

# Sterilization of FDM- Manufactured Parts



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Fused Deposition Modeling™ (FDM®) can be used to produce an array of medical devices; however, for such devices to be practical, they must be manufactured using sterilizable materials. Nine FDM materials were tested using four methods of sterilization: autoclave, ethylene oxide, hydrogen peroxide, and gamma radiation. Sterility testing was performed by incubating the samples in Tryptic Soy Broth for 14 days. The majority of the materials were sterilizable by all four methods while deformations were caused by autoclaving. Results from this research will allow medical staff to sterilize an FDM-manufactured device using a suitable method.

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## INTRODUCTION

Fused Deposition Modeling (FDM) is an additive manufacturing technology that is used in industry for development of three-dimensional models. The automotive industry, for example, uses FDM to produce functional prototypes to make design and testing more efficient processes<sup>[1]</sup>. Because of its ability to provide highly accurate models, FDM may offer many benefits to those wishing to use it for a range of medical applications. FDM can be used to build models that aid physicians in the surgical planning process or in the production of a surgical implant. It can be used to achieve a patient's agreement prior to surgery by providing a preoperative simulation device. During surgery, an FDM model can act as an orienting aid or a template for resection<sup>[2]</sup>. FDM can also be used to directly manufacture medical implants out of bioimplantable materials<sup>[3]</sup>. However, such surgical devices and implants will come into direct contact with sterile body tissues and fluids, and therefore, must be manufactured of sterilizable materials.

Construction of an FDM model is a multi-step process. First, a computer-aided design is made and is exported in ".stl" format. Models can also come from computed tomography scans, magnetic resonance imaging, or ultrasound<sup>[4]</sup>. The design is then processed by either Insight® (Stratasys®), the software used to set the desired build parameters. Once these have been established, the file is downloaded by the FDM system. A spool of solid material is fed into an extrusion head which heats it to a semi-liquid state. The head deposits the semi-liquid material one layer at a time. Because the temperature inside the envelope, the chamber in which the model is built, is lower than the material's melting temperature, the layers solidify as they are deposited. If necessary, the head can extrude a

support material that can later be removed, to hold the layers in place as they cool<sup>[1]</sup>.

The materials commercially available for use in FDM are thermoplastic polymers. Acrylonitrile butadiene styrene (ABS) is a commonly used material for construction in FDM.

Today, there are several derivatives of this material on the market. Five of the nine materials tested in this paper were ABS derivatives: ABSi, ABS-M30, ABS-M30i, ABS-ESD7, and PC-ABS. ABSi's translucent nature allows it to be used when monitoring material flow or transmission of light. This material is used mostly in the automotive industry. ABS-ESD7 has static dissipative properties which allow the material to be used in applications where products may be damaged as a result of static charge. This also prevents powders, dust, and fine particles from sticking to it. Because of this, it is used mostly in the assembly of electronic components. ABS-M30 is considered stronger than standard ABS. This makes the material ideal for achieving more realistic results in functional tests and higher quality end-use parts. ABS-M30i is biocompatible as per ISO® 10993 USP Class VI. This makes it a good candidate for use in the medical, pharmaceutical, and food packaging industries. PC-ABS is a polycarbonate-ABS blend. It is widely used because it offers the strength and heat resistance of PC as well as the flexibility of ABS. It is also used in the automotive, electronics, and telecommunications industries<sup>[5]</sup>.

The other four FDM materials tested in this paper are not ABS derivatives: PC, PC-ISO, PPSF, and ULTEM™ 9085 resin thermoplastic. PC is considered a true industrial thermoplastic due to its accuracy, durability, and stability in creating parts for functional testing. It is widely used in automotive, aerospace, and medical applications. PC-ISO, in its raw state, is also biocompatible

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as per ISO 10993 USP Class VI. It is, therefore, commonly used for food and drug packaging as well as medical device manufacturing. PPSF, or polyphenylsulfone, has the greatest heat and chemical resistance of all FDM materials. Parts built of this material are not only mechanically superior but also dimensionally accurate. It is an ideal material for use in aerospace, automotive, and medical applications. Finally, ULTEM™ 9085 resin is a flame retardant high performance thermoplastic resin. It can be used in the commercial transportation industry, especially in the manufacturing of aerospace, marine, and ground vehicles<sup>[5]</sup>.

Using the nine FDM materials described above, this paper describes sterilization testing on these materials using four methods of sterilization (autoclave, ethylene oxide gas, hydrogen peroxide gas plasma, and gamma radiation) that will be more fully described in the following section. A sterility test method was conducted to verify the success of each sterilization method in removing microorganisms from each material type. The samples were also visually analyzed to note any damage which may have been caused by the sterilization procedure. The following describes these methods of sterilization and the results from these tests in more detail.

## BACKGROUND ON STERILIZATION

### Techniques

Four sterilization techniques are commonly used to sterilize medical equipment: autoclave, ethylene oxide gas, hydrogen peroxide gas plasma, and gamma radiation. The Center for Disease Control's (CDC) Guideline for Disinfection and Sterilization in Healthcare Facilities<sup>[6]</sup>, released in 2008, describes each technique in the following way.

The basic principle of autoclave sterilization is exposing the items of choice to direct contact with steam at high temperature and pressure for a minimum amount of time. Two common sterilizing temperatures are 121°C and 132°C; both were tested in this paper. The advantage of steam sterilization is that it is nontoxic to the patient, medical staff, or environment.

Cycles are usually quick, as well as easy to control and monitor. It is rapidly microbicidal and sporicidal, meaning that it is not only destructive to microbes but also to their spores. It is least affected by organic or inorganic soils and easily penetrates medical packaging and device lumens. On the other hand, steam sterilization can damage heat-sensitive instruments, especially those exposed to the process repeatedly. It may leave instruments wet, causing rust. There is also a risk for burns to those handling the instruments. Flash autoclave sterilization is a cycle that is conducted at a higher temperature for a shorter amount of time. Typically items that are processed this way are not wrapped. Although this process saves some time, it is not recommended for routine sterilization. This is because the absence of packaging may lead to contamination following sterilization, possibly in transporting the items to the location where they are to be used. Also, the amount of heat exposure may not be sufficient in destroying all contaminants. Some facilities have taken specific actions to address these issues, such as placing sterilization equipment in close proximity to operating rooms and using packaging that permits steam penetration<sup>[6]</sup>.

Because not all materials can withstand heat and moisture, there are low-temperature methods of sterilization available. Such methods operate at temperatures below 60°C<sup>[7]</sup>. Ethylene oxide

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(ETO) is a colorless gas that is also microbicidal. Through alkylation of protein, DNA, and RNA, it prevents normal cellular metabolism and replication. An ETO cycle consists of five basic steps: preconditioning and humidification, gas introduction, exposure, evacuation, and air washes. ETO is compatible with most medical materials and is simple to operate and monitor. The gas can penetrate packaging materials and device lumens. On the other hand, ETO is flammable, toxic to humans, and carcinogenic. The process is, therefore, longer than steam sterilization because it requires aeration time to remove ETO residue<sup>[6]</sup>.

Another low-temperature sterilization method uses hydrogen peroxide gas plasma. The items are placed in a chamber under a deep vacuum. Gas plasma is generated when gas molecules are excited by electromagnetic waves. This produces free radicals that can interact with cell components, such as enzymes and nucleic acids, disrupting the metabolism of microbes. It is safe for the environment and leaves no toxic residues that can harm the patient. Because there is no aeration step, cycles are shorter than ETO. The process is simple and compatible with most materials except cellulose, linens, and liquids. It also requires that devices be wrapped in synthetic packaging such as polypropylene wraps or polyolefin pouches<sup>[6]</sup>.

The final method of sterilization tested was gamma radiation. The radiation produced by a radioisotope, such as cobalt-60, generates radicals which cleave carbon-carbon bonds like those found in cellular DNA. This, in turn, leaves cells non-viable<sup>[8]</sup>. It too is a low-temperature method suitable for heat-sensitive materials. However, sterilization by gamma rays is costly

and is, therefore, better suited for use in large-scale sterilization. Also, the radiation can cause oxidation in certain materials such as polyethylene and can be damaging to humans if accidental contact is made<sup>[6]</sup>.

## MATERIALS AND METHODS

### Sample Construction

The ASTM D638 Type I<sup>[9]</sup> was selected as the shape of choice for the samples used in these tests. These samples had a length of 165.1 mm, a maximum width of 19.05 mm, and a height of 3.175 mm (Figure 1). They were manufactured on a Fortus<sup>®</sup> 400mc 3D Production System (Stratasys). Build parameters were set using Insight 8.1 (Build version: 4268) (Stratasys): raster width at 0.508 mm, raster angle at 45°, contour width at 0.508 mm, and slice height at 0.254 mm. A T16 tip was used for the model material while a T12 was used for the support. The samples were built at the flattest orientation, with the height along the Z-axis at 3.175 mm. Thirty samples were built of each of the nine FDM materials: ABSi, ABS-M30, ABS-M30i, ABS-ESD7, PC-ABS, PC, PC-ISO, PPSF, and ULTEM<sup>™</sup> 9085 resin thermoplastic. Five test samples and one control were used for each method of sterilization.



Figure 1: Sample dimensions, (a) Top view (b) Side view

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## Autoclave

The samples were autoclaved using a SANYO® Labo Autoclave MLS-3750 (Panasonic Healthcare Company of North America, Wood Dale, IL) (Figure 2 a). This equipment allows the user to establish the temperature and duration of the cycle. The pressure is a consequence of these two settings. Two different cycles were tested for this procedure; five samples of each material per cycle. The first cycle was set at 121°C for 20 minutes. These samples were packaged according to material type in autoclavable bags (5 samples per bag). The maximum pressure reached in this cycle was 0.20 MPa. The second cycle was set at 132°C for 4 minutes. This cycle reached a maximum pressure of 0.12 MPa. This cycle is known as a “flash autoclave” cycle because it is faster. These samples were individually packaged in self-seal sterilization pouches (Alcan Packaging, Chicago, IL). Both cycles had heating and pressurization as well as cooling and depressurization periods that were approximately 30 minutes long.

## Ethylene Oxide Gas

Five samples of each material were sterilized by ethylene oxide in an Anprolene® AN74i sterilizer (Andersen Sterilizers, Inc., Haw River, NC) (Figure 2 b). Two cycles are available for this procedure: 12-hour and 24-hour. The 12-hour cycle was selected knowing that if this cycle failed, the 24-hour could be tested as well. These samples were individually packaged in self-seal sterilization pouches.

## Hydrogen Peroxide Gas Plasma

The third method of sterilization, hydrogen peroxide gas plasma, was done using a STERRAD® 100S sterilization machine (Advanced

Sterilization Products, Irvine, CA) (Figure 2 c). Again, five samples of each material were used. The cycle duration was 55 minutes at a maximum temperature of 55°C<sup>[10]</sup>. The cycle time is dependent on the type of STERRAD machine that is used. The 100S has only one cycle setting and changes cannot be made. This procedure was conducted at a different facility. The samples were individually wrapped in Tyvek® pouches so that they would remain sterile during transportation. Such pouches are permeable to hydrogen peroxide.

## Gamma Radiation

The last set of samples was submitted to the fourth and final method of sterilization, gamma radiation. This was conducted in a JS8900 Batch Gamma Irradiator by STERIS: Isomedix Services (Libertyville, IL) (Figure 2 d). The samples were also individually wrapped in self-seal sterilization pouches and shipped in a cardboard box with dimensions 323 x 279 x 45 mm. Once received by the processing center, the samples were not removed from their packaging and were sterilized within the box. The radiation dosage was approximately between 25 to 35 kGy. However, this is not a guaranteed dosage as the samples were submitted as a research run and no certificates of processing were provided.

## Sterility Test

The sterility test – performed to confirm the success of each sterilization process – was designed based on guidelines obtained from the U.S. Food and Drug Administration (FDA)<sup>[11]</sup> and Daniëlle Neut’s paper, “A Gentamicin-Releasing Coating for Cementless Hip Prostheses- Longitudinal Evaluation of Efficacy Using In Vitro Bio-Optical Imaging and its Wide-Spectrum Antibacterial Efficacy” which studies antibacterial

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coatings for hip-prostheses<sup>[12]</sup>. Bacto™ Tryptic Soy Broth (TSB) (BD, Franklin Lakes, NJ) was prepared and sterilized via autoclave at 121°C for 20 minutes. Once the test samples were sterilized by the corresponding method, the test was used to determine the success or failure of the sterilization process. The samples were taken into a biosafety cabinet (Purifier Class II, Labconco, Kansas City, MO) and removed from their packaging. They were inspected to note any visible changes caused by the sterilization procedure. They were then placed in 25 x 200mm Pyrex® glass tubes (Corning Incorporated, Corning, NY) in 60mL of TSB. A size 5 rubber stopper (Cole-Parmer, Vernon Hills, IL) was used to seal each tube and prevent contamination by exterior factors. The tubes were then incubated at 37°C and 90% humidity for 14 days. The control samples were treated to the same incubation procedure. However, prior to incubation, these did not undergo any type of sterilization. After the incubation period, the tubes were analyzed to see if any turbidity was present. All glassware, rubber stoppers, and tools were washed in chlorinated water and autoclaved at 121°C for 20 minutes prior to and between uses.

## RESULTS AND DISCUSSION

Prior to use, TSB is a transparent liquid. If the TSB is exposed to a microbial or fungal agent, the organism will reproduce, making the liquid turbid. Precipitation can accumulate at the bottom of the vessel if enough of the organism has settled (Figure 3). Turbidity and precipitation are indicators that the sterilization was not successful. Samples showing these characteristics are represented by a (+) sign in Table 1. No turbidity or precipitation in the TSB indicates there are no organisms present; therefore, sterilization was successful. These samples are represented by a (-) sign in Table 1. All but one of the control samples

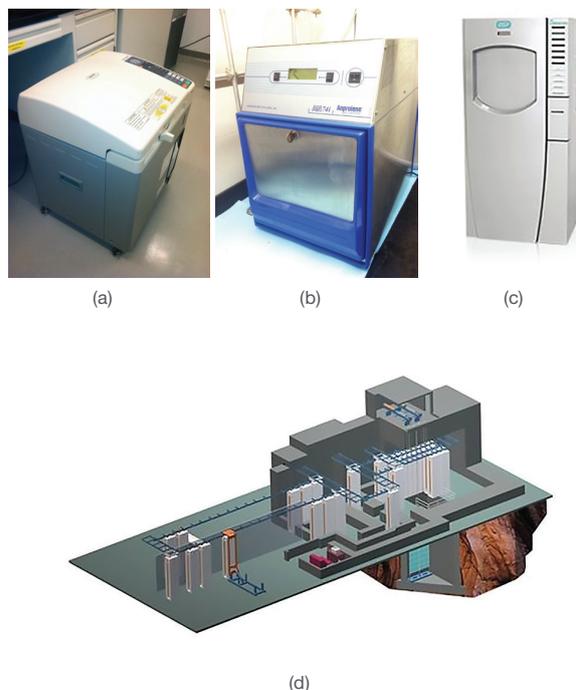


Figure 2: Sterilization equipment, (a) SANYO Labo Autoclave MLS-3750, (b) Andersen Sterilizers Anprolene AN74i Sterilizer, (c) Advanced Sterilization Products STERRAD 100S<sup>[10]</sup>, (d) STERIS: Isomedix JS8900 Batch Gamma Irradiator<sup>[13]</sup>

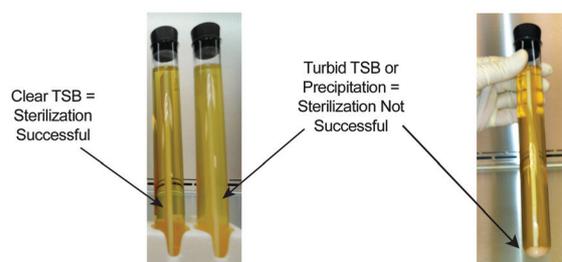


Figure 3: Determining success of sterilization procedure

**Table 1: Sterility Test Results**

	Autoclave	Flash Autoclave	Ethylene Oxide Gas	Hydrogen Peroxide Gas Plasma	Gamma Radiation
Material Type	Control Test Samples	Control Test Samples	Control Test Samples	Control Test Samples	Control Test Samples
	Success Rate	Success Rate	Success Rate	Success Rate	Success Rate
ABSi	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
ABS-ESD7	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
ABS-M30	+ 4 - 80%	+ 5- 100%	+ 4 - 80%	+ 5- 100%	+ 5- 100%
	1+		1+		
ABS-M30i	+ 3 - 60%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
	2+				
PC	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
PC-ABS	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
PC-ISO	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
PPSF	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%
ULTEM™ 9085 Resin	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%	+ 5- 100%

(ABS-ESD7, flash autoclave) yielded positive results. None of the samples were purposefully contaminated prior to testing. This means that any contamination seen on the samples may have been acquired during the manufacturing and transportation processes as these were conducted in a non-sterile way. Because all but one control showed positive contamination, any non-sterile test samples should also show contamination. Flash autoclave, hydrogen peroxide gas plasma, and gamma radiation were all 100% successful in sterilizing the test samples. The TSB remained clear because no contaminants were present in these tubes. A few test samples did, however, show contaminants. During the autoclave testing, one of five ABS-M30 samples was turbid. Two of the ABS-M30i samples were turbid as well. Of the samples sterilized with ETO, only one of five ABS-M30 samples was turbid. Because the occurrence of a positive result was so rare, it is possible these samples were contaminated following sterilization as they were being prepared for incubation. It is important to note, though, that contamination of ABS-M30 occurred for two sterilization methods.

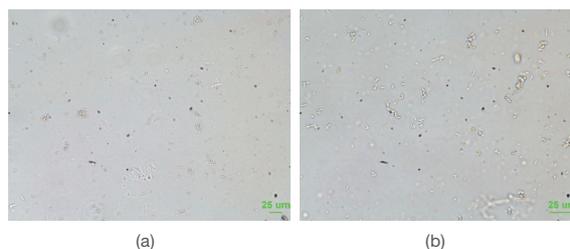


Figure 4: Microscopic view of precipitate, (a) 200x magnification, (b) 400x magnification

**Table 2: Materials Showing Visible Damage**

Material Type	Autoclave	Flash Autoclave	Ethylene Oxide Gas	Hydrogen Peroxide Gas Plasma	Gamma Radiation
ABSi	Yes	Yes	No	No	No
ABS-ESD7	Yes	Yes	No	No	No
ABS-M30	Yes	Yes	No	No	No
ABS-M30i	Yes	Yes	No	No	No
PC	No	Yes	No	No	No
PC-ABS	Yes	Yes	No	No	No
PC-ISO	No	No	No	No	No
PPSF	No	No	No	No	No
ULTEM™ 9085 Resin	No	No	No	No	No

Some of the precipitation visible in the control samples was analyzed using an inverted microscope (Leica DM IRB, Leica Microsystems, Germany). Figure 4 shows 200x and 400x magnification images of this precipitation. The white wave lines are pathogens, most likely bacterial, which can grow on surfaces of FDM-manufactured parts if they are not sterilized properly.

Although a large majority of the test samples showed they could successfully be sterilized by each of the methods, not all of them withstood the sterilization procedures themselves (Table 2). All 9 materials seemed unchanged after sterilization by ethylene oxide gas, hydrogen peroxide gas plasma, and gamma radiation. However, the high heat and moisture of the autoclave caused damage to some of the materials. For example, after the first autoclave cycle, bending and indentations were seen in ABSi, ABS-ESD7, ABS-M30, ABS-M30i, and PC-ABS (Figure 5), all of which are ABS derivatives. The indentations were caused by the metal basket used to hold the samples in the autoclave chamber. Some of the samples were also attached to one another as if melded together by the heat. For this reason, the samples were packaged individually for the flash autoclave cycle. However, the flash autoclave cycle also damaged all of the ABS derivatives (Figure 6). Bending was very apparent as well as indentation. Although some of the indentation was from the holding basket, there was also

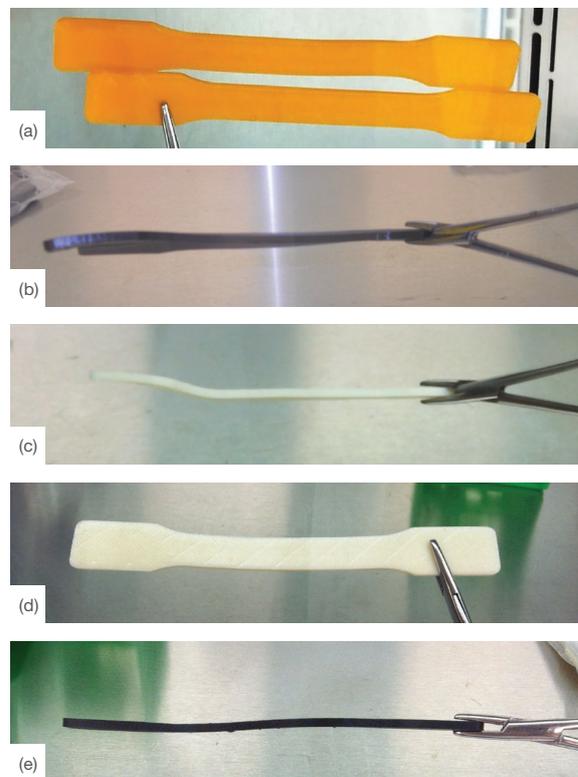


Figure 5: Material changes caused by autoclave, (a) ABSi: bending and multi-sample attachment, (b) ABS-ESD7: bending and multi-sample attachment, (c) ABS-M30: bending, (d) ABS-M30i: bending and indentations, (e) PC-ABS: bending

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some that appeared to be a result of the moisture retained by the bags post-sterilization. Color change was also noted. Slight bending of the samples was also seen in PC as a result of the flash autoclave cycle.

## CONCLUSION

FDM is a technique that is useful in a variety of applications, including some medical ones. The spectrum of medical applications can be expanded to include surgical tools or devices where direct contact with the patient is made. This can only be achieved if the FDM materials used to build such devices are deemed sterilizable. Based on the results obtained in this paper, it appears that each of the four methods tested are successful in sterilizing these materials. However, it is clear that ABS derived materials cannot resist the high temperatures of the autoclave. Although the autoclave is most likely the simplest sterilization method available, it is best to use more heat resistant materials, such as PC-ISO, PPSF, or ULTEM™ 9085 resin thermoplastic when using high temperature sterilization methods.

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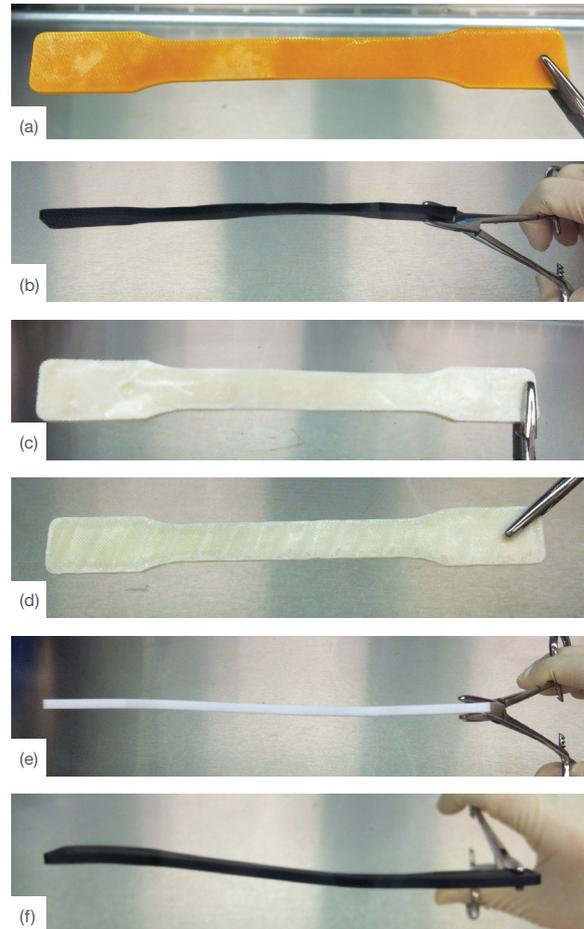


Figure 6: Material changes caused by flash autoclave, (a) ABSi: bending and color change, (b) ABS-ESD7: bending, (c) ABS-M30: bending and indentations, (d) ABS-M30: bending and indentations, (e) PC: bending, (f) PC-ABS: bending

Note: The FDA is the governing body for approval of medical devices in the United States. The guidelines that state which sterilization practices and sterility tests must be followed are specific to the device that is being submitted for approval. The tests conducted in this paper were preliminary tests used to narrow down the number of materials for future tests.

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