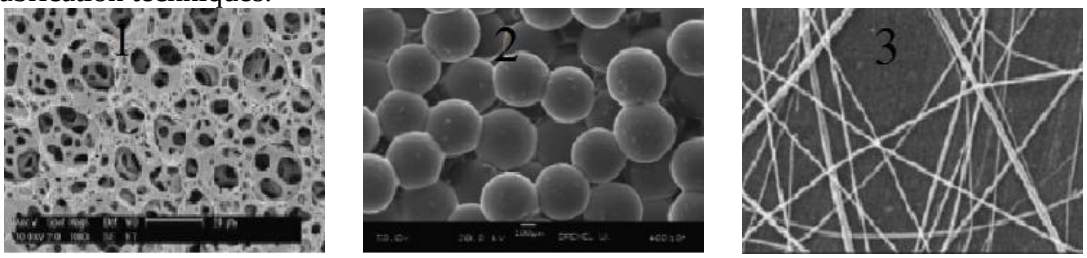

Fig.13: Porous three-dimensional structures developed from PLGA using:
1. Gas foaming (source Ref. 263),
2. Microsphere sintering (source Ref. 264) and
3. Electro-spinning (source Ref. 265).

6.2.1.4. Polydioxanone:

Multifilament sutures have a higher risk of infection associated with their use and causes a greater amount of friction when penetrating tissues. Polydioxanone (PDS) was the material of choice for

the first commercially developed monofilament suture under the trade name of PDS® in the 1980s. In addition to sutures, PDS has also been investigated for several orthopaedic applications as fixation screws for small bone and osteochondral fragments (Orthosorb Absorbable Pins®) [266]. The polymer exhibits a very low glass transition temperature ranging from -10 ˚C to 0˚C. Due to the high crystallinity and hydrophobicity of the polymer, it can be considered a slow to moderately degrading polymer. The polymer is known to lose its strength within 1–2 months and it's mass within 6–12 months by hydrolytic degradation [256].

5.1.1.5. Polycaprolactone:

Polycaprolactone (PCL) is semicrystalline polyester and is of great interest as it can be obtained by the ROP of a relatively cheap monomeric unit 'ε-caprolactone'. The PCL is highly processable as it is soluble in a wide range of organic solvents, has a low melting point (55–60 ˚C) and glass transition temperature (-60˚C) while having the ability to form miscible blends with wide range of polymers. The polymer undergoes hydrolytic degradation due to the presence of hydrolytically labile aliphatic ester linkages; however, the rate of degradation is rather slow (2–3 years). Due to the slow degradation, high permeability to many drugs and non-toxicity, PCL was initially investigated as a long-term drug/vaccine delivery vehicle. The long-term contraceptive device Capronor®, is composed of this polymer and has been developed for the long-term zero order release of levonorgestrel [267]. Due to its excellent biocompatibility, PCL has also been extensively investigated as scaffolds for tissue engineering. A study demonstrated the feasibility of using a composite matrix composed of PCL and hyaluronic acid as a potential meniscus substitute [268].

5.1.1.6. Poly(trimethylene carbonate):

High molecular weight PTMC has been investigated as a candidate implant material for soft tissue regeneration. Low molecular weight PTMC on the other hand, has been investigated as a suitable material for developing drug delivery vehicles. Unlike the previously described polyesters, PTMC undergoes surface degradation with the rate of *in vivo* degradation was found to be much higher than *in vitro* degradation. This is presumably due to the contribution of *in vivo* enzymatic degradation process [269]. The low mechanical performance of the homopolymer significantly limits its applications and consequently, several co-polymers were developed with other cyclic lactones. Thus, polyglyconates have been developed as block co-polymers of trimethylene carbonate and glycolides for use as flexible suture materials (Maxon®) and orthopaedic tacks and screws (Acufex®). BioSyn® is a terpolymer composed of glycolide, trimethylene carbonate and dioxane that has reduced stiffness and degrades within 3–4 months and has been used as suture materials.

5.1.1.7. Bacterial polyesters:

Bacterial polyesters are naturally occurring biodegradable polyesters produced by many bacteria as their energy source. The most common polymer among this class is poly(3-hydroxybutyrate) (PHB), which was discovered in 1920 as produced by the bacteria *''Bacillus megaterium''* (Fig.14a). Since then it was discovered that several other bacterial strains could produce the same polymer. PHB is a semi-crystalline isotactic polymer that undergoes surface erosion by hydrolytic cleavage of the ester bonds and has a melting temperature in the range of 160–180 ˚C [253]. The polymer shows glass transition temperature in the range of -5 to 20˚C. Both PHB and P(HB-HV) have been found to be soluble in a wide range of solvents and can be processed into different shapes and structures, such as films, sheets, spheres and fibres. Since the homopolymer PHB is a

tough, brittle polymer, the less brittle and tougher co-polymer has more potential as a biomaterial. Another unique property of P(HB-HV) is its piezoelectricity which makes it a potential candidate for orthopaedic applications since electrical stimulation is known to promote bone healing. It has also been investigated as a material for developing bone pins and plates [270].

5.1.2. Polyurethanes:

Biostable polyurethanes and poly(ether urethanes) have been extensively investigated as long term medical implants, such as cardiac pacemakers and vascular grafts due to their excellent biocompatibility and mechanical properties. Polyurethanes are generally prepared by the polycondensation reaction of diisocyanates with alcohols and/or amines [271]. Due to the toxicity of common diisocyanates such as 4, 40-methylenediphenyl diisocyanate (MDI) and toluene diisocyanate (TDI); other biocompatible aliphatic diisocyanates have been studied for the development of biodegradable polyurethanes. Lysine diisocyanate (LDI), and 1, 4-diisocyanatobutane (BDI) are a few examples. Degradable poly(ester urethanes) were developed by reacting LDI with polyester diols or triols based on D,L-lactide, caprolactone and other co-polymers having a wide range of properties [272]. In these biodegradable polyurethanes, aliphatic polyesters such as lactide/glycolide copolymers or polycaprolactones form the soft segments and polypeptides form the hard segments [273}. Another unique feature of the peptide-based polymer systems is that active moieties such as ascorbic acid and glucose can be incorporated into the polymer which could potentially promote cell adhesion, viability and proliferation without any adverse effect [274].

A biodegradable elastic poly(ester urethane) (Degrapol ®) is being used to develop highly porous scaffold for tissue engineering application [275]. A unique injectable, two component LDI-based polyurethane systems that cures in situ was recently developed for orthopaedic applications (PolyNovas). This self-setting system can be administered arthroscopically in liquid form and polymerizes at physiological temperature in situ to provide appropriate bonding strength and mechanical support comparable to or superior to widely used bone cements. This material has also been shown to promote favourable cell adhesion and proliferation [276].

5.1.3. Poly(ester amide):

Due to the hydrogen bonding ability of the amide bonds and biodegradability imparted by the ester bonds, these co-polymers have good mechanical and thermal properties. The degradation of poly(ester amides) has been shown to take place by the hydrolytic cleavage of the ester bonds, leaving the amide segments more or less intact. The good mechanical properties of poly(ester amides) derived from symmetrical bisamide-diols and succinyl chloride led to its investigation as a potential bioresorbable suture materials. Different water soluble bisamide-diols have also been prepared from glycolic acid and diaminoalkanes containing 2–12 methylene groups [277]. Attempts were also made to increase the degradation rate of poly(ester amides) by incorporating amino acid units in the polymer backbone. CAMEO® is a poly(ester amide) blend based on leucine or phenylalanine that is currently being developed for the site specific delivery of small hydrophobic drugs and peptides.

5.1.4. Poly(ortho esters):

The disadvantages of bulk eroding biodegradable polymers for use as drug delivery vehicles has led to the search for more hydrophobic polymers with hydrolytically sensitive backbones that could undergo surface erosion. Poly(ortho esters) were developed by the ALZA corporation (Alzamer®) as a hydrophobic, surface eroding polymer designed specifically for drug delivery applications. Although the ortho ester linkages are hydrolytically labile, the polymer is

hydrophobic enough such that its erosion in aqueous environments is very slow. The unique feature of poly(ortho esters) is that in addition to its surface erosion mechanism, the rate of degradation for these polymers, pH sensitivity, and glass transition temperatures can be controlled by using diols with varying levels of chain flexibility.

The pH sensitivity of the poly(ortho esters) has lead to the development of several drug delivery systems using this polymer. The rate of drug release is predominantly controlled by the rate of polymer hydrolysis through the use of acidic or basic excipients. By now four different classes of poly(ortho esters) have been developed [278].

5.1.5. Polyanhydrides:

Polyanhydrides can be considered as the most extensively investigated biodegradable surface eroding polymers specifically designed and developed for drug delivery applications. Polyanhydrides are one of the most hydrolytically labile polymers due to the highly sensitive aliphatic anhydride bonds on the polymer backbone. The hydrolytically labile backbone coupled with the hydrophobicity of the polymer precludes water penetration into the matrix allowing polyanhydrides to truly undergo surface erosion. The aliphatic polyanhydrides were developed in 1932 as a fibre forming polymer for textile applications [279]. Due to its hydrolytic instability and surface eroding nature, Langer et al., studies this class of polymers as materials for controlled drug delivery applications in 1980s [280]. In 1996, this material was approved by the US FDA, as a drug delivery vehicle following extensive in vitro and in vivo drug release and biocompatibility evaluations [248].

The most extensively investigated polyanhydrides is poly[(carboxy phenoxy propane)- (sebacic acid)] (PCPP-SA) (Fig.14 b). The polymer has been found to exhibit a zero order release of incorporated drug over periods of time ranging from days to years depending on the ratio of the co-monomers used and the molecular weight of the polymer. The degradation products of the polymers have been found to be non-toxic and biocompatible [248, 280-282]. This polymer was been approved by the US FDA for use as a localized delivery vehicle for the controlled delivery of the chemotherapeutic agent BCNU for the treatment of brain cancer (Gliadel®). A co-polymer of 1:1 sebacic acid and erucic acid dimer has been found to be useful as a potential delivery vehicle for gentamicine (Septacin®) in the treatment of osteomyelitis [283].

5.1.6. Poly(anhydride-co-imide):

Although polyanhydrides were found to be ideal candidates for drug delivery applications due to their surface eroding properties, the mechanical performance of these polymers were found to be less than optimal for load bearing applications, such as for orthopaedic implants. The Young's modulus for poly[1,6-bis(carboxyphenoxy) hexane] is only 1.3MPa, which is well below the modulus for human cancellous bone [284]. The search for high strength polyanhydrides with surface eroding properties has led to the development of poly(anhydrideco-imides) due to the imide segments in the polymer back bone imparting unusual strength. The poly(anhydride-co-imides) were found to undergo degradation via the anhydride bonds first, followed by the hydrolysis of the imide bonds [285]. Laurencin et al., have investigated the mechanical performance and biocompatibility of a wide range of poly(anhydride-co-imides), such as poly[pyromellitylimidoalanine-co-1,6-bis(p- carboxyphenoxy) hexane] (PMA ala:CPH) (Fig.14 c) as scaffolds for bone tissue engineering applications [286 -287].

5.1.7. Cross-linked polyanhydrides:

Another approach investigated to increase the mechanical strength of polyanhydrides is by incorporating acrylic groups in the monomeric units to form injectable photocrosslinkable

polyanhydrides. Injectable anhydrides can be used for filling irregularly shaped bone defects or for soft tissue repairs that require materials with a liquid or putty-like consistency, which can set and be molded into a desired shape under physiological conditions. Figure (14 d-f) shows the structure of the polymers poly(sebacic acid)(PSA), poly(1-3-bis(p-carboxyphenoxy) propane) (PCPP) and poly(1-6-bis(p-carboxy phenxoy)hexane) (PCPH). The hydrolytic degradation products of these polymers are nontoxic and composed of the corresponding diacid molecules and water-soluble linear methacrylic acid molecules. Different types of initiator-accelerator systems and energy sources have been investigated to develop crosslinkable matrices with appropriate thickness for orthopaedic applications. The most effective composition for the photopolymerization of these polymers was found to be 1.0wt% camphorquinone (CQ) and 1.0wt% ethyl-4-N,Ndimethyl aminobenzoate (EDMAB) with 150mW/cm^2 of blue light. As in the case of polyanhydrides, the mechanical strength and degradation rate of the crosslinked polyanhydrides has been found to depend on the nature of the monomeric units. A compressive strength similar to the lower range of cancellous bone (30–40Mpa) has been reported for this class of polymers [288].

5.1.8. Poly(propylene fumarate):

Another injectable biodegradable high-strength polymeric biomaterial developed for orthopaedic applications is the co-polyester poly(propylene) fumarate (PPF) (Fig. 14 g). Several routes, including the trans-esterification of fumaric diester, can be used to synthesize linear PPF. These synthetic procedures have been extensively reviewed by Peter et al [289].

Several attempts have been made to develop mechanically competent biodegradable systems for orthopaedic applications by cross-linking PPF or by developing composites using ceramic materials. Composites of PPF with ceramics, such as tricalcium phosphate or calcium sulfate, created high strength matrices (2–30MPa) suitable for orthopaedic applications [290]. These cross-linked polymer matrices also supported good cell viability and could function as a growth factor delivery system making them promising candidates for bone tissue engineering applications.

5.1.9. Pseudo poly(amino acid):

Poly(amino acid)s are ever-present, naturally-occurring biodegradable polymers; though, their application as a biomaterial has been limited due to immunogenicity and poor mechanical performances. To overcome these limitations, attempts have been made to develop pseudo amino acids composed of amino acids linked by non-amide bonds such as esters, imino-carbonates and carbonates. One of the most extensively studied system is the tyrosine-derived poly(amino acids) using desaminotyrosyl-tyrosine alkyl esters as the building blocks (Fig.14 h-k). Due to the aromatic backbone, these polymers show good engineering properties and therefore could serve as a mechanically-competent, biodegradable polymer system for load bear biomedical applications.

Tyrosine-derived polycarbonates are a versatile polymer class in which the glass transition temperatures (50-90˚C) and the mechanical properties (strength 50-70Mpa, stiffness 1-2Gpa) can be easily tailored by varying the pendant alkyl chain [291]. These polymers have been found to be amorphous, hydrophobic and undergo slow hydrolytic degradation at physiological temperature. One significant difference between PLLA and tyrosine-derived carbonates is in their water absorbtivity.

The tyrosine-derived polymers do not take up more than 5% water even at later stages of degradation and are able to maintain their shape for a longer period of time. PLLA on the other

hand swell significantly with time. Another unique advantage of tyrosine-derived carbonate is that because of their hydrophobic degradation products, the polymer experiences mass loss only at the very end of the degradation process. PLLA shows significant mass loss when the molecular weight reaches the threshold value of 20,000. The low acidity of the degradation products of tyrosine-derived carbonate compared to PLLA is another significant advantage. In a study that compared the acidic degradation products of different polymers, it was reported that poly(glycolic acid), poly(lactic acid), poly (DTE adipate) and poly(DTE carbonate) give rise to 15.5, 11.4, 6.4, and 2.6mEq of acid per gram of the polymer [292].

5.1.10. Poly(alkyl cyanoacrylates):

Poly(alkyl cyanoacrylate)s (PCA) form the major class of biodegradable acrylate polymers used for biomedical applications. Poly(alkyl cyanoacrylates) have so far been investigated as excellent synthetic surgical glue, skin adhesive and an embolic material. Poly(alkyl cyanoacrylates) can also be considered to be one of the first biodegradable polymers used for developing nanoparticles for drug delivery application (Fig. 14 l). These are neutral polymers prepared by the anionic polymerization of alkyl cyanoacrylic monomers with a trace amount of moisture as the initiator. The uniqueness of poly(cyano acrylates) is the instability of the carbon–carbon sigma bond on polymer backbone. The hydrolytic sensitivity of the backbone has been attributed to the high inductive activation of methylene hydrogen atoms by the electron withdrawing neighbouring groups.

Poly(cyano acrylates) are one of the fastest degrading polymers having degradation times ranging from few hours to few days. The degradation rate for these polymers depends on the length of the alkyl side groups. Due to the fast polymerization rate, these monomers have also been investigated as tissue adhesives. Dermabond®(2-octyl cyanoacrylate) has been approved by the US FDA as a tissue adhesive for topical skin application. Poly(alkyl cyanocrylates) have also been extensively studied for use as gene delivery vehicles and are considered to be unique matrices for the delivery of oligodeoxynucleotides (ODN) due to the unique hydrophobic interactions with ODN [293].

5.1.11. Polyphosphazenes:

In addition to organic polymers, several inorganic or inorganic–organic hybrid polymers have also been investigated as potential biodegradable biomaterials. Polyphosphazenes are hybrid polymers with a backbone of alternating phosphorus and nitrogen atoms containing two organic side groups attached to each phosphorus atom (Fig.14 m). Although polyphosphazenes were developed during late 1960s by Allcock et al [294-295], but biodegradable polyphosphazenes were developed only within the past two-three decades by the same group. Biodegradable polyphosphazenes are quite distinct from other biodegradable polymers due to its unprecedented functionality, synthetic flexibility and adaptability for various applications. The unique feature of the phosphorous-nitrogen backbone of polyphosphazenes is its unusual flexibility. Therefore, the side groups play a crucial role in determining the properties for these polymers. This allows for the possibility of designing and developing polymers with highly controlled properties such as extent of crystallinity, solubility, appropriate thermal transitions and hydrophobicity/hydrophlicity. In the case of biodegradable polyphosphazenes, the side groups control the rate of degradation for the polymers. Thus, polymers can be designed with appropriate degradation profile ranging from few hours to years by varying the side group chemistry [267].

Among the different classes of degradable polyphosphazenes investigated, poly[(amino acid ester) phosphazenes] have been met with the most success in terms of potential biomedical

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applications. Unlike polyesters, the amino acid ester polyphosphazenes undergo degradation to form neutral and non-toxic products such as phosphates, ammonia and the corresponding ester side groups. This unique property of polyphosphazenes has been recently utilized to form self-neutralizing blend systems by combining polyphosphazenes with poly(lactide-co-glycolide) [296].Many of the amino acid ester polyphosphazenes have shown excellent osteocompatibility and have been investigated as matrices for bone tissue engineering [297]. Recently a polyphosphazene-self setting calcium phosphate composite cement system has been developed by taking advantages of the favorable interactions between polyphosphazene side groups and calcium phosphate ceramics [298-301].

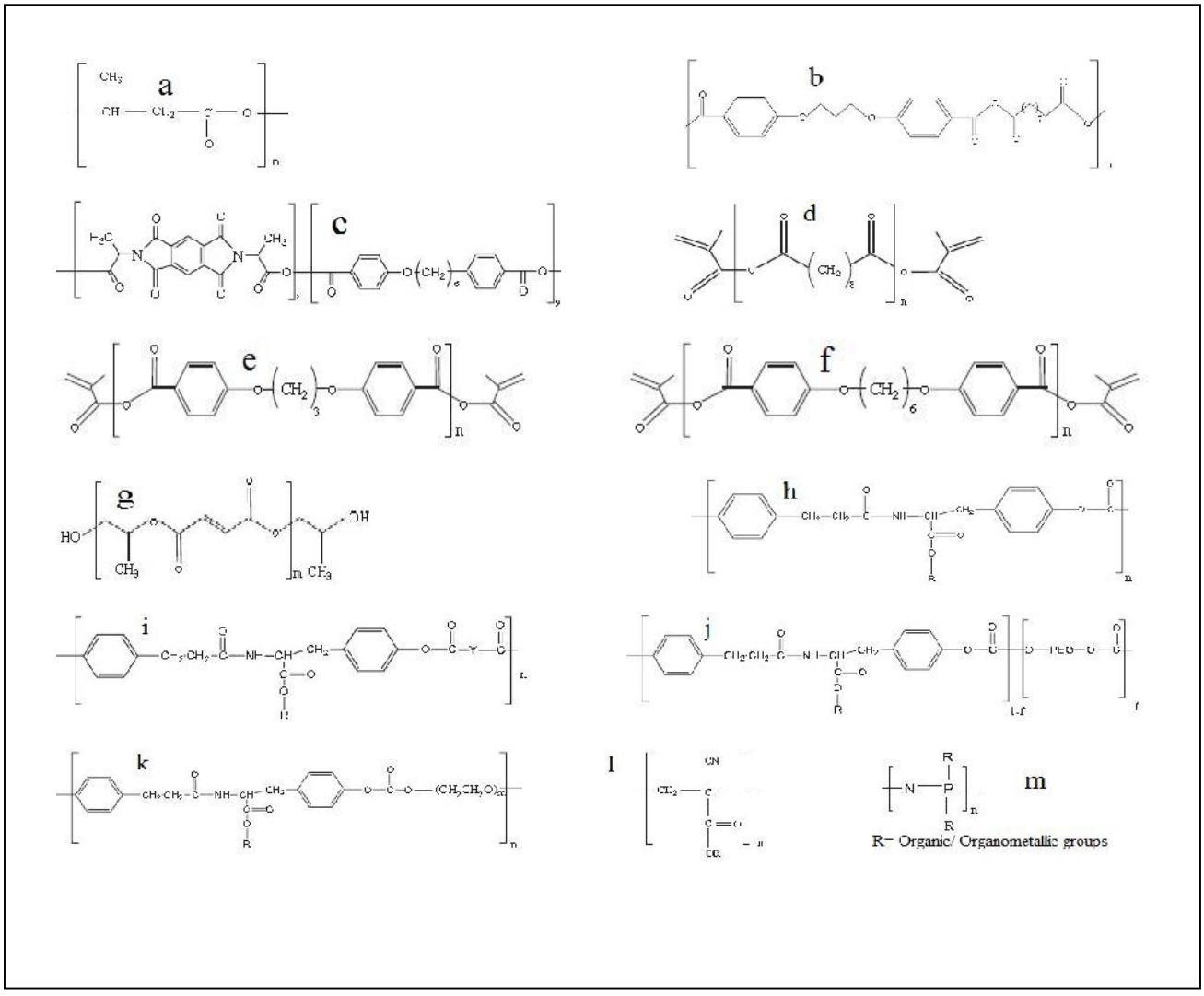

Fig.14: Structure of some commonly used polymers: **(a) poly(3-hydroxybutyrate) PHB, (b) Poly[(carboxy phenoxy propane)-(sebacic acid) P(CPP-SA), (c) Poly[pyromellitylimidoalanine-co-1,6-bis(p-carboxyphenoxy) hexane], (d) Poly(sebacic acid) (PSA), (e) Poly(1-3 bis(p-carboxyphenoxy)propane) (PCPP), (f) Poly(1-6 bis(p-carboxy phenxoy)hexane) (PCPH), (g) Polypropylene fumarate (PPF), (h) Tyrosine derived polycarbonates, (i) Tyrosine derived polyarylates, (j) Tyrosine containing poly(DTRPEG carbonate), (k) Tyrosine containing poly(DTR-PEG ether), (l) Poly(alkyl cyano acrylate) and (m) Polyphosphazene.**

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5.1.12. Polyphosphoesters:

Polyphosphoesters form another interesting class of phosphorus containing polymers developed as biomaterials. Polyphosphoesters were developed in 1970s by Penczek and his colleagues [302]. The unique property of the synthetic biodegradable polyphosphoesters is their good biocompatibility and similarity to biomacromolecules, such as nucleic acid. The pentavalency of the phosphorous atoms, as in the case of polyphosphazenes, allows for chemical linkages to be made between drugs or protein molecules and the polymer backbone, thereby enabling the development of novel polymer pro-drugs.

5.2. Enzymatically degradable polymers as biomaterials:

5.2.1. Proteins and Poly(amino acids):

Proteins the major structural components of many tissues are essentially amino acid polymers arranged in a three-dimensional folded structure and are one of the most important class of biomolecules. Being a major component of the natural tissues, proteins and other amino acid-derived polymers have been a preferred biomaterial for sutures, haemostatic agents, scaffolds for tissue engineering and drug delivery vehicles. Furthermore, protein based biomaterials are known to undergo naturally-controlled degradation processes [303].

The human body is capable of synthesizing a wide range of proteins in which the precursor molecules pass through four major stages in becoming functional proteins. The first step involves the formation of the primary structure where, a linear sequence of various amino acids is held together by peptide bonds. The constituent amino acids then participate in hydrogen bonding to form the secondary structure of protein. The linear primary structure arranges itself in the most stable structures an α-helices or β-pleated sheets. These secondary structures then join together to form three-dimensional tertiary structures which in turn interact with other protein chains to form the more refined three-dimensional quaternary structure of a multi-unit protein [304].

5.2.1.1. Collagen:

Collagen is the most abundant protein present in the human body being the major component of skin and other musculoskeletal tissues. Collagen is a rod-type polymer nearly 300 nm long with a molecular weight of 300,000. There have been more than twenty two different types of collagen identified so far in the human body, with the most common being Type I–IV. Type I collagen is the single most abundant protein present in mammals and is the most thoroughly studied protein. The Type I collagen is composed of three polypeptide subunits with similar amino acid compositions. Each polypeptide is composed of about 1050 amino acids, containing approximately 33% glycine, 25% proline and 25% hydroxyproline with a relative abundance of lysine.

The subunit chains of collagen are synthesized from free amino acids in the body and undergo transcription, translation and post-translation modification processes in appropriate cells such as fibroblasts and osteoblasts. The primary structure of these proteins is composed of repeating triplets of $(Glycine\text{-}X\text{-}Y)_n$, where X and Y are often proline and hydroxyproline. The repeating sequence is responsible for the helical structure and the inherent and predictable mechanical strength of collagen [305]. The glycine content accounts for the flexibility of the collagen chain; increased glycine gives rise to more flexibility. Ten of these polypeptide chains form the α-chain of collagen which arranges to form the right handed helical secondary structure. A left handed, triple helical tertiary collagen structure is formed from the arrangement of three secondary structures. The smallest repeating units (glycine molecules) have been found to cluster towards the inside of the triple helix [306].

Collagen undergoes enzymatic degradation within the body via enzymes, such as collagenases and metalloproteinases, to yield the corresponding amino acids. Due to their enzymatic degradability, unique physico-chemical, mechanical and biological properties collagen has been extensively investigated for biomedical applications. Collagen is mostly soluble in acidic aqueous solutions and can be processed into different forms such as sheets, tubes, sponges, foams, nanofibrous matrices, powders, fleeces, injectable viscous solutions and dispersions.

Studies have also shown that the degradation rate of collagen used for biomedical applications can be significantly altered by enzymatic pre-treatment or cross-linking using various cross-linking agents. Collagen is one of the primary initiators of the coagulation cascade and its high thrombogenicity has led to its application as a haemostatic agent. Several collagen-based hemostats are currently on the market or undergoing clinical trials for multiple surgical indications. Some of these include sealant consisting of bovine collagen and bovine thrombin (Sulzer-Spine® Tech) used for cardiovascular and spinal surgical procedures, CoStasis® Surgical Hemostat which is composed of bovine microfibrillar collagen, bovine thrombin combined with autologous plasma and Floseal®, a high viscosity gel haemostatic agent composed of collagen-derived particles and tropical bovine-derived thrombin.

An FDA approved bilayer skin substitute (Integra® dermal regeneration template), currently in the market for full thickness or deep partial thickness thermal injury, is composed of a dermal layer of crosslinked bovine collagen and glycosaminoglycan and an epidermal layer of polysiloxane [307 –308]. Other FDA approved collagen-based wound dressings are Biobrane® and Alloderm®, which are acellular collagen matrices obtained from chemically processed human cadavers.

Collagen has been extensively investigated for the localized delivery of low molecular weight drugs including antibiotics. Several collagen-based gentamicin delivery vehicles are currently on the market world-wide (Sulmycin®-Implant, Collatamp®-G). This delivery system shows a prolonged local delivery of antibiotics with very low systemic exposure. Another prolonged antibiotic collagen delivery system (Septocoll®) has been approved to prevent infection of collagen hemostatic sponge by incorporating two gentamicin salts having different solubilities [309]. Several composite systems of collagen and synthetic polymers are also currently under investigation as drug delivery devices [310].

Bioactive proteins, such as recombinant human bone morphogenic protein (rhBMP-2), were incorporated in collagen matrices to achieve sustained release of the protein due to favorable interactions of the collagen matrix with the protein [311]. In addition to its use as a protein delivery vehicle, collagen has also been investigated for gene and plasmid DNA delivery [312]. Due to the injectability of a collagen matrix, collagen has been shown to retain the gene vector/plasmid DNA and protect them from immunological or enzymatic reactions of the body. Similarly, a composite of fibrillar collagen, hydroxyapatite and tricalcium phosphate (Collagraft®) has been FDA approved for use as biodegradable synthetic bone graft substitute.

The major sources of collagen currently used for biomedical applications are bovine or porcine skin or bovine or equine Achilles tendons. One disadvantage of these collagen-based biomaterials, which is a limiting factor for the wide-spread clinical application is their mild immunogenicity imparted by the composition of the terminal region and the antigenic sites in the central helix. The immune response has been found to vary depending on the species from which collagen has been isolated, processing techniques and the site of implantation.

5.2.1.2. Natural poly(amino acids):

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Natural poly(amino acids) are biodegradable ionic polymers that differ from proteins in certain aspects. Natural poly(amino acids), such as cyanophycin, poly(ε-L-lysine) and poly-γ-glutamic acid are mainly composed of one type of amino acid. These molecules exhibit polydispersity and in addition to α–amide linkages, they exhibit other types of amide linkages that involve β- and γ-carboxylic groups as well as ε-amino groups [313]. Figure 15 (a-c) shows the structure of cyanophycin, poly(ε-L-lysine) and poly-γ-glutamic acid.

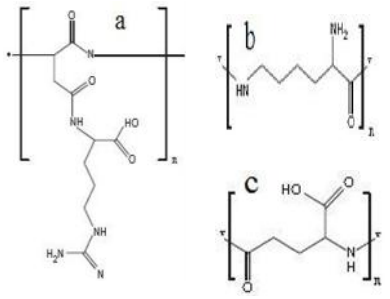

Fig 15: Structures of
(a) Cyanophycin,
(b) Poly(£-L-lysine) and
(c) Poly-¥-glutamic acid.

Poly-γ-glutamic acid (γ-PGA) is an anionic, water-soluble biodegradable homo-polyamide produced by microbial fermentation and is composed of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups. This biodegradable polymer was first isolated in 1937 by autoclaving capsules of *Bacillus anthracis* [314]. Further, several other Bacillus species were capable of secreting the polymer into culture growth medium as well as nematocysts of the eukaryotic organism *Hydra* (Hydrozoa, Cnidaria) [315-316].

Several modified forms of γ-PGA have been developed so far as drug delivery vehicles, tissue engineering scaffolds and as thermosensitive polymers. Poly(-γ-glutamic acid) benzyl ester (γ-PBG) was developed by Kishida et al. as a carrier vehicle for 5-fluorouracil; degrades very slowly in phosphate buffer (pH 7.4) and shows a diffusion-controlled release pattern [317]. Thermosensitive polymers have been shown to exhibit unique changes in their hydration properties by temperature changes and are considered to be nano-engineered intelligent materials for biomedical applications. Shimokuri et al [318], developed a thermosensitive polymer by controlled propylation of poly(-γ-glutamic acid); showed that, at appropriate levels of propyl esterification, the polymer could exhibit a hydrophobic-hydrophilic balance suitable for thermosensitivity.

Similar to to γ-PGA, poly L-lysine is of bacterial origin and is currently being investigated as scaffold material for tissue engineering and as drug delivery vehicles due to its ability to form biocompatible hydrogels [319]. Poly(L-lysine) is known to have antibacterial, antiviral and antitumour activity and is considered to be a potential candidate for developing drug carrier vehicles. However, cytotoxicity of the polymer due to the very high positive charge limits its applications.

Cyanophycin, is a comb-like polypeptide isolated from *cyanobacteria* that contains α–amino-α-carboxy- linked L-aspartic acid residues representing the poly(α-L-aspartic acid) backbone and

L-arginine residues bound to the β-carboxylic groups of aspartic acids making it a highly polydisperse polymer [320]. Though, studies on the applications of cyanophycin as a biomaterial have been limited.

5.2.1.3. Synthetic poly(amino acids):

i. Poly (L-glutamic acid):

Poly(L-glutamic acid) (L-PGA) is structurally different from γPGA and is composed of naturally occurring L-glutamic acid residues linked together through amide bonds. The triethylamine initiated polymerization of the N-carboxyanhydride (NCA) of γ-benzyl-L-glutamate is the most widely used route for synthesis of polymer [321]. Studies on developing a biosynthetic route to form monodisperse L-PGA have also been performed by expressing artificial genes encoding the polymer in bacterial strains [322]. The polymer is highly charged at physiological pH and has been identified as a unique gene/plasmid delivery vehicle. A recent study using a rodent model has shown that sodium poly-L-glutamate enhanced the expression of the reporter gene SEAP up to eight-folds after an intra-muscular injection when compared to the plasmid in saline. Using quantitative PCR analysis; the amount of plasmid retained was approximately three-fold higher for plasmid formulated with poly-L-glutamate compared to plasmids in saline after electrophoration-mediated DNA delivery [323].

The α−carboxylate side chains of L-PGA are highly reactive and can be chemically modified to introduce various bioactive ligands or to modulate the physical properties of the polymer. L-PGA has also been studied for developing polymeric drugs by conjugating anticancer drugs to the polymer backbone. The conjugation has been shown to significantly increase the aqueous solubility, plasma distribution time and tumor distribution of the drugs [321 & 324].

The high functionality of L-PGA also enabled the development of biodegradable MRI contrast agents [325]. Additionally, L-PGA has been investigated as an attractive biodegradable biological adhesive and hemostat, by chemically cross-linking gelatine [326]. L-PGA based adhesives demonstrated better soft tissue binding and hemostatic properties compared to fibrin glue in studies using animal models. Another promising bioadhesive is composed of porcine collagen and L-PGA and has been found to be superior to fibrin glue in sealing air leakage from the lungs [327].

ii. Poly(aspartic acid):

Poly(aspartic acid) (PAA) is synthesized from aspartic acid by thermal polymerization [328]. PAA is a highly water-soluble ionic polymer with a carboxylate content much higher than poly(glutamic acid) (Fig.16). PAA has also been found to undergo biodegradation by lysosomal enzymes. Several block copolymers with aspartic acid and other synthetic biodegradable polymeric moieties have been developed to form core forming micellar nanostructures for use as smart drug delivery vehicles [329]. Due to the polymer's high functionality, several chemically modified forms of PAA are also being considered as potential biomaterials. α, β– poly(N-2-hydroxyethyl)-D,L aspartamide (PHEA) is a synthetic water-soluble and biocompatible polymer extensively investigated as a plasma expander. This polymer was developed by simple aminolysis with an ethanolamine of a polysuccinimide (PSI). This polymer can also be converted

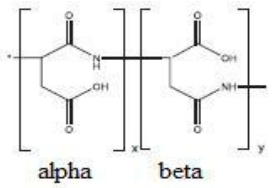

Fig. 16: Structure of Poly(aspartic acid) (PAA)

into a hydrogel by high energy radiations and is being investigated for various biomedical applications [330].

5.2.1.4. Elastin:

Elastin is a major protein component of vascular and lung tissue and is mainly responsible for the unusual elastic properties of these tissues. Elastin is a highly cross-linked insoluble polymer composed of a number of covalently bonded tropoelastin molecules. The tropoelastin molecules are produced intracellularly by smooth muscle cells and fibroblasts and are cross-linked extracellularly to form a secondary structure with β-turns. The tropoelastin is composed of several repeating sequences of the pentapeptide VPGVG, the hexapeptide APGVGV, the nonapeptide VPGFGVGAG and the tetrapeptide VPGG. Among these, the pentapeptide VPGVG recurs up to 50 times in a single molecule. In vivo biocompatibility studies have shown that elastin elicit immune response to the same extent as collagen implants. Elastin shows minimal interaction with platelets and hence has been evaluated as biological coatings for synthetic vascular grafts. To overcome the limitation of insolubility, synthetic elastin has been developed from recombinant human tropoelastin. The tropoelastin solution can be transferred to appropriate molds and allowed to coaservate and crosslink at 37 ˚C. The formed matrices were found to have good mechanical and biological properties making them promising elastic biomaterials for appropriate applications [331-333].

5.2.1.5. Elastin-like peptides:

Elastin-like polypeptides (ELP) are artificial polypeptides composed of the pentapeptide repeats (VPGXG) of human topoelastin except the fourth amino acid. The X in ELP stands for a guest residue that can be any amino acid except proline. The main properties of ELP are derived from the naturally occuring protein, elastin. ELPs have been found to have excellent biocompatibility, non-immunogenic properties and degradation products composed of natural amino acids that are non-toxic. Similar to topocollagen, ELPs also exhibit a reversible ITT. ELPs can also respond to other stimuli such as pH, ionic strength, and light by the incorporation of appropriate guest residues in the molecule at the fourth position [334]. ELPs can be recombinantly synthesized as monodisperse polymers in *E. coli* by the over expression of a synthetic gene. Due to its injectability and phase transition under mild conditions, ELP has been extensively investigated as a drug carrier vehicle [335]. The shear modulus of crosslinked ELP was found to be similar to normal cartilage and the dynamic shear modulus of the gel increased from 0.28 to 1.7 kPa after seeding with chondrocytes for 4 weeks in culture. This indicates the feasibility of remodeling ELP matrices by the deposition of functional cartilage extracellular matrix components [336].

5.2.1.6. Albumin:

Albumin is the most abundant protein in human blood plasma accounting to almost 50% of total plasma mass. Albumin is a water soluble-protein with a molecular weight of 66 kDa. The primary function of albumin is to carry hydrophobic fatty acid molecules around the blood stream and maintain blood pH. The preproalbumins are synthesized in the liver and undergo further processing before getting released into the circulatory system. The composition of albumin is characterized by a low content of tryptophan and methionine and a high content of cystine and charged amino acids, such as aspartic and glutamic acids, lysine and arginine. Studies have shown that almost all tissues in human body have the ability to degrade albumin, making it a highly preferred degradable biopolymer for medical applications [337].

Due to its solubility and the presence of functional groups along the polymer chain, albumin can be easily processed into various shapes and forms such as membranes, microspheres, nanofibres

and nanospheres. Due to its excellent blood compatibility, albumin has been extensively investigated as a carrier vehicle for intravenous drug/gene delivery. Albumin has also been investigated as coating materials for cardiovascular devices. Albumin based surgical adhesives have also been approved by the FDA for reapproximating the layers of large vessels, such as aorta, femoral and carotid arteries (CryoLife Inc.) and are composed of bovine serum albumin, gelatin and glutaraldehyde [338-339].

5.2.1.7. Fibrin:

Fibrin is a biopolymer similar to collagen that is involved in the natural blood clotting process. Fibrin is derived from fibrinogen, which is a 360kDa protein composed of three pairs of polypeptide chains. Fibrin is one of the earliest biopolymers used as biomaterials. This is due to the excellent biocompatibility, biodegradability, injectability and the presence of several extracellular matrix proteins, such as fibronectin, that favorably affects cell adhesion and proliferation. One of the first products developed from fibrin was a fibrin sealant. Various fibrin sealant products are being used clinically worldwide for hemostasis and tissue sealing applications in various surgical procedures. Due to its injectability and biodegradability, fibrin has also been investigated as a carrier vehicle for bioactive molecules. It has been found that proteins interact differently with fibrin clots, with certain growth factors demonstrating a strong interaction with fibrin matrices [340].

Fibrin matrices have also been found to be excellent cell carrier vehicles. Bioseed® is a fibrin-based product obtained by mixing keratinocytes with fibrin and is used to treat chronic wounds. A unique feature of fibrin-based cell carriers is that the matrix properties can be optimized for each different cell type [341].

5.2.2. Polysaccharides:

Polysaccharides are macromolecules formed from many monosaccharide units joined together by glycosidic linkages. Polysaccharides are gaining interest as biomaterials due to their unique biological functions ranging from cell signaling to immune recognition. This combined with new synthetic routes currently available to modify polysaccharides or synthesize oligosaccharide moieties, biodegradability and ability to fabricate appropriate structures, make them one of the most important and extensively studied natural biomaterials.

5.2.2.1. Polysaccharides of human origin:

i. Hyaluronic acid:

Hyaluronic acid (HA) was first isolated in 1934 from the vitreous humor of the eye by Meyer and Palmer (Fig. 17 a). Hyaluronic acid is a member of the glycosaminoglycan family, which are linear polysaccharides consisting of alternating units of N-acetyl-D-glucosamine and glucuronic acid, and are found in virtually every tissue in vertebrates. HA can be considered to be the largest glycosaminoglycan having molecular weights up to several millions. Unlike other members of the glycosaminoglycan family present in the human body (such as chondroitin sulfate, dermatan sulfate, keratin sulphate and heparin sulfate fig. 17 b-e), HA is not covalently bond to proteins. HA is water-soluble and forms highly viscous solutions with unique viscoelastic properties [342]. It has been reported to be present at high concentrations in synovial fluid and vitreous humor and significantly contributes to the viscoelastic properties of these tissues. Furthermore, HA plays an important structural role in a variety of tissues including articular cartilage, the nucleus pulposus, skin, the cervis, and the glycocalyx of endothelial cells. It has been reported that half of the

body's total HA content is present in the skin and the half-life of this polymer varies from a few minutes to weeks depending upon the tissue type. Studies have elucidated that within the cells, HA is synthesized on the cytosal surface of the plasma membrane under the direction of three glycosyl transferases: hyaluronan synthase-1 (Has-1, Has-2 and Has-3. Among these, Has-2 is the principal enzyme responsible for HA synthesis during embroyogenesis; however, specific roles played by Has-1 and Has-3 are not yet clear [343-344].

Since HA is produced by cells during early wound healing, this polymer has been extensively investigated for wound dressing applications. Other unique properties of HA include its ability to promote angiogenesis, to modulate wound site inflammation by acting as a free radical scavenger, and to be recognized by receptors on a variety of cells associated with tissue repair. Due to the high functionality and charge density of HA, it can be cross-linked by a variety of physical and chemical methods. Modified HA, such as esterified derivatives like ethyl/benzyl esters (HYAFF®) and cross-linked hyaluronic acid gels have been extensively studied for wound dressing application [345-346].

HA also plays an important role in tissue repair by promoting mesenchymal and epithelial cell migration and differentiation, thereby enhancing collagen deposition and angiogenesis. This property, in addition to its immunoneutrality makes HA an ideal biomaterial for tissue engineering and drug delivery applications. Its aqueous solubility allows HA to be fabricated into different types of porous and three-dimensional structures for these applications.

ii. Chondroitin sulfate:

Studies have shown that an important phase of wound healing involves the secretion of glycosaminoglycans by fibroblast cells to form a hydrophilic matrix suitable for remodelling while healing. A study using rat embryonic fibroblast cells showed that the majority of the glycosaminoglycan chains synthesized were chondroitin sulfate, suggesting the significance of this natural polymer for use in biomedical applications [347] (Fig. 17 b).

Chondrotin sulfate (CS) is the major component of aggrecan, the most abundant glycosaminoglycan found in the proteoglycans of articular cartilage. Studies have shown that CS can stimulate the metabolic response of cartilage tissue and has anti-inflammatory properties [348]. It is also involved in intracellular signaling, cell recognition and the connection of extracellular matrix components to cell-surface glycoproteins [349]. Due to its biocompatibility, non-immunogenicity and pliability, CS hydrogels have been extensively studied for wound dressing applications [350].

Similar to HA, several physical and chemical crosslinking techniques have been developed for CS to form hydrogels for biomedical applications. Since CS plays an important role in regulating the expression of the chondrocyte phenotype, it has been extensively investigated as a scaffolding material for cartilage tissue engineering [351].

5.2.2.2. Polysaccharides of non-human origin:

Apart from glycosaminoglycans present in the human body, other types of polysaccharide molecules have also raised interest as biodegradable polymeric biomaterials. The most important members among this class are the cationic polymer chitosan, which originates from crutacian skeletons, and the anionic polymer alginic acid, derived from brown algae, both of which are used as drug delivery vehicles [352]. One of the most extensively investigated polyelectrolyte complexes for biomedical applications involve chitosan and alginic acid. They are used as wound dressings, as drug, as well as cell delivery vehicles [353].

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i. Chitin and Chitosan:

Chitosan is derived from chitin which is a fully acetylated polymer and forms the exoskeleton of arthropod (Fig. 17 f-g). Chitosan is a linear polysaccharide consisting of β (1-4) linked D-glucosamine with randomly located N-acetylglucosamine groups depending upon the degree of deacetylation of the polymer. Chitosan has been found to be non-toxic after oral administration in humans and is an FDA approved food additive.

Enzymes, such as chitosanase, lysozyme and papain are known to degrade chitosan in vitro. The in vivo degradation of chitosan is primarily due to lysozyme and takes place through the hydrolysis of the acetylated residues. The rate of degradation of chitosan inversely depends on the degree of acetylation and crystallinity of the polymer. The highly deacetylated form exhibits the lowest degradation rates and may last several months in vivo [354-355]. Chemical modification of chitosan can significantly affect its solubility and degradation rate. Azab *et al*. studies the effect of cross-linking density on the in vivo degradation of chitosan gels; various concentrations of glutaraldehyde were used to develop gels having varying cross-linking densities. The degradation rates of the gels were investigated following subcutaneous and intraperitoneal implantations in a rat model. The gels with lower cross-linking density (FDG) showed significant weight loss after 14 days of implantation; approximately 80% (subcutaneous) and approximately 91% (intraperitoneal). No significant decrease in weight was observed for highly cross-linked gels (SDG) after 14 days of implantation [356]. Chitosan is soluble in weekly acidic solutions resulting in the formation of a cationic polymer with a high charge density and can therefore form polyelectrolyte complexes with wide range of anionic polymers [357].

Chitosan has structural similarities with glycosaminoglycans and hyaluronic acid present in human body and due to the presence of highly reactive amino groups along the polymer backbone, chitosan is susceptible to chemical or biological functionalization [358]. In fact, chitin and chitosan have shown to have stimulatory properties on macrophages, and chemo-attractive properties on neutrophils [359]. These properties, along with its antibacterial, hemostatic properties give chitosan enourmous potential as a natural polymer for wound healing applications [360-361].

The strong positive charges on chitosan makes it a very effective mucoadhesive as it can strongly interact with the negatively charged mucous membrane. Several chitosan-based bioadhesive drug/ vaccine delivery systems are under development [362]. Due to is aqueous solubility chitosan can be fabricated into various structures and forms, such as gels, nanofibers nanospheres, microspheres and combined with its pH sensitivity, excellent biocompatibility and biodegradability, makes chitosan a promising candidate for developing drug delivery devices and as scaffolds for tissue engineering. Chitosan has the ability to condense DNA to form complexes and extensive research has gone into developing non-viral gene delivery vehicles [362-363].

Chitosan has the ability to act as a permeation enhancer through its interaction with the cell membrane resulting in a structural reorganization of tight-junction associated proteins. This, along with its mucoadhesive property, makes it a suitable candidate for use in both oral and nasal vaccination formulations. As such, several solution and microsphere vaccine formulations based on chitosan have been developed. Chitosan offers another advantage by being able to form

micro/nanosphere formulations without the use of organic solvents, which maintains the immunogenicity of the antigens [364].

The high chemical reactivity of chitosan, has also led to several chitosan-drug conjugates for cancer therapy [365]. Chitosan was also used to develop injectable thermosensitive carrier material for biomedical applications [366-367]. Studies have shown that in the presence of certain phosphate salts, chitosan can undergo a temperature-controlled phase transition. Due to the mild gelling conditions, the hydrogel has been found to be a potential delivery vehicle for growth factors, small molecular weight drugs and cells for localized therapy.

ii. Alginic acid:

Alginic acid present within the cell walls and intercellular spaces of brown algae and has a structural role in giving flexibility and strength to marine plants. Due to its non-toxicity, alginate has been extensively used as a food additive and a thickener in salad dressings and ice creams. Alginate is a non-branched, binary copolymer of (1-4) glycosidically linked β-D-mannuronic acid and α-L-guluronic acid monomers.

Alginate is not a random copolymer but instead, it is a block copolymer composed of two uronic acid with different block lengths and sequential arrangement (Fig. 17 h). Alginates are extracted from the algae using a base solution and then reacted with acid to form alginic acid. They are high molecular weight polymers having molecular weights up to 500 kDa. Aqueous solutions of alginates show non-Newtonian behaviour similar to other glycosaminoglycans.

The high functionality of alginic acid makes it a favorable biopolymeric material for use in biomedical applications. The high acid content allows alginic acid to undergo spontaneous and mild gelling in the presence of divalent cations, such as calcium ions. This mild gelling property allows the encapsulation of various molecules or even cells within alginate gels with minimal negative impact [368]. Furthermore, the carboxylic acid groups of alginic acid are highly reactive and can be appropriately modified for various applications Fig.18 [369].

Even though alginates have been extensively investigated as biomaterials, one of the main disadvantages of using alginate-based materials is their inability to undergo enzymatic degradation by mammals. Studies are currently underway to develop degradable gels based on alginate. It has been found that ionically cross-linked alginates dissolve at neutral pH upon losing the divalent cross-linking cations and leads to uncontrolled and typically slow degradation in vivo.

Alginate has also been extensively investigated as a drug delivery device where in the rate of drug release can be varied by varying the drug polymer interaction as well as by chemically immobilizing the drug to the polymer back bone using the reactive carboxylate groups. The encapsulation of proteins and bioactive factors within ionically crosslinked alginate gels are known to greatly enhance their efficiency and targetability [370].

A disadvantage of using alginate-based gels, apart from their poor degradability, is poor cell adhesion on alginate gels. Recent studies; however, have shown the feasibility of developing alginate gels with good cell affinity. The modification of alginate with bioactive molecules, such as cell binding peptides is a versatile method for developing cell-binding hydrogels for use as scaffolds for tissue engineering.

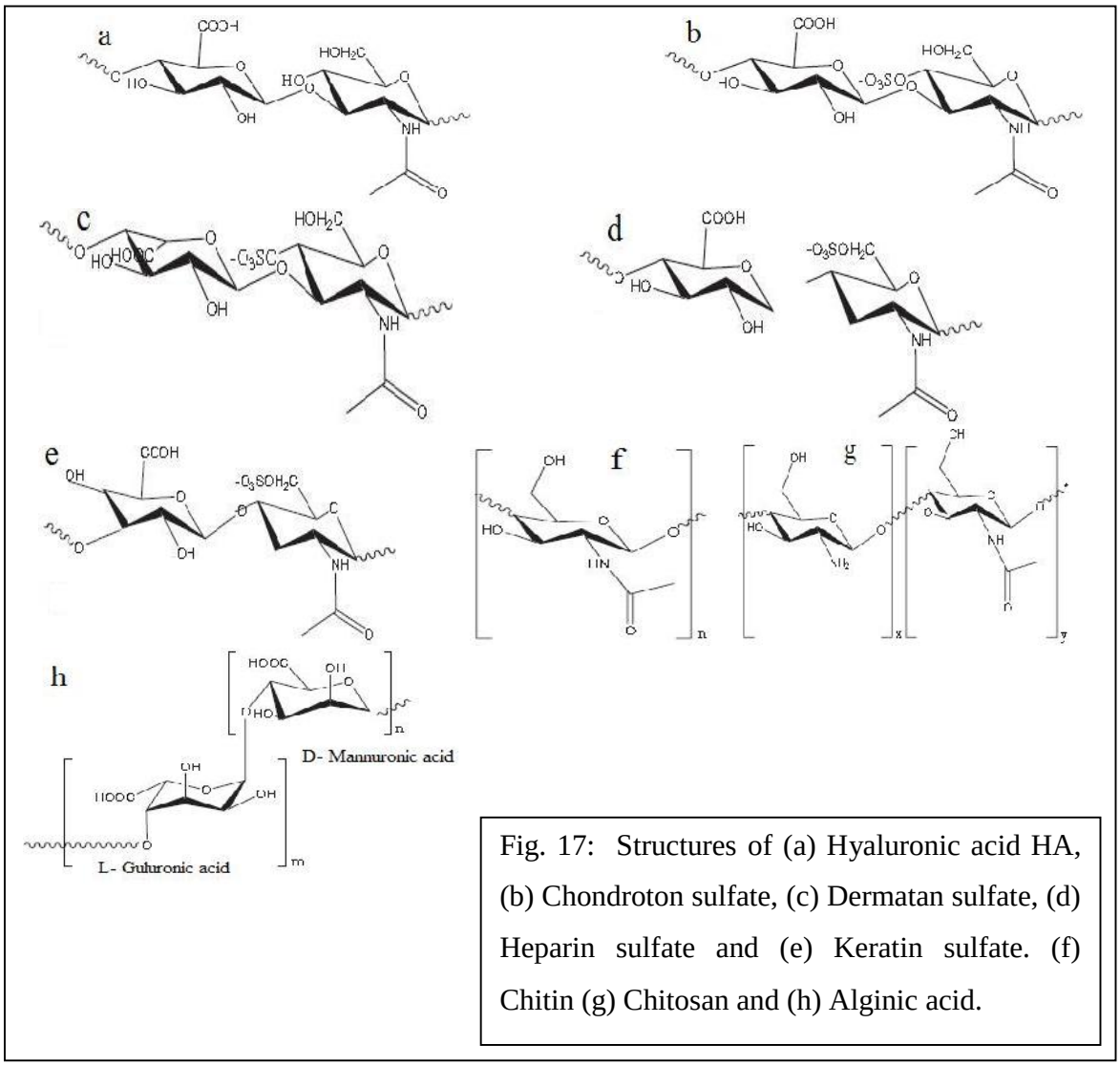

Fig. 17: Structures of (a) Hyaluronic acid HA, (b) Chondroton sulfate, (c) Dermatan sulfate, (d) Heparin sulfate and (e) Keratin sulfate. (f) Chitin (g) Chitosan and (h) Alginic acid.

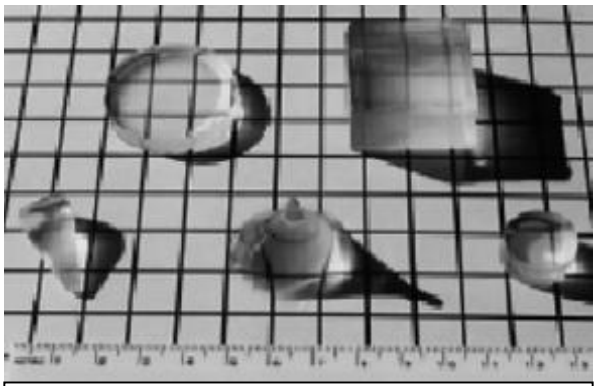

Fig.18: Various shapes developed from alginate via calcium crosslinking (source

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6. Conclusions:

As outlined above, a wide range of biodegradable polymers are currently available. Most of the biodegradable materials currently on the market are based on natural polymers such as collagen and synthetic polymers such as poly(aesters). Generally, natural biodegradable polymers are hydrophilic and low in mechanical strength, while synthetic biodegradable polymers are hydrophilic and have good mechanical properties. Advances in synthetic organic chemistry and novel bioprocesses are enabling the development of a wide range of novel polymeric materials as candidates for developing transient implants and drug delivery vehicles. The successes of biodegradable implants lie in our ability to custom design or modify existing biomaterials to achieve appropriate biocompatibility, degradation and physical properties to elicit favourable biological responses.

We have reviewed a large cross-section of biodegradable polymeric delivery systems for protein and peptides, pharmaceuticals and many other applications of biodegradable polymers in agriculture industry, in textile industry or even in daily life articles usage of it. Especially in case of delivery systems have unique challenges associated with both protein stability and protein release kinetics. Despite of numerous reports available in the scientific literature showed good results in preclinical models, very few of these systems have been converted or developed into viable products. For the meaningful results we have to looking on the continuous advances in biotechnology; which will produce more proteins and peptides that will be difficult to administer by conventional means, and an increased demand for various applications of biodegradable polymers is anticipated.

Today much of us consider that biodegradable polymers are very attractive and necessary for the co-existence of the human society with the nature, global production of biodegradable polymers is not as large as expected or required. The main reason for this seems to be not their poor properties as materials but their high production costs. Generally consumers do not want to pay much higher for conventional daily products even if they are urgently required to keep our environments both inside and outside the human body safe and clean. The greatest challenge to polymer scientists is to manufacture biodegradable polymers at a reasonably low cost; having well-balanced biodegradability and mechanical properties. The most appropriate biodegradable polymer for the targeted end use will be selected taking into account the ratio of polymer cost versus its performance

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