

DEFENCE MECHANISMS OF GINGIVA

Dr. Parag Hadge



- What do you mean by defense mechanisms?

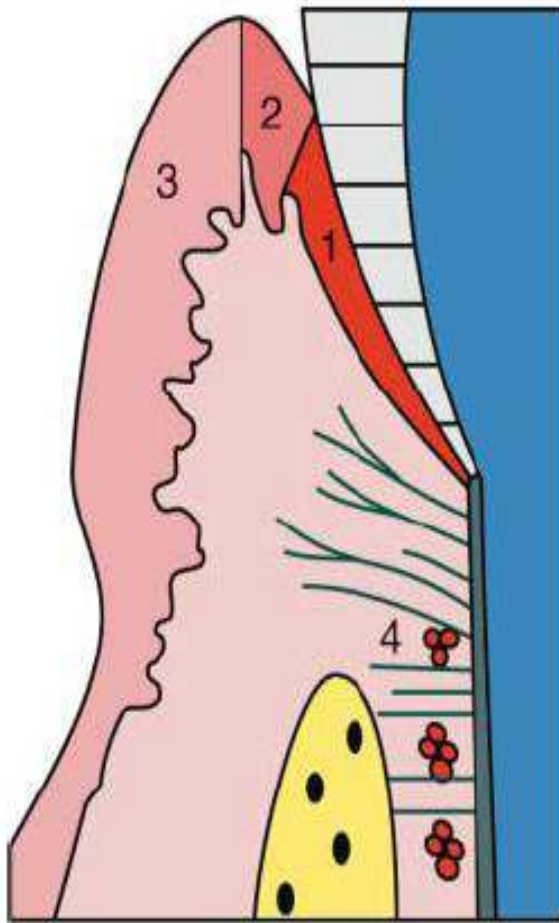
Defense mechanisms of gingiva

- Gingival epithelium
- Gingival crevicular fluid
- Leukocytes
- Saliva

Gingival epithelium

- The outer/ oral, sulcular and junctional epithelia are constantly renewing.
- Continuous desquamation of both the oral, sulcular and junctional epithelia may contribute to **the removal of bacteria**

Junctional epithelium



Dento-gingival junction

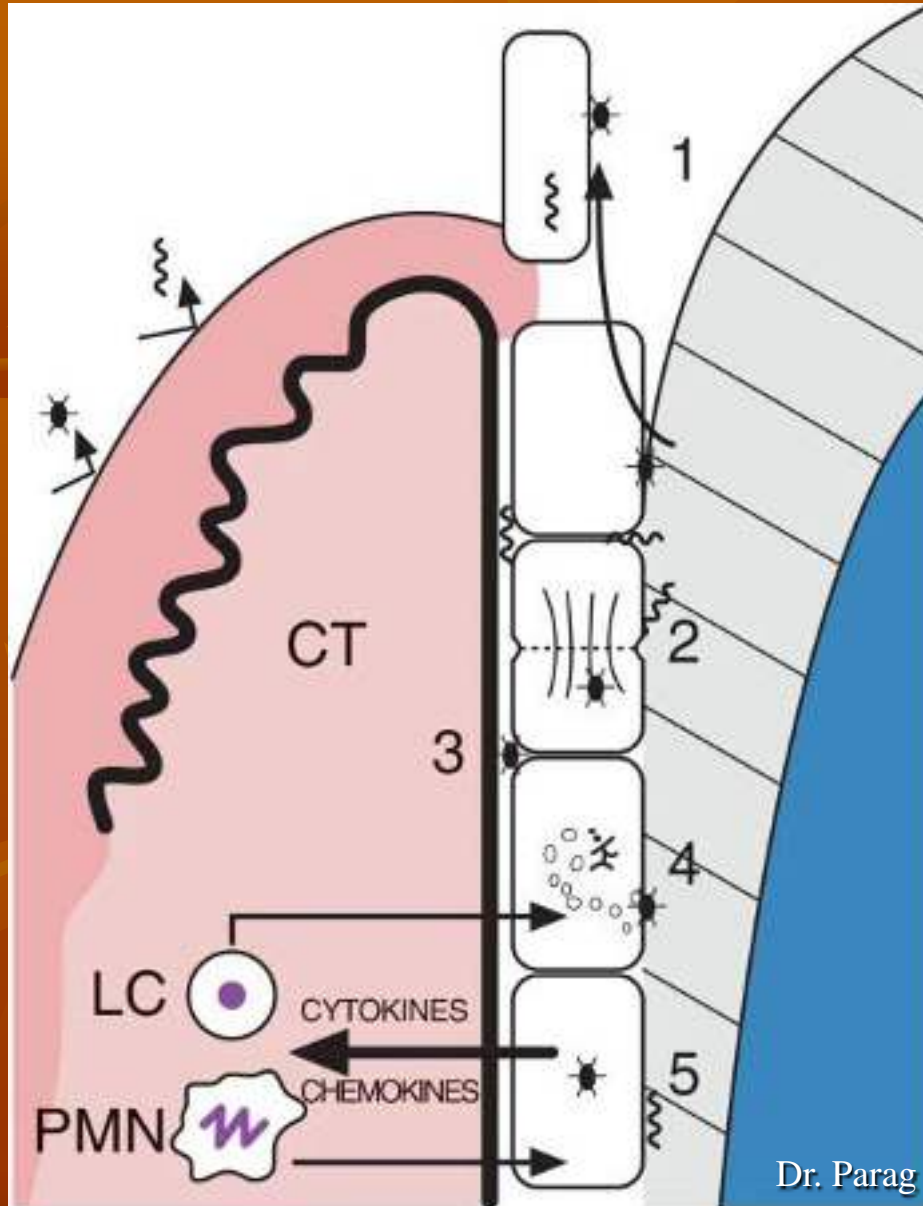
1. Junctional epithelium (JE)
2. Sulcular epithelium
3. Oral epithelium
4. Epithelial rests of Malassez

JE functions

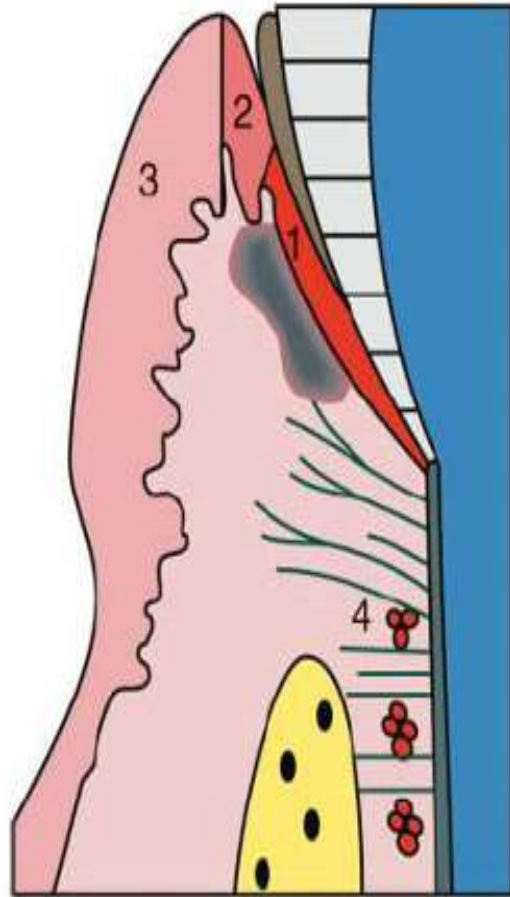
- attachment to tooth
- barrier
- rapid turnover
- antimicrobial defence
- GCF flow

The junctional epithelium (JE) exhibits a distinct phenotype that allows the tissue to attach to the tooth surface and participate in the host defence in a number of ways.

antimicrobial mechanisms



- quick cell exfoliation due to rapid turn over (1)
- Funneling effect (2)
- the (external) basal lamina forms an effective barrier (3)
- Production of antimicrobial substances, chemokines and cytokines (4)(5)



Problems in JE

- permeability
- degeneration
- detachment
- lateral and apical proliferation

The junctional epithelium (JE) is repeatedly or continually exposed to bacterial challenges, which may lead to the failure of the junctional epithelium and conversion of the gingival sulcus into a periodontal pocket.

GINGIVAL CREVICULAR FLUID

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History

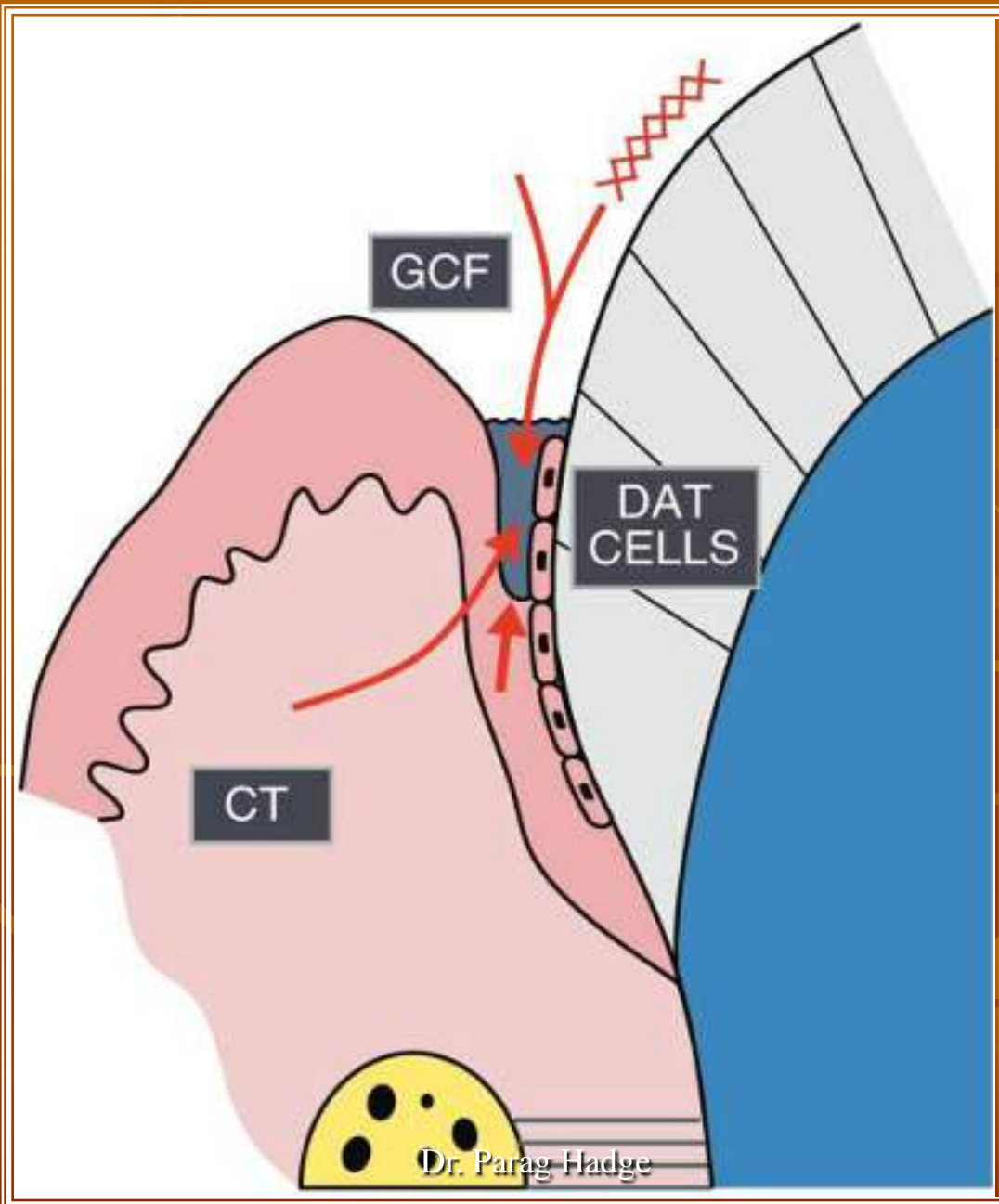
- Pioneer work by **Waerhaug and Brill** and **Krasse** in 1950's

- *Waerhaug (1950's)*: focused on the anatomy of the sulcus and its transformation into a periodontal pocket.
- Detected Fluid by *Serendipity*

- *Brill et al. (1962)* : physiology of GCF formation and its composition.
- This indicated passage of fluid from bloodstream into the sulcus by fluorescein dye.

- GCF, its constituents participate in the normal maintenance of function of the junctional epithelium. **(Adhesive function)**
- During inflammation the GCF flow increases. The increased GCF flow contributes to host defense by flushing bacterial colonies and their metabolites away from the sulcus. **(Flushing action)**

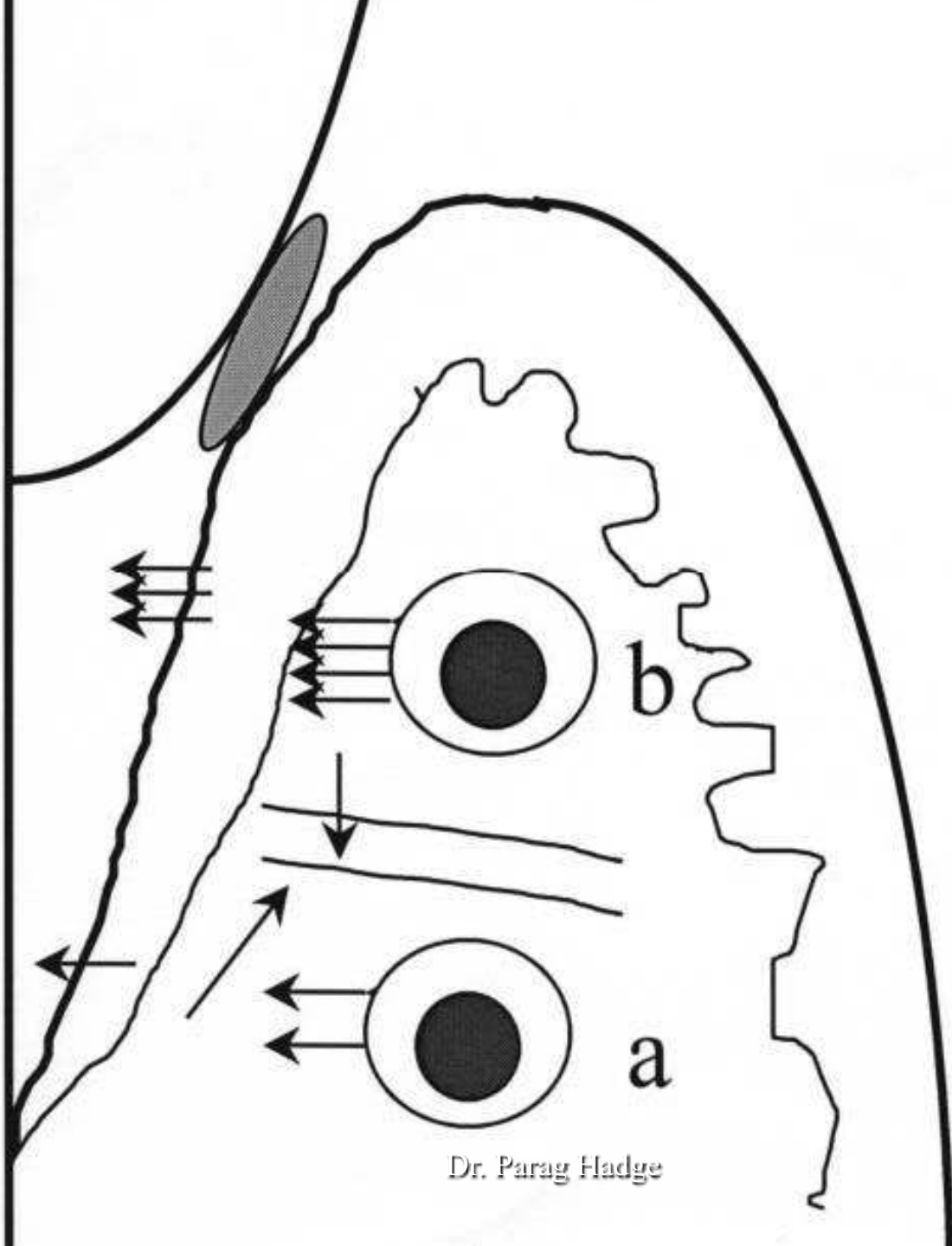
- provides sufficient **nutrients** for the DAT cells to grow. **(Nutritive Function)**
- Indeed, the GCF contains a wide variety of biologically active molecules with the potential capacity to affect the growth oral bacteria. **(antimicrobial action)**



GCF transudate or exudate?

- GCF production is governed by the passage of fluid from capillaries into the tissues (capillary filtrate) and the removal of this fluid by the lymphatic system (lymphatic uptake).
- When the rate of capillary filtrate exceeds that of lymphatic uptake, fluid will accumulate as edema and/or leave the area as GCF.

- The first studies by **Alfano** suggested that, at a clinically healthy gingival crevice, bacterial plaque would result in the accumulation of **high molecular weight molecules**.
- Here they would accumulate and produce an **osmotic gradient** which would induce the flow of interstitial fluid from the connective tissue to the gingival sulcus.



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- Thus, the ***initial fluid accumulation represents a transudate*** of interstitial fluid produced by an osmotic gradient and that the ***later fluid represents a true inflammatory exudate.***

- GCF protein concentration from inflamed gingivae - similar to that of serum - an inflammatory exudate of serum.
- GCF from healthy gingival crevices - similar to interstitial fluid.

*Methods of collection
and
measurement of the volume of GCF*

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- Gingival washing methods
- Capillary tubing or micropipettes
- Absorbent filter paper strips
- Preweighed twisted threads

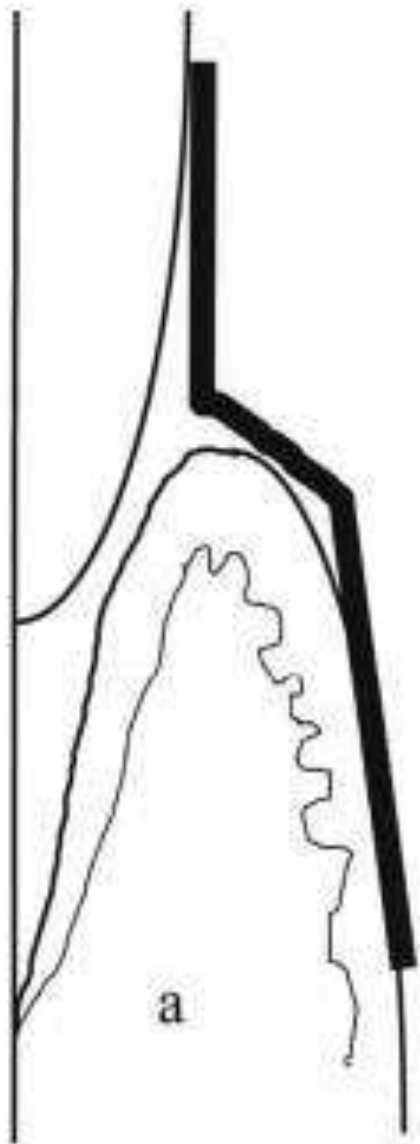
Absorbent filter paper strips

The **methods of collection** may be broadly divided into the –

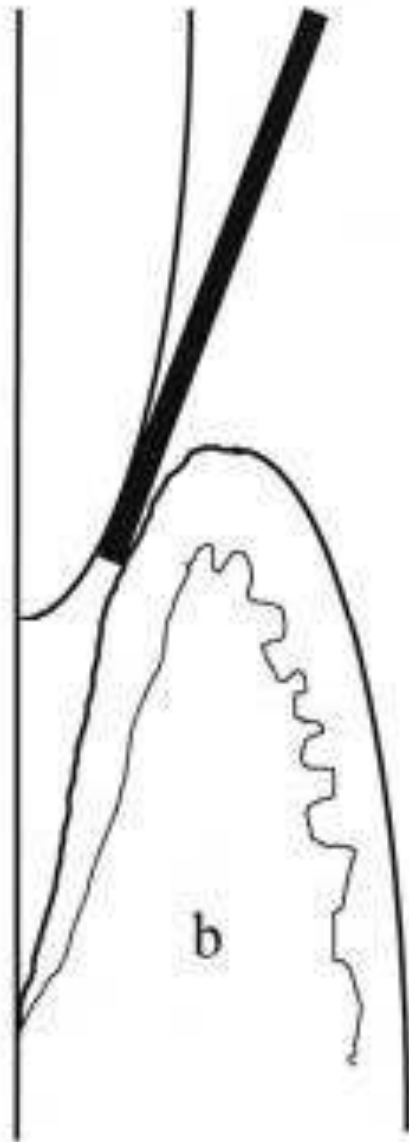
■ **Intracrevicular** :

- the strip is inserted just at the entrance of the crevice or periodontal pocket or
- whether the strip is inserted to the base of the pocket or ‘until minimum resistance is felt.’

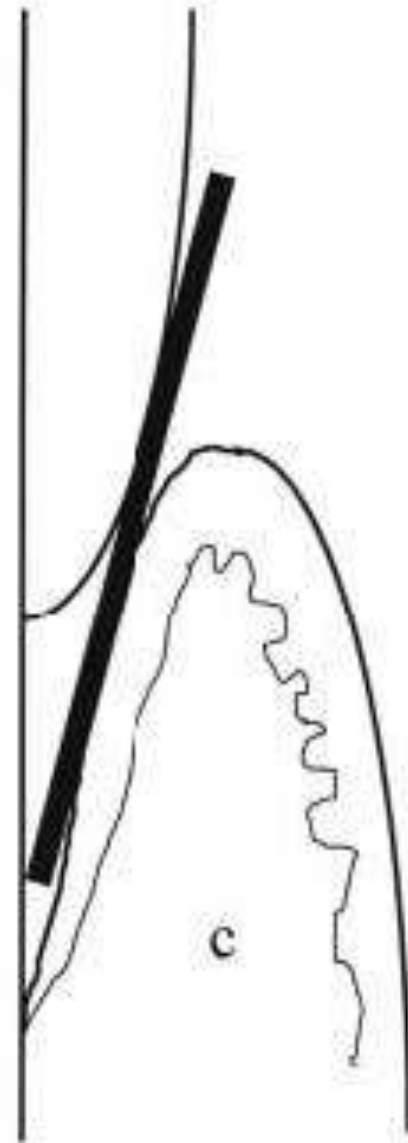
■ **Extracrevicular** : the strips are overlaid on the gingival crevice region in an attempt to minimize trauma.



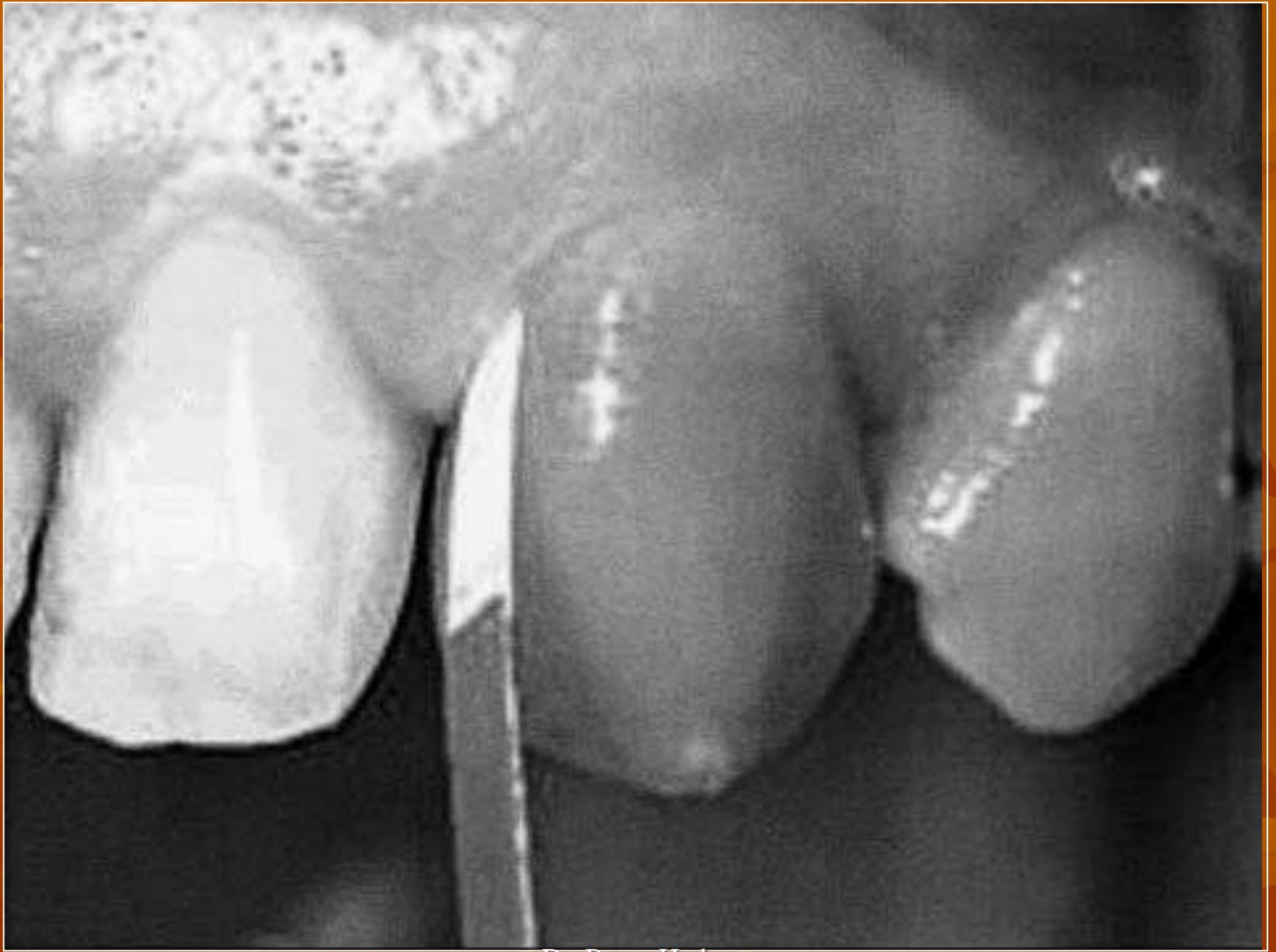
a



b



c



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The advantages :

- it is quick and easy to use
- can be applied to individual sites
- least traumatic.

Methods of estimating the volume collected

- by the distance the fluid migrated up the strip. **(simple linear measurement)**
- by assessing the **area of filter paper** wetted
- by staining the strips with **ninhydrin** to produce a purple color.
- **2g fluorescein** given systemically, 2hours prior to the collection of GCF, following which the strips were examined under ultraviolet light.

- The staining techniques discussed above have a number of disadvantages:
 1. not easily applied at the chairside.
 2. variation as a result of evaporation.
 3. prevents further laboratory investigations.

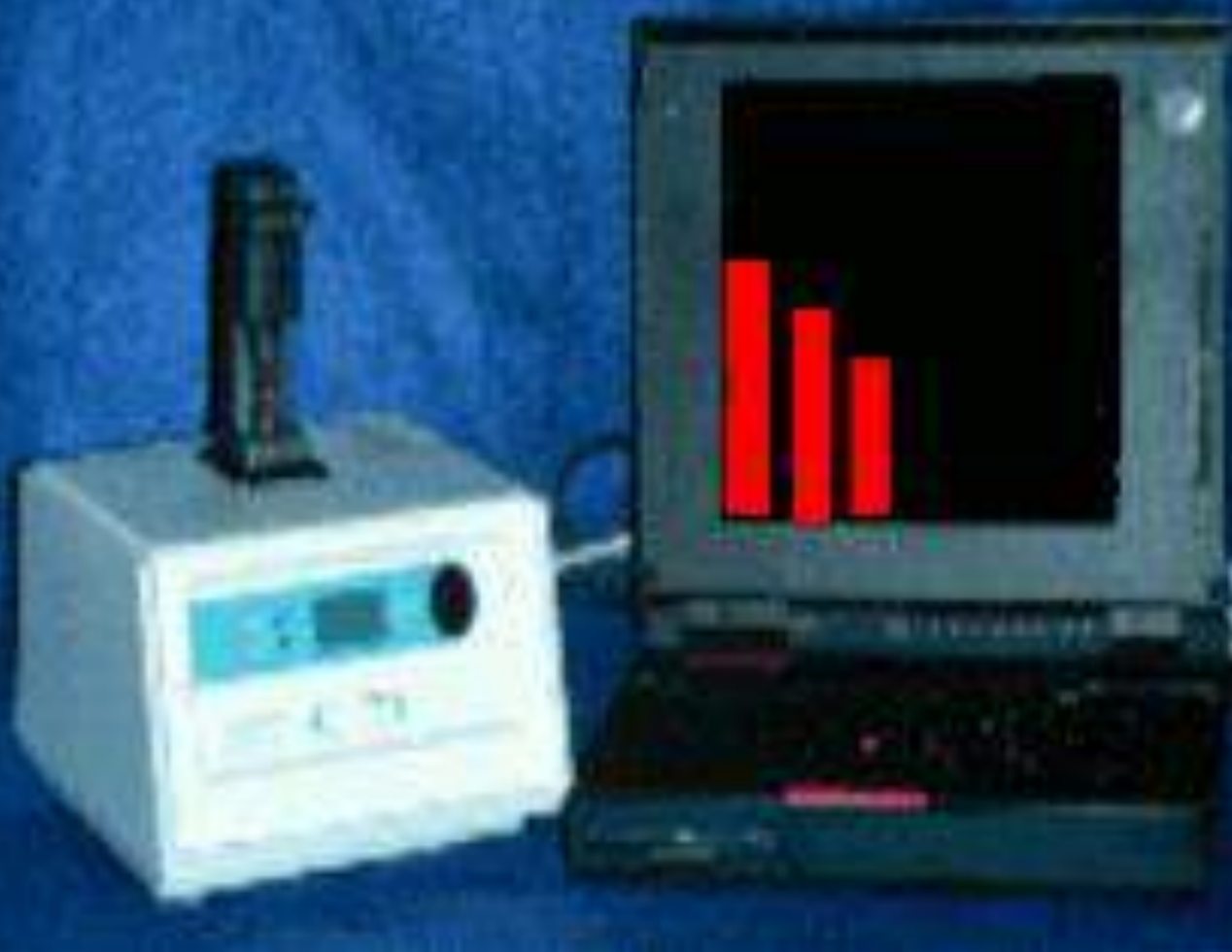
PERIOTRON 8000



Three
models of
Periotron® :
600, 6000
and now
the 8000

- **Periotron[®]**: electronic measuring device, allows accurate determination of the GCF volume and subsequent laboratory investigation.
- measures the effect on the electrical current flow of the wetted paper strips.

PERIOTRON SOFTWARE



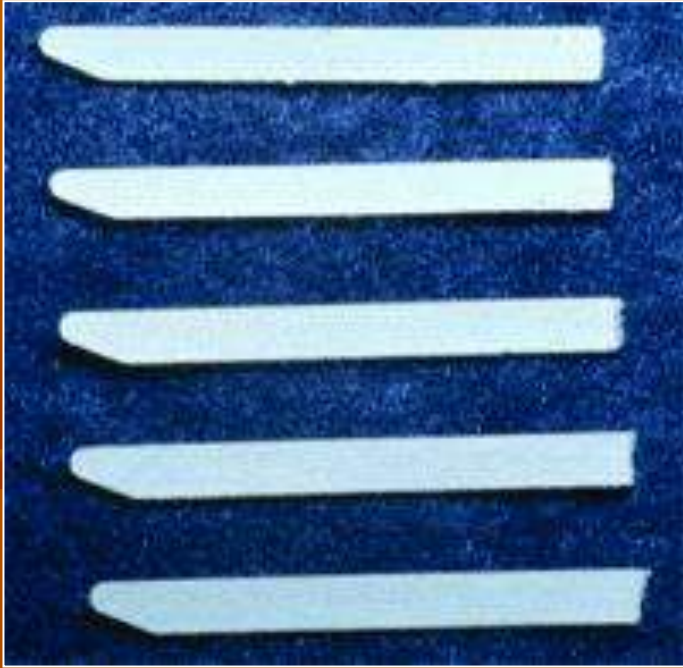
Limitation:

its inability to

measure

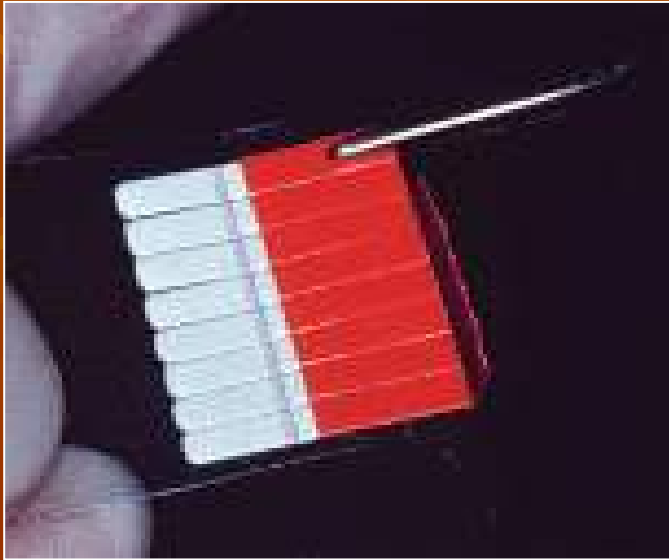
large volumes

of GCF



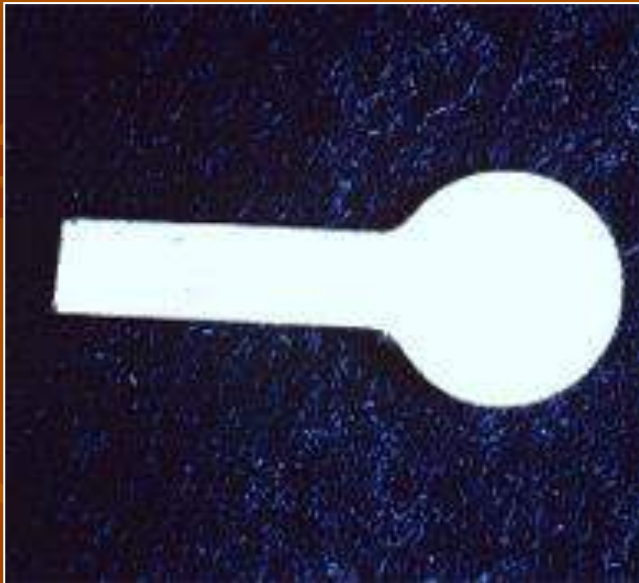
PerioCol Paper Strips

For collecting larger volumes
(0 - 2.0 micro-liters) of GCF fluid.
Package contains 10 sterilized strips.



PerioPaper Strips

absorb 0 - 1.2 microliters of fluid.
Box contains 24 sterilized packets.
Each packet contains 16 test strips.



SialoPaper Strips

For minor salivary gland
Collection and oral mucosal
wetness measurements. Each
strip collects 0 -3.0 μ l.
Package contains 10 sterilized
strips.

Weighing the strips

- An alternative approach, involving the weighing of strips before and after sample collection.

This has been successful

but

- Requires a very sensitive balance
- Chances of evaporative losses

Gingival washing methods

- In this technique the gingival crevice is perfused with an isotonic solution, such as **Hanks' balanced salt solution**, usually of fixed volume. The fluid collected then represents a dilution of crevicular fluid.



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Disadvantages

- difficulties of producing a technically satisfactory appliance for the mandibular arch.
- It is also a disadvantage that GCF from individual sites cannot be analyzed.
- all fluid may not be recovered during the aspiration and re-aspiration procedure. Thus accurate quantification of GCF volume or composition is not possible

Capillary tubing or micropipettes

- Following the isolation and drying of a site, capillary tubes of known internal diameter are inserted into the entrance of the gingival crevice.
- GCF from the crevice migrates into the tube by capillary action and can be accurately determined by measuring the distance which the GCF has migrated.



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- This technique provides an undiluted sample of 'native' GCF whose volume can be accurately assessed.
- However, it is difficult to collect an adequate volume of GCF in a short period, unless the sites are inflamed and contain large volumes of GCF.

Preweighed twisted threads

- The threads were placed in the gingival crevice around the tooth, and the amount of fluid collected was estimated by weighing the sample thread.

Problems with GCF collection and data interpretation

- Contamination
- Sampling time
- Volume determination
- Recovery from strips

Amount of GCF

- The amount of GCF collected is extremely small.
- Measurements performed by *Cimasoni (1983)* showed that a strip of paper 1.5-mm wide inserted 1 mm within the gingival sulcus of a slightly inflamed gingiva absorbs about 0.1 mg of GCF in 3 minutes.
- *Challacombe (1980)*: the mean GCF volume in proximal spaces from molar teeth ranged from **0.43 to 1.56 μ l**.



*Composition
Of
GCF*

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Enzymes and other compounds reported in the GCF

- Acid phosphatase
- Alkaline phosphatase
- α 1 antitrypsin
- α 2 macroglobulin
- Aryl sulfatase
- Aspartate aminotransferase
- β -glucuronidase
- Chondroitin sulfatase
- Citric acid
- Cytokines (IL- α , IL- β , IL-6, IL-8,)
- Immunoglobulins (IgG, IgA, IgM)

- Cystatins
- Exopeptidases
- Endopeptidases
 - Cathepsin
 - Elastase
 - Collagenase
 - Tryptase-like
 - Trypsin-like
- Glycosidase
 - Sialidase
 - Galactosidase
 - Mannosidase
- Hyaluronidase
- Lactate Dehydrogenase
- Lactoferrin
- Lactic acid
- Lysozyme
- Medullasin
- Myeloperoxidase
- PGE2
- Transferrin
- Thromboxane
- Fibrin
- Fibronectin
- Osteonectin

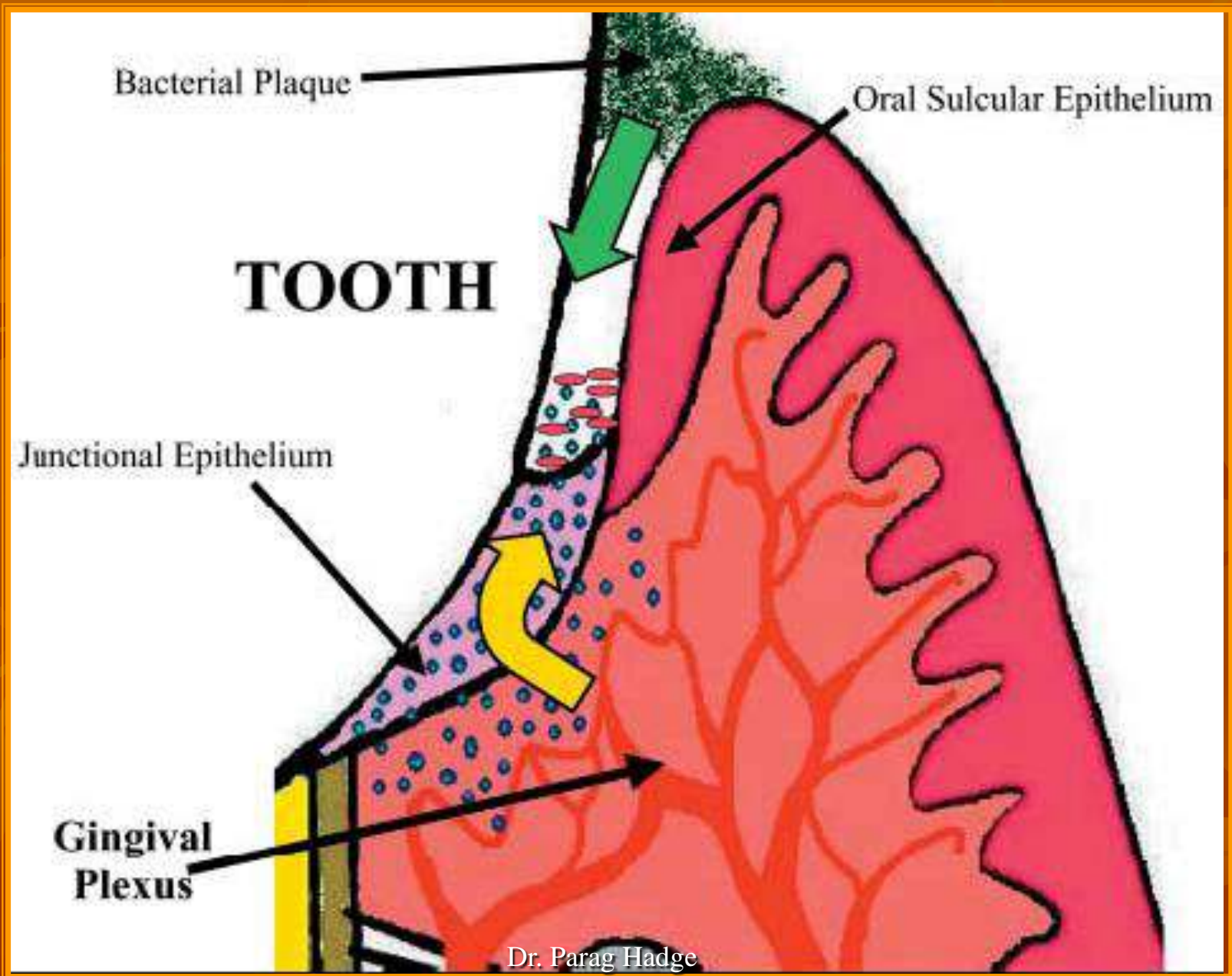
Compounds & enzymes of bacterial origin detected in GCF

- Acid phosphatase
- Alkaline phosphatase
- Phospholipase A
- Phospholipase C
- Lysophospholipase
- Aminopeptidases
- Betalactamases
- Collagenase
- Chondroitin sulfatase
- Aminopeptidases
- Chymotrypsin-like
- Trypsin-like
- DNase
- Fibrinolysin
- Glucosidases
- Hemolysin
- Hyaluronidase
- Iminopeptidases
- Immunoglobulinase

Cellular Elements

- Cellular elements found in GCF include bacteria, desquamated epithelial cells, and leukocytes (PMNs, lymphocytes, and monocytes/macrophages), which migrate through the sulcular epithelium.
- Approximately **30000 PMNs/minute** migrate from all junctional epithelium to gingival sulcus and ultimately into oral cavity

CELL TYPE	SOURCE	FUNCTION
Bacteria	Adjacent plaque mass	Etiologic factor for pdl disease; indicates host immune response
Epithelial cells	Oral SE & JE	Represents high turnover rate
Leukocytes	Gingival plexus of blood vessels	Effector cells of immune response, role in innate immunity & CMI
Erythrocytes	Blood vessels	Incidental finding



Electrolytes

- Potassium, sodium, and calcium have been studied in GCF.
- Most studies have shown a positive correlation of calcium and sodium concentrations and the sodium-to-potassium ratio with inflammation.

Kaslick RS (1970)

Organic Compounds

- Blood glucose levels do not correlate with GCF glucose levels; ***glucose concentration in GCF is three to four times greater than that in serum.***
- ***The total protein content of GCF is much less than that of serum.***

- The methodology used for the analysis of GCF
- Fluorometry
- Enzyme-linked immunosorbent assay (ELISA)
- Radio-immunoassays
- high-pressure liquid chromatography (HPLC) ;
- direct and indirect immunodots;

Clinical significance

- GCF is an inflammatory exudate. Its presence in clinically normal sulci can be explained by the fact that gingiva that appears clinically normal invariably exhibits inflammation when examined microscopically.
- The amount of GCF is greater when inflammation is present and is sometimes proportional to the severity of inflammation.

(Garnick JJ, 1979, Shapiro L, 1979.)

- **Circadian Periodicity**

There is a gradual increase in GCF amount from 6 AM to 10 PM and a decrease afterward.

- **Sex Hormones**

Female sex hormones increase GCF flow, probably because they enhance vascular permeability.

Pregnancy, ovulation, and hormonal contraceptives, all increase gingival fluid production.

- **Mechanical Stimulation**

Chewing and vigorous gingival brushing stimulate the flow of GCF. Even the minor stimuli represented by intrasulcular placement of paper strips increases the production of fluid. *Brill N: 1959.*

- **Smoking**

Smoking produces an immediate transient but marked increase in GCF flow.

- **Periodontal Therapy**

There is an increase in GCF production during the healing period after periodontal surgery. *Arnold R, 1966*

Drugs in Gingival Crevicular Fluid

- Drugs that are excreted through the GCF may be used advantageously in periodontal therapy.
- **Bader and Goldhaber (1966)** demonstrated in dogs that tetracyclines are excreted through the GCF; this finding triggered extensive research that demonstrated a concentration of tetracycline's in GCF when compared with serum.
- Metronidazole is another antibiotic that has been detected in human GCF.

AMA	DOSE (mg)	c serum ($\mu\text{g}/\text{ml}$)	c GCF ($\mu\text{g}/\text{ml}$)	t_{max} serum (h)	Half-life (h)
PENICILLIN	500	3	ND	1	0.5
AMOXICILLIN	500	8	3-4	1.5-2	0.8-2
DOXY CYCLINE	200	2-3	2-8	2	12-22
TETRA CYCLINE	500	3-4	5-12	2-3	2-3
CLINDAMYCIN	150	2-3	1-2	1	2-4

AMA	DOSE (mg)	c serum ($\mu\text{g/ml}$)	c GCF ($\mu\text{g/ml}$)	t_{max} serum (h)	Half-life (h)
METRO NIDAZOLE	500	6-12	8-10	1-2	6-12
CIPRO FLOXACIN	500	1.9-2.9	ND	1-2	3-6

Significance Of GCF

- GCF provides a unique window for analysis of periodontal condition.
- Various substances found in GCF possess a great potential for serving as indicators of periodontal disease progression and healing after therapy.
- Collection of GCF is a noninvasive and relatively simple procedure.

- Over 65 GCF components have been preliminarily examined as possible markers for the progression of periodontitis. These components fall into three general categories:
 1. Host-derived enzymes & their inhibitors
 2. Inflammatory mediators and host-response modifiers
 3. Tissue breakdown products

LEUKOCYTES

- Found in clinically healthy gingival sulcus.
- Appear extravascularly in the connective tissue adjacent to the bottom of sulcus, then travel across the epithelium to the gingival sulcus and expelled.

Differential count

- PMNs: 91.2-91.5%
- Mononuclear cells: 8.5-8.8%
 - B-cells: 56%
 - T- Cell: 24%
 - Phagocytes: 18%
- T cell: B cell ratio-1:3 (reversed)

- Leukocytes are present in absence of inflammation, bacteria and mechanical irritation
- Cells are: viable, have phagocytic and killing capacity.
- Thus, contribute in defence mechanism.

SALIVA

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According to Stedman's dictionary

Saliva is clear, tasteless, odourless slightly acidic (pH 6.8) viscid fluid, consisting of secretions from the parotid, sublingual and submandibular salivary glands and the mucous glands of the oral cavity

1. On basis of size

Major salivary glands

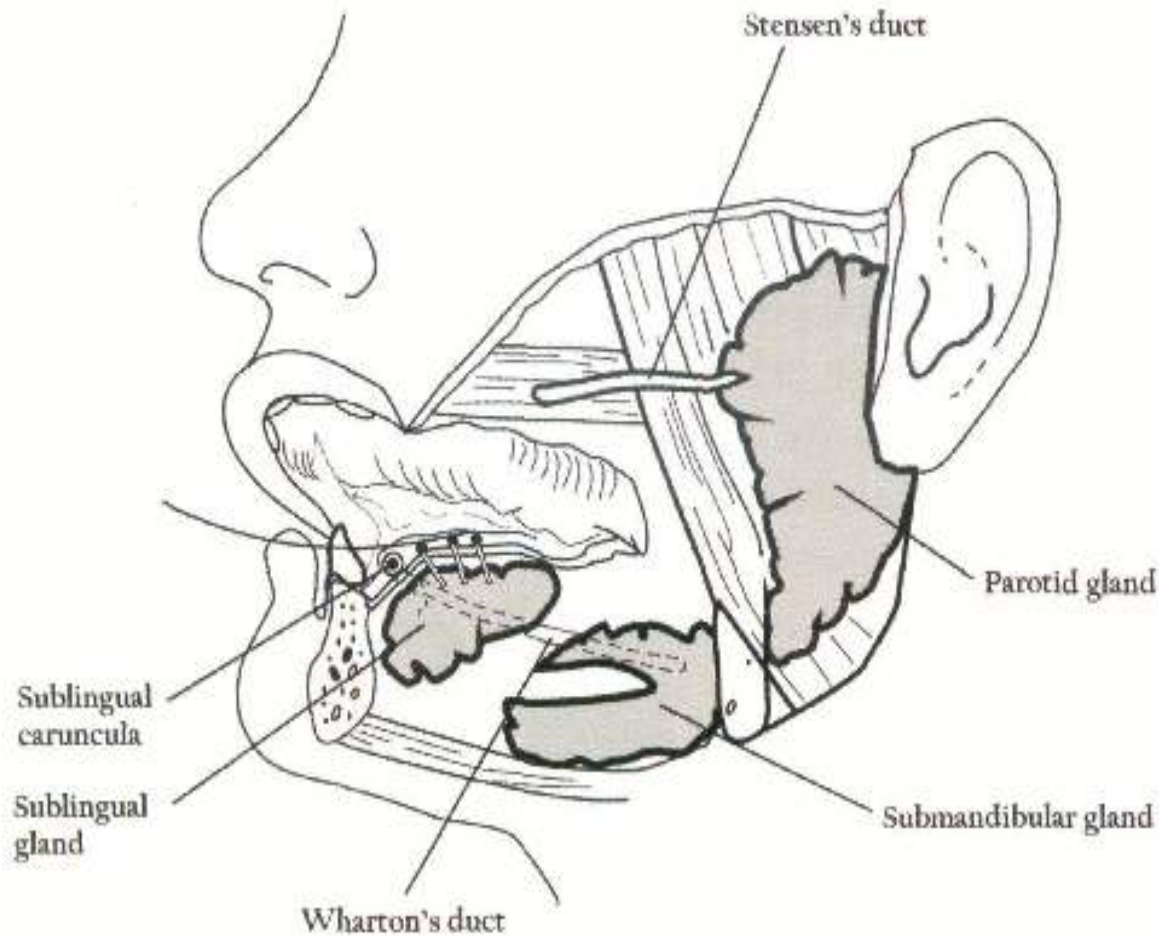
- Parotid gland
- Submandibular gland
- Sublingual gland

Minor salivary glands-

- Labial and buccal glands
- Glossopalatine glands or lingual glands

On basis of secretion

- Serous : Parotid
- Mixed –submaxillary glands (mainly mucous)
- Mucous-palatal salivary glands
- Mucous –sub lingual



● **Figure 1.** *The salivary glands.*

Human major salivary glands: the parotid, the submandibular, and the sublingual gland. The parotid duct (Stensen's duct) is about 5 cm long and opens into the mouth in the buccal mucosa opposite the second maxillary molar. The saliva from the submandibular and sublingual glands enters the mouth mostly through the around 5 cm long submandibular duct (Wharton's duct), which ends on the sublingual caruncula behind the mandibular incisors. But the sublingual gland also secretes through several small ducts along the sublingual fold in the floor of the mouth lateral to the side of the tongue.

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SALIVA

WATER (99.5%)

SOLIDS (0.5%)

ORGANIC (0.3%)

INORGANIC (0.2%)

GAMMA-GLOBULIN

PTYLIN

MUCIN

KALLIKREIN

BRADIKYNIN

LYSOSOME

IMMUNOGLOBULIN IgG

BLOOD GROUP ANTIGEN

NERVE GROWTH FACTOR

VIT C AND VIT K

UREA AND URIC ACID

CELLULAR COMPONENTS

CATIONS

SODIUM

POTASSIUM

CALCIUM

MAGNESSIUM

FLUORIDE

ANIONS

CHLORINE

BICARBON

PHOSPHAT

THIOCYNA

Characters of human saliva

Total amount: 1,200-1,500 ml in 24 hours a large proportion of this volume is secreted at meal time, when the secretory rate is highest

Consistency: slightly cloudy, due to presence of cells and mucin

Reaction: usually slightly acidic (ph 6.02-7.05)

Specific gravity: 1.002-1.02

Freezing point: 0.07 –0.34 degree centigrade

Role of saliva

1. Lubrication	Glycoproteins, mucoids	Coating
2. Physical protection	Glycoproteins, mucoids	Coating
3. Cleansing	Physical flow	Clearance of debris & bacteria

4. Buffering	Bicarbonate phosphate	Antacids
5. Tooth integrity maintenance	Minerals Glycoprotein pellicle	Mineralization Mechanical protection
6. Antibacterial action	Immunoglobulin A Lysozyme Lactoperoxidase	Control of bacterial colonization Breaks bacterial cell walls Oxidation of susceptible bacteria

Salivary antibodies

- Types
 - IgG
 - IgM
 - IgA (Predominant)
- Source
- Synthesis


Enzymes

- Source
- Major: salivary amylase
- Others: collagenase, hyaluronidase, lipase, chondritin sulphatase, beta-glucuronidase, decarboxylase, catalase, peroxidase.

- Proteolytic enzymes
- Role in periodontal disease progression
- Antiproteases present in saliva
- MMPs
- TIMPs

Leukocytes in saliva

- PMNs
- Orogranulocytes
- Correlation with severity of inflammation
- Reliable index

- 
- Role in periodontal pathology
 - Xerostomia
 - Hypertylism
 - Salivary analysis



THANK YOU

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