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General introduction

GENERAL INTRODUCTION

During the past few years, the biocompatibility of biomaterials (non-vital material intendedto interact with biological systems within or on the human body) has evolved into acomprehensive, complex, and independent discipline of biomaterials science.

Biomaterials can be derived either from natureor synthesized in the laboratory using a varietyof chemical approaches utilizing metalliccomponents,polymers, ceramics or compositematerials. They are often used and/or adoptedfor a medical application, and thus perform,augments, or replace a natural function. Suchfunctions may be benign, like being used fora heart valve, or may be bioactive with a moreinteractive functionality such as coated hipimplants. Biomaterials are also used every day indental applications, surgery, and drug delivery.For example, a construct with impregnatedpharmaceutical products can be placed intothe body, which permits the prolonged releaseof a drug over an extended period of time. Abiomaterial may also be an autograft, allograft orxenograft used as a transplant material.

An essential question that needs to be answered for the increasing need of biomaterials in safe human life is:how biomaterials can be evaluated to determine if they are biocompatible? And how biomaterials can be evaluated to determine whether they work appropriately in the in vivoenvironment?

In this thesis we will try to give answers to these questions, for this; in the chapter one we give some basic notion such as the definition of biomaterials; their properties and some their uses in the body, we give also definition for biocompatibility and their components.

In the chapter two we focus in methods of biocompatibility testing, and in the frame of it we present the performance requirements witch effectin,in orderto assess how the biocompatibility of a material process.

In the final of this chapter we summarize with a general conclusion.

Chapter 1
Biomaterials and biocompatibility

I.1 Introduction:

The field of biomaterials include medical devices has turn into an electrifying area because these materials improve the quality and longevity of human life. The first and foremost necessity for the selection of the biomaterial is the acceptability by human body; so biocompatibility [1].

I.2 Medical devices:

The term “medical devices” includes everything from highly sophisticated computerizedmedical equipment down to simple wooden tongue depressors. The intended primarymode of action of a medical device on the human body, in contrast with that of medicinalproducts, is not metabolic, immunological, or pharmacological**[2]**.

Several different international classification systems for medical devices are still in usein the world today. The World Health Organization, with its partners, is working towardsachieving harmonization in medical device nomenclature, which will have a significantimpact on patient safety. This is particularly important to be able toidentify adverse incident reports and recalls.

The Global Harmonization Task Force has proposed the following harmonized definitionfor medical devices; “Medical device” means any instrument, apparatus, implement, machine, appliance,implant, in vitro reagent or calibrator, software, material or other similar or relatedarticle, intended by the manufacturer to be used, alone or in combination, for humanbeings for one or more of the specific purposes of:

• Diagnosis, prevention, monitoring, treatment or alleviation of disease

• Diagnosis, monitoring, treatment, alleviation of or compensation for an injury

• Investigation, replacement, modification, or support of the anatomy or of aphysiological process

• supporting or sustaining life

• Control of conception

• Disinfection of medical devices

• providing information for medical purposes by means of in vitro examination ofspecimens derived from the human body and which does not achieve its primaryintended action in or on the human body by pharmacological, immunological ormetabolic means, but which may be assisted in its function by such means.

Note: An accessory is not considered to be a medical device. However, where anaccessory is intended specifically by its manufacturer to be used together with the‘parent’ medical device to enable the medical device to achieve its intended purpose,it should be subject to the same procedures and GHTF guidance documents as applyto the medical device itself.

Note: The definition of a device for in vitro examination includes, for example,reagents, calibrators, sample collection devices, control materials, and relatedinstruments or apparatus. The information provided by such an in vitro diagnosticdevice may be for diagnostic, monitoring or compatibility purposes. In somejurisdictions, reagents and the like may be covered by separate regulations.

Note: Products, which are considered to be medical devices in some jurisdictionsbut for which there is not yet a harmonized approach, are:

• Aids for disabled/handicapped people

• Devices for the treatment/diagnosis of diseases and injuries in animals

• Spare parts for medical devices

• Devices incorporating animal and human tissues which may meet the requirementsof the above definition but be subject to different controls**[2]**.

I.3 Biomaterials

I.3.1.History:

The first biomaterials used were gold and ivory for replacements of cranial defects. This was done by Egyptians and Romans. Biological materials such as placenta were used since the 1900s. Celluloid was the first man-made plastic used for cranial defects a polymethyl methacrylate (PMMA) was one of the first polymers accepted since World War II**[3]**.

Some of the earliest biomaterial applications were as far back as ancient Phoenicia where loose teeth were bound together with gold wires for tying artificial ones to neighboring teeth[1].

In the early 1900's bone plates were successfully implemented to stabilize bone fractures and to accelerate their healing[1].

While by the time of the 1950's to 60's, blood vessel replacement were in clinical trials and artificial heart valves and hip joints were in development[1].

600 B.C	Samhita	Nose construction
1893-1912	W.A.Lane	Steel screws for fixation
1912	W.D.Sherman	Use of Vanadium steel plate
1938	P.Wiles	First total hip replacement
1952	A.B.Voorhees	Blood Vessel

1953 A.KantrowitzIntraortic balloon pumping

1960 M.I.Edwards Heart valve

1980 W.J.Kolff Artificial Heart

I.3.2 Definition:

In general, a biomaterial is defined as any substance, except food and medications that can be used for a length of time as part of a system that aims to treat or to replace any tissue, organ, or body function. Few materials, if any, are totally inert from a physiological standpoint; most materials present a variety of components with potential toxic or irritating properties. In addition, chemical reactions that occur during setting of the material may also produce noxious effects [4]

Biomaterials need to satisfy a number of prerequisites before that can be used in applications, including biocompatibility. To verify this feature, its components should be subjected to different tests, performed as recommended by various organizations and federations. These tests consist of a sequence of research protocols, described and regulated in many countries, for correct use of experimental materials under evaluation, thereby determining their safety for clinical application in humans [4].

I.4Biocompatibility

I.4.1 definition:

Biocompatibility may be defined as:

"Ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response to that specific situation, and optimizing the clinically relevant performance of that therapy [4]."

Biocompatibility can also be defined as the relationship between a material and the organism so that neither produces undesirable effects. Biocompatibility has been mentioned in many works with increasing interest in evaluating the characteristics of medical and dental materials and devices and responses caused by its components. An ideal pattern for determining these properties has not yet been determined, however, various methods have been suggested for this purpose [4].

The most accepted definition of biomaterials is currently the one employed by the American National Institute of Health that describes biomaterial as ''any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual'' **[3]**.

I.4.2 Components of biocompatibility

In addition to the beneficial tissue response and the clinically relevant performance of a biomaterial, cytotoxicity, genotoxicity, mutagenicity, carcinogenicity and immunogenicity are considered to be the components which constitute “biocompatibility”

Toxicity of a material describes the ability to damage a biological system by chemical means.In higher organisms (animals, human beings), local toxicity – that is, adverse reactions emerging at the application site – is differentiated from systemic toxicity, in which adverse reaction appear in an area distant from the application site. Cytotoxicity refers to damage to individual cells, for example in cell cultures. Cells can die because of necrosis or apoptosis (programmed cell death).

Immunogenicity is referred to the ability of a substance to provoke an immune response or the degree to which it provokes a response. An allergic reaction to a substance can be triggered if the organism was previously sensitized to this substance. The concentrations that elicit a reaction in a previously sensitized person vary between subjects. The dose levels causing allergic reactions are generally significantly lower than those causing toxic reactions.

Genotoxicity describes an alteration of the basepair sequence of the genome DNA. Cells possess numerous mechanisms to repair genotoxic damages. Alternatively, a transfer of these genetic damages to subsequent generations of cells can be avoided by programmed cell death (apoptosis). Nonetheless, if theses genetic damages are passed on to the next generation, this effect is called mutagenicity.

Mutagenicity and carcinogenicity are not the same. Carcinogenicity means that alterations in the DNA have caused a cell to grow and divide inappropriately; in other words, alterations of DNA promoted the generation of malignant tumors.

Carcinogenicity results from several mutations. It is important to understand that not all mutagenic events lead to carcinogenesis. However, mutagenicity can be assessed as an indicator of “possible” carcinogenicity of substances that directly attack DNA [1,5].

I.4.3 Uses for Biomaterials:

One of the primary reasons that biomaterialsare used is to physically replace hard or soft tissuesthat have become damaged or destroyedthrough some pathological process.Although the tissues and structures of the bodyperform for an extended period of time in mostpeople, they do suffer from a variety of destructiveprocesses, including fracture, infection, andcancer that cause pain, disfigurement, or loss offunction. Under these circumstances, it may bepossible to remove the diseased tissue andreplace it with some suitable synthetic material**[6]**.

Orthopedics:

One of the most prominentapplication areas for biomaterials is for orthopedicimplant devices. Both osteoarthritis andrheumatoid arthritis affect the structure of freelymovable

(synovial) joints, such as the hip, knee,shoulder, ankle, and elbow. The pain insuch joints, particularly weight-bearing jointssuch as the hip and knee, can be considerable,and the effects on ambulatory function quitedevastating. It has been possible to replace thesejoints with prostheses since the advent of anesthesia,antisepsis, and antibiotics, and the reliefof pain and restoration of mobility is wellknown to hundreds of thousands of patients. A variety of metals, polymers, andceramics are used for such applications**[6]**.

Cardiovascular Applications:

In the cardiovascular, or circulatory system (the heartand blood vessels involved in circulating bloodthroughout the body), problems can arise withheart valves and arteries, both of which can besuccessfully treated with implants. The heartvalves suffer from structural changes that preventthe valve from either fully opening or fullyclosing, and the diseased valve can be replacedwith a variety of substitutes. As with orthopedicimplants, ceramics, metals, and polymers are used as materialsof construction.Arteries, particularly the coronary arteriesand the vessels of the lower limbs, becomeblocked by fatty deposits (atherosclerosis), andit is possible in some cases to replace segmentswith artificial arteries. Polymers are the material of choice for vascularprostheses **[6].**

Ophthalmics:

The tissues of the eye cansuffer from several diseases, leading to reducedvision and eventually, blindness. Cataracts, forexample, cause cloudiness of the lens. This maybe replaced with a synthetic (polymer) intraocularlens. Materials for contact lenses,because they are in intimate contact with the tissuesof the eye, are also considered biomaterials.As with intraocular lenses, they too are usedto preserve and restore vision**[6].**

Dental Applications:

Within the mouth,both the tooth and supporting gum tissues canbe readily destroyed by bacterially controlleddiseases. Dental caries (cavities), the demineralizationand dissolution of teeth associated withthe metabolic activity in plaque (a film of mucusthat traps bacteria on the surface of the teeth),can cause extensive tooth loss. Teeth in theirentirety and segments of teeth either can be replaced or restored by a variety of materials**[6]**.

Wound Healing:

One of the oldest uses ofimplantable biomaterials can be traced back tothe introduction of sutures for wound closure.

Types of Biomaterials Egyptians used linen as a suture asfar back as 2000 b.c. Synthetic suture materialsinclude both polymers (the most widely syntheticsuture material) and some metals (e.g.,stainless steels and tantalum)**[6]**.

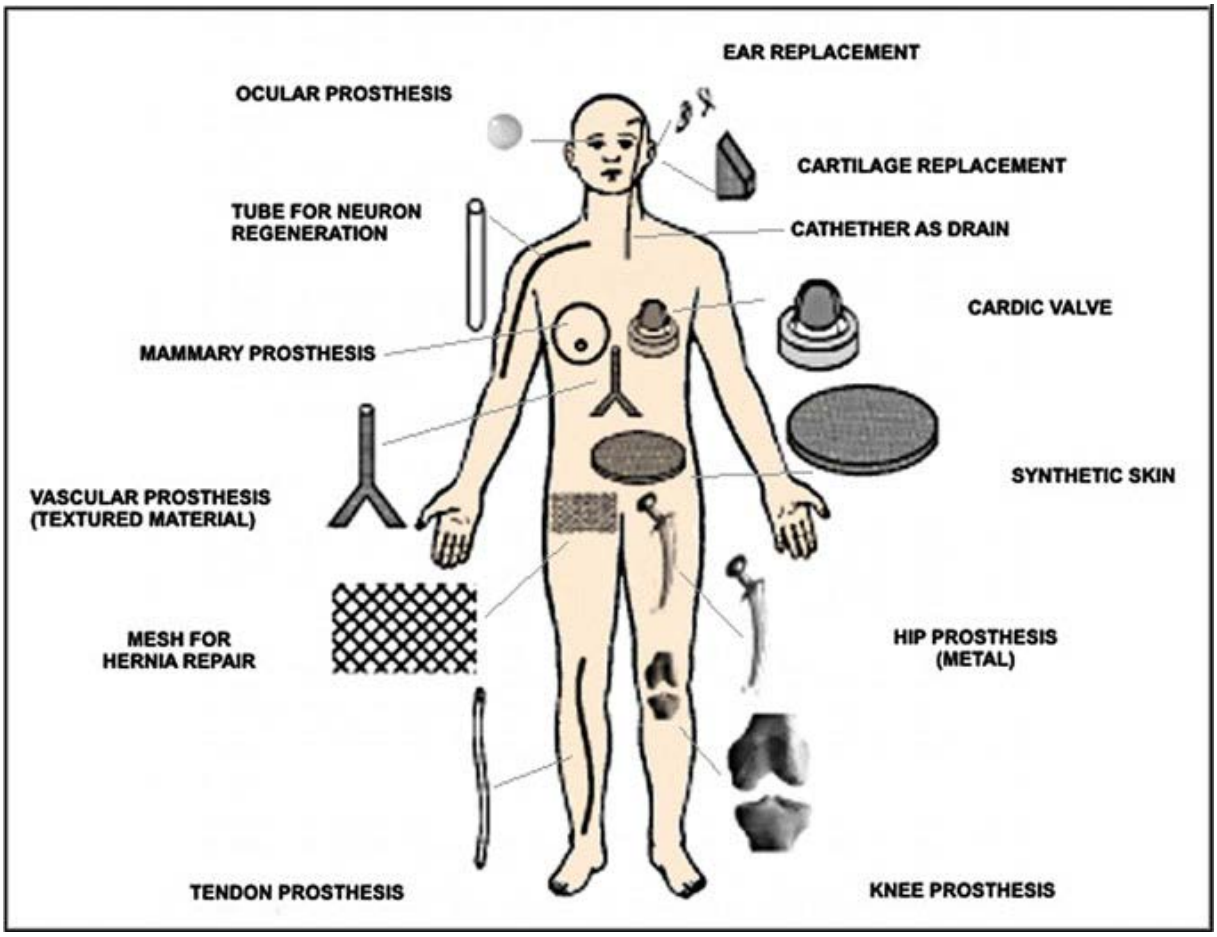

Fig.1 *Examples of biomedical materials[3]*

Some commonly used biomaterials:

Material categorizes	**Applications**
Silicone rubber	Catheters, tubing
Dacron	Vascular grafts
Cellulose	Dialysis membranes
Poly(methylmethacrylate)	Intraocularlenses, bonecement
Polyurethanes	Catheters, pacemaker leads
Hydogels	Ophthalmologicaldevices, Drug Delivery
Stainlesssteel	Orthopedicdevices, stents
Titanium	Orthopedic and dental devices
Alumina	Orthopedic and dental devices
Hydroxyapatite	Orthopedic and dental devices
Collagen (reprocessed)	Opthalmologic applications, wound dressings

Table1.1: materials and their applications [2]

I.4.4 Types of Biomaterials:

Most synthetic biomaterials used for implantsare common materials familiar to the averagematerials engineer or scientist. In general,these materials can be divided into the followingcategories: metals, polymers, ceramics,and composites.

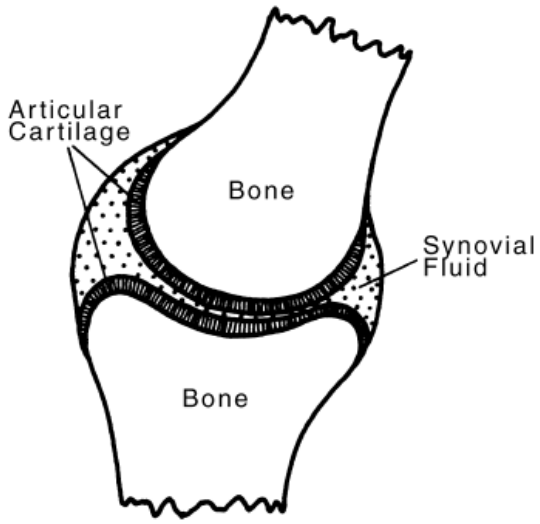

Fig.2: *Schematic showing key components of a natural synovial joint. It consists of layers of bearing material (articular cartilage) mounted on relatively hard bones forming the skeletal frame. The synovial fluid acts as a lubricant. In an artificial joint, lubrication is supplied by low-friction polymeric bearing materials [6]*

Metals:

As a class of materials, metals arethe most widely used for load-bearing implants.For instance, some of the most common orthopedicsurgeries involve the implantation ofmetallic implants. These range from simplewires and screws to fracture fixation plates andtotal joint prostheses (artificial joints) for hips,knees, shoulders, ankles, and so on. In additionto orthopedics, metallic implants are used inmaxillofacial surgery, cardiovascular surgery,and as dental materials. Although many metalsand alloys are used for medical device applications,the most commonly employed are stainlesssteels, commercially pure titanium and titaniumalloys, and cobalt-base alloys[6].

Polymers:

A wide variety of polymers areused in medicine as biomaterials. Their applicationsrange from facial prostheses to trachealtubes, from kidney and liver parts to heart components,and from dentures to hip and kneejoints review the useof polymers for these applications.Polymeric materials are also used for medicaladhesives and sealants and for coatings thatserve a variety of functions[6].

Ceramics:

Traditionally, ceramics haveseen wide scale use as restorative materials indentistry. These include materials for crowns,cements, and dentures. However, their use in otherfields of biomedicine has not been as extensive,compared to metals and polymers. For example,the poor fracture toughness of ceramics severelylimits their use for load-bearing applications; some ceramic materialsare used for joint replacement and bonerepair and augmentation. Review the uses of ceramics for nondental biomedicalapplications[6].

Composites:

The mostsuccessful composite biomaterials are used inthe field of dentistry as restorative materials or dental cements. Although carbon-carbon and carbon reinforcedpolymer composites are of greatinterest for bone repair and joint replacementbecause of their low elastic modulus levels,these materials have not displayed a combinationof mechanical and biological propertiesappropriate to these applications. Compositematerials are, however, used extensively forprosthetic limbs, where their combination oflow density/weight and high strength makethem ideal materials for such applications[6].

Natural Biomaterials:

Although the biomaterialsare syntheticmaterials, there are several materialsderived from the animal or plant world beingconsidered for use as biomaterials that deservebrief mention. One of the advantages of usingnatural materials for implants is that they aresimilar to materials familiar to the body. In thisregard, the field of biomimetics (or mimickingnature) is growing. Natural materials do notusually offer the problems of toxicity oftenfaced by synthetic materials. Also, they maycarry specific protein binding sites and otherbiochemical signals that may assist in tissuehealing or integration. However, natural materialscan be subject to problems of immunogenicity.Another problem faced by these materials,especially natural polymers, is their tendency todenature or decompose at temperatures belowtheir melting points. This severely limits theirfabrication into implants of different sizes andshapes.An example of a natural material is collagen,which exists mostly in fibril form, has a characteristictriple-helix structure, and is the mostprevalent protein in the animal world. Forexample, almost 50% of the protein in cowhideis collagen. It forms a significant component ofconnective tissue such as bone, tendons, ligaments,and skin.

There are at least ten differenttypes of collagen in the body. Among these,type I is found predominantly in skin, bone, andtendons; type II is found in articular cartilage injoints; and type III is a major constituent ofblood vessels.Collagen is being studied extensively for useas a biomaterial. It is usually implanted in asponge form that does not have significantmechanical strength or stiffness. It has showngood promise as a scaffold for neotissue growthand is commercially available as a product forwound healing. Injectable collagen is widelyused for the augmentation or buildup of dermaltissue for cosmetic reasons. Other natural materialsunder consideration include coral, chitin(from insects and crustaceans), keratin (fromhair), and cellulose (from plants)[6].

I.4.5 Biomaterials associated infection;

Biomaterials associated infection (BAI) is one of the most common complications associated with implantation of any biomaterial regardless of form or function. These infections usually involve bacterial colonization and biofilm formation on the biomaterial itself, rendering the infection impervious to antimicrobials and host defenses. In addition, it is becoming increasingly clear that infection of the surrounding tissues also plays an important role in BAI, and that the infection may be influenced by the composition and design of the implanted biomaterial. Advantages and disadvantages of biomaterials in some substances commonly used in implants are tabled below:

Biomaterials	**Advantages**	**Disadvantages**	**Types of Biomaterials**
Polymeric	Easy to make complicated items - Tailorable physical & mechanical properties - Surface modification -Immobilize cell etc. -Biodegradable	-Leachablecompounds - Absorb water &proteins etc. - Surface contamination - Wear & breakdown - Biodegradation - Difficult to sterilize	- PMMA - PVC - PLA/PGA - PE - PP - PA - PTFE - PET - PUR - Silicones
Bioceramic	- High compression strength - Wear & corrosion resistance - Can be highly polished - Bioactive/inert	High modulus (mismatched with bone) - Low strength in tension - Low fracture toughness - Difficult to fabricate	Alumina - Zirconia(partially stabilized) - Silicate glass - Calcium phosphate (apatite) - Calcium carbonate
Metallic	High strength - Fatigue resistance - Wear resistance - Easy fabrication - Easy to sterilize - Shape memory	- High moduls - Corrosion - Metal ion sensitivity and toxicity - Metallic looking	Stainless steel (316L) - Co-Cr alloys - Ti_6Al_4V - Au-Ag-Cu-Pd alloys - Amalgam (AgSnCuZnHg) - Ni-Ti - Titanium

Table 1.2: Advantages and disadvantages of biomaterials. **[2]**

I.5 Conclusion:

Biomaterials have found growing application inhealthcare technology industry including drugdelivery systems, active substance encapsulation,tissue scaffolds, wound care, implants, cosmeticsand diagnostics.There is a growing interest in the processing ofbiomaterials as developers find new applications.

A biomaterial is essentially a material that is used and adapted for amedical application. Biomaterials can have a benign function, suchas being used for a heart valve, or may be bioactive. Used for a moreinteractive purpose such as hydroxy-apatite coated hip implants andsuch implants are lasting upwards of twenty years.

Chapter2: Biocompatibility testing

II. Methods of biocompatibility testing:

II.1 Introduction:

Biomaterials are developed in order to evaluate, treat, augment or replace human tissue, organ or function. Biocompatibility is the main prerequisite for their safe use as medical devices. In order to assess the biocompatibility of a material, it is necessary to do a battery of tests, depending on the intended use, location and duration the material is to come in contact with the tissues. Biocompatibility is measured with 3 types of biologic tests: in vitro tests (level I), animal experiments (level II) and clinical tests (level III) [1].

To conduct these tests, it is necessary to involve health researchers for research methodology development and evaluation of the tissue and researchers for development of materials and their properties, such as engineers and chemists (Fig.2) [4].

Fig. 3 Plan of biocompatibility tests in order.

The common approach when testing the biological behavior of materials is to start with simple in vitro tests. If these experiments and investigations of a material's efficiency deliver promising findings, then more comprehensive studies on experimental animals (in vivo evaluation) will be performed. Clinical trials (usage tests) are the final step of this evaluation process.

II.2 In vitro and in vivo tests:

The ideal biological research methodology consists of in vivo experiments, despite the ethical aspects involved. Nevertheless, although in vitro studies provide responses limited 50 Polymerization by the absence of biological and physiological components that are impossible to reproduce entirely, they continue to be used and are suitable for determining whether a material contains significant quantities of extractable biologically harmful components.

Clinically, the results are divergent. Studies in vitro could report no damage, moderate damage or intense damage.This reinforces the effect of the physiology present in in vivo systems, but does not diminish the relevance of in vitro research. In vitro research remains important for pointing out pathways for studies of the adverse reactions recorded clinically.

In in vitro cell cultures, the complex physiology of an organism performing various functions simultaneously is not present. Thus, the buffer capacity of complex humoral and cellular systems in the intact organism is absent; a biomaterial may not work well in the in vitro test, but may be biocompatible in vivo.

This highlights the necessity of integrated in vitro and in vivo tests for valuable predictive estimation of the toxicity of complex materials [4].

II.2.1 In vitro tests

In vitro biocompatibility tests are less expensive ways to survey newly developed materials.They simulate biological reactions to materials when they are placed on or into tissues of the body. These tests are performed in a test tube, cell-culture dish, or otherwise outside of a living organism in which cells or bacteria are generally placed in contact with a material. For example, a strain of bacteria may be used to assess the ability of a material to cause mutations (the Ames test). The advantages of in vitro biocompatibility tests are, being experimentally controllable, repeatable, fast, relatively inexpensive and relatively simple.

Another major advantage is that these tests generally avoid the ethical and legal issues that surround the use of animals and humans for testing. The primary disadvantage of in vitro biocompatibility tests is their questionable clinical relevance [5].

The table below makes a comparison between advantages and disadvantages of in vitro tests;

Advantages	**Disadvantages**
-In vitro test, done in controlled experimental condition -Most rapid -Repeatable -Economical	-Lack of relevance to in vivo use of material -Lack of immune, inflammatory & circulatory system

II.2.2 Animal experiments

In animal experiments, the material is placed into an animal, usually a mammal. For example, the material may be implanted into a mouse or placed into the tooth of a rat, dog, cat, sheep, goat or monkey. Animal models allow the evaluation of materials over long time durations and in different tissue qualities (e.g. normal healthy or osteopenic bone) and ages.

Not only can the tissues in the immediate vicinity be assessed, but, tissues in remote locations of the implanted material can also be studied, which is particularly relevant to the study of wear particle debris. However, questions arise about the appropriateness of an animal species to represent the human response and that they are time-consuming and expensive. In animal experiments, ethical concerns and animal welfare issues are very important [5].

The table below makes a comparison between advantages and disadvantages of in vivo tests;

Advantages	**Disadvantages**
-Intact biologic system to respond to a material -Provide important bridge between in vitro environment & clinical use of material	-More expensive & difficult to control -Time consuming -Ethical concerns

II.2.3 Clinical tests

The clinical test is, by definition, the most relevant biocompatibility test. These tests are essentially clinical trials of a material in which the material is placed into a human volunteer in its final intended use. In a controlled clinical study, test and control materials are examined at the same time. Controlled clinical studies possess a higher level of significance/evidence compared with studies in which only one material is investigated.

Biocompatibility data from clinical studies are naturally of special interest for the clinician, since the examination was done on the target group of this material (patients). But this should not conceal the fact that clinical studies reveal limitations, too. An uncritical transfer of such results to patients in daily practice may result in problems, for instance, if data are not based on a blinded study. Therefore, at least treatment and subsequent assessment should be done by different persons. Many unwanted reactions appear only after chronic exposure. But clinical studies – in particular those with new materials – are frequently limited to comparatively short periods of time (some are only 6 months). In addition, only a small and often strictly selected group of patients is included in the study, for instance in a university hospital. The clinical studies are also expensive, time-consuming, extraordinarily difficult to control its variables, difficult to interpret and may be legally and ethically complex. Clinical tests are done only if satisfactory results are obtained in the in vitro and animal experiments [5].

The table below makes a comparison between advantages and disadvantages of clinical tests;

Advantages	**Disadvantages**
-Material placed in an environment clinically relevant to its use in clinical practice	-Extremely complex & difficult to perform -Exceptionally expensive & very time consuming -Ethical concerns

II.3 Biocompatibility testingprocesses:

Biocompatibility testing includes several specialized processes; some of them are explaining below:

II.3.1. Toxicology:

A biomaterial should not be toxic, unless it is specifically engineered for such requirements (for example, a “smart bomb” drug delivery system that targets cancer cells and destroys them). Since the nontoxic requirement is the norm, toxicology for biomaterials has evolved into a sophisticated science. It deals with the substances that migrate out of biomaterials. For example, for polymers, many low-molecular-weight “leachables” exhibit some level of physiologic activity and cell toxicity. It is reasonable to say that a biomaterial should not give off anything from its mass unless it is specifically designed to do so. Toxicology also deals with methods to evaluate how well this design criterion is met when a new biomaterial is under development [2].

II.3.2.Biocompatibility

Biocompatibility is determined as the ability of a material to co-exist and perform with a natural substance in a specific biological application. Since the material should be non toxic to perform with an appropriate host response, which having a biomaterial interface with human body are required to perform a particular physiological function such as that of stent, knee replacement or pacemaker. Biomaterials incorporated into medical devices are implanted into tissues and organs. Therefore, the key principles governing the structure of normal and abnormal cells, tissues and organs, the techniques by which the structure and function of normal and abnormal tissue are studied, and the fundamental mechanisms of disease processes are critical considerations.

Special processes are invoked when a material or device heals in the body. Injury to tissue will stimulate the well-defined inflammatory reaction sequence that leads to healing. Where a foreign body (e.g., an implant) is present in the wound site (surgical incision), the reaction sequence is referred to as the “foreign body reaction.”

The normal response of the body will be modulated because of the solid implant.Furthermore, this reaction will differ in intensity and duration depending upon the anatomical site involved. Asn understanding of how a foreign object alters the normal inflammatory reaction sequence remain an important concern [2].

II.3.3 Mechanical and Performance Requirements

An intraocular lens may go into the lens capsule or the anterior chamber of the eye. A hip joint will be implanted in bone across an articulating joint space. A heart valve will be sutured into cardiac muscle and will contact both soft tissue and blood.

A catheter may be placed in an artery, a vein or the urinary tract. Each of these sites challenges the biomedical device designer with special requirements for geometry, size, mechanical properties, and bioresponses.

Biomaterials and devices have mechanical and performance requirements that originate from the physical and /or electrochemical properties of the material. Such requirements vary in mechanical properties for example: Hip prosthesis must be strong and rigid, tendon material must be strong and flexible, heart valve leaflet must be flexible and tough, dialysis membrane must be strong and flexible, but not elastomeric, articular cartilage substitute must be soft and elastomeric. Then, mechanical performance varies based on a diverse range of requirements. Similarly the duration of contact also varies, for example: a catheter may be required for just 3 days; bone plate may fulfill its function in 6 months or longer, leaflet in a heart valve must flex 60 times per minute without tearing for the lifetime of the patient (realistically, at least for 10 or more years), hip joint must not fail under heavy loads for more than 10 years and so on. There are also other biophysical properties and other aspects of performance. The dialysis membrane has a specified permeability, the articular cup of the hip joint must have high lubricity, and the intraocular lens has clarity and refraction requirements. To meet these

requirements, design principles from physics, chemistry, mechanical engineering, chemical engineering, and materials science are to be accurately integrated **[2]**.

II.3.4. Regulation:

Patient care demands safe medical devices. To prevent inadequately tested devices and materials from coming on the market. Most nations of the world have medical device regulatory bodies. In addition the International Standards Organization (ISO) has introduced international standards for the world community. The costs to comply with the standards and to implement materials, biological, and clinical testing are enormous. As per regulatory requirement testing and other factors involved in biomaterials can be categorized as below [2]:

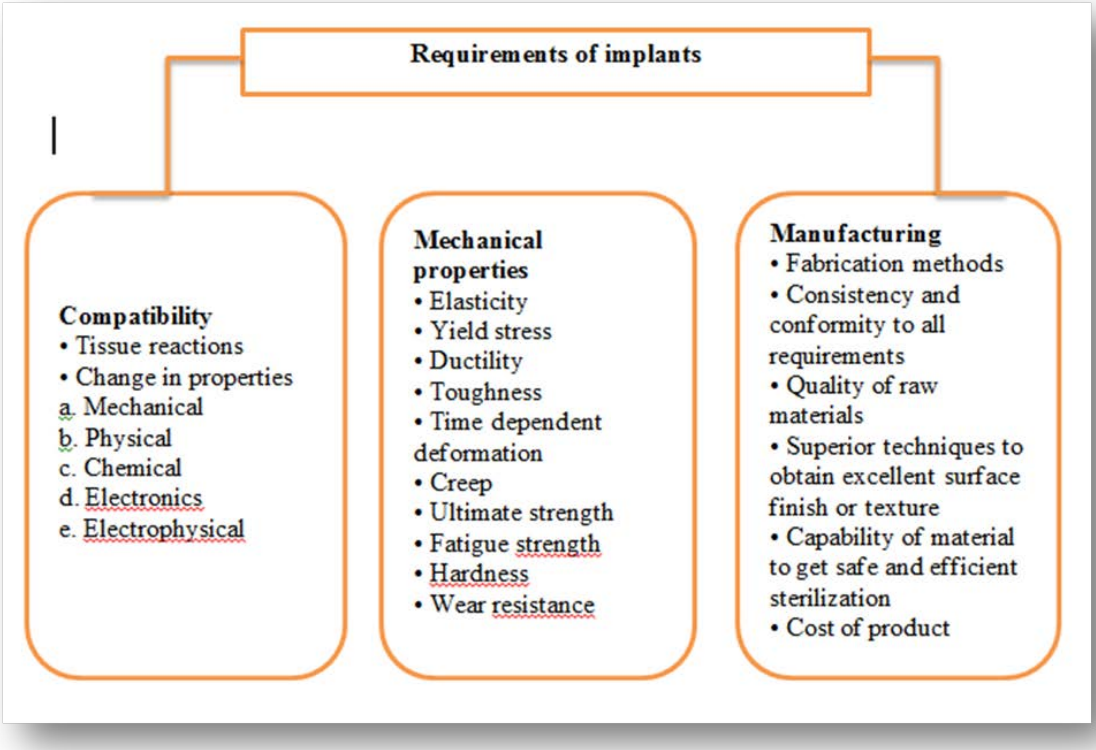

Table1.2: Requirements of Implants. [6]

II.4Standards for biomaterials and biocompatibility testing:

The starting point for understanding biocompatibility requirements is ISO Standard 10993, Biological Evaluation of Medical Devices. Part 1 of the standard is the Guidance on Selection of Tests, Part 2 covers animal welfare requirements, and Parts 3 through 19 are guidelines for specific test procedures or other testing related issues. (A list of the individual sections of ISO 10993 can be found in (Annexure A) and other relevant standards existed also.

The core of the ISO Standard is confirmation of the fitness of the device for its intended use. Although ISO develop policy and publishes standards related to conformity assessment, it does not perform conformity assessment activities.

ISO 17025 state the general requirement for the competence of testing and calibration laboratories. This is the main ISO standard used by testing and calibration laboratories (Annexure A) [2].

II.5Conclusion:

The purpose of the ISO Standard is to help our customers better understand biocompatibility and how establishes that a material or product is safe for skin contact applications.

After the comparison between in vivo and in vitro tests we conclude that only a combination of the two tests can provide an overview of the interaction of biomaterials with the host.

GENERAL CONCLUSION

As the number of available materials increases, it becomes more and more importantto be protected from unsuitable products or materials. This purpose is assured by the mean of biocompatibility tests.

For the biocompatibility of a material to be proved, it must be subjected to various studies ranging from in vitro assays to clinical trials and involving distinct areas such as pharmaceutics, biology, chemistry, and toxicology. The use of standardized tests allows better comparison between the results of different studies to clarify the behavior of the materials and their safety in relation to cells and tissues.

After the comparison realized in this work between the several methods of biocompatibility testing we found that:

- A combination of various in vitro and in vivo tests can provide an overview of the interaction of biomaterials with the host.

- The evaluation of biocompatibility is dependent not only on the tested biomaterial but also on the test method used. So clinicians need to be familiar with these methods.

ANNEXURE

ANNEXURE

Annexure A:

S.No	Standards	Title	Description
1	ISO 10993	Biocompatibility	This standard gives the basic guidelines of biocompatibility
2	ISO 10993-1:2009	Evaluation and testing in the risk of management process	-The general principle governing the biological evaluation of medical devices within a risk management process; -The general categorization of devices based on the nature and duration of their contact with the body; -The evaluation of exiting relevant data from all sources; -The identification of gaps in the available data set on the basis of a risk analysis; -The identification of additional data sets necessary to analyze the biological safety of the medical device; -The assessment of the biological safety of the medical device;
3	ISO 10993-2	Animal welfare requirements	The standard specifies the minimum requirements to be satisfied to ensure and demonstrate that proper provision has been made for the welfare of animals used in animal tests to assess the biocompatibility of materials used in medical devices.
4	ISO 10993-3	Tests for genotoxicity, carcinogenicity and reproductive toxicity	The standard specifies strategies for hazard identification and tests on medical devices for the following biological aspects: genotoxicity, carcinogenicity, and reproductive and developmental toxicity

5	ISO 10993-4	Selections of tests for interactions with blood	The standard provide general requirement for evaluation the intersections of medical devices with blood. It describes: -A classification of medical and dental devices that are intended for use in contact with blood, based on the intended use and duration of contact as defined in ISO 10993-1; -The fundamental principle governing the evaluation of the interaction of devices with blood; -The rationale for structured selection of tests according to specific categories, together with the principle and scientific basis of these tests. Detailed requirements for testing cannot be specified because of limitations in the knowledge and precision of tests for interactions of devices with blood. ISO 10993- 4:2002 describes biological evaluation in general terms and may not necessarily provide sufficient guidance for tests methods for a specific device.
6	ISO 10993-5	Tests for in vitro Cytocompatibility evaluation	The standard describes test methods to assess the in vitro Cytocompatibility evaluation of medical devices. These methods specify the incubation of cultures cells in contact with a device and/or extracts of a device either directly or through diffusion. These methods are designed to determine the biological response of mammalian cells in vitro using appropriate parameters.
7	ISO 10993-6	Tests for local effects after implantation	The standard specifies tests methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices. ISO 10993-6:2007 applies to materials

			that are: - Solid and non-biodegradable; - Degradable and/or reserbable; - Non-solid, such as porous materials, liquids, pastes and particulates. ISO 10993-6:2007 may also be applied to medical devices that are intended to be used topically in clinical indications where the surface or lining may have been breached, in order to evaluate local tissue responses.
8	ISO 10993-7	Ethylene oxide sterilization residuals	The standard specifies allowable limits for residual ethylene oxide (EO) and ethylene chlorohydrin (ECH) in individual EO-sterilized medical devices, procedures for the measurement of EO and ECH, and methods for determining compliance so that devices may be released. Additional background, including guidance and a flowchart showing how the standard is applied are also included in informative annexes. EO-sterilized devices that have no patient contact (e.g., in vitro diagnostic devices) are not covered by ISO 10993-7:2008.
9	ISO 10993-8	Selection of reference materials	The standard gives guidance on selection and qualification of reference materials for biological test (usually sent by the client).
10	ISO 10993-9	Framework for identification and quantification of potential degradation products	The standard provides general principle for the systematic evaluation of the potential and observed biodegradation of medical devices and for the design and performance of biodegradation studies. ISO 10993-9: 2008 consider both non-resorbable and resorbable materials
11	ISO 10993-10	Tests for irritation and delayed-type	The standard describes the procedure for the assessment of medical devices and

		hypersensitivity	their constituent materials with regard to their potential to produce irritation and skin sensitization. ISO 10993-10:2010 includes: -Pretest considerations for irritation in silico and in vitro methods for dermal exposure; -Details of in vivo (irritation and sensitization) test procedures; -Key factors for the interpretation of the results. Instructions are given for the preparation of materials specifically in relation to the above tests and several special irritation tests are described for application of medical devices in areas other than skin.
12	ISO 10993-11	Tests for systemic toxicity	The standard specifies requirements and gives guidance on procedures to be followed in the evaluation of the potential for medical device materials to cause adverse systemic reactions.
13	ISO 10993-12	Sample preparation and reference materials	The standard specifies requirements and gives on the procedures to be followed in the preparation of samples and the selection of reference materials for medical device testing in biological systems in accordance with one or more parts of ISO 10993. Specifically, ISO 10993-12:2012 addressers the following: -Test sample selection; -Selection of representative portions from a device; -Test sample preparation; -Experimental controls; -Selection of, and requirements for, reference materials;

			-Preparation of extracts. ISO 10993-12:2012 in not applicable to live cells, but can be relevant to the material or device components of combination products containing live cells.
14	ISO 10993-13	Identification and quantification of degradation products from polymeric medical devices	ISO 1099313:2010 provides general requirements for the design of tests in a simulated environment for identifying and quantifying degradation products from finished polymeric medical devices ready for clinical use.
15	ISO 10993-14	Identification and quantification of degradation products from ceramics	Biological evaluation of medical devices Part 14: Identification and quantification of degradation products from ceramics
16	ISO 10993-15	Identification and quantification of degradation products from metals and alloys	Biological evaluation of medical devices Part 15: Identification and quantification of degradation products from metals and alloys
17	ISO 10993-16	Toxicokinetic study design for degradation products and leachables	Biological evaluation of medical devices Part 16: Toxicokinetic study design for degradation products and Leachables
18	ISO 10993-17	Establishment of allowable limits for leachable substances	Biological evaluation of medical devices Part 17: Establishment of allowable limits for leachable substances
19	ISO 10993-18	Chemical characterization of materials	Biological evaluation of medical devices Part 18: Chemical characterization of materials
20	ISO 10993-19	Physicochemical, morphological and topographical characterization of materials	Physicochemical, Morphological and topographical characterization of materials Biological evaluation of medical devices Part 19: Physico-chemical, morphological and topographical characterization of materials

21	ISO 10993-20	Principles and methods for immunotoxicology testing of medical devices	Biological evaluation of medical devices Part 20: Principles and methods for immunotoxicology testing of medical devices

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