Published in final edited form as:
*Curr Top Med Chem*. 2008 ; 8(4): 270–280.

# SURFACE CHEMISTRY INFLUENCE IMPLANT BIOCOMPATIBILITY

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## Abstract

Implantable medical devices are increasingly important in the practice of modern medicine. Unfortunately, almost all medical devices suffer to a different extent from adverse reactions, including inflammation, fibrosis, thrombosis and infection. To improve the safety and function of many types of medical implants, a major need exists for development of materials that evoked desired tissue responses. Because implant-associated protein adsorption and conformational changes thereafter have been shown to promote immune reactions, rigorous research efforts have been emphasized on the engineering of surface property (physical and chemical characteristics) to reduce protein adsorption and cell interactions and subsequently improve implant biocompatibility. This brief review is aimed to summarize the past efforts and our recent knowledge about the influence of surface functionality on protein:cell:biomaterial interactions. It is our belief that detailed understandings of bioactivity of surface functionality provide an easy, economic, and specific approach for the future rational design of implantable medical devices with desired tissue reactivity and, hopefully, wound healing capability.

### Keywords

functional groups; biomaterials; biocompatibility; proteins; cells

## Introduction

An aging population, coupled with continued medical advances, has driven the increased utilization of medical implants and demand for new medical devices in the years ahead. Unfortunately, blood-contact medical devices may trigger a variety of iatrogenic reactions, including surface mediated thrombosis, complement activation, and device-centered infection [1–2]. There are also significant adverse reactions involved with tissue-contact medical implants. These include (1) local implant-associated inflammation, including temporomandibular [3–4] and other joint related implants [4–8], (2) phagocyte-mediated oxidation and ”stress cracking” of the implanted materials [9–10], (3) implant degradation and tissue fibrosis surrounding mammary prosthesis and many other types of implants [11–13], (4) poor integration of fibrotic tissues artificial ligaments using artificial (Woven Dacron®) or biological (such as collagen fiber) materials [14–16], (5) the formation of thick fibrotic capsules surrounding implants contribute to the failure of many types of devices, such as biosensors [17–18], joint implants [19–20], breast implants [21–22], encapsulated tissues/cells [23–25], drug delivery systems [26], and eye implants [27–29]. Unfortunately,

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the mechanisms governing biomaterial-mediated tissue responses and the possible role of surface properties affecting such responses are mostly unclear.

At first glance, these inflammatory responses to inert, non-immunogenic, and non-toxic materials are difficult to understand. We and many others have found that, shortly after implantation (minutes to hours), biomaterial implants are covered with a layer of host proteins prior to the accumulation of inflammatory cells [13,30–32]. Thus, it is generally believed that the composition and state of these adsorbed proteins play a pivotal role in subsequent host coagulation and immune reactions [13,30,32–33]. Therefore, the ability to control both (1) the amount and composition of host protein adhering to a surface, as well as (2) the degree of adsorbed proteins' conformational changes (exposing inflammatory epitopes), is the primary goal of modified biomaterial surfaces.

Many surface modification techniques have been developed during the past twenty years. The modifications of surface wettablility, hydrophobicity and surface charges have been shown to alter the extent of protein adsorption. Overall, increasing surface hyrdophilicity has been shown to improve the biocompatibility of the medical devices at least in vitro. Recently more effort has been placed on creating surfaces uniformly coated with different chemical functionalities in an attempt to determine more specifically what surface chemical entities, beyond hydrophobic or hydrophilic characteristics, can improve biomaterial compatibility. Though promising in vitro results have been widely documented in literature, surface functionalities have shown little success in exerting a significant influence on tissue responses in vivo. The purpose of this article is to review past and recent work in the area of surface group functional modification of biomaterials, and what effect these groups have on protein and cellular interactions with surface functionalities.

## How does biomaterial trigger foreign body reactions?

Commonly used biomaterials are mostly hydrophobic and have high affinity to a wide variety of proteins. Shortly after implantation, biomaterials are covered with a layer of plasma proteins, predominately albumin, fibrinogen, IgG, fibronectin, and von Willebrand factor [30,32–35]. These adsorbed proteins, possibly via hydrophobic interactions, tend to assume an altered conformation and to expose hydrophobic domains which become tightly adherent to hydrophobic biomaterial surfaces [36–41]. Such surface-mediated conformational changes of adsorbed proteins have been demonstrated by several different techniques including resistance to sodium dodecyl sulfate (SDS) elution [42–43], scanning angle reflectometry [12,44–45], 'scanning force' microscopy [46–48] and attenuated total reflectance Fourier transform infrared spectroscopy [37,49]. The conformational changes of adsorbed protein are thought to be responsible for initiating adverse reactions such as inflammation, coagulation, and foreign body response [39,50–52]. It is believed that these protein:biomaterial interactions prompt the exposure of hidden protein structures and sequences that serve as receptor sites for inflammatory cells, which then initiate the foreign body reactions (Figure 1).

Although the influence of material surface properties on subsequent tissue responses is not totally understood, it is widely documented that polymer surface properties can affect the amounts and types of bound proteins, as well as the conformation, orientation or binding strength of the adsorbed protein [12,31,38,53–54]. Adsorbed proteins can either retain a structure close to that in solution or may conformationally adjust in response to local environments [55]. This time-dependent conformational readjustment is generally believed to affect the cellular responses and subsequent tissue reactions [12,31,38,53]. In the case of fibrinogen, the resistance to SDS elution has been correlated with slow, progressive changes in state of the surface protein on biomedical polymers such as polyetherurethanes [36]. After polyethylene terephthalate (PET) film has been incubated with fibrinogen for 4 hours, more

than 60% of the protein is resistant to SDS elution [56]. Albumin and IgG behave similarly [57]. A recent study employing grazing angle infrared analysis was conducted to determine the interactions of fibrinogen and albumin adsorption onto hydrophobic and hydrophilic surfaces. Results showed within 1 hour of fibrinogen adsorption, changes in secondary structure (amide I band) could be seen suggesting that changes in fibrinogen structure were due to protein-surface interactions [58–59]. The degree of secondary structure alteration was shown to be dependent on the properties of the surface (hydrophilic versus hydrophobic) [60]. It has been shown that, upon adsorption to plastic tissue culture surfaces (which are not precisely analogous to most hydrophobic implant surfaces), fibrinogen changes conformation and exposes multiple receptor-induced binding sites (RIBS). Two of these RIBS epitopes have been localized to residues gamma112–119 (recognized by mAb 9F9) and Aα 95–98 (RGDF; recognized by mAb 155B16). Please see Figure 2 for the locations of some of these epitopes which are known to interact with immune cells. A similar exposure of these RIBS epitopes and fibrin-specific epitopes may also occur upon adsorption and conformational change of fibrinogen on implantable biomaterials [61–63].

Taken together, these findings indicate that upon interaction with implant surfaces, fibrinogen (i) becomes progressively more adherent, (ii) shows signs of changes in conformation (?denaturation?), particularly involving the D domain, and (iii) exposes previously occult sequences (e.g., RIBS, P1 and P2). Based on these earlier observations, it is likely that material properties can be modified to reduce the extent of neo-epitopes' exposure and subsequent cell and tissue responses.

## Influence of surface chemistry on biocompatibility

**Surface modification techniques—**In the search for "perfect" surfaces, material scientists have been modifying surface characteristics by a plethora of techniques including physical modifications, chemical modifications, and radiation [64–65]. In general, the modification of material surface properties (including chemistry, wettability, domain composition and morphology) has been shown to influence protein adsorption and subsequent cellular responses to biomaterials *in vitro.* Unfortunately, due to the lack of well-defined surfaces (that differ only in one or two properties) and well-characterized animal implantation models, many early studies have produced little insight into the influence of surface properties on the pathogenesis of foreign body reaction. For creating homogenous and well-defined surfaces, self-assembled monolayer techniques, chemical graft modification, and plasma medication techniques have been developed and used in many recent studies. Each method has its unique benefits and drawbacks.

Chemical graft modification has been employed to chemically immobilize compounds onto the surface of a biomaterial. This method usually involves covalent conjugation of either a protein or monomer to the surface to alter surface chemistry, avoiding drawbacks of protein/monomer detachment by providing long-term stability. The process involves activating the surface with reactive groups followed by grafting the desired functionality to the surface. Many different methods fall into this category and differ only by the way in which the surface is activated, including chemical reactions, UV, radiation exposure, plasma, and ozone exposure [66]. Chemical grafting has been used to mount heparin and PEO to surfaces to increase blood compatibility. Drawbacks, especially when grafting proteins, include the loss of protein mobility on the surface, and possibly, presentation of the protein in an unfamiliar conformation on the surface. Toxic monomer residues may also be left in the grafted surface. Some issues can be addressed by physical adsorption techniques, which usually involve dip coating a material to form a film with desired properties on the surface. While physical adsorption may help reduce toxic monomer residues, issues with binding between the materials arise as well as instability of immobilization when using proteins.

Addressing the drawbacks in chemical and physical modification, self-assembled monolayers (SAMs) were developed as a method to more precisely control the density and conformation of a single or multiple specific functional groups on a surface. The general process of producing SAMs is to first activate the bulk material surface, then graft polymerize onto the activated surface. SAMs can provide flat and chemically well defined surfaces [67], with additional benefits of a surface near thermodynamic equilibrium with closely packed, well-ordered functionalities on the surface [66]. SAMs tend to provide functionalities with control over pattern and densities. Selection of terminal group can also provide a site for further functionalization of the coated surface. Surface functionality via SAMs have been used to investigate in vivo inflammatory and foreign body responses of implanted biomaterials [68–69] as well as many other processes, giving insight into how a particular functional group effects a particular process. However, SAMs are limited to gold-coated or silver coated surfaces.

An increasing popular method of altering surface functionality of biomaterials is plasma modification. Plasma, which can be regarded as the fourth state of matter, is composed of highly excited atomic, molecular, ionic, and radical species. It is typically obtained when gases are excited into energetic states by radio frequency, microwave, or electrons from a hot filament discharge [70]. The process provides an economical and effective way to infer functionality to a surface, and is compatible with most materials currently being investigated in medicine including metals [71], polymers [72], and ceramics [73]. One of the great benefits of plasma modification is the ability to uniformly modify surfaces regardless of geometry, allowing for modification of micro and nanoparticles [74], films [72], or 3D components required in tissue engineering and artificial organs [75]. In addition, the use of pulsed plasmas, *in lieu* of the conventional continuous-wave operational mode, provides an exceptionally high level of film chemistry controllability during the coating process [76–80].

An increasing number of chemical functionalities have been placed on implant surfaces. The influence of these functionalities on protein adsorption, cellular and tissue reactions has also been investigated. Highlighted in the next section, is a summation of the recent understanding of surface chemistry as related to protein adsorption, cellular and tissue reactions.

**Effects of Surface Chemistry on Protein:Surface Interactions—**Surface properties affect the extent of protein adsorption, denaturation, and epitope exposure (specifically γ190–202 [P1] and γ377–395 [P2]) [38,81]. To reduce unwanted protein:surface interactions, intensive research efforts have been placed on engineering surfaces with uniform functional groups to “repel” protein. The typical characteristics of protein-resistant functional groups include: hydrophilic nature, presence of hydrogen bond acceptors, no hydrogen bond donors, and neutral charge [82], though there appear to be exceptions. It is generally believed that the identification of protein resistant functional coating can help to tailor material surfaces to control protein adhesion and their interactions following adsorption, and can thus enhance biocompatibility.

The effect of surface functionality on fibrinogen adsorption has been investigated vigorously in recent years [83–86]. Computer simulations have show that water is essentially able to successfully compete with fibrinogen over adsorption to hydroxyl coated surfaces, reaffirming results indicating that fibrinogen binds tightly to hydroxyl (-OH) surfaces [84]. It has also been suggested that an optimum concentration of -OH groups can enhance the affinity for albumin binding over fibrinogen [86]. Fibrinogen was however able to hydrophobically interact with methyl functionalized surfaces, in agreement with previous studies that suggest abundant adsorption of fibrinogen to methyl ($-CH_3$) functionalized surfaces [85]. Amine ($-NH_2$) surfaces were able to form hydrogen bonds with fibrinogen,

tethering it to the amine functionalized surface. In the case of carboxyl (-COOH) groups, fibrinogen was not able to out-compete water to interact with the carboxyl surface [81]. A similar result was also seen in another investigation in which positive, negative, hydrophobic, and hydrophilic surfaces were adsorbed with equivalent amounts of fibrinogen. Negatively charged surfaces, such as -COOH, formed little to no fibrin compared to other surfaces [83]. Further research involving mixed functionalities as well as increased in vivo investigations are needed to develop a more comprehensive understanding of surface functionality and protein interactions.

To determine the possible role(s) of functional group density in modulating protein:biomaterial interactions, we have generated surfaces bearing different densities of –OH and $–NH_2$ functional groups using Radio Frequency Glow Discharge plasma polymerization technique. As expected, increasing the density of surface -OH and $-NH_2$ improves surface hydrophilicity. In the case of surface:fibrinogen interactions, -OH bearing surfaces retained progressively less protein, whereas the $-NH_2$ surfaces adsorbed progressively more protein as the surface density of -OH and $-NH_2$ groups increased, respectively. This data confirms that the amount of spontaneously adsorbed protein is a function of surface chemistry. Subsequent studies have been done to determine whether the species and density of surface functional groups affect protein conformational changes (also called as denaturation). Our results confirm that even these modest changes in surface amine density strongly influence fibrinogen adsorption and P1/P2 epitope exposure.

Surface chemistry of biomaterials has also been shown to participate in complement cascades through both the classical and alternative pathway, leading to the recruitment and activation of phagocytes and the adherence and activation of leukocytes [12]. This was first identified in hemodialysis membranes [87] and activation was shown to occur on surface with many different functionalities. Results have suggested that IgG undergoes a more extensive denaturation on hydrophobic as opposed to hydrophilic surfaces, resulting in exposure of active sites which bind complement proteins [88]. It has also been suggested that the chemical functionalities themselves can covalently bind complement proteins, harboring them from regulatory proteins [89]. Hydroxyl (-OH) functionalized surfaces have been shown to selectively adsorb IgG from serum, ultimately leading to C3 deposition on the surface [90]. In contrast, surfaces functionalized with $-NH_2$ and -COOH showed only slight activation in comparison to -OH functionalized surfaces [69]. In vivo studies using SAMs with -OH functionality showed increased leukocyte response [91]. This process is composed of at least three continuous steps- the surface –OH-mediated complement activation, complement fragment C5a-triggered leukocyte activation, and leukocyte adhesion via binding to surface-bound complement fragment C3b [92].

## Influence of surface functional groups on cellular responses

Substantial research efforts have been placed on studying the influence of surface functionality in the cellular response to biomaterials. Surface functional groups can influence cell growth [93–94], likely due to the fact that surface chemical functionality affects adsorbed protein and subsequent protein:cell interactions. In general, hydrophilic functionality provides low interfacial free energy resulting in reduced protein adsorption, cell adhesion, and blood compatibility [54,95]. It is well established that proteins tend to bind to hydrophilic surfaces in a lower amount and less tightly than to hydrophobic surfaces [96–98]. Such reduction of protein adsorption affects subsequent cellular responses. For example, in an interpenetrating network study, it was seen that increasing hydrophilic monomers lead to a decrease in fibrinogen adsorption and an increase in albumin adsorption [99], as well as a gradual decrease in platelet adhesion [100]. Decreasing platelet adhesion has also been attributed to coatings that are non-ionic and neutral [101–102]. It has also been suggested that hydrophilic surfaces provide significant inhibition of leukocyte adhesion and

macrophage fusion [103], and may result in decreased cytokine secretion and attenuated inflammatory reactions [104]. In contrast, the addition of $–CH_3$ groups, imparting hydrophobic functionality, has been shown to increase leukocyte adhesion [105]. The adhesion of leukocytes to solid surfaces depends on many different factors such as surface chemistry, charge or hydrophilicity, and protein adsorption [105].

Though the influence of surface hydrophobicity on protein adsorption and cellular responses is well documented, recent studies have revealed that surface functional groups also affect protein and cell behavior [106–109]. The most common functionalities investigated with relation to biomaterial interactions are the carboxyl (-COOH), hydroxyl (-OH), amino ($-NH_2$), and methyl ($-CH_3$) groups. Many recent reports have investigated the effect of these groups on the binding of adhesive proteins and subsequent cellular interactions. The results of their findings are summarized below.

**Carboxyl (-COOH) functional group-bearing surface—**Biomaterial-bearing -COOH displays a negative charged functionality on material surfaces [109]. Studies on protein adsorption have shown that fibronectin and albumin are more easily eluted from surfaces coated with –COOH [93]. This was tied into the fact that this functionality, compared to other common surface functional groups, adsorbed FN and albumin at ratio significantly different to other coatings. Surfaces with –COOH also have shown an increase in cell growth. A more recent study showed that this phenomenon is dependent upon the concentration of –COOH on the surface, as an increase in functional group density results in a higher negative charge on the surface, which was shown to inhibit cell growth [94]. Carboxyl functional surfaces, pre-treated with FN, showed high levels of two FN domains ($\alpha_5\beta_1$ and $\alpha_v\beta_3$) associated with structural and signaling components related to focal adhesions [109]. On the other hand, -COOH-mediated $\alpha_v\beta_3$ exposure has been shown to inhibit osteoblast differentiation and mineralization. Similarly, increase in $\alpha_v\beta_3$ integrin expression on –COOH surfaces was correlated with a low level of myoblast differentiation though cell proliferation levels were high [110].

**Hydroxyl (-OH) functional group-coated surfaces—**The hydroxyl group functionality (-OH) represents a neutral, hydrophilic surface. Early research into surface functionality suggested that an increase in oxygen containing functionalities was proportional to cell growth [111], sparking further research in oxygen containing functional groups such as –OH. FN adsorbed onto -OH functional groups (in comparison with $–CH_3$ functional group) show high levels of $\alpha_5\beta_1$ levels leading to increased cell adhesion strength as well as increased levels of structural signaling components related to focal adhesions [109]. Further investigation with osteoblasts showed high levels of differentiation and mineralization with –OH functionality as opposed to other functional groups. Rather contradictory, -OH functionality has been shown to have reduced plasma protein adsorption and thus higher platelet compatibility [112]. It has been suggested that the charge neutrality and hydrophilic nature of the -OH functionality has low protein affinity, and thus protein repelling properties [113].

**Amine ($-NH_2$) functional group-rich surfaces—**Amine group ($-NH_2$) functionality displays a positive charge to the biomaterial surface. Studies using FN and osteopontin (OPN) show favorable protein conformations after adsorption to the positively charge $-NH_2$ surface [109,114]. Particularly, $-NH_2$ surfaces promote the exposure of high density bound receptors as well as focal adhesion components by adsorbed fibronectin [109]. These favorable protein adsorption profiles often lead to increased endothelial cell growth [114] and enhanced differentiation and mineralization of osteoblast cells [109,115]. Preferable adhesion, growth, and matrix formation was also shown with fibroblasts on $–NH_2$ surfaces compared with other coatings [116–117]. Further analysis showed than within 45 minutes,

cells cultured on -$NH_2$ had already begun the formation of focal adhesion plaques, linked to increased cell spreading on the surfaces [116]. However, in a study using myoblasts, -$NH_2$ functionality was shown to promote high levels of myoblast proliferation with low levels of differentiation [110].

**Methyl (-$CH_3$) functional group-bearing surfaces—**The -$CH_3$ group is the major component of commonly used polymeric materials and provides a hydrophobic surface on biomaterials. It is generally accepted that the hydrophobic –$CH_3$ functionality promotes protein adsorption, usually in conformations unfavorable for desired cellular interactions [109]. As a result, -$CH_3$ functionality has also shown increased fibrinogen binding, platelet accumulation and thus poor blood compatibility [112]. A study measuring the adhesion strength of fibrinogen, albumin, and IgG showed that all three proteins adhered with the highest strength to -$CH_3$ bearing surfaces [118]. Overall, these results suggest that -$CH_3$ surfaces will likely have unfavorable surface reactions with cells due to the magnitude of tightly bound proteins, and the likelihood that the bound proteins will expose sites responsive to inflammatory cells.

**Surfaces with Mixed Functionality—**In the recent years, research has progressed to the evaluation of mixed functional group chemistries on surfaces. The goal of these studies is to attempt to combine favorable properties of both functionalities onto one surface to enhance biocompatibility. Interestingly, the properties of each functional group seem to almost equally contribute to the overall properties of the surface. Using a SAM combining -$NH_2$ (positive charge) and -COOH (negative charge) functionality in different proportions, it was seen that at equal molar fractions the lowest platelet adsorption was seen [119]. This equal proportion of the functionalities left the surface with a near neutral charge. This finding highlights the possible importance of surface neutrality related to blood compatibility of biomaterials.

Another study, investigating -OH and -$CH_3$ mixed SAMs, found a decrease in platelet adhesion and activation as well as decreased fibrinogen adsorption with increasing hydrophilicity, attributed to the -OH functionality [120]. This study further highlights the importance of a wettable surface in decreasing blood platelet interactions. With regards to surface adhesion, it was seen that hydrophobic SAMs combining -COOH and -$CH_3$ groups had a higher adhesion than hydrophilic -COOH and -OH SAMs [121]. A study using -$PO_3H_2$ functionality with -$CH_3$ showed that though total number of platelets increased with increasing -$PO_3H_2$ content, the number of activated platelets decreased [122]. However, when considering -$PO_3H_2$ and -OH mixed SAMs, the number of activated platelets increased with increasing -$PO_3H_2$ with the lowest adhesion seen at almost equivalent proportion of the functionalities. Highlighting the importance of the fact that OH functionality has been shown to have higher platelet compatibility than -$CH_3$ due to its hydrophilic nature and neutral charge. This result also suggests that the platelet compatibility of -$PO_3H_2$ (low hydrophilic and anionic) is somewhere between -OH and -$CH_3$ [122]. At this time, there has been no work published with mixed SAMs in vivo. The in vivo interactions of mixed surface functionality would likely provide valuable information and fill a large information void on the effects of surface functionality in vivo.

## Effect of surface functionality on tissue responses

Despite a significant body of in vitro results, there are very few works which had focused on the effects of surface functional groups in vivo. Almost all in vivo studies were carried out in an acute implantation model. The results from those works have supported that surface functionality may alter biomaterial-mediated acute inflammatory responses in vivo [108,123–124]. Using an intraperitoneal implantation model with RFGD functionalized

disks, our early results have shown that surface functionality prompts different extents of acute inflammatory responses in the order: -$NH_2$ > $CH_3$ -$CF_x$ > -OH > siloxyl group [108]. A recent study was carried out using using SAMs to produce hydrophobic –$CH_3$ and hydrophilic -OH and -COOH surfaces. The results have shown that - $CH_3$ surface functionality slightly increases the thickness of the fibrous capsule formed around implants as well as higher recruitment of inflammatory cells in comparison to -OH and -COOH functionalities [68]. In a separate study it was seen that –$CH_3$ terminated SAMs lead to an increase in leukocyte and phagocyte migration to the implant site compared to the gold surface alone [124].

Surprisingly, almost all previous studies failed to show the effects of surface chemistry on chronic fibrotic responses in vivo [68,108,125]. We speculated that the relatively ineffectiveness of surface functionality on long-term in vivo cellular responses may simply reflect insufficient cell:surface interactions. Specifically, in an in vitro setting, cells are seeded on top of functional group and surface functionality, thus, exerting maximal effect on cellular responses. On the other hand, in an in vivo environment, the functional groups on non-porous implants can only interact with the first layer of the cells which represent only a small portion of the surrounding cells/tissue. These relatively poor cell-functional group interactions thus minimize the effect of surface functionality on cellular responses. Thus we hypothesized that possibly the influence of implant surface chemistry may be substantially enhanced by increasing the interaction between functional groups and cells in applications such as tissue scaffolds and drug releasing microspheres. To test this hypothesis, we have examined, in vivo, responses to microspheres bearing different, molecularly tailored, surface functionalities. Spherical particles were chosen for this purpose to maximize the surface to volume ratio of the implants and also to mimic microspheres used for controlled drug release. To test this hypothesis, we have recently reported a study using functionalized micron-sized particles, created and tested for their ability in modulating tissue responses to biomaterial implants. In this work, the surfaces of polypropylene particles were controllably coated with four different functional groups, specifically –OH, -$NH_2$, -$CF_x$ and –COOH, using a RFGD plasma polymerization technique [74]. The effect of these surface functionalities on host tissue responses were then evaluated in vivo. Surfaces with hydrophilic –OH and –$NH_2$ surface groups induced the thickest fibrous capsule accompanied with the greatest cellular infiltration into the implants. As previously mentioned, hydrophilic groups have been shown to reduce protein adsorption, though these studies used thin films consisting of functionality derived from SAMs. In contrast, surfaces with –$CF_x$ and –COOH exhibited the least inflammatory/fibrotic responses and cellular infiltrations. The results suggest the surface/volume ratio of the implant, as well as surface functionality, may play an important role in influencing tissue responses and biocompatibility [74].

## Role of Surface Chemistry in Tissue Engineering

Investigations into the effects of surface functionality in tissue engineering are still in its infancy. Recently groups have focused on the incorporation or modification of scaffold surfaces to enhance hydrophilicty. As previously mentioned, knowledge of how functional groups effect cell adhesion properties can be used to modify scaffold surfaces to enhance cell adhesion while also reducing inflammatory response to the scaffold material. Currently most research involving tissue engineering scaffolds is focused on the addition of ECM derived materials to enhance cell growth and differentiation (126). Surface functional groups are being used primary to facilitate the chemical addition of specific membrane ligands to promote cell adhesion. As knowledge of the in vivo effects of surface functional group coating of biomaterial surfaces expands, we will likely see increased research and application of surface group functionalized scaffolds.

Interestingly, the results from recent studies suggest that surface functionality can induce cell differentiation. A study using Caco-2 cells showed that there was a relationship between perfluoronated functional coating and degree of cell differentiation [127]. In a separate study, –COOH-mediated $\alpha_v\beta_3$ integrin exposure has been shown to inhibit osteoblast differentiation and mineralization [112]. Investigation of -OH functionality with adhered osteoblasts showed high levels of differentiation and mineralization with –OH, and -$NH_2$ functionality as opposed to other functional groups including -COOH, -$SO_4$, and –$CH_3$ terminal groups [109,112,115]. There remain many possibilities as to the characterization of protein adherence to functional coatings and the subsequent effects of these proteins and their configuration on cell differentiation.

## Functionalized surfaces to improve drug delivery

**Surface chemical effect on reducing diffusion barrier and improving drug release—**Like many other implantable medical devices, drug release implants are often surrounded by thick layer of collagenous fibrotic tissue a few days (weeks) after implantation. The fibrotic tissue may serve as diffusion barrier and, thus, reduce the drug transport from implanted drug release devices to the circulation (Figure 3). Therefore, it is generally believed that the reduction of biomaterial-mediated fibrotic reactions may improve the drug release properties [128–130]. Results using SAMs on gold have shown that implants coated with -COOH and -OH functionality produce thinner fibrous capsules than -$CH_3$ functionality [68]. Results from our laboratory using polypropylene functionalized microsphere implants also showed thin capsules for -COOH functionality. However, significantly increased fibrous capsule formation was found surrounding implants with -OH functionality [74]. More research is needed to determine the relationship between surface functional groups and resulting drug release properties.

**Surface functionality on improving drug cellular uptake—**A recent study has also suggested that surface functionality of drug delivery vehicles affects both the rate and mechanism of cell uptake. Using dendrimers with terminal groups of -$NH_2$, -OH, and PEG, results have shown that -$NH_2$ surfaces were able to enter cancer cells at a higher rate than -OH and PEG functionalized surfaces. It is suggested that the favorable interactions of the cationic -$NH_2$ surface with the cell membrane allow quicker passage of drug delivery vehicles into the cell. It is likely that the -OH and PEG functionalities, due to their anionic nature, increase affinity to the cell membrane, and then taken in by an endocytotic route [131]. Another study investigating -OH, -$NH_2$, and -COOH terminal groups on dendrimers showed that -COOH and -OH functional surfaces tend to have increased residence times in vivo, which may be attributed to their ability to resist recognition by the body through protein adsorption, as well as cell uptake properties due to surface functionality [132].

Surface coating has been shown to effect cellular uptake of nanoparticles [133–134]. In general, micro and nanoparticles with a hydrophilic shell show decreased protein adsorption and increased circulation time [135]. Particles modified with a PEG shell, with a hydrophilic –OH surface functionality, can reduce particle uptake by mononuclear phagocytes [136]. A recent study, examining the effects of altering surface charge on particle uptake, showed that increasing –$NH_2$ group functionality on the particle surface has a correlation to cell uptake [137]. This phenomenon was also shown to be cell dependant, with cancerous cell lines taking up more particles with an increase in –$NH_2$ group density on the surface. An interesting trend was seen with hydrophilic PVA surface modification with –OH functionality. In a study comparing uncoated PLGA versus -OH coated PLGA, it was seen that the addition of the hydrophilic coating led to a 2 fold increase in cellular uptake [138]. A recent study has shown that -COOH functional surface enhances cell uptake and the amount of nanoparticle uptake could be correlated to the amount of -COOH functionality on

the nanoparticle surface. Such interesting phenomenon might be due to favorable interactions of the cell with the negatively charged coating [139].

## Conclusion

A significant body of research clearly demonstrates that in vivo tissue compatibility may be modified by varying surface chemistry. Many recent works have uncovered that the species, density and composition of surface functional groups play an important role of controlling protein, cell and tissue reactions to material implants (Table 1). More in vivo studies are needed to define the interactions between surface functionality and host responses. It is our belief that such knowledge may help the development of molecularly tailored surface to improve the function and safety of many medical devices, including drug release devices, encapsulated cells and implantable sensors.

## Acknowledgments

This work was supported by a NIH grant RO1 GM074021, a Texas Higher Education Coordinating Board's Advanced Technology Program (003594-0003-2006), and an AHA Established Investigator Award.

## Abbreviations

| | |
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| **SDS** | Sodium dodecyl sulfate |
| **PET** | polyethylene terephthalate |
| **RIBS** | receptor-induced binding sites |
| **(SAMs)** | Self-assembled monolayers |
| **-OH** | Hydroxyl |
| **-$CH_3$** | methyl |
| **-COOH** | carboxyl |
| **-$NH_2$** | amine |

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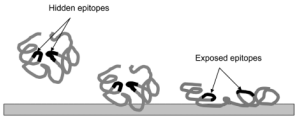


**Figure 1.**
Schematic drawing to depict the protein:biomaterial interactions which often lead to conformational changes of adsorbed proteins and the subsequent exposure of hidden proinflammatory epitopes.

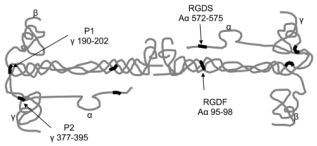


**Figure 2.**

Schematic drawing of fibrin(ogen) showing the approximate locations of some of the epitopes which have been implicated to be responsible for triggering foreign body reactions. The potential inflammatory epitopes include P1 (γ190–202), P2 (γ377–395), RGDS (Aα 572–575), and RGDF (Aα 95–98).

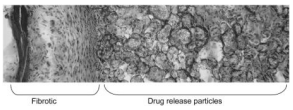


**Figure 3.**
Prominent fibrotic tissue formation was found surrounding poly-L-lactic acid particles which were subcutaneously implanted in Balb/C mice for 2 weeks. The presence of collagenous fibrotic tissue create diffusion barrier for drug transport from implanted particles to blood stream.

## Table 1

Effect of surface functionality on protein adsorption, cell behavior and tissue responses.

| Surface Functional Group | Protein, Cell, and Tissue Properties |
|---|---|
| Carboxyl (-COOH) hydrophilic, negatively charged | • Preferentially interacts with fibronectin and albumin [93].<br>• Enhances fibronectin adsorption and exposure of cell adhesive domains related to focal adhesion and cell growth [109].<br>• Increases nanoparticle uptake [139].<br>• Attenuates inflammatory responses and reduces fibrotic capsule formation *in vivo* [74]. |
| Hydroxyl (-OH) hydrophilic, neutral charge | • Reduces plasma protein affinity [113].<br>• Promotes fibronectin to expose cell adhesive domains related to focal adhesion [109].<br>• Enhances differentiation and increase mineralization of osteoblasts [109].<br>• Thickened fibrotic capsule with high levels of cell infiltration *in vivo* [74]. |
| Amine (-$NH_2$) hydrophilic, positive charge | • Possesses high fibronectin affinity leading to increased endothelial cell growth [114], differentiation and mineralization of osteoblasts [115], and myoblast proliferation [110].<br>• Promotes focal adhesion formation, cell spreading, and matrix formation with fibroblasts [116].<br>• Increases particle uptake [137].<br>• Triggers acute inflammatory responses, thick fibrotic capsule formation, and cell infiltration *in vivo* [74,108]. |
| Methyl (-$CH_3$) hydrophobic, neutral charge | • Tightly binds fibrinogen leading to platelet accumulation [112].<br>• Binds IgG more tightly than other surface functional groups [118].<br>• Leads to thick fibrotic capsule formation and high recruitment of inflammatory cells *in vivo* [68].<br>• Increased leukocyte adhesion and phagocyte migration [124]. |
| Mixed -$NH_2$ & -COOH positive & negative | • When mixed at equal molar fractions (near neutral charge), platelet adsorption is at its lowest compared to other fractional mixes of the functionalities [119]. |
| Mixed -OH & -$CH_3$ hydrophobic & hydrophilic | • As the fraction of -OH increases, platelet adhesion and fibrinogen adsorption decrease [120]. |
| Mixed -$PO_3H_2$ & -OH negative & neutral | • Equal molar fractions of -$PO_3H_2$ and –OH had the lowest number of adherent platelets [122]. |