INTRODUCTION

New capillary formation is indispensable for tissue regeneration\(^1\)\(^-\)\(^5\). The influences of metal ions released from metal prostheses on angiogenesis in oral tissue remain unclear\(^6\),\(^7\). The development of alloys containing metal ions with marked angiogenic effects may promote active oral tissue regeneration. In this study, we evaluated the influences of 8 types of metal ion (Ag, Au, Cu, In, Ni, Pd, Ti, and Zn), which are major components of gold-silver-palladium and nickel-titanium alloys used in the metallic dental biomaterials, on the in vitro formation of new blood vessels using a human angiogenesis kit (Kurabo, Japan)\(^8\)-\(^11\).

MATERIALS AND METHODS

1. Preparation of test solutions

Eight types of atomic absorption standard reagent (1,000 ppm, Ishizu, Osaka, Japan), were diluted in medium (Hu Media EG2, Kurabo) at 37°C to 10 ppm, further diluted in medium to 2.5 ppm, and used as test solutions.

2. New capillary formation test

The human umbilical vein endothelial cells (HUVEC) and diploid fibroblasts derived from human skin were used.
After changing each culture medium to a 500μL/well test solution in a CO₂ incubator, a culture was made therein for 11 days. Each test solution in the well was changed to a new solution after 4, 7 and 9 days of culturing. After 13 days of culturing, each test solution was removed by pipette, and each well was washed in PBS(-) and fixed for 30 minutes with -20°C ethanol solution. After ethanol solution was removed and the wells were washed again in 1 mL/well PBS(-) containing 1% BSA, the new capillary formation of new blood vessels was dyed with CD31 antibody (Kurabo). Then image data were obtained using a digital camera attached to a microscope, and the percent of area ratio and the vascular length was measured using an angiogenesis image analyzer software system(Kurabo).

RESULTS
Inverted microscopic images of stained new capillary formation for each ions are shown in Fig. 1. As shown in Fig. 2, the percent area ratio of new capillary formation for Zn ions was the highest, being 106.1% of that in the control group. The percent area for the other types of metal ion were lower than in the control group, decreasing in the order of Ag, In, Ti, Ni, Cu, Pd, Au and Zn ions. The percent area for Ag ions was the lowest,

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Fig.1a  Morphological observation of new capillary formation with Ag, Au and Cu ions.
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being 6.9% of that in the control group. As shown in Fig. 3, the new capillary length for Zn ions (95%) was also the greatest, being slightly smaller than that in the control group. The vascular length decreased in the order of Ag, Ni, In, Ti, Cu, Au, Pd, and Zn ions. The order of the types of metal ions differed between the percent area and length of new capillary formations, excluding Zn ions.

Fig. 1b  Morphological observation of new capillary formation with In, Ni, Pd, Ti and Zn ions.
showing the highest values and Ag ions showing the lowest values for both results.

**DISCUSSION**

The results of this study showed the highest angiogenesis % area and vascular length for Zn ions. We previously reported that the Zn ion concentration range increasing angiogenesis is very narrow\textsuperscript{12, 13}. DNA coping by Zn ions during cell division has attracted attention and has been studied. DNA is copied by various RNAs for new cells during cell division, and Zn ions are involved in transcription\textsuperscript{14-17}.

Conventionally, it was considered necessary to minimize metal ion release into the oral cavity when dental alloys are produced\textsuperscript{18-21}. However, if the release of a small amount of certain types of metal ion can be controlled, the rate of active new capillary formation may increase, resulting in earlier tissue re-
generation. In this study, measurement was performed using single types of metal ion, but studies on multiple types and the combination of organic matter and metal ions are also necessary.

Angiogenesis has been extensively studied in terms of regenerative medicine and tumor development. Angiogenesis-promoting factors include vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and peptides. Angiogenesis is complicated, involving various molecular or cellular mechanisms, and the entire process has not been clarified. There is a possibility that the results of this study are specific to the human angiogenesis kit. However, since Zn ions were less toxic than the other types of metal ions used in this study, it is also possible that a special mechanism promoting angiogenesis is involved.

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REFERENCES

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