Module -2

Polymeric Biomaterials: Introduction, polymerization and basic structure, polymers used as biomaterials, sterilization, surface modifications to for improving biocompatibility.

Composite Biomaterials: Structure, bounds on properties, anisotropy of composites, particulate composites, fibrous composites, porous materials, biocompatibility.

Biodegradable Polymeric Biomaterials: Introduction, Glycolide based biodegradable homopolymers polyesters, non-glycolide linear aliphatic polyesters, aliphatic and aromatic polycarbonates, and biodegradation properties of synthetic biodegradable polymers.

TISSUE DERIVED BIOMATERIALS: Structure and properties of collagen and collagen-rich tissues, biotechnology of collagen, design of resorbable collagen-based medical implant.

POLYMERIC BIOMATERIALS

Basic Structure

Polymers have very long chain molecules which are formed by covalent bonding along the backbone chain. The long chains are held together either by secondary bonding forces such as van der Waals and hydrogen bonds or primary covalent bonding forces through crosslinks between chains. The long chains are very flexible and can be tangled easily. In addition, each chain can have side groups, branches and copolymeric chains or blocks which can also interfere with the long-range ordering of chains. For example, paraffin wax has the same chemical formula as polyethylene (PE) [(CH2CH2)n], but will crystallize almost completely because of its much shorter chain lengths. However, when the chains become extremely long {from 40 to 50 repeating units [–CH2CH2–] to several thousands as in linear PE} they cannot be crystallized completely (up to 80 to 90% crystallization is possible). Also, branched PE in which side chains are attached to the main backbone chain at positions normally occupied by a hydrogen atom, will

not crystallize easily due to the steric hindrance of side chains resulting in a more noncrystalline structure. The partially crystallized structure is called semicrystalline which is the most commonly occurring structure for linear polymers.

The degree of polymerization (DP) is defined as an average number of mers, or repeating units, per molecule, i.e., chain. Each chain may have a different number of mers depending on the condition of polymerization. Also, the length of each chain may be different. Therefore, it is assumed there is an average degree of polymerization or average molecular weight (MW). The relationship between molecular weight and degree of polymerization can be expressed as: MW of polymer = DP × MW of mer (or repeating unit) .

The two average molecular weights most commonly used are defined in terms of the numbers of molecules, Ni, having molecular weight, Mi; or wi, the weight of species with molecular weights Mi as follows: 1. The number-average molecular weight, Mn, is defined by the equation, (39.4) 2. The weight average molecular weight, Mw, is defined by the equation.

An absolute method of measuring the molecular weight is one that depends on theoretical considerations, counting molecules and their weight directly. The relative methods require calibration based on an absolute method and include intrinsic viscosity and gel permeation chromatography (GPC). Absolute methods of determining the number-average molecular weight (Mn) include osmometry and other colligative methods, and end group analysis. Light-scattering yields an absolute weight-average molecular weight (Mw).

Polyvinylchloride (PVC)

- PVC is an amorphous, rigid polymer due to the large side group (Cl, chloride) with a Tg of 75~105°C.
- It has a high melt viscosity hence it is difficult to process. To prevent the thermal degradation of the polymer (HCl could be released), thermal stabilizers such as metallic soaps or salts are incorporated.

- Lubricants are formulated on PVC compounds to prevent adhesion to metal surfaces and facilitate the melt flow during processing. Plasticizers are used in the range of 10 to 100 parts per 100 parts of PVC resin to make it flexible.
- Di-2-ethylhexylphthalate (DEHP or DOP) is used in medical PVC formulation. However, the plasticizers of trioctyltrimellitate (TOTM), polyester, azelate.
- PVC sheets and films are used in blood and solution storage bags and surgical packaging. PVC tubing is commonly used in intravenous (IV) administration, dialysis devices, catheters, and cannulae.

Polyethylene

- PE is available commercially in five major grades: (1) high density (HDPE), (2) low density (LDPE), (3) linear low density (LLDPE), (4) very low density (VLDPE), and (5) ultra high molecular weight FIGURE 39.5 Change of volume versus temperature of a solid. The glass transition temperature (Tg) depends on the rate of cooling and below (Tg) the material behaves as a solid like a window glass.
- Applications Polyvinylchloride (PVC) Blood and solution bag, surgical packaging, IV sets, dialysis devices, catheter bottles, connectors, and cannulae. Polyethylene (PE) Pharmaceutical bottle, nonwoven fabric, catheter, pouch, flexible container, and orthopedic implants.
- **Polypropylene** (PP) Disposable syringes, blood oxygenator membrane, suture, nonwoven fabric, and artificial vascular grafts. **Polymethylmetacrylate** (PMMA) Blood pump and reservoirs, membrane for blood dialyzer, implantable ocular lens, and bone cement. Polystyrene (PS) Tissue culture flasks, roller bottles, and filterwares. **Polyethylenterephthalate** (PET) Implantable suture, mesh, artificial vascular grafts, and heart valve. Polytetrafluoroethylene (PTFE) Catheter and artificial vascular grafts. Polyurethane (PU).

Polypropylene (PP)

- Polypropylene (PP) PP can be polymerized by a Ziegler-Natta stereospecific catalyst which controls the isotactic position of the methyl group.
- Thermal (Tg:-12°C, Tm:125~167°C and density: 0.85~0.98 g/cm3) and physical properties of PP are similar to PE.
- The average molecular weight of commercial PP ranges from 2.2~7.0 × 105 g/mol and has a wide molecular weight distribution (polydispersity) which is from 2.6 to 12.
- Additives for PP such as antioxidants, light stabilizer, nucleating agents, lubricants, mold release agents, antiblock, and slip agents are formulated to improve the physical properties and processability.

Polymethylmetacrylate (PMMA)

- Commercial PMMA is an amorphous (Tg:105°C and density:1.15~1.195 g/cm3) material with good resistance to dilute alkalis and other inorganic solutions.
- PMMA is best known for its exceptional light transparency (92% transmission), high refractive index (1.49), good weathering properties, and as one of the most biocompatible polymers.
- PMMA can be easily machined with conventional tools, molded, surface coated, and plasma etched with glow or corona discharge. PMMA is used broadly in medical applications such as a blood pump and reservoir, an IV system, membranes for blood dialyzer, and in in vitro diagnostics.
- It is also found in contact lenses and implantable ocular lenses due to excellent optical properties, dentures, and maxillofacial prostheses due to good physical and coloring properties, and bone cement for joint prostheses fixation (ASTM standard F451).

Polystyrene (PS)

- PS is polymerized by free radical polymerization and is usually atactic. Three grades are available; unmodified general purpose PS (GPPS, Tg:100°C), high

impact PS (HIPS), and PS foam. GPPS has good transparency, lack of color, ease of fabrication, thermal stability, low specific gravity (1.04~1.12 g/cm3), relatively high modulus.

- HIPS contains a rubbery modifier which forms chemical bonding with the growing PS chains. Hence the ductility and impact strength are increased and the resistance to environmental stress-cracking is also improved.
- PS is mainly processed by injection molding at 180~250°C. To improve processability additives such as stabilizers, lubricants, and mold releasing agents are formulated.
- GPPS is commonly used in tissue culture flasks, roller bottles, vacuum canisters, and filterware.

Polyamides (Nylons)

- Polyamides are known as nylons and are designated by the number of carbon atoms in the repeating units. Nylons can be polymerized by step-reaction (or condensation) and ring-scission polymerization.
- They have excellent fiber-forming ability due to interchain hydrogen bonding and a high degree of crystallinity, which increases strength in the fiber direction.
- The presence of –CONH– groups in polyamides attracts the chains strongly toward one another by hydrogen bonding. Since the hydrogen bond plays a major role in determining properties, the number and distribution of –CONH– groups are important factors.
- For example, Tg can be decreased by decreasing the number of –CONH– groups . On the other hand, an increase in the number of –CONH– groups improves physical properties such as strength as one can see that Nylon 66 is stronger than Nylon 610 and Nylon 6 is stronger than Nylon 11

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- On the other hand, an increase in the number of –CONH– groups improves physical properties such as strength as one can see that Nylon 66 is stronger than Nylon 610 and Nylon 6 is stronger than Nylon 11. In addition to the higher Nylons (610 and 11) there are aromatic polyamides named aramids. One of them is poly (p-phenylene terephthalate) commonly known as Kevlar®, made by DuPont.
- This material can be made into fibers. The specific strength of such fibers is five times that of steel, therefore, it is most suitable for making composites. Nylons are hygroscopic and lose their strength in vivo when implanted.
- The water molecules serve as plasticizers which attack the amorphous region.
- Proteolytic enzymes also aid in hydrolyzing by attacking the amide group.
- This is probably due to the fact that the proteins also contain the amide group along their molecular chains which the proteolytic enzymes could attack. Fluorocarbon Polymers The best known fluorocarbon polymer is polytetrafluoroethylene (PTFE), commonly known as Teflon® (DuPont).

- Other polymers containing fluorine are polytrifluorochloroethylene (PTFCE), polyvinylfluoride (PVF), and fluorinated ethylene propylene (FEP). Only PTFE will be discussed here since the others have rather inferior chemical and physical properties and are rarely used for implant fabrication.
- PTFE is made from tetrafluoroethylene under pressure with a peroxide catalyst in the presence of excess water for removal of heat. The polymer is highly crystalline (over 94% crystallinity) with an average molecular weight of 0.5~5 × 106 g/mol.
- This polymer has a very high density (2.15~2.2 g/cm3), low modulus of elasticity (0.5 GPa) and tensile strength (14 MPa).
- It also has a very low surface tension (18.5 erg/cm2) and friction coefficient (0.1). Standard specifications for the implantable PTFE are given by ASTM F754.
- PTFE also has an unusual property of being able to expand on a microscopic scale into a microporous material which is an excellent thermal insulator. PTFE cannot be injection molded or melt extruded because of its very high melt viscosity and it cannot be plasticized.
- Usually the powders are sintered to above 327°C under pressure to produce implants.

Rubbers

- Silicone, natural, and synthetic rubbers have been used for the fabrication of implants. Natural rubber is made mostly from the latex of the Hevea brasiliensis tree and the chemical formula is the same as that of cis-1,4 polyisoprene.
- Natural rubber was found to be compatible with blood in its pure form.
- Also, cross-linking by X-ray and organic peroxides produces rubber with superior blood compatibility compared with rubbers made by the conventional sulfur vulcanization. Synthetic rubbers were developed to substitute for natural rubber.

- The Ziegler-Natta types of stereospecific polymerization techniques have made this variety possible. The synthetic rubbers have rarely been used to make implants.
- The physical properties vary widely due to the wide variations in preparation recipes of these rubbers. Silicone rubber, developed by Dow Corning company, is one of the few polymers developed for medical use.
- The repeating unit is dimethyl siloxane which is polymerized by a condensation polymerization.
- Low molecular weight polymers have low viscosity and can be cross-linked to make a higher molecular weight, rubber-like material.
- Medical grade silicone rubbers contain stannous octate as a catalyst and can be mixed with a base polymer at the time of implant fabrication.

Polyurethanes, Polyacetal, Polysulfone, and Polycarbonate

- Polyurethanes are usually thermosetting polymers: they are widely used to coat implants. Polyurethane rubbers are produced by reacting a prepared prepolymer chain with an aromatic di-isocyanate to make very long chains possessing active isocyanate groups for cross-linking.

- The polyurethane rubber is quite strong and has good resistance to oil and chemicals. These polymers have excellent mechanical, thermal, and chemical properties due to their stiffened main backbone chains.

- Polyacetals and polysulfones are being tested as implant materials, while polycarbonates have found their applications in the heart/lung assist devices, food packaging, etc.

- Polyacetals are produced by reacting formaldehyde. These are also sometimes called polyoxymethylene (POM) and known widely as Delrin® (DuPont).

- These polymers have a reasonably high molecular weight (>2 × 104 g/mol) and have excellent mechanical properties.

- More importantly, they display an excellent resistance to most chemicals and to water over wide temperature ranges.

Sterilizability

- Sterilizability of biomedical polymers is an important aspect of the properties because polymers have lower thermal and chemical stability than other materials such as ceramics and metals, consequently, they are also more difficult to sterilize using conventional techniques
- Commonly used sterilization techniques are dry heat, autoclaving, radiation, and ethylene oxide gas
- In dry heat sterilization, the temperature varies between 160 and 190°C. This is above the melting and softening temperatures of many linear polymers like polyethylene and PMMA.
- Steam sterilization (autoclaving) is performed under high steam pressure at relatively low temperature (125–130°C). However, if the polymer is subjected to attack by water vapor, this method cannot be employed. PVC, polyacetals,
- Chemical agents sometimes cause polymer deterioration even when sterilization takes place at room temperature. However, the time of exposure is relatively short (overnight), and most polymeric implants can be sterilized with this method.
- Radiation sterilization using the isotopic 60Co can also deteriorate polymers since at high dosage the polymer chains can be dissociated or cross-linked according to the characteristics of the chemical structures,

Surface Modifications for Improving Biocompatability

- Prevention of thrombus formation is important in clinical applications where blood is in contact such as hemodialysis membranes and tubes, artificial heart and heart-lung machines, prosthetic valves, and artificial vascular grafts.

- In spite of the use of anticoagulants, considerable platelet deposition and thrombus formation take place on the artificial surfaces.
- Heparin, one of the complex carbohydrates known as mucopolysaccharides or glycosaminoglycan is currently used to prevent formation of clots. In general, heparin is well tolerated and devoid of serious consequences.
- However, it allows platelet adhesion to foreign surfaces and may cause hemorrhagic complications such as subdural hematoma, retroperitoneal hematoma.
- Albumin-coated surfaces have been studied because surfaces that resisted platelet adhesion in vitro were noted to adsorb albumin preferentially.
- Fibronectin coatings have been used in in vitro endothelial cell seeding to prepare a surface similar to the natural blood vessel lumen Also, algin-coated surfaces have been studied due to their good biocompatibility and biodegradability.
- Recently, plasma gas discharge and corona treatment with reactive groups introduced on the polymeric surfaces have emerged as other ways to modify biomaterial surfaces.
- Hydrophobic coatings composed of silicon- and fluorine-containing polymeric materials as well as polyurethanes have been studied because of the relatively good clinical performances of Silastic®, Teflon®, and polyurethane polymers in cardiovascular implants and devices. Polymeric fluorocarbon coatings deposited from a tetrafluoroethylene gas discharge have been found to greatly enhance resistance to both acute thrombotic occlusion and embolization in small diameter Dacron® grafts.
- Hydrophilic coatings have also been popular because of their low interfacial tension in biological environments.
- Hydrogels as well as various combinations of hydrophilic and hydrophobic monomers have been studied on the premise that there will be an

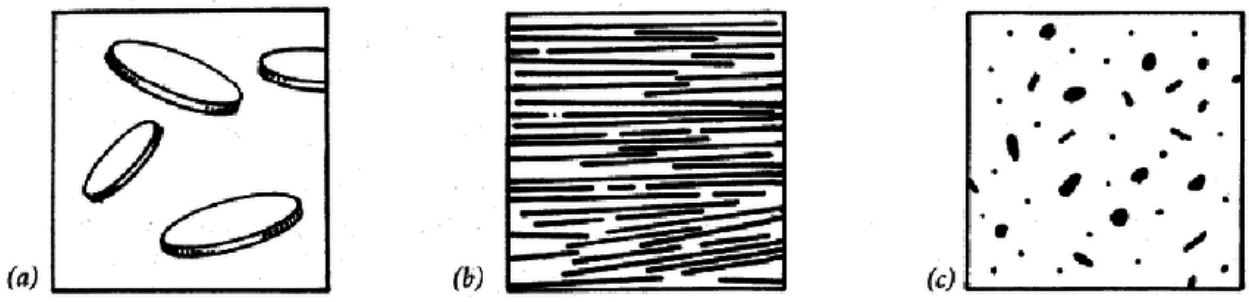
optimum polar-dispersion force ratio which could be matched on the surfaces of the most passivating proteins.

COMPOSITE BIOMTERIALS

STRUCTURE

The properties of composite materials depend very much upon structure. Composites differ from homogeneous materials in that considerable control can be exerted over the larger scale structure, and hence over the desired properties. In particular, the properties of a composite material depend upon the shape of the heterogeneities, upon the volume fraction occupied by them, and upon the interface among the constituents. The shape of the heterogeneities in a composite material is classified as follows. The principal inclusion shape categories are (1) the particle, with no long dimension, (2) the fiber, with one long dimension, and (3) the platelet or lamina.(FIG. ABC)

The inclusions may vary in size and shape within a category. If one phase consists of voids, filled with air or liquid, the material is known as a cellular solid. If the cells are polygonal, the material is a honeycomb; if the cells are polyhedral, it is a foam. It is necessary in the context of biomaterials to distinguish the above structural cells from biological cells, which occur only in living organisms. In each composite structure, we may moreover make the distinction between random orientation and preferred orientation.



BOUNDS OF PROPERTIES

- Mechanical properties in many composite materials depend on structure in a complex way, however for some structures, the prediction of properties is relatively simple. The simplest composite structures are the idealized Voigt and Reuss models.
- The dark and light areas in these diagrams represent the two constituent materials in the composite.
- In contrast to most composite structures, it is easy to calculate the stiffness of materials with the Voigt and Reuss structures, since in the Voigt structure the strain is the same in both constituents; in the Reuss structure the stress is the same.
- The Voigt relation for the stiffness is referred to as the rule of mixtures

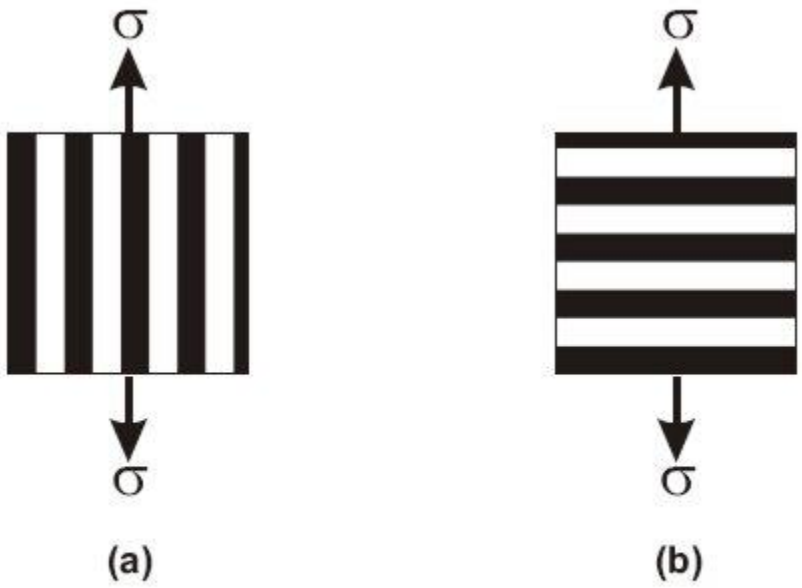


Figure 1.2: (a) Voigt and (b) Reuss composite model.

PARTICULATE COMPOSITES

- The stiffness of such a composite is close to the Hashin-Shtrikman lower bound for isotropic composites.

- Even if the spherical particles are perfectly rigid compared with the matrix, their stiffening effect at low concentrations is modest.
- Conversely, when the inclusions are more compliant than the matrix, spherical ones reduce the stiffness the least and platelet ones reduce it the most.
- Indeed, soft platelets are suggestive of crack-like defects.
- Soft platelets, therefore result not only in a compliant composite, but also a weak one.
- Soft spherical inclusions are used intentionally as crack stoppers to enhance the toughness of polymers such as polystyrene [high impact polystyrene], with a small sacrifice in stiffness.

FIBROUS COMPOSITES

- Fibers are mechanically more effective in achieving a stiff, strong composite than are particles.
- Materials can be prepared in fiber form with very few defects which concentrate stress.
- Fibers such as graphite are stiff (Young's modulus is 200 to 800 GPa) and strong (the tensile strength is 2.7 to 5.5 GPa).
- Composites made from them can be as strong as steel but much lighter.
- The stiffness of a composite with aligned fibers, if it is loaded along the fibers, is equivalent to the Voigt upper bound,.
- Unidirectional fibrous composites, when loaded along the fibers, can have strengths and stiffnesses comparable to that of steel, but with much less weight.
- However if it is loaded transversly to the fibers, such a composite will be compliant, with a stiffness not much greater than that of the matrix alone.

- While unidirectional fiber composites can be made very strong in the longitudinal direction, they are weaker than the matrix alone when loaded transversely, as a result of stress concentration around the fibers.
- If stiffness and strength are needed in all directions, the fibers may be oriented randomly. For such a three-dimensional isotropic composite, for a low concentration of fibers.
- The degree of anisotropy in fibrous composites can be very well controlled by forming laminates consisting of layers of fibers embedded in a matrix. Each layer can have fibers oriented in a different direction.
- One can achieve quasi-isotropic behavior in the laminate plane; such a laminate is not as strong or as stiff as a unidirectional one.
- Strength of composites depends on such particulars as the brittleness or ductility of the inclusions and the matrix. In fibrous composites failure may occur by (1) fiber breakage, buckling, or pullout, (2) matrix cracking, or (3) debonding of fiber from matrix. Short fiber composites are used in many applications.
- They are not as stiff or as strong as composites with continuous fibers, but they can be formed economically by injection molding or by in situ polymerization.
- Choice of an optimal fiber length can result in improved toughness, due to the predominance of fiber pull-out as a fracture mechanism. Carbon fibers have been incorporated in the high density polyethylene used in total knee replacements.
- The standard ultra high molecular weight polyethylene (UHMWPE) used in these implants is considered adequate for most purposes for implantation in older patients.
- A longer wear-free implant lifetime is desirable for use in younger patients. It is considered desirable to improve the resistance to creep of the

polymeric component, since excessive creep results in an indentation of that component after long term use.

- Representative properties of carbon reinforced ultra high molecular weight polyethylene
- .Enhancements of various properties by a factor of two are feasible. Polymethyl methacrylate (PMMA) used in bone cement is compliant and weak in comparison with bone.
- Therefore several reinforcement methods have been attempted. Metal wires have been used clinically as macroscopic “fibers” to reinforce PMMA cement used in spinal stabilization surgery
- The wires are made of a biocompatible alloy such as cobalt-chromium alloy or stainless steel.
- Such wires are not currently used in joint replacements owing to the limited space available. Graphite fibers have been incorporated in bone cement on an experimental basis.
- Significant improvements in the mechanical properties have been achieved.

POROUS COMPOSITES

- The presence of voids in porous or cellular solids will reduce the stiffness of the material. For some purposes, that is both acceptable and desirable. Porous solids are used for many purposes: flexible structures such as
- (1) seat cushions,
- (2) thermal insulation,
- (3) filters,
- (4) cores for stiff and lightweight sandwich panels,

- (5) flotation devices,
- (6) to protect objects from mechanical shock and vibration; and in biomaterials, as coatings to encourage tissue in growth. Representative cellular solid structures.
- The physical mechanism for the deformation mode beyond the elastic limit depends on the material from which the foam is made.
- Trabecular bone, for example, is a natural cellular solid, which tends to fail in compression by crushing.
- Many kinds of trabecular bone appear to behave mechanically as an open cell foam. For trabecular bone of unspecified orientation, the stiffness is proportional to the cube of the density and the strength as the square of the density, which indicates behavior dominated by bending of the trabeculae.
- For bone with oriented trabeculae, both stiffness and strength in the trabecular direction are proportional to the density, a fact which indicates behavior dominated by axial deformation of the trabeculae.
- Porous materials have a high ratio of surface area to volume. When porous materials are used in biomaterial applications, the demands upon the inertness and biocompatibility are likely to be greater than for a homogeneous material.
- Porous materials, when used in implants, allow tissue ingrowth [Spector et al., 1988a,b]. The ingrowth is considered desirable in many contexts, since it allows a relatively permanent anchorage of the implant to the surrounding tissues.
- There are actually two composites to be considered in porous implants: (1) the implant prior to ingrowth.
- In the case of the implant prior to ingrowth, it must be recognized that the stiffness and strength of the porous solid are much less than in the case of

the solid from which it is derived. Porous layers are used on bone compatible implants to encourage bony.

- The pore size of a cellular solid has no influence on its stiffness or strength (though it does influence the toughness), however pore size can be of considerable biological importance.
- Specifically, in orthopedic implants with pores larger than about 150 µm, bony ingrowth into the pores occurs and this is useful to anchor the implant.
- This minimum pore size is on the order of the diameter of osteons in normal Haversian bone. It was found experimentally that pores

BIOCOMPATIBILITY

- Carbon itself has been successfully used as a biomaterial. Carbon based fibers used in composites are known to be inert in aqueous (even seawater) environments, however they do not have a track record in the biomaterials setting
- If such composites are placed near a metallic implant, galvanic corrosion is a possibility.
- Composite materials with a polymer matrix absorb water when placed in a hydrated environment such as the body.
- Moisture acts as a plasticizer of the matrix and shifts the glass transition temperature towards lower values ,hence a reduction in stiffness and an increase in mechanical damping.
- Water immersion of a graphite epoxy cross-ply composite] for 20 days reduced the strength by 13% and the stiffness by 9%.
- Moisture absorption by polymer constituents also causes swelling.
- Such swelling can be beneficial in dental composites since it offsets some of the shrinkage due to polymerization.

BIODEGRABLE POLYMERIC MATERIALS

Structure of Collagen

- Collagen is a multifunctional family of proteins of unique structural characteristics.
- It is the most abundant and ubiquitous protein in the body, its functions ranging from serving crucial biomechanical functions in bone, skin, tendon, and ligament to controlling cellular gene expressions in development .
- Collagen molecules like all proteins are formed in vivo by enzymatic regulated step-wise polymerization reaction between amino and carboxyl groups of amino acids, where R is a side group of an amino acid residue.
- The simplest amino acid is glycine (Gly) (R = H), where a hypothetical flat sheet organization of polyglycine molecules can form and be stabilized by intermolecular hydrogen bonds.
- However, when R is a large group as in most other amino acids, the stereochemical constraints frequently force the polypeptide chain to adapt a less constraining conformation by rotating the bulky R groups away from the crowded interactions, forming a helix, where the large R groups are directed toward the surface of the helix .
- The hydrogen bonds are allowed to form within a helix between the Shu-Tung Li Collagen Matrix, hydrogen attached to nitrogen in one amino acid residue and the oxygen attached to a second amino acid residue.
- Thus, the final conformation of a protein, which is directly related to its function, is governed primarily by the amino acid sequence of the particular protein.
- Collagen is a protein comprised of three polypeptides (α chains), each having a general amino acid sequence of (-Gly-X-Y-)n, where X is any other amino acid and is frequently proline (Pro) and Y is any other amino acid and is frequently hydroxyproline (Hyp).
- Within the triple helix, glycine must be present as every third amino acid, and proline and hydroxyproline are required to form and stabilize the triple helix. To date, 19 proteins can be classified as collagen

- Among the various collagens, type I collagen is the most abundant and is the major constituent of bone, skin, ligament, and tendon.
- Due to the abundance and ready accessibility of these tissues, they have been frequently used as a source for the preparation of collagen.
- It is, however, of particular relevance to review some salient structural features of the type I collagen in order to facilitate the subsequent discussions of properties and its relation to biomedical applications.
- A type I collagen molecule (also referred to as tropocollagen) isolated from various tissues has a molecular weight of about 283,000 daltons.
- It is comprised of three left-handed helical polypeptide chains which are intertwined forming a right-handed helix around a central molecular axis.
- Two of the polypeptide chains are identical (α1) having 1056 amino acid residues, and the third polypeptide chain (α2) has 1029 amino acid residues.
- The triple-helical structure has a rise per residue of 0.286 nm and a unit twist of 108°, with 10 residues in three turns and a helical pitch (repeating distance within a single chain) of 30 residues or 8.68 nm Over 95% of the amino acids have the sequence of Gly-X-Y.
- The remaining 5% of the molecule does not have the sequence of Gly-X-Y and is therefore not triple-helical.
- These nonhelical portions of the molecule are located at the N- and C-terminal ends and are referred to as telopeptides (9~26 residues)
- (1) a tight fit of the amino acids within the triple-helix—this geometrical stabilization factor can be appreciated from a space-filling model constructed from a triple helix with (Gly-Pro-Hyp) sequence
- (2) the interchain hydrogen bond formation between the backbone carbonyl and amino hydrogen interactions; and
- (3) the contribution of water molecules to the interchain hydrogen bond formation. The telopeptides are regions where intermolecular crosslinks are formed in vivo.
- A common intermolecular crosslinks is formed between an allysine (the ε-amino group of lysine or hydroxy-lysine has been converted to an aldehyde)

of one telopeptide of one molecule and an ε-amino group of a lysine or hydroxylysine in the triple helix or a second molecule .

- Thus the method commonly used to solubilize the collagen molecules from crosslinked fibrils with proteolytic enzymes such as pepsin removes the telopeptides (cleaves the intermolecular crosslinks) from the collagen molecule.
- The pepsin solubilized collagen is occasionally referred to as atelocollagen.

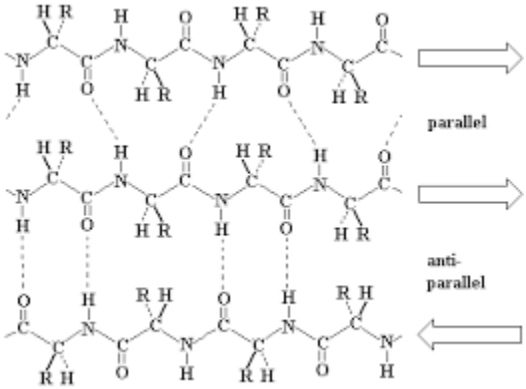


Fig. protein chain structure

Glycolide Based Biodegradable Homopolymers

- Polyesters PGA can be polymerized either directly or indirectly from glycolic acid. The direct polycondensation produces a polymer of Mn less than 10,000 because of the requirement of a very high degree of dehydration (99.28% up) and the absence of monofunctional impurities.
- For PGA of molecular weight higher than 10,000 it is necessary to proceed through the ring-opening polymerization of the cyclic dimers of glycolic acid. Numerous catalysts are available for this ring-opening polymerization.
- They include organometallic compounds and Lewis acids For biomedical applications, stannous chloride dihydrate or trialkyl aluminum are preferred.
- PGA was found to exhibit an orthorhombic unit cell with dimensions a = 5.22 Å, b = 6.19 Å, and c (fiber axis) = 7.02 Å. The planar zigzag-chain molecules form a sheet structure parallel to the ac plane and do not have the polyethylene type arrangement.

- The molecules between two adjacent sheets orient in opposite directions.
- The tight molecular packing and the close approach of the ester groups might stabilize the crystal lattice and contribute to the high melting point, Tm, of PGA (224–230°C).
- The glass transition temperature, Tg, ranges from 36 to 40°C.
- The specific gravities of PGA are 1.707 for a perfect crystal and 1.50 in a completely amorphous state .
- The heat of fusion of 100% crystallized PGA is reported to be 12 KJ/mole (45.7 cal/gram).
- A recent study of injection molded PGA disks reveals their IR spectroscopic characteristics, the four bands at 850, 753, 713, and 560 cm–1

Non-Glycolide/Lactide Based Linear Aliphatic Polyesters

- All glycolide/lactide based linear aliphatic polyesters are based on poly(α-hydroxy acids).
- Recently, there are two unique groups of linear aliphatic polyesters based on poly(ω-hydroxy acids) and the most famous ones are poly(ε-caprolactone) ,poly(β-hydroxybutyrate) (PHB), poly(β-hydroxyvalerate) (PHV) and the copolymers of PHB/PHV .
- Poly(ε-caprolactone) has been used as a comonomer with a variety of glycolide/lactide based linear aliphatic polyesters described earlier. PHB and PHV belong to the family of poly(hydroxyalkanoates) and are mainly produced by prokaryotic types of microorganisms like Pseudomonas olevorans or Alcaligenes eutrophus through biotechnology.
- PHB and PHV are the principal energy and carbon storage compounds for these microorganisms and are produced when there are excessive nutrients in the environment.

- These naturally produced PHB and PHV are stereochemically pure and are isotactic.
- They could also be synthesized in labs, but the characteristics of steroregularity is lost.
- This family of biodegradable polyesters is considered to be environmentally friendly because they are produced **from propionic acid and glucose and could be completely degraded to water**, biogas, biomass, and humic materials.
- The degradability of PHB could be accelerated by γ-irradiation or copolymerization with PHV. An interesting derivative of PHB**, poly(β-malic acid) (PMA), has been synthesized from β-benzyl malolactonate followed by catalytic hydrogenolysis. PMA differs from PHB in that the β-(CH3) substituent is replaced by –COOH .**
- The introduction of pendant carboxylic acid group would make PMA more hydrophilic and easier to be absorbed.

Aliphatic and Aromatic Polycarbonates

- Poly(BPA-carbonates) made from bisphenol A (BPA) and phosgene is non-biodegradable, but an analog of poly(BPA-carbonate) like poly(iminocarbonates) have been shown to degrade in about 200 days .
- In general, this class of aromatic polycarbonates takes an undesirably long period to degrade, presumably due to the presence of an aromatic ring which could protect adjacent ester bonds to be hydrolyzed by water or enzymes.
- Different types of degradation products of this polymer under different pH environments are produced.
- At pH > 7.0, the degradation products of this polymer are BPA, and ammonia and CO2, while insoluble poly(BPA-carbonate) oligomers were produced with pH < 7.0 .

- The polymer had good mechanical properties and acceptable tissue biocompatibility. Unfortunately, there is currently no commercial use of this class of polymer in surgery.

Theoretical Modeling of Degradation Properties

- The data showed a decrease in the rate of hydrolysis by about a factor of 106 with isopropyl α-substituents, but nearly a six-fold increase with t-butyl α-substituents.
- Electron withdrawing substituents α to the carbonyl group would be expected to stabilize the tetrahedral intermediate resulting from hydroxide attack, i.e., favoring hydroxide attack but disfavoring alkoxide elimination.
- Electron releasing groups would be expected to show the opposite effect. Similarly, electronegative substituents on the alkyl portion of the ester would stabilize the forming alkoxide ion and favor the elimination step.
- The data suggest that the magnitude of the inductive effect on the hydrolysis of glycolic esters decreases significantly as the location of the substituent is moved further away from the α-carbon because the inductive effect is very distance-sensitive.
- In all three locations of substitutions (α, β, and γ), Cl and Br substituents exhibited the largest inductive effect compared to other halogen elements

The Role of Free Radicals in Degradation Properties

- The biodegradation of synthetic absorbable sutures is closely related to macrophage activity through the close adhesion of macrophage onto the surface of the absorbable sutures
- It is also known that inflammatory cells, particularly leukocytes and macrophages are able to produce highly reactive oxygen species like superoxide (·O2 –) and hydrogen peroxide during inflammatory reactions toward foreign materials.

- Although the role of free radicals in the hydrolytic degradation of synthetic biodegradable polymers is largely unknown, a very recent study using absorbable sutures like Vicryl in the presence of an aqueous free radical solution prepared from H2O2 and ferrous sulfate, FeSO4, raised the possibility of the role of free radicals in the biodegradation of synthetic absorbable sutures .
- Surprisingly, the presence of surface cracks of Vicryl sutures treated in the free radical solutions did not accelerate the tensile breaking strength-loss as would be expected.
- Thermal properties of Vicryl sutures under the free radical and 3% H2O2 media showed the classical well-known maximum pattern of the change of the level of crystallinity with hydrolysis time.
- The level of crystallinity of Vicryl sutures peaked at 7 days in both media (free radical and 3% H2O2). The time for peak appearance in these two media was considerably earlier than Vicryl sutures in conventional physiological buffer media. Based on the Chu's suggestion of using the time of the appearance of the crystallinity peak as an indicator of degradation rate, it appears that these two media accelerated the degradation of Vicryl sutures when compared with regular physiological buffer solution.
- Based on their findings, Williams et al. proposed the possible routes of the role of ·OH radicals in the hydrolytic degradation of Vicryl sutures [Zhong et al., 1994]. Unfortunately, the possible role of OH–, one of the byproducts of Fenton reagents (H2O2/FeSO4), was not considered in the interpretation of their findings. OH– species could be more potent than •OH toward hydrolytic degradation of synthetic absorbable sutures.
- This is because hydroxyl anions are the sole species which attack carbonyl carbon of the ester linkages during alkaline hydrolysis. Since an equal amount of ·OH and OH– are generated in Fenton reagents, the observed changes in morphological, mechanical, and thermal properties could be partially attributed to OH– ions as well as ·OH

radicals. Besides hydroxyl radicals, the production of superoxide ions and singlet oxygen during phagocytosis has been well documented

- Although the role of superoxide in simple organic ester hydrolysis has been known since the 1970s its role in the hydrolytic degradation of synthetic biodegradable polyester based biomaterials has remained largely unknown. Such an understanding of the superoxide ion role during the biodegradation of foreign materials has become increasing desirable because of the advanced understanding of how the human immune system reacts to foreign materials and the increasing use of synthetic biomaterials for human body repair.
- Due to the extreme reactivity of the superoxide ion, it has been observed that the effect of superoxide ion-induced hydrolytic degradation of PDLLA and PLLA was significant in terms of changes in molecular weights and thermal properties.
- The superoxide ion-induced fragmentation of PDLLA would result in a mixture of various species with different chain lengths. A combined GPC method with a chemical tagging method revealed that the structure of oligomer species formed during the superoxide-induced degradation of PDLLA and PLLA was linear. The significant reduction in molecular weight of PDLLA by superoxide ion was also evident in the change of thermal properties like Tg.
- The linear low molecular species (oligomer, trimers, and dimers) in the reaction mixture could act as an internal plasticizer to provide the synergetic effects of lowering Tg by increasing free volume.
- The effect of the superoxide ion-induced hydrolytic degradation on molecular weight of PLLA was similar to PDLLA but with a much smaller magnitude.
- The mechanism of simple hydrolysis of ester by superoxide ion proposed by Forrester et al. was subsequently modified to interpret the data obtained from the synthetic biodegradable polymers.

- In addition to the PDLLA and PLLA, superoxide ions also have a significant adverse effect on the hydrolytic degradation of synthetic absorbable sutures.
- A significant reduction in molecular weight has been found along with mechanical and thermal properties of these sutures over a wide range of superoxide ion concentrations, particularly during the first few hours of contact with superoxide ions.
- For example, the PGA suture lost almost all of its mass at the end of 24 h contact with superoxide ions at 25°C, while the same suture would take at least 50 days in an in vitro buffer for a complete mass loss.
- The surface morphology of these sutures was also altered drastically. The exact mechanism, however, is not fully known yet; Lee et al. suggested the possibility of simultaneous occurrence of several main-chain scissions by three different nucleophilic species.
- They found that these γ-irradiated sutures retained better tensile breaking strength in the Fenton medium than in the regular buffer media.
- This mechanism is supported by the observed gradual loss of ESR signal of the sutures in the presence of the Fenton agent in the medium. Instead of the adverse effect of free radicals on the degradation properties of synthetic biodegradable polyesters
- A preliminary in vitro cell culture study of these new biologically active biodegradable polymers indicated that they could retard the proliferation of human smooth muscle cells as native nitric oxide.
- The full potential of this new class of biologically active biodegradable polymers is currently under investigation by Chu for a variety of therapeutic applications.

TISSUE DERIVED BIOMATERIALS

Structure and properties of collagen

- The function of collagenous tissue is related to its structure and properties. This section reviews some important properties of collagen-rich tissues.
- Physical and Biomechanical Properties The physical properties of tissues vary according to the amount and structural variations of the collagen fibers. In general, a collagen-rich tissue contains about 75–90% of collagen on a dry weight basis. It is a typical composition of a collagen-rich soft tissue such as skin. Collagen fibers (bundles of collagen fibrils) are arranged in different configurations in different tissues for their respective functions at specific anatomic sites.
- **Physiochemical Properties Electrostatic Properties**. A collagen molecule has a total of approximately 240 ε-amino and guanidino groups of lysines, hydroxylysines, and arginines and 230 carboxyl groups of aspartic and glutamic acids. These groups are charged under physiological conditions. Ion and Macromolecular Binding Properties. In the native state and under physiological conditions, a collagen molecule has only about 60 free carboxyl These groups have the capability of binding cations such as calcium with a free energy of formation for the protein-COO-Ca++ of about 1.2 Kcal/mol.
- **Cell Interaction Properties**. Collagen forms the essential framework of the tissues and organs. Many cells, such as epithelial and endothelial cells, are found resting on the collagenous surfaces or within a collagenous matrix such as that of many connective tissue cells. Collagen-cell interactions are essential features during the development stage and during wound healing and tissue remodeling in adults.
- Studying collagen-cell interactions is useful in developing simulated tissue and organ structures and in investigating cell behavior in the in vivo simulated systems

- **Immunologic Properties**. Soluble collagen has long been known to be a poor immunogen.
- A significant level of antibodies cannot be raised without the use of Freund's complete adjuvant (a mixture of mineral oil and heat-killed mycobacteria) which augments antibody response.
- It is known that insoluble collagen is even less immunogenic.
- Thus, xenogeneic collagenous tissue devices such as porcine and bovine pericardial heart valves are acceptable for long-term implantation in humans.

Biotechnology of collagen

- Isolation and Purification of Collagen There are two distinct ways of isolating and purifying collagen material. One is the molecular technology and the other is the fibrillar technology. These two technologies are briefly reviewed here.
- Isolation and Purification of Soluble Collagen Molecules The isolation and purification of soluble collagen molecules from a collagenous tissue is achieved by using a proteolytic enzyme such as pepsin to cleave the telopeptides.
- Since telopeptides are the natural crosslinking sites of collagen, the removal of telopeptides renders the collagen molecules and small collagen aggregates soluble in an aqueous solution.
- The pepsin-solubilized collagen can be purified by repetitive precipitation with a neutral salt. Pepsin-solubilized collagen in monomeric form is generally soluble in a buffer solution at low temperature.
- The collagen molecules may be reconstituted into fibrils of various polymorphisms.

However, the reconstitution of the pepsin-solubilized collagen into fibrils of native molecular packing is not as efficient as the intact molecules, since the telopeptides facilitate fibril formation.

- **Isolation and Purification of Fibrillar Collagen** The isolation and purification of collagen fibers relies on the removal of noncollagenous materials from the collagenous tissue. Salt extraction removes the newly synthesized collagen molecules that have not been covalently incorporated into the collagen fibrils.

- **Matrix Fabrication Technology**

The purified collagen materials obtained from either the molecular technology or from the fibrillar technology are subjected to additional processing to fabricate the materials into useful devices for specific medical applications

- **Solution Matrix** A collagen solution is obtained by dissolving the collagen molecules in an aqueous solution. Collagen molecules are obtained by digesting the insoluble tissue with pepsin to cleave the crosslinking sites of collagen (telopeptides) as previously described. The solubility of collagen depends on the pH, the temperature, the ionic strength of the solution, and the molecular weight. Generally, collagen is more soluble in the cold.

- **Filamentous Matrix** Collagen filaments can be produced by extrusion techniques. A collagen solution or dispersion having a concentration in the range of 0.5–1.5% (w/v) is first prepared.

Design of resorbable collegen based medical implant

- Designing a medical implant for tissue or organ repair requires a thorough understanding of the structure and function of the tissue and organ to be repaired, the structure and properties of the materials used for repair, and the design requirements.

- There are at present two schools of thought regarding the design of an implant, namely the permanent implant and the resorbable implant.

- The permanent implants are intended to permanently replace the damaged tissues or organs are fabricated from various materials including metals and natural or synthetic polymers.
- For example, most of the weight-bearing orthopedic and oral implants are made of metals or alloys. Non-weight-bearing tissues and organs are generally replaced with implants that are fabricated either from synthetic or natural materials.
- Material degradation can result from biological processes such as enzymatic degradation or environmentally induced degradation from mechanical, metal-catalyzed oxidation, and from the permeation of body fluids into the polymeric devices .
- The material degradation is particularly manifested in applications where there is repetitive stress-strain on the implant, such as artificial blood vessels and heart valves

Biocompatibility

- Biocompatibility of the materials and their degraded products is a prerequisite for resorbable implant development.
- Purified collagen materials have been used either as implants or have been extensively tested in clinical studies as implants without adverse effects.
- The meniscus template can be fabricated from purified type I collagen fibers that are further crosslinked chemically to increase the stability and reduce the immunogenicity in vivo.
- In addition, small amounts of noncollagenous materials such as glycosaminoglycans and growth factors can be incorporated into the collagen matrix to improve the osmotic properties as well as the rate of tissue ingrowth.
- **Physical Dimension** The physical dimension of a template defines the boundary of regeneration. Thus, the size of the collagen template should

match the tissue defect to be repaired. A properly sized meniscal substitute has been found to function better than a substitute which mismatches the physical dimension of the host meniscus

- . Thus, the apparent density is a direct measure of the empty space which is not occupied by the matrix material per se in the dry state. For example, for a collagen matrix of an apparent density 0.2 g/cm^3, the empty space would be 0.86 cm3 for a 1 cm^3 total space occupied by the matrix, taking the density of collagen to be 1.41 g/cm^3.

- The **apparent density** is also directly related to the mechanical strength of a matrix. In weight-bearing applications, the apparent density has to be optimized such that the mechanical properties are not compromised for the intended function of the resorbable implant as described in the mechanical properties section.

- **Pore Structure** The dimension of a mammalian fibrogenic cell body is on the order of 10–50 µm, depending on the substrate to which the cell adheres.

- In order for cells to infiltrate into the interstitial space of a matrix, the majority of the pores must be significantly larger than the dimension of a cell such that both the cell and its cellular processes can easily enter the interstitial space.

- In a number of studies using collagen-based matrices for tissue regeneration, it has been found that pore size plays an important role in the effectiveness of the collagen matrix to induce host tissue regeneration

- It was suggested that pore size in the range of 100–400 µm was optimal for tissue regeneration. Similar observations were also found to be true for porous metal implants in total hip replacement

- The question of interconnecting pores may not be a critical issue in a collagen template as collagenases are synthesized by most inflammatory cells during wound healing and remodeling processes.

- The interporous membranes which exist in the noninterconnecting pores should be digested as part of resorption and wound healing processes.
- Mechanical Property In designing a resorbable collagen implant for weight-bearing applications, not only the initial mechanical strength is important, but the gradual strength reduction of the partially resorbed template has to be compensated by the strength increase from the regenerated tissue such that at any given time point,
- The total mechanical properties of the template are maintained.
- In order to accomplish this goal, one must first be certain that the initial mechanical properties are adequate for supporting the weight-bearing application.
- For example, compressing the implant with multiple body weights should not cause fraying of the collagen matrix material.
- It is also of particular importance to design an implant having an adequate and consistent suture pullout strength in order to reduce the incidence of detachment of the implant from the host tissue.
- The suture pullout strength is also important during surgical procedures as the lack of suture pull strength may result in retrieval and reimplantation of the template.
- In meniscal tissue repair the suture pullout strength of 1 kg has been found to be adequate for arthroscopically assisted surgery in simulated placement procedures in human cadaver knees, and this suture pullout strength should be maintained as the minimal strength required for this particular application.
- **Hydrophilicity** Hydration of an implant facilitates nutrient diffusion. The extent of hydration would also provide information on the space available for tissue ingrowth.

- The porous collagen matrix is highly hydrophilic and therefore facilitates cellular ingrowth.
- The biomechanical properties of the hydrophilic collagen matrix such as fluid outflow under stress, fluid inflow in the absence of stress, and the resiliency for shock absorption are the properties also found in the weight-bearing cartilagenous tissues.
- Permeability The permeability of ions and macromolecules is of primary importance in tissues that do not rely on vascular transport of nutrients to the end organs.
- The diffusion of nutrients into the interstitial space ensures the survival of the cells and their continued ability of growth and synthesis of tissue specific extracellular matrix. Generally, the permeability of a macromolecule the size of the bovine serum albumin (MW 67,000) can be used as a guideline for probing accessibility of the interstitial space of a collagen template
- In Vivo Stability As stated above, the rate of template resorption and the rate of new tissue regeneration have to be balanced so that the adequate mechanical properties are maintained at all times.
- The rate of in vivo resorption of a collagen-based implant can be controlled by controlling the density of the implant and the extent of intermolecular crosslinking.
- The lower the density, the greater the interstitial space and generally the larger the pores for cell infiltration, leading to a higher rate of matrix degradation. The control of the extent of intermolecular crosslinking can be accomplished by using bifunctional crosslinking agents under conditions that do not denature the collagen.
- Glutaraldehyde, formaldehyde, adipyl chloride, hexamethylene diisocyanate, and carbodiimides are among the many agents used in crosslinking the collagenbased implants.

- Crosslinking can also be achieved through vapor phase of a crosslinking agent. The vapor phase crosslinking is effective using crosslinking agents of high vapor pressures such as formaldehyde and glutaraldehyde.
- The vapor crosslinking is particularly useful for thick implants of vapor permeable dense fibers where crosslinking in solution produces nonuniform crosslinking.
- In addition, intermolecular crosslinking can be achieved by heat treatment under high vacuum. This treatment causes the formation of an amide bond between an amino group of one molecule and the carboxyl group of an adjacent molecule and has often been referred to in the literature as dehydrothermal crosslinking.
- The shrinkage temperature of the crosslinked matrix has been used as a guide for in vivo stability of a collagen implant .
- The temperature of shrinkage of collagen fibers measures the transition of the collagen molecules from the triple helix to a random coil conformation. This temperature depends on the number of intermolecular crosslinks formed by chemical means.
- Generally, the higher the number of intermolecular crosslinks, the higher the thermal shrinkage temperature and more stable the material in vivo. A second method of assessing the in vivo stability is to determine the crosslinking density by applying the theory of rubber elasticity to denatured collagen.
- Thus, the in vivo stability can be directly correlated with the number of intermolecular crosslinks introduced by a given crosslinking agent.
- Another method that has been frequently used in assessing the in vivo stability of a collagen-based implant is to conduct an in vitro collagenase digestion of a collagen implant. Bacterial collagenase is generally used in this application. The action of bacterial collagenase on collagen is different from that of mammalian collagenase .

- In addition, the enzymatic activity used in in vitro studies is arbitrarily defined. Thus, the data generated from the bacterial collagenase should be viewed with caution. The bacterial collagenase digestion studies, however, are useful in comparing a prototype with a collagen material of known rate of in vivo resorption.
- Each of the above parameters should be considered in designing a resorbable implant. The interdependency of the parameters must also be balanced for maximal efficacy of the implant.
- Tissue Engineering for Tissue and Organ Regeneration Biomedical applications of collagen have entered a new era in the past decade.
- The potential use of collagen materials in medicine has increasingly been appreciated as the science and technology advances.
- One major emerging field of biomedical research which has received rigorous attention in recent years is tissue engineering.
- Tissue engineering is an interdisciplinary science of biochemistry, cell and molecular biology, genetics, materials science, biomedical engineering, and medicine to produce innovative three-dimensional composites having structure/function properties that can be used either to replace or correct poorly functioning components in humans and animals or to introduce better functional components into these living systems.
- Thus, the field of tissue engineering requires a close collaboration among various disciplines for success. Tissue engineering consists primarily of three components: (1) extracellular matrix, (2) cells, and (3) regulatory signals (e.g., tissue specific growth factors).
- One of the key elements in tissue engineering is the extracellular matrix which either provides a scaffolding for cells or acts as a delivery vehicle for regulatory signals such as growth factors.
- Type I collagen is the major component of the extracellular matrix and is intimately associated with development, wound healing, and regeneration.

- The development of the type I collagen based matrices described in this review article will greatly facilitate the future development of tissue engineering products for tissue and organ repair and regeneration applications.
- To date, collagen-based implants have been attempted for many tissue and organ repair and regeneration applications.