Designing a clay-based hydrogel ink for 3D printing of sterilizable medical devices for soft tissue repair

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INTRODUCTION

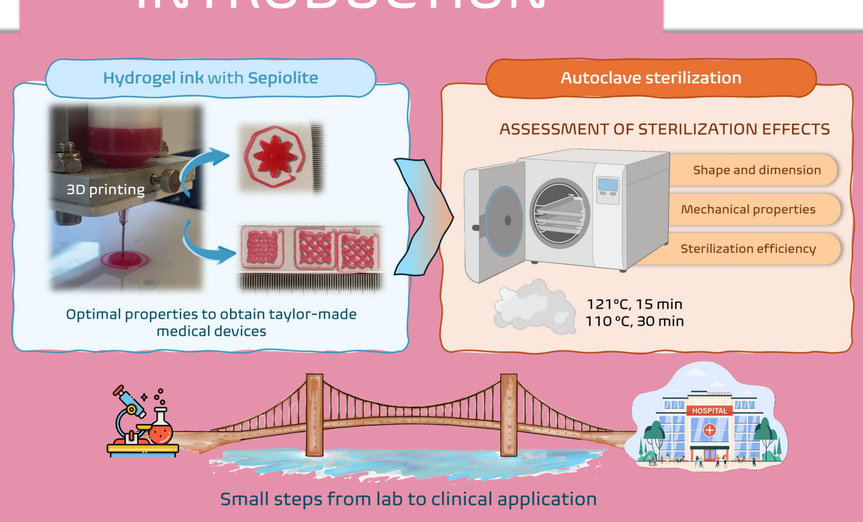


Fig. 1. Graphical abstract on the aim of this study.

A hydrogel-like ink containing sepiolite (fibrous clay mineral) was designed for pressure-assisted 3D printing (3DP) of medical devices for soft tissue regeneration, which require a compulsory sterilization step.

Despite the increasing interest in 3DP for tissue regeneration, the studies on sterilization are still scarce. It is not only necessary to determine the optimal sterilization conditions for each formulation and/or construct, but also how it affects their properties, performance and shape/dimensions.

The purpose of this study is **to define the conditions for effective autoclave sterilization and determine how they affect the final performance of the printed models**, thus bridging a gap between hydrogel-based 3DP and clinical practice (Fig. 1).

MATERIALS AND METHODS

PS9 hydrogel was printed in the EAZAO Bio (PAM), equipped with a 30 mL cartridge, using a 20 mm/s of printing speed (20G, metallic printing nozzle) for resolution line (Fig. 2A) and 90º rotated linear infill (90%) for cylinders (Fig. 2B).

In a pilot study, resolution lines (Fig. 2A) were printed, photographed (Euromex Microscopy B.V.) and sterilized at 121ºC and 110ºC. New photos were taken after sterilization processes and compared with their original dimensions. Measurements before and after sterilization were obtained with ImageJ.

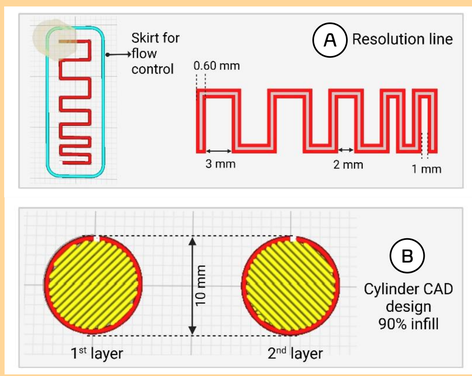


Fig. 2. Sliced CAD of printed cylinders

Mechanical changes after sterilization were studied through uniaxial compression tests on printed cylinders (Fig. 2B). A TA.XTplusC (Stable Micro Systems) was used. P/10 cylinder probe, 0.25 mm·s^{-1} until 50% of strain (1 compression cycle, n=6).

Cylinders (Fig. 2B) were inoculated with 50-100 µl of a bacteria/spores in saline solution and subsequently autoclaved at 110ºC for 30 min. Then, 3 mL of adequate culture medium were added and incubated for 14 days (Table 1).

Strain	Culture Medium	T (ºC)	Aerobic/ Anaerobic
B. subtillis	TSB	37	Aerobic
P. aeruginosa	TSB	37	Aerobic
S. aureus	TSB	37	Aerobic
G. stearotermophilus	TSB	65	Aerobic
P. camemberti	Potato Broth	28	Aerobic
C. sporogenes	Thioglycolate Broth	37	Anaerobic

Table 1. Bacteria and conditions for sterilization tests.

RESULTS AND DISCUSSION

The comparison of lines widths after sterilization (Fig. 3) reveals that sterilization at 121ºC produced significant changes in shape and dimension, compromising the specificity of the 3D printing structure produced. Therefore, 110ºC sterilization was selected.

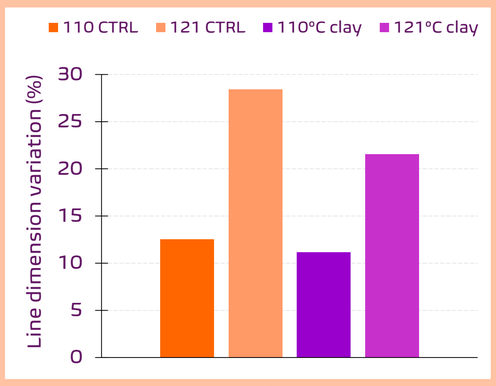


Fig. 3. Percentage of shape changes after sterilization of line resolution. CTRL: control (no clay).

Table 2. Sterilization tests results for PS9 and CTRL (without clay) cylinders. (*) Species included in the Ph. Eur. 2.6.1 – sterility

	PS9 cylinders				CTRL cylinders			
STRAIN	R1	R2	R3	C+	R1	R2	R3	C+
Bacillus sp.	-	-	-	+	-	-	-	+
Pseudomonas aeruginosa (*)	-	-	-	+	-	-	-	+
Staphylococcus aureus (*)	-	-	-	+	-	-	-	+
Geobacillus stearotermophilus	+	+	+	+	+	+	-	+
Penicillium camemberti	-	-	-	+	-	-	-	+
Clostridium sporogenes (*)	-	-	-	+	-	-	-	+

	Negative controls			
	TSB 37°C	TSB 65°C	PB	TB
PS9 cylinders	-	-	-	-
CTRL cylinders	-	-	-	-
No cylinder	-	-	-	-

- C+: positive control
- (+): positive growth
- (-): negative growth
- TSB: tryptic soy broth
- PB: potato broth
- TB: tioglycolate broth



Fig. 4. A) *G. stearotermophilus* test for PS9. B) *P. aeruginosa* test for PS9. Photos were taken after 14 days of culture.

The sterilization cycle at 110ºC was effective to sterilize both CTRL (without clay) and PS9 (with sepiolite) for those species included in Ph. Eur. and *Penicillium camemberti* (Table 2).

The mechanical studies revealed that the absence of clay (CTRL) showed no changes in mechanical performance. Nonetheless, the sterilized PS9 showed more resistance to deformation after sterilization. On the other hand, the distance to 50% deformation remains constant, suggesting that sterilization does not affect the deformation capacity/elasticity.

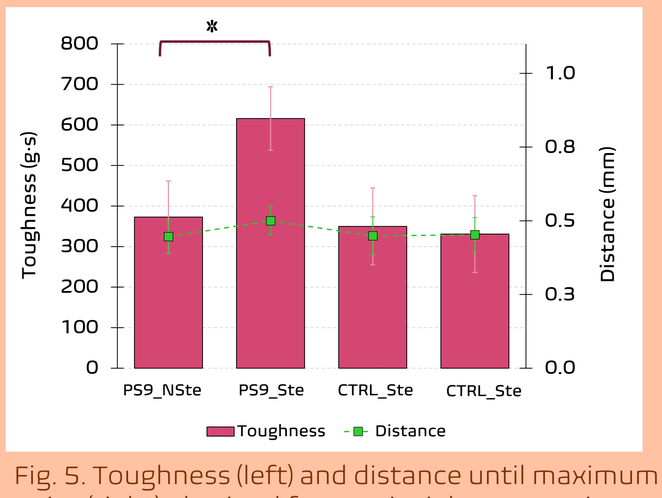


Fig. 5. Toughness (left) and distance until maximum point (right) obtained from uniaxial compression tests. Statistical differences were marked (one-way ANOVA, p-value ≤ 0.05).

Results on positive (C+) and negative (C-) controls validate the results. The strain *G. stearotermophilus,* included to monitor the effectiveness of the process, was able to grow in 5 out of 6 sterilized cylinders. This strain was not included in the Ph. Eur. sterilization test, but its growth demonstrates the limitations of the 110ºC sterilization cycle to destroy endospores of thermophilic bacteria.

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CONCLUSIONS

Based on the morphology (shape, dimension) of the original constructs before and after sterilization, mild autoclave conditions (110ºC, 30 min) are preferable since they exert less changes in final morphology, which is a crucial feature of tailor-made medical devices. Although the designed sterilization cycles were successful for bacterial strains included in the Eur. Ph. 11th Ed., the inclusion of *Geobacillus stearotermophilus* clearly highlights the limitations of the selected cycle against heat-resistant species.

The toughness of the printed constructs increased after sterilization (statistically significant). Although this change can be found as a positive feature, it could also change the therapeutic performance of the medical device, reason why further studies are still needed.