Development of Gentamicin-Loaded Bone Filling Material with Infection Control Function

○Tsuboi R[1], Kitagawa H[2], Kohno T[1], Imazato S[1,2]

[1]Department of Advanced Functional Materials Science, Osaka University Graduate School of Dentistry, Osaka, Japan

[2]Department of Biomaterials Science, Osaka University Graduate School of Dentistry, Osaka, Japan

Introduction

Since the surgical field is exposed to bacteria in the oral environment, antibiotics have been routinely administered after dental surgery to prevent infection at the surgical site and delayed wound healing. However, antibiotics administered per os show a low blood transfer rate, making it necessary to take an excessive amount to reach an effective concentration locally, and therefore the emergence and spread of drug-resistant bacteria is a major threat. We aimed to develop an antibiotics-containing bone filling material that exhibits an infection control function at the surgical site and contributes to better prognosis.

Objective

In this study, the experimental bone filling material containing gentamicin, which has been utilized for the commercial bone cement for artificial joint fixation, was fabricated and evaluated for its antibacterial effects against oral bacteria and bone forming ability.

Materials and Methods

Preparation of antibiotics-containing bone filling material

Cytrans Granules®
(GC Corp.)

The experimental bone filling material (GM) was prepared by immersing Cytrans Granules (size S, dia: 0.3-0.6 mm, GC Corp.) to the gentamicin solution (200 mg/ mL)

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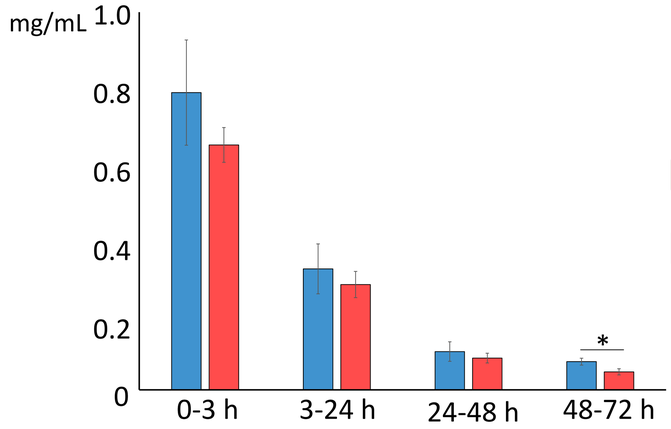
washed and freeze-dried

- **Agar diffusion test**: *Staphylococcus aureus* ATCC29213, *Streptococcus pyogenes* ATCC19615, *Streptococcus sanguinis* ST3R, *Streptococcus sobrinus* NCTC 12279, *Fusobacterium nucleatum* 1436, and *Parvimonas micra* GIFU7745
- **Bacteria contact test**: 1 mL of each bacterial suspension (1 x 10^6 CFU/mL) was cultured in the presence of Control or GM, and the number of viable bacteria was counted after 3 and 24 hours.
- **Implantation experiment**: A bone defect with a diameter of 5 mm and a depth of 8 mm was formed on the femoral head of the rabbit and filled with 300 mg of Control or GM. At 8 weeks after implantation, the femur was harvested and undecalcified sections were prepared from surgical tissue. The sections were stained with Villanueva Goldner. (Control: gentamicin-unloaded Cytrans Granules)

Results and Discussion

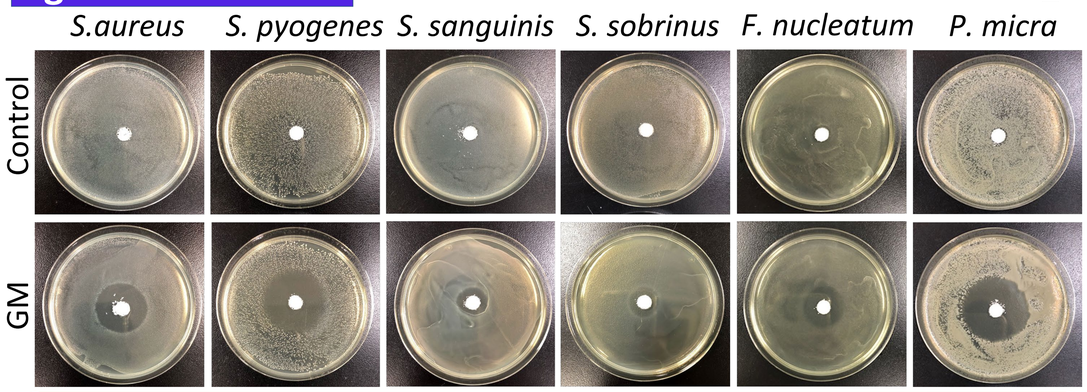
The concentration of gentamicin released



(* $p < 0.05$, Student's *t*-test, n=5, error bars indicate S.D.)

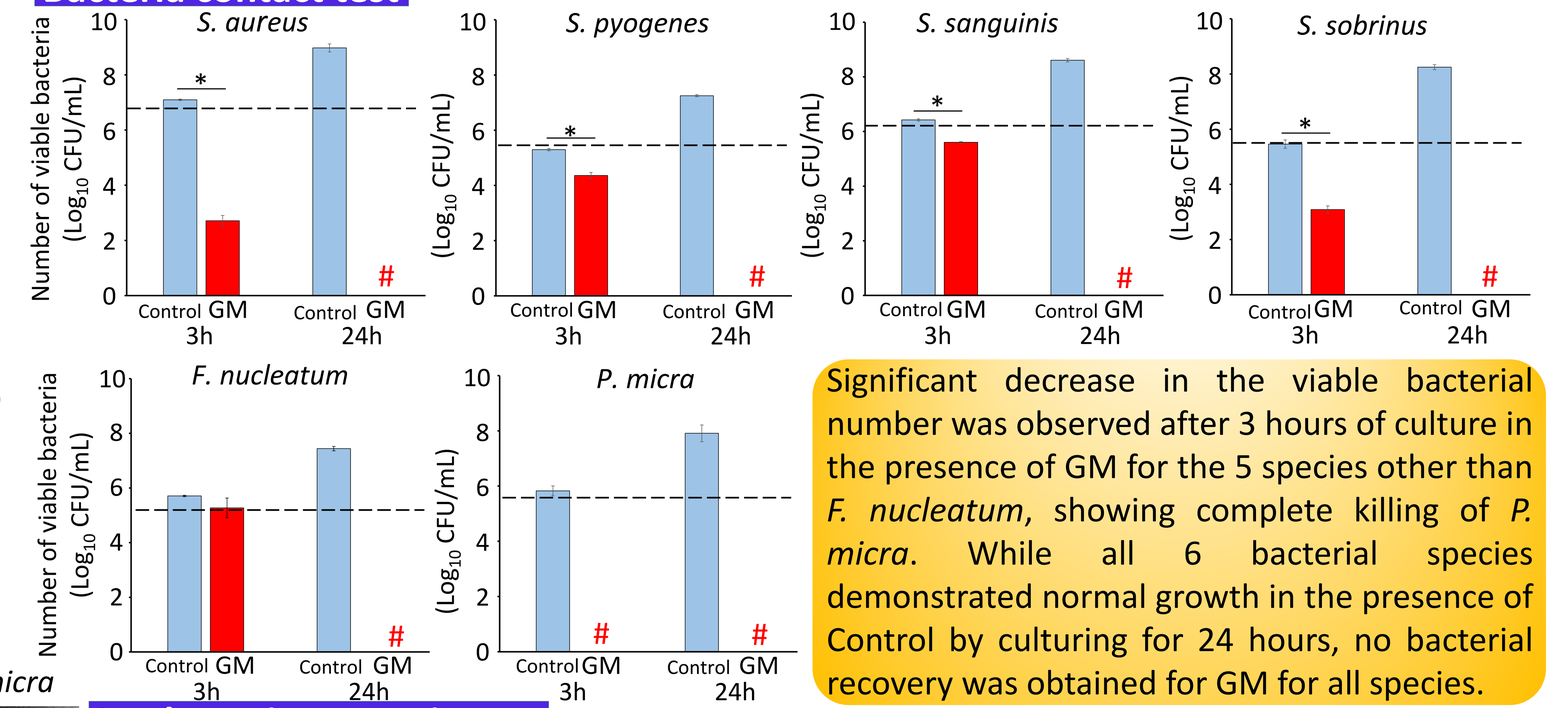
The concentration of gentamicin released from GM was equal to or lower than that of the commercially available gentamicin-loaded bone cement (Cobalt™ HV Bone Cement, Simmer Biomet), and the release kinetics were similar, indicating that GM is a biosafe material.

Agar diffusion test



GM produced inhibition zones against all 6 bacterial species. It showed high antibacterial activity not only against *S. aureus* and *S. pyogenes*, the most common causative agents of infections, but also against *F. nucleatum* and *P. micra,* the causative agents of severe dental infections.

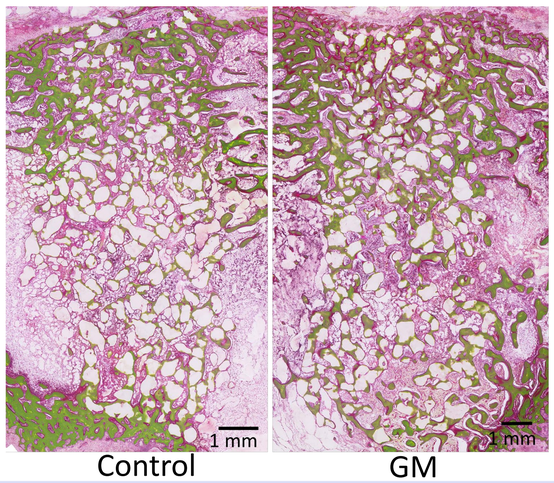
Bacteria contact test



Significant decrease in the viable bacterial number was observed after 3 hours of culture in the presence of GM for the 5 species other than *F. nucleatum*, showing complete killing of *P. micra*. While all 6 bacterial species demonstrated normal growth in the presence of Control by culturing for 24 hours, no bacterial recovery was obtained for GM for all species.

(Dashed line indicates initial bacterial number, # indicates zero, *$p < 0.05$, Student's *t*-test)

Implantation experiment



No irritation or biological reaction (i.e., inflammation, bone resorption, or hyperplasia) was observed for GM at the implantation site. There was no difference between Control and GM in the absorption state and bone formation. Additionally, no difference was found in the infiltration of inflammatory cells for both materials. After 8 weeks of implantation, GM demonstrated no negative influences on bone healing, suggesting that GM can be used effectively in clinical practice.

Newly formed bone exhibited a green color (Villanueva Goldner stain)

Conclusions

A bone filling material with an antibacterial effect was successfully fabricated by loading gentamicin to commercially available Cytrans Granules®. To ensure biosafety, the gentamicin release concentration was adjusted to slightly lower than that for the proprietary gentamicin-loaded bone cement clinically used. The experimental gentamicin-loaded bone filling material fabricated has a possibility to prevent intra- and post-operative infection by exerting a local antibacterial effect, leading to a better prognosis in terms of bone-forming ability.

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