WING-YEE YEUNG

Email Address:
wingyee.yeung@gmail.com

Education

University of Maryland School of Dentistry, Baltimore, MD July 2016– Present
Advanced Specialty in Endodontics
Master of Science (M.Sc.) in Biomedical Sciences

The Johns Hopkins Hospital, Baltimore, MD July 2015- June 2016
General Practice Residency (GPR) in Dentistry

Howard University College of Dentistry, Washington, D.C. July 2011- May 2015
Doctor of Dental Surgery (D.D.S)
Omicron Kappa Upsilon (OKU), National Dental Honor Society

Tulane University, New Orleans, LA October 2006
Freeman School of Business
Masters Certificates in Business Administration and Marketing

Smith College, Northampton, MA Sept 2001-May 2005
Bachelor of Arts (B.A.) – Double Major in Neuroscience and Biology
Graduated with honors, Sigma Xi, The Scientific Honor Research Society

Clinical Skills

University of Maryland School of Dentistry, Baltimore, MD July 2016– Present
Co-Chief Resident of Endodontics
Prospective Board Candidate of the American Board of Endodontics

Honors and Awards

American Association of Endodontics/Dentsply Sirona Resident Award, 2018
- Table clinic second place winner

American Association of Endodontics /Dentsply Sirona Resident Award, 2017
- Table clinic third place winner

Omicron Kappa Upsilon, Pi Pi Chapter, 2015
- Awarded to dental students who have demonstrated excellence in scholarship while demonstrating exemplary traits of character and potential qualities for professional growth and attainments.

Dr. Preston E. Lee Award, 2015
- Awarded to the female graduating dental student who has shown the greatest proficiency in Operative Dentistry

American Association of Endodontist Award, 2015

- Awarded to graduating dental student who has shown broad interest in Endodontics and demonstrated more involvment than is required.

Howard University Board of Trustees Academic Scholarship, 2012-2015

- Awarded after demonstrating academic excellence during academic school year.

Dr. Bernard C. and Mrs. Wilhelmina J. Parris Endowed Dental Student Scholarship, 2013-2015

- Awarded per Dean's nomination based on academic performance and services

Dental Net Tuition Scholarship, 2013-2015

- Awarded after demonstrating academic excellence

Howard E. Smith Endowed Scholarship, 2013-2015

- Awarded to student based on excellence in leadership, service and academic performance

Howard University Dean's List 2011-2015

- Awarded to top students of the respective class after demonstrated academic excellence

Sigma Xi, Scientific Research Honor Society, 2005

- Awarded to the graduating senior who have demonstrated excellence in research and scholarship

Smith College Dean's List 2004-2005

- Awarded to top students of the respective class after demonstrated academic excellence

Work Experiences

Sartorius Stedim Biotech, Bohemia, NY
North American Laboratory Science Marketing Manager November 2009 – August 2011

- Managed laboratory water, filtration, cell culture, microbiology and purification product lines to increase market share in North America by creating strategic and tactical marketing platforms
- Created tactical product support through brochures, data sheets, operating manuals, service manuals and new product launch packages

Millipore Corporation, Bioscience Division, Danvers, MA
Product Manager November 2006 – October 2009

- Responsible for $25M+ business and contributed to annual growth targets for the Cell Biology Biotools product portfolio. Product lines include Sterile Millex (syringe filters), Medical OEM Millex, and Virus Purification

- Drove new product development with project team. Developed market entry timing, product positioning and image, promotional strategy, distribution strategy, initial inventory levels and product price. Launched 5 new products in 2008 which contributed to over $2M in revenue for 2009

Fitzgerald Industries International/Research Diagnostics, Concord, MA
Technical Sales August 2005 - November 2006

- Sold antibodies, antigens, and research reagents internationally to research and diagnostic institutions and reached annual sales target of over $14M
- Provided technical and troubleshooting support to customers

Research Experiences

Howard University College of Dentistry, Washington, DC May 2015
Advisors: Melanie Ventocilla, DDS and Debra Jeffries DDS.

- Study the prevalence of pre-masticating food for weaning infants among mothers and caregivers in the Washington, DC area at Howard University Hospital
- Responsible for developing abstract and poster, data collection and analysis

Howard University College of Dentistry, Washington, DC May 2015
Advisors: Melanie Ventocilla, DDS and Debra Jeffries DDS.

- Case report following an 8-year-old Hispanic female with 47 XX + mar chromosome abnormality at location 14q11.2 to investigate the oral manifestation of the genetic deviation
- Responsible for literature research and creating paper and poster.

Wyeth, Women's Health and Bone Department, Cambridge, MA
Research Intern May 2004 - August 2004

- Researched potential drug targets for bone formation through cell culture
- Created and developed presentations on progress and results of the research

Cornell University, Department of Neurobiology and Behavior, Ithaca, NY
Research Intern June 2003 - August 2003

- Researched and identified the gene responsible for temperature sensitive paralytic fruit flies using molecular techniques

Smith College, Neuroscience Department, Northampton, MA
Research Assistant September 2001 - September 2004

- Performed oocyte extraction from *Xenopus* (frogs)
- Analyzed the effects of anesthetic molecules on neuronal ion channels in vitro
- Co-authored and published two scientific publications using experimental results

Publications and Presentations

American Association of Endodontics Foundation Research Grant July 2017-present

- Awarded research grant for $15,000 in support of research proposal on Utilizing Charged Membrane Technology for Endotoxin Removal from the Root Canal System.

Poster Presentation: Prevalence of pre-masticating food for weaning infants among mothers and caregivers in the Washington, DC area, May 2015 presented at the 2015 American Academy of Pediatric Dentistry annual session, Seattle, Washington.

Poster Presentation: 47 XX + Mar Chromosome Abnormality: A Case Report, May 2015 presented at the 2015 American Academy of Pediatric Dentistry Annual Session, Seattle, Washington.

Publication: Subunit-dependent block by isoflurane of wild-type and mutant <alpha>1 S270H $GABA_A$ receptor currents in Xenopus oocytes. Hall AC, Stevens RJ, Betts BA, Yeung WY, Kelley JC, Harrison NL. Neurosci Lett. 2005 Jul 15;382(3):332-7

Publication: Modulation of human $GABA_A$ and glycine receptor currents by menthol and related monoterpenoids. Hall AC, Turcotte CM, Betts BA, Yeung WY, Agyeman AS, Burk LA. Eur J Pharmacol. 2004 Dec 3;506(1):9-16.

JUNE, Journal of Undergraduate Neuroscience Education, published a picture from IHC research project on the cover, Fall 2003 Vol. 2. Issue 1

Leadership and Service

Edward C. Penick Endodontic Study Club, Washington, DC. Social organization centered in the Washington D.C. metropolitan area for dentists with an interest in endodontics to foster the study, advancement and interchange of knowledge in the art and science of Endodontics.

Howard University College of Dentistry Open House Screenings, Washington, DC. Created and organized new oral health screening event; directed dental students to take histories, radiographs and perform exams with appropriate documentation at the student-run event. 2013-2015

Student National Dental Association, Oral Cancer Walk, Washington, DC. Fund raised and participated in oral cancer awareness and provided free oral cancer screenings to the underserved population of Washington, DC. 2011-2015

American Dental Association, Give Kids a Smile, Washington, DC. Performed intraoral examinations and provided free preventative and restorative dental care to school-aged children of Washington, DC. 2011-2015

Student Academic Advisor, Smith College, Northampton, MA. Guided incoming new students with recommendations on appropriate course loads. Provided options for academic support. Fall 2004-Spring 2005

Teaching Assistant, Smith College, Northampton, MA**.** NSC 200, Experimental Methods in Neuroscience. Set up and break down of experiments, tutored students, and provided guidance for experimental designs. Fall 2004-Spring 2005

EMT-B certified as a basic EMT in Massachusetts. Spring 2003-Spring 2004

Kumon Math Tutor in Closter, NJ. Tutored students in elementary math to Calculus. Instructed students on SAT math strategies and tactics. Fall 1999-Spring 2001

Volunteer, Pascack Valley Hospital, Westwood, NJ**.** Volunteered over 150 hours in the Nursing, Radiology and Pediatric departments. 1999-2001

ABSTRACT

TITLE: Utilizing Charged Membrane Technology for Endotoxin Removal with Potential Use in Endodontic Procedures

Wing-Yee Yeung, Master of Science 2019

Thesis Directed By: Dr. Robert (Bob) K. Ernst, PhD. Professor and Vice Chair in the Department of Microbial Pathogenesis in the School of Dentistry at the University of Maryland

AIM: To examine the application of a positively-charged polyvinylidene fluoride (PVDF) membrane for removing liquids and endotoxins.

METHODOLOGY: Absorbency and endotoxin removal of paper points from various manufacturers was compared with PVDF membrane. The paper points and the PVDF membrane were evaluated for endotoxin binding using Limulus Amebocyte Lysate (LAL) assay. New paper points and 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: Our study showed that the 0.22µm PVDF membrane was significantly more absorbent and removed more endotoxins than paper points. Commercially available paper points were found to be contaminated with endotoxins and mechanical agitation of the PVDF membrane did not release endotoxin.

Utilizing Charged Membrane Technology for Endotoxin Removal with Potential Use in Endodontic Procedures

by
Wing-Yee Yeung

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore in partial fulfillment
of the requirements for degree of
Master of Science
2019

To my family who have sacrificed so that I could get to where I am today.

Acknowledgements

This study was supported by the Foundation for Endodontics with a research grant, #443148

Thank you to my research committee: Dr. Robert Ernst, Dr. Patricia Tordik, Dr. Elaine Romberg, Dr. Priya Chand, Dr. Michael Weir

Thank you to those who have also supported this in other ways: Kenneth Ludwig, Dr. Frederico Martinho, Dr. Heojin Kim, Dr. Steffi Estevez

Thank you/you're welcome to my co-residents for their help and support.

Patent Pending

Thank you to Emily who is an amazingly happy baby that laughs and smiles every day. It is such a joy to have you in our lives. Lastly, thank you to my husband, Dr. Alan H. Tieu, who's been my rock and stability, my partner both in life and on the courts, I really could not have done this without you.

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INTRODUCTION

Nonsurgical root canal treatment goals include cleaning, shaping, and obturation of the root canal system to prevent future ingress of bacteria. During the procedure, canals are irrigated to chemically kill bacteria and to clear debris from a root canal system. The irrigant is removed by suctioning and using paper points (PPs) to wick remaining fluids from canals. Drying canals is a necessary step, as the endodontic sealer can be affected by the degree of dryness inside a root canal (1) but if the PPs is dislodged or gets pushed into the periapical tissue, a foreign body reaction can be induced (2). Hosoya et al. has shown that the sealing ability of endodontic sealer (ZOE sealer) was found to be affected by the degree of dryness inside the root canal (1). Ehsani et al. also looked at the effects of moisture in the canals and they evaluated the amount of micro-leakage of different endodontic sealers in presence and absence of moisture of AH26, Excite DSC, MTA Fillapex, and ZOE sealers. AH26 provided the least apical micro-leakage under dry conditions while ZOE had the highest micro-leakage under moist conditions (3). Using PPs to dry canals have been a necessary step in the root canal procedure.

In a review by Nair, exogenous materials such as PPs fibers, were associated with foreign body reactions in non-healing periapical lesions (2). Others reported on consequences of cellulose fibers found in periapical biopsies of patients with a history of endodontic treatment (4, 5). Foreign body reactions initiated and perpetuated by cellulose fibers e.g. from disposable surgical gowns and drapes, gauze, ect. are well documented in the general medical literature (6). In a case report by Sedgley and Messer, a PP protruded through the apical foramen and a biofilm grew around the extruded PP, eventually leading to failure of the root canal treatment (5). This sustained and even intensified the apical periodontitis after root canal treatment eventually leading to a failure of treatment (5). 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 (7). 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 (8).

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. *Endodontically infected teeth is a polymicrobial infection that involves both gram + and gram – bacteria (9). In teeth that had pulpal exposure due to caries, the most prevalent species of bacteria present was Prevotella intermedia/nigrescens (g-), peptostreptococcus (g+), fusobacterium nucleatum (g-), Enterococcus faecalis (g+) (10).* Gram-negative bacteria such as *Prevotella*, *Fusobacterium*, and *Porphyromonas* are found in endodontic infections (9).

The cell walls of Gram-negative bacteria contain a lipopolysaccharide (LPS) that is capable of initiating a proinflammatory biological response (11). LPS is composed of three distinct regions: the O-specific polysaccharide, the common core, and the lipid membrane anchor lipid A (12). Lipid A, also known as endotoxin, is responsible for the biological activity of LPS and is recognized by the host innate immune system via the Toll-like receptor 4 (TLR4) complex. TLR4 allows macrophages to detect LPS which causes a homodimerization of extracellular proteins resulting in activation of Nuclear Factor Kappa Beta. NF-kB dependent responses including cytokine release resulting in inflammation (11). Endotoxins have been found to stimulate bone resorption in tissue culture and to possess the ability to attract osteoclasts to bone (11). During endodontic therapy, if endotoxins are released, a periapical inflammatory response and acute clinical inflammation may follow (11).

Review of the Literature

Using Membranes for Removal of Endotoxins in The Pharmaceutical Industries

Bacterial endotoxins are acknowledged by the pharmaceutical industry as potential causes of pyrogenic reactions in parenteral drug products. Filtration are means of removing endotoxins from biological solutions (13). In 1985, Gerba and Hou described the use of depth and membrane filters with charge-modified surfaces for the enhanced removal of bacterial endotoxin from solutions. They showed that charged nylon filters with a positive charge could aid in the removal of endotoxins in solutions (14) due to the strong negative charge residing on LPS. Bononi *et al*. demonstrated high levels of endotoxin retention using a membrane filter equipped with a positively charged membrane, which electrostatically attracts and/or retains the endotoxins (15). It was believed that endotoxin retention is mediated by electrostatic interaction forces. These membranes are normally composed of polyvinylidene fluoride, PVDF.

PVDF is a polymer discovered to have a high piezo and pyroelectric charge. PVDF is a semi-crystalline material that has a molecule conformation with repeating units of (-CF_2-CH_2-) that contains are large dipole moment. Membrane-based separations are very common in biotech processing because they can be used with a large variety of applications including: clarification, buffer exchange, purification, and sterilization (16). 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 (15). 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 (17).

Paper Points

PPs was patented in 1958 by Joseph N. Masci where he describes PPs to be made from hemp for its long and strong fibers. The fibers are oriented parallel to the long axis of the PPs to prevent breaking (18). Edwards and Bandyopadhyay evaluated 660 PPs to look at the physical and mechanical properties optimal for PPs and in October 1979, the endodontic absorbent PPs was accepted as a project for standardization by the International Standards Organization/Federation Dentaire Internationale (19).

In the latest edition on the “standards for PPs” released by the American National Standard and the American Dental Association published in December 2013, the PP is to be made from a textured absorbent material, odorless and with a lint-free surface with inspected visually without magnification. The materials and binders should be biocompatible. It defined two different types of PPs: the standard absorbent point, which has a standard dimension and a standardized taper of 0.02mm per millimeter of length, and the taper size absorbent point which is a point with dimensions and taper at the discretion of the manufacturer (20).

The standardized point should be uniformly tapered for the first 16mm as measured from the top and may have either a blunted, conical or rounded tip. The taper of the standardized point from a position located 16mm from its tip to its distal terminus shall not exceed the uniform taper of the first 16mm from the tip (20).

The points should be straight with an essentially circular cross section and a smooth surface. They should show no structural or other deficiencies likely to be detrimental to their intended use. *Regarding sterility, the points shall pass the test for sterility as given in US Pharmacopeia or any other prevailing national sterility requirements* (20).

The minimum length of the points should be 25mm and points should absorb testing solution to a height of not less than 10mm when tested. After being tested, the points should not disintegrate while in the water or when removed from the water with forceps (20).

Absorbency of Paper Points

According to the Standard, to test for absorption the PP is suspended vertically in a solution of FD&C Yellow #5 (0.04%) concentration such that the tip is immersed to a depth of 5mm while isolated from the container sides and other test pieces. The height to which the stain marking rises above the liquid after 60s is recorded and the reported value shall be the average result of 10 test pieces rounded to the nearest 0.5mm (20).

Absorbency of PPs has also been studied. Edwards et. al. studied the absorbency of PPs using a pin vise device which suspended the points vertically and 5mm of the tip was lowered into a 2% aqueous solution of mercurochrome. The rise in height of the solution by capillary action was noted after 60 seconds and an average of five readings was taken as the absorbency rate (19). In 2008, da Cunha Pereira et al. evaluated two different methods of measuring the absorbency of PPs. In one method, da Cunha Pereira et al. used digital balance with a precision of ±0.0001 to weigh the PP before and immediately after absorbing the dye. The second method had calibrated examiners measure the linear dye penetration on the PPs using a ruler under a stereomicroscope. The PP was placed into a holding device and lowered until 1mm of the point was in a dye solution of 1% Methylene Blue or 1% Rodamine B, for 10 seconds. It was concluded that using a digital balance to evaluate absorbency of PPs was the more reliable method because the digital balance is a calibrated and precise equipment (21).

PURPOSE

The purpose of this thesis is to examine the application of a positively -charged PVDF membrane for use in removing liquids, such as sodium hypochlorite and endotoxins, from root canals during endodontic therapy. Charged PVDF membrane points can absorb excess liquid from irrigation of the root canal system and the positive charge on the membrane can potentially remove endotoxins within the root canals system.

HYPOTHESES

The null hypotheses are:

1: there is no significant difference in absorbency between PPs and membranes.

2: there is no significant difference in absorbency of endotoxins between PPs and membranes.

3: there is no significant difference in the amount of endotoxin in the PPs or membranes.

The research hypotheses are:

1. Membranes have a higher absorbency than paper points.
2. A positively-charged rolled membrane will absorb more endotoxin than paper points.
3. There is no significant difference in the amount of endotoxin in the PPs or membranes.

RESEARCH DESIGN

Four studies were done to compare the absorbency and the amount of endotoxin removed by PPs or membranes. The first two studies compared the absorbency between unrolled and then rolled PPs with that of membranes. The third study compared the amount of endotoxin removed by PPs as compared to membranes. The fourth study was done to evaluate the endotoxins in commercially available PPs.

Charged PVDF membrane is currently commercially available. It was purchased for use in this study. Charged membrane is used to remove endotoxin in filtration and we are testing the membrane for a novel use to see if it would also remove endotoxins through absorption (FIGURE 1).

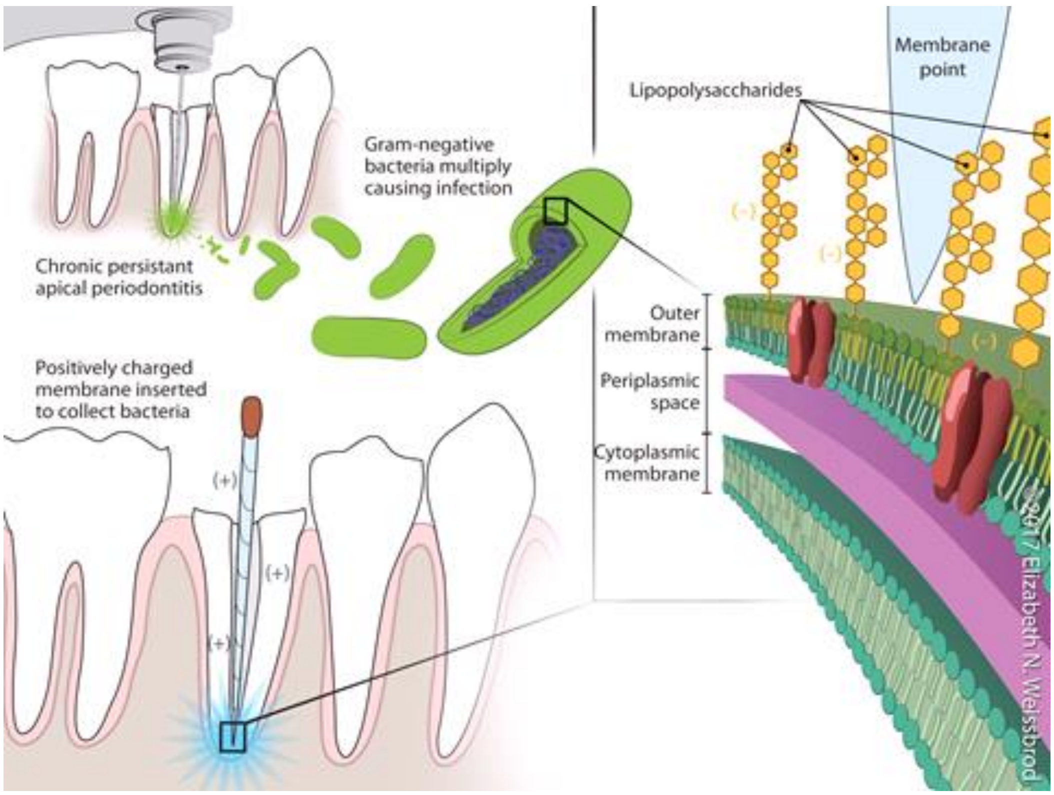

Figure 1. Schematic of gram-negative bacteria cell wall with negatively charged lipopolysaccharide and positively charged rolled membrane cone for endotoxin removal.

PREVIOUS WORK

Scanning electron micrographs were taken of the PPs and PVDF membrane (FIGURE 2). The long fibers of the PPs and the rolled layer design illustrates then nature of the PPs and the ability to fray with loose fibers. The scanning electron micrograph of the flat sheet PVDF membrane has a different makeup with no fibers. The membrane is a flat material with pores within the membrane.

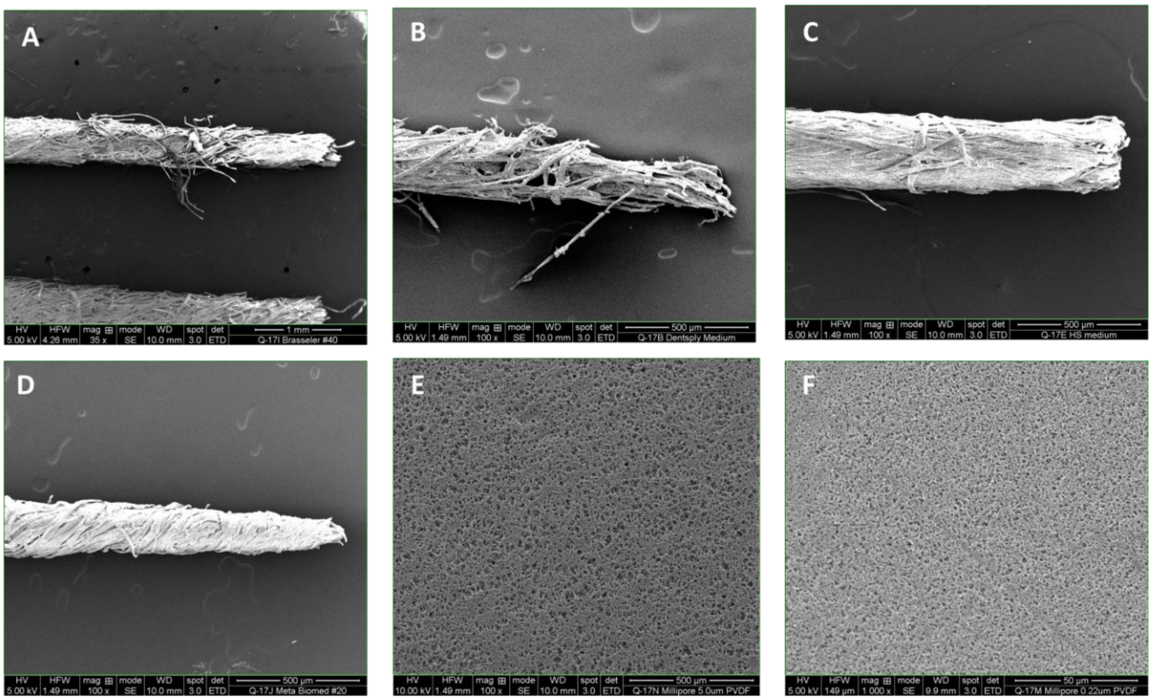

Figure 2. Scanning electron microscopic micrographs of (A) Brasseler #40 paper points at 35x magnification, (B) Dentsply medium paper point at 100x magnification, (C) Henry Schein medium paper point at 100x magnification, (D) Meta Biomed #20 paper point at 100x magnification, (E) 5.0µm PVDF membrane at 100x magnification, (F) 0.22µm PVDF membrane at 1,000x magnification

Introduction for Study #1: Evaluation of absorbency between unrolled PPs and PVDF membrane

To evaluate the absorbency of PPs, a study was carried out comparing commercially available PPs (Henry Schein and Dentsply medium and coarse, Meta Biomed and Brasseler, size #40) with that of flat sheet PVDF membrane (Millipore, pore size 0.22 or 5µm).

Methods and Materials for Study #1: Evaluation of absorbency between unrolled PPs and PVDF membrane

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 ™ (22) 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 PVDF membrane was cut to the same dimension as the Meta Biomed #40 PP. For analysis, five independent samples of each PP and PVDF type were prepared and weighed using a digital balance to a precision of ±0.0001 (21). Subsequently, each sample was placed in deionized water for 5 seconds, removed using tissue forceps and weighed again after absorbing water. The absorbency potential for each sample was calculated by subtracting the final weight from the initial dry weight and expressed as percent weight gain.

Statistical Analysis Study #1: Evaluation of absorbency between unrolled PPs and PVDF membrane

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 for Study #1 Evaluation of absorbency between unrolled PPs and PVDF membrane

Based on this study, absorbency of the 0.22µm and 5.0µm PVDF membrane was significantly greater than any of the PPs, p <.0005 (TABLE 1). The 0.22µm was the most absorbent of all the materials tested (FIGURE 3).

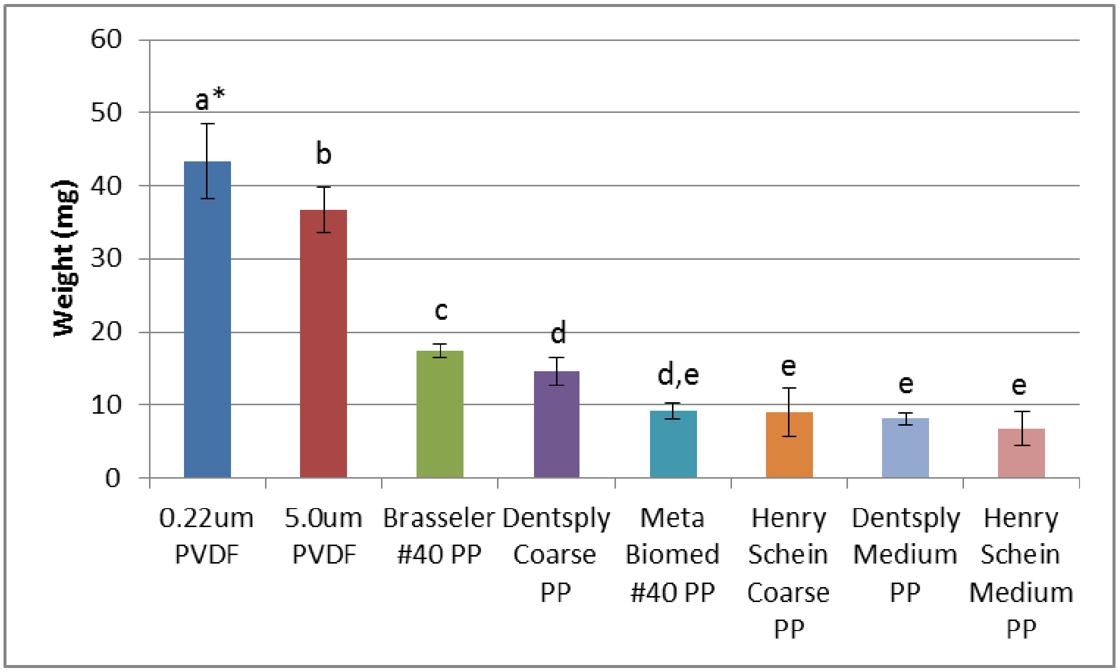

Figure 3. Average weight difference by similar dimension of unrolled paper point and membrane.

*a -e: absorbency materials with different letters are significantly different

Table 1. ANOVA analysis of absorbency weight difference by similar dimension of unrolled paper point and flat sheet membrane

Absorbency Material	N	Mean (SD)	F	p
0.22µm PVDF	5	43.32 (5.1) [a]	137.682	.0005
5.0µm PVDF	5	36.72 (3.1) [b]		
Brasseler #40	5	17.42 (3.3) [c]		
Denstply Coarse	5	14.56 (2.3) [c, d]		
Meta Biomed #40	5	9.17 (1.5) [d, e]		
Henry Schein Coarse	5	8.91 (1.9) [e]		
Dentsply Medium	5	8.1 (0.8) [e]		
Henry Schein Medium	5	6.6 (0.9) [e]		

a-e: absorbency materials with different letters are significantly different

Introduction for Study #2: Evaluation of absorbency between rolled PPs and PVDF membrane

A follow up study was done where rolled membranes were tested for absorbency. We only evaluated the 0.22µm pore size PVDF because it had higher absorbency than the 5.0µm pore size PVDF. PPs from Henry Schein size coarse and medium, Dentsply size coarse and medium, and Meta Biomed size #40 were unrolled and the dimensions were measured.

Methods and Materials for Study #2: Evaluation of absorbency between rolled PPs and PVDF membrane

The PVDF membrane was cut using a laser cutter (Epilog Laser cutter) to the exact dimensions of each of the PP counterparts, Henry Schein size coarse and medium, Dentsply size coarse and medium, and Meta Biomed size #40. The membrane was hand rolled and held in place by placing cyanoacrylate at the free end of the rolled membrane.

For analysis, five independent samples of each PP and PVDF type were prepared and weighed using a digital balance to a precision of ±0.0001 (21). Subsequently, each sample was placed in deionized water for 5 seconds, removed using tissue forceps weighed again after absorbing water. 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.

Statistical Analysis for Study #2: Evaluation of absorbency between rolled PPs and PVDF membrane

Using Power and Precision ™, the 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 2.76, power was equal to 100%. A t test was used to compare each PVDF with its counterpart PP and a p value $\leq .05$ was set as the threshold for significance.

Results for Study #2: Evaluation of absorbency between rolled PPs and PVDF membrane

Based on this study, absorbency of the 0.22µm PVDF membrane was significantly greater than any of the PP counterparts (FIGURE 4). The 0.22µm was the most absorbent of all the materials tested (TABLE 2).

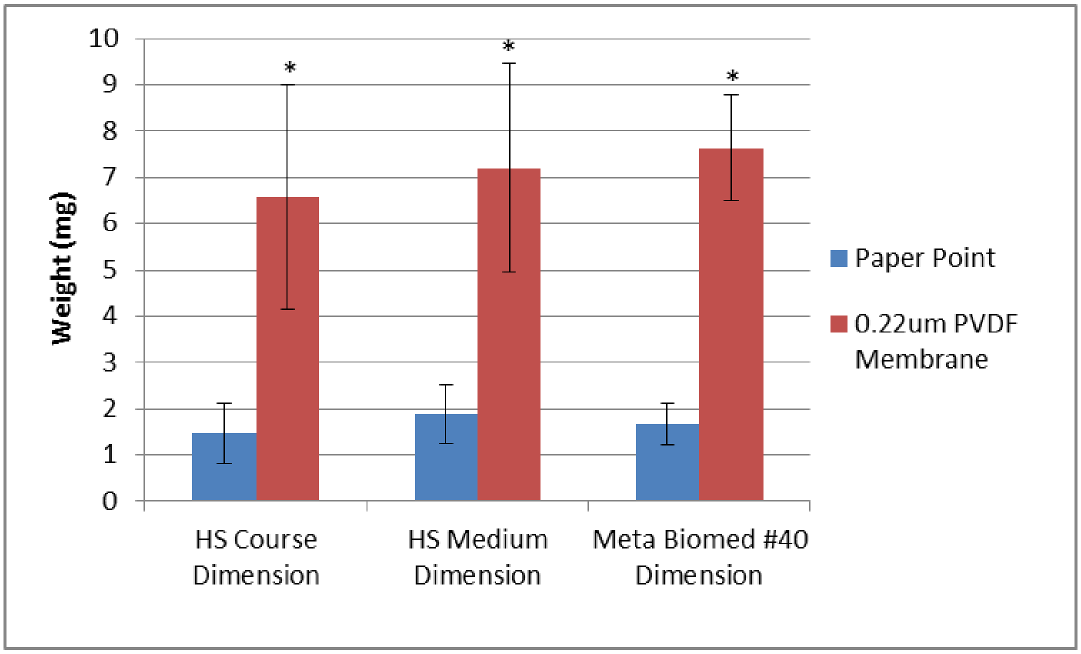

Figure 4. Average weight difference by same dimension of rolled paper point and membrane.

* Bars with the asterisk have significantly greater weight (mg) than their counterparts.

Table 2. t test analysis of absorbency weight difference by similar dimension of rolled paper point and rolled 0.22µm PVDF membrane.

Comparative Groups	n	Mean ± SD	t	p
Henry Schein Coarse	5	1.47 ± 0.64	4.562	.001
0.22µm PVDF rolled Henry Schein Coarse dimension	5	6.57 ± 2.4		
Henry Schein Medium	5	1.88 ± 0.64	5.064	.003
0.22µm PVDF rolled Henry Schein Medium dimension	5	7.20 ± 2.2		
Meta Biomed #40	5	1.66 ± 0.44	10.947	.0003
0.22µm PVDF rolled in Meta Biomed #40 dimension	5	7.64 ± 1.14		

Introduction for Study #3: Evaluation of the PVDF membrane for endotoxin binding using Limulus Amebocyte Lysate (LAL) test.

PVDF is a polymer discovered to have a high piezo and pyroelectric charge. PVDF is a semi-crystalline material that has a molecule conformation with repeating units of ($-CF_2-CH_2-$) that contains are large dipole moment. Endotoxins easily pass through the 0.2μm 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 (15).

To measure how much endotoxin is removed from samples a chromogenic assay kit was used. The Limulus Amebocyte Lysate (LAL) assay quantitatively determine the amount of LPS removed by the PVDF membrane. The key methodological principle of chromogenic assays is to reveal the presence of the analyte in a test sample via chemically-induced visible color changes. The resulting color is then measured using spectrophotometric methods to reveal the concentration of the analyte in the sample.

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.

We evaluated both the amount of endotoxin remaining in the wells after absorption with either PPs or membrane, and the amount of endotoxin released from the used PPs or membrane after mechanical agitation with vortex.

Methods and Materials Study #3: Evaluation of the PVDF membrane for endotoxin binding using Limulus Amebocyte Lysate (LAL) test.

The exact dimensions of the PPs were measured and the PVDF membrane was cut according to the same dimension as the Meta Biomed #40. The laser cutter (Epilog Laser Fusion M2) settings were: speed 75, power 5, and frequency 80. The cut PVDF membrane was hand rolled to a tight cone and Loctite Super Glue gel was used to hold the rolled membrane in a conical shape.

The LAL assay kit was used as directed by manufacturer. Endotoxins in concentrations of 50 EU/ml, 5 EU/ml, 0.5 EU/ml, 0.05 EU/ml, and 0.005 EU/ml and 0 EU/ml was created for the standard curve and 100ul of each concentration was placed into a well of a 96 well apyrogenic plate.

For the test samples, concentration of 5 EU/ml was placed into wells of a 96 well plate. For each test sample, a PP or PVDF membrane was weighed, then placed into the well for 30 seconds, removed and weighed again to measure how much liquid was absorbed. The amount of liquid removed was replaced by LAL Reagent water so that every well had 100ul of liquid.

In addition, after each PP or PVDF membrane was dipped into the well containing the endotoxin, the PP or PVDF membrane was suspended in 1 mL of LAL water and agitated in vortex for 60 seconds. *A sample of the supernatant, 100ul, was used in the LAL assay. This allowed us to see if the endotoxin absorbed into the PP or PVDF membrane could be released through mechanical agitation.*

The WinKQCL plate reader and software was used to measure the amount of endotoxin in each well. The plate was incubated at 37ºC ±1ºC for 10 minutes in a

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. During the kinetic test, the software continuously monitor absorbance at 405 nm in each microplate well and automatically calculates the log/log linear correlation between reaction time of each standard solution with corresponding *E.coli* endotoxin concentration standard. The difference in the positive control samples and the absorbed sample was used to determine endotoxin removal.

Statistical Analysis for Study #3: Comparison of Meta Biomed #40 PP and 0.22µm PVDF membrane for endotoxin binding using Limulus Amebocyte Lysate (LAL) test.

Data analysis was performed, using SPSS with a t test, with the significance level of 5%. Using Power and Precision ™ when no endotoxins were removed with PPs and 5 Endotoxin units removed with membrane, the power computation was performed. With an n of 5 in each group, a one-tail test, a $p \leq .05$, and a difference of at least 20% between the groups and an effect size of 2.50, power was equal to 100%. Therefore, an n of 5 was used in the two groups.

Results for Study #3, Part A: Evaluation of the PVDF membrane for endotoxin binding using Limulus Amebocyte Lysate (LAL) test – Endotoxins remaining in the wells

Based on this study, the amount of endotoxin that bound to the PVDF membrane after absorption was significantly greater than that of PPs. The amount of endotoxin remaining in the well was significantly less for membranes than PPs (t = 6.758 for a one-tailed test, p≤.0005 (FIGURE 5, TABLE 3).

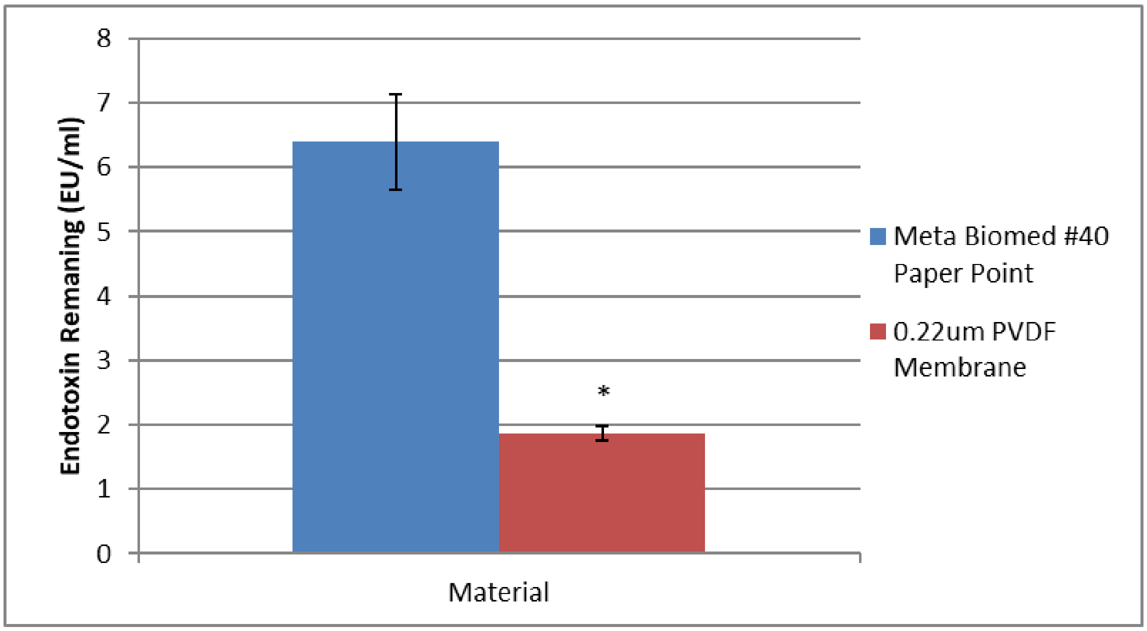

Figure 5. Average amount of endotoxins remaining in the well after absorption.

* Bar with the asterisk, the PVDF membrane, shows significantly less endotoxin remaining in the well than the Meta Biomed #40.

Table 3. t test analysis of remaining endotoxin in the wells after absorption.

Comparative Groups	n	Mean ± SD	t	p
Meta Biomed #40	5	6.40 ± 0.74	6.758	.0005
0.22µm PVDF rolled in Meta Biomed #40 dimension	5	1.87 ± 0.11		

Results for Study #3, Part B: Evaluation of the PVDF membrane for endotoxin binding using Limulus Amebocyte Lysate (LAL) test – endotoxins absorbed into the PP or membrane

Based on this study, the amount of endotoxin released (after vortexing) from the PPs was significantly more than from the membrane, t = 2.35, $p \leq .0005$ (FIGURE 6, TABLE 4).

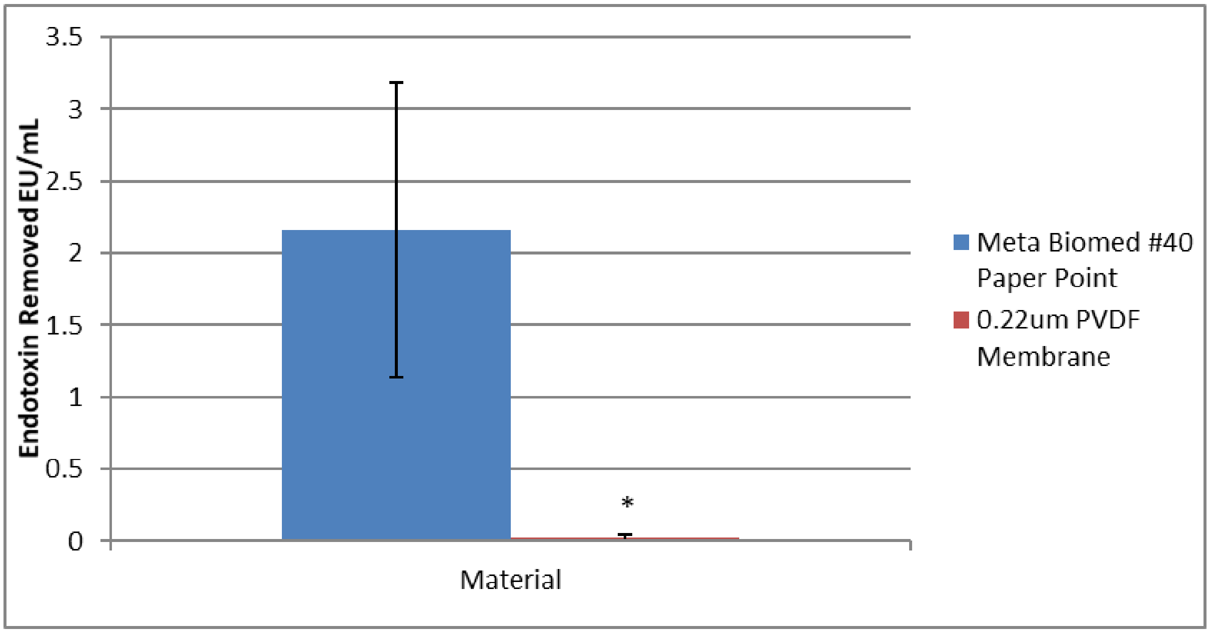

Figure 6. Average amount of endotoxins released from the used PP or the used PVDF membrane after vortexing.

* Bar with the asterisk, the PVDF membrane, shows significantly less endotoxin released than the Meta Biomed #40.

Table 4. t test analysis of endotoxins released from the used PP and PVDF membrane after vortexing.

Comparative Groups	n	Mean ± SD	t	p
Meta Biomed #40 PP	5	2.16 ± 1.02	2.35	.0005
0.22µm PVDF rolled in Meta Biomed #40 dimension	5	0.02 ± 0.11		

Introduction for Study #4: Evaluation for presence of endotoxin in four different brands of new unused PPs and one PVDF membrane

Commercially available PPs are packaged as sterile items. The amount of endotoxin in the four brands of PPs had not been evaluated. These four different brands were: Meta Biomed #40, Brasseler #40, Henry Schein coarse, and Dentsply coarse. The PVDF membrane used for this study was non-sterile. The amount of endotoxin in the PVDF membrane had also not been evaluated.

Methods and Materials for Study #4: Evaluation for presence of endotoxin in four different brands of new, unused PPs and one PVDF membrane

To evaluate the amount of endotoxin in PPs, a study was carried out comparing commercially available PPs (Henry Schein coarse, Dentsply coarse, Meta Biomed and Brasseler, size #40) with that of rolled 0.22μm PVDF membrane (Millipore). A new PP or rolled PVDF membrane was suspended in 1 mL of LAL water and agitated in vortex for 60 seconds and 100ul of the supernatant was used in the LAL assay, as described in Study #3. The LAL water was considered as the blank for all tests. The reason for vortexing the PPs or the membrane was to release the endotoxins.

During collection of the data for the Meta Biomed #40 PPs, the amount of endotoxin recovered from the Meta Biomed #40 PPs varied greatly among the five PPs tested resulting in a mean± standard deviation of 7.22±4.67. This caused a statistical anomaly when testing for significant differences. The large standard deviation might accurately reflect the variability in the manufacturing process. Due to the variability, no significant difference can be drawn (data is not shown).

Statistical Analysis for #4: Comparison of the amount of endotoxin in three different brands of new, unused PPs and one PVDF membrane

Since no previous studies had been completed on evaluating the presence of endotoxins in new, unused PPs and PVDF membranes, a post hoc analysis was done using the data collected in the study. Using Power and Precision ™ the following details the results of the post hoc power analysis. With an n of 5 in each of the four groups, a one-tail test, a $p \leq .05$, a difference of at least 20% between the groups, and an effect size of 20.98, power was equal to 100%. Therefore, an n of 5 was correct for use in this fourth experiment.

Data analysis was performed using SPSS with the significance level equal to 5%. Assumptions of the equality of variance and the normal distribution of errors was achieved. A one-way ANOVA and a Games-Howell significant difference test was used for intergroup analysis.

Results for Study #4: Evaluation for presence of endotoxin in new, unused PPs and PVDF membrane

The new, unused 0.22 PVDF membrane had significantly less endotoxins than any of the new, unused PPs. Denstply coarse had significantly more endotoxin than PVDF membrane, but significantly less than Brasseler#40 and Henry Schein coarse. Brasseler#40 has significantly more endotoxin than PVDF membrane and Dentsply coarse, but significantly less endotoxin than Henry Schein coarse. Henry Schein had the most amount of endotoxin compared to the other brands of PPs tested and the PVDF membrane. Because the amount of endotoxin was small and would not show up, the data was plotted under a log scale as seen in Figure 7.

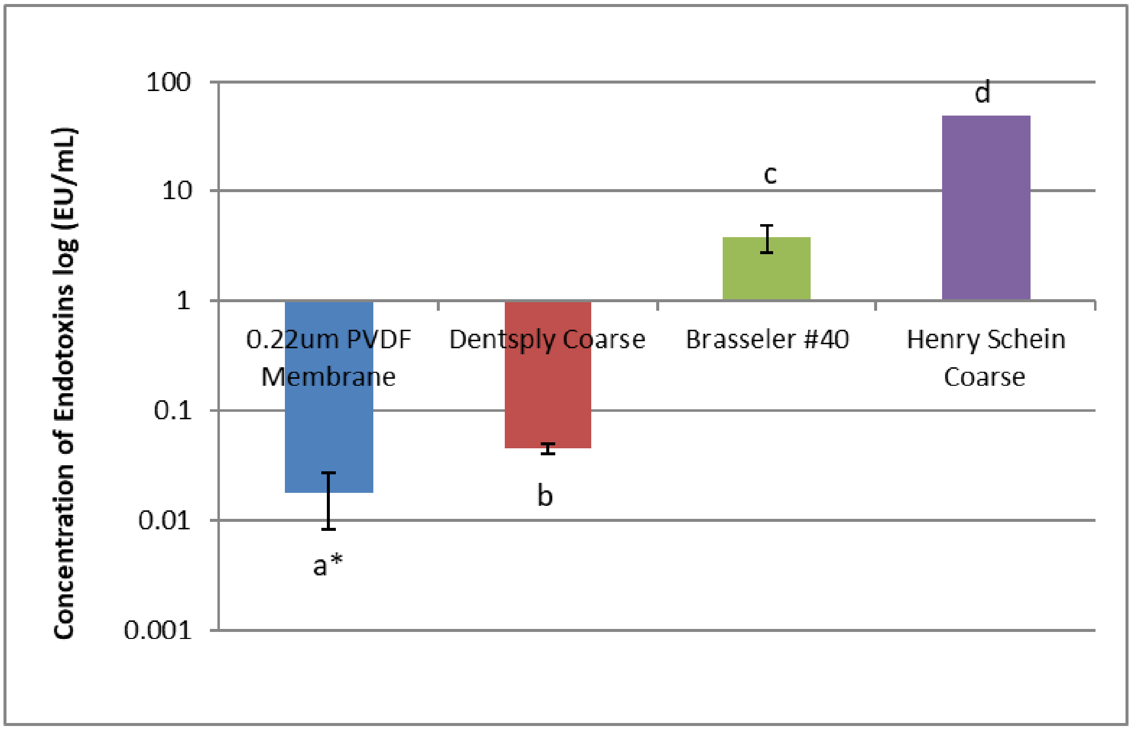

Figure 7. Average amount of endotoxins released from new unused PPs or PVDF membrane after vortexing, presented in $\log_{10}$.

* Bars with the asterisk are significantly less than their counterparts.

Table 5. ANOVA analysis of average amount of endotoxins released from new unused PPs or PVDF membrane after vortexing.

Absorbency Material	*N*	*Mean (SD)*	*F*	*p*
Henry Schein Coarse	*5*	*49.62 (.85)* ^{a}	*470.896*	*.0005*
Brasseler #40	*5*	*3.82 (1.07)* ^{b}		
Dentsply Coarse	*5*	*0.04 (.004)* ^{c}		
0.22µm *PVDF*	*5*	*0.017 (0.009)* ^{d}		

a-d: absorbency materials with different letters are significantly different

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 (4, 7). In a review by Nair, exogenous materials such as PP fibers, were associated with foreign body reactions in non-healing periapical lesions (2). Others reported on consequences of cellulose fibers found in periapical biopsies of patients with a history of endodontic treatment (4, 5). 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 Gram-negative bacteria contain a lipopolysaccharide (LPS) that is capable of initiating a proinflammatory biological response (11).

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.

To make a direct comparison with the PPs, study #2 was done where rolled membranes were also tested for absorbency. Since 0.22μm PVDF was the most absorbent form of the membrane tested, we used only the 0.22μm pore size PVDF membrane for this direct comparison study. PPs from Henry Schein size coarse and medium, Dentsply size coarse and medium, and Meta Biomed size #40 were unrolled and measured. The exact dimension of each of the PP was measured and the PVDF membranes were cut using a laser cutter (Epilog Laser cutter) to this exact same dimension and subsequently hand rolled to a conical shape. The free edge of the membrane was held in place with cyanoacrylate glue.

The results of the absorbency study, study #2, showed that in this direct comparison, every form of the 0.22μm PVDF membrane was significantly more absorbent than their PP counterpart. 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 our third study, we evaluated the amount of endotoxin binding to the PPs and PVDF membrane. We used the LAL chromogenic kit that revealed the amount of endotoxin remaining in the well after we dipped the PPs or the PVDF membranes. We saw that, not only that the 0.22μm PVDF membrane absorbed more water, as seen in study #2, it also removed more endotoxins from the wells. When we vortexed the used dipped PPs and PVDF membranes, we found that the mechanical agitation caused the release of the endotoxin. There were significantly more endotoxins released from the PPs than the membranes. A possible explanation for this is that the positive charge on the

PVDF membrane tightly binds to the negatively charged endotoxins so that mechanical agitation does not break that bond.

One of the interesting findings that we noted from our third study was that the amount of endotoxin remaining in the wells after dipping the PPs was at a higher concentration of endotoxin than the concentration at the beginning. In the last study, we did an assay where we vortexed brand-new PP and PVDF membranes and we found that there was endotoxin in the PPs. Although only some of the packages of the PPs claim to be sterile, none of the companies state that PPs are apyrogenic.

An ideal design of the membrane would be a solid cone of material that would be stiff enough to be navigated down the root canal system yet also flexible enough to negotiate any curvatures in the canals. 3D printing of PVDF was explored by a team of collaborators at The University of Texas at El Paso Department of Mechanical Engineering using a Fused Deposition Modeling 3D printing process. A 3D model of a 40/.02 membrane cone was designed using Tinkercad and PVDF pellets were made into a reel and the resin was printed to design. One of the limitations with 3D printing is the resolution of the 3D printer. The 3D printer was not able to consistently print as small as the PP and the printer was not able to print the whole cone. Another limitation of the 3D printer was that during the conversion of the PVDF pellet resins into a spool, the properties of the PVDF changed into a form that was not useful for dental purposes. The cones printed were hard and not flexible and more importantly, were no longer absorbent so the solid printed PVDF cones would no longer serve our needs.

LIMITATIONS

One of the variables in this study was the consistency of the rolled membrane. Since these are hand rolled and held in place by cyanoacrylate glue, there was variability in how tightly wound each of the test samples were. Despite the variability in how tightly wound each of the rolled membranes were, this should not have had an influence on our results since the rolled membranes were placed into the testing wells that were large enough to accommodate the multiple sizes.

Another variability in the study was the amount of cyanoacrylate glue used to hold the membrane in place. Using a glue that had a runny consistency was not ideal since it was hard to control the amount of glue used for each membrane. In addition, if the glue dripped down the surface of the membrane, it would change the amount of liquid it absorbed by creating a blockage on the surface of the membrane. Also, we noticed that using too much glue would cause the membrane to become brittle and break apart. To address this, we used a cyanoacrylate glue that came in a gel format. This allowed more control in the application and placement of the glue, but we still had to be careful to prevent any excess glue from touching any other surface of the rolled membrane.

Another limitation of the rolled membrane was that, due to the thicker characteristic of the membrane, rolling it created a larger cone size. A plastic tooth #8 was prepared to size 80/.04 using a rotary file and the rolled membranes was still too large to go down to the apex of the tooth.

FUTURE DIRECTIONS

1. Future studies of this material should include designing the PVDF membrane so it could be casted in a thinner dimension. Then, when the membranes are rolled, they will be smaller and able to be used root canals. 2. Optimization of the membrane ought to be done with a dedicated team of scientists and engineers. A researcher might see if the amount of charge on the membrane can be changed for a more effective means of binding with endotoxins. This might have a clinical impact in root canal therapy because it might have a higher binding potential with endotoxins. 3. In addition, the ideal design of the membrane would be a solid conical format with a tapering form. This would allow for easy adoption and ease of use within the root canal system. A solid cone would possibly allow for higher absorbency which would allow for a more efficient clinical procedure and workflow.

CONCLUSION

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.

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