3 Host Immune Response to Dental Implants

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3.1 Host Defense in Dental Implant Environments

3.1.1 Osseointegration and Host Response

Biocompatibility and biostability are the key properties of dental implants in the "oral environment," in order to optimize their performance before or after functional loading. Biomaterials involving dental implants should be able to directly interact with their oral environment and adapt to the needs of the living organ [1]. Clinical biocompatibility rather refers not to a generic property of a biomaterial, but of a biomaterial-host system [2]. Uneventful host defense to dental implant insertion is not necessarily "not at all a response in the tissue"; however, it starts with more a favorable immune response that promotes wound healing around the jawbone and soft mucosal tissue.

Dental implants are placed in the jawbone through surgical procedures that create an "implant wound" in the bone and soft tissue (Fig. 3.1). Attempts to minimize wound area and surgical trauma to bone and soft tissues are crucial to ultimately reduce the response in peri-implant tissues, thus leading to a faster wound healing and a more favorable host-biomaterial interaction (Fig. 3.2). Soft tissue healing is indicated by the formation of a mucosal barrier (biological seal) at the soft tissue-transmucosal interface, while a direct structural and functional connection between the bone and the implant interface is defined as "osseointegration," or has earlier been characterized as "functional ankylosis" [3].

Bone tissue healing around dental implants includes an initial homeostasis phase, a pro-inflammatory phase, a cell proliferation phase, and a final remodeling phase [4]. In experimental animal models, the inflammatory phase is initiated as soon as 2 h following implant placement and is characterized by recruitment of leukocytes, which is followed by an increased number of fibroblasts and presence of osteoclasts in the recipient bone, starting from at 4 days to 1 week (i.e., proliferative phase) [5]. Polymorphonuclear granulocytes are the dominating leukocytes on all surfaces followed by monocytes. Stabilization of the blood clot to the implant surface and cell adhesion are important steps for successful integration and mediated through protein adsorption to the implant surface [6]. Interestingly, in humans, there is no obvious osteoclastic activity during the proliferative phase. The remodeling

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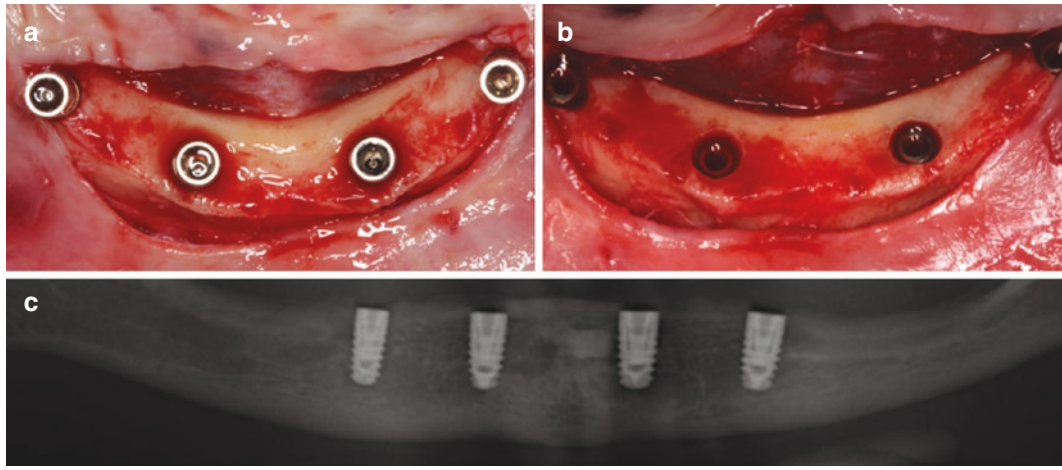

Fig. 3.1 Bone wound healing around implants. Achievement and maintenance of osseointegration and mucosal seal formation depends on establishment of a long-term equilibrium between host cells and titanium. Early and late host immune reactions to dental implant procedures are immune-inflammatory response, angiogenesis, and osteogenesis. Healing process that starts with the stabilization of the blood clot and respective occurrence of homeostasis, pro-inflammatory, cell proliferation, and finally remodeling phases eventually leads to osseointegration of the implants. (**a**) Insertion of four self-tapping implants to osteotomy sites at edentulous lower jaw; (**b**) surgical site following disconnection of transfer pieces; (**c**) radiographic images at the first-stage surgery

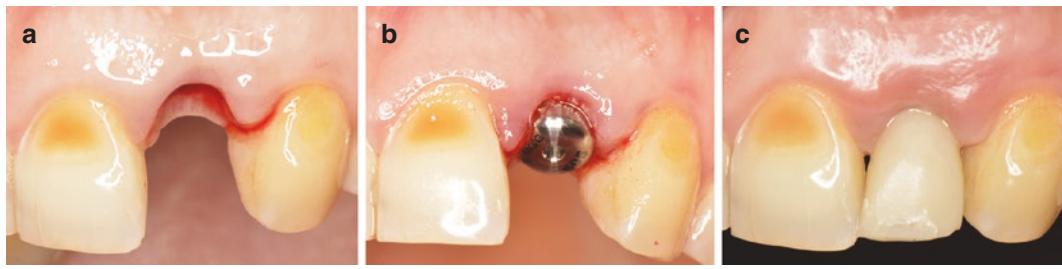

Fig. 3.2 Peri-implant soft tissue healing around healing abutments. (**a**) Edentulous space of upper left lateral incisor after 8 months of socket preservation and provisionalization; (**b**) healing abutment in place following placement of a dental implant via flapless guided surgery; (**c**) soft tissue healing around healing abutment over 3 days after immediate restoration with a temporary crown

phase begins at around 2 weeks by an increased number of osteoclasts and may extend to 12 weeks until most of the woven bone is replaced by lamellar bone. Transition to the remodeling phase in humans shows more delayed onset at least 2 weeks, compared with beagle dogs [7].

Implant wound healing in the jawbone is coordinated by structural and immune cells that interact with each other via growth factors, cytokines, chemokines, and matrix proteins [5, 8, 9]. Gene expression analysis of peri-implant tissue at early stages of tissue healing indicates that the pro-inflammatory response associated pathways are upregulated during the early stages of osseointegration around day 4. Thereafter, around day 14, these pathways are replaced with the upregulation of genes associated with osteogenesis-related mechanisms [10, 11]. Tissue-resident macrophages are an integral part of the osseointegration and wound healing process and can release several pro-inflammatory cytokines or growth factors in response to the injury caused by surgery

[12]. Multiple cytokine profiling of peri-implant crevicular fluid collected 2, 4, 8, 12, and 24 weeks following implant placement in humans showed that a vast array of cytokines peaked at week 2 after implant insertion, before decreasing at week 4 or week 8, then remaining steady at least until week 24, postoperatively [13, 14]. These findings highlighted that early weeks following implant insertion are crucial time points of successful wound healing around implants, and support the previously reported histological observations and gene expressions data.

This type of immune response during the early stages of osseointegration around implants can be considered as "sterile inflammation" that is resolved if there are no other complications. The immune response features of the bone-implant interface may be affected by several factors including implant surface characteristics/design, surgical procedure for implant bed preparation, or implant-abutment interface configuration [15]. Implant surface topography modifications may promote osteogenesis by osteoblasts, but much less is known about their potential effect on immune cell modulation and control of inflammation [16–19]. Hotchkiss and coworkers. [16] showed that macrophages cultured in vitro on implants with high surface wettability or implants with a combination of high-energy and altered surface chemistry produce an anti-inflammatory host response that reduces extended pro-inflammatory factor release. A better understanding of the effect of implant surface characteristics on a wound-healing microenvironment may enhance implant success and prevent early implant loss, which has also been postulated to be associated with a provoked foreign body reaction [20, 21]. Several other studies also reported foreign body or hypersensitivity reactions as a result of the implant material itself [22–26]. In healthy individuals with a maximum of 3 successfully restored titanium dental implants, blood levels of lactate dehydrogenase (LDH) and total protein levels were significantly higher 6 months after implant placement compared to baseline [22]. However, none of the levels were of clinical relevance. In addition, blood lymphocytes and monocytes from healthy individuals, baring or not dental implants, were isolated and their cell activity and cytokine production capacity to titanium were assessed in vitro [23]. T-cell proliferation was similar in both groups, but IL-1β, IL-6, and tumor necrosis factor (TNF)-α production was significantly lower in individuals with implants. Furthermore, several studies investigated the in vitro effect of blood on titanium and/or zirconia dental implants [24–26]. For this, unused implants were incubated in blood from a healthy donor and were harvested after 1, 8 , and 24 h to assess gene expression of IL-8 to the implant material [24, 26]. IL-8 gene expressions and IL-1β plasma protein levels were significantly increased, compared to baseline, irrespective of the implant type [24, 25].

On the other hand, dental implant coatings may wear off over time, leading to titanium corrosion and titanium particle release [27]. Berbel et al. showed that limited access to oxygen in the peri-implant defect environment reduces the resistance of implants to corrosion [28]. Although it is not clear whether such particle release can lead to a hypersensitivity reaction, there is evidence that titanium ions can induce inflammasome expression by macrophages and activate the release of pro-inflammatory cytokines [29]. This is in line with findings a greater number of macrophages containing titanium particles was found in the areas in close contact with the implant surface [30].

During wound healing following implant installation, bone modeling occurs that may result in some reduction of the marginal bone level coupled to immunological reactions. Early bone loss process can be a result of multifactorial factors, including intrinsic and extrinsic ones (Fig. 3.3). Among these, less traumatic osteotomy modalities for implant bed preparation may lead to the reduction of pro-inflammatory response at an early stage [31]. Piezoelectric surgery, a minimally invasive technique to prepare the implant bed has been shown to modify and reduce bone-destructive inflammatory molecules during implant osseointegration [13, 14, 32].

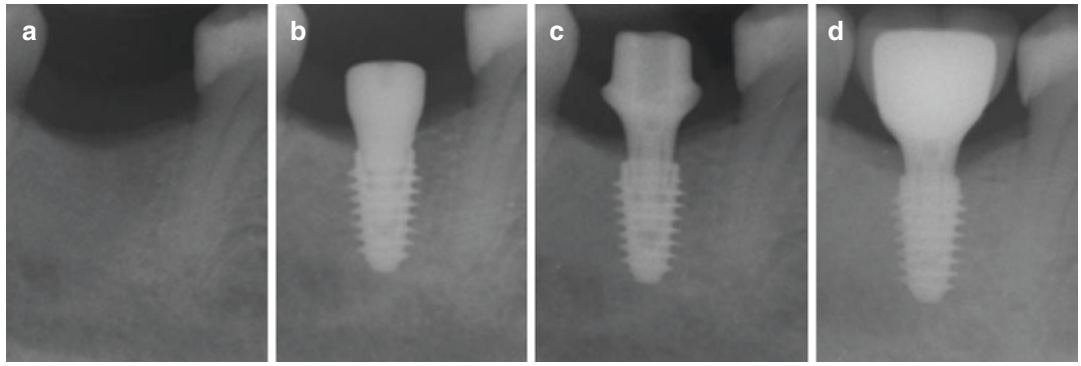

Fig. 3.3 Osseointegration and crestal bone levels around the implants. Early peri-implant crestal bone loss is a multifactorial phenomenon including several modifiable or avoidable factors related to patients, implant design, surgical and prosthetic interventions [31, 33]. Current strategies target achievement of minimal or no crestal bone loss around dental implants. (**a**) Healed lower molar extraction site; (**b**) healing abutment placed simultaneously immediately after guided flapless implant surgery; (**c**) implant was immediately restored with provisional crown and non-functionally loaded; (**d**) osseointegrated implant with definitive prosthesis. Note absence of crestal bone loss and maintenance of the successful osseointegration (Surgery Prof Ali Gurkan, Prosthetics Prof Bulent Gokce)

3.1.2 Peri-Implant Soft Tissue Integration and Host Response

The formation of a soft tissue barrier at implants is the result of a maturation process within the connective tissue and epithelial proliferation during wound healing. Peri-implant soft tissue healing is described as a "gingival seal" formation [34] (Fig. 3.4). When assessed at the microscopic level, the healthy peri-implant mucosa in humans can reach up to 3.6 mm height and consists of a 1.9 mm sulcular and junctional epithelium (keratinized and nonkeratinized) and a 1.7 mm underlying connective tissue [35]. While the apical part of peri-implant mucosa creates a connective tissue adhesion zone with limited vascularization, the coronal part consists of junctional and sulcular epithelium with some vascularity [12, 36]. Blood supply of peri-implant mucosa is provided solely by the supraperiosteal blood vessels [37]. Therefore, peri-implant mucosa may have an impaired immune response compared to gingiva around teeth [38].

Experiments in dogs and humans have documented the cellular events in the connective tissue interface portion of the peri-implant mucosa during the early stages of healing [12, 34]. Two hours after implant installation, blood coagulum was observed in the spaces between the mucosa and the implant and between the mucosa and bone. Following surgery at 4 days, there was an influx of the neutrophil granulocytes into blood cloth that degraded the coagulum and created a leukocyte-infiltrated fibrin network. It was demonstrated that macrophages were distributed in the connective tissue throughout the entire healing period. Acute inflammatory changes at week 1 were reflected as an increase in PICF volumes [38]. The PICF content showed higher expression of specific pro-inflammatory mediators in implants compared to teeth during post-operative healing, revealing a more robust response to surgical trauma in peri-implant compared to periodontal tissues [38, 39]. While T and B lymphocytes were densely packed in the connective tissue at 2 weeks of healing, then their numbers declined from 4 to 8 weeks of healing in parallel with reduced vascularity [12]. Furthermore, the first signs of epithelial proliferation were observed in specimens representing 1–2 weeks of healing and a mature junctional epithelium occurred after 6–8 weeks of healing. The collagen fibers of the mucosa were organized parallel to the implant surface after 4 or 6 weeks of healing without insertion into the implant surface. Collectively, the soft tissue attachment to implants is established after several weeks following surgery and induction and resolution of inflammation appear to be a hallmark for the healing process of the peri-implant mucosa (Fig. 3.4).

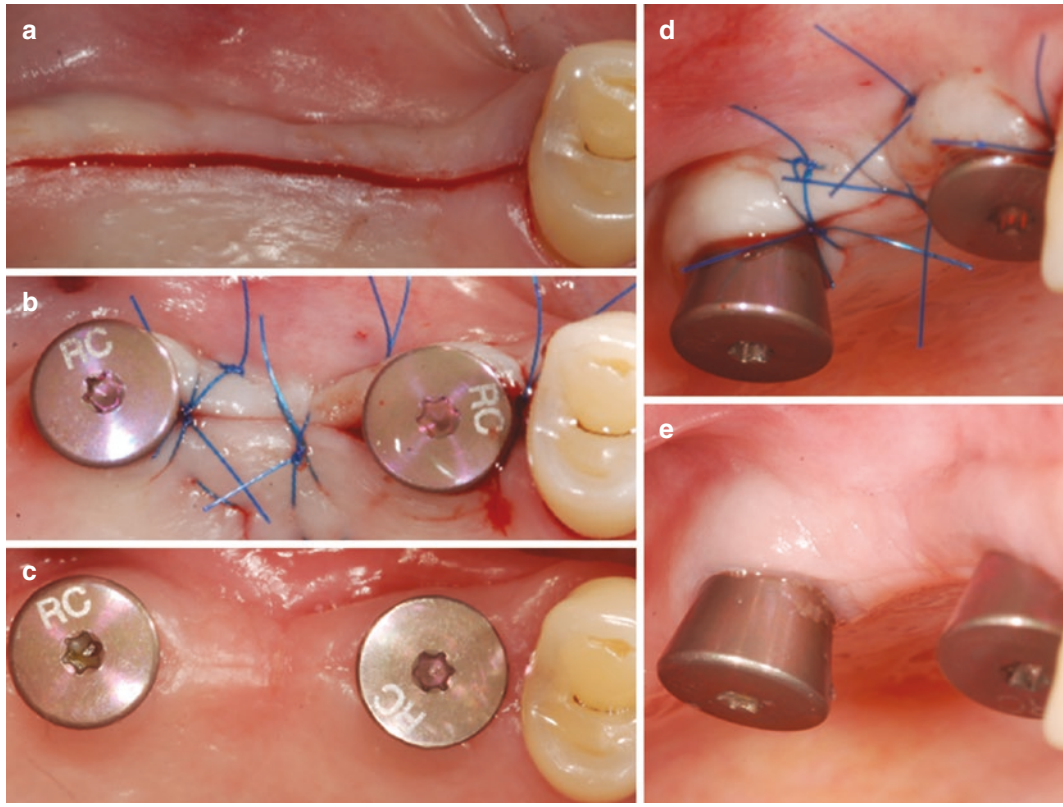

Fig. 3.4 Peri-implant soft tissue integration and host response around implants. During an uneventful wound healing period around implants, formation of a mucosal seal is characterized with proliferation of epithelium and maturation of connective tissue. The soft tissue undergoes pivotal changes including shift of the provisional matrix to a collagen fiber-dominated one and alterations in volume, cellular content, organization, and dimension and reaches its final characteristics within 6–8 weeks. (**a**) Mid-crestal incision prepared for second-stage surgery in order to uncover 2 implants left for submerged healing; (**b**) connection of healing abutments and primary closure of the flap; (**c**) post-op 2 weeks of undisturbed early peri-implant mucosal healing; (**d**) buccal view of the site at second-stage surgery; and (**e**) at post-op 2 weeks

3.1.3 Immune Responses to Biofilm Accumulation Around Implants

The nature of the peri-implant mucosa being exposed to the external and internal environment constitutes a challenge for the immune system to keep the homeostasis between oral microbial stimuli and an appropriate immune response. The lack of the periodontal ligament around implants creates a variety of biological disadvantages for the implant, compared to the periodontium of natural teeth including less physical barrier and reduced blood flow. Periodontal ligament provides the necessary biological niche for the production of immune cells and supports alveolar bone regeneration possibly via the presence of stem-like cells and epithelial cell rests of Malassez [40, 41]. Earlier studies in animal models seem to substantiate this theory that increased bone loss and osteoclasts in ligature-induced peri-implantitis related to the absence of periodontal ligament but not the cervical cementum in cynomolgus monkeys [42].

Biofilm formation in a newly exposed implant can happen as quick as 30 min from the existing species in the oral cavity [43]. Biofilm seems to be confined to the supra-mucosal area with the existence of a plaque and cell-free zone [12]. Similar to gingival tissue, peri-implant mucosa

harbor various features that help controlling biofilms including the flushing action of peri-implant crevicular fluid, the rapid epithelial turnover, an influx of innate immune response cells to the peri-implant tissue and the transmigration of neutrophils into the peri-implant sulcus [44, 45]. Stages of inflammatory events are described based on cellular and structural changes occurring during peri-implant mucositis development and progression in experimental studies. A prolonged exposure of the implant site to dental biofilms may induce both qualitative and quantitative changes of the inflammatory infiltrate around peri-implant mucosa, which is reversible upon reinstitution of plaque control similar to those in experimental gingivitis [46]. This response seems to be independent of implant type, at least based on experimental animal models [47]. The sequence of inflammatory events that take place in peri-implant mucositis is similar to those in experimental gingivitis, but potentially of inflammation border extends faster toward the alveolar bone [48]. In humans, experimental peri-implant mucositis lesion at 3 weeks is characterized by the presence of an inflammatory cell infiltrate within the connective tissue underlying oral epithelium [49]. The size of inflammatory lesion around the peri-implant mucosa can reach up to 0.14 mm^2, which is represented by increased proportions of T- and B lymphocytes [50].

The host response patterns in human peri-implantitis are qualitatively similar, yet more extensive, compared to periodontitis, resulting in a faster progression of tissue destruction [51, 52]. The information available on host-immune characteristics of peri-implantitis is derived from comparative studies using biopsy material from peri-implant mucosa and gingiva, as well as experimental studies in animal models. The switch to peri-implantitis from peri-implant mucositis is accompanied by a further influx of inflammatory cells into the affected area of the peri-implant mucosa, that now expands to reach the bone tissue [53, 54] (Fig. 3.5). Similar to advanced periodontitis lesion, apical migration of junctional epithelium, loss of collagen and a larger proportion of neutrophils, macrophages, T- and B-cells, osteoclasts as well as bone loss are the key features of peri-implantitis lesions. When quantified, the size of peri-implantitis lesion is double in size than periodontitis lesion (3.5 vs. 1.5 mm^2) [55]. Diseased tissue obtained from peri-implantitis sites is shown to exhibit higher expression of several mediators of inflammation,

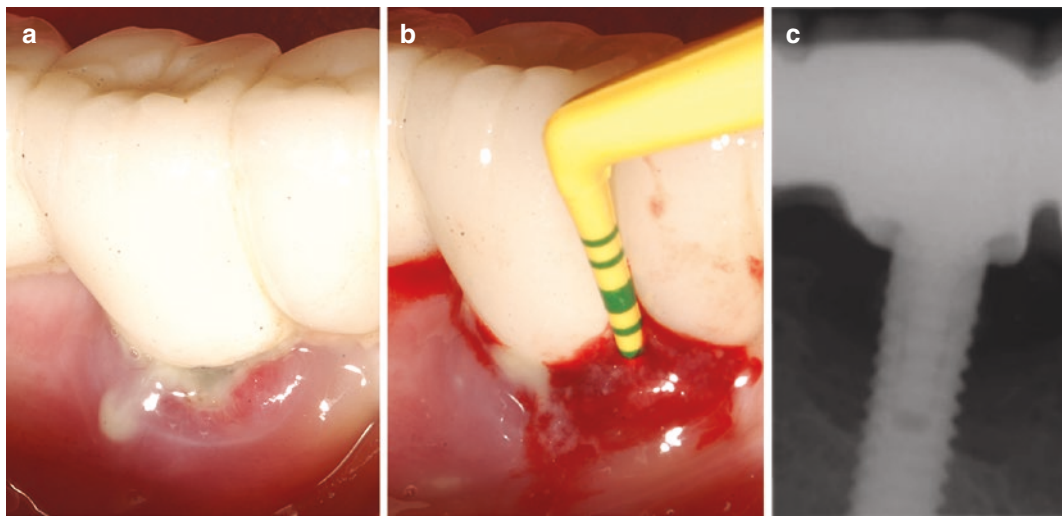

Fig. 3.5 Clinical and radiographic findings of a peri-implantitis case. (**a**) Presence of visual inflammatory changes around the peri-implant soft tissue evident by redness, swelling, ulceration, and suppuration. (**b**) Presence of bleeding on probing, increased probing depth and pus around the implant-supported prosthetic restoration. (**c**) Radiographic evidence of bone loss beyond crestal bone level around the implant

including pro-inflammatory cytokines interleukin (IL)-6, IL-8, and TNF-α, compared to healthy or peri-implant mucositis sites [56, 57]. A global gene expression profiling of peri-implant and gingival mucosa biopsies indicates that both shared and distinct mRNA expression patterns between peri-implantitis and periodontitis. Another high-throughput gene expression study by Liu et al. showed that the cyclooxygenase-2 pathway is the most upregulated biological process in peri-implantitis as compared to periodontitis. Their data also suggested that osteoclast differentiation-related pathways are comparatively more active in peri-implantitis indicated by higher receptor activator of NF-κB (RANK) ligand (RANKL) and osteoprotegerin ratio [58, 59].

Although limited animal models are available to compare peri-implantitis to periodontitis, it's in parallel condition, ligature models in beagle dogs and murine are the most studied ones [60, 61]. The placement of the ligature on the implants of experimental animals results in acute inflammatory reactions that involve tissue breakdown and bone loss, which resemble peri-implantitis in humans [62]. In general, ligatured-induced peri-implantitis presents with increased infiltration of T- and B-cells, neutrophils and macrophages and osteoclasts, while decreased the density of alveolar bone without "self-limiting" process [42, 54, 63–67]. In these models, after the removal of the ligatures, if plaque accumulation is allowed, progression of peri-implantitis occurs resembling natural history of periimplantitis in humans [68]. The lesions in ligatured-induced peri-implantitis appear earlier than they are in periodontitis. By placing ligatures in both tooth and implants of mice for 1 week, 1 month, or 3 months, Hiyari S et al. observed the more intensive bone loss lessons on peri-implantitis compared with periodontitis sites as early as 1 week, and this trend was intensified at later stages [64]. Interestingly, in murine ligature models, at 3 months, 20% of implants exfoliated due to peri-implantitis, but no natural teeth exfoliated in the case of periodontitis [66]. Additionally, removal of ligature leads to bone apposition in periodontitis cases whereas this is not the case in the peri-implantitis group [69]. At the molecular level, increased matrix metalloproteinase-8 (MMP-8), and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) expression seem to follow histopathological observations [64]. Experimental models using knockout mice strains suggested that in toll-like receptor (TLR) 2 and TLR4 mediate bone loss around implants [65, 70]. Similar models were also applied to evaluate the effect of implant type and implant surface characteristics in mediating immune response. The implants with doxycycline-treated surfaces resulted in significantly higher bone levels than the control surface in the peri-implantitis mice model, which showed this surface attenuated inflammatory response and progression [71]. In contrast, implant abutments with antibacterial coating or surface modification with a monolayer of multiphosphonate molecules in beagle dogs do not seem to prevent biofilm formation on the implant surfaces and do not attenuate host response in the adjacent peri-implant mucosa [72, 73].

3.1.4 Biological Fluids as a Reservoir of Inflammatory Mediators for Peri-Implant Mucositis and Peri-Implantitis and Their Diagnostic Potential

Although histopathologically peri-implant lesions are quite well described, the molecular determinants of these processes are not yet fully described. As indicated by many clinical studies that periodontal indices are not reliable diagnostic and prognostic tools for examining dental implants and determining treatment needs. Although probing clinical pocket depth, clinical attachment level and bleeding on probing (absence or presence) have been recognized as the dentist's most important tools in diagnosing periodontal health and disease, but probing depth around the implants is not as meaningful a diagnostic tool as the tooth. Regular probing around healthy implants could potentially result in trauma to the peri-implant soft tissues with consequent induced inflammation. Therefore, in current practice, probing around dental implants

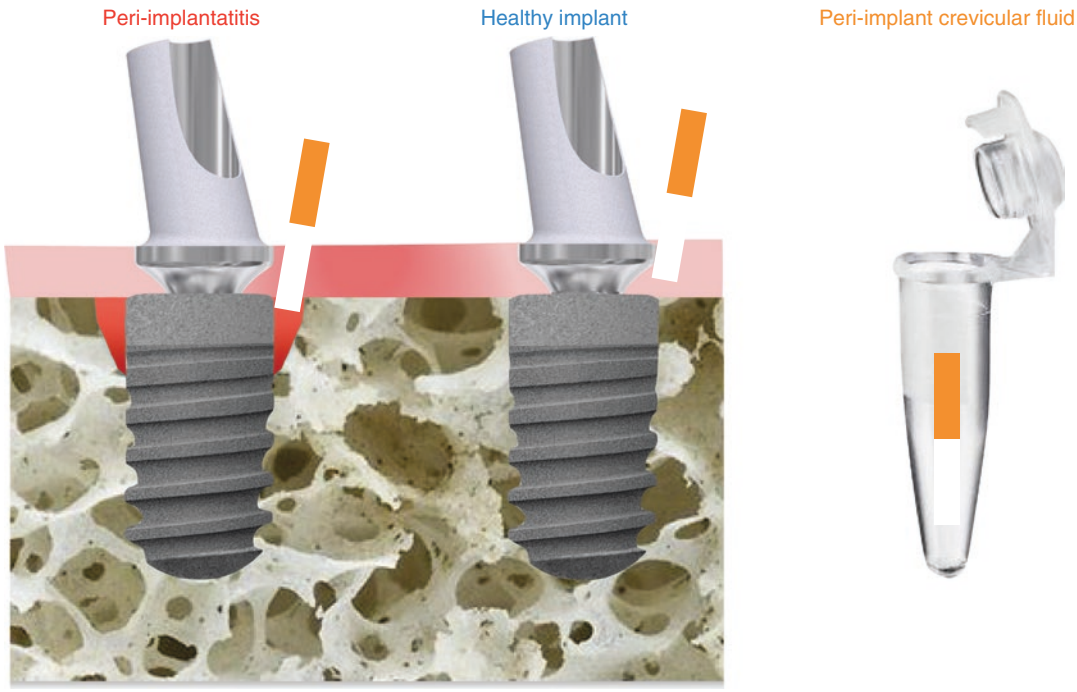

Fig. 3.6 Peri-implant crevicular fluid (PICF) collection in health and disease. The PICF is collected via paper strips after gentle insertion into the crevice for typically 30 s. Once the PICF is absorbed onto the paper strips, then eluted into buffer prior to analysis of the immunological content

cannot be performed until osseointegration is complete which may take up to 6 months [74]. Moreover, probing accuracy is more questionable around peri-implant mucosa as penetration seems to be more advanced at implants than at teeth [63, 75].

Presence bleeding in probing (BOP) around implants is also a poor indicator of progressive peri-implantitis, as BOP is constant both at sites with peri-mucositis and peri-implantitis or even stable peri-implantitis [76]. Ericsson et al. also reported the presence of BOP for the majority of the healthy peri-implant sites [76] potentially indicating a state of subclinical chronic inflammation in healthy peri-implant tissues. Therefore, contemporary, non-invasive diagnostic and prognostic tools based on "measurable biological indicators" of peri-implant diseases are needed to detect active disease and future disease progression and facilitate targeted treatment on a more rational basis. Peri-implant crevicular Fluid (PICF) and saliva are among the proximal sources of biomarkers for peri-implant health and disease. Both saliva and PICF can be obtained non-invasively in less than 5 min (Fig. 3.6). A narrative summary of the literature examining biomarkers in PICF, saliva, and in serum as potential diagnostic/prognostic tools for peri-implant diseases is provided in the following sections.

3.1.5 Peri-Implant Crevicular Fluid

The peri-implant crevicular fluid (PICF) is the inflammatory exudate of the peri-implant sulcus [14, 44]. Similarly, to gingival crevicular fluid (GCF), PICF is the outcome of increased permeability of the vessels within the underlying connective tissue, as an inflammatory response to the growing biofilm at the implant–tissue interface [77]. PICF is enriched with connective tissue breakdown products and inflammatory molecules [78–81]. Therefore, analysis of the PICF might

be suitable to evaluate the inflammatory status of peri-implant tissues, in a quantitative manner [45, 82].

In healthy implant tissues, the flow of PICF is minimum. However, in peri-implant mucositis and peri-implantitis, its volume is increased at a given site in response to biofilm accumulation [83, 84]. PICF protein content increases in experimentally induced peri-implant mucositis by the end of the 3 weeks and more interestingly, its volume is higher when compared with that of GCF [48]. Since the composition of PICF is modified along with the histopathological changes during the course of progressive peri-implant inflammation, its molecular analysis may support the early detection of clinically undetectable diseases [85]. The levels of pro-inflammatory cytokines including tumor necrosis factor alpha (TNF-α), Interleukin-1alpha, IL-1 beta (IL-1β) are increased in PICF collected from peri-implantitis-affected sites, compared to healthy controls or sites with periodontitis [86–90]. Further studies also showed that peri-implantitis treatment reduced the PICF levels of IL-1β [91] and TNF-α [92, 93]. Matrix metalloproteinases (MMPs) or their tissue inhibitors (TIMPs) are also found in high levels in PICF from peri-implantitis sites are elevated compared to healthy sites, and their enzymatic activity increases with disease severity or at sites with progressive bone loss risk [89, 94]. The regulation of osteoclastogenesis and osteogenesis-associated markers has also been studied in PICF [95, 96]. Similar to findings in GCF obtained from sites with periodontitis, there is increasing evidence for the association of the RANKL and its inhibitor OPG with the presence and severity of peri-implantitis [48, 97–100]. Higher PICF levels of cathepsin K, a collagenase that is mainly expressed by osteoclasts, have been shown to be associated with peri-implantitis [95, 96, 101]. Although further studies are needed, the current evidence suggests that the assessment of pro-inflammatory cytokines, that is, IL-1β, TNFα, MMP-8, or alveolar bone turnover/resorption molecules, that is, RANKL/OPG or Cathepsin-K in the PICF may be of value as predictors of peri-implant diseases.

3.1.6 Saliva

As an alternative to peri-implant crevicular fluid (PICF), saliva might be used to study host responses against dental implants. Saliva, a complex biofluid composed of minor and major salivary gland secretions, serum and salivary inflammatory mediators and components from the oral microflora, has the potential to reflect oral and systemic health and diseases and can be obtained noninvasively and in large quantities [102, 103]. Various pro- and anti-inflammatory molecules, proteolytic enzymes involved in tissue breakdown as well as markers for bone resorption have been studied in saliva in response to dental implants [104–113].

Significantly higher levels of IL-1β, IL-6, TNF-α, and procalcitonin were present in the saliva of individuals with peri-implantitis compared to healthy controls, and also peri-implant mucositis for procalcitonin [105, 107]. In addition, bleeding on probing positively correlated with salivary procalcitonin in peri-implantitis patients [6, 107]. On the contrary, levels of colony-stimulating factor 1 (CSF-1), IL-34, IL-1β, triggering receptor expressed on myeloid cells (TREM)-1, peptidoglycan recognition protein (PGLYRP)-1, MMP-8, tissue inhibitor of metalloproteinases (TIMP)-1 and MMP-8/TIMP1 ratio in saliva did not differ between peri-implantitis and peri-implant mucositis [9, 12, 110, 113]. Furthermore, CSF-1 in saliva and PICF were positively correlated. Both studies also evaluated the effect of concomitant periodontitis, resulting in significantly higher salivary levels of MMP-8 with both diseases present compared to peri-implantitis alone, while salivary levels of all other molecules were not affected. Furthermore, IL-1β levels in saliva of peri-implant mucositis patients with or without a previous history of periodontitis did not differ [5, 106]. However, in peri-implant mucositis patients without a previous exposure to periodontitis, salivary IL-1β predicted higher levels of IL-1β levels in PICF. Another study investigated peri-implant mucositis patients under regular peri-implant and periodontal therapy or not (controls) at baseline and 5 years after implant placement [108].

Salivary TNF-α was significantly elevated in patients without regular maintenance compared to patients under regular therapy, while IL-1β, IL-10, MMP-2/TIMP-2 complex, Receptor activator of nuclear factor-κB (RANK), osteoprotegerin (OPG), transforming growth factor (TGF)-β did not show any differences.

In a proof-of-concept study, a chewing gum detector was evaluated for its potential to measure MMP-8 activity in saliva by a peptide sensor which when cleaved releases a bitter substance [10, 111]. A significantly higher MMP-8 activity was detected in saliva from peri-implantitis and peri-implant mucositis patients compared to healthy controls. On the contrary, a commercial MMP-8 activity assay used as a control assay was not able to distinguish between healthy and diseased. Furthermore, a pilot study investigated pathogenic gene sets in the saliva of individuals with implant failure due to severe peri-implantitis using a whole-exome sequencing approach [109]. Significant enrichments were identified in gene sets for cytoskeleton, cell adhesion, and metal ion binding. The latter was also identified as a central functional group which, if misregulated, could interfere with cell morphology and adhesion and finally lead to implant failure.

Further studies investigated the host responses to restored and functional implants in the presence and absence of systemic diseases such as obesity [104] or type II diabetes [112]. Salivary IL-1β and IL-6 levels, as well as mean plaque, bleeding on probing, probing depth scores, and bone loss were significantly higher in obese than nonobese men [104]. The study on type II diabetes used an array-based multiplex assay to assess multiple inflammatory molecules at the same time, including IL-1b, IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, interferon (INF)-γ, C-reactive protein (CRP), macrophage inflammatory protein (MIP)-1α, MIP-1β, MMP-1, MMP-2, MMP-8, MMP-9, TIMP-1, TIMP-2, OPG, adiponectin, and procalcitonin (ProCT) [11, 112]. Salivary markers were measured at baseline and 1 year after implant placement and did not show big differences between the diseased and healthy groups. In patients with type II diabetes, IL-4, IL-10, and OPG were significantly decreased at the 1-year follow-up compared to baseline, while in healthy controls OPG was significantly increased after 1 year compared to baseline. Furthermore, in type II diabetes patients compared to healthy controls, OPG levels were already significantly higher at baseline. None of the other molecules were significantly affected.

3.1.7 Serum

The investigation of health and disease biomarkers in the blood is a standard method, but trends are turning towards other biofluids than blood such as saliva, which can be collected non-invasively and does not require specially trained personnel [103]. However, even though most inflammatory molecules in blood also seem to be detectable in saliva, the concentration of those molecules in the saliva is often substantially lower which might be due to the fluctuating salivary flow rate depending on the circadian rhythm [102, 103]. Hence, investigating the host response to dental implants using whole blood, serum, or plasma should not be neglected. Various studies, as described above for saliva, have investigated inflammatory molecules, proteolytic enzymes, and bone resorption markers in the blood in response to dental biofilm-driven peri-implantitis [112, 114, 115]. A cohort of patients with either successfully osseointegrated dental implants or with dental implants that failed to osseointegrate was investigated for serum IgG to *Actinomyces viscosus*, *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Staphylococcus aureus,* and *Streptococcus intermedius* [13]. Patients with failed implants presented with significantly lower levels of IgG to *S. aureus*, *P. gingivalis,* and *B. forsythus* compared to individuals with successful implants. Furthermore, patients with at least one failed dental implant due to pain, implant movement, or peri-implantitis were tested for IL-1 polymorphisms in the blood [115]. Six out of the 22 patients tested positive for the IL-1 genotype, but the genotype (IL-1 positive or IL-1 negative) did not differentially affect implant failure. However, in smokers, a positive IL-1 genotype resulted in a significantly higher implant

failure rate compared to IL-1 positive non-smokers. The study mentioned above investigating the host responses in saliva to functional implants in type II diabetes also analyzed the same molecules in serum at baseline and 1 year after implant placement by using an array-based multiplex assay [112]. Among all molecules, differences were only seen for serum MMP-1, which was significantly higher in healthy controls than type II diabetes patients.

References

1. Williams DF. Specifications for innovative, enabling biomaterials based on the principles of biocompatibility mechanisms. Front Bioeng Biotechnol. 2019;7:255.
2. Williams D. Revisiting the definition of biocompatibility. Med Device Technol. 2003;14(8):10–3.
3. Schroeder A, van der Zypen E, Stich H, Sutter F. The reactions of bone, connective tissue, and epithelium to endosteal implants with titanium-sprayed surfaces. J Maxillofac Surg. 1981;9(1):15–25.
4. Bosshardt DD, Salvi GE, Huynh-Ba G, Ivanovski S, Donos N, Lang NP. The role of bone debris in early healing adjacent to hydrophilic and hydrophobic implant surfaces in man. Clin Oral Implants Res. 2011;22(4):357–64.
5. Berglundh T, Abrahamsson I, Lang NP, Lindhe J. De novo alveolar bone formation adjacent to endosseous implants. Clin Oral Implants Res. 2003;14(3):251–62.
6. Eriksson C, Lausmaa J, Nygren H. Interactions between human whole blood and modified TiO_2-surfaces: influence of surface topography and oxide thickness on leukocyte adhesion and activation. Biomaterials. 2001;22(14):1987–96.
7. Abrahamsson I, Berglundh T, Linder E, Lang NP, Lindhe J. Early bone formation adjacent to rough and turned endosseous implant surfaces. An experimental study in the dog. Clin Oral Implants Res. 2004;15(4):381–92.
8. Trindade R, Albrektsson T, Wennerberg A. Current concepts for the biological basis of dental implants: foreign body equilibrium and osseointegration dynamics. Oral Maxillofac Surg Clin North Am. 2015;27(2):175–83.
9. Maenpaa J, Soderstrom KO, Salmi T, Ekblad U. Large atypical polyps of the vagina during pregnancy with concomitant human papilloma virus infection. Eur J Obstet Gynecol Reprod Biol. 1988;27(1):65–9.
10. Ivanovski S, Hamlet S, Salvi GE, Huynh-Ba G, Bosshardt DD, Lang NP, et al. Transcriptional profiling of osseointegration in humans. Clin Oral Implants Res. 2011;22(4):373–81.
11. Donos N, Hamlet S, Lang NP, Salvi GE, Huynh-Ba G, Bosshardt DD, et al. Gene expression profile of osseointegration of a hydrophilic compared with a hydrophobic microrough implant surface. Clin Oral Implants Res. 2011;22(4):365–72.
12. Tomasi C, Tessarolo F, Caola I, Piccoli F, Wennstrom JL, Nollo G, et al. Early healing of peri-implant mucosa in man. J Clin Periodontol. 2016;43(10):816–24.
13. Gurkan A, Tekdal GP, Bostanci N, Belibasakis GN. Cytokine, chemokine, and growth factor levels in peri-implant sulcus during wound healing and osseointegration after piezosurgical versus conventional implant site preparation: randomized, controlled, split-mouth trial. J Periodontol. 2019;90(6):616–26.
14. Peker Tekdal G, Bostanci N, Belibasakis GN, Gurkan A. The effect of piezoelectric surgery implant osteotomy on radiological and molecular parameters of peri-implant crestal bone loss: a randomized, controlled, split-mouth trial. Clin Oral Implants Res. 2016;27(5):535–44.
15. Ozturk VO, Emingil G, Bostanci N, Belibasakis GN. Impact of implant-abutment connection on osteoimmunological and microbiological parameters in short implants: a randomized controlled clinical trial. Clin Oral Implants Res. 2017;28(9):e111–e20.
16. Hotchkiss KM, Reddy GB, Hyzy SL, Schwartz Z, Boyan BD, Olivares-Navarrete R. Titanium surface characteristics, including topography and wettability, alter macrophage activation. Acta Biomater. 2016;31:425–34.
17. Alfarsi MA, Hamlet SM, Ivanovski S. The effect of platelet proteins released in response to titanium implant surfaces on macrophage pro-inflammatory cytokine gene expression. Clin Implant Dent Relat Res. 2015;17(6):1036–47.
18. Wang Y, Zhang Y, Sculean A, Bosshardt DD, Miron RJ. Macrophage behavior and interplay with gingival fibroblasts cultured on six commercially available titanium, zirconium, and titanium-zirconium dental implants. Clin Oral Investig. 2019;23(8):3219–27.
19. Hotchkiss KM, Ayad NB, Hyzy SL, Boyan BD, Olivares-Navarrete R. Dental implant surface chemistry and energy alter macrophage activation in vitro. Clin Oral Implants Res. 2017;28(4):414–23.
20. Albrektsson T, Dahlin C, Jemt T, Sennerby L, Turri A, Wennerberg A. Is marginal bone loss around oral implants the result of a provoked foreign body reaction? Clin Implant Dent Relat Res. 2014;16(2):155–65.
21. Trindade R, Albrektsson T, Galli S, Prgomet Z, Tengvall P, Wennerberg A. Osseointegration and foreign body reaction: titanium implants activate the immune system and suppress bone resorption during the first 4 weeks after implantation. Clin Implant Dent Relat Res. 2018;20(1):82–91.
22. Young CW, Lee JS, Le H, Smith RA. Surrogate markers of health after titanium dental implant placement. J Oral Maxillofac Surg. 2004;62(11):1413–7.

23. Thomas P, Iglhaut G, Wollenberg A, Cadosch D, Summer B. Allergy or tolerance: reduced inflammatory cytokine response and concomitant IL-10 production of lymphocytes and monocytes in symptom-free titanium dental implant patients. Biomed Res Int. 2013;2013:539834.
24. Quabius ES, Ossenkop L, Harder S, Kern M. Dental implants stimulate expression of Interleukin-8 and its receptor in human blood—an in vitro approach. J Biomed Mater Res B Appl Biomater. 2012;100(5):1283–8.
25. Harder S, Quabius ES, Ossenkop L, Mehl C, Kern M. Surface contamination of dental implants assessed by gene expression analysis in a whole-blood in vitro assay: a preliminary study. J Clin Periodontol. 2012;39(10):987–94.
26. Harder S, Quabius ES, Meinke F, Mehl C, Kern M. Changes in proinflammatory gene expression in human whole blood after contact with UV-conditioned implant surfaces. Clin Oral Investig. 2019;23(10):3731–8.
27. Albrektsson T, Buser D, Sennerby L. Crestal bone loss and oral implants. Clin Implant Dent Relat Res. 2012;14(6):783–91.
28. Kotsakis GA, Olmedo DG. Peri-implantitis is not periodontitis: scientific discoveries shed light on microbiome-biomaterial interactions that may determine disease phenotype. Periodontol 2000. 2021;86(1):231–40.
29. Pettersson M, Kelk P, Belibasakis GN, Bylund D, Molin Thoren M, Johansson A. Titanium ions form particles that activate and execute interleukin-1beta release from lipopolysaccharide-primed macrophages. J Periodontal Res. 2017;52(1):21–32.
30. Olmedo D, Fernandez MM, Guglielmotti MB, Cabrini RL. Macrophages related to dental implant failure. Implant Dent. 2003;12(1):75–80.
31. Tatarakis N, Bashutski J, Wang HL, Oh TJ. Early implant bone loss: preventable or inevitable? Implant Dent. 2012;21(5):379–86.
32. Atieh MA, Alsabeeha NHM, Tawse-Smith A, Duncan WJ. Piezoelectric versus conventional implant site preparation: a systematic review and meta-analysis. Clin Implant Dent Relat Res. 2018;20(2):261–70.
33. Capelli M. Surgical, biologic and implant-related factors affecting bone remodeling around implants. Eur J Esthet Dent. 2013 Summer;8(2):279–313. PMID: 23712347.
34. Berglundh T, Abrahamsson I, Welander M, Lang NP, Lindhe J. Morphogenesis of the peri-implant mucosa: an experimental study in dogs. Clin Oral Implants Res. 2007;18(1):1–8.
35. Tomasi C, Tessarolo F, Caola I, Wennstrom J, Nollo G, Berglundh T. Morphogenesis of peri-implant mucosa revisited: an experimental study in humans. Clin Oral Implants Res. 2014;25(9):997–1003.
36. Araujo MG, Lindhe J. Peri-implant health. J Periodontol. 2018;89(Suppl 1):S249–S56.
37. Berglundh T, Lindhe J, Jonsson K, Ericsson I. The topography of the vascular systems in the periodontal and peri-implant tissues in the dog. J Clin Periodontol. 1994;21(3):189–93.
38. Emecen-Huja P, Eubank TD, Shapiro V, Yildiz V, Tatakis DN, Leblebicioglu B. Peri-implant versus periodontal wound healing. J Clin Periodontol. 2013;40(8):816–24.
39. Khoury SB, Thomas L, Walters JD, Sheridan JF, Leblebicioglu B. Early wound healing following one-stage dental implant placement with and without antibiotic prophylaxis: a pilot study. J Periodontol. 2008;79(10):1904–12.
40. Xiong J, Gronthos S, Bartold PM. Role of the epithelial cell rests of Malassez in the development, maintenance and regeneration of periodontal ligament tissues. Periodontol 2000. 2013;63(1):217–33.
41. Eggert FM, Levin L. Biology of teeth and implants: host factors—pathology, regeneration, and the role of stem cells. Quintessence Int. 2018;49(6):497–509.
42. Schou S, Holmstrup P, Reibel J, Juhl M, Hjorting-Hansen E, Kornman KS. Ligature-induced marginal inflammation around osseointegrated implants and ankylosed teeth: stereologic and histologic observations in cynomolgus monkeys (Macaca fascicularis). J Periodontol. 1993;64(6):529–37.
43. Furst MM, Salvi GE, Lang NP, Persson GR. Bacterial colonization immediately after installation on oral titanium implants. Clin Oral Implants Res. 2007;18(4):501–8.
44. Belibasakis GN, Charalampakis G, Bostanci N, Stadlinger B. Peri-implant infections of oral biofilm etiology. Adv Exp Med Biol. 2015;830:69–84.
45. Belibasakis GN. Microbiological and immunopathological aspects of peri-implant diseases. Arch Oral Biol. 2014;59(1):66–72.
46. Liljenberg B, Gualini F, Berglundh T, Tonetti M, Lindhe J. Composition of plaque-associated lesions in the gingiva and the peri-implant mucosa in partially edentulous subjects. J Clin Periodontol. 1997;24(2):119–23.
47. Abrahamsson I, Berglundh T, Lindhe J. Soft tissue response to plaque formation at different implant systems. A comparative study in the dog. Clin Oral Implants Res. 1998;9(2):73–9.
48. Salvi GE, Aglietta M, Eick S, Sculean A, Lang NP, Ramseier CA. Reversibility of experimental peri-implant mucositis compared with experimental gingivitis in humans. Clin Oral Implants Res. 2012;23(2):182–90.
49. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. J Periodontol. 2018;89(Suppl 1):S257–S66.
50. Heitz-Mayfield LJA, Salvi GE. Peri-implant mucositis. J Clin Periodontol. 2018;45(Suppl 20):S237–S45.
51. Dionigi C, Larsson L, Carcuac O, Berglundh T. Cellular expression of DNA damage/repair and reactive oxygen/nitrogen species in human periodontitis and peri-implantitis lesions. J Clin Periodontol. 2020;47(12):1466–75.

52. Charalampakis G, Abrahamsson I, Carcuac O, Dahlen G, Berglundh T. Microbiota in experimental periodontitis and peri-implantitis in dogs. Clin Oral Implants Res. 2014;25(9):1094–8.
53. Gualini F, Berglundh T. Immunohistochemical characteristics of inflammatory lesions at implants. J Clin Periodontol. 2003;30(1):14–8.
54. Lindhe J, Berglundh T, Ericsson I, Liljenberg B, Marinello C. Experimental breakdown of peri-implant and periodontal tissues. A study in the beagle dog. Clin Oral Implants Res. 1992;3(1):9–16.
55. Carcuac O, Berglundh T. Composition of human peri-implantitis and periodontitis lesions. J Dent Res. 2014;93(11):1083–1088.
56. Duarte PM, de Mendonca AC, Maximo MB, Santos VR, Bastos MF, Nociti Junior FH. Differential cytokine expressions affect the severity of peri-implant disease. Clin Oral Implants Res. 2009;20(5):514–20.
57. Venza I, Visalli M, Cucinotta M, De Grazia G, Teti D, Venza M. Proinflammatory gene expression at chronic periodontitis and peri-implantitis sites in patients with or without type 2 diabetes. J Periodontol. 2010;81(1):99–108.
58. Liu Y, Liu Q, Li Z, Acharya A, Chen D, Chen Z, et al. Long non-coding RNA and mRNA expression profiles in peri-implantitis vs periodontitis. J Periodontal Res. 2020;55(3):342–53.
59. Belibasakis GN, Reddi D, Bostanci N. Porphyromonas gingivalis induces RANKL in T-cells. Inflammation. 2011;34(2):133–8.
60. Becker ST, Foge M, Beck-Broichsitter BE, Gavrilova O, Bolte H, Rosenstiel P, et al. Induction of periimplantitis in dental implants. J Craniofac Surg. 2013;24(1):e15–8.
61. Zitzmann NU, Berglundh T, Ericsson I, Lindhe J. Spontaneous progression of experimentally induced periimplantitis. J Clin Periodontol. 2004;31(10):845–9.
62. Schwarz F, Herten M, Sager M, Bieling K, Sculean A, Becker J. Comparison of naturally occurring and ligature-induced peri-implantitis bone defects in humans and dogs. Clin Oral Implants Res. 2007;18(2):161–70.
63. Schou S, Holmstrup P, Stoltze K, Hjorting-Hansen E, Fiehn NE, Skovgaard LT. Probing around implants and teeth with healthy or inflamed peri-implant mucosa/gingiva. A histologic comparison in cynomolgus monkeys (Macaca fascicularis). Clin Oral Implants Res. 2002;13(2):113–26.
64. Hiyari S, Wong RL, Yaghsezian A, Naghibi A, Tetradis S, Camargo PM, et al. Ligature-induced peri-implantitis and periodontitis in mice. J Clin Periodontol. 2018;45(1):89–99.
65. Yu X, Hu Y, Freire M, Yu P, Kawai T, Han X. Role of toll-like receptor 2 in inflammation and alveolar bone loss in experimental peri-implantitis versus periodontitis. J Periodontal Res. 2018;53(1):98–106.
66. Hiyari S, Naghibi A, Wong R, Sadreshkevary R, Yi-Ling L, Tetradis S, et al. Susceptibility of different mouse strains to peri-implantitis. J Periodontal Res. 2018;53(1):107–16.
67. Carcuac O, Abrahamsson I, Albouy JP, Linder E, Larsson L, Berglundh T. Experimental periodontitis and peri-implantitis in dogs. Clin Oral Implants Res. 2013;24(4):363–71.
68. Albouy JP, Abrahamsson I, Persson LG, Berglundh T. Spontaneous progression of ligatured induced peri-implantitis at implants with different surface characteristics. An experimental study in dogs II: histological observations. Clin Oral Implants Res. 2009;20(4):366–71.
69. Wong RL, Hiyari S, Yaghsezian A, Davar M, Casarin M, Lin YL, et al. Early intervention of peri-implantitis and periodontitis using a mouse model. J Periodontol. 2018;89(6):669–79.
70. Deng S, Hu Y, Zhou J, Wang Y, Wang Y, Li S, et al. TLR4 mediates alveolar bone resorption in experimental peri-implantitis through regulation of CD45(+) cell infiltration, RANKL/OPG ratio, and inflammatory cytokine production. J Periodontol. 2020;91(5):671–82.
71. Ding L, Zhang P, Wang X, Kasugai S. A doxycycline-treated hydroxyapatite implant surface attenuates the progression of peri-implantitis: a radiographic and histological study in mice. Clin Implant Dent Relat Res. 2019;21(1):154–9.
72. Almohandes A, Abrahamsson I, Dahlen G, Berglundh T. Effect of biofilm formation on implant abutments with an anti-bacterial coating: a pre-clinical in vivo study. Clin Oral Implants Res. 2021;32(6):756–66.
73. Sanz-Esporrin J, Di Raimondo R, Pla R, Luengo F, Vignoletti F, Nunez J, et al. Experimental peri-implantitis around titanium implants with a chemically modified surface with a monolayer of multi-phosphonate molecules: a pre-clinical in vivo investigation. Clin Oral Investig. 2021;25(6):3789–800.
74. Serino G, Turri A, Lang NP. Probing at implants with peri-implantitis and its relation to clinical peri-implant bone loss. Clin Oral Implants Res. 2013;24(1):91–5.
75. Lang NP, Wetzel AC, Stich H, Caffesse RG. Histologic probe penetration in healthy and inflamed peri-implant tissues. Clin Oral Implants Res. 1994;5(4):191–201.
76. Lekholm U, Adell R, Lindhe J, Branemark PI, Eriksson B, Rockler B, et al. Marginal tissue reactions at osseointegrated titanium fixtures. (II) A cross-sectional retrospective study. Int J Oral Maxillofac Surg. 1986;15(1):53–61.
77. Bostanci N, Belibasakis GN. Gingival crevicular fluid and its immune mediators in the proteomic era. Periodontol 2000. 2018;76(1):68–84.
78. Adonogianaki E, Mooney J, Wennstrom JL, Lekholm U, Kinane DF. Acute-phase proteins and immunoglobulin G against Porphyromonas gingivalis in peri-implant crevicular fluid: a comparison

with gingival crevicular fluid. Clin Oral Implants Res. 1995;6(1):14–23.
79. Kaklamanos EG, Tsalikis L. A review on peri-implant crevicular fluid assays potential in monitoring and predicting peri-implant tissue responses. J Int Acad Periodontol. 2002;4(2):49–59.
80. Golub LM, Raisanen IT, Sorsa T, Preshaw PM. An unexplored pharmacologic/diagnostic strategy for peri-implantitis: a protocol proposal. Diagnostics (Basel). 2020;10(12)
81. Thierbach R, Maier K, Sorsa T, Mantyla P. Peri-implant sulcus fluid (PISF) matrix metalloproteinase (MMP) -8 levels in peri-implantitis. J Clin Diagn Res. 2016;10(5):ZC34–8.
82. Esberg A, Isehed C, Holmlund A, Lundberg P. Peri-implant crevicular fluid proteome before and after adjunctive enamel matrix derivative treatment of peri-implantitis. J Clin Periodontol. 2019;46(6):669–77.
83. Guncu GN, Akman AC, Gunday S, Yamalik N, Berker E. Effect of inflammation on cytokine levels and bone remodelling markers in peri-implant sulcus fluid: a preliminary report. Cytokine. 2012;59(2):313–6.
84. Schierano G, Pejrone G, Brusco P, Trombetta A, Martinasso G, Preti G, et al. TNF-alpha TGF-beta2 and IL-1beta levels in gingival and peri-implant crevicular fluid before and after de novo plaque accumulation. J Clin Periodontol. 2008;35(6):532–8.
85. Schincaglia GP, Hong BY, Rosania A, Barasz J, Thompson A, Sobue T, et al. Clinical, immune, and microbiome traits of gingivitis and peri-implant mucositis. J Dent Res. 2017;96(1):47–55.
86. Curtis DA, Kao R, Plesh O, Finzen F, Franz L. Crevicular fluid analysis around two failing dental implants: a clinical report. J Prosthodont. 1997;6(3):210–4.
87. Severino VO, Napimoga MH, de Lima Pereira SA. Expression of IL-6, IL-10, IL-17 and IL-8 in the peri-implant crevicular fluid of patients with peri-implantitis. Arch Oral Biol. 2011;56(8):823–8.
88. Alassy H, Parachuru P, Wolff L. Peri-implantitis diagnosis and prognosis using biomarkers in peri-implant crevicular fluid: a narrative review. Diagnostics (Basel). 2019;9(4)
89. Wang HL, Garaicoa-Pazmino C, Collins A, Ong HS, Chudri R, Giannobile WV. Protein biomarkers and microbial profiles in peri-implantitis. Clin Oral Implants Res. 2016;27(9):1129–36.
90. Recker EN, Avila-Ortiz G, Fischer CL, Pagan-Rivera K, Brogden KA, Dawson DV, et al. A cross-sectional assessment of biomarker levels around implants versus natural teeth in periodontal maintenance patients. J Periodontol. 2015;86(2):264–72.
91. Bassetti M, Schar D, Wicki B, Eick S, Ramseier CA, Arweiler NB, et al. Anti-infective therapy of peri-implantitis with adjunctive local drug delivery or photodynamic therapy: 12-month outcomes of a randomized controlled clinical trial. Clin Oral Implants Res. 2014;25(3):279–87.
92. de Mendonca AC, Santos VR, Cesar-Neto JB, Duarte PM. Tumor necrosis factor-alpha levels after surgical anti-infective mechanical therapy for peri-implantitis: a 12-month follow-up. J Periodontol. 2009;80(4):693–9.
93. Faot F, Nascimento GG, Bielemann AM, Campao TD, Leite FR, Quirynen M. Can peri-implant crevicular fluid assist in the diagnosis of peri-implantitis? A systematic review and meta-analysis. J Periodontol. 2015;86(5):631–45.
94. Arakawa H, Uehara J, Hara ES, Sonoyama W, Kimura A, Kanyama M, et al. Matrix metalloproteinase-8 is the major potential collagenase in active peri-implantitis. J Prosthodont Res. 2012;56(4):249–55.
95. Strbac GD, Monov G, Cei S, Kandler B, Watzek G, Gruber R. Cathepsin K levels in the crevicular fluid of dental implants: a pilot study. J Clin Periodontol. 2006;33(4):302–8.
96. Yamalik N, Gunday S, Kilinc K, Karabulut E, Berker E, Tozum TF. Analysis of cathepsin-K levels in biologic fluids from healthy or diseased natural teeth and dental implants. Int J Oral Maxillofac Implants. 2011;26(5):991–7.
97. Rakic M, Lekovic V, Nikolic-Jakoba N, Vojvodic D, Petkovic-Curcin A, Sanz M. Bone loss biomarkers associated with peri-implantitis. A cross-sectional study. Clin Oral Implants Res. 2013;24(10):1110–6.
98. Bostanci N, Saygan B, Emingil G, Atilla G, Belibasakis GN. Effect of periodontal treatment on receptor activator of NF-kappaB ligand and osteoprotegerin levels and relative ratio in gingival crevicular fluid. J Clin Periodontol. 2011;38(5):428–33.
99. Reddi D, Bostanci N, Hashim A, Aduse-Opoku J, Curtis MA, Hughes FJ, et al. Porphyromonas gingivalis regulates the RANKL-OPG system in bone marrow stromal cells. Microbes Infect. 2008;10(14–15):1459–68.
100. Rakic M, Struillou X, Petkovic-Curcin A, Matic S, Canullo L, Sanz M, et al. Estimation of bone loss biomarkers as a diagnostic tool for peri-implantitis. J Periodontol. 2014;85(11):1566–74.
101. Yamalik N, Gunday S, Uysal S, Kilinc K, Karabulut E, Tozum TF. Analysis of cathepsin-K activity at tooth and dental implant sites and the potential of this enzyme in reflecting alveolar bone loss. J Periodontol. 2012;83(4):498–505.
102. Lee YH, Wong DT. Saliva: an emerging biofluid for early detection of diseases. Am J Dent. 2009;22(4):241–8.
103. Castagnola M, Scarano E, Passali GC, Messana I, Cabras T, Iavarone F, et al. Salivary biomarkers and proteomics: future diagnostic and clinical utilities. Acta Otorhinolaryngol Ital. 2017;37(2):94–101.
104. Abduljabbar T, Al-Sahaly F, Kellesarian SV, Kellesarian TV, Al-Anazi M, Al-Khathami M, et al. Comparison of peri-implant clinical and radiographic inflammatory parameters and whole salivary destructive inflammatory cytokine pro-

file among obese and non-obese men. Cytokine. 2016;88:51–6.
105. Abduljabbar T, Vohra F, Ullah A, Alhamoudi N, Khan J, Javed F. Relationship between self-rated pain and peri-implant clinical, radiographic and whole salivary inflammatory markers among patients with and without peri-implantitis. Clin Implant Dent Relat Res. 2019;21(6):1218–24.
106. Acharya A, Koh ML, Kheur S, Watt RM, Jin L, Mattheos N. Salivary IL-1beta and red complex bacteria as predictors of the inflammatory status in sub-peri-implant niches of subjects with peri-implant mucositis. Clin Oral Implants Res. 2016;27(6):662–7.
107. Algohar A, Alqerban A. Levels of procalcitonin in saliva and peri-implant crevicular fluid in patients with peri-implant diseases and health. Arch Oral Biol. 2020;120:104931.
108. Gomes AM, Douglas-de-Oliveira DW, Ferreira SD, Silva TAD, Cota LOM, Costa FO. Periodontal disease, peri-implant disease and levels of salivary biomarkers IL-1beta, IL-10, RANK, OPG, MMP-2, TGF-beta and TNF-alpha: follow-up over 5 years. J Appl Oral Sci. 2019;27:e20180316.
109. Lee S, Kim JY, Hwang J, Kim S, Lee JH, Han DH. Investigation of pathogenic genes in peri-implantitis from implant clustering failure patients: a whole-exome sequencing pilot study. PLoS One. 2014;9(6):e99360.
110. Lira-Junior R, Teixeira MKS, Lourenco EJV, Telles DM, Figueredo CM, Bostrom EA. CSF-1 and IL-34 levels in peri-implant crevicular fluid and saliva from patients having peri-implant diseases. Clin Oral Investig. 2020;24(1):309–15.
111. Ritzer J, Luhmann T, Rode C, Pein-Hackelbusch M, Immohr I, Schedler U, et al. Diagnosing peri-implant disease using the tongue as a 24/7 detector. Nat Commun. 2017;8(1):264.
112. Tatarakis N, Kinney JS, Inglehart M, Braun TM, Shelburne C, Lang NP, et al. Clinical, microbiological, and salivary biomarker profiles of dental implant patients with type 2 diabetes. Clin Oral Implants Res. 2014;25(7):803–12.
113. Teixeira MKS, Lira-Junior R, Lourenco EJV, Telles DM, Bostrom EA, Figueredo CM, et al. The modulation of the TREM-1/PGLYRP1/MMP-8 axis in peri-implant diseases. Clin Oral Investig. 2020;24(5):1837–44.
114. Kronstrom M, Svensson B, Erickson E, Houston L, Braham P, Persson GR. Humoral immunity host factors in subjects with failing or successful titanium dental implants. J Clin Periodontol. 2000;27(12):875–82.
115. Jansson H, Hamberg K, De Bruyn H, Bratthall G. Clinical consequences of IL-1 genotype on early implant failures in patients under periodontal maintenance. Clin Implant Dent Relat Res. 2005;7(1):51–9.