International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.8, pp 408-421, 2016

Protein and Carbohydrate Biopolymers for Biomedical Applications

Sudhanshu Verma[1], I. Manjubala[1], U. NarendraKumar[2]*

[1]School of BioSciences and Technology, VIT University, Vellore 632 014, Tamilnadu, India[#] Present address: IBM India Pvt Ltd., TPO, Panchshil, Yerwada, Pune 411006
[2] Department of Manufacturing, School of Mechanical Engineering, VIT University, Vellore 632014, Tamilnadu, India

Abstract : Now a day the strategies to use regenerative medicines are hugely concentrated on recovery of pathologically modified tissue architecture by implanting the cell of different type in a supportive 3-D structure well known as scaffold made up of different biomaterials. From last few decades the more importance is given to such materials which are biologically active, show biocompatibility and biodegradability. These materials provides analogous environment to the ECM and provide induced rate of synthesis or growth of the tissue. There are several natural and synthetic polymers are available which shows intrinsic bioactivity, biocompatibility and biodegradability. These can be used in the application of drug delivery, trauma, wound healing, tissue engineering, and in the designing of different implantable medical devices. This review discusses different protein and carbohydrate biopolymers which are used in different tissue engineering applications.
Keywords : protein biopolymers, carbohydrate biopolymers, Biomedical.

Introduction:

Due to several major advancements in technology the field of tissue engineering has increased dramatically in last two decades. From 1990 tissue engineering is emerged as a promising alternative for the treatment of damaged tissue or organs. It provided an alternative way to treat the patient in case of unavailability of appropriate number of donors. It has also reduces the trauma and the inflammatory response complications *[1-3]*. Another important aspect for using these materials is that availability of these materials in a large scale at low cost *[4, 5]*. Due to all these properties the great efforts had been put down to make protein and polysaccharides as biomaterials for tissue engineering and drug delivery application *[6-9]*. Protein biopolymers can be used for different purposes like provide a matrix for specific kind of cells for controlled growth *[10, 11]*, for drug delivery on specific sites to save it from first metabolic digestion and from unusual side effect on other tissues *[12-14]* or it can also act as a carrier to carry some specific kind of proteins or other compound to the specific target. Like protein there are several carbohydrate polymers which possess good biocompatibility, bioactivity and biodegradability. They also provide lot of option that to be changed according to our wish. All these quality of carbohydrates attracted the researchers specifically associated with regenerative medicine and tissue engineering.

Here in this review we had tried to summarize the use of different proteins and polysaccharides which are found naturally as biomaterials which are extensively been used in tissue engineering applications, as drug delivery systems.

Protein Biopolymers:

Collagen:

It is a fibrous protein containing three long chains of peptides, by the formation of intra-molecular hydrogen bond between Gly and Hyp of adjacent chains they forms triple helical structure *[15]*. Connective tissue of the mammals is highly consists of collagen and it is the most abundant protein which is found in mammals. It constitute about 25-35% of the body protein content *[16]*. Inside the body a bundle of collagen fibrils forms a collagen fiber. Collagen fibers are the major component of ECM which also provides support to nearly all tissue. Collagen possess high tensile strength, and is the major constituent of cartilage, bone, skin, tendons, ligaments and fascia.

As biomaterial for biomedical application collagen provides excellent biocompatibility and the immunogenicity is also negligible. It also shows very good bio- absorbability. Collagen is also a non-toxic material *[17]*. The partial hydrolysis of collagen under mild conditions provide separation of all three strands random coils, globular, give rise to the production of gelatin. Gelatin shows low antigenicity in comparison to collagen.

In research of last few decades collagen had been evaluated for several applications like in bone or cartilage constructs, in the treatment of various vascular diseases, for skin and ocular applications, neural application, in urogenital disease treatment, wound healing and in drug delivery.

Collagen based porous scaffolds in conjunction with cross linking agent hydroxyapatite *[18-20]* or bhrusite *[21, 22]* is used in when osteochondral defect reach above a certain extent. Some researches show that some amount of autologous chondrocytes grows on type I and type II collagen without any important difference *[23, 24]*. Collagen has also shown promising material for the decellularization of a very complex structure like meniscus as a replacement scaffold in optimal condition *[23, 24]*.

In vascular disease collagen can also be used as a biomaterial. The major problems associated with vascular diseases are there are some irregularities or improper functioning of heart and atherosclerosis. At present tissue engineering application only depends on colonization of acellular matrix and implantation of some specific part. This all is because of very complex structure of heart *[25, 26]*. Although the xenogenic heart valves are used frequently, but it is not a full proof treatment, because it possesses the tendency of getting calcified and it is also immunogenic *[27]*. Decellularization of complete heart by perfusion *[28]*, heart valves and veins production by using matrix of human vein *[29]* would lead the development in the field of regenerative medicine for cardiovascular disease based on collagen.

Collagen can also be used as biomaterial for different skin and ocular based regenerative medicine. For example, in the treatment of ulcer and wound caused due to burning collagen based material are used from long time *[30-32]*. With melanocytes models of tissue engineered skin have been developed *[33]*. Several collagen based skin, dressing and dermal substitute like Integra^{R}, Alloderm^{TM} are available commercially. Stem cell in combination with collagen biomaterial is also a good strategy which can be used for the treatment of defects in cornea. For the delivery of limbal epithelial cells for the treatment of damaged cornea collagen scaffolds were extensively examined in last decade *[34-39]*. Collagen scaffolds can also be used for urogenital complications. Patient's own urothelial cells which are populated on the collagen composite are also a promising way for augmentation of bladder *[40-42]*. Collagen based biomaterial is also proved as a very good nerve guide *[43-45]*. Collagen is also a promising biomaterial in the field of delivery systems. In the reconstruction of abdominal wall *[46-48]* and in the ulcer treatment *[49]* the delivery system based on collagen composite had displayed a great delivery potential.

Fibrin:

It is a fibrillar protein which is incorporated in blood clotting and made up of fibrinogen. Due to its cross-linking properties it has attracted the researcher to use fibrin in the formation of 2-D and 3-D mesh for drug delivery and tissue engineering *[50-53]*, which is plastic and biodegradable. The cross linking in fibrin is driven by activated factor III. This factor three catalyses the linking of ϒ-ϒ dimers by catalyzing the covalent lysyl-Glu bond formation *[54, 55]*. Cell attachment and cell proliferation is also promoted by fibrin and its degraded product. Beside all these qualities fibrin has certain drawbacks also. The materials made from fibrin

for tissue engineering application shows low mechanics. There are some reasons like instability and fibrinolysis. Due to these reasons fibrin usually appears as films which is used for cell adhesion, proliferation and differentiation, or as coating materials for the 3-D scaffolds for the repair of cartilage and neural injuries *[55, 56]*. Fibrin is used as a cell carrier in combination of fiber based scaffold for cardiovascular tissue engineering application and when it is compared with conventional method of seeding the cell after six weeks, fibrin based ECM appeared larger *[57]*. Fibrin is also used to fabricate renal proximal tubule for artificial kidney and it was also tested in vitro which shows correct polarization for the absorption of desired solutes from the glomerular filtrate *[58]*. Fibrin is also used in bone tissue engineering, two scaffolds of composite fibrin and thrombin were prepared for bone tissue engineering one has high thrombin concentration and other one has low thrombin concentration the result shows that the scaffold having low thrombin concentration and high fibrin concentration provide much better environment *[59]*. Scaffold of composite fibrin, fibronectin, gelatin and growth factors had shown a steady cell proliferation, good cell proliferation and it does not allow the dedifferentiation of endothelial cells in vitro *[60]*.

Keratin:

It is the family of tough, insoluble and structural protein which several cytoplasmic epithelia and epidermal appendageal structures like hair, horn, hooves and nails. Keratin is formed by a specialized cell known as keratinocytes. Keratin monomers are fibrous and they are twisted or wrapped around each other to for an intermediate filament. Analysis done for amino acid content of keratin had shown extraordinary high quantity of sulfur containing amino acids. Among others cysteine, which a sulfur containing amino acid was found in large quantity, it was ranging between 7-20% of the total amino acids. Cysteine forms inter and intra-molecular disulfide bonds which provides flexibility and tenacious property to the tissue *[61]*. Keratin shows good biocompatibility, bioactivity and biodegradability. Keratin owns its own cell adhesion sequences such as Arg-Gly-Asp (RGD) and Leu-Asp-Val (LVD) *[62, 63]*. It can be classified into two distinct groups on the basis of their function and regulation, (i) hard and (ii) is soft. Hard keratin form ordered arrays of IFs embedded in a matrix of cysteine rich proteins and contribute to the tough structure of epidermal appendages. Whereas Soft keratin normally forms loosely-packed bundles of cytoplasmic IFs and provide mechanical resilience to epithelial cells *[64-66]*. Several works are going on to make 3-D porous scaffolds and films for various biomedical applications. The major issue of keratin scaffold is that the scaffolds are very fragile that's why it cannot be used lonely in tissue engineering application. In recent years the studies has shown keratin as a promising biomaterial for biomedical application. The research on keratin is very less in comparison to other protein biopolymers. So till now very limited tissue engineering application are available. Keratin is used in different biomedical applications like in wound healing, ocular implants, nerve guiding or regeneration and in drug delivery. Keratin based film was developed in conjunction with 1-3% glycerol which act as softening agent.this film was for reconstruction of ocular surface. It was tested in vitro, which shows a very good result and its mechanical stability, bioactivity was also better *[67]*. It can also be used for the repairing of the nerves. Hill et al. prepared a keratin based hydrogel scaffolds for the repairing of peripheral nerve defects in rabbits. Although their scaffold was not able to repair the conduits concisely, but keratin shows better result in term of conduction delay in comparison to empty conduit and autograft *[68]*. Keratin based hydrogel has also demonstrated the capability of enhancing the regeneration and neuroinductivity in peripheral nerve injury model of mouse *[69, 70]*.

Zein:

Like starch, zein is also a storage protein. Zein is abundantly found in corn and constitutes about 40-50% of the total endospermic proteins. It belongs to the prolamines family of proteins which are soluble in the mixture of alcohol-water mixture. Solubility of zein in alcohol-water mixture is 60-95%. The solubility property of proteins depends on their amino acid composition. Zein contains huge amount of hydrophobic uncharged amino acid and have amino acids such as leucine (20%), proline (10%), and alanine (10%). Zein having molecular weight of 44 KDa is a heterogeneous mixture of amino acids which are linked by disulfide bonds. Zein contains about 50-60% α-helix, 15% β-sheets and remaining molecules are aperiodic *[71]*. It is used as coating agent in pharmaceuticals and food industry from long time. Zein is also used as adhesive, biodegradable plastics, chewing gum, and inks *[71, 72]* . It can be used as scaffold for tissue repairing, drug delivery vehicle in the treatment of various diseases, as a carrier of DNA for gene delivery. In 2009 J. Tu et al. fabricated a zein based scaffold by salt leaching method and seeded rabbit mesenchymal stem cells or investigating the bone formation. The scaffold was tested in vivo and they found the complexes of zein scaffold

and mesenchymal stem cell of rabbit could go for ectopic bone formation in nude mice in the region of thigh muscle pouches *[73]*. Zein is also used as drug carrier, in 2011 Karthikeyan et al. prepared zein microsphere by emulsification and solvent evaporation method and these microsphere were loaded with Aceclophenac. The stability of these microsphere were in gastric pH was checked in vitro. The result shows the retarded release of drug in this condition which reduces the risk of gastric injury in a result there will be no inflammation and side effect of the drug. The release of the drug was checked at intestinal pH in in vitro condition was 72 hours *[74]*. Zein can also be used as DNA delivery vehicle, M.C. Regier et al had fabricated zein based nanospheres in which the DNA can be entrapped and can be delivered at specific location. They prepared nanospheres which had encapsulated to DNA by using a coacervation technique without any use of harsh solvent and temperature so that the integrity of DNA will not disturbed. The particle size is ranging between 154- 410 nm *[75]*.

Casein:

It is a phosphoprotein which is found in milk and cheese. It constitutes nearly 80% of total milk proteins. Casein contains high number of proline peptide bond and lacks of disulfide bonds. That's why there are less secondary and tertiary structural elements. Due to its hydrophobic property it is less soluble in water. It present as suspension in the milk. It is used in the manufacturing of adhesives, binders, protective coatings, fabrics and food additives *[10, 76]*. For the tissue engineering purpose casein inexpensive, readily available, non-toxic and highly stable material. Casein microsphere is used as drug delivery vehicle. In compare to collagen, silk fibroin, keratin, fibrin, casein has very limited or specific area in which it can be used as biomaterial for biomedical application. It has very few work in the area tissue engineered bone or cartilages. Ritzoulis et al. prepared a porous scaffold of composite caseinate and hydroxyapetite for the application of bone tissue engineering. But in this caseinate was used to stabilize the emulsions of oil in water which made the link of hydroxyapetite on the surface of oil droplet by interaction of caseinate-calcium phosphate *[77]*. F. Song et al. prepared hydrogel of casein for controlled delivery of drugs in which the uses genipin as a cross linking agent. They used different concentration of genipin for evaluating its effect on the hydrogel formation. They observed that as they are increasing the concentration of genipin the gelling time is getting elongated whereas it increases the strength of the hydrogel. They also observed that temperature as an important parameter which affects the gelling. The genipin also stimulate the drug or protein release in different manner at gastrointestinal conditions *[78]*.

Albumin:

It is globular protein, which is soluble in water. When heat is exposed to albumin, it gets coagulated. It is frequently found in egg white, blood serum, milk and several other plant and animal tissues. Albumin of serum is one of the most abundant proteins which constitute about 55% of total blood plasma protein. The main application of albumin as biomaterial is microspheres used in pharmaceuticals. The size of these pharmaceutical microspheres varies from nanometer to micrometers. Albumin microspheres was first prepared by Rhodes and Zolle in mid of twentieth century, and the size of those microspheres was ranging from 5-15 µm. it contains ϒ radiation source, which was used for the determination of abnormalities in pulmonary circulation *[79, 80]*. Till now these microsphere have been used in targeted drug delivery to various tissues and organs *[81, 82]*. Like casein albumin also not so much used in the field of bone or cartilage tissue engineering, but is extensively used in the field of drug delivery, cell culture for different tissue formations. S. R. Lyu et al fabricated albumin based scaffold for promoting the neocartilage formation. They seeded and cultivated the porcine knee chondrocytes in a matrix composed of polyethylene oxide, chitin and chitosan which are coated with albumin. Although the quantity of did not shows any effect on the viability of the porcine cell, but it enhances the adherence of the cell to the 3-D matrix *[83]*. Albumin is also capable for delivering the drug as a carrier for the treatment of several diseases. It is used to form the nano size micelles for carrying the drugs. Y. Wu et al. developed the micellar based drug delivery vehicle for the treatment of cancer by loading it with anti-cancerous drug. They made the micelles from polycationic albumin precursor protein cBSA-147, and encapsulated doxorubicin (DOX) an anti cancerous drug. These micelles show a good stability in a wide range of pH and different physiological buffers. Their uptake into A549 cells was reported very well after the incubation period of 1 hour. They observe five times more cytotoxicity in comparison to free DOX. Intracellular drug release was also very efficient *[84]*. Albumin's applicability as a biomaterial had been also seen in the bone and cartilage tissue engineering applications. Earlier the mineralized scaffolds were used for procuring the defect of the bone by the means of an assumption that mesenchymal stem cells will grow and they will remodel the defect. But it was not a sure shot treatment. For this Weszl et al. used pre seeded scaffolds of freeze dried human or bovine bone graft or

hydroxyapetite which were seeded by human mesenchymal stem cell isolated from either bone marrow or dental pulp. Under standard culture condition it shows very less productivity of the cells, the coating of collagen and fibronectin improves the efficiency but they are still not sufficient. On the same time they uses human albumin coating, it provided potency of both seeding and proliferation and it also has no effect on the mechanical property of the scaffolds *[85]*.

Silk Fibroin:

It is a fibrous protein like keratin and collagen which is produced by insects like silk worm Bombyx mori *[86]*. It is the repeating protein sequences which has structural role in the formation of cocoon, trap, safety lines and the protection of eggs. It is being extensively investigated for many biomedical applications due its some good properties like easy in processing, poses good mechanical strength, stability, biocompatibility, controllable biodegradability, flexibility in morphology, and one unique property is that it can go for modification in its side chain of amino acid for the immobilization of growth factors *[86-88]*. Scaffold of silk fibroin are highly porous, also connectivity of pores is quite good. It shows the pore size ranging between 100-1000 μm. The porosity was more than 90%, better biodegradability and biocompatibility, and other important mechanical properties are characterized for these scaffolds *[89-92]*. Nanometric scaffold of silk fibroin possess very close similarity to ECM had been also demonstrated. It can also up-regulate integrin-β1 expression for inducing the pathway of adhesion of epithelial cells with micro fibrous scaffold sample *[93]*. Because of its low processing conditions, silk fibroin can be used as active biomaterials for drug delivery. Y. Gotoh et al. prepared lactose and silk fibroin based scaffold for the attachment of the hepatocyte cells and the observed that it is quite comparable to the collagen based scaffolds for the same. In term of morphological assessment it was different to the collagen *[94]*. H. Liu et al prepared a combined knitted silk scaffold and micro porous silk sponge for ligament tissue engineering. This combined structure was loaded with human mesenchymal stem cells. They observed that adherence, growing and cellular function was better in comparison to knitted scaffold *[95]*.

Carbohydrate Biopolymers:

In carbohydrate there are several polysaccharide polymers which are frequently used for different biomedical applications. Polysaccharides are large molecules having high molecular weights, which are made up of repeating units of same or different monomers. The use of the polysaccharides possesses some advantages like its wide availability, cost effective processing and huge range of properties and structures. Beside all these, due to the presence of reactive functional group it can be easily modified along with their polymeric chain. It also shows good account of biocompatibility and biodegradability. Most of the polysaccharide polymers are soluble in water. All these properties have attracted to use different polysaccharides for several biomedical applications. The polysaccharide biopolymers which are most frequently used are cellulose, starch, alginic acid, hyaluronic acid, chitin and chitosan, dextran.

Cellulose:

Cellulose is one of the most abundant polysaccharide which is found in nature. It forms the structural frame of the plants. It is the major constituent of the plant cell wall. It is composed of repeating units of D-glucose monomers. By enzymatic degradation it degrades in D-glucose units. It is a linear compound and soluble in many common solvents, the reason behind this is the presence of strong hydrogen bonding between polymeric chains. Cellulose possesses hydroxyl group which is very reactive and it can be easily functionalized. Several derivatives of cellulose like methyl cellulose, carboxy methyl cellulose, hydroxypropylcellulose, hydroxypropyl methyl cellulose are used in different biomedical applications. These derivatives are soluble in several common solvent and can be easily processed for various forms like films, sponges and nano or micro fibers. The membrane of cellulose possesses very high diffusion permeability to most of the toxic materials so it is used frequently as heamodialysis membrane in the dialysis *[96]*. F.A. Müller et al. fabricated cellulose based scaffold for cartilage repair. The scaffold was prepared by non-woven cellulose II. The scaffold was coated by calcium phosphate layer, which was obtained from the precipitate of super saturated physiological solution. The activation of scaffold is done by $Ca(OH)_2$. The chondrocytes cell adherence and proliferation of the scaffold was tested in vitro. In comparison to uncoated scaffold they found better response *[97]*. C. Remunan Lopez et al had designed the chitosan micro particles with the coating cellulose polymers like cellulose acetate butyrate or ethyl cellulose for controlled drug delivery system. The major issue with non-coated chitosan micro particles was that they dissolve easily in the stomach due to their hydrophilic properties. They used sodium diclofenac

(SD), fluorescein isothiocyanate-labeled bovine serum albumin (FITC-BSA) as a model compound for the investigation. The properties which were being investigated were entrapment of drug and its controlled release. The result shows that the entrapment ability was very good and its release can be also regulate *[98]*.

Starch:

It is basically reserve carbohydrates of the plants. After cellulose and chitin it is third most abundant polysaccharide found in nature. It can be usually isolated from wheat, potato, rice, sweet potato etc. It is basically a combination of amylase and amylopectin. Starch had about 20-30% of amylase which is linear in configuration and 70-80% of amylopectin which is branched polymer. Like cellulose is composed of D-glucose, amylase and amylopectin both also are consist D-glucose repeating units. The only difference in both is that amylase exhibits α (1-4) linkage whereas amylopectin exhibit α (1-6) linkage. In enzymatic degradation of starch the (1-4) linkage is attacked amylase and (1-6) linkage is attacked by glucosidases. By the processing of starch different structures like films, scaffold and fibers can me modified easily for different biomedical applications. Starch also shows a good biocompatibility and biodegradability. Contramid is the trade name of a product is marketed in the market which is made up of amylase rich starch. Bio adhesive drug delivery system for nasal protein delivery starch microsphere has been investigated *[99]*.For starch based scaffold in biomedical application several novel fabrication techniques had been developed *[100]*. The issues related with starch as a biomaterial is that, the construct made up of starch are very brittle *[101]*, and its difficult processing. Beside all these it is used as biomaterials for different biomedical applications. I. Pashkuleva et al. fabricated scaffold of 50% by weight starch scaffold which was highly porous and had interconnected mesh. The purpose of their research was to overcome to the drawbacks which were faced by the previously designed scaffold of starch like thermal degradation and entrapment of starch in material bulk etc. The topographical observations show rough surfaces which enhances cell attachment and growth. In vitro test which was done by seeding the osteoblast cell on the scaffold shows high cell number and increased culture time. Model modification by plasma was also carried out for the versatility of scaffold for further improvements *[102]*. M.I. Santos et al. prepared a scaffold of starch and poly(caprolactone) (SPCL). Their main goal of this research to demonstrate the interaction of endothelial cells (EC) and SPCL fiber meshes. They found that both micro and macro ECs which are growing on the SPCL mesh are able to maintain a normal expression of EC specific genes/proteins. This indicates the potential of this mesh for vascularization process of bone in vivo *[103]*.

Alginic Acid:

It is obtained from brown algae's cell wall, and is a linear hetropolymer. It consist of D-mannuronic acid and l-guluronic acid. It available as sodium salt, sodium alginate commercially. There is block copolymer region which has huge amount of mannuronic and guluronic acid and also had copolymer regions of both these sugars. In presence of divalent cations alginate can form gels due to occurrence of carboxyl groups along with the polymer chain. High water absorption capacity and porosity of calcium alginate gel and the haemostatic potential of alginates make them an attractive candidate for developing wound dressings materials *[104]*. Cell immobilization in alginate is very well established technology which is frequently being used in field of biomedical and biotechnology. AlgiDERM, Algisite, Hyperion, and Kaltostat are the different trade name under which the alginate based wound dressing materials are commercially available. Because the formation of scaffolds for encapsulation of cells or drugs in alginic acid is very feasible, fast and simple so it is extensively used as matrices for cell encapsulation and for artificial organ and for drug delivery*[105]*. Lishan Wang et al fabricated a novel wound dressing material from chitosan-alginate polyelectrolyte complex (PEC). The MTT assay and NMR results of this membrane demonstrated that they are non-toxic for mouse and human fibroblast cells. These membranes shows accelerated wound healing in a model of rat in comparison to conventional guaze dressing *[106]*. Gianni Ciofani et al demonstrated the efficiency of controlled protein delivery by alginate microsphere for neural regeneration. They loaded Netrin-1 which is an axon guidance protein. The release efficiency of these microspheres was tested in vitro experiment. They basically assessed action of axonal guidance on neuronal cell of embryo. The result shows that the grow of neuronal axon approaching Netrin-1 sources *[107]*. Z. Li et al. fabricated a scaffold based on chitosan and alginate and compared its properties with chitosan scaffold. The result shows that the hybrid scaffolds possess higher mechanical strength. The attachment and proliferation of osteoblast cell was also better *[108]*.

Hyaluronic Acid:

Like above all other polysaccharides, it is also a naturally occurring polysaccharide. It is linear and anionic polysaccharide which consists of repeating disaccharide units. Hyaluronic acid is the major compound and component of articulating cartilage. It is important component of connective tissue and is widely distributed in synovial as well as vitreous fluids of mammals. Its main function in synovial joints is to provide viscosity to the synovial fluid, so that friction between the bones can be avoided. In the mesenchymal embryo hyaluronic acid is also present. The chemical composition of hyaluronic acid is D-glucuronic acid and 2-acetamido-2-deoxy-D-glucose monosaccharide units. It is highly soluble in the water and provides very viscous solutions. It had many properties which make it an ideal candidate for wound dressing application *[109]*. It act as scavengers for free radicals at wound sites, in response it modulates the inflammation, the other important thing which make it unique is that it can interact several other biomolecules. The cells which are associated with the repairing of the tissue has a receptor for several antigens like bacteria, hyaluronic acid provide a bacteriostat which can be recognized by those cells. Several hyaluronic acid derivatives are like ethyl/benzyl (HYAFF) esters are used very frequently in wound dressing. The degradation rate and solubility of HA can be very effectively controlled by the quantity of esterification *[110]*. Hyaluronic acid is also known for increasing the rate of collagen deposition and angiogenesis by enhancing the migration and differentiation of mesenchymal and epithelial cells *[109]*. Hyaluronic acid possesses several properties which an ideal biopolymer is supposed to own. In the result of this it is used very frequently in many biomedical applications. It has attracted researcher as an application for biomedical application due to its mucoadhesive property and safety. H.S. Yoo et al. prepared a scaffold of PLGA and PEG then modified it by hyaluronic acid. After that seeding of osteoblast cell was done. They observed that hyaluronic modified PLGA scaffold shows better attachment of osteoblast cell in comparison to unmodified one. They also observed the increase in the synthesis of glycosaminoglycans and total collagen by modified scaffold *[111]*. S. Yamane et al. fabricated a scaffold which is hybrid of chitosan and hyaluronic acid by wetspinning method. They seeded rabbit chondrocytes on that and compared its cell adhesivity, differentiation, morphology, and synthesis of the extracellular matrix with native chitosan scaffolds. They observed that hybrid scaffold shows better results for each parameter *[112]*. M. Halbleib et al. made a hyaluronic acid based scaffold for tissue engineering of white adipose tissue. They seeded human adipocyte precursor cells which were obtained from enzymatic digestion of adipose tissue sample by collagenase. After the attachment of the cells their differentiation was induced with different defined adipogenic factors under serum free culture conditions. The result shows high degree of cell attachment and differentiation *[113]*.

Chitin and Chitosan:

It is a naturally available polysaccharide which is the major constituent of exoskeleton of crustaceans, insects and also found in the cell wall of the fungi. After cellulose chitin is the second most abundant polysaccharide which is found in nature. It consist of β (1-4) linked glycan, which had 2-acetamido-2-deoxy-D-glucose. The presence of N-acetyl glucosamino group in chitin shows its structural similarity with hyaluronic acid which had a very good wound healing property, so it is obvious that chitin will also show the wound healing property and in actual it possesses. Chitin mats, scaffolds, films and fibers are frequently used for wound healing. Chitin is insoluble in several common solvent so its deacetylated derivative chitosan is extensively used and studied polymer for biomedical applications. Chitosan is a linear polymer which is present in semi-crystalline form. It consist of β (1-4) linked D-glucosamine along with N-acetylglucosamine group which is randomly distributed. It is completely soluble in acidic water, or in solution whose pH is less than 5 *[114]*. In, in-vivo condition it degraded by lysozyme and its byproduct is also non-toxic. The degree of acetylation and crystallinity of the chitosan decides its degradation rate *[114]*. The processing of chitosan is very easy and it has very good biodegradability, biocompatibility and bioactivity so it has attracted researcher for several biomedical application. In fact chitosan is used very commonly for wound especially for burnt wound as dressing material. It is because of its applicability, permeability to oxygen, water absorbing property and it has also ability to induce the secretion of interleukin-8 from fibroblasts which is basically involved in the migration of endothelial and fibroblast cells *[115]*. The activity of antibacterial drugs which is used for wound dressing shows better result when it is used in conjunction of chitosan based dressing materials *[116]*. It can be used as biomaterial for tissue engineering, drug delivery and wound healing also. AA. Abbas et al. prepared porous PVA-chitosan based hydrogel which will act as ECM scaffold cartilage regeneration. They seeded chondrocytes which were derived from New Zealand white rabbits. The result shows enhanced chondrogenesis of the seeded cells, which shows that PVA- chitosan based hydrogel, has a huge potential of carrying cells *[117]*. T. Tanabe et al. prepared a keratin chitosan composite film for biomedical application. It was compared

with chitosan and keratin films. They observed that the composite film shows good attachment and proliferation of fibroblast cells and come to conclusion that it can be a good substrate for mammalian cell culture *[118]*. Min Sup Kim et al. fabricated polycaprolactone-chitin based nanofibrous mats for tissue engineering application. By the addition of chitin the porosity of the scaffold was increased and the contact angle with water decreases as there is increase in the quantity of the chitin increases in the scaffold. The mechanical strength was also higher in the hybrid scaffold. The in vitro studies showed that the viability of human dermal fibroblast was also higher in composite *[119]*.

Conclusion:

As in above discussion, the wide range of availability of protein and carbohydrate biopolymers had significantly affected the growth rate of use of these biopolymers for different biomedical applications. Beside all this tissue engineering is a field is in infanticide which had a vast potential of growth and it will nourished by the development cell and molecular biology. This will requires novel biomaterials which will not possess only good physical and mechanical properties but also shows a good interaction with biological components because tissue engineering is a field which has strong integration with biology. Similarly field of drug delivery either it is targeted or stimuli-sensitive will requires functionalized biopolymers which had better biocompatibility and biodegradability.

References:

1. Langer, R. and J. P. Vacanti(1993); Tissue engineering; Science; Vol.260, No.5110, pp 920-926.
2. Laurencin, C. T., Y. Khan, M. Kofron, S. El-Amin, E. Botchwey, X. Yu, and J. A. Cooper, Jr.(2006); The ABJS Nicolas Andry Award: Tissue engineering of bone and ligament: a 15-year perspective; Clinical orthopaedics and related research; Vol.447, pp 221-236.
3. Hunziker, E. B.(2002); Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects; Osteoarthritis Cartilage; Vol.10, No.6, pp 432-463.
4. Wang, Xianyan, Hyeon Joo Kim, Cheryl Wong, Charu Vepari, Akira Matsumoto, and David L. Kaplan(2006); Fibrous proteins and tissue engineering; Materials Today; Vol.9, No.12, pp 44-53.
5. Hannachi Imen, E., M. Nakamura, M. Mie, and E. Kobatake(2009); Construction of multifunctional proteins for tissue engineering: epidermal growth factor with collagen binding and cell adhesive activities; Journal of Biotechnology; Vol.139, No.1, pp 19-25.
6. Quaglia, F.(2008); Bioinspired tissue engineering: the great promise of protein delivery technologies; International Journal of Pharmaceutics; Vol.364, No.2, pp 281-297.
7. Wang, Xiaoqin, Esther Wenk, Xiao Hu, Guillermo R. Castro, Lorenz Meinel, Xianyan Wang, Chunmei Li, Hans Merkle, and David L. Kaplan(2007); Silk coatings on PLGA and alginate microspheres for protein delivery; Biomaterials; Vol.28, No.28, pp 4161-4169.
8. Kottke-Marchant, K., J. M. Anderson, Y. Umemura, and R. E. Marchant(1989); Effect of albumin coating on the in vitro blood compatibility of Dacron arterial prostheses; Biomaterials; Vol.10, No.3, pp 147-155.
9. Maltais, Anne, Gabriel E. Remondetto, and Muriel Subirade(2009); Soy protein cold-set hydrogels as controlled delivery devices for nutraceutical compounds; Food Hydrocolloids; Vol.23, No.7, pp 1647-1653.
10. Abu Diak, O., A. Bani-Jaber, B. Amro, D. Jones, and G. P. Andrews(2007); The manufacture and characterization of casein films as novel tablet coatings; Food and Bioproducts Processing; Vol.85, No.3 C, pp 284-290.
11. Yamauchi, K., H. Hojo, Y. Yamamoto, and T. Tanabe(2003); Enhanced cell adhesion on RGDS-carrying keratin film; Materials Science and Engineering C; Vol.23, No.4, pp 467-472.
12. Wenk, E., A. J. Wandrey, H. P. Merkle, and L. Meinel(2008); Silk fibroin spheres as a platform for controlled drug delivery; Journal of Controlled Release; Vol.132, No.1, pp 26-34.
13. Ruszczak, Z. and W. Friess(2003); Collagen as a carrier for on-site delivery of antibacterial drugs; Advanced Drug Delivery Reviews; Vol.55, No.12, pp 1679-1698.
14. Mandal, B. B., S. Kapoor, and S. C. Kundu(2009); Silk fibroin/polyacrylamide semi-interpenetrating network hydrogels forÂ controlled drug release; Biomaterials; Vol.30, No.14, pp 2826-2836.

15. Zeugolis, D. I., S. T. Khew, E. S. Y. Yew, A. K. Ekaputra, Y. W. Tong, L. Y. L. Yung, D. W. Hutmacher, C. Sheppard, and M. Raghunath(2008); Electro-spinning of pure collagen nano-fibres - Just an expensive way to make gelatin?; Biomaterials; Vol.29, No.15, pp 2293-2305.
16. Di Lullo, G. A., S. M. Sweeney, J. KÃ¶rkkÃ¶, L. Ala-Kokko, and J. D. San Antonio(2002); Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen; Journal of Biological Chemistry; Vol.277, No.6, pp 4223-4231.
17. Thumann, G., A. Viethen, A. Gaebler, P. Walter, S. Kaempf, S. Johnen, and A. K. Salz(2009); The in vitro and in vivo behaviour of retinal pigment epithelial cells cultured on ultrathin collagen membranes; Biomaterials; Vol.30, No.3, pp 287-294.
18. Ciardelli, G., P. Gentile, V. Chiono, M. Mattioli-Belmonte, G. Vozzi, N. Barbani, and P. Giusti(2009); Enzymatically crosslinked porous composite matrices for bone tissue regeneration; Journal of Biomedical Materials Research - Part A; Vol.92, No.1, pp 137-151.
19. Dubey, D. K. and V. Tomar(2009); Role of the nanoscale interfacial arrangement in mechanical strength of tropocollagen-hydroxyapatite-based hard biomaterials; Acta Biomaterialia; Vol.5, No.7, pp 2704-2716.
20. Susan, Liao, Ngiam Michelle, K. Chan Casey, and S. Ramakrishna(2009); Fabrication of nano-hydroxyapatite/collagen/osteonectin composites for bone graft applications; Biomedical Materials; Vol.4, No.2, pp 025019.
21. 21. Tamimi, F., B. Kumarasami, C. Doillon, U. Gbureck, D. L. Nihouannen, E. L. Cabarcos, and J. E. Barralet(2008); Brushite-collagen composites for bone regeneration; Acta Biomaterialia; Vol.4, No.5, pp 1315-1321.
22. Jayaraman, M. and M. V. Subramanian(2002); Preparation and characterization of two new composites: Collagen-brushite and collagen octa-calcium phosphate; Medical Science Monitor; Vol.8, No.11, pp BR481-BR487.
23. Tebb, T. A., S. W. Tsai, V. Glattauer, J. F. White, J. A. M. Ramshaw, and J. A. Werkmeister(2007); Development of porous collagen beads for chondrocyte culture; Cytotechnology; Vol.52, No.2, pp 99-106.
24. Glattauer, V., J. F. White, W. B. Tsai, C. C. Tsai, T. A. Tebb, S. J. Danon, J. A. Werkmeister, and J. A. M. Ramshaw(2010); Preparation of resorbable collagen-based beads for direct use in tissue engineering and cell therapy applications; Journal of Biomedical Materials Research - Part A; Vol.92, No.4, pp 1301-1309.
25. Tedder, M. E., J. Liao, B. Weed, C. Stabler, H. Zhang, A. Simionescu, and D. T. Simionescu(2009); Stabilized collagen scaffolds for heart valve tissue engineering; Tissue Engineering - Part A; Vol.15, No.6, pp 1257-1268.
26. Eitan, Y., U. Sarig, N. Dahan, and M. MacHluf(2010); Acellular cardiac extracellular matrix as a scaffold for tissue engineering: In vitro cell support, remodeling, and biocompatibility; Tissue Engineering - Part C: Methods; Vol.16, No.4, pp 671-683.
27. Van Nooten, G., P. Somers, C. A. Cuvelier, F. De Somer, M. Cornelissen, E. Cox, M. Verloo, and K. Chiers(2009); Gamma radiation alters the ultrastructure in tissue-engineered heart valve scaffolds; Tissue Engineering - Part A; Vol.15, No.11, pp 3597-3604.
28. Ott, H. C., T. S. Matthiesen, S. K. Goh, L. D. Black, S. M. Kren, T. I. Netoff, and D. A. Taylor(2008); Perfusion-decellularized matrix: Using nature's platform to engineer a bioartificial heart; Nature Medicine; Vol.14, No.2, pp 213-221.
29. Teebken, O. E., C. Puschmann, I. Breitenbach, B. Rohde, K. Burgwitz, and A. Haverich(2009); Preclinical development of tissue-engineered vein valves and venous substitutes using re-endothelialised human vein matrix; European Journal of Vascular and Endovascular Surgery; Vol.37, No.1, pp 92-102.
30. Yannas, I. V., J. F. Burke, D. P. Orgill, and E. M. Skrabut(1982); Wound tissue can utilize a polymeric template to synthesize a functional extension of skin; Science; Vol.215, No.4529, pp 174-176.
31. Doillon, C. J. and F. H. Silver(1986); Collagen-based wound dressing: Effects of hyaluronic acid and firponectin on wound healing; Biomaterials; Vol.7, No.1, pp 3-8.
32. Peters, W. J.(1980); Biological dressings in burns. A review; Annals of Plastic Surgery; Vol.4, No.2, pp 133-137.

33. Regnier, M., M. J. Staquet, D. Schmitt, and R. Schmidt(1997); Integration of Langerhans cells into a pigmented reconstructed human epidermis; Journal of Investigative Dermatology; Vol.109, No.4, pp 510-512.
34. Levis, H. and J. T. Daniels(2009); New technologies in limbal epithelial stem cell transplantation; Current Opinion in Biotechnology; Vol.20, No.5, pp 593-597.
35. Schwab, I. R.(1999); Cultured corneal epithelia for ocular surface disease; Transactions of the American Ophthalmological Society; Vol.97, pp 891-986.
36. Zakaria, N., C. Koppen, V. Van Tendeloo, Z. Berneman, A. Hopkinson, and M. J. Tassignon(2010); Standardized limbal epithelial stem cell graft generation and transplantation; Tissue Engineering - Part C: Methods; Vol.16, No.5, pp 921-927.
37. Shortt, A. J., G. A. Secker, R. J. Lomas, S. P. Wilshaw, J. N. Kearney, S. J. Tuft, and J. T. Daniels(2009); The effect of amniotic membrane preparation method on its ability to serve as a substrate for the ex-vivo expansion of limbal epithelial cells; Biomaterials; Vol.30, No.6, pp 1056-1065.
38. Dravida, S., S. Gaddipati, M. Griffith, K. Merrett, S. Lakshmi Madhira, V. S. Sangwan, and G. K. Vemuganti(2008); A biomimetic scaffold for culturing limbal stem cells: a promising alternative for clinical transplantation; Journal of Tissue Engineering and Regenerative Medicine; Vol.2, No.5, pp 263-271.
39. Grueterich, M., E. M. Espana, and S. C. Tseng(2003); Ex vivo expansion of limbal epithelial stem cells: amniotic membrane serving as a stem cell niche; Survey of Ophthalmology; Vol.48, No.6, pp 631-646.
40. Bouhout, S., E. Perron, R. Gauvin, G. Bernard, G. Ouellet, V. Cattan, and S. Bolduc(2010); In vitro reconstruction of an autologous, watertight, and resistant vesical equivalent; Tissue Engineering - Part A; Vol.16, No.5, pp 1539-1548.
41. Atala, A., S. B. Bauer, S. Soker, J. J. Yoo, and A. B. Retik(2006); Tissue-engineered autologous bladders for patients needing cystoplasty; Lancet; Vol.367, No.9518, pp 1241-1246.
42. Magnan, M., F. Berthod, M. F. Champigny, F. Soucy, and S. Bolduc(2006); In vitro reconstruction of a tissue-engineered endothelialized bladder from a single porcine biopsy; Journal of Pediatric Urology; Vol.2, No.4, pp 261-270.
43. Chamberlain, L. J., I. V. Yannas, H. P. Hsu, G. Strichartz, and M. Spector(1998); Collagen-GAG substrate enhances the quality of nerve regeneration through collagen tubes up to level of autograft; Experimental Neurology; Vol.154, No.2, pp 315-329.
44. Archibald, S. J., C. Krarup, J. Shefner, S. T. Li, and R. D. Madison(1991); A collagen-based nerve guide conduit for peripheral nerve repair: an electrophysiological study of nerve regeneration in rodents and nonhuman primates; The Journal of comparative neurology; Vol.306, No.4, pp 685-696.
45. Colin, W. and R. B. Donoff(1984); Nerve regeneration through collagen tubes; Journal of Dental Research; Vol.63, No.7, pp 987-993.
46. Liyanage, S. H., G. S. Purohit, J. N. Frye, and P. Giordano(2006); Anterior abdominal wall reconstruction with a Permacol implant; Journal of plastic, reconstructive & aesthetic surgery; Vol.59, No.5, pp 553-555.
47. Bellows, C. F., W. Jian, M. K. McHale, D. Cardenas, J. L. West, S. P. Lerner, and G. E. Amiel(2008); Blood vessel matrix: a new alternative for abdominal wall reconstruction; Hernia; Vol.12, No.4, pp 351-358.
48. Ansaloni, L., F. Catena, S. Gagliardi, F. Gazzotti, L. D'Alessandro, and A. D. Pinna(2007); Hernia repair with porcine small-intestinal submucosa; Hernia; Vol.11, No.4, pp 321-326.
49. Sun, W., H. Lin, B. Chen, W. Zhao, Y. Zhao, Z. Xiao, and J. Dai(2010); Collagen scaffolds loaded with collagen-binding NGF-beta accelerate ulcer healing; Journal of Biomedical Materials Research - Part A; Vol.92, No.3, pp 887-895.
50. Bootle-Wilbraham, C. A., S. Tazzyman, W. D. Thompson, C. M. Stirk, and C. E. Lewis(2001); Fibrin fragment E stimulates the proliferation, migration and differentiation of human microvascular endothelial cells in vitro; Angiogenesis; Vol.4, No.4, pp 269-275.
51. Jegoux, F., E. Goyenvalle, M. Bagot D'arc, E. Aguado, and G. Daculsi(2005); In vivo biological performance of composites combining micro-macroporous biphasic calcium phosphate granules and fibrin sealant; Archives of orthopaedic and trauma surgery; Vol.125, No.3, pp 153-159.
52. Yamada, Y., J. S. Boo, R. Ozawa, T. Nagasaka, Y. Okazaki, K. Hata, and M. Ueda(2003); Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold; Journal of cranio-maxillo-facial surgery; Vol.31, No.1, pp 27-33.

53. Le Guehennec, L., E. Goyenvalle, E. Aguado, P. Pilet, M. Bagot D'Arc, M. Bilban, R. Spaethe, and G. Daculsi(2005); MBCP biphasic calcium phosphate granules and tissucol fibrin sealant in rabbit femoral defects: the effect of fibrin on bone ingrowth; Journal of Materials Science. Materials in Medicine; Vol.16, No.1, pp 29-35.
54. Ehrbar, M., S. C. Rizzi, R. Hlushchuk, V. Djonov, A. H. Zisch, J. A. Hubbell, F. E. Weber, and M. P. Lutolf(2007); Enzymatic formation of modular cell-instructive fibrin analogs for tissue engineering; Biomaterials; Vol.28, No.26, pp 3856-3866.
55. Ju, Y. E., P. A. Janmey, M. E. McCormick, E. S. Sawyer, and L. A. Flanagan(2007); Enhanced neurite growth from mammalian neurons in three-dimensional salmon fibrin gels; Biomaterials; Vol.28, No.12, pp 2097-2108.
56. Willerth, S. M., K. J. Arendas, D. I. Gottlieb, and S. E. Sakiyama-Elbert(2006); Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells; Biomaterials; Vol.27, No.36, pp 5990-6003.
57. Mol, A., M. I. van Lieshout, C. G. Dam-de Veen, S. Neuenschwander, S. P. Hoerstrup, F. P. Baaijens, and C. V. Bouten(2005); Fibrin as a cell carrier in cardiovascular tissue engineering applications; Biomaterials; Vol.26, No.16, pp 3113-3121.
58. Ng., Chee Ping, Yuhang Zhuang., Alex Wei Haw Lin., and Jeremy Choon Meng Teo.(2013); A Fibrin-Based Tissue-Engineered Renal Proximal Tubule for Bioartificial Kidney Devices: Development, Characterization and In Vitro Transport Study; International Journal of Tissue Engineering; Vol.Volume 2013, pp Article ID 319476, 319410 pages.
59. Karp, J. M., F. Sarraf, M. S. Shoichet, and J. E. Davies(2004); Fibrin-filled scaffolds for bone-tissue engineering: An in vivo study; Journal of Biomedical Materials Research - Part A; Vol.71, No.1, pp 162-171.
60. Wang, W., B. Li, Y. Li, Y. Jiang, H. Ouyang, and C. Gao(2010); In vivo restoration of full-thickness cartilage defects by poly(lactide-co-glycolide) sponges filled with fibrin gel, bone marrow mesenchymal stem cells and DNA complexes; Biomaterials; Vol.31, No.23, pp 5953-5965.
61. Katoh, K., T. Tanabe, and K. Yamauchi(2004); Novel approach to fabricate keratin sponge scaffolds with controlled pore size and porosity; Biomaterials; Vol.25, No.18, pp 4255-4262.
62. Tachibana, A., Y. Furuta, H. Takeshima, T. Tanabe, and K. Yamauchi(2002); Fabrication of wool keratin sponge scaffolds for long-term cell cultivation; Journal of Biotechnology; Vol.93, No.2, pp 165-170.
63. Tachibana, A., Y. Nishikawa, M. Nishino, S. Kaneko, T. Tanabe, and K. Yamauchi(2006); Modified keratin sponge: binding of bone morphogenetic protein-2 and osteoblast differentiation; Journal of Bioscience and Bioengineering; Vol.102, No.5, pp 425-429.
64. Moll, R., W. W. Franke, D. L. Schiller, B. Geiger, and R. Krepler(1982); The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells; Cell; Vol.31, No.1, pp 11-24.
65. Fraser, R. D., T. P. MacRae, D. A. Parry, and E. Suzuki(1986); Intermediate filaments in alpha-keratins; Proceedings of the National Academy of Sciences of the United States of America; Vol.83, No.5, pp 1179-1183.
66. Coulombe, P. A., O. Bousquet, L. Ma, S. Yamada, and D. Wirtz(2000); The 'ins' and 'outs' of intermediate filament organization; Trends in Cell Biology; Vol.10, No.10, pp 420-428.
67. Reichl, S., M. Borrelli, and G. Geerling(2011); Keratin films for ocular surface reconstruction; Biomaterials; Vol.32, No.13, pp 3375-3386.
68. Hill, P. S., P. J. Apel, J. Barnwell, T. Smith, L. A. Koman, A. Atala, and M. Van Dyke(2011); Repair of peripheral nerve defects in rabbits using keratin hydrogel scaffolds; Tissue Engineering - Part A; Vol.17, No.11-12, pp 1499-1505.
69. Sierpinski, P., J. Garrett, J. Ma, P. Apel, D. Klorig, T. Smith, L. A. Koman, A. Atala, and M. Van Dyke(2008); The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves; Biomaterials; Vol.29, No.1, pp 118-128.
70. Apel, P. J., J. P. Garrett, P. Sierpinski, J. Ma, A. Atala, T. L. Smith, L. A. Koman, and M. E. Van Dyke(2008); Peripheral nerve regeneration using a keratin-based scaffold: long-term functional and histological outcomes in a mouse model; The Journal of hand surgery; Vol.33, No.9, pp 1541-1547.
71. Lai, H. M., P. H. Geil, and G. W. Padua(1999); X-ray diffraction characterization of the structure of zein–Oleic acid films; Journal of Applied Polymer Science; Vol.71, No.8, pp 1267-1281.

72. Shukla, Rishi and Munir Cheryan(2001); Zein: the industrial protein from corn; Industrial Crops and Products; Vol.13, No.3, pp 171-192.
73. Tu, J., H. Wang, H. Li, K. Dai, J. Wang, and X. Zhang(2009); The in vivo bone formation by mesenchymal stem cells in zein scaffolds; Biomaterials; Vol.30, No.26, pp 4369-4376.
74. Karthikeyan, K., Rachita Lakra, Rama Rajaram, and Purna Sai Korrapati(2012); Development and Characterization of Zein-Based Micro Carrier System for Sustained Delivery of Aceclofenac Sodium; AAPS PharmSciTech; Vol.13, No.1, pp 143-149.
75. Regier, M. C., J. D. Taylor, T. Borcyk, Y. Yang, and A. K. Pannier(2012); Fabrication and characterization of DNA-loaded zein nanospheres; Journal of Nanobiotechnology; Vol.10, pp 44.
76. Somanathan, N., M. D. Naresh, V. Arumugam, and R. Sanjeevi(2000); Mechanism of failure of hydrolyzed casein films; European Polymer Journal; Vol.36, No.11, pp 2485-2490.
77. Ritzoulis, C., N. Scoutaris, K. Papademetriou, S. Stavroulias, and C. Panayiotou(2005); Milk protein-based emulsion gels for bone tissue engineering; Food Hydrocolloids; Vol.19, No.3, pp 575-581.
78. Song, Fei, Li-Ming Zhang, Chuan Yang, and Li Yan(2009); Genipin-crosslinked casein hydrogels for controlled drug delivery; International Journal of Pharmaceutics; Vol.373, No.1â€“2, pp 41-47.
79. Rhodes, B. A., I. Zolle, J. W. Buchanan, and H. N. Wagner, Jr.(1969); Radioactive albumin microspheres for studies of the pulmonary circulation; Radiology; Vol.92, No.7, pp 1453-1460.
80. Zolle, I., B. A. Rhodes, and H. N. Wagner Jr(1970); Preparation of metabolizable radioactive human serum albumin microspheres for studies of the circulation; The International Journal of Applied Radiation and Isotopes; Vol.21, No.3, pp 155-156.
81. Iemma, Francesca, U. Gianfranco Spizzirri, Francesco Puoci, Rita Muzzalupo, Sonia Trombino, Roberta Cassano, Sonia Leta, and Nevio Picci(2006); pH-Sensitive hydrogels based on bovine serum albumin for oral drug delivery; International Journal of Pharmaceutics; Vol.312, No.1-2, pp 151-157.
82. Gan, Chee-Yuen, Lai-Hoong Cheng, Eng-Tong Phuah, Pei-Ni Chin, Abbas F. M. AlKarkhi, and Azhar Mat Easa(2009); Combined cross-linking treatments of bovine serum albumin gel beadlets for controlled-delivery of caffeine; Food Hydrocolloids; Vol.23, No.5, pp 1398-1405.
83. Lyu, Shaw-Ruey, Yung-Chih Kuo, Min-Hsio Lin, Wen-Hsin Hsieh, and Chia-Wei Chuang(2012); Application of albumin-grafted scaffolds to promote neocartilage formation; Colloids and Surfaces B: Biointerfaces; Vol.91, pp 296-301.
84. Wu, Y., E. K. Shih, A. Ramanathan, S. Vasudevan, and T. Weil(2012); Nano-sized albumin-copolymer micelles for efficient doxorubicin delivery; Biointerphases; Vol.7, No.1-4, pp 5.
85. Weszl, Miklós, Gábor Skaliczki, Attila Cselenyák, Levente Kiss, Tibor Major, Károly Schandl, Eszter Bognár, Guido Stadler, Anja Peterbauer, Lajos Csönge, and Zsombor Lacza(2012); Freeze-dried human serum albumin improves the adherence and proliferation of mesenchymal stem cells on mineralized human bone allografts; Journal of Orthopaedic Research; Vol.30, No.3, pp 489-496.
86. Vepari, Charu and David L. Kaplan(2007); Silk as a Biomaterial; Progress in polymer science; Vol.32, No.8-9, pp 991-1007.
87. Fuchs, Sabine, Antonella Motta, Claudio Migliaresi, and Charles James Kirkpatrick(2006); Outgrowth endothelial cells isolated and expanded from human peripheral blood progenitor cells as a potential source of autologous cells for endothelialization of silk fibroin biomaterials; Biomaterials; Vol.27, No.31, pp 5399-5408.
88. Unger, R. E., M. Wolf, K. Peters, A. Motta, C. Migliaresi, and C. James Kirkpatrick(2004); Growth of human cells on a non-woven silk fibroin net: a potential for use in tissue engineering; Biomaterials; Vol.25, No.6, pp 1069-1075.
89. Kim, U. J., J. Park, H. J. Kim, M. Wada, and D. L. Kaplan(2005); Three-dimensional aqueous-derived biomaterial scaffolds from silk fibroin; Biomaterials; Vol.26, No.15, pp 2775-2785.
90. Kim, Hyeon Joo, Ung-Jin Kim, Gordana Vunjak-Novakovic, Byoung-Hyun Min, and David L. Kaplan(2005); Influence of macroporous protein scaffolds on bone tissue engineering from bone marrow stem cells; Biomaterials; Vol.26, No.21, pp 4442-4452.
91. Hofmann, S., H. Hagenmuller, A. M. Koch, R. Muller, G. Vunjak-Novakovic, D. L. Kaplan, H. P. Merkle, and L. Meinel(2007); Control of in vitro tissue-engineered bone-like structures using human mesenchymal stem cells and porous silk scaffolds; Biomaterials; Vol.28, No.6, pp 1152-1162.
92. Wang, Y., D. D. Rudym, A. Walsh, L. Abrahamsen, H. J. Kim, H. S. Kim, C. Kirker-Head, and D. L. Kaplan(2008); In vivo degradation of three-dimensional silk fibroin scaffolds; Biomaterials; Vol.29, No.24-25, pp 3415-3428.

93. Bondar, B., S. Fuchs, A. Motta, C. Migliaresi, and C. J. Kirkpatrick(2008); Functionality of endothelial cells on silk fibroin nets: comparative study of micro- and nanometric fibre size; Biomaterials; Vol.29, No.5, pp 561-572.
94. Gotoh, Y., S. Niimi, T. Hayakawa, and T. Miyashita(2004); Preparation of lactose-silk fibroin conjugates and their application as a scaffold for hepatocyte attachment; Biomaterials; Vol.25, No.6, pp 1131-1140.
95. Liu, H., H. Fan, Y. Wang, S. L. Toh, and J. C. Goh(2008); The interaction between a combined knitted silk scaffold and microporous silk sponge with human mesenchymal stem cells for ligament tissue engineering; Biomaterials; Vol.29, No.6, pp 662-674.
96. von Baeyer, Hans, Annemarie Lajous-Petter, Wolfgang Debrandt, Hannelore Hampl, Frank Kochinke, and Rolf Herbst(1988); Surface reactions on blood contact during haemodialysis and haemofiltration ith various membrane types; Journal of Membrane Science; Vol.36, pp 215-229.
97. Muller, Frank A., Lenka Muller, Ingo Hofmann, Peter Greil, Magdalene M. Wenzel, and Rainer Staudenmaier(2006); Cellulose-based scaffold materials for cartilage tissue engineering; Biomaterials; Vol.27, No.21, pp 3955-3963.
98. Remunan-Lopez, C., M. L. Lorenzo-Lamosa, J. L. Vila-Jato, and M. J. Alonso(1998); Development of new chitosan-cellulose multicore microparticles for controlled drug delivery; European journal of pharmaceutics and biopharmaceutics; Vol.45, No.1, pp 49-56.
99. Illum, L., I. Jabbal-Gill, M. Hinchcliffe, A. N. Fisher, and S. S. Davis(2001); Chitosan as a novel nasal delivery system for vaccines; Advanced Drug Delivery Reviews; Vol.51, No.1-3, pp 81-96.
100. Salgado, A. J., M. E. Gomes, A. Chou, O. P. Coutinho, R. L. Reis, and D. W. Hutmacher(2002); Preliminary study on the adhesion and proliferation of human osteoblasts on starch-based scaffolds; Materials Science and Engineering: C; Vol.20, No.1â€“2, pp 27-33.
101. Lee, Eun-Jung, Dong-Keon Kweon, Bong-Kyung Koh, and Seung-Taik Lim(2004); Physical characteristics of sweet potato pulp/polycaprolactone blends; Journal of Applied Polymer Science; Vol.92, No.2, pp 861-866.
102. Pashkuleva, Iva, Paula M. LÃ³pez-PÃ©rez, Helena S. Azevedo, and Rui L. Reis(2010); Highly porous and interconnected starch-based scaffolds: Production, characterization and surface modification; Materials Science and Engineering: C; Vol.30, No.7, pp 981-989.
103. Santos, M. I., S. Fuchs, M. E. Gomes, R. E. Unger, R. L. Reis, and C. J. Kirkpatrick(2007); Response of micro- and macrovascular endothelial cells to starch-based fiber meshes for bone tissue engineering; Biomaterials; Vol.28, No.2, pp 240-248.
104. Horncastle, J.(1995); Wound dressings. Past, present, and future; Medical Device Technology; Vol.6, No.1, pp 30-34, 36.
105. Mumper, Russell J., Allan S. Huffman, Pauli A. Puolakkainen, Lisa S. Bouchard, and Wayne R. Gombotz(1994); Calcium-alginate beads for the oral delivery of transforming growth factor-β1(TGF-β1): stabilization of TGF-β1 by the addition of polyacrylic acid within acid-treated beads; Journal of Controlled Release; Vol.30, No.3, pp 241-251.
106. Wang, L., E. Khor, A. Wee, and L. Y. Lim(2002); Chitosan-alginate PEC membrane as a wound dressing: Assessment of incisional wound healing; Journal of Biomedical Materials Research; Vol.63, No.5, pp 610-618.
107. Ciofani, G., V. Raffa, A. Menciassi, S. Micera, and P. Dario(2007); A drug delivery system based on alginate microspheres: mass-transport test and in vitro validation; Biomed Microdevices; Vol.9, No.3, pp 395-403.
108. Li, Zhensheng, Hassna R. Ramay, Kip D. Hauch, Demin Xiao, and Miqin Zhang(2005); Chitosan - alginate hybrid scaffolds for bone tissue engineering; Biomaterials; Vol.26, No.18, pp 3919-3928.
109. Lloyd, L. L., J. F. Kennedy, P. Methacanon, M. Paterson, and C. J. Knill(1998); Carbohydrate polymers as wound management aids; Carbohydrate Polymers; Vol.37, No.3, pp 315-322.
110. Williams, D.(1997); Size and shape really matter: the influence of design on biocompatibility; Medical Device Technology; Vol.8, No.9, pp 8-12.
111. Yoo, H. S., E. A. Lee, J. J. Yoon, and T. G. Park(2005); Hyaluronic acid modified biodegradable scaffolds for cartilage tissue engineering; Biomaterials; Vol.26, No.14, pp 1925-1933.
112. Yamane, S., N. Iwasaki, T. Majima, T. Funakoshi, T. Masuko, K. Harada, A. Minami, K. Monde, and S. Nishimura(2005); Feasibility of chitosan-based hyaluronic acid hybrid biomaterial for a novel scaffold in cartilage tissue engineering; Biomaterials; Vol.26, No.6, pp 611-619.

113. Halbleib, M., T. Skurk, C. de Luca, D. von Heimburg, and H. Hauner(2003); Tissue engineering of white adipose tissue using hyaluronic acid-based scaffolds. I: in vitro differentiation of human adipocyte precursor cells on scaffolds; Biomaterials; Vol.24, No.18, pp 3125-3132.
114. Khor, Eugene and Lee Yong Lim(2003); Implantable applications of chitin and chitosan; Biomaterials; Vol.24, No.13, pp 2339-2349.
115. Ishihara, Masayuki, Kuniaki Nakanishi, Katsuaki Ono, Masato Sato, Makoto Kikuchi, Yoshio Saito, Hirofumi Yura, Takemi Matsui, Hidemi Hattori, Maki Uenoyama, and Akira Kurita(2002); Photocrosslinkable chitosan as a dressing for wound occlusion and accelerator in healing process; Biomaterials; Vol.23, No.3, pp 833-840.
116. Mi, F. L., Y. B. Wu, S. S. Shyu, J. Y. Schoung, Y. B. Huang, Y. H. Tsai, and J. Y. Hao(2002); Control of wound infections using a bilayer chitosan wound dressing with sustainable antibiotic delivery; Journal of Biomedical Materials Research; Vol.59, No.3, pp 438-449.
117. Abbas., AA, SY Lee., L Selvaratnam., N Yusof., and T Kamarul(2008); Porous PVA-Chitosan Based Hydrogel as an Extracellular Matrix Scaffold for Cartilage Regeneration; European Cells and Materials; Vol.16, No.2, pp page 50.
118. Tanabe, Toshizumi, Naoya Okitsu, Akira Tachibana, and Kiyoshi Yamauchi(2002); Preparation and characterization of keratinâ€“chitosan composite film; Biomaterials; Vol.23, No.3, pp 817-825.
119. Kim., Min Sup, Sang Jun Park., Bon Kang Gu., and Chun-Ho Kim.(2012); Polycaprolactone-Chitin Nanofibrous Mats as Potential Scaffolds for Tissue Engineering; Journal of Nanomaterials; Vol.2012, pp Article ID 635212, 635219 pages.

*****