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ORIGINAL ARTICLE

Haematological and Biochemical Alterations Following 180-Day Subcutaneous Implantation of Cobalt-Chromium-Molybdenum (CCM) Alloy in Rats

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Abstract

Objectives: This study aims to assess the impact of Cobalt-Chromium-Molybdenum (CCM) alloy on various haematological and biochemical parameters after 180-day subcutaneous implantation in rats. **Methods:** A total of 60 Wistar rats divided into 4 groups of 15 animals: Control Male (G1), Test Male (G2), Control Female (G3), and Test Female (G4) were used for the 180-day study via subcutaneous implantation. Test groups were implanted with CCM alloy dental abutments of human equivalent dose, whereas negative control groups were implanted with High-density polyethylene (HDPE) strips. At the end of the experimental period, blood samples were collected from all the animals via orbital sinus puncture. These samples were further used for analysis of a bunch of various haematological and biochemical parameters. **Findings:** In both males and females, no clinically significant difference was observed in the biochemical or haematological parameters in CCM alloy implanted animals compared to the negative control group. **Novelty:** Long-term effects of CCM alloy on animal models are limited. This study analyzed haematological and biochemical parameters following 180-day subcutaneous implantation of CCM in rats. The findings provide valuable insights into the safety profile of CCM implants, specifically regarding their impact on blood.

Keywords: Biochemical; Haematological; Subcutaneous implantation; Dental abutment; CCM

1 Introduction

International Organization for Standardization (ISO) guidelines have classified orthopedic and dental implants as long-term implantable medical devices. Cobalt- chromium

-molybdenum (CCM) alloy is a potential material used to manufacture such implants. Due to wear, tear, and corrosion, there are chances for metal ions to get released from these alloys into the bloodstream [1]. This is one of the major limitations of CCM [2]. The released metal ions can alter the haematological parameters and accumulate in the vital organs, resulting in the alteration of biochemical parameters too [3]. An increased level of Co and Cr ions was found in the serum of patients with spinal implants [4] and also in the blood samples of patients with metal-on-metal total hip arthroplasty [5]. In some other studies, there was no elevation of metal ions in the serum of patients with Co-Cr coronary stents [6].

Despite the widespread use of CCM alloys in dental and orthopedic applications, standardized long-term preclinical safety data are limited, particularly concerning systemic effects on blood chemistry and haematological health. Even though several literatures support and oppose the release of metal ions from the implants, the effects of these on the haematology and biochemical nature of blood are not explored in detail. The variability in reported findings (presence vs. absence of elevated metal ion levels) indicates a need for standardized, controlled animal studies to eliminate confounding clinical variables. The lack of data on the long-term toxic effects of CCM implants is another limitation, as the available literature is either case studies or short-term studies.

A potential use of CCM alloy is in the manufacture of dental abutment, which is a connector used to connect a crown, bridge, or denture to a dental implant, which is surgically implanted in a patient [7]. The oral environment differs from other biological systems due to several reasons, like constant exposure to external elements, pH changes, microbial diversity, constituents of saliva, and constant movements due to grinding and chewing [8]. All these properties may accelerate the corrosion and wear and tear of dental implants. Hence, dental implants should undergo crucial testing than any other orthopedic implants.

Biocompatibility assessment for such devices requires addressing multiple endpoints, including systemic toxicity, as outlined in ISO 10993 guidelines. Long-term implantation studies using animal models could provide detailed systemic toxic effects of CCM implants [9]. Animal models, particularly rodent models, offer reproducibility and relevance for preclinical toxicity screening of long-term implant materials like CCM. Metal ion release is influenced by implant location and duration, with soft tissue environments like subcutaneous tissue offering a controlled yet clinically relevant model to study systemic distribution.

Haematological and biochemical changes can serve as early biomarkers of subclinical toxicity, preceding visible organ damage or functional impairment [10]. Therefore, in the present paper, we are focusing on biochemistry and haematological changes following CCM subcutaneous implantation in rats. However, there is a lack of standardized long-term *in vivo* studies systematically assessing the potential haematological and biochemical alterations induced by CCM implants, making it difficult to conclusively determine their systemic biocompatibility. These data will be helpful in literature-based risk assessments and for building the weight of evidence when evaluating the biocompatibility of medical devices.

2 Methodology

The animal model selected for the current study is Wistar rats, due to the availability of large background data. The study was performed based on ISO 10993-2:2022 [11], ISO 10993-6:2016 [12], ISO 10993-11:2017 [13], and ISO 10993-12:2021 [14]. The ISO and OECD guidelines, along with various regulatory authorities, have recommended Wistar rats as an appropriate animal model for chronic toxicity studies. The study was performed with the Institutional Animal Ethics Committee (IAEC) approval of GLR Laboratories Private Limited. In addition to haematology and biochemistry, other chronic toxicity data were collected from the experiment but presented elsewhere.

2.1 Implants

The CCM alloy implants measured 10 mm in length and 2 mm in diameter. High-density polyethylene (HDPE) strips of identical dimensions (10 mm $\times$ 2 mm) were used as negative controls. Both the test items and controls were steam-sterilized at 121°C for 15 minutes prior to implantation.

2.2 Experimental Animals

Young adult male and young adult, nulliparous, non-pregnant female Wistar rats were obtained from VAB Biosciences (Hyderabad, India) for the study. Upon arrival, the animals were acclimatized to laboratory conditions for seven days. During this period, routine veterinary examinations were conducted, and animals were monitored for clinical signs, body weight, and overall health status. Only healthy rats with body weights within $\pm$20% of the group mean were selected for the experiment. The final body weight range of selected animals was 200–300 g. All animals were housed individually in clean polypropylene cages and provided commercial rodent pellet feed and water *ad libitum*. A total of 60 rats were used for this study. They were divided into 4 groups of 15 animals each - Control Males (G1), Test Males (G2), Control Females (G3), and Test Females (G4).

2.3 Dose Calculation

The dose of the CCM alloy was calculated based on the human equivalent dose (HED) for rats. Assuming an average adult human body weight of 60 kg, the maximum dose corresponds to 0.34 implants/kg (assuming a maximum of 20 implants per human). Using the rat conversion factor (6.2), the animal equivalent dose was determined as 2.11 implants/kg. Given an average rat body weight of 250 g, this translates to 0.53 implants/rat. Applying a safety factor of 10, the final dose was adjusted to 6 implants per 300 g rat, representing a worst-case exposure.

2.4 Implantation

All surgical procedures were performed by veterinary surgeons approved by the Institutional Animal Ethics Committee (IAEC). Animals were anesthetized via intraperitoneal injection of ketamine (70–100 mg/kg) and xylazine (8–12 mg/kg). Implants were placed into subcutaneous pouches (three on either side of the vertebral column) on the dorsal region with minimal tissue disruption and closed using absorbable sutures. Each test animal received six implants, while control animals received six HDPE implants. Post-operative care included intramuscular administration of anti-inflammatory drugs and antibiotics for seven days. Veterinary care was provided as needed. Animals were monitored daily for clinical signs, morbidity, and mortality, while feed and water intake were recorded weekly.

2.5 Haematology

At the end of the 180-day experimental period, animals were anesthetized via intraperitoneal injection of ketamine (70–100 mg/kg) and xylazine (8–12 mg/kg). Blood samples were collected from the orbital sinus by trained veterinarians and stored in appropriate vacutainers. Haematological assessments included haematocrit, haemoglobin concentration, red blood cell (RBC) count, white blood cell (WBC) count, WBC differential count, platelet count, and coagulation parameters such as prothrombin time (PT) and activated partial thromboplastin time (APTT). Haematology analyses were performed using a BC-5000 veterinary haematology analyser (Mindray, Shenzhen, China), while coagulation parameters were measured with a C2000-2 semi-automated coagulation analyser (Mindray, Shenzhen, China). Commercially available kits and reagents were used according to the manufacturer's instructions.

2.6 Clinical Biochemistry

Clinical biochemistry analyses were conducted on blood serum to assess potential systemic toxicity, with particular focus on liver and kidney function. The parameters measured included albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, chloride, cholesterol, creatinine, gamma-glutamyl transferase (GGT), glucose, inorganic phosphorus, potassium, sodium, total bilirubin, total protein, triglycerides, and blood urea nitrogen.

Before sample analysis, the electrolyte analyser was validated through a quality control (QC) procedure using three levels of standard electrolyte controls: ISEQC Level 1 (high), ISEQC Level 2 (medium), and ISEQC Level 3 (low) (Golden Harvest, India). Each QC level was run to ensure accuracy and consistency of the analyzer. Successful QC performance across all three levels—defined by values falling within the manufacturer-specified acceptable range—confirmed the instrument's readiness for analysis. Upon validation, test samples were subsequently loaded and analyzed for sodium, potassium, and chloride levels using the same electrolyte analyzer.

Biochemical parameters were analyzed using the BS-120 biochemistry analyzer (Mindray, Shenzhen, China). Prior to sample analysis, instrument performance was verified through quality control (QC) procedures using reconstituted lyophilized powder-Clinchem Multi Control Level 1 (normal range) and Level 2 (abnormal range). Each level was run to ensure precision and reliability of the analyzer. QC validation was confirmed when the measured values for both levels fell within the manufacturer's specified reference ranges.

Following successful QC validation, the serum samples and required reagents were loaded into their respective designated slots on the analyzer. A total of 14 biochemical parameters were measured according to standard operating procedures and the manufacturer's instructions.

2.7 Statistical Analysis

Statistical differences in body weight, haematological, and biochemical parameters between the respective control and test groups were assessed using an unpaired t-test with Welch's correction, considering a 95% confidence interval (CI). All statistical analyses were performed using GraphPad Prism software (version 10.2.3).

3 Results and Discussion

The results of clinical observations, haematological and biochemical changes following subcutaneous implantation of CCM alloy for 180 days are presented below.

3.1 Mortality and Morbidity

All the animals of both the control and the test groups were healthy and alive until the end of the experiment. This indicated that the CCM alloy implants had little or no adverse effects.

3.2 Body weight recording

After an early drop in body weights (data not shown) following implantation surgery, there was a gradual increase in weekly body weight in all the animals. A summary of body weight recorded for the animals treated and control groups is provided in Table 1.

Table 1. Body weights of male and female control and test groups following 180-day subcutaneous implantation of Cobalt-chromium-molybdenum alloy

Duration	Control Male (Mean ± SD; grams)	Test Male (Mean ± SD; grams)	Control Female (Mean ± SD; grams)	Test Female (Mean ± SD; grams)
Day of Implantation	278.51 ± 23.03	268.31 ± 28.04	230.43 ± 19.76	236.99 ± 27.49
Week 4	314.16 ± 29.76	311.91 ± 42.08	243.46 ± 21.25	256.76 ± 40.56
Week 8	329.46 ± 38.00	331.12 ± 47.30	251.65 ± 21.21	266.24 ± 43.60
Week 12	336.34 ± 38.45	336.90 ± 48.56	265.85 ± 20.31	270.57 ± 44.39
Week 16	347.46 ± 40.09	346.04 ± 50.85	262.79 ± 20.59	275.59 ± 45.13
Week 20	355.91 ± 42.09	356.42 ± 53.84	269.72 ± 21.42	280.13 ± 45.57
Week 24	362.89 ± 45.24	363.04 ± 55.28	276.84 ± 24.46	284.61 ± 46.72
Week 26	364.47 ± 45.83	365.51 ± 55.65	280.67 ± 25.86	288.05 ± 47.34

Values represents the mean body weight ± SD of both control and test groups of both male and female. Statistical analysis was performed on these data using Welch's t-test, and no significant differences were observed between control and test groups ($P < 0.05$).

3.3 Feed and Water Consumption

The feed and water intake of all the animals, both male and female, of both groups was normal. However, there was a low intake in the first week post-surgery, which is quite normal. There were no significant changes in the feed and water intake observed in test group animals when compared with the control groups.

3.4 Clinical Observation

None of the animals showed any clinical signs of toxicity throughout the study.

3.5 Haematology and Coagulation

A summary of haematological and coagulation measurements following 180 days of CCM alloy implantation in rats is given in Table 2. No significant changes in haematology or coagulation were observed following implantation with CCM alloy compared to negative HDPE controls.

RBC, Red Blood Cell count; Hb, Haemoglobin; HCT, Haematocrit; WBC, White Blood Cell count; MCH, Mean Corpuscular Haemoglobin; MCHC, Mean Corpuscular Haemoglobin Concentration; MCV, Mean Corpuscular Volume; PT, Prothrombin Time; APTT, Activated Partial Thromboplastin Time. Statistical analysis was performed on these data using Welch's t-test, and no significant differences were observed between control and test groups ($P < 0.05$).

Table 2. Haematology and coagulation measurements at the end of 180-day subcutaneous implantation of Cobalt-Chromium-Molybdenum alloy in control and test groups

Parameters	Control Male (Mean ± SD)	Test Male (Mean ± SD)	Control Female (Mean ± SD)	Test Female (Mean ± SD)
RBC ($10^6/\mu$L)	7.57 ± 0.63	7.45 ± 0.35	7.30 ± 0.58	7.21 ± 1.43
Hb (g/dL)	14.23 ± 1.08	13.75 ± 0.90	13.81 ± 0.87	13.51 ± 2.79
HCT (%)	41.33 ± 2.08	41.69 ± 2.79	41.52 ± 2.66	41.91 ± 8.51
WBC ($10^3/\mu$l)	12.59 ± 1.53	12.34 ± 1.39	12.34 ± 1.31	12.19 ± 2.57
Platelet ($10^3/\mu$l)	815.27 ± 70.86	836.00 ± 84.04	837.47 ± 87.81	832.07 ± 178.44
Lymphocytes (%)	76.83 ± 4.46	78.31 ± 4.50	78.54 ± 5.28	76.94 ± 15.70
Monocytes (%)	6.89 ± 1.35	6.71 ± 1.42	7.35 ± 1.43	7.00 ± 1.85
Neutrophils (%)	14.53 ± 3.55	14.05 ± 4.40	13.29 ± 5.67	15.24 ± 5.13
Eosinophils (%)	0.67 ± 0.14	0.69 ± 0.12	0.70 ± 0.15	0.71 ± 0.18
Basophils (%)	0.11 ± 0.09	0.13 ± 0.09	0.11 ± 0.09	0.11 ± 0.08
MCH (pg)	18.92 ± 2.14	18.49 ± 1.36	19.02 ± 1.83	17.64 ± 3.92
MCHC (g/dL)	34.53 ± 3.23	33.10 ± 3.05	33.41 ± 3.39	32.34 ± 6.64
MCV (fL)	54.83 ± 3.57	55.99 ± 3.54	57.29 ± 6.59	54.67 ± 11.68
PT (sec)	11.32 ± 1.05	11.59 ± 1.02	11.72 ± 1.06	11.53 ± 1.03
APTT (sec)	21.41 ± 2.23	21.16 ± 1.99	21.21 ± 1.47	20.65 ± 1.71

3.6 Clinical biochemistry

The results of biochemistry following 180 days of CCM alloy implantation in rats are given in Table 3. No significant changes were observed in biochemistry were observed following CCM alloy implantation. Small changes were observed that were not considered clinically relevant. Serum electrolytes, liver function, and renal function tests were all normal.

Table 3. Biochemical Parameters in Male and Female Control and Test Groups

Parameters	Control Male (Mean ± SD)	Test Male (Mean ± SD)	Control Female (Mean ± SD)	Test Female (Mean ± SD)
Sodium (mEq/L)	150.00 ± 3.90	151.88 ± 3.96	153.30 ± 3.69	150.89 ± 2.97
Chloride (mEq/L)	96.35 ± 4.26	94.88 ± 4.23	95.66 ± 4.43	93.80 ± 4.42
Potassium (mEq/L)	4.72 ± 0.22	4.62 ± 0.33	4.59 ± 0.31	4.67 ± 0.29
Glucose (mg/dL)	109.81 ± 6.78	108.88 ± 5.41	111.62 ± 5.94	109.56 ± 4.89
Cholesterol (mg/dL)	72.56 ± 6.36	73.73 ± 5.78	70.10 ± 5.84	69.95 ± 5.95
Total Bilirubin (mg/dL)	0.07 ± 0.03	0.06 ± 0.03	0.05 ± 0.03	0.05 ± 0.03
Total Protein (g/dL)	6.75 ± 0.45	6.87 ± 0.36	6.77 ± 0.42	6.88 ± 0.42
Albumin (g/dL)	3.46 ± 0.28	3.59 ± 0.25	3.62 ± 0.30	3.47 ± 0.28
Urea Nitrogen (mg/dL)	17.54 ± 1.83	17.66 ± 1.90	18.12 ± 2.15	17.85 ± 2.04
Creatinine (mg/dL)	0.38 ± 0.08	0.38 ± 0.07	0.42 ± 0.08	0.42 ± 0.06
Calcium (mg/dL)	14.71 ± 3.79	14.39 ± 4.34	14.90 ± 2.37	14.25 ± 3.17
Phosphate (mg/dL)	8.00 ± 0.70	7.92 ± 0.63	7.86 ± 0.66	7.93 ± 0.64
Triglycerides (mg/dL)	70.57 ± 9.97	70.13 ± 9.33	68.09 ± 15.55	70.39 ± 14.41
Aspartate Aminotransferase (AST) (IU/L)	123.63 ± 6.20	120.61 ± 7.58	124.39 ± 7.22	120.18 ± 9.10
Alanine Aminotransferase (ALT) (IU/L)	63.00 ± 4.86	59.84 ± 6.03	58.03 ± 4.58	60.45 ± 6.53
Alkaline Phosphatase (ALP) (IU/L)	196.42 ± 16.67	196.72 ± 24.05	198.66 ± 37.34	213.56 ± 32.60
Gamma-Glutamyl Transferase (GGT) (IU/L)	0.77 ± 0.14	0.78 ± 0.13	0.79 ± 0.12	0.81 ± 0.14

Statistical analysis was performed on these data using Welch's t-test, and no significant differences were observed between control and test groups ($P < 0.05$).

3.7 Statistical Analysis

Statistical analysis was performed on the body weight, haematological, and biological parameters. No statistically significant difference was observed ($p<0.05$) in any of the parameters, and the values were within the normal physiological range. Therefore, these changes were considered sporadic and unrelated to the CCM alloy. Similarly, in the biochemistry analysis, small but sporadic changes were observed in some parameters, but all values were within the normal physiological range, and therefore the statistical values are not presented.

CCM alloys are commonly used in permanent implantable medical devices. Therefore, evaluating their biocompatibility using *in vivo* methods is essential. One of the major concerns is the potential toxicity resulting from the leaching of constituent chemicals from the alloy[15]. Since these devices remain in the body over extended periods, leaching can occur slowly throughout the lifetime of the implant. Chemicals leaching from CCM alloys can migrate away from the implant site and reach other parts of the body via systemic circulation[16]. This raises concerns about systemic toxicity, which refers to adverse effects occurring beyond the site of contact due to the absorption and distribution of these substances throughout the body. Systemic toxicity is typically classified based on the duration of exposure: acute, subacute, or chronic. Chronic toxicity refers to harmful effects resulting from repeated or continuous exposure to a substance over a substantial portion of the organism's lifespan. Assessing chronic toxicity is especially important for evaluating the long-term safety of implantable medical devices[17].

In this study, we performed a chronic toxicity evaluation of CCM alloys using the subcutaneous route of implantation. While several administration routes exist for evaluating implantable devices - including subcutaneous, intramuscular, and bone implantation - the choice of route significantly influences the exposure profile[18]. The intramuscular route often leads to the formation of a dense fibrous capsule around the implant, which can limit the release of leachables into the systemic circulation[19]. In contrast, the subcutaneous route does not typically form such an encapsulating barrier, allowing for a more accurate assessment of potential systemic exposure[20]. Therefore, we selected the subcutaneous implantation route in this study to better simulate the *in vivo* environment, facilitate chemical leaching from the alloy, and allow for systemic distribution of any released substances.

In this manuscript, we present body weight changes, haematological and biochemical parameters from this chronic toxicity study. The other endpoints of this study are presented elsewhere. There was an expected increase in monthly body weights of all animals, both in the test and control groups. Animal body weights are measured during *in vivo* animal studies as it is directly linked to the overall health of animals[21]. Failure to maintain an increasing body weight indicates abnormalities such as signs of distress[22]. Body weight, the behavior of locomotion, climbing, and eating considerably decrease during the first few weeks of the surgery[23]. We did observe a similar result in our study, however, the male and female animals of both control and test groups depicted an increase in body weight from week 4 until the end of the experiment. Generally, males tend to gain more weight than females, and the same was observed in the current study. This shows that the animals were healthy and were not under any distress throughout the period. The feed and water intake of all the animals, both male and female, of both groups was normal. None of the animals showed any clinical signs of toxicity throughout the study. Furthermore, no clinically relevant changes in haematological and biochemical parameters were observed.

Cobalt and Chromium are known to cause haematological abnormalities[24]. We didn't observe any abnormalities in the haematological counts of test group animals when compared to those of control groups, probably because there was no significant leaching of chemicals from the CCM alloy. Therefore, it's safe to say the CCM didn't cause any adverse effects on the blood parameters under the conditions of this test. Several studies have associated leucocyte counts with metal implantation. Exposure to Cr causes leukocyte and platelet abnormalities[25]. Reduction of lymphocytes was observed in patients with Cobalt-chromium MoM Implants in one study[26]. In our study, no such changes were seen, as a comparable range of leukocyte, lymphocyte, and platelet counts between the treated and control groups. The mean MCV, MCH, and MCHC were comparable with the similar past studies[27]. The clotting potential between the treated and control groups of both males and females is comparable. Biochemical profiling revealed isolated fluctuations in select parameters; nevertheless, all readings were consistent with established physiological norms. As such, the liver function and renal function tests were not affected by the implantation of CCM alloy.

The current study provides unique insights into the biocompatibility of CCM alloy, widely used in orthopedic and dental implants, particularly its long-term effects. The highlight of this work is the comprehensive evaluation of both haematological and biochemical parameters following subcutaneous implantation of the abutment. This combination analysis further enhances the multidimensional view of how the body reacts to such implants. By observing them over a 180-day chronic toxicity period, the research explores the alloy's potential for causing systemic and localized health effects. The other endpoints of the study

provide crucial data on biocompatibility and long-term safety, which are not discussed in the current paper. This approach gives a thorough assessment of cumulative toxic effects, bridging the gap between material science and its clinical application. All these findings are crucial for safety risk assessments and optimizing the design of implants.

4 Conclusion

In the present study, no haematological and biochemical changes were observed in rats subcutaneously implanted with CCM alloy for 180 days.

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6 Contributory statement

- Ms. Mariya George: Conceptualized the study problem and conducted data generation.
- Ms. P. Pavithra: Assisted with data generation.
- Dr. K. Kayathri and Dr. Gayathri Satheesh: Veterinarians who performed the surgical procedures on the animals.
- V. Bhuvana: Carried out the blood sample analysis for the study.
- Dr. N. Mohana: Interpreted the results and performed statistical analysis.
- Dr. V.N. Sangeetha and Dr. S. S. Murugan: Contributed to data analysis, and facilitating research.
- Dr. T.N. Sathya: Designed the experimental framework, and interpretation of results. Provided overall research guidance.
- Dr. T. S. Kumaravel: Facilitating research activities, reviewed and edited the manuscript, ensuring accuracy and coherence. Provided overall research guidance.

References

1) Sun CWY, Lau LCM, Cheung JPY, Choi SW. The potential carcinogenicity of orthopaedic implants – a scoping review. *BMC Cancer*. 2024;24(1):1–12. Available from: https://bmccancer.biomedcentral.com/articles/10.1186/s12885-024-13279-2.
2) Bellouard M, Gasser M, Lenglet S, Gilardi F, Bararpour N, Augsburger M, et al. Toxicity and Metabolomic Impact of Cobalt, Chromium, and Nickel Exposure on HepaRG Hepatocytes. *Chem Res Toxicol*. 2022;35(5):807–816. Available from: https://pubmed.ncbi.nlm.nih.gov/35442019/.
3) Bhandari M, Guyatt G, Schemitsch E, Zura R, Frihagen F, Guerra-Farfán E, et al. Total Hip Arthroplasty or Hemiarthroplasty for Hip Fracture. *N Engl J Med*. 2019;381(23):2199–2208. Available from: https://pubmed.ncbi.nlm.nih.gov/31557429/.
4) Mathew SE, Xie Y, Bagheri L, Claton LE, Chu L, Badreldin A, et al. Are Serum Ion Levels Elevated in Pediatric Patients With Metal Implants? *J Pediatr Orthop*. 2022;42(3):162–168. Available from: https://pubmed.ncbi.nlm.nih.gov/34619722/.
5) Szczęsny G, Kopec M, Politis DJ, Kowalewski ZL, Łazarski A, Szolc T. A Review on Biomaterials for Orthopaedic Surgery and Traumatology: From Past to Present. *Materials (Basel)*. 2022;15(10). Available from: https://pubmed.ncbi.nlm.nih.gov/35629649/.
6) Stone GW, Maehara A, Ali ZA, Held C, Matsumura M, Kjøller-Hansen L, et al. Percutaneous Coronary Intervention for Vulnerable Coronary Atherosclerotic Plaque. *J Am Coll Cardiol*. 2020;76(20):2289–2301. Available from: https://pubmed.ncbi.nlm.nih.gov/33069847/.
7) Benakatti V, Sajjanar J, Aditya A. Dental implant abutments and their selection—A review. *J Evol Med Dent Sci*. 2021. https://doi.org/10.14260/jemds/2021/622.
8) Rowińska I, Szyperska-ślaska A, Zariczny P, Pasławski R, Kramkowski K, Kowalczyk P. The Influence of Diet on Oxidative Stress and Inflammation Induced by Bacterial Biofilms in the Human Oral Cavity. *Materials*. 2021;14(6):1444. Available from: https://www.mdpi.com/1996-1944/14/6/1444/htm.
9) Norman GAV. Limitations of Animal Studies for Predicting Toxicity in Clinical Trials: Is it Time to Rethink Our Current Approach? *JACC Basic Transl Sci*. 2019;4(7):845. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6978558/.
10) Vaicelyte A, Janssen C, Borgne ML, Grosgogeat B. Cobalt–Chromium Dental Alloys: Metal Exposures, Toxicological Risks, CMR Classification, and EU Regulatory Framework. *Crystals*. 2020;10(12):1151. Available from: https://www.mdpi.com/2073-4352/10/12/1151/htm.
11) ISO 10993-2:2022 - Biological evaluation of medical devices — Part 2: Animal welfare requirements. 2022. Available from: https://www.iso.org/standard/78866.html.
12) ISO 10993-6:2016 - Biological evaluation of medical devices — Part 6: Tests for local effects after implantation. 2016. Available from: https://www.iso.org/standard/61089.html.
13) ISO 10993-11:2017 - Biological evaluation of medical devices — Part 11: Tests for systemic toxicity. 2017. Available from: https://www.iso.org/standard/68426.html.
14) ISO 10993-12:2021 - Biological evaluation of medical devices — Part 12: Sample preparation and reference materials. 2021. Available from: https://www.iso.org/standard/75769.html.
15) Khan AA, Kim JH. Recent advances in materials and manufacturing of implantable devices for continuous health monitoring. *Biosens Bioelectron*. 2024;261:116461. https://doi.org/10.1016/j.bios.2024.116461.

16) Fernandes C, Taurino I. Biodegradable Molybdenum (Mo) and Tungsten (W) Devices: One Step Closer towards Fully-Transient Biomedical Implants. *Sensors*. 2022;22(8):3062. Available from: https://www.mdpi.com/1424-8220/22/8/3062/htm.
17) Kokova V. METHODS FOR DETERMINING SUB-ACUTE, SUB-CHRONIC, AND CHRONIC TOXICITY OF CHEMICAL COMPOUNDS. *Knowledge - International Journal*. 2023;59(4):257–261. Available from: https://www.ceeol.com/search/article-detail?id=1298786.
18) Wychowański P, Starzyńska A, Adamska P, Słupecka-Ziemilska M, Sobocki BK, Chmielewska A, et al. Methods of Topical Administration of Drugs and Biological Active Substances for Dental Implants—A Narrative Review. *Antibiotics*. 2021;10(8):919. Available from: https://www.mdpi.com/2079-6382/10/8/919/htm.
19) Hernandez JL, Park J, Yao S, Blakney AK, Nguyen HV, Katz BH, et al. Effect of tissue microenvironment on fibrous capsule formation to biomaterial-coated implants. *Biomaterials*. 2021;273:120806. https://doi.org/10.1016/j.biomaterials.2021.120806.
20) Audouard E, Rousselot L, Folcher M, Cartier N, Piguet F. Optimized Protocol for Subcutaneous Implantation of Encapsulated Cells Device and Evaluation of Biocompatibility. *Front Bioeng Biotechnol*. 2021;9:620967. Available from: http://clinicaltrials.gov/.
21) Ghasemi A, Jeddi S, Kashfi K. The laboratory rat: Age and body weight matter. *EXCLI J*. 2021;20:1431. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC8564917/.
22) Talbot SR, Biernot S, Bleich A, Dijk RMV, Ernst L, Häger C, et al. Defining body-weight reduction as a humane endpoint: a critical appraisal. *Laboratory animals*. 2020;54(1):99–110. Available from: https://journals.sagepub.com/doi/abs/10.1177/0023677219883319.
23) Baumans V, Bouwknecht JA, Boere H, Kramer K, Lith HAV, Weerd HAVD, et al. Intra-Abdominal Transmitter Implantation in Mice: Effects on Behaviour and Body Weight. *Animal Welfare*. 2001;10(3):291–302. https://doi.org/10.1017/S0962728600024064.
24) Ray RR. Adverse hematological effects of hexavalent chromium: an overview. *Interdiscip Toxicol*. 2017;9(2):55. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5458105/.
25) Su Z, Zhang Y, Hong S, Zhang Q, Xu J, Hu G, et al. Relationships between blood chromium exposure and liver injury: Exploring the mediating role of systemic inflammation in a chromate-exposed population. *Journal of Environmental Sciences*. 2024;143:22434. https://doi.org/10.1016/j.jes.2023.08.014.
26) Honkasaari N, Lainiala O, Laine O, Reito A, Eskelinen A. No association between blood count levels and whole-blood cobalt and chromium levels in 1,900 patients with metal-on-metal hip arthroplasty. *Acta Orthop*. 2020;91(6):711–716. Available from: https://pubmed.ncbi.nlm.nih.gov/33003969/.
27) Kampfmann I, Bauer N, Johannes S, Moritz A. Differences in hematologic variables in rats of the same strain but different origin. *Vet Clin Pathol*. 2012;41(2):228–234. Available from: https://pubmed.ncbi.nlm.nih.gov/22551195/.