· Research Article ·

Titanium-copper alloys with nanotubular coatings increase antibacterial abilities and osteoblast functions

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Abstract

BACKGROUND: Implant infection is still a serious adverse event after orthopedic surgery. Copper (Cu) is a currently known metal that has antibacterial properties. Studies have shown that nanostructured metals prepared by nanotechnology can promote the adhesion, proliferation and osseointegration of osteoblasts *in vivo*. *Staphylococcus aureus*, the most common implant-related pathogen in clinical practice, is used to test antibacterial properties of titanium-copper alloy nanotubes.

OBJECTIVE: To observe the effect of antibacterial properties of titanium-copper alloy nanotubes on the function of osteoblasts.

METHODS: Mouse osteoblasts (MC-3T3-E1) were co-cultured with different materials, including pure titanium, titanium dioxide nanotubes, and titanium-copper alloy nanotubes with a copper content of 5%, for 6 and 24 hours. Cell adhesion and proliferation on the scaffold were observed. Antibacterial properties of titanium-copper alloy nanotubes, titanium dioxide nanotubes and pure titanium were compared. Biocompatibility of osteoblasts co-cultured on different material surfaces was detected, and antibacterial properties of different materials to *Staphylococcus aureus* were measured.

RESULTS AND CONCLUSION: (1) Under scanning electron microscope, we observed good cell adhesion onto the surface of titanium dioxide nanotubes and titanium-copper alloy nanotubes, and the adherent cells had good cell morphology and regular arrangement. Cell proliferation of osteoblasts was better in the two nanotube groups than in the pure titanium group, but there was no significant difference between the titanium-copper alloy nanotube and titanium dioxide nanotube groups. (2) Higher bacteria counts were observed in the pure titanium and titanium dioxide nanotube groups than the titanium-copper alloy nanotube group. In conclusion, the titanium-copper alloy nanotubes have good inhibitory effect on bacterial adhesion, and have no influence on the bio-functions of osteoblasts.

Subject headings: Tissue Engineering; Titanium; Staphylococcus aureus; Osteoblasts

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INTRODUCTION

In recent years, the number of orthopedic patients needing implants continuous to rise. The key success of implantation within humans depends on the biocompatibility and antibacterial properties of the implant materials. Currently, the clinical application of biological implants is strictly based on aseptic processing and aseptic operation, but infection of implants is inevitable. The infection rate of artificial joints is 2%−4%[1-3]. This increase is due to the changes in the prevalence of joint diseases as a result of our growing population during the last several decades[4]. Moreover, the infection rate of the needle passage used in implants of external fixators is as high as 30%−50%[5, 6]. Two thirds of all orthopedic implant infections are due to staphylococcus aureus infection. Once infection occurs in implants, it is often difficult to eradicate it, and antibiotic use is mostly ineffective[5, 7]. Biological biofilm formation is considered to be the main reason why infections of the implant are difficult to treat [5, 7]. Gilbert and other scholars[6] in their study indicated that a very high dose of antibiotics is required to kill bacteria in the biofilm, which may be more than 1 000 times of the antibiotic dose required to kill bacteria in similar suspension. These show that implant materials must possess antibacterial ability in order to mitigate infection risk.

Titanium dioxide nanotubes have a diameter ranging from several nanometers to hundreds of nanometers. Various controversies exist on the effect of nanotube diameter on the functions of osteoblasts and bone marrow stem cells. The main controversy is on the effect of larger diameters (50−100 nm) on cells.

Park et al.[8-10] concluded that the diameter of titanium dioxide nanotubes affects various behaviors of osteoblasts, such as adhesion, proliferation, differentiation and apoptosis.

Earlier studies have confirmed that loading antibiotics on the metallic surfaces of titanium nanotubes can efficiently exert antimicrobial effect both *in vivo* and *in vitro*, as well as have a good biocompatibility[8-12]. Our previous studies have shown that titanium dioxide nanotubes have good biological compatibility, although its loaded drugs are released for a short time (less than 24 hours), which shows that titanium dioxide nanotubes has a poor antibacterial ability[13].

Researchers have been looking for other suitable materials as drug carriers to prevent infections. In the biomedical fields, implanted biomaterials are required to stay for a long time within the body, such as titanium plates used in the fixation of fractures, spinal fracture fixation, as well as orthopedic external fixation system. These biological materials in most cases are often needed to stay in place for a long time (1−2 years), sometimes even for life. Clinically, the delayed blood-borne bacteria may cause postoperative infections of implants[14, 15]; however by drug-loading in the nanotube, it is still very difficult to reach a long-term antibacterial effect[13]. Development of current biomaterials focuses on improving their long-term antibacterial properties and biocompatibility.

In order to reach the long-term antibacterial properties and biocompatibility, antibacterial ions such as copper[16-19], zinc oxide[20], silver[21, 22] have been introduced into biological materials. Copper, because of its effective antimicrobial effect, has attracted attention in today's medical field of biological materials[16-19]. Addition of an antimicrobial substance such as copper and silver to existing metal implants can increase the antibacterial properties of the original materials. In this study, anodization process is used to fabricate titanium-copper alloy nanotubes, with a copper content of 5%. The biocompatibility and antibacterial properties of the nanotubes were tested by co-culturing with osteoblasts and *Staphylococcus aureus*.

MATERIALS AND METHODS

Design

A cytology experiment.

Time and setting

The study was completed at the Third Affiliated Hospital of Jinzhou Medical University in China from January 2016 to October 2017.

Materials

Titanium-copper alloy nanotubes preparation: 95% of titanium metal powder with high purity (99.99%) and 5% of copper powder with high purity (99.99%) were ball milled for 6 hours, and then thermocompressed into samples under a pressure of 15−30 MPa at 850−1 050 ^{o}C. Finally, cooling was allowed to room temperature in an oven.

Commercial pure titanium metal was purchased from Alpha Company in the United States, with the purity of 99.5%. The 1-mm thick metal plates were cut into metal discus of 2 cm in diameter using a metal cutting machine, and then soaked in 5% hydrofluoric acid for 5 minutes in order to remove the metal oxide layer on the surface of the titanium. Ultrasonic cleaning was done three times with anhydrous ethanol, each lasting for 5 minutes. The samples were finally rinsed three times with distilled water and later dried.

The preparation of titanium dioxide nanotubes: Preparation of titanium dioxide nanotubes was done using anodization technology, using 0.5% HF as electrolyte, oxidation time of 30 minutes, and oxidation voltage of 20 V. All experiments were conducted at room temperature. The nanotubes used in this experiment were 80 nm in diameter.

Titanium-copper alloy nanotubes preparation: First, the titanium-copper alloy with copper content of 5% was prepared by scorification. Then using anodization technique, the titanium-copper alloy nanotubes were produced, using 0.5% HF as electrolyte, oxidation time of 30 minutes, and oxidation voltage of 20 V.

Microtopographic observation of samples: Surface topography observation was done using scanning electron microscopy (S4800, Japan) of both titanium dioxide nanotubes and titanium-copper alloy nanotubes produced by anodization. At the same time, by means of the energy dispersive X-ray (EDX) system on the scanning electron microscopy, the composition of the titanium dioxide and titanium copper alloy nanotubes was analyzed.

Methods

MC-3T3-E1 cell culture

Culturing of mouse osteoblasts MC-3T3-E1: MC-3T3-E1 cells were cultured in a medium containing α-MEM of 10% fetal bovine serum and then cultured in a 5% CO_2, 37 ^{o}C constant temperature incubator. α-MEM culture medium was replaced every 2 days. When the mouse osteoblasts grew and covered about 80% of the culture bottle, 0.25% non-EDTA containing trypsin was used for digestion. After digestion, cell passage was done in a ratio of 1:3. After sterilization of the prepared titanium-copper alloy nanotubes, titanium dioxide nanotubes and commercial titanium using ethylene oxide, the samples were placed into 6-well plates, and α-MEM cell culture medium was added. MC-3T3-E1 cell suspension with a cell density of 10^4/cm^2 was then added to the culture medium. The cell culture medium was replaced once every 2 days.

Observation of co-cultured cells: Titanium-copper alloy nanotubes, titanium dioxide nanotubes and commercial titanium were respectively co-cultured with MC-3T3-E1 cells at a cell density of 10^4/cm^2 for 6 hours and 24 hours respectfully. After each specified culture period, the samples were removed from the culture medium and fixed with 2.5% glutaraldehyde for 60 minutes. Through volume fraction of 75%, 85%, 90%, 95%, 100% ethanol gradient, dehydration was done on the respective samples. The samples were vacuum-dried and sprayed with gold. Cell adhesion and proliferation were observed by scanning electron microscope (S4800, Japan) and cell count completed.

Antibacterial properties testing

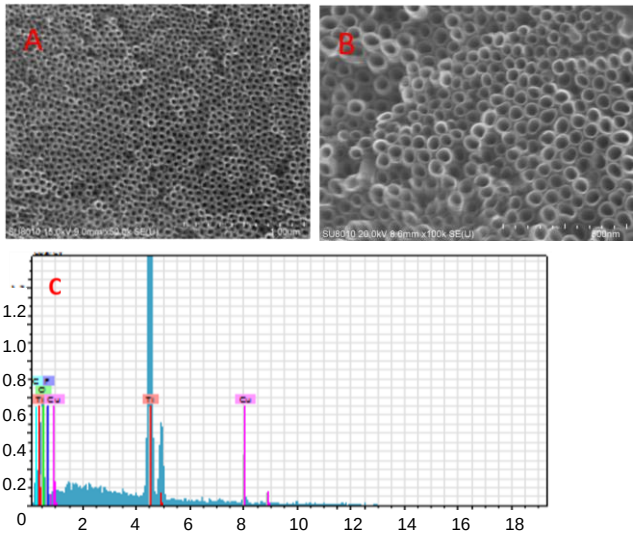
Staphylococcus aureus (ATCC 25923) were used to detect the antibacterial properties of titanium-copper alloy nanotubes. *Staphylococcus aureus* was cultured in LB medium before bacteria inoculation. Bacteria dilution was done in LB culture medium and placed in a bacterial incubator for 24 hours, bacteria concentration was

determined through optical density measurements. The bacterial concentration used in this *in vitro* antibacterial property experiment was 10^7 CFU/mL. Bacteria were seeded at the concentration of 10^7 CFU/mL onto the titanium-copper alloy nanotubes, titanium dioxide nanotubes and commercial titanium metal surface, then cultured in LB medium for 6, and 24 hours. Six-hour cultures were divided into two groups. The samples were then removed at the end of culture time and rinsed three times with PBS in order to remove any non-adherent bacteria. Samples from one group were stained with SYTO9/PI live/dead bacteria detecting kit for 15 minutes in the dark. Bacteria adhesion on the nanotubes was observed under a confocal laser scanning microscope. Samples from the other 6-hour group were fixed with 2.5% glutaraldehyde, dehydrated, sprayed with gold and observed under scanning electron microscope for bacteria density and morphology. The effect of commercial titanium, titanium dioxide nanotubes, titanium-copper alloy nanotubes on osteoblasts were compared.

RESULTS

Scanning electron microscope observation of titanium dioxide and titanium-copper alloy nanotubes

Figure 1A and **B** shows regular arrangements of produced titanium dioxide nanotubes and titanium-copper alloy nanotubes of about 80 nm in diameter under the scanning electron microscope. The nanotubes were perpendicular to the titanium base. **Figure 1C** shows the EDX analysis of the chemical composition of titanium-copper nanotube surface substances under the scanning electron microscope.

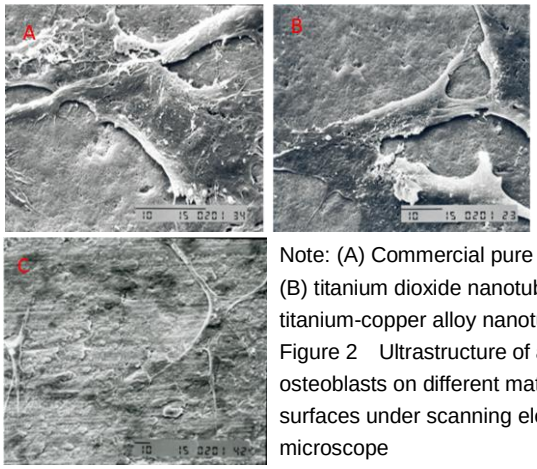


Note: (A) Titanium dioxide, scale bar=1 μm; (B) titanium-copper alloy, scale bar=500 nm; (C) energy dispersive X-ray spectroscopy (EDX) image of titanium-copper alloy. X-axis of EDX indicates energy (KeV), and y-axis indicates X-ray intensity.

Figure 1 Scanning electron microscope observation of titanium dioxide and titanium-copper alloy nanotubes

Adhesion and proliferation of mouse osteoblasts on different material surfaces

Figure 2A−C shows the proliferation condition of mouse osteoblasts on commercial titanium, titanium dioxide nanotubes and titanium-copper alloy nanotubes after 24 hours of culture. The images show good osteoblasts morphology on the titanium nanotubes and titanium copper alloy nanotubes (Figure 2B and C). A large number of cellular pseudopods were attached to the compounds, indicating that titanium-copper alloy nanotubes have good biocompatibility. Cell count also showed that titanium-copper alloy nanotubes and titanium dioxide nanotubes had significantly better osteoblasts proliferation capacity than the ordinary titanium metal.

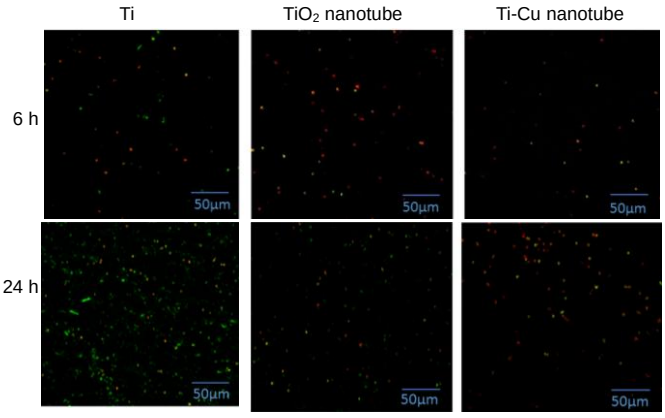


Note: (A) Commercial pure titanium; (B) titanium dioxide nanotube; (C) titanium-copper alloy nanotubes.

Figure 2 Ultrastructure of adherent osteoblasts on different material surfaces under scanning electron microscope

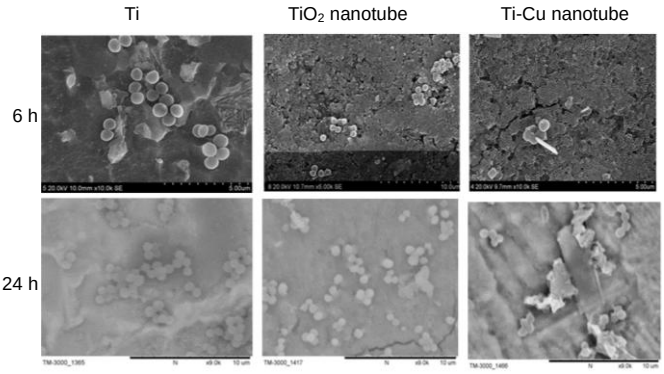
Antibacterial property testing

Staphylococcus aureus was co-cultured with titanium-copper alloy nanotubes, titanium dioxide nanotubes and titanium for 6 and 24 hours respectively, and confocal laser scanning microscope (**Figure 3**) and scanning electron microscope (**Figure 4**) were then used for bacterial adhesion. There was greater bacteria quantity on the titanium metal and titanium dioxide nanotubes compared to that on the titanium-copper alloy nanotubes (**Figure 5**). This shows that titanium-copper alloy nanotubes not only have good biocompatibility, but also reduce bacterial adhesion.



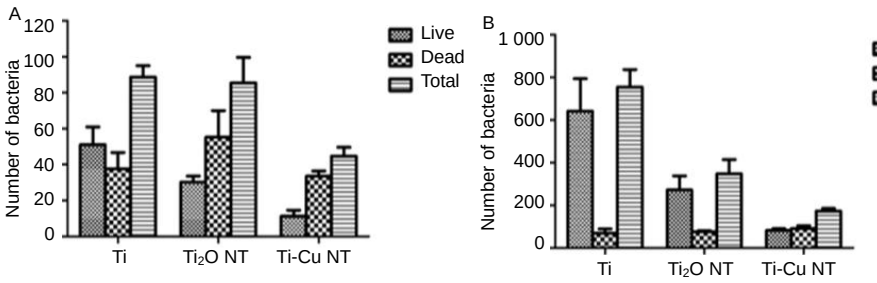
Note: Ti: Titanium; TiO_2: titanium dioxide; Ti-Cu: titanium-copper alloy; h: hours.

Figure 3 Antibacterial properties of different materials under laser confocal scanning microscopy (immunofluorescence staining, scale bars=50 μm)



Note: Scale bar=5 μm for Ti 6 h and scale bar=10 μm for other images. Ti: Titanium; TiO_2: titanium dioxide; Ti-Cu: titanium-copper alloy; h: hours.

Figure 4 Antibacterial properties of different materials (immunofluorescence staining; scanning electron microscopy)



Note: (A, B) Bacteria count after 6 and 24 hours. The results show greater bacteria quantity on the titanium metal and TiO_2 nanotubes compared to that on the Ti-Cu alloy nanotubes. Ti: Titanium; TiO_2: titanium dioxide; Ti-Cu: titanium-copper alloy.

Figure 5 *Staphylococcus aureus* density on different material surfaces after culture

DISCUSSION

Fabrication of nanotubes

Any implant used in the body must meet certain requirements, which must be biologically stable, able to promote osseointegration, and able to prevent bacteria adhesion and growth. Biocompatibility of implants depends on many factors, which include but not limited to the type of material used, size of the nanoparticles of the material, methods used in the preparation of the materials, surface treatment techniques and conditions under which the materials are produced or treated. Studies have shown that, as the size of nanoparticles is reduced, cellular and material interaction is enhanced and has also helped reduce bacteria colonization[23-25]. This reduction in size as small as few nanometers is achieved by the fabrication method used. Some methods also lead to more biostability of some materials than others. As the size of material is reduced to the nanoscale, the surface properties of the material change noticeably[23]. These surface properties primarily determine the interaction between the materials and proteins in the biological environment as well as interactions with the cells and tissues[26]. Azam et al.[23] reported the effect of nanoparticles on bacteria, and assumed that the properties of nanometer sized materials change with size. From their experiment, it was concluded that nano-materials can act as anti-microbial agents, and that their antimicrobial activity is size dependent. The surface properties of nanomaterials vary not only with size of particles but also with the technique used to produce the material as well as the surface treatment technique used. Anodization method is one that has been used and is preferred by many due to the size of nanotubes and physico-chemical properties produced. Titanium produced by anodization has yielded good effects in many researches. There is a change in surface chemistry after surface nano-modification by anodization and heat treatment of Titanium in many studies[25, 27-29]. Studies have shown that the nanotubes produced by the anodization have fluorine on their surfaces. This flourine is hypothesized to have affinity for bacteria. The trapped flourine can be removed by heat treating the nanotubes which may significantly impact the antibacterial property of the material surface. The titanium-copper alloy nanotubes used in our study were fabricated by anodization method. The nanotubes which had a diameter of 80 nm were heat treated by sintering at 850−1 050 ^{o}C during their fabrication. This led to the loss of the flourine contents on their surfaces. EDX analysis based on the scanning electron microscopy showed the surface chemical composition of titanium-copper nanotubes. It can be seen from the EDX analysis results that a new copper phase was synthesized after sintering which is seen as a peak two separate peaks on the graph. Therefore, expected alloys of titanium and cooper were successfully prepared in our study.

Effect of nanotubes on osteoblast adhesion and proliferation

Cells are said to be sensitive to biochemical, mechanical and topological cues[30]. The responses produced by the cells to biomaterial interfaces vary with the environment in which they exist. Since *in vivo* and *in vitro* conditions are different, the cells response differently in each environment. When an implant is placed in the host, many responses occur to initiate bone healing. This bone healing starts with protein adsorption and attachment, followed by adhesion of various biomolecules and cells onto the surface, which are fully influenced by the physico-chemical properties of the implant material[31]. The most crucial cells in this bone healing process are Osteoblasts. Osteoblast adhesion, morphology and functionality are all affected by biocompatibility of the implanted material. Studies have shown that the biocompatibility of the biomaterial is enhanced with reduction of the biomaterial size to the nanometer scale. As the size of nanoparticles is reduced, cellular and material interaction is enhanced[23-25]. Recent advances in nanotechnology have made it possible to prepare nanostructured materials using various manufacturing methods that are extensively used in modern implants. Titanium and its alloys have been extensively used as biomaterials in bone surgeries in recent

decades because of their generally good biocompatibility which is mainly due to its mechanical properties that make it better adapted to those of bone as well as because its surface is always covered by passive nanometer layer that produces its corrosion resistance and bioinert *in vivo* [32].

In this study, mouse osteoblasts adhesion and proliferation were investigated on commercial titanium, titanium dioxide nanotubes and titanium-copper alloy nanotubes. Osteoblasts showed good adhesion and morphology on the titanium nanotubes and titanium copper alloy nanotubes under the scanning electron microscope. A large number of cellular pseudopods were attached to the titanium-copper alloy nanotubes, indicating that adhesion of osteoblasts and their morphology are not affected by the physico-chemical properties or topological cues of the material. Compared to the other surfaces, titanium-copper alloy nanotubes have better biocompatibility. Zhang and his colleagues[13, 16], in their study reported the improved osteoblast adhesion onto nanostructured titanium compared to commercially pure titanium. They also found improved cytocompatibility of the nanostructured titanium based on MTT assay, indicating that the nanotubular surfaces have no significant impairment to osteoblasts function. This improved biocompatibility of titanium nanotubes has also been supported by many other investigations[27, 32-33].

The enhanced biocompatibility in our study was not only due to the titanium nanotubes, but also resulted from the copper components. In a study conducted by Ren et al.[34], they reported that Cu^{2+} ions released from the copper stainless steel implant played a key role in multi-biofunction of the stainless steel, including osteogenesis. Their results indicated that Cu^{2+} ions from Copper stainless steel could promote the osteogenic differentiation by stimulating alkaline phosphatase enzyme activity and osteogenic gene expressions, thereby enhancing the adhesion and proliferation of osteoblasts cultured on its surface[34]. Their study further proved that more new bone tissue formed around the implant with more stable bone-to-implant contact and satisfied biocompatibility[34]. Our study clearly demonstrates that the biocompatibility of titanium nanotubes combined with copper can be enhanced in the form of an alloy. We can therefore conclude that titanium-copper alloy nanotubes are a potential material for implants due to their enhanced biocompatibility.

Antibacterial effect of titanium-copper alloy nanotubes

Despite proper aseptic techniques and sterilization methods used in daily medical practice, the incidence of bacterial infections still remains a big challenge. Bacterial infection of implants is one of serious complications encountered in orthopedic surgery and it is also one of the main reasons for implant failure and revision surgeries. The most common implant-related pathogenic bacteria is Staphylococcus aureus. A good biomaterial is not only biocompatible to cells but also able to resist infection by preventing bacterial adhesion. It is postulated that there is competition between osseointegration of an implant and bacterial adhesion to the implant surface just after implantation. This means that if bacterial adhesion occurs before osseointegration or if the bacteria overcome the defensive mechanism of the body, colonization is bound to occur, and failure of the implant is possible especially if the colonization leads to biofilm formation. Antibiotic therapy during an implant infection only relieves symptoms caused by floating single planktonic bacteria shed from biofilms[35]. Infection control should therefore be geared towards primary prevention of bacterial adhesion to the implant surfaces. Type of the material used in implants and the physico-chemical properties of the material make it susceptible to bacterial adhesion[36]. Titanium and copper nanotubes have been separately investigated for their antibacterial effects, and some studies have achieved positive results.

In this study, we compared the antibacterial properties of titanium-copper alloy nanotubes with those of pure titanium nanotube and pure titanium. The study results on both confocal scanning laser and scanning electron microscopes show greater bacteria quantity on the titanium metal and titanium dioxide nanotubes compared to that on the titanium-copper alloy nanotubes. Although the number of adhered bacteria was greatest in the pure titanium metal group, bacterial adhesion was observed on all sample surfaces. In the 6-hour culture, pure titanium and titanium dioxide nanotube surfaces had the highest total number of adhered bacteria among all the groups, with live bacteria being more than dead bacteria. The titanium-copper alloy nanotube surface had lower number of adhered bacteria. The number of dead bacteria on the titanium-copper alloy nanotube surfaces was also more than that of live bacteria. This means that titanium-copper alloy nanotubes had the strongest bactericidal effect. Similar trend was also noted in the 24-hour culture. As the bacteria culture time increased from 6 to 24 hours, bacterial growth increased in all groups. Titanium copper alloy nanotubes compared with the other two kinds of materials had the less number of adhered bacteria, therefore having the strongest bactericidal effect. Scanning electron microscopy observation was done under various amplifications on some samples during the 6-hour culture. It was observed according to the results that commercial titanium had a large number of adhered bacteria onto its surface. There were many bacteria in clusters, most of which were live bacteria that were morphologically intact in structure with little aggregation. Few bacteria were dead with destructive cell structure. Bacteria adhered to the titanium nanotube surfaces were more scattered in their adhesion pattern, most of which had morphologically intact structures. Some bacteria in cluster had no morphology, which were dead bacteria. The titanium-copper alloy nanotube surfaces had the least number of adhered bacteria. Dead bacteria with incomplete cell morphology in clusters accounted for most of the adhered cells. Only a handful of bacteria with intact cellular structure were seen on their surfaces.

Both dead and live bacteria were able to attach to the surfaces. This shows that bacteria adhesion and growth is still possible even on a nanostructure material. The presence of dead bacteria also shows that the nanotubes were able to exert antibacterial effects, which was more evident in the titanium-copper alloy nanotube group. Nanotubes produced by anodization are said to leave their surfaces with inorganic residual ions which are toxic to bacteria, thereby exerting their antibacterial effect.
In this study, titanium-copper alloy nanotubes had a

reduced bacteria adhesion compared to the other two materials. This shows that the bactericidal ability was enhanced by the combination of two different materials, titanium and copper. Azam et al.[23] in their study found out that, the release of ions from the materials was due to the interactions between particles of cupric oxide used in their study and the solution. This interaction causes alterations in the bacteria membranes and ultimately leads to a bactericidal activity. In our study, the bactericidal effect of titanium and copper was enhanced with a higher component of copper in the alloy. This was manifested in the least number of adhered bacteria that was seen in the titanium-copper alloy nanotube group.

Under the scanning electron microscope, impaired bacteria morphology was shown on the titanium dioxide nanotube and titanium-copper alloy nanotube surfaces, but this was more prominent in the titanium-copper nanotube alloy surfaces. The dead bacteria had impaired cell morphology which was likely to result from the material solution interaction process. As reported by Zhang et al.[13], matrix constituents on the surfaces of the nanotubes are mainly responsible for bacteria adhesion to the surfaces. Nanotubes that are covered with different matrix constituents form a micro environment, which is beneficial for bacterial adhesion and survival[13]. This in turn may impair the antibacterial ability of the nanotubes. This is also responsible for the live bacteria adhered to all the surfaces, though lesser in the alloy nanotube group.

Since the main goal after implant surgery is to enhance implant osseointegration as well as to reduce or eliminate bacteria adhesion, the material used in implants must be able to promote osseointegration as well as be able to resist bacteria adhesion. Many factors are to be considered when choosing such material. The factors which have been identified to play major roles in biofunction of osteoblasts include the type of material used, size of the nanoparticles of the material, physicochemical properties of the material, as well as the microenvironment. All of these factors greatly influence osteoblasts adhesion to the implant surfaces. The same factors are also responsible for bacteria material interaction.

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钛铜合金纳米管形貌能够降低细菌活性并促进成骨细胞功能

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文章快速阅读：

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文题释义：

钛-铜合金纳米管：将使用熔融法铸造钛铜合金后采用阳极氧化技术制备钛铜合金纳米管，在钛铜合金纳米管形貌表面种植成骨细胞检测其生物相容性，在钛铜合金纳米管表面种植金黄色葡萄球菌检测其抗菌性能。

金黄色葡萄球菌：是人类的一种重要病原菌，隶属于葡萄球菌属(*Staphylococcus*)，有“嗜肉菌”的别称，是革兰阳性菌的代表，可引起许多严重感染。

摘要

背景：植入物的感染仍然是严重的骨科术后不良反应。铜是一种目前已知具有抗菌性能的金属。研究表明，通过纳米技术制备纳米结构金属可以促进成骨细胞在体内的黏附、增殖和骨结合。临床上最常见的重要植入物相关病原菌-金黄色葡萄球菌，是用来测试抗菌性能的钛-铜合金纳米管。

目的：观察钛-铜合金纳米管的抗菌能力及对成骨细胞功能的影响。

方法：实验将不同材料(纯钛、钛纳米管及5%-钛铜合金纳米管)与小鼠成骨细胞(MC-3T3-E1)共培养6，24 h，观察细胞在支架上的黏附和增殖。比较了钛-铜合金纳米管、纯钛纳米管和纯钛的抗菌性能。在各组材料形貌表面种植成骨细胞检测其生物相容性，在各组材料表面种植金黄色葡萄球菌检测其抗菌性能。

结果与结论：①扫描电镜可见，钛纳米管和钛-铜合金纳米管表面良好的细胞黏附性，小鼠成骨细胞形态良好，排列规则；纳米管组细胞增殖情况优于纯钛组，而钛-铜合金纳米管与钛纳米管组差异无显著性意义；②细菌黏附实验显示，与钛铜合金纳米管相比，钛金属和 TiO_2 纳米管上的细菌数量更多；③结果证实，钛-铜合金纳米管对细菌黏附具有良好的抑制作用，不影响成骨细胞的生物功能。

关键词：

钛铜合金纳米管；抗菌性能；成骨细胞；金黄色葡萄球菌；组织工程骨材料；内植物；组织工程

主题词：

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