ORIGINAL ARTICLE

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

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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 (^{1}H-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: ^{1}H-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.[2] 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.[2] 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.[3]

Vascular endothelial growth factor (VEGF) is a molecule identified in the regulation of intraocular neovascularization, increasing vascular permeability and ignition of ocular inflammation.[4] 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.[5-7] derived from Chinese Hamster ovary cells.[5] Consequently, it is known to neutralize or inhibit the biological activity of VEGF.[6,7]

SiO tamponade is a sound medical device used in complex vitreoretinal surgical procedures; however, it cannot solely prevent retinal or iris neovascularization post-operative.8 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.[8-12] 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.[10-12] 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.[3] In this case, the SiO environment may alter the pharmacodynamics of the anti-VEGF monoclonal antibody in comparison to the vitreous-filled eyes.[13]

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.[13] 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.[14] 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 (^{1}H-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.[15]

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 ^{1}H-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,[11,14] 2.5 mg or 0.1 mL,[10] and 5 mg or 0.2 mL.[16] 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,

Table 1. Information about the drug and the medical device

Drug or Medical Device	Marketed Product name; Company	Composition	Indication; Dosage form	Reference
Silicone oil tamponade (class IIb of Medical Device: MDR)	PDMS Lot#M665-4218; Micromed s.r.l. Fonte Nuova, Italy	Polydimethylsiloxane	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	Micromed PDMS Product information (https://www.micromedoftalmologia.it/pdms/?lang=en)
Bevacizumab (drug) is a recombinant humanized anti-VEGF monoclonal antibody produced by DNA technology in Chinese Hamster ovary cells.	Avastin® 100 mg/4mL Genentech Inc., San Francisco, CA, USA (US Biotech unit of Roche Registration GmbH, Grenzach-Wyhlen, Germany)	Trehalose dihydrate, sodium phosphate, polysorbate 20, water for injections	Proliferative diabetic retinopathy; Solution (Each mL of concentrate contains 25 mg of bevacizumab)	Avastin® Product information European Medical agency (http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000582/WC500029271.pdf) http://www.cptech.org/ip/health/avastin.html

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/)

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. [17] 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 ^{1}H 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[17]
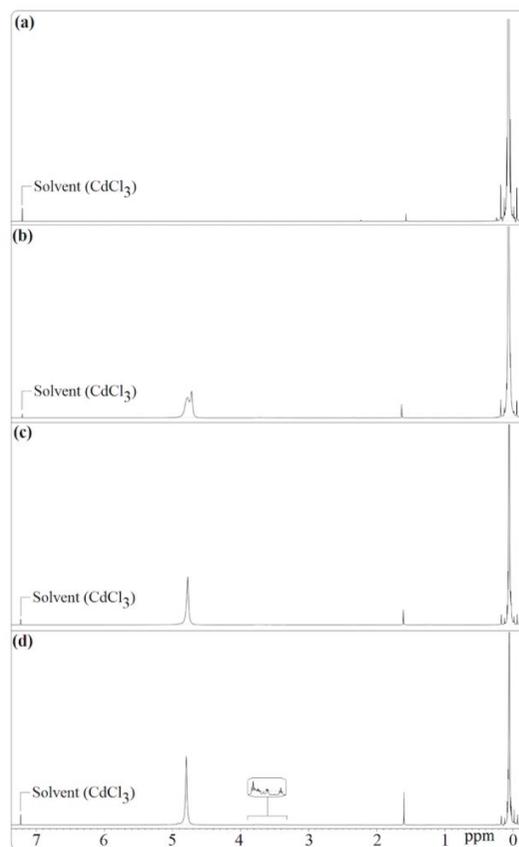
Fig. 1. Proton nuclear magnetic resonance spectra of a Silicone oil (SiO) tamponade between -0.3 and 7.0 ppm, (a) baseline control, (b) Group 1, (c) Group 2, (d) Group 3

Table 2. ^{1}H-NMR spectral interpretations of interactions between silicone-oil tamponade and Avastin®

Groups	Silicone-oil tamponade/humanized Anti-VEGF monoclonal antibody	CH₃ (1) group (δ)	Signal type	Reference or interpretation of CH3 (1) group of PDMS	CH₃ (2) group (δ)	Signal type	Reference or interpretation of CH3 (2) group of PDMS
Control	1 mL SiO tamponade with no addition	0.03 ppm	Singlet	Citation No.[17,18]	0.17 ppm	Doublet	Citation No.[17,18]
Group 1	1 mL SiO tamponade + 1.25 mg/0.05 mL Avastin®	0.03 ppm	Singlet	The decreasing intensity was observed with no chemical shift	0.17 ppm	Almost singlet	This group was disturbed by a different H atom and the shoulder peak was observed with the decreasing intensity. Chemical shift was not observed.
Group 2	1 mL SiO tamponade + 2.5 mg/0.1 mL Avastin®	0.03 ppm	Doublet	Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.	0.17 ppm	Singlet	This group was disturbed by a different H atom and the singlet peak was observed together with the decreasing intensity. Chemical shift was observed.
Group 3	1 mL SiO tamponade + 5 mg/0.2 mL Avastin®	0.03 ppm	Doublet	Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.	0.17 ppm	Doublet	Splitting into two lines was observed in this group together with the decreasing intensity. Chemical shift was observed.

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 CH₃ (1) functional groups (approx. δ = 0.03), and Si was bound with two oxygen atoms in CH₃ (2) functional groups (approx. δ = 0.17). The atoms of Si- CH₃ (1) functional groups were observed as a single coincident peak. In contrast, the atoms of Si-CH₃ (2) functional groups were seen as doublets in the signals due to the electronegativity of the oxygen atoms. The protons of the Si-CH₃ (1) and Si-CH₃ (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®. The water phase of the Avastin drug emerged in Groups 1–3 (seen as a suppressed signal at approx. δ = 4.7 ppm) (Fig. 1). ^{1}H-NMR spectral interpretations of Groups 1–3 are summarized in Table 2. After interacting with Avastin®, Si-CH₃(1) and Si-CH₃(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-CH₃(1) functional group gradually decreased with the increased Avastin® concentrations
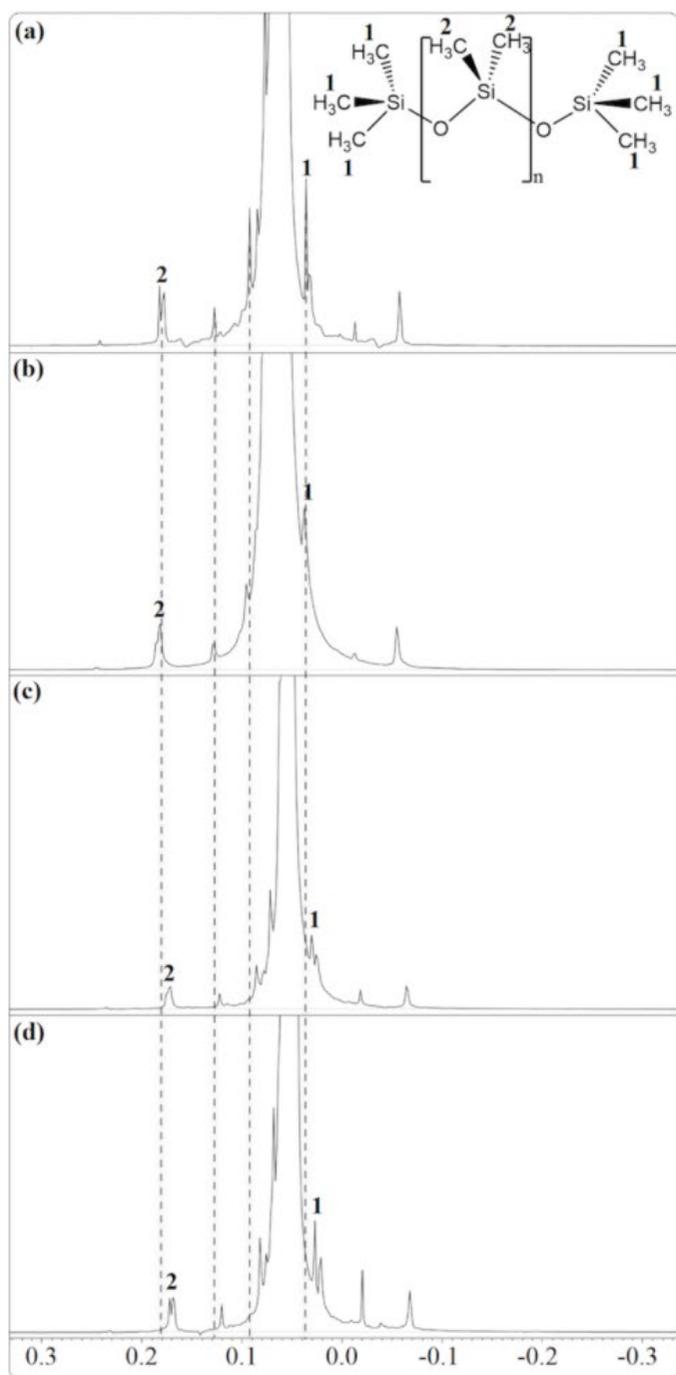
Fig. 2. Proton nuclear magnetic resonance spectra between −0.3 and 0.3 ppm; (a) baseline control, (b) Group 1, (c) Group 2, (d) Group 3. Numeric assignments refer to the chemical shifts of the functional groups of polydimethylsiloxane. Blank assignments refer to their chemical shifts in (c) and (d)

(approx. δ = 0.04). 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 =
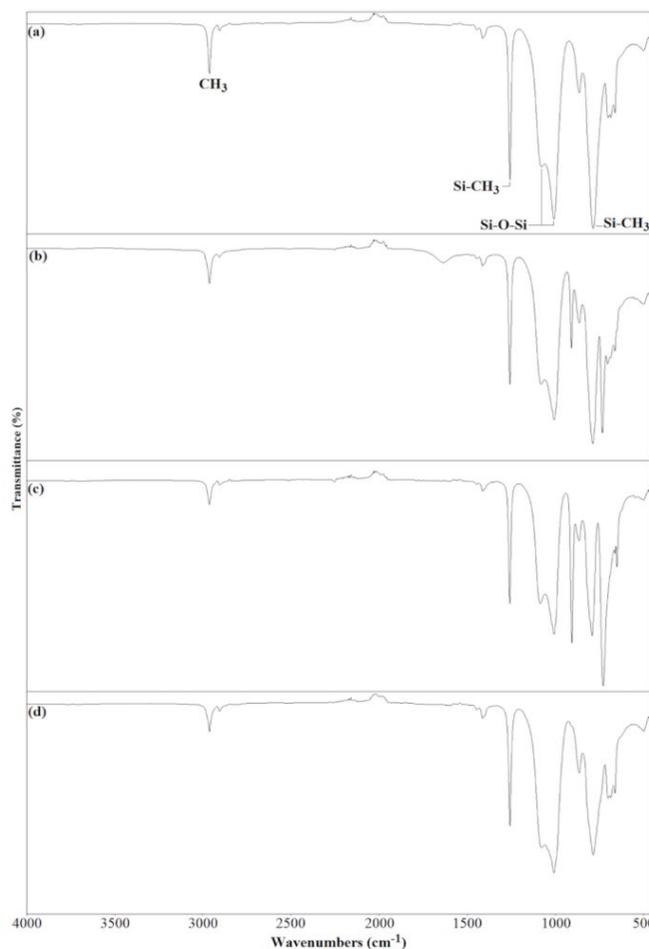
Fig. 3. Fourier transform-infrared spectra of Groups. (a) baseline control, (b) Group 1, (c) Group 2, (d) Group 3

0.17). This functional group was disturbed by a different H atom, and a shoulder peak and a singlet peak were observed with decreasing intensity in Groups 1 and 2 (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$_3$) 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).

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

Table 3. FT-IR spectral interpretations of interactions between silicone-oil tamponade and Avastin®

Groups	Silicone-oil tamponade/humanized Anti-VEGF monoclonal antibody	Si-CH$_3$	Reference or interpretation of Si-CH$_3$	Si-O-Si	Reference or interpretation of Si-O-Si	Si-(CH$_3$)$_2$	Reference or interpretation of Si-(CH$_3$)2
Control	1 mL SiO tamponade with no addition	1257 cm^{-1}	Citation No.[19-21]	1079 and 1008 cm^{-1}	Citation No.[19-21]	782 cm^{-1}	Citation No. [19-21]
Group 1	1 mL SiO tamponade + 1.25 mg/0.05 mL Avastin®	1257 cm^{-1}	No shift or no intensity change was observed in groups	1079 and 1008 cm^{-1}	The decreasing intensity was observed at 1008 cm^{-1}. No shift or no intensity change was observed at 1079 cm^{-1}	782 cm^{-1}	Chemical shifts with the decreasing intensities were observed in this group.
Group 2	1 mL SiO tamponade + 2.5 mg/0.1 mL Avastin®	1257 cm^{-1}	No shift or no intensity change was observed in groups	1079 and 1008 cm^{-1}	The decreasing intensity was observed at 1008 cm-1. No shift or no intensity change was observed at 1079 cm^{-1}	782 cm^{-1}	Chemical shifts with the decreasing intensities were observed in this group.shifts with the decreasing intensities were observed in this group.
Group 3	1 mL SiO tamponade + 5 mg/0.2 mL Avastin®	1257 cm^{-1}	No shift or no intensity change was observed in groups	1079 and 1008 cm^{-1}	The decreasing intensity was observed at 1008 cm-1. No shift or no	782 cm^{-1}	Chemical shifts with the decreasing intensities were observed in this group.shifts with the decreasing intensities were observed in this group.shifts with the decreasing intensities were observed in this group.

SiO: Silicone-oil tamponade; PDMS: Polydimethylsiloxane viscous gel at a viscosity of 1000-Centistoke; FT-IR: Fourier-transform infrared.

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-CH$_3$ 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,[11,14] 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. Salman[11] 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.[16] injected 5 mg/0.2 mL of Avastin® into vitreous-filled-rabbit eyes and observed no retinal toxicity. The level of the Avastin® used in the current work agreed with the reported levels used in clinical practice.[10,11,14,16]

The SiO tamponade presents hydrophobic surroundings for the aqueous drug formulations.[13] Overall, monoclonal antibodies are surface-active proteins, and they spontaneously adsorb to hydrophobic surfaces of the SiO–water interface.[17] When adsorbed onto SiO surfaces, the monoclonal antibodies partly unfold to link their hydrophobic groups.[17] It has been noted that this interaction with adjacent molecules forms viscoelastic pellicle-like aggregates at the SiO-water interface.[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.[19,20] Grzybowski et al.[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.[21]

Mainly, SiO degradation reactions and by-products (water-soluble silanols) due to hydrolysis have been previously reported.[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 ^{1}H-NMR spectroscopy method.[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.[15,22] Accordingly, the presented chemical shifts of the functional groups Si-CH$_3$ (1) and Si-CH$_3$ (2) were in agreement with the previous studies.[15,23-25] and, alike, FT-IR absorption bands of the organosilicon compound were in agreement with the previous studies.[15,26,27] 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® due to interacting

with the high dose dependent.

Recently, there has been an increasing focus on the interaction between SiO and therapeutic proteins.[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.[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® interaction, an approach was chosen wherein ascending Avastin® doses were exposed to SiO tamponade that presented approximately 6.5-7 times the interfacial area to which Avastin® may be exposed within a SiO-filled eye.[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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References

1. Cazabon S, Hillier RJ, Wong D. Heavy silicone oil: A "novel" intraocular tamponade agent. Optom Vis Sci 2011;88:772–5.

2. Chen Y, Kearns VR, Zhou L, Sandinha T, Lam WC, Steel DH, et al. Silicone oil in vitreoretinal surgery: Indications, complications, new developments and alternative long-term tamponade agents. Acta Ophthalmol 2021;99:240–50. [CrossRef]

3. Gallemore RP, McCuen BW. Silicone oil in vitreoretinal surgery. In: Ryan SJ, editor. Retina. 4th ed. Maryland Heights: Mosby; 2006. p. 2211–34. [CrossRef]

4. Nayak K, Misra M. A review on recent drug delivery systems for posterior segment of eye. Biomed Pharmacother 2018;107:1564–82. [CrossRef]

5. Avastin® Product information European Medical Agency. Available from: https://www.ema.europa.eu/docs/en_gb/document_library/epar_product_information/human/000582/wc500029271.pdf. Accessed Mar 30, 2023.

6. Smit DP, Meyer D. Intravitreal bevacizumab: An analysis of the evidence. Clin Ophthalmol 2007;1:273–84.

7. Mesti T, Bouchemal N, Banissi C, Triba MN, Marbeuf-Gueye C, Cemazar M, et al. Nuclear magnetic resonance metabolic fingerprint of bevacizumab in mutant IDH1 glioma cells. Radiol Oncol 2018;52:392–8. [CrossRef]

8. Yeh PT, Yang CM, Lin YC, Chen MS, Yang CH. Bevacizumab pretreatment in vitrectomy with silicone oil for severe diabetic retinopathy. Retina 2009;29:768–74. [CrossRef]

9. Falavarjani KG, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: A review of literature. Eye (Lond) 2013;27:787–94. [CrossRef]

10. Falavarjani KG, Modarres M, Nazari H. Therapeutic effect of bevacizumab injected into the silicone oil in eyes with neovascular glaucoma after vitrectomy for advanced diabetic retinopathy. Eye (Lond) 2010;24:717–9. [CrossRef]

11. Salman AG. Intrasilicone bevacizumab injection for iris neovascularization after vitrectomy for proliferative diabetic retinopathy. Ophthalmic Res 2013;49:20–4. [CrossRef]

12. Alishiri A, Naderi M, Jadidi K, Mosavi SA. Efficacy of simultaneous usage of bevacizumab and silicone oil injection after vitrectomy in diabetic tractional retinal detachment. Razavi Int J Med 2017;5:e43217. [CrossRef]

13. Xu Y, You Y, Du W, Zhao C, Li J, Mao J, et al. Ocular pharmacokinetics of bevacizumab in vitrectomized eyes with silicone oil tamponade. Invest Ophthalmol Vis Sci 2012;53:5221–6. [CrossRef]

14. Baek SK, Lee MW, Lee YH. Effect of intrasilicone bevacizumab injection in diabetic tractional retinal detachment surgery: A retrospective case-control study. J Clin Med 2020;9:3114.

15. Güngel H, Menceoğlu Y, Yildiz B, Akbulut O. Fourier transform infrared and ^{1}H nuclear magnetic resonance spectroscopic findings of silicone oil removed from eyes and the relationship of emulsification with retinotomy and glaucoma. Retina 2005;25:332–8. [CrossRef]

16. Manzano RP, Peyman GA, Khan P, Kivilcim M. Testing intravitreal toxicity of bevacizumab (Avastin). Retina 2006;26:257–61.

17. Li Y, Pan D, Nashine V, Deshmukh S, Vig B, Chen Z. Understanding protein-interface interactions of a fusion protein at silicone oil-water interface probed by sum frequency generation vibrational spectroscopy. J Pharm Sci 2018;107:682–9. [CrossRef]

18. Mehta SB, Carpenter JF, Randolph TW. Colloidal instability fosters agglomeration of subvisible particles created by rupture of gels of a monoclonal antibody formed at silicone oil-water interfaces. J Pharm Sci 2016;105:2338–48. [CrossRef]

19. Mahler HC, Friess W, Grauschopf U, Kiese S. Protein aggregation: Pathways, induction factors and analysis. J Pharm Sci 2009;98:2909–34. [CrossRef]

20. Rosenberg AS. Effects of protein aggregates: An immunologic perspective. AAPS J 2006;8:E501–7. [CrossRef]

21. Grzybowski A, Told R, Sacu S, Bandello F, Moisseiev E, Loewenstein A, et al. 2018 update on intravitreal injections: Euretina expert consensus recommendations. Ophthalmologica 2018;239:181–93. [CrossRef]

22. Degradation of Silicone Polymers in Nature, Ref. No 01-1113-01; Environmental Information Update. In: Health Environment and Regulatory Affairs (HERA) Dow Corning Green Flow Ltd; 1998. Available from: bit.ly/3PpKMUs. Accessed Mar 30, 2023.

23. Malmstrøm J. Quantification of silicone oil and its degradation products in aqueous pharmaceutical formulations by ^{1}H-NMR spectroscopy. J Pharm Sci 2019;108:1512–20. [CrossRef]

24. Cai G, Weber WP. Synthesis of terminal Si-H irregular tetra-branched star polysiloxanes. Pt-catalyzed hydrosilylation with unsaturated epoxides. Polysiloxane films by photo-acid catalyzed crosslinking. Polymer 2004;45:2941–8. [CrossRef]

25. Louis E, Jussofie I, Kühn FE, Herrmann WA. Karstedt catalyst-catalyzed stepgrowth co-polyaddition of 1,9-decadiene and 1,1,3,3-tetramethyldisiloxane. J Organomet Chem 2006;691:2031–6. [CrossRef]

26. Smith AL. Infrared spectra-structure correlations for organosilicon compounds. Spectrochim Acta 1960;16:87–105.

27. Crompton T. Analysis of organosilicon compounds. In: Rappoport Z, Patai S, editors. The Chemistry of Organic Silicon Compounds. New York: John Wiley and Sons; 1989. p. 393–444. [CrossRef]

28. Perera HA, Lu T, Fu L, Zhang J, Chen Z. Probing the interfacial interactions of monoclonal and bispecific antibodies at the silicone oil-aqueous solution interface by using sum frequency generation vibrational spectroscopy. Langmuir 2019;35:14339–47. [CrossRef]

29. Probst C. Characterization of protein aggregates, silicone oil droplets, and protein-silicone interactions using imaging flow cytometry. J Pharm Sci 2020;109:364–74. [CrossRef]

30. Dandekar R, Ardekani AM. Monoclonal antibody aggregation near silicone oil-water interfaces. Langmuir 2021;37:1386–98.

31. Kannan A, Shieh IC, Negulescu PG, Suja VC, Fuller GG. Adsorption and aggregation of monoclonal antibodies at silicone oil-water interfaces. Mol Pharm 2021;18:1656–65.