主　論　文　の　要　約

論文題目　**Fabrication of Hydrophilic Thin Film on Metallic Biomaterials for Improvement of Osteoconductivity**
(骨伝導性向上のための金属系生体材料への親水性皮膜の作製)

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論　文　内　容　の　要　約

Metals and their alloys have played vital role in structural biomaterials in the reconstructive surgery, particularly the orthopedics. With the progress of the clinically application of various metallic biomaterials, the relevant issues associated with osteointegration, immune-based reactions, long-term inflammation, etc., have also emerged.

I tried to prepare a protective film that is highly hydrophilic in different treatment methods and suppresses the release or decrease the release rate of metal ions by surface modification, and aimed to improve bone conductivity and reduce deterioration of hydrophilicity. The metallic materials used included the Ti alloys, AZ31 Mg alloys, and austenitic stainless steels including 316L and stainless steel. As the surface modification method, the protective film was prepared by hydrothermal treatment at high temperature and high pressure, electrochemical anodizing treatment, immersion treatment in an acid with oxidizer activity. The chemical composition, roughness, hydrophilicity, metal ions release, protein adsorption and so on were characterized for thin films formed by surface treatments. The EIS and polarization methods were adopted to analyze the anti-corrosion properties of the AZ31 Mg alloy specimens. The specific metal ions released from stainless steel in PBS solution were measured to analyze the possible toxicity of the specimens. In addition, *in vivo* tests were employed to evaluate osteoconductivity and protein adsorption influence on bone formation.

It has been reported that hydrophilicity and hydrophobicity of implants influenced the bioactivity. However, it is hard to maintain the hydrophilicity in case of being stored in air. So, it is critical to find a way to maintain implants' hydrophilicity. In general, silicate has been known to contribute the hydrophilicity. I have prepared the silicate-containing $CaTiO_3$ films on Ti substrates by two-step treatment for biomaterial applications. The hydrophilicity, osteoconductivity and protein adsorption of treated specimens have been investigated. The 1st step treatment for Ti is to form TiO_2 as precursors, either by anodizing in sulfuric acid solution at 25 ^{o}C, liquid phase oxidation in nitric acid solution with hydrogen peroxide at 80 ^{o}C, or thermal oxidation at 400 ^{o}C in air. Hydrothermal treatment in silicate containing alkaline solution is the 2nd step to convert TiO_2 to silicate containing $CaTiO_3$ films. The SEM, XRD, XPS, WCA (water contact angle) investigations and protein adsorption measurements have been carried out to characterize the surface properties. This surface maintained 10 deg. in WCA after 7 d exposure in air, while the specimen without silicate has WCA of more than 40 deg. The osteoconductivity is evaluated based on the contact ratio of formed hard tissue on the implanted specimens (abbreviated as $R_{B\text{-}I}$) after 14 d implantation in rats' tibia at *in vivo* test. The as-prepared film not only has exhibited smooth and superhydrophilic surface, but also has achieved high osteoconductivity and great protein adsorption capacity.

The water contact angle (WCA) value of the Ti substrate surfaces was successfully controlled to super-hydrophilicity by the treatment of UV light irradiation, atmospheric plasma or hydrothermal treatment in distilled water, and stored in different environments to control the variation in WCA over time. Proteins with various molecular mass were used to investigate the protein adsorption on the Ti substrate controlled WCA. ATR-FTIR was employed to quantify the protein adsorption amount. It was found that all proteins had relatively higher adsorption amount on superhydrophilic surface. The adsorption amount of proteins was similar under the same WCA value. PEGs, as organic molecules without peptide bonds, was unable to adsorb on Ti with WCA $<$ 60 deg., while adsorb only on the hydrophobic surfaces with WCA $>$ 90 deg. In particularly, I had carried out *in vivo* tests upon the protein pre-adsorbed Ti specimens. The *in vivo* tests indicated that the Ti substrate adsorbed proteins with high molecular mass had much higher osteoconductivity than that without protein. On the other hand, the PEGs adsorbed substrates showed no effect on osteoconductivity. This was due to its lack of peptide bonds. It was considered that implants adsorbed polypeptides can greatly enhance the osteoconductivity, regardless the mass and type of molecules.

Magnesium alloys have been widely studied as biodegradable implants for orthopedic applications due to their more suitable mechanical and biological properties. However, Mg and its alloys themselves don't have high hydrophilicity considered to be effective in improving the osteoconductivity of implanted materials. The drastic degradation rate of Mg alloys would also cause potential harm to the human body. With the aim to promote the hydrophilicity and corrosion resistance of Mg alloys, thin film consisting of magnesium hydroxide with silicate was synthesized by hydrothermal treatment in the alkaline solution of sodium hydroxide and sodium silicate at 150 ^{o}C. The physical properties of thin films were estimated by SEM, XRD, XPS, tape peeling test and water contact angle (WCA), and corrosion properties were evaluated by polarization curves and impedance measurements (EIS) in PBS solution. $Mg(OH)_2$ was found to be the main composite of the film with little of MgO coexisted. Si was detected on the surface of the film layer, based on XPS results. The addition of silicate during hydrothermal treatment would greatly improve the hydrophilicity and its stability in the air environment. The $Mg(OH)_2$ film containing Si also indicated higher corrosion resistance.

Stainless steels used as metal implants in medical field have also been attracting intensive attention, due to their advantages in mechanical properties, anti-corrosion properties and cost effectiveness. Good osteoconductivity, low toxicity and low inflammatory reactions are essential to stainless steel implant *in vivo*. However, there are few cases about the surface modification performed for searching the metal ion release behavior, and there are few researches on the relationship between the surface properties of stainless steel and osteoconductivity when used as implant. I employed 316 L and 304 stainless steel for surface modification including hydrothermal treatment after acid immersion, and anodizing treatment, while the as-polished stainless steel was used as control substrates. Metal ion release behaviors, protein adsorption properties and osteoconductivity of these specimens were intensively investigated *in vitro* and *in vivo*. It was found that specimen subjected to hydrothermal treatment at 230 °C after immersion in 18 M H_2SO_4 had the lowest metal ions release, while the anodized specimen had highest release of Fe and Cr due to corrosion. The protein adsorption amount of the specimens was positively related to the osteoconductivity, suggesting protein adsorption is the prerequisite for good osteoconductivity. The osteoconductivity decreased firstly and then increased with the increase in water contact angel (WCA) value. Specimen with surface modified by hydrothermal treatment after acid immersion had the highest protein adsorption amount and best osteoconductivity, due to its super-hydrophilicity property. The protein adsorption capacity and osteoconductivity for stainless steel tended to be the same as Ti alloys studied before, indicating the surface hydrophilicity property of the implanted metals was the dominant factor affecting the osteoconductivity. Thus, this research can provide new insight into the application of austenitic stainless steel for implanted material purpose.

In general, my research provided new insight into the relationship between metallic implanted biomaterials and the biocompatibility: Firstly, hydrothermal treatment was advantageous to kinds of metallic materials including Ti alloys, Mg alloys, and stainless steel, in the scope of higher hydrophilicity with corresponding higher protein adsorption, higher osetoconductivity. Secondly, the addition of silicate during the hydrothermal process was quite effective in increasing the hydrophilicity property of the specimens, which can exhibit the super-hydrophilic property, and also effective in enhancing the anti-corrosion property, which might be due to the formation of self-assembled monolayer (SAM) on the surface of metallic materials. Also, the method straightforward improves osteoconductivity is to attach polypeptide bones with amino functional groups, regardless of the amount and type of adsorbed molecules. Moreover, the WCA value (index to reflect hydrophilicity property) has direct relationship with the protein adsorption amount and osetoconductivity, regardless of what kind of metallic material the specimen is.

Keywords: Mg alloys, Ti alloys, stainless steel, metal implant, osteoconductivity, hydrophilicity, biocompatibility, surface modification, hydrothermal treatment.