Biocompatibility Results

MPU 100: Printing & Processing Protocols for Carbon M Series Printers

The protocols described in this document were used by Carbon for printing parts from MPU 100 material that were evaluated and found to meet the requirements of ISO 10993-5 and ISO 10993-10 and the USP VI designation for plastics. These tests are typically performed in order to establish suitability of materials in prolonged skin contact (more than 30 days) and short-term mucosal-membrane contact (up to 24 hours).

Resin Dispensing

MPU 100 is a two-component material supplied in a dual-chamber, light-resistant cartridge. The A and B components are mixed in a 10:1 ratio by volume using a static mixer tip attached to the end of the cartridge is installed into a Albion motorized dispensing unit. The initial volume (~10 mL) of resin is burned into a waste container to prevent off-stoichiometry. For these tests, the A and B components were mixed in a 10.59:1 ratio by weight (10:1 by volume) using a planetary centrifugal mixer. An appropriate volume of the mixed resin (as specified by the print planner software) was then dispensed into the printer cassette and the cassette was placed on the optical deck.

Note: When switching between materials, the cassette was cleaned with isopropanol (IPA) to ensure that residual resin from the previous print was not mixed with the MPU 100 material.

Printing

A cleaned build platform was installed onto the platform latch and the print process initiated by uploading the STL, entering run parameters (resin type and print orientation) and requesting print initiation. Print speed and light intensity are controlled by Carbon's proprietary software to ensure part accuracy and degree of UV network cure.

Part Removal from Build Platform

Once the "green" state part (only the UV network is cured) was built, the build platform was removed from the printer, the part gently removed from the build platform using a variety of scrapers, tweezers and blades.

Washing

The parts were washed with mild agitation in **Vertrel XM™**, an azeotropic mixture of 1,1,1,2,2,3,4,5,5,5-Decafluoropentane and methanol (91-93 to 9-7, w/w, Chemours™) for 2 minutes. Agitation can be achieved by placing the parts in a stainless steel small-parts basket and rotating the basket at 5-20 rpm in sufficient Vertrel XM™ to cover or using the Carbon Smart Part Washer (SPW). In the latter case, the washer will provide the proper wash cycle. *It is also recommended that for biocompatibility studies that the part be washed in freshly distilled Vertrel XM™ or in a SPW used exclusively for MPU 100*.

Thermal Cure

The parts were placed on a non-stick tray and placed in a clean, dedicated convection oven at 100°C for 4 hours (actual temperature between 94 – 116°C).

Biocompatibility Testing

Parts printed and processed as outlined in this document were provided to NAMSA for evaluation in accordance with ISO 10993-5, *Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity*, and ISO 10993-10, *Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization (GPMT)*. The results for all tests indicated that MPU 100 passed the requirements for biocompatibility according to the above tests. Additionally, parts were provided to NAMSA for testing in accordance with *USP Classification of Plastics (USP Biological Reactivity Tests In Vivo).* The results indicated that MPU 100 passed the requirements for biocompatibility according to the above tests.

Carbon makes no representation and is not responsible for the results of any biocompatibility tests other than those specified above.

Device Categories			Biological Effect										Product Types
Body Contact		**Contact Duration** A = Limited (<24hours) B = Prolonged (24 hours - 30 days) C = Permanent (> 30 days)	Cytotoxicity	Sensitization	Irritation/Intracutaneous	Acute Systemic Toxicity	Subchronic Toxicity (Subacute toxicity)	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Product Examples
Surface Devices	Skin	A	X	X	X								Radiotherapy boluses
		B	X	X	X								Prosthetics, Hospital bed
		C	X	X	X								Prosthetics, Hearing aids, Plagiocephaly bands
	Mucosal Membrane	A	X	X	X								Dental surgical guide
		B	X	X	X	O	O		O				Temporary crown
		C	X	X	X	O	X	X	O		O		Aligners
	Breached or Compromised Surfaces	A	X	X	X	O							Wound drainage devices, cosmetic microneedles
		B	X	X	X	O	O		O				
		C	X	X	X	O	X	X	O		O		
Externally Communicating Devices	Blood Path, Indirect	A	X	X	X	X				X			
		B	X	X	X	X	O			X			
		C	X	X	O	X	X	X	O	X	O	O	CPAP Mask
	Tissue/Bone/Dentin	A	X	X	X	O							Orthopedic guides, trials, sizers
		B	X	X	X	X	X	X	X				
		C	X	X	X	X	X	X	X				
	Circulating Blood	A	X	X	X	X		O*		X			Extracorporeal loop components (oxygenators)
		B	X	X	X	X	X	X	X	X			Vascular access port (drug delivery)
		C	X	X	X	X	X	X	X	X	O	O	Dialysis access ports
Implant Devices	Tissue/Bone	A	X	X	X	O							Very few examples
		B	X	X	X	X	X	X	X				Very few examples
		C	X	X	X	X	X	X	X		O	O	IOLs, Resorbable Buttress
	Blood	A	X	X	X	X	X		X	X			
		B	X	X	X	X	X	X	X	X			
		C	X	X	X	X	X	X	X	X	O	O	Resorbable stent

X = Tests per ISO 10993-1

O = Additional tests that may be applicable in the U.S.

Note - tissue includes tissue fluid and subcutaneous spaces

Note* - For all devices used in extracorporeal circuits

= Permitted

= Not Permitted

= MPU 100 biocompatibility data

Sterilization Results

MPU 100 Sterilization Sensitivity

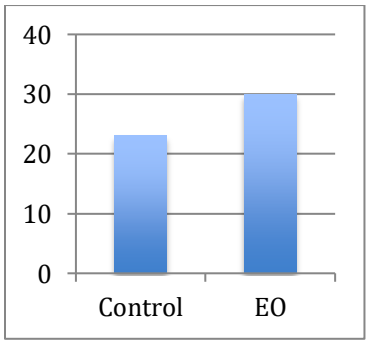
MPU 100 is a versatile material, which may be suitable for a range of medical applications. MPU 100 shows excellent response to ethylene oxide (EtO) sterilization with moderate change in physical properties and rapid reduction in EtO levels post-sterilization. For ionizing radiation sterilization (gamma and e-beam) MPU 100 shows some change in mechanical properties. These changes in mechanical properties can be accommodated for through design changes or accounted for early in the design stage.

Ethylene Oxide (EtO) Sterilization:

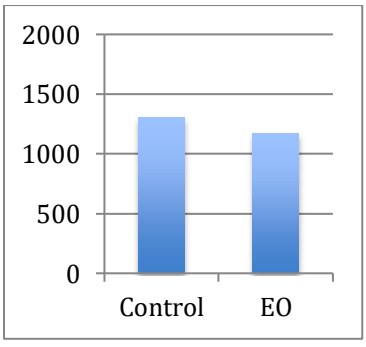
MPU 100 is compatible with EtO sterilization, showing a 30% increase in elongation at break and 10% drop in modulus with negligible impact on ultimate strength. Furthermore, there is a rapid reduction in EtO levels post-sterilization, with all EtO levels below limits for prolonged contact after the standard 24 hours of aeration.

Carbon prepared n=10 test specimens and provided these samples to Nelson Laboratories for EtO exposure and extraction studies. The samples were conditioned at 52°C, 55% relative humidity, and 1.3 psi for 60 minutes. The samples were then exposed to 100% EtO at 52°C for 240 minutes. The samples were allowed to aerate for 24 hours and residuals were measured every hour for 170 hours post sterilization.

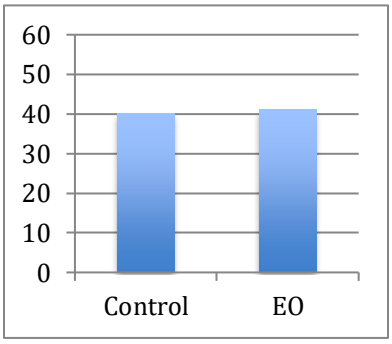
% Elongation at Break



Modulus (MPa)



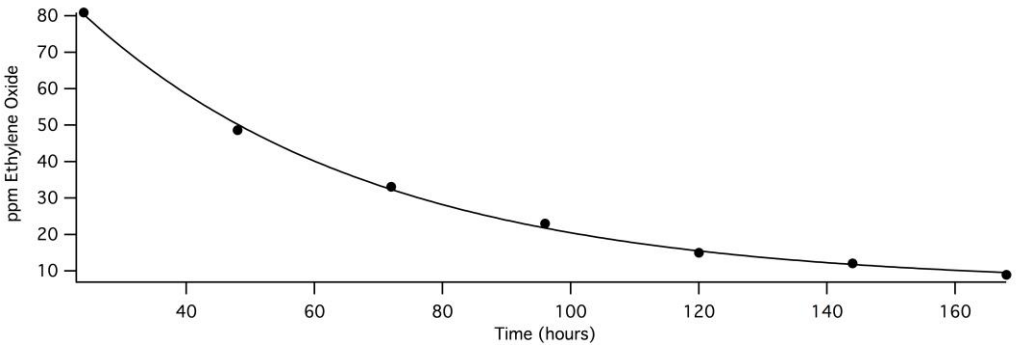
Ultimate Strength (MPa)



Post Ethylene Oxide Sterilization Cytotoxicity

After 1 cycle of sterilization, samples were tested for cytotoxicity per ISO 10993-5, Biological evaluation of medical devices – Part -5 Tests for in vitro cytotoxicity. The results show that post-sterilization, there is no observed cytotoxicity.

Ethylene Oxide Dissipation



Time axis: hours post sterilization (first time point at 24 hours after exposure)

Fitting equation:

$$ppm\ EtO = \ 6.38 + 124.9\ e^{-0.022t}$$

Mean Lifetime = 45.8 hours (based on exponential decay model, ppm EtO released versus time in hours).

EtO release for a hypothetical 100 g device would be 3 mg at 24 hours and cumulative (30 days) of 7.4 mg. Average release rate is 0.25 mg/day averaging from day 2 to 30, and 0.093 mg/day averaging from day 4 to 30. Per *ISO 10993-7: Ethylene oxide sterilization residuals*, release is required to be < 4mg total for limited (< 24h contact), 60 mg/30days (> 24h M 30 days) and no more than 2.5 g lifetime. MPU 100 meets these requirements based on the tests described here.

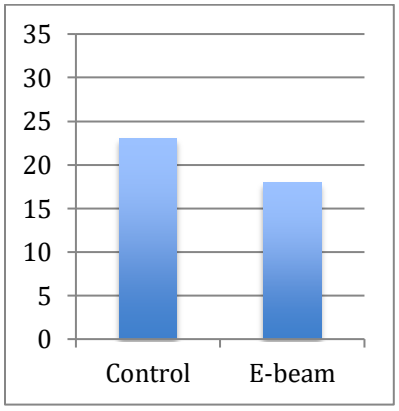
The samples showed no observable change in color post-EtO sterilization. The samples had a yellowness index (E313, D65/10) of 18.0 pre-sterilization (control) and 17.7 after EtO sterilization.

E-beam Sterilization:

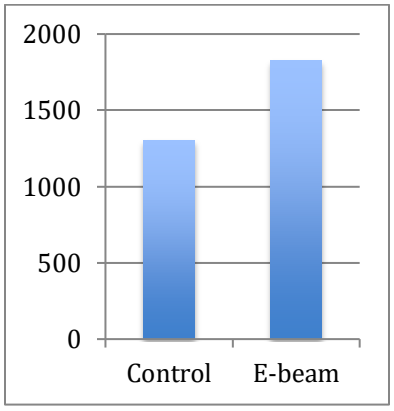
When exposed to e-beam sterilization, MPU 100 demonstrates a 22% reduction in elongation at break and a 40% increases in modulus. The samples also show a 23% increase in ultimate strength. The test specimens show some yellowing post-sterilization. The samples had a yellowness index (E313, D65/10) of 18.0 pre-sterilization (control) and 22.5 after e-beam sterilization.

Carbon prepared n=10 test specimens and provided these samples to Steris for e-beam sterilization. The samples were exposed to 33.9 – 36.6 kGy e-beam radiation (measured by dosimeter).

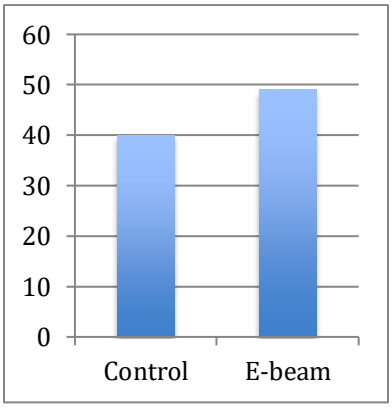
% Elongation at Break



Modulus (MPa)



Ultimate Strength (MPa)



Post e-Beam Sterilization Cytotoxicity

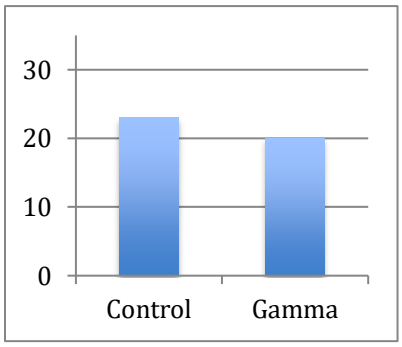
After 1 cycle of sterilization, samples were tested for cytotoxicity per ISO 10993-5, Biological evaluation of medical devices – Part -5 Tests for in vitro cytotoxicity. The results show that post-sterilization, there was no observed cytotoxicity.

Gamma Sterilization:

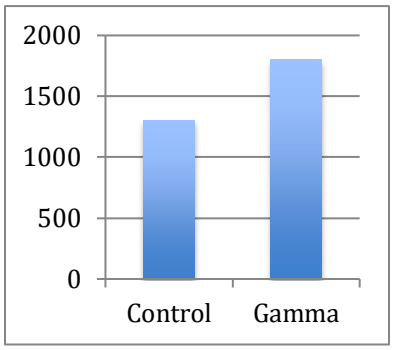
When exposed to gamma sterilization, MPU 100 demonstrates a 13% reduction in elongation at break and a 38% increase in modulus. The samples also show a 25% increase in ultimate strength. The test specimens show some yellowing post-sterilization. The samples had a yellowness index (E313, D65/10) of 18.0 pre-sterilization (control) and 22.6 after gamma sterilization.

Carbon prepared n=10 test specimens and provided these samples to Steris for e-beam sterilization. The samples were exposed to 34.12 – 35.61 kGy gamma radiation (measured via dosimeter).

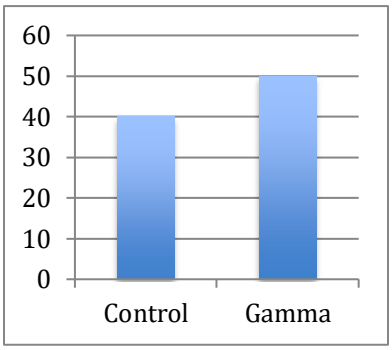
% Elongation at Break



Modulus (MPa)



Ultimate Strength (MPa)



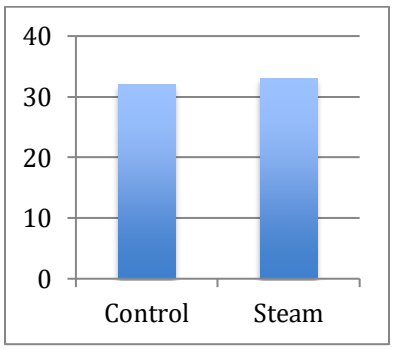
Post Gamma Sterilization Cytotoxicity

After 1 cycle of sterilization, samples were tested for cytotoxicity per ISO 10993-5, Biological evaluation of medical devices – Part -5 Tests for in vitro cytotoxicity. The results show that post-sterilization, there was no observed cytotoxicity.

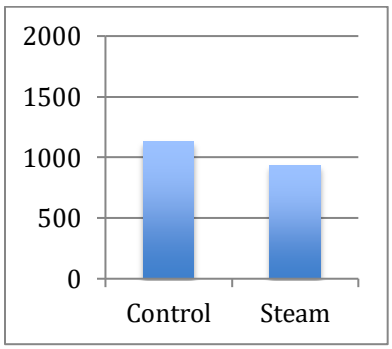
Steam Sterilization:

Carbon prepared n=10 samples which underwent 5 cycles of sterilization consisting of autoclave at 131°C followed by 3 min wash in 5% Deconex LIQ.

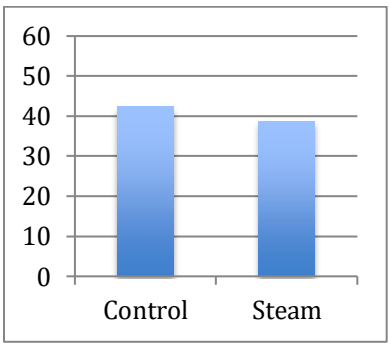
% Elongation at Break



Modulus (MPa)



Ultimate Strength (MPa)



When exposed to autoclave conditions, MPU 100 demonstrates a 6% increase in elongation at break, a 18% drop in modulus and a 9% drop in ultimate strength. **Despite these small changes in mechanical properties, parts readily deformed immediately after autoclave and remained soft for several hours afterwards. Carbon does not recommend high temperature sterilization for MPU 100.**

Sample Preparation

All test specimens were prepared by printing using the standard print planner. Following the printing, samples were washed for 2 minutes in Vertrel XM, then cured for 240 min at 100-110°C.

Disinfectant Compatibility

MPU 100 Compatibility with Common Disinfectants

MPU 100 is compatible with a range of commonly used hospital cleaning chemicals including ethanol, bleach, chlorohexidine gluconate, and benzalkonium chloride. Carbon evaluated MPU100's compatibility with these solvents under two conditions: (1) wipe and dry, and (2) 24 hour soak and dry. In this evaluation, compatibility was evaluated based on change in weight, color, and tensile properties of Type V specimens according to ASTM D638.

Sample Geometry:

Type V specimens were used according to ASTM D638 with a gage length of 10mm and a cross section of 4.00mm by 3.18mm.

Procedure:

All samples were printed in the z-direction on an M1 printer in the same build, per standard processes. Samples were weighed before and after exposer to solvent, as well as immediately before testing.

The following concentrations (% by weight) were used:

(1) 5% Bleach
(2) 70% EtOH (ethanol)
(3) 5% Chlorhexidine gluconate
(4) 0.13% Benzalkonium chloride

A set of n=3 control samples were also tested.

Wipe and dry condition:
Carbon prepared n=3 tensile samples per solution to test under the wipe and dry condition. Samples were wiped front and back using a saturated cotton swab, then rinsed in tap water and wiped dry 20 times every 5 minutes. The samples were then left to dry overnight.

24hr soak and dry condition:
Carbon prepared n=3 samples per solution to test under a 24 hour soak condition. Samples were left completely submerged in each solution for 24 hours. After 24 hours, the samples were rinsed in tap water, wiped dry, and left to dry overnight. This condition is meant to represent a worst case scenario.

Results:

Overall, the results show that MPU100 is compatible with the four tested disinfectants, with most cases causing only minor impacts to tensile properties and no change in mass, dimensions, or color.

Dimensional, weight, and color changes:
There was no notable color change in the samples before and after exposure. Samples weighed between 2.97 and 3.05g both before and after exposure in all cases. There was no significant variation in cross sectional area in all cases (cross sectional area is measured for each sample before tensile testing).

Tensile properties:
The following tensile properties were obtained.

Wipe & Dry (MPa)

	Modulus	Ultimate Tensile Strength	0.2% Offset Strength at Yield	Elongation to Break (%)
BLEACH AVG	1126.7	42.9	14.8	34.0
ETOH AVG	1213.3	41.4	15.4	31.0
CG AVG	1066.7	41.2	14.2	31.7
BC AVG	1100.0	41.0	14.0	32.0
Control	1176.7	45.6	14.5	35.7

Soak & Dry (MPa)

	Modulus	Ultimate Tensile Strength	0.2% Offset Strength at Yield	Elongation to Break (%)
BLEACH AVG	992.0	39.6	12.7	32.7
ETOH AVG	678.0	35.6	7.0	36.3
CG AVG	985.3	40.3	11.6	34.0
BC AVG	970.0	45.2	12.8	38.3
Control	1176.7	45.6	14.5	35.7

In the wipe & dry condition, the most noticeable effect was the chlorhexidine gluconate effect on modulus, which decreased by approximately 9%. However, this value, as with the other evaluated parameters, were within the noise of the control.

In the soak and dry condition, we see a consistent decrease in modulus by up to 17.5% in bleach, chlorohexidine gluconate, and benzalkonium chloride, while ethanol caused a more significant 42% decrease in modulus. With the other properties, we see minor impacts from all the disinfectants, except ethanol, which knocked down UTS by 22%, and yield strength by almost 52%. However, ethanol did not seem to impact elongation.

Overall, these solvents did not impact weight, color or dimensions of MPU100, and had almost no effect on material properties in the wipe & dry condition. While the 24 hours soak saw minor decreases for all parameters except elongation, the knockdowns were not major except in the case of ethanol. The most notable impact resulted from a 24 hours soak in ethanol, but wiping with ethanol did not produce similar effects.

Disclaimer
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