# Benefits of Polidocanol Endovenous Microfoam (Varithena®) Compared with Physician Compounded Foams

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## Abstract

### OBJECTIVE

To compare foam bubble size and bubble size distribution, stability, and degradation rate (DR) of commercially available polidocanol endovenous microfoam (Varithena®) and physician compounded foams (PCFs) using a number of laboratory tests.

### METHODS

Foam properties of polidocanol endovenous microfoam and PCFs were measured and compared using a glass plate method and a Sympatec QICPIC image analysis method to measure bubble size and bubble size distribution, Turbiscan™ LAB for foam half time and drainage, and a novel biomimetic vein model to measure foam stability. PCFs composed of polidocanol and room air (RA), $CO_2$, or mixtures of oxygen and carbon dioxide ($O_2$:$CO_2$) were generated by different methods.

### RESULTS

Polidocanol endovenous microfoam was found to have a narrow bubble size distribution with no large (>500 μm) bubbles. PCFs made with the Tessari method had broader bubble size distribution and large bubbles, which have an impact on foam stability. Polidocanol endovenous microfoam had a lower degradation rate (DR) than any PCFs, including foams made using RA ($p<0.035$). The same result was obtained at different liquid to gas ratios (1:4 and 1:7) for PCFs. In all tests performed, $CO_2$ foams were the least stable and different $O_2$:$CO_2$ mixtures had intermediate performance. In the biomimetic vein model, polidocanol endovenous microfoam had the slowest DR, and longest calculated dwell time, which represents the length of time the foam is in contact with the vein, almost twice that of PCFs using RA and eight times better than PCFs prepared using equivalent gas mixes.

### CONCLUSION

Bubble size, bubble size distribution, and stability of various sclerosing foam formulations show that polidocanol endovenous microfoam results in better overall performance compared with PCFs. Polidocanol endovenous microfoam offers better stability and cohesive properties in a biomimetic vein model compared to PCFs. Polidocanol endovenous microfoam, which is indicated in the United States for treatment of great saphenous vein system incompetence, provides clinicians with a consistent product with enhanced handling properties.

## Introduction

Physician-compounded foams (PCFs) have been introduced in vein treatment with the aim of increasing efficacy and treating larger varicose veins relative to liquid sclerosants.[1, 2] However, foams are not all the same and, in fact, they can be dramatically different from each other. The performance of foams is highly dependent on their physical characteristics: gas composition, the different absorption rates of nitrogen and carbon dioxide in the bloodstream, and bubble size.[3-5]

PCFs offer several advantages over traditional liquid sclerosants. When injected into a vein, a cohesive foam displaces the blood (rather than mixing with it), creating better contact with the vein wall. Foam treatment offers the possibility of using lower sclerosant concentrations.[6] This, in turn, increases the safety of foam treatment as shown in clinical trials.[7-9] Furthermore, foam is echogenic, which improves visibility and treatment accuracy.[10] Also, foam treatment can be performed in an outpatient setting without need for sedation or tumescent anesthesia.[11]

Foam treatment also presents challenges. Room air (RA) forms stable foam, but because the nitrogen it contains does not dissolve efficiently in blood, nitrogen bubbles may persist and can cause adverse effects.[12, 13] Carbon dioxide ($CO_2$) foams can be made, but the increased solubility results in foams that coarsen rapidly, leading to drastically reduced stability. PCF methods may generate large gas bubbles that may be potentially problematic in the circulation. Strategies such as using $CO_2$ rather than RA and limiting the injected volume of PCF to less than 10 mL have been proposed to reduce the incidence of serious complications, since significant neurological events have occurred after injections of as little as 4 mL of PCF. These neurological events have been attributed to nitrogen/air.[14-16] In fact, two reports have documented that all patients injected with PCF for the treatment of venous varicosities have gas bubbles visible in the right heart chambers, and some patients, such as those with patent foramen ovale (PFO) or other right-to-left shunts, have gas bubbles in the left heart chambers.[17, 18]

The two most popular techniques that clinicians use to generate PCFs are the Double Syringe System (DSS) and the Tessari method.[19, 20] DSS involves passing the sclerosant liquid and gas between two syringes joined by a simple straight connector (Figure 1a). The Tessari method is similar, but the straight connector is replaced with a 3-way valve (Figure 1b). Although these two methods are very similar, the DSS method is felt to produce slightly better foam.[21] Polidocanol endovenous microfoam (Varithena® [polidocanol injectable foam 1%], Provensis

Ltd, a BTG International group company) is a new product designed to overcome the challenges associated with PCFs. Polidocanol endovenous microfoam is generated by a proprietary device that produces consistent, pharmaceutical-grade low nitrogen (<0.8%), $O_2$:$CO_2$ (65:35) foam (Figure 1c). In a recent case series of sixty patients with middle cerebral artery bubble emboli during or after treatment with PEM, no evidence of subclinical cerebral injury was found on MRI.[22] In addition, in two pivotal Phase 3 clinical trials using polidocanol endovenous microfoam, there were no clinically meaningful neurologic adverse events observed.[23]

Methods for measuring bubble size and size distribution include the Sympatec image analysis sensor QICPIC (Sympatec Ltd., Bury, Lancashire, UK) and Turbiscan™ LAB apparatus (Formulaction SAS, L'Union, France) (Figure 2). Sympatec provides a bubble size distribution of flowing microbubbles in deionized water, whereas Turbiscan™ provides dynamic information on foam. The speed at which liquid separates from the body of foam has been used as a measure of foam stability.[24] Foam drainage time (FDT) and rate are good measures of foam stability.[21] Methods for measuring foam stability and cohesiveness include the Turbiscan™ LAB operated in the scanning detector mode for foam drainage kinetics and foam half time (FHT), and operated in the fixed detector mode for FDT determination. The novel biomimetic analysis system was developed for the quantification of foam properties under clinically relevant conditions to establish a robust method for comparative characterization of polidocanol endovenous foam and PCFs. The high resolution computational video analysis system allows accurate quantification of foam dynamic behavior, including foam plug expansion rate, degradation rate and dwell time (Figure 3).[25]

This paper reports the results of a number of tests performed to compare foam bubble size and bubble distribution, stability, and degradation rate of polidocanol endovenous foam and PCFs. These foams were also investigated in the biomimetic vein model to relate stability to clinical function.

# Materials and Methods

## Foam Production and Characterisation

Full details about foam production and characterisation methods are provided in the Supplementary Information sections S1 and S2, respectively.

For preparation of PCFs, one percent (1%) aqueous buffered polidocanol solution was used throughout these studies. Foam densities were at a liquid to gas ratio of 1:7 for direct comparison with PEM or at 1:4 to represent commonly used formulations. DSS and Tessari methods were used to create PCFs.

Polidocanol endovenous microfoam consists of a proprietary $O_2$:$CO_2$ (65:35) gas mixture with ultra-low nitrogen content (<0.8%) and 1% polidocanol solution contained within a pressurized canister and combined on discharge as uniform microfoam. Sterile canisters of the product were used to generate 5 mL of microfoam for experimentation.

Foam properties of polidocanol endovenous microfoam and PCFs were measured and compared using glass plate method and Sympatec QICPIC image analysis to measure bubble size and bubble size distribution, Turbiscan™ LAB apparatus for foam half time (FHT) and foam drainage time (FDT), and the biomimetic vein model to measure foam stability. These are summarised in Table I, and methodological details are reported in the Supplementary Information section S2.

## Statistical Analysis

One-way analysis of variance (ANOVA) was performed to analyze the differences between group means using OriginPro (Origin Lab Corp., Northampton, MA) software package. Pairwise comparison tests were performed using the Bonferroni method. The significance level was set to 0.05 (i.e., differences were considered to be statistically significant for p-value < 0.05). The number of experimental repeats for each test is reported in Table II. Selected relevant p-values for comparison between PEM and PCFs using the different methods are shown in Table III.

# Results

## Comparison of Methods Used to Measure Bubble Size Distribution

Figure 4 provides a visual comparison of the bubble images captured using the glass plate and the Sympatec methods and the frequency of bubble size measured by these two methods for polidocanol endovenous microfoam. The glass plate method shows a closely-packed foam (Figure 4a) and measures a relatively tightly distributed bubble size distribution with no bubbles >~300 μm in diameter (Figure 4b). The captured image from the Sympatec shows the bubbles are no longer in close contact with one another at the point of measurement, having been separated in the flowing carrier liquid (Figure 4c). This method over-reports the true bubble size with bubbles up to ~600 μm, and bubbles smaller than 15 μm in diameter are not detectable because of foam coarsening during the time taken to administer the foam into the instrument, and loss of smaller bubbles before they reach the detector in the instrument (Figure 4d).

## Bubble Size Distributions of Various Foam Formulations

Figure 5 shows the distribution of bubble sizes (in terms of volume fraction) generated using the Sympatec method for PCFs (liquid:gas ratio of 1:7) produced using RA, $O_2$:$CO_2$ (35:65) and $CO_2$, and the DSS and Tessari foam preparation methods. Initial bubble size distribution for prepared foams is narrowest for RA<$O_2$:$CO_2$<$CO_2$ for PCFs produced by either preparation method. The Tessari method clearly generates more large bubbles compared to the DSS method regardless of the gas mixture used.

When polidocanol endovenous microfoam bubble size distribution is compared to a PCF made with the same gas mixture [$O_2$:$CO_2$ (65:35)] and liquid:gas ratio (1:7) using either the DSS or Tessari methods, there is clearly a narrower bubble size distribution for polidocanol endovenous microfoam when measured using the Sympatec method 40 and 115 seconds after foam preparation (Figure 6). Generally, the contrast is more pronounced when polidocanol endovenous microfoam is compared with the other $O_2$:$CO_2$ PCFs made with higher $CO_2$ content (Figure 6a versus Figure 5b-5f). A greater number of larger bubbles were present for PCFs, particularly using the Tessari method. At 40 seconds, polidocanol endovenous microfoam bubble size distribution is similar to RA (maximum bubble size <500 μm by this method) and at 115 seconds only slightly broader than RA for foams produced using the DSS method (Figure 6a

versus 5a) and without the large bubbles present for RA made by the Tessari method (Figure 6a versus 5d).

**Foam Drainage Time (FDT)**

Foam drainage time (FDT) is an important indicator of foam stability.[21] Essentially, if the foam drains rapidly, it will coarsen and degrade more rapidly; the cohesiveness of the foam will be of short duration and, thus, there will be less time for the drug to be in contact with the vessel wall because the degrading foam will not be able to properly displace the blood in the vessel lumen. In this test method, the FDT is the time at which light transmission is detected at the bottom of the foam column. Figure 7a shows the percent transmission of light through the foam over a period of time. For 100% $CO_2$, the foam drains very quickly and so light passes through the liquid layer almost immediately as indicated by the blue curve in the image. At a 30:70 $O_2$:$CO_2$ composition (red curve), the resulting foam also drains very quickly, but the curve does not inflect upward until the 45 second mark, as the foam is more stable and therefore drains more slowly than foam made with 100% $CO_2$. RA foam (black curve) is most stable and takes approximately 165 seconds before light begins to transmit through the draining liquid. FDT is shown for polidocanol endovenous microfoam compared with the DSS vs Tessari foams produced with different gas compositions, and 1:7 liquid:gas ratio (Figure 7b). In general, FDT is longer for DSS-prepared PCFs against the Tessari, with the difference becoming greater as the $CO_2$ levels in the foams decrease and the foams become more stable. The FDT of polidocanol endovenous microfoam is greater than any combination of gas mixture and method of preparation, with the exception of RA using the DSS method. Figure 7c shows the FDT for PEM and PCFs produced using the DSS method with two different liquid-to-gas ratios (1:7 and 1:4). The FDT increases in the order RA>polidocanol endovenous microfoam>$O_2$:$CO_2$>>$CO_2$-only, with foams of a liquid:gas ratio of 1:7 consistently more stable (longer FDTs) than those prepared with a 1:4 ratio. Polidocanol endovenous microfoam has a statistically significantly higher FDT (close to that of RA) when compared to all $CO_2$-containing PCFs at either liquid:gas ratio, prepared by either the DSS or Tessari methods ($p<0.035$).

**Foam Half Time (FHT)**

The comparison of FHT for various PCF formulations made using the DSS and Tessari methods (1:7 liquid:gas ratio) relative to polidocanol endovenous microfoam was generated by the Turbiscan™. Polidocanol endovenous microfoam displayed longer foam half time compared to all $CO_2$-containing PCFs, indicating that PEM had greater stability (Figure 8).

**Degradation Rate (DR)/Dwell Time (DT)**

Degradation Rate (DR) was evaluated using a biomimetic vein model to assess the ability of the foam to displace a blood substitute. Dwell time (DT) is a more clinically meaningful expression of degradation rate as it represents the amount of time that the foam is in contact with the vein wall and can act on the endothelium. DT is derived from DR and is calculated as the inverse of the DR using the following mathematical expression (refer to Figure 3c):

$$DT = \frac{(t_2 - t_1)}{\left[x(t_1) - x(t_2)\right]} \quad (1)$$

The experimental set-up consisted of a segment of polytetrafluorethylene (PTFE) tubing (either 4 mm or 10 mm in diameter) (Thermo Scientific Inc., USA) filled with a blood substitute and fixed to a platform with an adjustable inclination angle (Figure 3a). On initial foam injection, a foam plug was formed, which displaced the blood substitute as it travelled upwards along the tubing (*plug expansion phase)* (Figure 3b), while real time video images were captured simultaneously. Individual foam plugs were transiently stable and then entered the *plug degradation phase*, during which the plug interface receded towards the initial injection site (Figure 3c), ending in complete plug degradation. Videos obtained from both plug expansion and degradation phases were analyzed computationally (see Supplementary section S2.4).[25]

PCFs of various gas formulations were prepared by DSS or Tessari methods and introduced into the biomimetic vein model. The performance of PCFs (1:7 liquid:gas ratio) was compared with polidocanol endovenous microfoam, which demonstrated that $CO_2$-containing PCFs, prepared by either method and regardless of gas formulation, had DRs faster (12.51 ± 4.49 to 25.81 ± 3.09

mm/sec) than that of polidocanol endovenous microfoam (3.43 ± 0.29 mm/sec, $p < 0.05$) (Figure S3.1a). Foams prepared using RA were also less cohesive and degraded more rapidly (6.05 ± 1.79 to 11.22 ± 1.72 mm/sec) than polidocanol endovenous microfoam, although for RA made using DSS, this trend was not statistically significant (Figure S3.1a). Polidocanol endovenous microfoam was also compared with selected PCF formulations produced using the DSS method at liquid:gas ratios of 1:4 and 1:7 (Figure S3.1b). Again, polidocanol endovenous microfoam had a statistically lower DR compared with all other $CO_2$-based PCFs regardless of gas ratio ($p<0.04$) and a non-statistical trend for lower DR than the RA PCFs. In general, in this model, the PCFs made with 1:4 ratios had a trend to lower DR than the corresponding PCFs made at 1:7 ratio. This is contrary to the findings for FDT for the equivalent PCFs. Polidocanol endovenous microfoam had the longest DT, almost twice that of PCFs using RA and approximately eight times better than PCFs prepared using equivalent gas mixtures (Figures 9a and 9b).

## Discussion

For many years, physicians have compounded RA foams. However, lack of foam homogeneity can affect the viscosity and stability of these products. Broad bubble size distributions promote foam coarsening and degradation, whilst the lack of solubility of nitrogen in the blood results in isolated bubbles persisting in the circulation. Foams with smaller, more uniform bubble size possess a lower degradation rate, indicative of a more cohesive and stable foam that should ensure better contact with the endothelium of the vessel wall when injected into the vein. The ideal foam, then, should be durable enough to allow injection before separating into its gas and liquid components, yet short-lived enough to break down once injected. In this study, we compared methods for determining bubble size and bubble size distribution of foam formulations commonly used in vein treatment as well as methods to establish how the foam bubble size characteristics are related to the stability and cohesive nature of the foam. The optical image analysis method is an established method for static foam bubble sizing and bubbles size distribution measured for freshly-generated foam within its delivery syringe. The Sympatec method is a convenient tool for generating foam bubble size distribution and permits multiple measurements with differing time delays, while Turbiscan™ permits the measurement of foam coarsening on a continuous basis.

Regardless of measurement technique, we noted consistent differences between foam formulations. RA PCFs produce foams with smaller bubbles, which are inherently more stable, but due to the insolubility of nitrogen carry a higher risk of transient ischemic attack (TIA).[26] Replacement of RA with $CO_2$ results in PCFs with increased initial bubble size distribution and increasingly rapid coarsening with increasing $CO_2$ content. Polidocanol endovenous microfoam produces foams of smaller bubble size and narrower distribution, which is more comparable with RA than $CO_2$-containing PCFs made by either the DSS or Tessari method (Figures 5 and 6). Therefore, polidocanol endovenous microfoam may have the safety benefit of absorbability because of the absence of nitrogen and the efficacy of stable small bubble foam. Polidocanol endovenous microfoam produces no large bubbles (Figure 6), unlike the DSS and Tessari methods. The neurologic disturbances reported with foam sclerotherapy might to be related to gas embolisms that originate from the foam[12, 15, 27] although this might not be the only factor, as recent evidence suggests a role for the release of endothelin-1[28] and histamine[29] in these disturbances but remains hypothetical. In contrast, when $CO_2$ replaced air the frequency of bubbles seen on transcranial Doppler did not reduce but the symptoms were almost eliminated,[27] and in the few reported cases where urgent CT scanning was performed following onset of neurological incident, gas was found replacing the contents of the vertebral or middle cerebral artery and in the cerebral venous drainage;[15, 16] the causal relationship seems inescapable. Similarly in a study by Regan *et al.* where $O_2$:$CO_2$ foam was used in patients with proven right-to-left shunt, despite many patients with bubble emboli, no significant neurological events occurred[22] illustrating the benign nature of small rapidly absorbing bubbles. To complete the picture, a similar study needs to be conducted using air based foam.

In the foam drainage studies, polidocanol endovenous microfoam performed similarly to RA PCFs (Figures 7b and 7c), consistent with previous observations of similarities in bubble size and distribution for both foam types.[25] For PCFs containing higher proportions of $CO_2$, initial foam drainage was rapid (Figure 7a). This leads to initial high percentages of liquid drainage (first phase) and rapid attainment of an equilibrium position (just tens of seconds to reach the slower phase), whereas the relatively dry foam consisting of large bubbles has an inability to sustain the higher drainage rates warranted by the larger bubble growth. Figure 7c shows the relationship between gas composition of the foam prepared by the polidocanol endovenous foam technique, DSS, or Tessari methods and with two different liquid to gas ratios (1:7 and 1:4) and

foam drainage time. Drier foams (1:7 liquid:gas ratio) take longer to drain than corresponding wetter foams (1:4 ratio), which are most frequently used clinically. Wetter foams will contain bigger fluid channels between the bubbles, which pose less resistance to fluid flow under gravity; capillary forces will also be lower, resulting in faster drainage.

Foam Half Time (FHT) results showed relative consistency with FDT. The influential variables again were the methods used to generate the foams (polidocanol endovenous microfoam vs DSS vs Tessari) and the foam gas formulations, which followed the same trends observed for foam drainage, i.e. reduced stability with increasing $CO_2$ content (Figure 8). The reproducibility of the results using Turbiscan™ was good, with relatively small standard deviation in the data. This validates the Turbiscan™ as a useful and convenient tool for generating FHT data in addition to dynamic foam drainage. Foam stability measurements using a vertically standing column of foam, however, only partly convey the physical requirements for useful foam. When injected into an incompetent vein, the sclerosing foam must ensure good contact with the vessel endothelial lining while displacing blood volume.

It is recognised that the characteristics of sclerosing foams for the treatment of varicose veins may be a major determinant of efficacy and safety,[3] a unique *in vitro* biomimetic model was therefore developed to determine the behavior of foam under clinically relevant conditions. This model allows for an assessment of the liquid-displacing capability of the foam and its subsequent rate of degradation within the vessel. In other measures of stability, RA foam performed best, but in the biomimetic model polidocanol endovenous microfoam had the slowest DR, almost half that of RA and eight times better than DSS and Tessari-equivalent gas mixtures (Figure S3.1). Dwell time (DT), a more meaningful expression of these data, characterizes the length of time the foam plug stays in contact with the vein wall. Polidocanol endovenous microfoam had a DT twice as long as PCF generated with room air (Figure 9). In a previous report, sufficient practical details were disclosed so that this method whether manually performed or with the aid of computerized image analysis could be reproduced, introducing a new parameter - degradation rate - to be used as a standard to quantify the cohesiveness of foams.[25] In addition, the biomimetic analysis system may be of value to researchers and clinicians to gain a deeper understanding of the physical parameters governing foam performance, ultimately leading to the determination of optimal foam for differing vein diameters and venous disorders.

## Technical Limitations and Future Perspectives

In the present study, we performed a range of experiments to compare the performance of PCFs with PEM. There were some practical limitations to the study due to the time required to manipulate the foam and place it into the instrument before taking a measurement. This was generally 30-40 seconds for most techniques which meant the foam had already undergone some degradation; this is however, likely to be the time it would take a physician to administer the foam into the vein in clinical practice. Despite the wide range of experimental conditions investigated, the effect of several parameters has not been examined and could be the subject of future investigations. One such area involves the effect of the physical properties of carrier fluids on the stability/cohesiveness of sclerosing foams. Experiments using fluids of varying viscosity or physiological fluids (i.e., plasma or whole blood) may be performed and could be of interest due to the deactivation effects of biological fluids on sclerosants. However, this would require optimization of existing techniques for characterising foam stability. Another area of possible investigation involves the effect of clinically relevant parameters such as foam injection rate on foam stability/cohesiveness. A third avenue of research could involve a more extensive investigation of the effect of different sclerosing agents (i.e., such as sodium tetradecyl sulphate or alcohol) and their concentration on foam stability/cohesiveness.

## Conclusion

Polidocanol foams are not all the same, and it is difficult to compare clinical results unless characteristics are known and reproducible. Air foams have good performance but have associated risks, with persistent nitrogen bubbles in the circulation. Small bubbles and narrow bubble size distribution, with slow drainage and separation times, improves foam performance by enhancing stability. The biomimetic vein test produces a new measure of foam performance that demonstrates the low degradation rate and longer dwell time of polidocanol endovenous microfoam compared to PCFs. The polidocanol endovenous microfoam with $O_2$:$CO_2$, low nitrogen gas composition and proprietary foam generation device results in better overall performance than PCF in a variety of tests, without the associated risk of high-nitrogen room air bubbles.

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**Competing interest:** JH and MA worked for Biocompatibles Ltd. (a BTG International group company) on the development of PEM. VOB and ALL are employees at Biocompatibles Ltd. (a BTG International group company) and they are working on the development of PEM, although they were not directly paid by this research project.

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**Contributorship:** DC, VOB: protocol design and development, execution of experiments, and data analysis; DNA, JH, XueZ, MA: execution of experiments; MH, XunZ, ALL, DDIW: conceived the study, critical revisions, data analysis.

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## Figure Captions

**Figure 1** Methods for producing physician compounded foams (PCFs) and polidocanol endovenous microfoam. In the DSS method, syringes are connected by a Combidyn® adapter (a), while in the Tessari method, they are connected by a three-way valve (b). In both techniques, the foam was produced by passing the polidocanol solution (liquid phase) from one syringe, ten times into and out of the other syringe initially containing the gas or gas mixture (gaseous phase). Foam was produced at room temperature (20°C-22°C). The proprietary canister system for generating polidocanol endovenous microfoam (Varithena®) is shown in (c).

**Figure 2** Methods for measuring bubble size distribution. Sympatec QICPIC image analysis sensor (a) and Turbiscan™ LAB apparatus (b).

**Figure 3** Schematic of the biomimetic vein model set-up (a). Foam is injected into the tube over time $t_1$ to form a column of length $x$ (mm) (b). On completion of the injection at $x = L_1$, the foam degrades over time $t_2$ to a length of $x = L_2$, whereby the degradation rate (DR) and dwell time (DT) may be attained (c). CFAS = computational foam analysis system.

**Figure 4** Comparison of glass plate and Sympatec method analyses of polidocanol endovenous microfoam. (a) Image of polidocanol endovenous microfoam from the optical image analysis method and (b) bubble size distribution measured for this foam; compared to (c) a single frame image of the same sample of polidocanol endovenous microfoam captured from the Sympatec dynamic image capture method and (d) the bubble size distribution measured by this method (over a 15s period, corresponding to 375 image frames). Note how the Sympatec over-reports the true bubble size.

**Figure 5** Size distributions of physician compounded foams (DSS vs Tessari) with liquid:gas ratio 1:7, obtained using the Sympatec method. Bubble size distribution curves for PCFs using different gas formulations (a+d RA; b+e: $O_2$:$CO_2$ of 35:65; c+f 100% $CO_2$) for both the DSS and Tessari methods 40s and 115s after foam preparation. Arrows highlight existence of larger bubbles in the PCF. RA = room air. n = 5.

**Figure 6** Comparison of bubble size distributions at 40s and 115s for polidocanol endovenous microfoam (a) compared to PCF $O_2$:$CO_2$ (65:35) made by DSS (b) and Tessari (c) methods. Arrows highlight existence of large bubbles in the foam. n = 5.

**Figure 7** Example foam drainage time curves used to measure FDT (a); FDT for DSS versus Tessari, and compared with polidocanol endovenous foam (b); FDT for different PCFs made using the DSS method at 1:4 and 1:7 liquid to gas ratios, and compared with polidocanol endovenous microfoam (c). RA = room air; PEM = polidocanol endovenous microfoam; FDT = foam drainage time. Standard deviation ranged from 0.37% to 5.58% of the mean (n = 4).

**Figure 8** Comparison of the foam half time (Turbiscan™) for various physician compounded foam (PCF) formulations made using DSS and Tessari methods (1:7 liquid:gas ratio) and foam half time for polidocanol endovenous microfoam. Polidocanol endovenous foam displayed a longer FHT than $CO_2$-containing PCFs. FHT = foam half time; RA = room air; PEM = polidocanol endovenous microfoam. n = 5.

**Figure 9** Polidocanol endovenous microfoam had the longest calculated dwell time, almost twice that of PCFs using RA and approximately eight times better than PCFs prepared using equivalent gas mixtures in a biomimetic model. RA = room air; PEM = polidocanol endovenous microfoam. n = 4.

## List of Tables

**Table I** – Summary of the methods of foam characterization employed in the present study.

| Equipment | Analysis | Supplier |
|---|---|---|
| Glass plate method | Bubble Size and Bubble Size Distribution | In-house method developed at BTG |
| Sympatec QICPIC | Bubble Size and Bubble Size Distribution | Sympatec Ltd, Bury, Lancashire, UK |
| Turbiscan™ LAB | FHT & FDT (foam stability) | Formulaction SAS, L'Union, France |
| Biomimetic Vein Model | Foam Dwell Time/Degradation Rate (foam stability) | In-house method developed at University of Southampton |

**Table II** – Number of experimental repeats for each foam characterization experiment performed.

| Experiment | Number of repeats (N) |
|---|---|
| Foam Bubble Sizing | 5 |
| Foam Half Time | 5 |
| Foam Drainage Time | 4 |
| Foam Dwell Time | 4 |

**Table III** – Selected p-values obtained from pairwise statistical comparisons between PEM and PCFs foams (p-value << 0.01 indicates values lower than 0.001).

| Foam formulations | p-values |
|---|---|
| **Foam Drainage Time (FDT, Figure 7)** | |
| PEM *vs* DSS (1:7) | 0.01 < p < 0.05 (=0.033) |
| PEM *vs* Tessari (1:7) | p << 0.01 |
| PEM *vs* DSS (1:4) | 0.01 < p < 0.05 (=0.0012) |
| PEM *vs* Tessari (1:4) | p << 0.01 |
| **Foam Half Time (FHT, Figure 8)** | |
| DSS (combined) *vs* Tessari (combined) | 0.01 < p < 0.05 (=0.045) |
| PEM *vs* DSS RA (1:7) | p << 0.01 |
| PEM *vs* DSS 65:35 $O_2$:$CO_2$ (1:7) | p > 0.05 |
| PEM *vs* DSS 35:65 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 30:70 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 23:77 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 100 $CO_2$ (1:7) | p << 0.01 |
| **Dwell Time (DT, Figure 9)** | |
| PEM *vs* DSS RA (1:7) | 0.01 < p < 0.05 (=0.018) |
| PEM *vs* DSS 65:35 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 35:65 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 30:70 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 23:77 $O_2$:$CO_2$ (1:7) | p << 0.01 |
| PEM *vs* DSS 100 $CO_2$ (1:7) | p << 0.01 |
| PEM *vs* Tessari RA (1:7) | p << 0.01 |
| PEM *vs* Tessari $O_2$:$CO_2$ (1:7) | p << 0.01 |

| | |
|---|---|
| PEM *vs* Tessari 35:65 $O_2$:$CO_2$ (1:7) | $p << 0.01$ |
| PEM *vs* Tessari 30:70 $O_2$:$CO_2$ (1:7) | $p << 0.01$ |
| PEM *vs* Tessari 23:77 $O_2$:$CO_2$ (1:7) | $p << 0.01$ |
| PEM *vs* Tessari 100 $CO_2$ (1:7) | $p << 0.01$ |
| PEM *vs* DSS RA (1:4) | $p < 0.01$ (=0.0054) |
| PEM *vs* DSS 65:35 $O_2$:$CO_2$ (1:4) | $p << 0.01$ |
| PEM *vs* DSS 35:65 $O_2$:$CO_2$ (1:4) | $p << 0.01$ |
| PEM *vs* DSS 100 $CO_2$ (1:4) | $p << 0.01$ |

## List of Figures

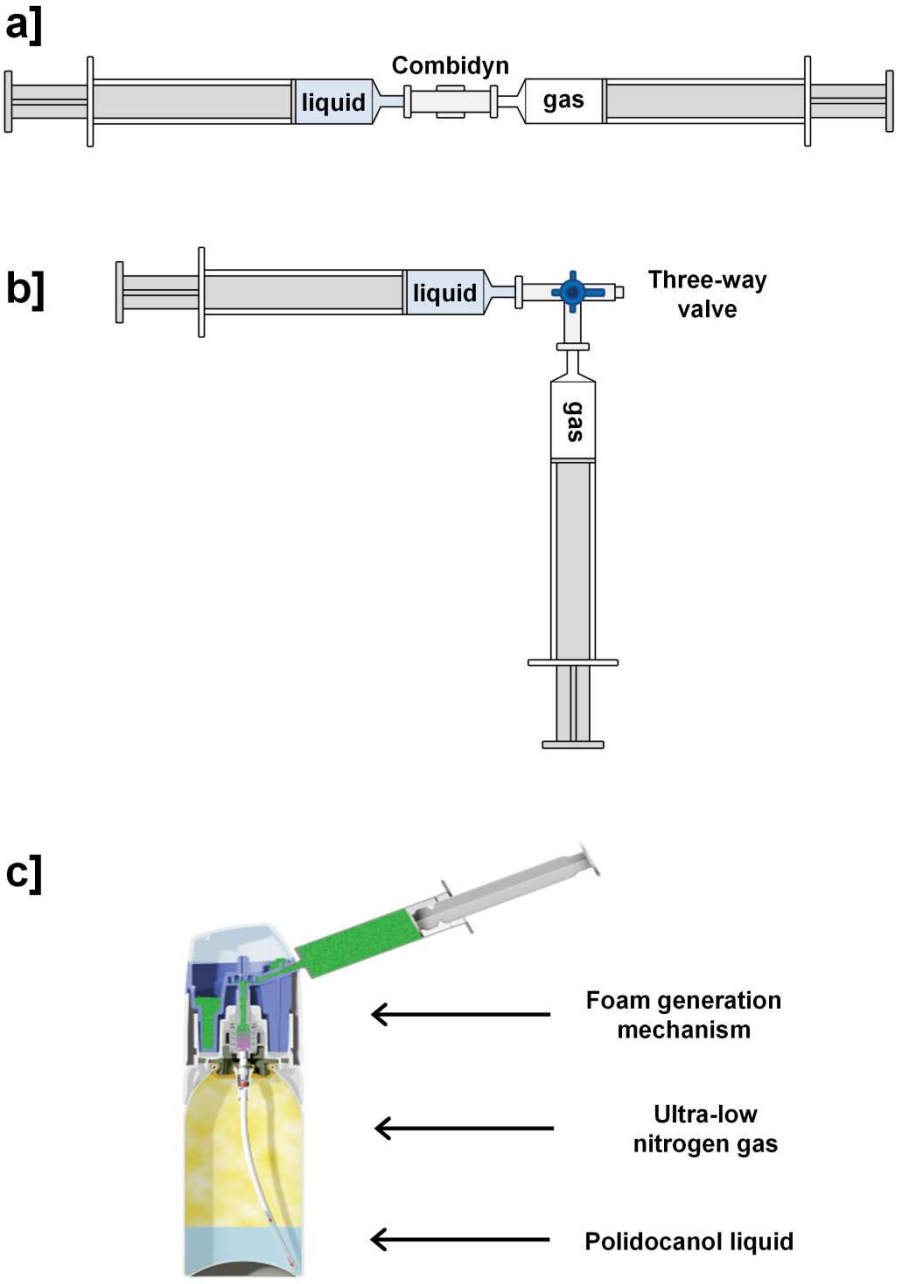


**Figure 1**

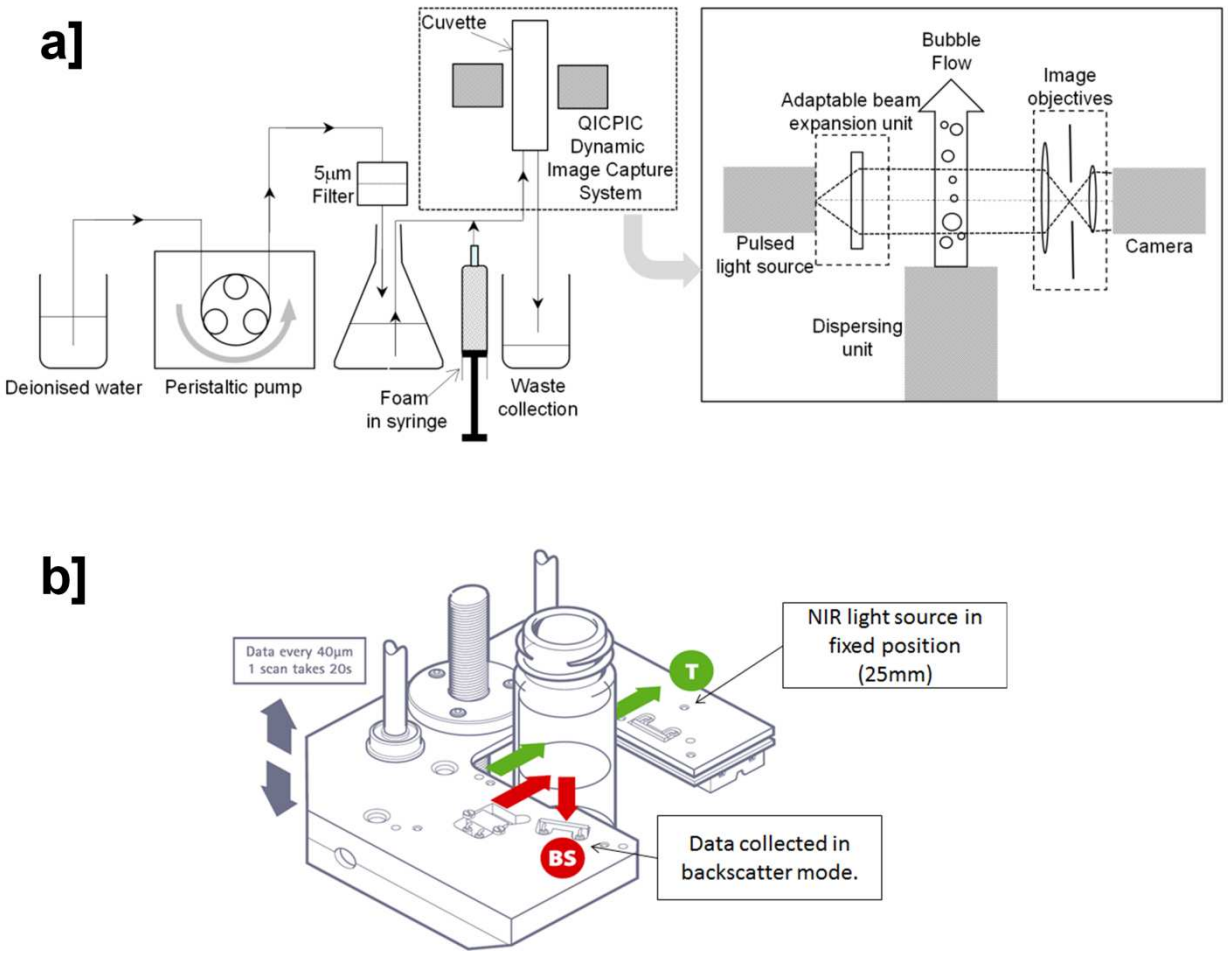
a]
Cuvette
QICPIC
Dynamic
Image Capture
System
5µm
Filter
Deionised water
Peristaltic pump
Foam
in syringe
Waste
collection
Bubble
Flow
Adaptable beam
expansion unit
Image
objectives
Pulsed
light source
Camera
Dispersing
unit
b]
Data every 40µm
1 scan takes 20s
NIR light source in
fixed position
(25mm)
T
BS
Data collected in
backscatter mode.

**Figure 2**

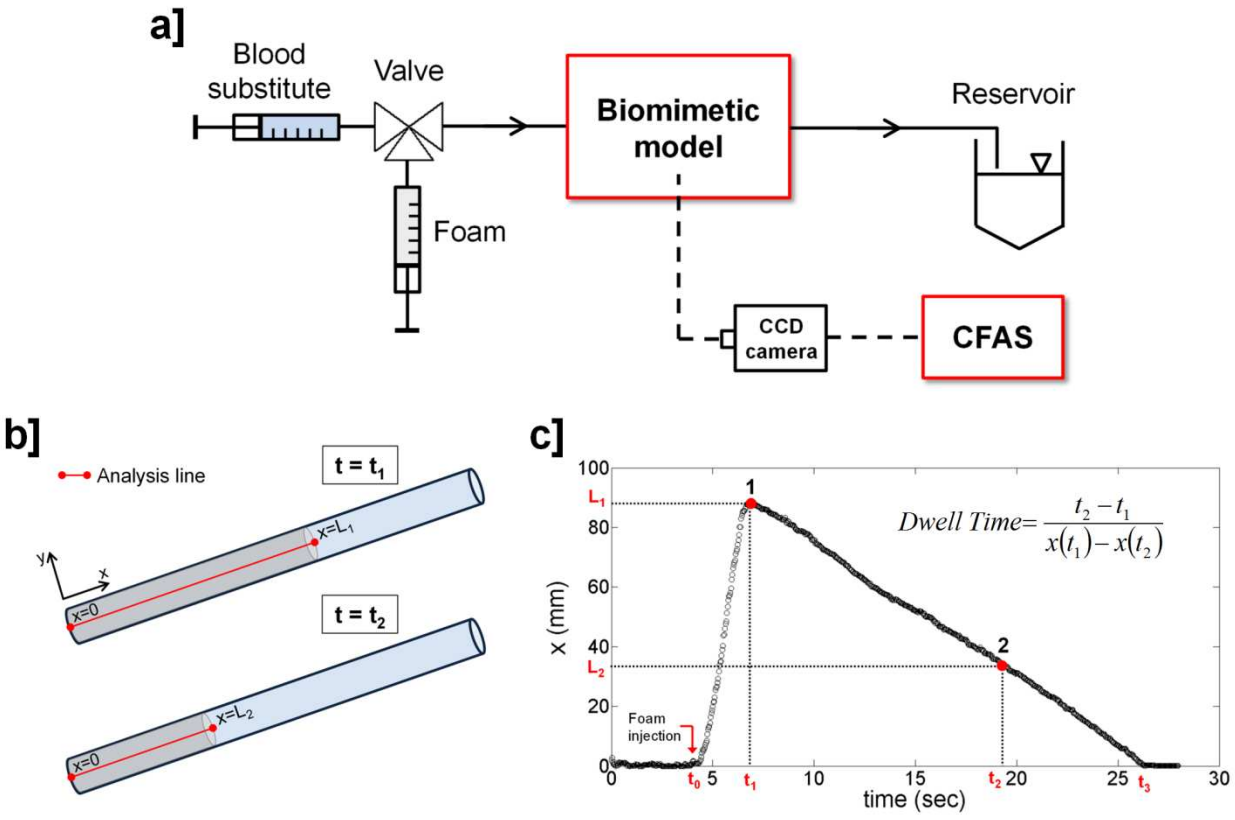
a]
Blood substitute
Valve
Biomimetic model
Reservoir
Foam
CCD camera
CFAS
b]
Analysis line
t = t1
x=L1
y
x
x=0
t = t2
x=L2
x=0
c]
100
L1
80
60
40
L2
20
0
x (mm)
1
2
Dwell Time= t2 − t1 / x(t1) − x(t2)
Foam injection
t0 5 t1 10 15 t2 20 25 t3 30
time (sec)

**Figure 3**

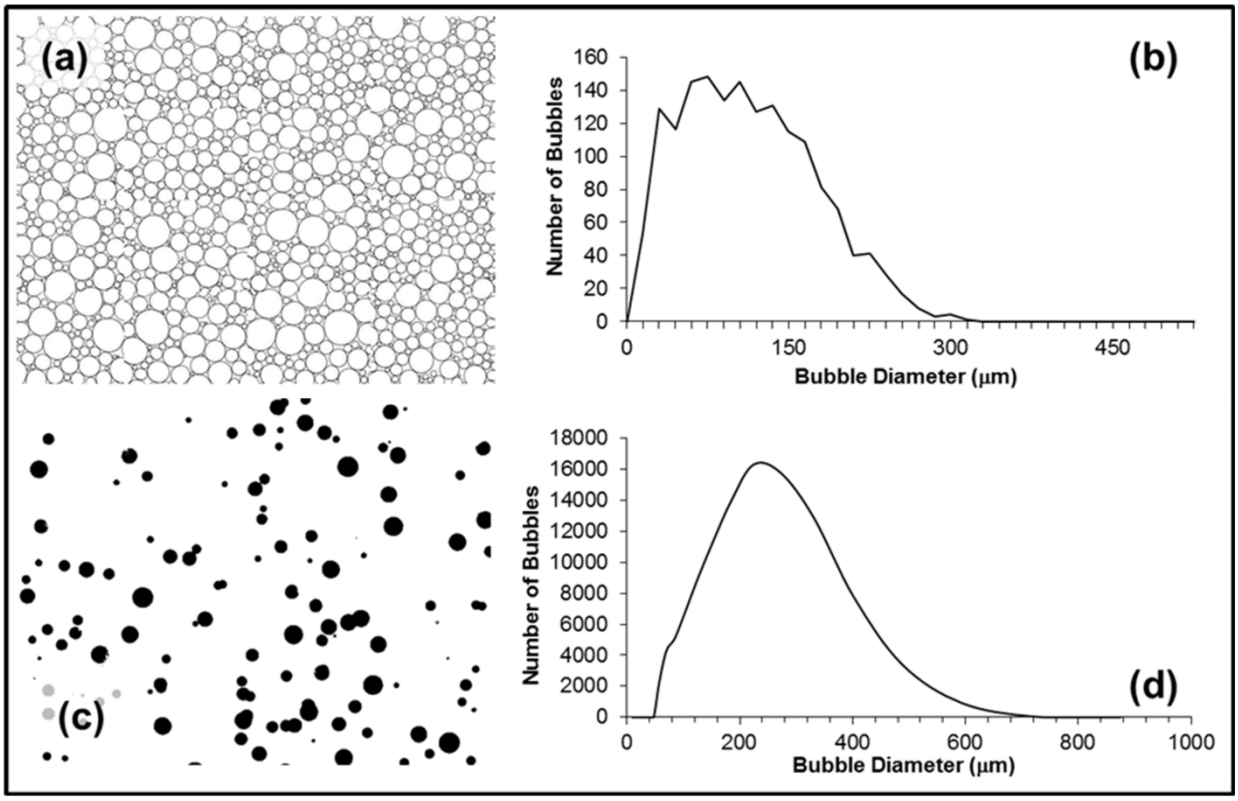
(a)
(b)
Number of Bubbles
160
140
120
100
80
60
40
20
0
0
150
300
450
Bubble Diameter (μm)
(c)
(d)
Number of Bubbles
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
200
400
600
800
1000
Bubble Diameter (μm)

**Figure 4**

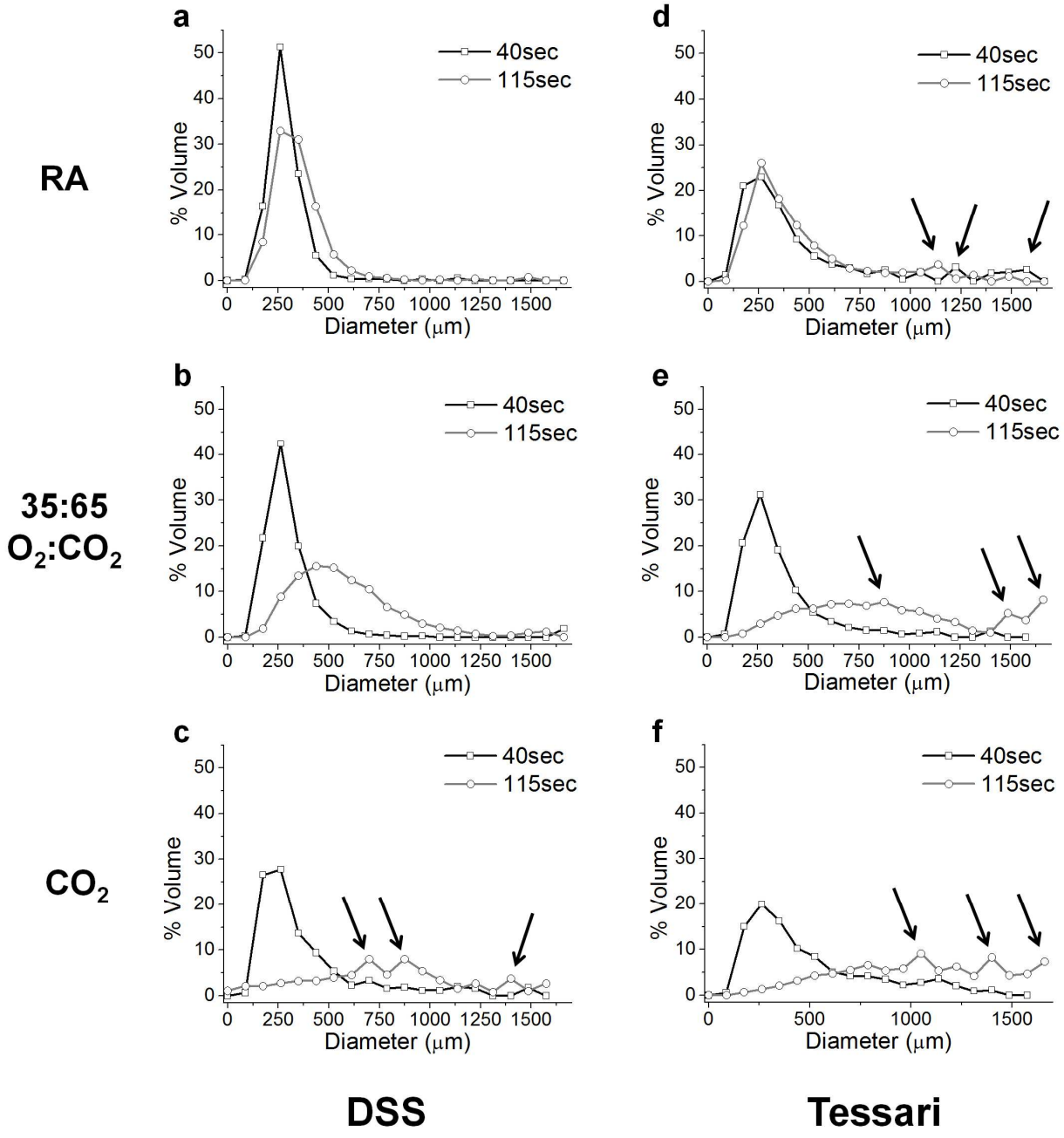
RA
35:65 $O_2$:$CO_2$
$CO_2$
DSS
Tessari
a
b
c
d
e
f
40sec
115sec
% Volume
Diameter (μm)

**Figure 5**

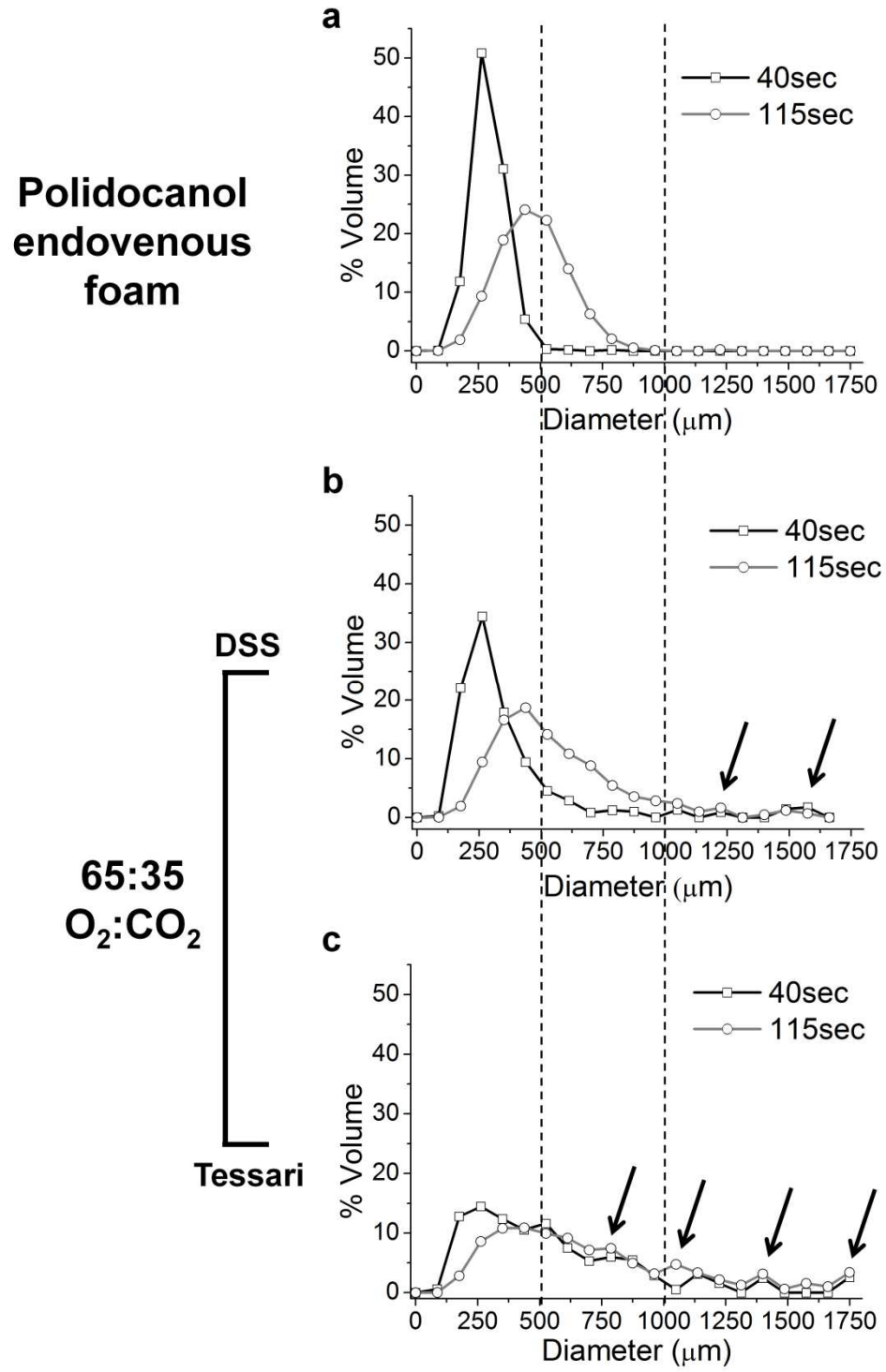
Polidocanol endovenous foam
65:35 $O_2$:$CO_2$
DSS
Tessari
a
b
c
40sec
115sec
% Volume
Diameter (μm)
0
10
20
30
40
50
0 250 500 750 1000 1250 1500 1750

**Figure 6**

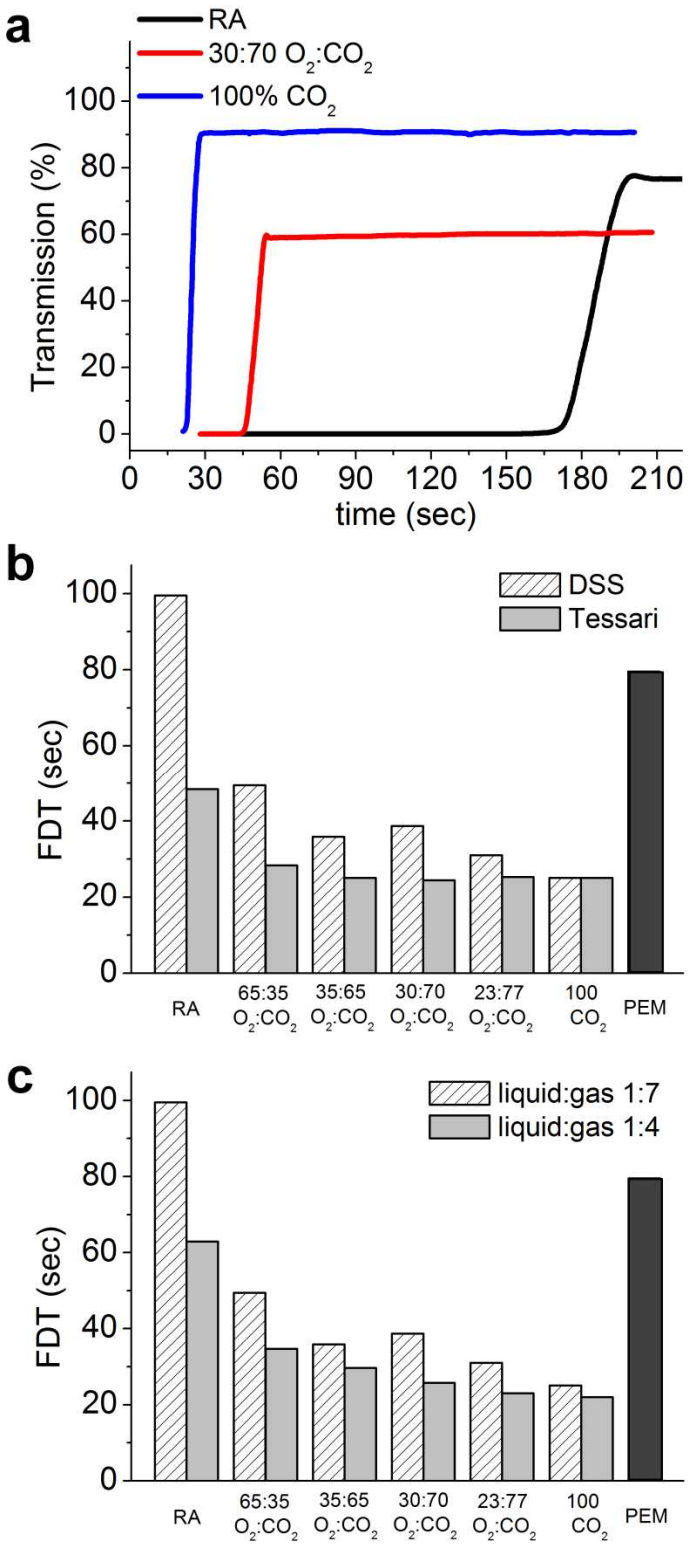
a
RA
30:70 O2:CO2
100% CO2
Transmission (%)
time (sec)
b
DSS
Tessari
FDT (sec)
RA
65:35 O2:CO2
35:65 O2:CO2
30:70 O2:CO2
23:77 O2:CO2
100 CO2
PEM
c
liquid:gas 1:7
liquid:gas 1:4
FDT (sec)
RA
65:35 O2:CO2
35:65 O2:CO2
30:70 O2:CO2
23:77 O2:CO2
100 CO2
PEM

**Figure 7**

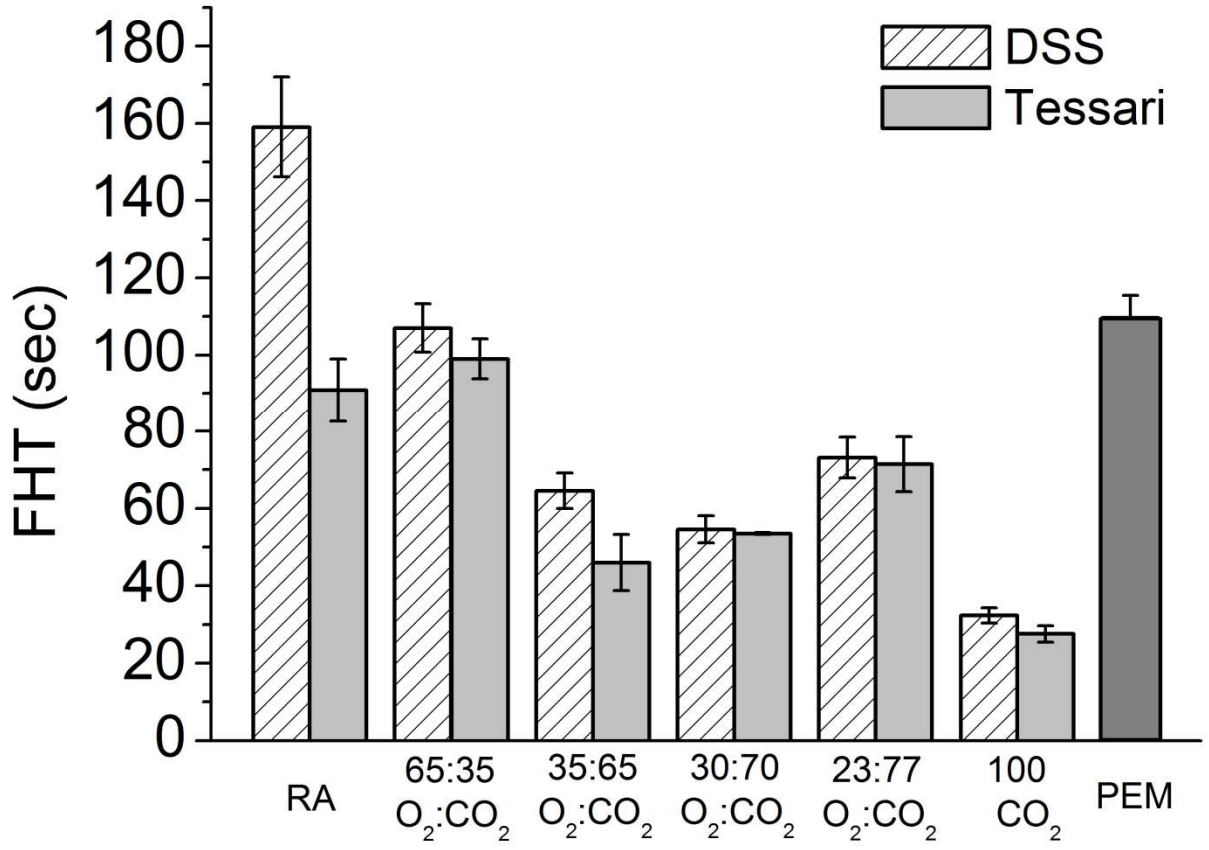
DSS
Tessari
FHT (sec)
180
160
140
120
100
80
60
40
20
0
RA
65:35 $O_2$:$CO_2$
35:65 $O_2$:$CO_2$
30:70 $O_2$:$CO_2$
23:77 $O_2$:$CO_2$
100 $CO_2$
PEM

**Figure 8**

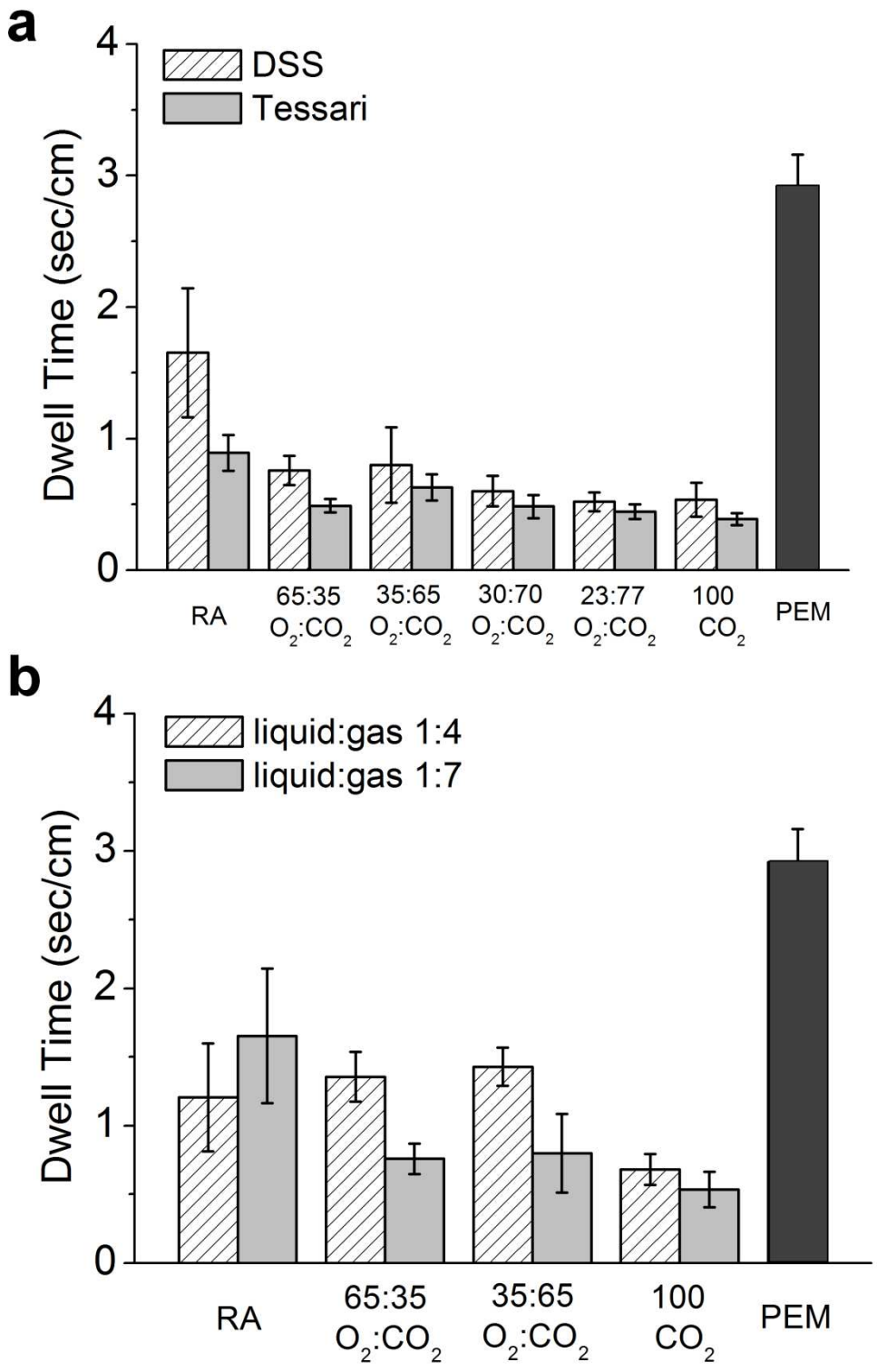
a
DSS
Tessari
Dwell Time (sec/cm)
0
1
2
3
4
RA
65:35 O2:CO2
35:65 O2:CO2
30:70 O2:CO2
23:77 O2:CO2
100 CO2
PEM
b
liquid:gas 1:4
liquid:gas 1:7
Dwell Time (sec/cm)
0
1
2
3
4
RA
65:35 O2:CO2
35:65 O2:CO2
100 CO2
PEM

**Figure 9**

# Supplementary Information for:

# Benefits of Polidocanol Endovenous Microfoam (Varithena®) Compared with Physician Compounded Foams

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## S1 Methods of foam preparation

### S1.1 Preparation of Physician Compounded Foams (PCFs)

Polidocanol (Shasun Pharma Solutions Ltd, Dudley, Northhumberland, UK) was formulated as a 1% buffered saline solution containing 4.2% ethanol and was used as a detergent-type sclerosing agent throughout these studies. PCFs were produced by either Double Syringe System (DSS) or Tessari methods. The DSS-Tessari method (DSS method for short from herein) is a variation of the Tessari method developed by Lorenzo Tessari [1].

The DSS foam was produced by passing 1 mL polidocanol from a 5 mL syringe (Discardit™ II, Becton Dickinson, Erembodegem, Belgium), ten times into and out of a 10 mL syringe containing 7 mL of gas. Syringes were interfaced *via* a straight connector (equivalent, Female-to-Female Luer Lock Connector, QOSINA Edgewood, NY, USA). The Double Syringe System remains a popular method employed by the physician to produce sclerosing foams [2]. The physician may use a combination of 2, 5 or 10 mL syringes when making the foam. A photograph of the Double Syringe System (DSS) is shown in figure S1.1.

**Figure S1.1**: Image of the Double Syringe System (DSS).

For the Tessari method, the straight connector is replaced with a 3-way valve (BD Connecta™ 3-Way Stopcocks, Becton Dickinson, Erembodegem, Belgium). A further modification involves setting the valve tap at a 30º angle to increase shear when passing the foam between the syringes [3][4], which was adopted in the present study. As with the DSS method, 5 and 10 mL syringes were used to prepare the foam. A photograph of the Tessari system is shown in figure S1.2.

**Figure S1.2**: Image of the Tessari system.

A 5 $\mu$m filter is sometimes placed between either the 5 or 10 mL syringe and the straight connector (in the DSS method) or 3-way valve (in the Tessari method) [5][6]. A filter was not used in the preparation of PCFs in these studies.

**S1.2 Preparation of Polidocanol Endovenous Microfoam (PEM)**

PEM is a combination drug device product in development by Provensis Ltd. (a BTG International Group Company, London, UK) consisting of a proprietary 65:35 $O_2$:$CO_2$ gas mixture with ultralow nitrogen content (<0.8%) and 1% polidocanol solution (no additional stabilisers are added), contained within a pressurised canister and combined on discharge from the canister as a uniform microfoam. Sterile canisters of the product were used as per the instructions for use (IFU), to generate 5 mL of microfoam for experimentation. The microfoam was drawn from the canister *via* a Microfoam Transfer Unit (MTU) into a 10 mL Norm-Ject syringe (Henke-Sass Wolf, Tuttlingen, Germany). All the analysis was done on the foams recovered from the canisters as described. Every effort was used to minimise the time between the discharge of the foam into the syringe and the analysis.

[1] Tessari L. Tessari method for foam sclerotherapy (10 years of history of technology that changed the world of phlebology). *XVI World Congress of the Union Internationale de Phlebologie*, Monaco, 2009 abstract # AP1.7-7

[2] Hamel-Desnos C, Desnos P, Wollmann JC, et al. Evaluation of the efficacy of polidocanol in the form of foam compared with liquid form in sclerotherapy of the greater saphenous vein: Initial results. *Dermatol Surg*. 2003; 29:1170–5

[3] Cavezzi A, Tessari L. Foam sclerotherapy techniques: different gases and methods of preparation, catheter versus direct injection. *Phlebology*. 2009; 24(6):247-51

[4] Mauriello J, Foam Sclerotherapy with other Gases. www.angioadvancements.com

[5] Shirazi AR, Goldman MP. The use of a 5-micron filter hub increases foam stability when using the double syringe technique. *Dermatol Surg* 2008; 34:91–2

[6] Hill D. Effect of a 5 micron filter on CO2 sclerosant foam stability. *XVI World Congress of the UIP*. Monaco, 31 August - 4 September 2009

## S2 Methods of foam characterization

### S2.1 Glass plate method

Optical image analysis of 2D foams is a well described method of capturing and measuring bubble size and bubble size distribution [1]. An aliquot of freshly generated foam (49 $\mu$L) was placed on a glass plate and immediately covered by another. The plates are thick enough not to be distorted and are separated by 32 $\mu$m. A flattened monolayer was created, of flat cylindrical bubbles 32 $\mu$m high. A light microscope and camera (AxioCam ICc 1, Carl Zeiss Microscopy, Cambridge,UK), with lighting adjusted to create sharp images of circular boundaries, were employed to capture sequential image fields. A built-in software was used to "stitch" fields together. Each individual bubble was identified and diameter measured using the image analysis (AxioVision, Zeiss) programme with bespoke BubbleSizerMeasure macro. In this way between 2000-3000 bubbles were measured. The diameter of these flattened bubbles ($d_f$) was automatically converted to spherical equivalent diameters ($d_s$), as follows:

$$d_s = \left[\frac{1}{4} x 6 d_f^2 + 3 d_f x(\pi - 4) + x^2(10 - 3\pi)\right]^{1/3} \quad \text{(Eq.1)}$$

This expression is valid for bubbles greater than the plate separation (which was calibrated before each measurement run). Bubbles less than the plate separation remain spherical and for these no adjustment was made. A data sheet containing a list of each bubble diameter was created for each run. The data are presented as a histogram showing the percentage bubbles from a series of images within 15 $\mu$m bin size ranges. The Limit of Detection (LOD) for the method was set at 11 $\mu$m.

The transfer of foam to the glass plate and the application of the second plate was completed in approximately 10 seconds; as the foam was flattened there was no drainage of liquid, and bubble size coarsening by gas transfer was greatly reduced such that the time taken to capture a series of images was not an issue. Since the purpose of the glass plate method is to capture static images and measure bubble size immediately after foam generation, it is unsuitable for the measurement of foam dynamics [2].

[1] Pugh RJ. Experimental techniques for studying the structure of foams and froths. *Advances in Colloid and Interface Science*. 2005; 114-115:239-251

[2] Cheng HC and Lemilch R. Errors in measurement of bubble size distribution in foam. *Industrial and Engineering Chemistry Fundamentals*. 1983; 38:105-109

## S2.2 Sympatec QICPIC

Bubble size distribution was assessed using a particle size and shape analyser (supplied by Sympatec UK, Bury, Lancashire). A 10 mL BD syringe containing either PCF or PEM was placed in a syringe pump (Harvard Apparatus PHD/ULTRA, Holliston, MA) and secured. The stream of water that carried the bubbles past the detector was driven by peristaltic pump (Watson Marlow 505S, Falmouth, UK) set at 35 rpm, which was turned on prior to any analysis to clear all the larger air bubbles from the system. The prepared foam was injected from the syringe pump at the maximum rate (37.6 mL/min) into the stream of deionised water conveyed through a 2 mm cuvette where image analysis software captured images of the foam (at 25 frames per second with the detector positioned in the middle of the cuvette). A distribution plot of bubble size was reported in the form of a histogram.

The analysis comprised 5 replicates of 15 second intervals of analysis of the bubbles travelling through the cuvette. The time taken from filling of the syringe with foam to beginning of the analysis was approximately 35-40 seconds. An initial measurement of bubble size was taken immediately after injection of the foam (approximately 40s post foam generation), which is clinically-relevant as foam is likely to be injected into the vein within that timeframe. We also took an additional measurement at 115s in order to better demonstrate how the different foams were coarsening, although this time is less relevant clinically as most physicians would make efforts to administer the foam sooner after its generation.

## S2.3 Turbiscan™ LAB

The Turbiscan™ LAb (Formulaction SAS, L'Union, France) is an optical analyser that is capable of multiple light scattering measurements [1]. The system consists of a pulsed near-infrared light single wavelength source (880 nm) which penetrates the sample, and is detected by transmission and backscatter detectors. The level of backscatter (BS) is related to the photon mean free path through the foam, and using a software algorithm [2] it can be used to

calculate the Sauter Mean Diameter ($d_{32}$) of the bubble size distribution of the foam using the equation:

$$BS = \left[ \frac{2d_{32}}{3\phi(1-g)Q_S} \right]^{-1/2} \quad \text{(Eq.2)}$$

Where $\phi$ is the gas volume fraction, $g$ and $Qs$ are optical factors depending upon $d_{32}$ and refractive index [3]. The refractive index of the polidocanol solution used was 1.456 [4] and the one of the gas or gas mixture in question was calculated from gas refractive indices available from the UK National Physical Laboratory [5].

The foam was added carefully to the glass vial *via* a 21G needle to avoid the introduction of additional large bubbles. The light source was fixed at 25 mm above the bottom of the vial which represents the middle of the foam sample in the vial. The level of backscatter was recorded every second over 2 minutes. Using the Lab$^{Expert}$ software, $d_{32}$ values for the foam were derived from this backscatter data.

Using the Turbiscan$^{TM}$ LAB the height of liquid accumulated in the vial (55 mm) was measured automatically scanning the vial every 30 seconds and plotted over time; the time to 50% drainage (or Foam Half Time, FHT) could be read from the resultant graphs.

We defined the Foam Drainage Time (FDT) as the time at which liquid first appears at the base of the Turbiscan vial. A full vial of foam is placed in the Turbiscan in fixed mode lined up with the base of the vial, and the moment of first transmission across the vial detected.

[1] Mengual O, et al. TURBISCAN MA 2000: multiple light scattering measurement for concentrated emulsion and suspension instability analysis. *Talanta*. 1999; 50(2):445-56

[2] Mengual O, Meunier G, and Snabre P. Optical characterization of concentrated dispersions. On-line process monitoring and control. *Recentes progres en genie des procedes*. 2001; 15(84):61-67

[3] Balerin C, et al. Effect of formulation and processing factors on the properties of liquid food foams. *Journal of Food Engineering*. 2007; 78:802-809

[4] *Sigma Aldrich MSDS Sheet for Polyoxyethylene 4 Lauryl Ether (Polidocanol)*. Available from: http://www.chemistry.mcgill.ca/msds/msds/9002-92-0.pdf

[5] *National Physical Laboratory*. Available from: www.kayelaby.npl.co.uk/general_physics/2_5/2_5_7.html

## S2.4 Biomimetic Vein Model

*S2.4.1 Experimental set-up*

The cohesiveness of sclerosing foams was investigated within a biomimetic model (Figure S2.1). Details of the model specifications have been previously reported [1]. The model comprised a segment of 4 mm inner diameter (ID) polytetrafluoroethylene (PTFE) tubing (Thermo Scientific Inc., USA) lodged in a straight etching within a rigid bespoke platform at a fixed, 25º inclination angle. The great saphenous vein varies in diameter from 2.3 - 4.4 mm [2], and therefore we selected 4 mm diameter tubing as this is the upper size and more typical of an incompetent vein. A three-way stopcock (Baxter, USA) was placed at the lower end of the tube, for sequential tube filling, foam injection and tube flushing. A ruler was attached to the platform surface for image calibration, and a high speed CCD camera was used to capture real time videos of foam plug expansion and degradation, at a temporal resolution of 30 ms.


**Figure S2.1** Photograph of the experimental set-up. PTFE tubing in a platform (*1*) stabilised within a manifold (*2*). Platform angle was measured by a digital inclinometer (*3*). A three-way stopcock at the lower end of the tube allowed sequential tube filling, foam injection and tube flushing (*4*). (Taken from Ref. [1])

*S2.4.2 Experimental protocols*

The tube was filled with a blood substitute (30% v/v glycerol in purified water), with dynamic viscosity of 0.003 Pa×sec and density of 1078 kg/m$^3$, which are comparable with the bulk values for blood [3]. The average injected volume of foam was 1.29 ± 0.18 mL. Upon initial foam injection a foam plug was formed, which displaced the blood substitute as it travelled upwards along the tubing, and real time video images were captured simultaneously. Individual foam plugs were transiently stable, followed by degradation during which the plug interface receded towards the initial injection site, until complete plug degradation. Videos obtained from both plug expansion and degradation phases were transferred to a personal computer (PC) and analysed offline as described below.

*S2.4.3 Computational foam analysis system*

An in-house software was developed using MATLAB (The Mathworks Inc., USA) to determine foam degradation rate from the acquired experimental videos. Details about the software have been reported in a previous publication [1], and are briefly described below.

- A video in Audio Video Interleave (AVI) format was loaded and each individual frame was automatically extracted.
- Two reference points on the ruler were manually selected by graphical input function, allowing for precise determination of tube inclination angle, image rotation and dimensional calibration (conversion from pixel units into physical units; e.g., millimetres).
- A Region Of Interest (ROI) on the images was selected for processing, which contained only the segment of the tube where the foam plug was present.
- Linear mapping was performed to optimise image contrast. Images were subsequently converted to black and white (B/W) binary format by thresholding. The resulting foam plug then appeared as a white surface in a black background.
- An analysis line was manually defined for the detection of the plug-fluid interface. This was located between the tube centreline and tube base. The code automatically read pixel intensity values along the analysis line and located the foam-fluid interface at the point of intensity discontinuity (i.e., pixel value varied from 1 to 0 at the

interface). This step allowed accurate determination of plug length and the calculation of plug volume.

- The plug volume–time trend was plotted automatically after completion of the video processing. By manually selecting two points on the degradation curve, the code calculated the plug degradation rate (DR, mm/sec) by linear interpolation of the experimental data points located within the selected interval. The interpolating function for the degradation phase was determined by least square method. Dwell time (DT) was then calculated as the inverse of DR.

[1] Carugo D, et al. A novel biomimetic analysis system for quantitative characterisation of sclerosing foams used for the treatment of varicose veins. *J Mater Sci Mater Med*. 2013; 24(6):1417-1423.

[2] Spivack DE, Kelly P, Gaughan JP, van Bemmelen PS, Mapping of superficial extremity veins: normal diameters and trends in a vascular patient-population. *Ultrasound Med Biol.* 2012; 38(2): 190-4.

[3] Pries A, Neuhaus D, Gaehtgens P. Blood viscosity in tube flow: dependence on diameter and hematocrit. *Am J Physiol*. 1992; 263(6):H1770–8.

## S3 Foam degradation rate (DR)

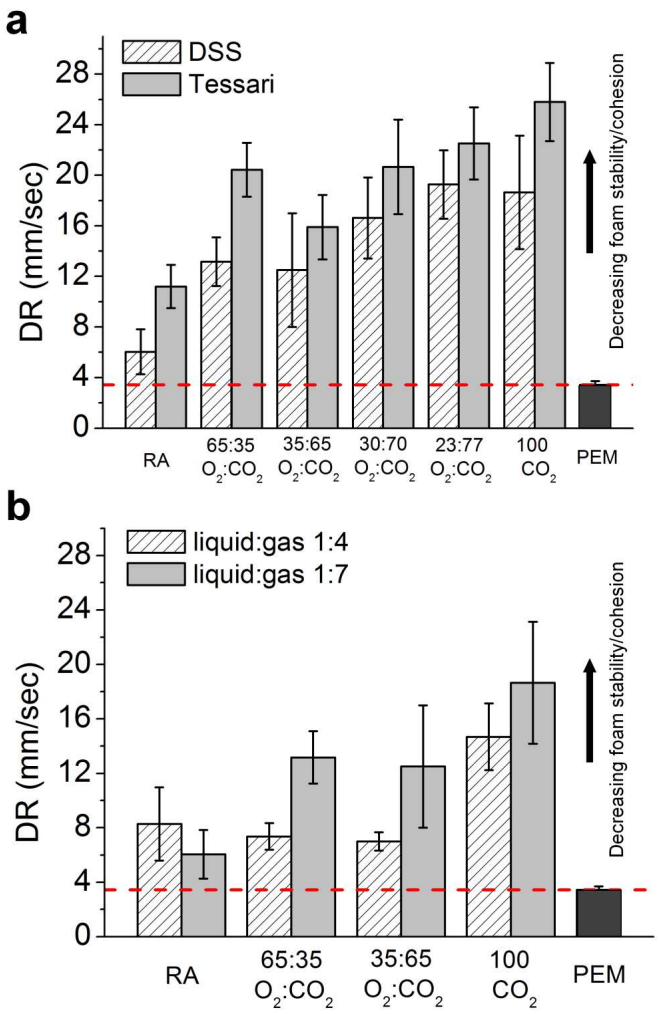
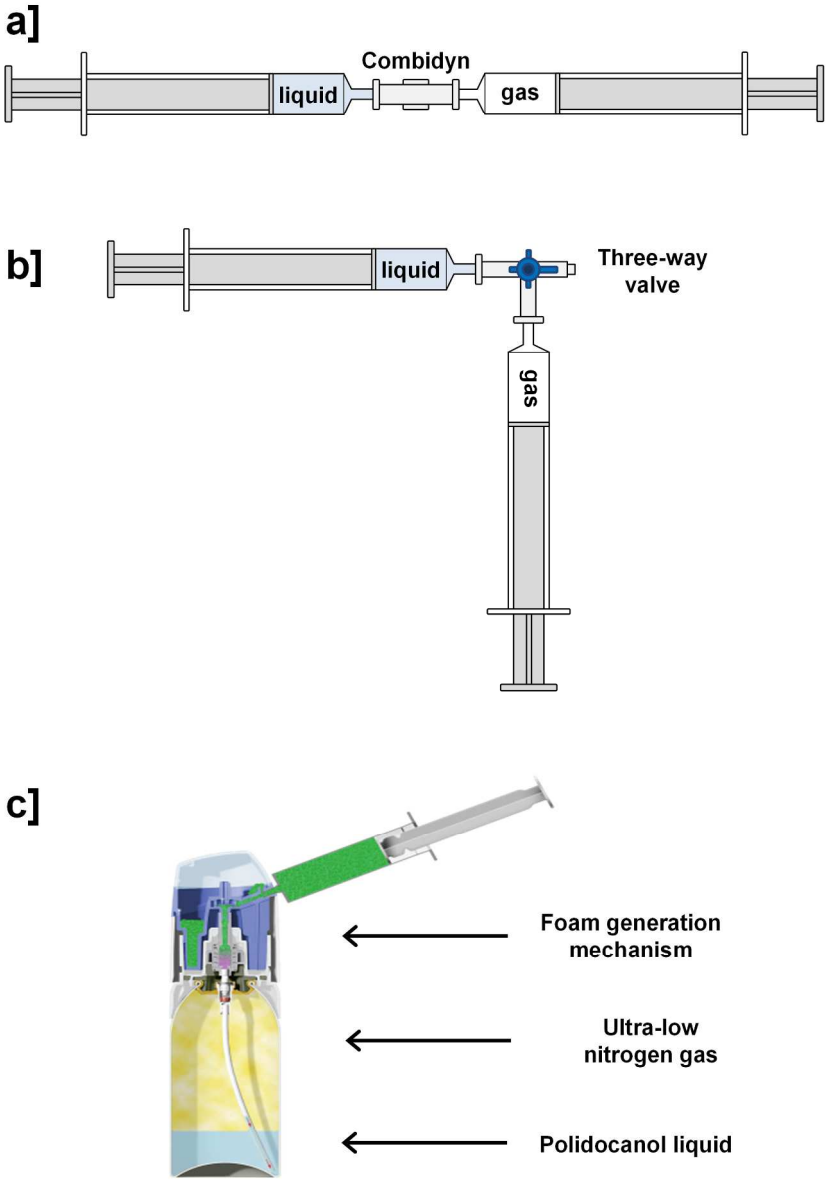
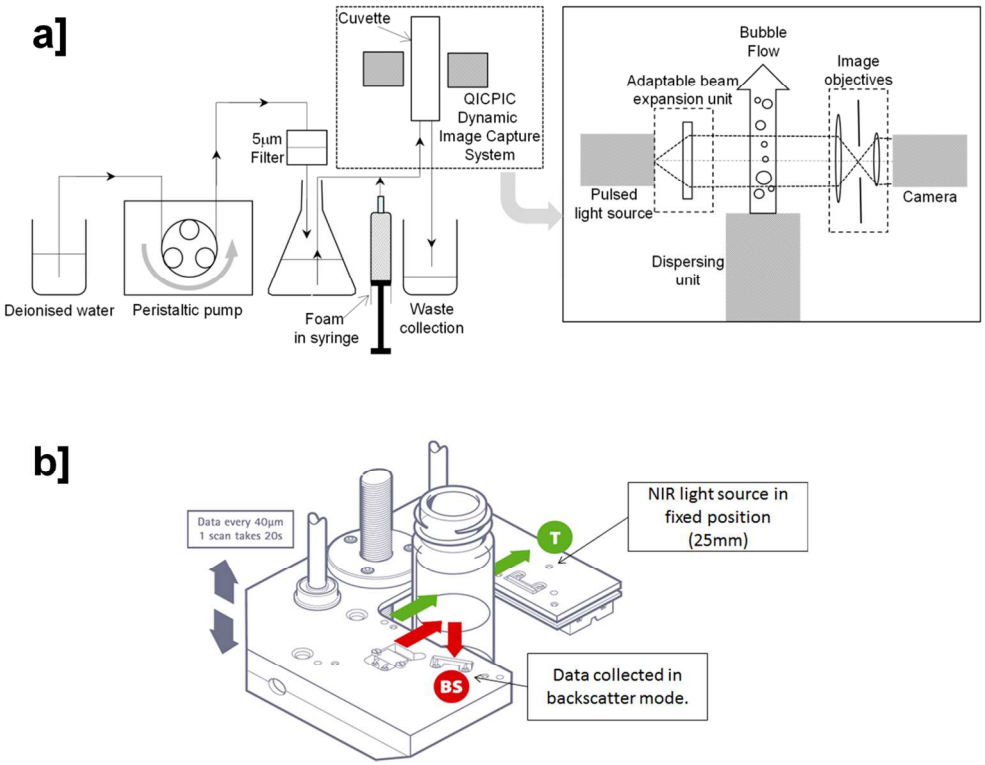


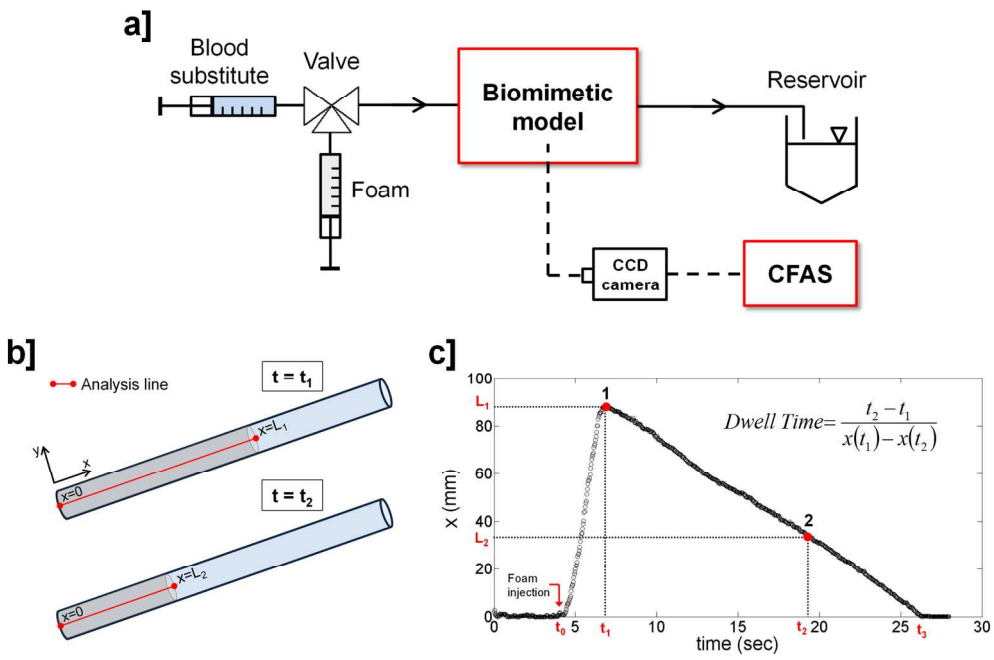

**Figure S3.1** Polidocanol endovenous foam has a lower DR than any PCF, including foams made using RA ($p<0.035$) (a). The same result was obtained at different liquid to gas ratios (1:4 and 1:7 liquid:gas) using the DSS method (b). 100% $CO_2$ foams were least stable in all tests performed and different $O_2$:$CO_2$ mixtures had intermediate performance. The Tessari method produced consistently less stable foams than DSS method. Polidocanol endovenous foam was more stable than foam made by either PCF method. RA = room air; DR = degradation rate; PEM = polidocanol endovenous microfoam.



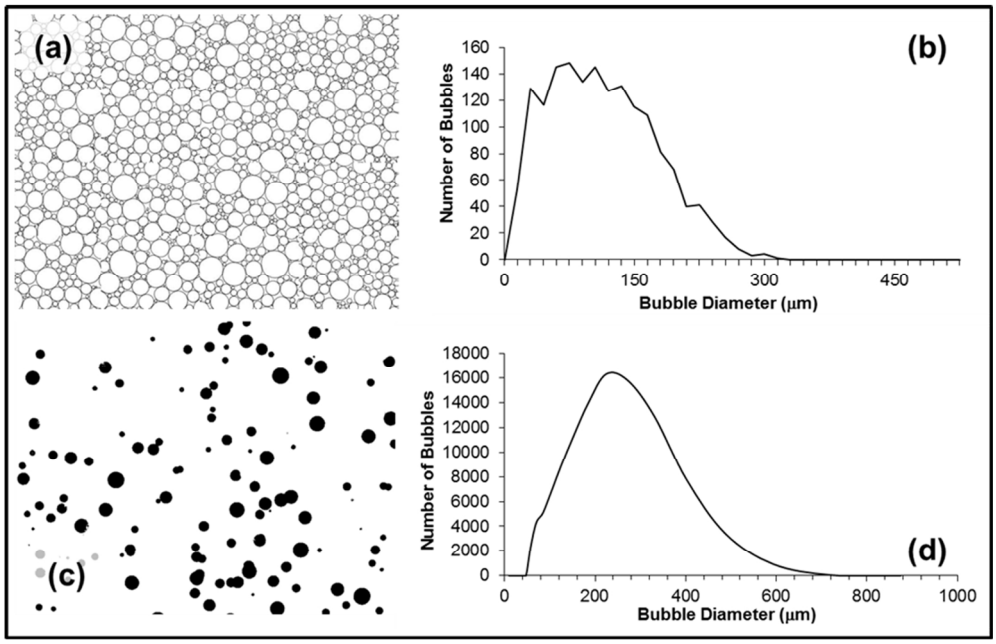

Methods for producing physician compounded foams (PCFs) and polidocanol endovenous microfoam. In the DSS method, syringes are connected by a Combidyn® adapter (a), while in the Tessari method, they are connected by a three-way valve (b). In both techniques, the foam was produced by passing the polidocanol solution (liquid phase) from one syringe, ten times into and out of the other syringe initially containing the gas or gas mixture (gaseous phase). Foam was produced at room temperature (20°C-22°C). The proprietary canister system for generating polidocanol endovenous microfoam (Varithena®) is shown in (c).
184x262mm (300 x 300 DPI)



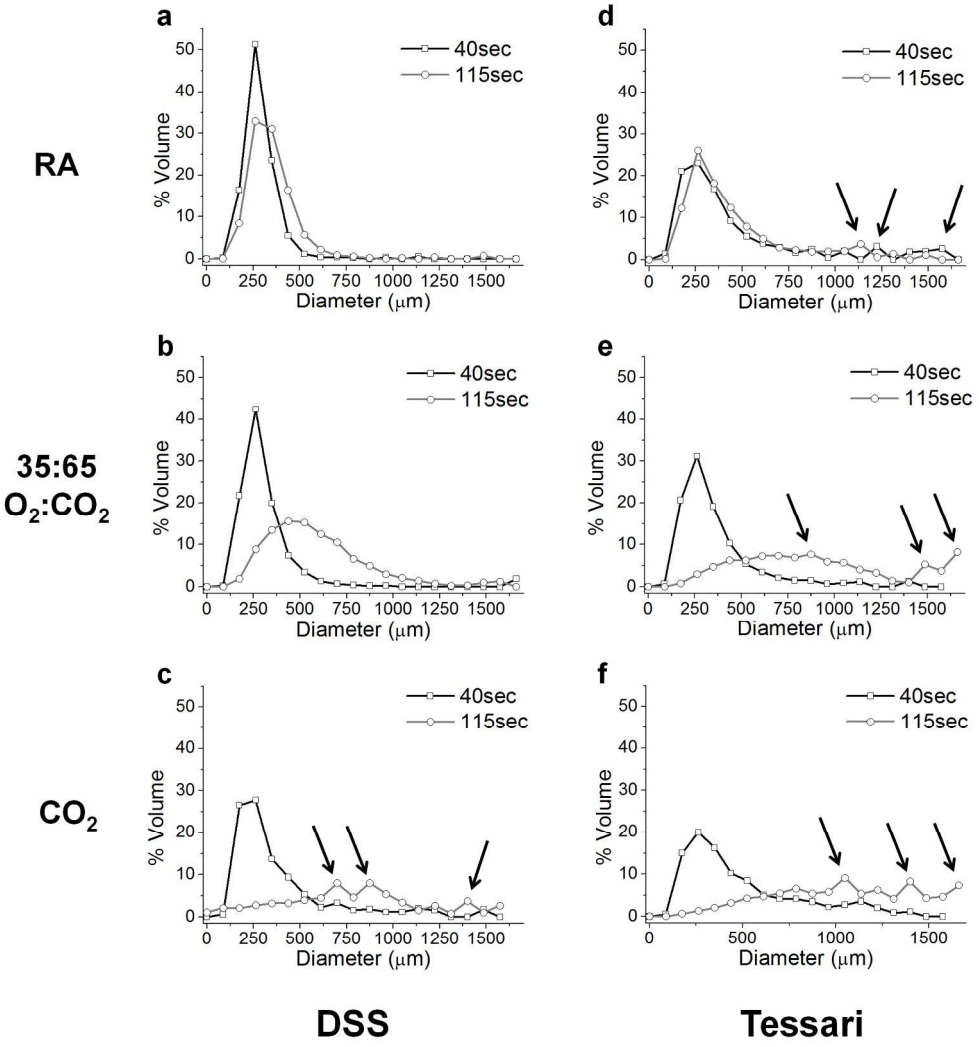

Methods for measuring bubble size distribution. Sympatec QICPIC image analysis sensor (a) and Turbiscan™ LAB apparatus (b).
142x110mm (300 x 300 DPI)



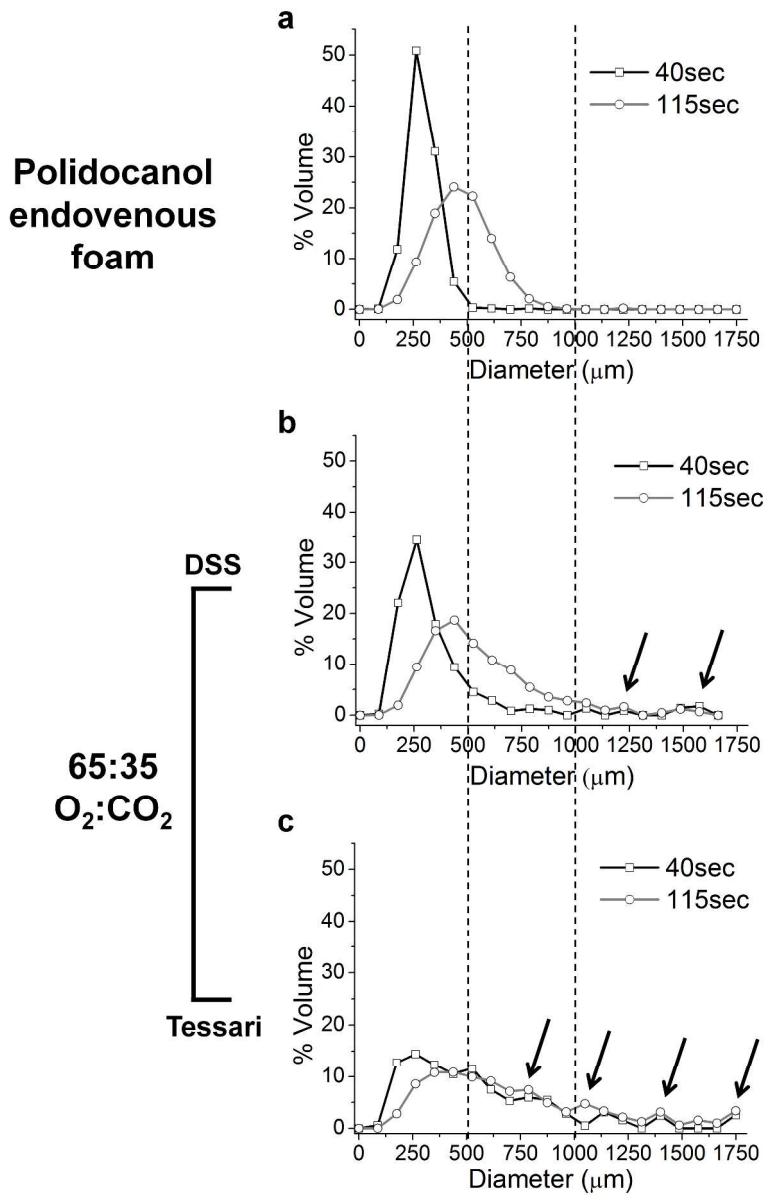

Comparison of bubble size distributions at 40s and 115s for polidocanol endovenous microfoam (a) compared to PCF O2:CO2 (65:35) made by DSS (b) and Tessari (c) methods. Arrows highlight existence of large bubbles in the foam. n = 5.
184x120mm (300 x 300 DPI)



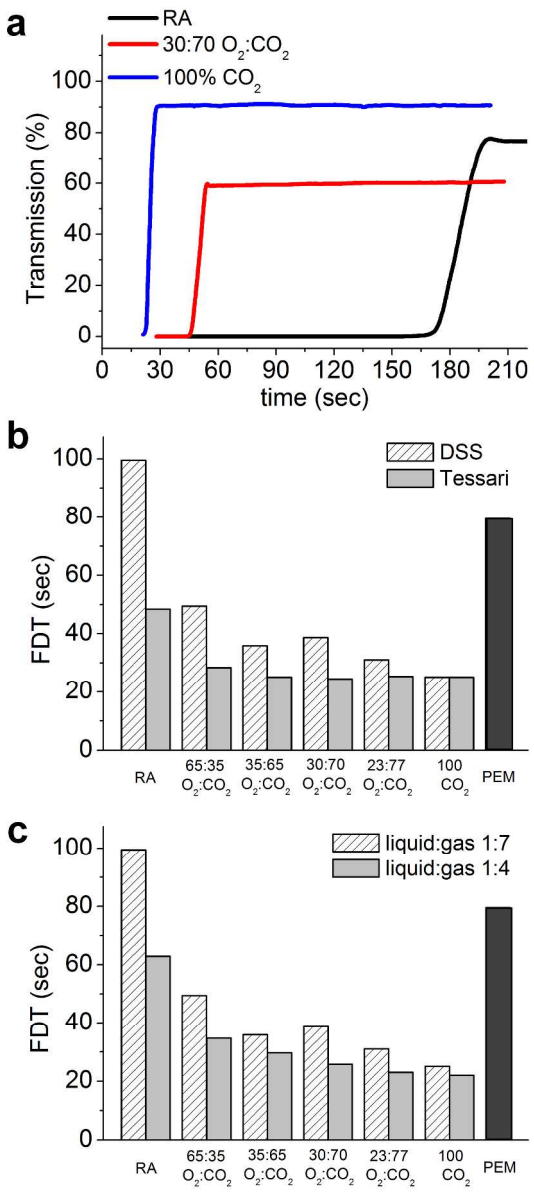

Comparison of glass plate and Sympatec method analyses of polidocanol endovenous microfoam. (a) Image of polidocanol endovenous microfoam from the optical image analysis method and (b) bubble size distribution measured for this foam; compared to (c) a single frame image of the same sample of polidocanol endovenous microfoam captured from the Sympatec dynamic image capture method and (d) the bubble size distribution measured by this method (over a 15s period, corresponding to 375 image frames). Note how the Sympatec over-reports the true bubble size.
184x119mm (300 x 300 DPI)



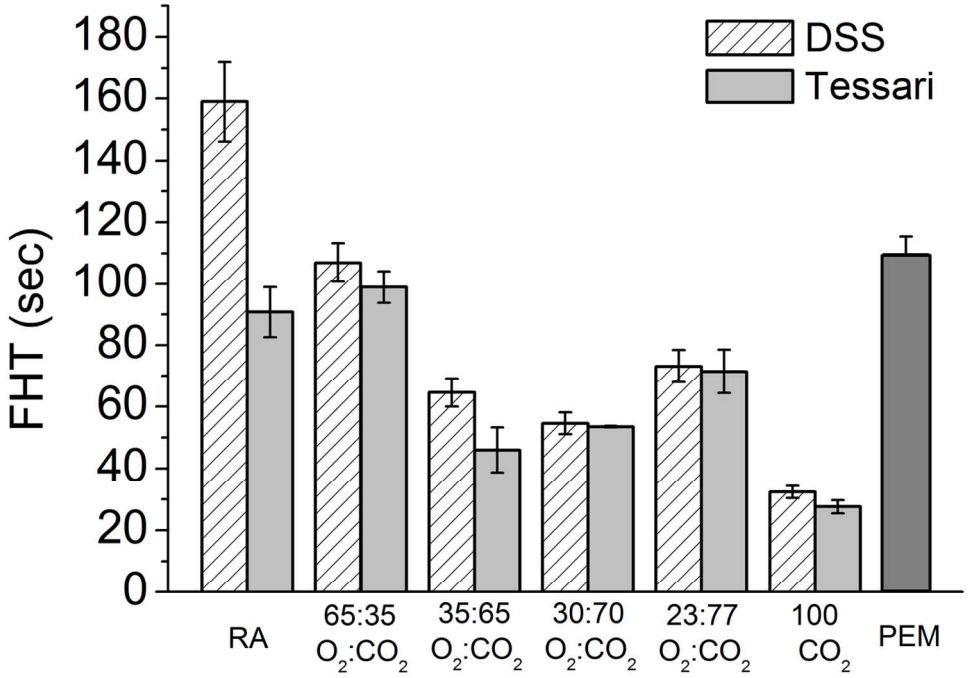

Size distributions of physician compounded foams (DSS vs Tessari) with liquid:gas ratio 1:7, obtained using the Sympatec method. Bubble size distribution curves for PCFs using different gas formulations (a+d RA; b+e: O2:CO2 of 35:65; c+f 100% CO2) for both the DSS and Tessari methods 40s and 115s after foam preparation. Arrows highlight existence of larger bubbles in the PCF. RA = room air. n = 5.
185x195mm (300 x 300 DPI)



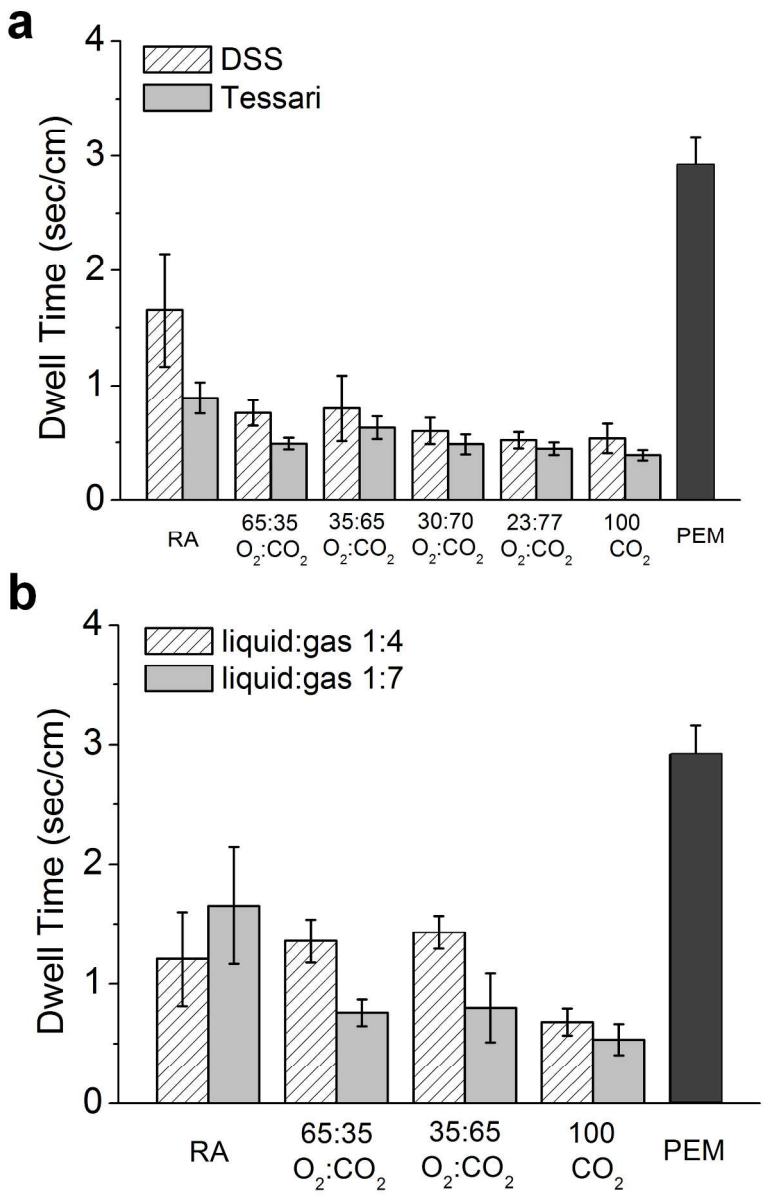

Comparison of bubble size distributions at 40s and 115s for polidocanol endovenous microfoam (a) compared to PCF O2:CO2 (65:35) made by DSS (b) and Tessari (c) methods. Arrows highlight existence of large bubbles in the foam.
271x425mm (300 x 300 DPI)




Example foam drainage time curves used to measure FDT (a); FDT for DSS versus Tessari, and compared with polidocanol endovenous foam (b); FDT for different PCFs made using the DSS method at 1:4 and 1:7 liquid to gas ratios, and compared with polidocanol endovenous microfoam (c). RA = room air; PEM = polidocanol endovenous microfoam; FDT = foam drainage time. Standard deviation ranged from 0.37% to 5.58% of the mean (n = 4).
270x584mm (300 x 300 DPI)




Comparison of the foam half time (Turbiscan™) for various physician compounded foam (PCF) formulations made using DSS and Tessari methods (1:7 liquid:gas ratio) and foam half time for polidocanol endovenous microfoam. Polidocanol endovenous foam displayed a longer FHT than CO2-containing PCFs. FHT = foam half time; RA = room air; PEM = polidocanol endovenous microfoam. n = 5.
130x91mm (300 x 300 DPI)




Polidocanol endovenous microfoam had the longest calculated dwell time, almost twice that of PCFs using RA and approximately eight times better than PCFs prepared using equivalent gas mixtures in a biomimetic model. RA = room air; PEM = polidocanol endovenous microfoam. n = 4.
282x433mm (300 x 300 DPI)