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#### SILICONIZATION: BLOOD-SURFACE INTERACTION

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Siliconization is defined as a surface treatment process, the objective of which is to prevent undesirable interactions between the treated surface and biological macromolecules. Literature review reveals that siliconization encompasses several different methods which produce surfaces with different characteristics. The potential for variation in biological interaction as a result of different methods is demonstrated for whole human blood. Polydimethylsiloxane, octadecylsilsesquioxane, chlorinated dimethylsiloxane oligomer and several organosilane treated surfaces were compared for relative blood protein adsorption and hemolysis.

#### INTRODUCTION

Siliconization is a surface treatment process, the objective of which is to prevent undesirable interactions between the treated surface and biological macromolecules. More generally siliconization can be defined as imparting silicone-like properties to a substrate material by addition or reaction of silicon containing compounds which results in formation of a thin surface film. The implication is that silicone-like properties are desirable and well-defined. The biocompatibility of silicone elastomers is well examined, but siliconized substrates demonstrate a broader range of bio-interaction and have only received fragmentary consideration. A review of literature reveals that siliconized compositions and methodology of applications are generally

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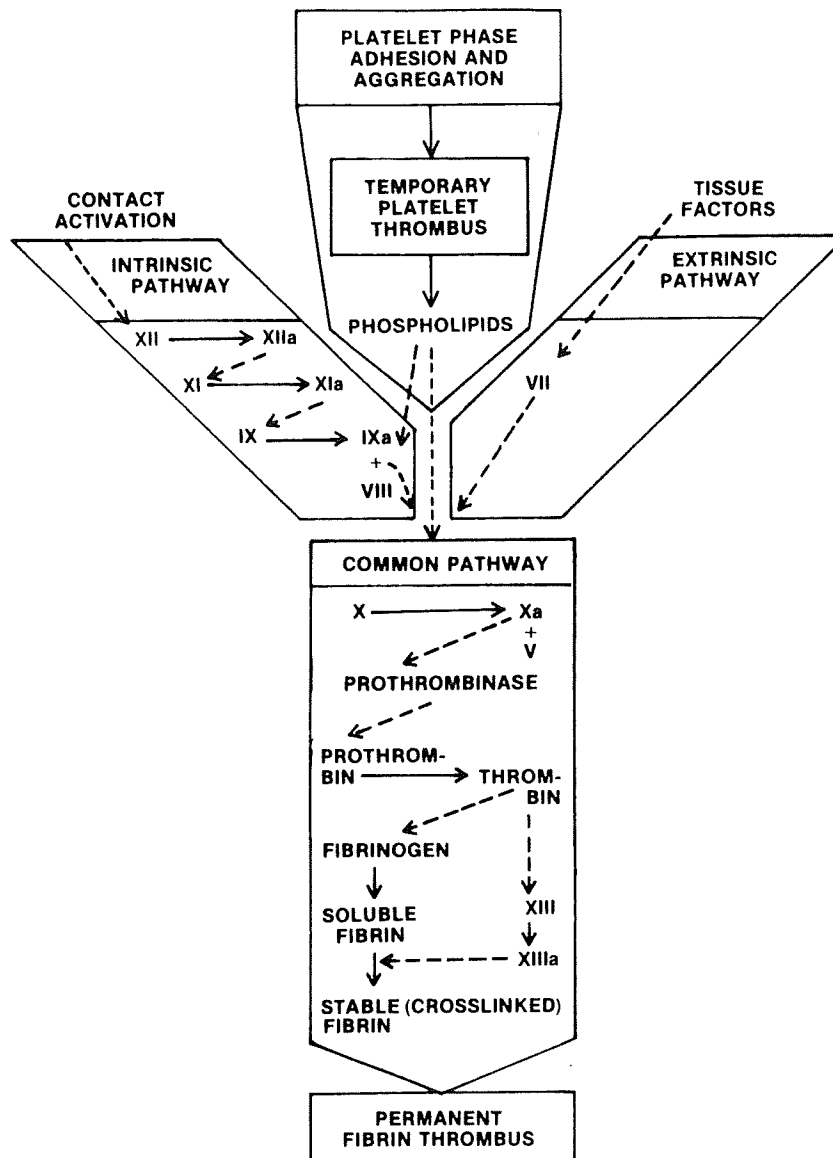
poorly described. The simple descriptor "siliconized" is not sufficient to define surface characteristics where biological interaction is concerned. This paper is a short review which distinguishes different siliconization procedures and presents new data documenting the need to better define and distinguish the term "siliconization."

Biomaterial science has emphasized development and characterization of materials with a dynamic biological interaction: implants, invasive and extracorporeal support devices. Materials which have "ex vivo" contact with biological tissues have been largely ignored. While siliconization of substrates is employed in a variety of protein and carbohydrate techniques, the most challenging area is in blood contact. In this context, glass is an extremely important substrate which is generally overlooked. Annual use of blood collection tubes, for example, exceeds one billion. Diagnostic analyses, both in terms of accuracy and time effectiveness, are affected by the condition of the glass surface, which is often subjected to siliconization. Literature is rampant with the term siliconized, but only a few well conducted examinations of protein interaction, for example, have been reported. In particular, studies by Brash and Mizutani deserve recognition. Unfortunately, these investigators reach different conclusions on the relative adsorption of serum albumin and other proteins at low phosphate concentrations. Mizutani reports almost no adsorption of serum albumin at moderate concentrations<sup>1,2</sup> while Brash reports a relatively high steady state adsorption.<sup>3,4,5</sup> Mizutani is quite clear on the compositional aspect of siliconization. While not clear on composition, Brash's careful reporting immediately indicates a quite different treatment. Mizutani's siliconization is a polydimethylsiloxane, solvent dispersed and baked. Brash's siliconization is an octadecylsilsesquioxane via micellar deposition. Norde<sup>6</sup> references another siliconization technique using dimethyldichlorosilane monomer and reports results at high phosphate concentration which show albumin adsorption similar to Mizutani's. Well over 90% of the references to siliconized substrates in experimental reports, however, do not reveal the type of siliconization employed.

The interaction of blood with foreign surfaces in the absence of inhibitors of coagulation is a rapid and sensitive series of events. Storage containers for specimens under ideal circumstances should resemble vascular endothelium - a nonthrombogenic surface which is resistant to platelet adhesion and aggregation. On the less than ideal surfaces presented to blood by biomaterials, a sequence of events is triggered: adsorption of plasma proteins, adhesion of platelets and leukocytes, activation of platelets by the release reaction and recruitment of nearby plat-

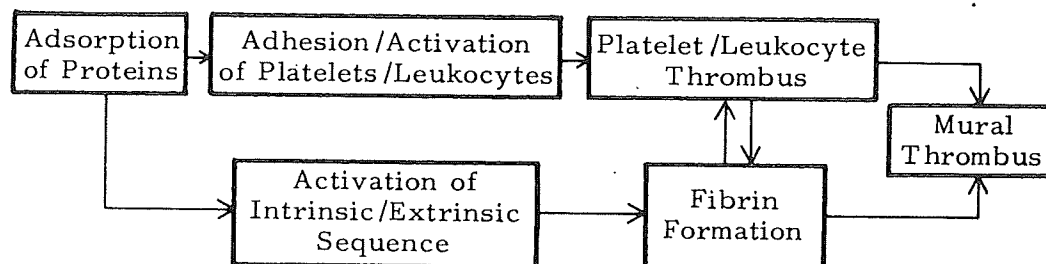
elets with the eventual formation of a thrombus. Subsequent formation of cross-linked fibrin stabilizes the thrombus. This is depicted in Figures 1 and 2.

Figure 1.



## Blood Coagulation Cascade

Figure 2.

**SYNOPSIS OF BLOOD/MATERIAL INTERACTIONS**

Complement activation which mediates immunological response to foreign substances is a separate group of factors which controls long term reaction to foreign surfaces. Thrombosis and complement activation at prosthetic interfaces has been recently reviewed. Upon contact, some plasma proteins are adsorbed on synthetic substrates. The specific proteins which are adsorbed dictate the course of events in the thrombogenic cascade. Desorption and the extent of protein denaturation during adsorption may also be important factors.

The total protein concentration in blood plasma is about 70 mg/ml. Albumin, globulin and fibrinogen account for 50, 10, and 3 mg/ml respectively.<sup>22</sup> Adsorption of other minor proteins such as Factor XII or kininogen may be more important for determining thrombogenic events.<sup>28</sup> Interaction of cellular elements is also critical in determining the relative stability of blood in contact situations. Platelet and erythrocyte interaction have been studied. Erythrocytes represent 40% of blood in terms of packed cell volume. Hemoglobin found within erythrocytes represents 140 mg/ml of protein in whole blood, yet less than .01 mg/ml of hemoglobin is found in plasma. Thus a blood contacting situation which causes hemolysis effects a profound change in the characteristics of blood. The ideal biomaterial should not activate the blood coagulation cascade, the complement system, platelet<sup>19</sup> interaction or hemolysis. The parameters proposed by Merrill,<sup>19</sup> for low platelet retention serve as a good physical description of polyethers in general and polydimethylsiloxane in particular: 1) absence of ionic groups 2) absence of hydrogen atoms that can form hydrogen bonds 3) relatively small nonpolar

groups 4) regular alternation of the non-polar groups with a polar group 5) segmental mobility 6) surface activity.

A review of literature reveals the following significant siliconization techniques. In general, industrial siliconization in North America is likely to involve polydimethylsiloxane. Research techniques vary widely, but octadecylsilsesquioxane siliconizations seem to dominate.

#### SILICONIZATION TECHNIQUES:

##### 1a. Polydimethylsiloxane, Baked Siliconization<sup>1,8,27,29</sup>

Clean dry surfaces are either sprayed with or dipped into polydimethylsiloxane. General industrial practice is to use neat fluid with a 100-350 centistoke viscosity. Alternately, higher viscosity fluids, 500-2000 centistoke are prepared in 5-10% solutions of aromatic or chlorinated solvents. The coated surfaces are baked at 265-325°C for 5-15 minutes.

##### 1b. Polydimethylsiloxane, Unbaked Siliconization<sup>8,18</sup>

The same practice as (1a) is followed but the resulting coating is not baked.

##### 2a. Polydimethylsiloxane Emulsion, Baked Siliconization<sup>8</sup>

Polydimethylsiloxane emulsion is reduced to 0.5-1.0% concentration in distilled water and used as a washing machine media. Following treatment in the washing machine, parts are baked at 265-275°C for 20-60 minutes.

##### 2b. Polydimethylsiloxane Emulsion, Unbaked Siliconization<sup>9</sup>

The same procedure as (2a) is followed, but after the wash, parts are dried for 1 hour at 120-125°C.

##### 3. Octadecylsilsesquioxane Siliconization<sup>3,10,11</sup>

A solution of octadecyltrialkoxysilane in t-butanol and diacetone alcohol is diluted to 0.1-0.5% final concentration in water. The mixture is an aqueous dispersion of silane micelles. Dipped parts are cured at 100°C for 3-5 minutes or at room temperature for 24 hours.

##### 4. Chlorinated Dimethylsiloxane Oligomer Siliconization<sup>12,23,26</sup>

A mixture of chlorine terminated polydimethylsiloxane olig-

omers is dissolved in an aprotic solvent such as toluene, methylene chloride or hexane at 1-10% concentration. Parts are immersed for 5-20 minutes, then are air-dried for twenty-four hours or heat cured at 100-110°C for 20 minutes.

### 5. Siliconization by Silylation<sup>6,13</sup>

Chlorinated silanes such as dimethyldichlorosilane or trimethylchlorosilane are either used neat or in aprotic solvent. Surfaces to be treated are either immersed in the silane and treated at reflux for 4-24 hours, immersed in alcoholic solutions for 10-15 minutes, or are subjected to vapor at elevated temperatures. The surfaces are rinsed with methanol and water, then dried prior to use.

A summary of these techniques is presented in Table 1.

Table 1. Siliconization Techniques

<u>Chemical Basis</u>	<u>Deposition</u>	<u>Cure</u>
POLYDIMETHYLSILOXANE FLUID	neat solvent	baked, unbaked
(DC360, KF96, PS041.5, SF96, Simethicone, PDMSO)*		
POLYDIMETHYLSILOXANE EMULSION	aqueous	baked, unbaked
(DC24A, DC365, PS053.5)*		
OCTADECYLSILSESQUIOXANE	aqueous	low temp.
(Siliclad, Glassclad 18, Aquasil, Prosil <sup>28</sup> )*		
CHLORINATED SILOXANE OLIGOMER	solvent	baked, unbaked
(Dri-film, SC87, Glassclad 6C, Sufasil)*		
SILICONIZATION by SILYLATION	solvent, vapor	low temp.
(dimethyldichlorosilane (DMDCS), trimethylchlorosilane (TMCS), various.*		

\* Acronyms and tradenames

An experimental body of data concerned with the interaction of blood elements with siliconized surfaces has evolved. While demonstrating the efficiency of silicones, it is difficult to draw correlative conclusions. A selected summary of siliconization studies that could be directly or indirectly defined follows:

Polydimethylsiloxane Siliconization, Methods 1a, 1b, 2a, 2b:

Albumin, hemoglobin, lysozyme<sup>1</sup>  
Platelets<sup>19</sup>

Octadecylsilsesquioxane Siliconization, Method 3:

Fibrinogen, albumin<sup>3,4,5</sup>  
Platelets<sup>24</sup>

Chlorinated Dimethylsiloxane Oligomers, Method 4:

Lipoproteins<sup>23</sup>

Siliconization by Silylation, Method 5:

Albumin<sup>6</sup>  
Erythrocytes<sup>25</sup>

A review of literature leads to the conclusion that siliconized surfaces are among the best blood contacting surfaces available in terms of eliciting minimal blood interaction. At the same time it is evident that there is a selectivity in the interaction and even at low levels this is critically important in determining subsequent thrombogenic and immunological events. Siliconization, however, is not a well-defined technique and reports with conflicting conclusions may be partly accounted for by the different techniques.

The importance of siliconization in blood contacting applications encouraged us to carry out a series of experiments in order to determine the extent to which different siliconized surfaces affect some of the parameters of stored whole blood. A representative series of siliconizations were applied to glass substrates which were evaluated in contact with whole human blood.

We now report the results of hemolysis and blood protein adsorption for whole human blood. Contact angles of water on the treated substrates are also reported.

## EXPERIMENTAL

### Surface Preparation:

Borosilicate glass tubes (Kimax) and borosilicate glass slides (Pyrex) were treated with 5% hydrochloric acid for 24 hours and then thoroughly rinsed with distilled water prior to treatment.

### Siliconization by silylation with chlorosilanes:

Dipropyldichlorosilane, t-butyltrichlorosilane and dimethyloctadecylchlorosilane were prepared as 2% solutions in warm (40-60°C) ethanol. The substrates were dipped and withdrawn several times from the solution to insure even wetting. Total immersion time was 10-15 minutes. They were then rinsed once with ethanol and allowed to air dry before being placed in an oven at 105°C for 3-5 minutes.

### Chlorinated Siloxane Oligomer Siliconization:

A mixture of chlorine terminated dimethylsiloxane oligomers containing primarily trisiloxane and tetrasiloxane designated Glassclad 6C (Petrarch) was prepared as a 5% solution in hexane. The parts were immersed for 15 minutes and then cured for 20 minutes at 105°C. They were then rinsed with alcohol and dried.

### Octadecylsilanesiloxane Siliconization:

A solution of Glassclad 18 (Petrarch), a 20% octadecyltrialkoxysilane concentrate, was prepared as a 1% solution in distilled water to give an active concentration of 0.2% octadecylsilane. Glass surfaces were immersed 5-10 seconds with gentle agitation and then dipped briefly into distilled water. The coating was cured at 105°C for two minutes.

### Alkoxysilane Siliconization:

A mixture of 2% N-trimethoxypropyl-N,N,N-trimethylammonium chloride and 5% water in methanol was prepared. The solution was warmed to 30-40°C and the parts immersed for 5-10 minutes. They were then dried 5 minutes at 105°C and rinsed with methanol.

### Contact Angle Measurements:

Contact angle of water was determined by goniometer. The range reported is several determinations of spreading and receding angles observed on treated glass slides.

### Blood:

Blood was freshly drawn from volunteers into evacuated B-D vacutainers containing sodium citrate.

### Hemolysis:

Approximately one ml of whole blood, which was drawn in heparin, was added to each test tube to be studied. A pediatric



red top vacutainer coated with "silicone(B-D)" was used as the control test tube. Day 0 plasma was obtained and stored in microhematocrit capillary tubes sealed at one end with Critoseal. Samples on the other days were drawn into microhematocrit capillary tubes, peripheral blood smears were obtained, and the tubes were then centrifuged in an Adams Autocrit Centrifuge. The tube was scored and snapped at the packed cell/plasma interface, and the plasma was stored in the tube which was then resealed at one end with Critoseal. The amount of Hb in the plasma was determined according to the method of Levinson & Goldman<sup>20</sup> with slight modifications: Either 1 or 2  $\mu\text{L}$  of plasma was added to 100  $\mu\text{L}$  3,3',5,5'-tetramethylbenzidine (Aldrich) in 90% (v/v) acetic acid. After addition of 100  $\mu\text{L}$  0.1%  $\text{H}_2\text{O}_2$  (v/v) the samples were incubated for 20 minutes before diluting<sup>2</sup> with either 1 or 2 mL 10% (v/v) acetic acid. The absorbance of the samples at 660 nm was then determined. A standard curve was prepared with each assay. The whole blood samples were stored in a 4°C cold room throughout the study.

#### Blood Protein Adsorption:

Blood was dispensed into treated tubes and stored at 4°C for 72-100 hours. The blood was then agitated with a vortex mixer and aspirated. Saline was added, the tube inverted 2-3 times and aspirated. This was repeated twice.<sup>21</sup> Biuret analysis for protein was used as described by Gornall.<sup>21</sup> This process was elected over Coomassie Blue, since the basic conditions of the former facilitate debonding of organic silicon compounds thus releasing proteins. The latter technique did not debond proteins and gave false positive results for protein against long chain alkyl treated glass. Bovine serum albumin was employed for calibration. The average absorption from 530 to 550 nm was determined on a Hewlett Packard UV/Vis Model 8450A.

## RESULTS

### Contact Angle

All siliconization techniques produced hydrophobic surfaces although with considerable variability in the degree of hydrophobicity. Chlorinated siloxane oligomer and octadecylsilsesquioxane exhibited the greatest range of contact angles but were generally the most hydrophobic of the surfaces evaluated. The silylated ammonium quat was the exception, giving the only readily wettable surface, and was deliberately included in the study for this purpose. See Table 2.

Table 2.

#### Contact Angle of "Siliconized" Glass Surfaces

Trimethylchlorosilane	76-85
Chlorinated Siloxane	75-90
Octadecylsilsesquioxane	65-100
Dipropyldichlorosilane	62-80
t-Butyltrichlorosilene	67-73
N-Trimethoxysilylpropyl- (trimethylammonium) chloride	10-20
Dimethyloctadecylchlorosilane	65-85

### Hemolysis

All surface treatments elicited extremely low levels of hemolysis, less than 0.005%. Results in Table 3 (below) are reported relative to a 5% HCl washed, deionized water rinsed control. The plasma level of hemoglobin at day one for the control was defined as 1. By day four all surface treatments, except the ammonium quat, demonstrated less hemolysis than the untreated control.

Table 3.

Relative Hemolysis		
	Day 1	Day 4
Control (untreated)	1	3.5
Trimethylchlorosilane	0.8	1.2
Octadecylsilsesquioxane	0.7	1.5
Dipropyldichlorosilane	1.0	2.6
t-Butyltrichlorosilane	1.3	2.0
Chlorinated dimethylsiloxane	0.8	2.4
(N-Trimethoxysilylpropyl)- trimethylammonium chloride	1.2	3.5

#### Blood Protein Adsorption

Little obvious correlation between surface modification and blood protein adsorption was observed. The ammonium quat silane and dimethyloctadecyl treated surfaces produced the highest level of protein adsorption. The octadecylsilsesquioxane treatment produced one of the lowest. The least protein adsorption was observed with t-butyltrichlorosilane and dipropyldichlorosilane treatments. In terms of absolute numbers, the protein adsorption is reported in  $\mu\text{g}$  per  $100\text{ mm}^2$  of treated surfaces. The results are tabulated in Table 4.

Table 4.

Relative Protein Adsorption	
Treatment	$\mu\text{g Protein}/100 \text{ mm}^2$
Trimethylchlorosilane	8
Chlorinated Siloxane	20-25
Octadecylsilsesquioxane	6
Dipropyldichlorosilane	2
t-Butyltrichlorosilane	4
N-Trimethoxysilylpropyl- (trimethylammonium) chloride	20-50
Dimethyloctadecylchlorosilane	40-60

### CONCLUSIONS

It is evident that different siliconization methods have profound effects on protein adsorption. Beyond the broad description of being non-ionic, non-hydrogen bonding, possessing regular alternation of polar and non-polar groups and segmental mobility, it is difficult to further define parameters which result in low protein adsorption. The long chain non-polar structures provided by the dimethyloctadecyl chlorosilane treatment gave the greatest level of protein adsorption, while the octadecylsilsesquioxane treatment which was more hydrophobic provided a surface with lower protein adsorption. On the other hand, the dimethylsiloxane structure which has short alkyl (methyl) substitution and is also one of the more hydrophobic treatments, gives relatively high levels of protein adsorption. The surfaces treated with dipropyldichlorosilane and t-butyltrichlorosilane exhibit the lowest protein adsorption. In comparison to the octadecylsilane treatments these treatments are relatively sterically closed and the groups would be expected not to extend far from the glass substrate. Both factors should reduce the opportunity for extended hydrophobic interaction with proteins.

Effects of siliconization on hemolysis are less dramatic with all coatings generally effective in reducing hemolysis. In general, siliconizations that caused the least hemolysis also induced the least protein adsorption.

It is clear that various "siliconizations" have different interactions with blood proteins. Total protein adsorption (this study) and protein adsorption patterns (cited studies) show significant variations. A particular siliconization technique can present a passive surface to one blood component system of interest while presenting an active surface to another. The potential for significant variations in experimental results arising from different siliconization techniques must be considered. Siliconization techniques employing silanes with short, relatively bulky substitutions appear to give lower levels of protein adsorption and should be further evaluated.

1. T. MIZUTANI, Adsorption of Proteins on Silicone-coated Glass Surfaces. J. Colloid Interface Sci. 82, p. 162, (1981).
2. T. MIZUTANI, Estimation of Protein and Drug Adsorption onto Silicone Coated Glass Surfaces. J. Pharm. Sci. 70, p. 493, (1981).
3. J. L. BRASH, S. UNİYAL, Dependence of Albumin-Fibronogen Simple and Competitive Adsorption on Surface Properties of Biomaterials. J. Poly-Sci: Poly Symp 66, p. 377, (1979).
4. J.L. BRASH, S. UNİYAL, B.M.C. CHAN, A. YU, Fibrinogen-Glass Interactions in "Polymeric Materials and Artificial Organs" C.G. Gebelein ed. American Chemical Society; Washington p. 45, (1984).
5. S. UNİYAL, J.L. BRASH, Patterns of Adsorption of Protein from Human Plasma onto Foreign Surfaces Thromb. Hemostasis 47, p. 285, (1982).
6. P. VAN DULM, W. NORDE, The Adsorption of Human Plasma Albumin on Solid Surfaces. J. Colloid Interface Sci 91, p. 248, (1983).
7. M. SZYCHER, Biocompatible Polymers Metals and Composites (Technomic Publishing/Soc. Plast. Engrs. Lancaster, PA., 1983).
8. C. RIFFKIN, Siliconization: As Applied to Containers and Closures. Bul. Parenteral Drug Assoc. 22, p. 66, (1968).

9. G.G. SMITH, T.L. GRIMES, D.E. FONNER, J.C. GRIFFIN, New Process for the Treatment of Parenteral Closures, Bul. Parenteral Drug Assoc. 30, (1976) Chap. 2, p. 53.
10. "GLASSCLAD 18" Commercial Brochure, Petrarch Systems, Bristol, PA.
11. E. SAKO, O. KAWAMURA, Polycarbonate Container for Medical Applications, U.S. Patent 3,746,196, (1973).
12. S. JEVONS, P. BARTON, Biochemistry of Blood Platelets: Interaction of Activated Factor X with Platelets Biochemistry 10, p. 428, (1971).
13. F. DEYHIMI, J. COLES, Rapid Silylation of a Glass Surface Helv. Chem. Acta 65, p. 1752, (1982).
14. W. NOLL, H. WEISSBACH, Zement-Kalk-Grps. 9, p. 476, (1956).
15. D.K. OWENS, R.C. WENDT, J. Apply Polym. Sci. 13, p. 1791, (1969).
16. W. NOLL "Chemistry and Technology of Silicones," p. 443-452, (Academic Press" New York, 1968).
17. A. NAGATA, J. IYODA, West German Patent 2,647,389, (1975).
18. R. MERKLER, et al "The Heparinization of Silicone Rubber Using Aminoorganosilanes: in Proc. Artificial Heart Prof. Conf. U.S. Dept. H.E.W., Washington, (1969).
19. E.W. MERRILL et al Platelet Retention on Polymer Surfaces in "Biomaterials: Interfacial Phenomena and Applications," edited by S.L. Cooper, N.A. Peppas, American Chemical Society. Washington, D.C., p. 35, (1982).
20. S. LEVINSON, J. GOLDMAN, Clinical Chemistry 28, p. 471, (1982).
21. A. G. GORNALL, C.J. BARADAWILL, M.M. DAVID, J. Biol. Chem. 177, p. 751, (1949).

22. C. LONG, E. J. KING, W.M. SPERRY, Biochemist's Handbook, p. 841, (VanNostrand, NY 1961).
  23. B.W. SHEN, Lipid Protein Interaction at Solid-Water Interface, J. Biol. Chem. 260, p. 1032, (1985).
  24. R. ROSENSTEIN, L.R. ZACHARSKI, R.D. ALLEN, Quantitation of Human Platelet Transformation. Thrombos Haemostasis, 46, p. 521, (1981), Chap. 2.
  25. B. ARKLES, A. MILLER, W. BRINIGAR, Whole Cell and Cell Organelle Immobilization on Siliceous Surfaces in "Silylated Surfaces" edited by D.E. Leyden and W. Collins. (Gordon & Breach, New York, 1980).
  26. H. BROCKMAN, J. LAW, F. KEZDY, J. Biol. Chem. 248, p. 4965, (1973).
  27. R.C. LEWIS, H.I. GLUECK, A Micromethod for Determining Clotting Time. J. Lab & Cln. Med. 52, p. 299, (1958).
  28. L. VROMAN, A. ADAMS, G. FISCHER, P. MUNOZ, M. STANFORD, Clot-Promoting Surfaces in "Bio-materials: Interfacial Phenomenon and Applications," Edited by S. L. Cooper, N.A. Peppas, American Chemical Society, Washington, D.C., p. 265, (1982).
  29. R. GOLDMAN, Drain-Clean Containers, U. S. Pat. 2,504,482, (1950).
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