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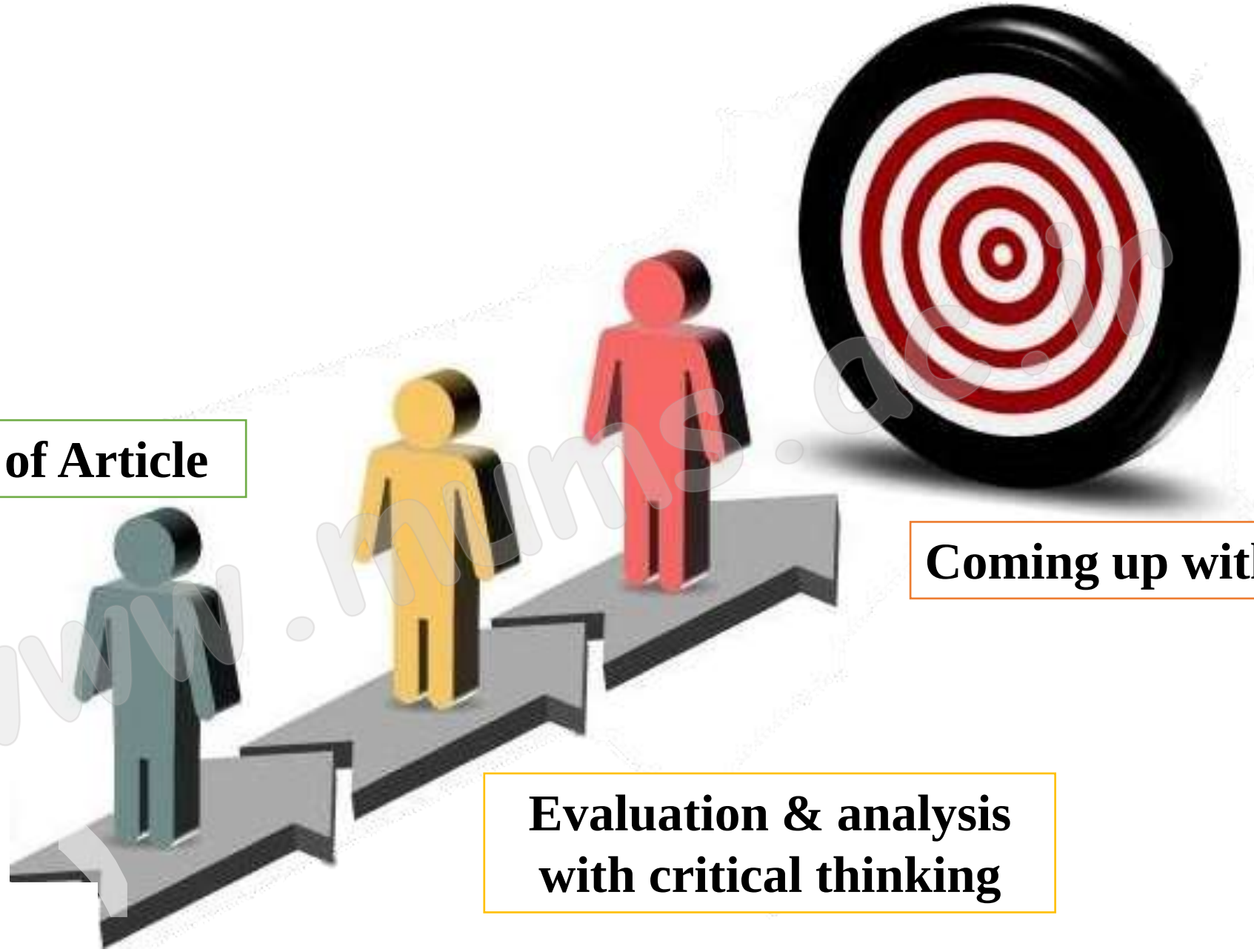
Impacts of sleep on the characteristics of dental biofilm

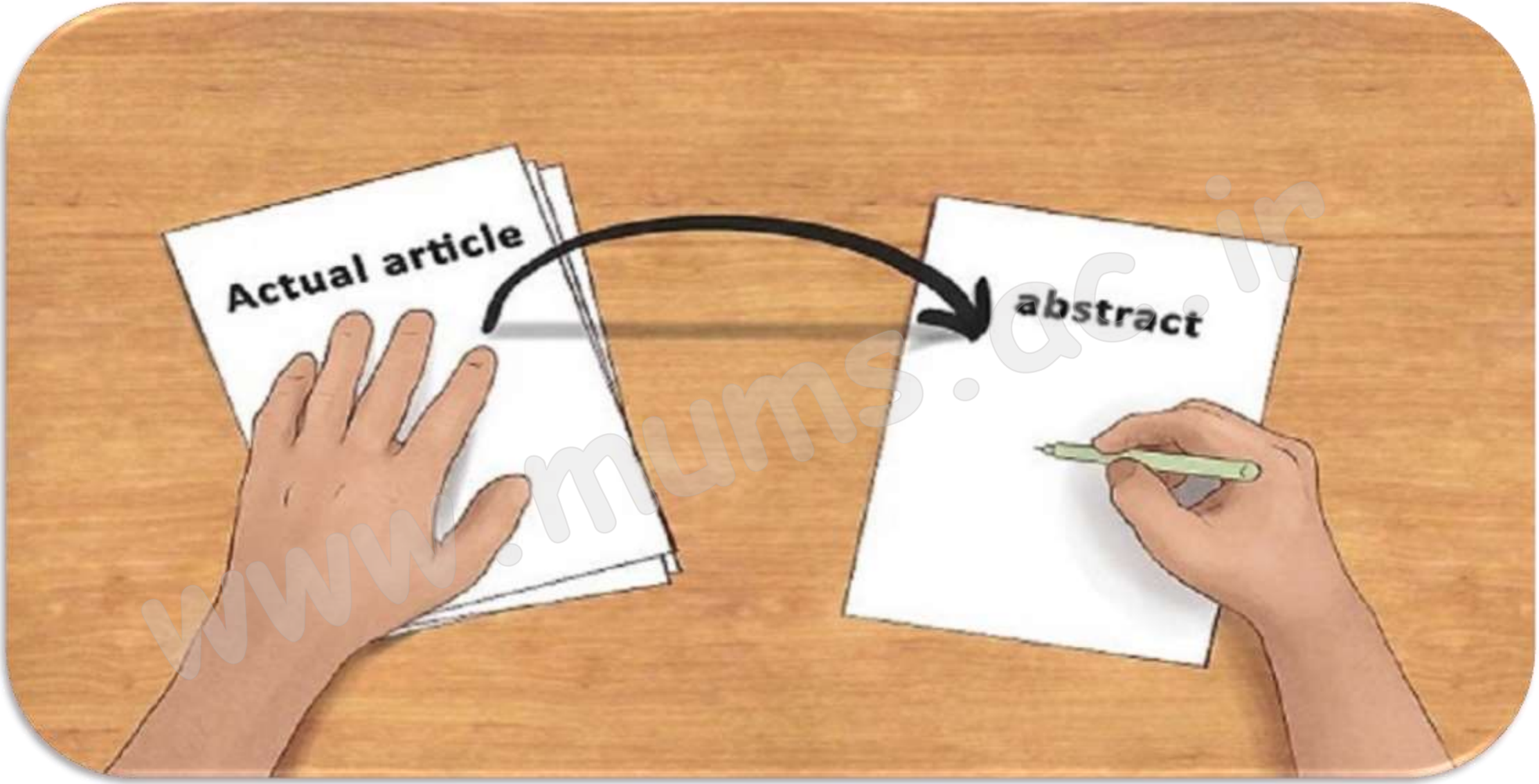
Presented by: Elham Khosravi

The pattern of Article



Coming up with an idea

Evaluation & analysis with critical thinking



انگیزه و بیان مسئله

Dental biofilm present on the tooth surface is associated with oral diseases, such as dental caries and periodontal disease. Because bacterial numbers rapidly increase in saliva during sleep, oral care before sleeping is recommended for the prevention of chronic oral diseases. However, temporal circadian changes in the quantity and quality of dental biofilms are poorly understood. This study aimed to investigate the impacts of sleeping on dental biofilm amounts and compositions by using an in situ model. The use of this in situ model enabled us to investigate dental biofilm formed in the oral cavity and to perform a quantitative analysis. Subjects began wearing oral splints in the morning or before

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نتایج / دستاوردها

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نتیجه گیری

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The human body is inhabited by an enormous number of bacteria, which form a structure known as a biofilm in habitats such as the skin, airways, gut, vagina, and oral cavity[1]. More than 700 species of bacteria inhabit the human oral cavity[2,3]; they form biofilms on various locations, including the tooth surface, hard and soft palate, buccal mucosa, and gingiva. The Human Microbiome Project (HMP, 2007–2017) was established to investigate the characteristics of the human microbiome and its relationship to disease. In this project, the microbiomes of various habitats in the human body were comprehensively investigated by performing a 16S rRNA sequence analysis[1]. The oral cavity is reportedly one of the most microbiologically diverse sites within the human body[4]. Most bacteria detected in the oral cavity belong to one of the following five phyla: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes, and Fusobacteria[5,6]. The diversity of the salivary and dental plaque microbiome differs widely among individuals. Because the oral cavity acts as the primary entrance to the human digestive tract, it tends to be influenced by host behaviors, such as toothbrushing and mouth washing[7]. In addition, the microbiome in the oral cavity is also affected by many other factors, including saliva pH, enzymes, host immunity, and antibacterial agents[8].

Dental biofilm that forms on the tooth surface is thought to be associated with oral diseases, such as dental caries and periodontal diseases[9,10], which are chronic infectious diseases found in many patients worldwide[11]. An imbalance of the microbiome (dysbiosis) contributes to some diseases, e.g. intestinal diseases, diabetes mellitus, and metabolic disease[12–14]. Recently, the dysbiosis of microbial communities in the oral cavity was found to be associated with oral infections[15,16]. For example, dental caries were found to result from a dysbiosis with acidogenic and aciduric bacteria, whereas periodontitis is triggered by dysbiosis with proteolytic, obligate anaerobic, and alkaliphilic bacteria[17]. Therefore, the prevention of these oral diseases requires monitoring and control of the microbial communities within dental biofilm. Biofilms, including dental biofilm, are composed of bacterial cells and exopolysaccharide (EPS)[8]. Importantly, biofilm-forming bacteria exhibit gene expression patterns that differ from those of planktonic bacteria[18,19]; moreover, a subset of bacteria in biofilms behave as persister cells, characterized by slow growth[20,21]. These mechanisms enable biofilms to tolerate exposure to typical

Salivary flow is reportedly lower during sleep than during the daytime[25]; additionally, because the number of bacteria in saliva increases rapidly at night, it is highest upon awakening[26]. Accordingly, it is generally recommended that oral care (including toothbrushing) should be performed before sleeping. Patients and clinicians generally agree on the need for oral care before sleep. However, this practice is recommended solely based on the number of bacteria in saliva, and it does not consider the role of dental biofilm in the onset of oral disease. The salivary microbiome is reportedly associated with circadian oscillation[27]; however, there remains a lack of information regarding the relationship between dental biofilm and the circadian rhythm, as well as regarding the roles of changes in the abilities of dental biofilms to cause oral disease between waking and sleeping hours. Unfortunately, the effects of sleep on the characteristics of dental biofilm have not been sufficiently investigated owing to the difficulty of such experiments.

Subject number	Sex	Age (years)	DMF	CPI
1	F	30	14	0
2	M	30	0	0
3	F	28	12	0
4	F	30	2	0
5	M	32	3	0
6	M	27	1	0
7	M	27	4	0
8	M	32	7	0
9	M	31	0	0
10	M	31	8	0

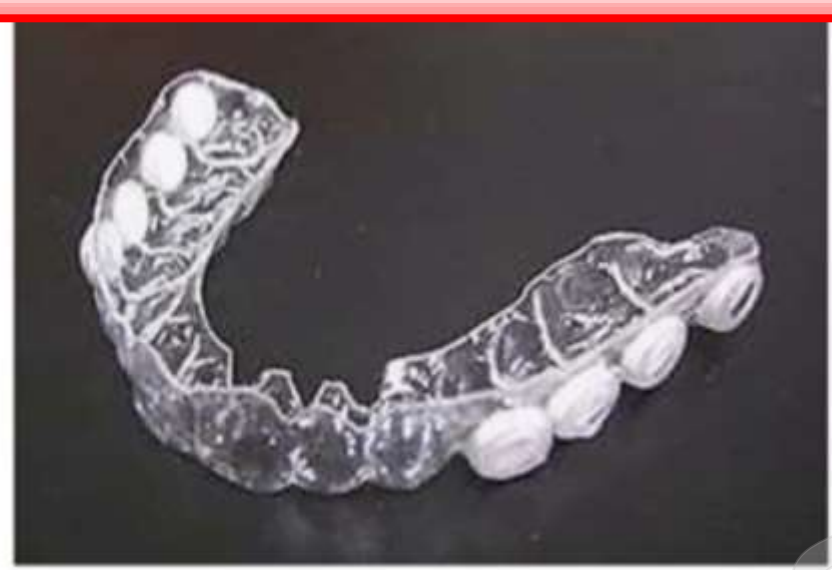
Table 1. Subject characteristics. The subject characteristics are shown. F, female; M, male. A quantification of the experience of dental caries as the total number of teeth that are decayed, missing, or filled (DMF) was used as an index of dental caries. The Community Periodontal Index (CPI) was used as an index of periodontal disease. No clinical signs of caries, gingivitis, or periodontitis were detected, and no systemic disease was observed, in any of the subjects.

Methods

Selection of study subjects. Ten healthy volunteers (seven men and three women, 27–32 years old) were recruited from among the students and staff of Osaka University Graduate School of Dentistry. Healthy subjects were defined as previously reported[38]. No clinical signs of caries, gingivitis, or periodontitis were detected, and no systemic disease was observed in any of the subjects. The total number of decayed, missing, or filled teeth (DMF) of each participant was recorded as an index of dental caries, and the Community Periodontal Index (CPI) of each participant was recorded as an index of periodontal disease. A summary of the subject characteristics is shown in Table 1. The subjects were asked to avoid using antibiotics for 3 months before beginning this study.

Ethics declarations. Written informed consent was obtained from all subjects. The study design was reviewed and approved by the Ethics Committee of the Osaka University Graduate School of Dentistry (H29-

Figure 1. In situ dental biofilm model. Schematic of the in situ dental biofilm model. Oral appliances with HA disks inserted into the buccal sides were used to observe the formation of experimental biofilms.



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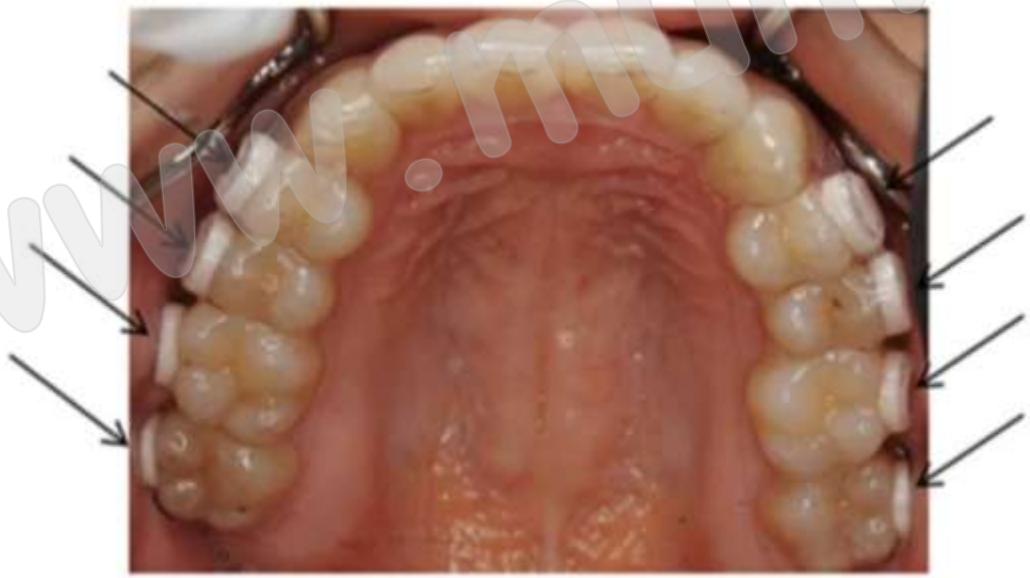
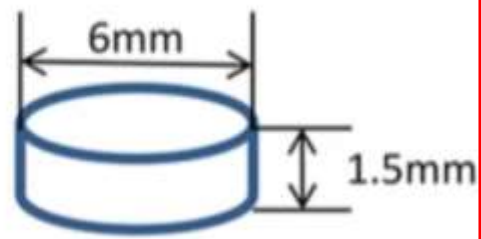
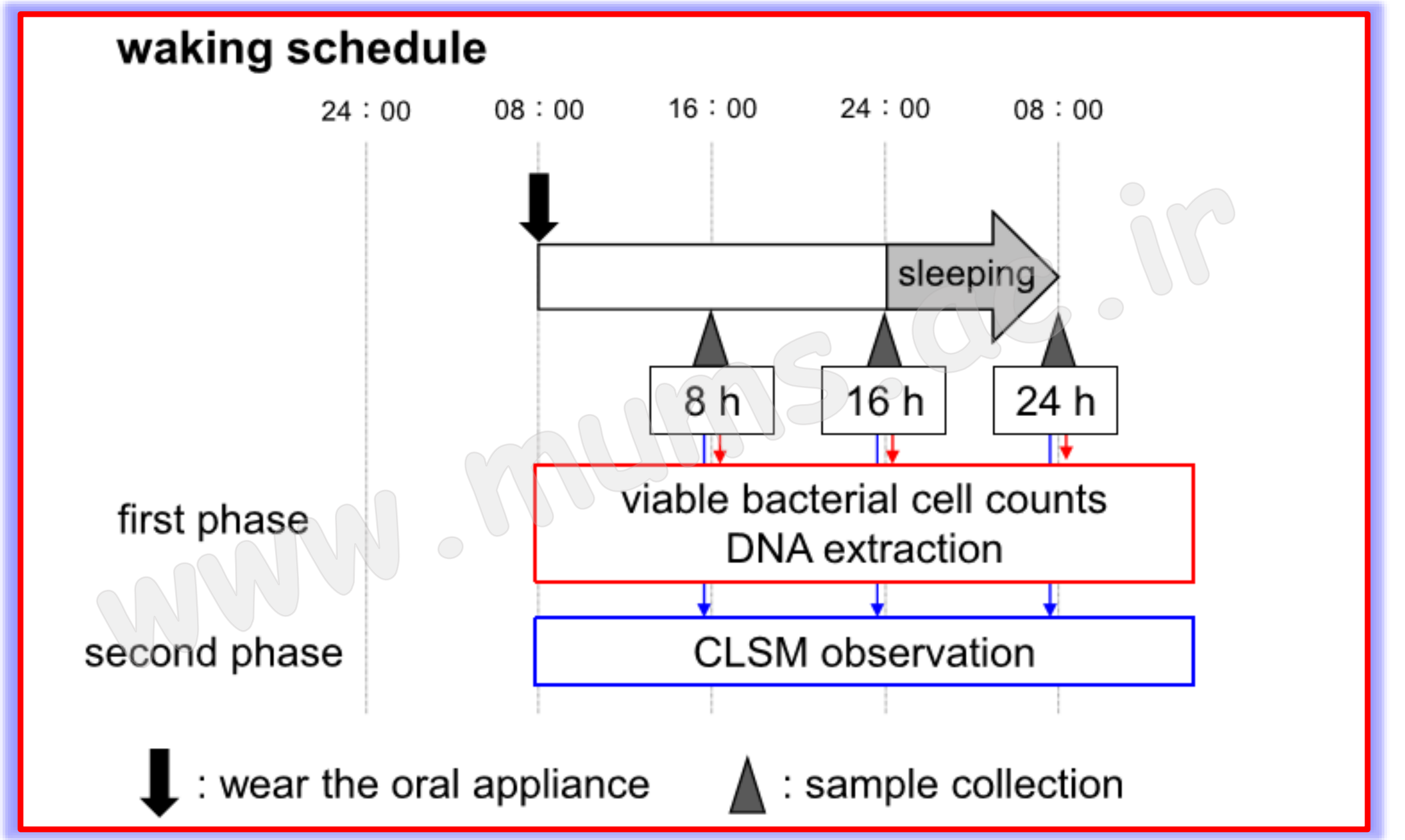
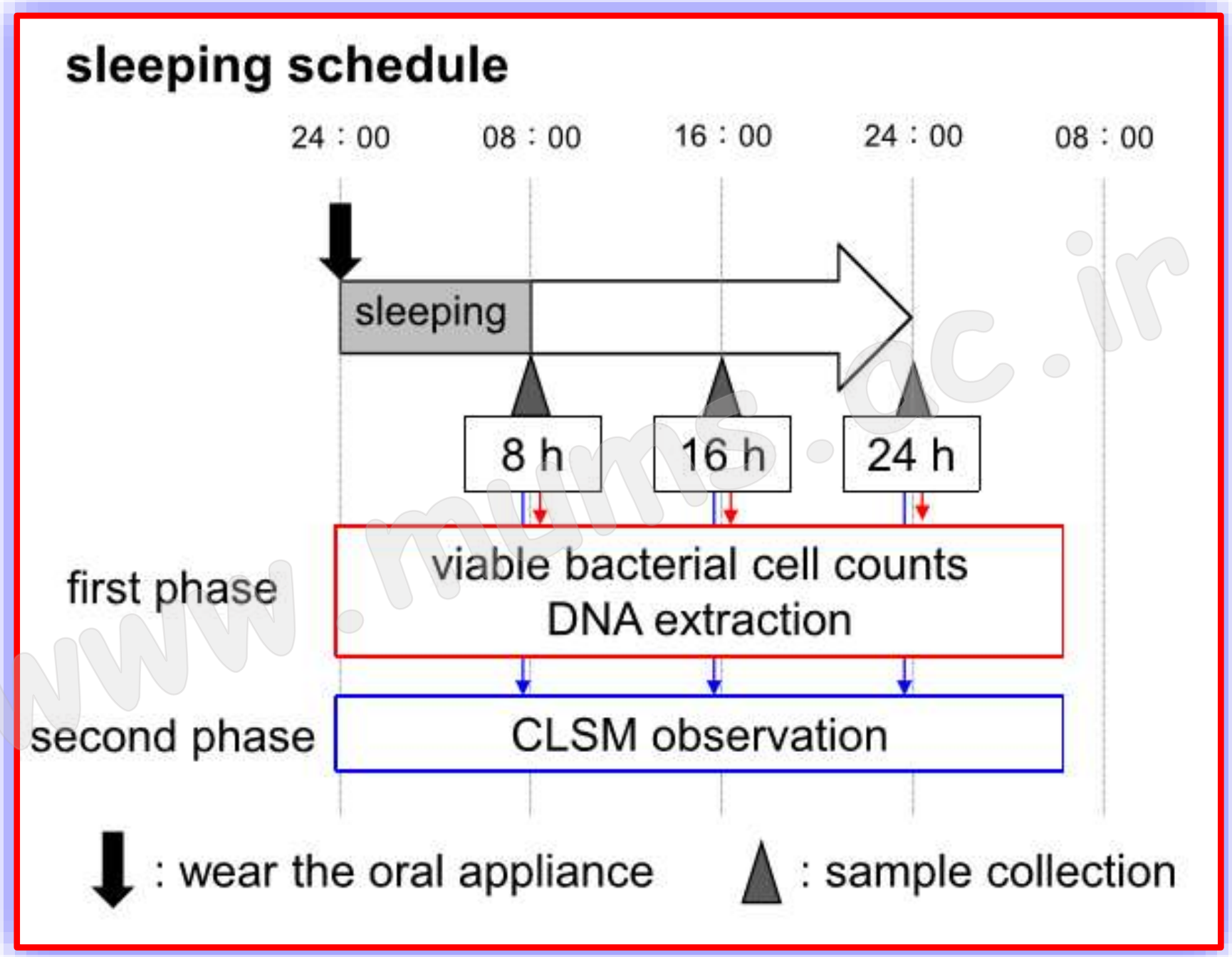


Hydroxyapatite disks






sleeping schedule
24 : 00
08 : 00
16 : 00
24 : 00
08 : 00
sleeping
8 h
16 h
24 h
first phase
viable bacterial cell counts
DNA extraction
second phase
CLSM observation
: wear the oral appliance
: sample collection

Viable bacterial cell counts. Biofilm samples were sonicated for 5 min in sterile distilled water. The resulting bacterial suspension was diluted and spread onto Colombia blood agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and incubated in aerobic conditions for 24 h or anaerobic conditions using Anaero Pack Kenki (Mitsubishi Gas Chemical Company, Inc. Tokyo, Japan) for 48 h. Three plates were used for each concentration and each condition. The numbers of biofilm-forming bacteria were calculated by counting colonies.

Aerobic → **24 hr**

Anaerobic → **48 hr**

Quantification of total biofilm-forming bacteria by real-time PCR. Bacterial DNA was extracted with a DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). Real-time PCR was performed in a total volume of 20 µl, composed of 10 µl of Power SYBR Green Master Mix (Applied Biosystems, Foster City, CA, USA), 1 µl of DNA, and the following universal bacterial 16S rRNA primers: 27F (AGRGTTTGATCMTGGCTCAG[39,40]) and 338R (TGCTGCCTCCCGTAGGAGT[41]). The final concentration of forward and reverse primers was 900 nM. The Applied Biosystems 7500 fast real-time PCR system (Thermo Fisher Scientific, Foster City, CA, USA) was used to estimate the numbers of biofilm-forming bacteria via a calibration curve method. The calibration curve was prepared using *Streptococcus mutans* ATCC 25175 genomic DNA.

16S rRNA gene sequencing. The V1–V2 region of 16S rRNA was amplified by using the primer set 27F and 338R. The Illumina library was prepared with the tailed PCR method, in accordance with the instructions of the "Illumina 16S Metagenomic Sequencing Library Preparation Guide". Sequencing was performed with a MiSeq instrument (Illumina Inc.). The sequences were processed and clustered into operative taxonomic units (OTUs) with a 97% similarity cutoff using the Green Gene database. The results of sequences were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) pipeline.

Confocal laser scanning microscopy observations. A modified biofilm staining method was used as previously described[42]. To analyze the structure of the biofilms, bacterial cells were stained with 4′,6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific), and EPS was stained with fluorescein isothiocyanate (FITC)-labeled concanavalin (Con A; Thermo Fisher Scientific) and FITC-wheat germ agglutinin (WGA; Thermo Fisher Scientific). The final concentrations of fluorescent labeling reagents were 125 ng/ml (FITC-ConA and FITC-WGA) and 105 ng/ml (DAPI). Biofilm samples on HA disks were immersed in aliquots of fluorescent labeling reagent for 30 min. A confocal laser scanning microscope (LSM 700, Carl Zeiss, Oberkochen, Germany)

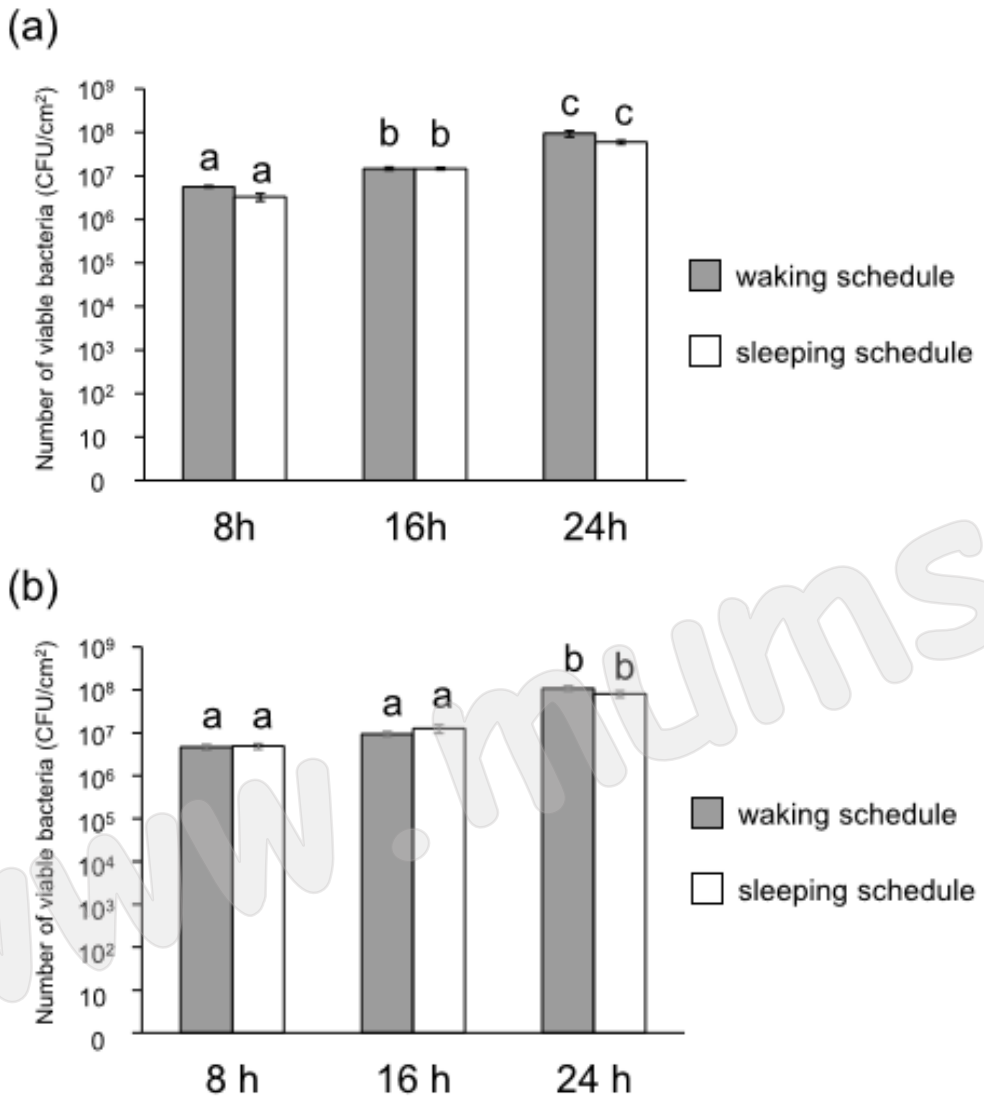


Figure 3. Numbers of viable biofilm-forming bacteria. (**a**,**b**) Numbers of viable biofilm-forming bacteria over time in dental biofilms from the waking and sleeping schedules, grown under aerobic (**a**) or anaerobic (**b**) conditions, as measured by counting colonies. Significant differences are represented by different letters (Friedman's test at $p < 0.05$).

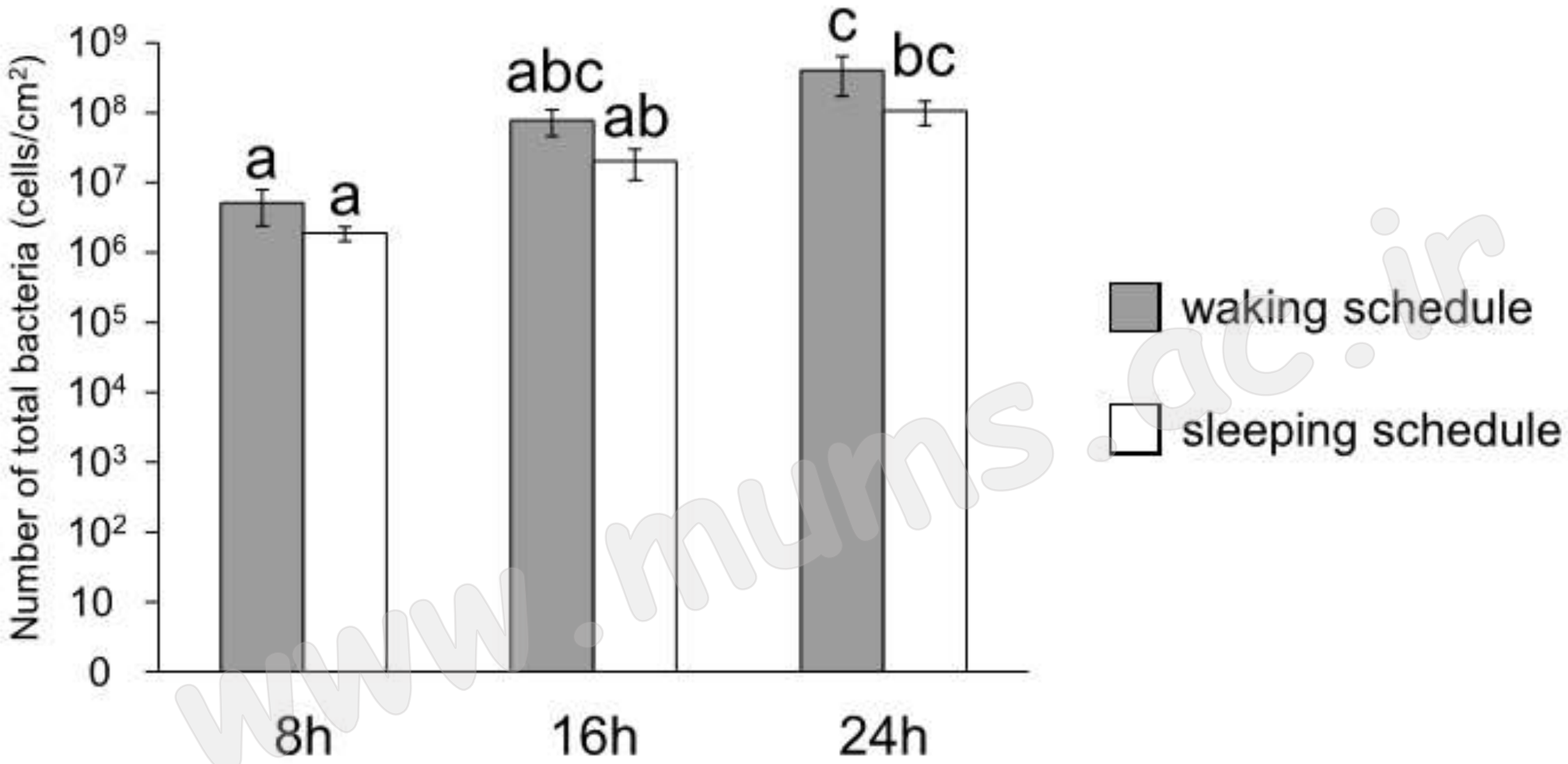
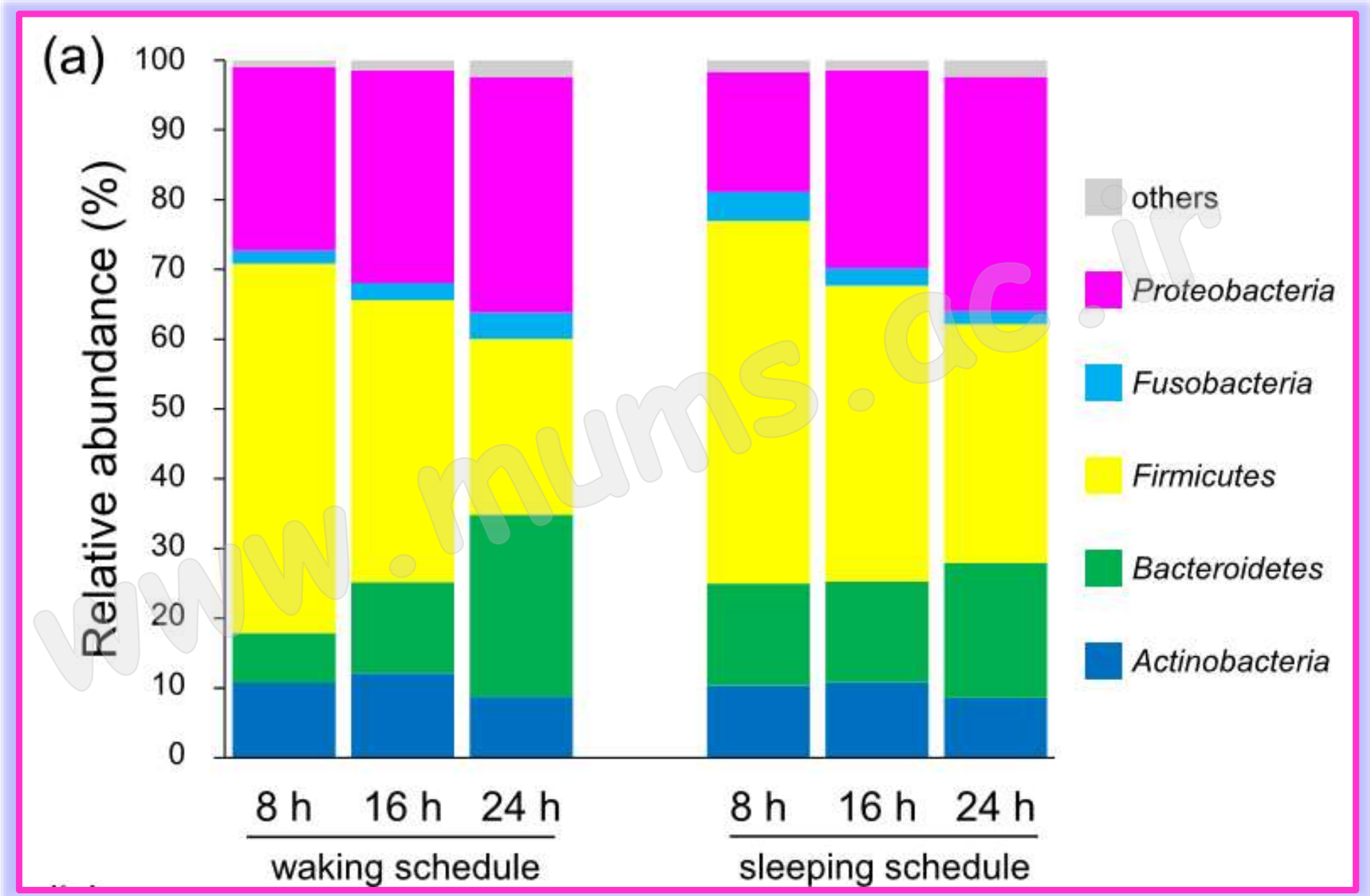
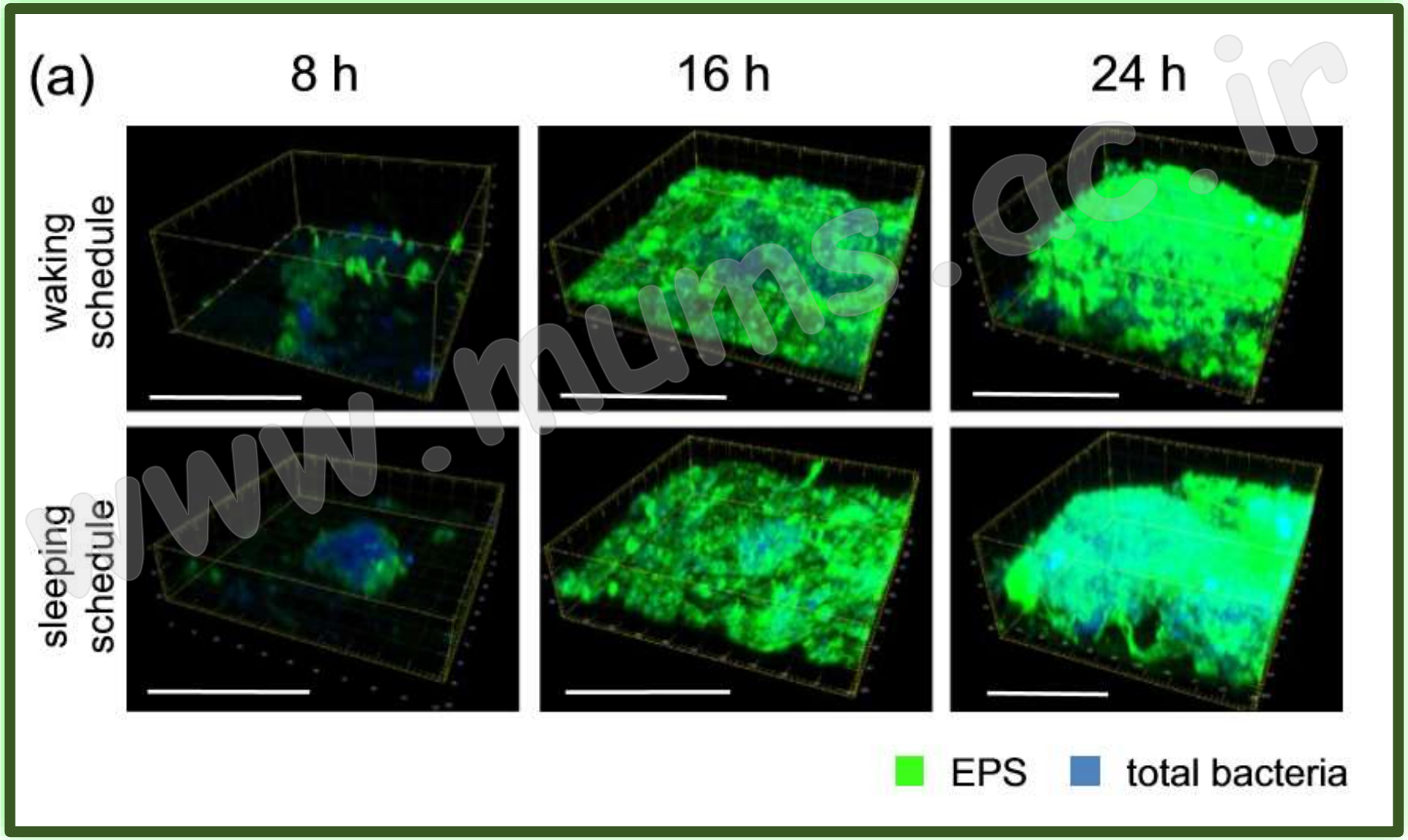


Figure 4. Numbers of total biofilm-forming bacteria. Numbers of total biofilm-forming bacteria over time in dental biofilms from the waking and sleeping schedules, as measured by real-time PCR. Significant differences are represented by different letters (Friedman's test at $p < 0.05$).

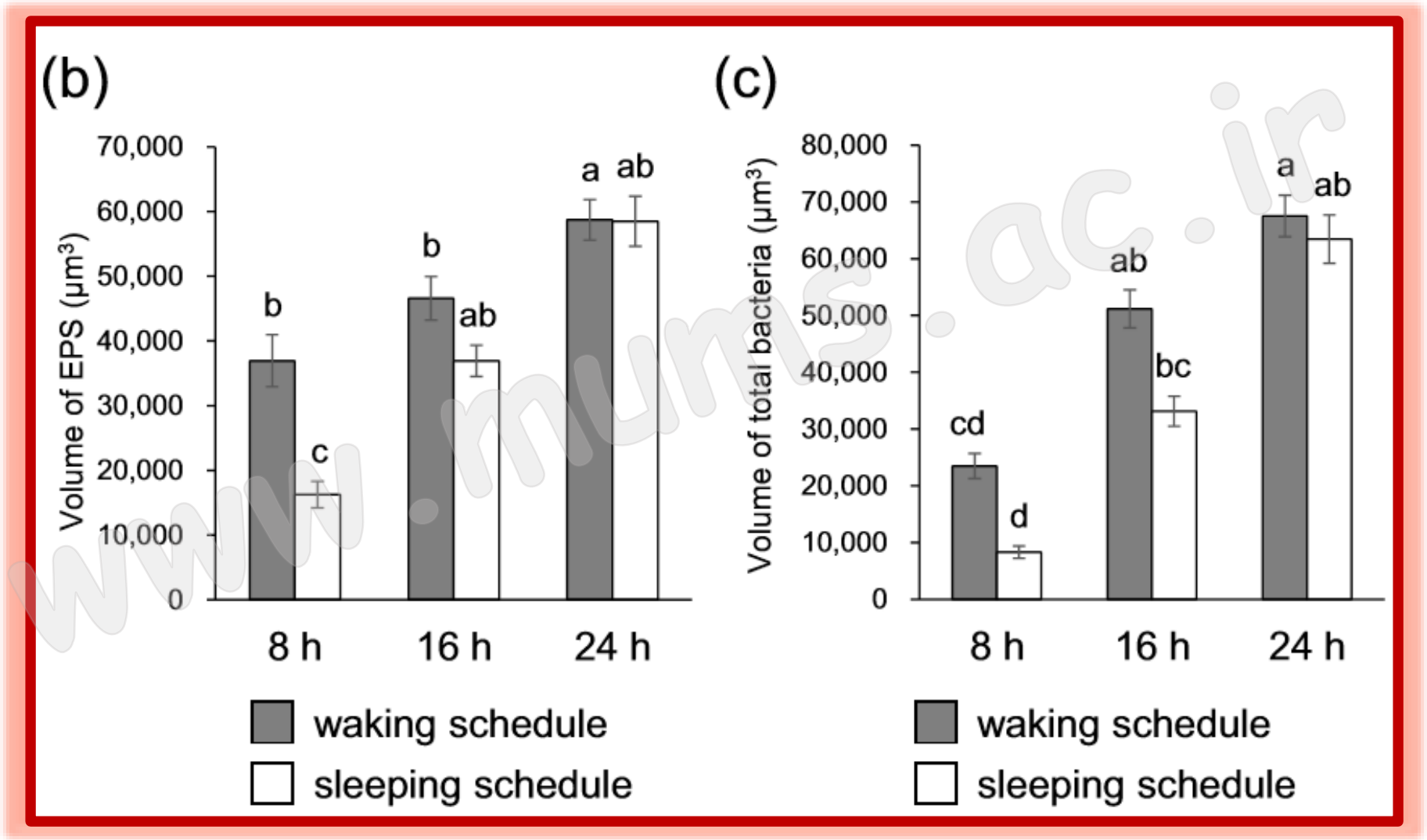
The relative abundance at the phyla (a)



Confocal laser scanning microscopy images of in situ dental biofilm and the volumes of components in EPS-total bacteria staining. (a) Exopolysaccharide (EPS) labeled with FITC-ConA and FITC-WGA is depicted in green, and total bacteria labeled with DAPI are depicted in blue.

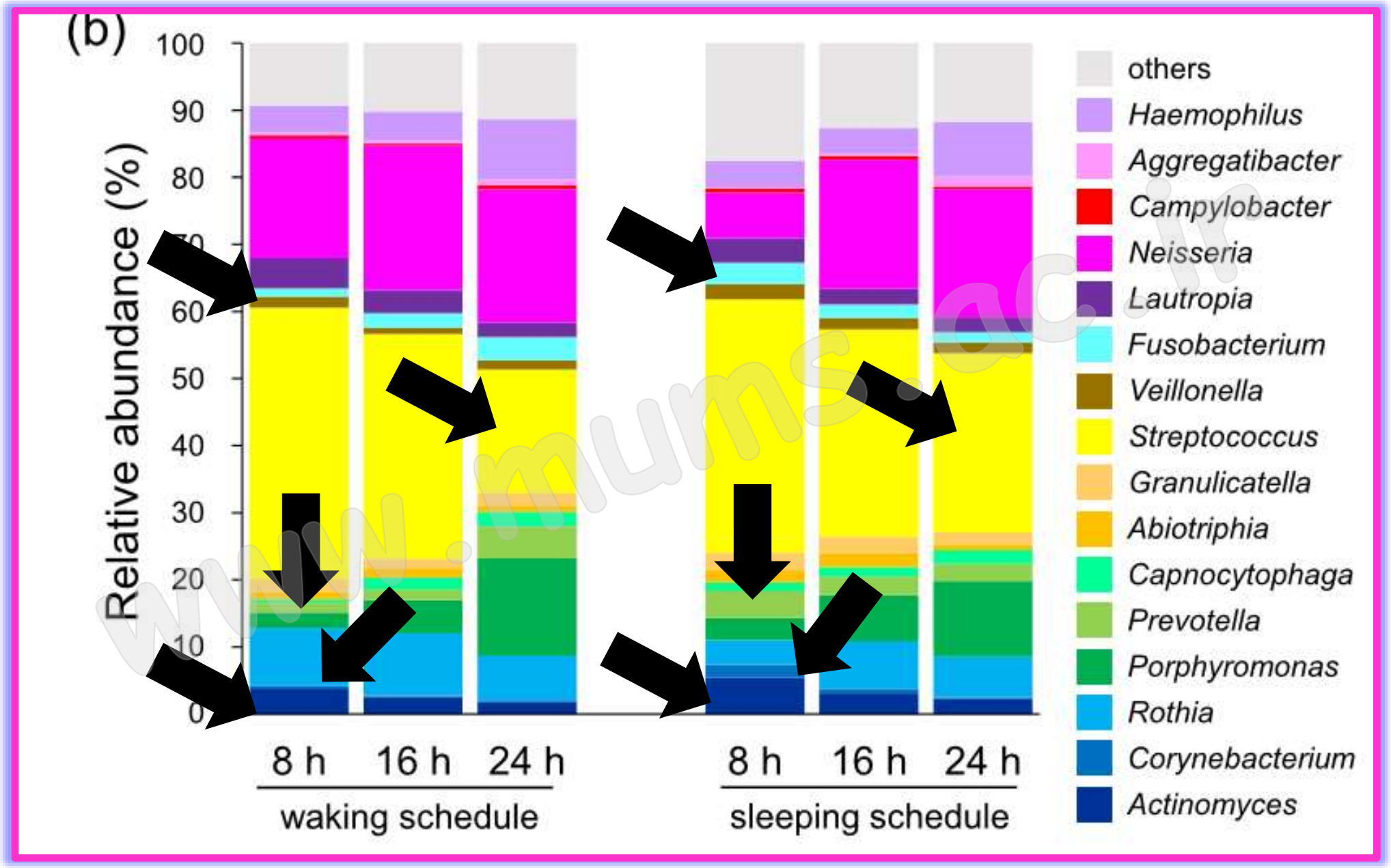


The volumes of EPS (b) and total bacteria (c). Significant differences are represented by different letters (Friedman's test, $p < 0.05$).

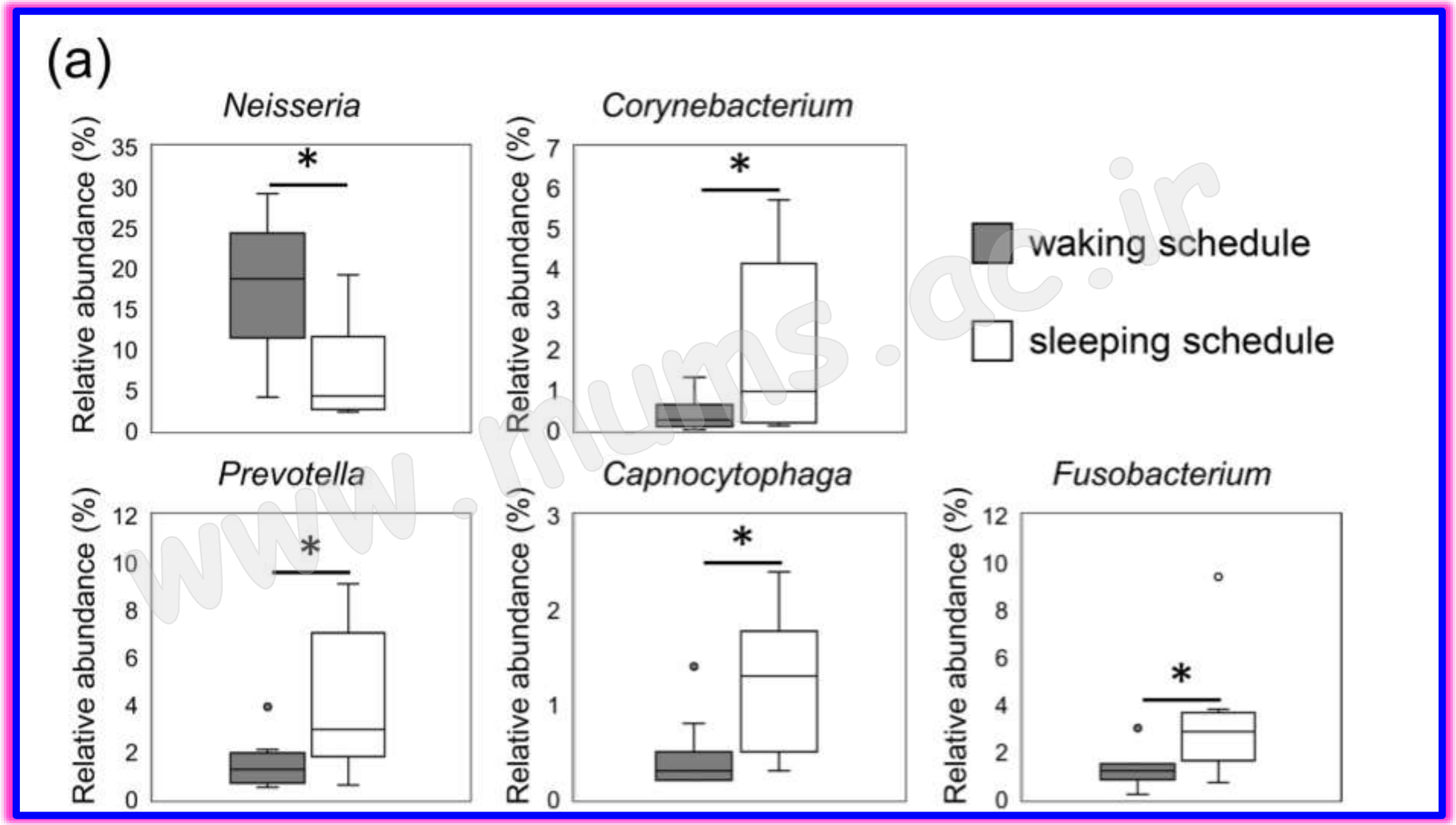



Discussion

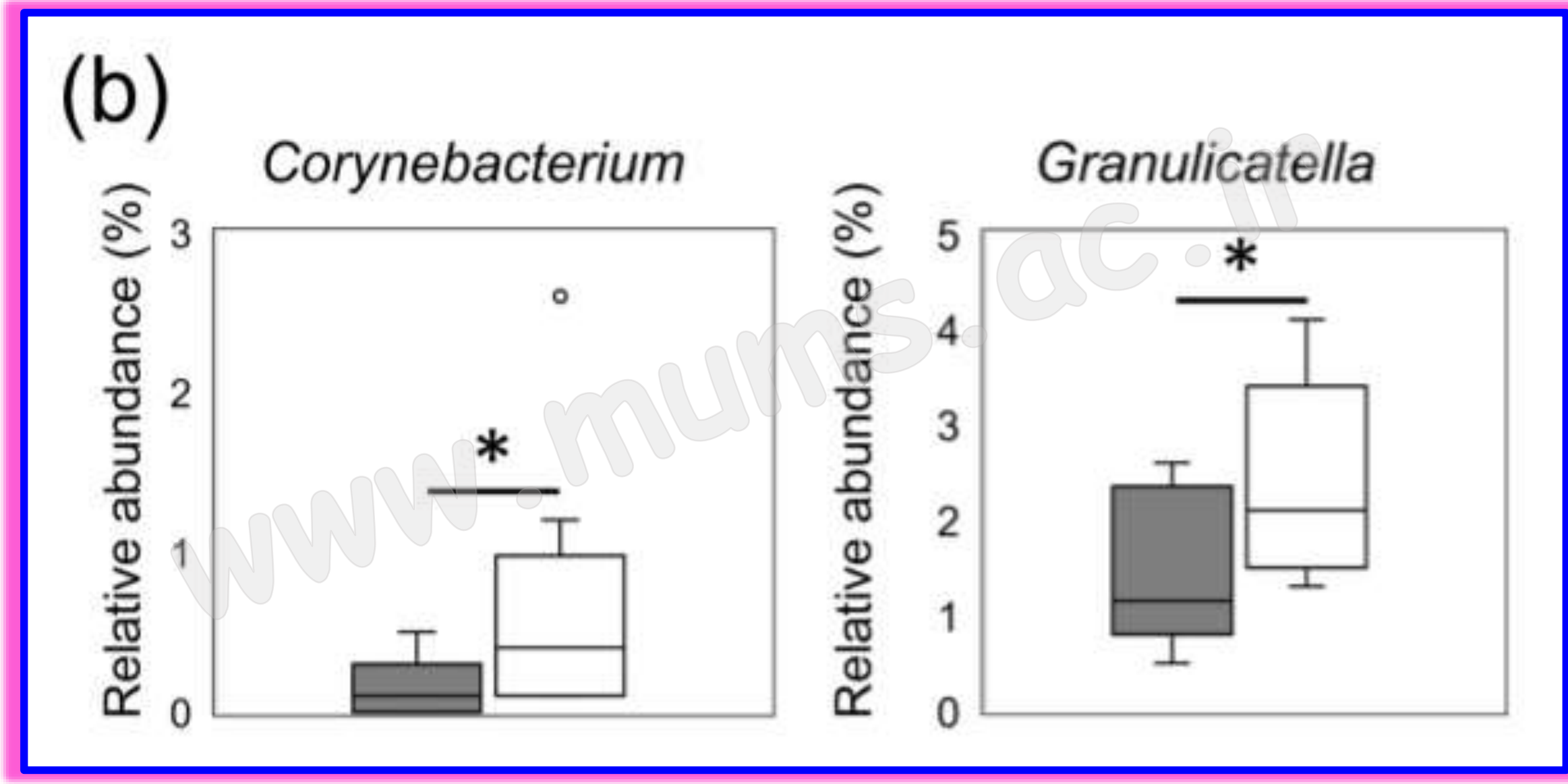
The relative abundance at the and genus



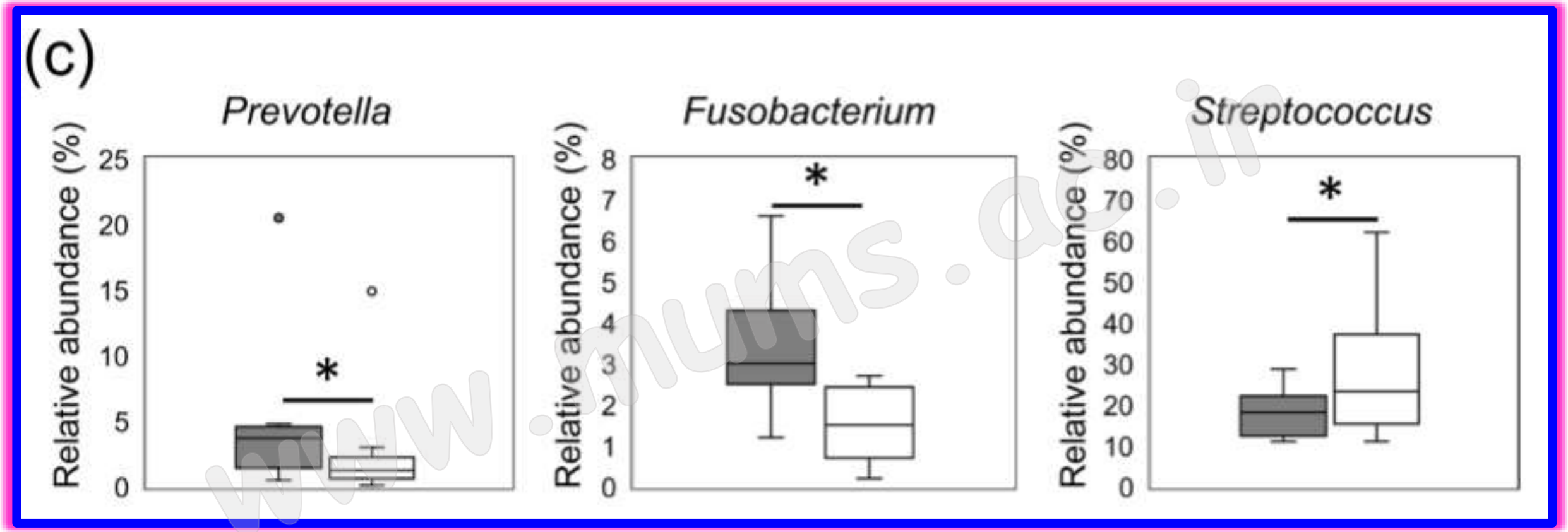
8 h (a)



16 h (b)



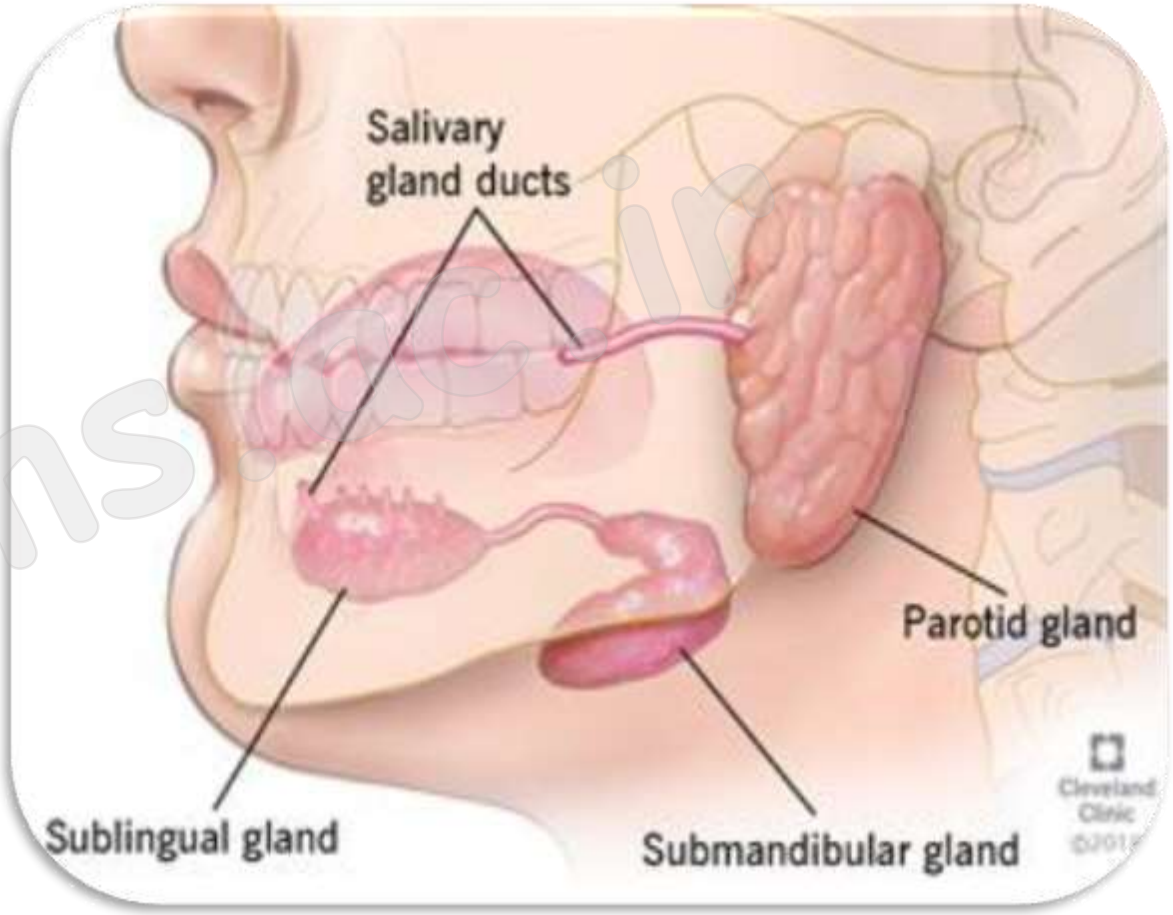
24 h (c)



The results of the present study suggest that the number of biofilm-forming bacteria is not affected by sleep whereas the relative abundances of obligates anaerobes (e.g. *Fusobacterium* and *Prevotella*) are higher after waking than during the daytime. Importantly, obligate anaerobes in dental biofilm are associated with periodontitis[10]. Therefore, effective periodontitis prevention may involve the removal of dental biofilm that contains more obligate anaerobes upon awakening. However, the pathogenic characteristics of obligate anaerobes in dental biofilm were not investigated in this study, so further analysis is required.

There are some limitations in this study. First, this research focused on only healthy subjects without dental caries or periodontitis. For establishing methods of controlling such oral diseases in individuals suffering from them, additional investigations into the characteristics of dental biofilm in patients with oral diseases are needed. Second, this study used 16S rRNA sequencing to examine the bacterial composition in dental biofilm at the genus level. To better clarify the differences in biofilm properties between periods of sleep and wakefulness, future work will need to evaluate function, especially pathogenic properties or metabolic activity, and conduct a species-level analysis. Third, sleep was self-reported and was not monitored because it is difficult to accurately monitor sleeping. The biofilm composition may be different in individuals with sleep disorders, such as insomnia and sleep apnea, or who breath through their mouths rather than their noses during sleep; if these issues can be clarified by sleep monitoring, it could allow for oral care to be tailored to individual patients.

Why didn’t they choose lower jaw?



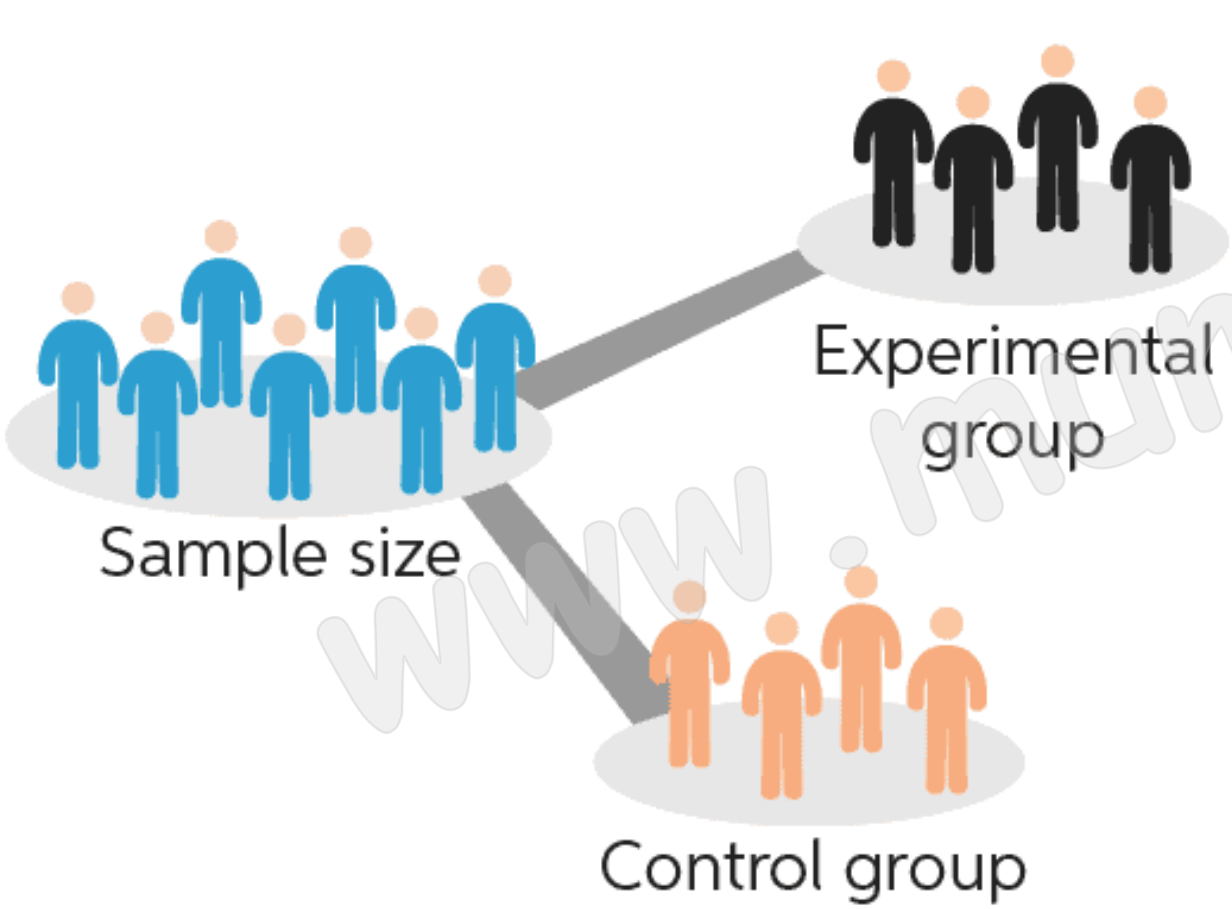
All subjects brushed their teeth, without using toothpaste or mouthwash, before they inserted the oral appliance.

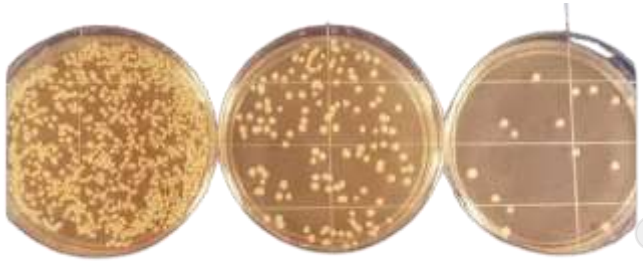
After meals using toothpaste or mouthwash…

All ten subjects were participated in both experimental schedules (waking and sleeping).

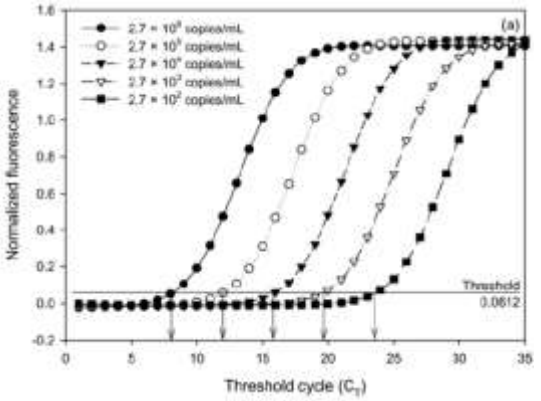
How many people gave up?

Control group and experimental group





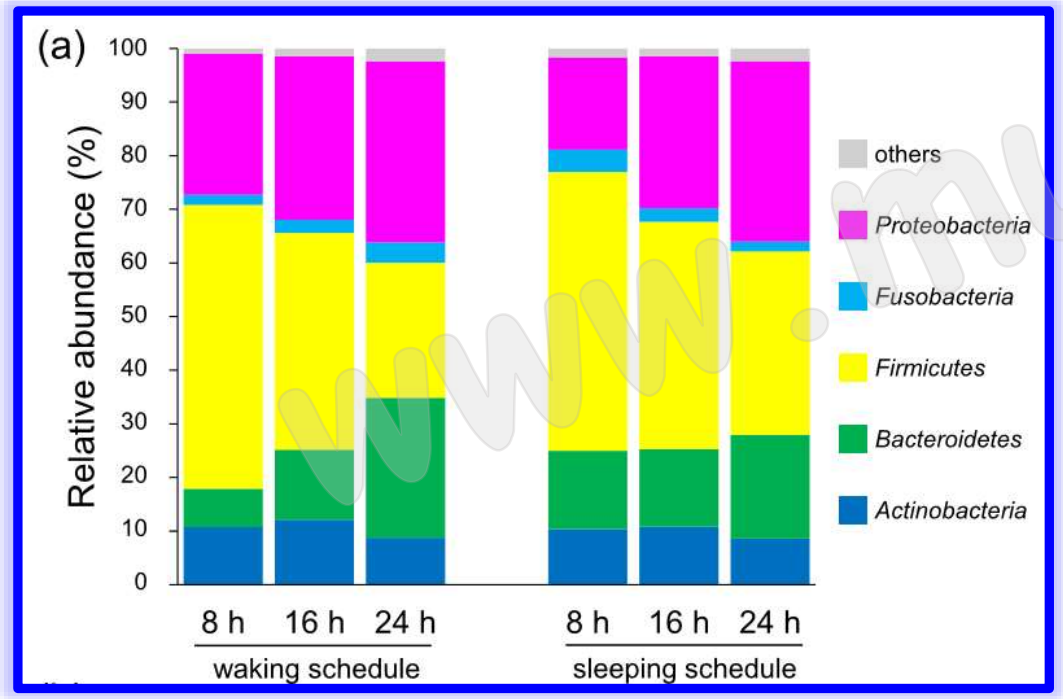
- **Viable bacterial cell counts**
- **Quantifcation of total bioflm-forming bacteria by real-time PCR**

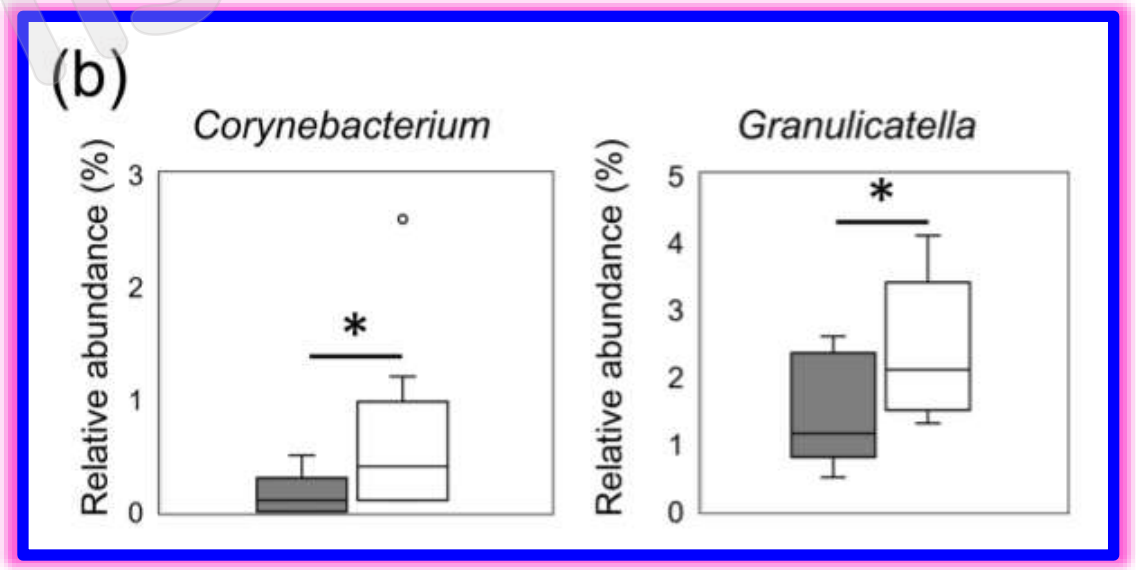


- **16S rRNA gene sequencing**

- **Confocal laser scanning microscopy observations**

Tables & Types of charts





However, these in vitro models can neither simulate the environment of the oral cavity nor reflect the influence of host factors. Although these problems could potentially be solved by collecting dental biofilm directly from the tooth surface, the structure of collected biofilm would be disturbed by the instruments used for their collection. In previous studies, the growth rate and growth patterns of dental biofilm formed during the daytime and nighttime were recorded by taking photos, which subsequently were used to calculate the area covered by dental biofilm[35,36]. However, these experiments did not provide an accurate assessment of the amount of dental biofilm.

In our previous work, we developed an in situ dental biofilm model[37] that enables the formation of experimental dental biofilm on hydroxyapatite (HA) disks in the oral cavity, which facilitates quantitative analysis. The use of this in situ dental biofilm model enables the collection of samples of dental biofilm formed in oral cavity without disturbing the biofilm structure and investigations of the amount of biofilm per unit area. We previously studied the temporal dynamics of experimental dental biofilm by using this in situ dental biofilm model for 96 h.

- **Informed consent**
- **Entry and non-entry criteria**
- **A rest period of least 2 weeks (waking and sleeping)**
- **Recorded the time of the meal while experimental time.**
- **Avoid using antibiotics for 3 months before beginning this study**

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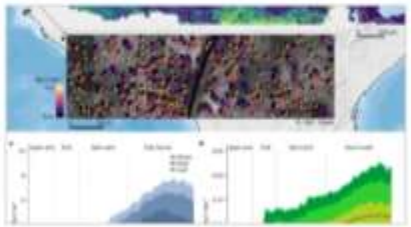

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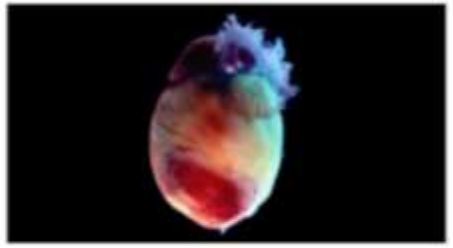

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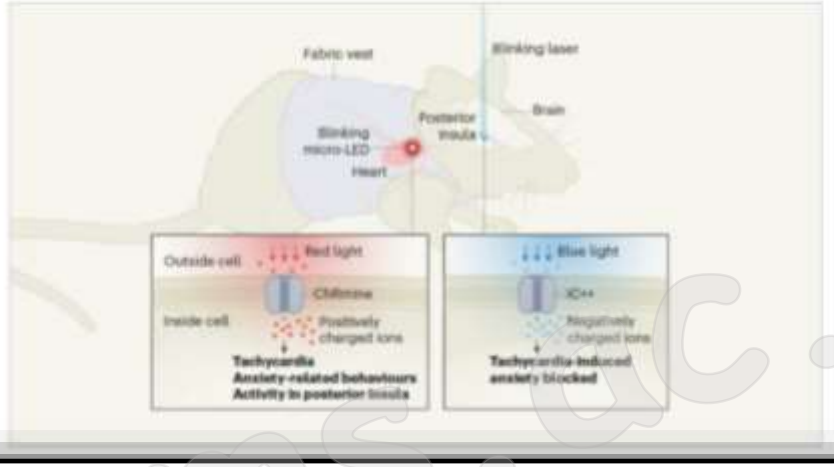
How an anxious heart talks to the brain

During periods of anxiety, the brain affects the heart, but does a racing heart also talk to the brain to cause anxiety-related behaviour? Use of a light-stimulated pacemaker in mice shows that it does, and pinpoints a brain region involved.



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How an anxious heart talks to the brain

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Yoni Couderc & Anna Beyeler

Have you ever been so anxious that you could feel your heart racing in your chest? This tachycardia is one of the main symptoms of anxiety[1], and can be so intense that the person

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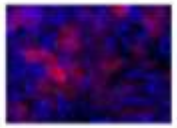

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Postdoctoral Associate- Alzheimer's Disease

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Houston, United States

Vice Chancellor for Research, Rutgers University–Camden

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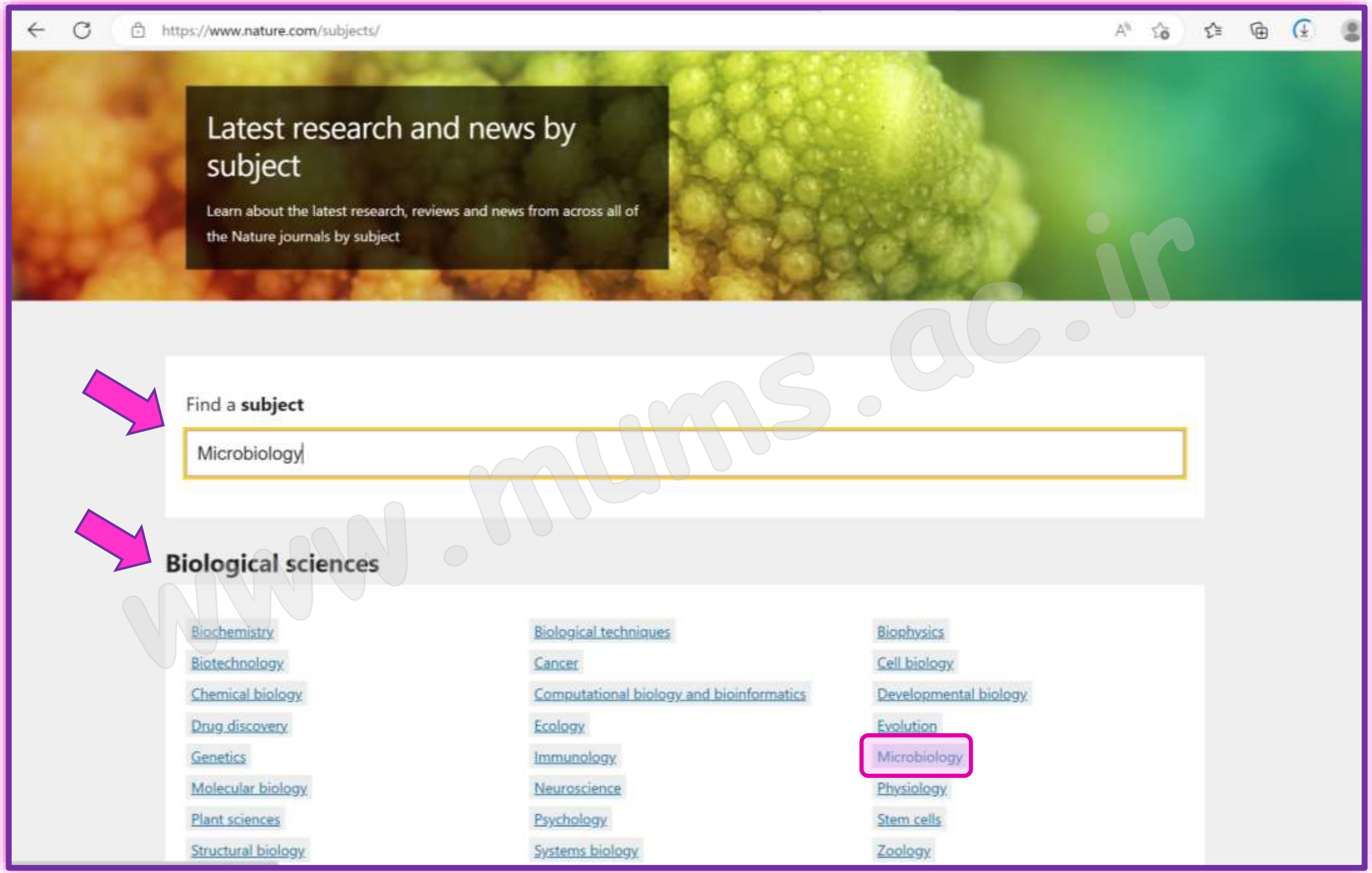

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Microbiology is the study of microscopic organisms, such as bacteria, viruses, archaea, fungi and protozoa. This discipline includes fundamental research on the biochemistry, physiology, cell biology, ecology, evolution and clinical aspects of microorganisms, including the host response to these agents.

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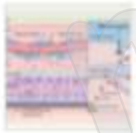

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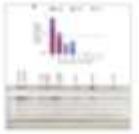

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Biofilms

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Biofilms are communities of microorganisms that attach to each other and to surfaces, for example by bacterial adherence. Biofilms consist of both the cells and the extracellular matrix produced by the cells. Biofilms can be problematic in certain places, for example inside pipes or on medical implants.

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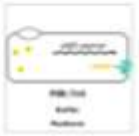

Alona Frenkel, Eli Zecharia & Rakefet Schwarz

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Amir Arastehfar, Farnaz Daneshnia & David S. Perlin

Nature Communications **14**, 1183

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Antimicrobials

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Antibiotics are substances that inhibit the growth of microbes such as bacteria, microscopic fungi or protozoans.

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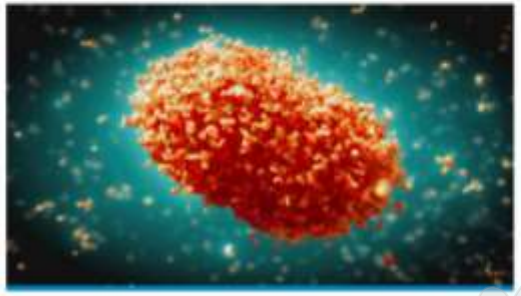

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