Investigation of the chemical structure of silicone oil interacting with bevacizumab by spectroscopic methods: A dose recommendation

Feride Tuncer Orhan
Department of Ophthalmology, Eskisehir City Hospital, Eskisehir, Türkiye

Abstract

Purpose: To investigate the effect of the therapeutic doses of bevacizumab, recombinant humanized anti-vascular endothelial growth factor monoclonal antibody, on the silicone oil (SiO) using nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopies.

Methods: Freshly opened 1000-centistoke SiO (polydimethylsiloxane, Micromed s.r.l. Fonte Nuova, Italy) was mixed with three different therapeutic doses of bevacizumab/Avastin (US Biotech unit of Roche Registration GmbH, Grenzach-Wyhlen, Germany), respectively as 1.25 mg/0.05 mL, 2.5 mg/0.1 mL, and 5 mg/0.2 mL. Coupled spectroscopic methods were employed to determine potential chemical shifts in the SiO: In the proton hydrogen-1 NMR (¹H-NMR) spectroscopy, the internal standard and the solvent were tetramethylsilane and chloroform, respectively. The ¹H spectrum was recorded at a frequency of 500 MHz. In the FT-IR spectroscopy, the attenuated total reflectance method was used for sampling, and spectral bandwidth was 4000 to 400 cm⁻¹ at 4 cm⁻¹ resolution.

Results: ¹H-NMR spectroscopy revealed a minor change in the functional groups of SiO after the interaction of the dose of Avastin at 1.25 mg/0.05 mL with no chemical shifts. In contrast, the high therapeutic doses caused remarkable changes in the Si-CH₃ functional groups. FT-IR spectroscopy identified decreasing the absorption peaks intensity of Si-O-Si bending at 1008 cm⁻¹ in the high therapeutic doses. The spectral findings showed the breakdown potential of the SiO due to hydrolytic degradation in the aqueous environment of Avastin (at 2.5 mg/0.1 mL, and 5 mg/0.2 mL doses).

Conclusion: SiO could undergo structural change due to the interaction of aqueous Avastin, especially at high-therapeutic doses. In case of Avastin™ is required, the quantity of 1.25 mg/0.05 mL of Avastin should be used in SiO-filled eyes to minimize its unknown chemical effect.

Keywords: Bevacizumab; retina; silicone oils.

Silicone oil (SiO) is used as a long-term intravitreal tamponade solution to make contact with the retina and prevent the passage of aqueous through the broken or damaged site.[1] Furthermore, SiO tamponade is used to displace aqueous from the surroundings of the damaged area, as aqueous includes a proinflammatory...
milieu responsible for the proliferative development of vitreoretinopathy. Polydimethylsiloxane (PDMS) is the main component of the polymer structure of SiO. The main advantage of the PDMS in the water environment is that it enhances less solubility (highly hydrophobic) and high viscosity to intravitreal tamponade. Currently, SiO tamponade is used in the posterior eye segment in cases with a complex proliferative vitreoretinal disease anticipated for failure with conventional vitreoretinal surgery.

Vascular endothelial growth factor (VEGF) is a molecule identified in the regulation of intraocular neovascularization, increasing vascular permeability and ignition of ocular inflammation. These types of therapeutics consisting of biological molecules are used in the posterior segment of the eye. Bevacizumab (Avastin®; Genentech, South San Francisco, CA, USA) is a transparent, sterile solution of a recombinant humanized anti-VEGF monoclonal antibody and binds all isomers of VEGF derived from Chinese Hamster ovary cells. Consequently, it is known to neutralize or inhibit the biological activity of VEGF.

SiO tamponade is a sound medical device used in complex vitreoretinal surgical procedures; however, it cannot solely prevent retinal or iris neovascularization post-operative. In this case, the clinical success of the peri-operative or post-operative application of humanized anti-VEGF monoclonal antibody into the SiO-filled eyes has been shown in previous studies. More specifically, Avastin® injection into the SiO-filled eye is a safe and effective method in the management of “neovascular iris” and “neovascular glaucoma” seen after the vitrectomy surgery of advanced proliferative diabetic retinopathy cases. However, some concerns regarding applying Avastin® to the SiO tamponade-filled eyes have been noted in the literature. The drug delivery of Avastin® or its local concentration may become unpredictable when injected into the SiO tamponade-filled eyes. In this case, the SiO environment may alter the pharmacodynamics of the anti-VEGF monoclonal antibody in comparison to the vitreous-filled eyes.

Furthermore, the path of the Avastin® up to the vitreoretinal interface is more complex in the presence of SiO tamponade than in the vitreous environment due to the droplets of Avastin® remaining suspended in tamponade for 24–72 h. To the best of our knowledge, there is no data available investigating the interaction of the molecular structure of 1000-centistoke SiO tamponade after interacting with bevacizumab. In addition, there is still no consensus regarding the dosage for intrasilicone Avastin® injection in clinical practice. To eliminate the gaps in the literature, this study aimed to investigate the effect of the therapeutic doses of Avastin®, humanized anti-VEGF monoclonal antibody, on the SiO using nuclear magnetic resonance (NMR) and Fourier transform infrared (FT-IR) spectroscopies.

**Materials and Methods**

**Ethical Statement**

Any patient or human subject was not involved in the presented laboratory-based study. Therefore, written informed consent or ethical approval is not applicable. Ethical approval is not applicable to this study.

**Materials**

Proton hydrogen-1 NMR (1H-NMR) and FT-IR spectroscopies were used as the method, respectively, to analyze the interaction between PDMS and different doses of Avastin® as suggested for analyzing PDMS interactions in a previous study. A recombinant humanized anti-VEGF monoclonal antibody was purchased by Genentech, Inc (US Biotech unit of Roche Registration GmbH, Grenzach-Wyhlen, Germany). 1000-Centistoke of SiO tamponades were purchased by Micromed SRL (PDMS 1000 Lot#M665-4218, Fonte Nuova, Italy). Information about the purchased ophthalmic drug and the medical device are listed in Table 1.

The unused medical devices and drugs were kept at 4°C until 1H-NMR and FT-IR spectroscopy experiments. The effects of bevacizumab in the SiO tamponade were examined in the presence of three reported bevacizumab/Avastin® doses as 1.25 mg or 0.05 mL, 2.5 mg or 0.1 mL, and 5 mg or 0.2 mL. The experimental groups were as follows (n=3):

- **Baseline control**: 1 mL SiO tamponade with no addition.
- **Group 1**: 1 mL SiO tamponade + 0.05 mL Avastin® (1.25 mg bevacizumab)
- **Group 2**: 1 mL SiO tamponade + 0.1 mL Avastin® (2.5 mg bevacizumab)
- **Group 3**: 1 mL SiO tamponade + 0.2 mL Avastin® (5 mg bevacizumab)

**Methods of the 1H-NMR Spectroscopy Investigation**

5-mm borosilicate Wilmad LabGlass NMR tubes for 500 MHz frequency (Lot #Z272019, Merck KGaA, Darmstadt,
Germany) were used in sampling. Chloroform (CdCl3) (Merck KGaA, Darmstadt, Germany) was the solvent.

To assess the chemical shifts of SiO in the CdCl3, previously reported proportion of solvent for SiO was employed. All groups experimentally interacted as given in the following: Avastin® doses were transferred on a suitable glass vial (minimum volume 5 mL) filled with a 1 mL SiO and mixed for 1 min on a vortex mixer. Then, the mixtures were dissolved by adding 3 mL solvent (CdCl3) by mixing for 1 min on a vortex mixer. Finally, five milliliters of each phase were transferred to a 5-mm NMR sample tube using a glass pipette (Pasteur pipette). Tetramethylsilane was the internal standard.

Sampling tubes were placed on the autosampler (JEOL Ltd., Tokyo, Japan). The ¹H spectrum was recorded using the JNM-ECZR NMR instrument (JEOL Ltd., Tokyo, Japan) at a frequency of 500 MHz.

**Methods of the FT-IR Spectroscopy Investigation**

FT-IR spectrum of the groups was recorded using PerkinElmer Spectrum Two range instrument (PerkinElmer Inc., Waltham, MA, US) from spectral bandwidth of 4000 to 400 cm⁻¹ at 4 cm⁻¹ resolution. For sampling, the attenuated total reflectance technique was utilized at room temperature (23±1°C). Previously used parameters and sampling methods were employed for FT-IR spectroscopy.\textsuperscript{17}

**Table 1.** Information about the drug and the medical device

<table>
<thead>
<tr>
<th>Drug or Medical Device</th>
<th>Marketed Product name; Company</th>
<th>Composition</th>
<th>Indication; Dosage form</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicone oil tamponade (class IIb of Medical Device: MDR)</td>
<td>PDMS Lot#M665-4218; Micromed s.r.l. Fonte Nuova, Italy</td>
<td>Polydimethylsiloxane</td>
<td>Intraocular use for long term buffering in place of the vitreous humor, in the surgical treatment of retinal detachment; Viscous gel at a viscosity of 1000-Centistoke</td>
<td>Micromed PDMS Product information (<a href="https://www.micromedoftalmologia.it/pdms/?lang=en">https://www.micromedoftalmologia.it/pdms/?lang=en</a>)</td>
</tr>
</tbody>
</table>

VEGF: Vascular endothelial growth factor; PDMS: Polydimethylsiloxane; MDR: Medical Device Regulation- Class IIb Medical Devices (https://www.medical-device-regulation.eu/tag/class-ii-b/)
Table 2. \(^1\)H-NMR spectral interpretations of interactions between silicone-oil tamponade and Avastin\(^\text{a}\)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Silicone-oil tamponade/humanized Anti-VEGF monoclonal antibody</th>
<th>(\text{CH}_3) (1) group ((\delta))</th>
<th>Signal type</th>
<th>Reference or interpretation of (\text{CH}_3) (1) group of PDMS</th>
<th>(\text{CH}_3) (2) group ((\delta))</th>
<th>Signal type</th>
<th>Reference or interpretation of (\text{CH}_3) (2) group of PDMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1 mL SiO tamponade with no addition</td>
<td>0.03 ppm</td>
<td>Singlet</td>
<td>Citation No.(^{[17,18]})</td>
<td>0.17 ppm</td>
<td>Doublet</td>
<td>Citation No.(^{[17,18]})</td>
</tr>
<tr>
<td>Group 1</td>
<td>1 mL SiO tamponade + 1.25 mg/0.05 mL Avastin(^\text{a})</td>
<td>0.03 ppm</td>
<td>Singlet</td>
<td>The decreasing intensity was observed with no chemical shift</td>
<td>0.17 ppm</td>
<td>Almost singlet</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>1 mL SiO tamponade + 2.5 mg/0.1 mL Avastin(^\text{a})</td>
<td>0.03 ppm</td>
<td>Doublet</td>
<td>Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.</td>
<td>0.17 ppm</td>
<td>Singlet</td>
<td>This group was disturbed by a different H atom and the singlet peak was observed together with the decreasing intensity. Chemical shift was observed.</td>
</tr>
<tr>
<td>Group 3</td>
<td>1 mL SiO tamponade + 5 mg/0.2 mL Avastin(^\text{a})</td>
<td>0.03 ppm</td>
<td>Doublet</td>
<td>Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.</td>
<td>0.17 ppm</td>
<td>Doublet</td>
<td>Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.</td>
</tr>
</tbody>
</table>

SiO: Silicone-oil tamponade; PDMS: Polydimethylsiloxane viscous gel at a viscosity of 1000-Centistoke.

Results

\(^1\)H-NMR Spectral Interpretation

\(^1\)H-NMR spectral data of a SiO baseline control are shown in Figure 1. To observe functional groups of PDMS molecule, the chemical shifts were interpreted between −1.0 and 1.0 ppm (Fig. 2). The Si atom was seen to attach to a single oxygen atom in \(\text{CH}_3\) (1) functional groups (approx. \(\delta = 0.03\)), and Si was bound with two oxygen atoms in \(\text{CH}_3\) (2) functional groups (approx. \(\delta = 0.17\)). The atoms of Si-\(\text{CH}_3\) (1) functional groups were observed as a single coincident peak. In contrast, the atoms of Si-\(\text{CH}_3\) (2) functional groups were seen as doublets in the signals due to the electronegativity of the oxygen atoms. The protons of the Si-\(\text{CH}_3\) (1) and Si-\(\text{CH}_3\) (2) functional groups of PDMS will be used for forthcoming interpretations in all groups.

In Groups 1–3, protein-related signals were not interpreted due to a low concentration of the Avastin\(^\text{a}\). The water phase of the Avastin drug emerged in Groups 1–3 (seen as a suppressed signal at approx. \(\delta = 4.7\) ppm) (Fig. 1). \(^1\)H-NMR spectral interpretations of Groups 1–3 are summarized in Table 2. After interacting with Avastin\(^\text{a}\), Si-\(\text{CH}_3\) (1) and Si-\(\text{CH}_3\) (2) yielded protons were observed at 0.03 and 0.17 ppm, respectively. Chemical shifts were seen in both functional groups. Accordingly, minor shifts and intensity changes were revealed in both functional groups of Group 1. The intensity of the Si-\(\text{CH}_3\) (1) functional group gradually decreased with the increased Avastin\(^\text{a}\) concentrations.
In Groups 2 and 3, splitting into two lines was observed in Si-CH$_3$(1) with decreasing intensity (approx. δ = 0.03). As a remarkable finding, the Si-CH$_3$(1) functional group was revealed as doublets in Groups 2 and 3.

The intensity of the Si-CH$_3$(2) functional group gradually decreased with groups 1, 2, and 3, respectively (approx. δ = 0.18 and 0.17, respectively). In Group 3, splitting into two lines was observed in Si-CH$_3$(2) with decreasing intensity (approx. δ = 0.17). As a remarkable finding, the Si-CH$_3$(2) functional group was revealed as doublets in Group 3.

**FT-IR Spectral Interpretation**

The FT-IR spectral data of groups are given in Figure 3. The absorption bands of baseline control were as follows: The symmetric deformation vibrations of the Si-(CH$_3$)$_n$ groups were observed at 782 and 1257 cm$^{-1}$, respectively. The Si–O–Si vibrational modes were observed between 1008 and 1079 cm$^{-1}$. The C-H stretching mode of CH$_3$ was observed at 2962 cm$^{-1}$ (Fig. 3a).

Fig. 3. Fourier transform-infrared spectra of Groups. (a) baseline control, (b) Group 1, (c) Group 2, (d) Group 3

FT-IR spectral interpretations of Groups 1–3 are summarized in Table 3. A noteworthy intensity change and chemical shifts were observed in the Si-CH$_3$ group at 782 cm$^{-1}$. The intensity of the Si-CH$_3$ group gradually decreased with
Groups 1, 2, and 3, respectively. The decreasing intensity was observed in the vibration band belonging to the Si-O-Si bending at 1008 cm⁻¹. The intensity of the Si-O-Si group gradually decreased with groups 1, 2, and 3, respectively. In contrast, no shift or intensity change was observed at 1079 cm⁻¹. No chemical shift or intensity change was observed in the vibration modes of the Si-CH₃ groups at 1257 cm⁻¹ (Fig. 3).

Discussion

In this study, the effects of reported Avastin® (Bevacizumab, humanized anti-VEGF monoclonal antibody) doses on SiO tamponade (a prefilled syringe PDMS 1000 cSt) were investigated. The effects of bevacizumab in the SiO tamponade were examined in reported three common ophthalmic doses of Avastin®: 1.25 mg/0.05 mL,⁴¹,¹⁴ 2.5 mg/0.1 mL,¹⁰ and 5 mg/0.2 mL.¹⁶ In addition, nuclear magnetic resonance and FT-IR spectroscopies were used as the method, respectively, to analyze the interaction between PDMS and different doses of Avastin®.

The theoretical concentration of Avastin® includes 25 mg/mL humanized anti-VEGF monoclonal antibody (bevacizumab).⁵ In the literature, there is no consensus on the ideal doses of bevacizumab or Avastin® injections into SiO-filled eyes. Salman¹¹ reported the regression of neovascularization in 12 eyes on Avastin® injection (1.25 mg/0.05 mL) in SiO-filled eyes. Furthermore, Baek et al.¹⁴ injected 1.25 mg/0.05 mL of Avastin® into SiO-filled eyes and observed no retinal toxicity. Falavarjani et al.¹⁰ injected 2.5 mg/0.1 mL of bevacizumab/Avastin® into SiO-filled eyes
and observed no retinal toxicity or other complications. Manzano et al.\textsuperscript{[16]} injected 5 mg/0.2 mL of Avastin\textsuperscript{®} into vitreous-filled-rabbit eyes and observed no retinal toxicity. The level of the Avastin\textsuperscript{®} used in the current work agreed with the reported levels used in clinical practice.\textsuperscript{[10,11,14,16]}

The SiO tamponade presents hydrophobic surroundings for the aqueous drug formulations.\textsuperscript{[13]} Overall, monoclonal antibodies are surface-active proteins, and they spontaneously adsorb to hydrophobic surfaces of the SiO–water interface.\textsuperscript{[17]} When adsorbed onto SiO surfaces, the monoclonal antibodies partly unfold to link their hydrophobic groups.\textsuperscript{[17]} It has been noted that this interaction with adjacent molecules forms viscoelastic pellicle-like aggregates at the SiO–water interface.\textsuperscript{[18]} Injected drugs into the hydrophobic SiO tamponade environment, the existence of the aggregates may affect the quality and the efficacy of the bevacizumab, or the aggregates may lead to occur immunogenic reactions.\textsuperscript{[19,20]} Grzybowski et al.\textsuperscript{[21]} reported that an eye filled with SiO presents difficulties in case an intravitreal drug injection is needed. Especially, the pharmacokinetics of intravitreally injected drugs were reported to show differences in previously vitrectomized eyes (e.g., SiO-filled eyes), decreasing half-life times, and faster drug clearance.\textsuperscript{[21]}

Mainly, SiO degradation reactions and by-products (water-soluble silanols) due to hydrolysis have been previously reported.\textsuperscript{[22]} In addition, the hydrolytic degradation of SiO is drawn in Figure 4. In a recent study, SiO and its hydrolytic degradation products in an aqueous 10 mg/mL protein antibody environment have demonstrated with the \textsuperscript{1}H-NMR spectroscopy method.\textsuperscript{[22]} However, the effect of the bevacizumab interaction on SiO was demonstrated for the first time in this study.

The presented non-destructive spectroscopic methodology was employed to monitor the 1000-centistoke SiO tamponade material and of interaction with a human recombinant monoclonal antibody-included drug. The presented methodology was in agreement with the materials and the presented interaction model.\textsuperscript{[15,22]} Accordingly, the presented chemical shifts of the functional groups Si-CH\textsubscript{3} (1) and Si-CH\textsubscript{3} (2) were in agreement with the previous studies and, alike, FT-IR absorption bands of the organosilicon compound were in agreement with the previous studies.\textsuperscript{[15,23-25]} In the presented findings, the remarkable chemical shifts were seen predominantly in functional groups of PDMS in Groups 2 and 3. Consequently, PDMS molecular structure was mostly changed in 2.5 mg/0.1 mL and 5 mg/0.2 mL Avastin\textsuperscript{®} due to interacting with the high dose dependent.

Recently, there has been an increasing focus on the interaction between SiO and therapeutic proteins.\textsuperscript{[28-31]} The adsorption or aggregation of some recombinant humanized monoclonal antibodies at SiO–water interfaces due to the interactions have been demonstrated in recent studies.\textsuperscript{[28-31]} Accordingly, the author noted that adsorption, aggregation, or the colloidal stability of bevacizumab, after interaction with SiO, might also be questionable. However, the presented findings cannot answer the question of how the bevacizumab interacts in the presence of SiO in this study. The possible different conformations or orientations of bevacizumab after interaction with SiO may be interpreted by detailed analysis by advanced approaches in further studies.

To better specify the effects of SiO tamponade and Avastin\textsuperscript{®} interaction, an approach was chosen wherein ascending Avastin\textsuperscript{®} doses were exposed to SiO tamponade that presented approximately 6.5–7 times the interfacial area to which Avastin\textsuperscript{®} may be exposed within a SiO-filled eye.\textsuperscript{[4]} However, this design may not represent actual SiO tamponade-filled eye exposure levels. A dose-dependent interaction model was employed and clearly described in the presented study to test the hypothesis. Therefore, the present study does not simulate real eye conditions. Consequently, a laboratory level of experimental group interactions could be considered a limitation of this study. Creating a new study design on animal eyes (i.e., a rabbit eye model) having advanced spectroscopic or chromatographic methodology may provide better information about the interaction nature by simulating real-life conditions in further studies. The interaction of Avastin on SiO was investigated in a single time variable. However, after intravitreal drug injection, it remains in the eye for a certain period and changes in the structure of the SiO may continue during this period. This change can occur in different amounts at different Avastin doses. Therefore, further longitudinal studies investigating the changes in SiO at different times after Avastin injection will increase the value of this study. This could be a limitation of the study.

Intravitreal injections, as one of the most common intraocular procedures worldwide with increasing numbers every year, have many different approaches in clinical practice. Therefore, it is important to consider the relevant factors in each approach when analyzing the results.

**Conclusion**

The present study describes the effect on the chemical
structure of SiO after interacting with Avastin® through $^1$H-NMR and FT-IR spectroscopies. Within the limitations of this current study, it was concluded that: SiO could undergo structural change due to the interaction of aqueous Avastin®, especially at high-therapeutic doses. In case of Avastin® is required, the quantity of 0.05 mL of Avastin® should be used in SiO-filled eyes to minimize its unknown chemical effect. The findings of this study provide a spectral dataset to enhance the current knowledge of intravitreal monoclonal antibody interactions with SiO tamponade in the ophthalmology and pharmaceutical industry.

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Conflict of Interest: None declared.

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