Bystander Activation: A Germane Mechanism in the Pathogenesis of Periodontitis

[1]Dr. B. Ramya Bharathi, [2]Dr. C. Srikanth, [3]Dr. M. Navya, [4]Dr. P. Usha

[1]Post Graduate Student, [2]Professor and Head, [3]Professor
[1]Department of Periodontology,
[1]Mallareddy Institute of Dental Sciences, Hyderabad, India

Abstract: **It is characterized by autoreactive B and T cells that undergo activation in an antigen-independent manner. Activation of such includes inflammatory milieu, co-signaling ligands and interaction with neighboring cells in periodontal diseases. The evasion of periodontal pathogens in the host causes immune dysregulation and further activates the enzymes that could directly or indirectly trigger the innate immune cells, e.g. pattern recognition receptors. The significant variations occurring in the infected cell, as well as an adjacent uninfected cell to produce inflammatory mediators through autocrine or paracrine signaling cascade, is called Bystander activation. This review aims to summarize the host and oral microbiota changes involved in the physio-pathogenesis of periodontitis that has been described as bystander activation which is contributing to overall responses causing disease.**

Index Terms: **Bystander activation, Autoimmunity, Innate immunity.**

I. INTRODUCTION

The indirect non antigenic -specific phenomenon termed bystander effect, occurs simultaneously, appears as overall, and transtimulated response to specific and nonspecific immune events.

Bystander activation was first described in 1996 by Tough et al.(1) in an attempt to understand the massive clonal T-cell expansion that occurs after a heterologous viral infection. Indeed, they hypothesized that T cells could proliferate and expand in a non-specific, T cell receptor (TCR)-independent manner. They demonstrated that type I interferons (IFNs) could act as an adjuvant and could induce T-cell proliferation with no-TCR involvement.

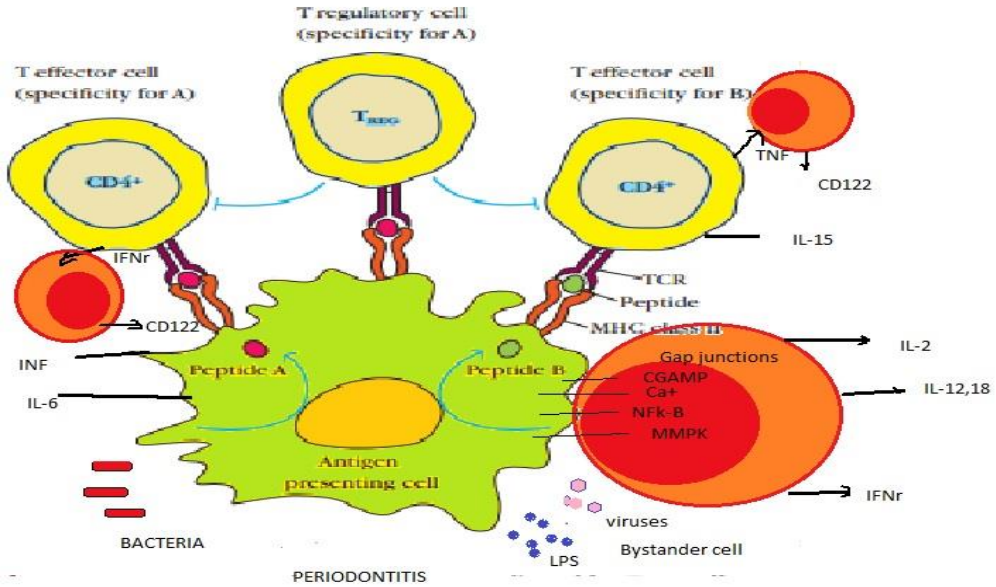
Bystander activation is mediated by indirect signals that favor an inflammatory events such asligands of co-signaling receptors, cytokines, chemokines, pathogen-associated molecular patterns, and extracellular vesicles with microbial particles(2). We understand periodontitis as the result of dysbiosis of the oral microbiota guided by inflammophilic bacteria, leading to an altered resolution of inflammation and lack of regulation of the inflammatory responses(3), Thus, this mechanism, although without a demonstrated specific involvement in periodontal disease, hypothetically could participate in the pathogenesis of periodontitis from the activation of host responses to dysbiotic changes, which involve innate immunity, as in the case of dysregulation of TLRs and all the branches of immunity as in the hyperproduction of cytokines

II. MECHANISM OF BYSTANDER ACTIVATION

An adaptation mechanism of innate immunity has been described as bystander activation. This mechanism may allow the immune system to overcome the pathogen's ability to disarm the immune signaling pathways in infected cells. The mechanism has been described in viral and bacterial infections. In addition to communication through microorganisms, cytokines or pathogen-associated molecular patterns, macrophages have been described in vitro as releasing active inflammasomes to establish contact. The presence of bystander effect well executed by T-Cell and B-cell activation in various studies.

Recent evidence could indicate that there is an underestimation of the role of innate pathogen-associated receptors in T cells, since they have been shown to have non-classical activation patterns. That goes beyond the exclusive reliance on antigenic recognition through TCR. Both the gd T lymphocytes and the mucosal associated invariant T (MAIT) cells, and the conventional ab CD4+ and CD8+ T-cells in mice and humans express TLRs, demonstrating that the cells of adaptive immunity also use these innate signaling pathways leading to the promotion of T helper cell-dependent inflammation through TLRs; this makes us think of these receptors as important regulators of the disease during infection. This process of activation independent of TCR antigen presentation, also occurs by bystander activation, and was described more than 2 decades ago based on the T cell expansion observed in viral infections(4).

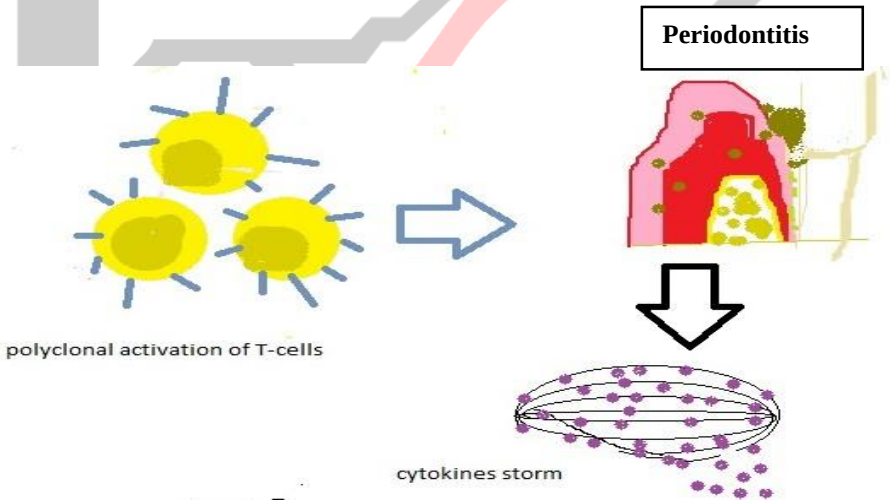
Cells infected by bacteria or viruses can induce activation of uninfected cells through soluble signals such as cytokines, coreceptor expression (example TLR, CD122, and NKG2D), and intercellular communication mediated by GAP junctions(5). Lipopolysaccharides (LPS) can activate T lymphocytes nonantigen specific. Furthermore, they can activate dendritic cells by up-regulation of CD86 and IFN-g production. Innate immunity mediators such as DC and natural killer cells (NK), induce bystander activation of T cells in response to TLRs agonists through the production of IFN-a/b/g(6)



Bystander activation mechanism

Multiple autoimmune diseases are associated with bystander activation: RA, SLE, type 1 diabetes, MS, autoimmune hepatitis, and autoimmune thyroid disease, among others(5). Specifically, for direct activation of T cell signaling through TLRs, there is evidence indicating that recognition of T cells by TLR3, TLR4, TLR7, and TLR9 is related to disease aggravation in models of infection, cancer, and autoimmunity(7).

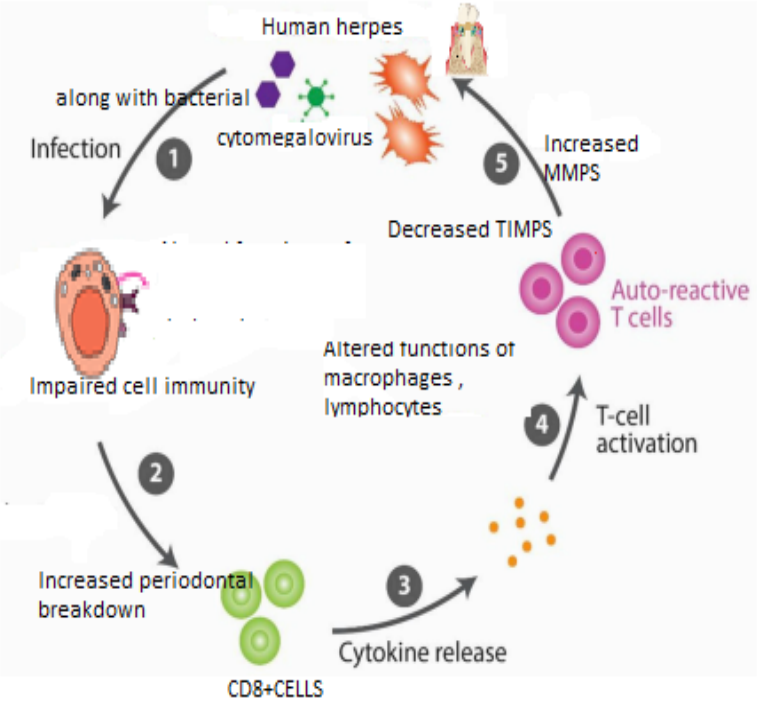
All the mechanisms described above converge in the bystander activation mechanisms of T cells, since it involves the participation not only of innate receptors but of other factors such as superantigens by polyclonal activation of T cells, which in turn triggers a storm of cytokines. Additionally, molecular mimicry, dual TCR signaling, and virtual memory T cells(8) (which has been observed in experimental animals) are other none classical T cell activation pathways(7). Thus, this mechanism, although without a demonstrated specific involvement in periodontal disease, hypothetically could participate in the pathogenesis of periodontitis from the activation of host responses to dysbiotic changes, which involve innate immunity, as in the case of dysregulation of TLRs and all the branches of immunity as in the hyperproduction of cytokines.



T-cell activation

III. VIRAL PATHOGENS AS BYSTANDERS:

The ability of herpes viruses to advance chronic periodontitis in its active phase and the synergism of cytomegalovirus with Porphyromonas gingivalis in aggressive periodontitis cases refute the assumption that viruses are just bystanders in periodontal disease. The clinical need for further antiviral medications is still disputed because most viruses are inhibited after mechanical debridement. However, if a latent virus is awakened, it can cause sickness. As a result, using antiviral prophylaxis and treatments with caution can help manage advanced untreatable cases of aggressive and chronic periodontitis. Considering the prevailing evidence of high copy counts of viruses like EBV and Cytomegaloviruses in severe periodontitis, it's unlikely that these viruses are just silent bystanders. (9)



Viruses as bystanders

IV. BACTERIAL PATHOGENS AS BYSTANDERS:

Bystander activation is triggered by soluble signals or in response to bacterial PAMPS or infection. It was discovered that in response to LPS and viral PAMPs, a limited number of early responder cells release IFN, which promotes antimicrobial gene expression in additional cells, resulting in a population-level response(10). Single-cell analysis, either through fluorescence microscopic study of RNA expression or single-cell RNA sequencing in combination with various approaches to chemically or physically disrupt cell–cell contact, was necessary to uncover this response. Fluorescence microscopy and flow cytometric analysis of epithelial cells infected by GFP-expressing L. monocytogenes were used in a study evaluating the immunological response produced against L. monocytogenes infection.

The ability of directly infected cells to produce the chemokines CXCL2 and CXCL5 was decreased, showing that L. monocytogenes suppresses chemokine production. Instead, these chemokines were largely produced in non-infected epithelial cells next to infected epithelial cells, and this cell–cell communication was not mediated by gap junctions or cytokine release. Instead, paracrine stimulation of nearby bystander cells was mediated by reactive oxygen intermediates generated by NADPH oxidase (NOX) 4 in infected cells (11). It's unclear how NOX4 is activated in response to L. monocytogenes infection, but it appears to require NOD2 and other cytosolic innate immune receptors detecting L. monocytogenes-derived ligands.

Another example of bystander activation is when infected cells create a restricted repertoire of soluble inflammatory cytokines that can activate bystander cells to produce cytokines and therefore increase and diversity the immune response. There is evidence that infected cells emit innate immune signalling machinery that can be picked up by bystander cells in addition to cell–cell communication via cytokines or bacterial PAMPs. Activated inflammasome complexes are released by macrophages after in vitro stimulation, according to two separate investigations (12). Without the need for further inflammasome-triggering stimuli, these premade inflammasome complexes were internalised and promoted IL-1 maturation in nearby bystander cells.

Bacterial lipopolysaccharide (LPS) has been shown to activate non-antigen-specific T lymphocytes in studies (1). In addition, by upregulating CD86 and producing IFN-, LPS can activate other immune system cells such as DCs. In turn, bystander activation of T cells is induced by innate immunological mediators such as DCs and NK cells in response to TLR agonists via the generation of IFN-/. Microbial receptors (i.e. TLRs) and soluble signals (i.e. cytokines) are both required for T cell activation by bystanders. Co-receptors expressed in these cells can be determined by signals from surrounding cells or the inflammatory microenvironment. Natural killer group 2D (NKG2D), for example, is an activating receptor that is highly expressed on NKs but less frequently expressed on specific CD8+ and CD4+ T cell subsets. Recent research has linked the receptor NKG2D to T cell activation by

bystanders. The effects of NKG2D signalling are comparable to those of TCR signalling, such as phosphorylation of ZAP70. (13) Bystander-activated memory CD8+ T cells, for example, use NKG2D to prevent Listeria monocytogenes infection (5).
In some bacterial infections, infected cells can employ gap junctions to transfer pathogen recognition signals to bystander cells, promoting the bystander effect. Connexin (Cx) generates gap junctions by forming channels that allow low-molecular-weight molecules to pass between neighbouring cells. TLR-2 produced on epithelial cells during Pseudomonas aeruginosa infection of the respiratory airway can activate MAPK signalling, resulting in Ca2+ flow and NF-B translocation, which increases the expression of cytokines and chemokines such as CXCL8, culminating in neutrophil recruitment. According to Martin et al., Ca2+ flux can be transferred to nearby bystander cells via gap junctions (Cx43), increasing epithelial CXCL8 synthesis (14).

In another example, Shigella flexneri-infected cells activate the MAPK (ERK, JNK, and p38) signalling cascade, which can then be passed on to bystander uninfected cells via gap junctions, increasing their IL-8 production (15). 2.2.3. Soluble mediators activate bystanders to control bacterial infection Legionella pneumophila uses a type IV secretion system (T4SS) to successfully infect cells. The T4SS injects complexes of bacterial proteins into the host cytosol and inhibits host protein synthesis, among other things. IL-1 and IL-1 circumvent this suppression in infected macrophages and adjacent cells thanks to MyD88 signalling (16).
Uninfected bystander cells, unlike infected cells, can produce IL-6, TNF, and IL-12, which are important for host protection. Furthermore, the absence of IL-1 receptor signalling reduces the production of bystander cytokines, suggesting that bystander activation is mediated by IL-1 and IL-1 receptor secretion (17). By avoiding the effects of pathogen-derived effector proteins released by the T4SS, this bystander mechanism enables for the formation of an efficient immune response. Chlamydia trachomatis is another example of bystander innate activation. IFN- plays a critical role in the antibacterial immune response in this infection, as it upregulates the production of indoleamine-2,3-dioxygenase-1 (IDO1), which catalyses host cell tryptophan elimination.
Chlamydia trachomatis survival requires tryptophan, which is likewise required for T-cell growth. To avoid this anti-bacterial immune response, bacteria can suppress IFN-production by inhibiting STAT1 translocation into the nucleus, which prevents IDO1 from being induced. Bystander cells will play a key role in limiting bacterial spread by compensating for the loss of IFN- and subsequent IDO1 production and tryptophan catabolism caused by the lack of IFN-. 2.2.4. Particle exchange activation of bystanders Exosomes containing bacterial proteins are released by macrophages infected with Bacillus Calmette-Guérin or Mycobacterium tuberculosis (M. tuberculosis) and increase cytokine production in naive macrophages. Extracellular vesicles generated by M. tuberculosis-infected macrophages also promote TLR-2 signalling and cytokine production in non-infected macrophages, which aids bacterial clearance (18).
Bystander cells have been shown in certain investigations to be able to internalise active inflammasome complexes formed by infected cells. Inflammasome sensors such as AIM2 or NLRP3 are activated by microbial chemicals, which stimulate the polymerization of the ASC adapter and the creation of an ASC speck. Through the triggering of caspase-1, the generation of IL-1, and the death of cells by pyroptosis, this multimeric complex contributes to innate immune responses. Bystander macrophages phagocytoze ASC specks generated by infected cells in the extracellular spaces, causing lysosomal damage and IL-1 production in adjacent cells (19). In this way, IL-1 promotes the quick clearance of bacteria transmitted through macrophage activation by bystanders.

V. FUNGAL PATHOGENS AS BYSTANDERS:

Several fungal species have been isolated from the periodontal pockets of periodontitis patients, the most common of which being Candida albicans. The prevalence of Candida albicans has been linked to the severity of periodontitis (20). It's unclear whether it's just colonising the environment and not actively pathogenic, but accumulating data suggests it has the ability to interact with periodontal pathogens and impact their behaviour. The anaerobes F. nucleatum and P. gingivalis are two bacteria that regularly co-isolate with Candida albicans in periodontal pockets. Surprisingly, fungi have been demonstrated to rapidly deplete oxygen in mixed species habitats, which may explain why obligate anaerobes and yeasts are found together (21).
P. gingivalis, for example, modifies and increases C. albicans germ tube formation, whereas F. nucleatum has been shown to hinder C. albicans hyphal morphogenesis (3). Other investigations have found that P. gingivalis has an antagonistic effect on the yeast-hyphal transition in Candida albicans, with P. gingivalis downregulating hyphal-related genes ALS3, HWP1, and SAP4 in particular (22). The virulence factor InlJ from the internalin protein family, which interacts with the C. albicans adhesin ALS3, has recently been shown to enhance P. gingivalis attachment to C. albicans. Furthermore, co-adhesion specific interactions were observed, in which adhesive connections between these pathogens appear to stimulate P. gingivalis' type 9 secretion system, which is characterised by increased community pathogenicity (23)
Bystander activation has been linked to the start or relapse of various autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), multiple sclerosis (MS), autoimmune hepatitis (AIH), and autoimmune thyroid disease (AITD). Bystander activation of auto-reactive cells may play a role in this activation. It takes just the migration of these cells to the site of inflammation, followed by their unspecific activation and cytokine production, to cause autoimmune disease.

VI. Conclusion

To conclude bystander phenomena might not have a precise role in the effector phase of the immune response and might simply reflect the existence of a network of connections between the different cellular elements of the immune system. These connections could be maintained by the cytokine network or cell surface molecule interactions. Altogether these interactions may participate in the homeostasis of the immune system. We expect that there will be diverse mechanisms driving bystander activation, and that many pathogens will evade or manipulate mechanisms of bystander activation for their own advantage. Understanding the role for uninfected bystander cells in infectious pathologies is not only important for the advancement of our understanding of the host:pathogen biology, but it also will continue to drive the forefronts of medicine, evolutionary biology and the vast study of

infectious diseases. These treatments should be directed against bystander actors such as proinflammatory cytokines, membrane co-receptors, and signaling molecules, among others. Also, they should aim to attenuate the chronic inflammatory milieu in chronic periodontitis patients to avoid the perpetuation of disease, often with a relapsing-remitting nature, in a bystander manner.

References:

1. Tough DF, Borrow P, Sprent J. Induction of Bystander T Cell Proliferation by Viruses and Type I Interferon in Vivo. Science. 1996 Jun 28;272(5270):1947–50.
2. Jara PI, Boric MP, Saez JC. Leukocytes express connexin 43 after activation with lipopolysaccharide and appear to form gap junctions with endothelial cells after ischemia-reperfusion. Proc Natl Acad Sci. 1995 Jul 18;92(15):7011–5.
3. Bor B, Cen L, Agnello M, Shi W, He X. Morphological and physiological changes induced by contact-dependent interaction between Candida albicans and Fusobacterium nucleatum. Sci Rep. 2016 Sep;6(1):27956.
4. Suárez LJ, Garzón H, Arboleda S, Rodríguez A. Oral Dysbiosis and Autoimmunity: From Local Periodontal Responses to an Imbalanced Systemic Immunity. A Review. Front Immunol. 2020 Dec 8;11:591255.
5. Pacheco Y, Acosta-Ampudia Y, Monsalve DM, Chang C, Gershwin ME, Anaya J-M. Bystander activation and autoimmunity. J Autoimmun. 2019 Sep;103:102301.
6. Matsue H, Yao J, Matsue K, Nagasaka A, Sugiyama H, Aoki R, et al. Gap Junction-Mediated Intercellular Communication between Dendritic Cells (DCs) Is Required for Effective Activation of DCs. J Immunol. 2006 Jan 1;176(1):181–90.
7. Whiteside SK, Snook JP, Williams MA, Weis JJ. Bystander T Cells: A Balancing Act of Friends and Foes. Trends Immunol. 2018 Dec;39(12):1021–35.
8. Van Kaer L. Innate and virtual memory T cells in man. Eur J Immunol. 2015 Jul;45(7):1916–20.
9. Aggarwal T, Lamba AK, Faraz F, Tandon S. Viruses: Bystanders of periodontal disease. Microb Pathog. 2017 Jan;102:54–8.
10. Patil S, Fribourg M, Ge Y, Batish M, Tyagi S, Hayot F, et al. Single-cell analysis shows that paracrine signaling by first responder cells shapes the interferon-b response to viral infection. :13.
11. Dolowschiak T, Chassin C, Ben Mkaddem S, Fuchs TM, Weiss S, Vandewalle A, et al. Potentiation of Epithelial Innate Host Responses by Intercellular Communication. Cossart P, editor. PLoS Pathog. 2010 Nov 18;6(11):e1001194.
12. Baroja-Mazo A, Martín-Sánchez F, Gomez AI, Martínez CM, Amores-Iniesta J, Compan V, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. Nat Immunol. 2014 Aug;15(8):738–48.
13. Crosby EJ, Goldschmidt MH, Wherry EJ, Scott P. Engagement of NKG2D on Bystander Memory CD8 T Cells Promotes Increased Immunopathology following Leishmania major Infection. Sacks DL, editor. PLoS Pathog. 2014 Feb 27;10(2):e1003970.
14. Martin FJ, Prince AS. TLR2 Regulates Gap Junction Intercellular Communication in Airway Cells. J Immunol. 2008 Apr 1;180(7):4986–93.
15. Kasper CA, Sorg I, Schmutz C, Tschon T, Wischnewski H, Kim ML, et al. Cell-Cell Propagation of NF-κB Transcription Factor and MAP Kinase Activation Amplifies Innate Immunity against Bacterial Infection. Immunity. 2010 Nov;33(5):804–16.
16. Asrat S, Dugan AS, Isberg RR. The Frustrated Host Response to Legionella pneumophila Is Bypassed by MyD88-Dependent Translation of Pro-inflammatory Cytokines. Roy CR, editor. PLoS Pathog. 2014 Jul 24;10(7):e1004229.
17. Copenhaver AM, Casson CN, Nguyen HT, Duda MM, Shin S. IL-1R signaling enables bystander cells to overcome bacterial blockade of host protein synthesis. Proc Natl Acad Sci. 2015 Jun 16;112(24):7557–62.
18. Athman JJ, Wang Y, McDonald DJ, Boom WH, Harding CV, Wearsch PA. Bacterial Membrane Vesicles Mediate the Release of *Mycobacterium tuberculosis* Lipoglycans and Lipoproteins from Infected Macrophages. J Immunol. 2015 Aug 1;195(3):1044–53.
19. Franklin BS, Bossaller L, De Nardo D, Ratter JM, Stutz A, Engels G, et al. The adaptor ASC has extracellular and "prionoid" activities that propagate inflammation. Nat Immunol. 2014 Aug;15(8):727–37.
20. Canabarro A, Valle C, Farias MR, Santos FB, Lazera M, Wanke B. Association of subgingival colonization of *Candida albicans* and other yeasts with severity of chronic periodontitis. J Periodontal Res. 2013 Aug;48(4):428–32.
21. Lambooij JM, Hoogenkamp MA, Brandt BW, Janus MM, Krom BP. Fungal mitochondrial oxygen consumption induces the growth of strict anaerobic bacteria. Fungal Genet Biol. 2017 Dec;109:1–6.
22. Thein ZM, Samaranayake YH, Samaranayake LP. Effect of oral bacteria on growth and survival of Candida albicans biofilms. Arch Oral Biol. 2006 Aug;51(8):672–80.
23. Sztukowska MN, Dutton LC, Delaney C, Ramsdale M, Ramage G, Jenkinson HF, et al. Community Development between *Porphyromonas gingivalis* and *Candida albicans* Mediated by InlJ and Als3. Biswas I, editor. mBio [Internet]. 2018 May 2 [cited 2022 Feb 27];9(2). Available from: https://journals.asm.org/doi/10.1128/mBio.00202-18