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Introduction
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Introduction

The human skeleton consists of 206 bones. We are actually born with more bones (about 300) but many of them fuse together as a child grows up. These bones support our body and allow us to move. The longest bone in our body is the femur (thigh bone). The smallest bone is the step bone inside the ear. Each hand has 26 bones in it. Our nose and ears are not made of bone; they are made of cartilage, a flexible substance that is not as hard as bone. Bones are connected to other bones at joints. There are many different types of joints, including: fixed joints (such as in the skull, which consists of many bones), hinged joints (such as in the fingers and toes), and ball-and-socket joints (such as the shoulders and hips). Bones contain a lot of calcium. Bones manufacture blood cells and store important minerals. Like liver, kidney and muscle; bone is a living tissue that responds to its environment. Two basic processes take place in bone as it responds to physiological demands. Bone modeling occurs primarily in children and young adults and results in bone growth; both in length and in cross-sectional area. The growth of bones through the addition of material to the endosteum or periosteum which is the result of the modeling process, can also continue throughout life. Bone remodeling involves the removal and in general replacement of bone. This process allows for the continual recycling of bone and in healthy tissue it prevents the accumulation of micro-cracks that could lead to fatigue failure of the structure. The same general processes are seen in fracture healing.

Bone fracture

Bone fracture is a medical condition in which there is a break in the continuity of the bone. A bone fracture can be the result of high force impact or stress or trivial injury as a result of certain medical conditions that weaken the bones, such as osteoporosis, bone cancer or osteogenesis imperfecta where the fracture is properly termed a pathologic fracture.
Surgical implants and other foreign materials have emerged as a common and often life-saving materials to improve the function of the human body.

**Orthopaedic implant**

An orthopaedic implant is a medical device manufactured to replace a missing joint or bone or to support a damaged bone [1]. The aim of Orthopaedic implant is to maintain stability until fusion or fracture healing has occurred. Major plus points for their penetration are the mechanical strength and proven biocompatibility. Success in orthopaedic implant surgery depends, in part on the quality of the material used to make the implant, its manufacturing routes, mechanical properties, biological stabilization and biocompatible surface coating [2].

The implants market is an age driven market, with demand arising mostly from the elderly section of the population. In the market there is large potential in the worldwide market for these implants. Metal and alloys fit large range of biomedical applications, including devices for fracture fixation, partial and total joint replacement, external splints, braces and traction apparatus, as well as dental amalgams [3].

Presently major medical devices for different applications like, Orthopaedics, ENT, Cardiovascular, Dental etc. are made up of metals SS 316L, Co-Cr alloy, Ti6Al4V and Titanium. Orthopaedic implants can be for the hip, knee, spine, ear or for the extremity joints that include the fingers, feet and shoulder.

Orthopaedic implant made of 1) Metallic or 2) Polymer 3) Ceramic or ceramic composite [4].

**Metallic implant**

Among commonly available materials like Austenitic stainless steels, Cobalt-Chromium alloys and Titanium and its alloys, 316L SS are used as an implant material due to the availability and easy fabrication, superior inherent mechanical properties, reasonable corrosion resistance, biocompatibility, suitable density for load bearing purpose and low cost [5] (fig 1.01). The chemical composition of surgical grade of type 316L SS the specimen is (wt %): Cr (18.00), Ni (12.00), Mo (2.50), Mn (1.70), Cu (0.026),
Si (0.15), C (0.02) and Fe (balance) [6]. The nominal chemical composition of the pure Titanium is (wt %): H (0.015), C (0.15), N (0.03), O (0.18), Fe (0.20) and Ti (balance) [7]. It is considered the universal material for permanent implants, such as endosseous dental implants. In other applications requiring higher mechanical strength, titanium based alloys or Co-Cr alloys are preferred [8]. Biocompatibility of the implant with body and bone tissue is essential to allow adequate new bone ingrowth into the synthetic prosthesis (Osseo integration or Osteogenesis) and makes a vital contribution towards the health of the patient [2].

![Figure 1.01: Different types of orthopaedic implants](image)

**Bio-degradable polymers for implant material**

Most of the commercially available biodegradable devices are polyesters composed of homopolymers or copolymers of Glycolide and Lactide [9]. The majority of results indicate that these polymers are sufficiently biocompatible. Currently biodegradable implants are used for stabilization of fractures, osteotomies, bone grafts and fusions particularly in cancellous
bones, as well as for reattachment of ligaments, tendons, meniscal tears and other soft tissue structures [10]. Low molecular weight polyglycolic acid was synthesized by Bischoff and Walden in 1893 [11]. The first synthetic absorbable suture was developed from polyglycolic acid (PGA) by American Cyanamid Co. in 1962 [16]. The 90:10 copolymer of glycolide and lactide - polygalactin 910 - has been applied as the competitive suture 'Vicryl' since 1975 [17]. Since then sutures of polyglycolide and polylactide have been used for many years and no carcinogenic, teratogenic, toxic or allergic side effects have been observed [18]. The only adverse reaction reported has been a mild non specific inflammation [19-20]. Use of PGA as reinforcing pins, screws and plates for bone surgery was first suggested by Schmitt and Polistina [21] in 1969. Since then there has been a lot of development in manufacturing biodegradable implants with properties appropriate for osteosynthesis.

Crystalline polymers slowly degrade due to orderly arrangement of molecules and amorphous polymers are easily degrade due to random structure [22]. This biodegradable polymer excretes from the body via body's natural metabolic actions. In the process of degradation, the polymeric chains are cleaved by hydrolysis and enzymatic degradation process results in decrease of molecular weight to form monomeric acids and are eliminated from the body through the Krebs cycle (or TCA cycle), primarily as carbon dioxide and water in urine and release drug to local area. Polymeric coating on metallic implant prevents corrosion up to some extent [27-29].

![Biodegradation mechanism of biodegradable polymers](image)

**Figure 1.02: Biodegradation mechanism of biodegradable polymers**
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**Poly L-Lactide (PLLA)**

Lactide is the cyclic dimer of lactic acid that exists as two optical isomers, d and l. L-Lactide is the naturally occurring isomer. The homopolymer of L-Lactide is a semi-crystalline polymer. This type of material exhibits high tensile strength and low elongation and consequently has a high modulus that makes them more suitable for load-bearing applications such as in orthopaedic fixation and sutures. Poly (L-Lactide) is about 37% crystalline with a melting point of 175-178°C and a glass-transition temperature of 60-65°C [30]. The degradation of PLLA is much slower than that of PDLLA, requiring more than 2 years to be completely absorbed [31]. Copolymers of L-Lactide and DL-Lactide have been prepared to disrupt the crystallinity of L-Lactide and accelerate the degradation process.

**Polyglycolide (PGA)**

PGA is the simplest linear aliphatic polyester and is prepared by ring opening polymerization of a cyclic lactone, glycolide. It is highly crystalline, with a crystallinity of 45-55% and thus is not soluble in most organic solvents. It has a high melting point (220-225°C) and a glass transition temperature of 35-40 °C [34]. PGA has excellent mechanical properties but its biomedical applications are limited due to its low solubility and its high rate of degradation yielding acidic products. Consequently, copolymers of glycolide with caprolactone, lactide or trimethylene carbonate have been prepared for medical devices [35].

![Structure of PGA](image)

*Figure 1.03: Structure of PGA*
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Polyvinyl Pyrrolidone

Polyvinyl Pyrrolidone (PVP) is a hygroscopic, amorphous polymer available in form of white, free flowing crystalline powder or in clear aqueous solution and available in several molecular weight grades. It is soluble in water and insoluble in esters, ethers, ketones and hydrocarbons. It is highly adhesive. Polyvinyl Pyrrolidone has been shown to be biocompatible; UV cured films of PVP copolymers have been proposed as a potential bioadhesive wound dressing matrix. Due to its lubricity and viscous properties, PVP is applied to coat tissue contacting surfaces [36].

![Figure 1.04: Structure of PVP](image)

**Poly (D,L- Lactide)**

Poly (D, L- Lactide) (-(C₆H₈O₄)n-(C₂H₄O₂)-(C₆H₈O₄)n-, CAS No: 2680-10-4) is a pale yellow colored semicrystalline polymer having glass transition temperature of 55-60°C and melting point of 174- 184°C which is soluble in acetone, dichloromethane and dimethyl formamide [37]. PDLLA is a racemic mixture of D-and L- enantiomers of lactic acid and severs as a biodegradable coating of medical implants [28]. In the human body, the L-isomer exists in carbohydrate metabolism and the D-isomer is found in acidic milk.

In order to regulate the drug delivery rate, biodegradable polymers are widely used due to their excellent biocompatibility and low toxicity. Poly (D, L-Lactide) is a biocompatible, bioabsorbable, osteoconductive and biodegradable polymer that is used previously to formulate many
types of implantable and injectable drug delivery systems for humans and other animals. Poly (D, L- Lactide) can degrade due to its amorphous structure.

![Figure 1.05: Structure of poly (D, L- Lactide)](image)

**Polycaprolactone (PCL)**

ε-caprolactone is a relatively cheap cyclic monomer. A semi-crystalline linear polymer is obtained from ring-opening polymerization of ε-caprolactone in presence of tin octate catalyst. PCL is soluble in a wide range of solvents. Its glass transition temperature is low, around -60 °C and its melting point is 60 – 65 °C. PCL is a semi-rigid material at room temperature has a modulus in the range of low-density polyethylene(LDPE) and high-density polyethylene(HDPE), a low tensile strength of 23 MPa and a high elongation to break (more than 700%). Due to its low Tg, PCL is often used as a compatibilizer or as a soft block in polyurethane formulations [39,40].

![Figure 1.06: Structure of Polycaprolactone](image)
Bone infection and antibiotic

Bone infection

Like other parts of the body, bones which usually are well protected from infection due to the skin barrier to outside contaminants can become infected by bacteria and fungi directly through open fractures, during bone surgery or from contaminated objects that pierce the bone [41]. The introduced infecting micro-organisms adhere and grow to form a bio-film on medical device or bio-material and may spread to the bone from nearby skin or muscles or from another part of the body through the bloodstream. Failure of orthopaedic implant, mainly femoral hip replacement, due to infection is of increasing medical importance [42]. To eradicate infection the body needs to deliver antibiotics, antibodies and infection-fighting cells to the bacteria-infected areas. Infection may be mild or severe. In case of mild infection, antibiotic therapy is required for 6 weeks. Severe infections require more than 6 weeks of antibiotic therapy to adequately eradicate any remaining bacterial cells in the bone and bloodstream that is specific to the bacteria that were present in the bone. Several procedures for treatment of bone infection include removal of the infected medical device, long term systemic antibiotic therapy with all it side effects and sometimes require
removal and further implantation through surgery [43]. This is a serious concern for the patients as well as surgeons. Osteomyelitis is a bone related infection usually caused by bacteria, including mycobacterium particularly staphylococcus aureus, but sometimes caused by fungi. Staphylococcus aureus is the bacteria most commonly responsible for bone and tissue related infection[44]. It is an infective process, which encompasses all of the bone (osseous) components, including the bone marrow, can destroy healthy tissue, multiply and spread through blood. When it is chronic it can lead to bone sclerosis and deformity and can persist intermittently for years. Orthopaedic infections can become chronic, without prompt treatment. Thus, even a small scratch on the fingertips has the potential to permanently disable your finger, hand or worse. Fortunately, early diagnosis, appropriate antibiotic therapy and surgical intervention can cure most infections and prevent permanent problems [45].

**Risk factor for bone infection**

An infection may be red, warm and inflamed. The affected area may be stiff, drain pus and lose range of motion. Infections can give fever and chills. Infants may act irritable and lethargic, refuse to eat or vomit. One may limp or refuse to walk. Infections pose special risks to young children for a number of reasons:

Children under age of 3 are easily infected. Their immune systems are not fully developed and they tend to fall down a lot, opening the skin to infection. Infections spread quickly through a young child's circulation system and bone structure [49]. Damage to bones and joints caused by infection can harm a child’s growth and lead to severe physical dysfunction. Infection of child’s hip joint is a surgical emergency.

Having certain chronic diseases (i.e., HIV, hepatitis, rheumatoid arthritis, diabetes mellitus, hemophilia, systemic toxicity, sickle cell anemia, the patient with history of previous surgery and infection) puts at greater risk for infections. Often infections get in through breaks in the skin; especially puncture wounds and other injuries that are difficult to clean. Sometimes joint infections develop from an internal hip or knee replacement device (prosthesis). The most commonly infectable joint is Knee joint [50].
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If having symptoms such as fever and bone pain, it is good to go to doctor as soon as possible because osteomyelitis can quickly get worse and become much more difficult to get rid of. The doctor after physical examination may ask questions about any recent injuries to the area where you have pain. It’s likely, too, that the doctor will perform blood tests to see whether your White Blood Cell (WBC) count is elevated (a sign of infection) and to look for signs of possible inflammation or infection at local side of injury in the body. The doctor collects medical history by asking about any concerns and symptoms you have, your past health, your family’s health, any medications you’re taking, any allergies you may have and other issues. Often, the doctor will go for a bone X-ray, which may show whether an infection in the bone is present or present. However, X-rays are often negative if someone has had osteomyelitis for only a short while. If the doctor strongly suspects osteomyelitis, he may suggest a test called a bone scan that provides a more detailed look at the bone. An MRI (magnetic resonance imaging) can produce much more detailed images than X-rays because of its ability to separate different types of soft tissues. MRI not only can diagnose osteomyelitis, but also can help establish how long the bone has been infected.

**Conventional therapy for bone infection**

Conventional therapy with systemic antibiotics (orally or parentally) for bone infection often becomes unsuccessful because they are not evenly distributed in the body, which frequently results in subtherapeutic regional drug concentrations, particularly in areas of poor vascularization, including wound sites [56]. Systemic antibiotic therapy alone does not eradicate bacteria because of poor penetration into bone [57]. Treatment of osteomyelitis depends on the severity of the infection and whether it is acute (recent) or chronic (has been present for a longer period of time). The doctor may use a needle to remove a bacteria sample from the bone to help identify the bacteria responsible for the infection. This is known as needle aspiration [58]. After this doctor prescribes correct antibiotic to treat that particular infection. In some cases, Osteomyelitis will become severe and a cavity or hole will develop in the bone. Pus is a large collection of bacteria and white blood cells which may have formed in this cavity. If this happens, the doctor
may perform a surgical procedure to remove the pus out of the bone so that the bone can heal properly. Osteomyelitis can be difficult to treat. After being diagnosed with Osteomyelitis, patient may be admitted to the hospital for a short stay so that intravenous (IV) antibiotics can be given (directly into a vein) to fight the infection. Once the condition improves, patient is discharged but may continue to need IV or oral antibiotics at home for several more weeks.

Osteomyelitis often requires prolonged antibiotic therapy, lasting a matter of weeks or months, and may require surgical debridement. Severe cases may lead to the loss of a limb. Following steps are to be taken to overcome [59].

• Long term parenteral administration of antibiotics.
• Multiple revisions with radical debridement.
• Local administration of drugs using antibiotic loaded Orthopaedic implant, bone cements, antibiotic impregnated collagen sponges or beads.
• Removal of infected device

Often long term systemic administration of antibiotics is not advisable due to its high level in body fluids and toxicity towards various vital organs. Certain antibiotics are highly nephrotoxic, particularly if multiple doses accumulate over a course of treatment [60]. Thus, there is a need to achieve prolonged drug delivery that can persist at least over a month, to prevent post operative Orthopaedic infections.

Commercially available drug delivery system for bone infection and disadvantage

Local drug delivery is a well known concept in the medical industry (devices include drug coated coronary stent, delivery catheters, drug loaded beads, drug loaded cements for Orthopaedic applications etc.) to provide drug directly to the target area without exceeding higher drug levels in different parts of body. Local delivery of antibiotics via a degradable carrier has the potential for high local antibiotic levels and avoids systemic toxicity. Several bio-materials such as antibiotic loaded polymethylmethacrylate (1.08), collagen sponge (1.09), apatite-wollastonite glass ceramic blocks,
hydroxyapatite blocks, polylactide/polyglycolide implants and polylactate polymers have been proposed to prevent and treat early surgery-related bacterial colonization of prosthetic surfaces [64-67]. Most of them are available with gentamicin.

Biomaterial-associated infection is the second most common cause of implant failure. Commercially available gentamicin impregnated polymethylmethacrylate beads deliver high levels of local antibiotics without significant systemic levels and residual antibiotic also released from beads detected after five years. Long-term release may lead in antibiotic resistance [73]. As PMMA implants are not degradable, surgical removal of these implants is recommended (or necessary) when the drug is depleted because the studies showed the presence of gentamicin-resistant staphylococci growing on the beads themselves [74]. Biofilm formation makes eradication of these microorganisms almost impossible, unless the implant is removed.
Figure 1.09: Scanning electron micrographs of gentamicin-loaded PMMA beads removed from patients after an implantation period of 2 weeks. The bead surface is covered with bacteria (cocci) [74]

Figure 1.10: Collagen sponge

The collagen sponges impregnated with gentamicin have the disadvantage compared to PMMA of adding no solidity to the fracture site and not allowing filling of bone defects or voids and the concentration is higher than the
recommended values. The release of gentamicin from collagen sponge is only for few hours [75]. That means the antimicrobial effect exists only for a few days, such high doses and/or lengthy treatment (orally or parentally in high dose) with antibiotics often lead to adverse effects like ear and kidney damage [76].

Several researchers have focused on use of drug impregnated ceramic materials such as tri-calcium phosphate or hydroxyapatite, since their chemical composition is similar to bone [77] but having low load bearing capacity, they are not widely used. Recently, peptide based implants, the so called RGD (arginine, glycine, aspartic acid)- peptide, have been reported to stimulate the adhesion of osteoblasts and therefore, to improve the osteointegration of RGD-coated implants [82]. Controlled release of antimicrobial drugs from the implanted objects thus represents an alternative to conventional systemic therapy [83].

For targeted delivery of a selected drug we need some carriers that can take drug to the site and release it at a specified pre-determined rate to the adjacent cells to prevent events that leads to infection. Literature suggests that biodegradable polymers can be used as a carrier for delivering drug to the targeted site. Biodegradable polymers are currently being used by the pharmaceutical industry to produce a variety of biodegradable sutures, staples, fixation rods, screws and clips. The biomaterials are constituted by biodegradable polymers from glycolide, lactide and ε-caprolactone monomers. These three cyclic monomers for use in medical applications can be assembled into a wide variety of polymers and copolymers.

**Antibiotic**

Antibiotics are used to treat infections caused by bacteria. Bacteria are microscopic organisms, some of which may cause illness. The search for antibiotics began in the late 1800s, with the growing acceptance of the germ theory of disease, a theory which linked bacteria and other microbes causing a variety of diseases. The first discovered antibiotic was penicillin. Such penicillin-related antibiotics as ampicillin, amoxicillin and benzylpenicillin are widely used today to treat a variety of infections; these antibiotics have been in use around for a long time because of the large number of penicillin
resistant strains. There are several different types of modern antibiotics and they are only available with a doctor's prescription in developed countries. The cephalosporins are a class of β-lactam antibiotics and became one of the most used classes of antibiotics in the prevention and the treatment of orthopaedic infections. Other classes of antibiotics that are used today in the treatment of orthopaedic infections are the aminoglycosides (gentamicin, tobramycin, amikacin), the glycopeptide antibiotics (vancomycin) and the quinolones (ciprofloxacin, ofloxacin).

The antibiotic prophylaxis duration should be for one day. A longer duration of the antibiotic prophylaxis increases the risk of microbial resistance and the cost of the treatment.

Wound contamination with both gram-positive and gram-negative microorganisms occurs; therefore, the antimicrobial treatment should be effective against both types of germs. Currently, systematic combination therapy using a first-generation cephalosporin, which is active against gram-positive bacteria and an aminoglycoside, which is active against gram-negative germs, appears to be optimal, although other combinations may also be effective. Substitutes for aminoglycosides include quinolones, aztreonam and third-generation cephalosporines. Ampicillin or penicillin should be added to the antibiotic regimen when there are conditions favoring the development of anaerobic infections, such as clostridial myonecrosis. The results of cultures obtained after debridement and of antibiotic-sensitivity testing may help in selecting the best agents for subsequent surgical procedures or in case of an early infection. Patzakis and Wilkins reported that the combination therapy (cephalosporin + aminoglycoside) was associated with a 4.6% infection rate, whereas administration of only cephalosporin was associated with a 13% infection rate. Quinolones are a promising alternative to i.v. antibiotics because they offer broad-spectrum antimicrobial coverage, are bactericidal, can be administrated orally with less frequent dosing than i.v. antibiotics and are well tolerated clinically.

The most common bacteria were: Staphylococcus (60.9%): S. aureus (35.8%), S. epidermidis (24.5%), Enterobacter (12.3%), Acinetobacter (9.6%),
Escherichia coli (7.2%), Klebsiella (2.4%). The bacteria isolated from the infection sites had the following antibiotic sensitivities:

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphiloccocus aureus</td>
<td>Amoxiclav, Ceftriaxon, Ciprofloxacin, Vancomycin</td>
</tr>
<tr>
<td>Staphiloccocus epidermidis</td>
<td>Amoxiclav, Gentamycin, Ciprofloxacin, Vancomycin</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>Ciprofloxacin, Amikacin, Gentamycin, Amoxiclav</td>
</tr>
</tbody>
</table>

Type I and II open fractures: Ceftriaxon 2 g/ day i.v. + Gentamycin 80mg/day i.v. + ATPA + Metronidazol 500 mg/day., for 2 days (20)

Some means of local drug delivery available today in the market are
- Gentamycin beads: Septopal Beads, PMMA+Gentamycin (Biomet)
- Gentamycin impregnated Gelfoam: Septocol (Biomet)
- Bone cement with gentamycin: PMMA+Gentamycin and Arithromycin. (Styker Howmedica)
- Antibiotic coated pacemakers (Guidant Corporation)

**Loosening of implants and bio-compatible ceramic material Loosening of Implants**

Orthopaedic implants which were used to be inserted in late 19th century and the first half of the 20th century were destined to failure, both as a result of metallic corrosion and of structural failure. Mechanical failure at the prosthesis/bone interface termed as loosening, is always caused by insufficiency of the substance adjacent to the implant. An interface without a separating fibrous membrane is the only guarantee for long term clinical success [102]. The success of a biomaterial or an implant is highly dependent on three major factors (i) the properties (mechanical, chemical and tribological) of the biomaterial in question (ii) biocompatibility of the implant and (iii) the health condition of the recipient and the competency of the surgeon.

The Second most frequent drawbacks in Orthopaedic surgery are loosening of implants, corrosion of metal, leaching of metal ions and foreign body adverse reactions particularly due to wear debris [103]. When Implant is placed into the body, it is either press-fit into the bone, or cemented into
position. Either way, it is fit tightly into the bone and pelvis so that the implant cannot move [105]. The implants face severe corrosion environment which includes blood and other constituents of the body fluid which encompass several constituents like water, sodium, chlorine, proteins, plasma, amino acids along with mucin in the case of saliva [106]. Concentrations of corroded particles in the tissue near the implants and other parts of the human body such as kidney, liver etc. [108, 109]. In spite of the fact that there is no histological evidence to show the slow release of metallic ions due to corrosion, the discoloration of the surrounding tissue and the foreign body reactions clearly indicate that this is due to corrosion of implants [111]. Generally implants have to be tight with surrounding bone tissue after surgery. When implants loosen, the implant can begin to move small amounts. Implant or Prosthesis loosening occurs over time and can cause problems with the normal function of the replacement prosthesis [113]. Usually this is associated with increasing pain and loss of motion experienced by the patient. Loosening of the implants results in a loss of stability that might lead to non-union or loss of reduction [115]. The fixation of orthopaedic implant depends on major factors viz. the quality of the bone and the design and size of the implant. Chemical stability, mechanical behavior and biocompatibility in body fluids and tissues are the basic requirements for successful application of implanted materials in bone fractures and replacements [116]. Diagnosis of implant loosening is based on clinical Symptoms like
  ó Pain during walking and during turning the body in bed.
  ó Bone Scan: 3-Phase –scintigraphy shows increased activity.
  ó Plane radiography: Bone resorption around the implant.
  ó During Surgery: Incomplete stability on direct manipulation.

**Biomaterials**

Ceramics, particularly alumina was first introduced by a french orthopaedic surgeon as structural orthopaedic biomaterials in the late 1960, where failures of the biomaterials in use got exploited then material such as steel, cobalt alloys and poly methyl methacrylate began to be detected [130]. However, limitations in processing technology and lack of quality control led
to materials with higher than desired levels of impurities and imperfections, including high porosity levels. These defects caused a further reduction in the strength of ceramics in tensile or shear loading, resulting in premature failure in a number of clinical cases [130].

Hence, attention was directed to ceramic materials in an attempt to find good bone integration features. Ceramics are now commonly used in the medical fields as dental, and bone implants [131-132]. The ceramic materials used are not the same as porcelain type ceramic materials. Rather bio-ceramics are closely related to either the body's own materials or are extremely durable metal oxides. Artificial teeth and bones are relatively commonplace. Joint replacements are commonly coated with bioceramic materials to reduce wear and inflammatory response. Examples of medical uses of bio-ceramics are in pacemakers, kidney dialysis machines and respirators [135]. Bio-ceramics fulfil a unique function as biomedical materials. The development of biomaterials and manufacturing techniques has broadened the diversity of applications within the human body.

Various Bio-ceramics like-alumina, zirconia, pyrolytic carbon, bioglass, silica, calcium phosphate group etc. have been matter of interest for scientists for their higher biocompatibility over metals [136,137]. The ceramic-based biomaterials have been accepted after biological evaluation through several in vivo and in vitro tests. Bio-ceramics are either bioinert, biodegradable or bioactive [138]. Bio-inert materials form a fibrous capsule around the implant. Bioactive materials on the other hand do an interfacial bond with the implant, whereas bioresorbable (biodegradable) materials are replaced with the new tissue as the implant dissolved [139]. Due to their brittle nature and low load bearing capacity, they are not widely popular to be used as prosthesis and alternatives for metallic implants [140].

**Alumina**

Since 1975 alumina ceramic has proven its bio-inertness. An alumina ceramic has characteristics of high hardness and high abrasion resistance. The reasons for the excellent wear and friction behavior of Al$_2$O$_3$ are
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associated with the surface energy and surface smoothness of this ceramic. There is only one thermodynamically stable phase, i.e. Al\(_2\)O\(_3\) having a hexagonal structure with Aluminum ions at the octahedral interstitial sites. Abrasion resistance, strength and chemical inertness of alumina have made it to be recognized as a ceramic for dental and bone implants [141]. The biocompatibility of alumina ceramic has been tested by many researchers. The results showed no signs of implant rejection or prolapse of the implanted piece. Loosening is the most frequently observed long-term complication following joint replacement. The reason is thought to be foreign-body reaction of the tissue against wear particles of various biomaterials. Relationship between the size and type of biomaterials and tissue reaction has not been clarified completely. When alumina ceramic (Al\(_2\)O\(_3\), 3.9 \(\mu\)m) was surgically inserted in the knee joints of Japanese white rabbits, the consequent histological reaction was examined. Alumina ceramic induced weak tissue reaction [142]. These properties are exploited for implant purposes, where it is used as an articulating surface in hip and knee joints. Its ability to be polished to a high surface finish make it an ideal candidate for this wear application, where it operates against materials such as ultra high molecular weight polyethylene. Porous alumina has also been used as a bone spacer, where sections of bone have had to be removed due to disease.

In this application, it acts as a scaffold for bone ingrowth. Single crystal alumina or sapphire has also been used in dental applications, although its use in this application is declining with the advent of more advanced materials such as resin-based composites.

**Pyrolytic carbon**

Pyrolytic carbon is a bioactive biomaterial commonly used in synthetic heart valves and has been the most popular material for this application for the last 30 years. Properties that make this material suitable for this application include good strength, wear-resistance and durability and most importantly, thrombo-resistance or the ability to resist blood clotting. Pyrolytic carbon is also used for small orthopaedic joints such as fingers and spinal inserts [152].
Zirconia is an inert biomaterial that has a bright future because of its high mechanical strength and fracture toughness. Zirconia ceramics have several advantages over other ceramic materials due to the transformation toughening mechanisms operating in their microstructure that can be manifested in components made out of them. The research on the use of zirconia ceramics as biomaterials started about twenty years ago and now zirconia is in clinical use in total hip replacement (THR) and developments
are in progress for application in other medical devices [145]. Today’s main
application of zirconia ceramics is in THR ball heads. The biocompatibilities
of polarized partially stabilized zirconia (PSZ) ceramics were examined using
osteoblastic cell cultivation. The proliferation of adhesive cells were
accelerated on the negative charge surface and decelerated on the positive
charge surface [146]. The osteointegration of zirconia was investigated in
normal and osteopenic rats by means of histomorphometry. The data
showed that the tested material was biocompatible in vitro and confirmed
that bone mineral density is a strong predictor of the osteointegration of an
orthopaedic implant and that the use of pathological animal models is
necessary to completely characterize biomaterials [147]. The cytotoxicity of
polycrystalline zirconia was speculated in L cell line culture. The study
revealed its non-cytotoxicity [148].

**Calcium phosphate ceramics**

There are several calcium phosphate ceramics that are considered
biocompatible. Of these, most are resorbable (biodegradable) and dissolve
when exposed to physiological environments [149]. Some of these materials
include, in order of solubility, Tetracalcium Phosphate (Ca$_4$P$_2$O$_9$) >
Amorphous calcium Phosphate > alpha-Tricalcium Phosphate (Ca$_3$(PO$_4$)$_2$) >
beta-Tricalcium Phosphate (Ca$_3$(PO$_4$)$_2$) >> Hydroxyapatite (Ca$_{10}$(PO$_4$)$_6$(OH)$_2$)

Unlike the other calcium phosphates, hydroxyapatite does not break down
under physiological conditions. In fact, it is thermodynamically stable at
physiological pH and actively takes part in bone bonding, forming strong
chemical bonds with surrounding bone. This property has been exploited for
rapid bone repair after major trauma or surgery [150,151].
While its mechanical properties have been found to be unsuitable for load-
bearing applications such as orthopaedics, it is used as a coating on
materials such as titanium and titanium alloys, where it can contribute its
‘bioactive’ properties, while the metallic component bears the load.

**Bioglass**

Bioglass was introduced to the scientific world in the late 1960s by Dr.
Hench. These glassceramics, which contained varied proportions of SiO,
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Na$_2$O, CaO, P$_2$O$_3$, CaF$_2$, and B$_2$O$_3$ were designed to interact with the normal physiology of bone to allow strong bone bonding. The bonding mechanism was found to depend on the composition of the glass, and this has sparked the development of other variations of glass-ceramics. Glass-ceramics have low tensile strength and fracture toughness, limiting their use in bulk form to applications subject to purely compressive loading. Attempts have been made to use these materials as part of composite structures to increase their application. The most common method is to coat a ceramic or metallic implant with the glass to create an osteo-inductive surface. The coating may be applied in a pure layer of glass or as an enamel coating with embedded glass particles. For the enamel systems, it is important to ensure that the components of the enamel do not interfere with the bone-formation process. The glass coating is still a brittle material and must be handled with care; any substantial impact may lead to failure of the entire coating system. Glass composites have also been investigated using stainless steel fibers (50 to 200 μm thick) to reinforce the glass-ceramic goal of these composites follows that of other fiber-reinforced materials—to increase their resistance to fracture by blunting crack growth and introducing a residual compressive stress within the material. This procedure was found to make the material significantly more ductile and stronger, thus reducing its tendency to fail catastrophically. In addition, the elastic modulus was reduced from that of the pure glass, bringing it closer to the ideal properties for bony replacement [153].

At present, glass-ceramics have been used clinically for only limited applications. These include material for filling bony defects along the lines of a bone graft [154], reconstruction of the ossicular bones [155], spine reconstruction [156] and dental reconstruction [157].

**Bone composition**

Bone is essentially a composite of organic and inorganic components namely; collagen and hydroxyapatite. Collagen is a protein with a high tensile strength and viscoelastic properties, whereas hydroxyapatite is a calcium phosphate compound with properties similar to that of a ceramic. Hydroxyapatite crystals are needlelike structures with a size of angstrom
unit and embedded in the sides of long collagen fibers. The collagen fibers are then arranged in sheets as parallel structures, which in turn are layered in concentric circles with the collagen fiber orientation varying between layers. The dimension about which these concentric layers of composite or lamellae are formed differs with the type of bone involved. The composition of the mineral phase varies between different parts of the bone and over time, but the main constituent of bone mineral is Hydroxyapatite [158].

**Improve metallic implant property by bioactive ceramic coating**

Most metallic orthopaedic and dental implants are bio-inert and do not bond chemically to bone. If the implant does not integrate well with the surrounding bone or is not held rigidly with a fastening device, the implant will be subjected to micro movement, and surrounding bone will remodel. This may lead to implant loosening over a period of time [159].

Metals and polymers can offer good load-bearing but are biologically inactive when implanted in the human body. Although bioactive biomaterials can form a strong biochemical bond with the bone and soft tissues, the mechanical properties of the materials themselves are usually unsuited for load-bearing applications. One approach to solve this problem is to combine them with a fracture tough material to produce a composite. The other alternative is to apply these materials as coatings on mechanically tough substrates.

In the past 30 years, hydroxyapatite (Ca$_5$(PO$_4$)$_3$(OH)) ceramic implants have attracted much attention as an alternative substance for bare metallic implants like stainless steel (S.S.), cobalt-chromium (Co-Cr) alloy and titanium Alloy used for orthopaedic implants [160]. Hydroxyapatite (HAp) is widely used as a bioactive ceramics since it forms a chemical bonding to bone. Hydroxyapatite coatings have been shown to achieve a very strong bond with living bone, in a relatively short period, even under loaded condition [161-171].
Many biocompatibility studies have proved that HAp has very similar chemical composition like the inorganic part of human hard tissue, such as bone and teeth. The most important advantage of HAp being a bioactive material is that bone will form a direct chemical bonding to HAp implant without forming a collagen interface layer which is usually found in many bare metallic devices and other bio-inert materials after implantation [173]. Owing to the inferior mechanical properties of HAp and poor osteo-inductive properties of titanium, SS, Co-Cr alloy implant, bioactive coating on metallic...
implant such as hydroxyapatite (HAp) has been tried to enhance osseo integration and initial fixation property of implant in dentistry and orthopaedic [174].

**Hydroxyapatite**

Hydroxyapatite \(\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2\) – CAS Number: 7758-87-4) is synthetic white odorless inorganic powder, free form biological contamination they do not elicit foreign body reaction when implanted. It is insoluble in water. Naturally occurring apatite may have brown, yellow or green colorations compared to the discolorations of dental fluorosis. Hydroxyapatite (HAp) is widely used as a bioactive ceramics since it forms a chemical bonding to bone [175].

![Structure of hydroxyapatite](image)

**Figure 1.12: Structure of hydroxyapatite**

**Mechanism of action of hydroxyapatite is described by Gorbunoff as follows [176]**

- After implantation, calcium phosphate solid-solution equilibrium are established by calcium and phosphate ions which are released from implant and surrounding bone.
- This means slight dissolution of HAp or bioglass is very important for the so-called bioactivity of these bioactive materials.
- This process results in calcium and phosphate ions super-saturation in the surrounding body fluid, and then carbonate apatite crystallites epitaxially reprecipitate on the surface of the HAp.
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- These modified surfaces are known to accommodate protein adsorption and cell adhesion more rapidly; in particular, cells (osteoblasts) associate with bone bonding.

- In six months, mineralization within the implant sites is comparable to the surrounding bone.

- TEM image analysis of dense HAp bone interfaces show almost perfect epitaxially alignment of some growing bone crystallites with the apatite crystals in HAp implant.

- Due to this chemical bonding interface, the bonding strength of HAp and bone is much higher than bare metallic implants.

- Thereby the relative micro-movement between the implant and bone is dramatically reduced by this direct bonding, and no fibrous tissue capsule can be found between the implant and bone.

- This is important for the patient’s recovery in the early period after implantation.

**Hydroxyapatite coated medical devices available in market:**

In market the hydroxyapatite coated different types of medical devices are available viz. middle ear prosthesis, cardio vascular Stent, bio-eye, ophthalmic implants etc. by various manufacturer are

- a) JRI UK
- b) Plasma Biotel Ltd
- d) CAM Implants
- e) Biomet Ltd.
- c) Zircotech
- d) Simpler Implants
- e) Life Core Medical

**Advantages and disadvantages of Hydroxyapatite [179]**

- Hydroxyapatite shows great biocompatibility with the human body, its applications are limited to non load-bearing areas and coatings due to its low mechanical properties.

- The most important advantage of HA being a bioactive material is that bone will form a direct chemical bonding to HA implant without forming a
collagen interface layer, which is usually found in many other bio-inert materials after implantation.

- The beneficial biocompatible properties of Hydroxyapatite are well documented.
- It is rapidly integrated into the human body, while at the same time the body is none the wiser as to the invasion by a foreign body, albeit a friendly invasion.
- Active bioceramic hydroxyapatite is ideal for using as material at artificial tooth root and total hip joints. HA provide a biocompatible compatibility of strong chemical bond with natural bone. In addition, it can induce frame tissue in growth and future formation of chemical bonding to achieve fixed function and its mechanical properties of low strength and high brittleness restrict the application only on bone repair.

- Increased bond strength
- Improved corrosion Resistance
- Extended Implants lifetime
- Accelerated healing rates
- No adverse inflammatory response
- No significant change in the blood chemistry

**Disadvantages of hydroxyapatite**

The main disadvantage of hydroxyapatite is poor mechanical properties (in particular fatigue properties) mean that hydroxyapatite cannot be used in bulk form for load bearing applications such as orthopaedics. In short, it provides assurance to the surgeon that the coated implant will take care of the fracture and there is no need for additional antibiotic therapy.

In present work, coating of anti-bacterial drug on metallic implants using biodegradable polymer Poly (D, L- Lactide) as a carrier in multi layers control release has been investigated (Chapter 3). The objective of the present investigation is to develop and examine drug loaded biodegradable polymer films that can bind to the orthopaedic implants and prevent bacterial infection through controlled release of the drug. The release kinetics should be such that the drug is delivered at least for a period of one
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month so that no complications arise during the osteointegration period. Coating should be non-cytotoxic, with minimum platelet adhesion and non-pyrogenic.

Figure 1.13: Schematic diagram of antibiotic drug eluting metallic implant (chapter 3)

The main disadvantage of hydroxyapatite lies with its poor mechanical properties, particularly fatigue properties it means hydroxyapatite can not be used in bulk for load bearing application such as orthopaedics [180]. To develop hydroxyapatite coating various coating techniques like Electrophoretic deposition, Electrochemical deposition, Sol-gel, Plasma spray and brush coating were used (chapter 4). The hydroxyapatite coating should be biocompatible, non-pyrogenic, having sufficient adhesion to implant surface and Ca/P ratio nearest to 1.67.

Figure 1.14: Schematic diagram of hydroxyapatite coated metallic implant (Chapter 4)
The final product “Antibiotic Eluting Hydroxyapatite coated Orthopaedic Implant” was prepared in chapter 3 and 5. Scanning electron microscopy (SEM), Drug release, Antimicrobial activity test, Bacterial Endotoxin test were employed to evaluate performance.

Figure 1.15: Schematic diagram of antibiotic drug eluting hydroxyapatite coated implant (chapter 5)
Antibiotics drugs for loading

**Gentamicin**

The open fracture contaminated with both gram-positive and gram-negative pathogens needs suitable antibiotic. Gentamicin (CAS Number: 1405-41-0) is a mixture of the sulfates of antimicrobial substances produced by micromonospora purpurea. It is a white powder, freely soluble in water, practically insoluble in chloroform, ethanol (95%), ether and hygroscopic in nature. Gentamicin is broad spectrum bactericidal antibiotic drug of the amino glycoside antibiotic group and can actively treat many different types of severe bacterial infections, particularly gram-negative infection. This drug was approved by the FDA in 1966 [84]. The mechanism that allows these drugs to enter the bacterial cell is an oxygen-dependent pump. Once inside the bacterial cell, they bind to the 30S/50S ribosomal subunit interface to prevent protein synthesis thus causing the genetic code to be misread resulting in prevention of bacterial growth [88].

![Structure of Gentamicin](image)

*Figure 1.16: Structure of Gentamicin*
Table No 1.02: Composition of gentamicin

<table>
<thead>
<tr>
<th>Gentamicin</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁</td>
<td>CH₃</td>
<td>NHCH₃</td>
<td>H</td>
</tr>
<tr>
<td>C₂</td>
<td>CH₃</td>
<td>NH₂</td>
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</tr>
<tr>
<td>C₁ₐ</td>
<td>H</td>
<td>NH₂</td>
<td>H</td>
</tr>
<tr>
<td>C₂ₐ</td>
<td>H</td>
<td>NH₂</td>
<td>CH₃</td>
</tr>
</tbody>
</table>

Gentamicin has been widely used with good success as locally applied antibiotic in orthopaedic surgery. Its good bone penetration, the broad antimicrobial spectrum including most bacteria commonly involved in osteomyelitis and its bactericidal effect, even on non-proliferating microorganisms make it favorable for local application. Gentamicin is not absorbed orally, serum concentrations are unpredictable after IM injection and IV infusion taking over 15-30 minutes is the usual mode of administration [89]. It maintains high drug concentration via local delivery which is the most advance technique to minimize the microbial infection using coated implants.

**Action Mechanism of Gentamicin:**
The gentamicin sulphate has general pattern of action which may be described in two main steps:
a) Transport of the gentamicin through the bacterial cell wall and cytoplasmic membrane.
b) Binding to ribosomes resulting in inhibition of protein synthesis.

Transport of gentamicin into bacteria is a multistep process. They diffuse through the outer coat of gram negative bacteria through porin channels. Once inside the bacterial cell gentamicin bind to 50S subunit as well as to 30S-50S interface, they freeze initiation of protein synthesis, prevent polysome formation and promote their disaggregation to monosomes so that only one ribosome is attached to each strand of mRNA. Binding of Gentamicin to 30S-50S juncture causes distortion of mRNA codon recognition resulting is misreading of the code [90].
Figure 1.17: Binding of Gentamicin with ribosomal sub units [93]

Figure 1.18: Transportation of gentamicin in the bacterial cell is an oxygen dependent

**Cefazolin**

Mainly used to treat bacterial infections of the skin. It can also be used to treat moderately severe bacterial infections involving the lung, bone, joint, stomach, blood, heart valve and urinary tract. It is clinically effective against infections caused by staphylococci and streptococci species of gram positive bacteria. May be used for surgical prophylaxis; if required metronidazole
may be added to cover B. fragilis. A semisynthetic cephalosporin analog with broad-spectrum antibiotic action due to inhibition of bacterial cell wall synthesis. It attains high serum levels and is excreted quickly via the urine.

![Figure 1.19: Molecular structure of cefazolin](image)

Cefazolin, CAS no is 25953-19-9, chemical formula: \( \text{C}_{14}\text{H}_{14}\text{N}_8\text{O}_4\text{S}_3 \) and M.W. is 454.5. Cefazolin (also known as cefazolin or cephazolin) is a semi-synthetic first generation cephalosporin for parenteral administration. Cefazolin has broad-spectrum antibiotic action due to inhibition of bacterial cell wall synthesis. It attains high serum levels and is excreted quickly via the urine. In vitro tests demonstrate that the bactericidal action of cephalosporins results from inhibition of cell wall synthesis. By binding to specific penicillin-binding proteins (PBPs) located inside the bacterial cell wall, it inhibits the third and last stage of bacterial cell wall synthesis. Cell lysis is then mediated by bacterial cell wall autolytic enzymes such as autolysins. Cefazolin is present in very low concentrations in the milk of nursing mothers. Cefazolin is excreted unchanged in the urine. In the first six hours approximately 60% of the drug is excreted in the urine and this increases to 70%-80% within 24 hours.