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Investigating of the Effects of Ultrasonic Frequency, Cleaning Solution Temperature, and Cleaning Time on the Bioburden of Machined Stainless-Steel Orthopedic Implants

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Abstract

Subtractive manufacturing has gained widespread adoption in biomedical and advanced manufacturing applications due to its ability to produce components with high dimensional accuracy, improved surface finish, and enhanced mechanical integrity. The use of advanced multi-axis machining technologies, including CNC sliding-head (Swiss-type) machines and vertical machining centres, enables the precise and efficient manufacturing of complex biomedical components while reducing machining time and improving production efficiency compared with conventional machining processes.

In the present investigation, nine products manufactured from medical-grade 316LRM stainless steel conforming to ISO 5832-1 were produced using high-precision machining equipment. A Taguchi L9 orthogonal array was employed to investigate the influence of selected cleaning parameters on the bioburden level of the machined products. The effects of cleaning parameters were evaluated through bioburden testing, and the optimum parameter combination was determined using analysis of variance (ANOVA) and signal-to-noise (S/N) ratio analysis. The optimized cleaning conditions were identified as an ultrasonic frequency of 40 kHz, chemical solution temperature of 50 °C, and cleaning time of 10 min. Under these conditions, the bioburden was reduced to 11 CFU/device from an initial average level of 23 CFU/device prior to cleaning. The findings demonstrate that systematic optimization of the cleaning parameters can effectively reduce the microbial load on machined biomedical components and provide a controlled approach for improving the cleaning process.

Kaywords: Implant, Manufacturing, 316LRM Stainless Steel, Processing Parameters, Cleaning, Bioburden and Taguchi orthogonal Array L9.

Introduction

Orthopaedic implants and surgical instruments are essential medical devices used in the treatment and surgical management of musculoskeletal disorders. Temporary implants such as nails, plates, and screws provide mechanical stabilization during bone healing, while instruments such as guide wires, awls, tissue protectors, and sleeves assist in implant placement and surgical manipulation. Orthopaedic implants are commonly manufactured from biocompatible materials, including 316L/316LRM stainless steel, titanium alloys, cobalt–chromium alloys, and polymers such as PEEK. The selection of implant type and material depends on factors such as the anatomical site, bone condition, and patient characteristics.[1–5] Examples of commonly used orthopaedic implants and their applications are presented in Figure 1.

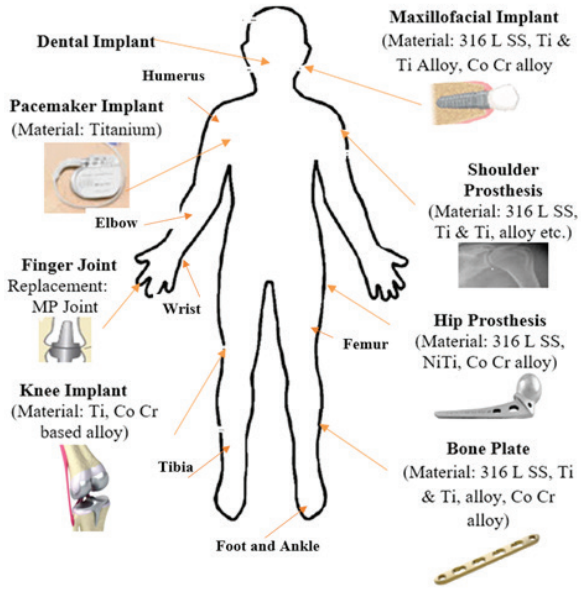

Figure 1: Distinct biomaterials and their application.[1]

Similarly, the global orthopedic implant market has shown consistent growth, with an estimated annual growth rate of approximately 6.1%.[6,7] This increasing demand highlights the need for reliable manufacturing processes and stringent quality control of orthopedic implants. Since implant quality directly influences patient safety and clinical performance, appropriate control of material selection, manufacturing, and cleaning processes is essential. The major categories of orthopedic implant materials and their classification are presented in Figure 2(a).

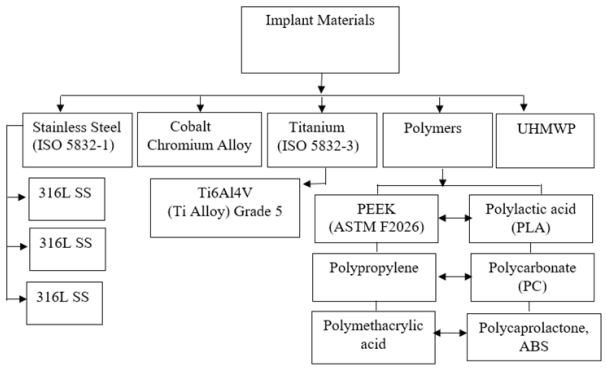

Figure 2 (a). Distinct orthopedic implant materials[8]

Various conventional and advanced manufacturing techniques, including turning, milling, and additive manufacturing, are employed for the production of orthopedic implants depending on the required dimensional accuracy, mechanical properties, geometry, and manufacturing efficiency. In the present study, high-precision CNC sliding-head (Swiss-type) machines were selected because of their capability to produce complex components with close dimensional tolerances and high repeatability, which are particularly important for orthopedic applications.[9–11] Following machining, effective cleaning of orthopedic implants is essential because residual manufacturing contaminants may adversely affect product safety, biocompatibility, and clinical performance. This is particularly important for components with complex geometries, where manual cleaning may not adequately remove machining residues, particles, oils, and other contaminants. Ultrasonic cleaning provides an effective approach by combining cavitation with a suitable cleaning solution to remove contaminants from difficult-to-access surfaces. The effectiveness of a cleaning process should be established through appropriate process controls and validation. Evaluation may include bioburden, organic and inorganic residue, particulate, and other relevant cleanliness tests. Cleaning validation should demonstrate that process residues and cleaning-agent residues are consistently reduced to acceptable levels and that the process remains capable of achieving the intended cleanliness requirements.[12–14] Among these parameters, bioburden is particularly important because microorganisms remaining on a medical device may contribute to infection risk, while microbial residues may also be associated with endotoxin-related concerns.

The level of contamination can be influenced by raw materials, machining operations, handling, cleaning agents, process water, storage, and environmental conditions. Both water-soluble contaminants, such as salts and detergent residues, and non-water-soluble contaminants, such as oils and greases, may remain on the device surface if the cleaning process is not adequately controlled.[15,16] Therefore, optimization and routine monitoring of cleaning parameters are essential to ensure consistent removal of contaminants and to support the safety and performance of orthopedic implants. The major sources of viable contamination associated with medical devices are illustrated in Figure 2(b).

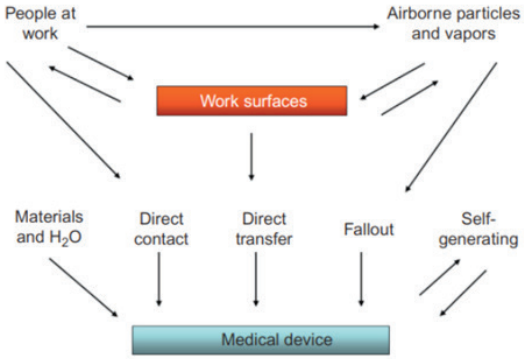

Figure 2 (b). Sources of viable contaminants[5]

Effective development, validation, and control of the cleaning process are essential for minimizing viable and nonviable contamination of medical devices. Appropriate personnel training, material selection, process monitoring, and defined cleanliness criteria are important components of an effective contamination-control strategy. Cleaning processes should be validated to demonstrate consistent removal of manufacturing residues and cleaning-agent residues to predefined acceptable levels. Where applicable, alert and action limits, routine monitoring, and trend analysis can further support process control and risk reduction.
During orthopedic implant manufacturing, cleaning is performed prior to packaging and sterilization to minimize residual contaminants that may affect product safety and biocompatibility. A combination of appropriate cleaning methods and analytical evaluations can therefore provide evidence of process effectiveness and consistency.
In the present investigation, nine orthopedic bone screws were manufactured from medical-grade 316L stainless steel using high-precision CNC sliding-head (Swiss-type) machines. A Taguchi L9 experimental design was employed to optimize the

selected ultrasonic cleaning parameters. Bioburden testing was performed to evaluate the microbial contamination remaining on the screw surfaces after cleaning. The experimental results were subsequently analysed to identify the optimum cleaning conditions and establish an appropriate approach for controlling bioburden in machined orthopedic implants.

Research Questions:

1. Whether the process parameters (ultrasonic frequency, chemical temperature, cleaning time, etc.) on the stainless-steel part will enhance the cleaning efficiency or not?
2. Which individual cleaning parameter has the maximum contribution to reducing bioburden on 316LRM stainless steel implants?
3. What is the combined effect of ultrasonic cleaning parameters on the bioburden properties of the manufactured part? Hence, the current experimental investigation aims to examine the effect of ultrasonic cleaning process parameters on the bioburden level of a 316LRM stainless steel-manufactured device. Thus, the low-cost biocompatible stainless-steel samples can be employed in distinct biomedical applications, such as bone systems.

Material and Method

The current research follows the following steps: (a) Selection of 316LRM SS material; (b) Selection of an advanced manufacturing machine (sliding head machine, Swiss type). (c) Part manufacturing and selection of cleaning process parameters (d) Cleaning of products using an ultrasonic machine as per selected parameters (e) Process parameter optimization; (f) Bioburden Testing of products (g) Suggested the best combination of parameters and results.

Selection of Material

The material used in the current investigation was 316LRM stainless steel in solid rod form, having a thread head of 4.55 mm, head dia. 9.00mm and a length of 133mm. At present, the 316LRM SS biocompatible material is used in many applications and industries, such as medical implants, surgical tools, textile tubing, pharmaceutical equipment, domestic jewelry, rapid prototyping, etc. The reason for its selection can be found in its reasonable cost, ease of fabrication, resistance to corrosion and fatigue, as well as malleability. An iron-based alloy known as stainless steel (SS) has chromium with a minimum amount of 12%, which resists the formation of rust in an unpolluted environment.[17-23] The mechanical properties given as per as per ISO 5832-1 (Table 1) and comparison of element composition as per ISO 5832-1: 2024 and after spectro chemical test analysis of material are given in Table 2 (a, b), respectively.

Table 1. Mechanical Properties of 316LRM SS material

Tensile Strength (N/mm2)		Percentage Elongation (50mm G.L.)	Proof Strength (0.2%)	Hardness (HRC)
Min.	860	12%	690	-
Max.	1100	-	-	-
Actual	941	30%	725	29

Note: Cast analysis: 26.526 (Required 26 min.)

Table 2. Elemental composition percentage comparison (a) 316LRM SS material as per ISO 5832-1: 2024 (cold worked implant) [24] (b) Spectro chemical test analysis of material.

a). 316LRM SS material as per ISO 5832-1: 2019		b). Elemental composition after spectro chemical test analysis of material.
Element Present	Actual composition%	Actual composition%
Carbon (C)	0.030 max.	0.011
Silicon (Si)	1.0 max.	0.340
Manganese (Mn)	2.0 max.	1.480
Phosphorus (P)	0.025 max.	0.0200
Sulfur (S)	0.010 max.	0.0030
Nitrogen (N)	0.10 max.	0.0715
Chromium (Cr)	17-19 max.	17.880
Molybdenum (Mo7	2.25-3.0 max.	2.620
Nickel (Ni)	13-15 max.	13.140
Copper (Cu)	0.5 max.	0.050
Iron (Fe)	Bal.	Bal.

Selection of manufacturing machine

The advance technological sliding head machine (Swiss type) make in japan was used to fabricate cannulated screw, having dimension 133 mm as per design specification.

There are many different models and brands machines available in the market. It is highly important to compare different models and brands to find the one that best suits by considering needs. Some popular brands of Swiss machines include Citizen, Star, etc. In the current investigation the sliding head machine (Swiss Type) were considered because of machines offer superior accuracy and repeatability, less cost, less skilled operator is required, ability to produce complex geometries, take less time to manufacture the implant and manufacture small parts with tight tolerances, which is prime important for orthopedic implant manufacturing application.

Part manufacturing operations & selection of cleaning process parameters

The different operation was performed during the manufacturing of bone screws such as turning, drilling, threading, head & size making, de-burring, rounding, punching etc. In addition, stages to manufacture the screws in departmental wise up to the end of cleaning stage as shown in Fig. 3.

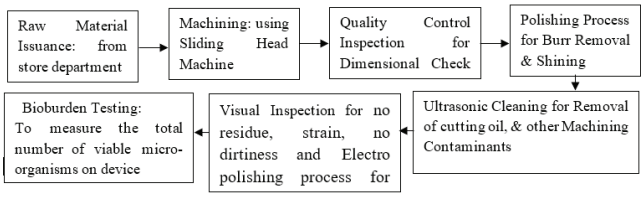

Fig. 3. Screw manufacturing stages

The actual images of manufactured samples before ultrasonic cleaning are shown in Figure 4 (a).

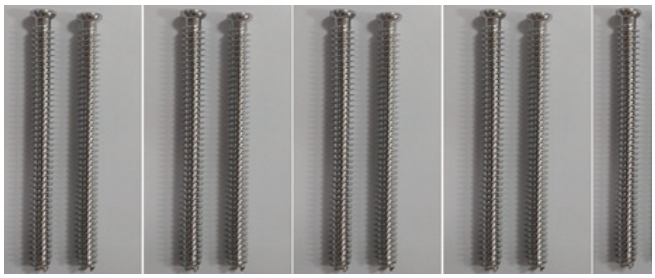

Fig.4 (a). Actual images of manufactured cannulated screws before ultrasonic cleaning

The bone screws (fully cannulated & threaded type) were considered as a worst case in the current investigation because of critical from the point of the cleaning. Such as cannulation in throughout the length, complete threads, larger length, and higher product sale without any complaint. Apart from this the visual inspection (for no residue, strain, no dirtiness and shining) were performed 100 percent on all the implant. However, for dimensional inspection of parts (During first part approval 100 percent dimensional check & at the time of in process check (manufacturing stage) follow the sampling plan (ISO 2859-1 General inspection level I) i.e. two samples out of 09 are dimensionally check. pThus, as per design specification results after measurement found to be within tolerance limit. For sample selection, the 3 samples of 3 consecutive batches of cannulated screw are considered for cleaning and 100% visual inspection is carried out on all the 09 samples. In addition, cleaning process parameters of variable types (i.e. ultrasonic frequency, ultrasonic temperature dipping time, rinsing time & drying time) etc. were selected based upon extensive literature survey.

Cleaning of products using ultrasonic machine as per selected parameters

Ultrasonic cleaners work on the principle of cavitation, which means the formation of bubbles in a liquid. Ultrasonic transducers create multiple bubbles in the ultrasonic solution, and these bubbles exhibit upthrust (upward force) when they come into contact with the device and cleaning action takes place. The use of validated proper cleaning process parameters and procedures (ultrasonic frequency, cleaning agent heating temperature, dipping time, DM water, chemical concentration, etc.) plays an important role in the proper cleaning of medical devices. For example, proper direction of hanging of product either horizontally or vertically by considering the formation of ultrasonic frequency (direction and speed of ultrasonic bubble formation) as well as shape of product, product quantity that are used during cleaning, condition of material (less or more dirty), hanger material or tray design, use of DM water quality, method of rinsing after ultrasonic cleaning, drying methods of products, application of powder-free gloves during handling or packaging, etc. By considering these factors above all, we can surely improve patient safety by reducing the risk of various types of harm, such as infection, etc.

In the current investigation, the cannulated screws were hanged vertically as well as by considering the cannulation. Screws are separated approx. 5mm from one another to avoid placing one screw over another. 09 samples were used during cleaning (quantity can increase by considering the tank and tray size), 09 optimum combinations of parameters during cleaning, SS/Ti hanger or tray material, used overflow DM water (deionized water) during rinsing and preparing a blend for ultrasonic cleaning, used recirculating hot air for drying (hot air oven) after rinsing through hot DM water, and used powder-free gloves (nitric gloves) during handling/packaging, etc. Hence, every manufacturer must consider all the above factors to improve patient safety and reduce various types of cleaning harm, such as infection, etc. The actual images of bone screws after ultrasonic cleaning are shown in Figure 4 (b).

Fig.4 (b). Actual images of cannulated screws after ultrasonic cleaning

In this investigation the demineralized water (DM) or deionized water (impurity free) test was also completed as per IS 1070:1992 standard, shown in Table 3 (a).

Table 3 (a). Demineralized water test

S. No.	Parameters	Test Method	Results	Requirement Limit
1	Ph value at 25°C	IS: 3025 (Part 11) 1983	6.5	-
2	Specific conductivity at 25°C	IS: 3025 (Part 14) 1984	0.1µmhos/cm	0.1 max.
3	Solid Dissolved (TDS)	IS: 3025 (Part 16) 1984	0.02 PPM	500 max.

The different cleaning regents can be employed for the cleaning of medical, surgical, and surgical instruments, such as, TU Caustic, TU Ultra Clean, 3M Rapid Enzyme Cleaner, etc. These cleaning agents not only remove the chips and cutting oil dirt that are left after machining but also remove the polishing and buffing compounds. In addition, different materials, such as metals (Stainless Steel and Titanium) and polymers (Cages, Ceramics, and Glass), can be cleaned using the mentioned chemical regents. The selection of these chemical residues depends on the quantity of contaminants present and their field of applications. In this investigation, based upon the quantity of contaminants, the cleaning regents' blend (i.e., TU Caustic + TU Ultra Clean solution) along with DM water was used for the cleaning of the 316LRM stainless steel bone screws. In addition, the different testing methods as per ISO 19227:2018(E) can be used to examine the contaminants (that are chemical, biological, or physical substances on the device surface that can impair the performance or safety of the medical device) on the device surface using the bioburden test, organic (total organic carbon, total hydrocarbon), and inorganic contaminants tests (ion chromatography) as per ISO 19227:2018, the Bacterial Endotoxins Test (BET) as per United States Pharmacopeia (USP) Chapter 85, 161, etc. Fig. 5. below represents the actual image of

(a) Ultrasonic chemical regent tank (b) Hot water rinsing and (C) foil testing after cleaning of implants.

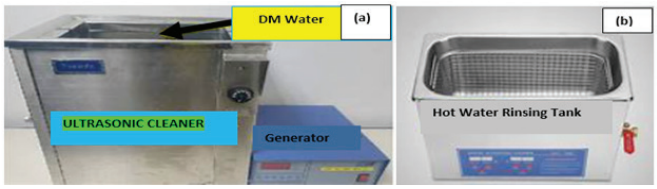

Figure 5: Ultrasonic cleaning machine (a) Ultrasonic chemical regent tank (b) Hot water rinsing tank

C) Foil testing after the hot water rinsing of implants. D) Cleaning Solution (3M Multi Enzymatic Cleaner). E) Cleaning Solution tube

The few examples of different testing for cleaning of orthopedic implant materials and their standard are given in Table 3 (b).

Table 3 (b). Represent the different cleaning testing of orthopedic implant materials and their standard

Test Name	Purpose	Standard/Testing Methods
Bioburden test	To check the viable microorganism on the surface of implant.	ISO 11737-1
Total organic carbon (TOC)	Examine the water-soluble orgpanic residue	UPS 643, ISO 19227 For extraction ISO 19227:2018 & recommended limit after testing i.e. 0.5mg/implant max.
Total hydro carbon (THC)	Examine the non-polar organic residue (i.e. oil, greases etc.)	ASTM D7066, ISO 19227 & recommended limit after testing i.e. 0.5mg/implant max.
Ion chromatography	Determination of fluoride, bromite, chloride, nitrite, sulfate, & phosphate.	ISO 10304
Bacterial Endotoxin Test (BET)	Test to detect or quantify bacterial endotoxins of gram-negative bacterial origin using an amoebocyte lysate prepared from blood corpuscle extracts of horseshoe crab.	As per United States pharmacopeia (USP) Chapter 85.,161

Ultrasonic cleaning efficiency and cavitation performance were visually verified using an aluminum foil erosion test in accordance with standard practice. Aluminum foil samples placed in the bath (Figure 5c) exhibited uniform pitting and perforation, confirming consistent ultrasonic intensity across the cleaning tank. Furthermore, thorough surface cleaning serves as an essential pre-treatment step for biomedical implants prior to subsequent surface modifications or biocompatible coatings.[27]

Process parameters optimization

Design of cleaning process comprises; selection of appropriate tank size & shape, selection of appropriate size tray, selection of transducer, selection of cleaning water, selection of chemicals, selection of medical devices, cleaning, testing & selection of optimize process parameters. The following below mentioned parameters were determined using mini tab software. The 09 samples were cleaned using nine different combinations in an Ultrasonic cleaning machine as given in Table 4.

Table 4. Optimum combination of parameters utilized during the cleaning of 316LRM stainless steel samples

Sample No.	Ultrasonic Frequency (40 KHz)	Chemical temperature (°C)	Cleaning Time (minutes)
1	25	40	10
2	25	50	20
3	25	60	30
4	40	40	20
5	40	50	30
6	40	60	10
7	68	40	30
8	68	50	10
9	68	60	20

Microbial testing or biological evaluation of samples

Bioburden Testing

Routine bioburden testing is an important component of contamination control in medical device manufacturing. Bioburden represents the population of viable microorganisms present on a product prior to sterilization and may include aerobic bacteria, anaerobic microorganisms, bacterial spores, yeasts, and Molds. The level of bioburden is influenced primarily by the manufacturing environment, material handling, processing conditions, cleaning practices, and personnel activities. Effective control of bioburden is therefore essential for maintaining consistent product cleanliness and supporting the effectiveness of subsequent sterilization processes.

In the present investigation, bioburden testing was performed in accordance with ISO 11737-1 following the in-process ultrasonic cleaning of the orthopedic bone screws. The membrane filtration method was employed to recover and enumerate viable microorganisms from the test samples. Following filtration, the membrane was transferred to an appropriate culture medium and incubated under specified conditions for the detection of bacterial and fungal growth. The resulting colonies were enumerated and reported as CFU/device. Appropriate aseptic precautions were maintained throughout sample handling and testing to minimize the possibility of external contamination and false-positive results.

As part of the cleaning process, the screw surfaces were wiped using lint-free cloths to remove residual coolant, oil, and chemical

residues. Particular attention was given to critical and difficult-to-clean areas, including cannulated regions, holes, and screw insertion features, using lint-free swabs. A 5× magnifying lens was used for visual inspection of the surfaces for visible particles, residues, and other contaminants prior to bioburden evaluation.

Design of experiment for cleaning parameters selection

The cleaning of implant is mainly affected by the processing variables used during implant cleaning. Hence, in the present study, 3 parameters (Ultrasonic Frequency (40 KHz), Chemical temperature (°C) and Cleaning Time (minutes) were used to analyze their impact on the bioburden characteristics of test implants. The distinct cleaning process parameters and their levels used are shown in Table 5.

Table 5: Process variables and their level

Parameters	Notation	Level 1	Level 2	Level 3
Ultrasonic Frequency (KHz)	A	25	40	68
Chemical temperature (°C)	B	40	50	60
Cleaning Time (minutes)	C	10	20	30

The above parameters and their level were selected based on the previous studies of researchers.[28] Further, the "Taguchi's orthogonal array of fractional factorial design" (L9) with Minitab software was used to set the experiments as given in Table 6.

Table 6: Taguchi orthogonal array L9

Sample No.	Ultrasonic Frequency (KHz)	Chemical temperature (°C)	Cleaning Time (minutes)
1	25	40	10
2	25	50	20
3	25	60	30
4	40	40	20
5	40	50	30
6	40	60	10
7	68	40	30
8	68	50	10
9	68	60	20

Results and discussion

Optimization of Cleaning Process Parameters

The bioburden of the manufactured samples after cleaning must have a smaller value; therefore the 'smaller is the better' equation of the S/N ratio is utilized. The results of bioburden examination and S/N ratio are given in Table 7.

Table 7: Results of bioburden test

Run	Ultrasonic Frequency (KHz)	Chemical temperature (°C)	Cleaning Time (minutes)	Bioburden (cfu/implant)	SNRA1	MEAN1
1	25	40	10	15	-23.5218	15
2	25	50	20	14	-22.9226	14
3	25	60	30	11	-20.8279	11
4	40	40	20	16	-24.0824	16
5	40	50	30	17	-24.6090	17
6	40	60	10	18	-25.1055	18
7	68	40	30	14	-22.9226	14
8	68	50	10	16	-24.0824	16
9	68	60	20	18	-25.1055	18

The maximum value of bioburden of 18cfu/device was obtained with run 6th & 9th, while the minimum value of bioburden was 11cfu/device obtained with the 3rd run. The bioburden level of the test samples is in Figure 6.

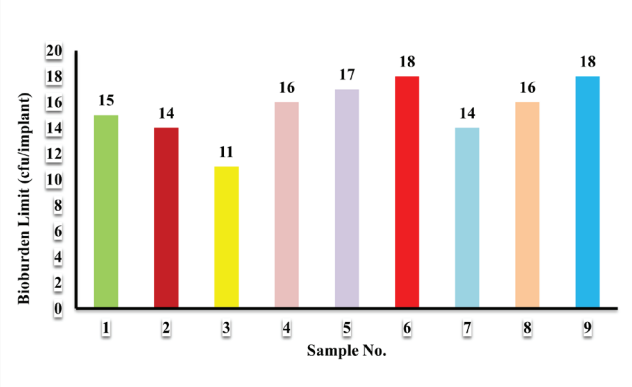

Fig. 6. Bioburden limit of test samples

In addition, the response table for SN Ratio and response table for the mean are shown in Table 8 and Table 9 respectively.

Table 8: Response table for the signal-to-noise ratio Smaller is better

Level	Ultrasonic Frequency (KHz)	Chemical temperature (°C)	Cleaning Time (minutes)
1	-22.42	-23.51	-24.24
2	-24.60	-23.87	-24.04
3	-24.04	-23.68	-22.79
Delta	2.17	0.36	1.45
Rank	1	3	2

Table 9: Response table for mean

Level	Ultrasonic Frequency (KHz)	Chemical temperature (°C)	Cleaning Time (minutes)
1	13.33	15.00	16.33
2	17.00	15.67	16.00
3	16.00	15.67	14.00
Delta	3.67	0.67	2.33
Rank	1	3	2

However, the optimum parameters are determined by selecting the minimum S/N ratio for each factor, which is selected to minimize the bioburden limit.

In addition, the main effect plot for the mean and SN ratio is shown in Figure 7 and Figure 8 respectively.

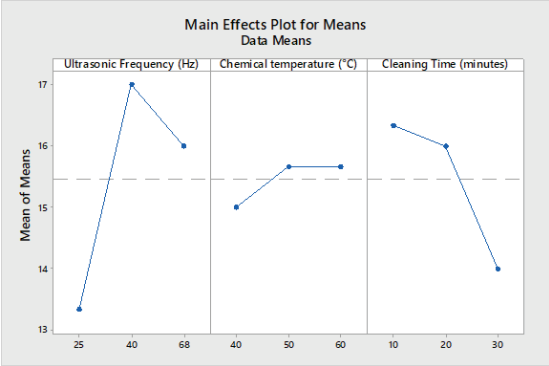

Figure 7. Main effects plot for mean using Minitab software for ultrasonic frequency, chemical regent temperature and cleaning time (dipping time in tank).

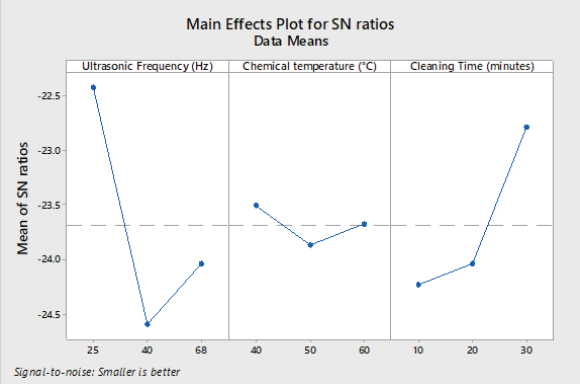

Figure 8. Main effects plot for SN ratio using Minitab software for ultrasonic frequency, chemical regent temperature and cleaning time (dipping time in tank).

Thus, based on the graph the optimum set of parameters are A2B2C1.

Where the optimum set of the parameters A2B2C1" mean- The English alphabet A2 denotes the ultrasonic frequency when the optimum value is 40KHz, B2 denote the chemical temperature, when the optimum value is 50°C & C1 represent the cleaning time in chemical regents, when the optimum value is 10 minutes.

The SN ration, mean and p value tables and graph as shown below-

Ultrasonic Frequency (KHz)	Chemical temperature (°C)	Cleaning Time (minutes)	Bioburden (Cfu/Implant)	SNRA1	MEAN1
25	40	10	15	-23.5218	15
25	50	20	14	-22.9226	14
25	60	30	11	-20.8279	11
40	40	20	16	-24.0824	16
40	50	30	17	-24.6090	17
40	60	10	18	-25.1055	18
68	40	30	14	-22.9226	14
68	50	10	16	-24.0824	16
68	60	20	18	-25.1055	18

The General Linear Model: SNRA1 versus Ultrasonic Frequency, Chemical temperature & Cleaning Time
Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Ultrasonic Frequency (KHz)	Fixed	3	25, 40, 68
Chemical temperature (°C)	Fixed	3	40, 50, 60
Cleaning Time (minutes)	Fixed	3	10, 20, 30

Further, the ANOVA analysis of the SN ratio of bioburden limit is performed as summarized in Table 10.

Table 10: Analysis of Variance for SN ratio of bioburden test

Source	DF	Adj SS	Adj MS	F-Value	P-Value	% contribution
Ultrasonic Frequency (KHz)	2	7.6469	3.82345	2.54	0.282	52.528937
Chemical temperature (°C)	2	0.1972	0.09860	0.07	0.938	1.3546
Cleaning Time (minutes)	2	3.7060	1.85301	1.23	0.448	25.4576
Error	2	3.0073	1.50366			20.6580
Total	8	14.5575				100

The ANOVA results presented in Table 10 indicate that none of the investigated process parameters had a statistically significant effect on the SN ratio at the 5% significance level ($P > 0.05$). Among the evaluated parameters, Ultrasonic Frequency exhibited the highest percentage contribution (52.53%), followed by Cleaning Time (25.46%) and Chemical Temperature (1.35%). Although Ultrasonic Frequency and Cleaning Time showed relatively higher contributions to the observed variation, their effects were not statistically significant at the 95% confidence level. The optimum combination of process parameters was selected based on the SN ratio response and is presented in Table 11.

Table 11: Combination of Cleaning Process Parameters Process parameters

Parameter Notation	Optimum level	Selected value
Ultrasonic Frequency (KHz)	1	40
Chemical temperature (°C)	2	50
Cleaning Time (minutes)	1	10

*Note: For % contribution of each factor divides the Adj SS value/Total Adj SS X 100

Conclusions

In the current investigation, the bioburden testing after the ultrasonic cleaning process on 316LRM stainless steel implant material manufactured by the advanced machining method was examined experimentally using variable and fixed process parameters. Based on the experimental results, the following

conclusions were drawn:

1. ANOVA results indicate that ultrasonic frequency and cleaning time accounted for the largest percentage contribution to bioburden variation, contributing 52.53% and 25.46% respectively, while chemical temperature contributed 1.35%.
2. The Taguchi S/N ratio analysis identified A2B2C1 (40 kHz frequency, 50°C chemical temperature, and 10 minutes cleaning time) as the overall optimal parametric combination, achieving a reduced bioburden level of 11 cfu/device (matching the lowest individual experimental run of 11 cfu/device obtained in run 3).
3. The microbial limits after bioburden testing on all the bone screws were found satisfactory (i.e., within the acceptable limit of 0–50 colony-forming units per gram (or milliliter) as per United States Pharmacopeia (USP) chapter 1111), and they also showed a shining surface after cleaning.

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