Bending strength and Biological properties of PLA-HA composites for femoral canine bone fixation plate

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Abstract. This research is based on the purposes to find suitable hot compression conditions of composites materials, also to optimize mechanical and biological properties of the plate specimen. The type of composites materials under this investigation are bio-polymers (polylactic acid: PLA) and bio-ceramic (Hydroxyapatite: HA). In this research, the term “The design of the experiment (DOE)” was implemented to find the relation between forming factors including the ratio of HA ratio, molding temperature, and molding pressure. The result showed that the optimum conditions of the bending test were at 5%wt of HA, 160 °C, 7 MPa of molding temperature and pressure respectively. Biodegradation rate of a specimen was 0.0175 g/day and the specimens acquired a good biocompatibility and they were non-toxic to living cells.

1. Introduction

The hind limb of canines is one of the areas which are interesting to veterinary orthopedic surgeons. In the surgical treatment of long bone diaphyseal fracture in canines, it is consistently attractive to use the bone plates [1,2] which are normally produced from metal such as stainless-steel grade 316L or titanium alloys. However, the strength of metal plate which is greater than the femoral bone may cause cracking around the surgical area. Furthermore, released ions from metal surface may lead to infection, inflammation, and swelling of the adjacent tissues [3]. According to the stated issues, this research shall find alternative materials for repairing tissues without toxicity. Thus, the compelling alternative materials in this research are bio-polymer: polylactic acid (PLA) and bio-ceramic: Hydroxyapatite (HA).

PLA is one of the most extensive thermoplastic aliphatic polyesters which is derived from a lactic acid monomer and the natural products of fermentation as sugar or corn. It becomes fusible at a temperature average 180 to 220 °C and at average 60 to 65 °C glass transition temperature (Tg). PLA has a good tensile strength, rigidity, and stiffness. Moreover, PLA can be degraded easily by a hydrolysis reaction between the ester group which occurs in the formation without injury and toxicity [4,5] and its properties are excellent for optimization with a biomedical application, for instance; internal plates or screw fixation of damaged or fractured bone [6].

HA is a fundamental inorganic component of bones and teeth because of its properties such as bone bonding ability and greater bioactivity to stimulate the formation of new cells surrounding
damaged tissues. Therefore, HA can be applied in many fields; for example, coating on metallic implants and bone substitutes [7]. However, the disadvantage of HA is low biodegradability. Therefore, it is not suitable to use in bone scaffolds or cells promotion [8]. With references to the subject mentioned above, this research has attempted to combine HA with polymer composite to use in the biomedical application. As a polymer matrix composite has the advantage of being very adaptable, allowing the accommodation for its properties thus, this shall allow the accommodation to access its properties [6,9].

Factorial designs have been commonly used in experiments about several factors, which it is necessary to investigate a response in each effect of the factor. The $2^k$ design is basically the term which we use in the primary steps of experimental processes because it contributes to the fundamental number of runs from all experiments and complete factorial design could be studied in each k factor. Therefore, for optimal experiments, it is necessary to use these designs to verify this matter [10].

Then, this research aims to investigate and identify optimal forming conditions and degradation rates of PLA-HA composites. The fabrication method of PLA-HA compact specimens for canine’s femoral bone plates is based on the $2^k$ factorial design with the center point. The optimal conditions for mechanical property was reported in terms of bending strength. Furthermore, Biodegradation was reported by calculating weight loss from the specimen in PBS solution and biocompatibility was tested with osteoblast cell lines. Finally, the actual determined bending, biodegradation and biocompatibility properties of the specimen from this research have benefits to apply for using in canine femoral in the future.

2. Materials and Methods

In this research, researcher manufactured PLA-HA composites based on the design of experiment, theory and fabricated all of specimen by hot compression molding.

2.1 Material Preparation
Polylactic acid (Molecular weight $\approx 60,000-80,000$) was purchased from Natureworks®. Polymer pellets was reduced in particle size by Universal Milling machine with liquid nitrogen, then, we sieve the pellets to become powder and keep them in an oven under atmospheric temperature [11].

Hydroxyapatite powder (HA) was prepared from bovine bones. The first step is to reduce the bovine bones into small pieces by cutting or grinding method, then, use hydrogen peroxide to soak these bones to remove organic tissues. Afterwards, remove their collagen and organic substances with boiling water for a moment. Next, keep these bones to reduce moisture content in the hot air oven under ambient temperature. Finally, calcine all dry bones at 900°C for 3 hours and reduce particle size less than 20 μm [3] with a high-speed ball milling machine.

Add PLA and HA from fixing ratio (from table 1) into the ball mill grinder for 24 hours after, then, remove their moisture content in the vacuum oven at 80 °C for 12 hours.

2.2 Methods
All samples were fabricated by hot compression molding process. Taking 12.6 grams of composite powder to ensure fully gravity of die casting compression mold which is pre-heated average 80°C before forming a specimen. A specimen was fabricated under HA proportion 5-10 %wt. with the molding temperature of 140-160 °C and with molding pressure 3-7 MPa [12]. Also, the factorial design was applied with center-point and set up following table 1.

2.3 $2^k$ Full factorial design
The experiment was designed for the various levels of composites forms according to $2^k$ factorial design with the center point. The factor in this experiment which regards to three factors contains HA proportion, temperature, and pressure following (table 1) repeat all of experiment twice in each condition then created a $2^k$ factorial table with software as Minitab18. The table showed 20 experiments include 16 factorial designs and 4 center points. The responses of all experiment were reported on bending strength, biodegradation, and biocompatibility
| Factors                  | Levels     |
|-------------------------|------------|
| HA (%)                  | Low (-1)   | Medium (0) | High (+1)  |
| Molding Temperature (°C)| 5          | 15         | 160        |
| Molding Pressure (MPa)  | 3          | 5          | 7          |

2.4 Characterizations

All specimens were characterized on the mechanical property: bending strength. The specimen of optimum bending strength was tested to biological properties: biodegradation rates and biocompatibility. Minitab18 was used to consider a statistically significant variability.

2.4.1 Bending strength

The bending strength of PLA-HA composites in each condition was measured by Universal Testing Machine (Instron 5566) with Three-point bending under the guidance of ASTM D790 at a loaded cell 100 N. Stress-strain curves were analyzed to find bending strength in each sample [13].

2.4.2 Biodegradation

The PLA-HA specimens were cut on cubic size 5 mm., into 36 pieces, then carried out these samples into the Phosphate Buffer Saline solution, pH ≈ 7.4, volume 1 ml. All samples were kept on disc gathered in the oven at 37 °C for up to 1, 3, 7, 14, 30, and 50 days. At daily intervals, these samples were removed from the Phosphate Buffered Saline (PBS) solution and dried at room temperature, then calculated equation \[\frac{100(W_i - W_f)}{W_0}\] to find the percentage of weight loss where \(W_i\) is the initial sample weight and \(W_f\) is the weight of dried residual [14].

2.4.3 Biocompatibility

PLA-HA samples were tested with osteoblast cell following on ASTM F895-11 and ASTM F1903-10. The percentage of cell viability was measured with the microplate reader at wavelength 570 nm. Finally, we calculated the following equation \[\frac{\text{OD of treated cell}}{\text{OD of control cell}} \times 100\] and compared with osteoblast cells without Dulbecco’s Modified Eagle Medium (Control sample).

2.4.4 Statistical analysis

\(2^k\) factorial design with center point was used to investigate the effect of hot compression molding and the statistical significance value between three factors in this experiment, which was identified by ANOVA. Statistically, signification was considered with P-value less than 0.05 at 95 confidential levels. Minitab18 software was employed for the statistical analysis.

3. Result and Discussion

All specimens were investigated to find a suitable condition of the mechanical property in term of bending strength, which we found at 5%wt. of HA, 160°C, 7 MPa. Biodegradation property of the specimen showed that it can be degraded in 0.0175 g/day or average 15 years. In addition, biocompatibility also showed cell viability at 88.97%, this can be concluded that this specimen is appropriate to use in femoral canine bone.

3.1 Bending strength

According to the experiment, we found the results of bending strength of three factors include; HA ratio, molding temperature, molding pressure to fabricate PLA-HA composites which were analyzed by minitab18 software. The results showed in table 2 and 3 that founded the highest bending strength was found at 5%wt. of HA ratio, temperature 160 °C and pressure 7 MPa. Thus, this highest bending value was nearly range with bending strength of cortical bone following in table 4. At higher HA ratio over 5%wt, the dispersal of HA particles reduced, which was the causes from higher viscosity than lower HA proportion in PLA matrix, and finally the bending strength of PLA-HA composites to get decreased [7]. In addition, the highest temperature appeared better distribution of the HA through PLA surface [15]. Researcher can assume that bending strength strongly depended on the effect of HA ratio.
and temperature because of its present p-value of both less than 0.005, R-sq and R-sq adjust are 90.07%, 82.85% respectively. Nevertheless, molding pressure of all experiment in each level of factor show the result which P-value reached more than 0.05. This indicates that it is not significantly different to bending strength test because of hot compression machine has limitation to be able do that.

| No. | HA (wt.%) | Temperature (°C) | Pressure (MPa) | Bending Strength (MPa) |
|-----|-----------|------------------|----------------|-----------------------|
| 1   | 5         | 140              | 3              | 73.092                |
| 2   | 15        | 140              | 3              | 41.670                |
| 3   | 5         | 160              | 3              | 63.711                |
| 4   | 15        | 160              | 3              | 58.701                |
| 5   | 5         | 140              | 7              | 48.857                |
| 6   | 15        | 140              | 7              | 51.627                |
| 7   | 5         | 160              | 7              | 70.336                |
| 8   | 15        | 160              | 7              | 49.591                |
| 9   | 5         | 140              | 3              | 64.889                |
| 10  | 15        | 140              | 3              | 47.915                |
| 11  | 5         | 160              | 3              | 61.627                |
| 12  | 15        | 160              | 3              | 55.432                |
| 13  | 5         | 140              | 7              | 41.001                |
| 14  | 15        | 140              | 7              | 55.766                |
| 15  | 5         | 160              | 7              | 75.275                |
| 16  | 15        | 160              | 7              | 42.400                |
| 17  | 10        | 150              | 5              | 56.292                |
| 18  | 10        | 150              | 5              | 50.889                |
| 19  | 10        | 150              | 5              | 46.252                |
| 20  | 10        | 150              | 5              | 47.084                |

**Table 3.** The estimated effects and coefficients bending strength from minitab18 software

| Term            | Coef | SE Coef | T     | P     |
|-----------------|------|---------|-------|-------|
| Constant        | 56.368 | 1.069  | 52.75 | 0.000 |
| HA ratio        | -5.980 | 1.069  | -5.60 | 0.000 |
| Temperature     | 3.266 | 1.069  | 3.06  | 0.011 |
| Pressure        | -2.011 | 1.069  | -1.88 | 0.087 |
| HA ratio*Temp   | -2.123 | 1.069  | -1.99 | 0.072 |
| HA ratio*Pressure| 1.470 | 1.069  | 1.38  | 0.196 |
| Temp*Pressure   | 1.778 | 1.069  | 1.66  | 0.124 |
| HA ratio*Temp*pressure | -6.772 | 2.390  | -2.61 | 0.024 |

**Table 4.** The average bending strength of materials and cortical bone.

| Bending Strength (MPa) |
|------------------------|
| Cortical Bone          | 61 [16]  |
| Stainless Steel (316L)| 645 [16] |
| Polylactic Acid        | 108 [17] |
| Hydroxyapatite         | 40 [18]  |

**3.2 Biodegradation**

Biodegradation of PLA-HA composite specimen at 5%wt. of HA ratio, molding temperature 160 °C and molding pressure 7 MPa. Figure 1. showed a relative between the percentage of degradation and the number of days to degrade that find its value from the graph. Predicted the percentage of degradation using least-square on Regression model to fit data to find the rate of an equation. The percentage of degradation Y in term of day of degradation X was explained by Y = 0.0175X + 0.0376 which means that a specimen can be degraded in 0.0175 g/day. We assumed that PLA-HA composite plate can be used appropriately in canines because of its bending property values nearly canines’ bone and degrade in average 15 years.

**3.3 Biocompatibility**

PLA-HA sample was tested on cell viability with osteoblast cell for 24 hours and repeated in each condition 3 times to prove that all these composites samples are non-toxic. As a result, figure 2 showed PLA-HA samples have the percentage of cell viability at 88.97 (more than 80% when compared with a control sample) that means it is possible to be applied in the living body.
4. Conclusion
In this study, researcher purpose is to fabricate a composites plate containing 2 kinds of biomaterial; polylactic acid and hydroxyapatite, by hot-compression molding method. All of experiment in this study, researcher employed 23 factorial design with the center point and 2 times repeated. The variable conditions that we observed were HA ratio (5, 10, 15 %wt.), molding temperature (140,150, 160°C) and pressure (3, 5, 7 MPa). Bending strength was investigated by a universal testing machine (UTM) and biodegradation rate was taken in PBS solution and Lysozyme for 1 3 7 14 and 50 days. The result showed that the optimum conditions of bending strength was found at the 5%wt. of HA, 160°C, 7 MPa. Moreover, biodegradation rate of specimen is 0.0175 g/day. In future works, in order to ensure the suitable femoral canine bone, biomechanical property including value comparison with finite element analysis shall be analysed. The investigation of biocompatibility shall be conducted to certify that composites plate are nontoxic and can be used in living canine’s body without danger.

Acknowledgement
This research was supported in financial sponsorship by the National Research Council of Thailand (NRCT) under Project ID 270376. The researchers would like to thank Small Animal Hospital, Faculty of Veterinary Medicine and Graduate School, Chiang Mai University for their facility support.

Reference
[1] Pfeil, D. J. V., Déjardin, L. M., DeCamp, C. E., Meyer, E. G., Lansdowne, J. L., Weerts, R. J., & Haut, R. C. (2005). In vitro biomechanical comparison of a plate-rod combination–construct and an interlocking nail–construct for experimentally induced gap fractures in canine tibiae. American journal of veterinary research, 66(9), 1536-1543.
[2] Shahar, R., & Banks-Sills, L. (2002). Biomechanical analysis of the canine hind limb: calculation of forces during three-legged stance. The veterinary journal, 163(3), 240-250.
[3] Pitjamit, S., Srirapha, P., & Nakkiew, W. (2016) Suitable forming condition of hydroxyapatite and bioactive glass composites for a bone fixation plate using Taguchi experimental design. Engineering and Applied Science Research, 43, 466-469.
[4] P Pawar, R., U Tekale, S., U Shisodia, S., T Totre, J., & J Domb, A. (2014). Biomedical applications of poly (lactic acid). Recent Patents on Regenerative Medicine, 4(1), 40-51.
[5] Wachirahuttapong, S., Thongpin, C., & Sombatsompop, N. (2016). Effect of PCL and compatibility contents on the morphology, crystallization and mechanical properties of PLA/PCL blends. Energy Procedia, 89, 198-206.
[6] Kasuga, T., Ota, Y., Nogami, M., & Abe, Y. (2000). Preparation and mechanical properties of polylactic acid composites containing hydroxyapatite fibers. Biomaterials, 22(1), 19-23.
[7] Shen, L., Yang, H., Ying, J., Qiao, F., & Peng, M. (2009). Preparation and mechanical properties of carbon fiber reinforced hydroxyapatite/poly lactide biocomposites. Journal of Materials Science: Materials in Medicine, 20(11), 2259-2265.
[8] Wattanuchariya, W., Ruennareenard, J., & Suttakul, P. (2016). Appropriate forming conditions for hydroxyapatite-bioactive glass compact scaffold. Engineering Journal, 20(3), 123-133.

[9] Nainar, S. M., Begum, S., Ansari, M. N. M., & Anuar, H. (2014). Tensile Properties and Morphological Studies on HA/PLA Biocomposites for Tissue Engineering Scaffolds. International Journal of Engineering Research, 3(3), 186-189.

[10] Montgomery, D. C. (2017). Design and analysis of experiments. John wiley & sons.

[11] Kunioka, M., Ninomiya, F., & Funabashi, M. (2006). Biodegradation of poly (lactic acid) powders proposed as the reference test materials for the international standard of biodegradation evaluation methods. Polymer degradation and stability, 91(9), 1919-1928.

[12] Watcharaprapapong, P., Nakkiew, W., Wattanuchariya, W., & Pitjamit, S. (2018). Effect of forming conditions of poly-lactic acid/hydroxyapatite to tensile strength of canine bone fixation plate using full factorial experimental design. MATEC Web of Conferences (Vol. 192, p. 01049, 1-4.

[13] Standard test methods for flexural properties of unreinforced and reinforced plastics and electrical insulating materials. ASTM; D790.

[14] Wu, T. M., & Wu, C. Y. (2006). Biodegradable poly (lactic acid)/chitosan-modified montmorillonite nanocomposites: preparation and characterization. Polymer Degradation and Stability, 91(9), 2198-2204.

[15] Charles, L. F., Shaw, M. T., Olson, J. R., & Wei, M. (2010). Fabrication and mechanical properties of PLLA/PCL/HA composites via a biomimetic, dip coating, and hot compression procedure. Journal of Materials Science: Materials in Medicine, 21(6), 1845-1854.

[16] Kharazi, A. Z., Fathi, M. H., & Bahmany, F. (2010). Design of a textile composite bone plate using 3D-finite element method. Materials & Design, 31(3), 1468-1474.

[17] Hartmann, M. H. (1998). High molecular weight polylactic acid polymers. Biopolymers from renewable resources (pp. 367-411).

[18] Chen, Q., Zhu, C., & Thouas, G. A. (2012). Progress and challenges in biomaterials used for bone tissue engineering: bioactive glasses and elastomeric composites. Progress in Biomaterials, 1(1), 2.