

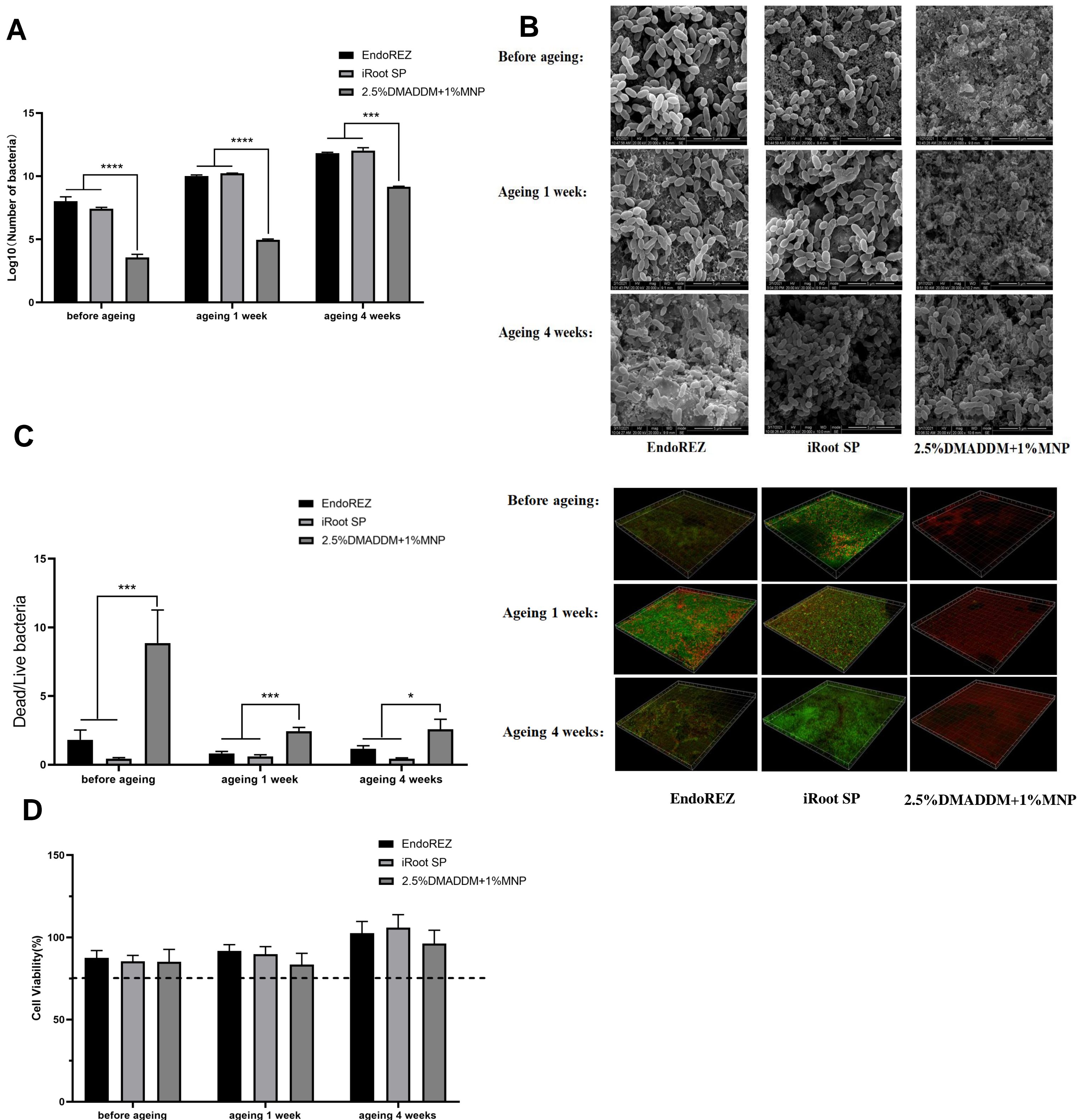
## INTRODUCTION

Periodontal apical periodontitis is a bacterial infectious disease that occurs in the pulp and tissues around the apex. At present, the most common treatment method in clinical is root canal therapy, and the key to success is to thoroughly remove the infectious substances in the root canal and to fill the root canal tightly. Single-cone technique (SCT) is increasingly used in clinical treatment due to its low technical sensitivity, short operation time, and strong anti-root fracture performance. Now, bioceramic sealers (such as iRoot SP) are the most common sealers used for SCT, but they have some disadvantages such as high solubility, non-lasting antibacterial properties, and intolerance to high temperature. In this experiment, Dimethylaminododecyl methacrylate (DMADDM) and Magnetic nanoparticles (MNP) were used to modify EndoREZ, a commercial root canal sealer. We did a study for its long-term antibacterial property, sealing property and permeability in Single-cone technique (SCT).

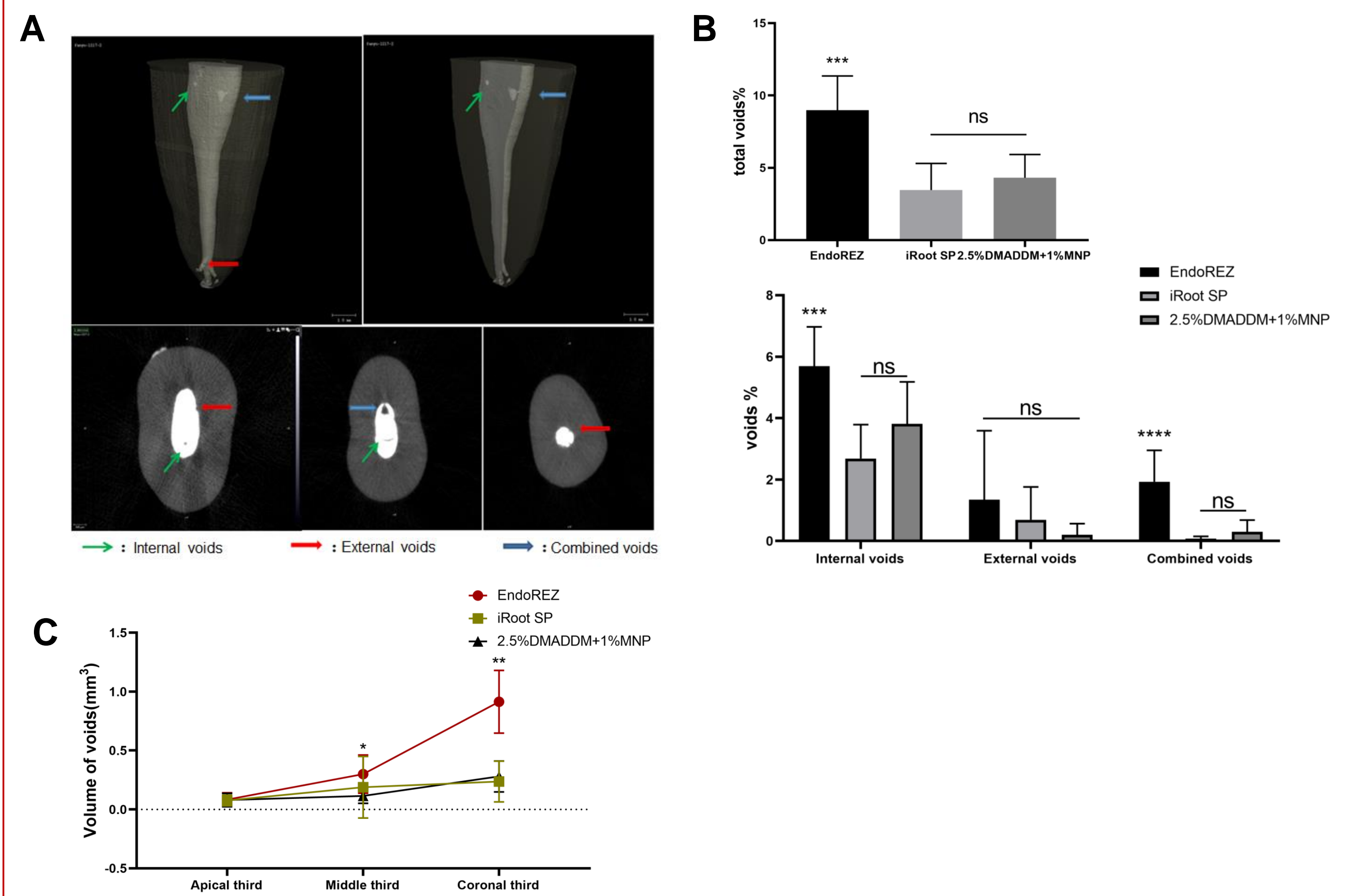
## MATERIALS & METHODS

- The long-term antimicrobial effects of modified root canal sealer were tested *in vitro* before aging and after aging 1 and 4 weeks by the single-strain enterococcus faecalis biofilm model.
- 30 freshly extracted human single-root maxillary premolars were selected and instrumented with nickel-titanium rotary file and the final file size was # 40/06. They were then randomly assigned into three groups according to the applied root canal sealers (modified root canal sealer, EndoREZ and iRoot SP) by SCT. Every specimen was then scanned using micro-CT to analyse void fraction and void volumes, scanned using Confocal Laser Scanning Microscope (CLSM) to study the dentin permeability in the apical, middle, and coronal thirds.

## RESULTS



**Figure 1.** The long-term antimicrobial effects of three sealers. EndoREZ, iRoot SP and modified root canal sealer (EndoREZ with 2.5%DMADDM and 1%MNP). (A) Colony-forming unit counts of biofilms formed on each root canal sealer disk before aging, and after 1 and 4 weeks of aging. Every values is shown as mean  $\pm$  SD (n = 4); (B) Representative images of biofilms by scanning electron microscopy (SEM); (C) Representative images and statistical analysis of biofilms (live bacteria - stained green; dead cells - stained red) in different groups; (D) Cytotoxicity assay of sealer eluents with mouse fibroblast. Every values is shown as mean  $\pm$  SD (n = 6). \* $p < 0.05$



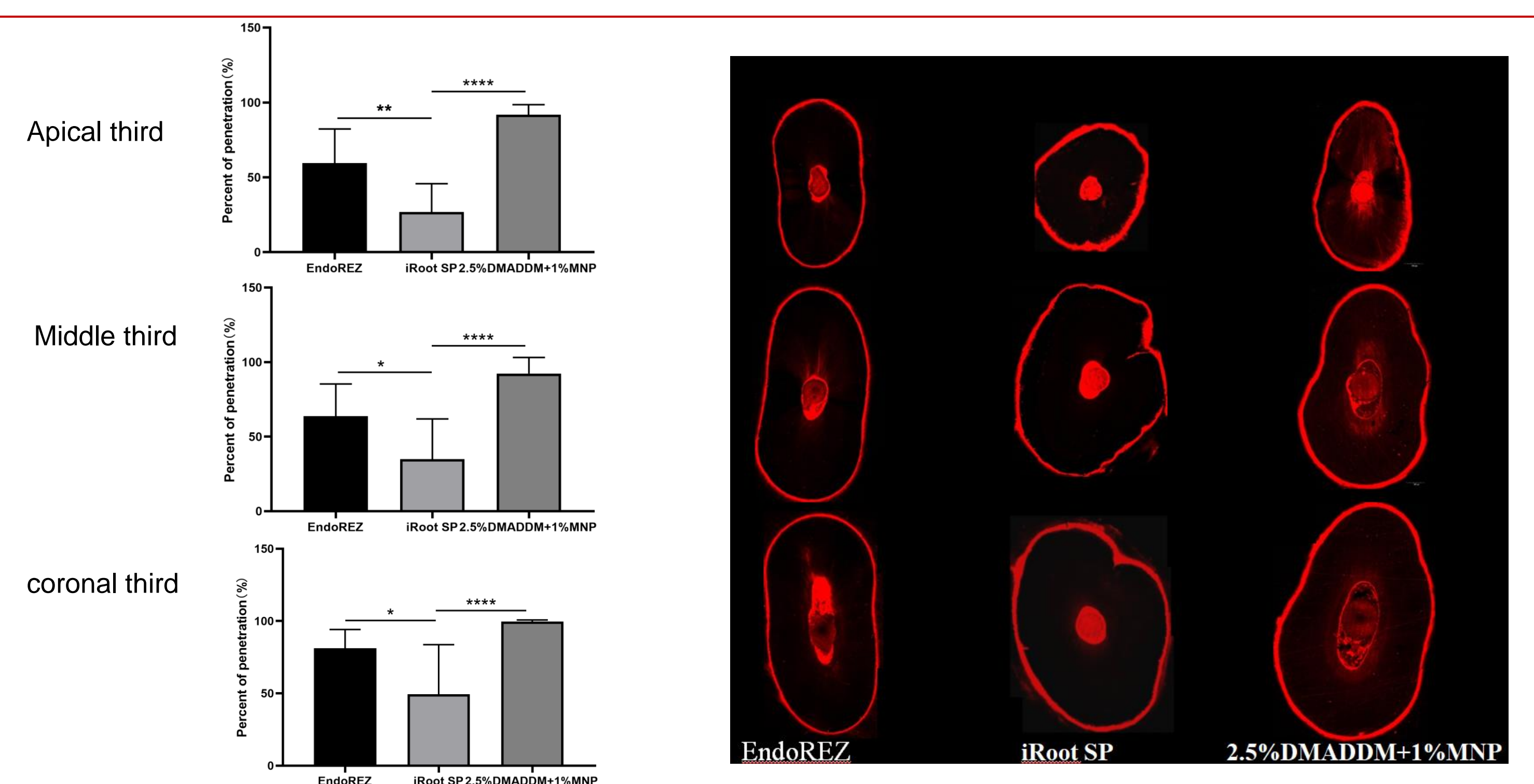
**Figure 2.** The sealing property of three sealers by Micro-CT. (A) Renderings of three different filling materials scanned with micro-CT (voxel size = 13 $\mu$ m) showing the 3D volumes of voids after the root canal filling treatment. (B) The void fraction (internal, external and combined) in the whole root canal filling materials. Every values is shown as mean  $\pm$  SD (n = 10); (C) The void volumes (apical, middle, coronal thirds). Every values is shown as mean  $\pm$  SD (n = 10). \* $p < 0.05$

**Table 1.** An overview of the root filling rate and the void fraction (internal, external and combined) in the whole root canal filling materials in micro-CT images

Root canal filling material	n	Mean(%)	SD	Statistical analysis
Root filling rate				<i>p</i> value Pairwise comparisons
2.5%DMADDM+1%MNP (1)	10	95.684	1.516	$P > 0.05$ 1-3
EndoREZ (2)	10	91.027	2.259	$P < 0.05$ 2-3
iRoot SP (3)	10	96.544	1.750	
Internal voids				<i>p</i> value Pairwise comparisons
2.5%DMADDM+1%MNP (1)	10	3.815	1.366	$P > 0.05$ 1-3
EndoREZ (2)	10	5.694	1.278	$P < 0.05$ 2-3
iRoot SP (3)	10	2.688	1.104	
External voids				<i>p</i> value Pairwise comparisons
2.5%DMADDM+1%MNP (1)	10	0.204	0.366	$P > 0.05$ 1-3
EndoREZ (2)	10	1.347	2.243	$P > 0.05$ 2-3
iRoot SP (3)	10	0.692	1.071	
Combined voids				<i>p</i> value Pairwise comparisons
2.5%DMADDM+1%MNP (1)	10	0.298	0.384	$P > 0.05$ 1-3
EndoREZ (2)	10	1.933	1.022	$P < 0.05$ 2-3
iRoot SP (3)	10	0.076	0.074	

**Table 2.** The void volume of the root canal filling materials in micro-CT images at different root regions (apical, middle and coronal thirds)

Region	Root canal filling material	n	Mean(mm <sup>3</sup> )	SD	Statistical analysis
Apical third	2.5%DMADDM+1%MNP (1)	10	0.080	0.057	$P > 0.05$ 1-3
	EndoREZ (2)	10	0.084	0.058	$P > 0.05$ 2-3
	iRoot SP (3)	10	0.076	0.045	
Middle third	2.5%DMADDM+1%MNP (1)	10	0.115	0.063	$P > 0.05$ 1-3
	EndoREZ (2)	10	0.300	0.162	$P < 0.05$ 2-3
	iRoot SP (3)	10	0.189	0.261	
Coronal third	2.5%DMADDM+1%MNP (1)	10	0.28	0.131	$P > 0.05$ 1-3
	EndoREZ (2)	10	0.914	0.266	$P < 0.05$ 2-3
	iRoot SP (3)	10	0.237	0.173	



**Figure 3.** The dentinal tubule penetration of three sealers by CLSM. Representative images and statistical analysis of dentinal tubule penetration (dentinal tubule penetration = A/B. A: The portions of the canal circumference in which tubule penetration was seen were measured and added; B: the total circumference of the canal wall). Every values is shown as mean  $\pm$  SD (n = 10). \* $p < 0.05$

## CONCLUSIONS

- Modified root canal sealer could effectively inhibit enterococcus faecalis biofilm.
- The voids of modified root canal sealer was not significantly different from the iRoot SP group, but was lower than the EndoREZ group. It suggested that the modified root canal sealer had good sealing performance.
- The permeability of the modified root canal sealant was significantly higher than that of the other groups. This indicated that the antibacterial sealant could enter deeper into the dentin tubule and kill bacteria located deep in the dentin tubule.