

Effect of Silicone on the Collagen Fibrillogenesis and Stability

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ABSTRACT: Collagen, the most abundant protein in mammals, is able to form fibrils, which have central role in tissue repair, fibrosis, and tumor invasion. As a component of skin, tendons, and cartilages, this protein contacts with any implanted materials. An inherent problem associated with implanted prostheses is their propensity to be coated with host proteins shortly after implantation. Also, silicone implants undergoing relatively long periods of contact with blood can lead to formation of thrombi and emboli. In this paper, we demonstrate the existence of interactions between siloxanes and collagen. Low-molecular-weight cyclic siloxane (hexamethylcyclotrisiloxane—D3) and polydimethylsiloxanes (PDMS) forming linear chains, ranging in viscosity from 20 to 12,000 cSt, were analyzed. We show that D3 as well as short-chain PDMS interact with collagen, resulting in a decrease in fibrillogenesis. However, loss of collagen native structure does not occur because of these interactions. Rather, collagen seems to be sequestered in its native form in an interlayer formed by collagen–siloxane complexes. On the other hand, silicone molecules with longer chains (i.e., PDMS with viscosity of 1000 and 12,000 cSt, the highest viscosity analyzed here) demonstrate little interaction with this protein and do not seem to affect collagen activity. © 2015 The Authors. *Journal of Pharmaceutical Sciences* published by Wiley Periodicals, Inc. and the American Pharmacists Association *J Pharm Sci* 104:1275–1281, 2015

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INTRODUCTION

Silicone polymers are assumed to be chemically stable in living systems because of their intrinsic properties such as thermal stability and non-adhesiveness to tissues. Therefore, silicones have been used in the medical field for many years as biocompatible biomaterials, ranging from intraocular lenses¹ to breast implants.² Silicone fluids are widely used as surgical tamponade in severe cases of vitreoretinal pathology³ and in compounds of bioactive organic–inorganic materials⁴ and drug delivery systems.^{5,6} As silicone has a long history as a biomaterial, its safety has been carefully investigated. Nevertheless, some of research works, specifically those that focused on implanted silicone prostheses, suggest that silicone is an adjuvant to the human immune system⁷ and may induce conformational changes and aggregation of proteins.^{8,9} Furthermore,

patients with silicone prostheses display a variety of nonspecific syndromes that usually disappear after removal of the implant.^{10–12} An inherent problem associated with implanted prostheses is their propensity to be coated with host proteins such as fibrinogen and fibronectin shortly after implantation. Implants can then act as a colonization surface to which bacteria readily adhere to, thanks to their protein binding receptors. Also, silicone implants undergoing a relatively long contact with blood can lead to the formation of thrombi and emboli.³ Studies by other authors^{13,14} also suggest that the reason for the observed host's tissue damage and native immune response can be caused by low-molecular-weight silicones (LMWS) consisting of both cyclic and linear compounds. These silicone fluids are used in the manufacturing of many high-molecular-weight silicone polymers including those used as biomaterials and can migrate from the implantation site to surrounding tissues and distant organs in the body.¹⁵

The adsorption of proteins onto materials' surfaces plays an important role in determining the design of biomaterial substrates for biotechnology and tissue engineering applications. The governing force contributing to this adsorption is either a hydrophobic or electrostatic interaction. In research works, the interaction of silicone fluids with any biological fluid is largely controlled by the adsorbed fibrinogen, bovine serum albumin (BSA), or mioglobin protein layer.^{2,16} Some reports found that in the presence of the LMWS, fibrinogen and fibronectin were shown to undergo denaturation.^{17–19} At the same time, the albumin–silicone interactions do not lead to conformational changes in the protein.¹⁹ This behavior is closely associated

Abbreviations used: CI, collagen type I; cSt, centistokes; D3, hexamethylcyclotrisiloxane; FITC, fluorescein isothiocyanate; LMWS, low-molecular-weight siloxanes; PDMS, polydimethylsiloxane; $T_{1/2}$, collagen fibrillogenesis half-time.

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with the protein structure and concentration, physical and chemical properties of the silicone surface, solution ionic environment, operating conditions, and consequent conformation at the water/silicone fluid interface.

So far no studies have been reported on investigating the effect of cyclic and linear siloxanes on the structure and function of collagen. Collagen is the most abundant protein in mammals and the major structural protein in the human body. Its molecules consist of three polypeptide chains with a significant amount of hydrophobic domains that are coiled around one another into a triple-helical conformation. Collagen molecules spontaneously form fibrils *in vitro* which shows that collagen fibrillogenesis is a self-assembly process.²⁰ This spontaneous process occurs *in vitro* because purified collagen molecules are free to bind to other collagen molecules, and only collagen molecules are present. *In vivo*, there are many binding partners that can stimulate or alter the fibrillogenesis process generating the diversity of fibril patterns. Because silicone is a hydrophobic polymer consisting of polar siloxane segments (Si-O-Si) and non-polar methylene groups (Me₂Si),³ adsorption of collagen onto the hydrophobic surface may occur by hydrophobic-hydrophobic interactions. It is likely that depending on the nature of the silicone molecules, the binding will be different in quantity as well as quality and lead to different interaction forces as well as amounts bound. If the collagen interacts with siloxanes from implants, the silicones can locally act as another “binding partner” in the fibrillogenesis process *in vivo*. This can lower the effective concentration of collagen monomers available to form fibrils and can disturb the local homeostasis.

There was a dual purpose of this study: first, to examine the physical nature of interactions between collagen and different silicones, and the resulting effect on the fibrils’ formation under *in vitro* conditions; second, we were interested in determining whether protein-silicone interaction mediates collagen conformational changes. Potential interactions between collagen and silicone surfaces would lower the effective concentration of collagen monomers available to form fibrils and also lead to conformational changes in the collagen molecules.

EXPERIMENTAL

Materials and Reagents

In all experiments, commercially available collagen type I from rat tails was used (Sigma-Aldrich, Poznan, Poland). Hexamethylcyclotrisiloxane (D3) and PDMSs (20, 100, 1000, and 1200 cS) were purchased from Sigma-Aldrich, Poznan, Poland). For measuring the native collagen concentration, “Sircol Collagen Assay” (Biocolor, County Antrim, UK) was used. For total collagen concentration measurement by the Lowry method, the phenol Folin-Ciocalteu reagent (Sigma-Aldrich, Poznan, Poland) was used. Collagen purification after fluorescence labeling was performed with the use of Sephadex G-25 Medium bed (GE Healthcare, Warsaw, Poland). Other reagents (unless stated otherwise) were purchased from Sigma-Aldrich (Poznan, Poland).

Methods

Preparation of Native Collagen Solution

About 2 mg of collagen was placed in a 2 mL vial. Next, 1 mL of 0.1% acetic acid solution in water was added. The solution was

incubated at 4°C with shaking overnight. Next day, the concentration of the native collagen fraction was measured using section *Measurement of Native Collagen Concentration* (below). The solution was diluted with the use of 0.1% acetic acid to achieve the final concentration of 1 mg/mL. Such solution was stored at 4°C and used for further analysis.

Measurement of Native Collagen Concentration

For native collagen concentration determination, a commercially available kit from Biocolor was used. The native collagen concentration was determined using a modified manufacturer’s protocol: 100 µL of the collagen solution, corresponding amount of siloxane and 500 µL of “Sircol Dye” was added to a 2 mL Eppendorf type vial. The vial was incubated for 30 min at room temperature with shaking. Next, the sample was centrifuged for 10 min (15,000 *g*). The supernatant was discarded and 500 µL of ethanol was added to the remaining sediment and vortexed. After shaking, the sample was again centrifuged at 15,000 *g* for 2 min, the supernatant was discarded and the sediment was suspended in 500 µL of Alkali reagent. Next, the sample was incubated in a thermal mixer for 10 min at room temperature. The solution obtained was analyzed by spectrophotometry at 540 nm. For the determination of the final concentration, a 5-point calibration curve was created (at each concentration point three measurements were performed). The calibration was performed using external standards in the range of 0.05–0.4 mg/mL. Each measurement was referenced to a blank test sample prepared in an analogous way to the prepared sample. The experiment was repeated three times and averages, SDs, relative SD, standard errors were calculated.

Measurement of Total Collagen Concentration

For total collagen concentration determination, modified Lowry method was used.²¹ Fifty microliters of collagen solution, corresponding amount of siloxane, 50 µL of acetic acid solution, 90 µL of solution A, and 10 µL of solution B were added to a 2 mL vial. The vial was then incubated in a thermal mixer for 20 min at 50°C with constant shaking (500 rpm). After cooling down to room temperature, 300 µL of Folin-Ciocalteu reagent was added to the sample. Next, the sample was vortexed for about 15 s. Then, the vial was incubated in a thermal mixer for 10 min at 20°C with constant shaking (500 rpm). The obtained solution was analyzed by spectrophotometry at 650 nm. For determination of the final concentration, a 5-point calibration curve was created (at each concentration point three measurements were performed). The calibration was performed using external standards in the range of 0.01–0.2 mg/mL. Each measurement was referenced to a blank sample prepared in an analogous way to the prepared sample. The experiment was repeated three times and averages, SDs, relative SD, standard errors were calculated.

Collagen Fibrillogenesis

To monitor collagen fibrillogenesis, 80 µL of 0.2 mg/mL collagen solution was placed in a 2 mL vial. Next, 80 µL of fibrillogenesis buffer [60 mM NaH₂PO₄, 1.4% NaCl (w/v), pH 7.5] was added. The sample was mixed with the use of an automatic pipette and immediately transferred to a quartz cuvette. The cuvette was placed in a thermostat measurement chamber. The PerkinElmer LS 55 Fluorescence Spectrometer was used at the temperature of 32°C in the cuvette. The excitation and

emission wavelengths were set to 550 nm so that the analysis of light scattered by the incubated solution was possible. Measurements were conducted in 3 min intervals until an equilibrium was reached (signal intensity remained at a constant level). Each experiment was repeated three times and fibrillogenesis halftimes, signal amplitudes were estimated.

Collagen FITC Labeling

For collagen fluorescent labeling, a modified Antonio Baici method²² was used. Three milliliters of native collagen solution was placed in a dialysis bag. The dialysis bag was then placed in a beaker containing 500 mL collagen labeling buffer 1 (0.25 M sodium bicarbonate, 0.4 M NaCl, pH 9.5) for 1 h at 4°C. Dialysis was repeated for 24 h and for 1 h in the same as above conditions. Twenty milligrams of fluorescein isothiocyanate (FITC) was dissolved in 200 mL of collagen labeling buffer 1. Next, the dialysis bag containing collagen was placed in the prepared buffer for 24 h in darkness, at 4°C with stirring. To remove excess of the fluorescent marker, the dialysis bag was then placed in 500 mL of collagen labeling buffer 2 [0.2% (v/v) acetic acid, pH 4.0] for 1 h under the same conditions as mentioned above. Dialysis was repeated twice for 24 h under the same conditions, using 500 mL of collagen labeling buffer 2 for each dialysis. The labeled collagen solution was applied to a Sephadex G-25 bed. Separation was performed at 0.2 mL/min flow. Fractions (1 mL) were collected. Fractions containing the highest protein concentration were transferred to a centrifuge vial. Collagen was precipitated by slow addition of NaCl to the final concentration of 5% (w/v). The sample was centrifuged afterwards for 1 h (50,000 g) at 4°C. The supernatant was discarded and the sediment was suspended in 3 mL of collagen labeling buffer 2 and left for 24 h in darkness at 4°C with constant stirring. Next the concentration of native collagen was measured.

Influence of Siloxanes on Stability and Activity of Collagen

In each case of protein–silicone analysis, the incubation was performed according to the following procedure. An excess of silicone relative to collagen was placed in a tube, then the buffer was added. The sample was sonicated and incubated under experimental conditions (30°C) overnight to achieve an equilibrium. The next day, collagen was added to the incubation mixture, final concentration of 0.2 mg/mL. In predefined intervals, a sample from the incubation mixture was collected in order to determine the concentration of the native collagen fraction (section *Measurement of Native Collagen Concentration*), total collagen concentration (section *Measurement of Total Collagen Concentration*), and to monitor fibrillogenesis (section *Collagen Fibrillogenesis*).

Influence of Silicones on Thermal Inactivation of Collagen

In the first stage, the incubation of collagen with silicones was performed in accordance to section *Influence of Siloxanes on Stability and Activity of Collagen* to obtain protein–silicone complexes. This fact was confirmed by microscopic observations of the incubation mixtures. In the next step, obtained complexes were transferred to a higher temperature (37°C) and incubated for 72 h. In predefined intervals, a sample from the incubation mixture was collected in order to determine the concentration of the native collagen fraction (section *Measurement of Native Collagen Concentration*), total collagen concentration (section

Measurement of Total Collagen Concentration), and to monitor fibrillogenesis (section *Collagen Fibrillogenesis*).

Detection of Collagen–Siloxane Complexes by ELISA

In the first stage, the incubation of collagen with silicones was performed in accordance to section *Influence of Siloxanes on Stability and Activity of Collagen* to obtain protein–silicone complexes. In predefined intervals, a sample from the incubation mixture was collected and centrifuged. Layers containing collagen and siloxane were transferred to the wells of 96-well microtiter plates in order to determine the concentration of collagen by ELISA. Primary antibodies against collagen were then added to the wells and mixed. After 60 min of incubation at 37°C, plates were washed with phosphate-buffered saline (PBS) containing 0.05% Triton X-100. Non-specific binding was blocked with BSA (1%) in PBS by 60 min incubation at 37°C. The mixtures were incubated with secondary antibodies conjugated with horseradish peroxidase for 1 h at 37°C. Then, the plates were washed and developed with 50 µL TMB and incubated for 5–10 min at 37°C. The reaction was terminated by adding 50 µL 1 M H₂SO₄ and optical density was measured at 450 nm using an ELISA plate reader (Wallac Victor2 1420).

FTIR Spectroscopy

The incubation of collagen with silicones was performed in accordance to section *Influence of Siloxanes on Stability and Activity of Collagen* to obtain protein–silicone complexes. Subsequently samples were centrifuged and top layers containing collagen and siloxane were applied to CaF₂ windows. Collagen films were obtained by drying at ambient temperature and pressure for 24 h according to procedure by Usharani et al.²³ Control films of native and thermal-denatured collagens were prepared in analogous way. FTIR spectra were obtained on Bruker IFS66 spectrophotometer; 256 coadded scans were collected (resolution of 4 cm⁻¹, range 1000–4500 cm⁻¹).²⁴

RESULTS AND DISCUSSION

Interactions Between Collagen and D3

Because of the fact that cyclic siloxanes are often used as precursors for silicone polymers and it was reported that cyclic siloxanes are present in bloodstream of patients with silicone implants, we examined the interaction between the main building protein of the human organism—collagen, and the smallest cyclic siloxane—D3. During incubation of collagen with D3, a significant difference in fibrillogenesis parameters was observed, that is, change in the amplitude of optical density and change in the $T_{1/2}$ time (time in which the amplitude reaches half of its maximum value) (Fig. 1a). In the sample containing collagen and D3, the $T_{1/2}$ was increased and this increase was nearly linear. Such phenomenon was not observed in the control sample containing only collagen (Fig. 1b).

Subsequently, a decrease in ΔH amplitude was observed (data not shown). This could indicate thermal denaturation of collagen molecules due to the interactions with D3. To investigate this hypothesis, native collagen concentration in the incubation mixture and fibrillogenesis were subsequently assayed. These analyses were performed using two methods. One was tertiary structure specific and allowed to determine the concentration of the native protein only, and the other determined the total concentration of both, denatured and native protein. No significant difference in native collagen concentration during

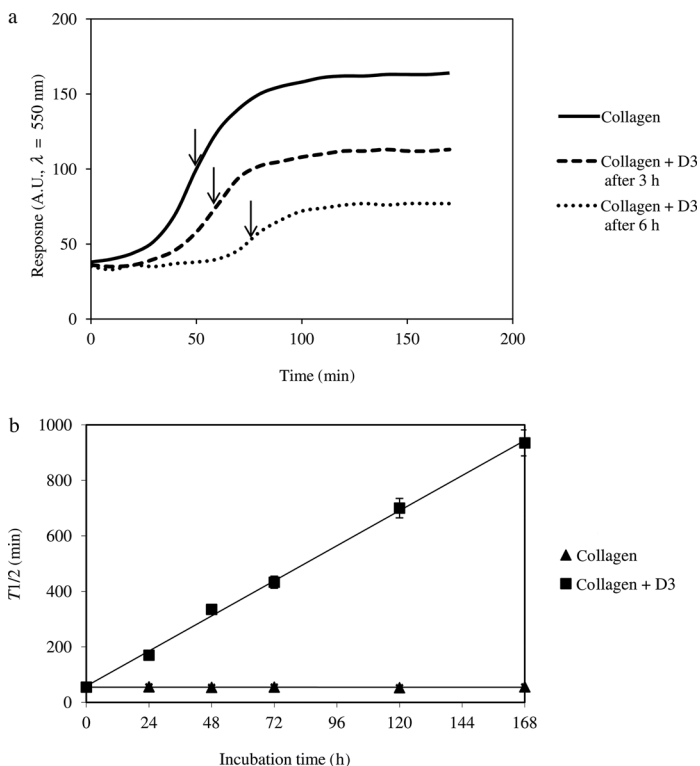


Figure 1. (a) Collagen fibrillogenesis curves for native collagen solution (—), collagen and hexamethylcyclotrisiloxane (D3) after 3 h incubation (---), collagen and D3 after 6 h incubation (.....), collagen fibrillogenesis half-time— $T_{1/2}$ (\downarrow); (b) incubation of collagen with D3 leads to increase in fibrillogenesis time (measured as $T_{1/2}$).

Table 1. Stability of the Native Collagen Incubated with Hexamethylcyclotrisiloxane

Incubation Time (h)	Native Collagen [$\mu\text{g/mL}$ ($\pm\text{RSD}^a$)]	Total Collagen [$\mu\text{g/mL}$ ($\pm\text{RSD}^a$)]
0	100.0 (1.0)	100.0 (0.8)
24	99.7 (0.8)	101.7 (0.9)
72	100.5 (1.1)	99.8 (1.0)
168	99.9 (1.2)	100.2 (0.8)

^aPercentage RSD ($n = 6$).

incubation with D3 was observed (Table 1). This result demonstrates that fibrillogenesis intensity loss was probably due to collagen interaction with silicone that does not induce protein denaturation.

Subsequently, micro and macroscopic changes were observed in the incubation mixture. On a macro scale, a distinct turbidity in the sample containing collagen and D3 was observed. This turbidity did not occur in control solutions lacking either collagen or D3. With the use of a light microscope, silicone micelles were observed. They were only present in the silicone–collagen mixture (Fig. 2). This led us to a hypothesis that collagen stimulates and maintains the polysiloxane emulsification process in an aqueous environment through interlayer interactions.

Interactions Between FITC-Labeled Collagen and D3

Fluorescent labeling of collagen was performed to reveal the interaction site between collagen and silicones. FITC-labeled collagen retains its natural ability to form fibrils, so the ob-

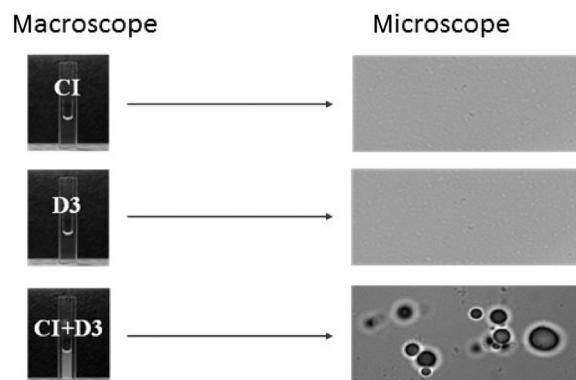


Figure 2. Turbidity changes in incubation mixtures containing control collagen solution (CI), control hexamethylcyclotrisiloxane solution (D3), collagen and hexamethylcyclotrisiloxane mixture (CI + D3).

tained protein was incubated with D3 in an analogous way to the previous experiment. The result indisputably shows an accumulation of fluorescently labeled particles on the interlayer formed through silicone–protein interactions (Figs. 3a and 3b). These results were confirmed using confocal microscopy, the fluorescent material was accumulated only on the surface of

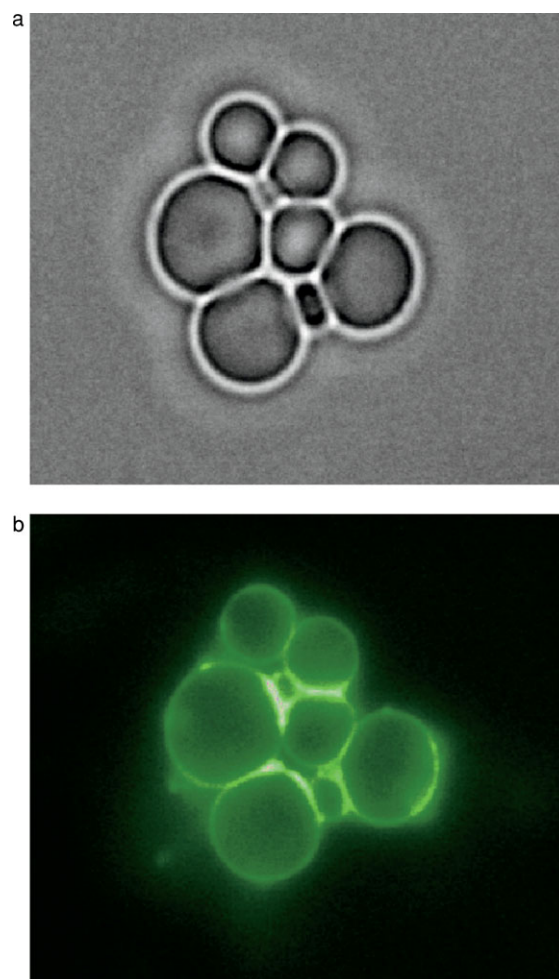


Figure 3. Micrographs of FITC-labeled collagen-hexamethylcyclotrisiloxane micelles after 14 day incubation (a) under light microscope and (b) under fluorescence microscope.

silicone micelles. Inside the micelles there were no fluorescence observed (Supporting Information). It can be reasoned, that such collagen–silicone interactions lead to the reduction in free collagen concentration in the solution. This leads to the observed gradual drop in the rate of fibrillogenesis while at the same time collagen bound with D3 does not lose its tertiary structure (the concentration level of the native form of collagen was constant during the incubation).

Interactions Between Collagen and Polydimethylsiloxanes of Different Viscosities

Low-molecular-weight silicones are usually found in human body as a production impurity and/or as a degradation product. Polydimethylsiloxanes (PDMSs) are used as implantable materials. In this case PDMSs interact with human tissues for many years. Therefore, we examined if any interactions exist between collagen and PDMSs of different viscosities (different chain lengths).

First, the kinetics of collagen–siloxane complex formation for D3 and 20 centistokes (cSt) PDMS were determined using an ELISA assay. In case of D3, the amount of collagen in the siloxane fraction was increasing with time of incubation, and reached about 90% of total collagen in samples after 20 days of incubation. When collagen was incubated with 20 cSt of PDMS, it was observed that the final yield of collagen in the siloxane fraction reached about 70% of the total collagen in the samples (Fig. 4). It was correlated with the increase in turbidity of the incubation solution. The accumulation of collagen in siloxane fractions was confirmed by SDS-PAGE (data not shown). Therefore, the ELISA assay confirmed that PDMS, which contrary to cyclic D3 are linear molecules, might interact with collagen as well.

Next, the effect of PDMS chain length on fibrillogenesis half time was investigated. In the case of chain siloxanes, a correlation was observed—the shorter the chain length, the higher the level of interaction with the protein, as measured by increase in fibrillogenesis half time. The highest level of interaction was observed between collagen and PDMS with viscosity of 20 and 100 cSt. PDMS with viscosity of 12,000 cSt (the longest of the polysiloxanes analyzed here) after 5 weeks of incubation demonstrated only a low level of interaction (Fig. 5).

The interactions between collagen and PDMSs manifest also in the turbidity of the incubation mixture. It is very important because emulsification is an inherent problem of silicone oil use in vitreoretinal surgery. Our results suggests that the possible mechanism of emulsification of the implanted silicone oil could

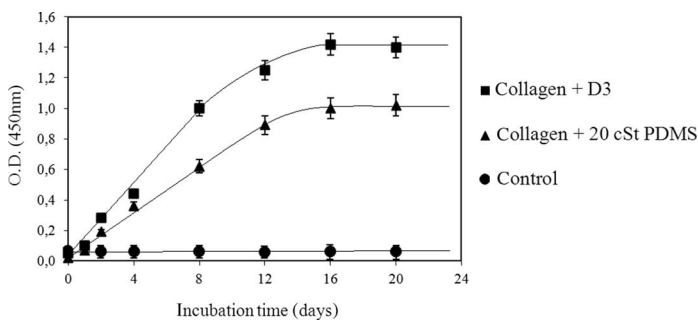


Figure 4. Kinetics of collagen–siloxane complex formation determined by ELISA. Optical density of the reaction mixtures of collagen and hexamethylcyclotrisiloxane (■), 20 cSt PDMS (▲), and control (●).

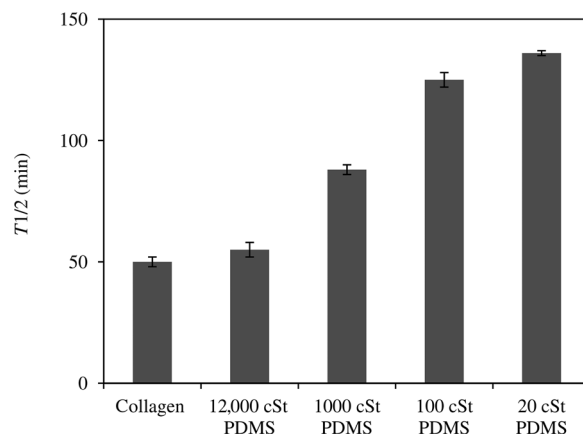


Figure 5. Fibrillogenesis halftimes, $T_{1/2}$, of collagen in the presence of different polydimethylsiloxanes (with increasing viscosity): 20 cSt PDMS, 100 cSt PDMS, 1000 cSt PDMS, and 12,000 cSt PDMS (mean SD = 4.4%).

rely on collagen–PDMS interactions as collagen physiologically exists in the vitreous body and thus might come into contact with PDMS.

Analysis of Secondary Structure Changes in Collagen after Incubation with Siloxanes

The FTIR spectra of collagen–silicone layers obtained in the present study show a specific band for collagen (Amide I with maximum at 1664 cm^{-1} and Amide II with maximum at 1555 cm^{-1}) and silicones (with maximum at 1259 cm^{-1}). The absorption spectrum of thermal denatured collagen shows a shift in peak maximum to shorter wavenumbers which is not observed in case of native collagen and collagen incubated with silicones (Fig. 6).

Amide I band for native collagen displayed a maximal peak centered at 1664 cm^{-1} which corresponds to alpha helix conformation (especially triple helix in collagen). In case of thermal denatured collagen, the intensity of peak at 1664 cm^{-1} is much lower and the highest peak is centered at 1653 cm^{-1} (Fig. 6). This shift refers to the conformation change from alpha helix to random coil.^{25,26} A shift of the spectrum to shorter wavenumbers in Amide II band for denatured collagen was also observed. Incubation with siloxanes does not change the peak maximum in Amide II band. Collagen bound to siloxanes has a FTIR peak profile of collagen without a characteristic shift for denatured forms, which proves that collagen remains in non-denatured conformation.

These observations were confirmed by the analysis of deconvoluted spectra of Amide I band. Native collagen possesses an intense band at 1664 cm^{-1} (Supporting Information, Fig. S3) which relates to the collagen native triple helix.²⁷ Heat-denaturation of collagen leads to decrease the intensity at 1664 cm^{-1} . After incubation of collagen with D3 the intensity of peak at 1664 cm^{-1} was slightly higher than in native collagen. We have also observed a decrease of intensity of 1633 cm^{-1} peak, which could be due to loss of water from collagen molecule.²⁷

Collagen–Silicone Complexes Protect form Thermal Inactivation of Protein

Collagen is the main building protein of the human organism. Subsequently it is prone to thermal inactivation. Under

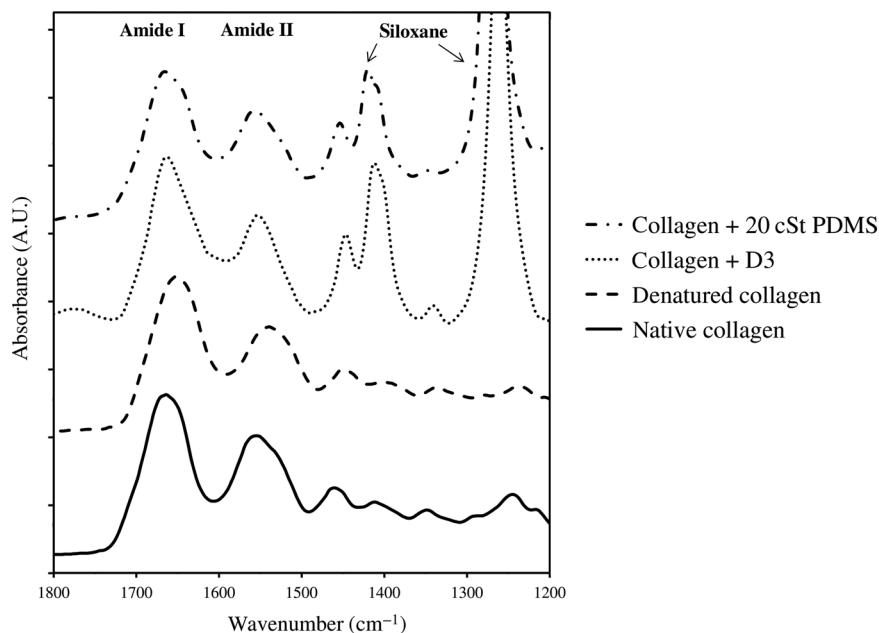


Figure 6. FTIR spectra of native collagen, denatured collagen, collagen incubated with 20 cSt PDMS, and collagen incubated with D3.

elevated temperature conditions this protein loses its trihelical structure and this prevents proper fibrillogenesis. To investigate if the protein–silicone formed complexes can act as protective agents, collagen was incubated with D3 and with PDMS (viscosity 20 cSt). After assembly of the complexes under standard assay conditions (30°C), the temperature of the incubation mixture was raised to 37°C (thermal stress). Incubation at the raised temperature was continued when conducting tests determining the concentration of the native collagen fraction, total collagen concentration, and monitoring fibrillogenesis. For the control sample, that is, containing only collagen, an almost complete denaturation was observed (native collagen concentration was 1 µg/ml, while at the beginning of the experiment it was 100 µg/mL). In the case of samples containing both, collagen and silicone, a certain fraction of collagen exhibited native structure and activity. A correlation between the concentration of polysiloxane in the incubation mixture and the fraction of native collagen was found, namely the higher the silicone excess, the higher the protection from denaturation (Fig. 7). When a 100-fold excess of siloxane was used, about 50% of the collagen fraction remained in its native conformation. If the excess was 50-fold, the level of protection from denaturation was lower—about 40% of collagen remained in trihelical form in case of D3 and about 35% in case of 20 cSt PDMS. Still, even though this kind of collagen–silicone interaction protects from thermal denaturation, it leads to lowering the concentration of free native collagen and thus causes a decrease in the rate of fibrillogenesis.

CONCLUSIONS

We show an existence of interactions between collagen and siloxanes. These interactions manifest themselves by decreasing the collagen fibril forming rate, which is usually taken as the indicator of collagen physiological activity. The decrease in collagen fibrillogenesis we observed was not caused by a change in physicochemical conditions in the incubation mixture or the

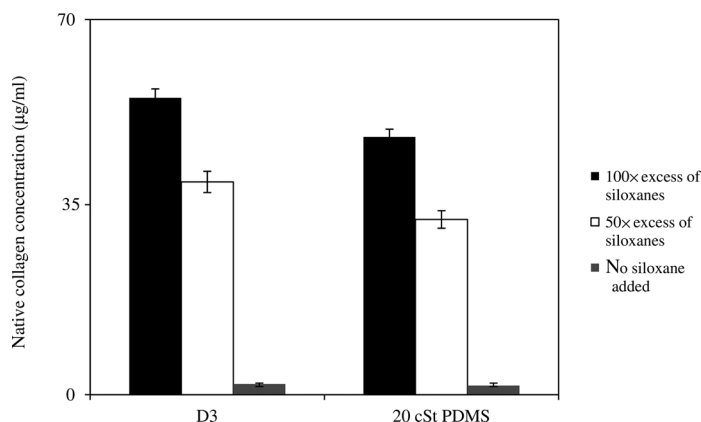


Figure 7. Protection from thermal denaturation of collagen in the presence of hexamethylcyclotrisiloxane and 20 cSt PDMS (nominal concentration of collagen at the beginning of experiment—100 µg/mL; excess of siloxane against collagen was 50-fold or 100-fold).

change in concentration of the two key compounds—silicone and collagen. If a drop in collagen native fraction concentration would occur, it would suggest a change in the tertiary structure stimulated by interactions with silicones (in analogy to results obtained by our group in the previous years for fibrinogen and hemoglobin). If the observed change in fibrillogenesis rate was a result of binding collagen to the walls of a vial, a drop in the collagen concentration would be observed for both, native and total concentration fractions. However, concentration of collagen assayed during incubation did not demonstrate any significant changes, which enabled us to hypothesize that the drop in the rate of fibrillogenesis is a result of exclusion of a certain fraction of collagen because of its direct interaction with polysiloxanes. Confirmation of this hypothesis was obtained by microscopic observations that indicated that the silicone–protein interaction induces emulsification of the incubation mixture. With the use of fluorescently labeled collagen, we have demonstrated

that this interaction is taking place on the silicone–protein interlayer. This result may explain the clinically observed cases of eye lens or vitreous silicone implant turbidity.

We have also shown that in the case of long polymer silicone chains the influence of silicones on collagen is inversely proportional to the length of the silicon–oxygen chain.

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