Original Article

The Effect of Sterilization on Antibiotic Beads against MRSA after 6 and 12 Months of Storage: An Experimental Study

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Background: Antibiotic beads are commonly used to treat osteomyelitis, but many of the antibiotic beads, which are custom-made, are left unused after preparation.

Objective: To compare the inhibitive effectiveness against MRSA of vancomycin and fosfomycin beads sterilized with gamma irradiation and ethylene oxide after 6 and 12 months of storage.

Materials and Methods: In this *in vitro* study, antibiotic beads were made locally from a mixture of 40 grams of polymethylmethacrylate and 4 grams of an antibiotic, either vancomycin or fosfomycin, using sterile techniques. Six groups of antibiotic beads were prepared including three groups made with vancomycin (a control group, a group sterilized with gamma radiation, and a group sterilized with ethylene oxide) and a similar set of three groups made with fosfomycin. All beads were placed in sterile packages at room temperature (24 to 37°C) and the sterilized bead groups were stored for either 6 or 12 months. After storage, the beads from each of the six groups were tested for inhibitive effectiveness against MRSA using a modified disc diffusion technique for 6 weeks.

Results: After 12 months of storage, there was no statistically significant difference in inhibitive effect against MRSA among the six groups: the control group and the two sterilized groups (gamma radiation and ethylene oxide) with beads made of either vancomycin or fosfomycin.

Conclusion: Either gamma radiation or ethylene oxide can be used as a sterilization method for antibiotic beads against MRSA without deterioration in inhibitive effect after 12 months of storage. The inhibitive effect of antibiotic beads against MRSA sterilized with either gamma radiation or with ethylene oxide is not reduced after 12 months of storage.

Keywords: Antibiotic beads, Sterilization, Gamma radiation, Ethylene oxide, MRSA

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Chronic osteomyelitis is one of the most difficult and challenging orthopedic problems. This disease can be a significant cause of morbidity and mortality as well as creating a burden stemming from associated high medical costs and prolonged hospitalization. The most common cause of osteomyelitis is open fractures(1-3) and the most common pathogen is *Staphylococcus aureus*. It has become more serious as evidenced by the increasing incidence of methicillin resistance *Staphylococcus aureus* [MRSA](4-9).

Antibiotic beads play an important role in the treatment of osteomyelitis(10-14). Current treatment protocol involves insertion of antibiotic beads into the wound. The beads are removed from the osteomyelitis site after 4 to 6 weeks. At present, there are no effective

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commercially available antibiotic beads for treatment of MRSA, so local production of custom-made vancomycin or fosfomycin beads is the treatment of choice(15-20). The preparation of antibiotic beads for a single patient requires 30 to 60 minutes; often more beads are made than are needed, so many beads remain unused.

There are two methods available for sterilization of antibiotic beads. The first method, gamma irradiation(21), is a standard sterilization method in medical settings but it is not feasible for use in general hospitals because of the cost and radiation control. The second method is a conventional sterilization method using ethylene oxide(22), which is widely available in general hospitals. Antibiotic beads can be used even up to one year after preparation(23); however, there have been no previous studies documenting the inhibitive effect of sterilization on antibiotic beads after a period of storage.

The objective of this study was to investigate the inhibitive effect of sterilized vancomycin and fosfomycin beads against MRSA after 12 months of storage.

Materials and Methods

In this experimental study, antibiotic beads were made from a combination of 4 grams of an antibiotic, either vancomycin (Hospera, US) or fosfomycin (Meiji Seika Kaisha, Japan), with 40 grams of plain powder of polymethylmethacrylate [PMMA] polymer and liquid PMMA monomer (Palacos®, Germany). The ingredients were mixed by gentle stirring with a spatula under sterile conditions. The antibiotic beads were formed into 5 mm diameter spheres using a sterilized template and were then placed in a sterilized plastic bag. The antibiotic beads were divided into 6 groups, 3 with vancomycin and 3 with fosfomycin: two control groups without sterilization, two gamma radiation groups, and two ethylene oxide groups. All antibiotic beads were kept at room temperature (24 to 37°C) for either 6 or 12 months. Ten vancomycin and ten fosfomycin beads from each of the 6 groups were tested in Muller-Hinton agar for their inhibitive effect against MRSA using the modified disc diffusion technique. The zone of inhibition was recorded daily for 6 weeks using a digital Vernier caliper.

Statistical analysis

Generalized estimating equations were used to test each comparator at the different time points using Epidata software.

Results

The inhibitive effect of the antibiotic beads, both the gamma irradiated and ethylene oxide sterilized beads, had not declined after 12 months of storage indicating that antibiotic beads can be kept at room temperature for 12 months without further sterilization. Both the sterilized vancomycin and fosfomycin beads were able to inhibit MRSA for at least 6 weeks. The mean inhibition zones of vancomycin and fosfomycin beads against MRSA after storage are shown in Figure 1 to 4.

Discussion

Both the vancomycin and the fosfomycin beads were able to inhibit MRSA after 6 and 12 months of storage. This study showed that gamma irradiated and ethylene oxide sterilized beads can be stored for up to 12 months before use. There were no statistically

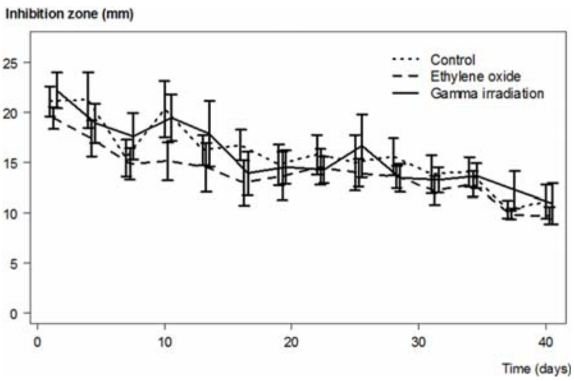

Figure 1. Comparison of vancomycin inhibition zone against MRSA between the control and two sterilized groups after 6 months of storage.

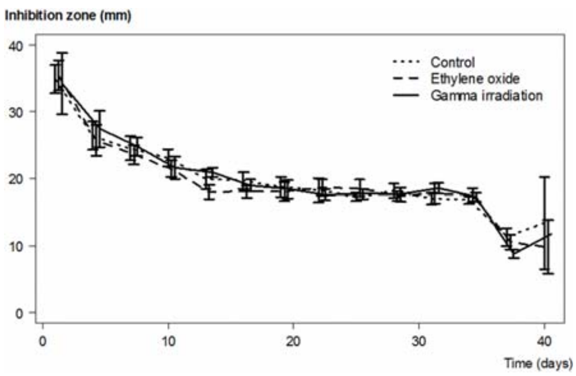

Figure 2. Comparison the fosfomycin inhibition zone against MRSA between the control and the two sterilized groups after 6 months of storage.

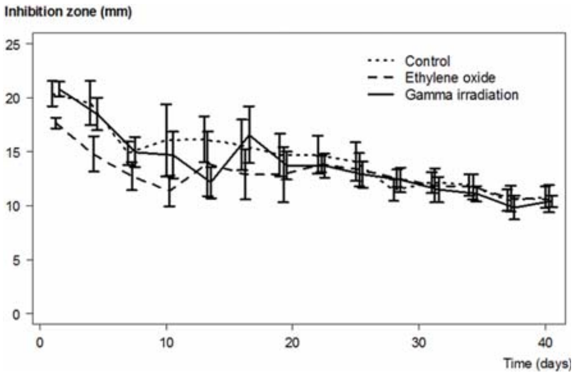

Figure 3. Comparison of vancomycin inhibition zone against MRSA between the control and the two sterilized groups after 12 months of storage.

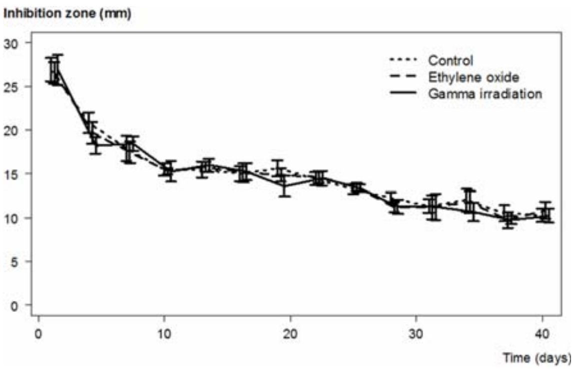

Figure 4. Comparison of fosfomycin inhibition zone against MRSA between the control and the two sterilized groups after 12 months of storage.

significant differences in the inhibitory effect on MRSA between the two sterilized groups and the control group with either the vancomycin or the fosfomycin beads after 6 and 12 months of storage.

Our study confirms the efficacy of ethylene oxide sterilization, a standard sterilization available in most general hospitals, of antibiotic beads for use against MRSA. Sterilization of unused beads or mass production of antibiotic beads can minimize operative time and reduce the cost of involved in preparing antibiotic beads for each individual patient.

A limitation of this study is that we could not compare the efficacy of vancomycin with fosfomycin. That comparison was not done because modified disc diffusion is a semi-quantitative technique which uses different cut-points with fosfomycin and with vancomycin. We decided to use a modified disc diffusion technique because it is a standard technique for evaluation of the efficacy of local antibiotic beads and because there is no available drug concentration measurement for fosfomycin. In additional, there was a linear correlation between the modified disc diffusion technique and drug concentration. These findings have clinical implications as ethylene oxide sterilization is a good option for sterilizing vancomycin and fosfomycin beads and is available in most general hospitals. It can reduce operative time and the cost of treatment due to the long shelf life of the beads which precludes the need to prepare new beads for each patient.

Conclusion

Either gamma radiation or ethylene oxide can be used as a sterilization method for antibiotic beads for MRSA and the beads can be stored at room temperature without deterioration of the antibiotic effect for up to 12 months.

What is already known on this topic?

Custom-made vancomycin and fosfomycin beads can be used effectively against MRSA. Antibiotic beads can be sterilized with either gamma irradiation or ethylene oxide.

What this study adds?

This study shows that both gamma radiated and ethylene oxide sterilized antibiotic beads can stored for up to one year without deterioration of effectiveness in inhibiting MRSA.

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Potential conflicts of interest

The authors declare no conflict of interest.

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