1.0 Introduction

1.1 Biomaterials:

According to the American National Institute of Health, the latest accepted definition of biomaterials is “any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual”.

The biomaterials can be classified into three major classes namely, polymeric, metallic and ceramics. The biomaterials can also be synthesized by combination within the classes or among the classes to give newer hybrid biomaterials. The key factor that is desirable for a material to be considered as biomaterial is biocompatibility. Biocompatibility means the ability of a material to perform with an appropriate host response in a specific situation that implies the material should be non-toxic. However more extended criterion includes biodegradability and biofunctionality of the materials for specific applications.

Metallic biomaterials include certain metal and alloys or in different combination of metals such as stainless steel (316L), Co-Cr alloys, Ti6Al4V, Au-Ag-Cu-Pd alloys, amalgam (AgSnCuZnHg), Ni-Ti, Titanium etc. Metals as biomaterials have many advantages such as higher strength, fatigue resistance, wear resistance, easy fabrication, easy sterilization, shape memory properties etc. However there are certain disadvantages such as high modulus, corrosion, metal ion sensitivity and toxicity, metallic looking etc. Ceramics based biomaterials includes alumina, zirconia (partially stabilized), silicate glass, calcium phosphate (apatite), calcium carbonate etc. The advantages with ceramics are high compression strength, wear & corrosion resistance, highly polished surface and bioactive/inert etc. except for their brittle behavior and fabrication difficulties. Long term disadvantage includes fracture and corrosion. Polymer based biomaterials include both natural and synthetic polymers. Polymers are endowed with inherent flexibility in processing, modifying and functional tuning of physical and mechanical properties of various tissues and organs of the body.

Among the classes of biomaterials, polymers form a versatile class of biomaterials that have been extensively studied for biomedical applications. The polymeric biomaterials
can further be classified in to two categories based on the sources of polymers as natural and synthetic polymer based biomaterials. The synthetic polymers have certain advantages such as easier fabrication to complicated shapes, tailorable physical & mechanical properties, immobilize cell, biodegradable and biocompatible at least in some cases. However they have certain demerits, such as they are easily leachable, absorb water & proteins, undergoes surface contamination, prone to wear & breakdown, undesirable biodegradable products and difficult to sterilize etc. The natural polymer based biomaterials or the bio based biomaterials are endowed with biocompatibility, biodegradability and with no or minimal cyto-toxicity etc. Also they have biomimicking ability, natural bioactivity, at least for few of the naturally derived biopolymers.

The major applications of biomaterial can be classified into a) Tissue engineering scaffolds, b) Cardiovascular medical devices (stents, grafts and etc.), c) Orthopedic and dental applications d) Implants, e) Ophthalmologic applications (contact lenses, retinal prostheses etc.), f) Bio-electrodes and biosensors, g) Burn dressings and skin substitutes, h) Sutures and i) Drug delivery systems etc. Fig.1.1 gives a pictographic representation of the major application of biomaterials as functional substitutes for different organs.
None of the biomaterial satisfies all the criteria for different bioapplication due to diversity in requirement of physiology within a particular individual as well as diversity among individuals for specific bioapplication. This necessitates looking for newer biomaterial for specific application and also creates opportunity for existing biomaterial to find suitable application. The determining factor of biomaterial for suitable application remains in its functionality. Therefore suitable functional tuning remains the key to address the limitation within existing biomaterials. Polysaccharides have recently attracted enormous research interest for synthesising natural/semi-synthetic hydrogel and thus providing a suitable alternative as starting material for making different polysaccharide based hydrogels as biomaterials.

Amongst various chemical modification techniques, graft copolymerisation is one of the widely employed technique for functionality tuning of polysaccharide backbone forming hydrogels with desirable properties for different biomedical applications. The graft
copolymers are macromolecular chains with one or more species of the block connected to the main chain as side chain(s) and can be described as having the general structure, where the main polymer backbone, commonly referred to as the trunk polymer, has branches of another polymeric chain emanating from different points along its length. This approach enabled to achieve novel polysaccharide-based materials with improved properties including all the expected usefulness of these biomaterials. The graft copolymerisation involves, the ‘grafting from’ technique which involves the growth of polymer grafts directly from the polysaccharide backbone with a conventional free radical process. Radicals can indeed be conveniently generated along polysaccharide backbones in the presence of chemical initiators or by applying irradiation affording the straightforward preparation of polysaccharide-based graft copolymers through ‘grafting from’ free radical polymerization [Tizzotti et al. 2010].

This thesis involves study of mainly polymer based biomaterials especially from natural renewable polymers and therefore the following section deals with a brief discussion about polymeric biomaterials.

1.2 Polymer based biomaterial

The polymer based biomaterials can be categorised into synthetic and natural polymer based biomaterials. Both naturally derived and synthetic polymers have been investigated for scaffolding ability. Naturally derived polymers, e.g., protein and polysaccharides, have been exploited for tissue regeneration with the potential advantage of biological recognition which supports cell development. The main drawback of natural polymers is related to their natural variability. The proteins also have additional disadvantages apart from natural variability, and it elicit immunogenic responses. Collagen for example, has been used for tissue regeneration and scaffolding materials owing to its suitable surface chemistry for cell growth and differentiation [Mizuno et al. 1997]. However, collagens are associated with potential immunogenicity and pathogen transmission, along with poor mechanical properties, biodegradability and complications in handling.
1.2.1 Synthetic polymer based biomaterials:

The synthetic polymers have received considerable attention as biomaterials for tissue engineering because of their flexibility in composition, easier fabrication for specific needs with optimized features, high reproducibility, and predictable properties. For instance, biological activity and biodegradability can be imparted to polymers by modification involving chemical, physical or/and processing techniques. In addition to easier chemical modification of polymers for desirable characteristics, composite materials are designed to meet the various requirements in tissue engineering that single polymers cannot fulfill [Zhang et al. 1999; Liu et al. 2004; Zhanpeng et al. 2013]. The disadvantage associated with most of synthetic polymers is their lower biodegradability. There are however, certain synthetic polymer mostly belongs to polyester family such as polyglycolides and polylactides which degrade in vivo. However, they still show poor biocompatibility, loss of mechanical properties during degradation and release of acidic degradation products [Gunatillake et al. 2003].

The synthetic polymers used in biomaterials include hydrophilic materials such as poly (ethylene glycol) (PEG), poly(vinyl alcohol) (PVA), and poly (acrylamide) (PAAM) and hydrophobic polymers, such as poly(n-butyl acrylate), poly-(α-esters), are also widely employed. Amphiphilic polymers such as (PEG-b-PPO-b-PEG) and thermosensitive polymers namely poly(N-isopropylacrylamide) (pNIPAAM) have also been widely employed owing to their lower critical solution temperatures, which afford thermal sensitivity to control microstructure formation, drug delivery, and cell adhesion. Among these polymers, PEG in particular has widely been used as a biomaterial because of its solubility in a range of organic solvents, ease of end-functionalization, low polydispersity index (PDI), and reasonable cost [Zalipsky et al. 1995]. Consequently, functionalization of PEG with small molecule drugs, peptides, and proteins has been widely employed, with benefits such as increased circulation lifespan, reduced elimination pathways, and improved efficacy [Hamidi et al. 2006; Baldwin et al. 2010].

1.2.2 Natural biopolymer based biomaterials:

The different natural biopolymers used as biomaterials include polysaccharides and proteins in different modified form and/or in conjugation with other bioactive molecules. Apart from their natural advantages of biocompatibility and biodegradability, they are in general cheap and renewable, anti-immunogenic. They are also associated with certain demerits such as low mechanical strength, difficulty in processing, batch-to-batch variation
etc. However, the advantages of natural polymer over synthetic polymer are their compositional uniqueness that simulates a specific cellular response, bioactivity etc. that overrides the advantages of synthetic polymers. Both the class of natural polymers have been extensively investigated for biomedical application. However amongst natural polymers, polysaccharides have the advantages over protein as they do not elicit any immunogenic response as observed with protein based biomaterials. Further the natural biopolymers form important class of biomaterials namely hydrogels.

Amongst natural polymers, polysaccharides offer huge potential as biomaterial and have oriented the researchers’ attention towards itself for newer avenues in biomedical applications. Polysaccharides unlike proteins and nucleic acids, contain repetitive structural features, which are polymers of monosaccharide residues joined by glycosidic linkages. Among the macromolecules, polysaccharides offer the huge capacity for carrying biological information because they have the greatest potential for structural variability. The functional diversity of polysaccharides endows upon to their structural diversity which allows them for different binding affinities with biomolecules involved in several important physiological and pathological processes. Polysaccharides are itself important biomolecules involved in a various physiological events such as cell signalling and adhesion. The interaction of polysaccharide to proteins and other signalling molecules modulate their activity, thereby influencing fundamental biological processes [Silva et al. 2014]. Polysaccharides are structurally diverse class of macromolecules, plays diverse and important roles in many biological processes. Apart from serving as energy stores (e.g. starch and glycogen) and structural components (e.g. chitin in arthropods and cellulose in plants), polysaccharides and its derivatives participate in cell signalling and communication along with important roles in the fertilization, pathogenesis prevention, blood clotting, immune system, and system development [Naveen et al. 2011; Zong et al. 2012; Raman et al. 2005; Wang et al. 2010]. Moreover, polysaccharides are endowed with a large number of reactive groups, have wide range of molecular weights, and varying chemical composition, which contribute to their diverse structural/functional properties. Due to the presence of various derivable groups on molecular chains (e.g. hydroxyl), polysaccharides can be easily modified chemically, resulting in many kinds of polysaccharide derivatives. Due to their dominant role in biological processes and their versatile functionality, natural biocompatibility, biodegradability, environmental friendliness, polysaccharides can be considered to be the “sleeping giant” of biotechnology.
1.3 Hydrogel based biomaterials

Hydrogel have been used as biomaterials and extensively investigated owing to its biomimicking ability. Hydrogels are three-dimensional macromolecular networks crosslinked by chemical or physical interactions that can imbibe large amount of water and can be designed to mimic the mechanical and chemical properties of natural tissue environments [Peppas et al 2000]. Due to their high water content, porosity and soft consistency, they closely simulate natural living tissue, more so than any other class of synthetic biomaterials. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve [Peppas et al. 2000]. Hydrogels can be made from different water-soluble polymer, encompassing a wide range of chemical compositions and bulk physical properties. Also, hydrogels can be made in to a variety of physical forms, such as slabs, microparticles, nanoparticles, coatings, and films enabling hydrogels to be used in clinical practice and experimental medicine for a wide range of applications, including tissue engineering and regenerative medicine, diagnostics, cellular immobilization, separation of biomolecules or cells, and barrier materials to regulate biological adhesions [Lee et al. 2001; Jen et al. 1996; Bennett et al. 2003; Hoare et al. 2008].

The control of hydrogel structure offers wide range of opportunity that meet various needs in specialized fields and therefore has been extensively studied [Nie et al. 2014]. Hydrogels are typically modified with drugs for controlled release, as well as with cellular adhesion domains for soft tissue regeneration. Further, dehydrated rigid scaffolds, dense polymeric materials; have been employed in hard tissue-engineering applications [Baldwin et al. 2010].

The ‘physical’ or ‘reversible’ gel involves molecular entanglements by secondary interaction such as as ionic, H-bonding or hydrophobic forces for network formation. Physical gels are reversible and often can be dissolved by changing environmental conditions, such as pH, and the ionic strength of solution or temperature. The ‘permanent’ or ‘chemical’ gel involves covalent interactions for network formation, Theses gels may be charged or neutral depends on the functional groups present in the polymeric network. The charged hydrogels usually exhibit changes in swelling upon variations in pH, and it is known that they can undergo changes in shape when exposed to an electric field [Alvarez-Lorenzo et al. 2013]. Chemical hydrogels are commonly prepared in two different ways: ‘three-dimensional polymerization’, in which a hydrophilic monomer is polymerized in the presence of a polyfunctional cross-linking agent, or by direct cross-linking of water-soluble polymers.
Polymerization is usually initiated by free-radical generating compounds such as benzoyl peroxide, 2,2-azo-isobutyronitrile (AIBN), and ammonium persulphate or by using UV, gamma or electron beam-radiation [Peppas et al. 2000].

1.3.1 Polymer hydrogels

Polymer hydrogels have been widely explored as therapeutic delivery matrices because of their ability to present sustained, localized and controlled release of bioactive factors. Different materials both synthetic and natural polymers are used for synthesis of hydrogels. The synthetic polymers such as water soluble polymers includes polyacrylic acid, polyvinyl alcohol, polyvinyl pyrrolidone, polyethylene glycol, polyacrylamide and natural polymers include polysaccharides (e.g. alginate, chitosan, hyaluronic acid, gelatin, heparin etc.) and proteins (e.g. gelatin, silk fibroin, collagen, keratin, elastin etc.) are the most common systems used to form hydrogels [Khan et al 2013, Silva et al 2014]. These water-soluble polymers are nontoxic and widely used in various pharmaceutical and biomedical applications. The hydrogels made by cross-linking of natural polymers (such as gelatine or agar) and synthetic polymers [such as polyvinyl pyrrolidone (PVP) or polyvinyl alcohol (PVA)] which were cross-linked by gamma radiation for the production of sterile hydrogels used in wound care [Enrica et al. 2014].

Bioactive factor delivery from injectable biopolymer hydrogels provides a versatile approach to treat a wide variety of diseases, to direct cell function and to enhance tissue regeneration. The innovative design of drug delivery systems based on hydrogels involves both natural-[e.g., alginate, chitosan, hyaluronic acid, gelatin, heparin (HEP), etc.] and synthetic-[e.g., polyesters, polyethyleneimine (PEI), polyethylene glycol (PEG), polyglycolic acid (PGA), polyvinyl alcohol (PVA), polylactic acid (PLA) etc.] polymers has been applied for release of loaded therapeutics for different diseases. [Nguyen et al. 2014; Nair et al. 2006].

1.3.2 Applications of hydrogel:

Hydrogels have been extensively studied for different biomedical application owing to their ability to absorb water and other physiological fluids thus enabling for bio-mimicking. Bioactive factor delivery is a promising strategy to treat a variety of human diseases and enhance tissue regeneration. The term ‘bioactive factor’ refers to small-molecule drugs like anticancer drugs [Elstad 2009], genetic agents [Manaka et al. 2011; Huang et al. 2005; Dang et al. 2006] and proteins such as growth factors (GFs) [Lee et al. 2011; Tessmar et al. 2007], which have been used to treat human diseases, guide and direct cell functions
and/or enhance tissue regeneration [Nguyen et al. 2014]. Hydrogels hold great potential in pharmaceutical and biomedical applications due to their ability to locally deliver entrapped therapeutics at the sites of interest in vivo in a spatiotemporally controlled and sustained fashion.

1.3.2.1 Hydrogels in drug delivery

Hydrogels have unique property of response to aqueous environment by swelling and therefore attracted researcher’s interest for using them in drug delivery applications. The highly porous network structure of hydrogels can be easily modified by controlling the cross-linking density of the gel and the affinity of the hydrogels for the aqueous environment. Their porosity facilitates loading of drugs into the gel matrix and subsequent release of drugs at a coherent rate with the diffusion coefficient of the small molecule or macromolecule through the porous gel network. The benefits of hydrogels for drug delivery may be mostly pharmacokinetic specifically that a release depot is created from which drugs slowly elute out, maintaining a high local concentration of drug at the desired site than in the surrounding tissues over an extended period. Also the hydrogels were used for sustained release devices and for systemic delivery. Further owing to high water intake capacity, hydrogels are generally highly biocompatible and physiochemical similarity of hydrogels to native extracellular matrix makes them suitable for in-vivo use. Biodegradability or dissolution may be designed into hydrogels via enzymatic, hydrolytic, or environmental (e.g. pH, temperature, or electric field) pathways; however, degradation is not always desirable depending on the time scale and location of the drug delivery device [Hoare et al. 2008; Peppas et al. 2001]

1.3.2.2 Hydrogels in tissue engineering

Regenerative medicine is promising as a means to improve the standard of healthcare because transplants or medical prosthetics are although available, but they offer only partial solution in comparison to the healthy, undamaged physiological state. Therefore continuous attempts are being made by researchers in regenerative medicine to look for medical options and to improve them. Therefore, the physical building blocks or materials utilized in tissue engineering must be carefully chosen to be inherently safer and have closer similarities with natural tissues. In terms of material requirements for tissue scaffolds or as therapeutic delivery systems, hydrogels have attracted attention owing to their innate structural and compositional similarities to the extracellular matrix and their structural framework for cellular proliferation and survival. Hydrogels possesses with unique biocompatibility and their flexible methods of synthesis apart from range of constituents, and desirable physical
characteristics, which enables it as material of choice for applications in regenerative medicine. In regenerative medicines, hydrogels have been used as scaffolds that provide structural integrity and bulk for cellular organization and guides morphogenesis, serves as tissue barriers and bio-adhesives, acts as drug depots, delivers bioactive molecules for neo-tissue constructs. Many hydrogel types with vastly different chemical and physical properties have been developed over the last several decades from a wide variety of chemical building blocks and using an array of synthetic techniques. This expanse of hydrogel knowledge allows for scaffold properties, such as cellular attachment, molecular response, structural integrity, biodegradability, biocompatibility, and solute transport to be carefully engineered to meet the proliferative demands of the construct [Slaughter et al. 2009; Lee et al. 2001]. However still there are challenges in designing suitable materials for different tissue construct as there is no such materials which suites for regeneration of all tissue types due to variation in physiological condition and requirements of different tissue constructs. This offers opportunity for development of new materials that have necessary characteristics for tissue regeneration. In this regard, the potential of polysaccharides neither have been well explored nor exhausted as suitable materials in tissue regeneration with necessary modification to achieve desired characteristics.

The thesis involves the study of polysaccharide based biomaterials used for different bioapplications. Therefore the following section deals with the natural, renewable material namely polysaccharides, from concept to application of polysaccharides based biomaterials.

1.4 Polysaccharides

Polysaccharides are the diverse class of polymeric materials of natural (plant, animal & algal) origin formed via glycosidic linkages of monosaccharides [Shukla et al. 2012]. Polysaccharides can have a linear or branched architecture that depends upon the nature of the monosaccharide unit. Besides the structural diversity, polysaccharides have a number of reactive groups, including hydroxyl, amino, and carboxylic acid groups, providing the possibility for chemical modification [Liu et al. 2008]. Moreover, the molecular weight of polysaccharide can vary between hundreds to thousands Daltons, further increasing their diversity [Saravanakumar et al. 2012]. Compared to many synthetic polymers, polysaccharides have very low toxicity [Dang et al. 2006; Ratner et al. 2004; Chen et al. 1995; Mizrahy et al. 2012; Zhang et al. 2013]. Polysaccharides have a general formula of $C_x(H_2O)_y$ where $x$ is usually a large number between 200 and 2500. Considering that the
repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can also be represented as \((C_6H_{10}O_5)_n\) where \(40 \leq n \leq 3000\) [Aminabhavi et al. 1990].

Biopolymers like polysaccharides, proteins, and nucleic acids differ in chemical structures and their properties make them suitable for various applications [Meyers et al. 2008]. In particular, polysaccharides have shown great potential for several biomedical and pharmaceutical applications including tissue engineering, post surgical treatments, wound healing, and controlled release of drugs/proteins [Malafaya et al. 2007]. Due to renewability and biological origin (algae and plants, cultures of microbial strains or through recombinant DNA techniques), polysaccharides have wide utility for various applications. Further their biological origin offers advantages of being biocompatible, non-toxic and biodegradable [Dumitriu 2005]. Moreover, polysaccharides are characterized by diversity in structures that lead to versatile properties that are hardly matched by synthetic materials [Rinaudo et al. 2008]. By chemical structure, polysaccharides are carbohydrates formed by repeating sugar units linked together by glycosidic bonds. These polymers consist of thousands of sugar moieties and they can have both linear and branched structures. Polysaccharides can further form secondary structure (e.g. gellan and scleroglucan have double or triple helix conformations respectively) which gives diversity in their physicochemical properties. Moreover, the diverse functional groups present in the monomer units open opportunities for chemical derivatization and functionality manipulation [Baldwin 2010; Tizzotti, 2010] to tailor make the physical and biological properties of the parent polysaccharide. The mechanical properties can be modified by introducing crosslinks between the polymer chains that provide a network structure. [Peppas et al. 2000].

Different types of delivery systems based on several polysaccharides such as sodium alginate, chitosan, xanthan gum, guar gum, gellan gum and pectin etc. have been employed either alone or in combination with their native or modified forms to control the drug release [Shukla et al. 2012]. Various chemical and physical modifications such as cross-linking, grafting, oxidation, esterification, and combination with a variety of other molecules can be performed to produce newer composite materials with desirable functionality. Additionally, polysaccharides have advantages of bio-adhesion, especially for mucosal surfaces, which has been exploited for targeting specific organs or cells and prolonging drug residence time [Khan et al. 2015]. The interactive force between delivery devices and the glycoproteins of the mucosa is mainly through hydrogen bonding. Therefore, materials containing H-bonding
domains such as high density of carboxyl and hydroxyl group appear promising for therapeutic delivery. Polysaccharides and their derivatives promote mucoadhesion and have potential for newer and efficient therapeutic delivery systems. All of these qualities have led to the growing use of polysaccharides in drug delivery systems. Also the polysaccharides are considered to be a promising material for tissue engineering application due to their hydrogel forming ability. The polysaccharide based hydrogels provide aqueous environment, and bio mimicking ability, a porous micro architecture which enables oxygen and nutrient permeation for cell growth and thus satisfying ideal scaffold properties for tissue regeneration.

1.4.1 Different type of polysaccharides (Based on origin: - Plant, animal, microbial, Algae, sea weed etc.)

Polysaccharides can be classified in many possible ways, such as on the basis of structure, chemical composition, solubility, sources, and applications. With regard to the chemical composition, the polysaccharides are classified into two types, i.e. homopolysaccharides or homoglycans, which are made up of a single type of monosaccharide, for example, cellulose and glycogen consist of glucose; and hetero-polysaccharides or heteroglycans, which consist of more than one type of monosaccharide, such as heparin which consists of, α-L-idopyranosyluronic acid 2-sulfate and 2-deoxy-2-sulfoamino-α-D-glucopyranose 6-sulfate [Xiao et al. 2011]. According to the glycosides linked onto the glycan, polysaccharides can also be classified as proteoglycans, glycoproteins, glycolipids, and glycoconjugates [Berg et al. 2012]. Based on the origins, polysaccharides can be classified as derived from plant (e.g. (a) dietary fibers (b) herbs and (c) wood plants), algae and lichen, and polysaccharides derived from animals (e.g. heparin, chondroitin sulfate, and hyaluronan) and possess similar structural features [Liu et al. 2015].
### Table 1. Classification of polysaccharides. (Courtesy: Backavoka et al. 2014).

<table>
<thead>
<tr>
<th>Classification criteria</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function</strong></td>
<td></td>
</tr>
<tr>
<td>Storage</td>
<td>starch, glycogen</td>
</tr>
<tr>
<td>Structural</td>
<td>cellulose, alginate, chitin, agar</td>
</tr>
<tr>
<td>Secreted</td>
<td>dextran, xanthan gum, pullulan, gellan gum, welan gum, diutan gum</td>
</tr>
<tr>
<td><strong>Chemical composition</strong></td>
<td></td>
</tr>
<tr>
<td>Homoglycans</td>
<td>starch, glycogen, cellulose, chitin</td>
</tr>
<tr>
<td>Heteroglycans</td>
<td>alginate, carrageenans, gellan, xanthan, agars, arabinoxylans, glycosaminoglycans</td>
</tr>
<tr>
<td><strong>Structure</strong></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>glycosaminoglycans, cellulose, amylose, pectin, agarose, alginates</td>
</tr>
<tr>
<td>Branched</td>
<td>glykogen, amylopectin, xanthan gum, arabic gum, arabinoxylan</td>
</tr>
<tr>
<td><strong>Electrical charge</strong></td>
<td></td>
</tr>
<tr>
<td>Neutral</td>
<td>Cellulose, amylose, amylopectin,</td>
</tr>
<tr>
<td>Anionic</td>
<td>alginites, carrageenans, xanthan gum, gellan, gum arabic,</td>
</tr>
<tr>
<td>Cationic</td>
<td>Chitosan</td>
</tr>
<tr>
<td><strong>Source</strong></td>
<td></td>
</tr>
<tr>
<td>Animals</td>
<td>Mammals</td>
</tr>
<tr>
<td>Non-mammals</td>
<td>Chitin</td>
</tr>
<tr>
<td>Plants</td>
<td>Higher plants</td>
</tr>
<tr>
<td>Algae</td>
<td>agars, alginites, carrageenan, fucoidan</td>
</tr>
<tr>
<td>Microorganisms</td>
<td>gellan gum, xanthan gum</td>
</tr>
<tr>
<td><strong>Modification</strong></td>
<td></td>
</tr>
<tr>
<td>Pristine</td>
<td>cellulose, alginate, chitin, tamarind kernel polysaccharide</td>
</tr>
<tr>
<td>Derivative</td>
<td>carboxymethyl cellulose, carboxymethyl tamarind, propylene glycol alginate, chitosan</td>
</tr>
<tr>
<td><strong>Degradability in humans</strong></td>
<td></td>
</tr>
<tr>
<td>Degradable</td>
<td>dextran, glycogen, glycosaminoglycans</td>
</tr>
<tr>
<td>Non-degradable, slowly degradable</td>
<td>cellulose, chitosan, alginate, agar</td>
</tr>
</tbody>
</table>
1.4.2 Various polysaccharide used for bioapplications

The following section involves a brief structural description and application of few polysaccharides.

1.4.2.1 Starch:

Starch is a biopolymer synthesized in a granular form by green plants and consists of two major components, i.e. linear amylose with $\alpha-(1\rightarrow4)$-D-glucopyranose, and branched amylopectin with $\alpha-(1\rightarrow4)$-D glucopyranose backbone and 5–6% of $\alpha-(1\rightarrow6)$-branch linkages [Liu et al. 2015]. It is naturally produced in the form of semicrystalline granules with different size and composition, depending on the source of origin. Starch is an excellent material for industrial uses due to its non-toxic, renewable and biodegradable properties. However, the intrinsic properties such as thermal, mechanical, and biological properties and poor processability of starch have limited its direct applications [Khan et al. 2013]. Starch can be modified for superior properties and can be processed to form shapes like 3D porous scaffolds, microparticles, and bone cements [Khan et al. 2013].

1.4.2.2 Alginate:

Alginate is a hydrophilic polysaccharide extracted from marine brown algae such as Laminaria hyperborea or soil bacteria such as Azobacter vinelandii and consists of blocks of alternating $\beta$-D-mannuronic acid (M block) and $\alpha$-L-guluronic acid (G block) residues with (1→4)-linkages [Rowley et al. 1999; Khan et al. 2013]. The alginates have broad distributions of molecular weights of 10–1000 kDa depending on source and processing. Alginate is a commonly used polymer for encapsulation of therapeutic agents [Goh et al. 2012; Remminghorst et al. 2006]. Alginate is well known for application in tissue engineering and cell immobilization fields, due to its gelling and stabilizing properties, in which the G-blocks serve to introduce a steric hindrance and to provide folded and rigid structural conformation [Khan et al. 2013; Rinaudo 2008; Spiller et al. 2011]. However, the drawbacks of inadequate mechanical properties, uncontrollable degradation profiles, and lack of cell recognition signals have limited its medical application. Notably, carboxyl groups and hydroxyl groups along the alginate backbone enable various tailor made modification for
desired physicochemical, biological, mechanical properties [Oliveira et al. 2011; Liu et al. 2015].

1.4.2.3 Fucoidan:

Fucoidan is a general term for sulfated polysaccharides (MW: average 20 000) derived from brown seaweeds and some marine invertebrates, like sea urchins and sea cucumbers [Holtkamp et al. 2009]. The main skeletons of fucoidan essentially consist of linear backbone composed of α-1-3-linked sulfated L-fucose (usually referred as α-L-fucopyranose) along with a possible repeating sequence of alternating α(1→3) and α(1→4) glycosidic bonds in some cases. Sulfation of α-L-fucose residues may occur at positions C-2 and/or C-4 and, though it is rare, also at position C-3; but the structure and sulfation pattern of the sugar-backbone are species-related. The biological activities of fucoidan and its oligosaccharides have been extensively studied and these include antitumor effect, antiviral, anticoagulant and anti-inflammatory activities [Kwak 2014; Taylor et al. 1991; Jung et al. 2011; Cumashi et al. 2007; Cunha et al. 2016].

1.4.2.4 Gellan gum:

Gellan gum is a polysaccharide derived from the microbial fermentation product of Sphingomonas elodea. Gellan gum is a linear anionic polysaccharide composed of tetrasaccharide repeating units (1,3-β-D-glucose, 1,4-β-D-glucuronic acid, 1,4-β-D-glucose, 1,4-α-L-rhamnose) [Jansson et al. 1983]. Gellan gum is available in two isoforms, an acylated form that produces soft hydrogels and a deacylated form that produces hard and brittle gels. Both forms are in random-coil conformation at high temperature and upon cooling there is a transition to double-helix conformation [Oliveira et al. 2010]. The double-helices self assemble in clusters of anti-parallel structures called junction zones which are joined together in a three-dimensional structure by untwined regions of the polymer chain [Gasperini et al. 2014].
1.4.2.5 Pullulan:

Pullulan seems to be the single commercially produced EPS of fungal origin, with marketed pharmaceutical applications. This biopolymer is a neutral linear homopolysaccharide, consisting almost of regularly repeating $\alpha$-(1→4)–maltotriosyl units (3-D-glucopyranosyl) joined through $\alpha$-(1→6). Its present applications as a pharmaceutical ingredient are based on its distinct binding and film-forming properties, as well as on its strong oxygen impermeability. Such properties make it very suitable for granulation and coating tablets, non-animal capsules, oral and wound care products [Mocanu et al. 2011; Moscovici 2015].

1.4.2.6 Carrageenan:

Carrageenan is a water-soluble anionic polysaccharide derived from the Rhodophyceae red algae by alkali extraction. Carrageenan is a galactan, like agarose, and it consists of repeat sequences of $\beta$-D-galactose and $\alpha$-D-galactose with variable proportions of sulfate groups. In carrageenan, the $\beta$-galactose is D while in agarose it is L. Commercially available carrageenan can be divided into three families based on the position and number of
sulfate groups: κ-(kappa), ι-(iota) and λ-(lambda) carrageenan carrying 1, 2 and 3 sulfate groups, respectively [Hossain et al. 2001]. Aqueous solutions of κ- and ι-carrageenan can reversibly form hydrogels in the presence of cations, while λ-carrageenan does not undergo a sol–gel transition. In fact, carrageenan in solution has a random-coil conformation and upon cooling κ- and ι-conformation becomes a double helix with the sulfate groups pointing outwards, while the higher sulfate content of λ-carrageenan inhibits the formation of the helicoidal structure [Campo et al. 2009; Gasperini et al. 2014]. Carrageenan have shown several potential pharmaceutical properties including anticoagulant, anticancer, antihyperlipidemic, and immunomodulatory activities [Campo et al. 2009; Wijesekara et al. 2011; Liang et al. 2014].

1.4.2.7 Chitin

Chitin is a hydrophobic linear polysaccharide derived from many natural sources including the exoskeleton of arthropods (e.g., shells of crabs and shrimp) and in the cuticles of insects. It is the second most abundant natural polysaccharide next to cellulose [Baldwin 2010]. Chitin comprises a polysaccharide consisting of (1→4)-β-N-acetyl-D-glucosamine units, while the modified N-deacetylated derivative, chitosan, is a mixture of N-acetyl-D-glucosamine and D-glucosamine [Kumar et al. 2000]. Partial deacetylation of chitin under alkaline conditions or by chitin deacetylase yields the most important derivative of chitin, i.e. chitosan. Chitin is chemically similar to cellulose, another structural polysaccharide, with a hydroxyl position at C-2 being replaced by an acetylamide group [Kumar et al. 2000]. Generally, when the number of N-glucosamine units is higher than 50%, the term chitosan is used [Khor et al 2003]. The deacetylation process has been shown to reduce the molecular weight from an average range of 1000–2500 kDa to one of 100–500 kDa. The
partial acid hydrolysis product of chitin and chitosan were recognized for their bioactivity, including hemostatic action, anti-inflammatory effect, anti-tumoral, antibacterial, and fungicidal properties, eliciting chitinase, and regulating plant growth [Rinaudo et al. 2008]. The active groups or the possible reaction sites of both chitin and chitosan mainly include the free amino groups, primary and secondary hydroxyl groups, and acetamido groups. The increased water solubility of chitosan and its amine functionality has enabled its use as a biomaterial polymer conjugate; furthermore, chitosan has also been shown to be degradable by enzymatic hydrolytic cleavage of the \( \beta-(1\rightarrow4) \) saccharide linkage [Hirano et al. 1989]. Chitosan has many biomaterial applications including wound-dressing materials, drug-delivery vehicles, and tissue-engineering scaffolds.

1.4.2.8 Hyaluronan

Hyaluronan, or Hyaluronic acid (HA), which is mainly derived from plant and animal tissues. It is a hydrophilic linear glycosaminoglycan composed of alternating \((1\rightarrow4)\)-\(\beta\)-D-glucuronic acid and \((1\rightarrow3)\)-\(\beta\)-N-acetyl-D-glucosamine, and the backbone contains both hydroxyl and carboxylic acid functionalities [Chong et al. 2005]. HA was discovered initially in the vitreous body of cattle eye, and found later on in the extracellular matrix (ECM) and synovial fluids [Lapčík et al. 1998]. The molecular weight of HA has a broad range depending on its origin. Although HA can be isolated by extraction from living tissues, it is produced mainly via microbial fermentation, due to the reduced risk of cross species viruses, infection, and contamination [Chong et al. 2005]. HA is an essential component of the extracellular matrix, mediating cellular signaling, wound repair, morphogenesis, and matrix organization and thus HA and its derivatives have been clinically used for medical applications, such as visco-supplementation, eye surgery, and drug delivery.
for decades [Prestwich et al. 2011; Baldwin 2010; Liu J et al. 2015]. For example, hyaluronan, a major extracellular component with unique hygroscopic, rheological, and viscoelastic properties, has been extensively developed for tissue repair purposes due to its physicochemical properties and specific interactions with cells and extracellular matrix. It is generally accepted that hyaluronan plays multifaceted roles in the mediation of the tissue repair process and is involved in all the stages of wound healing, i.e. inflammation, granulation tissue formation, reepithelialization, and remodeling. Derivatives of hyaluronan, such as cross-linked, esterified or other chemically modified products have also been developed for tissue repair or wound healing purposes [Anilkumar et al. 2011, Chen et al. 1999].

1.4.2.9 Chondroitin Sulfate

Chondroitin sulfate (CS) is composed of a linear polysaccharide chain consisting of \((1\rightarrow3)\-\beta\-N\-acetyl-D-galactosamine alternating with \((1\rightarrow4)\-\beta\-glucuronic acid presenting sulfates and hydroxyl and carboxylic acid functionalities [Volpi et al. 2006]. Various forms of chondroitin sulfate have been discovered and are designated by the site of sulfation of \(N\)-acetyl galactosamine in the 4-O and 6-O positions and subsequent other sulfation or epimerization of glucuronic acid [Volpi et al. 2006]. CS is mainly found attached to proteoglycans in connective tissue matrices, functioning as a structural component, or on cell surface and basement membranes, functioning as a receptor [Silbert et al. 2002]. Commercially available CS is obtained via extraction and purification from many sources including shark and whale cartilage, and bovine or porcine tissues [Volpi et al. 2006]. An important function of CS is in articular cartilage, where on the order of one hundred chains of CS are conjugated per protein, as in aggrecan [Watanabe et al. 1998]. The high negative charge of CS, coupled with the high density of CS chains on aggrecan, creates a charge gradient that swells the cartilage and enhances the ability of the tissue to absorb load [Chahine et al. 2005]. This natural role of CS has directed its use in tissue-engineering scaffolds for cartilage repair [Wang et al. 2007]. Modified CS has been used as adhesive cartilage repair scaffolds and ECM for chondrogenic differentiation [Wang et al. 2007; Varghese et al. 2008]. Modification of CS has yielded CS-based adhesives for sealing corneal incisions with minimal inflammatory responses and scar tissue formation while maintaining high burst pressures [Baldwin 2010].
1.4.3 Applications of polysaccharides

a) Biomedical application (Drug delivery, Tissue engineering, wound healing)

b) Other industrial applications (e.g. waste water treatment, textile, paint and pigment, food industry)

Natural polysaccharides from different sources have long been studied and widely used in different areas, such as food, textile, paint and pigment, paper making, medicine and pharmaceutics. In recent decades, there has been an increased interest in the utilization of polysaccharides, particularly bioactive ones, for various novel applications owing to their biocompatibility, biodegradability, non-toxicity, and some specific therapeutic activities. Polysaccharides are the most abundant group of biopolymers, involved in many biological processes, such as cell–cell communication, embryonic development, infection of bacteria and/or virus, and humoral and cellular immunity [Cooke et al. 2007; Dube et al. 2005; Varki et al. 1993]. Therefore, polysaccharides together with polynucleotides, proteins, and lipids constitute the most important bio-macromolecules in life science. The brief discussion about each of the biomedical applications pertaining to polysaccharides has been discussed in this thesis.

1.4.3.1 Biomedical applications of polysaccharides

Polysaccharides and their derivatives have advantages over the synthetic polymers, because they are non-toxic, biodegradable, biocompatible, and less expensive compared to their synthetic counterparts. All these advantages of polysaccharides and their derivatives makes them suitable for broad spectrum of applications in different areas, such as in biomedical or pharmaceutical, food and cosmetic industry. Moreover, polysaccharides are also used in traditional disease control and health care apart from many newer application areas such as in tissue engineering, in drug delivery, in wound healing, in cancer prevention, diagnosis, and therapy, and in treatment of bacterial and viral diseases [Khan et al. 2013; Lindblad et al. 2007; Sandra et al. 2009; Liu et al. 2015]. The succeeding section discusses the major areas of recent research activities of polysaccharides- tissue engineering, wound dressing/healing, and drug delivery applications.

1.4.3.2 Polysaccharide in tissue engineering

Natural origin materials have been demonstrated to promote tissue regeneration at a faster rate and are expected to exhibit greater compatibility with human tissues. Exploitation
of polysaccharides and their derivatives for tissue engineering applications, such as biological signaling, cell adhesion, cell differentiation, cell proliferation, cell responsive degradation, and remodeling, is attracting scientist’s interest in biomedical research for guiding and promoting new tissue regeneration or to define the shape and structure of cell growth [Khan et al. 2013]. Various types of polysaccharides, such as alginate, chitosan, cellulose, hyaluronic acid, chondroitin sulfate, starch and their derivatives, have been developed as biomaterials for tissue engineering applications [Oliveira et al. 2011]. Application of polysaccharides as scaffolds in tissue engineering needs to fulfil the requirements like biocompatibility and nontoxicity, biodegradability with controllable degradation rate, appropriate porosity, and structural integrity [Khan et al. 2013].

Since last few decades, number of biomaterials have been proposed as “ideal” scaffolds for cell growth yet only few have proved to have clinical efficacy. Biomaterials irrespective of their source, either natural or synthetic, must be biocompatible with a necessary structural integrity and also compatible with native tissue to fulfil their desired role in tissue regeneration. The essential role of material is to provide mechanical stability, cell anchorage sites, and structural guidance and within an in vivo environment, provides the interface to respond to physiological and biological changes and in turn to integrate with the surrounding native tissue [Khan et al. 2013, Chen H et al. 2014]. Ideally, biomaterials designed for tissue engineering should perform the structural and biochemical functions of the natural extracellular matrix (ECM), which provides cells with mechanical, topological, and chemical cues until the cell produced ECM remodelled [Ma et al. 2004]. Thus, materials that can form porous and solid three-dimensional structures are utilized in tissue engineering to make tissue scaffolds, while at the same time are biodegradable in synchronous with the cell modelling/remodelling rate and neo tissue formation process. Additionally, biocompatibility and bioactivity are desirable to facilitate and enhance cell adhesion, migration, proliferation, and differentiation. The material stiffness can also affect differentiation process and, often a determinant factor influencing material choice [Engler et al. 2006].

Extracellular matrix (ECM) should have certain desirable biophysical properties that are critical to their application in tissue engineering purposes. Different aspects such as surface topography, charge distribution, stiffness, wettability etc. are important physical parameters of hydrogels which control the cellular responses such as adhesion, spreading, growth and further differentiation of cells and thus define the biocompatibility of any specific
surface or matrix [Lima et al. 2015; Cigognini et al. 2013; Bidan et al. 2013, Cha et al. 2011]. In the context of bone tissue engineering, the bone cells have been reported to be extremely sensitive in terms of their precise ECM or surface requirements as cell adhesion-mediated signalling events play a major role in the survival and chemotaxis events [Lee 2006]. Non-compatible surfaces are known to induce loss of cell adhesion leading to apoptosis in osteoblasts and can cause major bone defects [Grigoriou et al. 2005]. During bone morphogenesis and homeostasis, cells experience various signals in their environments, including gradients of physical and mechanical stimulation, chemical cues etc [Faria-Torres et al. 2014; Cigognini et al. 2013]. Often such requirements are not only essential for the cellular survival and differentiation but also are subtle and difficult to replicate at in-vitro level. These limit the use of different natural ECM or “synthetic scaffolds” as potential surfaces for bone tissue engineering [Lee et al. 2014; Zhu et al. 2010; Drury et al. 2003]. In this context polysaccharide can play pivotal role that can address the above mentioned issues, however it requires certain necessary and desirable modification that suits scaffolding requirement for various tissues.

So far several techniques and materials have been employed for targeting cells with osteogenic properties [Venkatesan et al. 2012]. For example, materials made of expensive and/or heavy metals such as titanium, zirconia, ferritic stainless steel, etc. were tried without much success, either owing to high cost, or heavy metal toxicity or other non-desired material properties [Hefti et al. 2010; Malheiro et al. 2011; Krause et al. 2000]. Materials originated from hydroxyapatite, gelatin, chitosan, alginate, heparin, hyaluronan etc. were also extensively tried as scaffold for bone tissue repair without much desired success enforcing the quest for alternative material which is suitable for both osteogenic and nonosteogenic cells [Azami et al. 2012].

While the biocompatible polymers are useful because they do not specifically interact with biological systems, however non-interaction with biological systems hindered their use where interactions are desired to manipulate biological responses such as growth factor binding or enzymatic degradation. Certain biopolymers in particular, at least some polysaccharides have innate bioactivity. Further polysaccharides can be conjugated to synthetic polymers to impart desired bioactivity [Baldwin 2010]. Currently there are available techniques both in-situ and ex-situ through radical polymerisation and addition reactions that offers opportunity to conjugate polysaccharides to synthetic polymers such as aldehyde, epoxide, carbodiimide, hydrazide and active ester [Hermanson 1996]. These methods of
covalent conjugation of functional polymers with polysaccharides that do not produce any cytotoxic reactive species and are active under physiological conditions offers newer avenues for polysaccharide based biomaterials for bioapplication [Baldwin et al. 2010].

1.4.3.3 Polysaccharide in wound healing

Due to their inherent biocompatibility, low toxicity, and pharmaceutical biomedical activity, various polysaccharides, such as chitin, chitosan, hyaluronan, cellulose, and alginate have been widely used to prepare wound healing materials [Liu et al. 2015; Czaja et al. 2006; Czaja et al. 2007; Hrynyk et al. 2012]. Notably, wound healing-promoting activity of the materials is also important for other bioapplication such as designing of materials for tissue engineering. Maintaining a moisturized environment during healing helps to diminish the incidence of scars [Bryan et al. 2004; Jewo et al. 2009]. Hydrogels are suitable for wound dressings because they keep a moist environment and provide a cooling sensation diminishing the pain because high water retention capacity of hydrogels allows them to be applied in swollen state and still absorb residual fluid from the wound site representing ideal dressing characteristics. Their capacity of high water uptake allows them to be applied in the swollen state and still absorb some fluid from the wound site, presenting some characteristics of an ideal dressing [Wittaya-areekul et al. 2006]. The swelling also allows some debris and bacteria entrapment, apart from acting as a barriers and preventing bacterial penetration [Jones et al. 2005; Oliveira et al. 2014]. Sun et al reported dextran based hydrogel with certain modification used for burn wound treatments [Sun et al. 2011].

1.4.3.4 Polysaccharides in drug delivery

The potential of polysaccharides in drug delivery and controlled release applications owes to their advantages such as biocompatibility, low immunogenicity, and minimal cytotoxicity. Further polysaccharides have a wide range of molecular weights and a large number of functional groups for chemical modification; and thus are an attractive choice as a polymeric backbone in polymer conjugates. The pharmacokinetics of polysaccharide conjugates are greatly influenced by their charge, molecular weight, extent of chemical modifications, polydispersity and structure. This offers opportunity to conjugate drug to polysaccharide and develop a desired drug delivery system (DDS). Many polysaccharide-based drug delivery systems have been developed for specific targeted delivery or controlled release, for protection from premature degradation of drugs, for improving intracellular penetration and transpotation, for enhancing stability and bioavailability of drugs, or for the
delivery of biomolecules such as genes, antigens, and small interfering RNA [Liu et al. 2015; Csaba et al. 2009; Mao et al. 2010; Mizrahy et al. 2012; Valo et al. 2011].

Polysaccharides are also attractive candidates for smart drug delivery systems (DDS) because polysaccharides, in particular polyelectrolyte polysaccharides, respond to different stimuli such as pH, ionic strength of medium, temperature and concentration of certain molecules (such as lectins) etc. The release of the entrapped drugs or certain molecules can be triggered by the change of pH, ions, electrical or magnetic field, light, temperature, redox potential, or certain molecules [Alvarez-Lorenzo et al. 2013]. The stimuli responsiveness of polysaccharides or functionalised modified polysaccharide can be exploited for smart DDS. In contrast to non-responsive DDSs that releases the drug according to a pre-established pattern, the smart or intelligent DDSs releases the drug in the affected tissues or cells as and when desired in response to certain physiological events [Alvarez-Lorenzo et al. 2008]. Additionally, some polysaccharides have unique features that enable nanocarriers with surface properties which suitably regulate the interactions at target sites of contact during absorption and biodistribution, namely mucosa, blood, and target cells. Polysaccharides have the ability of biomimicking the surface of both eukaryotic cells and prokaryotes and facilitate the recognition and binding to desirable surfaces, while escaping from opsonization and complement activation [Vauthier et al. 2007; Tsai et al. 2011]. Thus, integration of the polysaccharide features in nano/micro/macro-hydrogel networks is particularly attractive to obtain novel biocompatible, responsive and even targetable DDSs, suitable to be administered via almost any route [Coviello 2007]. Polysaccharide networks can be obtained through crosslinking assisted by interactions of variable strength, from weak physical entanglements to irreversible covalent bonds [Rana et al. 2011; Alvarez-Lorenzo et al. 2013].

1.4.3.5 Polysaccharides for synthesis of metal polymer nanocomposites

Metallic nanoparticles (AgNPs and AuNPs) are the focus of extensive research over several decades due to wide range of possible applications, such as antimicrobial agents, catalysts and optical sensors. For example, silver NPs (AgNPs) have been used for wound dressings, bone cements and implants [Chaloupka et al. 2010]. Gold NPs (AuNPs) have anticancer property and used for targeted cancer therapy [Patra et al. 2010; Schröfel et al. 2014]. The “stabilization” and “controlled-size” parameters of NPs are important prerequisites for specific end-use applications and such objectives can be achieved by choosing suitable reductant and capping agent in aqueous medium. The preparation of uniform and stable colloidal NPs, free from agglomeration and precipitation, is still a major
challenge because the NPs are generally short-lived in an aqueous media owing to their natural tendency of agglomeration [Zahran et al. 2014]. Recently, many green synthesis processes have been reported as suitable alternative strategies for preparing AgNPs. For example, β-D-glucose has been used as a reducing agent for green synthesis of Ag nanoparticle where starch acts as a stabilizer [Raveendran et al. 2003]. Extract from acacia where acacia extract acted as both reducing and stabilizing agent has also been used for green synthesis of AgNP [Mohan et al. 2007]. Furthermore, different polymeric stabilising agents, dendrimers, latex particles and microgels has been studied for controlling size parameter and to achieve better stabilization of silver nanoparticles [Bardajee et al. 2012]. Limited yet significant efforts have been channelized to explore new synthetic methodology involving in-situ reduction and capping of AgNPs, especially for in situ synthesis of AgNPs within the polymeric network architectures leading to new hybrids or composite systems [Rao et al. 2011]. In this context, the carriers, for example polymers, dendrimers or microgels, act as ‘nanoreactors’ that immobilize the particles and provide an easy handling. Different microorganisms (bacteria, fungi, virus), DNA and proteins have also been used for the synthesis of AgNPs and this is due to the strong affinity of Ag ions towards –SH, –NH2 and –COOH groups [Hulkoti et al. 2014]. However, the microbial DNA and proteins exhibit immunogenic allergic reactions, thus restricting their usage for biomedical applications [Roy et al. 2003]. In this context, hydrogels are mostly safe, biologically inert and offer large free spaces between the cross-linked networks in the swollen stage that can act as “nanoreactors” for the nucleation and growth of the AgNPs [Vimala et al. 2009; Stojkovska et al. 2014]. However, the approach too has not been very successful due to the long reaction times, use of chemical and usually toxic reagents, low efficiency in converting the silver cations (Ag⁺) to nano-Ag, and lack of control over the size of AgNPs. In this context polysaccharides offers suitable environment for the synthesis of Ag & Au nanoparticles and different other metallic nanoparticle based nanocomposites due to their almost limitless availability, low price, and diversity in functionalities giving versatility to polysaccharides.

1.4.3.6 Polysaccharides for antimicrobial applications

Polysaccharides such as chitosan, carrageenans, heparin, alginate and their sulfated derivatives, fucan (brown algae), and ulvan (green algae) etc have been reported for their antimicrobial properties. Cimino et al. reported that rosacelose isolated from the aqueous extract of the marine sponge Mixylla rosacea is a new anti-HIV polysaccharide composed of glucose and fucose sulphate. [Coma et al. 2013; Wang et al. 2012].
1.4.3.7 Other industrial applications

For decades polysaccharides have been used for various industrial applications, e.g. electronics, pharmaceuticals, food stuff and nutrition, and biofuels, wastewater treatment, textiles and other allied industries [Suginta et al. 2013; Crini et al. 2005; Coma et al. 2013]

1.5 Polysaccharides based biomaterials.

Polysaccharide based biomaterial includes polysaccharides and their synthetically modified polysaccharides that are biocompatible used in biomaterial applications. The polysaccharides are mainly classified into (1) mammalian polysaccharides; and (2) non-mammalian polysaccharides.

Both polysaccharides derived from mammalian or non-mammalian sources offer opportunity for imparting biological activity into materials by facile methods. Non-mammalian polysaccharide includes chitin, alginate, and dextran possesses similar saccharide structure (Fig.1.2) despite their diverse origins. The simpler extraction and purification of these polysaccharides yield large quantities of material at low cost, coupled with lower immunogenicity, drives interest in these materials. Additionally, their chemical functionality possesses ionic charge, enables them for non-covalent cross-linking, provides routes for degradation, and allows for suitable modifications by cross-linking or grafting. Mammalian polysaccharides, such as the glycosaminoglycans, chondroitin sulfate, hyaluronan, and heparin, possess chemical similarities to their non-mammalian counterparts (Fig.1.3) and thus have similar routes for modification and activity. Despite their more difficult isolation process than that of the non-mammalian polysaccharides, they exhibit specific biological functionality, apart from their specific binding modules with multiple proteins. This has driven continued interest for mammalian polysaccharides in their application as biomaterials. However they are limited by batch-to-batch variation, the possibility of pathogen transmission, and immunogenicity related complication for general use of these materials. Therefore the non-mammalian polysaccharides attracted renewed interest for application in biomaterials.
As reported by Baldwin et al 2010, the incorporation of mammalian polysaccharides into biomaterials offers advantages over the incorporation of non-mammalian polysaccharides as mammalian polysaccharides elicits specific interactions with mammalian cells or physiological environments. The use of glycosaminoglycans, such as hyaluronan, heparin, and chondroitin sulfate, has been most widely explored and has yielded a range of highly useful biomaterials [Khan et al. 2013]. Polymeric networks containing multiple polysaccharides have better mimicking ability in the complicated environment of the ECM, and therefore hydrogel scaffolds are moulded using multiple functionalized polysaccharides and proteins cross-linked with synthetic polymers. The incorporation of various functional polysaccharide components provides a useful ECM-mimetic environment, both in cell culture and in vivo, compared to natural extracted matrices, with less concern over batch-to-batch variation, pathogen transmission, and immunogenicity [Baldwin et al. 2010; Serban et al. 2008].

1.5.1 Biomimicking Polysaccharide

Conjugation of polysaccharides, such as heparin, to polymeric biomaterials has proven beneficial, as important growth factors can be stabilized, sequestered, and activated. The remarkable achievement to produce diverse and useful material with desirable biological response and physico-chemical properties lies with the ability to develop newer polysaccharide-polymer conjugates that enables better mimicking the natural ECM. The
polysaccharide-polymer conjugates makes the new and important class of material that respond to the biological environment, including cell-demanded growth factor release or degradation that can further increase the efficacy of materials as tissue replacements. has led to important new classes of environmentally sensitive materials The future of polysaccharide-polymer conjugates will offer newer opportunity to tailor made polysaccharide based material with improved and controlled bio-chemical cues for bio-mimicking the natural ECM and will facilitate new generations of modified polysaccharide-based materials with controlled function, offering expanded options for guiding cellular fate for applications in tissue replacement and drug delivery [Krishna et al. 2009; Baldwin et al. 2010].

1.6 Motivation of the research project:

The use of polymeric materials as biomaterials has evolved over the past several decades, encompassing an expanding synthetic toolbox and many biomimetic approaches. Both synthetic and natural polymers have been used as components for biomaterials, as their unique chemical structures can provide specific functions for desired applications. The integration and widespread use of polymers as biomaterials has significantly expanded owing to advances in the synthesis of polymers with controlled and functional architectures, which has improved the range of materials possible, as well as their biocompatibility [Peppas et al. 1994; Uhrich et al. 1999]. Novel methods of extraction and purification have also enabled the use of many natural polysaccharides as biomaterials for multitude of uses, especially as drug-delivery vehicles and tissue-engineering scaffolds [Reis et al. 2008]. Biocompatible polymeric delivery vehicles with appropriate design have afforded controlled release of drugs, such as small molecules, peptides, or proteins, both systemically and locally to a target via molecular recognition [Langer 1990]. Tissue-engineering scaffolds also often utilize controlled drug delivery, with the added complexity of incorporation of cellular adhesion to the matrix and mimicry of the mechanical properties of target tissues [Lee et al. 2001; Baldwin et al. 2010]. However the polysaccharides are not well explored for their potential in mitigating many complexities arises in biomedical field despite many natural advantages such as biocompatibility, biodegradability, non-cytotoxicity, and innate bioactivity observed at least in case of few polysaccharides.

For decades polysaccharides have been used for various industrial applications, e.g. pharmaceuticals, biomaterials, food stuff and nutrition, and biofuels, however newer findings and better understanding about polysaccharides has drawn increased interest by researchers for deeper investigations of the importance of polysaccharides in life science as potential
biomolecules. The biological activities of polysaccharides are influenced by their chemical structure and chain conformations. However, the macromolecular structures of plant cell wall polysaccharides, especially hetero-polysaccharides or so-called hemicelluloses, are extremely complex due to the presence of different monosaccharides as building blocks, which usually are isobaric stereo isomers, variations in sequence, linkage, branching, and distribution of side chains [An et al. 2011; Cancilla et al. 1998; Mäki-Arvela et al. 2011]. Besides, the polysaccharides in microorganisms (fungi, yeasts, and bacteria), algae, plants, and animals are always physically and/or chemically entangled together with other biomolecules, e.g. proteins, polynucleotides, lipids, lignin, and some inorganic mineral substances [Yang et al. 2009]. A comprehensive understanding is required for exploring the potential of bioactive polysaccharides in life science and other bioapplications [Colegate et al. 2008]. Therefore it is worth exploring the polysaccharides with necessary modification that induces bioactivity and tailor made properties for different bioapplications.

1.7 Objectives of the work

1. To synthesise newer biomaterial from natural polysaccharide with desired physico-chemical properties.
2. To characterise the synthesised biomaterial for different physico-chemical properties such as swelling ability, hydrophobic-hydrophilic balance, degree of crystallinity, surface charge variation, micro-structural change, and spectroscopic characterisation.
3. To study the effect of increasing hydrophilicity/hydrophobicity on physico-chemical properties by incorporating hydrophilic synthetic monomer into natural biopolymer namely tamarind kernel polysaccharide.
4. To investigate the effect of incorporating hydrophilicity on biomedical application such as drug release ability and on bio-adhesion property.
5. To investigate the effect of incorporation of dual synthetic monomer aiming at creating hydrophilic-hydrophobic balance on biopolymer backbone and the consequent effect on the biomedical application such as drug release kinetics and cell adhesion.
6. To explore the potential of tamarind based polysaccharide in synthesis of polymer inorganic hybrid nanocomposites and to assess their potential application in biomedical field.
Since this thesis involves study about tamarind based polysaccharides for various biomedical applications, the following sections deals with necessary information pertaining to structural and extraction of tamarind kernel polysaccharides.

1.8 Tamarind kernel polysaccharide

Tamarind Kernel Polysaccharide (TKP) has been extensively used for different non-biomedical application such as thickener, binder, textile, paint and pigment etc. Recently however its biomedical application has explored in the area drug delivery devices. From ancient times TKP has been used for medicinal purposes and believed to have excellent anticancer properties [Arvind et al. 2012]. *Tamarindus indica*, a widely grown tree mainly in South East Asia including India, and its fruit have been used for human life as a food component, spice, and snack. The seeds of Tamarind are used as an antidiarrheal, anthelmintic, and an emetic agent, and the seed coat is used to treat burns and aid in wound healing as well as an antidysenteric agent [Farnsworth et al. 1992; Arvind et al. 2012].

Despite of different application of TKP, it needs further modification for certain properties that limit its various bio-applications. The biopolymer matrix lacks desirable mechanical strength required for application in drug delivery devices and tissue engineering scaffolds e.g. hard tissues such as bone. Therefore it is necessary to increase the mechanical property of polysaccharides without affecting other properties such as hydrophilicity which has paramount importance in bio-application. The mechanical strength of matrix can be enhanced to certain degree with increasing the network density by introducing cross-links to the network structure, however the cross-links beyond certain limit affects the hydro-swelling ability which in turn may have detrimental effect on its bio-adhesion property and that may affect its scaffolding potential. Making of hydrogels through grafting of different synthetic monomer [Bhattacharya et al. 2004] on the polymer backbone has been one of the suitable alternative approaches that have been taken up by different researchers to make network polymers with enhanced bio-adhesion and bio-mimicking potential to find suitable application in drug delivery and tissue engineering.

Tamarind kernel polysaccharides (TKP) is a galactoxyloglucan, a natural plant polysaccharide, obtained from the endosperm of *Tamarindus indica* seeds [Kaur et al. 2012a; Kaur et al. 2012b; Bhattacharya et al. 1991]. Tamarind trees are widely found in tropical climate of mostly South East Asian countries like India, Bangladesh, Myanmar, Srilanka, and Thailand. TKP is composed of (1→4)-β-d-glucan backbone substituted with side chains of
α-d-xylopyranose and β-d-galactopyranosyl (1→2)-α-d-xylopyranose linked (1→6) to glucose residues (Fig. 1.3) [Jana et al. 2013]. Tamarind kernel polysaccharide is a monomer of glucose, xylose and galatose in molar ratio of 3: 2: 1 [Kaur et al. 2012, Ray et al. 2002]. Tamarind polysaccharides have been investigated for its diversified application in pharmaceutics [Kulkarni et al. 1998, Babu et al. 2003]. Tamarind Kaernel Polysaccharide has been reported to be noncarcinogenic, biocompatible and stable enough even in acidic pH range [Pal et al. 2012]. It is used as binder, thickener, gelling agent, suspending agent, emulsifier, and release modifier in different pharmaceutical formulations [Avachat et al. 2011; Khanna et al. 1997; Kulkarni et al. 1997; Kulkarni et al. 2002; Mishra et al. 2011; Prajapati et al. 2013]. Further tamarind kernel polysaccharide is used in combination with other polysaccharide such as xanthan gum for controlled release of poorly soluble drugs [Razavi et al. 2014]. Moreover, it finds its use in the development of various types of mucoadhesive drug delivery systems due to its hydrophilic and mucoadhesive properties [Nayak et al. 2014].

![Figure 1.3](image_url)  
**Figure 1.3** Chemical structure of TKP. It is composed of (1 → 4) -β-d-glucan backbone substituted with side chains of α-d-xylopyranose and β-d-galactopyranosyl (1→2)-α-d-xylopyranose linked (1→6) to glucose residues.
The different steps involved in extraction of tamarind kernel polysaccharide powder from its original source, the plant *Tamarindus Indica L* has been shown in Fig. 1.3. The tamarind fruit was dried and the husk was removed to obtain the tamarind kernels. The tamarind kernels were ground to fine powder to give tamarind kernel powder which was repeatedly washed with ethanol to remove the unwanted impurities to give pure TKP.

**Figure 1.4** Pictographic representation of the extraction of tamarind kernel powder (TKP) from the plant *Tamarindus indica*. 