LAB®-TEST 2: MICROFLORA AND PERIODONTAL DISEASE

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Periodontitis represents a destructive chronic inflammatory disease with a bacterial infection resulting from the complex actions of a small subset of periodontal pathogens (1).

From a pathological point of view, periodontitis can be defined as the presence of gingival inflammation at sites where there has been a pathological detachment of collagen fibres from the cementum and the junctional epithelium has migrated apically (2). The inflammatory response of the periodontal tissues to infection is influenced by environmental factors as well as by genetic factors (3). However, the resulting inflammatory events associated with connective tissue attachment loss lead to the resorption of coronal portions of tooth supporting alveolar bone (4).

The primary microbial factor contributing to periodontitis is a shift in the content of the oral microflora, while the primary immunological factor is the destructive host inflammatory response (5).

The microbiota associated with periodontal health and disease has been intensely studied for well over a century by several generations of skilled scientists and clinicians (6, 7). Oral microbiota is an enormously complex and dynamic entity that is profoundly affected by perpetually changing local environments and host-mediated selective pressures (8). The presence of a commensal microbiota, including potential pathogens, is essential for the proper development of mucosal immunity (9).

The microorganisms live in hard-to-study biofilms comprising organized polymicrobial communities attached to biotic or abiotic surfaces and adapted to thriving and surviving in the multiple micro-ecosystems of the oral cavity (5, 8). As a surface becomes colonized with individual cells, the bacteria form microcolonies, which then secrete a sticky extracellular polymeric substance that helps the bacteria adhere to the surface. Consequently, the biofilm matures by becoming larger and taking on a distinctive architecture (10). In the absence of mechanical or chemical removal of oral bacteria this biofilm can consequently lead to gingival infections, periodontitis and loss of alveolar bone and teeth.

The normal oral flora is hence in a balance between pathogens and commensals that requires regular cleaning to be maintained. A decrease in oral hygiene is quickly followed by the build-up of oral biofilms on tooth surfaces and, if left untreated, will progress to gingival inflammation and possibly periodontitis, alveolar bone loss and loss of teeth. It is likely that differences in host-defence mechanisms, including antimicrobial protein profiles, determine whether bacterial colonization progresses to overt disease (11).

Recent data estimates that the oral cavity may contain up to 19 000 bacterial phylo-types (12), but each individual will only have a rate of the total numbers of pathogens. Indeed, there is a substantial diversity in the content of the microflora between individuals (13) and between different oral sites within the same individual (14, 15). Research has indicated that dietary changes combined

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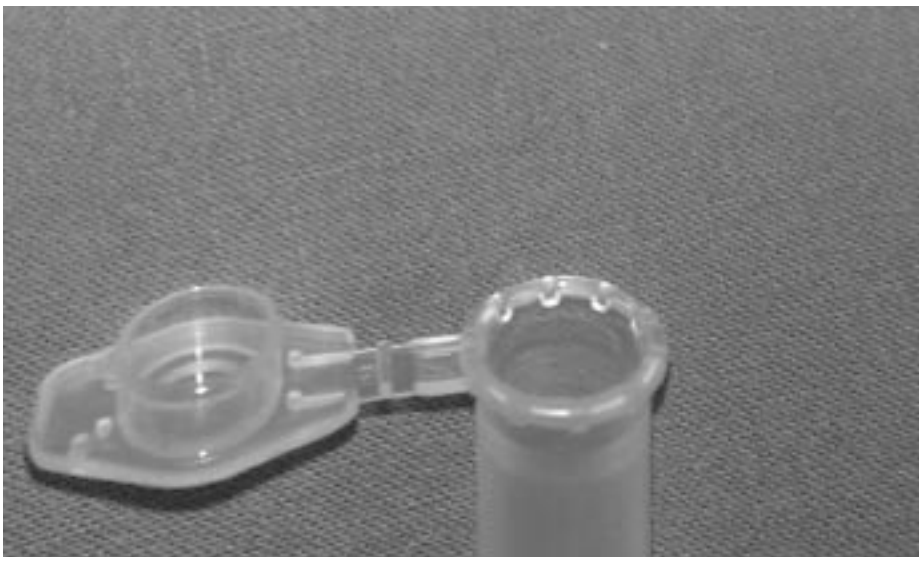

Fig. 1. *Microtube and coned shaped paper for crevicular fluid collection*

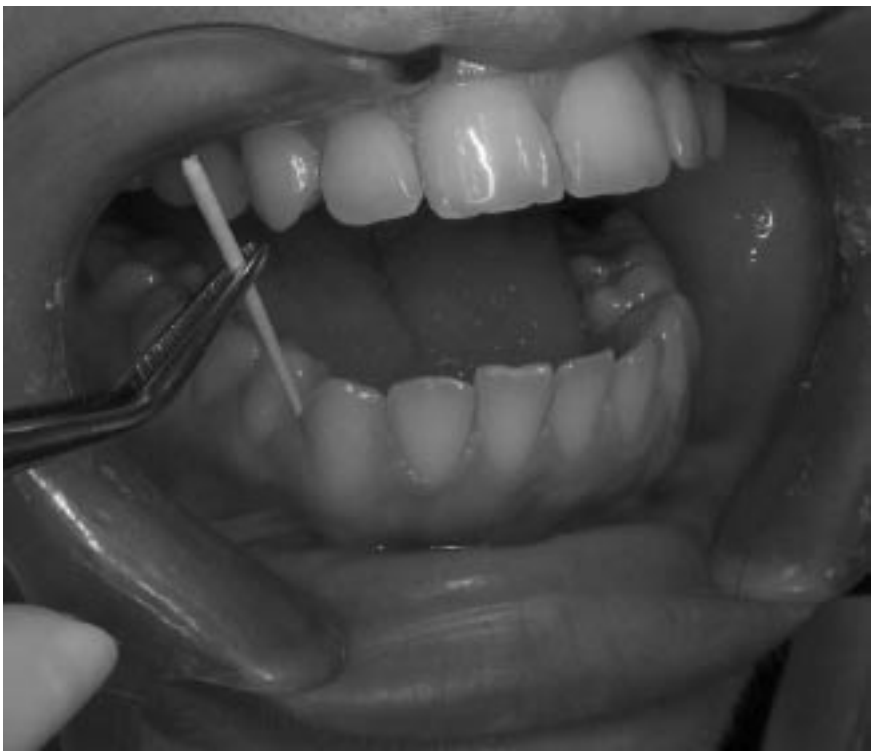

Fig. 2. *Collection of crevicular fluid in a patient affected by periodontitis*

with poor hygiene can cause a shift in the composition of the oral bacteria (15, 16). Moreover, some evidence in recent studies suggests that the oral microbiome changes as humans age and the dysbiosis in the oral cavity can lead to periodontitis (5).

Several methods have been used for microbiological testing in periodontitis (17). However, many techniques have not been fully accepted due to low sensitivity or specificity, moreover sometimes they are slow, expensive and laborious. In our laboratory (LAB SRL, Codigoro,

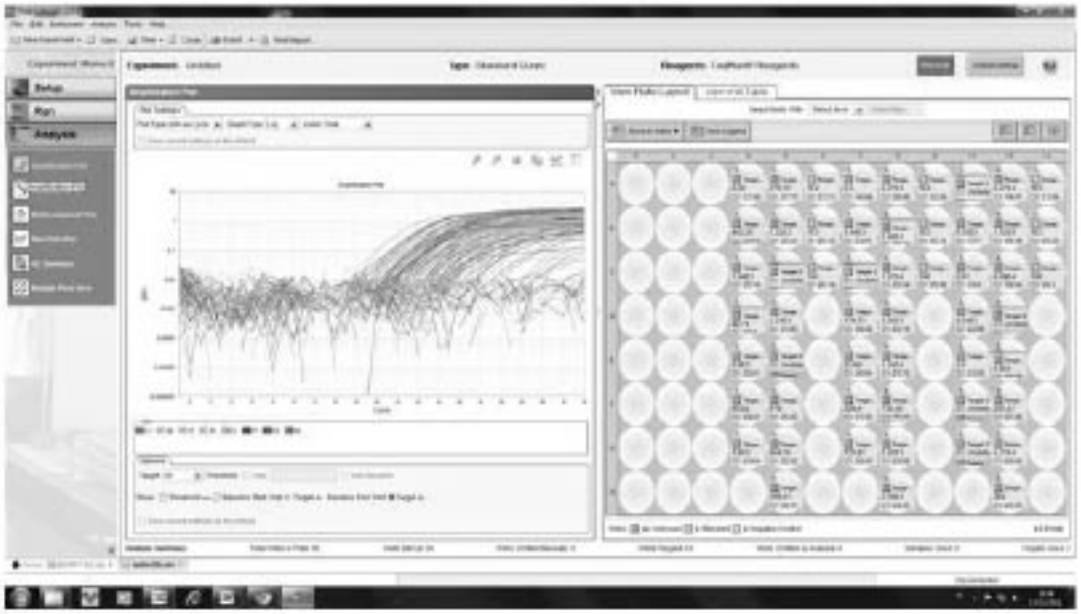

Fig. 3. *Bacterial analysis in PCR- Real Time.*

Ferrara, Italy), we developed an analysing strategy based on the detection of eight bacterial species isolated in the crevicular fluid of patients affected by periodontitis (Fig. 1 and 2): *Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythia*, *Treponema denticola, Eubacterium saphenum, Atopobium rimae, Porphyromonas endodontalis, Treponema lecithinolyticum* and *Capnocytophaga ochracea.*

Both *P. gingivalis* and *T. denticola* occur concomitantly with the clinical signs of periodontal destruction. They appear closely 'linked' topologically in the developing biofilm, shown an in vitro ability to produce a number of outer membrane-associated proteinases and are considered the first pathogens involved in the clinical destruction of periodontal tissues. Moreover both them and *T. forsythia,* show an higher prevalence in disease than in health suggesting that these bacterial are associated with the local development of periodontitis (18). *P. gingivalis* and *P. endodontalis* belong to the genus *Porphyromonas* and it has been suggested that they are key pathogens in adult periodontitis and in root canal infections, respectively (19).

A. actinomycetemcomitans is a Gram negative cocobacillus strongly associated with aggressive periodontitis, but it can also be found in patients with chronic periodontitis or in healthy individuals (12). The disease onset dependens on *A. actinomycetemcomitans* virulence and host susceptibility (20). *C. ochrace,* a non-sporing, microaerophilic, Gram negative bacillus, *has* also been recognized as pathogen of periodontitis based on its presence and relative numbers in healthy *vs.* diseased sites (21-23). *E. saphenum* and *A. rimae,* are both anaerobic Gram positive bacteria but the first is associated with chronic periodontitis whilst the latter is more prevalent in periodontally healthy subjects than in subjects with the disease (21). *T. lecithinolyticum* belongs to the phylogenetic group of oral spirochetes that are frequently found in chronic and aggressive periodontitis.

The presence and the level of these pathogens can be effectively revealed carrying out a Real Time analysis using bacterial species-specific primers and probes.

Our findings support the hypothesis that quantification of bacteria in biofilm microflora could be an appropriate tool for the diagnosis and prognosis of periodontitis.

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