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REVIEW

Non-covalent Surface Interactions between Silicone and Biological Macromolecules Yield Bioreactive Substances

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While most organometallics enter the environment through their industrial release, silicones are organometallic compounds purposefully introduced in high volume directly into healthy humans. A chemically centered study of the behavior of silicones in the biological environment reveals numerous degradative reactions and surface interactions that can produce bioreactive substances. Data from a variety of disciplines suggest that the preponderance of evidence supports the argument that silicone is a toxic organometallic.

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INTRODUCTION

While the value of environmental chemistry has been recently underscored with the 1995 Nobel Prize in Chemistry to Paul Crutzen, F. Sherwood Rowland and Mario Molina,[1] the concepts of this field are still foreign to most casual observers. In part, the lack of understanding of environmental chemistry by the general public is linked to a strong belief in an earlier environmental model. Difficulty in changing the existing perspective is often due to the limitations afforded by the available technology to demonstrate the reality of the chemical phenomena.[2] For example, the Nobel Prize-winning modeling work of Rowland and Molina was performed in 1974 but was not supported by fieldwork until 11 years later.[3] Furthermore, even today the data remain highly controversial.

The medical experience with silicones, when framed as an environmental chemistry problem, is exemplary of the general case. While the chemical and biological interactions of silicones with the biological environment are fully consistent with fundamental principles of modern chemistry, the implied consequences are in contrast to long-held beliefs. Consequently, the vestiges of old paradigms create barriers to an appreciation of the real properties of an extraordinary polymer.[4–9] Not surprisingly, the lack of understanding is also linked to the limitations afforded by the available technology. In the United States, difficulty in appreciating the toxic biophysical properties of silicone by many who might otherwise be technically competent may be related to derivative ethical and medico-legal issues.[10–12]

This paper explores the biological and chemical behavior of silicones within a unique environmental compartment—the mammalian body. It assembles data from a wide variety of disciplines, and shows how the biophysical and biological factors interact. It is organized in sections describing:

- —silicones of biological significance (a brief introduction);
- —the methods by which they are introduced into the environment;
- —relevant elements of the biological environment;
- —the chemistry of silicone–environment interactions;

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— the biological consequences of those interactions; and
— data from a study testing the working model of silicone toxicity.

The thrust of this paper is that non-covalent surface interactions between silicone and biological macromolecules yield bioreactive toxic substances.

SILICONES OF BIOLOGICAL SIGNIFICANCE

Although first synthesized and ironically mischaracterized as 'silicon-substituted ketones' early in the 20th century by Kipping,[13] silicones were not apparently introduced into the human body until the 1940s.[14] Rowes animal experiments at Dow Chemical and Dow Corning over the following decade showed that silicones were far better tolerated by the mammalian body than other then-available synthetic polymers. Evidence cited included the lack of grossly evident necrosis following injection and the lack of apparent toxicity after oral ingestion.[15,16] Today, it may seem to be a stretch for Rowe to have concluded from the preceding that silicone was 'inert' but, relative to other polymers at the time, there was no overt exuberant acute inflammatory reaction. Early human studies reached similar conclusions, and silicone became a preferred medical polymer.[17]

For the past 40 years, the medically relevant silicones have been synthesized mostly from starting blocks of octamethylcyclotetrasiloxane, more commonly written as D_4. The D_4 cyclic is cracked open and polymerized by a catalyst to approximately 90% equilibrium favoring the polymer—an oil.[18] The balance remains as a mixture of cyclics and linear oligomers. The polydimethylsiloxane (PDMS) oil (chemically, α-(trimethylsilyl)-ω-methylpoly[oxy(dimethylsilylene)], CAS-9006-65-9, 'Dimethicone') is then cured or vulcanized in the manufacture of gels, elastomers, rubbers and solids.[19]

Injectable silicones, used for syringe lubricants as well as direct soft tissue repair or augmentation, consist of PDMS oils of various viscosities. PDMS oils mixed with 20 nm silicon dioxide particles and formulated to viscosities of about 3600–4000 cP, marketed under the name 'simethicone', have been used as antifoaming agents for extracorporeal blood circulation [heart–lung by-pass machines].[20]

Devices designed for implantation consist of elastomers, or, when used as breast or testicular implants, gels, oils and nanoparticulate (fumed) silica, such as Dow Corning Degussa Dust (source: Alexander R@http://seamless.com/alexanderlaw/txt/dow.html). In the production of elastomer, which is used to form the shell of both gel- and saline-filled devices, about 95% of the PDMS polymer chains are cross-linked. Fumed silica, which may comprise up to 30% of the elastomer shell by mass, is added for strength. The remaining 5% silicone oil content in the shell provides suppleness and elasticity. The cured gel in the devices is composed of a loose network of chains that trap the oil molecules. The actual oil content in commercially sold medical devices ranges from about 9% to 80% (Fig. 1).[18]

Pharmacologically active silicone, considered at one time for use as an oral estrogen, consists of a 2,6-*cis*-biphenyl-substituted D_4. This compound, whose pharmacological activity was completely unanticipated,[21,22] was synthesized at the Dow Corning Corporation in Midland, Michigan, and a full evaluation of the synthetic

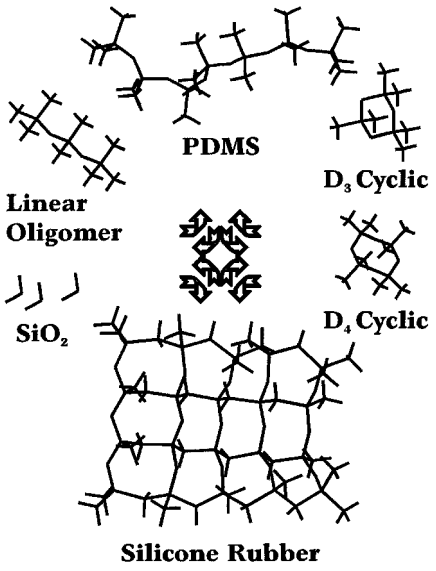

Figure 1 The fate of silicone in relationship to its use in medical applications. While the mineral form, SiO_2, may comprise up to 30% of the weight of silicone elastomers, the bulk is first converted into octamethylcyclotetrasiloxane (D_4), from which it is cracked and polymerized to form the polydimethylsiloxane oils that are then further treated to produce cross-linked gels such as PDMS (polydimethylsiloxane) and vulcanized elastomers. (After P. Wilkerson.[18])

estrogen was conducted in cooperation with KABI, Stockholm.[23]

METHODS BY WHICH SILICONES ARE INTRODUCED INTO THE ENVIRONMENT

Silicones are present in a broad range of commercial and industrial products.[24,25] They are used as lubricants, hydraulic oils, electrical insulators, moldings, adhesives, food release agents, paints, enamels, varnishes, cosmetics and pharmaceuticals.

Dow Corning's PDMS silicone fluid, Syltherm 800, is a competitive product in the high-temperature heat-transfer fluid market.[26] In environments which utilize operating temperatures of up to 400 °C, Syltherm 800 has been used because it appears to resist thermal stress and is non-coking.[27] In 1993, Dow Chemical agreed to market Dow Corning's Syltherm globally.[28] The high operating temperatures and volatile nature[3] of the lower-molecular-weight cyclics such as D_3 and D_4 may yield silicone vapors (for example, Syltherm 800, a PDMS of high molecular weight, is in an equilibrium state with volatile D_3, D_4 and PDMS oligomers), but there are few data on human exposure to gaseous silicones.

Silicones are used extensively in textile work and personal-care products such as hair sprays because of their antistatic and anticling properties.[29] Since nebulization of silicone-containing solutions is common in these fields, inhalation of microdroplet silicone is a possible route of introduction. Again, data quantifying the amount of silicone 'fog' inhaled' are not readily available. Silicones are also components of pharmacologically active over-the-counter antiflatulence medicaments; in the pharmaceutical industry, PDMS is usually called 'dimethicone' and is a common ingredient in antiflatulence medicaments such as Maalox and Mylanta. In these applications, oral ingestion is the most common route of introduction. Oral intake was also the preferred route for the administration of the estrogenically active 2,6-*cis*-biphenyl-substituted D_4, which was marketed under the trade-names 'Quadraosilan' and 'Cisobitan'.[30,31]

There are three routes of direct introduction of silicones into the body that by-pass the physiological skin, pulmonary and gastrointestinal barriers (Fig. 2). The first is direct mixing of silicones with blood in the vascular system. Antifoaming formulations of PDMS were rou-

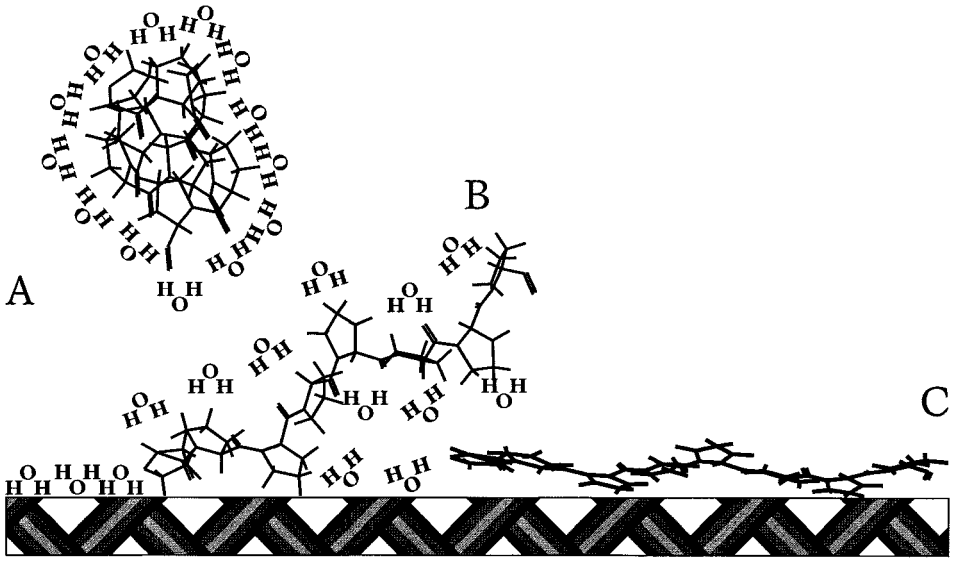

Figure 2 Surface-induced conformational denaturation. As water, a biological plasticizer (A), is driven off the polymer surface and out of the biological macromolecule through both enthalpic and entropic effects (B), irreversible conformational denaturation ensues (C). The structure–function relationship and the immunospecificity of conformation principles of biology dictate that the physiological consequences of molecular denaturation may include loss of function and loss of immunorecognition.

tinely added in the 1960s to the circulating volumes of blood oxygenators used to sustain life during cardiac by-pass surgery.[20] The second route is by way of direct injection of PDMS into the tissues. PDMS for soft-tissue augmentation, while most commonly injected immediately under the skin, would also occasionally be injected inadvertently directly into the vascular system. PDMS injections have also been performed directly into the globe (eyeball) to treat retinal detachment, and the emulsification of the oil by body proteins has facilitated its systemic distribution.[32] Injection into the globe requires eventual removal, and there are many medical complications associated with the removal procedure.[33]

A third route, used to introduce the bulkier medical devices, is surgical implantation. As a historical reference, the silicone gel-filled breast implant was first developed in 1963. It was offered as a substitute for the then standard direct intramammary PDMS injections for women with small breasts who sought breast augmentation.[34]

Because of the mechanics of breast implants and the inherent properties of the silicone elastomer, the implanted gel-filled devices function as slow-release reservoirs for the seepage, or 'bleeding', of low-molecular-weight silicones.[18,35] Recent measurements of the silicon content of breast tissues have been made. In their native state, after exposure to the silicone rubber of saline-filled implants, and after exposure to both the rubber and bleeding gel of gel-filled implants, the tissues contained 0.1, 8 and 9900 μg of silicon per gram of tissue.[36]

Engineering efforts to reduce the 'bleed' have consisted of several design and material changes in the composition of breast implants. The first effort was the deployment of a technological innovation originally introduced to reduce the adverse effects of scarring.[37] This was effected by adding a saline barrier around the entire device and encasing it within a second hollow silicone rubber outer shell. The second effort was aimed at reformulating the outer shell into a laminate composed of platinum-cured dimethyl- and diphenyl-silicone elastomers.[38] An unfortunate consequence of the latter action was that, while the new elastomers were more bleed-resistant, they showed poorer resistance to fold flaw failure.[39]

The mechanical strain on an implant is greatest at the periphery of the implant or points of folding of the shell where sudden change of shape occurs.[40] The combination of weakened polymer challenged by mechanical strain made failure of the implant shell almost inevitable. Time appears to be the principal variable separating low-molecular-weight silicone bleed through an intact elastomer shell from gross silicone gel spillage through a ruptured shell.[41–46]

ELEMENTS OF THE BIOLOGICAL ENVIRONMENT

The biological environment with which with various silicones interact is a complex aqueous colloid comprising dissolved salts and gases, dispersed proteins and lipids, and suspended cellular elements. Many of the proteins exhibit degradative enzymic action, and most of the cellular elements contain specialized packets of degradative and oxidative enzymes. In addition, there are both circulating cells and dedicated organs whose primary job is the collection and destruction of non-native materials through oxidative and enzymic pathways. Last, there is a complicated higher-order defense network that may exhibit the anthropomorphized property of immunological 'memory'. In the context of immunology, 'memory' describes the empirical phenomenon that an immune response to an organic stimulant is often much more aggressive and directed upon re-exposure than it is upon primary exposure. Immunological memory is the explanation offered to account for the apparent success of vaccines.

A solution of dissolved salts and gases

Blood, and its hyperfiltrate cousin, tissue fluid, constitute approximately 40% of the total body fluid. They are both based on a salt solution composed of monovalent and divalent ions and dissolved gases balanced at a pH of between 7.3 and 7.4. The principal ions are sodium, potassium, calcium, magnesium, phosphate, chloride and bicarbonate. The principal gases are oxygen, carbon dioxide and nitrogen.

A colloid of dispersed proteins and lipids

Added to the salt solution of both blood and tissue fluid are colloidal solids comprising a wide

range of functional proteins and lipids. The total protein content of blood plasma is between 20 and 45 mg/100 ml and is less than one-tenth of that concentration in tissue fluids. The mass of organic acids is approximately 20% of the mass of protein in blood plasma and is approximately three times the mass of protein in tissue fluid.[47] Among the most surface-active of the blood proteins are fibrinogen and fibronectin. They play a key role in inflammation.

A suspension of cellular elements

Rounding out the colloid are suspended elements. These include the oxygen-carrying red cells, the defense-oriented white cells, and the blood-clot-promoting platelets. While certainly more numerous in the blood stream, the cellular elements, especially select white blood cells, are also present in the tissue fluids.

Oxidative and degradative promoters

Many of the molecules secreted by inflammatory white blood cells function to provide logistical support for the inflammatory process by regulating blood flow and blood vessel permeability. Other products secreted by the cells have oxidative and degradative actions. They include lysozyme, alkaline phosphatase, collagenase, acid hydrolases, various neutral proteases, and the oxygen reduction products O_2^-, H_2O_2 and $OH^\cdot$. The latter, in turn, may combine with NO to produce toxic NO derivatives.[48]

Dedicated filter cells and organs

The tasks of identifying and removing debris, destroying damaged native components and foreign agents, and recruiting help for the aforementioned activities fall upon the white blood cells. Members of the while blood cell family include neutrophils, lymphocytes and macrophages. Neutrophils are very effective against bacteria. Lymphocytes and macrophages are much more effective against chronic persistent agents and therefore are especially relevant to the biological reaction to silicones.

Lymphocytes and macrophage precursors circulate in the blood, while tissue-based macrophages may be found randomly dispersed in the tissue space. In addition, there are dedicated organs that filter either blood or lymph flow where both lymphocytes and macrophages tend to aggregate. Among these organs are the spleen, liver, lung, kidney and the numerous lymph nodes scattered throughout the body.

Higher-order defense network—inflammation and the immune system

Inflammation is the reaction of vascularized living tissue to injury. The defenses mounted by the body against most forms of injury are mediated by direct attack consequent to immediate injury. Key blood proteins such as fibrinogen and fibronectin facilitate the earliest phases of the biological response. However, when an injurious agent is persistent, a more complex integrated defense system becomes overlaid upon the generic effectors. Among the cells that mediate important functions in the immune system are macrophages and lymphocytes.

Macrophages play a central role in that they serve as ‘scouts’ to advise the immune system of the persistence of noxious stimulants. Macrophages are also critical in helping the immune system develop a conformationally specific picture of the stimulating target. In turn, specialized B-lymphocytes called ‘plasma cells’ produce conformationally specific antibody that binds the target of interest and helps the macrophages destroy it. Various blood proteins can be activated by antibody to further aid macrophages in their mission. (Medicine has its roots in medieval philosophy. A commonly used term for antibody is ‘humoral immunity’.)

Meanwhile, T-lymphocytes play a major regulatory role and help macrophages by secreting products that increase their metabolic activity. T-lymphocytes may also suppress immune activation—especially when the target suggested by macrophages tends to be ‘self’. Finally, other lymphocytes become repositories of ‘memory’ and serve as the nucleus of a rapid response force, should the target ever return.

Individual lymphocytes are specialized in that they are committed to respond to a limited set of structurally related targets (antigens). Macrophages, on the other hand, are functional mercenaries and show little discrimination. They are good at collecting information, and very good at destroying targets. They are especially good when aided by antibody or specific lymphocyte activation, and they rely on the lymphocytes to control the overall process.

CHEMISTRY OF SILICONE–MACROMOLECULAR INTERACTIONS

In biomedical applications, environmental stability tends to be particularly important in three areas—processing, sterilization and long-term implantation. Because silicones were initially felt to be inert and therefore capable of lifetime implantation, the long-term implantation consequences of chemical and hydrolytic degradation are of primary interest.[49]

Being hydrophobic polymers, silicones rapidly perturb their immediate microenvironment. The chemical consequences of these perturbations are two-fold. First, the environment responds to the perturbation in a purely thermodynamic manner by slowly hydrolyzing the siloxane bond. The contribution of specific cells with their oxidative and degradative enzymes is still not well established. Second, the relatively large self-aggregating particles and droplets of silicone interact non-covalently with surrounding macromolecules, adsorbing them to their surfaces, and denaturing them. The consequences of the latter reactions are primarily biological.

Degradation of silicones

While it appeared that silicones might be resistant to degradation, the first evidence to the contrary appears to have been uncovered during metabolic studies of 2,6-*cis*-biphenyl D_4. By 1980, it was apparent that the cyclic silicone oligomer could be degraded into oligomeric diols. Its two major metabolites, identified in both man and monkey, were dimethylsilanediol and phenylmethylsilanediol. Both of these silanediols are more hydrophilic than the parent compound.[30] Recent studies have reaffirmed the hydrolytic degradation potential of silicones and have established the activation energy at between 17.2 and 19.3 kJ mol^{-1}.[50] Other degradative mechanisms, including oxidation, have been proposed, but recent *in-vivo* data suggest that, irrespective of the pathway, a family of reactive silicon intermediaries are established.[51–53] Recent studies using ^{29}Si NMR offer evidence that these reactive intermediaries ultimately yield in humans a full range of silicon-based molecules, including native PDMS, hydrolyzed silicone, silica gel and highly coordinated silicon.[54]

The exact elements that promote degradation are not well established. Hydrolysis simply by being in the presence of the aqueous biological environment appears to be sufficient and can generate reactive hydroxyl moieties; however, even fungi have been shown to contribute to the erosion of implanted elastomeric materials.[55–60]

Other mechanisms of silicone degradation may be related, in part, to the absorption of foreign macromolecules.[61,62] Studies of the mechanical properties of silicone rubber during static, non-loaded subcutaneous or intramuscular implantation showed an 8% reduction in tensile strength after only 24 months. At the other extreme, silicone subjected to drastic cyclic long-term fatigue massively absorbs lipids and can become substantially embrittled.[63]

Denaturation of adsorbed macromolecules

In an aqueous environment, the hydrophobic residues of typical biomolecular species are essentially buried, on average, with the exception of a small residual area that is approximately constant. The moderately polar residues and the very polar residues also appear to bury a characteristic fraction of their available surface by going from the stochastic standard state to the folded state.[64] Upon contact with a hydrophobic material such as silicone, both adsorption and molecular denaturation are both enthalpically and entropically favorable and therefore inevitable (Fig. 2).[65]

Evidence of biologically significant molecular denaturation following surface adsorption is abundant.[66–83] Molecular denaturation following adsorption to silicone is no exception to the general rule and has long been the subject of concern.[84] There is indirect evidence of molecular denaturation on silicone through studies of changes in enzyme function and molecular loss of standard antigenicity.[85–90] There are assays of cell function that show differences in cell behavior depending on the chemistry of the substrates upon which the protein is adsorbed.[91–93] Last, there is direct evidence through conformational studies of molecular structure. The data show that for certain proteins, the β secondary structure is more stable on hydrophobic surfaces such as silicone.[94]

With respect to the latter, aqueous FTIR/ATR data show that fibronectin undergoes significant conformational changes with an approximate 10% reduction in the ratio of β-sheets to β-turns after adsorbing to siliconized surfaces.[95] On the other hand, the cationic apolipoprotein (apo) B/E

receptor binding regions of ApoB acquire increased ratios of β-sheets to β-turns after adsorbing to siliconized surfaces.[96] Two structural studies of fibrinogen adsorbed to siliconized surfaces using the scanning force microscope showed two different conformations of adsorbed fibrinogen as a function of surface chemistry. When adsorbed to silicone, there were 40 nm globular structures and a 60 nm trinodular structure. In addition, 10–20 nm surface asperities were observed.[97] In contrast, when adsorbed to a hydrophilic surface, the fibrinogen assumed an end-to-end linear network matrix.[98] Last, the incubation of either fibrinogen or fibronectin with D_4 induces conformational changes demonstrable by both fluorescence quenching and circular dichroism spectroscopy.[99]

BIOLOGICAL CONSEQUENCES OF SILICONE

The realm of potential biological activities is great. There are pharmacological properties that may affect various organ systems. There are physical properties that may promote infection and possibly cancer induction. There are surface chemical properties that, in conjunction with non-covalent macromolecular surface interactions, appear to stimulate inflammation. Sorting these potential factors and determining their actual relationship to disease states, and the prevalence thereof, is a highly controversial matter.

What is clear is that silicones are toxic organometallics with broad biological activity. The data in this regard are overwhelming. What is not clear is the prevalence of the various disease states elicited by silicone exposure. The data in this regard are drawn largely from the field of epidemiology, and it is in this limited domain that the controversy is strongest.

Pharmacological properties

A number of different pharmacological and toxicological properties have been observed following the administration of various silicones. Pharmacological properties seem to involve the central nervous system primarily. Toxicological properties are more diverse.

Central nervous system activity

The most studied pharmacological property of 2,6-*cis*-biphenyl-D_4 is its estrogenic effect. Only this aforementioned geometric configuration of the four possible isomers possesses hormonal activity. Significant feminizing activity has been observed in rabbits, rats, dogs and rhesus monkeys. The mode of action is primarily through the central nervous system with the primary activity in the hypothalamus and the anterior pituitary.[23,30,100] Although the mechanisms are not quite understood, silicones also appear to trigger an increase in central nervous system dopamine content.[101]

Other cellular and organ toxicity

A film of silicone alone is markedly cytotoxic to a broad range of cells.[102,103] In rats, silicone stimulated increased activity of serum β galactosidase, a non-collagenous protease significantly elevated in specific types of fibrotic disease processes such as scleroderma.[104–106] In mice, exposure to intraperitoneal silicone fluids, gels and rubber, for 180 days, produced significant changes in immune cell behavior.[107] The bone marrow had a significantly increased number of granulocyte–macrophage colony-forming units, there was a significantly increased hepatic macrophage uptake of radio-labeled sheep erythrocytes, and there was a significant dose-dependent reduced responsiveness of NK cells against YAC target cells.

While all implanted materials create a risk of infection, some authors have suggested that silicones create a disproportionately greater risk.[108] The cancer-promoting properties of silicones have been studied in many animal models. In addition to the classical glandular cancer of the breast, there are continued suggestions that rare squamous cell cancers[109] and desmoid tumors of the breast,[110] and plasma cell lymphomas, may be related to silicone exposure.[111] The issue of the malignant potential of breast implants is beyond the scope of this surface chemistry article, and readers are directed to a recent review of the subject.[112]

Consequences of non-covalent macromolecular interactions

When PDMS is present in the blood stream, there is interfacial denaturation of the plasma proteins by intramolecular energies. The biological effects of these denatured proteins, particularly, are emboli and sludge leading to vascular stasis,

morbidity and death.[113] Adsorbed proteins, especially fibrinogen and fibronectin, also appear to facilitate subsequent bacterial attachment and infection.[114] Outside the blood stream, inflammation and immunological activation are the principal consequences of silicone's interaction with body proteins.[115] Irrespective of the organ system exposed, silicones cause inflammation. In part, the magnitude of the inflammation appears to be related to the amount of D_4.[116] It also appears to be related to the physical dimensions of the silicone, with high-surface-area micron and submicron droplets evoking the greatest reactions.[65]

Immunity

Various organosilicon fluids have long been known to potentiate the formation of humoral antibody, modulate cell-mediated immunity and promote the induction of interferon by stimulation of the immune system.[117] Humoral immunity that is enhanced by the non-covalent bonding of macromolecules to silicone has been observed repeatedly in small-animal studies.[118–121] Cellular immunity to silicone-associated molecules has been demonstrated in guinea pigs, sheep and humans.[92,122,123] With respect to humans, T cell proliferation studies in response to stimulation from silicone dioxide, elemental silicon, or silicone gel consistently show greater degrees of reactivity in patients with silicone breast implants.[124] Moreover the activity of lymphocytes in patients who have undergone breast augmentation for aesthetic reasons seems to be greater than the responsiveness of lymphocytes in patients who underwent breast implantation for reconstructive purposes.[125]

Autoimmunity

In autoimmunity, the immune reaction is directed against native or 'self' macromolecules. It is in this context that the binding of native macromolecules to surface-active silicones may produce their most significant biological consequence.

Mechanisms to suppress autoimmunity and induce immunological tolerance to self are among the most fundamental properties of the immune system. While the ability to make antibodies that react with self exist, under normal circumstances, antibodies to native proteins are not made.[126] However, tolerance can be broken if the body becomes immunized to a foreign molecule that mimics a native molecule. In principle, a protein could engender autoimmunization if it contained an epitope that closely resembled an epitope in the host protein. Such a protein could trigger a cross-reaction on the part of the host's T cells. Classic examples of molecular-mimicry pairs and disease, including rheumatological disease,[127] abound (Table 1).

Experimental autoimmunity has been induced in animals by stimulating them with silicone mixed with the connective tissue protein, collagen.[128] In humans, antibodies reacting with molecules that are considered markers of classical autoimmunity have been reported in abnormal concentrations and distributions in patients with breast implants.[129,130] Antibodies reacting against other (denatured) proteins not commonly associated with classical autoimmune

Table 1. Molecular mimicry and autoimmune disease

Molecular mimic[a] eliciting antibodies	Self or native macromolecule targeted (or cross reacted with) by antibodies	Disease caused by antibody attack
Streptococcal M protein	Cardiac myosin	Rheumatic heart disease
Mycobacterium tuberculosis	Cartilage glycan	Adjuvant arthritis
Hepatitis D polymerase	Myelin basic protein	Neuropathy
Penicillin-bound denatured red blood cell membrane complex	Red blood cell membrane	Hemolytic anemia
Retrovirus 120/41 glycoprotein, *env*, and *gag*	70-kDa protein of the U1-snRNP	Mixed connective tissue disease[b]
Silicone-bound denatured native protein	Native protein	Silicone-associated disease

[a] R. S. Schwartz, In: W. E. Paul, In: *Fundamental Immunology*, 3rd edn, Wallace, D. J. and Hahn, B. H. (eds), Lea and Febiger, Philadelphia, 1993, pp. 442–454.
[b] Ref. 127

disease have also been observed.[131–133] The significance of the latter statement is that one of the main discriminating serological features of drug-induced autoimmunity is the absence of many of the autoantibodies usually seen in patients with classical autoimmune diseases.[134]

For example, in a recently completed study of more than 640 women, the frequency of novel autoreactive antibodies to silicone surface-associated antigens [anti-SSAA(x)] was measured in healthy control patients, symptomatic patients with breast implants, asymptomatic patients with breast implants, and control patients with classical rheumatological diseases. The frequencies of elevated anti-SSAA(x) antibodies in 310 symptomatic breast implant patients were 17.4% anti-SSAA(fibronectin), 12.9% anti-SSAA(collagen 1), and 7.4% anti-SSAA(collagen 3) and 7.1% anti-SSAA(fibrinogen) [Normal ($n=173$) = 0.6% for all four tests] ($P<0.005$). In 11 asymptomatic breast implant patients, the frequencies of elevated values for the same anti-SSAAs were 0%, 9%, 0%, and 0% respectively, while in 50 patients with rheumatoid arthritis, the frequencies were 4%, 0%, 6% and 2% respectively. The anti-SSAA(x) profile for symptomatic patients with breast implants was different from the profile for control healthy patients ($P<0.005$ on all eight tests) but differed significantly by two measures, anti-SSAA(fibrinogen) and anti-SSAA(collagen 3), from the profile for the 19 patients with systemic lupus erythematosus.[135]

SILICONE-ASSOCIATED DISEASE STATES

Medical investigators frequently use epidemiological studies to uncover statistical associations, and therefore potential causal connections, between etiological agents and various disease states. There are two major challenges to conducting meaningful epidemiological studies on silicone-associated diseases. First, studies of silicone-associated diseases have been conducted in the absence of what is considered to be the single most important feature that determines the reliability of a study—well-defined, generally recognized diagnostic criteria.[136] Second, it is generally accepted that modern epidemiology is not sufficiently robust to identify subtle increased risks of disease—even if they may have potentially huge impacts on public health.[137] It should therefore come as no surprise that the growing number of epidemiological studies in this field have, on the whole, been of limited value.

A number of studies conducted at different institutions have failed to find an association between silicone exposure and specific and well-characterized classical autoimmune diseases.[5,138–142] In general, these studies have been criticized for being incapable of finding moderate risks of disease for the reasons cited above.[143,144] Other criticisms of these studies have focused on conflicts of interests and other potentially confounding variables.[10,11]

A smaller number of studies conducted at some of the same institutions as above, on the other hand, have found at least a moderately increased risk of various rheumatological diseases.[145,146] At the same time, experts have argued that silicones cause disease states that constitute a distinct clinico-pathological entity other than classical rheumatological disease.[147–154] While the reported spectrum of symptoms has confounded epidemiological investigations, the breadth of potential symptoms is consistent with diseases caused by other known environmental agents (Table 2).

EXPERIMENTAL STUDY

Experimental summary

The adjuvant activity of silicone in eliciting immunity against porcine insulin was studied in the rabbit model. After 14 weeks, silicone co-injected with insulin evoked a specific and statistically significant elevation in anti-insulin antibodies, while silicone injected alone induced a statistically significant elevation in a broad range of autoreactive antibodies.

Background

The barrels of disposable syringes are lubricated with silicone. Previous studies have shown that approximately 200 μg of silicone fluid are present in a 0.5 ml syringe, and that approximately 2 μg can be expected to be discharged into distilled water each time the plunger is depressed.[155] An additional amount may be expected if the syringe is filled with a drug that exhibits mild detergent activity. Insulin is such a drug, and it shows great avidity for hydrophobic

Table 2. Biological effects of environmental agents

	Ethanol[a]	Silicone
Range of biological effects	Early: cognitive impairment and sedation. Liver: insignificant histological changes, alcoholic hepatitis and scarring/cirrhosis (approximately 10% of alcoholics actually progress to cirrhosis). Other: heart failure, muscle wasting, nerve conduction failure, structural changes in the brain, pancreatitis, and testicular atrophy.	Brain: cognitive impairment and sedation (chronic lethargy). Breast: insignificant histological changes to the tissues around a breast implant, silicone mastitis, and scarring/capsule formation and contracture (approximately 40% of breast implant patients have severe capsule formation). Other: muscle and joint pain, skin and hair changes, and other symptoms
General biophysical mechanisms	Direct: ethanol. Indirect: one of the few metabolic products generated by liver enzyme processing of this simple two-carbon compound. Ethanol or one of its metabolites stimulates the liver's *p*-450 microsomal oxidative system.	Direct: silicone. Indirect: one of the numerous metabolic products generated by liver enzyme processing of this complex polymer. Silicones stimulate the livers *p*-450 microsomal oxidative system.
Immunological mechanisms	Both alcoholic hepatitis[b,c] and alcoholic heart muscle[d] diseases may be immunological reactions.[e]	Some of the manifestations may be due to immunological reactions against native proteins denatured by silicone.

[a] J. Scholmerich and A. Holstege, *Drugs* **40** (Suppl 3), 3 (1990)
[b] B. Ruhland, L. Walker, A. O. Wollitzer and C. M. Peterson, *Alcoholism, Clin. and Exp. Res.* **15**, 745 (1991).
[c] S. Mishiro, Y. Hoshi, K. Takeda *et al.*, *Lancet* **50**, 152 (1990).
[d] V. R. Preddy and P. J. Richardson, *Br. Med. J.* **50**, 152 (1994).
[e] F. Paronetto, *Sem. Liv. Dis.* **13**, 183 (1993).

surfaces.[156] In fact, it has been calculated that 25–50 g of silicone are administered over a lifetime to patients such as diabetics who repeatedly inject themselves therapeutically with silicone-lubricated syringes.[5]

Hypothesis

If silicone were administered in the presence of one particular molecule, such as insulin, it would surface-associate with that molecule (adsorb) and promote humoral immunity against that same molecule. On the other hand, if silicone were administered alone where it would be free to associate with molecules of all types within the host environment, it would induce broad humoral immunity. Using porcine insulin as the specific molecule of choice, we measured the ability of silicone to elicit antibodies against insulin (when co-injected with insulin) and autoreactive antibodies (when injected alone) in the rabbit model.

Method

The study was performed on 16 New Zealand White rabbits in accordance with institutional (UCLA) and National Institutes of Health (NIH) guidelines regarding the humane use of research animals.

Injection of insulin and control formulations

From each of the 16 rabbits, 5 ml of blood was drawn for baseline antibody studies and the sera were stored frozen at $-70\,^{\circ}C$ for later analysis.

The animals were divided into groups of four and each group underwent subcutaneous injection with 2 ml of one of the following four materials:

—Two units (2 U) of porcine insulin (Eli

Lilly, Indianapolis, IN, USA) diluted in phosphate-buffered saline (*negative control*);
- Two units (2 U) of porcine insulin (Eli Lilly) diluted and well mixed in silicone oil (Dow Corning 200 fluid, Midland, MI, USA);
- Two units (2 U) of porcine insulin (Eli Lilly) diluted in Freund's Incomplete Adjuvant (Sigma, St Louis, MO, USA) (*positive control*);
- Silicone oil (Dow Corning 200 fluid) only (*vehicle control*).

An international unit of insulin (U) is a standard dilution containing sufficient quantity of dispersed functional insulin capable of reducing the fasting serum glucose of a sample of rabbits by a specified amount. In general, 100 U will contain less than 0.65 mg of extractable nitrogen (source: *US Pharmacopeia*).

The solutions for injection were divided into 1 ml aliquots and injected in two different sites, i.e. subcutaneously between the shoulder blades (1 ml) and intramuscularly in the hind leg (1 ml). Repeated injections were administered on weeks 2, 4, 7, 8 and 12 for a total of six 2 ml injections over the course of the 14-week study. Blood samples of 5 ml were collected from each animal immediately after the injections each week and at the end of the 14-week study, and the sera were stored as noted above.

Two of the four animals injected with insulin–saline, and one of four animals injected with insulin–silicone, died over the course of the experiment. Autopsy disclosed pneumonia as the cause of death. Although their antibody titers were not used in the antibody calculations presented below, interim analysis showed that their values were not significantly different from the values of other members of their group.

Antibody assay

Serum antibodies were analyzed using an enzyme-linked immunosorbent assay [ELISA]. Standard polystyrene 96-well microtiter plates (Falcon 3913 microtest III, Becton Dickinson, Franklin, NJ, USA) were cleaned with 1.2 M HCl for 1 h to remove all contaminants bound to the plate surfaces. The plates were then neutralized with three washes of buffered saline, and the wells were coated with 250 ml of 50 mM D-(+)cellobiose in 0.1 M sodium bicarbonate, pH 7.4, and incubated overnight at 4 °C. The cellobiose film tends to conserve the native aqueous conformation of surface-bound macromolecules (antigens).[157]

After excess cellobiose was removed gravitometrically, macromolecular antigens for antibody avidity assays were then added to the cellobiose-coated microtiter plates as follows, on a per-well basis.

- porcine insulin (Eli Lilly) at a concentration of 1 U per 125 ml of 0.1 M sodium bicarbonate;
- myelin basic protein (Sigma) at a concentration of 10 mg in 125 ml of distilled water;
- fibronectin (Calbiochem, La Jolla, CA, USA) at a concentration of 10 mg in 125 ml of distilled water;
- myosin (Sigma) at a concentration of 10 mg in 125 ml of 10 mM potassium phosphate, pH 6.8.

The plates were incubated at 4 °C, after which the wells were again emptied gravimetrically. A blocking solution of 250 ml 2% bovine serum albumin in phosphate-buffered saline (PBS) was added to each well and incubated for 3 h at room temperature. The plates were washed with three successive 250 ml aliquots of wash buffer (pH 7.4/1.91 mM KH_2PO_4/8.09 mM Na_2HPO_4/150 mM NaCl/0.5% v/v Tween) and then sera from the rabbits were added in triplicate dilutions (1 ml in 124 ml of PBS per well per plate) and incubated overnight at 4 °C.

The following morning, the plates were washed in triplicate after which 125 ml of secondary goat anti-rabbit alkaline phosphate conjugate (Sigma) diluted 1:9000 in 50 mM Tris, pH 7.4, was added to each well and incubated for 50 min at 25 °C. Following three additional washes, 125 ml of freshly prepared pNPP substrate (*p*-nitrophenol tablets; Sigma; 5 mg tablets in pH 9.80/1.0 M diethanolamine/0.5 mM $MgCl_2$/0.02% NaN_3 to a concentration of 1 mg ml^{-1}) was added to each well and allowed to incubate at 25 °C in the dark for 10 min. The colorimetric reaction was quantified by the absorption of light at 405 nm (BioRad Model 3550 microplate reader, Hercules, CA, USA). The absorption values were corrected for plate-to-plate variation by subtracting the measured values from absorption values for a standard solution of pooled native rabbit sera. A total of 1092 data points were collected. The statistical significance of the observed differences in antibody avidity, as

indicated by the absorption intensity, for each of the four macromolecular antigens in each of the four groups of animals over the 14-week period was calculated using Students *t*-test for means or paired samples.

The change in antibody (IgG) avidity to the four different macromolecular antigens differed significantly among the various groups (Fig. 3). The two surviving animals injected with insulin admixed with PBS, a putative negative control, showed no statistically significant change in antibody avidity to any of the antigens. While not statistically significant owing to a great deal of variance in the response, insulin when admixed with Freund's adjuvant, a putative positive control, elicited some degree of increased humoral avidity to myosin, myelin, insulin and fibronectin.

On the other hand, insulin when admixed with silicone elicited a significant increase in IgG binding avidity to insulin (two-tailed $P = 0.032$) and silicone alone elicited a significant increase in IgG binding avidity to fibronectin (two-tailed $P = 0.022$).

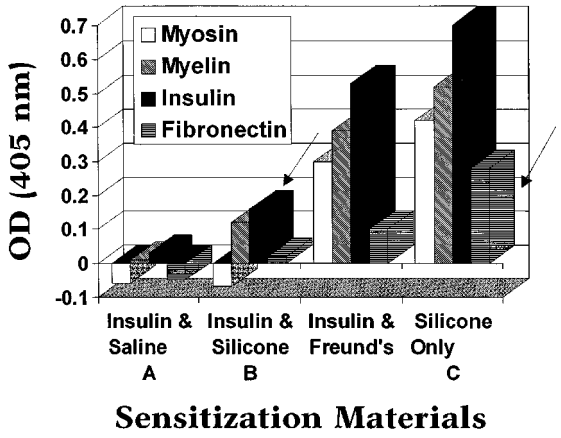

Figure 3 Change in IgG binding avidity to four different macromolecules, myosin, myelin, insulin and fibronectin, after 14 weeks of sensitization, demonstrating the immune sensitization potential of silicones against native or self molecules. Rabbits 'sensitized' with insulin admixed with PBS showed no increased antibody avidity (A). Thus porcine insulin alone is a poor immune sensitizer. Animals 'sensitized' with insulin admixed with silicone showed a specific increased avidity to insulin ($P < 0.05$) (B), while animals sensitized with silicone alone showed an increased avidity to fibronectin ($P < 0.05$) (C). These data also show that both Freund's adjuvant and silicone alone elicited a range of antibody reactivity indicative of generalized inflammatory activation. Overall, the data suggest that silicones have the potential for triggering both specific autoimmunity as well as a general increase in autoreactive antibodies.

SUMMARY

The biological effects of (dimethyl)silicones are classical environmental chemistry phenomena. While the association of silicone with a distinct human disease state remains an epidemiological challenge, there are ample doubts as to the medical safety of silicones. There are four primary driving forces for these persistent concerns.

(1) There is no evidence to support claims of safety. Both the medical and regulatory standards require that the known risks be understood and that the risks must not outweigh the benefits. To date, the recent epidemiological studies either suggest a moderate risk or cannot rule out a moderate risk of specific rheumatological diseases. There are not epidemiological studies of other types of diseases.
(2) There is a wealth of empirical small-animal experimental research *demonstrating* a broad range of adverse biological effects and existing environmental chemistry models supporting a cause-and-effect relationship.
(3) There is a wealth of basic physicochemical data providing strong evidence that silicones *should* cause inflammatory and immunological reactions.
(4) There is a growing body of evidence that humans exposed to silicone, primarily in the form of breast implants, have *both* inflammatory and immunological reactions.

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