

BIOFILMS PRODUCED BY CANDIDA YEASTS AND ITS CONSEQUENCES: A REVIEW

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Abstract:

This paper aimed to gather information regarding mechanism of biofilm formation and development of resistance of the fungi mainly the candida species to antifungal therapy from literature through 1996-2012. We have carried out a detailed search in the National Library of Medicine's PubMed database, Google search and Science Direct to include all studies and reviews published in English language. The key words used for the search were biofilm, *Candida*, antimicrobial resistance, denture stomatitis and Candidiasis. This paper tries to explain the mechanism of biofilm formation, factors which initiate and propagate biofilm production and certain special features like thigmotropism and quorum sensing which are responsible for development of resistance in these yeasts.

Keywords : Biofilms by Candida, quorum sensing, thigmotropism, antifungal resistance, candidal colonisation, denture stomatitis

Introduction :

Infections due to fungal biofilms are usually refractory in nature and do not show positive response to antimicrobial therapy as they have developed resistance to conventional antifungals^{1,2}. A biofilm is defined as a structured community of micro-organisms surrounded by a self produced polymeric matrix and is adherent to an inert or living surface³. It is seen that a biofilm is a resilient group of microbes in an organized form, in which properties such as drug resistance is acquired by individual cells. We have seen that many fungal species are capable of forming aggregates in the form of flocs, mats, biofilms, etc., but *C. albicans* is one of very few fungal species which is efficient

enough to form biofilms in a healthy mammalian host . This paper aimed to gather information regarding mechanism of biofilm formation and development of resistance of the fungi mainly the

Candida species to antifungal therapy from 1996-2012. We have carried out a detailed search in the National Library of Medicine's PubMed database, Google search and Science Direct to include all original studies and reviews. The key words used for the search were biofilm, *Candida*, antimicrobial resistance, denture stomatitis and Candidiasis.

Denture stomatitis and candida biofilm :

The fitting surface of the maxillary denture and soft lining materials are the main reservoirs of *Candida albicans* and related *Candida* species. Soft denture liners are used to provide comfort to complete denture wearers where retention is compromised and cannot tolerate dentures due to irregular alveolar bone and thin fragile mucosal covering. Colonization by *Candida* and subsequent formation of biofilm on denture materials leads to development of denture stomatitis⁵ The surface of the acrylic denture has a biofilm of mixed species formed which contains large numbers of bacteria, and yeasts⁶ It is seen that candidal colonisation is common in immune

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compromised individuals, denture prosthesis and soft liners provide refuge to *Candida* fungi rapidly and lead to formation of either single species or multispecies biofilms. Also it is seen that *Candida* yeast species can adhere very strongly to denture materials⁷.

Candidiasis a superficial form denture stomatitis affects 65% of edentulous individuals, the development of which is an outcome of biofilm formation. Polymethylmethacrylate, which is used in construction of dentures, and silicone elastomers were the main bio prosthetic materials where biofilm formation is seen and infection becomes re-established after treatment. Biofilms grown on the irregular surface of polymethylmethacrylate (PMMA) have a biphasic structure compared to, those grown on flat hydrophobic surfaces such as silicone elastomer. This is composed of an adherent blastospore layer covered by sparser hyphal elements embedded in a deep layer of extracellular material⁸. Insufficient oral hygiene and stress factors leads to fungal proliferation and biofilm formation with respect to soft denture liners mainly in the maxillary denture bases⁹.

Van der Waals forces facilitate adherence of *Candida albicans*. Absence of ionic surface on PMMA minimizes the adsorption of defence molecules on denture surfaces. Edentulous patients mostly wear dentures during the night which is a significant factor for development of denture stomatitis. Risk of general health complications and denture wearing increases with age. Reduced saliva, the natural protectors of oral cavity along with poor oral hygiene are directly related to higher *Candida* colonization on dentures due to increased medications in such patients¹⁰.

Immediately after inserting into the oral cavity, the denture surface will be coated by salivary components which change the physicochemical property of the surface, adding specific receptors for microbial adhesion and hence influences fungal adhesion. With an increase in mucin thickness the density of adhering *C. Albicans* is also seen to increase, mucin thus has an important role in rigidity of the biofilm¹¹. Superior adhesion to acrylic resin surfaces is seen

with *C. glabrata* when compared to *C. albicans*. *Candida* growth was higher on the polyamide resin than on the PMMA material. Complexity and phenotypic heterogeneity explains the difference in adherence capacity. Heterogeneity is a result of variation in surface hydrophobicity, formation of hyphae, presence or absence of extracellular proteases or thigmotropism, all of which influence adhesion of *Candida* to plastic surfaces¹². Environmental factors which contribute to the initial surface attachment flow of the surrounding medium (urine, blood, saliva, and mucus), pH, temperature, osmolarity, bacteria, presence of antimicrobial agents, and host immune factors¹³. Fungal colonisation on denture lining materials may also be promoted by salivary and serum pellicles, mainly mucinous glycoprotein of human saliva by sugar-specific interactions¹.

Mechanism of biofilm formation and its function:

When 'planktonic' bacteria which are unattached individual cells adhere to a surface, there is beginning of biofilm development. Mechanism of adherence varies depending on the surface to be attached and microbes, physiological adaptations are induced as adherent cells grow and divide in proximity to the surface, including secretion of exopolysaccharides which creates a protective matrix enclosing the cells¹⁵. Sophisticated intercellular communication systems are utilized for formation of a biofilm, it also involves extracellular polymeric matrix formation and depends on adhesion both to substrates, adhesion from cell to cell, and can be composed of multiple cell types. Biofilms by microbes represent the first step in the evolution of multicellularity in higher eukaryotes¹⁶. Biofilm is defined as a community of microorganisms attached to a surface, forming three-dimensional structures containing exopolymeric matrix and cells that exhibit distinctive phenotypic properties¹⁷.

Biofilm formation follows certain characteristic phases, beginning with initial adherence of individual or single cells to the substratum. Later there is formation and growth of micro colonies in the form of trabeculated monolayer which grows confluent. There is a network formed

consisting of hyphae, yeast cells and pseudohyphae which become enclosed by extracellular matrix¹⁸. Initial colonization is in the form of yeast cells, this is followed by formation of germ tubes 3-6 hrs later. The basal layer is formed by adhering yeast cells which is responsible for firm attachment of basal layer to the substratum. Subsequent germination brings about bulk of biofilm. Biofilm which is mature contains mycelia, yeast and pseudo mycelia and are seen after 48 hrs. Yeasts form only basal layer therefore form a thin bio film, mycelia forms a loose adherent attachment to the surface¹⁷?. In *C. albicans* biofilm formation, cells adhere to the substratum resulting in formation of basal layer which further divide and produce compartmentalized hyphae. Intertwining in the upper region of the biofilm is brought about by these long tubular projections¹⁶. The strongly adherent yeasts can cause cavitations on the surface of substratum mainly the mucosal and epithelial surfaces. Tip growing hyphae also arise from yeast cells, grooves ridges and pores of the substratum guide the direction of hyphal growth, this type of contact guidance is called thigmotropism and holds the responsibility of hyphal invasion in areas of weak surface or membrane integrity²⁰.

Biofilms with characteristic three-dimensional structures is formed by *C. albicans* cells on solid surfaces. For a successful biofilm formation, appropriate cell surface molecules for adhesion should be expressed. Also genes responsible for protein synthesis in high levels should be expressed. Biofilm formation by adherence of *Candida glabrata* is mediated in part by a cell surface protein Epa6p¹⁷. Efg1p another cell surface protein promotes formation of biofilm and a protein Czf1p antagonizes bio film formation. *C. albicans* cells must sense more than one feature on the surface of substratum to produce a correct response such as invasive filamentation or biofilm formation ,which are distinct processes occurring on different types of surfaces²¹. Cell wall protein Hwp1 is required for the formation of biofilm in case of *C. Albicans* both in vitro and in vivo. SUN41, a cell wall-related gene has a major role to play in formation of biofilm, integrity of cell wall and virulence in oropharyngeal and disseminated

candidiasis. Limited oxygen at base of substratum causes expression of gene SUN 41, which augments biofilm attachment to substratum²²

C. albicans, has the ability to switch between different morphological forms. It can grow as blastospore or pseudo hyphae or hyphae, it is therefore considered a pleomorphic or polymorphic organism. The blastospores are converted to mycelial or filamentous forms by production of germ tubes. Yeast cells growing by budding elongate and do not detach from adjacent cell but undergo polarised cell division to form pseudo hyphae. Round, retractile spores with a thick cell wall called chlamydo spores are transformation forms of *Candida* in non optimal growth conditions. Various such transformation capacity of *Candida* permits it to adapt to different biological niches^{19,23}.

SEM studies have shown the emergence of germ tube appendages which produce adhesins for plastic surfaces. They also possess binding sites for serum protein components like fibrinogen, fibronectin and complement factor. Greatest blastospore-substrate adhesion was seen on the serum-coated specimens indicating presence of adhesin receptor- like mechanisms in the presence of serum. Fibronectin and Con-A binding material were involved in *Candida* biofilm development with saliva and serum. Therefore biofilm formation is a complex phenomenon which involves mannan-binding protein, fibronectin, glycoproteins and IgG or IgM¹ .

Profuse biofilm was produced by *C. albicans* when compared to *C. parapsilosis* and *C. Krusei*. Compared to *C. albicans*, biofilms of *C. parapsilosis* and *C. Krusei* were of reduced thickness, less profuse, and devoid of hyphal elements while *C. albicans* biofilms showed development of a dense mass stacked in a palisadic manner devoid of intracellular spaces. *C. tropicalis* strain produced a biofilm without a basal blastospore layer. *C. glabrata* isolates produced scant population of blastospores^{24,25}. Extracellular matrix of polymeric substances should be released by cells in the developing biofilm , adhesion plays a major role in development of biofilm. Adhesion must first

bind cell to one another and to the substratum. As a second step, the hyphae must be bound to each other to stabilize the maturing biofilm¹⁶. These hyphae are essential elements which give structural integrity to fully mature biofilm. Hyphal formation in *C. albicans* occurs as a response to distinct environmental stimuli. The hyphal formation is controlled by complex regulatory networks. Filamentation also is critical in the development of the spatially organized architecture of a mature biofilm²⁶

Population in a biofilm can be developed from a single species or multiple microbial species forming a community. Protection from the environment, new genetic traits acquisition, availability of nutrient source and metabolic cooperation are the ecologic advantages for the microbes in a biofilm. Early (0–11 h), intermediate (12–30 h), and mature (38–72 h) are three distinct developmental phases of a biofilm^{17,27}. Biofilms form a protective reservoir or safe sanctuary for oral microbes^{3,13}. Performing special cellular functions and production of specific extracellular materials is the characteristic nature of a true biofilm. *C. albicans* are capable of biofilm formation both in yeast and hyphal forms, hence biofilm growth is not morphology specific³. Adherence and dimorphic transition are major steps in initiation of biofilm development²⁸

Stages in formation of biofilm Based on in vitro studies carried out (Baillie and Douglas, 1999; Chandra et al., 2001; Douglas, 2003; Hawser and Douglas, 1994; Nobile et al., 2009; Uppuluri et al., 2010a, 2010b)
1. Attachment and colonization of yeast-form (nearly spherical) cells to a surface
2. Growth and proliferation of yeast-form cells to allow formation of a basal layer of anchoring micro colonies
3. Growth of pseudo hyphae (ellipsoid cells joined end to end) and extensive hyphae (chains of cylindrical cells) concomitant with the production of extracellular matrix material
4. Dispersal of yeast-form cells from the biofilm to seed new sites.

A typical bio film formed on flat, hydrophobic surfaces, like silicone elastomer and polyvinyl chloride, has a biphasic distribution of components: A blastospore layer, adherent to substrate which in turn is covered by a sparse layer of hyphal elements embedded in EPS. Water channels exist

between hyphal cells to facilitate the diffusion of nutrients from the environment through the biomass and from there to the bottom layers, it also permit waste disposal, resistance of physical and chemical stress, a community-based regulation of gene expression, forms micro-niches in the bio film, and host oxygen radical and protease defences and potential for dispersion via detachment^{13,26,27,30}. Mature bio films are elaborate structures which appear as pillars rising up from mats of jumbled cells, fluid-filled micro channels for permeation. They are dynamic communities which can spread across surfaces, they can also incorporate particulates and other microbes from the surrounding and continue to shed new planktonic cells. On dentures formation of bio film is a result of complex interactions among yeast, bacteria, nutrients, and saliva or even serum proteins¹⁵

Constitution of biofilm and factors influencing its formation:

Universal, complex, interdependent communities of surface-associated microorganisms enclosed in an exopolysaccharide matrix are called biofilms and can occur on any surface¹.The cells embedded in extracellular polymeric material which showed an amorphous granular appearance .On close examination the structures in the biofilm showed coaggregating *C. albicans* cells³. Recently biofilm network in *C. albicans* has undergone relatively an extensive evolutionary changes where "old" genes are underrepresented and "young" genes are enriched in the biofilm circuit .Extracellular polymeric material of the planktonik as well as from bio films showed presence of carbohydrate, protein, phosphorus, hexosamine, glucose in a proportion higher than mannose, galactose, extracellular DNA and uronic acid^{6,13,31}.

Candida cells in the bio film show heterogeneity, which may be a response to environmental conditions, like differences in pH, oxygen availability, and redox potential. A bio film has been described as "heterogeneous mosaic model", since it contains extracellular polymeric substances that hold stacks of bacterial micro colonies together. A layer of cells (5 mm thick) underlie and attach to

the substratum below these stacks. Morphogenesis of cells in the bio film is dependent on factors such as carbon source, substratum, and species. Maturation of Candida bio films formed in in-vivo models is faster and of greater thickness than the ones grown in in-vitro models. Substratum, nutrient, presence of saliva, availability of oxygen, EPS and Candida species are factors which influence Candida bio film formation²⁷.

Substratum used in in-vitro model systems are acrylic, silicone elastomeric catheter disks, cellulose cylindrical filters, polymethylmethacrylate, plastic, Biotic surfaces, such as those in an engineered human oral mucosa model and glass²⁷. Surface properties of materials, surface roughness, hydrophilicity, physiochemical properties, surface topography and chemical properties also play a critical role in varied bio film formation^{11,27}.

Glucose, fructose, galactose and lactose favour *C. albicans* bio film formation compared to sucrose and maltose. Bio film formation is also linked to the (over)expression and polysaccharide matrix production by adhesins¹¹.

The surface turns hydrophilic once it is coated with saliva, which causes reduced adhesion of hydrophobic strains at the same time enhances hydrophilic strains. Mucin among salivary components acts as a receptor for Candida adhesion and subsequent biofilm formation. Increased yeast counts and risk of candidosis is associated with dry mouth²⁷.

Temperature changes, ionic stress, changes in osmolarity, and oxidative stress are a range of some physiological stresses. Various receptors sense these stresses and elicit responses via signalling pathways. One such response is mitogen-activated protein kinase (MAPK) Mck1p, which is activated by contact stress, is involved in biofilm development¹³.

Low levels of oxygen near the central core area favors biofilm architecture. biofilm growth under dynamic conditions were higher than static conditions after 48 h irrespective of the environmental oxygen content in isolates of *C. albicans* which produced a dense, compact,

multilayer biofilm with clusters of blastospores under aerobic dynamic condition and an extensive hyphal network under anaerobic conditions³². *Candida* is able to grow either aerobically or anaerobically²⁷. Positive impact of dynamic conditions on microbial biofilm growth is a result of better perfusion of oxygen and nutrients into the innermost part of the biofilm³². Cell type, growth conditions, and the abiotic surface properties affect the tenacity of biofilm adherence¹⁸.

Variation in biofilms of different species is observed, more confluent biofilms are seen with *C. albicans*, *C. dubliniensis*, and *C. krusei*. Wild-type *Candida* strains produce healthy and confluent biofilm. Greater biofilm-forming ability is seen with non-*albicans* species than for *albicans* species²⁷. *C. parapsilosis* formed less thicker biofilm that showed distinct clumping and consisted of irregular groupings of blastospores devoid of extracellular matrix material and hyphae, complex biofilms are formed by *C. albicans* which consists of confluent basal blastospore layer covered by a thick matrix composed of extracellular material and hyphal elements²⁵. *C. krusei*, *C. tropicalis*, *C. parapsilosis*, *C. kefyr* and *C. guilliermondii* showed higher biofilm growth under anaerobic conditions in a static environment, only *C. krusei* species formed less biofilm in anaerobic atmosphere. Low oxygen and nutrient starvation promotes the hyphal growth hence nature of gaseous environment is responsible for the phase transition in the dimorphic fungus *C. albicans*³².

Slowly growing or nongrowing microorganisms are the contents of a biofilm and are protected by their inactivity. The biofilm mode of growth is characterised by slow growth and limited nutrition. microbes in the biofilm are killed slowly compared to their planktonic varieties, the rate of killing decreases with increase in the thickness of the biofilm.³³ Tyrosol and Farnesol which are quorum sensing molecules promotes hyphal formation during the early and intermediate stages of biofilm development, they also stimulate yeast cells to be released from the biofilm and permit spread of the microbe to colonize new surfaces³. Composition of medium, substratum, flow

conditions, and quorum sensing cause different plasticity in biofilm structure suggesting different locations within the host calls for different properties of biofilms¹⁷.

Factors responsible for development of resistance in biofilm :

Candida associated with biofilm often show resistance to conventional antifungal agents like amphotericin B, nystatin, chlorhexidine, and fluconazole^{13,16,13,35,37}. Resistance of biofilm cells may due to their anaerobic growth conditions, mainly as seen with tooth decay and periodontal disease and these anaerobic cells lead to “persister” cells, which remain viable in biofilms even after being treated with antimicrobial agents²⁹. *C. krusei*, *C. glabrata*, *C. lusitanae*, and the newest *Candida* species, *C. dubliniensis* are a few species of *Candida* which are less susceptible to commonly used antifungals. Clinical resistance is defined as persistence or progression of an infection despite appropriate antimicrobial therapy. If the organism shows drug resistance before exposure to the drug then it is called as primary resistance, whilst resistance shown by the microbial community is a result of exposure to drug, it is termed as secondary resistance¹. Biofilms have cells with reduced ergosterol content in comparison to cells of liquid culture. Also major expression of efflux pumps like adenosine triphosphate-binding cassette (ABC) transporters encoded genes encoding drug efflux determinants or multidrug resistance (MDR) transporters CDR1 and CDR2 are expressed by cells in biofilm with transient expression of MDR1 by the adherent cells^{11,23,16,8,17,35,37}. Overexpression of genes encoding efflux pumps are regulated differentially during development and exposure to antifungal agents and are employed in nutrient uptake and cellular detoxification^{13,35}. They are important in maintaining homeostasis within complex environments and confer self protection against acute toxicity. During early to intermediate phases of biofilm development these efflux pumps are used but are conceded as ECM achieves maturity and are then used to “soak” and consume antifungal agents¹³. Non-efflux pump confers drug resistance to mature biofilms².

Resistance to azole and polyene-derived antifungal agents can be explained based on the alteration of ergosterols in biofilm membranes¹³. There is a significant decrease in the ergosterol levels and diminished levels of ergosterol biosynthetic gene expression of intermediate and mature phases of biofilm, this diminished levels of ergosterol in sessile *C. albicans* reflects a physiological state that is more conducive to resistance in these cells^{35,37}. Cells in biofilms also have shown to express higher levels of genes involved in amino acid and nucleotide metabolism, protein synthesis, other metabolic functions, and subcellular localization¹⁷.

Bacterial matrix material may restrict penetration of drugs by acting as a barrier to fluconazole penetration in mixed species biofilms of *C. albicans* and *S. epidermidis* & substantially more matrix material are produced in biofilms subjected to a liquid flow than the ones incubated statically. *C. tropicalis* in contrast with *C. albicans* synthesize large amounts of extracellular material under static conditions, with many cells hidden by the enveloping matrix³¹. The matrix which envelops sessile communities of cells, formed by merging of initial founder microcolonies are composed mainly of slowly growing cells act as a barrier to the diffusion of antimicrobials and/or as an ion-exchange resin to bind charged antimicrobial molecules. Drug resistance develops over time, coincident with biofilm maturation^{35,37}. Cell density, membrane sterols and cell wall glucans may also be the reason behind this resistance development^{17,36}. PH, temperature, oxygen availability, and many such factors like environmental stresses will lead to alter the architecture of the biofilm, as well as antifungal sensitivity¹³.

Phenotypic changes or phenotypic switching resulting from a decreased growth rate or nutrient limitation, cellular heterogeneity and reduction of growth rates under different environmental pressures can benefit the biofilm. Fungal biofilm resistance is an inducible phenotype which is a part of a highly evolved series of molecular pathways responsible of regulating development and homeostasis of biofilm^{13,13,17,31}. Thigmotaxis, the directional response

of a cell or tissue to touch, or physical contact with a solid object¹⁷ reorients the direction of hyphal growth in response to contact with features of the physical environment. On a solid surface, activation of the cell integrity pathway contributes to biofilm structure and expression of the drug resistant phenotype²¹.

Quorum sensing, which is a cell-density dependent communication and coordination of microbial behaviour via signalling molecules, relies on production of acylhomoserine lactone (AHL) signal molecules which freely diffuse across cell membranes^{15,17}. It is fundamental to microbial biofilm formation because it benefits the biofilms' well being by preventing unnecessary overpopulation. It also controls the competition for nutrients and has important implications in the infectious process, particularly for dissemination and for the establishment of distal sites of infection³⁵. The fungal pathogen *Candida albicans* was the first eukaryotic microorganism shown to exhibit quorum sensing³. Farnesol, a quorum sensing molecule is produced by continuously growing cells of *Candida albicans* and accumulates to a level similar to cell number and inhibits the filamentation of yeast cells thereby inhibiting its growth. It is also seen that incubation of cells in the presence of farnesol leads to reduced formation of biofilms and mature biofilms are affected by the presence of these molecules. Therefore the formation and stability of the biofilm is regulated by farnesol^{17,35}.

A small number of drug-tolerant or "persister" cells which usually account for 0.1 to 1% of the biofilm population can remain viable even at high concentrations of antimicrobial agents^{17,35,36}. *C. albicans* persisters are phenotypic variants of the wild type that arise in a clonal population of genetically identical cells³⁷. Biofilm exopolymer is an important component of recalcitrance of persister cells because of restricted penetration of drugs through it²⁸. Biofilms of *C. krusei* and *C. Parapsilosis* appear to harbor persister cells, while biofilms of *C. glabrata* and *C. tropicalis* are devoid of such cells^{17,36}. Tyrosol (2-[4-hydroxyphenyl] ethanol), a derivative of tyrosine, was subsequently

identified as a second quorum-sensing molecule in *C. albicans*. Tyrosol accelerates the formation of germ tubes³.

Calcineurin, a Ca²⁺-calmodulin-activated serine/threonine-specific protein phosphatase has also been implicated in mediating resistance to the azoles in both in vitro and in vivo models of biofilm formation. Beta-1,3, glucan is responsible for sequestering azoles, by acting as a "drug sponge" to confer resistance on *C. albicans* biofilms, they are also responsible for sequestering echinocandins, pyrimidines, and polyenes¹³. Quiescence (state of stillness or inactivity) is thought to be a factor in drug resistance, but the majority of cells in *C. albicans* biofilms are seen to be metabolically active^{37,8}. Biofilms of *C. albicans* which were grown under glucose and iron limited conditions were highly resistant to amphotericin B and those biofilms grown under anaerobic conditions also showed that *C. albicans* biofilms were resistant to the high levels of amphotericin B and different azole antifungals¹³.

Discussion :

A biofilm is defined as a structured community of microorganisms surrounded by a self produced polymeric matrix and is adherent to an inert or living surface. It is universal, complex, interdependent communities of surface-associated microorganisms enclosed in an exopolysaccharide matrix. Immuno compromised patients such as those with cancer or HIV infection are often the most susceptible ones. Biofilms are most notorious and difficult to be eliminated and are often source of recalcitrant infections. Increased use of azole antifungals which are fungistatic has resulted in the development of resistance to these drugs¹.

Biofilm formation and development of resistance to antimicrobials are closely associated. Mature *C. albicans* biofilms show a complex three-dimensional architecture with extensive spatial heterogeneity, and consist of a dense network of yeast, hyphae and pseudo hyphae encased within a matrix of exopolymeric material. *Candida* associated denture stomatitis is a result of adherence of *Candida* directly or via an intermediary layer of plaque-forming bacteria to denture acrylic

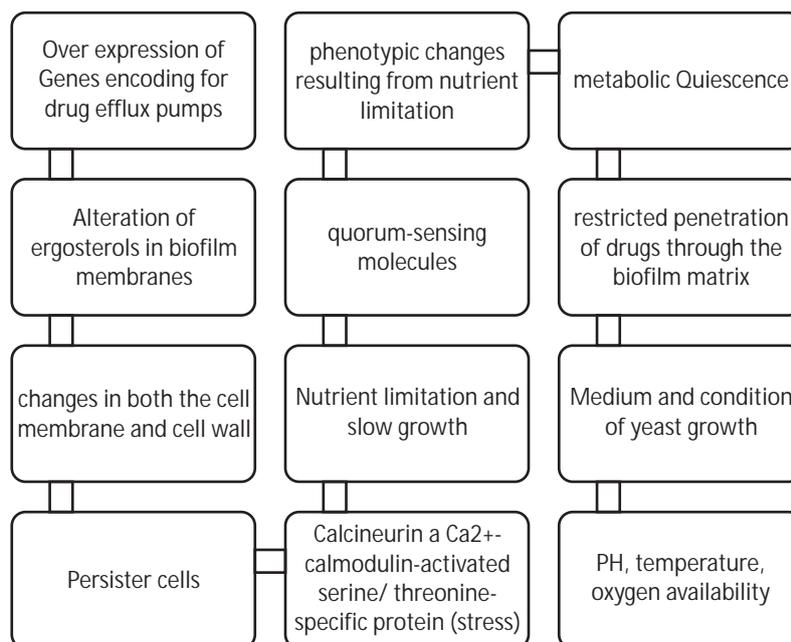
(polymethylmethacrylate). Complex biofilm in the form of denture plaque serve as a protected reservoir of *C. albicans*. The most important factor in the pathogenesis of denture stomatitis is growth of large numbers of *Candida* on the fitting surface of the appliance and acid production by grown yeasts. This leads to direct cytotoxicity and activation of acid proteinase. Production of phospholipase by these yeasts promotes adhesion of *Candida* and successful colonization⁵. Close fitting removable maxillary complete dentures are most commonly colonized by *Candida* due to large surface area available.

Nonspecific interactions like hydrophobic and electrostatic interactions, and specific interactions like like adhesin or integrin-receptor interactions, serum proteins and salivary factors regulate co-adhesion and adherence which results in colonisation and subsequent formation of biofilm. Thigmotropism and fungal biofilm formation are also closely related where the yeast growth follows surface irregularities⁷. Pathogen is protected by virtue of biofilms from host defences and antibiotics, biofilms also provide pathogen spatial stability and autonomy in controlling its own microenvironment. As a mechanism to protect cell propagation in a hostile environment, *C. albicans* biofilm formation has preceded host colonization in the evolution of the organism⁶.

Conclusion :

Reduced manual dexterity, hyposalivation, Xerostomia, increased medications, immune compromised situations, poor oral hygiene, all these are factors which predispose the elderly population to the risk of biofilm formation and development of denture stomatitis. Development of resistance due to biofilm formation is a serious problem to be combated. Several studies have been carried out to use herbal products or combination of various antimicrobials to overcome the resistance developed by the fungi.

Characteristic features like dimorphism, Quorum sensing, thigmotropism, metabolic quiescence all put together result in formation of a biofilm which is stubborn and prevents the penetration of antifungal drugs and thus a resistant form of the yeast in the biofilm, well protected from the host defence mechanism as well as the treatment rendered. It is a field open for further research to find mechanisms of preventing these causative factors and to discover or invent formulations targeting these areas which are responsible for developing resistance in the yeast cells.



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