

# Microbial Indicators Related to the Management of Advanced Dentinal Caries



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## Introduction

Successful management of advanced caries remains a significant challenge in clinical dentistry. Today's standard treatment for a deep carious lesion is to remove all the affected dental tissue, causing great weakening of the remaining tooth structure.

But is this complete caries removal really necessary or could affected dentine undergo healing/repair under the right circumstances? We are trying the concept of Minimal Intervention by leaving infected matrix behind and sealing the cavity. In order to gain dental tissue repair, an arrest of bacterially-induced tissue destruction would be necessary.

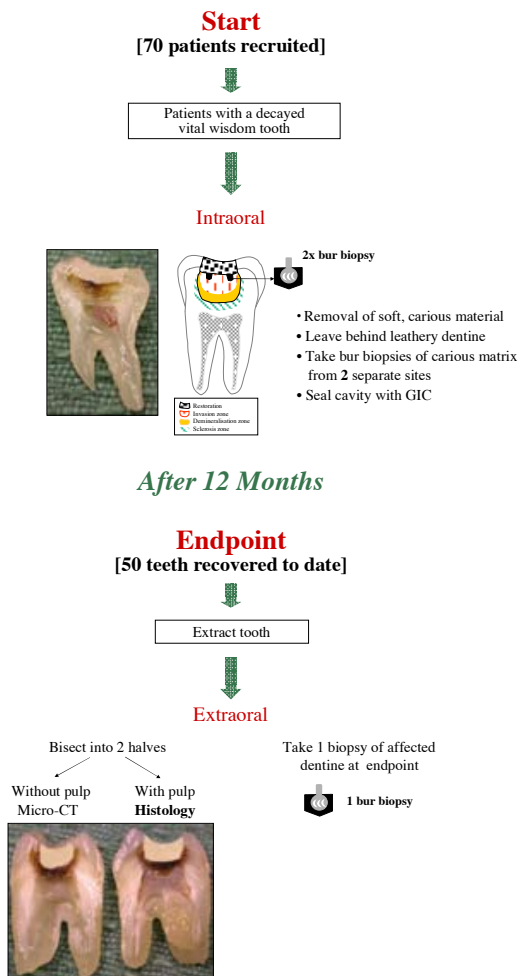
Whether bacteria left in the lesion will fail in their metabolism, preventing progression of the lesion OR Whether they might persist, progress and cause pulpal infection depends on the microbial composition?

The hypothesis to be tested is that depending on the bacterial species present, some lesions will have a more favourable outcome and will respond better to conservative management than others.

## Materials and Methods

- Bacterial DNA-extraction from biopsies taken from the carious lesion
- PCR analysis of bacterial DNA to disclose 9 major groups detected in advanced caries
- Extraction and vertical bisection of the study teeth into 2 halves, one half containing the pulp
- Tooth half with pulp is prepared for histology: fixation, decalcification, embedding in resin
- Slides stained for histopathological analysis of the pulp and the dentinal lesion using FISH

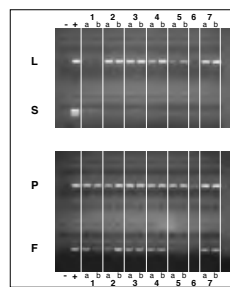
## Clinical Study



## Results

A. In preliminary trials biopsy pairs (see methods) were demonstrated to represent the microbial load of the lesion as shown below:

- bacterial DNA is extracted from each biopsy
- DNA is amplified by PCR to disclose the 9 major groups of bacteria detected in advanced carious lesions



Full cycle Polymerase Chain Reaction (PCR) results, showing presence / absence of 4 bacterial genera in a carious lesion

- L=Lactobacillus
- S=Streptococcus
- P=Prevotella
- F=Fusobacterium

Correspondence between the paired biopsies taken before restoration is > 90%

The amplicons detected in pre-restoration biopsies will next be compared to amplicons detected in the corresponding endpoint biopsy in order to detect changes in the microflora as a result of the treatment.

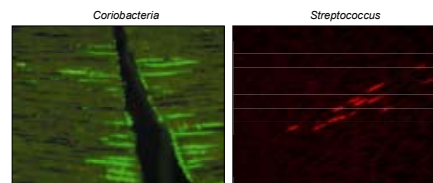
B. Tooth half containing the pulp

- will be processed for histology
  - serial sections will be cut to perform histopathological analysis
- of the dentinal lesion and its microbial load and of the pulp

To detect the different microbial species we use Fluorescent *in-situ* Hybridization (FISH). With FISH we can:

- correlate bacteria from within the carious dentine from pre-restoration biopsies to the end point situation
- detect and define tissue changes
- use high resolution histopathology to determine the status of pulpal responses

### Staining with FISH



FISH is used to detect all present and live bacteria. Specifically coloured probes are used to stain different types of bacteria. Here *Coriobacteria* are detected by a green fluorescent probe, *Streptococcus* by a red one.

C. Findings

Of the 70 patients treated only 4 patients presented with signs of irreversible pulpitis. These teeth were extracted at the time and processed. No adverse symptoms have been reported by the other 66 patients up to the nominated endpoint.

## Conclusion

We are aiming to find a pattern of behaviour that is related to the specific types of bacteria; that is a relationship between the character and abundance of bacteria and the tissue response they provoke.

Completion of the present analysis will define those bacterial consortia associated with favourable response to conservative management and those groups that lead to unfavourable response and progression of the lesion.

A successful outcome will provide the basis for development of chair-side diagnostic tests to inform the clinician of the best treatment choice.

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