Sains Medika: Jurnal Kedokteran dan Kesehatan

journal homepage: http://jurnal.unissula.ac.id/index.php/sainsmedika/

RESEARCH ARTICLE

Measurement of Pterygium Tissue Dry Weight Using Two Different Tissue Preparation Techniques in Freeze-Dry Method

Mohd Radzi Hilmi[1], Khairidzan Mohd Kamal[2], Mohd Zulfaezal Che Azemin[3]

[1] Department of Optometry and Vision Sciences, Kulliyyah of Allied Health Sciences, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia
[2] Department of Ophthalmology, Kulliyyah of Medicine, International Islamic University Malaysia (IIUM), Kuantan, Pahang, Malaysia
[3] Faculty of Optometry and Vision Science, Segi University, Petaling Jaya, Selangor, Malaysia
* Corresponding author: khairidzan@gmail.com

ARTICLE INFO

Keywords:
Pterygium
Freeze-dried
Dry weight
Corneal astigmatism

ABSTRACT

Due to pterygium tissue compression on the corneal surface, it has been hypothesised that the degree of corneal astigmatism caused by pterygium can vary. This study aims to develop a freeze-dried method for estimating net pterygium tissue mass (NPTM) as dry weight. A single surgeon (KMK) excised 60 primary pterygium using the controlled partial avulsion technique and divided them into two groups: formalin-fixed (n=30) and unfixed (n=30). After determining the weight of each sterile container, 5 mL of 5% buffered formaldehyde was added to the formalin-fixed group and stored for one week, while 5 mL of distilled water was added to the non-fixed pterygium group. Each container was pre-frozen for 12 hours before being freeze-dried (-24 hours at -80 degrees Celsius). The result is referred to as the net pterygium tissue mass (NPTM). Using an independent T-test, a comparison of wet and dry weight and percentage of NPTM was conducted between groups. Wet weights for formalin-fixed and non-fixed pterygium were 253.33 82.17 g and 255.17 63.52 g, respectively, while dry weights were 184.92 84.31 g and 179.54 72.85 g. Formalin-fixed pterygium tissue revealed a slightly higher percentage of NPTM than non-fixed pterygium tissue (69.39 13.29% vs. 67.75 13.29%, $p = 0.792$), but this difference was not statistically significant. The *freeze-dried* method can be utilised to quantify the NPTM of pterygium fibrovascular tissue and investigate the influence of pterygium translucency on predicting induced-corneal astigmatism.

1. Introduction

Pterygium is defined as an abnormal wing-shaped growth of fibrovascular tissue that emerges from the conjunctiva and advances towards the cornea (Errais *et al.*, 2008; Ang, Chua and Tan, 2012; Manzar and Mahar, 2013). Pterygium is one of the most prevalent ophthalmic disorders, and its pathogenesis has been closely linked to chronic ultraviolet (UV) radiation exposure (Marmamula *et al.*, 2013; Nangia *et al.*, 2013; Jiao *et al.*, 2014; Chen *et al.*, 2015). Recent studies have discovered that the pathogenesis of pterygium is also closely linked to angiogenesis factors found in epithelial cells, endothelial cells, fibroblasts, inflammatory cells, and connective tissues (Ribatti *et al.*, 2009; Dzunic *et al.*, 2010; Liu *et al.*, 2013; Park *et al.*, 2015).

Based on a corneal topography map, previous research Errais *et al.* (2008) identified compression on the corneal surface. This demonstrates the presence of the corneal curvature coupling effect, which causes one region to become more slanted and the other region to become flatter. Recent research (Hilmi *et al.*, 2017; Hilmi *et al.*, 2018; Hilmi *et al.*, 2019) demonstrates that the whitish appearance of the pterygium plays an

https://doi.org/10.30659/sainsmed.v13i2.2958

important role in corneal curvature and oculovisual function changes. This postulate asserts that the quantity of fibroconnective tissue can be translated into the tissue's physical appearance. Tan *et al*. (1997) first characterised the whitish appearance. To the best of our knowledge, very limited information found on the usage of freeze-dried in deterring biological tissue mass (Libera *et al*, 2008; Connon *et al*, 2010; Benders *et al*, 2010), with only one study done on ocular tissue (Frota *et al*, 2008). In addition, the fibroconnective components of the pterygium have not been analysed and quantified. Due to these obstacles, the purpose of this study is to investigate the use of freeze-dried pterygium tissue to determine the net pterygium tissue mass (NPTM) (dry weight).

2. Materials and Methods

2.1. Research design

Sixty eyes from sixty patients with primary pterygium were enrolled. The Institutional Research Ethics Board (IIUM/310/G13/4/4-125) approved this study on the basis that it complied with the Declaration of Helsinki. The consent form was distributed to all participants. Inclusion criteria included a confirmed diagnosis of primary pterygium, both sexes, ages between 20 and 70 years, and a lack of ocular trauma, ocular surgery, or contact lens use (Hilmi *et al.*, 2017). Excluded were patients with significant ocular surface diseases, such as recurrent pterygium, corneal opacity, or irregularities caused by diseases other than pterygium. Two steps comprise the determination of net pterygium tissue mass components (NPTM): surgical excision and freeze-drying.

2.2. Surgical procedure

One surgeon (KMK) performed all of the procedures as outpatient procedures using topical anaesthesia and 0.5ml to 1ml of subconjunctival anaesthesia. The body of the pterygium was dissected 4 mm from the limbus and reflected over the cornea, followed by removal of the pterygium head using a Beaver No. 64 surgical blade (Becton Dickinson, Waltham, Massachusetts) and a blunt dissection technique. Excessive tenons were removed from the bare area, and the limbus was polished and smoothed using a 3.3 diamond burr (Katena Ophthalmic Burr (K2-4920), Katena Diamond Burr 3.3 Diameter Ball (K2-5923), Katena Product Inc., New Jersey, US). In all eyes, a thin, free conjunctival graft was dissected superficially from the superior bulbar conjunctiva, avoiding the underlying Tenon's layer. Grafts were premeasured to be 1 mm larger horizontally and vertically than the sclera defect itself. Graft margins were adhered to the sclera using fibrin glue (Tisseel™, Baxter AG, Vienna, Austria). All patients received 0.18% (0.3 ml) preservative-free Vismed® artificial tears (TRB Chemedica International SA, Geneva, Switzerland) qid for three (3) weeks after surgery.

2.3. Quantification process of formalin-fixated and non-fixed pterygium tissue utilizing Freeze-dried

For the formalin-fixed group, 30 fresh pterygium tissue samples were placed in sterile specimen bottles and appropriately labelled. Each sterile bottle containing a pterygium sample and 5 mL of 5% formaldehyde (formalin) is stored at room temperature for one week (Mohamed *et al.*, 2012). The remaining 30 fresh pterygium tissue samples were placed in individual sterile bottles and appropriately labelled. Each sterile bottle was filled with 5 mL of distilled water before the sample was added. All fresh pterygium was measured in micrograms (g) using an analytical balance (MS204S Analytical Balance, Mettler Toledo™, Greifensee, Switzerland) and a separate scale. For each formalin-fixed pterygium sample, the wet weights of pterygium tissue samples were determined after a week of fixation using the same analytical balance (MS204S Analytical Balance, Mettler Toledo™, Greifensee, Switzerland) (Benders *et al*, 2010). A single operator performed three measurements of wet weight, and the average of these measurements was used to determine the wet weight of pterygium tissue.

Then, all sample bottles were prefrozen for 24 hours and freeze-dried at -80 °C in a freeze dryer (Sahin *et al.*, 2006; Mohamed *et al.*, 2012) (Alpha 2-4 LDplus, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). All samples were weighed using an analytical balance (MS204S Analytical Balance, Mettler Toledo™, Greifensee, Switzerland) after being freeze-dried for 24 hours. A single operator performed three measurements on freeze-dried pterygium samples, and the average of these measurements was taken as the net pterygium tissue mass (NPTM), which represents the dry weight of pterygium. All freeze-dried pterygium samples were stored in a 5.0 ml Eppendorf® tube for archival purposes.

2.4. Statistical analysis

All data were presented in mean ± SD. Normality of data was tested using ratio of kurtosis and skewness of ± 2.50 (George and Mallery, 2010). Comparison between non-fixed and formalin-fixed net pterygium tissue mass was assessed using independent T-test. The alpha significance level was set at $p < 0.05$. Statistical analysis was performed using IBM SPSS (Predictive analytics software) (Version 12, SPSS Inc., Chicago, IL, USA).

https://doi.org/10.30659/sainsmed.v13i2.2958

Table 1. Comparative analysis between formalin-fixed and fresh pterygium tissue via freeze-dried method based on independent T-test

Variables/Group	Formalin-fixed pterygium tissue	Non-fixed tissue	p-value (95% CI)
Wet weight (µg)	253.33 ± 82.17	255.17 ± 63.52	p = 0.923 (-36.12, 39.79)
Dry weight (µg)	184.92 ± 84.31	179.54 ± 72.85	p= 0.792 (-46.10, 35.34)
Net Pterygium tissue mass (%)	69.39 ± 13.29	67.75 ± 13.27	p= 0.635 (-8.50, 5.23)
Water content (%)	30.61 ± 13.29	32.25 ± 13.27	p= 0.635 (-5.23, 8.50)

3. Results

3.1. Descriptive analysis

The overall mean and SD for formalin-fixed and fresh pterygium wet weight were 253.33 ± 82.17 µg (n = 30) and 255.17 ± 63.52 µg (n = 30) respectively. Whereas the overall mean and SD for formalin-fixed and fresh pterygium dry weight were 184.92 ± 84.31 µg (n = 30) and 179.54 ± 72.85 µg (n = 30) respectively. In terms of percentage of fibroconnective components, formalin-fixed group revealed slightly higher percentage compared to fresh pterygium tissue with 69.39 ± 13.29 % and 67.75 ± 13.27 % respectively.

3.2. Comparative analysis

Based on the results, we found that the differences in pterygium dry weight and percentage of net pterygium tissue mass between formalin-fixed and non-fixed freeze-dried were statistically insignificant (all $p > 0.05$). These results suggest that both methods were comparable and interchangeably. A summary of comparative analysis between all parameters are shown in Table 1.

4. Disscussion

This study aimed to describe a method for determining the dry weight of pterygium using the freeze-dried method. We have separated 60 samples of pterygium into two groups: non-fixed and formalin-fixed. The selection of fresh tissue assumed that it would be free of external impurities, such as chemical exposure. This is significant because the objective of this study was to quantify the net pterygium tissue mass (NPTM) components, which infers the fibroconnective components of pterygium. In contrast, samples fixed with formalin undergo a chemical reaction. Consequently, we doubt that using formalin-fixed tissue would influence the measurement of pterygium tissue mass. It's possible that the effect was negligible; however, to be certain, we decided to compare the dry weight values of pterygium between these two methods.

This study discovered that both methods were comparable; however, the formalin-fixed group had a higher wet weight for pterygium. This may be due to the nature of formalin as a fixative, which replaces water with formaldehyde, thereby increasing its original weight and, consequently, its water content (Birkl *et al.*, 2016). The non-fixed group had a higher percentage of water content than the formalin-fixed group, which indirectly led to a greater NTPM. As suggested by a previous study, we hypothesise that the differences in increased percentage of NPTM and decreased percentage of water content in the formalin-fixed group indicate a slight increase in protein weight due to formalin fixation of tissue (Birkl *et al.*, 2016).

After surgical excision, it is common practise to store pterygium samples in formalin. Formalin, also referred to as formaldehyde, is a well-known fixative used to preserve biological tissues for future research. However, numerous studies have reported that formalin's chemical reactions cause some structural changes in biological tissues (Thavarajah *et al.*, 2012; Birkl *et al.*, 2016). Nonetheless, our findings suggest that the effects of additional aldehyde molecules on the dry weight of pterygium may be negligible.

However, the freeze-drying technique could mitigate this disadvantage by removing moisture and ensuring chemical-free conditions, so that the actual dry weight of pterygium could be determined while the tissues remained functional. This is significant given that the pterygium tissue mass consists of fibroblasts and connective tissues (Dunic *et al.*, 2010). Clinically, the actual mass of pterygium is thus indirectly determined by having a dry tissue. Previous studies (Rodriguez-Ares *et al.*, 2009; Allen *et al.*, 2013) have demonstrated that the freeze-dried method is reliable for determining the tissue content of an ocular tissue and is superior to the desiccation method for preserving ocular tissue (Frota *et al.*, 2008). As a result, we were unable to compare our findings to those of other studies.

Based on our findings, we hypothesise that the physico-biological properties of pterygium may play a significant role in causing corneal astigmatism. This is because NPTM represents fibroconnective components, which could serve as the total mass, that cause mechanical traction on the cornea and result in the previously described local corneal flattening (Errais *et al*, 2008; Hilmi *et al*, 2017). Our hypothesis was that the quantity of NPTM could indicate the level

of angiogenesis activity (Qi *et al.*, 2012; Sato *et al.*, 2014; Che Azemin *et al.*, 2014; Azemin *et al.*, 2015; Azemin *et al.*, 2016; Kumagai *et al.*, 2016), which served as an important indicator for fibrous tissue growth, which in this case leads to various degree of induced corneal astigmatism. This may explain why some pterygium cause significant corneal astigmatism while others do not.

5. Conclusions

In conclusion, we discovered that freeze-drying could provide a consistent method for measuring the dry weight of pterygium. As there is no statistically significant difference between the two tissue preparation methods, both formalin-fixed and non-fixed methods can be used in the future for this purpose. The dry weight of pterygium tissue, also known as NPTM, represents the actual fibroconnective components of pterygium tissue, which inferentially represent the mass of pterygium. This is the first report to our knowledge that describes a method for quantifying pterygium fibroconnective components. This study may serve as a foundation for future research into the effect of physico-biological properties of pterygium on changes in corneal curvature, which result in corneal astigmatism.

Acknowledgement

This research is financially supported by International Islamic University Malaysia (IIUM) under Publication-Research Initiative Grant Scheme (P-RIGS) P-RIGS18-035-0035.

Declaration of Interest

The authors report no conflicts of interest

References

Allen, C. L., Clare, G., Stewart, E. A., Branch, M. J., McIntosh, O. D., Dadhwal, M., Dua, H. S., & Hopkinson, A. (2013). Augmented Dried versus Cryopreserved Amniotic Membrane as an Ocular Surface Dressing. *Plos One*, 8(10), e78441. https://doi.org/10.1371/journal.pone.0078441

Ang, Leonard PK., Chua, Jocelyn LL., Tan, Donald TH. (2007). Current concepts and techniques in pterygium treatment. *Current Opinion in Ophthalmology* 18(4): 308-313. DOI: 10.1097/ICU.0b013e3281a7ecbb

Azemin, M.Z.C, Hilmi, M.R., Kamal, K.M. (2014). Supervised Pterygium Fibrovascular Redness Grading Using Generalized Regression Neural Network, *Frontiers in Artificial Intelligence and Applications*, 265: 650-656. DOI: 10.3233/978-1-61499-434-3-65

Azemin, M.Z.C. *et al.*, 2015. GLCM texture analysis on different color space for pterygium grading, *ARPN Journal of Engineering and Applied Sciences*, 10: 6410-6413. https://www.researchgate.net/publication/283229914_GLCM_texture_analysis_on_different_color_space_for_pterygium_grading

Azemin, MZC., Tamrin, Izzuddin M., Hilmi, MR., Kamal, KM. (2016). Inter-grader reliability of a supervised pterygium redness grading system, *Advanced Science Letters,* 22(10): 2885-2888. https://doi.org/10.1166/asl.2016.7125

Benders, K.E., Malda, J., Saris, D.B.F., Dhert, W.J.A., Steck, R., Hutmacher, D.W., Klein, T.J. (2010). Formalin fixation affects equilibrium partitioning of an ionic contrast agent-microcomputed tomography (EPIC-μCT) imaging of osteochondral samples, *Osteoarthritis Cartilage* 18:1586-1591.

Birkl, C., Langkammer, C., Golob-Schwarzl, N., Leoni, M., Haybaeck, J., Goessler, W., Fazekas, F., and Ropele, S. (2016). Effects of formalin fixation and temperature on MR relaxation times in the human brain. *NMR in Biomedicine*, 29: 458– 465. doi: 10.1002/nbm.3477.

Chen, T., Ding, L., Shan, G., Ke, L., Ma, J., Zhong, Y. (2015) Prevalence and Racial Differences in Pterygium: A Cross-Sectional Study in Han and Uygur Adults in Xinjiang, China. *Investigative Ophthalmology & Visual Science,* 56(2):1109-1117. doi: https://doi.org/10.1167/iovs.14-15994.

Connon CJ., Doutch J., Chen B., Hopkinson, A., Nakamura, T., Kinoshita, S., Meek, KM. (2010). The variation in transparency of amniotic membrane used in ocular surface regeneration. *British Journal of Ophthalmology* 94:1057-1061. http://dx.doi.org/10.1136/bjo.2008.153064

Džunić, B., Jovanović, P., Veselinović, D., Petrović, A., Stefanović, I., & Kovačević, I. (2010). Analysis of pathohistological characteristics of pterygium. *Bosnian Journal of Basic Medical Sciences* 10(4): 307-303. https://doi.org/10.17305/bjbms.2010.2677

Errais K, Bouden J, Mili-Boussen I, Anane R, Beltaif O, Meddeb Ouertani A. (2008). Effect of Pterygium Surgery on Corneal Topography. *European Journal of Ophthalmology.* 18(2):177-181. doi:10.1177/112067210801800203

Frota, A. C. de A., Lima Filho, A. A. de S., Dias, A. B. T., Lourenço, A. C. dos S., Antecka, E., & Burnier Jr., M. N.. (2008). Freeze-drying as an alternative method of human sclera preservation. *Arquivos Brasileiros De Oftalmologia,* 71(2): 137–141.

George, D. & Mallery, M., 2010, *SPSS for Windows Step by Step, A Simple Guide and References*, Boston: Pearson.

Hilmi MR., Che Azemin MZ., Mohd Kamal K., Mohd Tamrin MI., Abdul Gaffur N., Tengku Sembok TM. (2017). Prediction of Changes in Visual Acuity and Contrast Sensitivity Function by Tissue Redness after Pterygium Surgery. *Current Eye Research.* 2017 42(6):852-856. doi: 10.1080/02713683.2016.1250277.

Hilmi MR., Kamal, KM., Azemin, MZC., Ithin, MH., Mystafa, MMSM., Yusof, F., Ahmad, N. (2018). Corneal Curvature Measurements Utilizing a New Swept-source Optical Coherence Tomography Tomey OA-2000® and Comparison With IOL Master® 500 in Pterygium Patients. *Sains Medika: Jurnal Kedokteran dan Kesehatan*, 9(1):11-17. doi:http://dx.doi.org/10.26532/sainsmed.v9i1.2918

Hilmi MR., Khairidzan MK., Azemin ZC., Azami MH., Ariffin, AE. (2018). Measurement of Contrast Sensitivity Using the M&S Smart System II Compared with the Standard Pelli–Robson Chart in Patients with Primary Pterygium. *Makara Journal of Health Research* 22(3): 169-171.

Hilmi, M.R., Khairidzan, M. K., Mohd Zulfaezal, C. A., & Azrin, E. A. (2019). Corneo-pterygium total area measurements utilising image analysis method. *Journal of Optometry* 12(4): 272-277. https://doi.org/10.1016/j.optom.2019.04.001

Jiao, W., Chengchao Zhou, Ting Wang, Shaoyuan Yang, Hongsheng Bi, Liping Liu, Yan Li, Lihua Wang. (2014). Prevalence and Risk Factors for Pterygium in Rural Older Adults in Shandong Province of China: A Cross-Sectional Study. *BioMed Research International*, Vol. 2014, Article ID 658648: 1-8. https://doi.org/10.1155/2014/658648

Kumagai, A., Kondo, F., Sano, K., Inoue, M., Fujii, T., Hashimoto, M., Watanabe, M., Soejima, Y., Ishida, T., Tokairin, T., Saito, K., Sasajima, Y., Takahashi, Y., Uozaki, H., & Fukusato, T. (2016). Immunohistochemical study of hepatocyte, cholangiocyte and stem cell markers of hepatocellular carcinoma: the second report: relationship with tumor size and cell differentiation. *Journal of Hepato-Biliary-Pancreatic Sciences,* 23(7), 414-421. https://doi.org/10.1002/jhbp.356

Libera, R. D., Melo, G. B. de ., Lima, A. de S., Haapalainen, E. F., Cristovam, P., & Gomes, J. Á. P.. (2008). Assessment of the use of cryopreserved x freeze-dried amniotic membrane (AM) for reconstruction of ocular surface in rabbit model. *Arquivos Brasileiros De Oftalmologia,* 71(5): 669-673. https://doi.org/10.1590/S0004-27492008000500011

Liu, T., Liu, Y., Xie, L., He, X. & Ji Bai. (2013). Progress in the Pathogenesis of Pterygium. *Current Eye Research* 38 (12): 1191-1197. DOI: 10.3109/02713683.2013.823212

Mahar PS and Manzar N. (2013). Pterygium recurrence related to its size and corneal involvement. *Journal of the College of Physicians and Surgeons Pakistan* 23(2):120-3. https://www.jcpsp.pk/archive/2013/Feb2013/06.pdf

Marmamula, S., Khanna, C.K., Rao, G.N. (2013). Population-Based Assessment of Prevalence and Risk Factors for Pterygium in the South Indian State of Andhra Pradesh: The Andhra Pradesh Eye Disease Study. *Investigative Ophthalmology & Visual Science* 54(8):5359-5366. https://doi.org/10.1167/iovs.13-12529.

Mohamed, A., Sangwan, V.S. & Augusteyn, R.C. (2012). Growth of the human lens in the Indian adult population: Preliminary observations, *Indian Journal of Ophthalmology* 60 (6): 511–515. DOI: 10.4103/0301-4738.103775

Nangia, V., Jonas, J. B., Nair, D., Saini, N., Nangia, P., & Panda-Jonas, S. (2013). Prevalence and Associated Factors for Pterygium in Rural Agrarian Central India. The Central India Eye and Medical Study. *Plos One*, 8(12): 1-19. https://doi.org/10.1371/journal.pone.0082439

Park, Y. M., Kim, C. D., & Lee, J. S. (2015). Effect of Bevacizumab on Human Tenon's Fibroblasts Cultured from Primary and Recurrent Pterygium. *The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology* 19(4): 357-363. https://doi.org/10.4196/kjpp.2015.19.4.357

Qi, X., Zhang, D., Yuan, J., Yang, Z., Sun, Y., Wang, T., Ye, H., & Ling, Q. (2012). Relationship between angiogenesis and lymphangiogenesis in recurrent pterygium. *International Journal of Ophthalmology* 5(6): 655-660. https://doi.org/10.3980/j.issn.2222-3959.2012.06.01

Ribatti, D., Nico, B., Perra, M. T., Maxia, C., Piras, F., Murtas, D., Crivellato, E., & Sirigu, P. (2009). Correlation between NGF/TrkA and microvascular density in human pterygium. *International Journal of Experimental Pathology* 90(6): 615-620. https://doi.org/10.1111/j.1365-2613.2009.00665.x

Rodríguez-Ares, M.T., López-Valladares, M.J., Touriño, R., Vieites, B., Gude, F., Silva, M.T. and Couceiro. J. (2009). Effects of lyophilization on human amniotic membrane. *Acta Ophthalmologica* 87: 396-403. https://doi.org/10.1111/j.1755-3768.2008.01261.x

Sahin, S., Karabey, Y., Kaynak, M.S., Hincal, A.A. (2006). Potential Use of Freeze-Drying Technique for Estimation of Tissue Water Content, *Methods Findinding Experimental and Clinical Pharmacology*, 28(4): 211–215. DOI: 10.1358/mf.2006.28.4.990200

Sato, Y., Ojima, H., Onaya, H., Mori, T., Hiraoka, N., Kishi, Y., Nara, S., Esaki, M., Shimada, K., Kosuge, T., Sugihara, K. and Kanai, Y. (2014), Hypervascular cholangiocellular carcinoma. *Hepatology Research.* 44: 1119-1129. https://doi.org/10.1111/hepr.12236

Takahiro Nakamura, Makoto Yoshitani, Helen Rigby, Nigel J. Fullwood, Wakana Ito, Tsutomu Inatomi, Chie Sotozono, Tatsuo Nakamura, Yasuhiko Shimizu, Shigeru Kinoshita. (2004). Sterilized, Freeze-Dried Amniotic Membrane: A Useful Substrate for Ocular Surface Reconstruction. *Investigative Ophthalmology & Visual Science* 45(1):93-99. doi: https://doi.org/10.1167/iovs.03-0752.

Tan, D.T., Chee, S.P., Dear, K.B. & Lim, A.S. (1997). Effect of pterygium morphology on pterygium recurrence in a controlled trial comparing conjunctival autografting with bare sclera excision, *Archive Ophthalmology* 115(10):1235–1240. https://doi.org/10.1001/archopht.1997.01100160405001

Thavarajah, R., Mudimbaimannar, V. K., Elizabeth, J., Rao, U. K., & Ranganathan, K. (2012). Chemical and physical basics of routine formaldehyde fixation. *Journal of Oral and Maxillofacial Pathology: JOMFP,* 16(3), 400-405. https://doi.org/10.4103/0973-029X.102496