**Title**: The potential influence of Schirmer strip variables on dry eye disease characterisation, and on tear collection and analysis.

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**ABSTRACT**

**Purpose:** The use of the Schirmer strip as a tool in the characterisation of dry eye disease, depends upon the quantitative assessment of tear production and constituents. The aim of this study was to ascertain the extent to which the properties of commercially available SSs can vary and the way in which this baseline information may relate to their comparability in clinical use.

**Methods:** Five Schirmer strips from different manufacturers were analysed: Clement Clarke®, TearFlo®, Bio Schirmer®, Omni Schirmer® and JingMing®. Various aspects of their physical appearance and physicochemical behaviour were measured, including size, width, weight, and thickness together with surface morphology (assessed by electron microscopy) and aqueous uptake and release behaviour (including the influence of each strip on protein retention and eluent osmolarity).

**Results:** All physical parameters varied between the strips studied: Clement Clarke was the largest, thickest, and heaviest Schirmer strip assessed in this study. Most of the strips had an inked ruler and one of them also contains fluorescein (JingMing®). SEM images showed that each of the Schirmer strips had unique surface morphologies. Statistically significant differences among the strips were found for uptake ($p=0.001$) and release volume ($p=0.014$). Clement Clarke absorbed the highest volume over a fixed time period ($23.8\pm1.6$ µl) and Omni the lowest ($19.3\pm0.5$ µl). Clement Clarke SS showing the highest eluent osmolarity value ($5.0\pm0.0$ mOsm) and TearFlo the lowest ($2.8\pm0.4$ mOsm).

**Conclusion:** The five strips investigated in this study indicate that there is no standardisation of commercial strips, despite the fact that the need for standardisation was recognised over fifty years ago. The comparative study of the structural variability of Schirmer strips described here provides useful baseline information relating to their comparability in clinical use.

**Keywords**: Schirmer strip characterisation; tear flow measurement, dry eye diagnosis, tear sampling; albumin uptake and release.

Highlights

- Shirmer strips are used to characterise dry eye disease, eqspecially aqueous deficiency
- They can also be used to sample the tear film for constituent analysis

- The properties of commercially available Schirmer strips are not all equal
- The SS properties influence the volume and constituents analysis obtained clinically
- Additional clinical guide aids such as inked rulers and fluorescein also have an impact of the readings obtained.

## 1. Introduction

It is estimated that between 5-30% of the population suffer from this condition and symptoms of dryness are very commonly reported by patients in eye care clinics [1-3]. Aqueous tear deficiency, which is related to a reduction of the lacrimal tear secretion and dysfunction, is one of the two main categories of DED and Schirmer strips (SS) are still widely used today to measure tear production for DED diagnosis [4-7]. The test was first described in 1903 by Otto Schirmer [8]. It uses absorbent filter paper strips, which are inserted into the temporal lower conjunctival sac and after 5 minutes the length of wetting of the strip is recorded in millimetres. A more recent application of the Schirmer strips has been to collect tear samples for analysis of ocular biomarkers, the advantage of this approach being the fact that the device is well-established in clinical ophthalmic practice [9-11]. Accurate quantitation of tear components in tear fluid is not only important in understanding the physiological properties of tears, but also affords valuable diagnostic opportunities for the clinician [12]. It is only by recognition of the sources of error and in particular the variability of Schirmer strips in clinical practice, however, that the well-recognised problem of Schirmer reproducibility can be understood and minimised [13].

Standardisation of procedure has an important influence on the results obtained with the Schirmer test [14-17]. Differences are caused, for example, by variations in the eye gaze position, with higher results obtained when the Schirmer test is performed with an inferior gaze [14]. Similarly, differences arise when the test is performed with open, in contrast to closed, eyes; closed eyes result in lower values, but these are likely to be more reliable, as eyelid margin effects, eyelash stimulation and local environmental conditions can alter the tear turnover rate [15, 16]. It is equally important to recognise and quantify the effects of variations in the Schirmer strip material on the results obtained. Initially the Schirmer test used blotting paper which was cut into strips measuring 35 by 5 mm. Subsequently, litmus paper, cigarette paper and a number of other blotting papers were investigated [18-20]. Two standardised materials for fabrication of Schirmer strips have been proposed: Whatman standard No. 41 filter paper in 1953 [6] and Black Ribbon No. 589 in 1961

[21]. Although Whatman standard No. 41 or Black Ribbon No. 589 are still widely used in Schirmer strip fabrication today, the majority of manufacturers do not declare the origin or source

| Schirmer strip | Manufacturer | Box details | Physica |
|---|---|---|---|
| **Clement Clarke** | Haag-Streit Clement Clarke Intl. (UK) | 50 pouches, each with 2 strips | |
| **TearFlo** | HUB Pharmaceuticals, LLC (USA) | 100 individually packed strips | |
| **Bio Schirmer** | Biotech Vision Care (India) | 100 individually packed strips | |
| **Omni Schirmer** | Omni Lens PVT. Ltd (India) | 100 individually packed strips | |
| **JingMing** | Tianjin JingMing New Technological Development Co. Ltd (China) | 50 pouches, each with 2 strips | |

of their strips.

There are, currently, many commercially available Schirmer strips, and even a simple visual inspection indicates that there are differences between them. The aim of this study was to collate comparative data on the relative behaviour of commercially available Schirmer strips using a variety of characterisation techniques relevant to the assessment of tear volume and the analysis of tear components. Dissimilarities between the strips have the potential to influence tear production measurements and affect diagnostic assessments. They may also affect the retrieved volume and perceived constituents of sampled tears.

## 2. Materials and methods

A representative sample of five commercially available SS was selected and assessed. (Table 1). They including one fluorescein incorporated strip, one without ruler markings and a spread of geographically sourced strips.

Table 1. Selected Schirmer strips.

### 2.1. Physical Parameters

The physical characteristics: appearance, size, thickness and weight of the five different SS were measured. Precise length was measured using a jeweller's eye piece with a 0.001 cm sensitivity. A microbalance with a microgram sensitivity range was used to measure weight, and a micrometer

with an accuracy of 0.001 cm was used to measure thickness. Five individual strips of each type were measured.

### 2.2. Uptake and release: volume

To measure the uptake volume, an 80 µl aliquote of phosphate buffed saline (PBS) was added to a round bottomed 2 ml microcentrifuge tube. The tip of each strip was dipped into the PBS reservoir for 1 min which enabled the capillary action of the strips, which is responsible the in-eye wicking action used to 'fill' the strip, to be assessed. The wetted strip was then placed into a smaller 0.5 ml centrifuge tube in which a hole had been made at the base using a 0.6 mm gauge microlance. A microcentrifuge tube piggyback centrifugal set-up was then used. This was done by placing the 0.5 ml centrifuge tube into a larger 1.5 ml centrifuge tube and both were then centrifuged at 10,000 rpm for 5 mins. The volume remaining in the 2 ml microcentrifuge tube was measured with a micropipette having a volume accuracy of 0.1 µl. (Uptake volume = starting volume (i.e. 80 µl) – remaining volume). The wetting length in millimetres was also recorded where applicable. The volume released was collected in the 1.5 ml centrifuge tube and measured immediately, also using the micropipette.

### 2.3. Osmolarity

Five strips of each type were immersed in 1 ml of deionized (DI) ultrapure water (Purite: resistivity 18.2 MΩ.cm) or PBS and placed on a shaker at room temperature for 24 hours. After the 24 hr soak the strips were placed individually into a 0.5 ml microcentrifuge tube; the microcentrifuge tube centrifugal piggyback set-up (Sec 2.2) was again used. 100 µl of the resultant eluate was collected for osmolarity measurement. Each sample was measured on the automatic micro-digital osmometer (Type 6, CamLab, Cambridge, UK). Six measurements were performed with both DI and PBS separately. The osmometer was calibrated using known standards solutions (DI = 000 mOsm; PBS ≈285 mOsm). As a control, 1ml of calibrant (DI or PBS) was aliquoted to an individual vial in the absence of a SS.

### 2.4. Uptake and release: protein concentration

For simplicity in these initial in vitro studies stages, a single protein species was chosen to investigate the potential interaction between the strip and tear proteins. Human albumin, which is

upregulated in tears on SS insertion [22], was the obvious choice. It is a negatively charged protein with a molecular weight in the region of 66 kDa. An 80 µl aliquote of 1 mg/ml of human serum albumin was added to 2 ml microcentrifuge tube. The tip of each strip was dipped into the albumin solution reservoir for 1 min to mimic the in-eye wicking and capillary action used to 'fill' the strip. The individual strips were then placed into a 0.5 ml microcentrifuge and the microcentrifuge tube piggyback centrifugal set-up was again used. The resultant eluate was collected for total protein concentration measurements.

The volume remaining in the original 2 ml microcentrifuge tube and the volume released were measured with a micropipette. These volumes were used to calculate the actual microgram weight of protein (as opposed to mg/ml concentration which would be volume dependent). Presenting the results in terms of weight negated volume dissimilarities between strips. Protein levels in the blank strip were also measured as a control by extracting the SS separately with deionised water and PBS.

Total protein concentration was measured using a microBCA Protein Assay Kit (Thermo Scientific, Rockford, USA), in accordance with the kit instructions. Briefly, 150 µl of standard/sample and 150 µl of working reagent were added to each designated well, of a 96 well plate. The plates were covered and incubated at $37^0$C for 2 h. Absorbance at 562 nm was measured with a UV-Vis spectrophotometer (SpectraMax M2, Molecular Devices, Sunnyvale CA, USA). All analytes were measured in duplicate. Sensitivity limits are quoted at 2 µg/ml.

*2.5. Scanning electron microscopy*

The porosity of the each of the SS was evaluated with a scanning electron microscope (SEM) (Stereoscan 90, Cambridge Instruments) at an accelerating voltage of 25 KV. A section of each SS was cut and sputter-coated under vacuum in an inert atmosphere for two minutes with gold. The surface of each strip was examined. Images of each surface at a magnification in the region of x720 were obtained.

*2.6. Statistical analysis*

The statistical analysis was conducted using SPSS v.21 (SPSS Inc., Chicago, IL, USA). As normal distribution of the limited measurements should not be assumed, non-parametric tests were used. Kruskal-Wallis test was used to compare the data obtained from all the SSs regarding physical parameters, uptake and release volume, uptake and release total proteins and osmolarity. When differences among all the SSs were found, Mann-Whitney test was used post hoc. Spearman's rank correlation was used to assess the association of solution uptake with measured wetted length and area. P-values lower than 0.05 were considered as statistically significantly different.

## 3. Results and Discussion

### 3.1. Physical parameters

The appearance, size, thickness and weight were distinctly and significantly different among the SS investigated in this study (Table 2). Each strip has its own particular combination of characteristics. All the SS investigated, with the exception of the Clement Clarke strip, have a millimetre ruler measure printed on the strip to assist wetting length value readings used to determine tear flow in the clinic. The JingMing SS also has fluorescein embedded to visually enhance this measurement.

Table 2. Physical parameters of each of the five Schirmer strips (mean ± standard deviation).

| Parameter | Clement Clarke | TearFlo | Bio Schirmer | Omni Schirmer |
|---|---|---|---|---|
| **Total length (cm)** | 4.59±0.03 | 3.98±0.03 | 4.07±0.03 | 4.40±0.00 |
| **Total width (cm)** | 0.59±0.03 | 0.50±0.00 | 0.51±0.03 | 0.51±0.01 |
| **Length in contact with the eye (cm)** | 0.70±0.00 | 0.50±0.00 | 0.41±0.03 | 0.35±0.00 |
| **Weight (mg)** | 21.95±1.29 | 16.20±0.65 | 18.20±0.61 | 19.25±0.60 |
| **Thickness (mm)** | 0.23±0.01 | 0.21±0.01 | 0.21±0.00 | 0.18±0.02 |
| **Ruler marked** | No | Yes | Yes | Yes |
| **Fluorescein** | No | No | No | No |

*Kruskal Wallis test

In terms of strip weight there are clear differences: the heaviest strip is the Clement Clarke SS with a mass of ~ 22 mg compared with the lightest, the TearFlo SS, at ~16 mg. The total width is the same in all strips except Clement Clarke, which is a 1 mm wider and this strip is also the thickest.

Importantly, statistically significant differences were also found in the size of the area that is in contact with the eye. The non-standardised nature of the area of the strip in contact with the eye, (where these strips are placed in the temporal lower conjunctival sac of the patient) are not ideal. The contact area differential can potentially affect wetting rate.

| Schirmer Type | Uptake (µl) | Release (µl) | Wetting length (mm) |
|---|---|---|---|
| Clement Clarke | 23.8±1.6 | 11.8±1.8 | - |
| TearFlo | 21.5±0.8 | 12.3±1.5 | 25.5±1.6 |
| Bio Schirmer | 21.2±2.6 | 11.0±0.6 | 21.3±1.0 |
| Omni Schirmer | 19.3±0.5 | 8.5±1.8 | 22.0±2.0 |
| JingMing | 19.5±0.5 | 10.7±0.8 | 23.5±0.8 |
| p-value | 0.001* | 0.014* | 0.001* |

In short, the combination of all these physical differences will affect the wetting volume measurement and wetting length and in consequence strip to strip comparisons will be subject to some degree of error.

*3.2. Uptake and release: volume*

Statistically significant differences among the SS were found in relation to the uptake and release volume, as well as the actual wetted strip length (Table 3). The actual uptake volume varies somewhat from strip to strip, which is not surprising in view of their different absorbing surface areas. On average, an uptake difference of ~4.5 µl was determined between the highest uptake: Clement Clarke, and the lowest strip: JingMing. The volume absorbed by the Clement Clarke SS was significantly higher than the quantity absorbed by the TearFlo SS (p=0.07), the Bio Schirmer (p =0.007), the JingMing SS (p=0.003) and the Omni Schirmer (p=0.003). The release data did not always parallel uptake; the lowest volume was released by Omni Schirmer, significantly less than JingMing SS (p=0.039).

Table 3. PBS uptake and release volume measurements and the related wetted length reading for the Schirmer strips (mean ± standard deviation) from an 80 µl starting volume, after 1 min insertion.

Differences in uptake volumes reflect different wetting rates and/or unequal specific absorption characteristics. Wetting lengths of obtained with strips with an imprinted ruler were higher for the TearFlo SS than the JingMing SS (p=0.021), Omni Schirmer (p=0.015), and Bio Schirmer strips (p=0.003). The Bio Schirmer showed the lowest Schirmer wetting values, with a statistically significant difference compared to TearFlo SS (p=0.003) and JingMing SS (p=0.003). The correlation between uptake volume and wetted length (r = 0.411, p = 0.046) was greater if strip width was taken into account in the form of wetted area (r = 0.523, p = 0.009). Clinically it is expected that the wetted length should be the same for patients with the same volume of tears, regardless of the physical properties of the strip (such as width or porosity).

Figure 1 shows the release profiles of the SS, showing PBS released relative to an uptake volume of 100%. Interestingly there is a vast difference in the ability of the strip to release the PBS, or in other words some strips have a stronger retention ability for PBS. The percentage release values all lie around the 50%, which is quite low. TearFlo releases the highest relative volume, corresponding to ~57% release, whereas JingMing releases only ~ 44% of the PBS uptake volume.
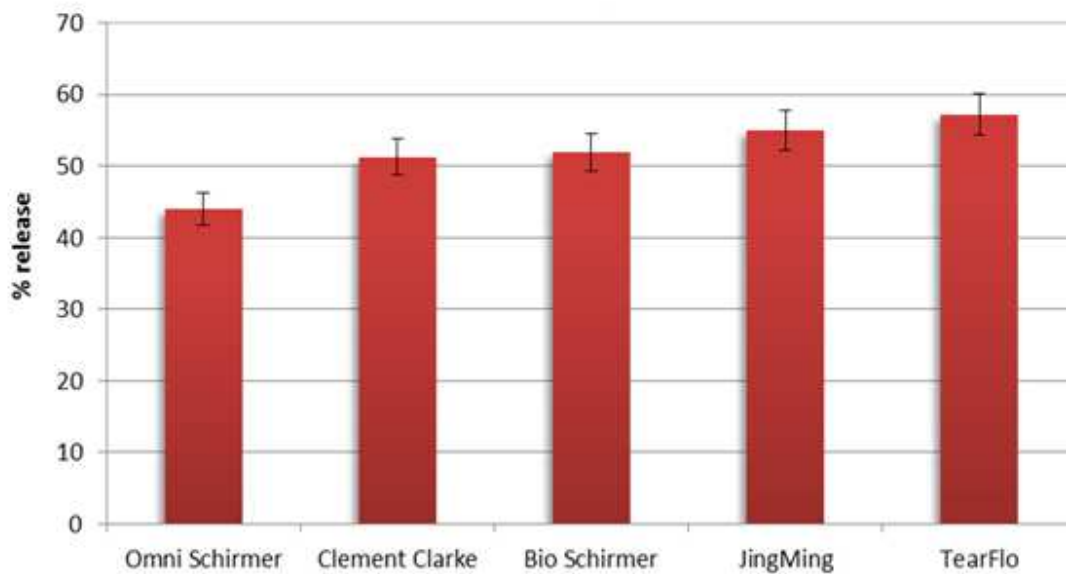
Figure 1. The percentage of PBS released, by volume, relative to the original uptake volume. Error bars = 1 S.D.

These results also have an implication for use of the SS to collect tear samples, when it is important that as much sample as possible is recovered from the SS, with no influence of the absorbing material on the concentration or composition of the sample. Another aspect is extraction efficiency. In tear analysis an extraction step is generally required so that the resultant eluate can be assayed and the clearly disparate extraction efficacy of the SS studied here will be very important in tear component analysis.

### 3.3. Osmolarity

Osmolarity provides a measure of the number of solute particles per unit volume of solution (mOsm). This measurement gives information about the level of solutes/ions in individual SS. SS are one of many tools used in the assessment of dry eye; osmolarity measurement is another and the purpose of this evaluation was to assess if the strips could also be used as a vector for osmolarity assessment.

After the 24 hour soak the Clement Clarke SS exhibited the highest baseline, with an osmolarity value of ~5 mOsm, while the TearFlo strip exhibited the lowest value at ~3 mOsm (Table 4). These

levels are minimal. The osmolarity value obtained with Clement Clarke SS was statistically significantly higher than those obtained with all the other SS (p<0.05). Similarly, the results obtained with TearFlo were statistically significantly lower than those obtained with Clement Clarke (p=0.001) and Omni Schirmer (p=0.001). PBS has an osmolarity close to levels found in tears and was used as a surrogate to assess the potential of each strip as a means of collecting tear samples for osmolarity measurement. In general, the differences inherent with the osmolarity values for DI (although small) were also exhibited after PBS interaction. However, the fact that a +7 mOsm change can be directly associated with the strip alone must be acknowledged as it could have a significant effect on clinical diagnosis. In general, values of tear osmolarity greater than 308 mOsms/l are considered as mild DE and values greater than 312 mOsms/l are indicative of moderate to severe DE [23]. Further studies are needed to determine how the osmolarity of SS influences the assessment of tear osmolarity of clinical samples.

Table 4. Osmolarity values for the blank Schirmer strips post DI and PBS soak, expressed as mean ± standard deviation.

| Schirmer Type | Osmolarity DI (mOsm) | Osmolarity PBS (mOsm) | Difference from PBS (284.0 ± 1.0 mOsm) |
|---|---|---|---|
| Clement Clarke | 5.0±0.0 | 291.0±2.2 | + 7 |
| TearFlo | 2.8±0.4 | 287.5±2.4 | + 3.5 |
| Bio Schirmer | 3.7±1.2 | 288.0±1.0 | + 4 |
| Omni Schirmer | 4.0±0.0 | 291.8±2.2 | + 7.8 |
| JingMing | 3.0±0.0 | 289.7±1.5 | + 5.7 |
| p-value | <0.001* | 0.075* | - |

*Kruskal Wallis test; DI: Deionised Water; PBS: Phosphate Buffered Saline

*3.4. Uptake and release: protein concentration*

The diagnosis of dry eye is not limited to physical factors such as tear flow, tear breakup time, tear meniscus height, and fluorescein staining tests, but with more sophisticated techniques available to the clinician greater attention is now being paid to actual tear composition. Tear protein analysis is central to this changing focus [24-27]. Accurate quantitation of proteins and other tear

components, and the identification of potential biomarkers in tear fluid is important in understanding the physiologic properties of tears. It also affords valuable diagnostic opportunities for the clinician for a multitude of ocular disorders in addition to contact lens wear ocular studies [28-30]. SS can be used to collect these tears and the advantages are two-fold; it is already approved for used in clinical practice and it needs no extra equipment or training. However, it is important that SS used in this way absorb and release the protein in an equivalent manner. Therefore, the purpose of this section was to assess the uptake and release profiles of the five strips in terms of protein quantification.

There was no statically significant difference in the total protein absorbed and released after soaking the strips in albumin (Table 5). The uptake levels in general for all strips were quite similar taking into account the standard deviation. However, the release patterns were different (Fig 2) and significantly they all exhibited quite low release percentages. This suggests that a marked percentage of the albumin was still adhere to the strip using these non-destructive extraction conditions. Residual levels of proteins were extracted from the 'blank' strips (Table 6), but this is only to be expected. Filter paper from which the strips are fabricated derives from trees and thus is likely to retain some proteinaceous content. The baseline protein content for the Clement Clarke strip was the highest with levels not too dissimilar from those determined for albumin release. Further studies are required looking at individual protein species with different sizes and charge in addition to a 'tear protein' mix, but the results do suggest that while albumin can be extracted from SS, a certain percentage remains on the strip.

Table 5. Uptake and release: albumin concentration (µg) for all five strips following a 1 minute insertion in 80 µl of a 1 mg/ml solution of albumin. Results expressed as mean ± standard deviation.

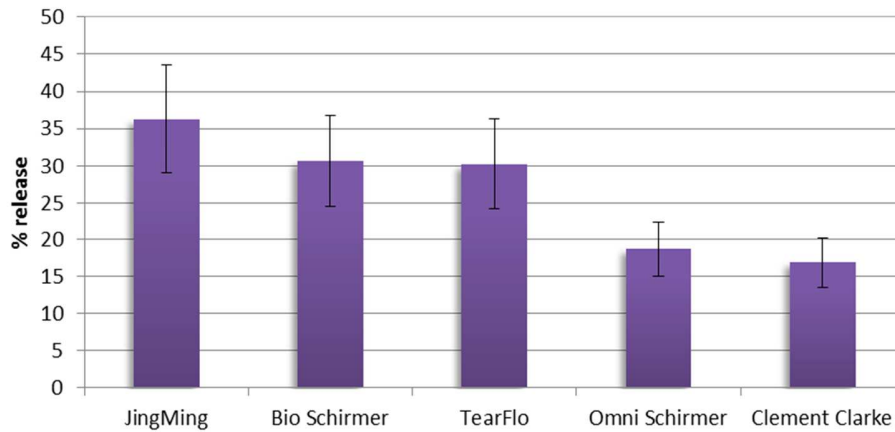| Schirmer Type | Up taken protein (µg) | Released protein (µg) |
|---|---|---|
| Clement Clarke | 29.7±5.6 | 12.4± 3.0 |
| TearFlo | 31.4±4.4 | 12.1±3.1 |
| Bio Schirmer | 31.6±4.3 | 12.3±2.0 |
| Omni Schirmer | 29.9±4.7 | 10.9±3.1 |
| JingMing | 30.9±1.3 | 14.6±3.4 |
| p-value | 0.862* | 0.303* |

*Kruskal Wallis test.

Figure 2. The percentage, by weight, of albumin released relative to the original uptake weight and the extractable protein background levels. Error bars = 1 S.D.

Table 6. Residual protein levels in μg in the blank strips (mean ± standard deviation).

| Schirmer | Residual protein (μg) |
|---|---|
| Clement Clarke | 7.4±3.2 |
| TearFlo | 2.6±0.5 |
| Bio Schirmer | 2.6±0.5 |
| Omni Schirmer | 5.3±0.6 |
| JingMing | 3.4±0.7 |

*3.5. Scanning electron microscopy*

The surface morphologies and porosities of the strips show significant differences, which will unmistakably affect the wetting rates and interaction with tear components (Fig.3). The pore size and structure of the surface for Clement Clarke, TearFlo and JingMing SSs are very different from that exhibited by the Bio and Omni Schirmer strips. It is known that the pore structure of the SS has influence on the particle retention and on the flow rate [31]. The Omni Schirmer absorbed the lowest volume and it had a quite compact porosity. Furthermore, it was noted that the SS with the higher pore size (Clement Clarke and TearFlo), absorbed the greatest volume of PBS. The JingMing SS showed less compact porosity and also displayed the lowest absorption quantity. It is possible that the characteristics of the inked ruler and the use of fluorescein influenced this result.
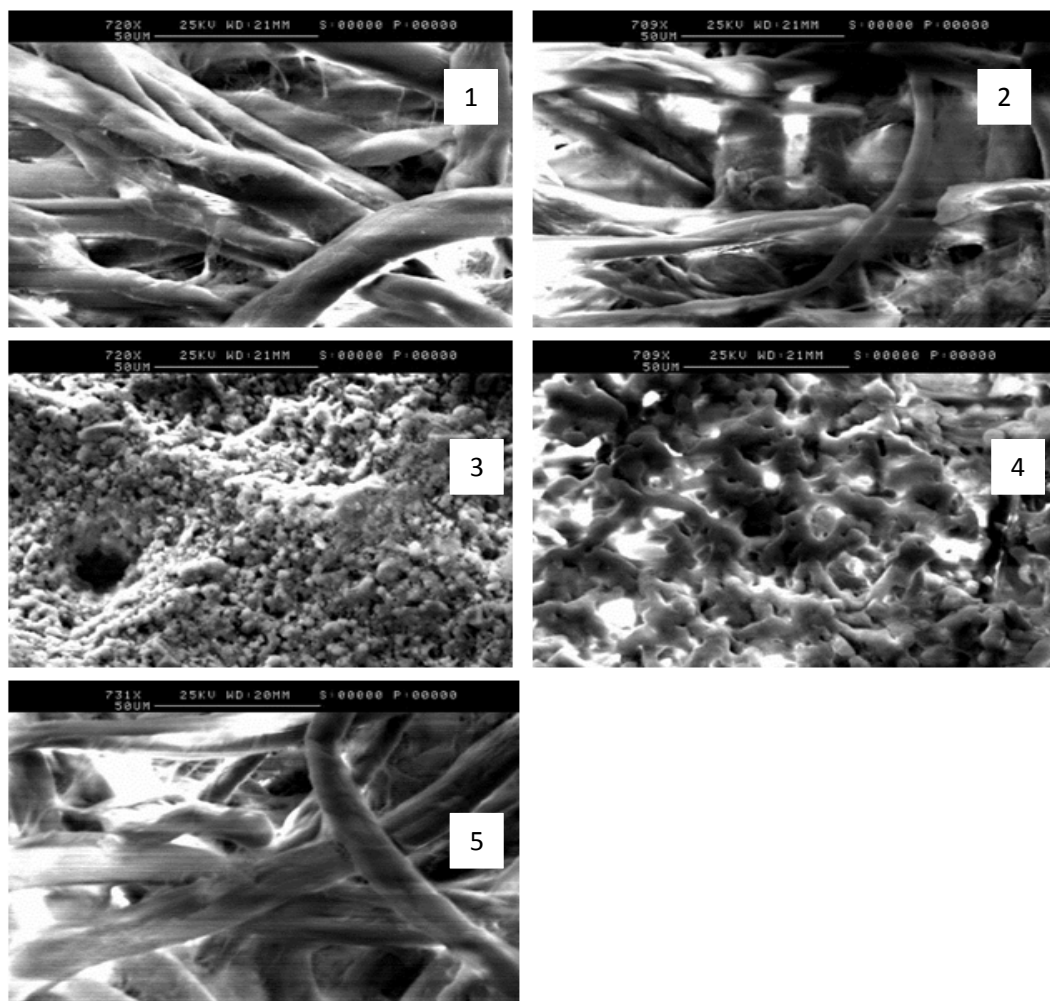
Figure 3. SEM images of the surface of the five SS at ~720 magnification. 1= Clement Clarke, 2 = TearFlo SS, 3= Bio Schirmer, 4= Omni Schirmer and 5= JingMing SS. A 50 µm scale bar is provided with each image.

## 4. Conclusions

The use of the Schirmer strip as a tool in the characterisation of dry eye disease, depends upon the quantitative assessment of tear production and constituents. This assessment depends upon the capillary uptake of liquid into the Schirmer strip structure driven by capillary action, which is dependent in turn upon the porosity of the material. It is evident, therefore that any variation in the capillarity of the Schirmer strip will affect the clinically-obtained result. The quantitative assessment of structural variability of Schirmer strips and the effect of this on tear uptake can only be carried out under carefully controlled in vitro conditions. The comparative study of the

structural variability of typical commercial Schirmer strips described here thus provides useful baseline information relating to their comparability in clinical use.

It is important to acknowledge that all the behavioural differences were determined in vitro under standardised controlled conditions. SS clinical use is invasive and the physical characteristics of the strip may affect the patient response, for example tear stimulation.

Across the five strips that could be accessed globally at the time this study was conducted, the range of behaviour observed indicates clearly that there is no standardisation of commercial strips. This is surprising in view of the fact that the need for standardisation of strip material was recognised over fifty years ago [6, 21]. Apart from the variety of clinical procedures used to perform the Schirmer test, it seems likely that one of the causes of the variability of the Schirmer test between studies is related to the use of SS having different sorption behaviour. The outcome of the palette of characterisation tests reported here suggests that of those studied, the optimal SS was the TearFlo strip. It may not have been 'leading' strip in all the experiments but it was the most consistent and performed satisfactorily throughout. This study highlights the importance of recording the SS used in clinical studies as the results are not comparable between strips. Choosing one strip and using it throughout the duration of a clinical evaluation – especially in multi-centre studies - is important.

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