applied sciences

MDPI

Article

Physical and Mechanical Properties of Composite Scaffolds with or without Collagen Impregnation

José Joaquín López Marcos [1,*,†], Vittoria Perrotti [2,*,†], Flavia Iaculli [2], Águedo Aragones [3], Cesar Augusto Magalhães Benfatti [4], Gabriel Leonardo Magrin [4], Adriano Piattelli [2] and Marco Aurélio Bianchini [1]

1 Department of Dentistry, Federal University of Santa Catarina (UFSC), s/n-Trindade, Florianópolis SC 88040-900, Brazil; bian07@yahoo.com.br
2 Department of Medical, Oral and Biotechnological Sciences, University G. d'Annunzio Chieti-Pescara, Via dei Vestini, 31, 66100 Chieti, Italy; f.iaculli@unich.it (F.I.); apiattelli@unich.it (A.P.)
3 Biocenter, Federal University of Santa Catarina (UFSC), s/n-Trindade, Florianópolis SC 88040-900, Brazil; aguedo@terra.com.br
4 Center for Education and Research on Dental Implants (CEPID), Federal University of Santa Catarina (UFSC), Florianópolis SC 88040-900, Brazil; cesarbenfatti@yahoo.com (C.A.M.B.); gabriel.magrin@posgrad.ufsc.br (G.L.M.)
* Correspondence: jjlmarcos@yahoo.com (J.J.L.M.); v.perrotti@unich.it (V.P.)
† These Authors contributed equally to this work.

Received: 11 September 2019; Accepted: 10 October 2019; Published: 12 October 2019

Abstract: This in vitro study aimed at evaluating the physical and mechanical properties of newly developed scaffolds of poly (lactic-co-glycolic acid) (PLGA) and biphasic ceramic (Hydroxyapatite HA + beta-tricalciumphosphate β-TCP) with or without collagen impregnation to be used for bone regeneration in the oral and maxillofacial district. Solvent casting and particle leaching techniques were used to produce the scaffolds, which were then divided into six groups according to PLGA/HA + β-TCP ratio and impregnation with collagen: G1 (50/50) + collagen; G2 (60/40) + collagen; G3 (40/60) + collagen; G4 (50/50); G5 (60/40); G6 (40/60). As control group, inorganic xenogenous bone was used. Structure and porosity were evaluated by scanning electron microscopy, and a chemical analysis was performed through an energy-dispersive spectrometer. Moreover, to evaluate the hydrophilicity of the samples, a wettability test was conceived, and finally, mechanical properties were examined by a compression test. High porosity and interconnectivity, resulting in a large surface area and great fluid retention capacity, were presented by the PLGA/HA + β-TCP scaffolds. In the composite groups, collagen increased the wettability and the mechanical resistance, although the latter was not statistically affected by the percentage of HA + β-TCP added. Further in vitro and in vivo studies are needed for a deeper understanding of the influence of collagen on the biological behavior of the developed composite materials and their potential, namely biocompatibility and bioactivity, for bone tissue regeneration.

Keywords: collagen; composite; hydrophilicity; polymers; wettability

1. Introduction

Polymeric materials are widely used as biomaterials because of their great flexibility in controlling their properties and processing through the manipulation of their composition and chemical structure [1–3]. Natural and mainly synthetic polymers are gaining a great deal of attention in the field of tissue engineering for bone and other mineralized tissue applications [1,2,4]. The synthetic polymers most commonly used for the production of scaffolds are polylactic acid (PLA), polyglycolic acid (PGA), and their copolymer polylactic-co-glycolic acid (PLGA).

PLGA is the most used because it offers better control of its properties by adjusting the monomer percentage [2,3]. In addition, it is nontoxic, as the products resulting from its degradation are lactic acid and glycolic acid, which are eliminated in the form of carbon dioxide and water [3,5–7]. However, before being metabolized, the acids can induce adverse reactions, such as inflammation, in the surrounding tissues by altering the pH of the region. A technique used to neutralize the acidity of degradation products is the addition of alkaline substances [8]. In this light, ceramic particles could be used as additives to PLGA in order to provide a pH buffering effect at the polymer surface and, thus, avoid the local reduction of pH, which could in turn damage bone cell health and hamper new bone growth [9].

Calcium phosphates, besides being alkaline materials, have calcium and phosphorus, which are natural constituents of the bone and, therefore, would neutralize the pH and increase the osteoconductive properties of PLGA [4,5,8,10–12]. The use of calcium phosphates combined with PLGA enhances alkaline phosphatase activity, which is crucial for osteoblastic differentiation [13] and acts as a reinforcement to improve the mechanical strength of the scaffold [3]. The main disadvantage of using PLGA is its hydrophobic nature, which prevents penetration and adhesion of the cells [14,15]. However, surface modification through the addition of natural polymers can be used.

Type I collagen could be a good option as it represents about 90% of the proteins in the bone extracellular matrix, is highly hydrophilic, it is considered a weak antigen [16], and an immune response against it is very rare [17]. Lynn et al. [16] reported that less than 3% of the patients presented adverse reaction, inflammation, and granuloma; hypersensitivity to collagen was found in 2% to 4% of patients, and only 1% presented allergy during the postoperative. Nevertheless, collagen could contribute to the hydrophilicity, biocompatibility, porosity, biodegradability, and osteoconduction of composite scaffolds. Collagen would interact with the various proteins mimicking the natural environment of the cells, and it could perform a structural role enhancing the mechanical properties [15,18–24]. Composites of collagen and Hydroxyapatite (HA) showed improved bioactivity and in vitro mineralization [25], higher bone formation, as well as enhancement of elastic modulus [26], when compared to collagen alone. Polymers coated with collagen have been shown to allow attachment, survival, and proliferation of human neural cells [17], increase and accelerate bone formation [27], and enhance the spreading of endothelial cells [28]. Some limitations, however, exist when working with collagen, as it is costly, it has a low biostability, owing to its rapid degradation in the biological environment, and there are problems related to its antigenicity [29].

The objective of this study was to develop a new class of composite porous scaffolds of PLGA and biphasic ceramic with or without collagen impregnation to be used for bone regeneration in the oral and maxillofacial district and to investigate their physical–mechanical properties in order to tailor their usage accordingly.

2. Materials and Methods

2.1. Production of PLGA/HA + Beta-Tricalciumphosphate (β-TCP) Composite Scaffolds

Resomer® LG 824 S poly (L-lactic-co-glycolic) 82:18 (Evonik, Essen, Germany) and Resomer® LT 706 poly(L-lactic acid-co-trimethylene carbonate) 70:30 (Evonik, Essen, Germany) were used as the matrix of the newly developed composite scaffold, with a ratio of 75:25. Whereas, biphasic calcium phosphate (70% HA 30% β-TCP) (Baumer, Mogi Mirim, São Paolo, Brazil), with a mean particle size of 50 μm, was used as the bioactive ceramic.

The composite scaffolds were produced following the methodology described in detail by Messias et al. [30] according to the solvent casting and particle leaching (SC/PL) method. Briefly, 10 g of polymer was dissolved in 100 mL of chloroform; after complete dissolution, HA-β-TCP was added in 40%, 50%, and 60% weight and homogenized. Then, sucrose, a porogenic agent with mean particle size of 500 μm (Merck®, Darmstadt, Germany), was added in the solution to produce scaffolds with 70% (w/w) of porosity. The obtained solution was poured into cubic molds. Once the solvent was

evaporated, the samples were washed with fully hydrolyzed poly (vinyl alcohol) (PVA) (P1763, Sigma Aldrich, Saint Louis, MO, USA) to remove the sucrose.

Six groups of newly developed composite scaffolds with different PLGA/HA+ β-TCP ratios were obtained, and inorganic xenogenic bone (Geistlich Bio-Oss Block®, Wolhusen, Switzerland) was used as control (Table 1).

Table 1. Experimental groups distribution.

Groups (PLGA/HA + β-TCP)	Number of Samples
G1 (50/50) + Collagen	$n = 13$
G2 (60/40) + Collagen	$n = 15$
G3 (40/60) + Collagen	$n = 15$
G4 (50/50)	$n = 13$
G5 (60/40)	$n = 13$
G6 (40/60)	$n = 15$
Control inorganic xenogenous bone	$n = 8$

[PLGA: polylactic-co-glycolic acid; Hydroxyapatite (HA) + beta-tricalciumphosphate (β-TCP): biphasic ceramic].

Half of the specimens were impregnated with collagen; specifically, a 10%vol solution of collagen was produced by diluting 10 g of hydrolyzed bovine type 1 collagen (Homeopatia Galênica Florianópolis, Santa Caterina, Brazil) in 100 mL of distilled water at 60 °C. After the solution reached room temperature, the samples were immersed in the liquid, being left under vacuum for 10 min, and then dried in the open air for approximately 72 h.

2.2. Structural Characterization and Chemical Analysis

The surface features, structure morphology, and interconnectivity of the pores of all scaffolds were analyzed by scanning electron microscopy (SEM). Briefly, one sample for each group (total: 7 samples) were cut with a scalpel and lightly sputter-coated with gold (SCD-050 Sputter Coater, New York, NY, USA) (average coating time: 2–3 min). The samples (surface and cross-section) were viewed with an SEM (HITACHI TM3030, Tokyo, Japan) with operating conditions of 15 kV accelerating voltage and mean working distance of 7 mm. The images (1280 × 1040 pixels) were captured with 3 scans using a frame-average technique.

To perform a compositional analysis for calcium and phosphorus concentration, an energy-dispersive spectrometer (EDS; TM3030, HITACHI, Tokyo, Japan) was used with an acquisition time of 10 s and accelerating voltage of 15 kV.

2.3. Evaluation of the Scaffold's Wettability

To assess the wettability of the composite scaffolds, the methodology described by Barbosa et al. was adapted, and the absorption of liquid was measured, taking into consideration that it has been demonstrated that the capillary effect can be used to verify the wettability of an implant [31]. The test consisted of introducing the 10 × 10 × 10 mm^3 scaffold into 1 mm of aqueous solution with methylthioninium chloride as a dye for 5 min. The parameters used to evaluate the wettability were the absorption time, the height reached by the liquid at the external surface of the sample, the ability of the liquid to reach the center of the scaffold, and the ability of the scaffolds to retain the liquid (measured as drop formation once the scaffolds were removed from the solution). A camera recorded all the parameters.

2.4. Mechanical Properties

The compressive strength and modulus of elasticity of each sample were measured dry and at room temperature using a ElectroForce®, 3300 Seria II (Bose, Eden Prairie, MN, USA). The samples with 10 × 10 × 10 mm^3 were compressed with a speed of 1 mm/min according to the ASTM D695-15 (Standard Test

Method for Compressive Properties of Rigid Plastics) [32]. For the composites, the maximum deformation adopted was 2 mm according to the ASTM D1621-16 (Standard Test Method for Compressive Properties of Rigid Cellular Plastics) [32] and maximum stress as the yield strength calculated with a deformation of 0.2%. In the control group, the maximum stress considered was the fracture stress. The modulus of elasticity (stiffness) was calculated along the elastic deformation portion.

2.5. Statistical Analysis

The Kruskall–Wallis non-parametric test followed by a non-parametric multiple comparison test was used to determine if the differences between the groups for both properties (compressive strength and modulus of elasticity) were statistically significant at a significance level of 5% ($p < 0.05$).

3. Results

3.1. Structure, Porosity, and Chemical Analysis of PLGA/HA+ β-TCP Scaffolds

Control scaffolds showed an interconnected pore structure with round morphology (Figure A1). The solvent casting and particle leaching technique (SC/PL) produced highly porous structures with heterogeneous pore sizes of 120, 220, and 680 µm. In addition to high porosity, the composites showed high interconnectivity, resulting in a larger surface area and greater fluid retention capacity than the control group. The ceramic particles were dispersed in the matrix forming agglomerations, which increased by increasing the amount of ceramic; these agglomerations were mainly detectable in groups G4, G5, and G6, whereas in groups G1, G2, and G3, a great portion of the ceramic particles was coated by collagen (Figure 1).

The collagen coating (groups G1, G2, and G3) covered the exposed ceramic particles and rounded the irregular pores within the PLGA matrix (Figure 2).

EDS analysis revealed the presence of carbon (C), oxygen (O), calcium (Ca), and phosphorus (P) (Figure 3). The carbon derived from the polymeric matrix as well as from the collagen (in G1, G2, and G3). Oxygen was present within the matrix and in the calcium phosphate particles. Calcium and phosphorus derived exclusively from calcium phosphates.

3.2. Wettability of PLGA/HA+ β-TCP Scaffolds

The presence of collagen (groups G1, G2, and G3) on the surface of the composite scaffold caused an increase in wettability, as shown in Table 2 and Figure 4, whereas the scaffolds without collagen (groups G4, G5, and G6) showed high hydrophobicity and did not allow the liquid to penetrate into their structures.

The ceramic/polymer ratio seemed to influence the wettability, mainly in the groups impregnated with collagen, and this was evident through the variation of the absorption time and drop formation (Table 2). G1 presented the greatest similarity with the control group (Figure A2), showing the absence of drop formation once it was extracted from the solution and complete absorption of the liquid.

3.3. Mechanical Properties

Both control and composites presented lower values of compressive strength than the bone tissue; however, the scaffolds coated with collagen showed values closer to the bone (Table 3). There was a statistically significant difference in terms of compressive strength between all the scaffolds and the control; moreover, groups with collagen (G1, G2, and G3) showed a significantly higher compressive strength than noncollagen groups (G4, G5, and G6) (Table 3), indicating that collagen improved the mechanical resistance of the scaffold materials.

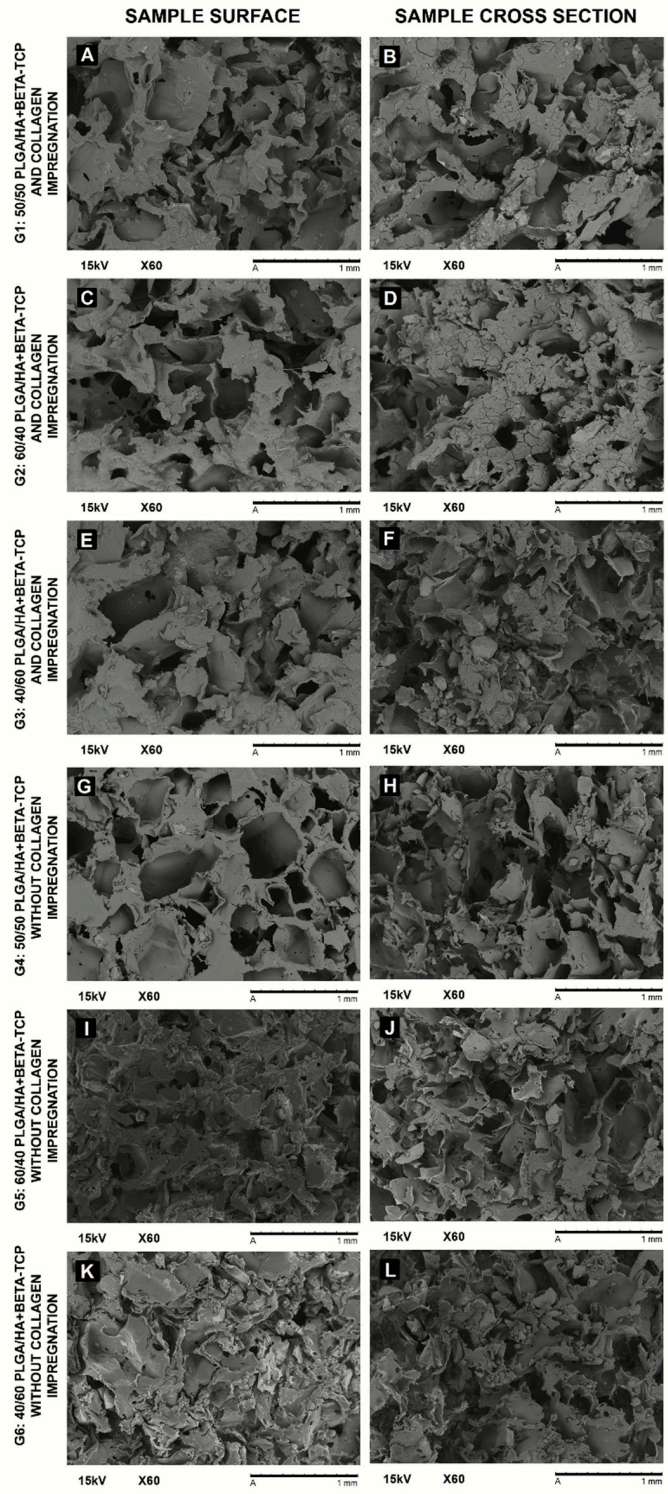

Figure 1. Scanning electron microscopy surface (**A**,**C**,**E**,**G**,**I**,**K**) and cross-section (**B**,**D**,**F**,**H**,**J**,**L**) images of the composite scaffolds made of polylactic-co-glycolic acid (PLGA) and biphasic ceramic (HA + β-TCP) at different ratios with G1: 50/50 (**A**,**B**), G2: 60/40 (**C**,**D**), and G3: 40/60 (**E**,**F**) and without G4: 50/50 (**G**,**H**), G5: 60/40 (**I**,**J**), and G6: 40/60 (**K**,**L**) collagen impregnation. In all groups, a structure with open pores and visible interconnectivity was produced. In groups G1, G2, and G3, collagen was able to coat both PLGA and HA + β-TCP, although group G3 exhibited several agglomerated ceramic particles. The groups without collagen coating presented a rougher morphology, and the HA + β-TCP particles seemed separated from the matrix; moreover, the agglomerates increased by increasing the amount of ceramic (G5 < G4 < G6). Magnification 60×.

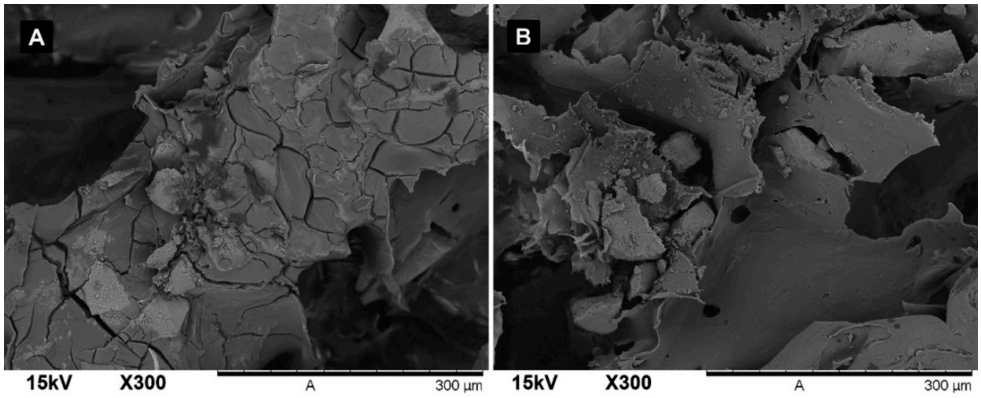

Figure 2. Scanning electron microscopy images of samples made of polylactic-co-glycolic acid (PLGA) and biphasic ceramic (HA + β-TCP) at a ratio of 60/40 in G2 with collagen (**A**) and 60/40 in G5 without collagen (**B**). (**A**) The ceramic particles within the matrix were covered by collagen, which created a bond holding the ceramic on the PLGA and resulting in higher mechanical properties. Isolated HA + β-TCP could be detected probably because of the sectioning of the samples by scalpel. (**B**) There is a lack of bond between PLGA and HA + β-TCP and ceramic scattered in the matrix is present. Magnification 150×.

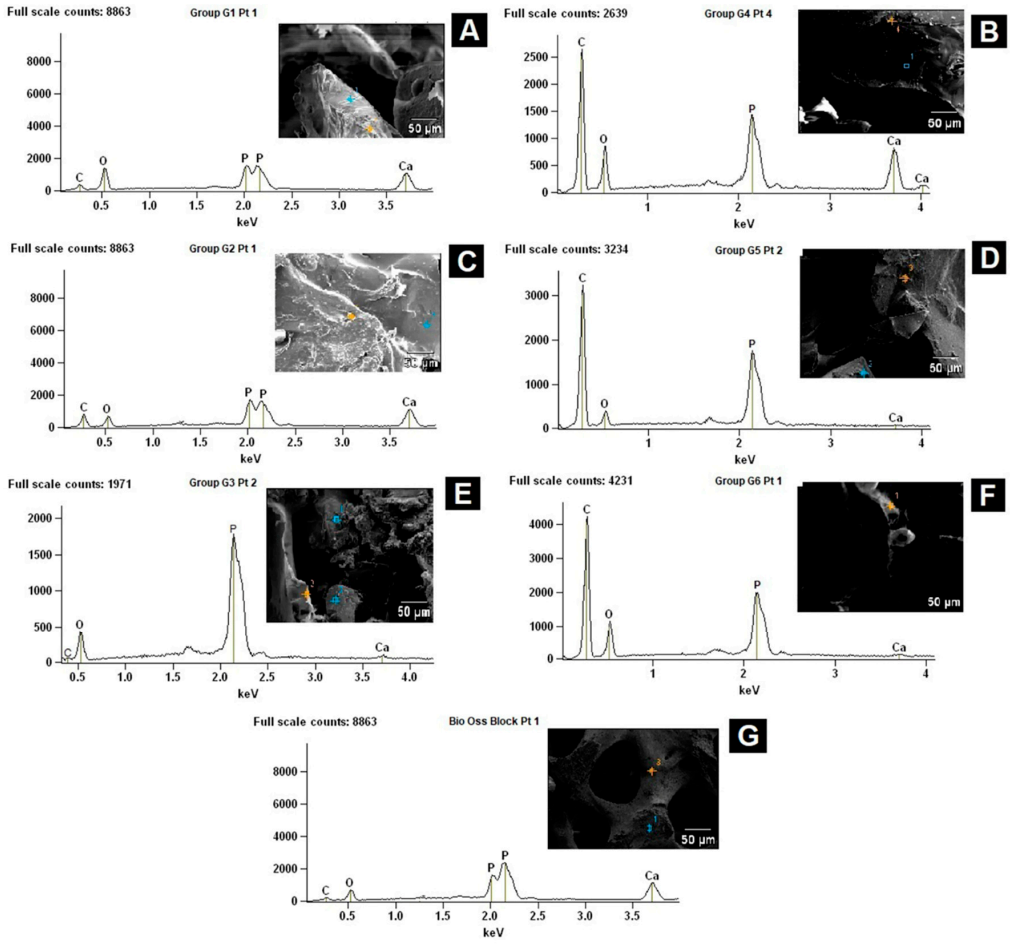

Figure 3. Chemical analysis by an energy-dispersive spectrometer (EDS) of all composite groups made of polylactic-co-glycolic acid (PLGA) and biphasic ceramic (HA + β-TCP) at different ratios: G1, 50/50 + collagen (**A**); G2, 60/40 + collagen (**C**); G3, 40/60 + collagen (**E**); G4, 50/50 (**B**); G5, 60/40 (**D**); G6, 40/60 (**F**); and control group (**G**), which revealed the presence of carbon (C), oxygen (O), calcium (Ca), and phosphorus (P).

Table 2. Characterization of the wettability of the composite and control groups. In the groups without collagen impregnation there is no liquid absorption (*).

Groups (PLGA/HA + β-TCP)	Absorption Time (s)	Liquid Height (mm)	Reached Sample Center	Drop Formation
G1 (50/50) + Collagen	129	10	Yes	No
G2 (60/40) + Collagen	146	5	Yes	Yes
G3 (40/60) + Collagen	157	3	Yes	Yes
G4 (50/50)	*	1	No	Yes
G5 (60/40)	*	1	No	Yes
G6 (40/60)	*	1	No	Yes
Control Bio-Oss Block®	2	10	Yes	No

[PLGA: polylactic-co-glycolic acid; HA + β-TCP: biphasic ceramic].

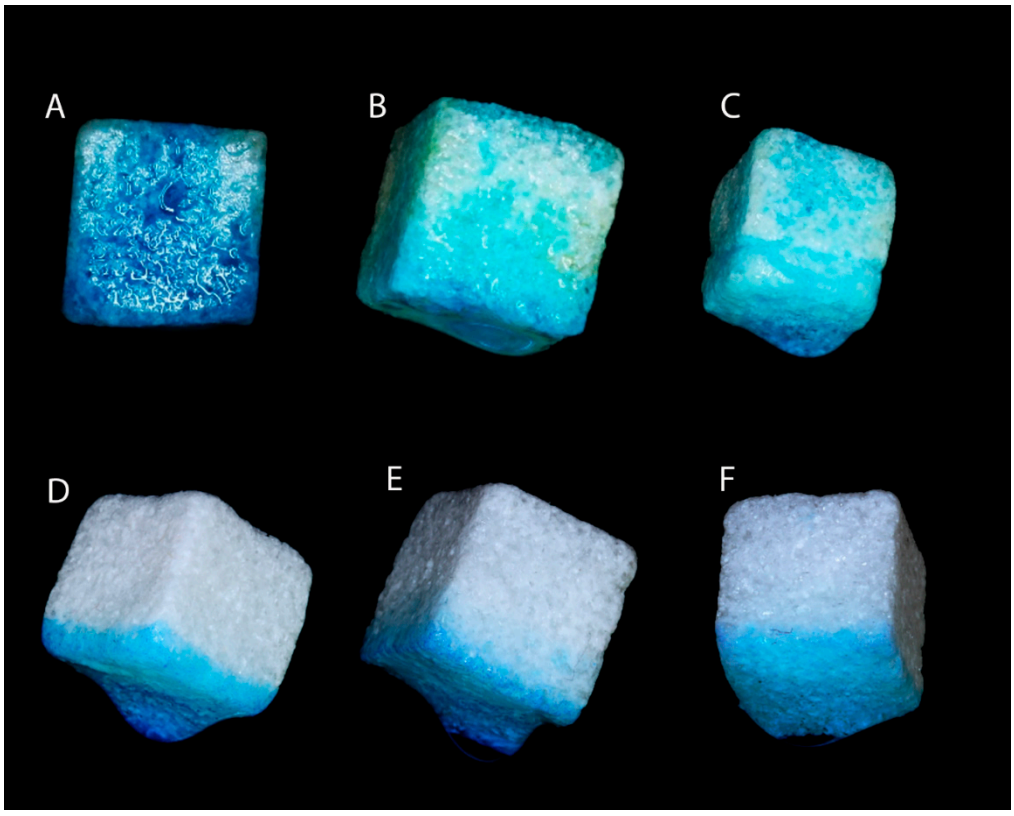

Figure 4. Wettability of the composites made of polylactic-co-glycolic acid (PLGA) and biphasic ceramic (HA + β-TCP) at different ratios with collagen impregnation—G1: 50/50 (**A**), G2: 60/40 (**B**), and G3: 40/60 (**C**)—and without collagen impregnation—G4: 50/50 (**D**), G5: 60/40 (**E**), and G6: 40/60 (**F**)—immediately after being extracted from the solution. Drop formation was evident in groups G2 and G3 and in all the groups without collagen, as there was no absorption of the liquid.

Equally, the elastic modulus was statistically higher in groups coated with collagen (G1, G2, and G3) than in noncoated groups (G4, G5, and G6); however, all the scaffolds exhibited a significantly lower stiffness than the control (Table 4). The mechanical properties of the evaluated scaffolds were not affected by the amount of biphasic ceramic added.

Table 3. Compressive strength values in the composite and control groups. There was a statistically significant difference between the composites and control group. Moreover, statistical analysis revealed significant differences in the compression strength between groups with (G1, G2, and G3) and without collagen impregnation (G4, G5, and G6).

Compression Strength (MPa)			
Groups (PLGA/HA + β-TCP)	**Median**	**Minimum**	**Maximum**
G1 (50/50) + Collagen	*/** 1.5	1.32	1.83
G2 (60/40) + Collagen	*/** 1.91	1.78	2.22
G3 (40/60) + Collagen	*/** 1.62	1.51	1.82
G4 (50/50)	* 0.96	0.84	1.02
G5 (60/40)	* 1.01	0.89	1.67
G6 (40/60)	* 1.15	0.76	1.29
Control Bio-Oss Block®	0.34	0.17	0.46
Trabecular Bone [10]		2–10	

* All presented a statistical difference with the control group. ** Groups G1, G2, and G3 presented statistical differences with groups G4, G5, and G6. [PLGA: polylactic-co-glycolic acid; HA + β-TCP: biphasic ceramic].

Table 4. Elastic modulus values in the composite and control groups. There was a statistically significant difference between the composites and control group. Moreover, statistical analysis revealed a significant difference in the elastic modulus between groups with (G1, G2, and G3) and without collagen impregnation (G4, G5, and G6).

Elastic Modulus (MPa)			
Groups (PLGA/HA + β-TCP)	**Median**	**Minimum**	**Maximum**
G1 (50/50) + Collagen	*/** 18.6	16.7	23.8
G2 (60/40) + Collagen	*/** 28.3	26.7	40.6
G3 (40/60) + Collagen	*/** 22.8	21.9	24.5
G4 (50/50)	13.2	11.4	17.3
G5 (60/40)	15.4	13.8	18.6
G6 (40/60)	15.7	9.5	21.6
Control Bio-Oss Block®	* 2882.6	1808.6	3018.9
Trabecular Bone [33]		1000	
PLGA [5]		2000	

* The control group presented statistical difference with all the other groups. ** Groups G1, G2, and G3 presented statistical differences with groups G4, G5, and G6. [PLGA: polylactic-co-glycolic acid; HA + β-TCP: biphasic ceramic].

4. Discussion

For a successful osteoconduction, the 3D structure of a scaffold should support cell penetration and proliferation as well as neovascularization, enabling an adequate diffusion of nutrients to the new cells and preventing the failure of bone repair [4,34–37]. Moreover, scaffolds should present interconnected pores of sizes and morphologies adequate to ensure the diffusion of waste products out of the scaffold without interfering with other surrounding tissues [34]. It has been demonstrated that large pores (250–600 μm) would increase scaffold permeability, cell colonization, as well as cell adhesion [14,38]. A relatively good pore interconnectivity, however, causes a decrease in mechanical properties, namely loss of the compressive strength and elasticity modulus. On the other hand, higher pore interconnectivity would increase new bone formation within the scaffolds [14]. Moreover, osteoblasts and fibrovascular tissue seem to exhibit a preference for binding to biomaterials with pore sizes significantly larger than the own cell size [39]. An ideal bone substitute material for oral and maxillofacial use should mimic the structure of human trabecular bone, acting as a proper support for vascularization and bone ingrowth [36]. The newly developed composites evaluated in the present study showed high interconnectivity that lead to a larger surface area and greater fluid retention capacity than control group. All the scaffolds without collagen presented agglomerates

of ceramic particles that did not bind to the PLGA matrix, probably indicating a lack of chemical interaction between ceramic and PLGA, which can also explain the lowest mechanical resistance found in those groups. On the other hand, the composites with collagen showed a tight interaction with the HA-β-TCP particles, and the presence of collagen induced an increase in the wettability of the scaffolds. Biodegradable synthetic polymers, such as PLGA, are hydrophobic and would inhibit liquid absorption, decreasing cell adhesion and proliferation, preventing bone tissue remodeling [14,15]. In vitro studies demonstrated that the enrichment of PLGA with collagen supported a greater adhesion and proliferation of cells if compared to the PLGA scaffolds alone [15,40,41]. Collagen-coated scaffolds showed a significantly higher compressive strength as well as a higher elastic modulus than noncoated ones. However, even if collagen improved the mechanical resistance of the biomaterials, all the scaffolds presented lower values of compressive strength and elasticity when compared to trabecular bone. The bone substitute materials should present mechanical properties similar to those of the host tissue. If the difference between elastic modules is wide, there will be a heterogeneous distribution of stresses, which may lead to failure of the bone or implant [35]. Regarding the mechanical resistance, this should allow the scaffold to be loaded with compressive and torsional tensions without any structural impairment. From a clinical point of view, the scaffolds should present minimum resistance, allowing their handling during implantation [35]. Indeed, the major disadvantage of Bio-Oss Block® in clinical use is its high attitude to fracture at the moment of fixation, due to its low mechanical strength and high stiffness, weaknesses that were confirmed by the results of the present study. On the other hand, the mechanical properties of the studied composites were not affected by the amount of biphasic ceramic added. Ebrahimian-Hosseinabadi et al. [42] and Shuai et al. [12] studied the effects of bioactive ceramics, BCP and HA, respectively, added to PLGA. In both studies, ceramic produced a large increase in the compressive strength and elastic modulus of the scaffolds; however, by increasing the amount of ceramic to more than 20%–30% by weight, the two properties decreased dramatically.

Further in vitro and in vivo studies are needed to investigate the biological behavior of the newly developed composite materials and their potential, namely biocompatibility and bioactivity, for bone tissue regeneration.

5. Conclusions

(1) The PLGA/HA + β-TCP scaffolds manufactured by the solvent casting and particulate leaching methods presented high porosity and interconnectivity, resulting in a higher surface area and fluid retention capacity when compared to control.

(2) Collagen promoted an increase in the wettability of the composite scaffolds.

(3) The mechanical properties were also enhanced by the presence of collagen, which lent higher compressive strength and elastic modulus values to the groups where it was added.

(4) The polymer/ceramic ratio (50/50, 60/40, and 40/60) did not statistically affect the mechanical properties; however, it affected the wettability mainly in the groups with collagen impregnation through the variation of the liquid absorption time and drop formation.

(5) The collagen enrichment of biomaterials tested in the present paper can be easily obtained for other bone substitute materials (BSBs) and could improve their mechanical properties and also their wettability. This, in turn, could enhance their regenerative potential by reducing material deformations under masticatory loading and by improving the ability to be permeated by blood clots, which is the first step of the bone cascade for bone regeneration. These data are of particular interest for basic researchers working on the betterment of biological and mechanical properties of BSBs and for clinicians in order to tailor the usage of this material to achieve the expected clinical response. Moreover, the present study may prove very useful for future collagen enrichment of different BSBs.

Author Contributions: Design of the study, conception and direction of the work, data interpretation, J.J.L.M.; Design of the study and contribution of data interpretation, V.P.; Draft of the work, F.I.; Supervising of the material development, Á.A.; Data interpretation and verification of the analytical methods, C.A.M.B.; Acquisition of data, G.L.M.; Critical revision and data interpretation, A.P.; Led and supervised the study, M.A.B.

Funding: This research received no external funding.

Acknowledgments: The authors wish to thank the Center for Research on Dental Implants (CEPID) for the financial support. G.L.M. holds a scholarship from the Coordination for Improvement of Higher Education Personnel (CAPES) and thank this support.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

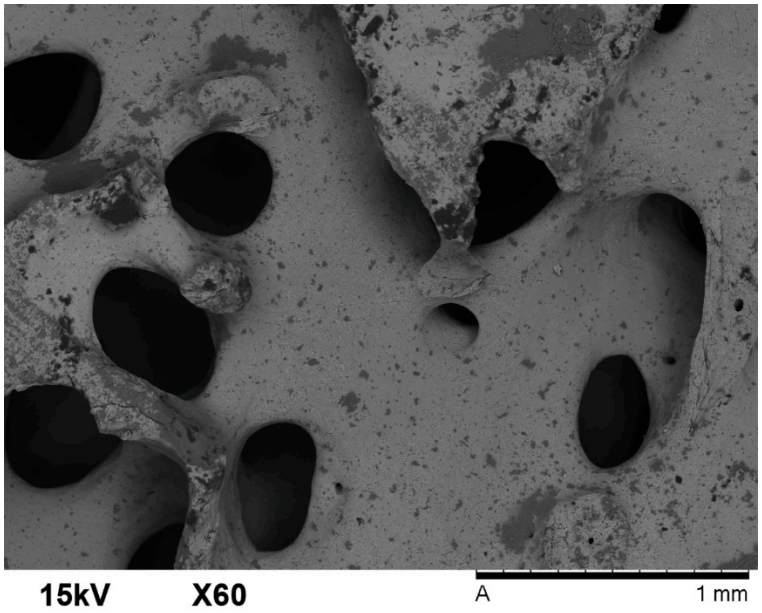

Figure A1. Scanning electron microscopy cross section of inorganic xenogenous bone (control group) showing the morphology and interconnectivity of the pores. Magnification 60×.

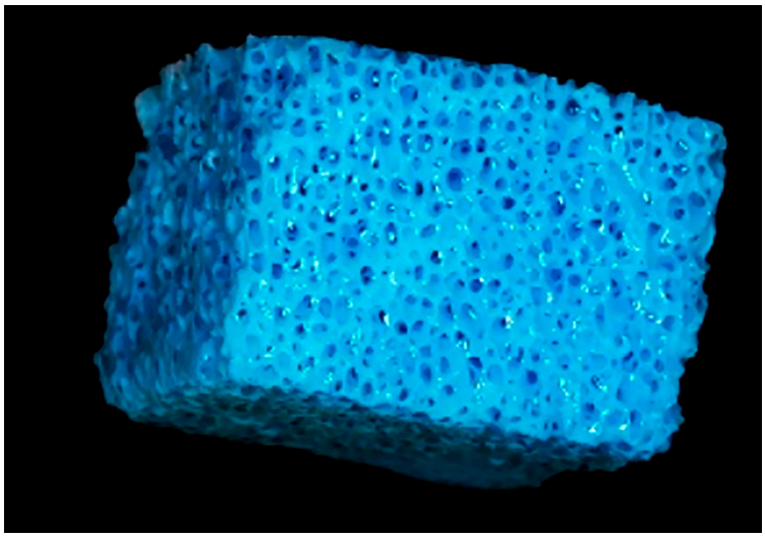

Figure A2. Wettability of the inorganic xenogenous bone (control group) immediately after being extracted from the solution. No drop formation was evident.

References

1. Lei, B.; Guo, B.; Rambhia, K.J.; Ma, P.X. Hybrid polymer biomaterials for bone tissue regeneration. *Front. Med.* **2019**, *13*, 189–201. [CrossRef] [PubMed]

2. Chocholata, P.; Kulda, V.; Babuska, V. Fabrication of scaffolds for bone-tissue regeneration. *Materials* **2019**, *12*, 568. [CrossRef] [PubMed]
3. Félix Lanao, R.P.; Jonker, A.M.; Wolke, J.G.; Jansen, J.A.; van Hest, J.C.; Leeuwenburgh, S.C. Physicochemical properties and applications of poly(lactic-co-glycolic acid) for use in bone regeneration. *Tissue Eng. Part B Rev.* **2013**, *19*, 380–390. [CrossRef] [PubMed]
4. Roseti, L.; Parisi, V.; Petretta, M.; Cavallo, C.; Desando, G.; Bartolotti, I.; Grigolo, B. Scaffolds for bone tissue engineering: state of the art and new perspectives. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2017**, *78*, 1246–1262. [CrossRef]
5. Gentile, P.; Chiono, V.; Carmagnola, I.; Hatton, P.V. An overview of poly(lactic-co-glycolic) Acid (PLGA)-based biomaterials for bone tissue engineering. *Int. J. Mol. Sci.* **2014**, *15*, 3640–3659. [CrossRef]
6. Ogueri, K.S.; Jafari, T.; Escobar Ivirico, J.L.; Laurencin, C.T. Polymeric biomaterials for scaffold-based bone regenerative engineering. *Regen. Eng. Transl. Med.* **2019**, *5*, 128–154. [CrossRef]
7. Turnbull, G.; Clarke, J.; Picard, F.; Riches, P.; Jia, L.; Han, F.; Li, B.; Shu, W. 3D bioactive composite scaffolds for bone tissue engineering. *Bioact. Mater.* **2017**, *3*, 278–314. [CrossRef]
8. Yang, F.; Cui, W.; Xiong, Z.; Liu, L.; Bei, J.; Wang, S. Poly(l,l-lactide-co-glycolide)/tricalcium phosphate composite scaffold and its various changes during degradation in vitro. *Polym. Degrad. Stab.* **2006**, *91*, 3065–3073. [CrossRef]
9. Liu, H.; Slamovich, E.B.; Webster, T.J. Less harmful acidic degradation of poly(lacticco-glycolic acid) bone tissue engineering scaffolds through titania nanoparticle addition. *Int. J. Nanomed.* **2006**, *1*, 541–545. [CrossRef]
10. Khojasteh, A.; Fahimipour, F.; Eslaminejad, M.B.; Jafarian, M.; Jahangir, S.; Bastami, F.; Tahriri, M.; Karkhaneh, A.; Tayebi, L. Development of PLGA-coated β-TCP scaffolds containing VEGF for bone tissue engineering. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2016**, *69*, 780–788. [CrossRef]
11. Jeong, J.; Kim, J.H.; Shim, J.H.; Hwang, N.S.; Heo, C.Y. Bioactive calcium phosphate materials and applications in bone regeneration. *Biomater. Res.* **2019**, *23*, 4. [CrossRef] [PubMed]
12. Shuai, C.; Yang, B.; Peng, S.; Li, Z. Development of composite porous scaffolds based on poly(lactide-co-glycolide)/nano-hydroxyapatite via selective laser sintering. *Int. J. Adv. Manuf. Technol.* **2013**, *69*, 51–57. [CrossRef]
13. Ignjatović, N.L.; Liu, C.Z.; Czernuszka, J.T.; Uskoković, D.P. Micro- and nano-injectable composite biomaterials containing calcium phosphate coated with poly(dl-lactide-co-glycolide). *Acta Biomater.* **2007**, *3*, 927–935. [CrossRef]
14. Pamula, E.; Filová, E.; Bacáková, L.; Lisá, V.; Adamczyk, D. Resorbable polymeric scaffolds for bone tissue engineering: The influence of their microstructure on the growth of human osteoblast-like mg 63 cells. *J. Biomed. Mater. Res. A* **2009**, *89*, 432–443. [CrossRef] [PubMed]
15. Khang, G. *Handbook of Intelligent Scaffolds for Tissue Engineering and Regenerative Medicine*, 2nd ed.; Pan Stanford Publishing: Singapore, 2017.
16. Busra, M.F.M.; Lokanathan, Y. Recent development in fabrication of collagen scaffolds for tissue engineering applications. *Curr. Pharm. Biotechnol.* **2019**. [CrossRef]
17. Parenteau-bareil, R.; Gauvin, R.; Berthod, F. Collagen-based biomaterials for tissue engineering applications. *Materials* **2010**, *3*, 1863–1887. [CrossRef]
18. Brodie, J.C.; Merry, J.; Grant, M.H. The mechanical properties of calcium phospate ceramics modified by collagen coating and populated by osteoblasts. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 43–48. [CrossRef]
19. Hoerth, R.M.; Kerschnitzki, M.; Aido, M.; Schmidt, I.; Burghammer, M.; Duda, G.N.; Fratzl, P.; Willie, B.M.; Wagermaier, W. Correlations between nanostructure and micromechanical properties of healing bone. *J. Mech. Behav. Biomed. Mater.* **2018**, *77*, 258–266. [CrossRef]
20. Kong, J.; Wei, B.; Groth, T.; Chen, Z.; Li, L.; He, D.; Huang, R.; Chu, J.; Zhao, M. Biomineralization improves mechanical and osteogenic properties of multilayer-modified PLGA porous scaffolds. *J. Biomed. Mater. Res. A* **2018**, *106*, 2714–2725. [CrossRef]
21. Kim, S.S.; Sun Park, M.; Jeon, O.; Yong Choi, C.; Kim, B.S. Poly (lactide-co-glycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* **2006**, *27*, 1399–1409. [CrossRef]
22. Naik, A.; Shepherd, D.V.; Shepherd, J.H.; Best, S.M.; Cameron, R.E. The effect of the type of HA on the degradation of PLGA/HA composites. *Mater. Sci. Eng. C Mater. Biol. Appl.* **2017**, *70*, 824–831. [CrossRef] [PubMed]

23. Ou, K.L.; Chung, R.J.; Tsai, F.Y.; Liang, P.Y.; Huang, S.W.; Chang, S.Y. Effect of collagen on the mechanical properties of hydroxyapatite coatings. *J. Mech. Behav. Biomed. Mater.* **2011**, *4*, 618–624. [CrossRef] [PubMed]
24. Wojak-Cwik, I.M.; Hintze, V.; Schnabelrauch, M.; Moeller, S.; Dobrzynski, P.; Pamula, E.; Scharnweber, D. Poly(L-lactide-co-glycolide) scaffolds coated with collagen and glycosaminoglycans: Impact on proliferation and osteogenic differentiation of human mesenchymal stem cells. *J. Biomed. Mater. Res. A* **2013**, *101*, 3109–3122. [CrossRef] [PubMed]
25. Gleeson, J.P.; Plunkett, N.A.; O'Brien, F.J. Addition of hydroxyapatite improves stiffness, interconnectivity and osteogenic potential of a highly porous collagen-based scaffold for bone tissue regeneration. *Eur. Cell. Mater.* **2010**, *353*, 218–230. [CrossRef]
26. Roether, J.; Bertels, S.; Oelschlaeger, C.; Bastmeyer, M.; Willenbacher, N. Microstructure, local viscoelasticity and cell culture suitability of 3D hybrid HA/collagen scaffolds. *PLoS ONE* **2018**, *13*, e0207397. [CrossRef]
27. Wojtowicz, A.M.; Shekaran, A.; Oest, M.E.; Dupont, K.M.; Templeman, K.L.; Hutmacher, D.W.; García, A.J. Coating of biomaterial scaffolds with the collagen-mimetic peptide GFOGER for bone defect repair. *Biomaterials* **2010**, *31*, 2574–2582. [CrossRef]
28. He, W.; Ma, Z.; Yong, T.; Teo, W.E.; Ramakrishna, S. Fabrication of collagen-coated biodegradable polymer nanofiber mesh and its potential for endothelial cells growth. *Biomaterials* **2005**, *26*, 7606–7615. [CrossRef]
29. Venugopal, J.; Prabhakaran, M.P.; Zhang, Y.; Low, S.; Choon, A.T.; Ramakrishna, S. Biomimetic hydroxyapatite-containing composite nanofibrous substrates for bone tissue engineering. *Philos. Trans. A Math. Phys. Eng. Sci.* **2010**, *368*, 2065–2081. [CrossRef]
30. Messias, A.D.; Aragones, A.; Duek, E.A. PLGA-Hydroxyapatite composite scaffolds for osteoblastic-like cells. *Key Eng. Mater.* **2009**, *396–398*, 461–464.
31. Barbosa, T.P.; Naves, M.M.; Menezes, H.H.M.; Pinto, P.H.C.; de Mello, J.D.B.; Costa, H.L. Topography and surface energy of dental implants: A methodological approach. *J. Braz. Soc. Mech. Sci. Eng.* **2017**, *39*, 1895–1907. [CrossRef]
32. Simon, C.G.; Yaszemski, M.J.; Ratcliffe, A.; Tomlins, P.; Luginbuehl, R.; Tesk, J.A. ASTM international workshop on standards and measurements for tissue engineering scaffolds. *J. Biomed. Mater. Res. B Appl. Biomater.* **2015**, *103*, 949–959. [CrossRef] [PubMed]
33. Keaveny, T.M.; Hayes, W.C. Mechanical properties of cortical and trabecular bone. *Bone* **1993**, 285–344.
34. Alaribe, F.N.; Manoto, S.L.; Motaung, S.C.K.M. Scaffolds from biomaterials: Advantages and limitations in bone and tissue engineering. *Biologia* **2016**, *71*, 353–366. [CrossRef]
35. O'Brien, F.J. Biomaterials and scaffolds for tissue engineering. *Mater. Today* **2011**, *14*, 88–95. [CrossRef]
36. Liu, X.; Jakus, A.E.; Kural, M.; Qian, H.; Engler, A.; Ghaedi, M.; Shah, R.; Steinbacher, D.M.; Niklason, L.E. Vascularization of natural and synthetic bone scaffolds. *Cell Transplant.* **2018**, *27*, 1269–1280. [CrossRef]
37. Roi, A.; Ardelean, L.C.; Roi, C.I.; Boia, E.R.; Boia, S.; Rusu, L.C. Oral bone tissue engineering: Advanced biomaterials for cell adhesion, proliferation and differentiation. *Materials* **2019**, *12*, 2296. [CrossRef]
38. Matsiko, A.; Gleeson, J.P.; O'Brien, F.J. Scaffold mean pore size influences mesenchymal stem cell chondrogenic differentiation and matrix deposition. *Tissue Eng. Part A* **2015**, *21*, 486–497. [CrossRef]
39. O'Brien, F.J.; Harley, B.A.; Yannas, I.V.; Gibson, L.J. The effect of pore size on cell adhesion in collagen-GAG scaffolds. *Biomaterials* **2005**, *26*, 433–441. [CrossRef]
40. Sulong, A.F.; Hassan, N.H.; Hwei, N.M.; Lokanathan, Y.; Naicker, A.S.; Abdullah, S.; Yusof, M.R.; Htwe, O.; Idrus, R.B.; Haflah, N.H. Collagen-coated polylactic-glycolic acid (PLGA) seeded with neural-differentiated human mesenchymal stem cells as a potential nerve conduit. *Adv. Clin. Exp. Med.* **2014**, *23*, 353–362. [CrossRef]
41. Tomlins, P. *Characterisation and Design of Tissue Scaffolds*, 1st ed.; Woodhead Publishing Series in Biomaterials: Cambridge, UK, 2015; ISBN 9781782420958.
42. Ebrahimian-Hosseinabadi, M.; Ashrafizadeh, F.; Etemadifar, M.; Venkatraman, S.S. Evaluating and modeling the mechanical properties of the prepared PLGA/nano-BCP composite scaffolds for bone tissue engineering. *J. Mater. Sci. Technol.* **2011**, *27*, 1105–1112. [CrossRef]