Dental Insights — Future bases for host-based diagnosis and host modulation therapy of periodontal disease?

1. Genetic mutations helps us understand periodontitis

NIH Clinical Center (the largest research hospital in the world): Many of their patients have rare single-gene mutations, which can be used for leveraging genetic syndromes to understand common diseases.

Plasminogen deficiency (PD) is a rare syndrome with mucosal immunopathology. PD gives low enzymatic activity in plasminogen, which causes widespread mucosal immunopathology (fx ocular disease, intenstinal tract, lungs and vagina), and severe periodontitis. PD patients will lose their teeth already in their teenage-years or even earlier.

Normal wound healing
Bleeding + Tissue injury + Bacterial infection —>
Red blood cells and neutrophils goes from the blood stream and enter the tissue —>
Fibrin network (the network mesh in blood clots) is created + Platelets to create a hemostatic plug/blood clot —>
Hemostatis —>
Removal of fibrin blood clot to initiate wound healing —>
Plasminogen creates plasmin, which degrades fibrin, and proper wound healing with tissue repair can start.

Neutrophils are the most abundant immune cell, and the first acuta immune cell on injury site, and its antimicrobial defense is by phagocytosis of microbes, degranulation and spitting out enzymes, and spill out of DNA that can trap microbes like a net (NETosis). Too much of these processes will become toxic.

Neutrophils surrounds the fibrin-lesion in a fibrin-neutrophil interaction.

Accumulation of fibrin will drive neutrophil activation and hence immunopathology.
Normal wound healing need fibrin to create hemostatis, and neutrophils to trap and fight against any microbes that want to invade the tissue. When hemostasis is accomplished, fibrin needs to be removed in order for the wound healing and tissue regeneration to happen.

The fibrin-neutrophil interaction mediates immunopathology.
Neutrophils alone will not by themselves drive inflammation, but they need a secondary activation factor. Fibrin allows for neutrophil adhesion and activation.

Defective wound healing in patients with plasminogen deficiency (PD)
The PD patients have defective wound healing with chronic inflammation and chronic wounds, because fibrin is not broken up and degraded after it's created hemostasis.
Fibrin also comes out of a leaky blood vessel, not only when there's a wound, but also when there's an injury, inflammation and infection.

Plasminogen-deficient mice (knock-out PD mice) phenocopy human disease with: ligneous conjuctivitis in the eyes, colitis in the intestines, enlarged spleen, enlarged submandibular lymph nodes, and ligneous periodontitis.

PD mice lose their teeth within 24 weeks, and they have tremendous fibrin accumulation.
Study with plasminogen-deficient and fibrin-deficient mice showed a 100% rescue of alveolar bone loss. This tells us that fibrin DOES drive immunopathology.

A study showed that plasminogen-deficient mice with inhibition of the fibrin-neutrophil interaction complete rescue of alveolar bone loss.

In PD patients fibrin keeps accumulating and neutrophils keep getting activated, and the wound will become chronic.

Genetic diseases can help us understand common diseases and potentially develop effective treatments.

2. Diagnostics using -omics approaches in periodontal research

Why use omics in periodontal research?
Omics are measures of all the molecules of a certain of a certain class in a certain habitat, fx all the expressed genes, or all the methylated genes in the DNA, or all the bacteria in an ecosystem.

To handle so many data points, you need huge amounts of statistical power.
Patients with periodontitis can be both patients with biofilm-associated periodontitis (slower, often in older patients) and non-biofilm associated periodontitis (aggressive, often in younger patients).

Why do some people get severe periodontitis, even if they are keeping good oral hygiene?
What causes periodontitis? Microbes + Host response.

Article: ”Molecular differences between chronic and agressive periodontitis”, Kebschull et al, 2013. Conclusion = Evidence for diagnostic imprecision of the classification.
Omics study on 120 healthy non-smokers with untreated chronic periodontitis or untreated aggressive periodontitis: collection of 240 diseased gingival tissue biopsies, and performing a genome-wide mRNA analysis.

Machine learning found new subgroups of periodontitis patients (new molecular classes).
Periodontal pockets had different gene expressions (= disease severity).

In 2017 a new classification system for periodontitis was agreed on the ”2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions”.

The new classification clusters didn’t overlap with the old classification for periodontitis.
Clinical phenotypes showed different clusters according to gender and periodontal parameters.

Our life style changes our DNA
Normal transcriptome process: Gene expression with DNA (modified by small miRNA and DNA methylation = epigenetics) —> mRNA —> Protein production.
DNA methylation can be changed by life style fx smoking, some bacteria and junk food. Generally both miRNA and DNA methylation leads to decreased gene expression.
miRNA = mikro RNA.

There are only 200-2000 miRNAs, but there are more than 500.000 DNA methylation CPG islands, which need a lot of statistical power to and computing power to investigate correlations.

The more methylated the DNA is, the less gene expression there is.

Omics research for studying the oral microbiome
Different spatial approaches to analyse one piece of tissue with different tissue and cell types:
1) Mathematic deconvolution,
2) Molecular cartography (geographical landmarks on the gingival tissue),
3) Single-cell sequencing.

Mathematics can handle large data sets of fx transcriptomes, and distill out results that are distinct for a certain population.

Molecular cartography (geographical landmarks) can create different color staining of the tissue. The deeper the periodontal pocket is, the more the inflammatory markers blow up in the color staining.

Sequencing of the microbiome can use meta-transcriptomes to identify what kind of oral bacteria are there AND what they are thinking and doing.

Studying the microbiome = QuantifyingDysbiosis (patented). Can create a Microbiome Dysbiosis Index, which means you get a single number within your regression for quantification of the dysbiosis that is increasing with probing depth. Getting a single number in the Microbiome Dysbiosis Index for what's going on the periodontal tissues can dramatically change the clinical diagnosis in the stratification of patients.

Summary:
1) Significant body of work on human tissue multi-omes,
2) Challenge with understanding the interactions between –omes (fx looking at the methalome at certain bacteria that methylate DNA and decreased gene expression),
3) Challenge with disentangling the "mixed bag" of tissue (fx looking at which cell population is responsible for what).

3. Anti-inflammatory and host-modulatory strategies in future therapy?

A new classification system for periodontal diseases (2017)
In 2017 a new classification system for periodontitis was agreed on the "2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions".

In the new periodontitis classification, there is a definition of health. Periodontal health is defined in 4 levels:
1) Pristine Periodontal health (in a structurally sound and uninflamed periodontium),
2) Well-Maintained Clinical Periodontal Health (on a structurally and clinically sound (intact) periodontium),
3) Periodontal Disease Stability (on a reduced periodontium),
4) Periodontal Disease Remission/Control (on a reduced periodontium).

A patient cannot go back to level 1, but should try to be converted back to a suitable healthy level.

Clinical health: health promoting biofilm (symbiosis), proportionate host response, low biomass, acute resolution of inflammation. Biomass = dental plaque.

Gingivitis: incipient dysbiosis (quorum sensing bacteria), proportionate host response, high biomass, chronic resolution of inflammation.

Periodontitis: frank dysbiosis (pathogenic biofilm), disproportionate host response (hyper-inflammatory), high biomass, failed resolution of inflammation, connective tissue and bone damage, chronic non-resolving inflammation.

Periodontal therapy in a nutshell: Periodontitis is a dynamic transition from health (symbiosis) to disease (dysbiosis): from clinical health —> gingivitis —> periodontitis. The goal of periodontal therapy is to restore tissue homeostasis.

Host modulation therapy of periodontal disease
Host modulation therapy: inflammation is the central role of periodontitis pathogenesis, and the main target of host modulation therapy is controlling the inflammation to control the infection, rather than the previous concept (last 40-50 years) that was focused solely on eliminating the infection to control the inflammation.

Host modulation therapy tool box of anti-inflammatory and pro-resolving agents: anti-cytokine and biological therapy (anti-IL1, anti-IL6, anti-TNFalfa, anti-CD20 (suppressor of T-cell activation to deplete B-cells)), corticosteroids, NSAIDs, Bisphosphonates, RANKL inhibitors, small molecule compounds (fx histone deacetylase inhibitors), low dose doxycycline, enamel matrix derivatives, lipoxins, resolvins, protectins, maresins (SPMs).

Periodontal recovery:
Periodontal inflammation —>
Granulation tissue —>
Resolution of granulation tissue and recovery of periodontal tissues —>
Wound healing.

Good inflammation graphic: from one of the Serhans reviews 2019.

Possible future supplement in treating periodontal disease:
Resolvin E1 (RvE1) can prevent periodontal disease, because it regulates inflammation, increases wound closure and restores tissue homeostasis. Periodontal regeneration is possible without tissue grafts, by just controlling the inflammation.

A study with Resolvin E1 (Hasturk et al 2007) showed that by inducing periodontitis in the test animal, it also created the hepatic portal vein problems in the liver. By treating periodontitis with Resolvin E1, it also treated the hepatic portal vein problems in the liver. By treating the disease, you also create an environment where the pathogenic bacteria cannot survive anymore, and the microbiome actually turns back to health-promoting bacteria after treatment with Resolvin E1.

A study showed that you can prevent IL-17 induced periodontal disease by inducing Resolvin E1.

Lipoxins can be regenerated from aspirins and omega-3 fatty acids.
Safety study with a oral mouthrinse with lipoxins.

A study showed that scaling + root planing + 900 mg EPA + DHA + aspirin resolved almost all the >7mm periodontal pockets.

What's next:
1) Optimize the best dose and delivery method,
2) Translate the knowledge from basic science and animal studies into clinic,

3) Develop into new drugs to evaluate the efficacy and the risks and benefits in large patient populations in randomized controlled trials,
4) Develop and incorporate new anti-inflammatory and pro-resolving host modulatory therapies to provide clinicians with new tools,
5) Target periodontal disease and other systemic inflammatory diseases as adjunctive agents.

Other oral diseases share common pathways as periodontal disease.

Discussion: Eskimos and Japanese people have better epigenetic function for metabolism of omega-3 fatty acids from food, than European people, who can't get enough omega-3 from eating fatty foods only (fx salmon).

Good resources

"Molecular differences between chronic and agressive periodontitis", Kebschull et al, 2013.
"2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions". Article: "Periodontal health and gingival diseases and conditions on an intact and a reduced periodontium: Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions", Chapple et al 2018.
"Molecular aspects of the pathogenesis of periodontitis", Meyle and Chapple 2015.
A study with Resolvin E1 (Hasturk et al 2007).
"Resolvin E1 reverses experimental periodontitis and dysbiosis", Lee et al 2016.
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"Resolvin E1 regulates Th17 function and T-cell activation", Oner et al 2021.
"RvE1 impacts gingival inflammatory infiltrate by inhibiting the T cell response in experimental periodontitis", Alvarez et al 2021.
"Host modulation and treatment of periodontal disease", Balta et al 2021.
"Osteoimmunology of oral and maxillofacial diseases: translational applications based on biological mechanisms" Alvarez et al 2019.

Top 3 Dental Insights — Key Take Aways

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Sources

EuroPerio10 15-18.06.2022

That was Dental Insights. Thank you for being here. ❤

Dental love, Anne Mette