

Biodegradable scaffolds for bone defect treatment

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Submitted: 27th September 2023

Accepted: 12th December 2023

Abstract

Purpose

Additive techniques in dog orthopedics has recently emerged as a valuable approach in fabricating individualized implants for receiver-specific needs. The scaffolds made by 3D printing are used to replaces bones damaged by injuries sustained in accidents, tumour resections and defects resulting from disease e.g. osteoporosis. In this way can promote the growth and reconstruction of bone defects structure. These implants should have the right properties to ensure the right conditions for bone fusion. It is also important to determine the time of degradation, which is associated with a significant loss of mechanical properties.

Methods

Polylactic acid (PLA) scaffolds with different dimensions were fabricated via fused deposition modeling (FDM) by CABIOMEDE for bone tissue reconstruction in veterinary application. In the research the mechanical properties and impact of the soaking process in Ringer's solution for 1, 3, 6 months and steam sterilization for 1, 2, 3 cycles of the scaffolds were assessed. The static compression and three-point bending tests, material wettability, and macro and microscopic observations were carried out.

Results

Compressive test results indicated that PLA scaffolds showed elastic-plastics deformation in longitudinal and transverse directions. The compressive strength of the scaffold in initial state were $R_{cw} = 5-6$ MPa in the longitudinal direction and $R_{cp} = 0.33 -1$ MPa in the transvere direction, depending on size. The flexular strength of th scaffolds were $R_g = 348-865$ MPa depending od size. The soaking and steam sterilization process increases the elastic modulus of the material and decreases the flexural strength. Hydrolytic degradation initiated by exposure to Ringer's solution resulted in a change in structure from amorphous to semicrystalline. This

indicates partial dissolution of the amorphous phase and thus an increase in the percentage of the crystalline phase, which caused the material to become brittle. Progressive degradation results in brittle fracture of the material, as can be observed in implants after 6 months of soaking.

Conclusions

The mechanical properties of the PLA scaffolds were close to the properties of cancellous bone. The PLA scaffolds can be anticipated as promising scaffold biomaterials for bone tissue engineering applications by virtue of their bone mimicking porous structure and good mechanical properties.

Keywords: 3D printing, bone defect, scaffold, Polylactic acid (PLA), veterinary medicine

1. Introduction

Many owners treat their dogs as a member of the family, so when their pet's health deteriorates, they visit the veterinarian immediately. The most dogs common cause of emergency visits to veterinarians is bone fractures. They predominate among injuries to the musculoskeletal system, which can be caused by many factors. is a preponderance of injuries due to traffic accidents [33], [17]. Fighting with other animals falls from heights, and intentional human actions are among the common causes of injuries. Injuries occur regardless of age, femur shaft fractures often are the result of genetic defects that only become apparent when the animal is growing age, breed, and size-eloping bone tumor. Osteochondrosis is one of the developmental disorders of the skeletal system in dogs, the development of which can result in the formation of cysts. They are most often located in the epiphyses of the long bones, the ulna, and the radius. At the site of cyst formation, the bone is weakened, which may result in pathological fracture [31]. Another skeletal disorder diagnosed in dogs is asynchronous forearm bone growth. It manifests itself as a deformation of the long axis of the limb in the area of the carpal bone and the distal epiphysis of the radius or elbow, shortening of the forearm length,

and lameness [12]. Among the most commonly diagnosed tumors that affect the canine skeletal system are osteosarcomas, accounting for approximately 85-98% of primary bone tumors [25]. Lesions are most often located in the long bones of the limbs, where the risk of developing them is approximately four times higher than in the bones of the axial skeleton. The second most common primary neoplastic lesions are chondrosarcomas, which account for 5-13% of oncologically treated dogs [5], [26]. Injuries and musculoskeletal conditions in animals require surgical intervention and external immobilization. The use of metal implants carries several possible complications, such as allergic reactions or reoperations performed to remove the implant. On the other hand, the use of a brace makes it difficult to move and also disturbs animals, so they try to get rid of it (often causing difficulties in wound healing). Therefore, the search for methods to eliminate the placement of metal implants and reduce the time of use of total external immobilization has been the subject of much research for years. The dynamic development of veterinary medicine and modern manufacturing techniques allows the use of bone implants manufactured through additive techniques [16]. Additive techniques in dog orthopedics has recently emerged as a valuable approach in fabricating individualized implants for receiver-specific needs. Additive technologies, also known as 3D printing, involve the creation of physical models, previously designed in CAD systems or obtained by reverse engineering [11]. The process of creating a 3D model takes place layer by layer and, depending on the fabrication technique and the polymer material used, the main techniques used in canine orthopaedics are stereolithography (SLA) and fused deposition modelling (FDM). The SLA method involves curing the liquid resin contained in the workspace. The curing process is made possible by photopolymerisation, performed by a computer-serrated laser. Models made using SLA technology are characterised by high detail resolution and accuracy (the smallest models can reach 50-200 μm). The SLA technology using epoxy resin has been used to produce pre-operative 3D biomodels [6, 13]. On the other hand, the FDM method involves applying a head

of molten material, working in the x and y axes. The downward movement of the table along the z-axis creates space for the next layer. The head first creates the contour of the layer and then creates the filling, the type of which is defined in the printer software. The most common materials used in this technology are ABS, PLA and PA12. ABS was also used for preoperative planning to produce a 3D model [3]. And with PLA, 3D models for two adult dogs with antebrachial limb deformity [15] and adult golden retriever dog with angular limb deformity [14]. However, all of the above-mentioned applications relate to the production of preoperative models. The use of additive techniques is increasingly being found in the innovative solution of implants made of biodegradable polymers that serve as a scaffold which can promote growth and reconstruction of bone structure. Scaffold implants are used to replace bones damaged by injuries sustained in mishaps, tumor resections, and defects resulting from diseases. And FDM 3D printing technology, allows control of the internal porosity, pore size and combined structure of biomaterials. Mainly PLA [1, 9], but PLA-based composites [19, 32] have found applications in tissue engineering. Currently, the most common implants in veterinary medicine are made of titanium alloys. While effective, these implants mechanical properties and in some cases need of additional surgical procedure for removal, especially in younger animals, can be a significant drawback. In contrast, PLA and its blends, although used primarily for Tibial Tuberosity Advancement procedures, offer a resorbable alternative, eliminating the need for implant removal and reducing the overall stress and risk to the animal.

Scaffolds should have the right properties to ensure the proper conditions for bone fusion. A key issue that determines the success of treatment is the geometric form of the implant and its porosity. In the case of polymer implants, it is also important to determine the degradation time, which is associated with a significant loss of mechanical properties [7]. Among other things, the forces acting on the implants are the main criterion for selecting implant properties. The forces that occur in the body can be divided into internal and external forces. Internal forces

include continuous nature, such as those developed by muscles, that are transferred to bones through tendons [4]. The consequence of prolonged exposure to these forces is overloading, resulting in the diagnosis of joint disorders that are observed most often in overweight dogs, as body weight puts additional stress on the skeletal system. External forces acting on the musculoskeletal system usually occur suddenly and their values exceed the forces generated during the animal's normal activity, resulting in bone fractures. Thus, the effectiveness of their use depends on the design itself and the properties of the material from which scaffolds made. The surface of the implant should ensure the adhesion and proliferation of bone tissue cells, while the scaffold should serve a supportive function until the bone is rebuilt and proliferated. Therefore, it is important to determine their mechanical properties depending on the function performed, the parameters of the preparation of the structure, and the condition of the surface. The implant should be able to carry the loads generated in the limb during the initial healing phase, when the bone is too weak even if there is a momentary increase in load, such as a total load on the limb when tripping or jumping. The gradual loss of mechanical properties should be temporally correlated with the ability of the growing bone tissue at the defect site to carry the load [2]. A key innovation of our PLA implants is possibility of easy incorporation of bone growth inhibitors like hydroxyapatite in a gel form, platelet-rich plasma gels or a gels with stem cells, which can be induced into the internal porosity of the cylinders. This feature not only aids in bone regeneration but also aligns with the natural healing process, enhancing the implant's effectiveness and integration with the animal's bone structure.

The purpose of this study was to determine the mechanical properties of polymeric implants used in veterinary medicine produced by incremental technologies and the effect of degradation on their change. Polylactide (PLA) implants have been manufactured by CABIOMEDE in a selection of dimensions, which can allow to be used in bone defects both for small and large dogs.

2. Materials and Methods

2.1.3D printing of PLA implants

CABIOMEDE's veterinary implants – figure 1. made from Evonik's polylactide (PLA) using FDM printing technology on CABIOMEDE's proprietary machine were studied. Machine used for scaffolds manufacturing was CABIOMEDE own modification based on open-source Prusa MK3S developed with full metal hot end, active heated chamber and modified layer cooling system. G-code consisting printing parameters (nozzle temperature - 215°C, bed temperature - 60°C) and machine paths were developed using previously developed Python script. The layer thickness was 0.1 mm and the diameter of the printing nozzle was 0.4 mm. The detailed construction and printing algorithm was proprietary to CABIOMEDE. The dimensions of the tested implants depend on the size of bone defect and the breed of dog – table 1.

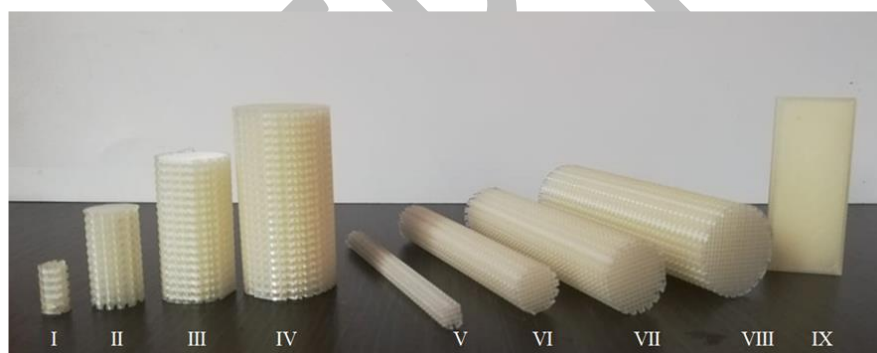


Figure 1 Implants by CABIOMEDE

Table 1 Dimensions of implants

GROUP		I	II	III	IV	V	VI	VII	VIII	PLATE
DIMENSION [mm]	D	6	12	18	24	6	12	18	24	20
	H	12	24	36	18	80	80	80	80	50
	B	–	–	–	–	–	–	–	–	5

The test material consisted of cylindrical specimens used to determine mechanical properties during static compression and bending tests, while rectangular plates were prepared to test the wettability and surface energy of the material – Figure 2. For each test series, 5 samples were prepared. To evaluate the mechanical properties of the implants after sterilization, they were packaged in paper-foil sleeves, and then steam sterilization was carried out at 121 °C, at 112

KPa using a SUN Class B 18L autoclave. The process was carried out in 1 (PS_1), 2 (PS_2), and 3 cycles (PS_3), 20 min each, respectively.

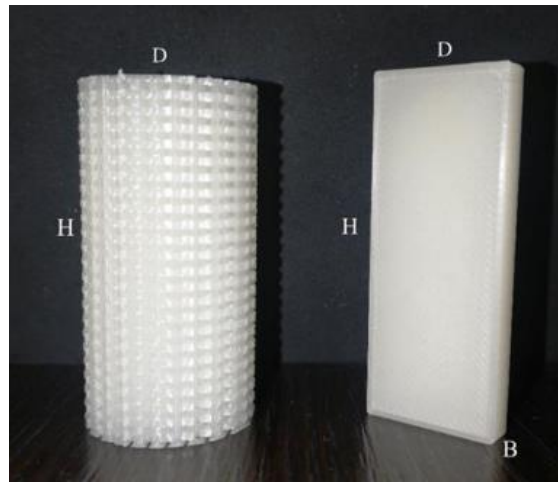


Figure 2 Geometric form and dimensions of the samples

2.2. *In vitro* tests

In order to assess the biodegradability of the implants and strength properties, implants were exposed for one (R1), three (R3) and six (R6) months in Ringer's solution simulating real conditions in the dog's body. The samples were placed in a thermal cycler at 38 ± 1 °C. After simulating the tissue environment in Ringer solution, pH=6,5 (8,6 g - NaCl, 0,3 g - KCl, 0,33 g - CaCl₂, 1000 dm³ water) the samples were subjected to a drying process at 23 ± 1 °C. The weight of the implants was then measured using a RADWAG electronic laboratory balance. Before the study, the weight of the implants was measured at the beginning of the study and after exposure to Ringer's solution.

2.3. Mechanical properties

Static uniaxial compression testing was performed according to the recommendations of PN-EN ISO 604:2006 [21] at a constant speed of $v = 1$ mm/min on an MTS Criterion testing machine in both longitudinal and transverse directions. The tests were performed at room temperature. Specimens of 6x12, 12x24, 18x36, and 24x48 [mm] (groups I - IV) were tested in

the initial state, for each sterilization cycle, as well as exposure to Ringer's solution. Five samples were tested for each variant. The static three-point bending test was carried out according to the recommendations of PN-EN ISO 178: 2019 [20] at a constant speed of $v = 5$ mm/ min at room temperature on an MTS Criterion testing machine. The distance between the crossbars was $l = 52$ mm and the diameter of the rollers on which the specimen was placed was $d = 5$ mm. The test was carried out for implants with a length of $L = 80$ mm and diameters D of 6, 12, 18, and 24 [mm], respectively (groups V- VIII) in the initial state, after each sterilization cycle and exposure to Ringer's solution. The bending test was performed on 5 samples of each variant.

2.4. Surface morphology

Observations were made on a Leica optical microscope at magnifications of 50x, 100x, 500x, and 1000x. Areas of delamination or cracks in the print path as well as samples in the initial state were observed. Scanning electron microscopy (SEM), TESCAN VEGA 4 under low vacuum conditions at 15 keV using a secondary electron detector SE was carried out. The observations were made for the initial state and the implants after static compression and bending tests.

2.5. Chemical composition test

Qualitative analysis of the chemical composition was performed using a scanning electron microscope equipped with Energy dispersive X-ray spectroscopy (EDS) equipment. The study was carried out at 15 keV. Qualitative analysis was performed for the implant at baseline and after exposure to a simulated tissue environment to identify the precipitate deposition from the Ringer's solution.

2.6. Wettability tests of PLA surfaces

To determine the surface wettability of the selected samples, wetting angle measurements were carried out using the sitting drop method, and surface free energy (SEP) measurements were carried out using the Owens-Went method. Young's equation was used to determine the surface energy. Measurements were made with demineralized water (w) and diiodomethane (d) at room temperature $T = 23 \pm 1$ °C. Polar and dispersion components for distilled water were $\gamma_{sp} = 51.0$ [mJ/m²] and $\gamma_{sd} = 21.8$ [mJ/m²] and for diiodomethane: $\gamma_{sp} = 6.7$ [mJ/m²] and $\gamma_{sd} = 44.1$ [mJ/m²]. Measurements were made on a test stand, which consisted of a SURFTENS UNIVERSAL goniometer from OEG and SURFTENS 4.5 software. Five drops of distilled water and 1 mm³ diiodomethane were applied successively to the surface of each sample. Angle measurements were taken 15 seconds after placing the drop on the test material to stabilize it. One measurement was made for 60 seconds with a sampling frequency of 1 Hz. The average values of the wetting angle and surface free energy were determined from the tests.

2.7 Statistical analysis

In the study, for the mechanical research method, the sample populations were presented as means with standard deviation (SD) for quantitative data. In order to determine the significance of differences for $p < 0.05$, the one-way analysis of variance (ANOVA) for the obtained results were used. Each test was performed on five different scaffold.

3. Results

3.1. *In vitro* testes

The results of the weight measurements are shown in Figure 3a. An increase in the weight of the samples after one and three months of soaking was observed as a result of salt deposition from the Ringer solution. Further exposure to Ringer solution resulted in a decrease in implant weight of the implants due to the detachment of the outer layers of the specimen, Figure 3b.

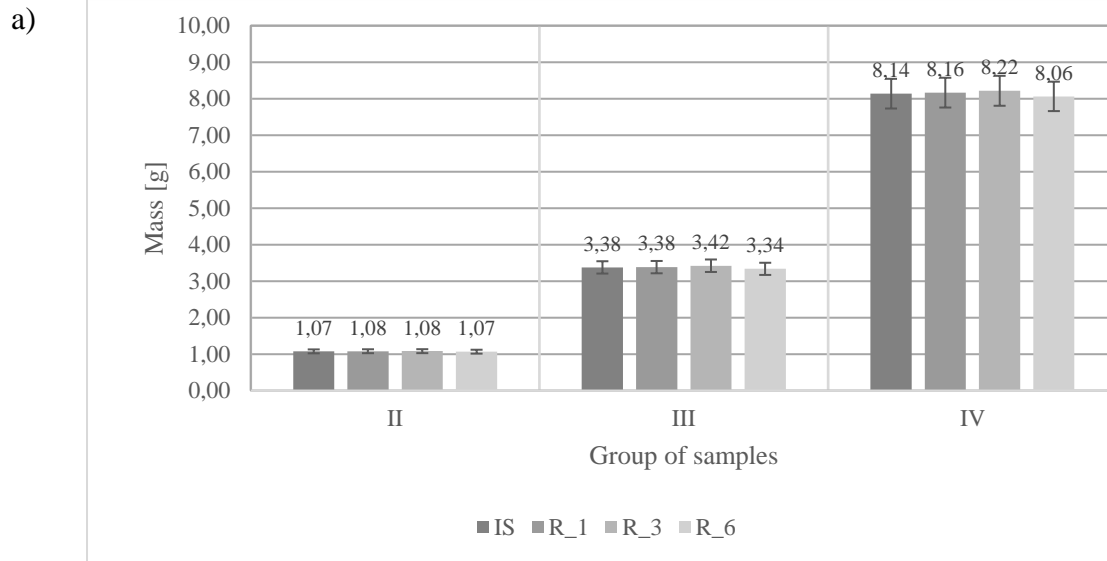


Figure 3 Implants: a) weight measurements,, b) breaking off the outer layer

3.2. Mechanical properties

The results of the static compression test in the longitudinal direction are shown in Table 2. The stress-strain relationship is shown in Figure 4.

Table 2 Results of the static compression test in the longitudinal direction

	12X24- GROUP II			18X36- GROUP III			24X48- GROUP IV		
	F_{max} [N]	R_{cw} [MPa]	E [MPa]	F_{max} [N]	R_{cw} [MPa]	E [MPa]	F_{max} [N]	R_{cw} [MPa]	E [MPa]
IS	704 ± 166	6 ± 1	476 ± 5	1300 ± 270	5 ± 1	408 ± 6	2160 ± 603	5 ± 1	422 ± 3
R_1	464 ± 25	4 ± 0.2	540 ± 1	2270 ± 220	9 ± 1	439 ± 5	5064 ± 112	11 ± 0.3	121 ± 5
R_3	1155 ± 170	10 ± 2	527 ± 20	2420 ± 230	10 ± 1	425 ± 11	4914 ± 124	11 ± 0.3	455 ± 3
R_6	123 ± 1	1 ± 0.01	48 ± 3	169 ± 85	1 ± 0.3	45 ± 16	277 ± 163	1 ± 0.4	48 ± 13
S_1	1219 ±	11 ± 2	534 ± 16	1158 ± 358	5 ± 1	461 ± 12	4206 ±	9 ± 0.8	470 ± 16

	195						374		
S_2	1134 ±	10 ± 1	594 ± 19	1965 ± 103	8 ± 0.4	489 ± 5	3766 ±	8 ± 0.6	495 ± 27
	156						246		
S_3	832 ± 158	7 ± 1	585 ± 4	1759 ± 246	7 ± 1	502 ± 1	2733 ±	6 ± 1	500 ± 3
							416		

In group II, after 1-month exposure to Ringer's solution, a decrease in compressive strength was observed (IS_gr_II R_{cw} = 6 MPa, R1_gr_II R_{cw} = 4 MPa), while after 3 months of soaking, the compressive strength of the implants increased compared to baseline (R3_gr_II R_{cw} = 10 MPa). Moreover, the effect of soaked after 1 and 3 months was significant ($p < 0.05$) for the value of mechanical properties for scaffold. For group III and IV samples, 1- and 3-month exposure to Ringer's solution resulted in an increase in compressive strength (R1_gr_III R_{cw} = 9 MPa, R3_gr_III R_{cw} = 10 MPa) compared to baseline (IS_gr_III R_{cw} = 5 MPa) ($p < 0.05$). For all groups of specimens, 6 months of exposure to Ringer's solution resulted in a decrease in longitudinal compressive strength to a value of about 1 MPa. Implants after sterilization, regardless of the group, showed higher compressive strength values (S1_gr_IV R_{cw} = 9 MPa, S2_gr_IV R_{cw} = 8 MPa, S3_gr_IV R_{cw} = 6 MPa) than the baseline condition (IS_gr_IV R_{cw} = 5 MPa) ($p < 0.05$), except for samples after 1 sterilization cycle from group III, where the maximum value of force F_{max} decreased by 1 MPa. The highest value of modulus of elasticity (S3_gr_III E = 502 MPa) ($p < 0.05$) is characterized by samples after 3 sterilization cycles, while the lowest value is for samples after 6 months of exposure to Ringer's solution (R6_gr_III E = 48 MPa) ($p < 0.05$).

The results for group I (6x12 mm) were not included in the analysis due to their miniaturized geometric form. The size of the specimens made it impossible to perform a static compression test due to the small contact area with the grips of the testing machine, especially for specimens that had degraded.

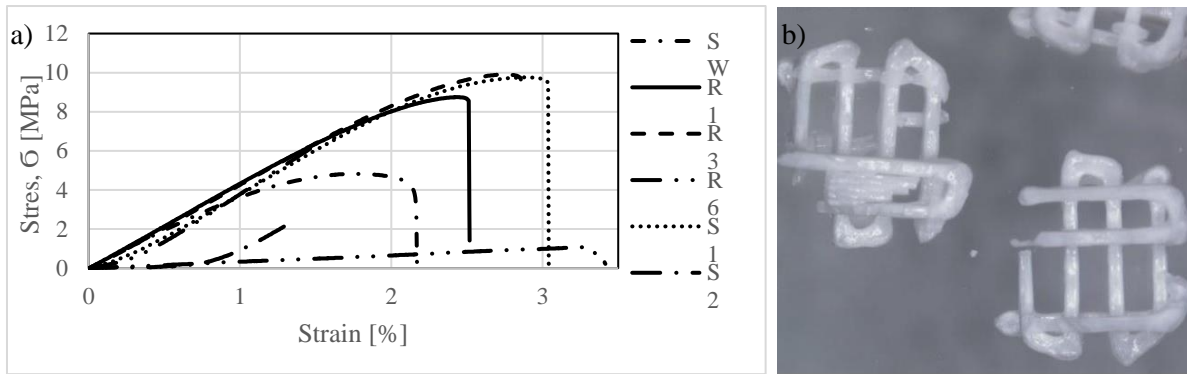


Figure 4 Results of static uniaxial compression test in the longitudinal direction: stress-strain relationship for samples with dimensions of 18x36 mm, b) implants damaged as a result of degradation (group I)

Comparison of the specimen at baseline and after 6-month exposure to Ringer's solution following a compression test in the longitudinal direction – Figure 5.

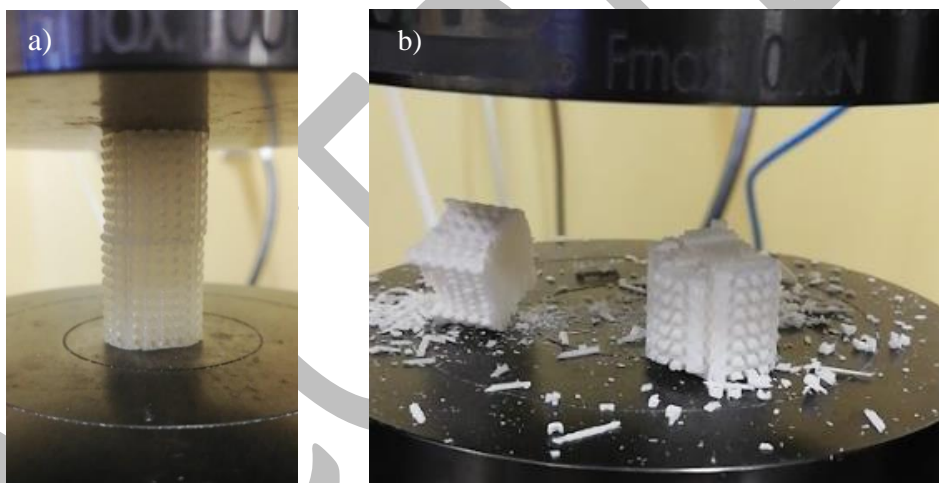


Figure 5 Samples for static uniaxial compression test in the longitudinal direction: a) initial condition (IS), b) after 6 months of exposure to Ringer's solution (R_6)

The results of the static compression test in the transverse direction are shown in Table 3. The stress-strain relationship is shown in Figure 6.

Table 3 Results of the static compression test in the transverse direction

	12X24- GROUP II			18X36- GROUP III			24X48- GROUP IV		
	F _{max} [N]	R _{cp} [MPa]	E [MPa]	F _{max} [N]	R _{cp} [MPa]	E [MPa]	F _{max} [N]	R _{cp} [MPa]	E [MPa]
IS	310 ± 80	1 ± 0.3	102 ± 6	1850 ± 100	2.8 ± 0.1	60 ± 19	384 ± 171	0.33 ± 0.14	9.3 ± 2
R_1	600 ± 110	2 ± 1.5	104 ± 19	2000 ± 190	3.5 ± 0.2	55 ± 10	2720 ± 268	2.36 ± 0.2	66 ± 12
R_3	480 ± 110	1.65 ± 0.4	73 ± 14	404 ± 300	0.6 ± 0.3	53 ± 8	1150 ± 576	1 ± 0.5	55 ± 9
R_6	40 ± 30	0.1 ± 0.1	13 ± 5	16 ± 11	0.03 ± 0.01	31 ± 5	160 ± 38	0.14 ± 0.03	17 ± 8

S_1	230 ± 90	0.2 ± 0.2	37 ± 19	1288 ± 264	2 ± 0.3	32 ± 6	2360 ± 340	2 ± 0.3	64 ± 2
S_2	180 ± 70	0.3 ± 0.02	60 ± 40	325 ± 86.4	0.5 ± 0.09	46 ± 11	420 ± 342	0.4 ± 0.3	31 ± 13
S_3	70 ± 10	0.3 ± 0.03	14 ± 2	59.3 ± 36.1	0.1 ± 0.04	35 ± 12	62 ± 7	0.05 ± 0.006	19 ± 0.4

The highest compressive strength was demonstrated by implants after 1 month of soaking in Ringer's solution (R1_gr_II R_{cp} = 2 MPa, R1_gr_III R_{cp} = 3.5 MPa, R1_gr_IV R_{cp} = 2.4 MPa) ($p < 0.05$). On the other hand, the lowest compressive strength in the transverse direction was found in group II and III implants after 6 months of exposure to Ringer solution (R6_gr_II R_{cp} = 0.1 MPa, R6_gr_III R_{cp} = 0.3 MPa) and in group IV samples after 3 sterilization cycles (S3_gr_IV_ R_{cp} = 0.05 MPa) ($p < 0.05$). In groups II and III, the lowest elastic modulus (E) was recorded for samples after 6 months of soaking in Ringer solution (R6_gr_II E = 13 MPa, R6_gr_III E = 31 MPa) while in group IV for samples in the initial state (IS_gr_IV E = 9.3 MPa) ($p < 0.05$).

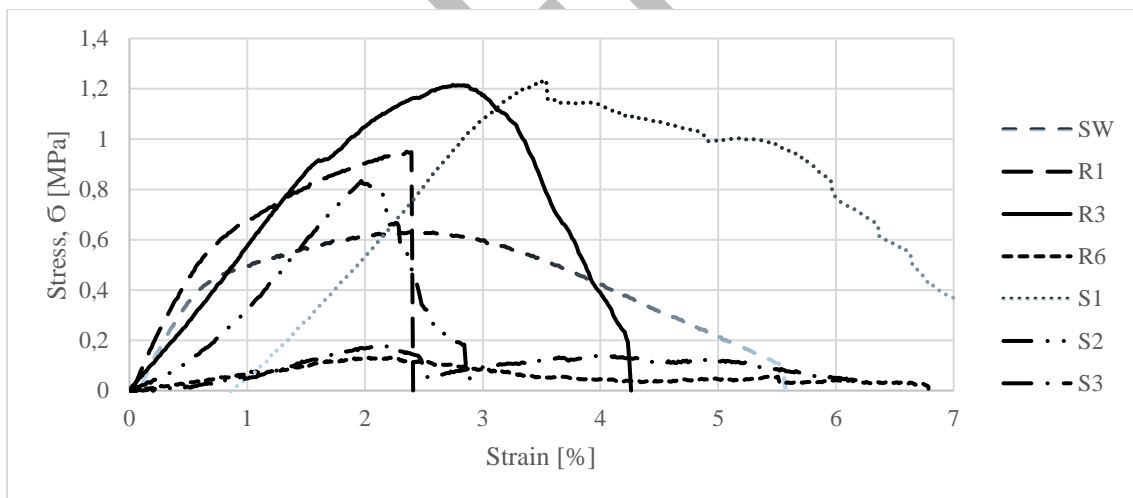


Figure 6 Static uniaxial compression test in the transverse direction: stress-strain relationship for samples with dimensions of 12x24 mm

A comparison of the sample at baseline and after 6 months of exposure to Ringer solution following a longitudinal compression test is shown in Figure 7.

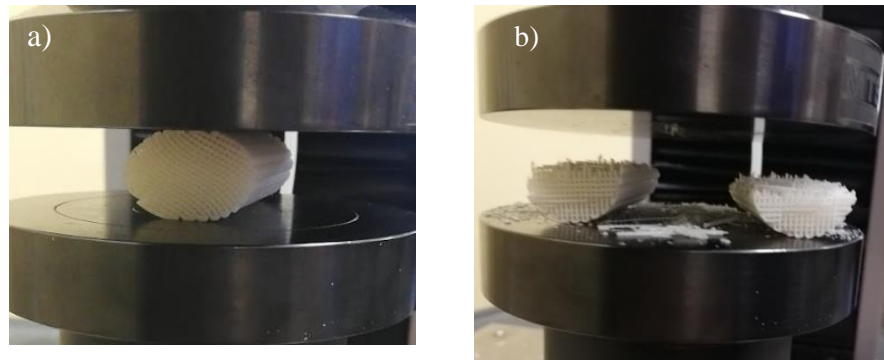


Figure 7 Samples after static uniaxial bending test in the transverse direction: a) initial condition (IS), b) after 6-month exposure to Ringer's solution (R_6)

The results of the static three-point bending test are shown in Table 4, while the samples after the test are shown in Figure 8.

Table 4 Results of the three-point bending test

	6X80 GROUP V			12X80 GROUP VI			18X80 GROUP VII			24X80 GROUP VIII		
	F _{max} [N]	R _g [MPa]	f [mm]	F _{max} [N]	R _g [MPa]	f [mm]	F _{max} [N]	R _g [MPa]	f [mm]	F _{max} [N]	R _g [MPa]	f [mm]
IS	9840 ±	348 ±	2.105	97748 ±	865 ±	1.4 ±	186160	732 ±	1.8 ±	350613	775 ±	2.7 ±
	1200	43	± 0.33	14665	129	0.35	± 26670	105	0.3	±	310	1.5
										140884		
R_1	9125 ±	322 ±	1.5 ±	92461 ±	818 ±	1.2 ±	134890	530 ±	0.99 ±	333615	738 ±	2 ±
	2300	80	0.4	8611	76	0.4	± 27680	109	0.3	± 46826	103	0.8
R_3	4349 ±	154 ±	0.67 ±	43675 ±	386 ±	0.5 ±	71690 ±	382 ±	0.57 ±	183621	406 ±	1.4 ±
	900	31	0.11	17123	151	0.2	13020	51	0.06	± 4776	11	0.06
R_6	2313 ±	81 ±	0.26 ±	796 ±	7 ± 1.9	0.2 ±	880 ±	3 ± 4	0.08 ±	1390 ± 0	6 ± 0	0.2 ±
	1200	40	0.2	210		0.06	110		0.04			0
S_1	4875 ±	173 ±	0.62 ±	40343 ±	357 ±	0.4 ±	66890 ±	236 ±	0.54 ±	218326	483 ±	1.1 ±
	900	31	0.08	8835	78	0.1	19110	75	0.1	± 12584	28	0.06
S_2	2513 ±	89 ±	0.47 ±	29830 ±	257 ±	0.3 ±	56790 ±	223 ±	0.44 ±	111971	248 ±	0.6 ±
	1300	46	0.42	6172	55	0.06	22530	87	0.2	± 15162	34	0.07
S_3	2352 ±	83 ±	0.47 ±	19206 ±	170 ±	0.02 ±	28310 ±	111 ±	0.32 ±	80263 ±	178 ±	0.5 ±
	600	20	0.27	3359	29	0.03	6220	24	0.1	7982	18	0.07

The highest flexural strength values were obtained for the samples in the initial state in all groups (IS_gr_V R_g = 348 MPa, IS_gr_VI R_g = 865 MPa, IS_gr_VII R_g = 732 MPa,

IS_gr_VIII $R_g = 775$ MPa) ($p < 0.05$). Soaking in Ringer's solution affected the decrease in flexural strength (R_g) in all groups of samples. The lowest flexural strengths are characterized by samples after 6-month exposure to Ringer's solution (R6_gr_V $R_g = 81$ MPa, R6_gr_VI $R_g = 7$ MPa, R6_gr_VII $R_g = 3$ MPa, R6_gr_VIII $R_g = 6$ MPa) ($p < 0.05$). In contrast, as the number of sterilization cycles increased, the flexural strength decreased (S1_gr_V $R_g = 173$ MPa, S2_gr_V $R_g = 89$ MPa, S3_gr_V $R_g = 83$ MPa) ($p < 0.05$). The highest values of the deflection arrow were recorded for the samples under the initial conditions, while the samples subjected to 6-month exposure to Ringer's solution have the lowest deflection arrow (IS_gr_VIII $f = 2.7$ mm, IS_gr_VIII $f = 0.2$ mm) ($p < 0.05$).

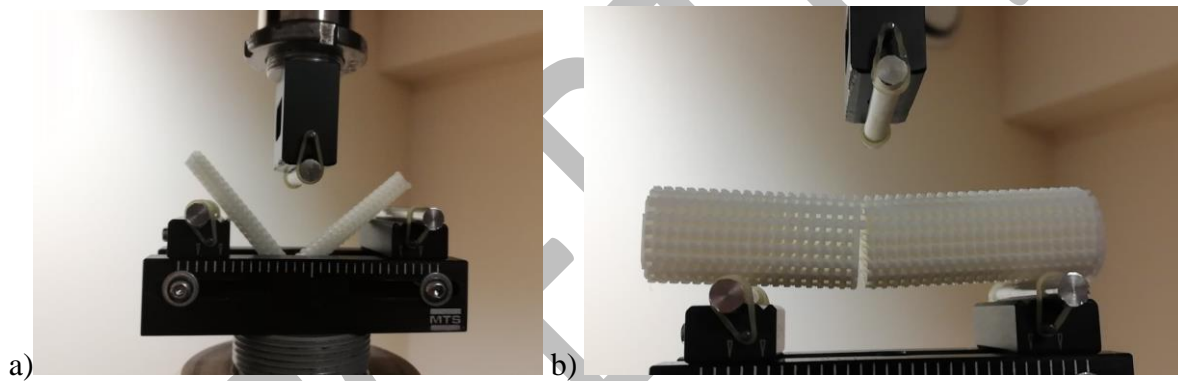


Figure 8 Samples after static three-point bending test: a) group VI, b) group VIII

3.3. Macroscopic and microscopic evaluation

Macroscopic observations of the surface of the PLA material from which the implants were made showed a change in the color of the implants after exposure to Ringer solution – Figure 9. Areas of delamination or cracks in the print path were observed. The pore size and the print path is shown in Figure 9, while the average pore size is shown in Table 5. Observations of the implants after a static compression test are shown in Figure 10 and after a three-point bending test in Figure 11.

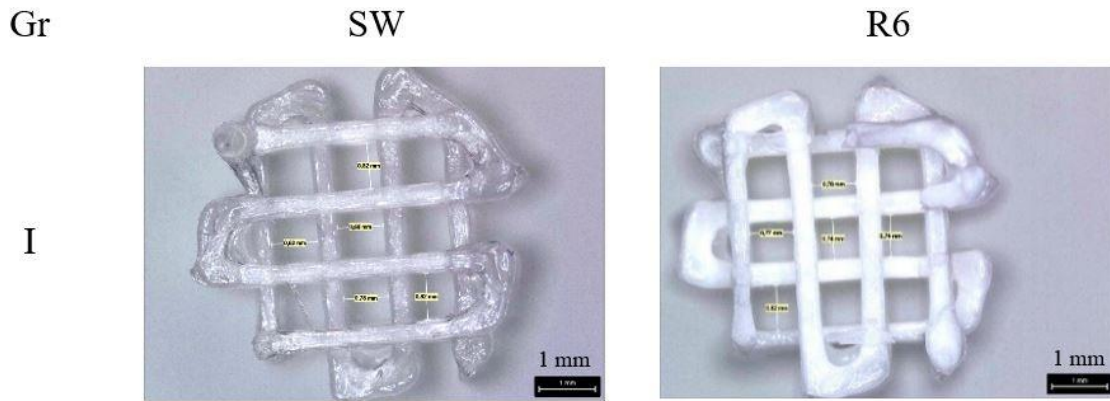


Figure 9 Measurement of pore size and print path thickness, visible color change of implants exposed to Ringer's solution, mag. 100x

Table 5 Average size of pores and implant printing paths depending on the variant

	GROUP I		GROUP II		GROUP III		GROUP IV	
	pores	path	pores	path	pores	path	pores	path
IS	0.8	0.4	0.8	0.38	0.8	0.38	0.79	0.39
R_1	0.76	0.38	0.79	0.39	0.8	0.38	0.78	0.38
R_3	0.76	0.37	0.8	0.38	0.8	0.39	0.77	0.39
R_6	0.77	0.39	0.78	0.4	0.78	0.38	0.79	0.39
S_1	0.77	0.37	0.77	0.39	0.8	0.39	0.79	0.39
S_2	0.8	0.38	0.8	0.38	0.8	0.38	0.79	0.39
S_3	0.81	0.38	0.79	0.38	0.8	0.38	0.78	0.39

SW_gr_IV

R1_gr_IV_SPSP

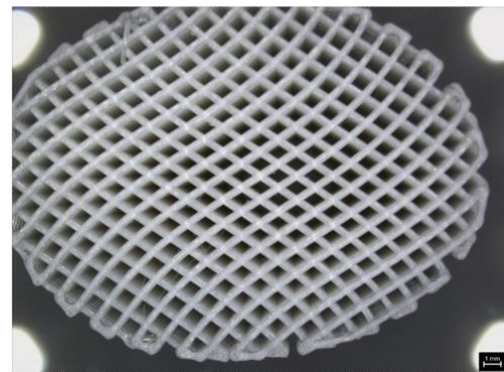
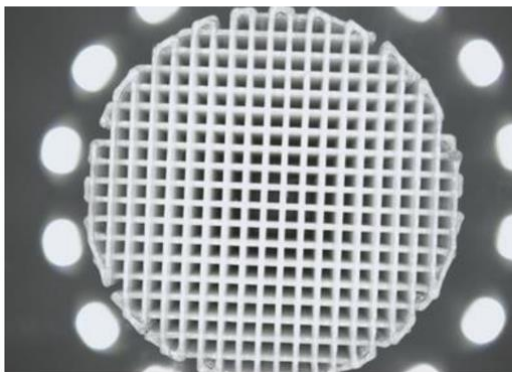


Figure 10 Samples (group IV) after a static compression test in the transverse direction, mag. 25x

In the design of the implants in the initial state, after one month of soaking and one sterilization cycle, no cracks were observed but they were deformed - there was a shift in the print paths under load. The largest deformation was observed in the sample after one month of exposure to Ringer's solution, which shows the highest compressive strength in the transverse direction (R_{cp}) in the group. Observations of the failure points of the samples after the static compression and three-point bending tests are shown in Figure 11.

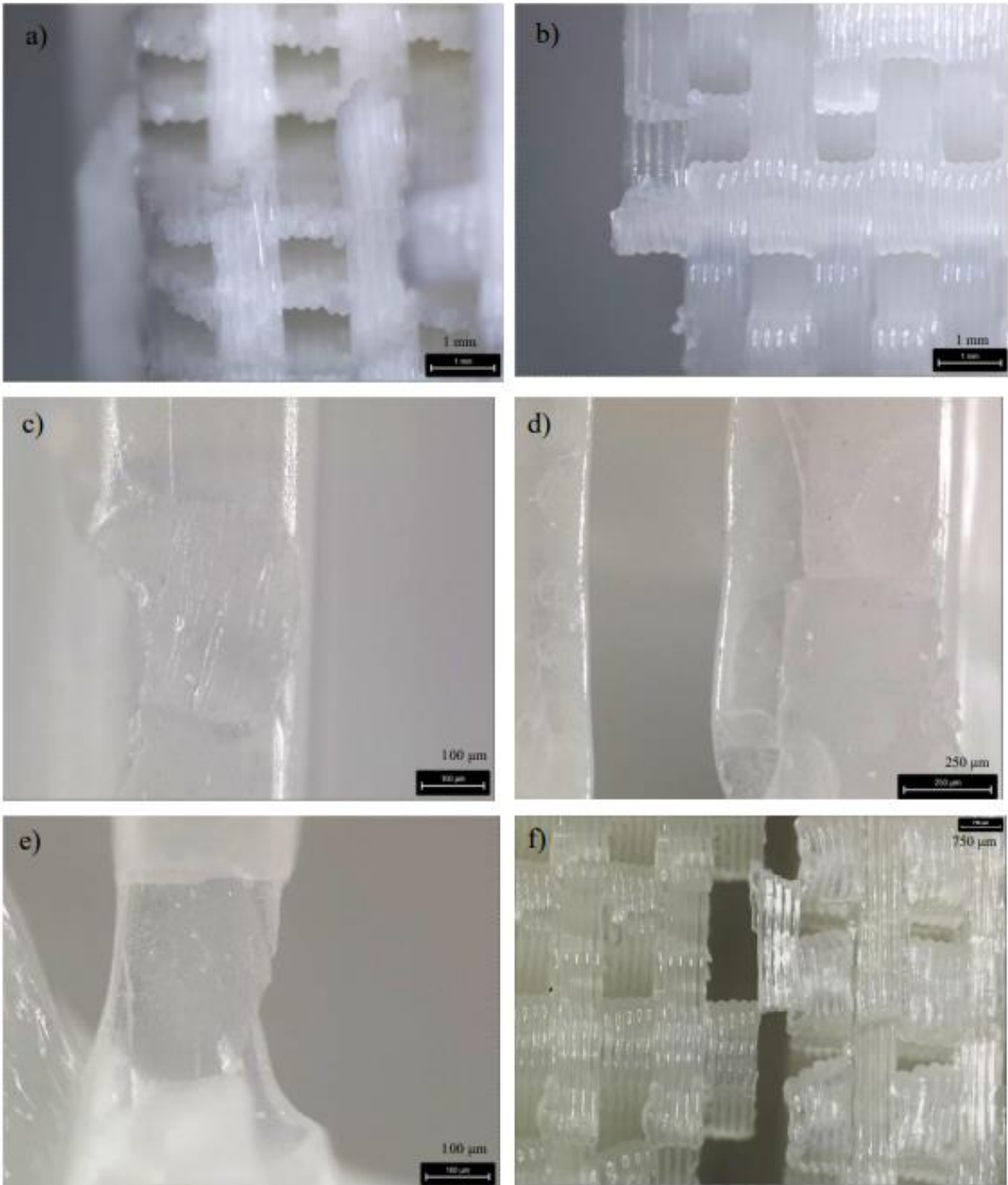


Figure 11 Damage after the three-point bending test: a) breakage- R6_gr_III sample, mag. 100x, b) cracking- S3_gr_IV mag. 100x, c) path breakage - S3_gr_I mag. 1000x, d) cracks paths- R6_gr_II mag. 500x, e) R6_gr_I mag. 1000x, f) IS_gr_VIII mag. 100x

Microscopic observations (SEM) of the samples in their initial state after static compression testing are shown in Figures 12-13. In the images, the irregularity of the print paths can be seen as where they are connected.

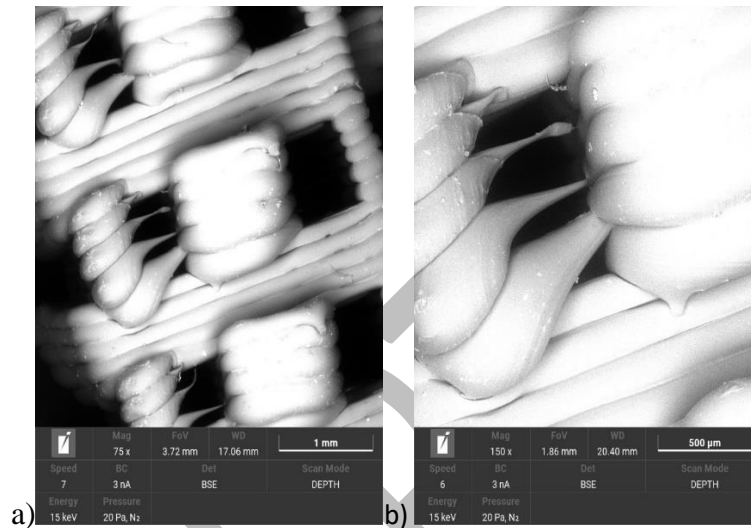


Figure 12 SEM observation of print paths in the initial state a) mag. 75x, b) mag. 150x

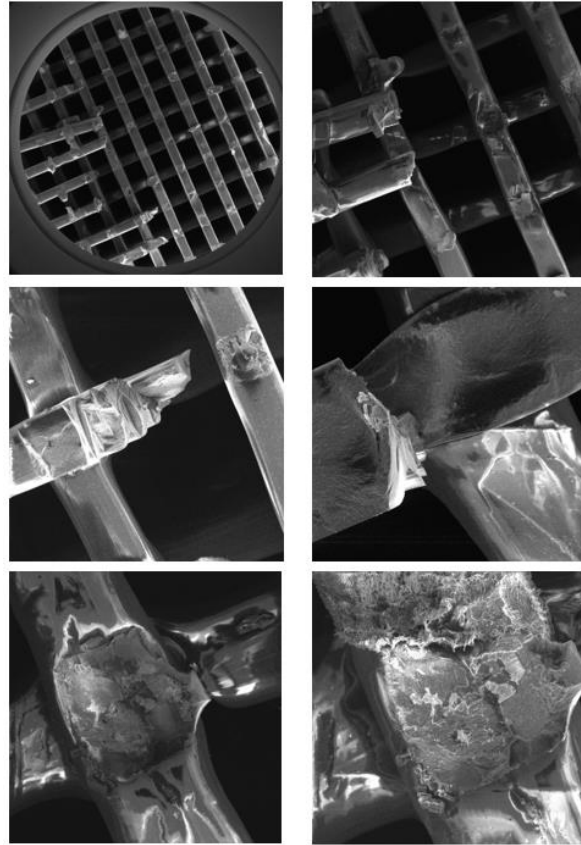


Figure 13 Microscopic observations of samples after 6-month exposure to Ringer's solution – SEM: a) mag.75x, b) mag. 100x, c) mag. 150x, d) mag. 250x, e) mag. 250x, f) mag. 300x

3.4. Chemical composition test

Figure 14 shows a qualitative analysis of the chemical composition of the implant in the initial state (a) and after 6 months of exposure to Ringer's solution (b). The EDS microanalysis of the implant surface in the initial state in the selected area (Figure 14a) showed the presence of carbon (55.7%) and oxygen (44.3%). Microscopic observation of the implant exposed to Ringer's solution additionally revealed crystals formed by salt deposition from the Ringer solution (Figure 14b). Their analysis revealed the presence of sodium (2.5%) and chlorine (1.4%).

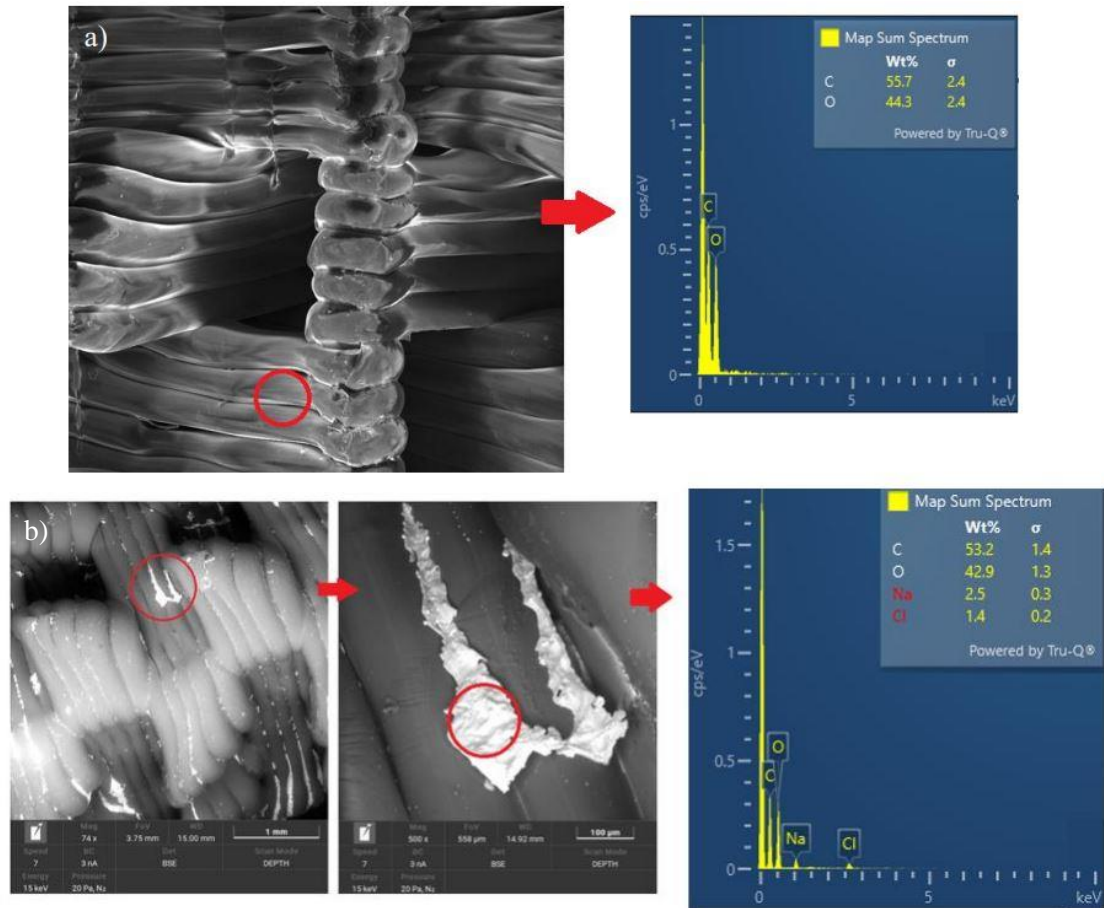


Figure 14 Basic microanalysis of implant chemical elements a) in the initial state, b) after six months of exposure to Ringer's solution

3.5. PLA surface wettability tests

The results of the wetting angle and surface energy of PLA surfaces are shown in Table 6. Both the implant surface at baseline and after one month of exposure to Ringer solution shows a hydrophilic character ($< 90^\circ$). The soaking process slightly affected the increase in the wetting angle ($p < 0.05$). Hydrophilicity is a desirable feature for tested implants, as it determines the rate of cell adsorption and adhesion, which is particularly important with scaffolds [27, 28].

Table 6 Results of contact angle and surface energy

Sample	Distilled water	Diiodomethane	γ_s^p , mJ/m ²	γ_{sd} , mJ/m ²	γ_s , mJ/m ²
	Contact angle, θ°	Contact angle, θ°			
IS	37.65 ± 2.82	44.29 ± 1.65	42.23 ± 3.50	16.27 ± 1.43	58.50 ± 2.10
R1	46.98 ± 1.65	45.33 ± 1.14	33.55 ± 1.99	18.13 ± 0.94	51.67 ± 1.17

4. Discussion

The additive techniques in dog orthopedics has recently emerged as a valuable approach in fabricating individualized implants for receiver-specific needs. The scaffolds by FDM methods made of biodegradable PLA materials simulating bone structure and function in physical and chemical properties can promote growth and reconstruction of bone structure. Scaffolds should have sufficient elastic modulus and compressive strength to support bone growth and should be characterised by a 3D pore porous structure, be able to degrade gradually with new bone growth [2, 32]. Our study introduces 3D printed PLA implants specifically designed for scenarios where biodegradability offers significant advantages. This is particularly relevant in young and growing animals (especially near joint regions), as well as in non-load bearing conditions (e.g. skull, or missing bone fragments in bridging plating). The biodegradable nature of PLA avoids the need for a second surgery to remove the implant, which is especially beneficial in growing animals where the dynamics of bone growth are a critical factor. For the proposed PLA scaffolds in different sizes, tests were carried out to meet the mechanical and degradation requirements of load-bearing bone scaffolds. The static compression and three-point bending tests were carried out. In this study, the functional properties of the implants were evaluated in the initial state, as well as after simulating the tissue environment in Ringer solution and after steam sterilization. Compressive strength is an important property of an implant due to the forces generated during the animal's gait and the loads associated with its weight. The tested implants show a higher compressive strength in the longitudinal direction ($R_{cw} IS_GR_III = 5$ MPa) than in the transverse direction ($R_{cp} IS_GR_III = 2.3$ MPa), based on which it can be concluded that they can be used successfully in long limb bones, where the dominant amount of load is transferred along the bone. The longitudinal compressive strength values of all tested implant variants are in the range of 1 to 11 MPa. According to the literature, the compressive strength of cancellous bone is 4-12 MPa [22]. The compressive strength R_c in the longitudinal and transverse directions increased after one month of exposure to Ringer's solution compared to baseline ($R_{cw} R1_GR_II = 9$ MPa, $R_{cp} R1_GR_III = 3.5$ MPa). In some cases, in the first phase of degradation, the mechanical properties of the material can be improved by additional cross-linked structures, as confirmed in their study by the authors of the paper [23]. A significant decrease in compressive strength ($R_{cw} R6_GR_II = 1$ MPa, $R_{cp} R6_GR_III = 0.03$ MPa) was observed for samples subjected to 6-month exposure, indicating progressive degradation of the material. The degradation of PLA during the sixth month of incubation in Ringer's solution is confirmed by the study of Morawska-Chochól et al., during which a change

in the pH of the solution and an apparent weight loss of the samples were noted after this period [18]. Our study also found a weight loss in implants exposed to Ringer's solution for six months ($R6_{gr_IV} = 8.062$ g), while the weight of implants exposed for three months ($R3_{gr_IV} = 8.216$ g) increased concerning the baseline condition ($IS_{gr_IV} = 8.136$). The sterilized samples exhibit higher values of compressive strength values in the longitudinal direction ($R_{cw} S3_{gr_III} = 7$ MPa) than the baseline condition ($R_{cw} S3_{gr_III} = 5$ MPa). In the case of transverse compression, the effect of sterilization on compressive strength (R_{cp}) cannot be determined. During the static longitudinal compression test, the highest value of elastic modulus (E) was characterized by samples after 3 sterilization cycles ($R3_{gr_II} = 585$ MPa, $R3_{gr_III} = 502$ MPa, $R3_{gr_IV} = 500$ MPa). In a study of the effect of sterilization parameters on the mechanical properties of PLA, the authors also described an increase in the elastic modulus of the samples after sterilization [24]. The lowest elastic modulus is characterized by samples after 6 months of exposure to Ringer's solution ($gr_II = 48$ MPa, $gr_III = 45$ MPa, $gr_IV = 48$ MPa). Progressive degradation caused the samples to become brittle [29, 30]. According to the authors [10], scaffolds show usefulness in bone surgery if their compression elastic modulus is between 0.4 and 350 MPa. In the case of longitudinal compression, implants in the initial state exceed this limit (about 450 MPa), but the results obtained for transversely compressed specimens fall within the mentioned range. Flexural strength determines the vulnerability of the implant in the event of bending forces acting in the direction perpendicular to it. Such loads can be generated, among other things, because of impact or fall. The flexural strength (R_g) of the specimens after each sterilization cycle was lower than the initial condition ($gr_VIII: IS = 775$ MPa, $S1 = 483$ MPa, $S2 = 48$ MPa, $S3 = 178$ MPa). A decrease in flexural strength after steam sterilization was also reported by Gogolewski et al. [8]. Microscopic observations and EDS analysis of the surfaces of the implants showed the presence of the elements sodium and chlorine, which resulted from soaking in Ringer's solution. Monthly exposure to Ringer's solution increased the hardness of PLA (74.8 ShA) compared to the baseline (73.4 ShA). In addition, the soaking process increased the degree of surface hydrophilicity. A decrease in surface energy values was also observed ($IS_{\gamma S} = 58.5$ mJ/m², $R1_{\gamma S} = 51.7$ mJ/m²). Measurement of the pore size and thickness of the print path showed no significant differences in the results depending on the condition of the implant. Implants in the initial state have an amorphous structure. After the exposure process in Ringer's solution, which caused hydrolytic degradation, a semicrystalline structure was identified. In addition, a color change in the implants was observed as a consequence of exposure to Ringer's solution. To further identify the effect of the degradability on the strength properties, the implants should be tested after prolonged exposure to Ringer's

solution. Measurements of the solution's pH should also be included in the study. The PLA implants described in our study are versatile. They can be used as final products in cases such as fracture non-union or for bone segment restoration post-tumor resection. Moreover, these implants can serve as semi-products for further CNC fabrication, offering a customizable solution based on the specific needs of each case. This adaptability extends the potential applications of these implants beyond current offerings in the market. CABIOMEDE 3D printed PLA implants represent a significant advancement in veterinary orthopedics, offering a biodegradable, customizable, and potentially more biocompatible alternative to traditional titanium implants. This innovation addresses specific needs in veterinary medicine, particularly for young and growing animals and in complex surgical procedures.

5. Conclusions

Based on the study, the following conclusions and observations were made:

- The implants have a higher compressive strength in the longitudinal direction than in the transverse direction. This is due to the geometry of the implant and the direction of the applied loads, as well as the orientation of the print.
- Hydrolytic degradation initiated by exposure to Ringer's solution resulted in a change in structure from amorphous (IS) to semicrystalline (R6). This indicates partial dissolution of the amorphous phase and thus an increase in the percentage of the crystalline phase, which caused the material to become brittle.
- Progressive degradation results in brittle fracture of the material, as can be observed in implants after 6 months of soaking.
- The steam sterilization process increases the elastic modulus of the material and decreases the flexural strength.
- The compressive strength values of PLA implants are similar to the properties of spongy bone.

Acknowledgements: The authors would also like to thank CABIOMEDE company for providing the scaffolds for testing.

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