How to Cite:
Anubhuti, A., Goudar, S., Ahmed, S., Rajesh, D., Karukola, R. K., & Goutam, M. (2022). Positively charged polyvinylidene fluoride (PVDF) membrane: A potential alternative for absorbent paper points in endodontics. *International Journal of Health Sciences*, *6*(S7), 2666–2671. https://doi.org/10.53730/ijhs.v6nS7.11924

Positively charged polyvinylidene fluoride (PVDF) membrane: A potential alternative for absorbent paper points in endodontics

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Abstract---Aim The purpose of the study was to evaluate the performance of Polyvinylidene Fluoride (PVDF) as an alternative to paper points (PPs) for endodontic treatments. Methodology The paper points and the PVDF membrane were evaluated for endotoxin binding using Limulus Amebocyte Lysate (LAL) assay. New paper points and

International Journal of Health Sciences ISSN 2550-6978 E-ISSN 2550-696X © 2022.
Manuscript submitted: 9 April 2022, Manuscript revised: 18 June 2022, Accepted for publication: 27 July 2022

the PVDF membrane were evaluated for the presence of endotoxins. Results Absorbency and endotoxin removal with the 0.22µm PVDF membrane was significantly greater than any of the paper points tested. There was significantly more endotoxin found in new paper points compared to the PVDF membrane. Conclusion In conclusion, our study showed that the 0.22µm PVDF membrane was significantly more absorbent and removed more endotoxins than PPs.

***Keywords*---**Absorbency, Endotoxin, Endodontics, lipopolysaccharide, Paper point, Root canal.

Introduction

Membrane technology has gradually become a popular separation technology over the past few decades. There are many significant advantages of using membranes for industrial processes, for example, no phase changes or chemical additives, modular which is easy to scale up, simple in operation, relatively low energy consumption, etc. Therefore, membrane technology has been widely applied to various fields such as water treatment [1,2], gas purification [3], food processing [4], pharmaceutical industry [5] and environmental protection [6]. The membrane is the key of the membrane separation technology, and it directly affects process efficiency and practical application value. At present, almost all membranes for industrial processes are made from inorganic materials and/or organic polymers, and the latter dominates the existing membrane market. Examples of organic polymers include polysulfone (PSF),poly(ethersulfone) (PES),polyacrylonitrile(PAN), polyamide, polyimide, poly(vinylidene fluoride) (PVDF)and polytetrafluoroethylene(PTFE).Therein, PVDF is one of the most used membrane materials and has been paid much attention by researchers and manufacturers in recent years [7]. Poly(vinylidene fluoride)(PVDF) is a promising polymeric membrane material due to its peculiar antioxidation activity, excellent chemical resistance, thermal stability, and good membrane-forming properties [8]. Nonetheless, the hydrophobic nature of the PVDF membrane makes it susceptible to contamination by proteins and some of the other impurities in water treatment systems, which causes a sharp drop in the water flux during the membrane filtration process [9]. To overcome this disadvantage, various modifications have been proposed to improve the performance of PVDF membranes, such as physical blending [10-13], plasma treatment [14], surface modification [15] and chemical grafting [8,16]. Among these methods, physical blending with organic or inorganic additives is facile and effective. Introducing additives into the membrane matrix can improve the hydrophilic character, water permeability and antifouling properties of the PVDF membrane. In a review by Nair, exogenous materials such as PPs fibers, were associated with foreign body reactions in non-healing periapical lesions [17]. Others reported on consequences of cellulose fibers found in periapical biopsies of patients with a history of endodontic treatment [18,19]. Foreign body reactions initiated and perpetuated by cellulose fibers e.g. from disposable surgical gowns and drapes, gauze, etc. are well documented in the general medical literature [20]. In a case report by Sedgley and Messer, a PP protruded through the apical foramen and a biofilm grew around the extruded PP, 2 eventually leading to failure of the root canal treatment. This sustained and even intensified the apical periodontitis after root canal treatment eventually leading to a failure of treatment [19]. Brown evaluated six different PP brands using an artificial simulate apical foramen and he found every brand of PP shed fibers during canal length confirmation [21]. In 1987, Koppang et al. investigated hematoxylinophilic birefringent foreign bodies of non-healing lesions and concluded that cellulose fibers from endodontic PPs were responsible for the chronic periapical lesions of endodontically treated teeth observed (4). Koppang later identified commonly occurring foreign material in post endodontic periapical granulomas and cysts and four types of foreign materials were observed: amalgam, endodontic sealer, calcium hydroxide and cellulose [22]. Despite documented association between plant cellulose and non-healing lesions, there has been no change to the clinical use of PPs. To solve this problem, we developed an innovative tool for drying the root canal system using a positively-charged polyvinylidene fluoride (PVDF) membrane currently used in the biopharmaceutical industry for sterile filtration and removal of endotoxins. The ability to remove endotoxins are a useful benefit in treating infected pulps. Endotoxins easily pass through the 0.2 µm pores of noncharged membrane filters, in which size exclusion is the only retention mechanism. Since endotoxins are negatively charged, the positively charged membrane may aid the removal of endotoxins [23]. Millipore produces charged PVDF membranes that are used in filtration cartridges designed for the removal of endotoxins from pharmaceutical-grade water systems. Due to the properties of a charged PVDF, they have found that a 0.2um pore sized filter was able to retain endotoxins during filtration [24].

Aim of The Present Study

This study compared the absorbency capacity of paper points (PPs) to positively charged and non-charged Polyvinylidene Fluoride (PVDF) membranes (PVDFMs) and investigated the ability of PPs and PVDFMs to bind and remove endotoxin.

Methodology

Three commercially available PPs were compared to PVDFM (Millipore Sigma, Burlington, MA, USA) prototype points. As PPs come in rolled format and the PVDF membrane is currently only available as a flat sheet, PPs were unrolled and the absorbency study was performed to minimize these differences. Using Power and Precision TM, a power computation was performed. With an n of 5 in each group, a two-tail test, a $p \leq .05$, and a difference of at least 20% between the groups, and an effect size of 13.19, power was equal to 100%. The initial dry weight for each PP and PVDFM using a digital balance to ±0.0001 precision for the absorbency were noted. PPs and PVDFMs were then immersed in deionized water and weighed to obtain the wet weight. The absorbency was calculated with the formula:

Percent increase = [(wet weight – dry weight)/dry weight] x 100.

For endotoxin removal, endotoxin remaining in wells were quantified after immersing PPs and PVDFMs in a 24 well-plate containing 10 endotoxin units/mL (EU/ml) of *E. coli* O55:B5 (Lonza, MD). Extraction and quantification of endotoxin was done from PPs and PVDFMs using the KQCL test. Data analysis was performed, using SPSS, with a significance level of 5%. Assumptions of the equality of variances and the normal distribution of errors was checked. A one-way ANOVA and Tukey's honestly significant difference test was used for intergroup analysis.

Results

Based on this study, absorbency of the 0.22µm and 5.0µm PVDF membrane was significantly greater than any of the PPs, p <.05. The 0.22µm was the most absorbent of all the materials tested. The absorbency potential for each sample was calculated by subtracting the final weight from the initial dry weight and expressed as percent weight gain. An independent t-test showed that all rolled forms of the 0.22µm PDVF membrane were significantly more absorbent than the PPs. Based on this study, absorbency of the 0.22µm PVDF membrane was significantly greater than any of the PP counterparts. The Limulus Amebocyte Lysate (LAL) assay quantitatively determine the amount of LPS removed by the PVDF membrane. If endotoxins are present in the sample, the subsequent enzymatic reactions of the LAL reagent cause a color change solution. The more endotoxin present, the more yellow the solution will become. This can be quantitated using a spectrophotometer or absorbance plate reader to reveal the specific endotoxin concentration. The WinKQCL plate reader and software was used to measure the amount of endotoxin in each well. The plate was incubated at 37°C ±1°C for 10 minutes in a 17 Kinetic-QCL (Lonza) reader, which is coupled to a microcomputer by means of the WinKQCL software. Next, 100 mL chromogenic reagent was added to each well. (Table 1) The absorbencies for the positively charged and non-charged PVDFMs were higher than the PPs (p<.05), with no difference between them (p>.05). The positively charged PVDFMs bound and removed endotoxin than non-charged PVDFMs and the PPs (p<.05). Moreover, the non-charged PVDFMs bound and removed more endotoxin than any PPs (p<.05).

Discussion

Paper points, the basic armamentarium used to remove moisture and contaminants from root canals, has not changed in 100 years. Endodontic PPs also are made of plant cellulose that cannot be degraded and PPs used in endodontics have been shown to shed fibers [18,19]. In a review by Nair, exogenous materials such as PP fibers, were associated with foreign body reactions in non-healing periapical lesions [17]. A prototype of a PP alternative for drying the root canal system was made using a positively-charged polyvinylidene fluoride (PVDF) membrane currently used in the biopharmaceutical industry for sterile filtration and removal of endotoxins. The ability to remove endotoxins are a useful benefit in treating infected pulps. Gram-negative bacteria such as Prevotella, Fusobacterium, and Porphyromonas are found in the oral cavity and in primary endodontic infections. The cell walls of Gramnegative bacteria contain a lipopolysaccharide (LPS) that is capable of initiating a proinflammatory biological response [25]. As PPs are primary used to remove irrigants from the root canal system, the absorbency of PPs and the PVDF membrane was compared in study #1. PPs from several manufacturers and PVDF in the 5.0µm and 0.22µm pore size were compared in an unrolled format. Both pore sizes of the PVDF membrane were found to be significantly more absorbent than the PPs. In comparing the absorbency between 5.0μm and 0.22μm pore size PVDF, the 0.22μm pore size PVDF was more absorbent than the 5.0μm pore size PVDF. This finding makes sense since a membrane with a smaller pore size would leave more surface area of material for absorption. A PVDF membrane with a higher absorbency than PPs would be useful clinically because using less material to absorb more irrigants from the root canal system would allow for a more efficient treatment workflow. In conclusion, our study showed that the 0.22μm PVDF membrane was significantly more absorbent and removed more endotoxins than PPs. Commercially available paper points were found to be contaminated with endotoxins and mechanical agitation of the PVDF membrane did not release endotoxin.

Conclusion

PVDFM prototype points are more absorbent than PPs. Moreover, the positively charged PVDFM points are more effective in binding and removing endotoxin than non-charged PVDFMs and PPs. This study suggests that positively charged PVDFMs with 0.22 μm pore size could potentially replace PPs used in endodontics.

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Tables

Table 1- ANOVA analysis of average amount of endotoxins released from new unused PPs or PVDF membrane after measurements.

Absorbent material	N	Mean ±SD	t	p
Paper points (PPs)	5	1.38±0.78	3.88	1.22
Positively charged PVDF	5	0.59±0.08	2.39	0.042
Uncharged PVDF	5	0.97±0.511	5.66	0.189