Advanced Ceramics Progress: Vol. 7, No. 3, (Summer 2021) 17-22

Materials and Energy Research Center
MERC

Contents lists available at ACERP

Advanced Ceramics Progress

Journal Homepage: www.acerp.ir

Original Research Article

In Vivo Assessments of the Poly(d/l)lactide/Polycaprolactone/Bioactive Glass Nanocomposites for Bioscrews Application

J. Esmaeilzadeh [a,*], S. Hesaraki [b], S. Borhan [c]

[a] *Assistant Professor, Department of Materials and Chemical Engineering, Esfarayen University of Technology, Esfarayen, North Khorasan, Iran*
[b] *Professor, Department of Nanotechnology and Advanced Materials, Materials and Energy Research Center (MERC), Meshkindasht, Alborz, Iran*
[c] *Assistant Professor, Department of Materials, Chemical, and Polymer Engineering, Imam Khomeini International University-Buin Zahra Higher Education Center of Engineering and Technology, Buin Zahra, Qazvin, Iran*

* Corresponding Author Email: esmaeilzadehj@esfarayen.ac.ir (J. Esmaeilzadeh)

URL: https://www.acerp.ir/article_136416.html

ARTICLE INFO

Article History:

Received 20 May 2021
Received in revised form 13 July 2021
Accepted 01 September 2021

Keywords:

PDLLA/PCL
Bioactive Glass Nanoparticles
6 Months Follow Up
Canine Animal Model
Bioscrews

ABSTRACT

In the present study, in vivo properties of poly (D/L) lactide (PDLLA)/polycaprolactone (PCL)/bioactive glass nanocomposites (PPB) and PDLLA/PCL blends (PP) were investigated up to six months. The in vivo results from the implants inserted on canine models indicated that the weight losses of PPB and PP were approximately 60 and 70%, respectively. In addition, the average molecular weight of both specimens decreased as a function of grafting times; however, such decrease in trend of blends was more considerable than that in nanocomposites. Moreover, the obtained histological images of the animal model up to six months of implantation distinguished the formation of the new bone within the implanted area, while no osteitis and osteomyelitis or structural abnormality were observed. Overall, the animal in vivo tests results of implants within a period of 180 days confirmed the good biocompatibility among them and appropriate degradation behavior of PPB, hence a proper candidate for Anterior Cruciate Ligament Reconstruction (ACLR) screws.

https://doi.org/10.30501/ACP.2021.286695.1061

1. INTRODUCTION

The stability of knee joint is ensured by four extremely strong ligaments: Anterior Cruciate Ligament (ACL) and Posterior Cruciate Ligament (PCL) prevent the tibia from slipping in sagittal planes; Medial Collateral Ligament (MCL) and Lateral Collateral Ligament (LCL) prevent the knee from bending in coronalplan [1]. Anterior Cruciate Ligament Reconstruction (ACLR) screws are the most popular implants among all orthopaedics implants used in fixation and reconstruction of damaged bones.

Currently, metallic screws are the most commonly used ligament graft fixation devices in ACLR. To eliminate some of the potential problems related to metallic ACL screws, the biodegradable ones were generated [2,3]. The biodegradable screws can be resorbed in body during the determined time after

Please cite this article as: Esmaeilzadeh, J., Hesaraki, S., Borhan, S., "In Vivo Assessments of the Poly(d/l)lactide/Polycaprolactone/Bioactive Glass Nanocomposites for Bioscrews Application", ***Advanced Ceramics Progress***, Vol. 7, No. 3, (2021), 16-21. https://doi.org/10.30501/ACP.2021.286695.1061

implantation, and degradation products disappear through metabolic routes [4]. As previously reported, the mechanical properties of PDLLA/PCL blends including tensile strength, tensile modulus, flexural strength, and flexural modulus [5] as well as creep and creep recovery [6] were enhanced by incorporating sol-gel-derived bioactive glass nanoparticles (BGn) into the matrix. This incorporation functions as a bone on growth agent and provides a reservoir of calcium and phosphate ions, thus accelerating the new bone formation and preventing voids after screw removal [7]. Moreover, adjacent bone can interact with screw and attach to the bioactive fillers of bioscrews while the polymeric matrix is simultaneously degraded [8].

With regard to biodegradable ACL screws, tailoring of degradation manner gains significance; therefore, there should be a harmonic trend between the mechanical properties of loosening that results fromdegradation of screw constructs and ligament healing process [9]. In vitro and preclinical animal in vivo studies have been extensively used to investigate the biocompatibility and degradation behaviors of biodegradable implants [10-12].

In preclinical in vivo tests, the animal model was selected based on the properties under study such as biocompatibility and biodegradation or biomechanical characterestics. The obtained results of in vivo tests were typically a combination of clinical examination, imaging (radiological, MRI, CT), macroscopic, histological evaluation, biomechanical, and physicalproperties (e.g., mass loss) [13-15].

A number of researchers have conducted in vivo assessments of biodegradable polymeric materials and polymeric-based composites [16,17]. For instance, the three-month follow-up for in vivo tests of PLA/hydroxyapatite (PLA/HA) and PLA grafts revealed that the PLA/HA nanocomposites were characterized by good biocompatibility and promising potential applications for bone implants [18].

The present research aimed to carry out in vivo studies including mass loss, molecular weight variations, and histopathological analysisfor the PDLLA/PCL/BGn as the nanocomposite and PDLLA/PCL as the control groups. In addition, afforts have been made to develop PDLLA/PCL/BGn nanocomposite using solvent casting followed by a hot pressing step. The osteoinductive potential of the nanocomposites was investigated in a preliminary in vivo study in a canine tibia bone. It was hypothesized that the bioactive glass nanoparticles could enhance the bioactivity and biocompatibility of PDLLA/PCL throughout in vivo assessments. To the best of our knowledge, no or at least very few studies have been reported on the animal in vivo studies of PDLLA/PCL/BGn triple nanocomposites for any bio implants applications.

2. MATERIALS AND METHODS

2.1. Specimens Preparation

The preparation details of PDLLA/PCL as the control (PP) and PDLLA/PCL/BGn as the nanocomposite (PPB) samples have been fully described in the previous paper [5]. Briefly, the control samples were produced by introduction of the PCL phase into the PDLLA matrix phase in a portion of 20:80 dissolving in chloroform solvent. The nanocomposite samples were also prepared by adding three wt% BGn into PDLLA/PCL bipolymeric solution. After homogenization by stirring, the mixtures were cast and dried at 50 °C and 80 °C in the oven and vacuum oven, respectively, to remove the solvent. Finally, the dried samples were poured into the molds and then, were compressed under 30 MPa at 180 °C followed by water-cooling to room temperature. Meanwhile, all nanocomposites were pressed under heating for less than three minutes.

2.2. In Vivo Animal Model

The animal model tests were conducted in the canine model. The control and nanocomposite samples were sterilized using gamma-ray with 25 K Gray energy for 10 hours. General anesthesia was given by an intramuscular injection of 0.1 mg/kg atropine and local anesthesia by 6–12 mg/kg of Zoletil. Local anesthesia was performed by an injection of lidocaine/epinephrine. The defects with the size of seven mm in diameter and three mm in depth were created with a trephine bur between methaphysis and diaphysis of tibia on canine. Bone defects were filled by PDLLA/PCL and PDLLA/PCL/BGn specimens, and an empty defect was used as a control (Figure 1). Samples (n=3) were harvested after each month up to six months. At each harvest time point, scalpel blade No.9 was used to collect the specimens that were immediately placed in 10% formalin.

2.3. Pathologic Procedure

After decalcification of the samples, the implanted samples were excised using scalpel blade No.9, and the prepared section of samples of 5-6 µm in diameters was stained by Hematoxylin and Eosin (H&E) staining procedure. Sections were then examined for evidence of biocompatibility and bone regeneration under a light microscope.

2.4. Degradation Assessments

After biopsy, the supernumerary tissue was removed from implanted samples, and the samples were soaked in 2.5 g/l collagenase-II and 2 g/l tryptase solutions for one hour, respectively. Then, the samples were rinsed by distilled water and dried in the air for 24 hours.

The animal in vivo degradation of samples at different intervals was identified by measuring the weight and molecular weight variations. The in vivo weight loss

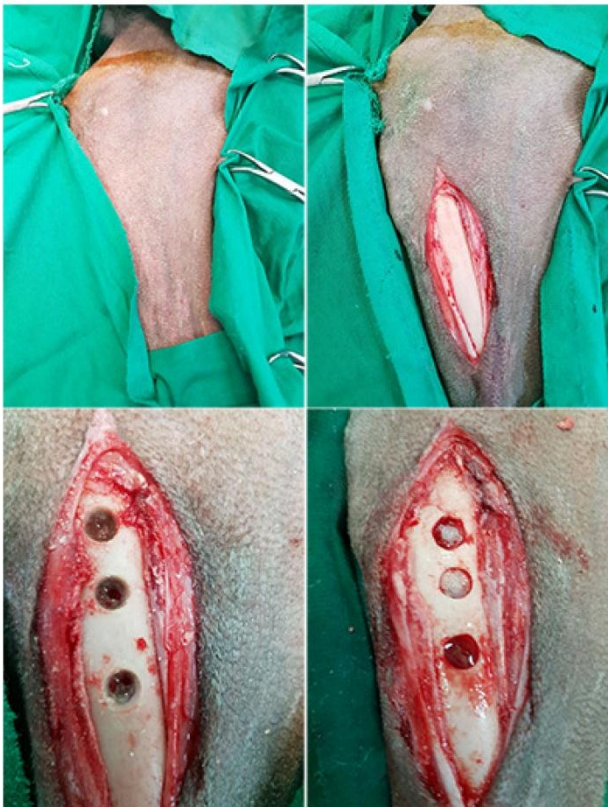

Figure 1. The surgery procedure and implantation site of PPB nanocomposites and PP blends implants into canine tibia bone

variations were estimated through the following equation:

$$W_t\% = \frac{W_f - W_i}{W_i} \times 100 \quad (1)$$

where W_i is the initial dry weight of the sample and W_f is the dry weight of the sample at studying time periods. Values are expressed as the average of three replicates. The molecular weights of the samples before and after implantation were obtianed using Size Exclusion Chromatography (SEC) supplemented by alltima columns. In this chromatography, tetrahydrofuran with a rate of 1 mL/min was used as the refractory coefficient detector. For each sample, 30 µL of tetrahydrofuran solution was used, and standard polystyrene was chosen for calibration.

3. RESULTS AND DISCUSSIONS

3.1. Degradation Behaviors

Figure 2a shows the weight loss variations of the implants for different time periods of implantation in the canine model. Both implants show a sharp trend at the early stage up to 30 days; then, the trend continues with a relative constant slop up to six month.

Due to the prescence of dynamic circumstance as well as sever activities of macrophages cells and immune cells through animal models, it is expected that the weight loss percentages during in vivo studies bemore than that in the simulated body solutions. Figure 2b shows the weight variations for PP and PPB specimens in Simulated Body Fluid (SBF) and Phosphated Buffered Sulin (PBS) during 180 days of immersion (equal to 4320 hours).

The results indicated that the weight variations for PP and PPB specimens in PBS biological solutions were greater than those in the SBF solutions during immersion time. In addition, the findings had 64% and 55 % weight losses for PP and PPB, respectively, at the end of immersion times. At the end of six-month follow-up of in vivo assessments, the weight loss values range from 70% to 75% for PP and 60% to 65% for PBB. Obviously, the remaining mass of PP and PPB implants was higher throughout the in vitro studies than that in the in vivo assay.

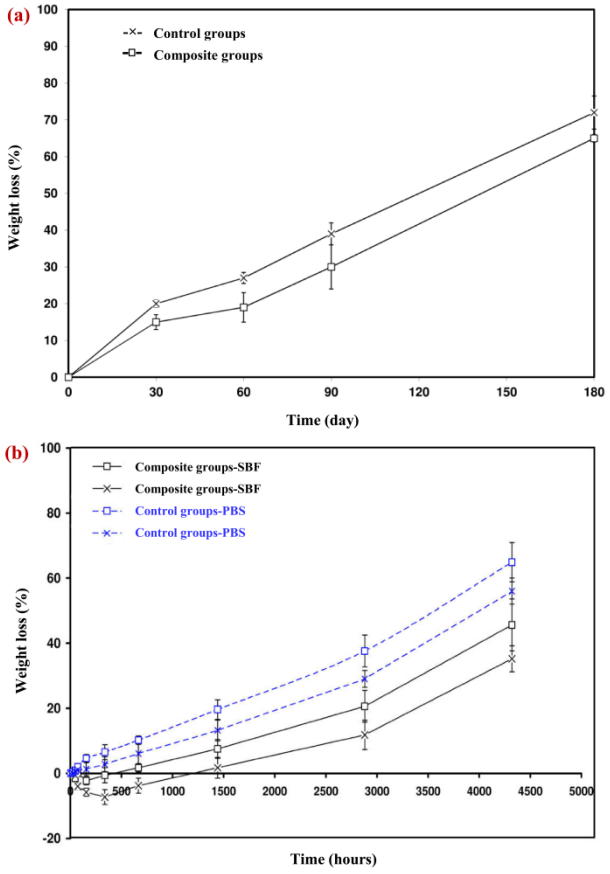

Figure 2. (a) Weight loss variations of PP and PPB for different time periods of implantation in canine model study, (b) Exhibitions of weight loss variations for PP and PPB specimens in SBF and PBS solutions during immersion times up to 6 months

It can be anticipated that the rate of weight loss would increase after six months since with the formation of some early prosities throughout the implants bulk, the exposure of the implants to the body fluid would significantly increase, thus leading to fast degradations.

The lower degradation rate of PPB compared to that of PP implants may be related to the presence of BGn and their appropriate distribution throughout the PDLLA/PCL matrix [5]. It is hypothesized that the bioactive glass can be significantly grafted to the bone tunnel due to its similarity to natural bone in terms of composition. Therefore, it can act as a barrier against further hydrolization of PDLLA/PCL phases. Further, BGn prevents the migration of the products resulting from PDLLA and PCL degradation. The acidity of implants caused by the acid release from products can be neutralized by releasing Ca-P ions of BGn. Therefore, the self-catalytic effects within polymer degradation are suppressed which lead to a greater decrease in the degradation rate of PPB implants than that of PP ones.

3.2. Molecular Weight Variations

The averages of molecular weight (M_w) variations for PP and PPB implants within different implantation time periods are presented in Figure 3.

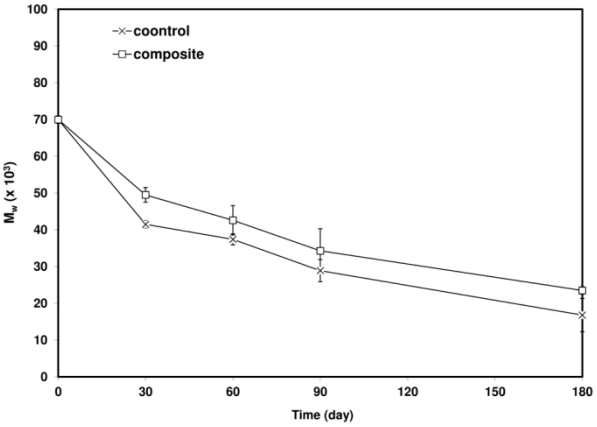

Figure 3. Averages of molecular weight (M_w) variations for PP and PPB specimens for 6 months of implantation time periods

These results are indicative of the accuracy of weight loss results. Both curves follow similar trends with those of the weight loss results, but inversely. Hence, at the first stage, both curves show a decreasing trend with a sharper slop rather than other stages.

At the whole interval evaluations, PP has lower M_w than PPB, while the initial M_w was the same for both implants. Up to 30 days, M_w would considerably decrease which may be due to release of residual monomers of specimens. The decrease in M_w indicates that the major part of degradation mechanism is attributed to polymer chains breakaging.

It can be concluded that the lower resorption and degradation rates of PPB than those of PP implants can play significat roles in manufacturing biodegrade internal fixation devices mainly because providing appropriate mechanical properties and optimum durability can be superior for an bioscrews applications. In addition, slow release of degradation products of PPB can enhance its biocompatibility [19].

3.3. Histopathological Analysis

The histological analysis of groups with no implant replacements harvested from canine tibia bone after 30 days and 180 days of follow-up stained with H&E is shown in Figures 4.

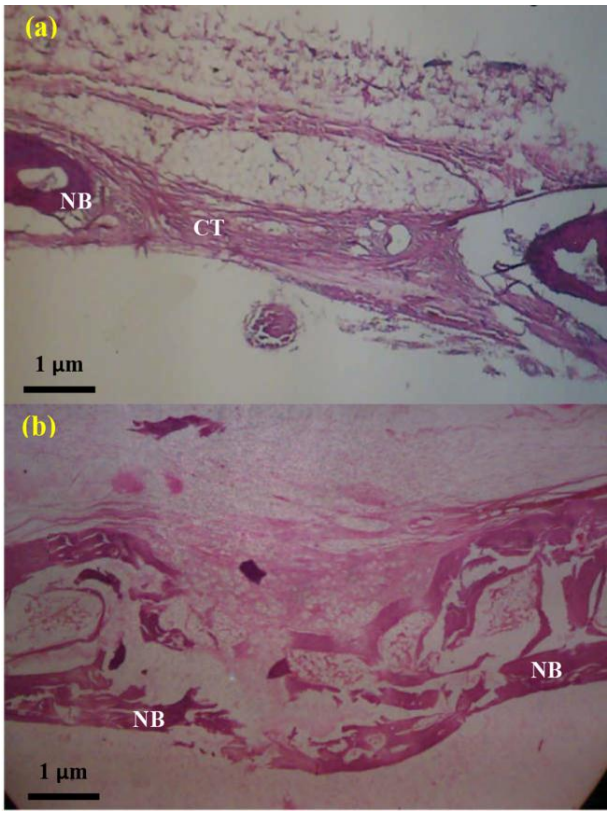

Figure 4. Histopathological images of cavities inserted into tibia bone with no implants after following up to 6 months, NB: New Bone, CT: Connective Tissue

The images show no adverse inflammation response after one-month follow-up. Moreover, ossifications in the vicinity of cavities are poor. After one month of implantation, the defect was filled by a mature connective tissue made up of lamellar collagen fibers and blood vessels.

As observed, the mineralized osteoid was converted to immature bone spicules. After six-month follow-up,

some parts of defect were replaced by connective tissues, and the remaining parts were exchanged by the new bone in both modular and cortical forms as well as osteocytes. It should be noted that the thickness of collagen fibers at the early formation stages of connective tissues is higher than that of mature connective tissue.

Figure 5 depicts the images of the decalcified area of defects inserted into the tibia of canine which was replaced by PP and PPB implants after 30, 60, 90, and 180 days of follow-ups.

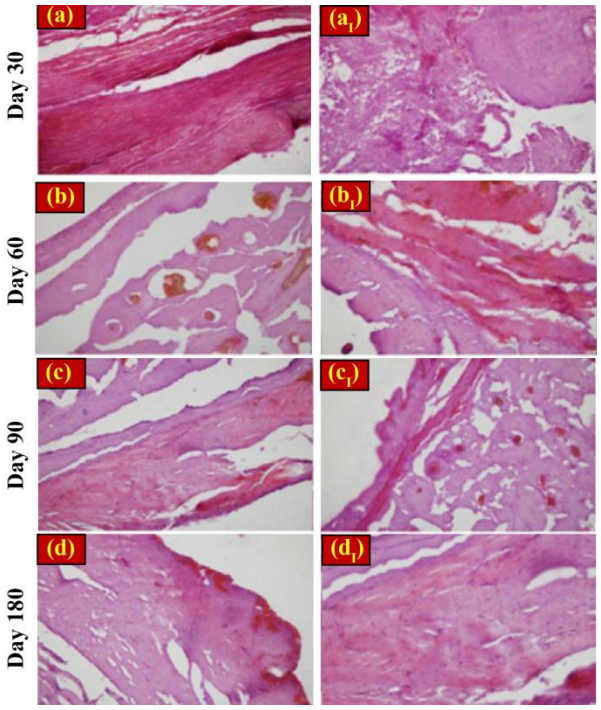

Figure 5. Images of decalcified area of defects inserted into tibia of canine and replaced by PP and PPB implants after 30, 60, 90 and 180 days follow ups

Some fibroblast cells as well as local calcium precipitates and blood vessels for PPB implants were observed in the first month after the surgery. However, for PP implants, plenty of inflammation cells without calcium precipitations were observed. For PPB implants in the second post-surgery month, the spheroid-like osteoblast cells wereorderly observed in vicinity of collagen fibers. After 3 months post-surgery, the morphology of osteoblast cells was converted to lamellar, indicating the new bone formation. For PP implants, not only is there no evidence for order configuration of osteoblast cells in the vicinity of collagen fibers but also osteoblast cells are randomly distributed in vicinity of collagen fibersafter 2 and 3 months. For PPB implants in the 6th month, the collagen fibers would be converted to the lamellar structure and trabecular bone tissue.

Overall, the histopathologic assessments confirmed that the novel formulation of PDLLA/PCL/BGn nanocomposites materials enhanced the bone reconstruction more efficiently than PDLLA/PCL. Of note, the degradation rate of PPB was lower than that of PP implants.

4. CONCLUSIONS

Based on the results of this study, the following concluding remarks can be made:

- The weight loss changes within the canine model throughout the in vivo study showed that both PPB and PP lost approximately 60% and 70% of their initial total weights, respectively.
- The average molecular weight variations as a function of grafting times illustrated that the decreasing trend of PP was more considerable than that of PPB.
- The histopathological results up to six months of implantation confirmed the formation of the new bone within the implanted area, while no osteitis, osteomyelitis, and structural abnormality were observed.
- The in vivo tests results of implants into tibia of the canine model during six months confirmed the good biocompatibility and appropriate degradation behavior of PPB which can promise it as a proper candidate for ACLR screws.

ACKNOWLEDGMENT

The authors wish to acknowledge Esfarayen University of Technology (EUT) and Materials and Energy Research center (MERC) for the all supports throughout this work.

COMPLIANCE WITH ETHICAL STANDARDS

(In case of Funding) Funding: The research leading to these results received funding from the Ministry of Industry Mine and Trade of Islamic Republic of Iran under Grant Agreement No. 93/41/5659. Partial financial support was also received from Esfarayen University of Technology(EUT).

CONFLICT OF INTEREST

The authors declare no conflict of interests.

REFERENCES

1. Esmaeilzadeh, J., Setayesh, H., “Bioabsorbable Screws for Anterior Cruciate Ligament Reconstruction Surgery: A Review”, ***Advanced Ceramic Progress***, Vol. 6, No.3, (2020), 31-46. https://doi.org/10.30501/acp.2020.110318

2. Luo, Y., Zhang, C., Wang, J., Liu, F., Chau, K. W., Qin, L., Wang, J., "Clinical translation and challenges of biodegradable magnesium-based interference screws in ACL reconstruction", ***Bioactive Materials***, Vol. 6, No. 10, (2021), 3231-3243. https://doi.org/10.1016/j.bioactmat.2021.02.032
3. Mao, G., Wang, C., Feng, M., Wen, B., Yu, S., Han, X., Yu, Z., Qiu, Y., Bian, W., "Effect of biodegradable Zn screw on bone tunnel enlargement after anterior cruciate ligament reconstruction in rabbits", ***Materials & Design***, Vol. 207, (2021), 109834. https://doi.org/10.1016/j.matdes.2021.109834
4. Ramos, D. M., Dhandapani, R., Subramanian, A., Sethuraman, S., Kumbar S. G., "Clinical complications of biodegradable screws for ligament injuries", ***Materials Science and Engineering: C***, Vol. 109, (2020), 110423. https://doi.org/10.1016/j.msec.2019.110423
5. Esmaeilzadeh, J., Hesaraki, S., Hadavi, S. M. M., Ebrahimzadeh, M. H., Esfandeh, M., "Poly (D/L) lactide/polycaprolactone/bioactive glass nanocomposites materials for anterior cruciate ligament reconstruction screws: The effect of glass surface functionalization on mechanical properties and cell behaviors", ***Materials Science and Engineering: C***, Vol. 77, (2017), 978-989. https://doi.org/10.1016/j.msec.2017.03.134
6. Esmaeilzadeh, J., Hesaraki, S., Ebrahimzadeh, M. H., Asghari, G. H., Kachooei, A. R., "Creep behavior of biodegradable triple-component nanocomposites based on PLA/PCL/bioactive glass for ACL interference screws", ***Archives of Bone and Joint Surgery***, Vol. 7, No. 6, (2019), 531-537. https://doi.org/10.22038/abjs.2019.30582.1796
7. Niemelä, T., Kellomäki, M., "Bioactive glass and biodegradable polymer composites", In Ylänen H. O. (ed.), ***Bioactive Glasses: Materials, Properties and Applications***, Woodhead Publishing Series in Biomaterials, Woodhead Publishing, Oxford, (2011), 227-245. http://www.woodheadpublishing.com
8. Meretoja, V. V., Tirri, T., Malin, M., Seppälä, J. V., Närhi, T. O., "Ectopic bone formation inand soft tissue response to P(CL/DLLA)/bioactive glass composite scaffolds", ***Clinical Oral Implants Research***, Vol. 25, No. 2, (2014), 159–164. https://doi.org/10.1111/clr.12051
9. Mayr, H. O., Hube, R., Bernstein, A., Seibt, A. B., Hein, W., von Eisenhart-Rothe, R., "Beta-tricalcium phosphate plugs for press-fit fixation in ACL reconstruction—a mechanical analysis in bovine bone", ***The Knee***, Vol. 14, No. 3, (2007), 239–244. https://doi.org/10.1016/j.knee.2007.01.006
10. Zhang, Z., Jia, B., Yang, H., Han, Y., Wu, Q., Dai, K., Zheng, Y., "$Zn_{0.8}Li_{0.1}Sr$—a biodegradable metal with high mechanical strength comparable to pure Ti for the treatment of osteoporotic bone fractures: In vitro and in vivo studies", ***Biomaterials***, Vol. 275, (2021), 120905. https://doi.org/10.1016/j.biomaterials.2021.120905
11. Sekar, P., Narendranath, S., Desai, V., "Recent progress in in vivo studies and clinical applications of magnesium based biodegradable implants – A review", ***Journal of Magnesium and Alloys***, Vol. 9, No. 4, (2021), 1147-1163. https://doi.org/10.1016/j.jma.2020.11.001
12. Jia, B., Yang, H., Han, Y., Zhang, Z., Qu, Xi., Zhuang, Y., Wu, Q., Zheng, Y., Dai, K., "In vitro and in vivo studies of Zn-Mn biodegradable metals designed for orthopedic applications", ***Acta Biomaterialia***, Vol. 108, (2020), 358-372. https://doi.org/10.1016/j.actbio.2020.03.009
13. Schmidmaier, G., Baehr, K., Mohr, S., Kretschmar, M., Beck, S., Wildemann, B., "Biodegradable Polylactide membranes for bone defect coverage: biocompatibility testing, radiological and histological evaluation in a sheep model", ***Clinical Oral Implants Researchs***, Vol. 17, No. 4, (2006), 439-444. https://doi.org/10.1111/j.1600-0501.2005.01242.x
14. Kontio, R., Ruuttila, P., Lindroos, L., Suuronen, R., Salo, A., Lindqvist, C., Virtanen, I., Konttinen, Y. T., "Biodegradable polydioxanone and poly(l/d)lactide implants: an experimental study on peri-implant tissue response", ***International Journal of Oral and Maxillofacial Surgery***, Vol. 34, No. 7, (2005), 766-776. https://doi.org/10.1016/j.ijom.2005.04.027
15. Waris, E., Ashammakhi, N., Lehtimäki, M., Tulamo, R. M., Kellomäki, M., Törmälä, P., Konttinen, Y. T., "The use of biodegrdable scaffold as an alternative to silicone implant arthroplasty for small joint reconstruction: an experimental study in mini pigs", ***Biomaterials***, Vol. 29, No. 6, (2008), 683-691. https://doi.org/10.1016/j.biomaterials.2007.10.037
16. Barbeck, M., Serra, T., Booms, P., Stojanovic, S., Najmand, S., Engel, E., Sader, R., Kirkpatrick, C. J., Navarro, M., Ghanaati, S., "Analysis of the in vitro degradation and the in vivo tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components - Guidance of the inflammatory response as basis for osteochondral regeneration", ***Bioactive Materials***, Vol. 2, No. 4, (2017), 208-223. https://doi.org/10.1016/j.bioactmat.2017.06.001
17. Guo, Z., Bo, D., He, Y., Luo, X., Li, H., "Degradation properties of chitosan microspheres/Poly(L-lactic acid) Composite in vitro and in vivo", ***Carbohydrate Polymers***, Vol. 193, (2018), 1-8. https://doi.org/10.1016/j.carbpol.2018.03.067
18. Nguyen, T. T., Hoang, T., Can, V. M., Ho, A. S., Nguyen, S. H., Nguyen, T. T. T., Pham, T. N., Nguyen, T. P., Nguyen, T. L. H., Thi, M. T. D., "In vitro and in vivo tests of PLA/d-Hap nanocomposites", ***Advances in Natural Sciences: Nanoscience and Nanotechnology***, Vol. 8, No. 4, (2017), 045013. https://doi.org/10.1088/2043-6254/aa92b0
19. Akagi, H., Iwata, M., Ichinohe, T., Amimoto, H., Hayashi, Y., Kannno, N., Ochi, H., Fujita, Y., Harada, Y., Tagawa, M., Hara, Y.,"Hydroxyapatite/poly-L-lactide acid screws have better biocompatibility and femoral burr hole closure than does poly-L-lactide acid alone", ***Journal of Biomaterials Applications***, Vol. 28, No. 6, (2014), 954–962. https://doi.org/10.1177/0885328213487754