

Polyelectrolyte Complexes (PECs) for Biomedical Applications

Manisha Buriuli and Devendra Verma

Abstract Polyelectrolytes are a class of macromolecules which spontaneously acquire a net positive or negative charge when dissolved in an appropriate polar solvent such as water. Polyelectrolytes can co-react in aqueous solutions and form polyelectrolyte complexes (PECs) or polysalts, which resemble general self-assembly processes. It is believed that PECs are formed due to increase in entropy, caused by the liberation of low-molecular-weight counter ions. PECs can be classified into three types: soluble, colloidally stable, and coacervate complexes. Depending on the compatibility between the reacting polyelectrolytes, the electrostatic interaction between the anionic and cationic groups is stronger than most secondary interactions. Hence, this avoids the use of various chemical cross-linking agents, which reduces the possibilities of toxicity and other harmful effects that may be caused by the agents. PECs combine unique physicochemical properties, which are different from those of the individual components. These find a wide range of applications, such as membranes, as coatings on films and fibers, and as micro-capsules for drug delivery, to name a few. PECs have immense potential uses in the field of ecology, biotechnology, medicine, and pharmaceutical technology.

Keywords Polyelectrolyte complexes · Wound healing · Drug delivery · Tissue engineering · Gene delivery · Bioadhesives

Abbreviations

2D	Two-dimensional
3D	Three-dimensional
ADA	Adenosine deaminase
AFM	Atomic force microscopy
AgSD	Silver sulfadiazine
APMA	N-(3-aminopropyl) methacrylamide hydrochloride

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ASC	Adipose-derived stem cell
BMP	Bone morphogenetic protein
cCD	6-carboxymethylthio- β -cyclodextrin
cDNA	Complimentary deoxyribonucleic acid
CH	Chitosan
CLSM	Confocal laser scanning microscopy
CMC	Carboxymethyl cellulose
CPP	Chitosan-polyphosphate
CS	Chondroitin sulfate
CTPP	Chitosan-tripolyphosphate
DE	Degree of esterification
DEP	Dielectrophoresis
DHEA	Dehydroepiandrosterone
DOPA	3,4-dihydroxyphenylalanine
DNA	Deoxyribonucleic acid
DTT	1,4-dithiothreitol
ECM	Extracellular matrix
EDC	1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride
EGFP	Enhanced green fluorescent protein
FE-SEM	Field-emission scanning electron microscopy
FGF	Fibroblast growth factor
FTIR	Fourier transform infrared spectroscopy
GAG	Glycosaminoglycan
GI	Gastrointestinal
HA	Hyaluronic acid or hyaluronan
HAp	Hydroxyapatite
HBPO	Hyper-branched polyether
HMC	Hydroxymethyl cellulose
HPMA	N-(2-hydroxypropyl) methacrylamide
HPMC	Hydroxypropyl methyl cellulose
HS	Heparan sulfate
iCMBA	Injectable citrate-based mussel-inspired bioadhesive
IPC	Interfacial polyelectrolyte complexation
LbL	Layer-by-layer
LPS	Lipopolysaccharide
MAP	Mussel adhesive protein
MSC	Mesenchymal stem cell
MTT	(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
NiTi	Nickel-titanium
OREC	Organic rectorite
PAA	Poly(acrylic acid)

PAH	Poly(allylamine hydrochloride)
PAMAM	Poly(amidoamine)
PBS	Phosphate-buffered saline
PDL	Poly-D-lysine
pDNA	Plasmid deoxyribonucleic acid
PEC	Polyelectrolyte complex
PEG	Poly(ethylene glycol)
PEI	Polyethylenimine
PEMU	Polyelectrolyte multilayers
PGA	Poly(glutamic acid)
PgA	Poly(galacturonic acid)
PLGA	Poly-L-glutamic acid
PLL	Poly-L-lysine
PNiPAM	Poly-N-isopropylacrylamide
PP	Polyphosphate
PTA	Transactivator of transcription-based polypeptide
PU	Polyurethane
Px	Piroxicam
RGD	Arginylglycylaspartic acid
SCID	Severe combined immunodeficiency
SDS-PAGE	Sodium dodecyl sulfate poly-acrylamide gel electrophoresis
SEM	Scanning electron microscopy
TAT	Transactivator of transcription
TEM	Transmission electron microscopy
TNF	Tumor necrosis factor
TPP	Triphosphate
UV-Vis	Ultraviolet-visible
VEGF	Vascular endothelial growth factor
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction

1 Introduction

Polyelectrolyte complexes (PECs) or polysalts are precipitates formed when two oppositely charged polyelectrolytes are allowed to co-react in aqueous solution. These PECs have been shown to display unique physical and chemical properties due to the considerably stronger electrostatic interactions compared to most other secondary binding interactions (Lee et al. [2002](#)). The main driving force of PEC formation is the increase in entropy due to the release of the low molecular counter

ions. Hydrogen bonding and hydrophobic interaction are also known to play a part (Chelmecka 2004).

Interest in PECs was fueled after it was discovered that PECs are naturally produced by some marine organisms such as the sandcastle worm (*Phragmatopoma californica*) (Stewart et al. 2004). The PEC functions as a proteinaceous cement to join and construct their mineralized tubes. When removed about 1 cm from their tubes and placed on a bed of 0.5 mm diameter glass beads, the worms worked extensively to rebuild their tubes by cementing the available glass beads. The cement was found to consist of polyelectrolytes having net opposite charges at physiological pH. The robust nature of adhesive to prevail in underwater conditions and adhesion to various substrates suggest its many possible biomedical applications such as in bone cement and fixation.

1.1 Formation of PECs

The formation of a PEC takes place in three sequential steps (Fig. 1):

Step 1: *Primary complex formation*. There is random bond formation between the polyelectrolytes.

Step 2: *Secondary complex formation*. Here, intracomplex correction and rearrangement of the bonds take place which results in the formation of an orderly secondary complex.

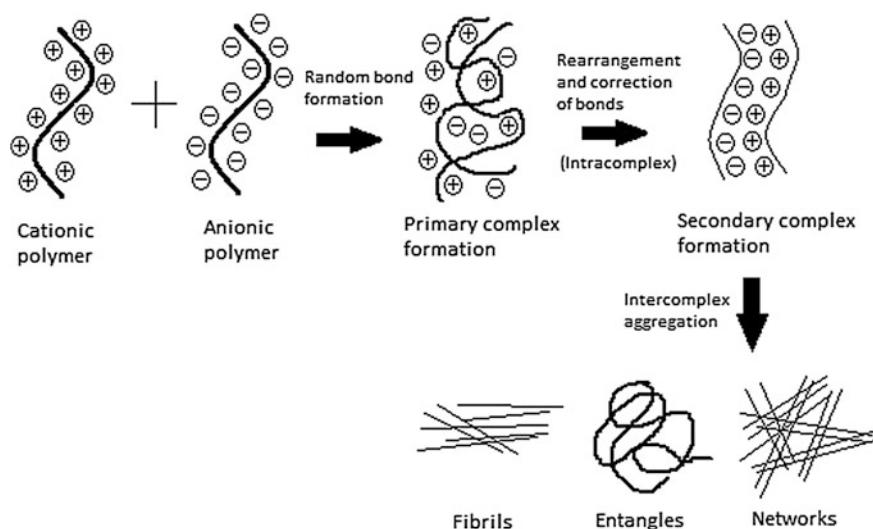


Fig. 1 Schematic representation of PEC formation

Step 3: *Intercomplex aggregation*. The hydrophobic interactions between the secondary complexes cause their aggregation. These complex aggregates may be in the form of large fibrils, entangles or networks.

1.2 Structure of PECs

PEC formation results in various structures, depending on the properties of individual polyelectrolytes and the external conditions provided during the reaction. As reported in the literature, the structure of any resultant PEC can be grouped into two models: ladder-like or scrambled egg (Michaels and Miekka 1961).

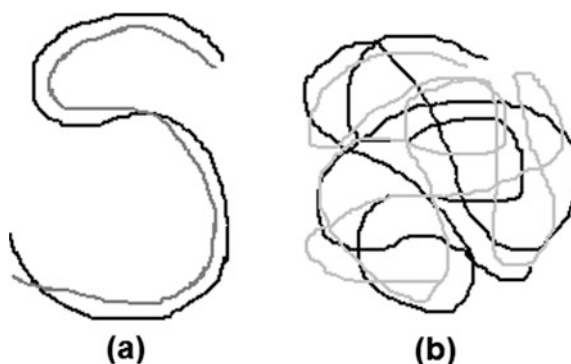
1.2.1 Ladder-like Structure

The ladder-like structure (Fig. 2a) is formed when a single-stranded hydrophilic segment interacts with a double-stranded hydrophobic segment. This phenomenon results from the intermixing of polyelectrolytes that have weak ionic entities and major differences in molecular dimensions (Hartig et al. 2007). The ladder-like structure is formed due to the “zippering action”, as the ionic site which has reacted first will most likely result in the reaction of the ionic site immediately next to it, giving rise to the zippering effect.

1.2.2 Scrambled Egg Structure

The scrambled egg structure (Fig. 2b) is generally formed when a polymer contains a high number of chains. This model signifies complexes that arise due to intermixing of polyions with strong ionic entities and corresponding molar masses. These yield insoluble and extremely aggregated complexes under a stringent 1:1 stoichiometry.

Fig. 2 Structural models of PEC: **a** Ladder-like, and **b** Scrambled egg



1.3 Types of PECs

PECs are generally classified based on their structure; the other classification being based on the main interaction forces acting during PEC formation (such as Coulomb's forces and van der Waal's forces).

Based on their structure, PECs are classified as, water-soluble, colloiddally stable, and coacervate complexes.

1.3.1 Water-Soluble PECs

Intermixing of polyelectrolytes having large differences in molecular weights, containing weak ionic entities, and addition in non-stoichiometric proportions under certain salt conditions, results in water-soluble PECs. Water-soluble PECs have a long host chain with some small guest chains (Kabanov 2003). These PECs assume a conformation which is similar to that of the ladder-like structure.

1.3.2 Colloiddally Stable PECs

PECs give rise to different types of colloidal systems depending on the compatibility between the polyelectrolytes. Polyelectrolytes with compatible charge densities give rise to very compact colloidal structures. On the other hand, polyelectrolytes with unsuitable charge densities result in colloidal particles with a high degree of swelling.

1.3.3 Coacervate PECs

A coacervate (spherical aggregate of colloidal droplets held together by hydrophobic forces) is formed when the interactive binding between the oppositely charged polyelectrolytes is mild due to low charge densities. They are known to exhibit viscoelastic nature (Spruijt et al. 2013).

1.4 Factors Influencing PEC Formation

There are numerous factors underlying PEC formation. Since the complexation results in a conformational loss, it has to be counter-balanced in order for the complexation to occur. The formation and stability of PECs depend on the following factors:

- Concentration of the polyelectrolytes
- Molecular weight of the polyelectrolytes

- pH of the medium
- Temperature of the medium
- Mixing order
- Mixing ratio
- Degree of ionization of each polyelectrolyte
- Charge density on the polyelectrolytes
- Position of ionic group on the polymeric chain.

1.5 Methods of Preparation of PECs

The simplest and the most widely used method of PEC formation is the polyelectrolyte titration, also called the drop-by-drop method. In this method, one polyelectrolyte is slowly added to a beaker containing the oppositely charged polyelectrolyte, under stirring conditions.

Though this method is the predominant method of PEC formation, it has certain disadvantages:

- The solvent which is present in the titrant dilutes the solution, and
- The polyelectrolyte (in the beaker) to which the titrant is added is continuously consumed in the complexation process.

As a result, the polyelectrolyte which is present in the beaker undergoes a gradual decrease in its concentration during the complexation process. To overcome this problem, many times the concentration of the polyelectrolyte in the beaker is taken 2–10 times higher than the other polyelectrolyte (Gärdlund et al. 2003).

The PEC solution can be easily cast into films by pouring in a container followed by air drying (Verma et al. 2012). Porous scaffolds can be fabricated using freeze drying and porogen leaching (Verma et al. 2009). PEC capsules are prepared by dropwise addition of one polyelectrolyte solution into another complementary charged polyelectrolyte solution (Amaiike et al. 1998). This results in a core-shell structure where the first polyelectrolyte occupies the core. The shell consists of PEC of both the polyelectrolytes. The structure of these capsules depended on the order of addition of the polymer solutions. For example, spherical droplets were formed in the gellan solution when cationic chitosan solution was added to anionic gellan solution. The capsules had PEC as a shell with chitosan at the core. The capsules were found to be stable after washing with water and 80% ethanol. They were also strong enough for pinching and magnetic stirring in distilled water and resistant to acid, alkali, and boiling water. When gellan solution was added to chitosan solution, hemispherical droplets were formed at the air-chitosan solution interface (Wan et al. 2004).

For the preparation of PEC fibers, two methods have been described in the literature: (1) self-propelling by gravity in air, and (2) wet spinning technique (Amaiike et al. 1998). The wet spinning technique with a roll-up apparatus is widely

preferred for formation of fibers, as it gives longer length fibers and ability to orient the fibers in a specific direction. The scaffolds generated by wet spinning technique followed by lyophilization do not cause any cytotoxicity and maintain cell growth (Shao and Hunter 2007).

As proposed by researchers, the fiber formation mechanism by interfacial polyelectrolyte complexation (IPC) includes four steps which are: (a) formation of PEC film (b) scattering of the complex (c) growth of nuclear fibers, and (d) coalescence of nuclear fibers (Wan et al. 2006). As the fiber is pulled off from the interface, the PEC at the interface is broken down into many individual domains. These domains act as nucleation sites for further complexation. As the nuclear fiber increases in size, the viscosity of the free excess components outside the fibers decreases, due to a decrease in concentration of the polyelectrolytes. Simultaneously, an increase in the ionic strength of the medium is observed, which results from the release of salt counter ions. The nuclear fiber eventually merges together. Regularly spaced gel droplets are observed along the fiber axis, which is formed by the excess polyelectrolytes. It was inferred that continuous fiber formation was related to the stability of the interface and the precipitation of a PEC in the solution. The dimensions of the fibers were found to be directly related to the area of contact between the two solutions. Also, it was seen that the draw rate affects the formation of fiber with beads. Lower the rate, lesser the beads in fiber. The bead formation is slow for viscous solutions. Lesser draw rates are required to form fibers at higher viscosities (Tuzlakoglu et al. 2004; Xu et al. 2015).

The layer-by-layer (LbL) self-assembly of polyelectrolytes has been widely used to prepare tailored ultrathin membranes with defined thickness, composition, and structure (Lvov et al. 1993; Shiratori and Rubner 2000). These membranes find their applications in bio-sensing, catalysis, filtration, and optics. For the preparation of membranes using LbL method, the substrate is alternatively dipped in complementarily charged polyelectrolytes. Strong electrostatic interaction at the interface of each layer stabilizes the structure. One advantage of the LbL method is that it allows nanometer-level control over the thickness and composition of the membranes. The thickness of the films can be controlled either through the number of layers that are deposited or by increasing the concentration of the polyelectrolyte in the solutions from which the films are formed.

A study reported the fabrication of PEC microfibers (Verma et al. 2011). The PEC microfibers were made from chitosan and alginate. PECs generally form nanoparticles when mixed at low concentrations ($\sim 0.1\%$ w/v). The PEC microfibers were made at concentrations of about 1% w/v of each constituent. The fibers were around $1\ \mu\text{m}$ in thickness. The homogeneous mixing of polyelectrolytes at this concentration was facilitated by sonication process. One of the important parameter, which affects final structure of the PECs is pH of the solutions. Sonication of polymer mixture at pH around 8 results in nanoparticles, whereas sonication at around pH 4 results in the formation of microfibers.

2 Applications of PECs

Morphologies of associated polyelectrolytes differ widely, depending on the balance of water, polymer, and salt ions within the complex (Porcel and Schlenoff 2009). The mixing of polycation and polyanion solutions results in dense precipitates. Once the bound water is removed (Porcel and Schlenoff 2009), the wet complexes are stiff or rubbery depending on their salt content (Michaels 1965). Most of the applications of PECs arise from their solid-like properties. Ionically bonded polymeric network structures, readily synthesized from linear polyelectrolytes, possess unusual physical and chemical properties not found in conventional polymers (Michaels 1965). PEC hydrogels, sponges, nanofibers, films, structure micro/nanoparticles, and coatings find various applications in biomedicine.

2.1 Wound Healing

2.1.1 Wound

Skin is the largest organ of the adult body which has a major role in protection from pathogens. A wound can be defined as an injury to the skin. This injury can be a cut, tear, puncture, burn, or even a bruise. The sources of the wound are many, but they relatively have the same effect. Injury to the skin can be fatal as it may act as an easy passage for the microorganisms which might be of pathogenic nature and can cause dreadful diseases. Wounds can be classified into various types. Depending on the source causing the wound, they can be of following types:

- Incision
- Lacerations
- Abrasions
- Avulsions
- Puncture wounds
- Penetration wounds
- Gun-shot wounds

Besides these, the wound can be *open* if cut by a sharp-edged object or *closed* if caused due to trauma by a blunt object. Wounds can have a profound effect on a specific region. Due to high regeneration capacity, skin wound heals quickly depending upon the degree of injury. However, sometimes it leads to serious consequences like infections, inflammations, scarring, and loss of function.

2.1.2 Phases of Wound Healing

Healing of wound is a spontaneous process which takes place by itself. Proper and faster wound healing is essential as it protects an individual from various kinds of pathogenic organisms and air-borne fungal infections. It is a complex and dynamic process in which the missing cells or tissue gets replaced by new cells. The healing process takes place in the following phases: hemostasis, inflammatory phase, proliferative or fibroblastic phase, and maturation or remodeling phase.

(i) *Hemostasis*

This is the first phase of wound healing which starts within few minutes from the time the person gets injured. After the injury, the sub-endothelium of the skin gets exposed, which is rich in collagen and certain tissue factors. Collagen and tissue factors are responsible for aggregation of platelets over the wounded area and form a plug to prevent further bleeding. The platelets get activated as soon as they get attached to the collagen of the exposed wound. The activated platelets start to degranulate, releasing many chemotactic factors which result in clot formation, leading to the conversion of soluble fibrinogen to insoluble fibrin and forms a mesh over the wound. Also, many growth factors (e.g. platelet derived growth factor) and vasoactive agents are also secreted at the wound site. These factors released by the platelets, result in attraction of inflammatory cells switching to next phase of wound healing.

(ii) *Inflammatory phase*

The cellular aspect of wound healing involves cells like neutrophils, macrophages, and lymphocytes which occurs within hours of injury. Neutrophils do not play a role in wound healing but are responsible for cleaning the wound site. They are responsible for engulfing bacteria and other infection-causing microorganisms. Macrophages are the most important cells responsible for wound healing. The major roles of macrophages in wound healing are:

- Phagocytose bacteria
- Secrete collagenases and elastases, which disintegrate the injured tissue and liberate cytokines
- Release cytokines that are responsible for growth and movement of fibroblasts and smooth muscle cells
- The release of compounds that attract endothelial cells to the wound site. This triggers their proliferation, which in turn promotes angiogenesis.

T-lymphocytes start to act within 72 h of injury. They release various lymphokines (e.g. basic fibroblast growth factor) which are responsible for promoting wound healing.

(iii) *Proliferative or fibroblastic phase*

In proliferative phase, the fibroblasts play a major role. Within 48–72 h of injury, the fibroblasts start to migrate towards the wound, marking the onset of proliferative phase and this migration may even start before the inflammatory phase ends. The proliferative phase consists of many steps which are not necessarily in a sequential manner: epithelialization, fibroplasia, angiogenesis, and contraction. A new network of blood vessels is built in the wound which comprises of collagen and extracellular matrix (ECM) (angiogenesis). The new granulation tissue formation is dependent upon fibroblasts. A healthy granulation tissue formation takes place when the fibroblasts have sufficient oxygen and nutrient supply from the blood vessels. There is a formation of an epithelial layer over the cut wound to protect it from the external environment (epithelialization).

(iv) *Maturation or remodeling phase*

This phase is initiated when collagen formation and degradation reaches an equilibrium state. In this phase, newly deposited type III collagen is converted to type I collagen. Rearrangement of collagen fibers takes place and lead to an increase in wound tensile strength. The actual tensile strength of the skin is not achieved. However, the newly replaced skin achieves a maximum of about 80% of its original tensile strength. Maturation phase can vary widely depending on the type of wound, from 3 days to 3 weeks, and in some cases even a year or longer.

2.1.3 Wound Dressings

Wound dressings are used beneath compression bandages in order to promote quick healing and to prevent the contact of the crude wound and the bandage. The range of dressings is becoming broader day by day. Modern dressings also have biological activities appended because microbial biofilm is believed to play a role in wound healing. There are four major functions a wound dressing should perform. They involve cleaning, absorbing, regulating, and adding medication. The choice of dressing mainly depends on the amount of wound exudate and the type of wound. The eight main categories of wound dressings are discussed below.

- *Gauze dressings*

These dressings are made of either woven or non-woven gauze. Gauze is non-occlusive and permeable and is the most readily available wound dressing. The drawback is that these types of dressings may promote desiccation in wounds with less exudate. Hence, under such conditions, they must be used along with a topical agent. They are inexpensive and are good for short term use. They come in different designs based on the comfort of the user.

- *Film dressings*

Film dressings, as the name suggests, are thin and flexible. They are made of clear polyurethane (PU) with an adhesive coating that adheres to the skin. The exudate and adhesive react and prevent the adhesion to wound bed while allowing the film to adhere to the dry skin around the wound. They can be used either as a primary or a secondary dressing. They are elastic in nature and adjust to body contours. Visualization of the wounds is possible with these types of transparent dressings.

- *Hydrogel dressings*

Hydrogels are gels that comprise of 80–90% water. Glycerin-based hydrogels are also available. Though they can absorb only less amount of fluid, they can keep the dry wounds moist because of the presence of ample amount of water in their structure. They cool the applied area and elevate pain. They are permeable to gas and water. Hence, they are not effective bacterial barriers. These are mostly non-adhesive and require a secondary dressing.

- *Foam dressings*

Foamed polymer solutions like PU contain small cells which can hold fluids and are used as dressings. They can be either layered in combination with other materials or they can be impregnated alone. They are non-adhesive to the wound and can easily be removed. They can be made with adhesive border and a transparent coating which can act as a bacterial barrier.

- *Alginate dressings*

Alginate is a polymer made from seaweeds. Alginate beads react with serum and wound exudate and convert it to a gel. The gel is moist and traps bacteria. The bacteria are washed off while changing the dressings. Alginate gels are non-occlusive and highly permeable. Hence, there must be a secondary dressing like gauze. Alginates are available as sheets, ropes, and alginate-tipped applicators. Hydrofiber dressings are prepared with carboxymethyl cellulose (CMC) sodium salt which is similar to alginates.

- *Composite dressings*

As the name suggests, composites are multilayer dressings. They are mostly made of three layers. The innermost layer is non-adherent and prevents trauma to the wound bed during a change of dressings. The middle layer prevents maceration by absorbing moisture and wicking it away from the wound bed. It also maintains a moist wound environment. The middle layer may be a hydrogel, foam, hydrocolloid or alginate. The outer layer acts as a bacterial barrier. It is made of a semipermeable film.

- *Hydrocolloid dressings*

Hydrocolloids contain hydrophilic colloidal compounds like gelatin, cellulose, and pectin. They have a strong film and foam adhesive back. They absorb surrounding exudate slowly and swell into a gel. Removal of hydrocolloid dressing may leave

some residue that gives foul odor. They insulate the wound thermally and have the least permeability to water, oxygen, and bacteria. These dressings have lower infection rates than other dressings.

- *Interactive dressings*

Wound dressings are classified as active, passive, and interactive. Passive dressings just have protective functions. Active dressings promote healing by creating a moist environment around the wound. Interactive wound dressings create a moist environment and also interact with the wound bed to enhance wound healing.

2.1.4 PEC-Based Wound Dressings

Alginate (or alginic acid) is an anionic biopolymer that is derived from acids obtained from brown seaweeds. Dressings made from alginate are not only biodegradable, they also have high absorbency rate (up to 20 times their weight). Depending on the type of seaweed species from which the alginate is made, the dressing may either gel or swell in the wound after absorption of wound fluid. To accelerate gel formation, an alginate dressing is incorporated with a mix of both sodium and calcium alginates. Calcium alginates tend to swell, whereas sodium alginates tend to dissolve or gel in the wound bed (Kent 2009). Alginate dressings promote new skin growth by keeping the wound moist, remove exudates, and promote hemostasis.

Chitosan and alginate dressings have been widely researched for wound healing applications. Chitosan, which is derived from deacetylation of chitin present in shrimps and other crustaceans, is extensively applied in biomedicine due to its low toxicity and high biocompatibility (Kathuria et al. 2009; Tripathi and Melo 2015). Alginates, when combined with a cationic biopolymer such as chitosan, can be made into a PEC-based wound dressing. PECs from alginate and chitosan can be prepared by adding chitosan solution dropwise to alginate solution. It can also be prepared by using a two-step process where calcium alginate beads are first formed, and then the gel beads are dropped into chitosan solution to form the PEC on the surface (Gåserød et al. 1998). Alginates have also been combined with silver, zinc, and CMC to improve the antimicrobial efficacy of the dressings.

Hydrogels are made up of three-dimensional hydrophilic material and contain about 90% water (Kumar and Tripathi 2012). Due to this, hydrogel dressings help control the exchange of fluids within the wound site. These dressings exhibit excellent biocompatibility because their surfaces produce low interfacial free energy when in contact with bodily fluids (Tsao et al. 2010, 2011). Hydrogels do not dissolve in water when crosslinkers are present, but these crosslinkers possess the significant risk of toxicity. Moreover, chemical crosslinkers form weak ionic interactions between various polymer chains. A PEC hydrogel can overcome these limitations.

A PEC membrane made from chitosan and γ -poly(glutamic acid) (γ -PGA), when tested for wound healing efficacy showed that the PEC provided an appropriate moisture content and exhibited good mechanical properties (Tsao et al. 2011). Since both these conditions were prevalent, the dressing could be easily removed from the wound without causing any damage to the newly regenerated tissue. The use of this dressing promoted more than 50% of re-epithelialization and regeneration of the wound, as revealed by histological examinations conducted on the newly regenerated tissue.

Chitosan and γ -PGA PEC matrices exhibit greater hydrophilicity, more favorable cytocompatibility, and a more extensive mechanical structure, compared with that of chitosan alone (Hsieh et al. 2005). Chitosan- γ -PGA hydrogel dressings have been reported to promote early new bone formation in the alveolar socket following tooth extraction (Chang et al. 2014). These dressings adhered better to wound surfaces than neat chitosan and were found to promote earlier as well as greater amounts of new bone formation than treatment with chitosan and gelatin sponge alone.

Chitosan-hyaluronic acid (HA) PEC hydrogel dressings were used to treat burn wounds (Vasile et al. 2012). HA is an anionic, non-sulphated glycosaminoglycan (GAG) found in the connective, neural, and epithelial tissues. It has very high molecular weight in the range of millions. It is known to have good biocompatibility, biodegradability, and gel-forming properties. Chitosan-HA PEC hydrogels are suitable for wounds healing applications as they combine and enhance the antimicrobial activity, prevent wound damage, and subsequently promote wound healing.

Chitosan and sodium alginate PEC sponge dressings impregnated with the drug, silver sulfadiazine (AgSD) as an antibacterial were used as a potential wound dressing material (Kim et al. 1999). The release of AgSD from the dressings was controlled by the number of repeated in situ PEC reactions between chitosan and sodium alginate. The dressing could protect the wound from bacterial invasion and the extent of cellular damage was found to reduce by the controlled release of AgSD from the sponge dressings. Granulation tissue formation and wound contraction were observed to be very fast in dressings containing AgSD and dehydroepiandrosterone (DHEA).

Chitosan-alginate PEC sponges have also been formed by incorporating curcumin (diferuloylmethane) found in turmeric to deter wound infection and accelerated healing (Dai et al. 2009). Curcumin is well known to have natural wound healing properties. In vivo animal testing revealed that adding curcumin into the sponge enhanced its therapeutic healing effect.

Wound dressings in the form of films were made from PECs of chitosan and alginate coacervates with calcium chloride (Wang et al. 2002). These dressings were found to increase incisional wound healing in model rats when compared to conventional gauze dressings. Post-operative observations showed the closing of the wound on day 14, extremely good remodeling on day 21 with thick collagen deposition and presence of mature fibroblast cells. Good biocompatibility and

wound-healing efficiency indicated that chitosan-alginate PEC film dressings have potential applications in wound healing.

Dressings were made by incorporating chitosan with polyphosphate (PP) to act as a procoagulant, and silver nanoparticles to act as an antimicrobial agent (Ong et al. 2008). Chitosan dressings were fabricated with different amounts and lengths of PP chains (45 or 65 phosphate units per chain). The PP-silver containing chitosan dressings were found to have superior hemostatic properties when compared to chitosan dressings. The PP present in the dressings was responsible for the accelerated production of sufficient amounts of thrombin to support earlier fibrin generation. Moreover, the presence of chitosan engaged RBCs to expand and solidify the growing thrombus, leading to the formation of a stable blood clot.

2.2 Drug Delivery

Drugs are constantly being developed to treat various diseases and abnormalities. In most cases, though the drugs perform very well in *in vitro* conditions, their performance *in vivo* is rather suppressed. This is due to deposition of the drug in non-specific locations in the system. Hence, drug delivery is considered to be one of the most challenging tasks in pharmacology. There are various methods that effectively deliver drugs in their respective locations, some of which include nano delivery vehicles like micelles and liposomes. One such delivery mechanism that has attracted recent interests involves encapsulation of the drug within PECs. These PECs act as reservoirs for drugs which are stored in the central region referred to as the core. The solid drug core is then surrounded by a capsule that consists of shells made of alternating anionic and cationic polyelectrolytes. The drug core can also be located in the shell region in some cases. The shells are designed such that they dissolve in specific locations in the system where the drug is deployed, depending on the local conditions prevailing there. Thus, multiple barriers that hinder drug delivery can be overcome by careful selection of layers that contribute to the shell.

Initially, studies were based on only strong polyelectrolytes in their fully charged states like polystyrene sulfonate, dextran sulfate, heparin, and polydimethyl diallyl ammonium chloride, with their pH maintained below 7. Later, the significance of weak polyelectrolytes such as poly(acrylic acid) (PAA), poly(galacturonic acid) (PgA), and alginate became known, since their charge densities can be controlled by adjusting the solution pH. The thickness of the layer in weak polyelectrolytes is the highest near the solution pK_a of the polyelectrolytes.

Two oppositely charged polyelectrolytes can be crosslinked (to form a PEC) through electrostatic interactions. Polyelectrolytes can also make a complex with surfactants and even biologically active micelles via electrostatic interactions. Hence, they find their use in various biomedical and pharmaceutical applications. PECs are ionic in nature with a hydrogel structure. They can also permeate body fluids. They possess many characteristics of proteins and this feature enables them to be less toxic. They are mostly hydrophilic with good mechanical strength and

have the ability to bind oppositely charged molecules. Thus, they are used for target specific applications.

PECs are formed by various techniques that involve mixing of solutions containing oppositely charged polymers. One such technique for preparing PECs is layer-by-layer (LbL) assembly technique. It involves the alternate deposition of ionic polyelectrolytes on a layer with opposite charge. The chemical composition and structure of the film are controlled to a better extent than other ultrathin film forming technologies and the polyelectrolyte is deposited in a predesigned layer-by-layer fashion. LbL is suitable for fabrication of films on flat surfaces with larger areas. Films with a thickness less than 100 nm are possible using LbL, and when many layers are formed to increase the thickness to micrometer levels, the loading capacity is increased to a greater extent. The increase in thickness also increases the mechanical robustness, which helps when nano-scaled hierarchical structures with integrated functions to be tailored. Various LbL assemblies like spin LbL, spray LbL, and exponential LbL has been developed to accelerate the LbL process. LbL methods offer flexibility and freedom to satisfy the needs of drug delivery systems with complex design considerations.

A study showed that crosslinking can be induced by varying the temperature to prepare non-detachable hydrogels (Khutoryanskaya et al. 2010). The hydrogels exhibited pH-dependent swelling properties. Another research prepared stable, single component multilayered PEC with cationic and anionic forms of the same polymer, chitosan (Bulwan et al. 2009). It exhibited good biocompatibility with bacteriostatic properties. The LbL technique can deposit polyelectrolytes on any substrate provided that the substrate does not dissolve in the coating solution, and the substrate has a charge in the coating solution.

2.2.1 Drug Loading

Loading and controlled release of small molecular weight drugs and small molecules are challenging goals that are required to be reached when it comes to drug delivery. LbL deposition of polyelectrolytes can elevate the efficiency of drug loading and targeting.

Ibuprofen, which is used for wound healing, incorporated in a PEC of poly (allylamine hydrochloride) (PAH) and dextran with hyaloplasm acid was formulated (Wang et al. 2009). This helped in the sustainable release of ibuprofen incorporated in surgical sutures. The quantity and release kinetics of the loaded drug can be adjusted by altering the parameters of LbL deposition without affecting the properties of the suture. Fibroblast growth factor (FGF) and heparin were loaded in aortic valves of porcine heart (De Cock et al. 2010). The growth factor activity was preserved and sustainable release of growth factor was also achieved. When prodrugs were loaded with thin LbL films, the desired anti-inflammatory effect was observed (Cao and He 2010).

Loading of hydrophobic drugs requires a hydrophobic core that can act as a reservoir for the drug to be loaded. A study synthesized a heparin-like sulfonated hyperbranched polyether (HBPO-SO₃) with an HBPO hydrophobic core and negatively charged sulfonic acid terminal groups (Hu and Ji 2010). This could self-assemble in aqueous media to form stable micelles with low cytotoxicity. These complexes with hydrophobic cores can be used as nano-reservoirs for hydrophobic guest molecules. The PEC could serve as a multifunctional coating that could help in anticoagulation and local drug delivery. The release rate of drugs depends on the size and solubility of the drug, the number of layers of polyelectrolyte deposition and thereby, the thickness of the shell and the types of polyions used for the LbL process. A study concluded that the shells act as a barrier between the core and the release conditions (Antipov and Sukhorukov 2004). When proper target conditions are achieved, the core dissolves and the shell becomes stable. A drug delivery assembly was described for dexamethasone in a reservoir-type control system with a semipermeable rate controlling membrane (Pargaonkar et al. 2005). The release profiles followed almost zero order kinetics. Hence, it provided necessary proofs that drug release is a diffusion-controlled mechanism.

The release of drugs like dexamethasone involves two processes: initially, the bulk solution diffuses into the capsules to dissolve drug crystals and then the dissolved drug molecules diffuse out of the capsules. The dissolution of the crystal cores proceeds from the surface towards the crystal center. The smaller crystals dissolve faster than the larger crystals.

Drugs can also be loaded by the use of salts. Salts affect the polyelectrolyte multilayer assembly and also affect the preformed multilayers. Salt ions will screen electrostatic charges and induce swelling of the multilayers. This increases the permeability (Radtchenko et al. 2002). The permeation is blocked at low ionic strength and if the salt concentration is increased further, the drug is released.

2.2.2 Drug Release Triggers

The release of the drug normally takes place through diffusion, a non-specific process. Specific drug release is carried out with chemical and biological triggers. pH is one of the most common triggers for the release of drugs specific to organs. The pH gradient ranges from 1 in the stomach to 7.5 in the small intestine, when it comes to the gastrointestinal (GI) tract. Neutral and anionic polymers do not respond at low pH. However, cationic polymers like chitosan are responsive even at very low pH values. Polymers and polyelectrolytes are chosen according to their pI values. Swelling occurs due to charge imbalance inside the hydrogel structure, thereby decreasing the charge density to balance the surrounding pH. When the charge density becomes low such that the polyelectrolyte cannot hold the complex, swelling or dissolution occurs.

PECs like N-succinyl chitosan/alginate hydrogels and chitosan-acrylamide grafted hydroxymethyl cellulose (HMC) find potential drug release applications in the GI tract. PECs like poly (N-isopropylacrylamide) (PNiPAM)-chitosan show

thermo-responsive behavior and hence, the drug release is affected by temperature (Gandhi et al. 2015). In certain cases, drug release can be controlled by enzymes that are specific to a particular locus being targeted. For example, the bacteria in GI tract produce enzymes such as amylase, pectinase, xylanase, etc. The polysaccharides that could be substrates for these enzymes can be complexed with polyelectrolytes for drug release in these areas. Enzymes also cause local pH deviations in the hydrogel microenvironment that can affect drug release. A study described that HA and poly-L-lysine (PLL) planar films are invaded by living cells which gradually digest them with the release of enzymes (Picart et al. 2005). This can help in the intracellular release of encapsulated therapeutics. Drug release can also be initiated by applying external electric field when PECs are used. The field is removed when the required amount of drug is released. This type of release is suitable for drugs in the dermal, epidermal, and subcutaneous regions.

Drug release can also be done by taking control of the swelling pressure. At elevated pH and temperature, the gels explode to release the drug. These are called self-exploding capsules (De Geest and De Smedt 2012). The microgels were synthesized with diameters larger than 100 μm to reduce the pressure required to overcome the capsule's tensile strength. Chitosan can form ionic complexes with multivalent counter ions like tripolyphosphate (TPP) and polyphosphate (PP), denoted as CTPP and CPP, respectively, that can be used to release acidic and water insoluble drugs. Drugs like 6-mercaptopurine were dispersed in a solution of chitosan in acetic acid and dropped into solutions of multivalent counter ions to obtain drug-loaded polyelectrolyte beads. The pH of the counter ion solutions was kept at extremes in order to prevent coacervation of the chitosan beads (Mi et al. 1999a, b). The pH of the medium was maintained below 6 so that the gelation was completely ionic. This increased the release rate of 6-mercaptopurine from CTPP and CPP gel beads in simulated intestinal fluid. Decreasing the pH increased the rate of release of drug from the complex. At simulated gastric fluid (pH 1.2), CTPP had slower release rates than CPP. Thus, by modifying the pH of the medium and the gelation, the release profiles of drugs from PECs can be controlled.

A study evaluated the drug release capabilities of matrix-based chitosan-alginate and chitosan-carrageenan systems (Tapia et al. 2004). The chitosan solution was prepared in 1% acetic acid solution, alginate in water, and carrageenan in 5.7% sodium chloride solution. The PEC was prepared by heating different ratios of the respective solutions at about 80 °C. It was then washed with distilled water and centrifuged at 10,000 rpm for 30 min. The PEC was then suspended in water at 9 °C, followed by vacuum drying, milling, and sieving. The PEC tablets were made by dry mixing the polymers with diltiazem hydrochloride, lactose, and magnesium stearate and were compressed by tableting machines to obtain 500 mg tablets. On testing the various ratios of polymers in PEC, it was found that the optimum ratio was the one in which the solution's viscosity and the supernatant's viscosity after centrifugation was the same. This was obtained with 30–40% chitosan in the mixture. The degree of swelling of the PEC made of chitosan-carrageenan was found to be higher than that of the normal polymer mixture in 0.1 N HCl, pH 1.2 (gastric pH) because of complete protonation of chitosan and electrostatic repulsions. However, the degree of swelling

of chitosan-alginate was very low, which is attributed to the presence of unionized alginate at a pH as low as 1.2. Thus, swelling in solution depended on the number of ionized groups present in the mixture of polymers. The drug release was characterized by dissolution studies. It showed that chitosan-carrageenan complexes had a low retardant capacity of drug release since carrageenan has the ability to promote entry of water into the tablet. When the concentration of carrageenan was increased to 70% v/v, the drug release was slowed down. Chitosan-alginate tablets had a high retardant capacity of drug release. This was due to the low degree of swelling of alginate gels. Thus, the swelling rate was found to affect the release of drugs in PECs. Thus, chitosan-alginate PECs were found to be better than chitosan-carrageenan PECs for drug release as lower concentrations of the former can result in a better-controlled drug release.

Nanoscale chitosan-carrageenan drug carriers were prepared by adding drops of diluted carrageenan into chitosan solution (Grenha et al. 2010). These nanocarriers had no cytotoxicity till 3 mg/mL of drug and had better resuspension in water. The electrical and mechanical properties of electrospun chitosan-carrageenan fibers were found to be enhanced by spinning them along with carbon nanotubes (Granero et al. 2010).

Hyaluronic acid (HA) is a high molecular weight GAG found throughout all tissues in living organisms. It is an ionic polysaccharide and can extensively form PECs with cationic biopolymers like chitosan. A study discussed the use of chitosan-HA PECs crosslinked with genipin for the controlled release of bone morphogenetic protein-2 (BMP-2) (Nath et al. 2015). BMP-2 along with BMP-7 has a strong capacity to induce bone formation. The chitosan-HA PECs were used to improve the half-life of BMP-2 and reduce the rapid clearance of BMP-2 from the body. Chitosan-HA PEC acted as a carrier by immobilizing BMP-2 for sustained and controlled drug delivery. Since chitosan-HA had both anionic and cationic biopolymers, they also provided better attachment and proliferation of pre-osteoblasts. The PEC solution was prepared by dissolving chitosan (1%) and HA (0.1–0.5%) in 1% acetic acid. To this solution, different amounts (1–4 mg per 50 mL chitosan-HA) of genipin (a gardenia fruit extract) was added. Genipin is a natural, non-toxic crosslinker, unlike glutaraldehyde which is toxic. The gelation was continued and the gel formed was freeze-dried to obtain porous PEC scaffolds. The degree of crosslinking increased with increasing genipin concentration, which in turn affected the *in vitro* swelling of the scaffold. In the absence of crosslinking, the degradation was high in the scaffolds. Scaffolds loaded with the highest amount of BMP-2 had the lowest initial burst release. BMP-2 release was gradual and sustained only after 3 days of drug loading. 1 μ g of BMP-2 was released for 4 weeks by the scaffolds. Also, the protein absorption was found to be higher in the scaffolds, which was due to the cooperative nature of HA in chitosan-protein interactions.

Another study showed that the stability of chitosan/TPP nanoparticles can be increased markedly by coating them with HA (Almalik et al. 2013). It also reduced the toxicity of negatively charged chitosan/TPP nanoparticles. Chitosan/HA

delivery system was found to be useful in drug delivery to the corneal epithelium and the ocular surface (de la Fuente et al. 2008).

Pectin is a natural biopolymer and has a characteristic high stability in the GI tract, but easily degrades in the colon. Hence, pectin based systems are mostly used in colon drug delivery. A study briefly discussed chitosan-pectin PEC-based carrier systems for colon drug delivery (Pandey et al. 2013). Theophylline was studied as the drug. Chitosan was dissolved in 1% acetic acid and pectin was dissolved in water. They were then homogenized by mixing with gentle agitation, and dried under vacuum to yield a powder. The final composition of chitosan:pectin was 1:5. The tablet was made of 100 mg theophylline with 4% sodium starch glycolate, magnesium stearate, and lactose monohydrate. The tablet was then coated with a solution of Eudragit S100 in isopropyl alcohol. The ex vivo release studies were carried out by dissolution in solutions of pH 1.2–7.4. Chitosan-pectin tablets showed pH-dependent swelling behavior in all the solutions. The release profile of theophylline depended on factors like the type of anionic and cationic polymer, the percentage of coating, and swelling behavior. Drug release was not found below a pH of 4.6 due to the coating. However, at pH 6.8, there was a maximum of 90.9% drug release at the colon site. This was related to the reduction in the ionic interactions between chitosan and pectin at the colon site.

Water-insoluble coatings like hydroxypropyl methyl cellulose (HPMC) on chitosan-pectin PECs can reduce their swelling properties, thereby retarding the release of drugs (Macleod et al. 1999). Use of physical crosslinkers like calcium and covalent crosslinkers like EDC/NHS reduced the water uptake of the PECs and improved the tensile strength and degradation profile of the composites (Chen et al. 2010). Physical crosslinkers are more acceptable than covalent crosslinkers because of their biocompatibility.

A few other examples of drug delivery using PECs are tabulated in Table 1.

2.3 Gene Delivery

Gene delivery refers to the process of introducing a foreign gene into a host organism for the treatment of many genetic disorders such as sickle cell disease, severe combined immunodeficiency (SCID), and adenosine deaminase (ADA) deficiency. The ‘vehicles’ that carry the foreign genes are called vectors. The traditionally used vectors for gene delivery are viral or non-viral such as plasmids and liposomes. Viral vectors can carry only a limited amount of deoxyribonucleic acid (DNA) material and are known to cause immune reactions in patients. Though non-viral vectors do not trigger immune responses in the host, they have low-efficiency rates.

Researchers are looking for alternative methods of gene delivery to combine the carrying capacity and immune advantages of plasmids, and the efficiency rates of the viral vector. One such method is the use of ‘viroosomes’. *Viroosomes* are liposomes having an outer covering of viral surface proteins. The viral proteins interact

Table 1 Polyelectrolyte complexes (PECs) in drug delivery applications

Polymers employed in the PEC	Method	Drug employed	Target	Reference
Poly (vinyl pyrrolidone)-carboxyvinyl polymer	Mixing of polymer solutions	Chlorpheniramine maleate and indomethacin		Takayama and Nagai (1987)
Poly (acrylamido-2-methyl-1-propanesulfonate sodium -co- methyl methacrylate)	Free radical solution polymerization	Labetalol HCl, propranolol HCl, verapamil HCl, diltiazem HCl and oxprenolol HCl	Oral	Konar and Kim (1998)
Chitosan-tripolyphosphate and chitosan-phosphoric acid	Ionotropic gelation	6-mercaptopurine	Intestine	Mi et al. (1999a, b)
Chitosan-poly (acrylic acid)	Ionic crosslinking, freeze-drying	Amoxicillin	Stomach	de la Torre et al. (2003)
Phosphorylated chitosan-tripolyphosphate	Ionotropic gelation	Ibuprofen	Intestine	Win et al. (2003)
Poly (vinyl pyrrolidone)-poly (acrylic acid)	Template polymerization	Ketoprofen	Transmucosal	Chun et al. (2004)
Methylcellulose-carboxy vinyl polymer	Solid dispersion	Phenacetin		Ozeki et al. (2005)
Chitosan-polycarbophil	Freeze-drying			Lu et al. (2008)
Carbopol-chitosan	Freeze-drying	Theophylline		Lee et al. (2008)
Gum kondagogu-chitosan	Mixing of polymer solutions	Diclofenac		Naidu et al. (2009)
Gelatin-sodium carboxymethyl cellulose	Emulsification, phase separation	Isoniazid	Tuberculosis	Devi and Maji (2009)
Chitosan-carboxymethyl tamarind kernel powder	Drying	Budenoside	Colon	Kaur et al. (2010)

(continued)

Table 1 (continued)

Polymers employed in the PEC		Method	Drug employed	Target	Reference
Guar gum-xanthum gum		Drying	Domperidone		Singh et al. (2011)
Chitosan-pectin		Drying	Carvedilol		Kaur and Kaur (2012)
Xanthum gum-guar gum		Freeze-drying	Metoclopramide HCl	Nasal	Dehghan and Girase (2012)
Chitosan-sodium alginate copolymers		Free radical polymerization	5-fluorouracil		Li et al. (2013)
Random copolymers		Heating of polymer mixtures	Rhodamine B, bovine serum albumin		Wan et al. (2013)
Chitosan-pectin		Mixing of polymer solutions	Theophylline	Colon	Pandey et al. (2013)
Poly-L-lysine-cellulose sulfate		Mixing of polymer solutions	Rifampicin and risedronate	Bones	Vehlow et al. (2016)

with the surface proteins of the target cell and this leads to the release of foreign gene contents into the host.

In the recent years, polymer-based advancements in gene delivery have gained momentum. The goal is to deliver the desired gene into the target cell in such a manner that it results in successful expression of the protein encoded by the DNA. The desired gene must be combined with a ‘transfection agent’ such as a cationic polymer, to facilitate entry into the cell and overcome cell-based hindrances in the processing of DNA (Luo and Saltzman 2000; Pack et al. 2005) (Fig. 3). An ideal transfection agent should possess the following properties:

- The complex it forms with DNA should be small enough to be taken up by the cells
- It should facilitate internalization of DNA into the cells by active transport processes such as endocytosis
- It should protect the DNA from degradation by intracellular enzymes, and
- It should release the DNA payload at the right time and location so that it is accessible for subsequent processing.

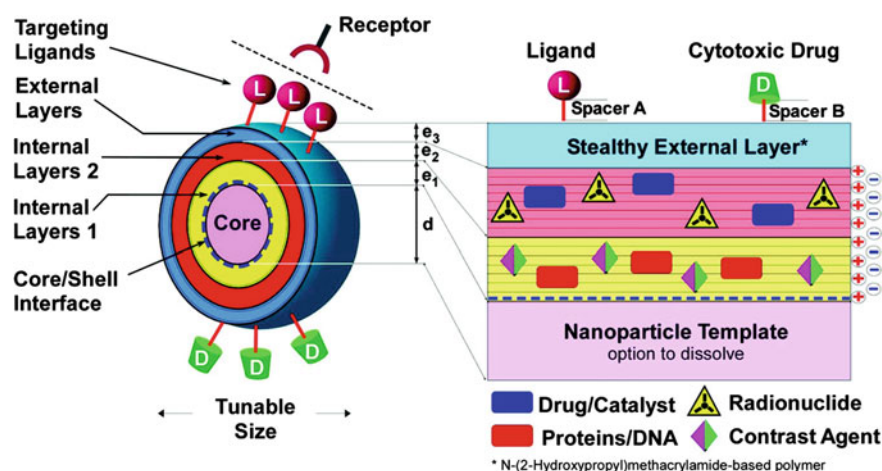


Fig. 3 A schematic depicting a new drug delivery system comprising of nanoparticles coated with multilayer shells. The shell is constructed in a stepwise manner using the layer-by-layer (LbL) polyelectrolyte multilayer (PEM) assembly method. The internal layers are divided into two compartments: Internal layer 1 (yellow) and red Internal layer 2 (red), indicating that different functionalities can be integrated into different layers. The Internal layer 1 is to serve as to compatible mediator between the core and the external layers. Both the internal layers can incorporate drugs, radionuclide for radiotherapy, proteins/nucleotides for bioactivity, or contrast agents for detection. The external layers carry functionalities such as enzymatically cleavable drugs or ligands for receptor mediated targeting, both of which must be accessible on the outside (Reprinted with permission from Schneider et al. (2009). Copyright 2009 American Chemical Society)

DNA itself is an anionic polyelectrolyte due to the presence of negatively charged sugar-phosphate backbone. Hence, the cationic polymers interact with negatively charged DNA readily and form PEC through electrostatic interactions. The formation of this PEC gives rise to complex coacervates nanoparticles in the order of 100–200 nm in size, which is ideal for size dependent internalization by cells. Also, if the proportions of cationic polymer and DNA are met aptly, they can form PECs with positive zeta potentials (Pack et al. 2005). Once it undergoes internalization through endocytosis, the PECs get packed inside tiny vesicles called *endosomes*. These endosomes are transported to other vesicles through lysosomes. Since the microenvironment inside endosomes and lysosomes are acidic, there are chances of DNA degradation. Moreover, lysosomes even contain enzymes that are capable of degrading DNA. In such cases, the success of an effective gene delivery depends on the ability of the polymer to deliver the DNA to the cytoplasm unharmed (Fig. 4).

Polyethylenimine (PEI) is a polymer having the highest cationic charge density potential. Once brought into the cytoplasm via endocytosis, protonation of PEI amines causes an influx of counter ions. This results in osmotic swelling, ultimately leading to polymer-DNA complex rupture and content release into the cytoplasm. A study successfully demonstrated that PEI caused luciferase gene transfection, indicating that its buffering capacity to pH changes inside endosomes and lysosomes (from pH 7–5) protected the DNA from nuclease degradation (Boussif et al. 1995). PEI was found to be highly efficient as a transfection agent.

Poly-L-lysine (PLL) was used for receptor-mediated gene transfer (Zauner et al. 1998). To promote internalization by receptor-mediated endocytosis, many cell

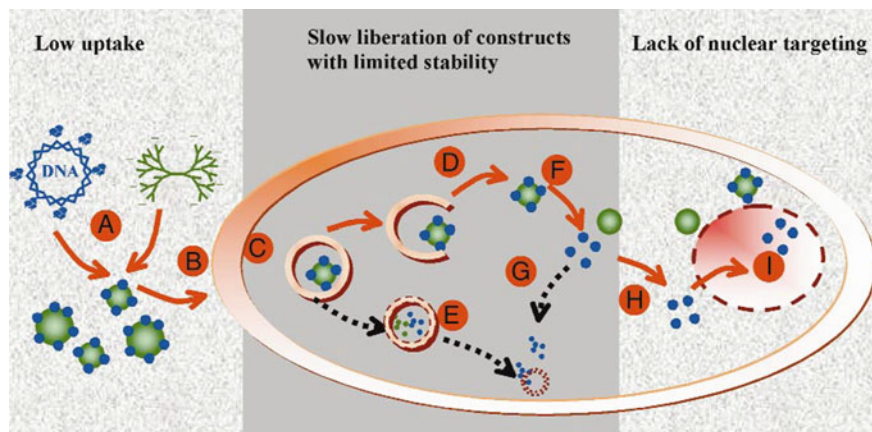


Fig. 4 Schematic drawing of DNA delivery pathways with three major barriers: low uptake across the plasma membrane, inadequate release of DNA molecules with limited stability, and lack of nuclear targeting: (A) DNA–complex formation (B) Uptake (C) Endocytosis (endosome) (D) Escape from endosome (E) Degradation (endosome) (F) Intracellular release (G) Degradation (cytosol) (H) Nuclear targeting (I) Nuclear entry and expression (Reprinted by permission from Macmillan Publishers Ltd: Nature Biotechnology, Luo and Saltzman, 18, 33–37, copyright 2000)

binding ligands for hepatocytes, T-cells, and transferrin were attached to PLL through covalent bonding. The addition of lysosomotropic agents (glycerol) and endosmolytic agents (membrane active peptides) to the transfection medium promoted the release of particles from internal vesicles. PLL-based gene transfer provided flexibility regarding the size of DNA and also facilitated receptor-ligand incorporation.

Nanospheres made from PECs of complimentary DNA (cDNA) and cationic biopolymers such as gelatin and chitosan were evaluated as non-viral gene delivery vehicles (Leong et al. 1998). Although these carriers showed lower transfection efficiency as compared to the two controls, lipofectamine and calcium phosphate, they showed increased expression of β -galactosidase. This gene delivery system possessed the following advantages: (1) conjugation of ligands to the nanosphere for receptor-mediated endocytosis was possible; (2) degradation of DNA in endosomes and lysosomes can be reduced by incorporating lysosomolytic agents; (3) nanospheres can encapsulate bioactive agents, apart from DNA (such as plasmids); (4) improved bioavailability of DNA due to shelter from degradation by serum nuclease; and (5) storage using lyophilization.

The layer-by-layer (LbL) assembly technique makes use of attractive electrostatic forces existing between oppositely charged polymers. When iterative dipping of a substance is carried alternately in cationic and anionic polymers, it gives rise to thin, multilayered polyelectrolyte films. This technique offers several advantages, such as control over film composition and thickness. Another practical advantage is the direct incorporation of DNA as an anionic layer, offering control over loading of DNA at the surface. Also, LbL allows incorporation of multiple layers of different types of DNA. This creates additional opportunities for internalization and uptake of the DNA by the cells.

The first report that applied multilayered polyelectrolyte thin films for gene delivery made use of alternate layers of anionic DNA and cationic poly(allylamine) (Lvov et al. 1993). Another study demonstrated that it is possible to develop DNA-containing multilayer polyelectrolyte films that offer controlled release of transcriptionally active DNA from surfaces (Zhang et al. 2004). Sustained release of functional plasmid (pDNA) under physiological conditions was promoted by deposition of alternating multilayers of anionic pDNA encoding for enhanced green fluorescent protein (EGFP) and a cationic synthetic degradable polyamine on planar silicon and quartz substrates using the LbL technique. The layers eroded gradually within 30 h when incubated in phosphate-buffered saline (PBS) at 37 °C. Characterization studies revealed that the DNA released had open circular topology and successfully promoted expression of EGFP.

A study described the erosion of multilayered films in a reducing environment (Blacklock et al. 2007). LbL films were made from DNA and transactivator of transcription (TAT)-based polypeptide (PTAT) having disulfide linkages in the backbone. Controlled disassembly of the multilayers was activated by 1,4-dithiothreitol (DTT) reduction of the disulfide bonds present in the PTAT backbone. When the study was carried out by replacing PTAT with PLL, the films

showed stability against DTT reduction. Hence, it was deduced that the presence of reducing condition is necessary to trigger DNA release from LbL films.

Ultrathin multilayered films with prolonged release of DNA were developed from pDNA and a cationic polymer having side chains capable of charge-shifting (Zhang and Lynn 2007). The erosion of pDNA-polymer films was due to the gradual hydrolysis of these side-chains. The assemblies were capable of releasing intact DNA up to a period of 3 months in PBS and promoted efficient transgene expression. This LbL assembly approach serves as an alternative to rapidly eroding thin films which incorporate polyamines that degrade hydrolytically or enzymatically.

Gene delivery systems have also been developed with ternary complexes. Initially, a binary complex of pDNA and protamine showed slight toxicity due to the presence of overall cationic charge (Kanda et al. 2013). A ternary complex of pDNA, protamine, and γ -poly(glutamic acid) (γ -PGA) when used as a transfection system not only showed no cytotoxicity, it also displayed a transfection efficiency as high as that of the binary pDNA-protamine system, although they possessed different zeta potentials. The endocytosis mechanism of both the complexes was also found to be different. The pDNA-protamine system was absorbed by caveolae-mediated endocytosis and the pDNA-protamine- γ -PGA system by clathrin-mediated endocytosis. Another ternary complex comprising of pDNA electrostatically assembled with a cationic polymer, polyamidoamine (PAMAM) dendrimers and an anionic polymer, chondroitin sulfate (CS) was developed for efficient gene delivery (Imamura et al. 2014). pDNA-PAMAM-CS ternary complex formed nanoparticles with negative zeta potential, expressed no cytotoxicity and agglutination, and showed high gene expression in the spleen of mice when injected intravenously.

2.4 Bioadhesives

A *bioadhesive* is a natural polymer that is produced or derived from living organisms and acts as an adhesive. Traditionally, starch and gelatin have been used as adhesives for general purposes, but these natural adhesives have well-known limitations such as low stability at higher temperatures. To overcome these disadvantages, natural adhesives were replaced with synthetic sealants like polyurethane (PU), epoxy, cyanoacrylate, and acrylic polymers. However, synthetic polymers pose biocompatibility issues. In the recent years, the bioadhesive market has expanded rapidly to the growing demands of bioadhesives in biomedical and other sectors. These bioadhesives are biocompatible, capable of retaining on the surface for a long period of time, and have fewer environmental concerns.

Bioadhesives are broadly classified into the following five categories:

(i) *Natural adhesives*

Natural adhesives are the oldest known adhesives to humankind. These adhesives are partially or fully synthesized from bio-based raw materials and are not substances used by biological systems as glues. Natural adhesives are easily available, cheap, easily degradable, non-toxic, and environmentally friendly. Examples of natural adhesives include rubber, gums, casein, bitumen, and animal-based adhesives such as collagen.

(ii) *Biological adhesives*

This type of bioadhesives are those which are naturally secreted by marine, water, and land organisms. Mollusks, worms, bacteria, fungi, spiders, insects, etc. are known to produce adhesives that adhere to various surfaces. One common example of biological adhesives is biofilm formation. *Biofilms* are groups of microorganisms that stick to each other and also adhere to a surface. The microorganisms attach via secretion of certain proteins and polysaccharides, and self-produce an extracellular matrix (ECM) within which the cells are embedded. Biofilm formation is the major cause of biofouling, which leads to corrosion of ship hulls and other major industrial systems.

(iii) *Biocompatible adhesives*

Biocompatible adhesives are specially designed for biomedical applications wherein interaction with living tissues takes place. This category can include both natural as well as synthetic adhesives. There is an increasing need of biocompatible adhesives for drug delivery and surgical (such as sutures) applications. Pectin, acacia gum, tragacanth, poly(vinyl alcohol) etc. are widely used biocompatible adhesives.

(iv) *Biomimetic adhesives*

Biomimetic adhesives are designed to closely resemble the biological structure and environment of a particular natural adhesive.

(v) *Bio-inspired adhesives*

These adhesives are designed by taking inspiration from nature's mechanisms and functions of adhesion.

This chapter will be focussing mainly only on the biocompatible, biomimetic, and bio-inspired adhesives used for biomedical applications.

2.4.1 Bioadhesives in Biomedical Applications

In the field of biomedicine, the most important application of bioadhesives is for the closure of surgical wounds. Among the various method applied for closing a wound, the most widely used ones are stitches (or sutures), surgical staples, and tapes.

In suturing, the doctor simply “sews” the ends of the skin for wound closure and secures a knot. The closure of wound promotes natural healing which may otherwise not happen. This method can be used for almost all types of internal and external wounds. The sutures used for this purpose may be absorbable or non-absorbable. The *absorbable* sutures are ones which start losing their strength as they are slowly broken down by the body with the passage of time and do not need a removal process afterward. *Non-absorbable* sutures require removal once the wound has healed. Sutures are often associated with wound infection and scar formation, not to mention the pain associated with it. Hence, the recent years have seen a rise in the use of bioadhesives for wound closure. These bioadhesives are biocompatible, low cost, easy to handle, firm, and avoid pain to the patient.

Tissue adhesives or *sealants* refer to a liquid or semi-liquid compounds which can be applied to tissue incisions to promote wound closure, hemostasis or adherence to soft tissues. An ideal tissue bioadhesive should possess the following properties:

- Non-toxic, sterilizable, easy to produce, affordable, cost-effective
- Possess flow properties for easy and precise application
- Exhibit rapid solidification under physiological conditions
- Should possess adhesiveness for sufficient period of time to allow tissue healing, and
- Possess mechanical properties throughout healing period.

The sandcastle worm, *Phragmatopoma californica*, is a marine polychaete worm which forms reefs. This worm forms a glue to build a protective tube by sticking bits of sand grains and broken seashells together underwater. The underwater glue is made up of different proteins having opposite charges, called as polyphenolic proteins (Stewart et al. 2004). This complex coacervate (or PEC) can be used to repair fractured bones instead of placing metal pins and screws. It can also be used as sealants for skin cuts and wound closure. The precursor proteins for the glue, Pc1, and Pc2, when isolated from the worm were found to contain repeated sequences of motifs which were rich in glycine, lysine, and 3,4-dihydroxyphenyl-L-alanine (L-DOPA) residues. The protein side chains possess phosphate and amine groups whose presence are known to promote adhesion under wet conditions.

Internal surgeries require strong wet tissue adhesion for sutureless closure. A study developed a citrate-based bioadhesive for sutureless wound closure, inspired from mussels (Mehdizadeh et al. 2012). The injectable citrate-based mussel-inspired bioadhesive (iCMBAs) pre-polymer was developed using citric acid and polyethylene glycol (PEG), functionalized with catechol-containing compounds, dopamine, and L-DOPA through a polycondensation reaction. The developed PEC-based iCMBAs were found to be superior to the traditionally used tissue bioadhesive, fibrin glue. It possessed controlled biodegradability and good elastomeric mechanical properties that closely resembled that of tissues. More importantly, they did not elicit any inflammatory response and facilitated wound healing.

Another mussel-inspired PEC-based bioadhesive was developed using poly (acrylic acid) (PAA) and DOPA (Wang et al. 2015). The PAA-DOPA, when metal chelated with ‘weak’ crosslinker Zn^{2+} at pH 4, imparted superior adhesion as well as good mechanical properties to the bioadhesive under both dry and wet conditions. However, when chelated with ‘strong’ metal crosslinker Fe^{3+} , the results were poor. It was proposed that zinc chelated DOPA through electrostatic interactions with anionic carboxylic groups of PAA. This resulted in the spontaneous generation of PEC coacervate. Moreover, injection at low pH and then the presence of higher pH contributed to gelation of the bioadhesive. This system can prove to be an ideal material for tissue adhesion and other medical purposes.

A recombinant hybrid mussel adhesive was developed from mussel adhesive proteins (MAPs) and hyaluronic acid (HA) (Lim et al. 2010). Since pure natural MAPs are difficult to obtain, a recombinant hybrid of MAPs (fp-151 or fp-131) and HA was produced by mixing in the ratio of 8:2. PEC was formed between the cationic fp-151 or fp-131 and anionic HA. The resulting coacervation process increased the adhesive strength in both dry and wet environments and showed viscoelastic properties. Oil microencapsulation of the fp-151/HA and fp-131/HA coacervates further indicated superior adhesive properties. This demonstrated the possible uses of recombinant MAPs as bioadhesive and self-adhesive drug carriers in tissue engineering applications.

Barnacles are often found clinging to ship hulls, nuclear submarines and even other animals. Scientists studying barnacles’ ability to stick to objects submerged in water found that in the final larval stage, the larvae (called *cyprids*) attach to different surfaces and undergo metamorphosis to become adult barnacles (Gohad et al. 2014). The adhesive plaques that are responsible for initial cyprid attachment contain proteins and peptides along with lipids. The adhesive system is essentially biphasic, having both a protein phase and a lipid phase. The presence of this biphasic system results in strong adhesion that can attach to virtually anything. This adhesive can be potentially used for various biomedical applications.

2.5 Tissue Engineering

A number of natural and synthetic polymers such as collagen, gelatin, fibrin, silk fibroin, alginate, chitosan, hyaluronic acid (HA), poly(glycolic acid), etc. are currently being employed for making scaffolds for tissue engineering applications (Kim and Lee 2016; Mousa et al. 2016; Yadav et al. 2015). Biological polymers contain functional groups which can be linked with cell adhesive proteins and growth factors, but they lack mechanical properties. On the other hand, synthetic polymers have superior mechanical properties and their structures can be controlled. However, it is a difficult task to link them with cell signaling molecules because of lack of functional groups. The binary blends of polymers have been explored for tissue engineering application because it is a cost-effective way of preparing new materials with the desired physicochemical and mechanical properties, as well as

coveted biological responses. Blends with synthetic and natural polymers can combine with the wide range of physicochemical properties of synthetic polymers as well as the biocompatibility and biological functionality of biopolymers (Costa and Mano 2014). Blends of complementary charged polymers, i.e., polyelectrolyte complexes (PECs) have also been investigated for tissue engineering application. Generally, PECs are prepared in combination with chitosan, a polycation. Poly-L-lysine (PLL) has also been explored as a polycationic polymer. As polyanionic polymers, various polymers have been explored such as gelatin, silk, poly(glutamic acid) (PGA), alginate, pectin, poly(galacturonic acid) (PgA), and hyaluronic acid (HA).

2.5.1 Alginate-Chitosan

Chitosan is the deacetylated derivative of chitin, composed of β -(1-4)-linked D-glucosamine units and a small amount of N-acetyl-D-glucosamine residues. Chitosan has been shown to exhibit excellent antimicrobial activity, biocompatibility, biodegradability, and accelerated wound healing property. Therefore, it is considered as a promising scaffold material in the regeneration of various tissues such as skin, bone, cartilage, liver, nerve, and cardiovascular tissue (Di Martino et al. 2005). The presence of amine groups imparts cationic nature to chitosan. Alginate is also a polysaccharide which has been extensively investigated for fabrication of scaffolds for tissue engineering. With its gelling property, biocompatibility, hydrophilicity and biodegradability under normal physiological conditions, it is an excellent polymer for tissue engineering. The carboxylate ions of alginate and amine groups of chitosan can bind together to form a PEC.

Both chitosan and alginate are highly hygroscopic and absorb the excess amount of fluid. This leads to a drastic decrease in mechanical properties of scaffolds made of only chitosan or alginate. A PEC of chitosan and alginate absorbs less amount of water as compared to the individual components and hence, shows the superior mechanical property. The fabrication of PEC scaffold is conducted at physiological conditions. Therefore, they can be easily incorporated with growth factors and cells. A porous scaffold made from chitosan and alginate polymers showed significantly better mechanical and biological properties compared to chitosan scaffolds (Li et al. 2005). Enhanced mechanical properties were attributed to the complex formation between chitosan and alginate. In vitro studies using osteoblasts showed that the scaffolds favored cell attachment, proliferation, and deposition of calcified matrix. In vivo study was conducted by implanting scaffolds in the muscles of rats. The results suggested that the alginate-chitosan scaffold does not cause a fibrotic response.

PEC can also be fabricated in fiber form. PEC fibers were prepared from alginate and chitosan by spinning highly concentrated alginate solution in diluted chitosan solution (Majima et al. 2005). Chitosan solution was used as a crosslinker. Mechanical tests showed that the prepared fibers displayed a good mechanical strength of above 200 MPa. Cell studies revealed superior adhesion of fibroblast cells and deposition of dense type I collagen on the fibers.

Mechanical properties and bioactivity of PEC scaffolds can be further enhanced by incorporation of hydroxyapatite (HAp) in the scaffolds. A study reported the fabrication of alginate-chitosan PEC scaffolds with and without the inclusion of HAp (Han et al. 2010). X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS) results revealed the formation of PEC scaffold by ionic interaction between NH_3^+ of chitosan and COO^- of alginate. Alginate-chitosan PEC scaffold exhibited significantly higher compressive strength compared to uncrosslinked alginate scaffold and Ca^{2+} crosslinked alginate scaffold. Considering the good biocompatibility of the PEC scaffold and better mechanical strength, alginate-chitosan scaffolds have the potential to be used for tissue engineering application.

To mimic ECM structure, PEC fibers have been synthesized and used for tissue engineering applications. PEC fiber formation was first reported in 1998 by Amaike and co-workers for the manufacturing of textile fibers (Amaike et al. 1998). In this fiber formation process, a fiber was drawn from the interface of two oppositely charged polymers. During this process, water-soluble polymers become insoluble in the form of PEC fibers at the interface. Another research further elucidated fiber formation mechanism. They found out that the PEC fibers coalesce in the 100 nm range and they combine to form a thicker fiber (Wan et al. 2006). This method of fiber formation has significant potential in tissue engineering because it is simple, toxic solvent-free, water-based method, and does not require high temperature. To avoid clumping of wet fibers, inorganic silica components were incorporated into the PEC fibers as crosslinkers.

In a study, a composite scaffold made of alginate, chitosan, collagen, and HAp was fabricated by electrospinning technique (Yu et al. 2013). In this method, alginate fibers were electrospun in a coagulation bath containing chitosan. These fibers were then dispersed in collagen and HAp solutions to coat fibers with the respective solutions. The distribution of each component was confirmed by confocal laser scanning microscope (CLSM) using fluorescent labeling of polymers. The distribution of different phases was also investigated using field-emission scanning electron microscopy (FE-SEM) and transmission electron microscopy (TEM). This new composite fiber showed better stability in collagenase solution in comparison to collagen films.

Since PEC preparation is done at room temperature and it does not require any toxic solvent, cells can be easily encapsulated within the PEC biomaterials. A PEC of alginate-chitosan with microencapsulated mouse osteoblast MC3T3-E1 cells combined with calcium phosphate cement was prepared (Qiao et al. 2013). The resultant construct was implanted subcutaneously in a nude mouse. The MC3T3-E1 cells were labeled with a fluorescent dye and traced *in vivo*. The encapsulated cells survived in the *in vivo* conditions for at least two weeks. Implanted construct promoted lamellar bone-like mineralization, deposition of new collagen, and showed angiogenesis after 4 weeks. At 8 weeks, the absorption of the bone cement and further deposition of collagen was observed.

Nano-ranged bioactive silica particles can form a tighter interface with the polymer matrix and enhance the mechanical strength of the composites.

Additionally, it can also improve biomineralization of the composite scaffolds. The synthesis and characterization of biocomposite scaffolds containing chitosan, alginate, and nano-silica for bone tissue engineering application was reported (Sowjanya et al. 2013). The scaffolds were fabricated using the freeze-drying (lyophilization) method. These nanocomposite scaffolds had a pore size of about 20–100 μm . The presence of nano SiO_2 in the scaffolds enhanced protein adsorption. The incorporation of nano silica did not affect biodegradability of the scaffolds but improved apatite deposition on these scaffolds. In vitro studies indicated no significant loss of cell viability of osteoprogenitor cells.

Chitosan/alginate-based PEC scaffolds were investigated for tissue regeneration after acute myocardial infarction (Deng et al. 2015). Scaffolds having different alginate to chitosan ratios were prepared by lyophilization. The prepared scaffolds showed high porosity and highly interconnected pores. In vitro evaluation using mesenchymal stem cells (MSCs) demonstrated biocompatibility, and ability to proliferate and maintain the paracrine activity of the MSCs. In vivo performance of seeded 3D PEC scaffolds with a polymeric ratio of 40/60 was evaluated in acute myocardial infarction model in rats. The results showed that the PEC scaffolds promoted a significant increase in the ejection fraction, improved neovascularization, attenuated fibrosis, and less left ventricular dilatation when compared to the control group.

Adhesions are abnormal fibrous connections between tissues and can occur following virtually any type of surgery. Adhesions develop after an injury to the normal peritoneal tissue. This injury can result from surgery, trauma, inflammation, infection, or foreign body placement in the peritoneal cavity. Post-operative adhesions develop due to inflammation, which is a part of body's normal healing process. Adhesions often require subsequent surgeries to remove them which is complicated process, not to mention time-consuming too. The most widely used method to prevent post-operative adhesions is to insert physical barriers. Most physical barriers have low success rates. A PEC membrane composed of chitosan and alginate was developed to prevent post-surgical adhesions in neurosurgery (Verma et al. 2012). The membranes were hygroscopic, possessed good mechanical properties, and controlled the release of the model drug, albumin. As fibroblast cells play an important role in adhesion formation, the adhesion and migration study using fibroblast and mixed neuronal cells were carried out. Keeping the concentration of alginate higher to impart the PEC an overall negative charge, the adhesion, and migration of fibroblasts and neuronal cells was stopped in vitro. Both fibroblasts cells and neuronal cells neither adhered nor migrated onto the PEC material after 5 days in vitro. Aqueous absorption study showed that PEC films were extremely hygroscopic and absorbed a significant amount of water. The amount of complex formed was dependent on the ratio of polyelectrolyte and it was found to be highest in films containing an equal amount of each polymer. Degradation study in lysozyme solution revealed that films were stable even after 1 month.

2.5.2 Silk-Chitosan

Silk is a natural polymeric biomaterial produced by silkworm. It has excellent mechanical properties, biocompatibility, and biodegradability. Silk consists of two protein layers: a hydrophobic fibroin inner layer and a hydrophilic sericin outer layer. Silk fibroin is known to be biocompatible and hence, it has been extensively investigated for many biomedical applications (Mandal et al. 2012; Mandal and Kundu 2008; Mobini et al. 2013). It is used in both native fiber form as well as in regenerated forms such as films, electrospun fibers, wet-spun fibers, hydrogels, and scaffolds.

The PEC formation between silk fibroin and chitosan is due to the ionic interactions between carboxylate moieties on silk fibroin and protonated amines on chitosan. In one study, researchers examined porous PEC scaffolds of silk fibroin and chitosan (Bhardwaj and Kundu 2011). The fabricated scaffolds showed pore sizes in the range of 100–160 μm , good interconnectivity, and high porosity. The scaffolds also exhibited reduced degradation rate in lysozyme when compared to pure chitosan scaffolds. Moreover, they also showed a higher compressive strength and modulus than scaffolds made from the individual components. Chitosan preserved its antibacterial effect when incorporated at the higher amounts in the blends. In vitro cytocompatibility tests demonstrated that the PEC scaffolds supported growth and adhesion of fibroblast cells.

In another study, silk fibroin-chitosan scaffolds were fabricated and investigated for cartilage tissue engineering (Bhardwaj et al. 2011). The researchers seeded bovine chondrocytes in the silk-chitosan scaffolds and cultured in vitro for 2 weeks. The constructs were analyzed for cell viability, histology, ECM components (GAG) and collagen types I and II, and biomechanical properties. The study demonstrated that silk fibroin-chitosan scaffolds support chondrocyte attachment and their growth. The chondrogenic phenotype was indicated by Alcian Blue histochemistry and showed relative expression of type II versus type I collagen. Fibroin-chitosan scaffolds with 1:1 ratio showed best results as they demonstrated the highest accumulation of GAG and collagen.

PEC-based scaffolds can also be loaded with growth factors for bone tissue engineering (Tong et al. 2016). In this study, vascular endothelial growth factor (VEGF) was directly added to silk fibroin-chitosan scaffolds. VEGF-containing scaffolds promoted significant human fetal osteoblast 1.19 cell growth and proliferation in the scaffolds.

Various studies have shown that mimicking nanofibrous structure of ECM has positive effects on cellular morphology and cellular activities including cell attachment, proliferation, and differentiation. Alignment of 3D nanofibrous silk fibroin-chitosan scaffolds was achieved using dielectrophoresis (DEP) (Dunne et al. 2014). DEP is a non-destructive electrokinetic mechanism which can be used to manipulate micro or nanoparticles such as DNA, proteins, nanotubes, and nanoparticles in aqueous solutions. The researchers studied effects of alternating current frequency, the presence of ions, silk fibroin to chitosan ratio, and post-DEP freezing temperature on fiber alignment. Highest alignment was achieved in silk fibroin-chitosan 50:50 samples prepared at 10 MHz with sodium chloride.

2.5.3 Pectin-Chitosan

Pectins are a family of polysaccharides rich in D-galacturonic acid, present in the primary cell walls of plants. Pectin is extracted mainly from apple pomace and peels of citrus fruits by means of an acidic aqueous extraction (Munarin et al. 2012). Commercial pectin consists predominantly of linear chains of α -(1-4)-D-galacturonic acid residues, partially methyl esterified. Pectin is classified depending on the degree of substitution of D-galacturonic carboxyl groups by methoxyl groups ($-\text{OCH}_3$), defined as the degree of esterification (DE). Pectins are either highly esterified ($\text{DE} > 50\%$) or low-esterified ($\text{DE} < 50\%$). Pectin has been investigated for colon-specific drug delivery systems because it can be degraded by enzymes produced by a large number of microorganisms present in the colon (Liu et al. 2003). Pectin has also been reported to exhibit anti-inflammatory and anti-carcinogenic properties (Tazawa et al. 1997).

A study reported the fabrication of pectin-chitosan porous scaffolds for bone tissue engineering (Coimbra et al. 2011). Elemental analysis showed that the final scaffolds were composed of approximately 30% w/w chitosan and 70% w/w pectin, irrespective of their initial proportions. Degradation studies conducted in PBS at pH 7.4 showed that the scaffolds lose approximately half of their weight after one month of study. SEM study revealed highly porous and irregular structure of the scaffolds. Assessment of its biocompatibility conducted on human osteoblast cells showed that the pectin-chitosan scaffolds supported cell adhesion and proliferation. MTT assay corroborated these findings.

Poly(galacturonic acid) (PgA), also known as pectic acid, is produced after degradation of pectin. PgA forms gels in the presence of calcium ions, similar to that of alginate. Porous, fiber-containing scaffolds comprising of PgA and chitosan were fabricated by optimizing freezing temperature and PEC concentration (Verma et al. 2009). At higher freezing temperature and concentration, scaffolds had sheet-like structure. It was inferred that the fiber formation occurred due to the assembly of PEC particles during the freezing process. The fiber formation occurred at 0.1 g/100 ml solution and -196°C freezing temperature. PgA-chitosan containing HAp was also prepared. In vitro studies on human osteoblast cells showed that PgA-chitosan scaffolds did not support cell adhesion. However, the addition of HAp significantly improved cell adhesion.

In another study, nanocomposite scaffolds consisting of HAp, chitosan, and PgA was reported (Verma 2008; Verma et al. 2010). HAp was synthesized in the presence of PgA and chitosan in solution. These biopolymers in solution acted as nucleating agents for crystallization of HAp. This method is similar to the way minerals deposit in living systems and hence, it is a biomimetic method of HAp synthesis. AFM images revealed the uniform distribution of HAp in the polymer matrix. There was nearly 100% improvement in elastic modulus of the chitosan-PgA-HAp nanocomposite in comparison to chitosan-HAp and PgA-HAp nanocomposite. Fourier transform analysis indicated that the increase in mechanical properties was attributed to the interfacial interactions between chitosan and PgA present in the nanocomposite. In vitro studies were conducted on human osteoblast

cells by culturing on both 2D and 3D structures of the nanocomposites. 2D structures were created simply coating on culture dishes and 3D scaffolds were prepared using lyophilization. The adhesion of osteoblast cells was found to be dependent on the amount of HAp present in the nanocomposite. Higher amounts of HAp favored better cell adhesion and proliferation. After few days of cell culture, osteoblast cells separated into colonies and subsequently into nodules. The formation of bone-like nodules was observed after 7 days of culture. The nodule size continued to increase with time and after 10 days of culture, nodules were in the range of 250–500 μm . Later, these nodules detached from the surface and coalesced together. SEM images showed the fibrous protein-like structure in the nodules.

A study reported nanocomposites made of chitosan, PgA, HAp, and sodium montmorillonite clay (Katti et al. 2010). The clay was first modified with three different unnatural amino acids before incorporating into the composite. XRD results showed an increase in the d-spacing as an indication of intercalation of amino acids into the d-spacing of the clay after being modified with the three unnatural amino acids. Cell culture experiments showed that the sodium montmorillonite clay modified with the three amino acids and the nanocomposite were biocompatible.

2.5.4 Gelatin-Chitosan

Porous scaffolds of chitosan and gelatin for dermal tissue engineering were fabricated by lyophilization (Tseng et al. 2013). These scaffolds were crosslinked using various crosslinking agents including glutaraldehyde, 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC), and genipin. Biocompatibility studies conducted on human fibroblast cells indicated that EDC-crosslinked scaffolds exhibited least cell toxicity as was evident from the highest number of cells that survived in the scaffold after four days of culture. EDC-crosslinked chitosan-gelatin scaffolds also showed superior mechanical properties in both dry and wet state. Moreover, the elastic modulus of EDC-crosslinked scaffolds was similar to that of commercial collagen wound dressings.

In another study, chitosan-gelatin porous scaffolds containing hyaluronic acid (HA) and heparan sulfate (HS) were fabricated using lyophilization technique. These scaffolds were prepared for neural tissue engineering (Guan et al. 2013). Electron microscopic images displayed that the chitosan-gelatin-HA-HS composite scaffolds had above 96% porosity and average pore size ranging from 90–140 μm . Cell viability assay and fluorescence microscopy observation revealed positive effects of HA and HS present in the scaffolds on adhesion and proliferation of neural stem and progenitor cells. Chitosan-gelatin-HA-HS composite scaffolds also promoted multilineage differentiation potentials of the cells with enhanced neuronal differentiation upon induction in comparison to chitosan-gelatin scaffolds without HA and HS.

One of the major challenges in tissue engineering is to mimic complex 3D structure of the native tissue. Bioprinting uses 3D printing technology that is used to

simultaneously deposit multiple types of cells and biomaterials to replicate complex tissue structures. Collagen has been primarily used for bioprinting purposes, but it suffers from poor printability and long crosslinking time. It becomes challenging to create a tissue construct with precise shape and configuration. To overcome this problem, chitosan/gelatin-based PEC was utilized for functional 3D bioprinting (Ng et al. 2016). Chitosan was complexed with the oppositely charged gelatin at a specific pH of 6.5 to form PECs. The fabricated polyelectrolyte blends were evaluated for their chemical interactions within the polymer blend, rheological properties, printing efficiency, and biocompatibility. The printed polyelectrolyte gels exhibited better shape fidelity and biocompatibility.

2.5.5 Poly(Glutamic Acid)-Chitosan

PEC of γ -poly(glutamic acid) (γ -PGA), a non-toxic, hydrophilic, and biodegradable polymer, with chitosan is widely used to enhance the hydrophilicity and cytocompatibility of chitosan-based biomaterials. γ -PGA contains carboxylate groups which form PEC with chitosan and has been successfully used in bio-glue and drug delivery systems (Hsieh et al. 2005). The authors used the powdered form of chitosan and added it to the γ -PGA solution to get a homogeneous solution, as mixing non-powdered chitosan and γ -PGA solution produced large aggregates at the interface. This study showed that the pores in the scaffolds were interconnected and the pore sizes were in the range of 30–100 μm . The addition of γ -PGA made the scaffolds more hydrophilic, cytocompatible and enhanced their load bearing capacity.

Porous poly-L-glutamic acid (PLGA)-chitosan PEC microspheres were developed through electrostatic interaction between the two polymers (Fang et al. 2014). Firstly, chitosan microspheres were prepared and then these microspheres were impregnated PLGA solution to form PEC microspheres at 37 °C. PEC microspheres showed better structural stability. The pore size of the microspheres was controlled by varying the solid content and freezing temperature. Chitosan microspheres with a concentration of 2% (w/v) and a freezing temperature of $-20\text{ }^{\circ}\text{C}$ exhibited an average pore size of $47.5 \pm 5.4\text{ }\mu\text{m}$. Due to strong electrostatic interaction, a large amount of PLGA (110.3 $\mu\text{g/mg}$) was homogeneously absorbed within the chitosan microspheres. These PEC microspheres retained their original size, pore diameter, and interconnected porous structure. They also promoted better chondrocyte attachment and proliferation when compared to chitosan microspheres. Chondrocytes containing PEC microspheres were injected into nude mice and found to produce significantly more cartilaginous matrix than on injection with chitosan microspheres.

Another study developed porous PEC-based scaffolds of PLGA and chitosan using lyophilization method (Yan et al. 2013). PLGA-chitosan scaffolds containing 2% polymer content and at a freezing temperature of $-20\text{ }^{\circ}\text{C}$ exhibited an interconnected porous structure with an average pore size of 150–200 μm . The scaffolds showed a swelling ratio of 700%. Resistance to degradation increased with increase

in chitosan concentration. The scaffolds exhibited gel-like behavior in both dry and wet state. In vitro culture conducted on rabbit adipose-derived stem cells (ASCs) indicated that the scaffolds supported cell attachment and growth. In vivo study was performed in rabbit articular cartilage repair model and indicated successful repair of articular cartilage defects after 12 weeks of implantation.

2.6 Cell-Based Therapy

Rejection of foreign cells by the immune system is a major hurdle in cell-based therapies and regenerative technologies. New technologies are emerging to prevent the rejection of transplanted cells by the immune system. These technologies involve placing the desired cells within a biocompatible material in an attempt to protect the cells from the host immune attack and prolong their function in vivo (Hennink and van Nostrum 2012; Nicodemus and Bryant 2008). Major functions of delivery materials are to provide structural support and proper environment for cells to function. Delivery of cells can be achieved using injectable matrices, soft scaffolds, membranes, and solid load-bearing scaffolds.

Articular cartilage has a limited regenerative capacity and this complicates the treatment of joint injuries and osteoarthritis. Newer strategies based on biomaterials and cells are being developed for the treatment of damaged cartilage. PECs-based on chondroitin sulfate (CS) and chitosan (CH) were used to encapsulate mesenchymal stem cells (MSCs) (Daley et al. 2015). MSCs were encapsulated in the PECs by the either of the following ways: using water-in-oil emulsification process, direct embedment of MSCs in PEC, or by co-embedding MSCs with PEC in agarose-based microbeads. Direct embedding of MSCs in PEC resulted in large particles. However, co-embedding of PEC particles with MSCs in agarose resulted in uniform microbeads of 80–90 μm in diameter. Cell viability was reported to be high irrespective of the embedding method. Both high and low CS:CH ratios resulted in more homogeneous microbeads than 1:1 formulation. Effect of PEC on chondrogenesis was evident from the higher expression of sulfated GAG and collagen type II in 10:1 CS:CH PEC-agarose microbeads compared to pure agarose beads.

In a recent study, series of polycations with different molecular weights of N-(3-aminopropyl) methacrylamide hydrochloride (APMA) and N-(2-hydroxypropyl) methacrylamide (HPMA) were prepared by reversible addition-fragmentation chain transfer (RAFT) copolymerization (Kleinberger et al. 2016). These polycations were complexed with alginate for cell encapsulation. Hydrogels with lower cationic charge density and lower molecular weight showed less cytotoxicity and cell adhesion. However, cells were found to be more mobile within alginate gels. This study suggests that cellular behavior also depends on the composition and molecular weights of the polyelectrolytes.

Cell-encapsulated spiral-shaped alginate fibers were produced through a combination of ionotropic gelation and a perfusion-based (LbL) technique (Sher et al. 2015). The spiral shape was achieved by reeling alginate fibers on cylindrical molds having different geometries and sizes. Chelation between alginate and chitosan altered the internal microenvironment of the 3D construct from solid to the liquefied state, while preserving the external geometry. Cell viability of encapsulated L929 cells by MTS cell proliferation and double-stranded DNA quantification assays suggested that liquefied 3D constructs favored survival of cells more than non-liquefied ones.

Besides mammalian cells, probiotic bacteria have also been encapsulated for biomedical applications. *Lactobacillus acidophilus* has beneficial effects on the health of the host. However, they must be protected from the acidic environment of the stomach and the bile in the small intestine. Microencapsulation of probiotics is a smart way to protect probiotic organisms against an unfavorable environment and maintain their metabolic active state. *L. acidophilus* was immobilized with xanthan-chitosan gel using extrusion method (Chen et al. 2015). The impact of various factors such as pH, concentration, and cell suspension-xanthan ratio on microencapsulation of *L. acidophilus* was investigated. Optimum pH and concentration of chitosan solution for *L. acidophilus* were 5.5 and 0.9%, respectively. Optimum xanthan concentration and cell suspension-xanthan ratio were 0.7% and 1:10, respectively. The optimum mixed bacteria glue liquid-chitosan ratio was 1:3.

In another study, hollow polymer microspheres were prepared to encapsulate *Escherichia coli* (Flemke et al. 2013). Hollow microspheres were based on porous calcium carbonate cores with an average size of 5 μm . The outer layer was prepared by LbL deposition of different polyelectrolytes and proteins onto the porous calcium carbonate cores. CLSM and microtiter plate fluorescence tests were used to investigate the effect of encapsulation process as well as polyelectrolytes on the survival rate of the cells. These studies indicated that approximately 40% of the cells survived the encapsulation process. The lag phase of cells treated with polyelectrolytes increased and the encapsulated *E. coli* cells were able to produce green fluorescent protein inside the microcapsules.

2.7 Coatings

Biomedical implants are coated with a variety of materials to improve their biocompatibility, tissue integration, and protection from body immune system. These coating can also be used as drug delivery systems.

Two types of multilayered coatings onto titanium were fabricated by electrostatic self-assembly (van den Beucken et al. 2006). In one of the coatings, DNA was used as the anionic polyelectrolyte and poly-D-lysine (PDL) was used as a polycationic polyelectrolyte. In the other coating, DNA was used as anionic polyelectrolyte and poly(allylamine hydrochloride) (PAH) was used as the cationic polyelectrolyte. The characterization of the coatings was done using ultraviolet-visible (UV-Vis)

spectrophotometry, AFM, XPS, contact angle measurement, and FTIR. Cell culture experiments were performed on titanium substrates with and without multilayered DNA-coatings to study cell proliferation, viability, and cell morphology. There was a progressive and uniform development of both the types of coatings on titanium, but the coating was more uniform on PDL/DNA when compared to PAH/DNA. The presence of DNA did not cause any mutagenic effect on cells and cell viability was not affected. However, an increase in proliferation of the cell was observed on both types of multilayered DNA-coatings compared to non-coated controls.

Preparation of biomaterial coatings based on polypeptides, poly-L-lysine and poly-L-glutamic acid multilayers possessing anti-inflammatory properties was reported (Benkirane-Jessel et al. 2004). In this study, piroxicam (Px), was incorporated into the coatings as an anti-inflammatory agent. In order to maximize the loading capacity, the drug was incorporated into the films in the form of complexes with a charged 6-carboxymethylthio- β -cyclodextrin (cCD). The anti-inflammatory properties of the multilayer construct with the drug were evaluated by determining the inhibition of tumor necrosis factor- α (TNF- α) production by human monocytic THP-1 cells. These cells were stimulated with lipopolysaccharide (LPS) bacterial endotoxin before the test. The results indicated that the drug maintained its anti-inflammatory property and its effect can be controlled by changing the film structure.

Highly uniform microporous thin films based on weak polyelectrolytes were assembled onto silicon substrates by sequential adsorption of an aqueous solution of poly(acrylic acid) and poly(allylamine) (Mendelsohn et al. 2000). These multilayers were then immersed briefly into acidic solution ($\text{pH} \approx 2.4$), resulting in microporous structures. These porous structures were stable against further rearrangement under ambient conditions. The authors proposed that these transformations are based on interchain ionic bond breakage and reformation in the highly protonating environment, leading to an insoluble precipitate on the substrate. This mechanism of change in the structure of the film was studied by FTIR, AFM, in situ ellipsometry, and SEM for monitoring the morphological changes. These types of porous films have applications in biomedical areas such as peritoneal dialysis.

PEC coatings based on hyaluronic acid (HA) and a recombinant fusion protein consisting of mussel adhesive motifs and the arginylglycylaspartic acid (RGD) peptide (fp-151-RGD) were coated on titanium (Hwang et al. 2010). HA and fp-151-RGD were effectively distributed over the titanium surfaces. The coatings supported the proliferation of osteoblast cells (MC-3T3) on complex-coated titanium and exhibited over 5 times greater cell proliferation than titanium coated with only HA or fp-151-RGD.

HA and chitosan were deposited using LbL assembly process to engineer bioactive coatings for endovascular stent application (Thierry et al. 2003). Polyethyleneimine (PEI) primer layer was adsorbed on nickel-titanium (NiTi) disks to initiate the sequential adsorption of the HA and chitosan. Multilayer-coated NiTi disks exhibited enhanced antifouling properties compared to unmodified NiTi

disks, as demonstrated by a decrease of platelet adhesion by in vitro assay. However, ex vivo assay revealed that the coated disks failed to prevent fouling by neutrophils in a porcine model. Sodium nitroprusside-doped multilayers were shown to further reduce platelet adhesion compared to standard multilayers.

Bacterial fouling of implants is a major clinical issue. Innovative technologies are being developed to counter bacterial infection through surface modification and surface treatment (Campoccia et al. 2013). One of the strategies is to prevent protein adsorption. In a study, adsorption of proteins was investigated on polyelectrolyte multilayers (PEMUs) consisting of synthetic polyelectrolytes and proteins, including serum albumin, fibrinogen, and lysozyme (Salloum and Schlenoff 2004). Effects of surface and protein charge, polymer hydrophobicity, and hydrophilic repulsion on the mechanism of protein charge were investigated. It was found that electrostatic interaction was the dominant interaction in protein adsorption, as proteins having a complementary charge to the PEMUs were adsorbed. Adsorption of proteins having the same charge as PEMUs occurred to a much lower extent and driven by non-electrostatic forces. The introduction of a diblock copolymer containing poly(ethylene oxide) further minimized protein adsorption. However, none of the samples completely prevented protein adsorption.

2.8 Other Applications

It is necessary that the surface of contact lenses must be hydrophilic in order to be wet by tears to facilitate unblurred vision. Traditionally, contact lenses were prepared from neutral monomers and/or polymers. Soft and hard contact lenses having ionic charges on their surface were treated with lens solution consisting of a polymer having opposite charges (Ellis and Salamone 1979). This resulted in the formation of a hydrophilic PEC hydrogel at the lens surface, which cannot be dissolved easily by the fluids present in the eye. Both cationic and anionic surfaces can be developed on the lens surface by incorporation of respective cationic (di-alkylaminoethyl acrylate or methacrylate, vinylpyridine, etc.) and anionic (acrylic and methacrylic acid, vinylsulfonic acid, etc.) monomer repeat units. Since the PEC hydrogel developed will be thin, lubricating, and oxygen-permeable, it provides a 'cushioning' effect between the eye and the lens. Also, it causes no irritation and avoids punctate staining. Therefore, the contact lens can be worn for up to 24 h.

PEC-based bandages have not only been developed for wound healing, but also for hemorrhage control. Uncontrolled hemorrhage is the leading cause of death in military combat and second leading cause of death of civilians. A study reported preparation of hemostatic bandage based on chitosan and organic rectorite (OREC)/sodium alginate (Zhang et al. 2015). The sponge was fabricated by solution intercalation and chemical crosslinking techniques. The structural and compositional analysis of the sponges showed uniform pore distribution and highly crosslinked sponges using both the methods, with and without the addition of OREC into them. They also exhibited biocompatibility and antibacterial properties.

The hemostatic performance of the sponges was evaluated in ear-artery, ear-vein, and liver injuries of rabbit model. Hemorrhage control study showed that addition of OREC into the chitosan-sodium alginate composite sponge significantly improved the hemostatic efficiency.

Electrospinning technique was employed to develop a pure chitosan nanofibrous mat to be used as a hemostatic material in combat settings (Gu et al. 2013). Since acidic chitosan is water-soluble, various alkaline solutions, namely Na_2CO_3 , NH_3 , and NaOH were used as neutralizing agents to avert their dissolution in aqueous conditions. The porosity of the nanofibrous mats was controlled by subjecting them to ultrasonication treatment for varying time duration. It was observed that the porosity of the chitosan mat increased with increase in ultrasonication time, and the water absorption time was found to reduce from 110 to 9 s. Various aspects of the chitosan mat such as hemoglobin binding efficiency, mechanical strength, contact angle measurement, and adsorption time of water droplets were also assessed. The nanofibrous chitosan mats thus developed proved to be an effective hemostatic wound dressing material for tissue engineering applications.

To develop biocompatible composite microspheres for novel hemostatic use, microspheres consisting of carboxymethyl chitosan, sodium alginate, and collagen were synthesized (Shi et al. 2016). In hemostatic function experiment, it was found that the composite could facilitate platelet adherence, platelet aggregation, and platelet activation *in vitro*. Moreover, the maximum swelling capacity of the composite submerged in PBS for 50 min was over 300% of that exhibited by commercial hemostatic compound microporous polysaccharide hemostatic powder. In addition, it also exhibited good biodegradability and non-cytotoxicity.

Hydraulic fracturing is a process used in gas wells to create fractures in the deep-rock formations to promote the easier flow of petroleum, natural gas, and brine. This is done by pumping millions of gallons of pressurised fluid underground to fragment the rocks and release the gas. The fracturing fluid is a slurry of water, proppant materials, and chemical additives. Their role is to increase the fracture and maintain the fracture after formation. Water-based polymer gels are usually employed in hydraulic fracturing to increase the viscosity of the fracturing fluid to maintain the fracture. However, after the fracturing process, the gel has to be degraded by various enzymes or gel breakers to a lower viscosity to facilitate its clean up. For this purpose, enzymes are either directly added to the hydraulic fluid or encapsulated in beads. It is necessary that the bursting and release of the enzymes is delayed enough to allow complete hydraulic fracturing. As PECs are widely used to delay drug release in pharmaceuticals, a study made use of PEI-dextran sulfate PEC to encapsulate the degrading enzyme, pectinase (Barati et al. 2011). The nanoparticle-encapsulated pectinase enzyme was able to successfully degrade borate-crosslinked guar and hydroxypropyl guar gels with delayed breaking. Not only was the encapsulated gel-breaking enzyme uniformly distributed throughout the gel, the delayed breakage allowed sufficient time for gelation of the gels to occur. This was supported by comparing the results of SDS-PAGE and viscosity measurements of the gels degraded with untrapped pectinase given sufficient time.

PECs have also been used as biocontrol agent carriers. In a study, *Trichoderma viride* spores were immobilized in a water-insoluble PEC of chitosan and poly (acrylic acid-co-maleic acid) as novel carriers of biocontrol agents (Gicheva et al. 2012). Three types of carriers were developed: PEC-coated chitosan beads, genipin-crosslinked chitosan beads, and PEC-coated crosslinked chitosan beads. *T. viride* spores were immobilized either in the bulk or on the surface of the beads. The spores were able to maintain their viability after immobilization and the microbiological tests showed that they successfully inhibited the growth of the plant pathogens *Alternaria* and *Fusarium*.

There is an increasing need to develop highly conducting fuel cells with reduced Ohmic losses and high efficiency. Proton exchange membranes were developed for direct methanol fuel cells from a nanostructured PEC hybrid of *N-p*-carboxy benzyl chitosan-silica-poly(vinyl alcohol) by sol-gel method (Tripathi and Shahi 2008). By crosslinking and the grafting of strong proton conducting $-\text{SO}_3\text{H}$ groups on inorganic segments and weak protonating $-\text{COOH}$ groups on the organic segments, high proton conductivity and stability was achieved. The crosslinking density and the amount of *N-p*-carboxy benzyl chitosan-silica were varied. It was found that optimization *N-p*-carboxy benzyl chitosan-silica content is necessary to control the chemical and mechanical stabilities as well as proton and fuel transport properties. The developed membranes were tested under direct methanol fuel cell operating conditions and found to be stable, flexible, had good water retention capacities, and low methanol permeability. A great advantage of these membranes was their capacity to be functional under high-temperature conditions.

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