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RESEARCH ARTICLE

Review of Selected Orthopaedic Implants for their Genotoxicity Potential

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Abstract

Background/Objectives: Orthopaedic implants are intended to be part of a biological system for a considerable amount of time, hence its essential to test for their genotoxicity. This review investigates the genotoxicity associated with various orthopaedic implant materials. **Methods:** We collected genotoxicity studies conducted on twenty different types of orthopedic implant materials in our laboratory from 2015 to 2022, along with their accompanying reports. Based on these reports, the implants were categorized into groups according to their materials of construction. This paper includes a thorough evaluation of the findings from the genotoxicity tests obtained from the Bacterial Reverse Mutation Assay, Chromosomal Aberration Assay, and Micronucleus Assay conducted on orthopedic implant materials in our laboratory. **Findings:** A total of 20 different orthopedic implants were tested in our laboratory for their genotoxic potential. These 20 implants were made of four different materials viz., titanium alloys, ultra-high molecular weight polyethylene, stainless steel and Cobalt chromium molybdenum alloys. All these implants were tested on Ames test, chromosome aberration test and or *in vivo* micronucleus tests. None of the materials showed any evidence of mutagenicity. **Novelty:** This is the first open paper highlighting the results of genotoxicity testing of selected orthopedic implant materials.

Keywords: Orthopaedic Implants; Genotoxicity; Chromosomal Aberration; Ames; Micronucleus Assay

1 Introduction

DNA mutations may arise spontaneously due to chemical actions of exogenous or endogenous agents. In eukaryotic organisms, the genetic damage in somatic cells may results in malignancy and the same in germ cells may adversely affect reproduction or induce heritable mutations[(1)]. Study of such agents that can cause DNA damage and chromosomes are known as genetic toxicology. Analysis of genotoxicity and mutagenicity have crucial role in the identification of hazardous effects of

therapeutic drugs, medical devices, cosmetics, food additives, industrial compounds, nanomaterials, agrochemical and natural toxins for regulatory purposes[2]. To evaluate genotoxicity and mutagenicity we have various *in vitro* and *in vivo* tests that evaluate different genotoxicological endpoints such as point mutation, numerical and structural chromosomal aberrations[3]. A minimum of two or three validated tests, including at least one *in vitro* test on cell cultures and one *in vitro* test on bacteria, make up the regulatory core battery of the standard genotoxicity testing[4].

Bone is a dynamic tissue that is constantly changing, in the event of any minor injuries bone has the potential to recover and regain its mechanical and biological characteristics. However, major injuries that affect the skeletal system like congenital malformations, disorders, and trauma cannot be healed on its own. The skeletal system's consequent fractures and abnormalities can increase mortality, although the degree to which this is true varies depending on which bones are affected[5].Such major fractures and breakages could be fixed with the help of medical intervention using orthopaedic implants and surgeries. Orthopaedic implants are intended to repair/replace a bone, joint, or cartilage that has been damaged or deformed. A patient could require an implant as a result of a congenital impairment, limb loss or fracture[6]. Osteointegration is the process by which an implant is directly anchored and integrated into living bone. The composition, form, and surface features of the implant, mechanical stress, surgical technique, and the location and regional condition of the host bone all affect the rate at which an implant osseointegrates. The ultimate objective is to create an interface matrix with biomechanical qualities similar to bone in terms of structure, content, and ability to bear initial mechanical loads[7]. Following a bone implantation, a new matrix is formed to bridge the space between the implant and bone, necrotic tissues are reabsorbed simultaneously. The spacing between the implant and the bone is crucial because it directly affects the primary bone healing process, which is the deposition of new bone at the interface[8]. Blood coagula initially fills the area, attracting multipotent mesenchymal cells from the surrounding vessels and environment as well as cells for debridement. Following their migration through the coagula to the implant surface, these cells lay down a thin layer of afibrillar tissue, which is quickly followed by a layer of collagen matrix. Within 4-6 weeks, a woven bone replaces this structure, creating a link between implant and surrounding bone[9].

Generally, three classes of biomaterials are used in the manufacturing of such implants- metals, ceramics and polymers, each material has its own unique advantages and limitations. Titanium alloy, Cobalt-Chromium alloy and stainless steel are commonly used metals due to their wear, corrosion, heat resistance, and strength. In most cases, the implant is put in position so that the bone may grow into it and strengthen it[10]. Surgical methods and type of implants are opted depending on the condition of the patient. The surgeon may use a variety of orthopaedic devices created specifically for the operation to remove the damaged joint and replace it with an orthopaedic implant. If the wrong material is used, the implants themselves may cause bone fractures, deformities, or incomplete bone healing[11]. The failure of the implantation would be further increased by bacteria-related late infection of the orthopaedic devices[12]. The two most common complications that arises after the implantations are aseptic loosening and prosthetic joint infection other than the toxicity[13]. In this paper, we are dealing with four types of orthopaedic implants – Titanium, Cobalt Chromium, stainless steel and Ultra-High Molecules Weight Polyethylene (UHMWPE). As orthopaedic implants are intended to reside in the body of host for a long period of time, they should be biologically inert. Titanium implants when implanted readily oxidizes and produce a very thin coating (bioinert) that serves as an overlay for the implant[14]. Orthopedic implants are often disinfected by gamma irradiation in ambient air prior to implantation. Chain cleavage caused by gamma rays results in the creation of free radicals. These free radicals may still be present in the polymer after gamma irradiation and interact with accessible oxygen species during storage or *in vivo* to cause harmful oxidation of UHMWPE[15].

It is essential to examine these implants for biocompatibility, particularly genotoxicity, as they are intended to be part of a biological system for a considerable amount of time[16]. In this paper, we discuss about the genotoxicity test results of various orthopaedic implants through data derived from Chromosomal aberration (CA), Bacterial reverse mutation test (Ames) and *in vivo* micronucleus test (MNT). Test item preparation, selection of extraction ratio, extraction vehicle and conditions, test system etc., were selected in a way that ensures compliance with the ISO 10993 Guidelines[17].

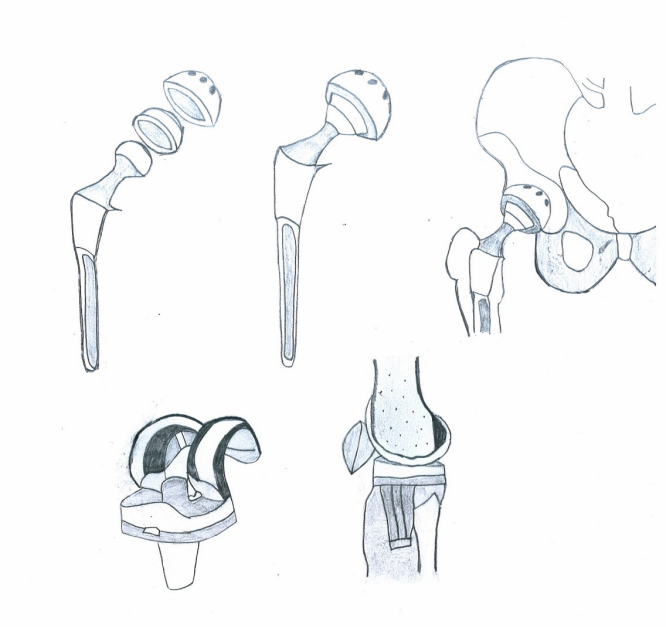

Fig 1. Implants used for Total Hip and Knee Replacement

2 Methodology

2.1 Grouping of Test Items

By comparing the various materials of a known orthopaedic implant, the proposed methodology to compute biocompatibility is put to the test. For the current investigation, all genotoxicity tests performed on orthopaedic implants at GLR Laboratories in Chennai between 2015 and 2022 were taken into account. 20 distinct orthopaedic implants' data models were examined. Test items were grouped based on the material of the implant and are classified into four groups summarized in Table 1.

Group A: Titanium of Material Grade: Titanium alloy (Ti-6AI-4V), ASTM F136 ELI Titanium, ASTM F67 Unalloyed Titanium, Titanium Grade 23, Titanium Grade 4, Ti/HA (Titanium hydroxyapatite coating), ISO 5832-3 (TITANIUM), ASTM F2063 Nitinol, Titanium Grade 5 (ISO 5832-3).

Group B: Ultra-High Molecules Weight Polyethylene.

Group C: Stainless steel of Material Grade: ISO 5832-1 (SS316LVM).

Group D: Cobalt chromium molybdenum alloy (ASTM F 1537)

In groups B, C and D, all the individual test items of the respective groups were made up of the same ingredient but the implants, purpose of use and brands were different.

Table 1. The table describes the different materials tested in this study

Material of implants	Abbreviation	No of test items
Titanium	Ti	8
Ultra-High Molecules Weight Polyethylene	UHMWPE	6
Stainless Steel	SS	4
Cobalt chromium molybdenum alloys	Co-Cr	2

2.2 Bacterial Reverse Mutation Test

The *Salmonella typhimurium* strains that require histidine are used in the bacterial reverse mutation test (also known as the Ames test), which measures the genetic activity in both the absence and presence of an exogenous metabolic activation system. This test is quick, accurate, and reasonably priced, and it can be used to determine a test item's mutagenic potential. The capacity

of this test to identify genetically active chemicals from the majority of chemical classes with 80%–90% sensitivity and specificity was established using a large database [18]. The following bacterial strains were used in the study (Table 2).

Table 2. Bacterial strains used in the study

Organism	Strain	Type of mutation in the histidine gene
S. typhimurium	TA98	Frameshift
S. typhimurium	TA100	Base-pair substitution
S. typhimurium	TA102	Base-pair substitution
S. typhimurium	TA1535	Base-pair substitution
S. typhimurium	TA1537	Frameshift

2.3 Chromosomal Aberration

The *in vitro* chromosome aberration test is used to identify substances that cause structural chromosomal aberrations in cultured mammalian cells. Primary cell cultures of human origin is used for this study. The cells are selected on the basis of growth ability in culture, stability of the karyotype (including chromosome number) and spontaneous frequency of chromosome aberrations. Cultured cells are then exposed to the test item extracts both with and without an exogenous source of metabolic activation unless cells with an adequate metabolizing capability are used. At an appropriate predetermined interval after the start of exposure of cell cultures to the test extract, they are treated with a metaphase-arresting substance colchicine, harvested, stained and metaphase cells are analyzed microscopically for the presence of chromatid-type and chromosome-type aberrations [19].

2.4 *In-Vivo* Micronucleus Test

The mammalian *in vivo* micronucleus test is used for the detection of damage induced by the test item to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents. The purpose of the micronucleus test is to identify substances that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. When a bone marrow erythroblast develops into a polychromatic erythrocyte, the main nucleus is extruded; any micronucleus that has been formed may remain behind in the cytoplasm. Visualization of micronuclei is facilitated in these cells because they lack a main nucleus. An increase in the frequency of micronucleated polychromatic erythrocytes (PCE) in treated animals is an indication of induced chromosome damage.

The mammalian *in vivo* micronucleus test is in particular relevant for assessing genotoxicity. This test is additive for further investigation of genotoxicity detected by an *in vitro* system.

3 Results and Discussion

Metallic biomaterials are widely used in medical devices for different medical applications due to their exceptional mechanical properties. There are several factors which influence the biocompatibility of medical devices. The function of the device and its durability are the two main factors affecting biocompatibility. The two major factors that affects the biocompatibility of a material are i) Reaction induced by the host towards the material ii) Degradation of the material by the body. *In vitro* research provides a number of benefits over *in vivo* research, including (1) strict environmental control (chemical and physical), (2) lower cost, (3) higher productivity, and (4) less animal usage [20]. Nevertheless, *in vitro* experiments do not accurately mimic the conditions of cells in an organism, as is the case with isolated and cultured primary cells that frequently differ significantly from the corresponding cell type in an organism, limits the usefulness of *in vitro* data in predicting *in vivo* behavior.

Genotoxicity is a vital aspect of studying the damage to genetic information within a cell, such as DNA strand breakages, chromosomal fragmentation, point mutations, and alterations of gene expression profiles. Genotoxins that interact with chromosomes directly during interphase and may bind with DNA molecules to prevent DNA replication or transcription are said to cause direct primary genotoxicity. Additionally, genotoxins may interact with chromosomes during mitosis, resulting in mechanical or chemical binding that results in chromosomal breakage (clastogenic effect) or loss (aneugenic effect). The release of toxic ions from soluble genotoxins during indirect primary genotoxicity is an indirect interaction that can affect proteins necessary for DNA replication, transcription, or repair as well as the mitotic spindle apparatus, centrioles, or the proteins that are connected to them. These interactions can render the proteins structurally inactive.

In the present study, data from 20 orthopedic implants were analyzed. Data model of Ames, CA and MNT of different types of orthopaedic implants are summarized in Table 3.

Table 3. Data from the Ames Test, Chromosomal Aberration Test, and Micronucleus Test of various types of orthopedic implants

TEST ITEM	AMES*	CA*	MNT*
Titanium alloy (Ti-6AI-4V)	x	x	x
ASTM F136 ELI Titanium, ASTM F67 Unalloyed Titanium,	x	x	-
Titanium Grade 23	x	-	-
Titanium Grade 4	x	-	-
Ti/HA (Titanium hydroxyapatite coating),	x	x	-
ISO 5832-3 (TITANIUM),	x	-	-
ASTM F2063 Nitinol	x	x	-
Titanium Grade 5 (ISO 5832-3).	x	-	-
Ultra-High Molecules Weight Polyethylene (No 1)	x	x	x
Ultra-High Molecules Weight Polyethylene (No 2)	x	-	-
Ultra-High Molecules Weight Polyethylene (No 3)	x	x	-
Ultra-High Molecules Weight Polyethylene (No 4)	x	-	-
Ultra-High Molecules Weight Polyethylene (No 5)	x	-	-
Ultra-High Molecules Weight Polyethylene (No 6)	x	-	-
Stainless steel ISO 5832-1 (SS316LVM) (No 1)	x	-	-
Stainless steel ISO 5832-1 (SS316LVM) (No 2)	x	-	-
Stainless steel ISO 5832-1 (SS316LVM) (No 3)	x	-	-
Stainless steel ISO 5832-1 (SS316LVM) (No 4)	x	-	-
Cobalt chromium molybdenum alloy (ASTM F 1537) (No 1)	x	x	-
Cobalt chromium molybdenum alloy (ASTM F 1537) (No 2)	x	x	-

* AMES, AMES Test; CA, Chromosome aberration test; MNT, Micronucleus test, 'x', genotoxicity test data available; and '-', endpoint was not studied.

None of the implant materials tested positive for genotoxicity out of the 20 reviewed here. This could be accredited to their inherent property and quality of preparation. However, it should be noted that biocompatibility tests on implant materials including tests for genotoxicity do not take into account the wear debris from these implants.

In a study by Figgit et al[21], the presence of genotoxicity and metal ion levels in 86 patients that had hip replacements was evaluated. Half the patients showed alterations in four chromosomes compared to the control. The authors indicated and early genotoxicity in the bladder and cautioned the use of metal-on-metal implants for their genotoxicity per se and their wear debris. In a recent review by Qin et al[22], which investigated data from experimental and human data on the genotoxicity of materials used in endoprostheses, induction of chromosomal aberrations was reported with certain implant materials *in vitro* and *in vivo*; this damage was accredited to the release of metal ions and particles from the implant. The authors concluded that patients hosting artificial implants could be at a higher risk of DNA damage that could probably initiate carcinogenicity.

The classical recall of the De Puy Synthes hip replacements owing to the high wear debris that caused complications such as metallosis in the patients who received them, reminds us of the fact that wear debris are to be critically assessed. There is a gap that needs to be addressed in the genotoxicity testing of orthopaedic implants; assessing whole implants and wear debris could be useful. In addition, assessment of metal concentration in blood could be an indirect assessment of an ongoing genotoxic event.

4 Conclusion

In the present study, we conducted a thorough review of the genotoxicity data from 20 orthopedic implants. It is important to note that all the results presented here are from *in vitro* studies, except for two *in vivo* micronucleus assays conducted on Titanium and UHMWP materials, respectively. According to the ISO 10993-3 requirements, conducting animal studies is not mandatory if two *in vitro* endpoints indicate that the biomaterial does not exhibit genotoxicity. The absence of genotoxicity in these materials could be attributed to the rigorous quality control measures during their manufacturing process and/or their inherent non-genotoxic properties.

Regulatory testing does not currently mandate the assessment of wear particles and site of contact genotoxicity from these materials. However, addressing this gap is crucial. Therefore, it is recommended to include an assessment of the genotoxicity of wear particles from implants as part of the implantation study. Additionally, it would be valuable to evaluate the concentration of metal ions in the blood and the potential genotoxic effects at the site of contact. Presently, this area remains relatively unexplored and requires extensive research to accumulate sufficient data and validation. By conducting such studies, we can gain a better understanding of the potential genotoxic risks associated with implant materials and ensure safer and more reliable medical devices in the future.

References

1) Phillips DH, Arlt VM. Genotoxicity: damage to DNA and its consequences. *Experientia Supplementum*. 2009;99:87–110. Available from: https://core.ac.uk/reader/29909583?utm_source=linkout.
2) Turkez H, Arslan ME, Ozdemir O. Genotoxicity testing: progress and prospects for the next decade. *Expert Opinion on Drug Metabolism & Toxicology*. 2017;13(10):1089–1098. Available from: https://www.tandfonline.com/doi/abs/10.1080/17425255.2017.1375097.
3) Kumaravel TS, Brabu B, Parthiban N, Murugan SS, Jha AN. Site of contact genotoxicity' assessment for implants - Potential use of single cell gel electrophoresis in biocompatibility testing of medical devices. *Toxicology Letters*. 2021;341:59–67. Available from: https://doi.org/10.1016/j.toxlet.2021.01.024.
4) Nesslany F. The current limitations of in vitro genotoxicity testing and their relevance to the in vivo situation. *Food and Chemical Toxicology*. 2017;106:609–615. Available from: https://pubmed.ncbi.nlm.nih.gov/27591928/.
5) Shrivats AR, Alvarez P, Schutte L, Hollinger JO. Bone Regeneration. *Princ Tissue Eng Fourth Ed*. 2013;p. 1201–1222. Available from: https://doi.org/10.1016/B978-0-12-398358-9.00055-0.
6) Kim T, See CW, Li X, Zhu D. Orthopedic implants and devices for bone fractures and defects: Past, present and perspective. *Engineered Regeneration*. 2020;1:6–18. Available from: https://doi.org/10.1016/j.engreg.2020.05.003.
7) Stanford CM. Surface Modification of Biomedical and Dental Implants and the Processes of Inflammation, Wound Healing and Bone Formation. *International Journal of Molecular Sciences*. 2009;11(1):354–369. Available from: https://pubmed.ncbi.nlm.nih.gov/20162020/.
8) Hadjidakis DJ, Androulakis II. Bone Remodeling. *Annals of the New York Academy of Sciences*. 2006;1092(1):385–396. Available from: https://pubmed.ncbi.nlm.nih.gov/17308163/.
9) Mavrogenis AF, Dimitriou R, Parvizi J, Babis GC. Biology of implant osseointegration. *Journal of Musculoskeletal and Neuronal Interactions*. 2009;9(2):61–71. Available from: https://www.ismni.org/jmni/pdf/36/01MAVROGENIS.pdf.
10) Kim T, See CW, Li X, Zhu D. Orthopedic implants and devices for bone fractures and defects: Past, present and perspective. *Engineered Regeneration*. 2020;1:6–18. Available from: https://doi.org/10.1016/j.engreg.2020.05.003.
11) Goodman SB, Yao Z, Keeney M, Yang F. The future of biologic coatings for orthopaedic implants. *Biomaterials*. 2013;34(13):3174–3183. Available from: https://pubmed.ncbi.nlm.nih.gov/23391496/.
12) Wang Y, Teng W, Zhang Z, Ma S, Jin Z, Zhou X, et al. Remote Eradication of Bacteria on Orthopedic Implants via Delayed Delivery of Polycaprolactone Stabilized Polyvinylpyrrolidone Iodine. *Journal of Functional Biomaterials*. 2022;13(4):195. Available from: https://doi.org/10.3390/jfb13040195.
13) Quinn J, Mcfadden R, Chan CW, Carson L. Titanium for Orthopedic Applications: An Overview of Surface Modification to Improve Biocompatibility and Prevent Bacterial Biofilm Formation. *iScience*. 2020;23(11):101745. Available from: https://doi.org/10.1016/j.isci.2020.101745.
14) Uhthoff HK, Bardos DI, Liskova-Kiar M. The advantages of titanium alloy over stainless steel plates for the internal fixation of fractures. An experimental study in dogs. *The Journal of Bone and Joint Surgery British volume*. 1981;63-B(3):427–484. Available from: https://online.boneandjoint.org.uk/doi/abs/10.1302/0301-620X.63B3.7263759.
15) Cowie RM, Pallem NM, Briscoe A, Jennings LM. Third Body Wear of UHMWPE-on-PEEK-OPTIMA^{TM}. *Materials*. 2020;13(6):1264. Available from: http://www.ncbi.nlm.nih.gov/pubmed/32168765.
16) Filip N, Radu I, Veliceasa B, Filip C, Pertea M, Clim A, et al. Biomaterials in Orthopedic Devices: Current Issues and Future Perspectives. *Coatings*. 2022;12(10):1544. Available from: https://doi.org/10.3390/coatings12101544.
17) ISO 10993-1:2018. Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process. 2018.
18) ISO 10993-12:2021. Biological evaluation of medical devices - Part 12: Sample preparation and reference materials. .
19) ISO 10993-3:2014. Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity. 2014.
20) Rodriguez-Garraus A, Azqueta A, Vettorazzi A, De Cerain AL. Genotoxicity of Silver Nanoparticles. *Nanomaterials*. 2020;10(2):251. Available from: https://doi.org/10.3390/nano10020251.
21) Figgitt M, Parry M, Maclean A, Blom A, Bannister G, Eastaugh-Waring S, et al. Genotoxicity of orthopaedic wear debris: A patient study. *Toxicology*. 2011;290(2-3):115. Available from: https://doi.org/10.1016/j.tox.2011.09.026.
22) Qin HM, Herrera D, Liu DF, Chen CQ, Nersesyan A, Mišík M, et al. Genotoxic properties of materials used for endoprostheses: Experimental and human data. *Food and Chemical Toxicology*. 2020;145:111707. Available from: https://doi.org/10.1016/j.fct.2020.111707.