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

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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 lenses[1] to breast implants.[2] Silicone fluids are widely used as surgical tamponade in severe cases of vitreoretinal pathology[3] and in compounds of bioactive organic–inorganic materials[4] 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 system[7] 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 halftime.

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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 (Me_2Si),[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 fibrilogenesis 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 NaH_2PO_4, 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

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