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Impact of the Surface Properties of Medical Implants on Host Response

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ABSTRACT: The aim of this literature review is to analyze various implant properties and their association with host response and biocompatibility. Based on the electronic search of journals, books published in pubmed, google scholar and Elsevier, necessary data was collected andanalyzed. The various properties of biomaterial and their modifications to make them acceptable by the body has been discussed. Various materials with different surface characteristics, design and modification techniques are analyzed. The overall study revealed, implant material need to have different properties based on the site of implant and intended use. Mostly metals with smooth surface showed good biocompatibility in dental and bone implants while ceramics and polymers which are highly porous had better results in vascular and implant site with more soft tissues.

KEYWORDS: Biomaterial, Biocompatibility, Surface properties, Porosity

INTRODUCTION

Medical implants include a wide range of man-made devices, tissues or products that are placed in the human body with an aim to support the process of healing or to replace the damaged organ or tissue.(1) Various attempts to replace the damaged or missing tissue with anartificial implant have been made and it has been a great challenge for biomedical engineers to develop implant that mimic the missing or replaced tissue. The major challenge in acquiring a perfect material to replace the missing tissue is to understand the interaction of that material with biological tissue at cellular level.(2) Material implanted in the body must match the mechanical properties of the replaced tissue and also should be biocompatible. Generally the property of any implant is categorized as bulk property and the surface property. The bulk property determines the mechanical strength while the surface properties determines the cellular response and biocompatibility of the implant.(3)

As the implant is placed inside the body various molecules and cells are activated within few seconds. This response includes the sequence of activities such as water absorption, protein adsorption, cellular adhesion, cellular growth and proliferation and the inflammatory response activation. But all these events depend on the type of the implant and the surface characterizations of the implant.(4) Although these events are mandatory for all implants their extent and activation time alters with various type of surface properties of the implant and the site of the implant. All these reactions occurring after the implant placement is termed as 'host response'.(5)

The major surface properties that affects the host response include the roughness, porosity, surface chemistry, wettability, surface energy, functional group, physiochemical components (surface charge). In order to achieve the desired host response, the surface properties can be modified as per the requirement of the implant region. Methods of surface modification can be either addition of some particles to the surface (increasing roughness, surface coating to alter the wettability, functional characterization reversal) or the removal of the surface material (increasing bumps).

With the modification techniques nanostructured materials can be designed that either attract the right proteins to the defect site from within the body, or that contain the proteins and deliver them directly to the site at a controlled rate. It is important to be able to study how proteins attach to materials and how cells respond to macro porous and nanostructured surfaces. This is only possible by combining advanced materials characterization techniques such as Scanning electron microscope (SEM), x-ray photoelectron spectroscopy (XPS), Contact Angle (CA), sessile drop and various other surface characterization techniques.(6) The different modification techniques has help to achieve the biocompatible implant with adhesion of favorable molecules and required cellular adhesion, growth and proliferation rate.

MATERIALS AND METHODS

In accordance to this study design, various papers published on the effect of surface properties and the experimental setup used in

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those articles were analyzed. The analysis for different surface properties have been done based on the articles available from pubmed, google scholar and Elsevier.

SURFACE TOPOGRAPHY

The implant surface has been described to be one of the six factors that influence the wound healing at the implantation site and ultimately affect the Osseo-integration. The smooth is used to describe the abutments while terms such as minimally rough (0.5 to 1 µm), intermediately rough (1 to 2µm) and rough (2 to 3µm) for the implant surface. But the majority of the research do not describe the roughness in such manner. Based on the average surface roughness (Sa), the implant surface is said to be smooth if $Sa \leq 1\mu m$ and is said to be rough if $Sa \geq 1\mu m$.(7) Nanoscale surface topography of the implant is known to play an important role in interaction with the biological surrounding. The overall effect of surface roughness on protein adsorption have not been described clearly till now. The amount of fibrinogen absorbed on the oxidized silicon nano-grooves was unaltered as compared to the smooth silicon.(8) On the similar research, Cai et al concluded that the amount of fibrinogen absorbed onto the nano rough surface was not much different than the smooth control titanium when treated for the increase in surface area with increasing the surface roughness.(9) Quartz crystal microbalance with dissipation monitoring (QCM-D) is a sensitive analysis of the amount of protein adhered to any implant surface. QCM-D studies of the fibrinogen adsorption to the colloidal nanostructures showed that there is lesser adsorption of fibrinogen in the surface with roughness of 11nm compared to the surface roughness of 7nm or 8nm.(10) So even for a similar type of protein there is no any general trend in the adsorption related to the surface roughness. In support to the altered adsorption of protein based on the roughness, the amount of fibronectin adsorbed to an acid graven titanium surface with average surface roughness of 70nm was found to increase as compared to the flat titanium surface.(11) Similarly, the rough titanium surface with the nano pit diameter of 40nm and depth 10nm showed greater platelets adhesion on it while there was not significant amount of platelets adhesion the smooth titanium implant. Another paper concluded that deposition of plaque on a smooth surfaced (Sa=0.1µm) titanium dental implant was ¼ of the total implant region while the rough surface (Sa=2µm) was completely covered by plaque.(12) Silicon substrate with nano rough surface with sharp tip ad the pit depth of 500-500nm formed a layer of short filopodia only on the tip of the pits while there were long filopodia developed from tip to the bottom of the pit when the pit depth was decreased to 50-100nm. Similar study using the silicon substrate with nano-pits of height 50- 100nm showed better adherence of fibroblast upto the bottom of the pits with regular patterns of cell adherence but the fibroblast failed to reach upto the bottom and the cell adherence did not conform the surface of the implant when the nano-pits of height of 500nm were used.

The alteration in the form of protein is also aided by the surface roughness. Study of stochastically rough surfaces revealed that the grooves size nearly equal to the size of protein do not cause any alteration in protein structure while the groves size much larger or much smaller than the size of the adhered proteins is significantly altered. Webster et al confirmed this effect as shape and size of vitronectin changed when coated over the nano-rough alumina surface, which was not observed when coated over the smooth alumina. Here the average surface roughness of alumina was 32nm and the size of the vitronectin molecule was 15nm.(7) Similar findings were observed from atomic force microscopy for fibrinogen of characteristic dimension of 46nm when interacted with platinum surface with mean roughness of 9nm.(13) The inflammatory response also increases with the increase in roughness of the surface. Greater the roughness, the available area for the cells to adhere is also greater. So, the number of cells adhering to the surface increases thus enhancing the inflammatory response. Parker and coworker confirmed the presence of higher number of inflammatory cells at the implant-tissue interface of surface with random roughness than the surface with continuous microgroove. They also reported the higher concentration of cytokines at those interfaces.(14)

Cellular response of various osteoblasts, fibroblasts, macrophages, neural cells and endothelial cells for different surface topographies are summarized.

SURFACE POROSITY

Porosity is defined as the percentage of void spaces in the material surface. The porosity and the pore size are the important factors that needs to be considered while preparing the scaffold as they play an important role in bone formation in vitro as well as in vivo. The porosity not only alters the cellular integration but also determines the mechanical strength of the implant so the pore size must be selected satisfying both the criterions.(15)

Different procedures can be applied for the calculation of porosity of the implant, one of the most used technique being gravimetry,

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which gives the porosity (P) from the equation,

$$P = 1 - \frac{\rho material}{\rho scaffold}$$

Where, ρmaterial is the density of the material of which the scaffold is fabricated and ρscaffold is the apparent density of the scaffold measured by dividing the weight by the volume of the scaffold.

Kuboki et al showed the necessity of porosity in the scaffold using a rat ectopic model and solid and porous hydroxyapatite for BMP-2 delivery. The results showed that no new bones were formed on the solid surface whereas in the porous scaffold direct osteogenesis was observed.(16) Additional support to this is provided from the porous coated and non-coated metal surfaces. In sheep tibial implant the titanium treated with sintered titanium beads increased porosity and enhanced the bone ingrowth and quick fracture healing while the same implant treated with hydroxyapatite did not provide porosity to the surface and hence resulted delayed fracture healing.

The pore size too plays an important role in wound healing. Hulbert et al. implanted the calcium aluminate cylindrical pallets in dog's femur. The porosity of the implant being 46% in all cases, the size of the pores was varied and studied. The larger pores (≈200µm) showed substantial bone ingrowth, the medium pores (75-100µm) resulted the ingrowth of unmineralised bone while the smaller pore (10-40µm) resulted the proliferation of fibrous tissue only.(17) So, they concluded that the minimum pore size for proper bone growth must be ≈100µm. However, the laser perforation technique using titanium implant contradicted with the conclusion of Hulbert et al. Titanium implant of four different pore sizes (50µm,75µm,100µm,125µm) were implanted in rabbit's femoral defects under non-load-bearing condition, whose results showed no any considerable difference in bone ingrowth for all the pore size.(16)

Some of the paper concluded that surface roughness is equally important along with the porosity for the osteogenesis. This was illustrated by the better osseointegration on the acid etched titanium implant (highest surface roughness) as compared to the grit blasted and fiber mesh coatings with average pore size of 400µm when implanted in rabbit's femur.(16) The hydroxyapatite ceramic rods with average pore size 200µm with smooth and dense pore walls failed to induce ectopic bone growth in dog while the same material with average pore size 400µm with rough and porous pore walls resulted in better bone growth.(18)

WETTABILITY

The wettability of the biomaterial determines the biological cascade of reactions at the implant –tissue interface. The wettability of any clinical implant can be analyzed using contact angle (CA) measurement technique. Different approaches can be used for the determining the CA, these include sessile drop method, captive bubble method and Wilhelmy plate method. During the measurement of CA, the shape of the drop is determined by the surface tension of the liquid, surface tension of the solid and the surface energy at the solid liquid interface.(19) This relation can be illustrated from the following figure.

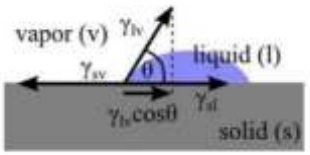

Figure 1: Schematic diagram for contact angle measurement

Based on the contact angle and the surface energy relation the surfaces can be categorized into the following four classes:

1. The contact angle ranges between 0^0 and 90^0, then the surface is said to have high wettability (hydrophilic).
2. The contact angle is 0^0, then there is spreading of water over the surface known as complete wetting (hydrophilic).
3. The contact angle ranges between 90^0 and 180^0, then the surface is said to have low wettability (hydrophobic).
4. If the contact angle is 180^0, then there is complete repulsion of water by the surface known as non-wetting (hydrophobic).

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So, based on the contact angle the surfaces can be either hydrophilic (water absorbents) or hydrophobic (water repellent). Now whether hydrophilic surfaces are better biocompatible or the hydrophobic surfaces adopt better in biological environment is challenging task. Some research suggest that hydrophilic surface show faster wound healing and better cellular and molecular adhesion while in contradiction to this other suggest that the surface must be hydrophobic for better cellular response.

In a study of wetting behavior of dental implant, Rupp et al concluded that the hydrophilic surface facilitates the initial interaction between the body fluid and the implant surface which is important phenomenon for wound healing and Osseo integration. Similar study on the hydrophilic titanium implant on mice showed high level of interleukin (IL-4, IL-10) activation around the implant.(20) The increased release of anti-inflammatory protein result in release of M2-activated macrophages which are responsible for the process of wound healing.(21) Similarly, it was found that the silicon wafer silanized with 0.1% DDS, a hydrophilic surface with water contact angle 70^0, yielded the higher cellular adhesion and protein adsorption. While the polyethylene surface with the contact angle 55^0 prepared by corona discharge gave the maximum cell adhesion.(22)

The chitosan surface with contact angle of $\approx 89^0$ when treated with N-stearoyl chloride and succinic anhydride altered the contact angle to $\approx 104^0$ (hydrophobic surface) and $\approx 56^0$ (hydrophilic). The behavior of protein adsorption to these surface was analyzed by treating them with the Bovine Serum Albumin (BSA) and lysosomes. The amount of adsorbed protein was measured with bicinchoninic acid (BCA) assay. It was found that the BSA adsorption to the N-stearoylchitosan was $4.86\mu g/cm^2$ and lysosome adsorption was $7.87\mu g/cm^2$ while for the N-succinylchitosan surface BSA adsorption was $7.3\mu g/cm^2$ and that for lysosome was $1.63\mu g/cm^2$.(23) So, it concluded that the amount of protein adsorption to the hydrophobic surface was much higher than that for the hydrophilic surface. Similar results were obtained when the Teflon (TFE), Polyvinyl chloride (PVC), Nylon-6,6 (N-6,6), Siliconized glass were treated with the human serum albumin (HSA), fibrinogen and immunoglobulin (Ig). The protein adsorption was higher for the relatively hydrophobic surface. But the exceptional case for siliconized glass was observed. Siliconized glass though being more hydrophobic than Teflon resulted lesser extent of protein adsorption. The adsorption sequence for the proteins analyzed was found to be Fibrinogen > IgG > HSA > BSA.(24,25)

SURFACE FUNCTIONAL GROUP

The surface functional group comprise of the chemical composition of the surface. The functional groups widely used in the biomedical implants includes the –OH, -COOH, -CH3 groups. The functional groups rich in oxygen such as –C–O– (hydroxyl or ether), -C=O (ketone or aldehyde), O=C-O- (carboxylic acid or ester) increased the hydrophilicity of the surface. The increased hydrophilicity hence increasing the amount of protein adsorption.(24) Adhesive proteins effectively replaced albumin and modified surfaces suitable for cell adhesion on mixed SAMs with water contact angles less than 50^0 for CH3/OH, and less than 90^0 for CH3/COOH and CH3/NH2 mixed SAMs. However, albumin adsorbed to highly hydrophobic surface resisted the displacement and resulted in lower cellular adherence. So it was concluded that the albumin adsorbed to hydrophilic SAMs allowed better cellular adhesion thus causing faster wound healing.

Silicon wafers coated with alkylsilanes by SAMs were used to create the substrate of different functional groups. The functional groups used as substrate were epoxide, COOH, CH_3, NH_2. These substrates were treated with extra cellular matrix (ECM) to quantify their adherence to fibronectin. After placing the substrate under controlled environment in the ECM for 30 minutes the order of fibronectin adsorption was found as OH < COOH < NH_2 < CH_3. Further study done on linking of fibronectin with integrin (alpha-5beta-1) concluded that the binding capacity is the function of SAM. The binding trend COOH≈CH_3≈NH_2 < OH proved that fibronectin adsorbed to hydrophilic substrate is more likely to bind with the integrin.(24)

PHYSIOCHEMICAL PROPERTIES

The physiochemical properties of the implant include their surface charge, energy and its chemical behavior. The study of physiochemical response varies with the type of charge the surface consists of. The response for the anodic (positively charged) surface and that for the negatively charged surface is completely different in the body.(26)

The negatively charged surface of titanium, silicon, polyurethane promote protein (Fibrinogen) adsorption in early phase of

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implantation and in the later stage fibrinogen are replaced by High molecular weight kininogens (HMWK). The process of replacement of fibrinogen with HMWK is termed as "Vroman effect'.(27) On the other hand, the hydrophobic and positively charged implant show no or negligible protein adsorption.(4)

To study the effect of physiochemical properties on cellular response the anodized titanium implant was used. The pure titanium disk was treated with the aqueous solution of (NH4)2O·5B2O3, (NH4)2SO4, (NH4)3PO4, which yielded an anionic concentration in the titanium implants with the pH values 4.5, 5.4, 7.6 respectively. In vitro test of these samples resulted in lower contaminations on the implant surface due to carbon adsorption. This resulted in better hydrophilic surface with lower contact angle. Also the cellular distribution on the anionic surfaces were more and widely distributed than that in the normal untreated titanium surface.(28)

CONCLUSION

Hence from the study of various research papers and journal, it can be concluded that the surface properties of the implants drive the inflammatory response. Though the implant as a whole is placed inside the human body, only its surface comes in contact to the body fluid (ECM, Blood). These body fluid consisting of various proteins and defensive cells recognize the implant as foreign invader. So, the cells like neutrophils, macrophages, monocytes and other inflammatory cells are activated. With the activation of these cells inflammation (redness, swelling, pain, loss in function, hotness) can be seen around the implant site. Since inflammation is the first step of wound healing, now the tissues around the implant site starts to heal the damaged cells or organs.

The rate and extent of the inflammatory response is guided by the surface characteristics. The type of surface present in the implant determines the variety of proteins that are adsorbed and the cell types that adhere to the injury site and their activation. These surface characters include the parameters such as roughness, porosity, wettability, functional groups, and physiochemical surface properties. With even a slight variation in any of the above mentioned parameters the cellular response and the wound healing mechanism varies to a great extent.

The roughness of the implant surface caused higher extent of protein adsorption on the surface. This also increased the rate of unfolding of the proteins which helped in the quick formation of biofilms around the implant that caused lower rate of rejection or the infection in the implant site. Although very rough surface may different response than the expected result of higher cellular adherence, as it may alter the rhythm of flow of blood and other body fluid. Similarly, the porosity of the implant allows better proliferation of the cells adhered on the surface. Above results concluded that the porous surface provides better bone ingrowth and Osseo integration than that for the implant of same material with lower level of porosity. There has not been a specific effect of wettability on cellular response. In some cases, the hydrophilic surfaces illustrated better protein adsorption and faster healing while in some cases the hydrophobic surfaces proved to be better. The wettability of the implant is site and protein dependent. Some proteins adsorption is enhanced in hydrophilic surface while hydrophobic surface is needed for some variety of proteins.

So from this review we can conclude that there is no any specific response to any of the surface properties of the implant. The host response widely varies with the implant site. So any implant must be characterized specifically for the specific implant site.

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