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ORIGINAL ARTICLE

Comparison of immune responses to zirconia, polyether ether ketone (PEEK), and stainless-steel in orthopaedic implants

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ABSTRACT

Aim Orthopaedic implants must meet specific criteria, including mechanical strength, durability, and biocompatibility. This study compares the immune response to zirconia, polyether ether ketone (PEEK), and stainless-steel implants *in vivo*, focusing on lymphocyte and fibroblast infiltration as indicators of immune activation.

Methods A total of 27 New Zealand white rabbits was used, with nine animals in each group. Implants of zirconia, PEEK, or stainless steel were surgically placed in the thigh and observed for 4 weeks. Histological analysis measured lymphocyte and fibroblast infiltration at the implant site using a microscope at 400x magnification. Statistical analysis included the Kruskal-Wallis test for group comparisons, followed by Mann-Whitney and Bonferroni correction for pairwise comparisons.

Results The Kruskal-Wallis test showed significant differences in lymphocyte ($p=0.002$) and fibroblast ($p=0.003$) counts among the groups. Zirconia exhibited significantly lower lymphocyte (median=0.5) and fibroblast (median=1.0) infiltration compared to stainless steel (lymphocytes: median=3.0, fibroblasts: median=2.0), and PEEK (lymphocytes: median=2.0, fibroblasts: median=3.0). Bonferroni correction confirmed that zirconia exhibited the least immune activation ($p<0.0167$).

Conclusion Zirconia offers superior biocompatibility with minimal immune response, making it an ideal material for orthopaedic implants, particularly for patients with metal sensitivities. PEEK showed moderate immune activation but is helpful for non-load-bearing applications. Stainless Steel induced the highest immune response due to the release of metal ions and corrosion. Zirconia is the most biocompatible material tested, making it a promising choice for orthopaedic implants.

Keywords: immunity, polyether ether ketone, prostheses and implants, stainless-steel, zirconia

INTRODUCTION

Orthopaedic implants are widely used in the treatment of various bone and joint disorders, including osteoarthritis, rheumatoid arthritis (RA), and fractures (1). As the global population ages, the demand for these implants, particularly joint replacement surgeries, has steadily increased. The materials used in orthopaedic implants must fulfil several critical criteria, including mechanical strength, durability, and, most importantly, biocompatibility (2). Biocompatibility refers to the ability of the implant material to interact with the surrounding tissues without inducing an adverse immune response or causing toxicity. A key determinant of biocompatibility is the material's ability to minimize inflammatory responses and promote tissue healing, which are essential for the long-term success of the implant (3).

One of the most significant factors influencing the long-term success of orthopaedic implants is the immune response to the implanted material (4). The immune system's response to foreign materials can lead to chronic inflammation, fibrosis, or even implant rejection. This response is particularly problematic with metal-based implants, such as those made from stainless steel, which can release metal ions into the surrounding tissue due to corrosion, potentially causing allergic reactions or irritation (4,5). Corrosion has been identified as a significant

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issue in metallic implants, as the release of metal ions (such as nickel, chromium, and cobalt) can stimulate a pro-inflammatory immune response and alter the material's biomechanical properties over time (6).

In contrast, alternative materials such as ceramics and polymers have garnered increasing attention in recent years. Zirconia (ZrO2) is a ceramic material known for its low cytotoxicity, excellent mechanical properties, and superior wear resistance. Zirconia's low immune reactivity makes it an attractive candidate for use in orthopaedic implants, especially in weight-bearing joints, as it minimizes immune activation and encourages better tissue integration (7,8). Furthermore, zirconia has a unique phase transformation ability that enhances its toughness, making it suitable for joint replacements that endure mechanical stress (9).

Polyether ether ketone (PEEK), a thermoplastic polymer, is valued for its chemical stability, low wear, and favourable mechanical properties. It is beneficial in non-weight-bearing applications such as spinal implants and trauma devices, where its radiolucency is advantageous for post-surgical imaging. While PEEK shows a moderate immune response, its biocompatibility has been well-documented, and surface modifications can further improve tissue integration and reduce immune activation (10-12).

Despite the promising properties of zirconia and PEEK, stainless steel remains one of the most widely used materials in orthopaedic implants due to its high mechanical strength and affordability. However, its use is often limited by corrosion issues, which can trigger local inflammation and immune activation. The release of metal ions from stainless steel implants has been shown to activate the complement system and induce chronic inflammation, ultimately compromising implant stability and patient outcome (1,13).

Given these concerns, this study was initiated to evaluate the immune responses triggered by different orthopaedic implant materials. Specifically, the aim was to compare the immune response to zirconia, PEEK, and stainless-steel implants *in vivo*, using a rabbit model. This comparison is crucial for understanding how these materials interact with the immune system, particularly in terms of lymphocyte and fibroblast responses around the implants. Understanding these immune mechanisms will provide valuable insights into the long-term success and complications associated with each material, ultimately guiding the development of more biocompatible and effective orthopaedic implants.

MATERIALS AND METHODS

Materials and study design

The study was conducted at the Animal Research Laboratory, Faculty of Medicine, Universitas Brawijaya, where the animal subjects were maintained, treated, sampled, and subsequently euthanized from July to September 2024. The research continued at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya, where measurements of lymphocyte and fibroblast cell parameters were performed from September to October 2024.

This study employed a post-test-only control group design to compare the immune response induced by zirconia, PEEK, and stainless-steel orthopaedic implants *in vivo*. The manufacture of implants in the form of chips follows ISO 19227:2018 and is assisted by PT. EKA ORMED INDONESIA.

The three materials used in the study were:

Zirconia (ZrO2): a bioceramic material with low cytotoxicity, excellent mechanical properties, and known for its superior wear resistance and biocompatibility.

Polyether Ether Ketone (PEEK): a thermoplastic polymer known for its chemical stability, radiolucency, and good mechanical properties.

Stainless-steel: a traditional material used in orthopaedic implants with good mechanical strength but associated with metal ion release and corrosion.

The New Zealand white rabbit was chosen as the animal model due to its reliable anatomical similarity to human tissue, particularly in the subcutaneous layer, and its widely accepted use in preclinical studies involving biocompatibility and immune response assessments. The inclusion criteria for the rabbits in this study were that they were healthy and actively moving, with no disabilities in their extremities, and weighed between 1.5 and 2.5 kg. Rabbits with extremity disabilities, infection, and those that died before the study began were excluded. All rabbits were housed in controlled conditions with a temperature of 22°C±2°C, a humidity of 60%±5%, and a 12-hour light/dark cycle. The animals were allowed to acclimate to the environment for 7 days before the procedure. They were fed standard rabbit chow and given access to clean water ad libitum.

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya, under approval number 113/EC/KEPK-PSPDS/05/2024 in May 2024.

Methods

Sample size calculation. The sample size was determined using the Federer formula (14), a commonly applied method in experimental designs to ensure sufficient statistical power while controlling for variability between groups. The Federer formula is suitable here, as it strikes a balance between providing an adequate sample size and minimizing animal use. The formula used is as follows:

(n - 1) (t - 1)≥15 where: n = required sample size per group, t = number of treatment groups

$$(n-1)(3-1) \geq 15$$

$$(n-1)(2) \geq 15$$

$$2n-2 \geq 15$$

$$2n \geq 17$$

$$n \geq \frac{17}{2}$$

$$n \geq 8.5$$

In this study, the number of treatment groups was three: Group 1, zirconia; Group 2, PEEK; and Group 3, stainless steel. Solving the equation yields required sample size of 9 rabbits per group. Therefore, a total of 27 rabbits were included in this study.

Surgical procedure. The surgical procedure was performed under general anaesthesia using intramuscular injection of 200 mg ketamine and 20 mg xylazine each. The thighs, legs, and upper back were shaved, and each lower extremity was prepared with 70% alcohol and 7.5% Povidone Iodine solution. Throughout the procedure, the animals breathed spontaneously and were monitored by a veterinary technician. The lower extremities of

each animal were covered with sterile adhesive surgical drapes and secured with towel clips. All surgical procedures were performed under sterile conditions, using a separate set of surgical instruments for each site. To minimize variability and ensure standardized surgical procedures, the same surgeon performed all operations. A 2 cm longitudinal incision was made over the lateral aspect of each thigh and extended to the muscle layer until the bone was reached. Group 1: rabbits implanted with a set of zirconia-based chips, Group 2 with a set of stainless steel-based chips, and Group 3 with a set of PEEK-based chips. The skin was approximated using a subcuticular stitch with non-absorbable 3-0 monofilament nylon sutures. Postoperatively, each animal was placed in a warm, oxygenated recovery room until complete recovery from anaesthesia was observed. Once in their cages, each animal was provided access to water and rabbit food. The animals were monitored daily for food intake, faeces, urine output, and behaviour. Observation of the animals continued for up to 4 weeks post-operation.

Tissue preparation for histological examination. After 4 weeks, euthanasia was performed using a lethal dose of 200 mg/kg intramuscular ketamine on the animals. Subsequently, the muscle and bone tissues of the rabbits were excised and prepared as samples for further microscopic histological analysis. The samples were stored in formalin before histological analysis. The rabbits, from which the thigh tissues were collected for the study, were then buried according to standard animal disposal procedures. The excised thigh muscles were stored in formalin bottles for the preparation of microscopic slides. Tissue processing involved immersion in graded alcohols for approximately 21 hours. The tissue was then embedded in paraffin. The paraffin-embedded tissues were sectioned using a microtome. The tissue sections were then mounted onto glass slides. Staining was performed using Harris Hematoxylin and Eosin (HE) stain. The sections on the slides were sequentially immersed in the following solutions: Xylene, Xylene, Xylene, 100% Alcohol, 100% Alcohol, Distilled Water, Hematoxylin, Distilled Water, Eosin, 96% Alcohol, 96% Alcohol, 96% Alcohol, 100% Alcohol, 100% Alcohol, Xylene, and Xylene. Mounting was performed using entellan to adhere the cover glass to the slide, and the slides were then stored in an incubator for approximately 24 hours.

The counting of lymphocytes and fibroblast cells around the implantation site was observed under a microscope with 400x magnification using a semi-quantitative counting method.

Statistical analysis

A descriptive analysis was conducted to obtain a general description of the research variables. The median value was chosen as the measure of central tendency because it is more representative of the data, especially when the distribution is not normal or contains outliers. The number of infiltrating immune cells (lymphocytes and fibroblasts) was compared among the three groups using the Kruskal-Wallis test. A $p<0.05$ was considered statistically significant. For post-hoc pairwise comparisons to determine the significant differences between the groups, the Mann-Whitney test was performed. Since multiple pairwise comparisons were made, a Bonferroni correction was applied to adjust the significance level.

RESULTS

The median value was chosen as the measure of central tendency because it is more representative of the data, especially when the distribution is not normal or contains outliers. As the data distribution in this study was not normal, the median was preferred over the mean for presenting the central tendency (Table 1).

Table 1. Median lymphocyte and fibroblast cell counts in stainless steel, zirconia, and polyether ether ketone (PEEK)

Groups (number of animals)	**Lymphocyte cell counts** (median)	**Fibroblast cell counts** (median)
Stainless steel (9)	3.0	2.0
Zirconia (9)	0.5	1.0
PEEK (9)	2.0	3.0

A statistically significant difference was found for lymphocyte ($p=0.002$) and fibroblast counts ($p=0.003$) between the groups. Post-hoc pairwise comparisons were conducted using the Mann-Whitney test to determine which specific group comparisons were significantly different. All comparisons between Stainless Steel, Zirconia, and PEEK showed statistically significant differences in both lymphocyte and fibroblast responses (Table 2).

Table 2. Statistical comparison of lymphocyte and fibroblast across stainless steel, zirconia, and polyether ether ketone (PEEK)

Group	**Lymphocytes**	**Fibroblast**
	p	**p**
Stainless steel - zirconia	0.003	0.018
Stainless steel - PEEK	0.042	0.027
Zirconia - PEEK	0.015	0.004

Since all group comparisons were significant, the Bonferroni correction was applied to determine which material performed best. Bonferroni correction was performed by dividing the desired significance level (α) by the number of comparisons being made. If a significance level of 0.05 was used and three comparisons were made, the corrected significance level was 0.0167. Following the application of the Bonferroni correction, the adjusted significance level was 0.0167 (Table 3). After applying the Bonferroni correction, zirconia showed significant differences compared to stainless-steel and PEEK for both lymphocytes and fibroblasts. Specifically, zirconia exhibited the least immune response, with significantly lower lymphocyte infiltration and minimal fibrosis compared to the other materials. On the other hand, stainless steel showed the highest immune response, while PEEK exhibited moderate immune activation (Table 3).

Table 3. Bonferroni correction

Group	**Comparisons of original significance with adjusted significance**	**Significance**
Lymphocytes		
Stainless steel - zirconia	0.003 < 0.0167	Significant
Stainless steel - PEEK	0.042 > 0.0167	Not significant
Zirconia – PEEK	0.015 < 0.0167	Significant
Fibroblast		
Stainless steel - zirconia	0.018 > 0.0167	Not significant
Stainless steel - PEEK	0.027 > 0.0167	Not significant
Zirconia - PEEK	0.004 < 0.0167	Significant

DISCUSSION

The results of this study demonstrate that zirconia implants induced the least immune response, with significantly lower lymphocyte infiltration and minimal fibrosis around the implant site. These findings align with previous research highlighting zirconia's excellent biocompatibility and low cytotoxicity in orthopaedic applications (15,16). Zirconia's bioinert nature contributes to its suitability for joint replacements, as it minimizes immune activation and encourages better tissue integration. Its fracture toughness and mechanical properties also make it an ideal choice for load-bearing applications, such as joint prostheses. Moreover, zirconia's ability to suppress the TH1 pathway may contribute to its reduced immune response, limiting macrophage activation and pro-inflammatory cytokine production, which are associated with chronic inflammation (17,18).

While PEEK showed a moderate immune response, it exhibited more favourable tissue integration than stainless steel. The material's chemical stability, radiolucency, and favourable mechanical properties, including high tensile strength and elasticity, make it particularly advantageous in non-load-bearing applications, such as spinal implants and trauma devices, where post-surgical imaging is crucial (19). Despite moderate immune activation, PEEK remains a viable material for applications that require high strength without load-bearing needs. Furthermore, surface modifications, such as plasma treatment or bioactive coatings, have been shown to enhance PEEK's biocompatibility and reduce immune activation, positioning it as a promising candidate for broader orthopaedic use in the future (20).

In contrast, stainless steel implants induced the most significant immune response, characterized by increased lymphocyte infiltration and extensive fibrosis around the implant sites. This is primarily due to the release of metal ions, such as nickel, chromium, and cobalt, which occur as a result of corrosion. These metal ions stimulate a pro-inflammatory immune response, leading to chronic inflammation, fibrosis, and poor osseointegration, which negatively affect the long-term performance of metallic implants. These findings underscore the challenges associated with using stainless steel in orthopaedic implants, particularly in terms of corrosion and long-term implant stability (21,22). Despite the widespread use of stainless steel due to its strength and affordability, the persistent issue of corrosion underscores the need for alternatives such as zirconia and PEEK, which offer improved biocompatibility and reduced immune activation.

The comparative analysis suggests that zirconia is the superior choice in terms of immune compatibility and tissue healing, especially for weight-bearing applications. Its low immune activation, excellent mechanical properties, and reduced risk of corrosion make it a promising alternative to stainless steel, particularly for patients with metal sensitivities or those at risk for chronic inflammation (15). However, PEEK remains a valuable option for non-load-bearing applications, where its favourable biomechanical properties and the potential for surface modifications provide significant advantages, particularly for spinal and trauma implants (10,23).

While the findings of this study are promising, several limitations should be addressed. The subcutaneous model used in this study may not fully replicate the dynamic mechanical forces or biological conditions that implants experience within joint spaces. Future studies could consider using joint-specific models, such as those simulating weight-bearing or dynamic loading conditions, to more accurately reflect the real-world conditions of implants. Additionally, this study observed the implants only for 4 weeks, which may not be sufficient to assess long-term wear, mechanical degradation, or chronic inflammatory effects. More extended observation periods would provide more insight into the long-term performance and potential complications associated with these implant materials, especially stainless steel. Furthermore, surface modification techniques for PEEK, such as bio functionalization or coating with bioactive agents, should be further explored to enhance its biocompatibility and minimize immune responses.

In conclusion, zirconia demonstrated superior biocompatibility compared to PEEK and stainless-steel, with a minimal immune response. PEEK showed moderate immune activation, while stainless-steel induced the most significant immune response due to metal ion release and corrosion. These findings suggest that zirconia is a promising material for orthopaedic implants, particularly for patients prone to metal sensitivities, while PEEK remains suitable for non-load-bearing applications.

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Conflicts of interest: None to declare.

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