Supplementary Information for:

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

Dario Carugo[1,2], Dyan N. Ankrett[2], Xuefeng Zhao[1], Xunli Zhang[1,5], Martyn Hill[2,5], Vincent O'Byrne[3], James Hoad[3], Mehreen Arif[3], David D.I. Wright[4], Andrew L. Lewis[3,*]

[1] Bioengineering Science Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, United Kingdom

[2] Electro-Mechanical Engineering Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton SO17 1BJ, United Kingdom

[3] Biocompatibles UK Ltd., a BTG International group company, Farnham Business Park, Weydon Lane, Farnham, Surrey GU9 8QL, United Kingdom

[4] BTG International Ltd., 5 Fleet Place, London EC4M 7RD, United Kingdom

[5] Institute for Life Sciences, University of Southampton, Southampton SO17 1BJ, United Kingdom

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.

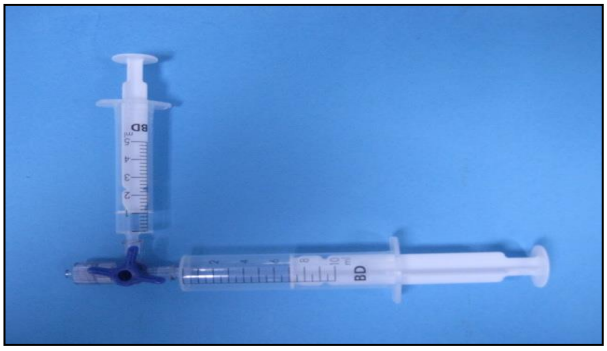

Figure S1.2: Image of the Tessari system.

A 5 μm 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 μL) 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 μm. A flattened monolayer was created, of flat cylindrical bubbles 32 μm 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:

$$d_s = \left[\frac{1}{4}x6d_f^2 + 3d_f x(\pi - 4) + x^2(10 - 3\pi)\right]^{1/3} \quad \text{(Eq.1)}$$

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 μm bin size ranges. The Limit of Detection (LOD) for the method was set at 11 μm.

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^{TM} 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 ϕ 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)

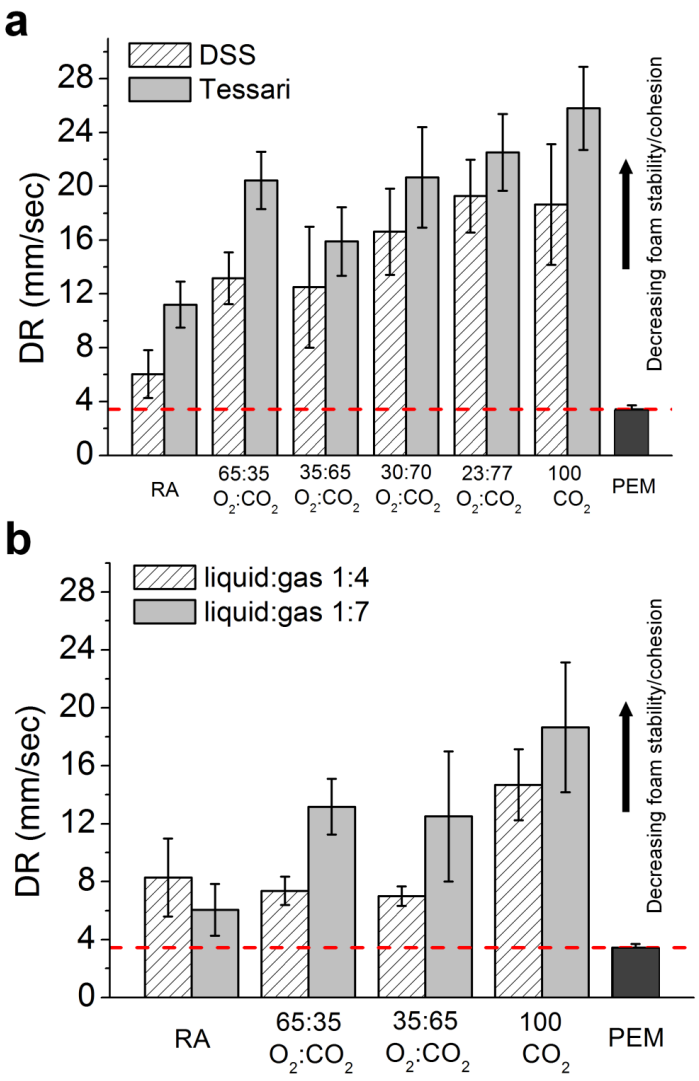

Figure S3.1 Polidocanol endovenous foam has a lower DR than any PCF, including foams made using RA ($p<0.035$) (a). The same result was obtained at different liquid to gas ratios (1:4 and 1:7 liquid:gas) using the DSS method (b). 100% CO_2 foams were least stable in all tests performed and different 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.