Uncultured Members of the Oral Microbiome

William Wade, BSc, PhD; Hayley Thompson, BSc, PhD; Alexandra Rybalka, BSc, PhD; and Sonia Vartoukian, BDS, FDS, PhD

Abstract Around one-third of oral bacteria cannot be cultured using conventional methods. Some bacteria have specific requirements for nutrients while others may be inhibited by substances in the culture media or produced by other bacteria. Oral bacteria have evolved as part of multispecies biofilms, and many thus require interaction with other bacterial species to grow. In vitro models have been developed that mimic these interactions and have been used to grow previously uncultivated organisms.

Definition of “Unculturability”

The human mouth is heavily colonized by microorganisms with all of the different types represented: bacteria, archaea, fungi, protozoa and viruses. This review will focus on the bacteria because of their importance in the common dental diseases, dental caries and periodontal diseases, and because the phenomenon of unculturability has been extensively investigated in bacteria. It has long been realized that not all bacteria that can be seen under the microscope can be grown.

Recent advances in culture have modified this estimate so that it is now considered that around two-thirds of oral bacteria can be cultivated. Although uncultivated bacteria cannot be grown on commonly used laboratory media, they clearly compete well in the bacterial communities found in the mouth and many are associated with oral disease. At present, their role in pathogenesis and contribution to antimicrobial resistance is unknown, which is why there is substantial interest in culturing uncultivated bacteria and subjecting them to detailed phenotypic and genomic analysis.

The aim of this review is to list the uncultivated members of the oral microbiome, discuss the reasons why some bacteria are difficult to culture in vitro and describe recent advances in culturing previously uncultivated oral bacteria.

Authors

William Wade, BSc, PhD, is a professor of oral microbiology at Queen Mary University of London with interests in the characterization of the human microbiome and development and evaluation of novel strategies for the prevention and treatment of microbiota-related disease. Conflict of Interest Disclosure: None reported.

Hayley Thompson, BSc, PhD, is a postdoctoral scientist with interests in oral microbiology, the virulence factors of pathogenic bacteria and uncultured oral bacteria. Conflict of Interest Disclosure: None reported.

Alexandra Rybalka, BSc, PhD, is a postdoctoral scientist with interests in oral microbiology and the development of novel model systems to study uncultured oral bacteria. Conflict of Interest Disclosure: None reported.

Sonia Vartoukian, BDS, FDS, PhD, is a specialist periodontist and lecturer in oral microbiology at Queen Mary University of London. Conflict of Interest Disclosure: None reported.
previously cultivated and characterized species. This does not mean, however, that all of these taxa cannot be cultured. Indeed, a comprehensive cultural analysis of the microbiota of severe early childhood caries revealed 45 species-level taxa that, at that time, had not been cultivated. In addition, it is often forgotten that in microbiome surveys, only DNA is detected, not living cells, and the detection of an organism’s DNA does not necessarily mean that the organism was viable at the time of sampling. Additionally, DNA extraction and PCR reagents are frequently contaminated with DNA from environmental bacteria and can make up a significant proportion of amplicon libraries, particularly when samples are taken from sites with low bacterial levels. A practical definition of unculturability will be used for this review. An organism will be regarded as uncultivated if there are no reports that it has been grown in previous cultivation studies.

Uncultured Oral Bacteria

When culture-independent methods were first used to study the composition of the oral microbiota and compared to cultural analyses of the same samples, it was clear that a substantial number of bacterial taxa could not be readily cultured. The Human Oral Microbiome Database (HOMD, homd.org) lists the bacteria found in the mouth. Many species-level taxa have yet to be named and are, therefore, assigned human oral taxon (HOT) numbers. HOMD release 13.2 includes 210 species-level taxa that have yet to be cultured. Many uncultivated taxa belong to genera whose members are predominantly cultivable. Because still relatively few oral bacteria have been cultured and identified by 16S rRNA gene sequence analysis, it is possible that these taxa are cultivable but representative strains have not yet been

![Figure 1. Phylogenetic tree showing uncultivated species-level oral taxa within the phylum Bacteroidetes. Tree prepared by the neighbor-joining method from a distance matrix constructed using the Jukes-Cantor algorithm and an alignment of 998 bases. Sequences representing type species of relevant genera are included for reference and colored red.](image-url)
encountered. Thus, the common genera Actinomyces, Prevotella, Streptococcus and Veillonella all include such taxa.

In contrast, many groups of uncultured taxa cluster in deep branches of the phylogenetic tree with no or few cultivated neighbors. **FIGURE 1** shows a phylogenetic tree of the uncultivated members of the phylum Bacteroidetes. It can be seen that Bacteroidetes genera G-3 and G-7 comprise a branch of six uncultured species-level taxa of which Bacteroidetes [G-3] HOT-281 is the most commonly detected taxon, while Bacteroidetes genera G-4 and G-5 constitute another deep branch with four uncultivated taxa. Similarly, the phylum Firmicutes includes a number of deep-branching lineages made up of uncultivated taxa (**FIGURE 2**). These include a major branch of the Peptostreptococcaceae with seven uncultivated taxa from five genera, four uncultivated taxa within the Ruminococcaceae and a number of uncultivated representatives of the Lachnospiraceae, Syntrophomonadaceae and Veillonellaceae.

The Fusobacteria phylum includes an uncultured branch consisting of Fusobacteria [G-1] with two taxa: HOT-210 and HOT-220, and one comprised of uncultivated Leptotrichia taxa HOT-212, HOT-215, HOT-217 and HOT-392. Interestingly, the majority of Leptotrichia taxa have yet to be cultured, but most branches of this genus include cultivated members.

*Bdellovibrio* HOT-039 represents a deep uncultured branch within the phylum Proteobacteria. *Bdellovibrio* are normally aerobic, predatory bacteria, feeding on other Gram-negative bacteria and it will be interesting to know if this taxon has a similar predatory lifestyle.

A substantial number of spirochetes have yet to be cultured. All oral
spirochetes belong to the genus Treponema, and of the 49 oral Treponema taxa, only 14 have been cultivated. In particular, one branch of 10 taxa has no cultivable representatives: HOT-250-256, HOT-508, HOT-517 and HOT-518.

The recently described phylum Synergistetes\(^9\) includes a large number of uncultured taxa. Oral members of the phylum form two clusters, denoted A and B.\(^{10}\) The majority of Cluster A taxa have yet to be cultured, while Cluster B includes the recently described species Jonquetella anthropi and Pyramidobacter piscolensis.\(^{11}\)

Until recently, the phylum Chloroflexi has had no cultivated representatives among the oral microbiota, although environmental relatives have been cultivated.\(^{12}\) Three strains of Anaerolineae bacterium HOT-439, an important taxon thought to serve as a biomarker for periodontitis,\(^{13}\) have recently been isolated from subgingival plaque samples and found to grow with the help of Fusobacterium nucleatum.\(^{14}\)

Three oral phyla have no, or very few, cultivated representatives: GN02, TM7 and SR1. Candidate division GN02 was first described to comprise a group of sequences identified in a study of the Guerrero Negro hypersaline microbial mat.\(^{15}\) Three oral taxa are found: HOT-871, HOT-872 and HOT-873, representing two class-level taxa. Interestingly, four related taxa were identified among the canine oral microbiome.\(^{16}\) Little is known regarding the genetic potential or functional capability of this group of organisms, although its ubiquity, albeit at low levels, suggests that it deserves to be the target of future studies.

Sequences representing candidate division SR1 were originally detected in sediments from Sulphur River in Parkers Cave, and were first classified within candidate division OP11.\(^{17}\) They are widely distributed, being found in anaerobic habitats such as deep-sea sediments, various extreme environmental sites, the cow rumen and the human mouth.\(^{18}\) SR1 comprises two lineages, BH1 and BD2-14, with the former found only in geothermal habitats. Human oral SR1 representatives are found in subgroup III of the BD2-14 lineage. Three species-level taxa belonging to the same genus-level taxon are found in the mouth: HOT-345, HOT-874 and HOT-875. Like GN02, SR1 is a rare member of the oral microbiome, and typically makes up around 0.01 percent of clone libraries. At this level, it will be challenging to detect individual cells by fluorescence in situ hybridization (FISH) or attempt direct isolation. Three species-level taxa of SR1 have been identified among the canine oral microbiome, but were only detected when a specific Bacteroidetes-TM7-SR1 primer was used.\(^{16}\)

The TM7 candidate division was named with reference to the Torf, mittlere Schicht, or peat, middle layer, in which it was first detected in a German peat bog.\(^{19}\) Subsequently, members of this division have been isolated from a wide range of environments including waste water and batch reactor sludges,\(^{20,21}\) fresh and sea water\(^{22-24}\) and soil. The name Candidatus Saccharibacteria has recently been proposed for organisms formerly described as TM7.\(^{25}\)

Saccharibacteria are found in a range of animals. In invertebrates, they have been detected in the microbiota associated with sponges and corals,\(^{26-28}\) termites\(^{29,31}\) and nematodes.\(^{32}\) They form part of the intestinal microbiota of mammals and appear to be a consistent member of the mammalian gut microbiome, having been found in mice,\(^{33,34}\) cattle,\(^{35-37}\) dogs,\(^{38}\) pigs,\(^{39}\) elephants, gazelle, bighorn sheep, takin, buffalo, bonobo and gorillas.\(^{40}\)

In humans, Saccharibacteria have been detected in several habitats, including the intestinal tract,\(^{41}\) skin,\(^{42}\) vaginal fluid\(^{43}\) and oral cavity\(^{44-46}\) although they typically make up less than 1 percent of the community at a given site. Saccharibacteria appear to be associated with oral disease, particularly those conditions associated with a mature anaerobic biofilm. For example, Paster et al.\(^{44}\) found 34 sequences representing the Saccharibacteria division among 2,522 cloned 16S rRNA genes from the subgingival plaque of healthy subjects and patients with periodontal disease, which were later identified as oral taxa HOT-346, HOT-347, HOT-349, 355 and HOT-356. Of these, only HOT-346 was found in health, while HOT-356 (represented by phylotype 1025, now recognized to belong to HOT-356) was associated with periodontitis, a finding confirmed in a study using oligonucleotide probes specific for HOT-356, in which the taxon was found in 50 percent of healthy subjects and 83 percent of patients with periodontitis.\(^{47}\) The same trend was seen in a polymerase chain reaction (PCR)-based study, with HOT-356 being detected in 91 percent of diseased sites and 71 percent of controls, although the difference was not statistically significant.\(^{48}\) In refractory periodontitis, Saccharibacteria HOT-346, HOT-356 and HOT-437 were detected in significantly higher proportions than
in patients whose periodontal treatment was successful or healthy controls in a study using the Human Oral Microbe Identification Microarray (HOMIM).\textsuperscript{49} Three \textit{Saccharibacteria} phylotypes were found in oral samples collected from subjects with halitosis and one of them, HOT-352, was significantly associated with the condition.\textsuperscript{45}

The culture of members of the division \textit{Saccharibacteria} has long been a goal. It was reported that \textit{Saccharibacteria} had been successfully cultured after 50-day aerobic incubation of low-nutrient solid media, with microcolonies visible to the naked eye.\textsuperscript{30} No further detail of this isolation has been reported, however. There have been a number of reports of the successful isolation of \textit{Saccharibacteria} bacteria in mixed culture. For example, microcolonies of \textit{Saccharibacteria} from soil were obtained using a soil substrate membrane system.\textsuperscript{54} After seven days incubation, several morphotypes were detected by means of FISH with the TM7-905 probe, although no pure cultures were obtained. Using the same method, Abrams et al.\textsuperscript{52} reported the isolation of microcolonies that included cells that reacted positively with the TM7 probe, but also others that were negative with the specific probe but positive for a universal bacterial probe. Rybalka\textsuperscript{53} found that \textit{Saccharibacteria} could be isolated in mixed culture with a variety of other species, including \textit{Slackia exigua} and \textit{Atopobium parvulum}, but could not be isolated in pure culture or even maintained as a mixture for more than a few subcultures.

The successful isolation of a pure culture of a \textit{Saccharibacteria} phylotype was reported from a sample of dental plaque but a culture was not deposited with a culture collection.\textsuperscript{54} A \textit{Saccharibacteria} strain successfully isolated and maintained from saliva, TM7x, was an extremely small coccus found in an exclusive physical and parasitic relationship with a strain of \textit{Actinomyces odontolyticus}.\textsuperscript{51} It would appear then that the \textit{Saccharibacteria} isolates studied thus far are only found in such close associations with other bacteria. It was further reported that association with TM7x caused the \textit{A. odontolyticus} host to change its morphology from relative short rods to filaments,\textsuperscript{56} although the growth phase and natural morphological variation of both partners in the interaction requires further investigation. The TM7x genome was found to be small at 705 kb and completing lacking

\begin{boxedminipage}{\textwidth}
\begin{center}
\textbf{Strictly anaerobic bacteria coexist with oxygen-consuming and -tolerant species and cooperate to protect each other from atmospheric stress.}
\end{center}
\end{boxedminipage}

in amino acid biosynthesis capability, perhaps explaining its need to parasitize other bacteria. Small genomes are a feature of a number of other Divisions yet to be cultured, including SR1, OD1 and WWE 3,\textsuperscript{57} suggesting that a limited metabolic repertoire and dependence on association with other organisms may be common features of phylum-level taxa with no or few cultivable representatives.

\textbf{Reasons for Unculturability}

If bacteria are able to grow in a particular environment but we are unable to cultivate them in the laboratory, then clearly at a basic level we are unable to reproduce the conditions that they need for growth. Understanding these conditions is key to the cultivation of previously uncultivated organisms.

Atmospheric conditions, particularly the presence or absence of oxygen, as well as the availability of CO\(_2\), are obviously extremely important. Some bacteria require specific nutrients for growth. \textit{Methanoseta} species, for example, are obligately acetotrophic; hence, the addition of acetone to media, which is slowly converted to acetate, will promote the growth of these otherwise slow-growing species.\textsuperscript{58}

Sometimes the medium itself can be toxic. It has been shown that autoclaving agar-containing culture media in the presence of phosphate can generate inhibitory levels of hydrogen peroxide, an effect that can be avoided by replacing agar with gellan gum.\textsuperscript{59}

Oral bacteria typically live as part of a multispecies community in densely packed biofilms. Within the biofilm, there are a variety of gradients of nutrients, signaling molecules and gases, due to the diffusion patterns of these substances and the metabolic activity of neighboring bacteria, such that conditions for individual cells, and groups of cells, can vary markedly.\textsuperscript{60,61} Despite the mouth being exposed to the atmosphere, about half of oral bacteria are obligate anaerobes. As oral biofilms develop, obligate aerobes and facultative anaerobes rapidly reduce the local oxygen concentration; four days of plaque formation in vivo in two subjects produced a mean redox potential at the tooth surface of –127 mV.\textsuperscript{62} Strictly anaerobic bacteria coexist with oxygen-consuming and -tolerant species and cooperate to protect each other from atmospheric stress.\textsuperscript{63}

In a similar way, there will be a concentration gradient within the biofilm for nutrients with their concentration decreasing with increasing depth of biofilm, while bacterial metabolic products will be increased. There can be direct interactions between species.
with one using the end products of another for growth. For example, *Veillonella* species use lactate produced by streptococci as a major carbon source.\(^{64}\)

Other possibly important factors in growth-regulating interactions between bacteria are bacterial signaling molecules. Gram-negative bacteria communicate by means of acyl homoserine lactones (AHLs),\(^{65}\) and Gram-positives use small diffusible peptides,\(^{66}\) but both systems are primarily intraspecies. In contrast, autoinducer-2 (AI-2), the product of the luxS gene, has homologues in a wide variety of organisms, both Gram-positive and Gram-negative and has been suggested to act across taxonomic boundaries.\(^{67}\) Bacterial signaling affects a number of functional aspects including expression of the biofilm phenotype, production of virulence factors and growth itself. Bacteria accustomed to growing in biofilms may thus require the presence of exogenous signals for growth. For example, a small, 5-amino acid

peptide, thought to be a signaling molecule

stimulated the growth of a previously uncultivated *Psychrobacter* strain.\(^ {68} \)

Resuscitation-promoting factor (Rpf) is a protein that was identified as being able to revive *Micrococcus* cells from dormancy.\(^ {69} \) Rpf is structurally similar to lysozyme\(^ {70} \) and cleaves peptidoglycan. Rpf is, therefore, likely to generate peptidoglycan fragments from the cell walls of intact bacteria, which might act as signaling molecules.\(^ {71} \) Muropeptide fragments are known to have signaling properties in *Bacillus subtilis*, where they bind to PkC, a serine/threonine kinase on the cell surface.\(^ {72} \) A specific muropeptide, a disaccharide-tripeptide with a meso-diaminopimelic-acid residue, typically found in Gram-positive bacteria is necessary for this activity. Rpf then may function by producing muropeptides from peptidoglycan with signaling, possibly growth-stimulating properties. Further work is required to investigate if this is a general method of growth regulation.

<table>
<thead>
<tr>
<th>Approach to cultivation</th>
<th>Examples</th>
<th>Reference number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Media supplementation, customization or modification</td>
<td>Addition of supplements to media e.g., siderophores, N-acyl homoserine lactones or growth factors; design of media selective for specific bacterial taxa using the SMART method; modified media preparation methods/substitution of agar with gellan gum as gelling agent, to limit growth inhibition by hydrogen peroxide.</td>
<td>14, 59, 68, 73, 74, 91, 92</td>
</tr>
<tr>
<td>Modification of growth conditions</td>
<td>Modified temperature, pH, O(_2) presence/absence, incubation time, gravity.</td>
<td>93, 94</td>
</tr>
<tr>
<td>Modification of sample handling</td>
<td>Dilution-to-extinction to achieve single-cell isolation; small inoculum for reduced microbial competition.</td>
<td>77, 78</td>
</tr>
<tr>
<td>Simulated natural environment</td>
<td>Diffusion chamber incubated within the natural environment allowing passage of growth-stimulatory chemical compounds across a membrane; hollow-fiber membrane chamber for in situ cultivation in the natural environment; I-tip in situ cultivation device permitting inward diffusion of natural chemical factors.</td>
<td>89, 95, 96</td>
</tr>
<tr>
<td>Microfluidic device</td>
<td>Encapsulation of subsets of the microbial community to form microdroplets that are exposed to signals or nutrients from external bacteria.</td>
<td>97, 98</td>
</tr>
<tr>
<td>Community culture and co-culture</td>
<td>Bacterial culture facilitated by chemical components produced by the main bacterial community separated from the target organism by a membrane, transwell insert or a well within the media plate; growth of bacteria in consortia, followed by detection and enrichment of specific bacterial targets using colony hybridization; coculture of bacterial strains with “helper” species on which they depend for provision of growth factors or for environment modification.</td>
<td>14, 99–103</td>
</tr>
<tr>
<td>High-throughput methods</td>
<td>I chip: A device comprised of hundreds of miniature diffusion chambers, within each of which a single cell is cultured; hollow-fiber membrane chamber device (see above) comprised of 48–96 chamber units.</td>
<td>96, 104</td>
</tr>
</tbody>
</table>

**Methods for Culture of Uncultured Bacteria**

A number of approaches have been taken in attempts to culture previously uncultured bacteria and these are summarized in the **TABLE**. Perhaps the most promising approach is the recognition that bacteria in nature frequently live in multispecies biofilms and are, therefore, in chemical contact with other bacteria. If chemical interactions can be maintained in vitro, then isolation of novel organisms should be possible. For example, D’Onofrio et al.\(^ {73} \) grew seawater sediment bacteria on agar plates in mixed culture at various dilutions. Because disproportionally more colonies were seen on plates that had been heavily inoculated, pairs of colonies growing within 2 cm of each other were subcultured and then grown together. Around 10 percent of these pairs showed evidence of the growth of one organism being dependent on its pair. The growth of many of the dependent isolates was
stimulated not only by its co-culture partner but also by *Escherichia coli*. A panel of *E. coli* mutants was, therefore, constructed and tested to determine the identity of the substance produced by *E. coli* that was stimulating the growth of the dependent isolates. Enterobactin, a siderophore, was found to be responsible and adding siderophores to culture media allowed a number of novel bacteria to grow.

A novel system has been developed that explores genomic data for information on specific carbon source requirements and antimicrobial resistance of particular bacteria, leading to the development of highly selective media designed by SMART — selective medium-design algorithm restricted by two constraints. Using this method, the authors prepared “selective” media for five plant-pathogenic bacteria and demonstrated accurate selection for the target bacterial species among a panel of 18 strains representing 10 species. The use of such systems may provide a rational basis for the development of novel culture media.

A range of compounds with siderophore activity have been screened for their ability to stimulate the growth of oral bacteria that are unable to grow in pure culture. Growth of *Prevotella* HOT-376 was more strongly stimulated by the siderophore pyoverdines-Fe, than by a culture filtrate of its helper *Fusobacterium nucleatum* (the positive control); to a lesser extent, it was also stimulated by ferric citrate, desferricoprogen, ferrichrome-Fe-free and salicylic acid. Likewise, growth of *Fretibacterium fastidiosum* of Synergistetes cluster A was consistently stimulated by desferricoprogen, salicylic acid and ferrichrome-Fe-free. Consequently, media used for culture of heavily diluted samples of subgingival plaque, were supplemented with siderophores pyoverdines-Fe or desferricoprogen, or a neat suspension of subgingival plaque, which led to the successful isolation of several previously uncultivated bacterial strains, including *Chloroflexi* taxon *Anaerolineae* bacterium HOT-439.

One method of achieving a pure culture of a slow-growing organism is the dilution-to-extinction method whereby dilution ensures that single cells are placed in a growth medium and have time to grow without being inhibited by other bacteria. A high-throughput version of the method was successful in cultivating a number of novel strains of the seawater organism SAR11 as well as representatives of the abundant, but previously uncultured, SAR116 clade. Dilution to extinction would appear to be a method most suited to samples such as seawater where bacterial concentrations are relatively low and the bacterial cells are found primarily in planktonic suspension and interactions between bacteria are, therefore, limited. The oral microbiome, conversely, is primarily made up of dense biofilms where this method may be less generally applicable, although some novel taxa have been recovered using this method.

Culture-independent surveys have made available 16S rRNA gene sequences for the microbiomes studied. These data can then be used to design specific oligonucleotide probes which can be used in FISH to visualize uncultured bacteria in samples of biomass. Thus, even though an organism cannot be grown, its morphology can be determined. This has been successfully performed for TM7, *Tannerella* BU063 and Synergistetes cluster A. An alternative labeling method is to use antibodies if it is possible to select an appropriate specific antibody. Because antibody labeling, unlike DNA probing, is nonlethal, it can be possible to obtain viable cells for culture, after sorting as described above; in this way fluorescent antibodies have been used to obtain viable cells after sorting. Flow cytometry can be used to isolate single cells from mixtures from which whole genome sequences can be obtained; this approach has been successfully used to sequence genomes of the health-associated taxon related to *Tannerella forsythia*, HOT-286 (BU063).

Mixed primary cultures frequently include representatives of bacterial species not yet cultured in isolation. Colony hybridization is a useful method for determining the location of specific taxa on solid media. By membrane blotting and the use of specific probes, target organisms can be localized to specific regions of replica plates, allowing their subculture and enrichment. This method was used to culture the first representative of Synergistetes cluster A: *Fretibacterium fastidiosum* from subgingival plaque in periodontitis.

The isolation of previously uncultivated bacteria may be a multistage process. Clearly, the agar plate is an alien environment for bacteria used to living in biofilms associated with mammalian tissues. One approach has been to establish biofilms in vitro, seeded with natural inocula. The Calgary Diofilm Device, a microplate-based system with plastic pegs coated with hydroxyapatite to mimic the tooth surface, has been successfully used to produce dental
with their natural environment. Agar while in chemical communication refers to a substrate that is placed in a chamber where communities can even be cultured in. Cultivated from Baikalian sponges a greater variety of the bacterial community, but also of the associated chemical compounds. They are developed as an in vitro cultivation device using the co-workers. 

This method was found to be of value for the culture of previously uncultivated oral bacteria and complementary to dilution and conventional plating. This method was found to be of value for the culture of previously uncultivated oral bacteria and complementary to dilution and conventional plating. 

Future Prospects

A number of approaches for the cultivation of previously uncultivated oral bacteria have been developed and successfully used to isolate representative strains. Progress has been slow, however, with only a small number of new species cultivated. Efforts should be directed toward developing high-throughput methods of detecting the growth of novel organi

promoting signaling molecules. The culture of an organism remains the key factor in determining its characteristics, including the production of virulence factors and resistance to antimicrobials. 

REFERENCES

32. Ladygina N, Johannson T, Tomacka B, Tuntil A, Hedlund K. Diversity of bacteria associated with grassland soil nematodes of
Practice Sales and Leasing

It is a Great Time to Sell! Inventory & Rates are Still Low & Buyer Demand is High! Call for a Free in Office Valuation!

ARCHADIA – (4) op comput G.P. Located in a well known Prof. Bldg. on a main thoroughfare. Cash/Ins/PPD pt. Annual Gross Collect $300K+ on a (3) day week. REDUCED

BAKERSFIELD #31 - Free Stand. Bldg. & Pract. (4) op comput G.P. w excell. exposure & signage. (3) ops eq’t/3’’ plumed. Annual Gross Collect $232K+Cash/Ins/PPD.

CLAREMONT – (3) op comput G.P. w newer eq’t. 2015 Collect $265K Cash/PPD. SOLD

GROVER BEACH – (3) op Turnkey Office w included charts (not guaranteed). (2) ops eq’t w newer eq’t. 3’’ plumed. Digital Pan & x-r. Dentrix. In a strip ctr. LL incentives. PENDING


OXNARD #9 - (3) op comput G.P. & a Prof Office Condo for sale. Located on a main thoroughfare. (3) ops eq’t’d. Annual Gross Collect $200K+ p.t. Cash/Ins/PPD/HMO $4.5K/mos.


THOUSAND OAKS & Comput. Annual Gross Collect. ~ $500K on a (3) day week. Absentee owned (6) op fully eq’t’d G.P. First floor street front location on a main thoroughfare. Cash/Ins/PPO/HMO. Cap Ck approx $7K/mos. 2015 Collect $1.4M+

SAN GABRIEL VALLEY - (4) op comput G.P. Located in an exclusive area of the Valley. (5) op comput G.P. located in a free stand bldg. w exposure/visibility & signage. VERY LIMITED COMPETITION. 2015 Collect $600K. Cash/Ins/PPD.

Digital x-rays & CT Scan. (6) sensors, Bldg. also available. Seller retiring. NEW

SAN FERNANDO VALLEY #9 - (8) op comput G.P. w modern eq’t. In a prof. bldg. on a main thoroughfare. Cash/Ins/PPD/HMO. Cap Ck approx $7K/mos. 2015 Collect $1.4M+ PENDING

SAN FERNANDO VALLEY #10 - Located in an exclusive area of the Valley. (5) op comput G.P. w high end buildout. Digital x-ray and CT Scan, Laser, Dentrix x/w & (5) year old eq’t. Gross Collect $1M+/yr. Cash/Ins/PPD pts. Reasonable overhead, high Net! NEW

SAN GABRIEL VALLEY - (4) op comput G.P. w newer P&Cas Equip, All the toys & whistles. Digital x-rays, Solaris Steril Ctr, Soprocara Intra Oral Camera, Velscope Cancer Screen, The Wand, Air Abrasion, Electric Hand Pieces, Laser, etc. FFS, 2015 Gross Collect. $881K+ on a 3/5 day week. (4) days of Hygiene. Seller retiring. NEW

SANTA ANA - absentee owned (6) op fully eq’t’d G.P. First floor street front location on a main thoroughfare. Exposure/visibility/signage. Cash/Ins/PPD. No HMO & No Denti-Cal. Pan & Eq’t & Comput. Annual Gross Collect. ~ $500K on a (3)/4’’ day week. BACK ON MARKET

THOUSAND OAKS (4) ops/(2) eq’t’d comput. Turnkey Office w included charts. Chart included but not guaranteed. Sirona Eq’t. Located in a condo in a Prof. Bldg. on a main thoroughfare. UP COMING PRACTICES:

Bakersfield, Beverly Hills, Central Coast, Covina, Downey, Duarte, Goleta, Oxnard, Pomona, San Gabriel, Palm Desert, Van Nuys, Visalia & West L.A.

D&M SERVICES:

Practice Sales and Appraisals  Practice Search & Matching Services
Practice and Equipment Financing Locate and Negotiate Dental Lease Space
Expert Witness Court Testimony Medical/Dental Bldg. Sales & Leasing
Pre - Death and Disability Planning Pre - Sale Planning

CA Representative for the National Association of Practice Brokers (NAPB)


THE CORRESPONDING AUTHOR: William Wade, BSc, PhD, can be reached at w.wade@nml.ac.uk.