Innovations in Sanitization for 3D-Printed Parts in Medical and Critical Applications

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Abstract: *Currently, there are no standards or other techniques in use at medical facilities to clean and sanitize 3d-printed parts, due to lack of studies and testing. This present study aims to help create a standard and ultimately awareness for properly cleaning and sanitizing prints. Sanitation effectiveness of 3D-printed parts for food and medical applications has been established in a 12-month lab study and controlled tests. The present study examined the continued use of sanitation techniques across 3 more months of testing and experimentation in household kitchens. Multiple specimens of the most common thermoplastics for Fused Filament Fabrication (FFF), were printed with a range of settings to test for pathogen contamination, biofilm production, bacteria, and other pathogens masking (hiding) in the layer lines, gaps, and other imperfections of said prints. This study investigates methods of sanitation and cleaning to reduce or eliminate pathogens along with their biofilms from the defects and interstitial spaces that naturally occur in FFF printing. Results from various testing methods used in hospitals and FDA approved microbial surface testing, indicate that 3D printed parts of PLA/PLA+ (Polylactic Acid), and PETG (Polyethylene terephthalate glycol) can be cleaned to safe levels using warm water (120 °F), and non-concentrated dish soap. The examination and verification of cleanliness were completed via Petri dish preparations, and protein residue testing. It was found that Colony Forming Units (CFU) and Plaque Forming Units (PFU) had been reduced by 90%. Experimental results indicate that using a pinch (2g) of baking soda, when used with soapy water, eliminates biofilms by chemical and physical action, neutralizes acidic bacteria, and removes mucus. It is suggested and tested by surgical technicians that a 2-minute room temperature bleach water soak (200ppm), after washing and rinsing could be done to ensure pathogens are at safe levels (recommended but not required). Acetic acid from vinegar was tested as well via petri dish for CFU reduction and can effectively eradicate biofilms due to the ability to penetrate the biofilm matrix and the cell membrane. Acetic acid is* ***not recommended*** *for disinfecting, only for biofilm reduction. It is noted to the reader that sanitation in this context refers to the method of bringing a surface or object to safe levels of cleanliness for food or medical preparation and storage. Furthermore, mass spectrometry readings indicate that no contamination from heavy metals, or other toxins are present in PLA+, and PETG before and after printing. Lastly, filaments made from a pull-trusion method from recycled soda or water bottles have been tested and found to be safe. When using 3D-printed items for liquids, it is highly recommended to coat the 3D-printed parts in resin. This is a simple, fast, safe, and very effective way to smooth parts to ensure easy cleaning. These findings contribute to the foundation of a standardized sanitation protocol for 3D-printed medical devices and food-contact items, addressing a critical gap in current regulatory frameworks.*

I. INTRODUCTION

This study builds upon the original, novel research by Matt Thomas et al [1]., extending its findings to further explore mass spectrometry for nozzle contamination inspection. In addition to incorporating aspects, text and images of the original work to provide context, this study introduces new details and expanded research on alternative methods for biofilm and pathogen removal. The testing metrics and experimental investigation were updated to adhere to the FDA microbial surface testing metrics. Testing and culture growth on petri dishes was redone following the standards we created. It has been found that Autoclaving 3D printed parts is not a viable, or feasible option for PLA, PLA+ or PETG due to the heat and pressure that autoclaves use. The parts will be destroyed.

With the rise of Covid and mass shutdowns during early 2020, effecting the production of Personal Protection Equipment (PPE), frontline workers found themselves in a big mess and used whatever they could get their hands on. Concerned makers decided to take matters into their own hands and 3D printed respirators, connectors, valves for ventilators, and even test swabs. This brought many questions to the table such as Can 3D printed parts be cleaned, sanitized and reused for food or medical applications? Common sense and instinct say yes, however, it must be documented, tested and approved for hospitals, where lives are at stake, to use 3D printed items.

Due to its increasingly widespread use in the medical field, there are growing concerns regarding risks associated with contamination. Prior to the pandemic, federal agencies have implemented regulations and guidelines for compliance of 3Dprinted medical devices [2]. However, regulations take time to be drawn up and implemented, and typically lag innovation and use. This may be the reason why, during the COVID-19 pandemic, the United States Food and Drug Administration (USFDA) provided emergency use authorizations, waiving the strict requirements for good manufacturing practices. This may have been done to address the urgent need for this equipment and supplies. Hence, cleaning and decontamination of 3D-printed parts is vital in ensuring its safe use.

One area of interest for 3D-printing, that has exponentially gained popularity, is the personal manufacturing of hands and limbs for children or those who have lost limbs due to poor living conditions, birth defects and other various reasons, and cannot afford or have insurance to cover the costs of prosthetics. These 3D-printed hands and limbs are not a one-time use device and creating a set of standards for cleaning and sanitizing will be very beneficial in keeping those recipients healthy and safe.

The most common bacteria found in kitchens and households that cause food bourn illness are E. Coli and Salmonella according to the Cleveland Clinic, a nonprofit multi-specialty medical education center established in 1921. Food poisoning happens when there has been a lack of cleanliness or cross contamination between items such as raw chicken and cooked foods.

This study builds upon my previous research on the sanitization of 3D-printed parts by refining existing methods and expanding contamination inspection techniques. While my earlier work established foundational techniques, this research introduces an extended analysis using mass spectrometry to assess contamination at a more detailed level. Additionally, new approaches for biofilm and pathogen removal are explored, providing a comparative assessment alongside established methods. These refinements contribute to a more comprehensive understanding of 3D-printed part sanitization, with potential applications in clinical and food industry settings.

The sanitization methods evaluated in this study have direct applications in both clinical and food industry settings, where proper decontamination of reusable equipment is essential. In clinical environments, 3D-printed medical tools, such as face shields, respirators and prosthetics must meet strict hygiene standards to prevent bacterial contamination and biofilm formation. The tested cleaning methods, particularly the combination of warm soapy water with baking soda followed by a bleach soak or IPA treatment, offer a cost-effective and scalable approach to ensuring sterility without **damaging** printed parts. Similarly, in the food industry, where 3D-printed molds, utensils, and processing components may come into contact with consumables, effective sanitization is critical for compliance with food safety regulations. The recommended cleaning protocol established from this study, aligns with established sanitation guidelines from governing bodies [11][12], providing a practical solution for maintaining hygiene in both sectors. Future studies should explore the long-term durability of 3D-printed parts subjected to repeated cleaning cycles to further validate their suitability for these applications.

II. PRELIMINARY INFORMATION

In this current study, the scope however, is to provide an understanding and a methodology of how 3D-printed objects from a variety of common thermoplastics, can be feasibly cleaned to levels that are safe for food-related and medical applications. In this regard, testing methods, findings and all procedures are documented in this journal.

A prevalent concept among users, groups, and healthcare professional's centers on the concern that the voids in the layer lines of 3D-printed objects, often referred to as dimples, represent a significant challenge in ensuring the food safety and cleanability of 3D-printed items. The primary argument poses that these minute crevices could potentially facilitate the growth and proliferation of bacteria, making thorough cleaning difficult due to their small dimensions. It's essential to consider that the typical bacterium responsible for foodborne illnesses measures between 2 to 5 microns in length and 0.5 to 1.5 microns in diameter. It is necessary to have adequate space for growth. To put it into perspective, think of it as the analogy of a goldfish attempting to thrive in a small fishbowl. Viruses, such as a respiratory virus (COVID-19) rely on the same principle for infection. If there is not enough space in the respiratory droplets exhaled by humans, the virus will not have enough viral content to allow replication and infection. These viruses are 0.125 microns in size for comparison.

Upon conducting an examination of 3D-printed items using a VEGA3 Tescan SEM imaging device, it was determined that the

smallest dimples measure 0.5 microns in size. These dimples constitute only 1-2% of all imperfections. Consequently, the bacteria responsible for causing food poisoning would encounter significant challenges in fitting into these minuscule 0.5-micron spaces. The accompanying figure (Figure.1) illustrates and provides a scanned image of the surface of a 3D-printed respirator mask for reference. The mask was printed at 0.16mm layer height, 3 walls thick (1.2mm total thickness)

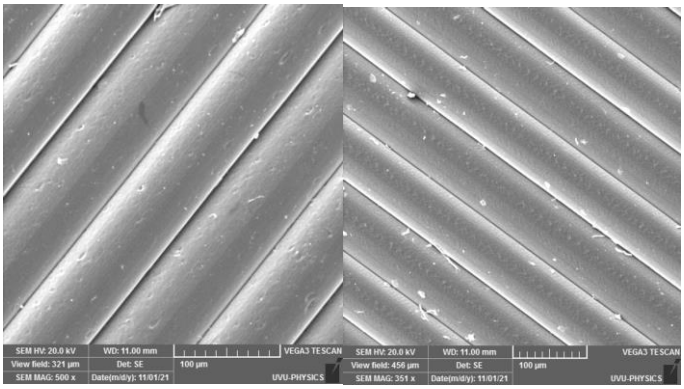

Fig 1: VEGA3 SEM Imaging Machine and Scanned Surface.

The remaining 98-99% of imperfections exceed the 0.5-micron threshold, boasting an average size of approximately 1 micron. While this dimension might appear modest to users, it proves relatively substantial to bacteria and pathogens. Much of the 3D printing community has expressed considerable concern, believing it to be a significant issue; however, we will soon discover that it doesn't pose as many challenges as initially anticipated. Larger crevices, while potentially accommodating bacterial growth, offer easier accessibility for cleaning.

Another safety concern in the realm of food applications has revolved around lead contamination. The Environmental Protection Agency [3] limit for lead in brass fittings and pipe is 0.25%. For non-wetted surfaces that will not corrode as easy as wetted surfaces, lead content in brass is 1.5%. This includes manufactures around the world (China) that the USA buys from.

Approximately 21% (0.63 grams) of a standard 0.4mm nozzle's 3-gram weight is removed to allow the filament to flow through.

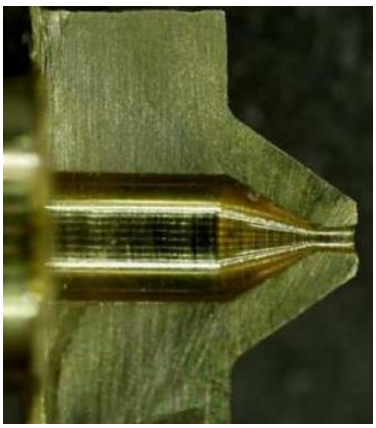

Fig 2: Cross Section of a Standard Brass Nozzle.

Of the remaining 2.37g of brass for the nozzle, only 1.5% is lead, resulting in a remaining quantity of 0.035 grams of lead within the nozzle. Furthermore, the filament encounters very little contact with this 21% of lead due to the very small surface area of the flow chamber of the nozzle, implying the possibility of direct contact with a mere 0.007 grams of lead. But how much of that 0.007g of lead is passed onto the filament over thousands of hours of printing? Lead in brass is bonded to the metal for easier machinability. Lead will not leach out of brass unless there is significant wear (friction) or corrosion due to water.

Lead tends to leach out in exceedingly minute quantities, typically stemming from handling or extended contact with water (corrosion) within a brass fixture. The potential for lead to be leached into or frictionally (wear and tear) transferred onto molten, free flowing filament is thus considered negligible. Comparing the masses of new nozzles with those of used nozzles (with 1000 hours of use) reveals either no mass loss due to printing or such a minuscule amount lost due to friction that it falls below detection limits (immeasurable). In essence, this indicates that an exceedingly small quantity of lead could have been frictionally transferred onto thousands of meters of filament over the course of 1000 hours of use. It's worth noting that studies have demonstrated that consumers employing standard brass keys may be exposed to lead levels 19 times higher than what is considered safe [4].

To push the study further and gain more accurate results, the VEGA3 Mass Spectrometer was used to view all the molecules and elements that make up thermoplastics. PLA/PLA+ as well as PETG were tested. No contamination from lead or other heavy metals and toxins were present. Figure 3 shows the output from scanning PLA+. Its 99% Calcium Carbonate with traces of Silica. The image is very small due to output resolution of the software used for mass spectrometry.

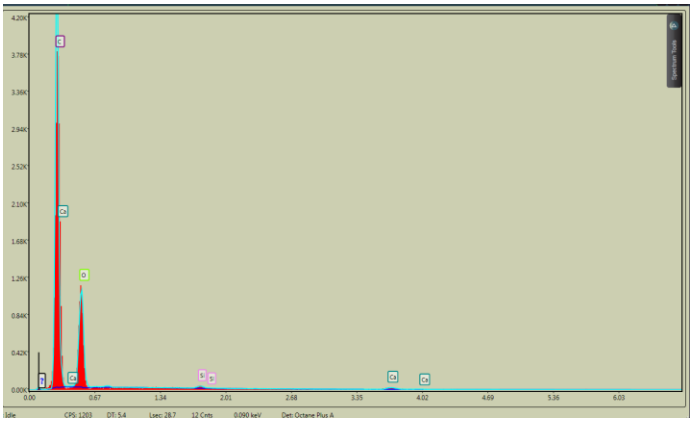

Fig 3: Mass Spectrometry Reading of E-sun PLA+. No lead or Metals

The last concern to address is the manufacturing process of the filament itself. The color additives may leach out of the filament when placed in a liquid. These colors that are added during the melting stage may not be food safe unless otherwise noted. During the manufacturing process, pigments are added to the melted plastic. Companies, to keep their color a trade secret, will avoid using any type of pigment that requires a listing on the MSDS therefore, pigments used in filaments are usually non-toxic and

inert to most treatments and foods. Usually listed under the *classification* category of the MSDS, it will say something similar to " Not dangerous according to Directive 67/548/EEC" as well as "No harmful ingredients". However, to avoid any possible risk, coating in resin adds an extra step of safety if the print will be in contact with aqueous substances. In most cases, the color of plastic does not bring any danger or changes the properties more significant than changing its melting point. As the chosen pigments are inert to most treatments, they do not need to be listed on the MSDS, and thus omitted, allowing the companies to keep them a trade secret that helps them compete against other companies for only they have this one specific color. To mitigate these concerns, coating a 3D-printed item in resin is safe according to title 21 volume 3 of the FDA [5]

In addition to pigments, filaments such as PETG, PLA, and PLA+ include various additives and binders that influence their properties. PETG often incorporates plasticizers to enhance flexibility and reduce brittleness, as well as UV stabilizers to improve durability in outdoor applications. PLA filaments generally include nucleating agents to control crystallinity and improve heat resistance, along with lubricants to ensure smooth extrusion during printing. PLA+, an enhanced version of PLA, often features impact modifiers to improve strength and durability, as well as adhesion promoters to enhance layer bonding. These additives are Generally Regarded as Safe (GRAS) by regulatory agencies such as the FDA, and are designed to maintain non-toxic characteristics.

Three types of testing were done: reduction percentage count of CFU, Adenosine Triphosphate (ATP) monitoring, and protein residue testing. The latter is the most accurate of the testing methods. Visual inspection is not nearly good enough for hospitals or food establishments such as CFU reduction counting due to human error. So, we must use other testing metrics to determine how clean surfaces are. Using an ATP monitor (very expensive) you can test how many light units reflect to the monitor. A statement from Bio Med Central reports [6] *"Measurement of ATP is nowadays frequently used to measure cleanliness of surfaces in hospitals. By measuring ATP, the presence of all kinds of organic material is measured; e.g. microbial contamination and organic contamination (skin flakes, bodily fluids, food scraps, etc.). As the amount of ATP is quantified, ATP measurements give insight into the level of environmental contamination within the healthcare setting. The ATP results are available within seconds which enable immediate feedback."*

ATP is present in every living cell. The cells use it as an energy source, and they leave it behind as they move about. With dead or non-living cells and contamination from microbial organisms, ATP monitoring will not be sufficient. Some disinfectants can interfere with ATP monitoring as well. Syscehm, a UK company states the following [7]: *"ATP Hygiene monitoring does not represent contamination itself. Therefore, microbial, or organic contamination will always be an indirect measurement when measured by ATP. On the other hand, however, Protein Residue tests directly measure organic/microbial contamination."*

III EXPIERMENTAL INVESTIGATION

To ensure proper testing, a set of print metrics and a list of equipment were constructed. Following these guidelines and procedures ensure that a proper conclusion can be made. According to the FDA guidelines for microbial surface testing, each lab must come up with their own set of metrics and methodologies to demonstrate that a surface or object is safe to use for food or medical applications.

A. Printer Settings and Metrics

Three different printers were used for the experiments;

1. Tevo Tornado

2. Anet A8

3. Custom built Prusa

The printer settings used were;

• 0.16-0.24mm layer height in increments of 0.02mm and 60mm/s speed

• Nozzle temperature 205-220°C in 5°C increments

• Bed temperature: 60°

Using these printers and settings will provide a wide range of surface qualities. The Anet A8 is a printer known for having issues with surface quality. However, the hypothesis is that parts with a higher quality surface will be the most difficult to clean, and that those with larger gaps will be the easiest.

B. Materials and Equipment

•Bunsen burner was used to clean tools and keep a positive convection updraft, to keep contamination from falling into the petri dishes or cultured broths.

• Petri dishes 100mm x 15mm (quantity 50 in total)

• 3 different plastic filaments were used in the study: Polylactic acid (PLA/ PLA+) and polyethylene terephthalate glycol (PETG).

• Cultured broth containing pathogens

• Autoclave • Inoculation loop

• Scale and or balance

• Culture tubes

• Incubator set to 37C (98.6F)

• Erlenmeyer flasks

• Single channel precision pipettor

• Yeast extract, peptone, beef extract, salt, glucose, and agar are all used to make nutrient rich broth and gel for petri dishes

• PH stabilizer

• Tryptic Soy Agar (TSA)

• Tevo Tornado 2019 Gold Edition 3D printer,1 custom-built Prusa and one Anet a8

• VEGA3 TESCAN Scanning Electron Microscope and Mass Spectrometer

• Gold and Carbon powder for SEM and Mass Spectrometer preparation

• Pathogens for testing

• Dawn dish soap

• 200 ppm molarity bleach water at room temperature for sanitation

• 70% IPA (Isopropyl Alcohol) Some people referrer to this as "rubbing alcohol" they are similar. Rubbing alcohol is IPA diluted with water.

• PRO-Clean Hygenia protein residue swabs

C. Pathogens for Culture on Specimen

The pathogens cultured on the printed specimen include the following with associated ATCC numbers: *Klebsiella pneumoniae 13883, Acinetobacter baumanii 19606, Pseudomonas aeruginosa 10145, Escherichia coli 11775, Shigella sonnei 29930 , Salmonella typhimurium 14028. Proteus mirabilis 29906, Citrobacter freundii 8090, Bacillus cereus 14579,* and *Streptococcus pyogenes 12344.*

For best testing results for normal household conditions, 3D-printed parts, such as a 3d printed respirator mask that has curves and contours, were soaked in chicken blood or livers for 24 hours, then allowed to dry another 24 hours once removed from the liquid. As aforementioned, the preferred method and gold standard for surface testing is to use the protein residue swabs for analysis and confirmation after a petri dish examination.

IV RESULTS AND DISCUSSION

When water is placed on a plastic, it will bead up and roll off. This is due to the surface tension of water as well as the typical hydrophobic nature of plastic surfaces. This is known as Flowability. Higher surface tension fluids do not flow well into small spaces unless they get some help from some sort of additive that loosens the tension of the hydrogen bonds. Think of a ball of elastic bands tightly bond together. If you stretch the bands, they will return to their normal position, but if you apply a small amount of heat, they will loosen and hold to a new shape. This idea and concept of loosening the bands is what we will take advantage of. To accomplish this an additive must be added to the water to loosen said bonds, this is where dish soap comes into play. Basic soap and detergents will dramatically weaken the hydrogen bonds of water, making it flow into very small spaces. Table 1 below shows some common surface tensions of liquids.

Table 1: Surface Tension Forces of Common Liquids.

Substance	**Dynes/cm (unit of force)**
Water	73
Bleach Water	70
Soapy Water (Dawn)	25
70% Rubbing Alcohol (IPA)	23

IPA and soapy water have very low surface tension and almost 0-degree wetting angle. Using a 0.1-micron filter paper, 70% IPA has complete flow into the filter medium instantaneously, and shows the grid pattern, indicating that it has flown into the 0.1-micron sized pores. Soapy water was then tested on the same medium yielding the same results as shown in figure 4. This indicates that soapy water can flow into spaces much smaller than the smallest bacteria that can cause food poisoning or the smallest imperfection of a 3D-printed item. For comparison, the size of a Corona virus is 0.125 microns. To prove the results, a single drop of water placed on the same filter material never showed any signs of flow. Then water was placed on a 0.45 micron filter paper, it took 5 minutes for half of the water to flow through the paper.

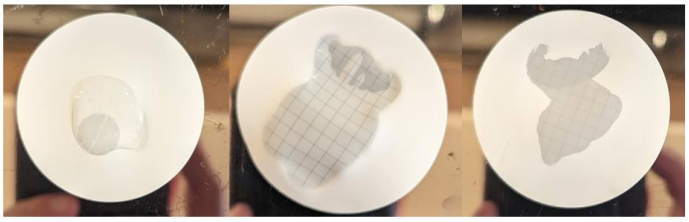

Fig 4: Flowability of Water, IPA, and Soapy Water on porous filter paper, indicating how well it can flow into spaces the size of a virus.

Preliminary testing and culture growth is step 1 of the process. A 3D-printed respirator mask, due to its curves and contours, would prove a worthy candidate for testing, that had not been sanitized for 90 days, with constant wearing during the COVID 19 pandemic, was used for the first preliminary test. The mask was swabbed with a clean, moist sterile swab and then spread onto a 100mm x 15mm Petri dish and allowed to incubate for 4 days in a room set at 83 °F. Clean glass tiles that were handled with "clean" (soap washed) and "dirty" (un-washed) hands were also swabbed and cultured for comparisons and controls. Lastly, "clean" fingernails and hands were swabbed and then spread onto a dish as well. This preliminary study was conducted to obtain an initial idea of how many Culture Forming Units (CFU) grow between what is "clean" relative to "dirty" conditions. Once the Petri dishes were incubating, the mask and glass tiles were then washed with basic soapy water using nonconcentrated soap. After the washing, each item was then swabbed and cultured again to determine a percent reduction in CFUs. It was found after cfu counting that a rough guess of 90% to 95% reduction had occurred. Using surface area contamination counting, it was found that 99% reduction of growth was reduced after washing with basic soap on the uncoated 3d printed mask contaminated with bacteria and virions (Figures 5, 6, and 7). This leads to the possible conclusion [8] that the 3D-printed object is safe for food use. From visual inspection, the growth had

been reduced, however, visual inspection is not conclusive enough. For this reason, swab testing is the standard method chosen.

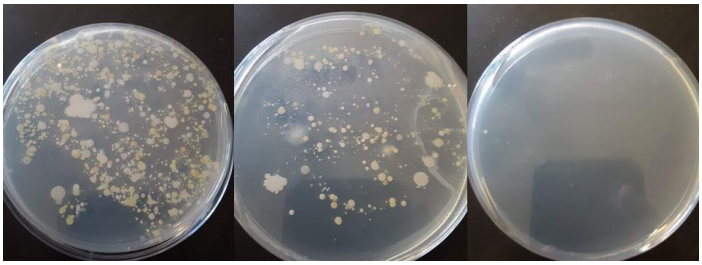

Fig 5: Non-resin-coated Mask Before and After Cleaning

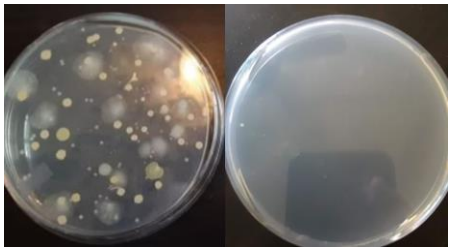

Fig 6-7: Glass Tiles Used as Control; Before and After Washing.

After the first set of preliminary tests, 3 groups of 3D-printed cubes of various wall thicknesses and layer heights were inoculated with different pathogens found in common household settings, such as E. coli or salmonella (chicken blood). The goal was to allow pathogen growth. These pathogens were then stressed by applying heat so that they would create biofilms, a protective blanket. These biofilms are what need to be removed during cleaning which allows cleansing agents to clear away the bacteria that were veiled by the biofilm. Groups 1, 2 and 3, all consisted of 4 single-walled prints, 4 double-walled prints, and 4 triple-walled prints. Each print varied from 0.16 to 0.24 mm layer height in 0.02mm increments, printed with different speeds and temperatures. All groups consisted of PLA, PLA+, and PETG as the choice of materials used. Each cube was inoculated and allowed to culture. Once cultured, each cube was then swabbed and spread onto a Petri dish. Each part in Group 1 was washed with warm 120 °F water, and non-concentrated dish soap for 30 seconds. Group 1 was then re-swabbed and tested on a Petri dish for visual comparison and percentage reduction in CFUs to determine if the number lies below the Infectious Dose (ID) number. To further prove the results, Group 1 underwent further testing with PRO-Clean Hygenia protein residue testing. Group 1 were re-inoculated, with a soak in chicken blood and other common bacteria found in food establishments. After the allotted time to grow and produce biofilms, group 1 was rewashed and allowed to air dry for 2 minutes. One printed object was left out of the cleansing group to be used as a control. This contaminated cube was tested to show a failure result as indicated in figure 8.

Fig 8: Control Swab for Protein Residues

The rest of the cubes from group 1 were tested using the residue method after being cleaned with basic soap and water, and each cube indicated a pass. Refer to figure 9 below.

Fig 9: Three Green Passes and 1 Purple Fail for The Control Group

To further test and study, each cube from group 1 was left out for 48 hours to see if any contamination would occur. They were re-tested and passed, however, after 3 hours, the catalyst in the vial had turned light purple, indicating contamination of samples. Groups 2 and 3 underwent the same inoculation and culturing. The cleaning process for Group 2 was the same method as Group 1, but with an added 2-minute soak in bleach water. This method has been known to help reduce and dissolve E-coli (table 2).

Table 2: BLEACH VS E. COLI. TNTC IS TOO NUMEROUS TO COUNT.

Concentration of Bleach	CFU of E. Coli
0.1%	***0***
0.01%	***6***
0.001%	***TNTC***
0.0001%	***TNTC***
0.0%	***TNTC***

It is required by the FDA that for all food establishments to be deemed food safe, dishes and surfaces must be soaked or wiped in a molarity of 200ppm (1 tablespoon bleach per gallon of H2O) of cold bleach water for at least 1 minute. It is also recommended for 2 minutes for best results. Since warm water deactivates bleach, this is typically performed using room temperature water. Group 3 used a small amount of baking soda on the 3D-printed part during

washing. 2g of baking soda was placed on the part and was scrubbed on using fingertips. The part was then washed with soapy water. O-Dobay et el [9] state that baking soda makes an excellent cleaning agent when you need to get rid of the biofilm. This is on account of the fact that it works by both chemical and physical means. The rough particles in the baking soda will dislodge any biofilm from the plumbing surfaces, while the basic pH will also help in chemically removing most of the waste. Using baking soda during the washing process of 3d printed parts, has proved to be the most effective at eliminating biofilms so that soap can then eliminate the bacteria and other pathogens by saponification and destroying cell walls. Biofilms are a slimy residue on the printed parts.

Once dry, the parts from Groups 2 and 3 were tested for protein residue and again these indicated a pass result. The 3D-printed parts were left out for 48 hours and then re-tested. Tests came back green, indicating a pass, and after 3 hours, had not turned light purple meaning, no re-contamination occurred. A 5th group was used for clarity to help determine the best methods for cleaning.

70% IPA was introduced to the list. It was found that only with physical agitation, such as rubbing, that IPA cleaned the parts to safe levels. However, due to recent studies [10], IPA could potentially increase biofilms with specific pathogens such as Staph. Therefore, 70% or higher IPA should be used AFTER cleaning as a precautionary sanitization step, as you would with bleach water. Figure 10 shows this possible increase of staph.

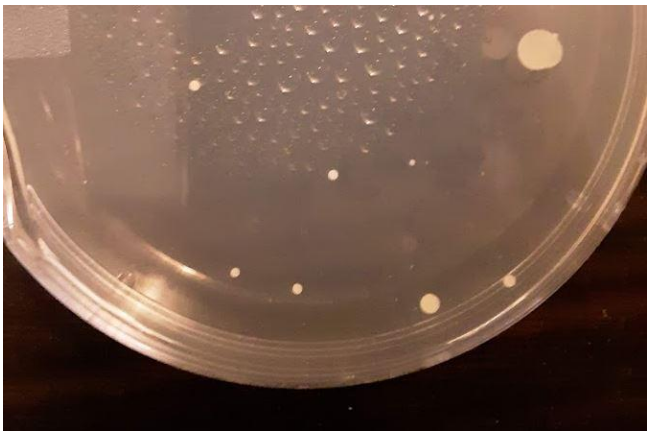

Fig 10: Possible Increase of Staph

For the next set of tests, a 3-walled cube was filled with chicken blood and water, then put under pressure to allow the fluids to seep into the print. After washing the part with soapy water and baking soda technique, sterile, distilled water was added to the cube and put under pressure to allow the water to flow into the gaps and layer lines, collecting any type of contamination that could be present. The water was then tested with the protein residue method. The catalyst in the vial turned green indicating a pass for safety (Figure 11). Waiting 3 hours, the liquid in the vial remained green. Further testing should be done with the more hazardous and uncommon pathogens such as Pseudomonas or others listed in our group.

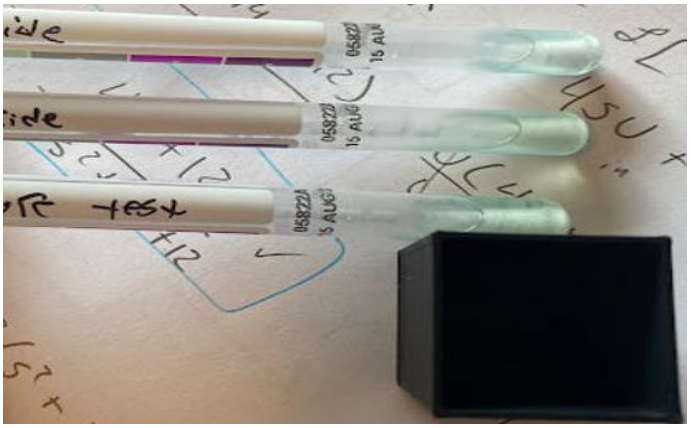

Fig 11: Pressure Test Contamination Results Indicate a Pass.

Before concluding the experiments and study, the 3D-printed parts, including a resin coated mask that were cultured for a percent reduction were compared with standard household items that had been washed with soapy water and swabbed for petri dish culturing. A plastic cutting board (85% reduction), a glass plate (99%), a standard spoon (98%) that when examined under a digital scope, had many holes, cracks, and scratches the same size range as 3D printed parts, and lastly, fingernails (40%) that had been recently washed. (Figure 12 and Chart 1).

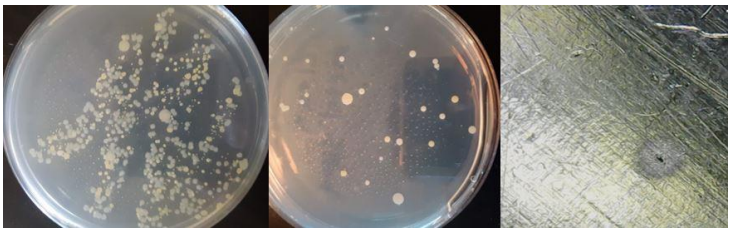

Fig 12: Metal spoon Before and After Washing. Imperfections in a Metal Spoon Roughly the Same Size Range as Imperfections In 3D-Printed Parts. Compare CFU reduction

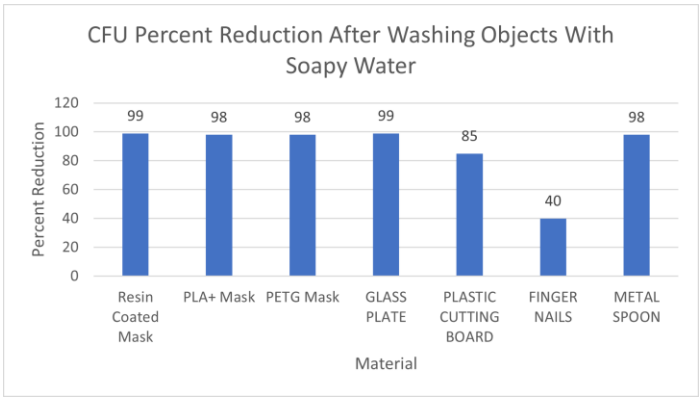

Chart 1: Displays the CFU Reduction of Common Kitchen Equipment vs 3D Printed Items after counting.

Fingernails seemed to have the lowest reduction count after handwashing. Examining figure 13, you can see how dirty clean fingers really are. The same fingers we eat foods with.

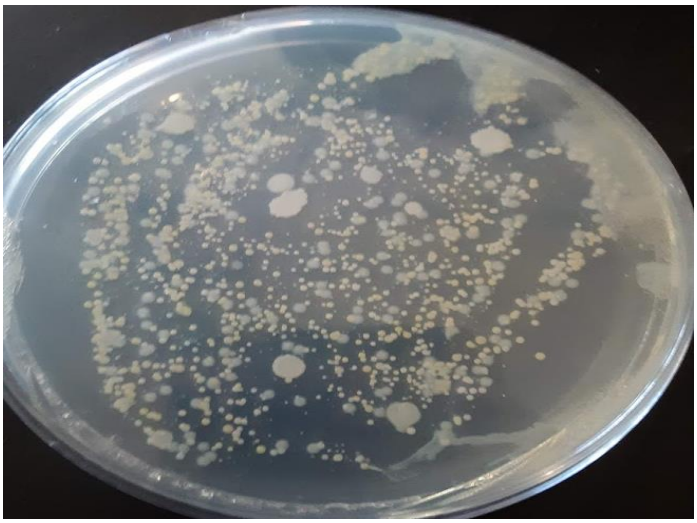

Fig 13: Clean Fingernails and fingers After Handwashing

Plastic cutting boards seem to have more contamination than 3D-printed parts. More CFU's grew on the petri dishes from a plastic cutting board that had been washed with soapy water, than 3D-printed parts that were washed with the same method.

Surgical technicians were contacted at two different hospitals (choose to remain anonymous) to test specimens for safe levels. Each specimen underwent a wash, rinse, and bleach sanitize in bleach water for 2 minutes. ATP and residue testing was performed on the parts and were found to be within specifications safe for food and medical establishments according to World Health Organization [11] as well as The Center for Disease Control[12]

To enhance the study and extend the testing results, a testing metric was formed using acetic acid from vinegar to cleanse 3D-printed parts. In order for acetic acid to be effective against biofilms, the pH of the weak acid in question must be below its pKa value. Vinegar has a pH of 2.5 and a pKa value of 4.6. This makes vinegar a decent choice for testing [13]. Vinegar, in certain situations is a good disinfectant, but for the majority of cleansing, it is not recommended for sanitizing or disinfecting. Use vinegar to help remove biofilms before washing with soapy water. Spritzing the 3D-printed part with full strength white vinegar, and leaving for 60 seconds, will help dissolve the biofilms covering the bacteria. Then proceed to use the baking soda and soapy water method to clean the prints.

In figure 13, you will see a petri dish that was divided into 3 equal parts: before washing, after washing and control. A swab test for culture growth was done on a standard home kitchen sink before washing and cleaning. Vinegar was then spritzed onto the surface of the sink, allowed to sit for 60 seconds, and then scrubbed with a paper towel. A final swab of the cleansed area was taken and spread onto the petri dish. The last section of the dish is the control, and nothing is to be done in this area. If nothing grows in the control area, then the procedure was done correctly.

Based on research and findings from Binu Kundukad [13][14], weak acids such as vinegar and citric acid do well at eradicating biofilms, but struggle to eliminate or kill drug resistant pathogens. As for using vinegar as a cleaning agent at home, more physical research and testing needs to be done according to the aforementioned reference.

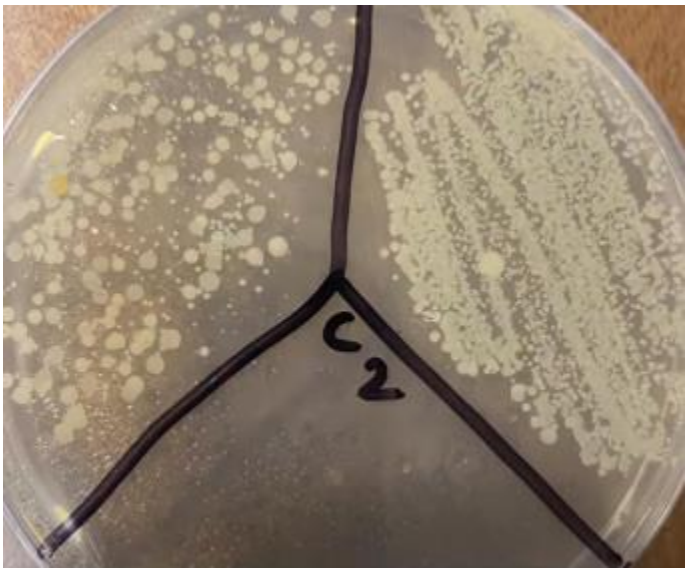

Fig 13: Disinfectant Efficacy of Vinegar is Low but Can Still Eradicate or Weaken Biofilms.

V. CONCLUSION

Experimental results confirmed that baking soda, when combined with soapy water, effectively eliminates biofilms through both chemical and physical action. Further testing demonstrated that a 2-minute room temperature bleach water soak (200 ppm) without a baking soda scrub successfully dissolved biofilms and pathogens to safe levels, as verified by surgical technicians. These findings align with established sanitation methods while offering a practical and accessible approach for 3D-printed part cleaning.

Based on the results, the most effective cleaning method involves washing 3D-printed parts with warm soapy water and a small (1/8 teaspoon or 2g) amount of baking soda on a dish rag, scrubbing for approximately 15 seconds with moderate force to prevent part damage. To ensure thorough decontamination, a precautionary step is recommended, either soaking in a 200 ppm bleach solution for 1-2 minutes (equivalent to 1 tablespoon of bleach per gallon of water) or applying 70% or greater isopropyl alcohol (IPA) by spraying and allowing it to air dry as per stated by medical professionals, however, this step requires further research due to the possibility of increased staph growth as previously mentioned in the data.

These findings provide a scalable and cost-effective cleaning protocol with potential applications in clinical and food industry settings, where sterilization of 3D-printed parts is critical. Furthermore, when using filaments containing colorants or non-food-safe additives, it is strongly recommended to apply a resin coating and allow it to fully cure before use in liquid-contact applications. This study refines previous work by providing specific, validated recommendations that enhance the safety and usability of 3D-printed parts across various industries.

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