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ORIGINAL ARTICLE

Scaffold based on castor oil as an osteoconductive matrix in bone repair: biocompatibility analysis

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Abstract

To analyze the biocompatibility of the scaffold produced from a natural polymer derived from castor oil through hemolytic activity and antimicrobial activity, to enable the clinical application. Three *in vitro* tests were performed: Hemolytic activity test - Polymer partially dissolved in contact with blood agar; Hemolytic activity test in sheep's blood - Polymer extract with red blood cells solution; Antimicrobial activity test - Solid polymer in direct contact with E. Coli and S. Aureus. For hemolytic tests, none of the samples showed hemolysis. Negative hemolytic activity is a good indicator, as the maintenance of the blood clot in the area of the lesion is essential for the formation of new tissue. For the antimicrobial activity test, no significant activity was observed against the bacteria used. The polymer is not toxic to red blood cells, being viable for clinical application as a matrix for tissue regeneration.

Keywords: *bone matrix, materials testing, tissue scaffolds.*

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1. Introduction

There is a vast amount of surgical procedures performed in an attempt to repair bone tissue damaged by disease or trauma. The field of tissue engineering research aims to develop biological substitutes that restore, maintain or improve the function of damaged tissue by combining body cells with biomaterials. Scaffolds, commonly produced from polymeric biomaterials, provide structural support for cell binding and subsequent tissue development[1].

Scaffolds produced from various biomaterials are used in the field in an attempt to regenerate different tissues and organs in the body. Regardless of the type of fabric, a number of considerations are important when designing or determining the suitability of scaffold for use in tissue engineering, this generally requires that the devices be equivalent in performance, biocompatibility, safety, stability and sterility to previously approved devices[2].

The characteristics that biomaterials must have are: a) biocompatibility: the material must be non-toxic, not promote an acute or chronic inflammation reaction, have a low tissue reactivity, that is, do not promote host rejection; b) bioabsorption: the material must have degradability that will accompany the formation of a new tissue; c) porosity: the material must have a pore density of around 75% with average sizes of 200 to 400 mm in diameter, to favor protein adhesion, in addition to increasing the collagen formation; d) chemotaxis: the material must attract mesenchymal cells and provide means of cell adhesion, facilitating cell proliferation and differentiation; e) angiogenesis: the material must promote vascularization, being hydrophilic, to absorb blood fluid and reinforce the initial coagulation after implantation; f) low cost: the material cannot exceed the value of the autograft, having abundant constituent materials and efficient sterilization[3-5].

Most tests performed on new scaffold devices follow the protocols of the International Organization for Standardization - ISO 10993, for the Biological Assessment of Medical Devices. Bearing in mind that biocompatibility is an important property for human use

of biomaterials, the need to conduct *in vitro* studies of cellular behavior at the interface with these materials is evident. The *in vitro* tests are fast, cost-effective, do not involve ethical problems and simulate the performance of the material in the body. Biocompatible biomaterials should not have a toxic or harmful effect on biological systems[6].

The object of study of this research was a scaffold produced from a natural polymer derived from castor oil, it is an innovative biomaterial produced at the Federal University of Piauí. This study aims to analyze the biocompatibility of the castor oil scaffold, with the main tests of toxic activity against red blood cells and antimicrobial activity, to enable the clinical application of this biomaterial as an osteoconductive matrix in the repair of bone injuries.

2. Materials and Methods

The production and characterization of the material were carried out at the Materials Physics Laboratory of the Federal University of Piauí (FISMAT-UFPI). The biocompatibility experiments were carried out at the Interdisciplinary Laboratory of Advanced Materials at the Federal University of Piauí (LIMAV/UFPI).

2.1 Production and characterization

The scaffolds were found from castor oil monoglyceride and characterized as described in previous studies[7-9]. The pure Castor oil was commercially acquired. Also, the reagents used in the production of monoacylglycerides (MAG) and its polymer (CPU) were glycerol (C3H8O3, Impex), lithium hydroxide (LiOH, Vetec), and Hexamethylene Diisocyanate (HDI) (C8H12N2O2, Sigma-Aldrich) for polymerization[7].

Glycerol was added to the castor oil in a heating bath at 140ºC. After 10 minutes of preparation, lithium hydroxide (0.05% w/w) was added and kept under stirring for 5 hours. To form the polymer in scaffolding format, the initial temperature of 80ºC was preserved. After this process, polyethylene glycol (2.5g) was added to the monoglyceride (5g) and stirred until complete dissolution. Hexamethylene diisocyanate (HDI) was added at a ratio of 1:4.5 (MAG:HDI), still under composition until completion of polymerization. During the temperature, it was possible to observe the formation of the spongy material, remaining 12 hours at the initial temperature until the end of the process[7,8].

Characterization was performed by Fourier Transform Infrared Spectroscopy (FTIS) and thermal analysis. To confirm the presence of the materials in the sample, spectroscopy was performed in a Thermo Fisher Scientific Nicolet iS5 apparatus, with a purge pump and a wavelength between 800 cm^{-1} and 4000 cm^{-1}, 128 accumulated cans, 4 cm^{-1} resolution, in attenuated total reflection. To verify the stability and thermal decomposition as a function of mass loss, the thermal analyzer TGA-51H, Shimadzu, standardized with a heating rate of 10 ◦C min^{-1} in a nitrogen atmosphere, up to a temperature of 600 ◦C and a sample mass of approximately 7 mg[7-9].

2.2 Hemolytic activity test on blood agar

Blood agar culture medium was used in 90 mm diameter Petri dishes for the test. Dilutions of the polymer were prepared in two different solvents (methanol and ethanol). In a 1: 1 ratio, 1.3 mg of the fragmented polymer was collected and added to 1.3 ml of solvent. Using the common concentration formula: $C = \frac{\text{mass of solute}\left(1,3\text{mg}\right)}{\text{volume of solution}\left(1,3\text{ml}\right)}$, 1.3 ml of solution in volume was obtained, at a concentration of 1 mg / ml, being stirred in a solution shaker (Model AP56 – Phoenix Luferco)[10].

After obtaining the two solutions, a dosing pipette with disposable tips, fixed at 40 µl, was used to soak sterile filter paper discs number 1 with a diameter of 7 mm. The solutions were divided into two groups, one with methanol solvent and the other with ethanol solvent. Each group was divided into two subgroups: Control group: Discs impregnated only with the solvent (Methanol or ethanol) and Experimental group: Discs impregnated with the solvent and polymer solution. For each group (Control and experimental), three discs were used, in a triplicate test[11].

After the natural evaporation of volatile solvents, the Petri dishes were opened in an oven, next to the Bunsen burner flame, to avoid contamination of the medium. The discs were inserted into the Petri dish using a toothless Adson forceps, and then incubated at 35 ° C for 24 hours. The analysis of hemolytic activity was performed macroscopically after the incubation time determined[10,11].

2.3 Blood compatibility evaluation

The hemolytic activity test in sheep's blood was performed as described by Grilo[12] and Hou et al.[13] with some adaptations.

This analysis consists of a colorimetric assay to measure the release of cyanomethemoglobin caused by hemolytic activity through spectrophotometry. Defibrinated sheep blood (Newprov®, Paraná, Brazil) was used to produce a 2% w/v suspension of red blood cells in the following steps: 1) Blood centrifuge at 4.000 rpm, for 15 minutes at 4ºC; 2) Removal of supernatant plasma with a micropipette; 3) Three successive washes with saline at 4ºC; 4) Weighing the red blood cell pellet; 5) Addition of saline to obtain a suspension at 2% w/v according to the formula: $[\%] = \frac{\text{pellet}\left(\text{g}\right)}{\text{solution volume}\left(\text{ml}\right)} * 100$.

To obtain an extracting solution, the biomaterial was weighed and mixed with saline solution to a concentration of 2mg/ml and taken to incubation at 37º for 1 hour. Then, 800µl of the extraction solution was mixed with 200µl of the 2% red blood cell solution in tubes. For the negative control, saline solution was used, for the positive control, distilled water. The tubes were slightly agitated and taken to incubation at 37°C for 1 hour, the test was performed in triplicate. After the incubation time, the tubes were centrifuged at 3.000 rpm for 10 minutes, the supernatant liquid was collected with a micropipette and taken for analysis in the DU® 800 UV/Visible Spectrophotometer at 545nm (Beckman Coulter, California, EUA). After

reading, the percentage of hemolysis was obtained using the formula: $\%H = \frac{AB - AS}{AW - AS} * 100$

Where AB, the absorbance of the tube with bioadhesive; AS, negative control absorbance (Saline solution) and AA, positive control absorbance (Distilled water).

2.4 Antimicrobial activity test

The antimicrobial activity test was performed as described by NCCLS[14] with some necessary adaptations due to the properties of the scaffold under study.

Strains of Escherichia coli (ATCC® 25922 ™) and Staphylococcus aureus (ATCC® 25293™) were used to perform the test. The strains were inoculated in liquid culture medium of Mueller Hinton in broth (MHB) and incubated at 37ºC until a turbidity corresponding to 0.5 of the McFarland scale (0.5 x 108 Colony Forming Units - UFC) was obtained, in Then, the mixture was diluted until a mixture was obtained at 0.5 x 105 CFU.

After obtaining the bacteria, 0.5 ml of the mixture of culture medium + bacteria was inoculated in each of the 8 vials, 4 for Escherichia coli and 4 for Staphylococcus aureus, distributed as follows: 1) Experimental Groups: Flasks 1 and 5: 4.5 ml of MHB culture medium + 1mg of polymer, Flasks 2 and 6: 4.5 ml of MHB culture medium + 10 mg of polymer and Flasks 3 and 7: 4.5 ml of MHB culture medium + 100 mg of polymer; 2) Control Groups: Flasks 4 and 8: 4.5 ml of MHB culture medium (positive control).

The tubes were fitted into an adapted device and incubated (Nova Ética 430-RDBP) with constant agitation and a temperature of 37ºC for 24 hours. After the determined time, the flasks were analyzed according to turbidity, using the control groups as a reference. To confirm the results obtained, 100 µl of the content of each flask was transferred to Petri dishes with MHA culture medium using a dosing pipette. The plates were incubated in an SPLabor SP-200 oven at 37ºC for 24 hours for analysis of bacterial development.

3. Results and Discussions

The spectra obtained by Fourirer transform infrared spectroscopy were compatible with what has already been reported in the literature, confirming the standardization of the scaffolds production. In the thermal analysis, the biomaterial also behaved in a predicted way, showing thermal degradation in three different stages, starting at 180ºC, going through 280ºC and ending at 380ºC with the greatest loss of mass (57%), according to previous studies[7-9].

The blood agar culture medium is widely used in hemolytic activity tests. This culture medium is a mixture of defibrinated sheep blood with a 1:20 base, red in color and PH 6.8 +/- 2. Being a medium rich in healthy erythrocytes, it contributes to the identification of hemolysis halos caused by toxic substances. The 2% red blood cell solution allows the material under study to come into direct contact with blood cells[10].

For the hemolytic diffusion test on blood agar, the partially dissolved samples did not show hemolytic ability, that is, none of the samples presented a hemolytic halo around the impregnated disc. As expected, the negative controls did not show a hemolytic halo. This result indicates that the castor polymer is not toxic against red blood cells.

The hemolytic test in defibrinated sheep blood confirmed the result obtained in the test with the blood agar culture medium. An average of the absorbance obtained was performed and the formula was applied to obtain the hemolysis percentage (Figure 1). According to the standard of hemolysis assay, samples with percentage hemolysis between 0-2% are classified as non-hemolytic[14]. The scaffold showed hemolytic activity below 1%, being considered a non-hemolytic material, this is an important factor for its application in bone tissue.

For the antimicrobial activity test, no significant activity was observed against the bacteria used, Escherichia coli and Staphylococcus aureus. All vials of bacteria in contact with scaffold had turbidity similar to the control vial, for both bacteria. After adding the mixtures in MHA culture medium, the presence of bacteria in the flasks was confirmed. Thus, the biomaterial has no antimicrobial activity against the bacteria under study.

The significant development of biomaterials has represented a powerful therapeutic tool in surgical activities, especially in the correction of critical bone defects. However, despite the proven benefits, its use requires careful clinical and ethical care from the professional in the analysis of the risks and benefits that each biomaterial may present[15].

The performance of *in vitro* tests is extremely important for the viability of biomaterials in the health sciences, toxicity tests can predict whether a material presents any type of damage to the cells[16]. The hemolytic activity test seeks to understand whether the biomaterial in which it is intended to be used in living organisms has toxic activity on erythrocytes, since the free hemoglobin molecule in plasma due to red cell lysis can cause elevation of plasma hemoglobin, inducing deleterious effects mainly in kidneys (Nephrotoxicity) and the cardiovascular system (vasomotor effect)[11].

Hemocompatibility is one of the main criteria that limit the clinical applicability of blood contact biomaterials. Adverse interactions between newly developed materials and blood

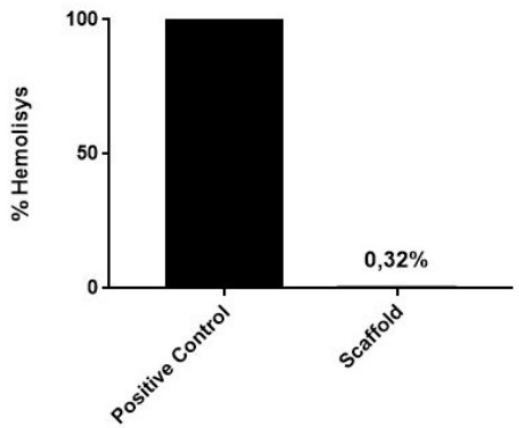

Figure 1. Percentage of hemolysis in the Positive control (Distilled water) and Scaffold group.

must be extensively analyzed to prevent the activation and destruction of blood components[12]. The negative result for the hemolytic activity test is relevant for the clinical application of the scaffold, since the maintenance of the blood clot in the area of the lesion is fundamental for the healing and formation of new tissue, as it contains elements that are essential to the bone regeneration process[17-19].

The scaffold under study was developed from the natural polymer of castor oil. Despite the high toxicity of castor seeds, castor oil is not toxic, since ricin, a toxic protein in seeds, is not soluble in lipids, the entire toxic component being restricted to pie[20-22]. That is, even though it originated from a toxic seed, the castor polymer scaffold did not present toxicity against blood cells.

For the negative result of antimicrobial activity, there is no implication in the clinical failure of the biomaterial, as this property would be desirable to avoid bacterial infections in the area to be repaired. However, in addition to the possibility of systemic antibiotic therapy, some studies have explored the hypothesis of using biodegradable antimicrobials impregnated in scaffolds to prevent infection and support new bone growth without contamination, avoiding several problems during healing[23-26].

The main function of the scaffold in the bone defect is to provide mechanical resistance to the lesion site and serve as an osteoconductive matrix, the antimicrobial activity would be an additional feature for the success of the therapy, but it is not essential, since the infection can be fought with association of scaffold with other materials and drug therapy[27,28].

4. Conclusions

With hemolytic activity tests, it was observed that the polymer does not present toxicity to blood cells, being, therefore, viable for clinical application as a matrix for bone tissue regeneration. The absence of antimicrobial activity observed during the tests does not compromise the clinical use of the material, since antibiotic therapy allows the control of bone infections. *In vivo* biocompatibility tests are required to confirm the biomaterial's biocompatibility.

5. References

1. Perier-Metz, C., Duda, G. N., & Checa, S. (2020). Mechano-biological computer model of scaffold-supported bone regeneration: effect of bone graft and scaffold structure on large bone defect tissue patterning. *Frontiers in Bioengineering and Biotechnology*, *8*, 585799. http://dx.doi.org/10.3389/fbioe.2020.585799. PMid:33262976.
2. Jeong, H., Gwak, S., Seo, K. D., Lee, S., Yun, J., Cho, Y., & Lee, S. (2020). Fabrication of three-dimensional composite scaffold for simultaneous alveolar bone regeneration in dental implant installation. *International Journal of Molecular Sciences*, *21*(5), 1863. http://dx.doi.org/10.3390/ijms21051863. PMid:32182824.
3. Fernandes, H. R., Gaddam, A., Rebelo, A., Brazete, D., Stan, G. E., & Ferreira, J. M. F. (2018). Bioactive glasses and glass-ceramics for healthcare applications in bone regeneration and tissue engineering. *Materials*, *11*(12), 2530. http://dx.doi.org/10.3390/ma11122530. PMid:30545136.
4. Sergi, R., Bellucci, D., & Cannillo, V. (2020). A review of bioactive glass/natural polymer composites: state of the art. *Materials*, *13*(23), 5560. http://dx.doi.org/10.3390/ma13235560. PMid:33291305.
5. Taale, M., Schütt, F., Zheng, K., Mishra, Y. K., Boccaccini, A. R., Adelung, R., & Selhuber-Unkel, C. (2018). Bioactive carbon-based hybrid 3d scaffolds for osteoblast growth. *ACS Applied Materials & Interfaces*, *10*(50), 43874-43886. http://dx.doi.org/10.1021/acsami.8b13631. PMid:30395704.
6. Keong, L. C., & Halim, A. S. (2009). In vitro models in biocompatibility assessment for 6 biomedical-grade chitosan derivatives in wound management. *International Journal of Molecular Sciences*, *10*(3), 1300-1313. http://dx.doi.org/10.3390/ijms10031300. PMid:19399250.
7. Moura, F. N., No., Fialho, A. C. V., Moura, W. L., Rosa, A. G. F., Matos, J. M. E., Reis, F. S., Mendes, M. T. A., & Sales, E. S. D. (2019). Castor polyurethane used as osteosynthesis plates: microstructural and thermal analysis. *Polímeros: Ciência e Tecnologia*, *29*(2), e2019029. http://dx.doi.org/10.1590/0104-1428.02418.
8. Pacheco, I. K. C., Reis, F. S., Carvalho, C. E. S., Matos, J. M. E., Argolo, N. M., No., Baeta, S. A. F., Silva, K. R., Dantas, H. V., Sousa, F. B., & Fialho, A. C. V. (2021). Development of castor polyurethane scaffold (Ricinus communis L.) and its effect with stem cells for bone repair in an osteoporosis model. *Biomedical Materials*, *16*(6), 065006. http://dx.doi.org/10.1088/1748-605X/ac1f9e. PMid:34416741.
9. Morais, J. P. P., Pacheco, I. K. C., Maia, A. L. M., Fo., Ferreira, D. C. L., Viana, F. J. C., Reis, F. S., Matos, J. M. E., Rizzo, M. S., & Fialho, A. C. V. (2021). Polyurethane derived from castor oil monoacylglyceride (Ricinus communis) for bone defects reconstruction: characterization and in vivo testing. *Journal of Materials Science: Materials in Medicine*, *32*(4), 39. http://dx.doi.org/10.1007/s10856-021-06511-z. PMid:33792773.
10. Merck. (1990). *Cultivation measurement manual*. Darmstadt: Merck KGaA.
11. Kalegari, M., Miguel, M. D., Dias, J. F. G., Lordello, A. L. L., Lima, C. O., Miyazaki, C. M. S., Zanin, S. M. W., Verdam, M. C. S., & Miguel, O. G. (2011). Phytochemical constituents and preliminary toxicity evaluation of leaves from Rourea induta Planch. (Connaraceae). *Brazilian Journal of Pharmaceutical Sciences*, *47*(3), 635-642. http://dx.doi.org/10.1590/S1984-82502011000300023.
12. Grilo, K. T. M. (2016). *Determinação da concentração de hemoglobina livre em concentrados de hemácias pela espectrofotometria direta: método de Harboe* (Dissertação de Mestrado). Universidade de São Paulo, Ribeirão Preto, Brasil.
13. Hou, Y., Wang, X., Yang, J., Zhu, R., Zhang, Z., & Li, Y. (2018). Development and biocompatibility evaluation of biodegradable bacterial cellulose as a novel peripheral nerve scaffold. *Journal of Biomedical Materials Research. Part A*, *106*(5), 1288-1298. http://dx.doi.org/10.1002/jbm.a.36330. PMid:29316233.
14. Clinical and Laboratory Standards Institute – CLSI. (2005). *Performance standards for antimicrobial susceptibility testing: fifteenth informational supplement CLSI/NCCLS document M100-S15*. USA: Clinical and Laboratory Standards Institute.
15. Shah, F. A., Thomsen, P., & Palmquist, A. (2018). A review of the impact of implant biomaterials on osteocytes. *Journal of Dental Research*, *97*(9), 977-986. http://dx.doi.org/10.1177/0022034518778033. PMid:29863948.
16. Weber, M., Steinle, H., Golombek, S., Hann, L., Schlensak, C., Wendel, H. P., & Avci-Adali, M. (2018). Blood-contacting biomaterials: in vitro evaluation of the hemocompatibility. *Frontiers in Bioengineering and Biotechnology*, *6*, 99. http://dx.doi.org/10.3389/fbioe.2018.00099. PMid:30062094.

17. Zhu, T., Cui, Y., Zhang, M., Zhao, D., Liu, G., & Ding, J. (2020). Engineered threedimensional scaffolds for enhanced bone regeneration in osteonecrosis. *Bioactive Materials*, *5*(3), 584-601. http://dx.doi.org/10.1016/j.bioactmat.2020.04.008. PMid:32405574.
18. Kawai, T., Shanjani, Y., Fazeli, S., Behn, A. W., Okuzu, Y., Goodman, S. B., & Yang, Y. P. (2018). Customized, degradable, functionally graded scaffold for potential treatment of early stage osteonecrosis of the femoral head. *Journal of Orthopaedic Research*, *36*(3), 1002-1011. http://dx.doi.org/10.1002/jor.23673. PMid:28782831.
19. Wang, G., Li, Y., Sun, T., Wang, C., Qiao, L., Wang, Y., Dong, K., Yuan, T., Chen, J., Chen, G., & Sun, S. (2019). BMSC affinity peptide-functionalized β-tricalcium phosphate scaffolds promoting repair of osteonecrosis of the femoral head. *Journal of Orthopaedic Surgery and Research*, *14*(1), 204. http://dx.doi.org/10.1186/s13018-019-1243-5. PMid:31272458.
20. Tan, A. C. W., Polo-Cambronell, B. J., Provaggi, E., Ardila-Suárez, C., Ramirez-Caballero, G. E., Baldovino-Medrano, V. G., & Kalaskar, D. M. (2018). Design and development of low cost polyurethane biopolymer based on castor oil and glycerol for biomedical applications. *Biopolymers*, *109*(2), e23078. http://dx.doi.org/10.1002/bip.23078. PMid:29159831.
21. Patel, V. R., Dumancas, G. G., Viswanath, L. C. K., Maples, R., & Subong, B. J. J. (2016). Castor oil: properties, uses, and optimization of processing parameters in commercial production. *Lipid Insights*, *9*, 1-12. http://dx.doi.org/10.4137/LPI.S40233. PMid:27656091.
22. Oryan, A., Alidadi, S., Moshiri, A., & Maffulli, N. (2014). Bone regenerative medicine: classic options, novel strategies, and future directions. *Journal of Orthopaedic Surgery and Research*, *9*(1), 18. http://dx.doi.org/10.1186/1749-799X-9-18. PMid:24628910.
23. McLaren, J. S., White, L. J., Cox, H. C., Ashraf, W., Rahman, C. V., Blunn, G. W., Goodship, A. E., Quirk, R. A., Shakesheff, K. M., Bayston, R., & Scammell, B. E. (2014). A biodegradable antibiotic-impregnated scaffold to prevent osteomyelitis in a contaminated in vivo bone defect model. *European Cells & Materials*, *27*, 332-349. http://dx.doi.org/10.22203/eCM.v027a24. PMid:24908426.
24. Yang, K., Han, Q., Chen, B., Zheng, Y., Zhang, K., Li, Q., & Wang, J. (2018). Antimicrobial hydrogels: promising materials for medical application. *International Journal of Nanomedicine*, *13*, 2217-2263. http://dx.doi.org/10.2147/IJN.S154748. PMid:29695904.
25. Kamaruzzaman, N. F., Tan, L. P., Hamdan, R. H., Choong, S. S., Wong, W. K., Gibson, A. J., Chivu, A., & Pina, M. F. (2019). Antimicrobial polymers: the potential replacement of existing antibiotics? *International Journal of Molecular Sciences*, *20*(11), 2747. http://dx.doi.org/10.3390/ijms20112747. PMid:31167476.
26. Pramanik, S., Ataollahi, F., Pingguan-Murphy, B., Oshkour, A. A., & Osman, N. A. A. (2015). In vitro study of surface modified poly(ethylene glycol)-impregnated sintered bovine bone scaffolds on human fibroblast cells. *Scientific Reports*, *5*(1), 9806. http://dx.doi.org/10.1038/srep09806. PMid:25950377.
27. Turnbull, G., Clarke, J., Picard, F., Riches, P., Jia, L., Han, F., Li, B., & Shu, W. (2017). 3D bioactive composite scaffolds for bone tissue engineering. *Bioactive Materials*, *3*(3), 278-314. http://dx.doi.org/10.1016/j.bioactmat.2017.10.001. PMid:29744467.
28. Qasim, M., Chae, D. S., & Lee, N. Y. (2019). Advancements and frontiers in nano-based 3D and 4D scaffolds for bone and cartilage tissue engineering. *International Journal of Nanomedicine*, *14*, 4333-4351. http://dx.doi.org/10.2147/IJN.S209431. PMid:31354264.

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