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Current status of dental plaque

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ABSTRACT

The review deals with global position of the dental plaque, development of dental plaque and species involved and their pathogenicity. Streptococci are the initial colonizers in development of the dental plaque. They attach to the acquired pellicle on tooth surface and later on they show receptors for bonding with the secondary colonizers and late colonizers The conditions leading to the development of dental plaque were discussed in detail. Role of oral *Streptococci* in development of dental plaque/ biofilm formation is explained in depth. Quorum sensing (QS) in plaque forming bacteria is considered as one of the factors responsible for maintaining the plaque structure. Therefore quorum sensing in biofilm and regulation of gene expression in dental plaque is considered as one of the important factors. Use of antibiotics with special reference to antimicrobial resistance in plaque bacteria in dentistry is suggested because the structure of a biofilm may restrict the penetration of the antimicrobial agent as the bacteria growing on a surface display a novel phenotype, which can result in a reduced sensitivity to inhibitors.

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INTRODUCTION

Bacteria have been the cause of some of the most deadly diseases and widespread epidemics of human civilization. Diseases such as tuberculosis, typhus, plague, diphtheria, typhoid, cholera, dysentery and pneumonia had affected a large toll of human population. On the contrary dental diseases are the problems of almost all the human beings for a long time. Mouth contains a number of bacteria as its normal micro flora and that oral bacteria cause diseases due to the changes occurring in the oral environment which lead to the development of microfilms in different parts of the oral cavity.

Dental plaque is the term commonly used for the biofilm formed on teeth surfaces however the term plaque has now been extended to encompass biofilms on all the oral surfaces. The plaque consists of complex microbial communities embedded in a matrix of polymers of bacterial and salivary origin and they are the primary cause of dental illnesses. Dental plaque develops naturally on teeth, and if not ecologically disturbed it forms part of the defence systems of the host by creating colonization resistance (preventing the colonisation of enamel by exogenous microorganisms). Once plaque forms, it protects the component species, in spite of regular minor environmental stresses posed by dietary components, oral hygiene practices, host defenses, diurnal changes in saliva flow, etc. This stability is termed as *microbial homeostasis* which donates a balance imposed by numerous microbial interactions, including synergism and antagonism (Marsh and Bradshaw, 1999).

The interest in the dental biofilm flora has increased in recent years because of the growing evidence that specific species are etiologic agents of dental caries and periodontal diseases. These diseases are among the most prevalent infections afflicting nearly all humans and the cost of their treatment exceeds that of any other infectious disease.

Global position of the dental plaque

Dental diseases occur widely in the world and are a costly burden to health care services. The treatment of dental caries is expensive for governments of both developed and developing countries and costs between 5-10% of total health care expenditures in the developed countries, exceeding the cost of treating cardiovascular disease, cancer and osteoporosis (Shani et al 2000). In most of the developing countries, the prevalence rate of dental caries is high enough but more than 90% of caries cases remains untreated because of certain financial problems.

The dental caries are pandemic in nature as the term *pandemic* is used for global disease outbreaks that are acute and fatal, such as the influenza epidemic of 1918 that killed tens of millions globally. Pandemic suggests an impact on populations of entire countries, continents, or much of the world. The term therefore implies to two elements, the global distribution and the severe consequence. By characterizing dental caries as a pandemic, World Health Organization has focused attention on caries as a highly prevalent disease around the globe. They have also implied that it has profound individual and societal significance because of its often severe, though non-fatal, consequences.

The prevalence of oral cancer also occur worldwide. It occurs mostly in south central Asia, Denmark, Germany, Scotland, central and eastern Europe, and to a lesser extent, Australia, New Zealand, Japan and the USA. In World Health Organization's (WHO, 2003) report on oral health. Petersen, 2003, Peterson, *et al.*, 1988 provided an overview of global caries epidemiology that confirmed its international pandemic distribution. Globally, WHO has reported caries prevalence in school age children at 60–90% and as virtually universal among adults in the majority of countries (Petersen *et al*., 2005). An index of severity of decayed, missing and filled teeth (DMFT) shows a clear pattern of higher

disease experience in North and South America, Western Europe, and much of Africa, more moderate disease experience in much of South America, Russia, and the former Soviet Republics, and low levels of disease in Eastern Africa, China, Australia, and Greenland. WHO has observed that developed countries have higher rates of caries experience, while developing countries have lower rates (Petersen, 2003). Findings of Centers for Disease Control and Prevention (CDC) in US released in August 2005 reveal high prevalence of dental caries in children, with 27% of pre schoolers, 42% of school-age children, and 91% of dentate adults having caries. The major oral and dental diseases found in Indian population are dental caries and periodontal diseases. Dental caries is a universal disease affecting all geographic regions, races, both the sexes and all age groups. The prevalence of dental caries is generally estimated at the ages of 5, 12, 15, 35–44 and 65–74 years for global monitoring of trends and international comparisons. Shah (2005) reviewed the work done by Indian workers and concluded that periodontal diseases are frequent in adult population of this country and are most frequent in children (Singh and Jain, 2011).

Available literature indicates that dental caries (tooth decay) and periodontal diseases are the most common among bacterial infectious diseases of mankind, together affecting almost the entire population of the world (Armfield *et al*., 2000).

Development of dental plaque, species involved and their pathogenicity

There have been two main thoughts on the role of plaque bacteria in the etiology of caries and periodontal diseases. The "Specific Plaque Hypothesis" proposed that, out of the diverse collection of organisms comprising the resident plaque microflora, only a few species are actively involved in disease (Loesche, 1976). In contrast, the "Non-Specific Plaque Hypothesis" considered that disease is the outcome of the overall activity of the total plaque microflora (Theilade, 1986). More recently Marsh (2003) proposed an alternative hypothesis i.e., "Ecological Plaque Hypothesis" that brings together the key elements of the earlier two hypotheses.

The specific species of bacteria in dental plaque believed to cause dental caries include, *Streptococcus mutans* and *Lactobacilli* (Hardie and Marsh, 1978; Rogers 2008). The main bacterial species associated with the initiation of dental caries are members of the 'Streptococcus mutans group', namely *Streptococcus mutans* and *Streptococcus sobrinus.* Both these species are strongly acidogenic and aciduric (Loesche, 1986; Dashper and Reynolds, 1992, 2000; Marsh, 2003). Other species such as *Lactobacillus acidophilus*, *Actinomyces viscosus*, *Nocardia spp* and *Streptococcus mutans* are most closely associated with caries, particularly the root caries.

Streptococci are the initial colonizers in development of the dental plaque. They attach to the acquired pellicle on tooth surface and later on they show receptors for bonding with the secondary colonizers and late colonizers (Table 1).

Conditions leading to the development of dental diseases

As defined earlier that the plaque consists of complex microbial communities and polymer matrix of bacterial or salivary origin and the components of food debris. The pits and fissure on the teeth and the point of contact between the two teeth etc. accumulate bacteria along with the food debris. The accumulated bacteria ferment glucose, fructose and sucrose components of food debris into acids by oxidative fermentation. The acid formation is considered as an important component in the category of the dental caries (Muntz, 1943; Stephan, 1943; Kleinberg, 1961; Geddess, 1975). Although it is stated that the flow of saliva in the mouth washes off the exogenic microflora from the tooth surfaces but helps in circulation of nutrients throughout the oral cavity. The eating of fast foods, chocolates and

drinks containing sugars, fruit juices enhances the food supply to the growing plaque forming microorganisms and the conditions favourable for development of pathogenic microorganisms.

Table 1
Receptors and coaggregation partners of the organisms involved in plaque formation

Adhering Bacteria	Receptors	Co aggregation partner
Early colonizers		
Streptococcus oralis	Galactose binding, Bacterial cell fragment	*Actinomycetes naeslundii, Capnocytophaga ochracea, Fusobacterium nucleatum, Hemophillus parainflunzae, Pervotella loscheli Streptococcus gordonii, Veillonella atypica,*
Streptococcus mitis	Galactose binding,	*Capnocytophaga ochracea, Fusobacterium nucleatum, S. gordonii*
S. gordonii	α-amylase, Proline rich protein, Bacterial cell fragment	*Fusobacterium nucleatum, Porphyromonas acene, Streptococcus mitis, S. oralis, S. sanguis*
S. sanguis	Bacterial cell fragment	*A. naeslundii, H. parainfluenzae, P. loescheli. S. gordonii, V. atypica,*
Intermediate colonizers		
F. nucleatum	Statherin	*Capnocytophaga sputigens, C. ochracea, S. oralis, S. mitis, P. acnes, S. gordonii, Capnocytophaga gingivalis, Actinomyces israelii, H. parainfluenzae, V. atypica, A. naeslundii, Actinobacillus mycetemcomitans.*
Veillonella atypica		*S. oralis, A. actinomycesnaeslundii, V. atypica*
Pervotella loescheli		*S. oralis, S. sanguis*

Adhering Bacteria	Receptors	Co aggregation partner
Actinomyces naeslundi	Proline rich protein	*S. gordonii, S. oralis, S. sanguis, F. nucleatum, V. atypica*
C.gingivalis		*Actinomyces israelii, F. nucleatum*
Late colonizers		
A. actinomycetemocomitans		*F. nucleatum*
Eubacterium		*F. nucleatum*
Treponema spp		*F. nucleatum, P. gingivalis*
P. gingivalis		*F. nucleatum*
Selenomonas flueggi		*F. nucleatum*

Adopted information given by Marsh and Nyvad (2003)

According to Loesch *et al.* (1972) facultative streptococci constitute a major percentage of microorganisms from dental plaque while their role in the formation of carious lesions has been emphasized by other workers (Keyes, 1968; Krasse *et al.,* 1968; Zinner and Jablon, 1968; Englander and Jordan 1972). Depending on type of the organisms involved in the plaque, plaque maturity caries lesions are classified variously as: (i) *Advanced lesions* (a high proportion of lactobacilli), (ii) *Dentinal lesions* (diverse microflora with many fastidious Gram positive bacteria such as *Actinomyces naeslundii, A. odontolyticus, Propionibacterium* spp., *Eubacterium* spp. and Gram negative bacteria such as *Fusobacterium* spp. *Capnocytophage* spp, *Veillonella* spp. (iii) *Root surface caries* (originally associated with *Actinomyces* spp) (iv) *Enamel caries* (*mutans streptococci* and *lactobacilli,* with possibly a role for *A. naeslundii)* and (v) *Rampant caries* (occur in xerostomic patients and in infants fed with high levels of sugar in pacifiers).

Dental plaque development is an orderly sequence of events, resulting in a structurally and functionally organized, species-rich microbial community (Marsh, 2004). Distinct stages in plaque formation include: (i) acquired pellicle formation; (ii) reversible adhesion involving physico-chemical interactions between the cell surface and the pellicle, which lead to stronger adhesin-receptor mediated attachment; (iii) co-adhesion resulting in attachment of secondary colonizers to already attached cells; (iv)multiplication and biofilm formation and, (v) detachment. Dental plaque accumulates preferentially at stagnant sites that afford protection from the vigorous removal forces that apply in the mouth. The details of distinct plaque phases of development as described by Marsh (2004) are as follows:

The biofilm development on teeth starts immediately following cleaning of the teeth (Al-Hashimi and Levine, 1989) and directly influences the pattern of initial microbial colonization. At first the acquired pellicle is formed. The receptors which were adsorbed on naked teeth surface include histidine and proline rich proteins, bacterial cell fragments, alpha amylase, statherine etc. These receptors constitute the acquired pellicle on which the primary colonizing bacterial species get attached with the receptors forming acquired pellicle (Vacca-Smith *et al*., 1994; Kopec *et al*., 2001; Marsh and Nyvad, 2003).

Weak, long-range physicochemical interactions between the microbial cell surface and the pellicle-coated tooth create a weak area of net attraction that facilitates reversible adhesion (Busscher and van der Mei, 1997): While the strong, short-range interactions

between specific molecules on the bacterial cell surface (adhesins) and complementary receptors in the pellicle can result in irreversible attachment (Jenkinson *et al*., 1996; Lamont and Jenkinson, 2000). Oral bacteria generally possess more than one type of adhesin on their cell surface and can participate in multiple interactions both with host molecules and similar receptors on other bacteria (coadhesion). Co-adhesion of later colonizers to already attached early colonizers involves specific interbacterial adhesin-receptor interactions and leads to an increase in the diversity of the biofilm and to the formation of unusual morphological structures, such as corn-cobs and rosettes (Kolenbrander *et al*., 2006). Co-adhesion facilitates the functional organization of dental plaque. Bacteria engage in a range of antagonistic and synergistic biochemical interactions (Marsh and Bradshaw, 1997). Cell division leads to confluent growth and, a three-dimensional spatially and functionally organized, mixed-culture biofilm. Extracellular polysaccharide production results in the formation of a complex extracellular matrix. Such a matrix is a common feature of biofilms and makes a significant contribution to the known structural integrity and general resistance of biofilms.

Role of oral Streptococci in development of dental plaque/ biofilm formation

The mouth provides an environment for microorganisms belonging to different taxa and that many of these are involved in formation of dental plaque. The mouth also provides a medium for growth of bacteria leading to dental decay. The species of several gram positive and gram negative bacteria that are found in oral cavity includes species of *Enterococcus, Peptostreptococcus, Streptococcus, Staphylococcus, Actinomyces, Corynebacterium, Eubacterium*, *Lactobacillus*, *Aggregatibacter* (formerly *Actinobacillus*), *Haemophilus, Bacteroides, Campylobacter, Leptotrichia, Prophyromonas, Capnocytophaga, Prevotella, Tannerella, Eikenella, Treponema, Fusobacterium*, and *Wolinella* (Marsh, 2000). It is also known that majority of accumulated bacteria in plaque belong to genus *Streptococcus*. Streptococci have been reported as the predominant inhabitants in the oral cavity and that some of them are the primary colonizers (i.e., *Streptococcus sanguis, S. oralis, S. mitis*) of dental plaque (Whiley and Beighton, 1998). In addition to these a number of other streptococci along with some species of other oral bacteria act as secondary colonizers of tooth surface. This indicates that oral streptococci are among those which help in the formation of dental plaque. The oral streptococci those produce green coloration after incubation on blood plates are known as viridans streptococci which comprises a large portion of normal oral flora. They have been associated with dental caries, endocarditis, septicaemia abcess formation etc. (Hardie and Marsh, 1978).

Quorum sensing in biofilm

Quorum sensing (QS) in plaque forming bacteria is considered as one of the factors responsible for maintaining the plaque structure. Biofilm formation by bacteria is a group behavior that is coordinated through a quorum-sensing (QS) system. In the biofilm mode (dental plaque) of growth, microorganisms exhibit increased resistance to antimicrobial compounds, environmental stresses and host immune defense mechanisms (Costerton *et al.,* 1999). Petersen *et al*. (2004) recently reported that competence stimulating peptide (CSP) has not molecules that are playing important role in QS signaling. It is reported that development of competence for natural transformation in the Gram positive bacterium *Streptococcus intermedius*, also favors biofilm formation. Synthetic CSP have not been reported to induce competence when cells were exposed to a transformable plasmid and significantly enhanced biofilm formation (Petersen *et al.,* 2004). Intriguingly, Petersen *et al.* (2004) also showed a tenfold increase in the proportion of CSP in competent cells in biofilms compared with planktonic cell fractions. Gram-

positive bacteria typically use secreted peptides (pheromones) as signal molecules and two-component regulatory systems (i) composed of a membrane-bound histidine kinase receptor and (ii) an intracellular response regulator) to detect the peptide and trigger the required changes in gene expression (Kleerebezem *et al.,* 1997).

This CSP-induced mechanism, along with many of its underlying genes, has been identified in other streptococcal species including various strains of the mitis group (including *S*. *pneumonia* and *Streptococcus gordonii*), anginosus (S. intermedius) and mutans (*Streptococcus mutans*) groups of streptococci (Havarstein *et al.,* 1997; Cvitkovitch *et al.,* 2003]. Natural transformation is thought to provide a selective advantage to bacteria by enabling the recipient to acquire novel genes, such as antibiotic-resistance elements and other virulence determinants, from the DNA of donor cells (Cvitkovitch *et al.,* 2003). This quorum sensing system also functions to regulate acid tolerance in *S. mutans* biofilms (Li *et al.,* 2002a). It has been proposed that *S. mutans*, upon exposure to low pH, could release CSP and initiate a co-ordinated 'protective response' among neighbouring cells to lethal stress (Kolenbrander *et al.,* 2002). Thus CSP molecule in many Gram-negative and Gram-positive bacteria, have emerged as an enticing target for fighting biofilm infections.

Regulation of gene expression in dental plaque

Bacteria in dental plaque display a phenotype that is distinct from that of their planktonic cells growing freely. The binding of bacteria to specific receptors can trigger significant changes in both bacterial and host cell patterns of gene expression (Abraham *et al*., 1998). Surface associated responses have been identified in plaque bacteria, although the magnitude of this shift in gene expression may be less than that observed in free-living species because of the absolute dependence of oral bacteria on a biofilm lifestyle (Burne, 1998). Studies carried out by Du and Kolenbrander (2000) and Li and Burne (2001) noted some changes in gene expression of *Streptococcus gordonii, S. mutans* and *Actinomyces spp*. as a result of changes in the state of bacteria from planktonic to biofilm state. However, Li and Burne (2001) were of opinion that exposure of *Streptococcus gordonii* to saliva results in the induction of genes *(sspA/B)* encoding adhesins that can bind to salivary glycoproteins and engage in co-aggregation with *Actinomyces* spp. (Du and Kolenbrander, 2000), similar changes are considered to occur during colonization in dental plaque. The altered phenotypic expressions are regulated by a change in local environmental conditions within the biofilm (e.g. sugar concentration, pH) rather than due to attachment per se (Li and Burne, 2001). Thus, biofilm growth can have both direct and indirect influences on gene expression by oral bacteria.

Gene transfer within the dental plaque

Cells communicate with one another in biofilms via horizontal gene transfer. Signaling molecules such as CSP are supposed to play an important role in biofilms to take up DNA (Li *et al*., 2002b). The transfer of conjugative transposons encoding tetracycline resistance between streptococci in model biofilms has been demonstrated by Roberts *et al*. (2001). Dowson *et al*. (1990) and Hakenbeck *et al*. (1998) recovered the resident *(S. mitis*, *S. oralis)* and pathogenic *(S. pneumoniae)* bacteria from the nasopharynx with penicillin resistance genes showing a common mosaic structure and confirmed that gene transfer can occur *in vivo*. Aforesaid findings suggest that plaque can function as a 'genotypic reservoir' by harbouring transferable mobile elements and genes (Loo, 2003). Such genetic exchange could have a wider significance as far as drug resistance in bacteria is concerned.

Use of antibiotic in dentistry

Dental practitioners prescribe antibiotics to treat the dental infections. Antibiotics commonly used in dental practice include,

Tetracycline (Tetracycline HCL , Doxycycline hyclate, Minocycline HCL), Erythromycin (Ethyl succinate , Stearate), Penicillin (Penicillin G, Penicillin VK, Ampicillin, Amoxicillin, Augmentin), Metronidazole, Cephalexin, Ciprofloxacin, Azithromycin, Clindamycin, Vancomycin, and some dentifrices such as Chlorhexidine and Benetidine etc.

It is reported that many antimicrobial classes are utilized by dentists (Longman & Martin, 1999; Epstein *et al*., 2000; Jaunay *et al*., 2000; Palmer *et al*., 2000a, 2000b; Palmer *et al*., 2001; Slots & Ting, 2002; Salako *et al*., 2004). For the proper selection of antibiotic for prescription the dentists normaly consider (i) the pharmacological characteristics of the antibiotics,(ii) the patient's safety, (iii) the nature of the probable infectious agent occurring at the site and (iv) the cost of the drug.

Some times the dentist's antimicrobials prescription attitude seems to be biased towards certain classes of antimicrobials, mainly penicillins and metronidazole (Epstein *et al*., 2000; Palmer *et al*., 2000b; Roy & Bagg, 2000). For example, penicillins and metronidazole prescription accounted for about 68 and 26% of the total antibiotic prescriptions issued by 10% of the dentists working in England (Palmer *et al*., 2000b). Metronidazole prescription issued by dentists accounted for 45% of all metronidazole prescriptions in UK (Committee SMA, 1988). Pallasch (2003) reported that the total dentist prescription contributed to about 7-9% of the total prescription issued for the community and that on an average, 159 antibiotic courses per year are prescribed by each dentist in UK (Sweeney *et al*., 2004). In addition to therapeutic usage some antibiotics are also prescribed by dentists as a prophylactic measure, even though they are not required for some dental illnesses. The use of untargeted, unwanted antibiotic intake by the patient may develop drug resistance in oral microflora.

Antimicrobial resistance in plaque bacteria

A major finding with respect to micro-organisms growing on a surface is their increased resistance to antimicrobial agents (Gilbert *et al*., 1997; Ceri *et al*., 1999; Gilbert *et al*., 2002, Singh et al., 2006, 2008). For example, *P. aeruginosa* growing on urinary catheter material can be 500–1,000 times more resistant to antibiotics than the same cells growing in liquid culture. Bacteria growing in dental plaque also display increased resistance to antimicrobial agents, including those used in dentifrices and mouth rinses (Marsh and Bradshaw, 1993; Kinniment *et al*., 1996; Wilson, 1996; Pratten and Wilson, 1999). Shani *et al*. (2000) observed that the biofilm inhibitory concentration for chlorhexidine and amine fluoride was 300 and 75 times greater, respectively, when *S. sobrinus* was grown as a biofilm and compared with the minimum bactericidal concentration of planktonic cells. The age of the biofilm has also been reported as a significant factor for drug resistance in *S. sanguinis* (Millward and Wilson, 1989). Oral bacteria in their biofilm state have been found to be show more resistance to antibiotics (e.g. amoxycillin, doxycycline, metronidazole) as compared to their planktonic state (Larsen and Fiehn, 1996; Larsen, 2002). Drug resistance in bacteria may be because of one or the other reasons such as (i) mutations affecting the drug target, (ii) the presence of efflux pumps, (iii) the production of modifying enzymes etc. However, the actual mechanisms behind the increased resistance of biofilms to antimicrobial agents are the subject of much research and debate (Gilbert *et al*., 2002). It is also suggested that the structure of a biofilm may restrict the penetration of the antimicrobial agent as the bacteria growing on a surface display a novel phenotype, which can result in a reduced sensitivity to inhibitors. Growth on a surface may also result in the drug target being modified or not expressed in a biofilm, or the organism may use alternative metabolic strategies. Bacteria grow slowly under nutrient depleted conditions in an established biofilm and, as a consequence, are much less susceptible than faster-dividing cells. In

addition, it has also been proposed that the environment in the depths of a biofilm may be unfavourable for the optimal action of some drugs (Gilbert *et al*., 2002).

A greater understanding of dental plaque may have considerably help clinical practice. Future research which may help in reduction of dental plaque can be: (a) the development of inhibitors and antiplaque agents that are more effective against surface-associated micro-organisms, coupled with more effective delivery systems for targeting specific bacteria (b) interference with communication networks that coordinate or regulate microbial activities within biofilms; (c) preventing colonization of selected organisms; (d) the development of process(es) to promote biofilm detachment from the tooth surface (Hillman, 1999; Tagg and Dierksen, 2003) (e) affecting biofilm architecture, for example, by the use of enzymes that can degrade the exopolymers that comprise the plaque matrix; (e) the neutralization of parameters that are implicated in disease (Marsh, 2003) and the identification of pathogenic clones that can help in improved diagnosis and might predict sites that are more susceptible to disease development. The literature indicated that streptococci are playing important role in the development of dental plaque (dental biofilm) and that the plaque formation over dent's besides providing protection against exogenous microflora may involve in the development of certain dental diseases. It is also evident that biofilms are posing resistance to antimicrobial substances. Overall very little information on these aspects are available from population of rural area of the country to determine the ability of constitutive microflora form biofilms. The hypothesis that bacteria in their biofilm state are more resistant to antimicrobial substance than their planktonic form has also to be tested.

CONCLUSION

A greater understanding of dental plaque may considerably help in clinical practice. Future research which may help in reduction of dental plaque can be: (a) the development of inhibitors and antiplaque agents that are more effective against surface-associated micro-organisms, coupled with more effective delivery systems for targeting specific bacteria (b) interference with communication networks that coordinate or regulate microbial activities within biofilms; (c) preventing colonization of selected organisms; (d) the development of process(es) to promote biofilm detachment from the tooth surface (e) affecting biofilm architecture by the use of enzymes that can degrade the exopolymers that comprise the plaque matrix; (e) the neutralization of parameters that are implicated in disease and the identification of pathogenic clones

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