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Osseointegration in Rabbit Model Using PEEK Coated by HA: Comparative Study for Histological and X-ray

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Abstract

Biocompatible implants are often proposed to improve osseointegration such as metal, polymer, and ceramic to increase bone growth. This study aimed to create a new interface to enhance osseointegration. This interface is between the polymeric part and bone tissue. In this work coat PEEK (polyether ether ketone) plastic material coated with an active biocompatible material (hydroxyapatite). Regarding biocompatibility, coated PEEK was implanted inside the specimen in rabbit bone, the specimens were X-rayed after a period, and a histological investigation was performed after the samples were extracted from the test animals. X-ray and histological examination results indicate increased bone formation in the HA-coated implant compared with the non-coated HA implant.

Introduction

Biomedical implants are designed to repair diseased internal tissues in orthopaedic and dental applications, to restore the patient's normal biomechanical activity. Effective outcomes are achieved by matching the implant device with the surrounding tissue using a variety of engineering materials. Which is a challenging task considering the complexity of biological systems (Liu et al., 2020). The thermoplastic (PEEK) polyether ether ketone has a modulus of elasticity that is half between trabecular and cortical bone. PEEK is preferred in a variety of therapeutic applications due to its reduced mechanical mismatches with bone tissue than metal implants, which may reduce stress shielding (Owonubi et al., 2017). This material's inert chemical structure with high-temperature resistance makes it appropriate for a range of sterilizing procedures, including gamma irradiation, ethylene oxide, and autoclave. Additionally, PEEK is radiolucent, providing excellent detection of peri-implant tissue recovery (Durham III et al., 2016). As a result, most implant applications started using polymers alongside metals. Some use a polymer as a material for coating the metal part and others use polymers as basic parts within the design (Torres et al., 2017). To improve the body's ability to integrate with the implanted part and increase the possibility of its acceptance. Also, reduce the inflammatory response (Tomaszewski et al., 2013) (Durham III et al., 2016).

Methods

Synthes of the Specimen

PEEK disks by (Vsmile, Hunan, China) with dimensions of 98 mm in diameter and 20 mm in length. Implants are substrates with a nominal density of 1.32 g/cm3. Using a lathe, the extruded disk was cut down to the required implant 2.5 mm diameter and 4 mm length. To

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minimize particle contamination, the surface of the substrate is cleaned with deionized water between grinding processes. The cylindrical rods were then immersed in isopropanol, acetone, and deionized water for 10 minutes before being ultrasonically cleaned. The substrates were compressed air-dried and kept in a sterile container. The specimen polymer is coated with hydroxyapatite (HA) from Sunkoo Chemical.LTD has a 545.5nm particle size by the dip-coated method. Magnetic stirrer model LMS-1003 (220v-50Hz) from Korea was used to mix the solution then the sample was then dipped in the liquid and then placed in the oven for drying.

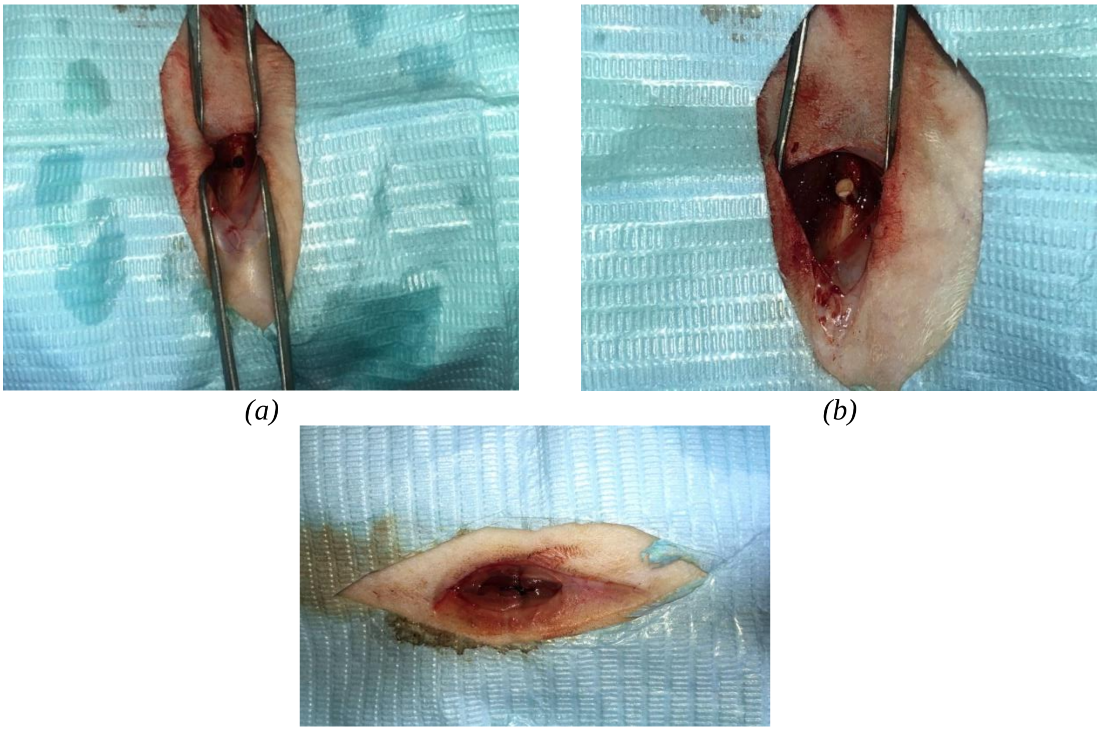
Sterilizing samples before a culture is an essential step in preventing transplant failure because of external substances. For this process, a dental autoclave sterilizer is used at a temperature of 134 Cº for 45 min. Where samples are stored in medically sterilized pouches, closed, and then inserted into the device. This device used steam Sterilization.

In Vivo Test

The biological reactivity of HA-coated implants was evaluated in vivo; all materials were biocompatible. The size and number of implants have an immediate impact on the animal species used for the experiment. In this study, PEEK implants were evaluated in four skeletally mature male New Zealand White rabbits called (albino laboratory animals). The subsequent surgical protocol was authorized and performed within the guidelines of the Biotechnology Research Center at Al-Nahrain University. Cylindrical shape implants were used. The rabbits aged (8-11) months old, weighed between 2 kg used in this study. The rabbits were separated into two groups by number. Group I, specimen PEEK without coated was inserted at the mid-shaft of the left femoral bone and regarded as a control group. In group II, the same PEEK specimen was crafted but with coated HA material which was previously been prepared and considered an active group. Implanted in right femoral bone in a rabbit model. The rabbit's observation in X-ray and for CBCT, varied periods. For the X-ray test after either 3 weeks, and the last in 7 week by cone-beam computed tomography.

All animals were injected with a mixture of xylazine (40mg/kg), and ketamine hydrochloride (90 mg/kg) and euthanized by inhalation anaesthesia. The left for group I and right for group II The skin was cleansed with a povidone-iodine surgical scrub after the hind limbs were shaved from the femur. The localized region of operation was surgically prepped, and the animal was placed in a lateral recumbence. A one-centimetre-long surgical incision was performed in the centre of the limb from the lateral side. The hole, initially 2 mm in diameter, was sequentially enlarged to a final diameter to match the implant geometry. Bone debris and marrow were flushed from the surgical site and the implant was placed (press-fit) into the femur, fig 1. After routine closure of the wound (resorbable sutures for subcutaneous tissues, Silk nonabsorbable for the skin incision). the procedure was repeated on the other rabbits.

Postoperatively, buprenorphine hydrochloride (0.05 mg/kg) was given twice daily for at least 48 h and carprofen (4–5 mg/kg) was administered every 12 to 24 h for up to 3 days post-surgery to control post-operative pain. Groups of animals were sacrificed 7 weeks after surgery. All animals were subjected to radiography every 3, 7 weeks and to evaluate the osteogenesis at the implant site, a histological investigation was performed on all specimens. The rabbits were acclimatization to the creature's place of Animal house. They were housed in very much ventilated rooms inside plastic enclosures and fed on standard pellets and drinking water not obligatory during tests. The room temperature was 25±2 Cº. Light/dull cycle along the test period for 12 hrs.



(a) (b)

(c)

Figure 1. Illustrated implantation steps, a:drilling the femur, b: inserting the specimen, c: Inner stitching

After the required interval for implantation, the animal is sacrificed, and the bones are extracted and washed in distilled water. The bones remain between six and eight hours in a 10% formalin solution (Suvarna et al., 2018). Then it is washed again with distilled water and then immersed in the folic acid, maintained to remove calcium and for a period of 3 to 5 days until it becomes soft tissue to prepare it for histological examination. Wound healing conditions were observed after surgery. X-ray evaluations were conducted post-operative and before euthanasia (3, 5) weeks post-operative, and cone beam computed tomography (CBCT) examination of bone after euthanasia

Results and Discussion

This study investigated the histological and radiological performance of PEEK coated by HA. To evaluate osseointegration. Examination of two groups, the control group without any coated material and the second group II with HA-coated at the surface of the PEEK specimen. Radiological examination after 3 weeks after implantation. Shows imaging of the two groups a difference in shape between the control group and the active group in terms of the level of healing. From the side section, it is apparent that the sample hole in a from, fig.2 is smaller than the specimen hole in b from, fig.2, indicating an increase in healing. When the longer the sample remains inside the bone, the greater the probability of its integration into the bone

a b

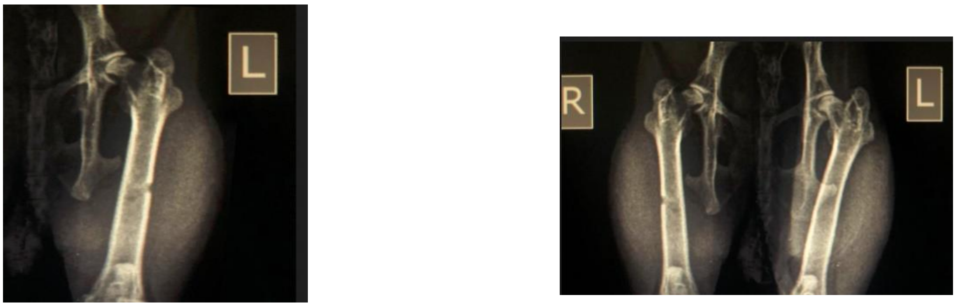


Figure 2. Shows group I in (a), and group II in (b)

After the animal has been sacrificed, the femurs are extracted. Before taking the samples for treatment and histological evaluation, CBCT is performed. The acquired images contain the control and active groups. The diameter of the implanted hole is measured after healing illustrated in, fig 3, where it is found that group II range in diameter after bone healing between 2.2 mm and 2.3 mm, while the control group is approximately 2.5 mm. It is possible that a simple healing event can't be measured in the control sample. During the period of the investigation, neither dermatitis either inflammatory effect, or bone deformation were identified using radiography in the rabbits. This indicates that the used biomaterials are non-toxic and suitable for application in vivo as bone repair or implant support materials

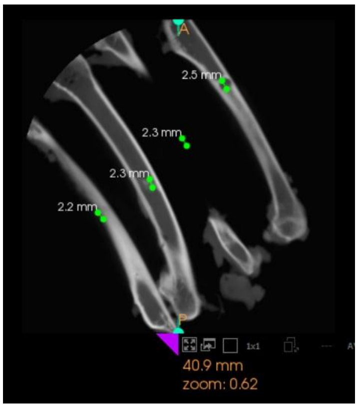
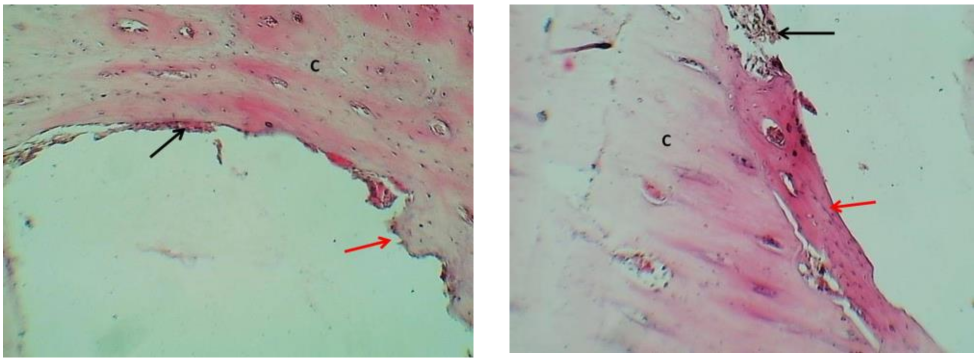


Figure 3. Dimension of the hole after.

On the other hand, the histological examination gives indicated the level of osseointegration between coated and uncoated samples with the bone. After finishing the period of implantation, several processing steps are performed to prepare for histological examination. Images are taken at different magnifications. A comparison is made between the first and second groups in bone formation. This comparison after 7 weeks of implantation in femoral rabbit bone in the same conditions for the two groups, fig.4 illustrated the control group.

Histopathological images for the control group. A normal appearance of cortical bone and the rim of the hall was revealed. Also illustrated fibrous osteogenic tissue around the most surface area of the hall with little mass of non-remodeled and remodeled newly formed bone formed around the little surface area (A, B) section from, fig 4. The image of circular oriented newly formed bone revealed fibro fibrous osteogenic tissue, the surface-active osteoblasts, osteoclasts, and mature osteocyte.

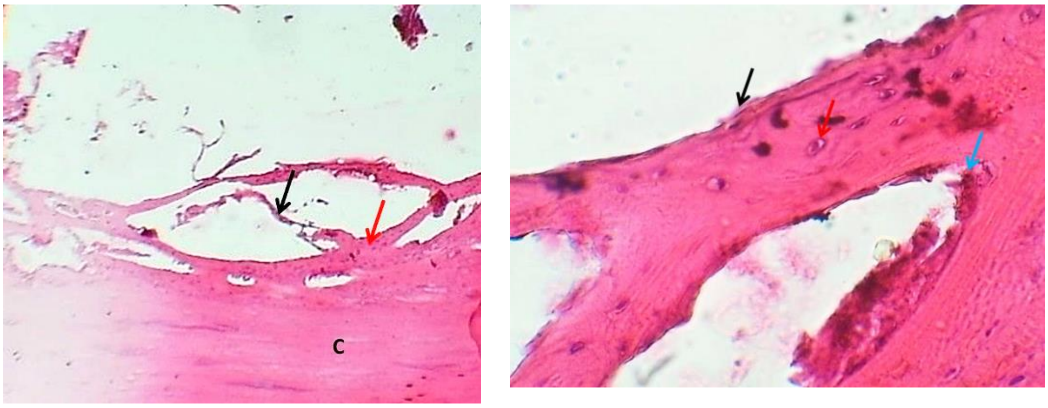


A: fibrous osteogenic tissue (Black arrows) & non-remodeling newly formed bone (Red arrows). H&E stain.100x.

B: fibrous osteogenic tissue (Black arrows) & remodeling newly circular oriented layer of bone (Red arrows). H&E stain.100x.

Figure 4. Demonstrate group I where A and B are for the different sides

The active group showed a normal appearance of cortical bone. All surface areas of the rim of the hall revealed a very little layer of fibrous osteogenic tissue covering well remodeled new bone in section (A). Which was composed of surface-active fibroblast, osteocyte, and osteoclast for part (B) from, fig 5. No significant inflammatory or necro response was observed. The sample was surrounded by or an interface with the lamellar bone, indicating excellent biocompatibility and osteoconductive.



A: a very thin layer of fibrous osteogenic tissue composed of inactive fibroblasts (Black arrows) thin new bone formation (red arrow) H&E stain.100x.

B: newly formed bone composed of surface-active fibroblast (Black arrow), osteocyte (Red arrow) & osteoclast (Blue arrow). H&E stain.400x.

Figure 5. Shows the third specimen from the active group

The PEEK is poor OI (Trindade et al., 2019). For this reason, the PEEK coated by HA is used to increase bone growth (Shekhawat et al., 2021). Through histological examination, a small percentage of new bone is observed around the control group implant. This is consistent with the previous study (Trindade et al., 2019), which showed the formation of primary bone tissue cells during the first 10 days of implantation and the maturation of these cells within 28 days. Compared with the same group, according to the study a fatty layer is formed around the implant. While in the implants used for this research, no fatty tissue was observed, whether in the sample without coating or with the coating material. Regarding the second group with HA-coated implants, an increase in the amount of bone formed surrounding the implant, and not found any fat layer. Immunity plays a major role in the healing process.

Conclusion

The results of the present study showed the presence of new bone formation in the PEEK/HA-coated samples compared to the control group. Illustrated by X-ray images for 3 weeks and 7 weeks of implantation. Also, this difference between the groups is shown through the histological examination after the end of the period specified for implantation. Where complete osteocytes were found in the active group, while differences in remodelling and non-remodelling were found in the control group. This is on the one hand. As for the ratio of the variance for osteoblast, osteoclast, and fibrous between the two groups. The reason is due to the HA-coated and the different healing responses of each animal in comparison to the other. Which produces a difference in the new bone production process.

References

Durham III, J. W., Montelongo, S. A., Ong, J. L., Guda, T., Allen, M. J., & Rabiei, A. (2016). Hydroxyapatite coating on PEEK implants: Biomechanical and histological study in a rabbit model. *Materials Science and Engineering: C*, *68*, 723-731.

Liu, Y., Rath, B., Tingart, M., & Eschweiler, J. (2020). Role of implants surface modification in osseointegration: A systematic review. *Journal of Biomedical Materials Research Part A*, *108*(3), 470-484.

Owonubi, S. J., Agwuncha, S. C., Fasiku, V. O., Mukwevho, E., Aderibigbe, B. A., Sadiku, E. R., & Bezuidenhout, D. (2017). Biomedical applications of polyolefins. In *Polyolefin Fibres* (pp. 517-538). Elsevier.

Shekhawat, D., Singh, A., Banerjee, M., Singh, T., & Patnaik, A. (2021). Bioceramic composites for orthopaedic applications: A comprehensive review of mechanical, biological, and microstructural properties. *Ceramics International*, *47*(3), 3013-3030.

Suvarna, K. S., Layton, C., & Bancroft, J. D. (2018). *Bancroft's theory and practice of histological techniques E-Book*. Elsevier health sciences.

Tomaszewski, P., Lasnier, B., Hannink, G., Verkerke, G. J., & Verdonschot, N. (2013). Experimental assessment of a new direct fixation implant for artificial limbs. *Journal of the mechanical behavior of biomedical materials*, *21*, 77-85.

Torres, J. L., Gehrke, S., Guirado, J. C., & Aristazábal, L. (2017). Evaluation of four designs of short implants placed in atrophic areas with reduced bone height: A three-year, retrospective, clinical and radiographic study. *British Journal of Oral and Maxillofacial Surgery*, *55*(7), 703-708.

Trindade, R., Albrektsson, T., Galli, S., Prgomet, Z., Tengvall, P., & Wennerberg, A. (2019). Bone immune response to materials, part II: copper and polyetheretherketone (PEEK) compared to titanium at 10 and 28 days in rabbit tibia. *Journal of Clinical Medicine*, *8*(6), 814.

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