2 Literature Review

2.1 An introduction to Oral Disease

Oral health can be defined as per **World Health Organization [WHO 2012]**:

"Oral health is essential to general health and quality of life. It is a state of being free from the mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal disease, tooth decay, tooth loss, and other diseases and disorders that limit an individual's capacity in biting, chewing, smiling, speaking, and psychosocial well-being."

Globally, about 100% of the adult population and 60–90% of school children have dental cavities. Moreover, 15–20% of middle-aged (35-44 years) population is suffering from severe periodontal (gum) disease, which may ultimately result in tooth loss. Nearly 30% of the total old-aged (65–74) people do not have natural teeth. Various risk factors for oral diseases include unhealthy diet, harmful alcohol use and poor oral hygiene, and social determinants (WHO, 2012).

According to WHO, **Dental caries** was the most prevalent condition (affecting 35% of the population), whereas **severe periodontitis and severe tooth loss** were the 6th and 36th most prevalent conditions affecting 11% and 2% of the population, respectively (WHO, 2012).

2.1.1 Tooth Anatomy

The dental unit comprises of the tooth and its supporting tissues. The teeth are considered to be the hardest part of the human body. The gingiva forms a tight collar around the neck of the tooth so that the contiguous areas of tooth and gum form almost an unbroken surface. Different parts of the teeth as shown in Figure 2.1 include:

- **Enamel:** It is the hardest, white outer part of the tooth. Enamel is mostly made of calcium phosphate, a rock-hard mineral.
- **Dentin:** It is the layer underlying the enamel. It is a hard tissue which contains the microscopic tubes. In case of damaged dentin, heat or cold can enter the tooth through these paths and cause sensitivity or pain.
- **Pulp:** The softer, living inner structure of the teeth is called as pulp. Blood vessels and nerves run through the pulp of the teeth.
- **Cementum:** Cementum is a layer of the connective tissue that binds the roots of the teeth firmly to the gums and jawbone.
- **Periodontal ligament:** These are the tissues that help to hold the teeth tightly against the jaw.

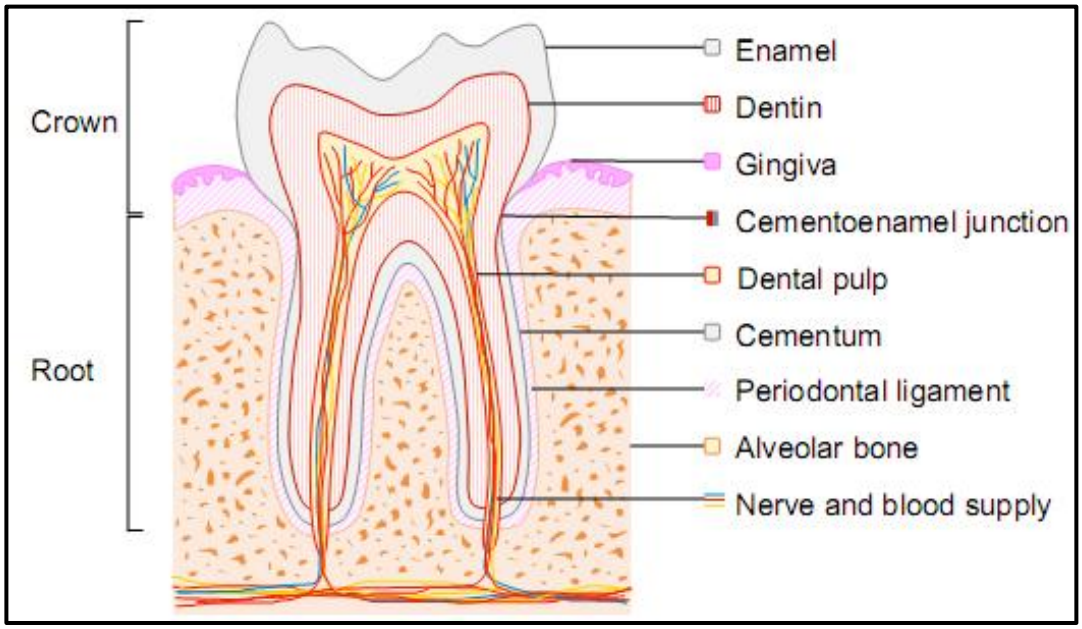


Figure 2.1 Schematic representation of a human molar [Li et al. 2017].

2.1.2 Oral diseases and its conditions

The most common oral diseases are dental cavities, periodontal (gum) disease, oral cancer, oral infectious diseases, trauma from injuries, and hereditary lesions.

- Dental cavities: Worldwide, 60–90% of school children and nearly 100% of the adults have dental cavities, often leading to pain and discomfort.
- Periodontal disease: Severe periodontal (gum) disease, which may result in tooth loss, is found in 15–20% of middle-aged (35-44 years) adults.
- Tooth loss: Dental cavities and periodontal disease are the major causes of tooth loss. Complete loss of natural teeth is widespread and particularly affects older people. Globally, about 30% of people aged 65–74 have no natural teeth.
- Oral cancer: The incidence of oral cancer ranges from one to 10 cases per 100 000 people in most of the countries. The prevalence of oral cancer is relatively higher in men, in older people, and among people of low education and low income. Tobacco and alcohol are known to be the major causal factors.
- Fungal, bacterial or viral infections in HIV: Almost half (40–50%) of the people who are HIV-positive have oral fungal, bacterial or viral infections. These often occur early in the course of HIV infection.
- Oro-dental trauma: Across the world, 16-40% of children in the age range 6 to12 years old are affected by dental trauma due to unsafe playgrounds, unsafe schools, road accidents, or violence.
- Noma: Noma is a gangrenous lesion that affects young children living in extreme poverty conditions primarily in Africa and Asia. Lesions are severe gingival disease followed by necrosis (premature death of cells in living tissue) of lips and chin. Many children affected by noma suffer from other infections such as measles and HIV. About 90% of these children die due to lack of sufficient treatment.

- Cleft lip and palate: Birth defects such as cleft lip and palate occur in about one per 500–700 of all the births. This rate varies substantially across different ethnic groups and geographical areas.

2.1.3 Periodontal disease

Periodontal disease is a pathological condition which is characterized by inflammation and degeneration of the tissues surrounding and supporting the teeth (periodontium) which include the gingiva, periodontal ligament, cementum, and alveolar bone. It initially leads to the formation of tiny pockets between gums and teeth, gingival inflammation, followed by degeneration of gums, loss of teeth supporting structures like alveolar bone, periodontal ligament, cementum, ultimately culminating in complete tooth loss [Reise et al. 2012, Zamani et al. 2010]. Figure 2.2 shows a comparative diagram of the diseased and the normal teeth.

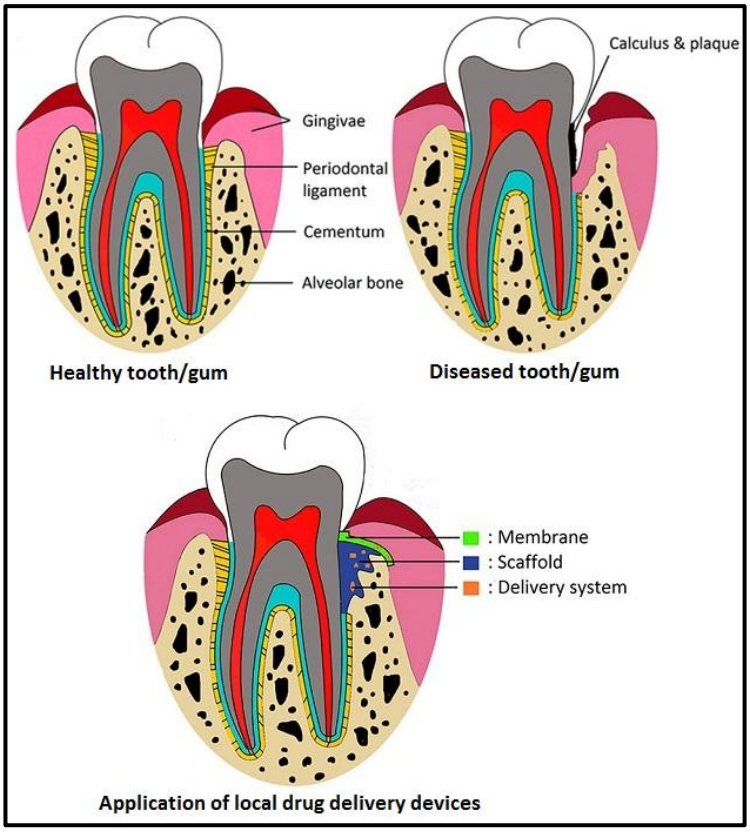


Figure 2.2 Image illustrating the normal periodontal tissue, diseased periodontal tissues and periodontal pocket with intra-pocket devices [Sun et al. 2017].

2.1.4 Etiology of Periodontal disease

The primary cause of periodontitis can be said to be poor oral hygiene which leads to the cumulation of bacterial matrices at the gum line and plaque formation. Plaque is a sticky film composed of salivary glycoproteins and extracellular polysaccharides and is almost entirely constituted by oral bacteria. A variety of anaerobic microbes and other gram-negative facultative anaerobic and aerobic rods of *Enterobacteriaceae* family harbor at the site [Maheshwari et al. 2006, Southard and Godowski 1998]. Bacterial load within a plaque are thousands of times more resistant to antibiotics than the free-living or "planktonic" bacteria. The periodontal pathogens residing in the plaque secrete cytotoxins, bacterial endotoxins and several enzymes responsible for breaking extracellular matrices as well as host cell membranes for producing nutrients for their growth. While doing so, they trigger host-mediated-response that leads to self-injury like inflammation (**gingivitis**), destruction of tissues, etc. [Jain et al. 2008]. Figure 2.3 describes the immunopathogenesis in the progression of periodontal disease. Further increase in the inflammation leads to the destruction of gingival fibers, separation of gum tissues from the tooth and creation of periodontal pocket which is called as periodontitis [Harvey and Emily 1993, Wiggs and Lobprise 1997]. **Moderate Periodontitis** can be indicated by conditions such as mild to moderate loss of attachment and pocket formation, up to 50% loss of bone support, slight tooth mobility, tenderness, swelling, sensitivity to cold and touch. Untreated moderate periodontitis eventually leads to **Advanced Periodontitis** which can be symptomized by severe pocket depth, bleeding on eating or brushing teeth, falling out of teeth and the need to be removed by dentist [Jain et al. 2008]. Another etiology of periodontitis is poor nutrition and underlying medical issues such as diabetes.

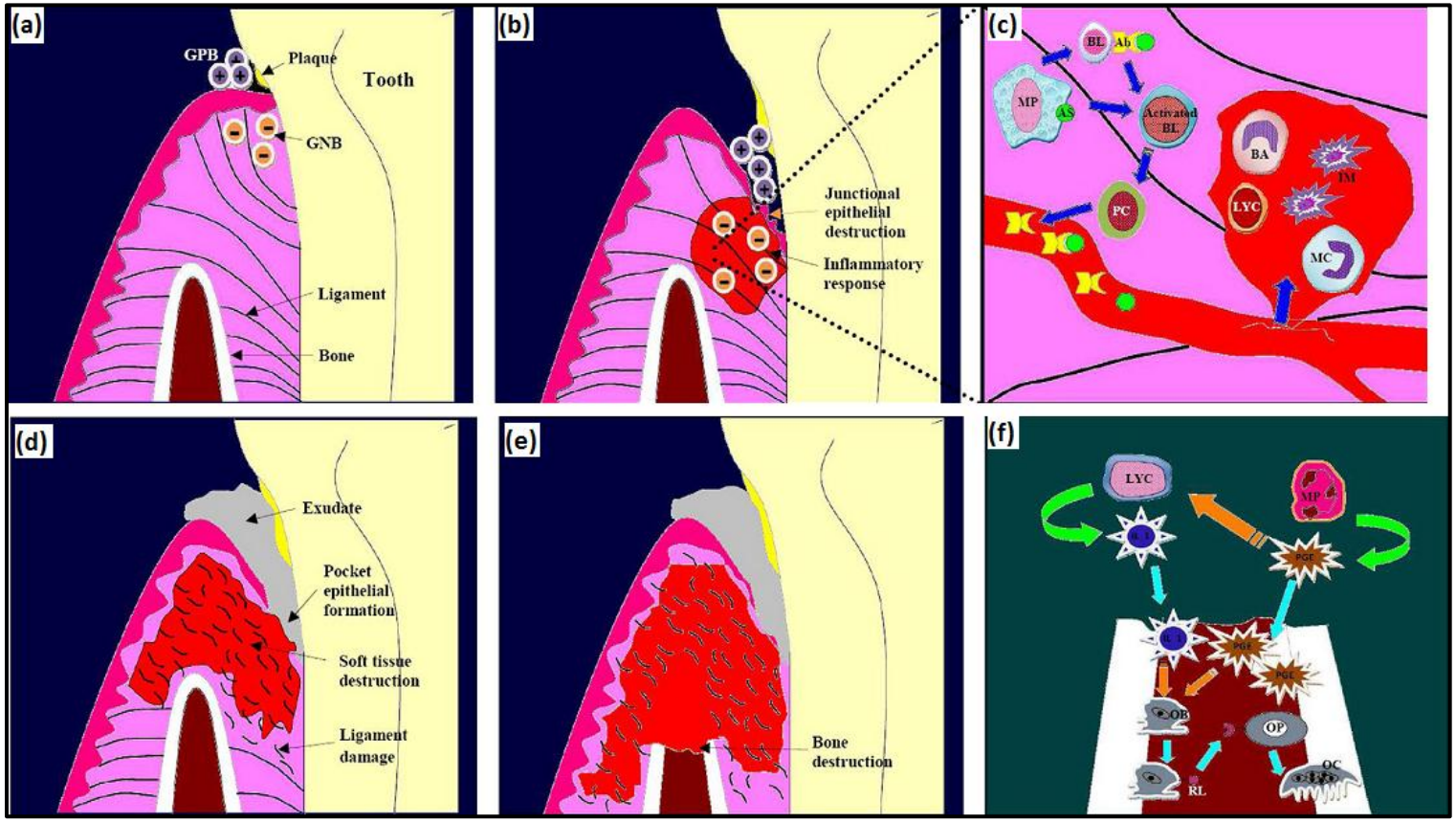


Figure 2.3 Immunopathogenesis in the progression of periodontal disease.

(a) Supragingival biofilm (plaque) formed by the Gram +ve bacteria (GPB) and subgingival biofilm by Gram −ve bacteria (GNB). (b) Junctional epithelial destruction: Appearance of gingival inflammation is noted. (c) Vasodilatation causes fluid escape to surrounding gingival tissue. It contains basophils (BA), mast cells (MC), lymphocytes (LYC), etc. Mast cells release the inflammatory mediators (IM). In the gingival tissue, the macrophages interact with B-lymphocytes and convert them to plasma cells which produce antibodies against the antigenic substrate. (d) Pocket epithelial formation: Inflammatory response stimulates immune response. Periodontal ligament fiber damage is commenced. (e) Severe periodontal ligament fiber damage. Destruction of bone is noticed (f) Immune response in bone destruction: Prostaglandin E and Interleukin-1 formed from macrophages and lymphocytes. They stimulate osteoblasts to express RANK-ligand. RANK-ligand binds to osteoclast precursor cells which finally undergoes fusion resulting in osteoclasts (osteoclastogenesis) and bone loss [Ali et al. 2011]

2.1.5 Treatments for periodontal disease

The ultimate aim of periodontitis treatment is to completely clean the pockets developed around teeth and prevent tooth loss. Treatment should be performed by a medical practitioner like a dentist. One has the best chance for successful treatment when one adopts a daily routine of good oral care. It can be classified into following categories:

2.1.5.1 Nonsurgical treatments

Nonsurgical treatments remain the preferred first-line treatments in primary stages of the disease with minor to moderate periodontitis. Nonsurgical treatments include the use of systemic antibiotics as an adjuvant to mechanical therapy [Roshna and Nandakumar 2012]:

a) Mechanical Antimicrobial Therapy

Scaling and Root Planing (SRP) involves physical removal of tartar and bacterial load by suitable instruments or an ultrasonic device. Although painful treatment procedure and recurrence of disease due to incomplete removal of the bacteria limits the use of this technique [Apatzidou and Kinane 2004].

Quirynen et al. framed another mechanical antimicrobial approach, i.e., one-stage full-mouth disinfection therapy. This therapy includes full-mouth debridement (SRP, rinsing of the mouth for with a 0.2% v/v chlorhexidine solution, brushing of the tongue and irrigation of periodontal pockets with 1% v/v chlorhexidine solution) [Quirynen et al. 1999].

b) Systemic drug delivery

A systemic antibiotic (like tetracycline, metronidazole, TNZ, amoxicillin) is administered with or without SRP and/or surgery and provides better clinical improvement.

c) Localized intra-pocket drug delivery

Localized intra-pocket devices loaded with antimicrobial drugs is also a novel treatment option especially in the case of deep pockets which not responding effectively to mechanical and systemic antibiotic treatments. The localized intra-pocket device delivers the drugs at high concentrations at the site of action as compared to that of systemic delivery devices. Additionally, this is a preferred route for the patients with intolerance to systemic administration of the drugs. Table 2.1 enlist various reason for preference of Localized intra-pocket drug delivery over Systemic drug delivery.

Table 2.1 Advantage of Localized intra-pocket drug delivery system over Systemic drug delivery

Localized intra-pocket drug delivery	Systemic drug delivery
Pocket achieves minimum effective concentration at low dose of drug	Pocket achieves minimum effective concentration at high dose of drug
Avoids exposure of drug to non-oral sites	The whole body is exposed to the drug
Relatively lower dose is required	Higher dose is required
Minimal side effects	More adverse effects
Avoids first pass metabolism of drugs	Inactivation of drugs due to first pass metabolism and excretion
Fast action	Slow action
Provides controlled and sustained release	Immediate release
More patient compliance as non-invasive and painless	Less patient compliance

2.1.5.2 Surgical Treatments

In some cases where advanced periodontitis does not respond to nonsurgical treatments and oral hygiene, surgical treatment becomes necessary. Various types of surgical treatments consist of flap surgery, a soft tissue implant, bone implant, guided tissue regeneration, etc.

2.2 Electrospinning

Electrospinning is a widespread technique used in tissue engineering and drug delivery because of its comparative ease of operation and flexibility. It is a most favored method of producing ultrafine fibers within nano-meter range and with controlled surface morphology. The characteristic high surface to volume ratio of electrospun nanofibers can enhance cell attachment, improve drug loading, and supports in the sustained and controlled release of drugs at the site of action. The drug release profile and the degradation rate of the electrospun membranes can be controlled by modifying the electrospinning parameters [Hu et al. 2014, Xue et al. 2014b].

2.2.1 Electrospinning: a method of choice for nanofiber production

Nanofibers can be produced using several methods drawing, electrospinning, force-electrospinning, phase separation, self-assembly, etc. as briefly listed in Table 2.2. Among the different known methods for nanofiber production, electrospinning has attracted incredible research and commercial interests due to its simplicity, versatility and distinctive capability to produce dry and continuous nanofiber with adjustable morphology and drug loading capacity in a single and efficient step. Electrospinning also produces a structure which mimics a natural extracellular matrix [Kim et al. 2009, Pelipenko et al. 2015].

Table 2.2 Brief description of the most commonly used methods for nanofiber production [Pelipenko et al. 2015].

Method	Description	Advantages	Disadvantages
Drawing	The fabrication of fiber is done by contacting a previously collected polymer solution droplet with a sharp tip and then drawing it as a liquid fiber, which is further solidified by solvent evaporation.	Simple process	Limited amount of product, discontinuous process
Electrospinning	Nanofibers are produced in one-step method from a viscoelastic solution of a polymer or its melt under applied high voltage.	Unlimited length, simple and core-shell nanofibers, great diversity of polymers and solvents used	High voltage, solvents required, numerous parameters affecting the process
Forcespinning	Fibers are produced using a very highly concentrated polymer solution or melt under applied centrifugal force.	Free from high voltage, simple method, high production yield	Thermal degradation of melts, fibers are usually much thicker than 1 μm in diameter
Phase separation	Firstly, a gel is formed by cooling the polymer solution to the gelation temperature. Afterwards, the gel is immersed in distilled water for solvent exchange, followed by removal from the distilled water, blotting with filter paper, and transfer to freeze-drying	Simple, no special equipment required	Numerous parameters, limited to a few polymers
Self-assembly	Amphiphilic molecules are used as basic building blocks that self-associate to produce nanofibers	Suitable for production of very thin nanofibers	Poor control over morphology and orientation of nanofibers
Template melt extrusion	The molten polymer is forced by an extruder screw through a mold or spinning die in the head of extrusion devices and then cooled to solidify. Anodic aluminum oxide (AAO) membranes are used as a template.	No need for solvents, homogenous fiber diameter	Short length of fibers, time-consuming
Template synthesis	Nanofibers are formed within the numerous cylindrical pores of a nonporous membrane by oxidative	Aligned nanofibers, homogenous and easily controlled fiber	Complex method

2.2.2 Electrospinning setups

In 1934, Formhals patented a process for the fabrication of polymer filaments using electrostatic force. When applied to spin fibers this way, the process is termed as electrospinning [Li et al. 2006]. A usual electrospinning setup, presented in Figure 2.4, comprises of four primary components: a spinneret (a syringe fitted with a blunt end metallic needle act as a nozzle), a high voltage power supply, an electrically conductive collector (a piece of aluminum foil or silicon wafer) and a syringe pump. The solution for spinning is loaded into a 5 mL plastic syringe which is fixed on a pump in order to maintain a constant and adjustable flow rate of the solution. For the production of nanofibers, a high voltage power (10-40 kV) is supplied to the end of the nozzle. At a lower voltage, the polymer solution starts oozing out from the nozzle. As the electric field increases, the hemispherical surface of the solution at the end of the nozzle start extending and as it crosses a threshold voltage, it creates a cone-shaped structure known as a ‘Taylor cone.' Further increase in the electric field leads to overcoming of the surface tension using repulsive electrostatic force, and the charged jet spurts out of the end of the Taylor cone towards the grounded collector. The continuous lengthening and whipping of jet results into the reduction of diameter from several hundred micrometers to as thin as tens of nanometers. At the same time, the jet thinning allows quick solvent evaporation and solidification of the polymer solution to form solid nanofibers collected on the grounded collector [Pelipenko et al. 2015, Reneker et al. 2000, Wang et al. 2009]

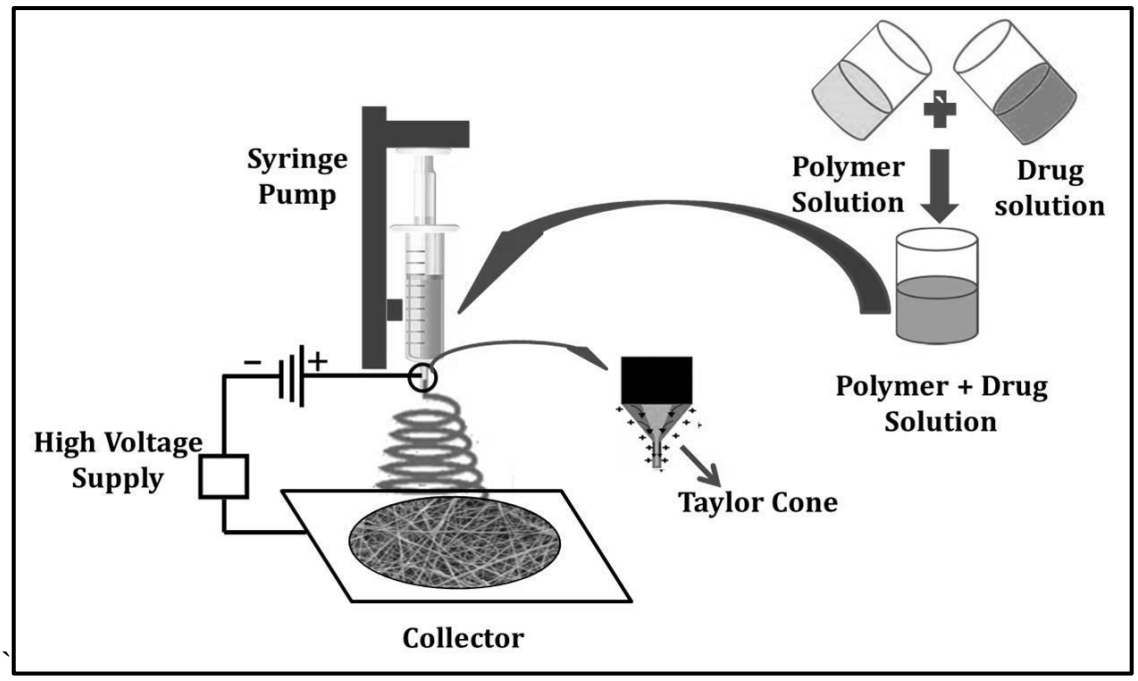


Figure 2.4 Schematic illustration of the basic setup for electrospinning

2.2.3 Parameters affecting the process of Electrospinning

Despite being apparently simple technique, the process of electrospinning is governed by several factors, which puts forward various challenges to be handled. A number of parameters governing the electrospinning can be grouped into three major headings: solution parameters, process parameters, and ambient parameters [Liu et al. 2009].

2.2.3.1 Solution parameters

Solution parameters are the most commonly considered parameters. These parameters which are determined by solvent and polymer characteristics include polymer(s) molecular weight, configuration and polyelectrolyte nature; concentration, rheological properties conductivity, surface tension and dielectric constant of the solution [Pelipenko et al. 2015].

1. Polymer characteristics

High molecular weights polymers (higher degrees of polymerization) have a significant effect on rheological properties (such as viscosity) and electrical properties (such as

surface tension, conductivity, and dielectric strength). These polymers are suitable for electrospinning to facilitate a sufficient number of intermolecular entanglements. However, it has been reported that if an efficient intermolecular interaction (like a hydrogen bond) is found between low molecular weight polymers, then entanglements criteria between high molecular weight polymers are not required. Additionally, electrospinning has better results when linear polymers are used instead of nonlinear polymers because of the latter form very viscous solutions or even gels already at low polymer concentrations. Finally, polymers with a polyelectrolyte nature are very difficult to electrospin, and thus uncharged polymers are preferable [Pelipenko et al. 2015].

2. Polymer concentration

Polymer concentration is the most commonly studied parameter. An optimum polymer concentration is required for nanofiber formation. It has been observed that at low solution concentration, discontinuous beaded fibers and elongated beads is obtained and as the solution concentration increases the discontinuous beaded fibers change to continuous cylindrical fibers.

3. Surface tension

Surface tension is the elastic tendency of a liquid surface that resists change due to an external force and depends on the characteristics of the solvent and solute. Surface tension is the main force acting against Taylor cone formation and further jet elongation [Zhang et al. 2005]. Low surface tension values result in the formation of fibers without beads, and low voltages can be applied in electrospinning. Surface tension can be manipulated by the addition of surface-active substances. However, a low surface

tension cannot solve the problems that occur due to the use of relatively low molecular weight polymer.

4. Rheological characteristics

Viscosity and viscoelastic properties of a polymer solution play an important role in electrospinning. It has been recognized that an increase in the solution viscosity results in the formation of thicker fibers and less bead generation [Zhao et al. 2005], whereas electrospinning a polymer solution with low viscosity does not enable the formation of continuous fibers.

5. Conductivity

A threshold surface charge is necessary for Taylor cone formation and hence for the electrospinning process. A solution with low conductivity lacks sufficient surface charge, hence preventing Taylor cone formation and on the other hand, very high conductivity of the solution leads to a depleted tangential electric field along the surface of the fluid droplet, preventing Taylor cone formation. It has been observed that high conductive solution usually results in thinner nanofibers and require lower applied voltage.

2.2.3.2 Process parameters

1. Applied voltage

An ideal value of the applied voltage is a prerequisite for Taylor cone formation. Solutions with low viscosity, low surface tension, and high conductivity, require lower voltages, and vice versa. A high voltage causes increased repulsive electrostatic forces, which leads to more extensive stretching of the electrospun jet, resulting in thinner fibers. When the applied voltage is too high the probability of bead formation in the electrospun product is much greater due to Taylor cone instability [Pham et al. 2006].

2. Nozzle tip-to-collector distance

It has been found that a minimal distance that assures sufficient time for the electrospun jet to dry before reaching the collector is required. If the distance is too short, the jet does not solidify before it reaches the collector, which results in nanofiber fusion and polymer film formation [Pelipenko et al. 2015].

3. Feed rate

Solvent volatility determines the feed rate of solution. A highly volatile solvent(s) system requires higher flow rate. However, a high electric field must be applied to ensure the electrically-induced extraction of the polymer solution from the nozzle tip. Some researchers indicate that increasing flow rate leads to the formation of thicker nanofibers due to thicker jet formulation [Bhardwaj and Kundu 2010]; while other reports indicate high flow rates may lead to the formation of beads or deposition of undried nanofibers [Pham et al. 2006].

4. Types of Collector

Types of collector used to determine the orientation and morphology of the electrospun nanofiber. The collector can be a flat surface, a rotating cylinder, or a wheel-like disk. A static flat collector results in randomly-oriented nanofiber membrane whereas rotating cylinder collector produce aligned nanofiber membrane.

2.2.3.3 Ambient parameters

Apart from solution and process parameters, there are some less commonly considered parameters like environmental temperature, humidity, etc. Environmental temperature affects the solution viscosity and rate of solvent vaporization during electrospinning and thus ultimately affecting the diameter of the produced nanofiber. Environmental

temperature also closely correlates with the relative humidity (RH). The effect of relative humidity can be explained by the combination of two effects, namely the solvent evaporation rate (solidification velocity) and the formation of bead on-a-string morphology, due to the capillary breakup of the viscoelastic fluid [De Vrieze et al. 2009, Pelipenko et al. 2015].

2.2.3.4 Advantages of Electrospinning [Chong et al. 2007]

- The mechanical, biological and kinetic properties of the scaffold can be easily manipulated by altering the polymer solution composition and the processing parameters, hence owing to these properties; there is increasing interest towards employing electrospinning for scaffold fabrication.
- Another advantage of using the electrospinning process is the ability to produce a non-woven nanofibrous structure, which has morphological and architectural features similar to that of the natural ECM present in the skin.
- Moreover, the scaffold structure changes dynamically over time as the polymer of the nanofibers degrades, allowing the seeded cells to proliferate and produce their own ECM.
- Apart from electrospinning of polymer materials, there are also several approaches which can be used to electrospun the natural biomaterials such as collagen and fibrinogen.

2.2.3.5 Applications

The applications include filtration, cosmetic mask, military protective clothing, nano-sensor, energy-related applications, wound dressings, drug delivery, enzyme immobilization, and tissue engineering scaffolds [Hu et al. 2014].

Nanofibers in drug delivery

Nanofibers have been investigated for over a decade as drug delivery systems for transdermal, oral, oromucosal, parenteral, and ocular application and numerous drugs have already been incorporated into nanofibers, namely antibiotics, analgesics, antimycotics, non-steroidal anti-inflammatory drugs, anti-cancer drugs, nucleic acids, and growth factors. The electrospinning process has also been used to prepare nanofibers with incorporated proteins and live cells [Pelipenko et al. 2015].

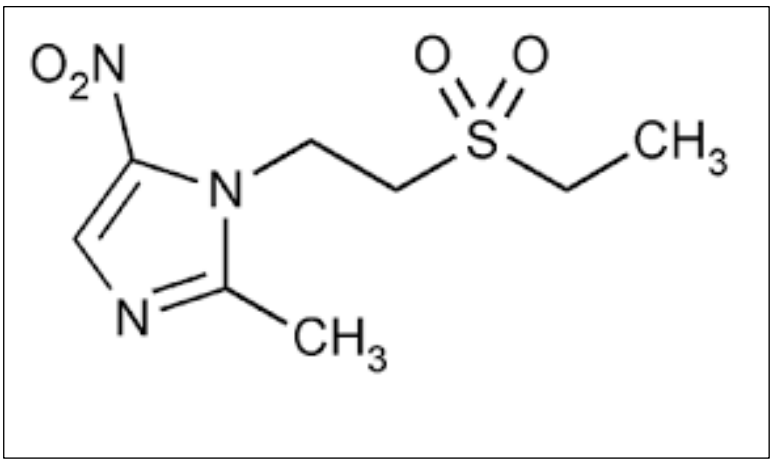
Nanofibers in tissue engineering

Regenerative medicine is a comparatively new, exciting, and continuously progressing research field. It enables the formation of functional tissue substitutes to repair or replace tissue or organ function lost due to aging, damage, disease, or congenital disabilities. Basic science in tissue engineering and regenerative medicine further aims to investigate the deposition, growth, and remodeling of tissues by exploiting the knowledge from a range of different disciplines. Table 2.3 enlist the different polymers which have been already investigated in tissue regeneration.

Table 2.3 Polymer nanofibers already investigated in tissue regeneration.

Target Tissue	Nanofiber Composition
Dermal Wounds	Chitosan nanofibers loaded with silver nanoparticles, chitosan/PEO nanofibers loaded with growth factors, polyurethane nanofibers, GE/polyurethane nanofibers loaded with silver-sulfadiazine, PVA nanofibers, PVP nanofibers with emodin, poly-*N*-acetyl glucosamine, and PCL/PEO nanofibers with human epidermal growth factor for diabetic wound healing
Skin	Core-shell GE/copolymer PLA–PLC nanofibers with encapsulated epidermal induction factors, PLA/chitosan, and PLA/chitosan/collagen nanofibers
Bone	Hydroxyapatite-containing chitosan nanofibers, polyamide-6/calcium lactate nanofibers, chitosan-based nanofibers, nanofibers made from PLA-PCL loaded with bone morphogenetic protein 2, PCL nanofibers with silica nanoparticles, cellulose and collagen, chitosan nanofibers reinforced with poly(butylene succinate)
Tendons and Ligaments	Braided PLA nanofibrillar scaffold, hybrid PLGA fibrillar scaffold with fibroblast growth factor, aligned poly(lactide-*co*-varepsilon-caprolactone)/collagen nanofibers, cellulose/collagen with genipin cross-linked nanofibers, PLGA nanofibers
Cartilage	Chitosan-based nanofibers, peptide nanofibers, PLLA nanofibers grafted with cationized GE, PVA/PCL nanofibers
Periodontal	PCL/chitosan nanofibers, PCL nanofibers with incorporated metronidazole benzoate, PLLA nanofibers
Muscle/Cardiac	Highly oriented PCL nanofibers with incorporated polyaniline, haemoglobin/GE/fibrinogen nanofibers cross-linked with phytic acid for enhancement of cardiomyogenic differentiation, oriented PCL/GE nanofibers for cardiac tissue engineering
Nerves	Aligned collagen, PLA–PCL/collagen, collagen/PLGA, PLGA/PCL, chitosan/PCL nanofibers, laminin functionalized PCL/chitosan nanofibers
Blood vessels	Spider silk fibroin/PCL/GE and spider silk protein/PCL/chitosan nanofibers for small-diameter vascular tissue engineering, PGA/collagen nanofibers

2.3 Tinidazole Specific Review



IUPAC Name **:** 1-[2-(ethylsulfonyl)ethyl]-2-methyl-5-nitro-1H-imidazole

Molecular Weight **:** Average 247.3

Chemical Formula **:** $C_8H_{13}N_3O_4S$

TNZ is a second-generation nitro-imidazole similar to metronidazole in almost all ways, except some of the characteristics such as [Tripathi 2013]:

- The metabolism of TNZ is slower leading to a relatively long half-life ($t_{1/2}$/~12 hr). Owing to longer half-life, the duration of action is also longer, and hence the resultant dosage schedules are simpler. Thus, the drug is more suited for the therapies consisting of a single dose or once-daily dosing frequency.
- Several comparative trials in amoebiasis have reported higher cure rates with TNZ as compared to that of metronidazole.
- TNZ appears to be tolerated in a better way and presents lower side effects as compared to metronidazole, some of the side effects experienced are: metallic taste (2%), nausea (1%), rash (0.2%).
- Economically, TNZ is found to be more expensive than metronidazole.

- TNZ is a synthetic antiprotozoal and antibacterial agent. The U.S. FDA approved the drug for the treatment of conditions such as amoebiasis, amebic liver abscess, giardiasis, and trichomoniasis in the year 2004.

Physical Properties of TNZ: [Drug-Bank , Indian-Pharmacopoeia 2014]

Color: It appears as white-creamy powder

Identification: Infrared spectrum, UV Spectroscopy (λ_{max} = 310 nm, in methanol)

Description: Pale yellow crystals or a crystalline powder

Odour: It has slight and characteristic odour

Solubility: Soluble in water (> 10 mg/ml) at 20 °C

Melting Point: 125-129 °C

Log P: -0.35

pK_a: 3.1

Mechanism of action: TNZ is a prodrug. The nitro group of TNZ molecule is reduced by a ferredoxin-mediated electron transport system inside the *Trichomonas.* The nitro free radicals which are generated as a result of this reduction process are supposed to be responsible for the antiprotozoal activity of the drug. It is suggested that the toxic nitro free radicals covalently bind to the DNA, hence causing irreversible damage to the DNA ultimately leading to the cell death. The mechanism through which TNZ moiety exhibits activity against *Giardia* and *Entamoeba* species is not known yet, though it might be probably similar.

Pharmacokinetics: TNZ is rapidly absorbed from the small intestine under fasting conditions. Moreover, a large part of the administered drug is absorbed in the small intestine, and the remaining unabsorbed drug reaches the colon. Administration of the drug along with food results in a delay of approximately 2 hr in the T_{max} of the drug and a decline of approximately 10% in the C_{max} and AUC ultimately obtained is

901.6±126.5 µg*hr/ml. The drug is widely distributed in the body, attaining a required therapeutic concentration in vaginal secretion, semen, saliva, and CSF. The drug is metabolized primarily in the liver by the oxidation and glucuronide conjugation mechanism and finally excreted in the urine. Plasma $t_{1/2}$ of the drug is reported as 12-14 hrs.

Pharmacodynamics: TNZ demonstrates both *in vitro* and *in vivo* activity against clinical infections which are caused by the following protozoans: *Trichomonas vaginalis*, *Giardia duodenalis* (also termed *G. lamblia*), and *Entamoeba histolytica*. Although, TNZ does not appear to have activity against most of the strains of vaginal lactobacilli.

Volume of distribution: 50 liters

T_{max}: 1.6 hr

Oral Bioavailability: > 90%

Protein binding: Plasma protein binding exhibited by TNZ is approximate 12%.

Metabolism: The metabolism of TNZ occurs mainly by hepatic means, especially via CYP3A4. Similar to metronidazole, TNZ is also significantly metabolized in humans before excretion. Partial metabolism of TNZ occurs through oxidation, hydroxylation, and conjugation. TNZ is the major drug-related constituent present in the plasma after human treatment, along with the presence of small amount of 2-hydroxymethyl metabolite.

Route of elimination: TNZ molecule crosses the placental barrier and is also secreted in the breast milk. TNZ is excreted predominantly by the liver and the kidneys mainly in the form of the unchanged drug (approximately 20-25% of the administered dose). Moreover, approximately 12% of the drug is excreted in the feces.

Elimination Half-life: Elimination half-life is 13.2±1.4 hr. Plasma half-life is 12 to 14 hr.

Toxicity: No overdose with the drug TNZ in humans has been reported. In acute studies with mice and rats, the LD_{50} for mice was found to be greater than > 3,600 mg/kg for oral administration and greater than > 2,300 mg/kg for intraperitoneal administration. In rats, the LD_{50} was greater than > 2,000 mg/kg for both oral and intraperitoneal administration (Drug Bank).

Indications: It is indicated for the treatment of trichomoniasis caused by *T. vaginalis* in both female and male patients. TNZ is also indicated for the treatment of giardiasis caused by *G. duodenalis* in adults as well as pediatric patients older than three years of age. It is also used for the treatment of amebic liver abscess and intestinal amebiasis caused by *E. histolytica* in both pediatric patients and adults. TNZ is also found to be active against gram-negative facultative anaerobic and aerobic rods of *Enterobacteriaceae* family including *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella enteritidis* and *Enterobacter cloacae* [Maheshwari et al. 2006].

Dose and administration: 2 grams administered orally daily

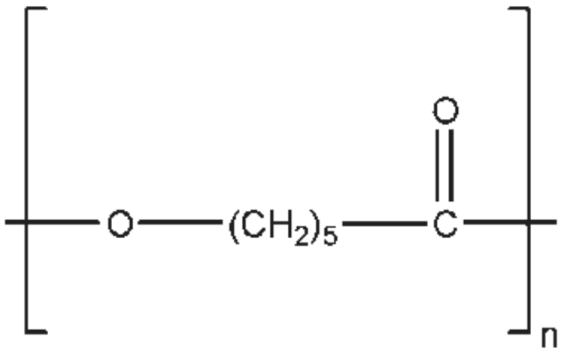
Storage: Store protected from light and moisture

Table 2.4 Reported development carried out by various research groups to improve the pathological condition of periodontitis.

S. No	Title of Research	Reference
1.	TNZ inhibitory and cidal activity against anaerobic periodontal pathogens	[Alou et al. 2009]
2.	Fabrication and properties of multilayer chitosan membrane loaded with TNZ	[Hong et al. 2007]
3.	Design and evaluation of TNZ dental implants	[Karunakar et al. 1994]
4.	Single-dose Concentrations of TNZ in Gingival Crevicular Fluid, Serum, and Gingival Tissue in Adults with Periodontitis	[Liew et al. 1991]
5.	TNZ concentration in human gingival crevicular fluid after insertion of biodegradable dental implants	[Nagaraju et al. 2003]
6.	Development and evaluation of in situ gelling system for treatment of periodontitis	[Patel et al. 2014a]
7.	Formulation and evaluation of in situ forming PLA implant containing TNZ for the treatment of periodontitis	[Qin et al. 2012]
8.	*In vitro* release of TNZ from polyvinyl alcohol and polyvinyl pyrrolidone strips as a local drug delivery system to treat periodontal pockets	[Reddy 2011]
9.	Controlled release of TNZ and theophylline from chitosan-based composite hydrogels	[Samanta and Ray 2014]
10.	Synthesis, characterization, and evaluation of TNZ-loaded mPEG–PDLLA (10/90) in situ gel forming system for periodontitis treatment	[Tian et al. 2016]
11.	Preparation and Its clinical Application of Compound TNZ Film	[Yuwei and Jing 2005]
12.	Efficacy of TNZ buccal tablet local using on periodontitis and pericoronitis	[Zhang and Ye 2012]
13.	Preparation of TNZ microcapsule	[Wangyu and Shengnan 1997]
14.	Controlled release of metronidazole benzoate from poly ε-caprolactone electrospun nanofibers for periodontal diseases	[Zamani et al. 2010]
15.	Evaluation of metronidazole nanofibers in patients with chronic periodontitis: A clinical study	[Chaturvedi et al. 2012]
16.	Release of metronidazole from electrospun poly (L-lactide-co-D/L-lactide) fibers for local periodontitis treatment	[Reise et al. 2012]
17.	A novel spatially designed and functionally graded electrospun membrane for periodontal regeneration	[Bottino et al. 2011]

18.	Doxycycline poly e-caprolactone nanofibers in patients with chronic periodontitis–a clinical evaluation	[Chaturvedi et al. 2013]
19.	Electrospinning of PLGA/gum tragacanth nanofibers containing tetracycline hydrochloride for periodontal regeneration	[Ranjbar-Mohammadi et al. 2016]
20.	Development and characterization of novel medicated nanofibers against periodontitis	[Joshi et al. 2015]
21.	Local delivery of resveratrol using polycaprolactone nanofibers for treatment of periodontal disease	[Zupančič et al. 2015]
22.	Periodontal ligament cellular structures engineered with electrospun poly (DL-lactide-co-glycolide) nanofibrous membrane scaffolds	[Inanç et al. 2009]
23.	Long-term sustained ciprofloxacin release from pmma and hydrophilic polymer blended nanofibers	[Zupančič et al. 2015]
24.	Effect of hydroxyapatite-coated nanofibrous membrane on the responses of human periodontal ligament fibroblast	[Park et al. 2008]
25.	Electrospun poly (L-lactic acid)/nano-hydroxyapatite hybrid nanofibers and their potential in dental tissue engineering	[Xu et al. 2007]

2.4 Poly (ε-caprolactone) (PCL)



Chemical name: 2-Oxepanone, homopolymer

Empirical Formula: It is a homopolymer of ε-caprolactone.

Functional Category: Bioabsorbable biocompatible and biodegradable material.

Applications in Pharmaceutical Formulation: PCL is as biocompatible and biodegradable carriers in many types of implantable or injectable drug-delivery systems for both human and veterinary use. Examples of implantable drug delivery systems

include rods, cylinders, tubing, films, fibers, pellets, and beads. Examples of injectable drug-delivery systems include microcapsules, microspheres, and nanoparticles.

Description: PCL is a synthetic homopolymer of ε-caprolactone. It is nontoxic and can easily be fabricated into a variety of novel devices, such as rods, screws, nails, and cylinders. The polymer is commercially available in varying molecular weights as copolymers.

Molecular weights: 20000 Da to 80000 Da.

Solubility: Practically insoluble in water; soluble in chloroform, acetone, methanol, tetrahydrofuran, ethyl acetate, dichloromethane, and hexafluoroisopropanol.

Melting point: 58-63 °C

Glass Transition temperature: -65 to -60 °C

Specific gravity: 1.11

Color: White

Inherent Viscosity (mpas): 1.0 – 1.3

Stability and Storage Conditions: Polymer is easily susceptible to hydrolysis in the presence of moisture. Hence, it should be packaged under high-purity dry nitrogen and properly stored in airtight containers, preferably refrigerated at below 8 °C. It is necessary to allow the polymers to reach room temperature in a dry environment before opening the container.

Safety and Handling precaution: PCL is regarded as biodegradable, biocompatible, and bioabsorbable materials. Its biodegradation products are non-toxic, non-carcinogenic, and non-teratogenic. In general, PCL exhibits very little hazard. Contact with eyes, skin, and clothing, and breathing the dust of the polymers should be avoided. Aliphatic polyesters produce acid materials such as hydroxyacetic and/or lactic acid in

the presence of moisture; thus, contact with materials that will react with acids, especially in moist conditions, should be avoided.

Regulatory Status: Listed as GRAS, and included in Canadian list of Non-medicinal ingredients. Its medicinal devices are FDA approved.

2.5 Gelatin (GE)

GE is a natural biopolymer and purified animal protein. GE is obtained by the partial hydrolysis of animal collagen which is the major extracellular matrix building protein and is also found to have a favorable influence on the cellular response of a material. GE has many integrin-binding sites (sequence of Arg-Gly-Asp (RGD) amino acid) responsible for cell adhesion and cell differentiation. Hence, in this way, GE increased the cellular attachment and enhanced spreading to the surface of the material [Dulnik et al. 2016, Xue et al. 2014b]

Properties of Gelatin

Storage temp: Generally stored at 2-8 °C

Water Solubility: 67 mg/ml at 50 °C, slightly hazy, slightly yellow

Physical form: Powder

pH: 4.0-6.0 (25 °C, 67 mg/ml in water)

Stability: Stable, hygroscopic and also incompatible with strong oxidizing agents

Category: Pharmaceutical aid (encapsulating agent; suspending agent; tablet binding and coating agent) (IP 2014).

Description: Light amber to faintly yellow, translucent flakes, sheets, shreds, powder or granules; has a slight odour. It is stable in air but is subjected to microbial decomposition when moist or in solution (IP 2014).

Chemical Composition and Structure

GE is a heterogeneous mixture of single-stranded or multi-stranded polypeptides, with extended left-handed proline helix conformations and containing about 50 - 1000 amino acids. The triple helix of type I collagen which is extracted from skin and bones consists of two α_1 and one α_2 chain, each with the molecular mass of ~95 kD, the width of ~1.5 nm and length of ~0.3 μm. GE molecules contain repeating sequences of glycine, proline and alanine amino acid triplets (Figure 2.5), which are responsible for the triple helical structure of GE. The triple helical structure supports for the easy chemical modification and also for the surface interaction of chemicals and drugs with a covalent bond which makes GE-based nanoparticles a favorable drug carrier system [Harrington and von Hippel 1961].

There are two types of GE are available namely A and B which are obtained from acid and alkali treatment of collagen with a distinct difference in isoelectric points. Type B GE has more carboxyl groups and lowers isoelectric point (4.8-5.0) as compared to Type A (7.0-9.0) [Peng et al. 2008, Zhang et al. 2006]. Alkaline pre-treatment of collagen leads to conversion of asparagine and glutamine residues to their respective acids and results in higher viscosity.

Similar to its precursor collagen, GE also contains about 51% of carbon, 25% of oxygen, 18% of nitrogen and 6% of hydrogen. Some traces of sulphur and phosphorus have also been found in GE but it is not certain whether they are an integral part of the molecule or present as an impurity. Most of the commercially available GE consists under 0.1% of sulphur. Also like collagen, GE consists of notably high content of the amino acids glycine, proline, and hydroxyproline. GE molecules are characteristically large and complex as those of other proteins. The average molecular weight of GE

ranges between 15,000 to 2,50,000. GE consists of about 18 different amino acids, which are linked in an orderly fashion. Table 2.5 shows the list of different amino acids that are a part of GE.

Table 2.5 Approximate amino acid composition of a typical GE

Components	Content	Components	Content
Glycine	26-28%	Lysine	4-6%
Proline	14-18%	Serine	3-4%
Hydroxyproline	14-16%	Threonine	2-3%
Glutamic acid	10-12%	Valine	2-3%
Alanine	9-11%	Tyrosine	1%
Arginine	8-9%	Histidine	1%
Aspartic acid	5-7%	Hydroxylysine	1%
Leucine	5-6%	Methionine	1%
Isoleucine	5-6%	Phenylalanine	1%

From above table it clear that GE contains many prolines, 4-hydroxyproline residues and glycine residues (almost 1 in 3 residues, arranged every third residue). A typical structure is -Ala-Gly-Pro-Arg-Gly-Glu-4Hyp-Gly-Pro-.

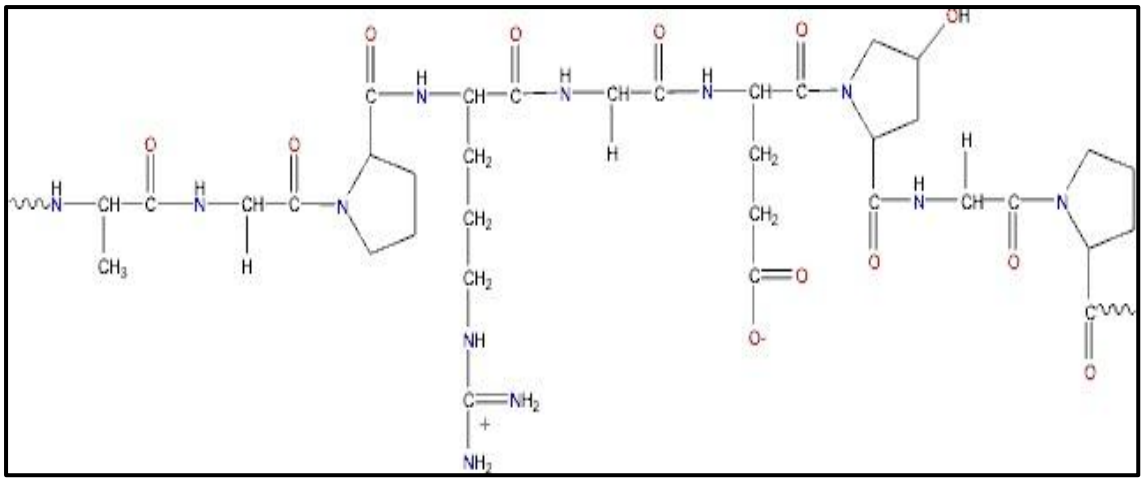


Figure 2.5 A typical structure of GE

Functionality and concerns

GE is primarily used as a gelling agent which forms a transparent elastic thermoreversible gel when cooled below 35 °C, which has the property to dissolve at low temperatures to give 'melt in the mouth' products with the desired flavor-release. In addition to these, the amphiphilic nature of the GE molecules endows them with useful emulsification (for example, whipped cream) as well as foam-stabilizing properties (for example, mallow foam). On dehydration, the irreversible conformational change that takes place might be used in the formation of surface films. Such films are found to be the strongest when they contain greater triple-helix content.

Uses

The use of GE majorly in foods, pharmaceutical preparations and photographic products depending mainly on its gel-forming ability, the viscosity of its water solutions, the ease of the reversible transition from gel to sol., and its effectiveness as a protective solid.

GE that is used in the manufacture of capsule shells or as a pharmaceutical aid in the manufacturing of tablets may contain suitable antimicrobial agents.

2.6 Chitosan

Synonyms : 2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; chitosani hydrochloridum; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine; poly-D-glucosamine; poly-(1,4-b-D-glucopyranosamine).

Chemical Name: Poly-b-(1, 4)-2-Amino-2-deoxy-D-glucose

CH is a polysaccharide comprising of copolymers of glucosamine and N-acetylglucosamine, usually derived from agricultural feedstock or crustacean shell wastes. CH is a deacetylated product of chitin to form soluble amine salts (Figure 2.6).

Greater than 80–85% degree of deacetylation required to obtain a soluble product. Chitin and CH are linear copolymers of D-GlcN and D-GlcNAc residues distributed randomly and are linked entirely in the E-1, 4 configurations. It has the affinity to form inter and intramolecular hydrogen bonding due to the presence of hydroxyl groups and highly reactive amino groups [Elieh-Ali-Komi and Hamblin 2016, Rinaudo 2006]. CH is biocompatible, biodegradable, and non-toxic biopolymers. It also has antimicrobial and hydrating properties. CH is commercially found in several different types and grades that vary in molecular weight by 10000–1000000 and also vary in the degree of deacetylation and viscosity.

Depending on the source of chitin, it is found in two allomorphs, namely α and β forms, that can be characterized by FTIR and solid-state NMR spectroscopy, together with PXRD Study. A third allomorph, i.e., γ-Chitin has also been described. The allomorphs differ in the orientation of the microfibrils [Elieh-Ali-Komi and Hamblin 2016].

Description: Odourless, white or creamy-white powder or flakes; may look 'cotton-like' due to the formation of fibers.

Functional Category: Coating agent; film-forming agent; mucoadhesive; disintegrant; viscosity increasing agent; tablet binder.

Acidity/alkalinity pH: 4.0–6.0 (1% w/v aqueous solution)

Density: 1.35–1.40 g/cm^3

Glass transition temperature: 203 °C

Moisture content: It absorbs moisture from the surrounding atmosphere, the amount of water adsorbed depending upon the initial moisture content and the temperature as well as the relative humidity of the air.

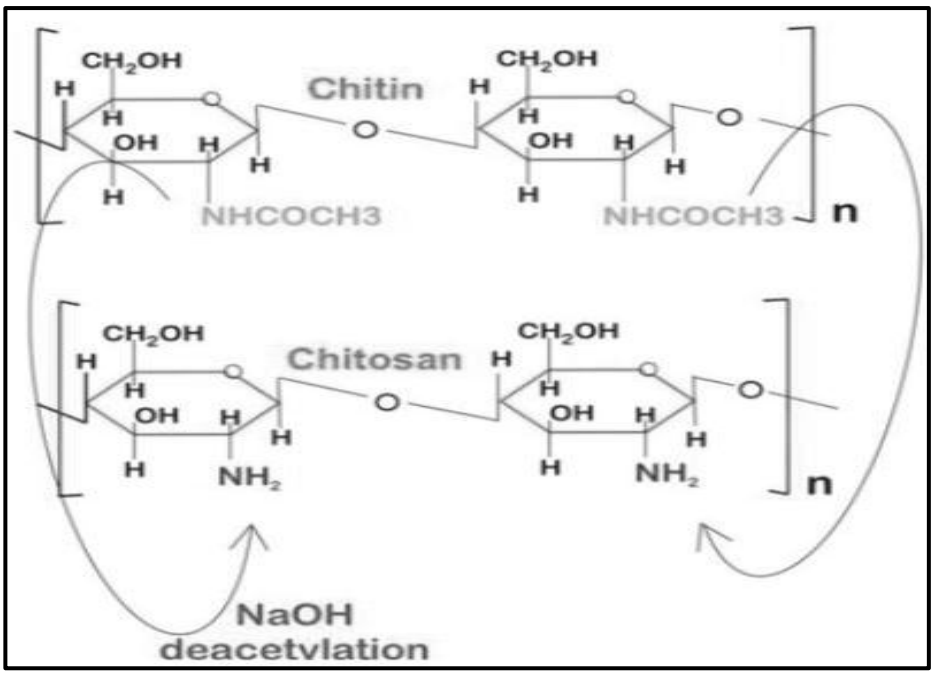


Figure 2.6 Structure of chitin and its deacetylated product, CH.

Particle size distribution: < 30 mm

Stability and Storage Conditions: Stable at room temperature, although hygroscopic after drying. CH should be stored in a tightly closed container in a cool, dry place. CH should be stored at a temperature of 2–88 °C.

Incompatibilities: CH is found to be incompatible with several strong oxidizing agents.

Typical Properties

CH is a cationic polyamine which exhibits a high charge density at pH < 6.5, and hence it shows the property of adherence to negatively charged surfaces and chelation of the metal ions. It is a linear polyelectrolyte with several reactive hydroxyl and amino groups which are available for chemical reaction as well as salt formation. The properties of CH are attributed to its polyelectrolyte and polymeric carbohydrate character. The presence of a number of amino groups in the molecule allows CH to react chemically with anionic systems, resulting in alteration of physicochemical characteristics of such combinations. The nitrogen present in CH is mostly in the form

of primary aliphatic amino groups. Hence, CH undergoes typical reactions of amines: for example, N-acylation and Schiff reactions. Almost all the functional properties of CH depend on the chain length, charge density, and charge distribution. Numerous studies have also explained that the salt form, degree of deacetylation, molecular weight as well as the pH at which CH is used, influences how this polymer is utilized in pharmaceutical applications.

Solubility: Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, as well as in neutral or alkali solutions at pH above approximately 6.5. CH also dissolves easily in dilute and concentrated solutions of most of the organic acids and dissolves up to some extent in mineral inorganic acids (except phosphoric and sulfuric acids). On dissolution, the amine groups present in the polymer gets protonated, and results in a polysaccharide (RNH_3) which is positively charged and CH salts (chloride, glutamate, etc.) that are soluble in water; the solubility is also affected by the degree of deacetylation. The higher the ionic strength, the lower is the solubility as a result of a salting-out effect, which ultimately leads to the precipitation of CH in the solution.

Viscosity (dynamic): A wide range of CH-based on viscosity types is commercially available. Owing to its high molecular weight, linear, unbranched structure, CH acts as an excellent viscosity-enhancing agent in the presence of acidic environment. It also acts as a pseudo-plastic material, hence exhibiting a decrease in the viscosity with an increase in the rates of shear. The viscosity of CH solutions increases with the increase in CH concentration, decrease in the temperature and increase in the degree of deacetylation.

Applications in Pharmaceutical Formulation

CH is used in several cosmetics and is currently under investigation for use in a number of pharmaceutical formulations. Several studies have assessed the suitability and performance of CH as a component of pharmaceutical formulations for drug delivery applications. These include applications such as controlled drug delivery applications, a component of mucoadhesive dosage forms, colonic drug delivery systems, rapid release dosage forms, and gene delivery. CH has been processed into various pharmaceutical forms including gels, films, beads, tablets, and coatings for liposomes. Furthermore, CH may also be processed into drug delivery systems using several techniques including spray-drying, coacervation, direct compression, and granulation processes [Elieh-Ali-Komi and Hamblin 2016, Jayakumar et al. 2010a].

Application in Tissue engineering

The basic purposes of tissue engineering, which involves the use of living cells are to repair, replace, maintain, or enhance the function of a particular tissue or organ. The primary requirement to design polymer scaffolds is high porosity, biodegradability, structural integrity, being non-toxic to cells, biocompatibility, interacting with the cells to promote cell adhesion, encouraging cell function [Jayakumar et al. 2010a, Jayakumar et al. 2010b].

Safety: CH is currently being investigated widely for use as an excipient in oral as well as other pharmaceutical formulations/ drug delivery systems. Also, it is used in various cosmetics. CH is considered to be a non-toxic and non-irritant material. It is biocompatible with both healthy and infected skin. CH has also been presented to be biodegradable. LD_{50} in mouse, when given orally, is found to be >16 g/kg.