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Paper Title: Exploration of biocompatibility and antibacterial
properties of silicon nitride ceramic oral restoration materials
based on a simple in vitro model

Exploration of biocompatibility and antibacterial properties of silicon nitride ceramic oral restoration materials based on a simple in vitro model

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Abstract: This project aims to explore the application potential of silicon nitride ceramics in response to the current issues of insufficient antibacterial properties and the need to improve biocompatibility of dental implant materials. The study systematically evaluated the antibacterial and biocompatibility properties of the material through in vitro experiments, using methods such as "Colony Wars" visualization comparison, "Acid-base Turbulence" pH monitoring, and "Seed Germination" vitality testing, and compared it with traditional Ti-alloys. The results showed that silicon nitride ceramics can effectively inhibit microbial growth and acid metabolism, and their extract has no negative effect on seed germination, demonstrating good biocompatibility. The conclusion is that silicon nitride ceramics have excellent antibacterial properties and biocompatibility, and have important development prospects in the field of dental implant restoration, providing experimental evidence for the development of high-performance dental implants.

Keywords: Silicon Nitride Ceramics; Biocompatibility; Antibacterial activity; Oral Environment

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1. Introduction

With the acceleration of population aging and the increasing demand for oral health, the performance requirements of oral restoration materials are constantly improving. Although traditional restorative materials such as Ti-alloys and Zirconia have been widely used, there are still issues with biocompatibility limitations and secondary caries caused by microbial attachment ^[1-3]. In recent years, silicon nitride ceramics have gradually become a research hotspot in the field of oral restoration due to their excellent mechanical properties, chemical stability, and potential biological activity.

However, as a new type of biomaterial with good biocompatibility and antibacterial potential, the long-term biocompatibility and antibacterial performance of silicon nitride ceramics in complex oral environments still need to be systematically evaluated, and traditional testing and experimental methods are costly and time-consuming ^[4,5]. Traditional testing includes in vitro cell/bacterial culture, animal implantation, and clinical trials. Its high cost lies in the large investment in animal procurement, surgical consumables, testing equipment, and manpower; If the cycle is long, animal experiments require several months of observation, complex hard tissue processing, and clinical trials require several years of follow-up, resulting in slow overall R&D iteration. So there is an urgent need for a low-cost, fast, and intuitive reliable testing and experimental

method to meet the large demand for biocompatibility and antibacterial testing verification of new materials, in order to develop new core performance excellent oral restoration materials faster and significantly improve patients' oral medical experience.

Therefore, this study will focus on investigating the biocompatibility and antibacterial properties of silicon nitride ceramics through a series of in vitro experiments. The aim is to explore the performance of this material in simulated oral environments through low-cost, rapid, and intuitive experimental methods, providing experimental basis and theoretical reference for the development of dental implants with greater application potential.

2. Materials and methods

The core objective of this project is to establish a low-cost, fast, and intuitive effective in vitro evaluation system to comprehensively evaluate the core performance of silicon nitride ceramics as dental implant materials. Therefore, this study designed three interrelated and progressive experiments, as shown in Fig. 1, to investigate the antibacterial performance, metabolic effects, and biocompatibility from three dimensions, with Ti-alloys widely used in clinical practice as the control benchmark.

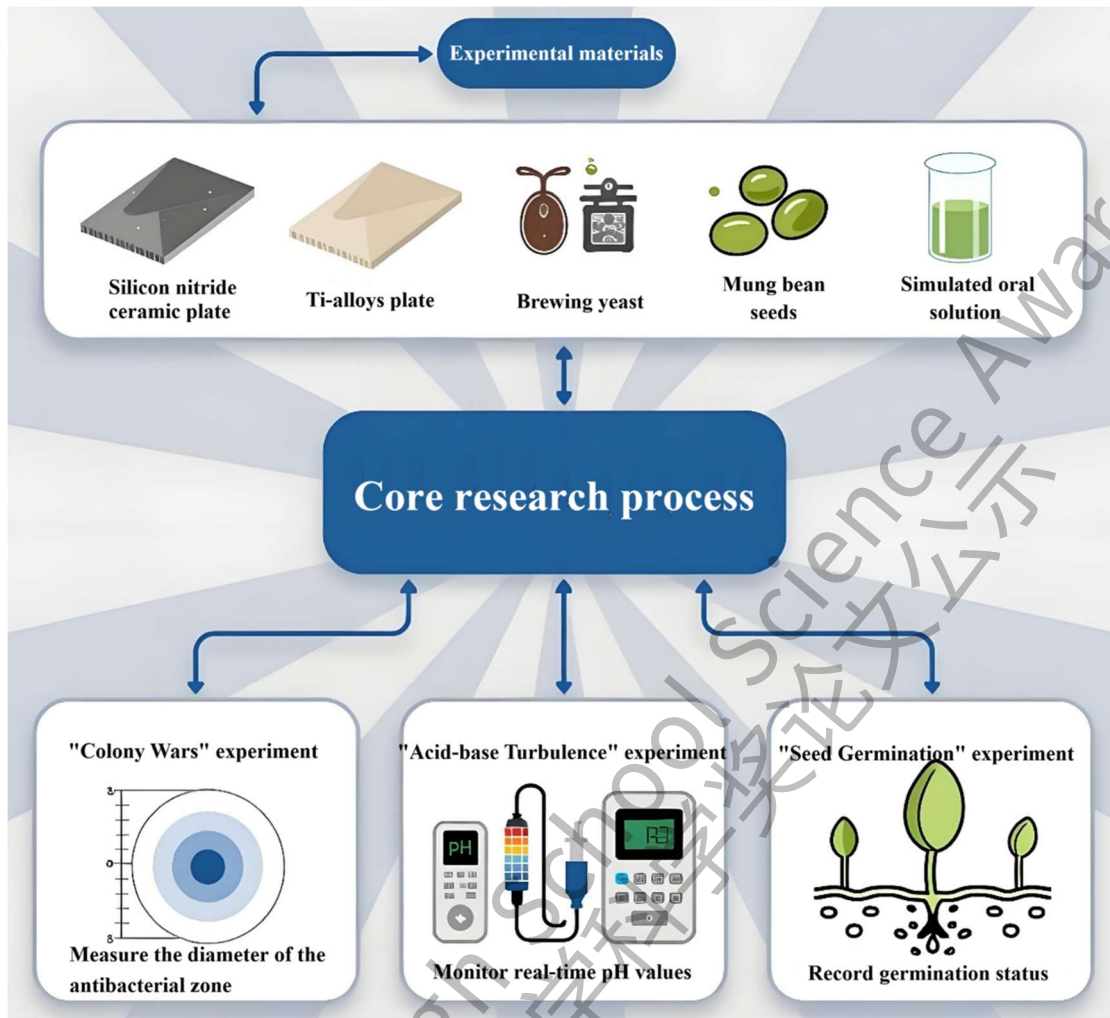


Fig. 1 The experimental materials and core research process of this project

2.1 Materials and simulation environment

Experimental materials:

Core testing material: Silicon nitride ceramic plate (size: 30mm × 30mm × 3mm, Hengyang Kaixin Special Materials Technology Co., Ltd.), surface polished to $Ra \approx 0.6 \mu m$ to simulate implant surface.

Reference material: Medical grade Ti-alloys plate (Ti-6Al-4V, same size as silicon nitride ceramic plate, Baoji Yingnaite Medical Titanium Co., Ltd.). The selection of Ti-alloys as a control is based on its status as the

current "gold standard" material for dental implants, which gives the research results clear clinical reference value.

Biological model materials: brewing yeast, mung bean seeds. The reason for choosing brewing yeast is that as a single-cell eukaryotic model organism, it has a short growth cycle, is easy to observe, and is often used for preliminary antibacterial screening experiments; The reason for choosing mung bean seeds is that their germination process is sensitive to environmental toxicity and is a classic plant model for initial screening of biocompatibility.

Chemical reagents: agar powder, sucrose, yeast extract powder, phosphate buffer solution, etc.

Construction of simulated oral environment: In order to simulate real oral conditions as closely as possible, this study prepared a simulated oral solution. This solution is based on phosphate buffer solution, with the addition of 5% sucrose (simulating residual sugar in the oral cavity, providing substrate for microbial metabolism) and 0.5% yeast extract (providing nitrogen source and other nutrients). All experiments were conducted in a constant temperature incubator at 37 °C to simulate human oral temperature.

2.2 Experimental methods

Experiment 1: "Colony Wars" - Visual Evaluation of Material Surface Antibacterial Ability

Experimental objective: To qualitatively and semi quantitatively compare the inhibitory ability of silicon nitride ceramics and Ti-alloys surfaces on microbial growth. By observing the formation of antibacterial zones, the contact antibacterial or dissolution antibacterial effects of the materials can be preliminarily determined.

Experimental steps:

A. Preparation of "Microbial Battlefield": Weigh a certain amount of agar powder and sucrose, dissolve them in a simulated oral solution, and heat to completely melt them. After cooling to about 45 °C (not hot but not solidified), add an appropriate amount of brewing yeast suspension, mix thoroughly, pour into a sterile culture dish with a thickness of about 3-4mm, and let it stand to solidify.

B. Placement of materials: On a solidified agar plate, use sterile tweezers to gently place the pre sterilized silicon nitride ceramic plate and Ti-alloys plate in the center of the plate. Ensure that the material is in close contact with the agar surface.

C. Cultivation and observation: After sealing the culture dish, place it in a 37 °C constant temperature incubator for cultivation. Observe and record at 24 and 48 hours, and place it on an orange cardboard for

observation at 48 hours.

D. Data collection: At the preset time point, remove the culture dish, observe and take photos. The main observation indicators are whether there is a transparent "bacteriostatic zone" (i.e. an area without yeast growth) around the material and its diameter size. Measure the diameter of the antibacterial zone using a vernier caliper, repeat three times for each sample, and take the average value.

Experiment 2: "Acid-base Turbulence" - The Effect of Materials on Microbial Metabolism and Acid Production

Experimental objective: To investigate whether silicon nitride ceramics affect the sugar metabolism process of microorganisms, especially by inhibiting their acid production ability. Oral bacterial acid production is the main cause of peri implantitis and caries, and this experiment aims to evaluate the material's ability to prevent this critical pathological process.

Experimental steps:

A. Grouping and inoculation: Prepare several sterile test tubes and divide them into three groups: ① Experimental group: Add 50mL of simulated oral solution and silicon nitride ceramic plate; ② Control group: Add 50mL simulated oral solution and Ti-alloys plate; ③ Blank control group: Only 50mL of simulated oral solution was added. Add an equal

amount of brewing yeast suspension to all test tubes.

B. Constant temperature oscillation cultivation: Place all test tubes in a 37 °C constant temperature shaker and oscillate at a speed of 150 rpm to ensure uniform and sufficient gas exchange of the solution.

C. pH monitoring: Measure the pH values of the solutions in each test tube using a calibrated precision pH meter at 0h, 6h, 12h, 24h, and 48h time points after the start of cultivation. Before each measurement, thoroughly clean the pH electrode with deionized water and dry it with filter paper.

D. Data recording and analysis: Record in detail the pH value measured each time, and use plotting software to draw the curve of pH variation over time. Analyze the inhibitory effect of different materials on microbial acid production process by comparing the decreasing trend and final pH value of three sets of curves.

Experiment 3: "Seed Germination" - Preliminary Simulation of Material Biocompatibility

Experimental objective: To simulate the potential effects of silicon nitride ceramic leaching solution on the growth of "biological tissue" cells and preliminarily evaluate its biological safety by utilizing the highly sensitive biological process of plant seed germination to toxic substances.

Experimental steps:

A. Preparation of material extraction solution: According to the international standard (ISO 10993-5), immerse silicon nitride ceramic plates and Ti-alloys plates in sterile deionized water (with a surface area to liquid volume ratio of 3 cm²/mL), and extract at 37 °C for 72 hours to obtain silicon nitride extraction solution and Ti-alloys extraction solution, respectively. Set up another set of pure deionized water as a negative control.

B. Seed sowing and cultivation: Select full and uniformly sized mung bean seeds, disinfect the surface with 1% sodium hypochlorite solution for 10 minutes, and then rinse with deionized water. Place 20 mung bean seeds in three sterile culture dishes, add equal amounts of the above three liquids (extraction solution or water), soak 1/3 of the height of the mung bean seeds, replenish the liquid every 12 hours, and cover with a lid.

C. Observation and recording: Place the culture dish in a 25 °C light incubator and observe and record the germination of the seeds daily. Observe continuously for 2 days, and make observation records at 12 hours, 24 hours, and 48 hours.

D. Indicator measurement: After the experiment is completed, calculate the final germination rate. At the same time, use a ruler to measure the root length and sprout length of the germinated seeds, and take the

average value. All indicators were subjected to three independent repeated experiments.

3. Results and discussion

Through the experimental design and method described above, the results clearly reveal the performance characteristics of silicon nitride ceramics:

3.1 Results of the "Colony Wars" experiment

The "Colony Wars" experiment drew inspiration from the classic Oxford Cup method principle. If the surface of the material or its dissolved components have antibacterial properties, they will diffuse into the surrounding agar, inhibit the growth of microorganisms in the contact area, and thus form an antibacterial zone. The size of the antibacterial zone is positively correlated with the strength of the material's antibacterial ability.

The comparison of experimental photos of "Colony Wars" is shown in Fig. 2. After 24 hours of cultivation, significant differences can be observed. The yeast growth on the agar plate around the Ti-alloys plate is dense, no different from other areas of the plate, and no antibacterial zone is observed. On the contrary, a clear and visible transparent antibacterial zone appeared around the silicon nitride ceramic plate. After 48 hours, this difference became more significant. The yeast around the Ti-alloys plate was highly dense, showing a darker light yellow color, while there were

still clear areas around the silicon nitride ceramic plate, forming a continuous antibacterial zone that was more prominent against an orange background. The measurement data shows that the average diameter of the antibacterial zone in the silicon nitride ceramic group is 43.8 ± 2.3 mm, while the diameter of the antibacterial zone in the Ti-alloys group is zero.

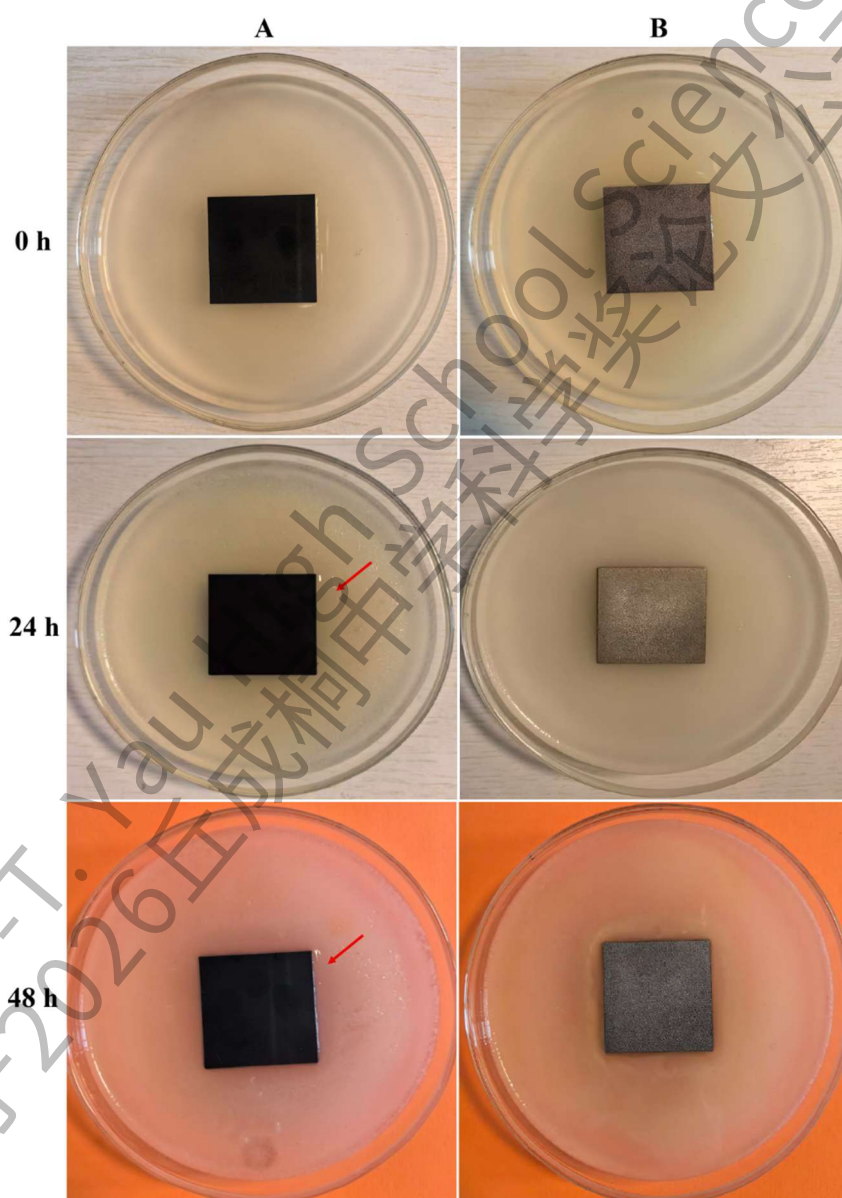


Fig. 2 Photos of the "Colony Wars" experiment. A: Silicon nitride ceramic group; B: Ti-alloys group. The red arrow points to the location of the antibacterial zone.

This result intuitively proves that the surface or dissolved components of silicon nitride ceramics have a significant inhibitory effect on the growth of brewing yeast, while Ti-alloys did not show this effect under the conditions of this experiment.

3.2 Results of the "Acid-base Turbulence" experiment

Microorganisms produce acidic substances (such as carbonic acid and organic acids produced by yeast) when using sucrose for metabolism, especially when simulating oral solutions containing organic substances such as yeast extract powder. Microbial metabolism is active, leading to a decrease in environmental pH. If the material can inhibit microbial growth or directly neutralize acidic substances, the pH value of its group will decrease significantly less than that of the blank control group and Ti-alloys group. This can indirectly but effectively reflect the ability of materials to maintain microenvironmental stability in dynamic environments.

The comparison of the pH monitoring experimental results of the "Acid-base Turbulence" is shown in the curve in [Fig. 3](#). The pH monitoring curve clearly shows the dynamic changes of the three groups of samples within 48 hours. The pH value of the blank control group solution rapidly decreased from the initial value of about 7.0, to around 5.5 at 24 hours, and to around 5.1 at 48 hours, indicating that the brewing yeast was

metabolically active in acid production under the action of sucrose and yeast extract powder in simulated oral solution. The pH change curve of the Ti-alloys group almost overlaps with the blank control group, and there is no significant difference in the downward trend and amplitude, indicating that Ti-alloys has no significant effect on microbial acid metabolism. The pH value of the silicon nitride ceramic group decreased significantly, with a pH value still above 6.2 at 24 hours and a final pH value of 6.0 at 48 hours, significantly higher than the other two groups.

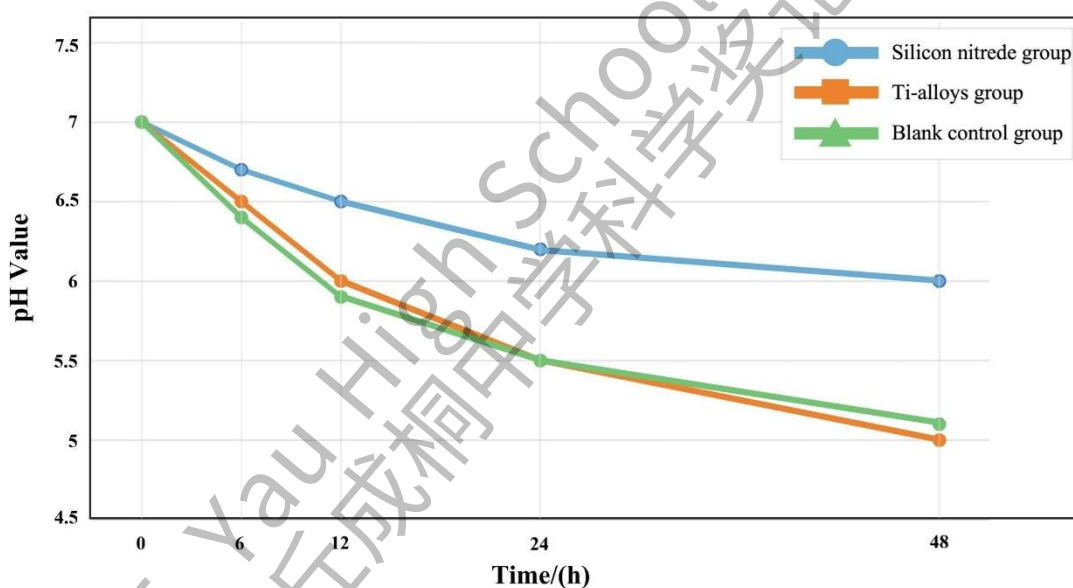


Fig. 3 Comparison of pH Monitoring Experiment Results of "Acid-base Turbulence"

Analysis of Variance (ANOVA) showed significant differences in pH values among the three groups at 24 and 48 h time points ($p < 0.05$), further confirming that the pH value of the silicon nitride ceramic group was significantly higher than that of the Ti-alloys group and the blank control

group.

3.3 Results of the "Seed Germination" experiment

Seed germination and early seedling growth are sensitive indicators for evaluating environmental stress and chemical toxicity. If the material contains biologically toxic components, they will be released into the water during the extraction process, thereby inhibiting seed germination and seedling growth. The data on germination rate, root length, and shoot length can quantitatively reflect the cytotoxicity level of the material, providing important preliminary references for more advanced mammalian cell experiments in the future.

The comparison of the experimental results of the "Seed Germination" vitality test (12 h, 24 h, 48 h) is shown in Fig. 4, and the comparison of the germination rate, average root length, and average bud length of different test groups is shown in Fig. 5.

In the 2-day germination experiment, the germination performance of the three groups of seeds was different at 12 h, 24 h, and 48 h. Overall, the negative control group (deionized water) was similar to the silicon nitride ceramic extraction solution group, but the germination performance of the Ti-alloys extraction solution group was slightly worse. At 48 hours, the germination rate of the negative control group (deionized water) reached 50.0%, and the seedling roots and stems were robust, with an average root

length of 28 mm and an average bud length of 10 mm. The germination rate of the silicon nitride ceramic extraction solution group was the highest, reaching 55.0%, slightly higher than the negative control group, with no significant statistical difference ($p>0.05$). The average root length of its seedlings was 31 mm and the average bud length was 11 mm, which were slightly higher than the negative control group, but the difference was not significant.

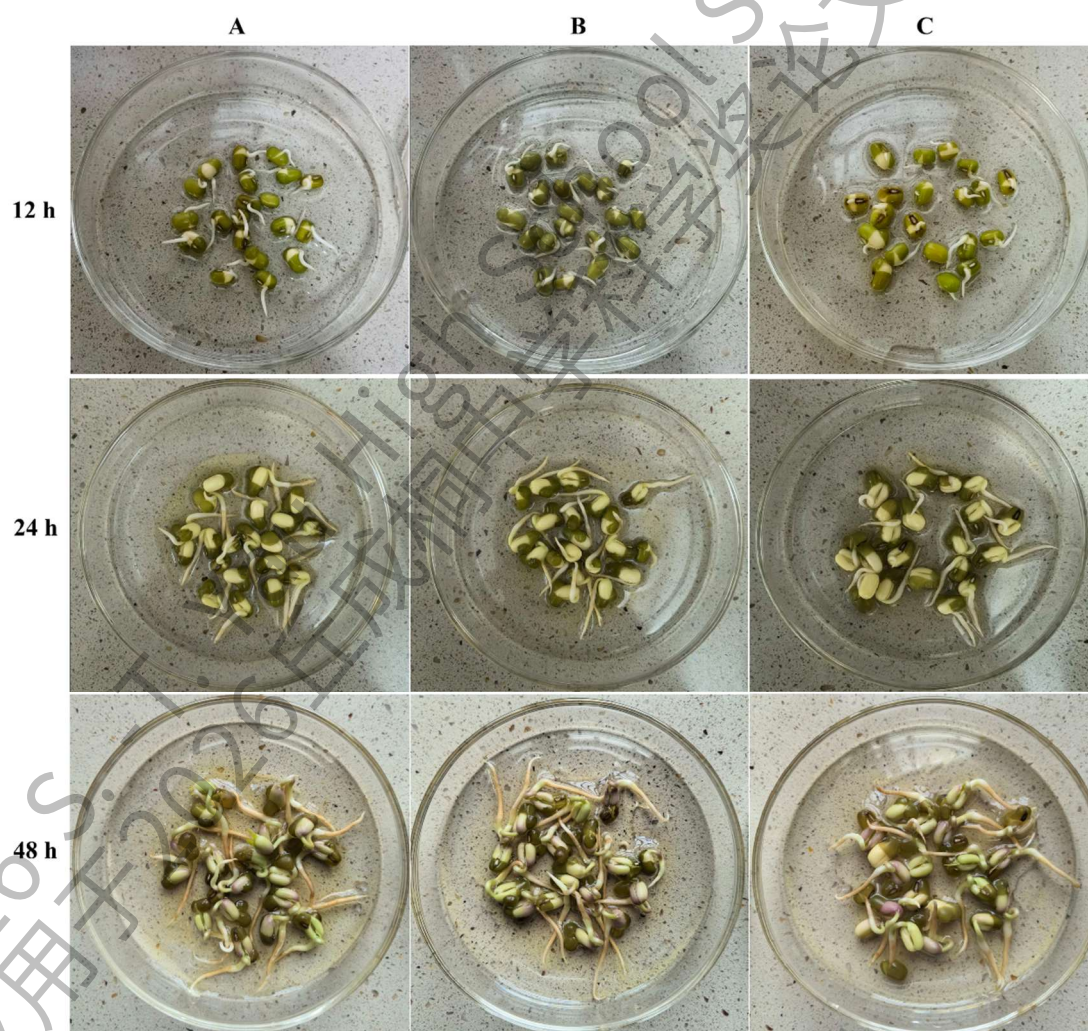


Fig. 4 Comparison of vitality test results for "Seed Germination" (12 h, 24 h, 48 h). A: Negative control group; B: Silicon nitride leaching solution group; C: Ti-alloys leaching solution group

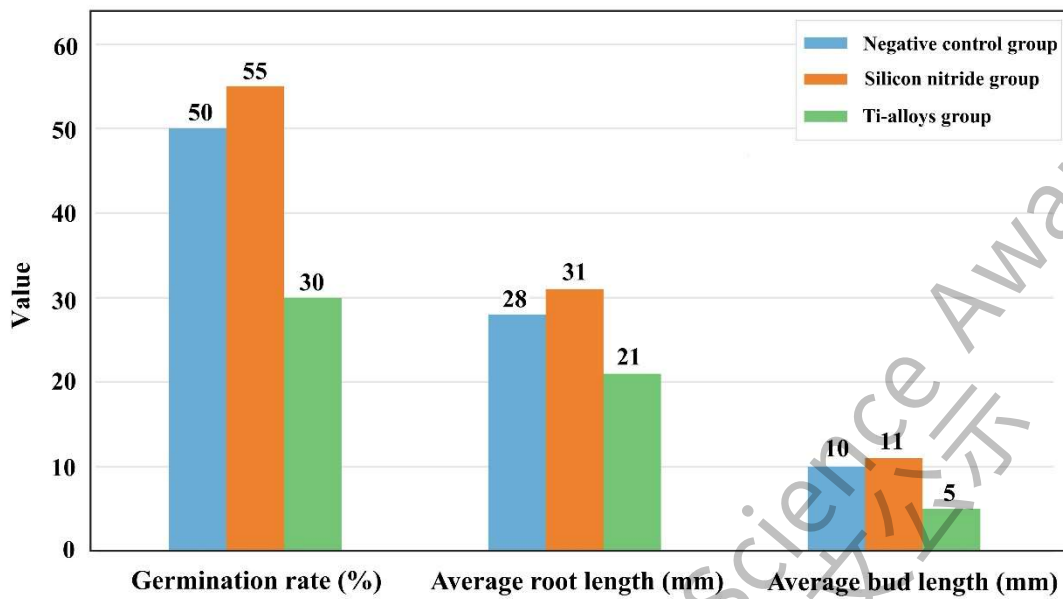


Fig. 5 Comparison of germination rate, average root length, and average bud length among different test groups in the "Seed Germination" vitality test experiment

The germination rate of the Ti-alloys extraction solution group was affected to some extent, only 30.0%, and the growth of its seedlings was also inhibited to a certain extent. The average root length of 21 mm and the average bud length of 5 mm were significantly lower than those of the negative control group and the silicon nitride ceramic group ($p < 0.05$). This result indicates that the leaching solution of silicon nitride ceramics does not have toxic inhibitory effects on the germination and early growth of mung bean seeds, and its biocompatibility is superior to Ti-alloys in this experimental model. The comprehensive results of this study indicate that silicon nitride ceramics exhibit superior potential in terms of antibacterial and biocompatibility compared to traditional Ti-alloys.

3.4 Exploration of antibacterial mechanism of silicon nitride ceramics

The observed antibacterial zone and pH stabilization effect in this study strongly suggest that silicon nitride ceramics have inherent antibacterial properties. The mechanism simulation diagram is shown in Fig. 6, which may originate from the following aspects: firstly, silicon nitride ceramics undergo slow surface hydrolysis in an aqueous environment, releasing trace amounts of silicate ions and ammonium ions. Previous studies have shown that these hydrolysis products can alter the permeability of microbial cell membranes, interfere with their energy metabolism, and thus inhibit their growth [6]. Secondly, the unique microstructure and hydrophobicity of silicon nitride ceramic surfaces may hinder the initial adhesion of microorganisms, reducing the starting point for biofilm formation [7].

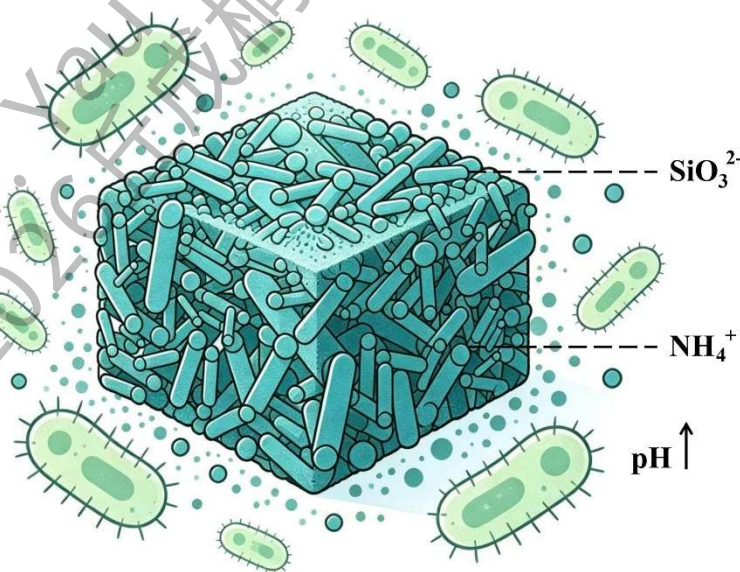


Fig. 6 Simulation diagram of antibacterial mechanism of silicon nitride ceramics

Finally, the slight increase in local microenvironment pH caused by surface hydrolysis (as observed in this experiment) is also detrimental to the growth of many acidophilic oral pathogens, such as *Streptococcus mutans*. In contrast, Ti-alloys, as a biologically inert metal, does not possess this chemical activity on its surface, making it difficult to actively inhibit microorganisms.

3.5 The implications of biocompatibility results and the significance of model selection

The results of the "Seed Germination" experiment showed that the silicon nitride ceramic extraction solution had no cytotoxicity, and even its seedling growth was significantly better than that of the Ti-alloys group, which is an encouraging finding. This provides preliminary evidence of the biocompatibility of silicon nitride ceramics as biomaterials. The growth of seedlings in the Ti-alloys group was slightly inhibited, as shown in [Fig. 7](#), which may be related to the leaching of extremely small amounts of metal ions (such as vanadium ions and aluminum ions). Although it is safe in the human body, differences may still be observed in highly sensitive plant models. In silicon nitride ceramics, both silicon and nitrogen elements exhibit positive biological activity. Silicon element can slowly release silicate ions under the hydration effect on the material surface, effectively promoting the proliferation and differentiation of osteoblasts, accelerating biological growth, and participating in the synthesis of collagen, which is

crucial for maintaining periodontal tissue health. Nitrogen can regulate the acid-base balance of local microenvironments in physiological environments, and its slow-release properties help to inhibit the respiration and metabolism of anaerobic bacteria, thereby endowing materials with natural broad-spectrum antibacterial ability. The synergistic effect of the two not only promotes the firm bonding between implants and biological tissues, but also significantly reduces the risk of oral infections, which is the core advantage of silicon nitride as a new generation of biomaterials. The selection of plant seed models as initial screening in this study is based on ethical, cost, and convenience considerations. It can quickly and effectively screen materials with potential toxicity, providing important decision-making basis for whether more complex and expensive animal or human cell experiments are worth conducting in the future [8].

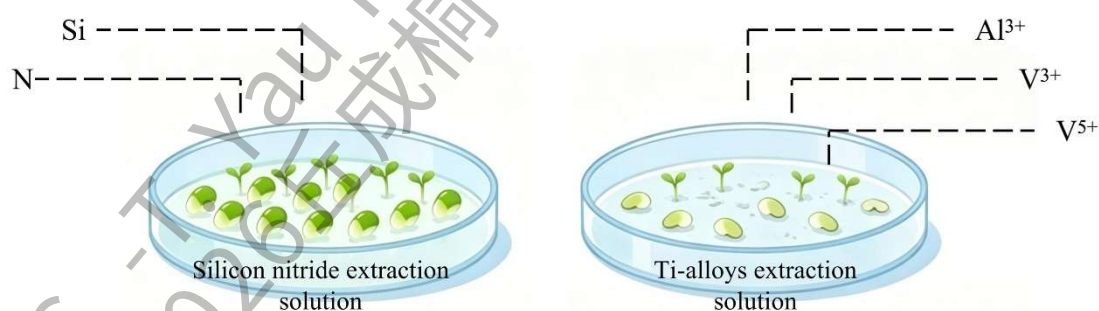


Fig. 7 Comparison of biocompatibility effects between silicon nitride ceramics and Ti-alloys

4. Conclusion

In summary, through a systematic in vitro experiment, this project has obtained strong evidence supporting the advantages of silicon nitride ceramics as dental implant materials. It exhibits significant antibacterial activity and the ability to maintain microenvironmental stability in simulated oral environments, while demonstrating good biocompatibility. These characteristics are of great significance for preventing peri implantitis in dental implants and improving long-term service effectiveness. Although this study has limitations such as model simplification, its results provide valuable preliminary experimental data and support for further in-depth research and clinical translation. In the future, research can move towards using more accurate oral bacterial models, conducting mammalian cell experiments, and surface modification of materials to further enhance their performance.

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Of course, research is not always smooth sailing. The detailed adjustment of experimental steps, fluctuations in data, and how to ensure comparability between the simulation system and the real oral environment have all been the focus of discussion and thinking in this study. Under the free and careful guidance of my advisor — Zhipeng Yang, I gradually

overcame these difficulties through researching and repeatedly verifying. During the paper writing stage, Advisor Yang also provided many constructive suggestions. Under his guidance, I learned how to transform complex scientific problems into actionable experimental plans, understood the importance of setting up control groups, improved my practical skills, and more importantly, cultivated a scientific attitude of daring to assume and carefully verify unknown problems. This experience undoubtedly planted a seed of passion for science and courage to explore in my heart. Thanks to Advisor Yang for his selfless guidance.

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In the process of this study, Yuanbao (version 2.83.10) was used as an intelligent research aid tool, mainly utilizing its professional semantic retrieval and literature organization capabilities to quickly sort out domestic and foreign literature on the biocompatibility and oral

antibacterial properties of silicon nitride ceramics; During the paper writing stage, it is mainly used for language polishing.

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