Bacteriological studies on endodontic paper points

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Four brands of endodontic paper points were subjected to tests for sterility, antibacterial activity, and suitability as vehicles for bacteriological sampling procedures. No brand showed growth in the sterility assay. One brand showed weak but reproducible antibacterial activity. Recovery of viable bacteria after absorption into the points varied and appeared to depend both on the brand of point and on the bacterial strain used. One brand appeared ineffectual for clinical bacteriological sampling procedures. □ *Antibacterial activity; bacteriological sampling; endodontics; sterility*

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Paper points serve several functions in endodontics: to dry root canals, to sample root canals for bacteriological studies, and, in some cases, to carry antiseptic dressings between sittings.

It is important that the points be sterile when used for any of the three purposes. Furthermore, to serve as vehicles for bacteriological samples, the points must not exert antibacterial properties possibly masking growth of bacteria infecting the root canal.

In the present work, four brands of endodontic paper points were subjected to conventional tests for sterility and for antibacterial activity. Moreover, the recovery of viable bacteria from contaminated paper points was evaluated in a simplified in vitro assay.

Materials and methods

Paper points

The brand names, manufacturers, description, and codes to be used in the text of the paper points tested are given in Table 1. The various brands were tested as delivered from the distributors.

Sterility assay

Tests for sterility were carried out in accordance with the U.S. Pharmacopeia XIX and as suggested for standard testing of endodontic paper points in the draft proposed by the International Organization for Standardization (ISO).

Two media were used, fluid thioglycollate medium (TG)(Difco) and tryptone soya

Table 1. Paper points tested

Name	Manufacturer	Size, type	Code
Absorbent points sterile	Johnson & Johnson East Windsor, N.J., USA	'Fine'	JJS
Pappersspetsar	Johnson & Johnson, Sollentuna, Sweden	'Coarse'	JJC
Absorbent paper points sterilized	A/S Norsk Dental Depot, Oslo, Norway	ISO Size 80	NDD
Absorbent paper points 'PD'	Produits Dentaire S.A., Vevey, Switzerland	'Coarse'	PD

broth (TSB)(Oxoid). Ten points of each brand were tested in the TG medium and 20 in the TSB medium. The TG medium with points and controls were incubated for 14 days at 32°C. The points in TSB medium were divided in two parts: one was incubated at 23°C and one at 37°C, both for 14 days. All tubes were inspected for growth at intervals during and at the end of the incubation period.

Gel diffusion test for antibacterial activity

Blood agar plates were flooded with whole saliva diluted 1:5 in phosphate-buffered saline. Duplicate paper points of each brand were placed on the seeded plates, which were then incubated for 24 h in an atmosphere of 95% N_2 and 5% CO_2 (GasPak; BBL). After incubation, the plates were inspected for inhibition of growth around any of the paper points.

Bactericidal effect

Three 1-g portions of each brand of paper points were cut in 1–2-mm pieces, and each incubated for 24 h in 2 ml saline. The ensuing extracts were filter-sterilized before use in the assay. *Streptococcus mutans* (OMZ 176) was grown overnight in Todd-Hewitt broth (THB)(Oxoid) and diluted 1:500 in sterile saline. To 1-ml portions of this dilute bacterial suspension were added 100 µl of paper point extract or saline as control. The extract/bacteria mixtures were incubated at room temperature under cover, and aliquots withdrawn at 15-min intervals over a 2-h period. The aliquots were tenfold serially diluted in saline, and the dilution series was seeded on blood agar plates, which were incubated for 24 h at 37°C in an $H_2 + CO_2$ atmosphere (BioMerieux). Any decline in the number of surviving bacteria in extract-containing mixtures during the 2-h incubation period was taken as a measure of bactericidal effect. Only two brands were tested with this procedure: JJS and PD.

Recovery of bacteria after absorption by paper points

Whole saliva was collected by chewing on Parafilm and was diluted 1:2 in reduced transport fluid (RTF) (1). Ten-microliter portions were dispensed into microtiter wells and absorbed (for 10 min) into duplicate points of each brand. Each pair of points was then transferred to screw-capped vials containing 4.5 ml RTF and 200 µl glass beads. Each vial was shaken for 30 sec, and the RTF was tenfold serially diluted, plated on blood agar plates and incubated for 24 h at 37°C in an $H_2 + CO_2$ atmosphere. Ten microliters of diluted saliva seeded directly into RTF served as a control. Duplicate or triplicate samples were tested, and each experiment run three times.

By substituting cells of *Strep. sanguis* ATCC 10556 or a laboratory strain of *Staphylococcus aureus* grown overnight in TSB for the diluted saliva, the same procedure was used to assess the recovery of these individual organisms.

A similar procedure was adopted for testing recovery of graded amounts of bacteria absorbed by the paper points. Whole saliva, centrifuged and diluted 1:2 in RTF as above, was further tenfold diluted in RTF down to 1:20,000. Ten-microliter portions of each dilution were absorbed by two paper points of each brand. After absorption, the points were aseptically transferred to screw-capped

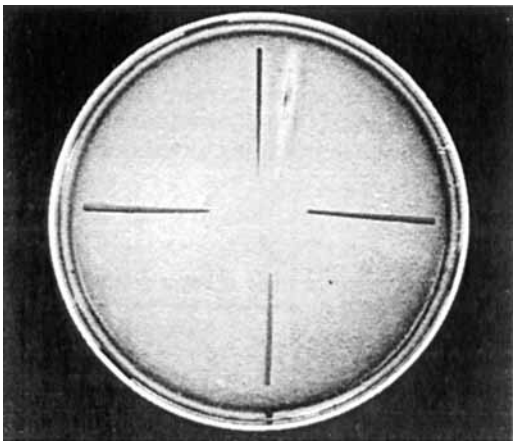

Fig. 1. Inhibition of salivary bacterial growth by gel diffusion of leachables from PD dental absorbent points (top). Clockwise from right: NDD, JJS, JJC. One point in each duplicate pair was removed for maximal visibility before photography.

tubes, each containing 4.5 ml TG medium, and incubated at 37°C for 14 days. For controls, 10-µl portions of salivary dilutions were inoculated directly into separate TG tubes. The tubes were inspected daily for growth. This experiment was carried out in triplicate.

Results

Sterility

No tube showed growth in the sterility assay.

Antibacterial activity

One brand, PD, showed a small but reproducible and clearly distinguishable zone of inhibition (Fig. 1). No inhibition of growth was observed for the other three brands tested.

Bactericidal effect

No bactericidal effect was observed for either of the two brands (PD and JJC) tested.

Recovery after absorption

With whole saliva, recovery of bacteria was always smaller from paper points than from controls (Fig. 2). Recovery from JJS was about 45%, from JJC 15–50%, from NDD 20–40%, and from PD 1–12%.

With *Strep. sanguis*, a similar pattern was observed (Fig. 3). The recovery from PD was nil in two out of three runs. Species differences were indicated, in that recovery of *Staph. aureus* from PD was greater and exceeded that from NDD in all experiments (Fig. 4).

Fig. 5 shows the results of recovery from the dilution series absorbed by the points. The heaviest inoculum contained 4×10^5

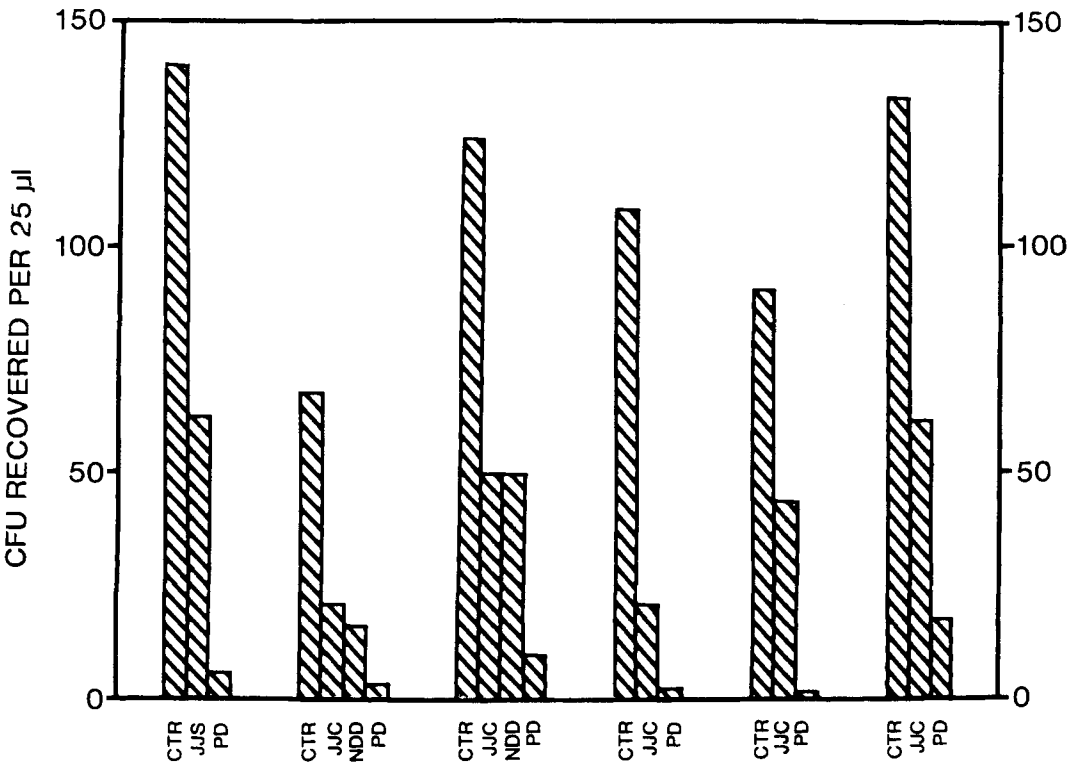

Fig. 2. Recovery of viable bacteria from 25 µl of whole saliva diluted 1:2 in RTF. CTR: recovery after direct inoculation of diluted saliva; JJS, JJC, NDD, PD: recovery after absorption onto paper point brands.

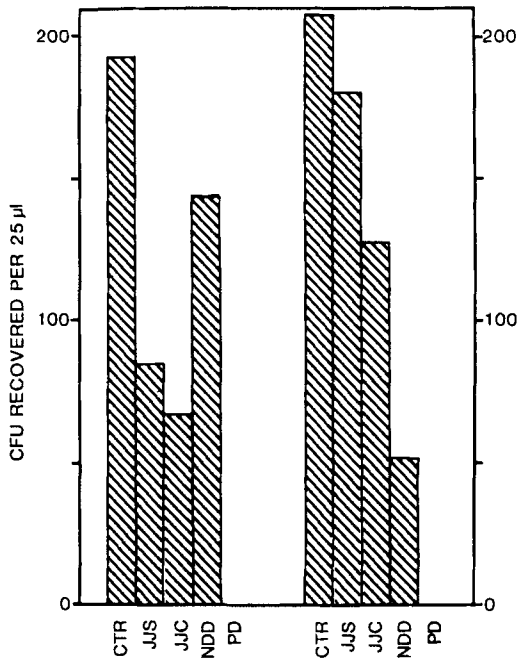

Fig. 3. Recovery of *Strep. sanguis* from paper points.

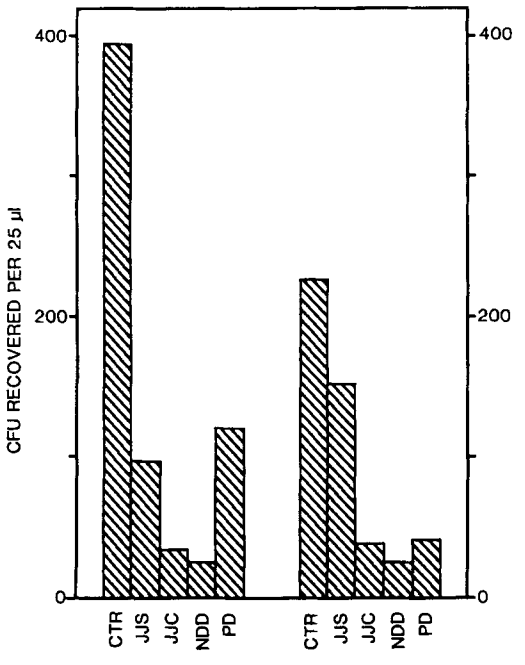

Fig. 4. Recovery of *Staph. aureus* from paper points.

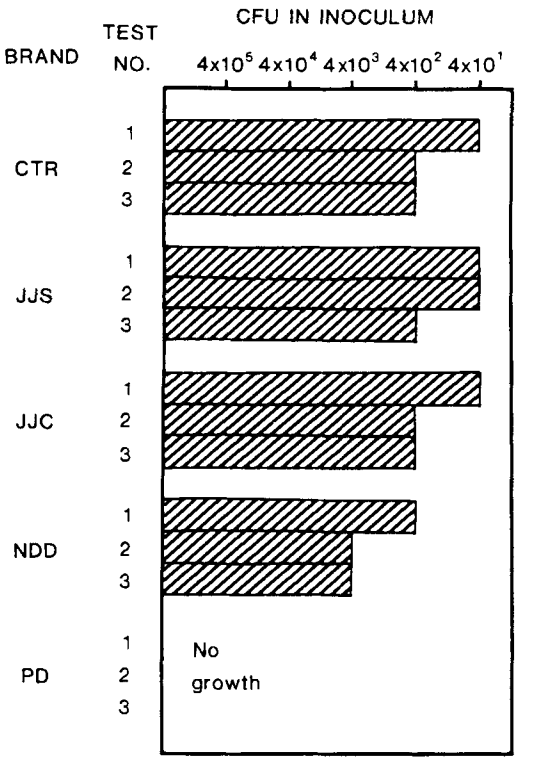

Fig. 5. Growth of salivary bacteria in increasing dilutions after absorption onto paper points. CTR: direct inoculation.

colony-forming units (CFU) of salivary bacteria. Again, PD was conspicuous in that no growth was seen even for this large number of organisms. NDD required 10 times as many bacteria as the control, whereas JJS and JJC gave growth at dilutions similar to the controls.

Discussion

The results from the sterility test documented adequate sterility in all brands, which is reassuring for the clinical usage of the paper points. The requirement for aseptic handling of the points in clinical practice naturally prevails, inasmuch as the present study was carried out with samples from previously unopened packages obtained directly from the distributors.

The experiments on the antibacterial

activity of and recovery of organisms from the paper points were aimed at clarifying the function of the points in bacteriological sampling procedures. One brand, PD, showed indications of antibacterial properties in the gel diffusion test. This indicates that some leachable, antibacterial substance is incorporated in the points. However, the antibacterial activity was not sufficiently strong to kill *Strep. mutans* in the test for bactericidal activity. On the other hand, this same brand has been shown, in previous experiments, to exhibit cytotoxic as well as hemolytic activity (2).

The experiments on recovery of bacteria after absorption into paper points indicated a variable but significant recovery of mixed salivary bacteria as well as of pure cultures. Again, the PD brand was exceptional in that recovery was usually less than from other brands. In two out of three experiments with *Strep. sanguis*, recovery was nil from PD. This may have one or both of two explanations. The antibacterial activity observed in gel diffusion may have reduced the number of the originally viable organisms absorbed by the points. On the other hand, retention of absorbed organisms may be greater for PD than for the other brands, causing a smaller number of bacteria to be released during shaking in the transport medium.

The experiment with absorption of a dilution series of salivary bacteria was designed to simulate the clinical procedures usually applied for bacteriologic sampling of root canals. The clinical approach is a qualitative one; that is, the results are scored either as growth or no growth from the root canal sample. From the present results, it would appear that some 40 to 400 salivary organisms is the minimum number of bacteria to give growth. JJS and JJC afforded adequate recovery, and NDD also gave growth at rather high dilutions. On the other hand, PD points would appear ineffectual for this clinical usage, since no growth was observed even for inocula as large as 4×10^5 CFU.

It is possible that recovery of specific endodontic pathogens may show a different behavior with the paper points tested. However, in the absence of information on specific pathogens, the present data indicate that some brands of paper points may be ineffectual in bacteriologic sampling procedures from root canals.

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