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Hydrogel Loaded Metronidazole as a Promising Drug Delivery System for Treatment of Some Oral Parasites in Orthodontic Patients

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By

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

«وَنُنَزِّلُ مِنَ الْقُرْءانِ مَا هُوَ شِفَآءٌ وَرَحْمَةٌ لِّلْمُؤْمِنِينَ وَلاَ يَزِيدُ الظَّلِمِينَ إِلاَّ خَسَارا»

صدق الله العلي العظيم

[الإسراء: 82]

Supervisor Certification

I certify that this Thesis, **(Hydrogel Loaded Metronidazole as a Promising Drug Delivery System for Treatment of Some Oral Parasites in Orthodontic Patients)** Submitted by (**Elaf Mushtaq Talb**) was prepared under our supervision at the Department of Biology / College of Science / University of Misan, as a partial fulfillment of the requirements for the degree of Master of Science in Biology.

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/ / 2023

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/ / 2023

Dedication

To my master Imam Mahdi (may Allah hasten his appearance) who did not tire of my supplication and was a balm for my wounds all this from his bounty.

To my parents and brothers who supported me in finishing my studies and stood with me in the most difficult circumstances, I could not have completed my research without their trust and love for me.

To my husband and companion of my heart and soul, who stood by my side and supported me with all love and respect.

Elaf

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Thank you, my dear father, beloved mother, my husband (Arshad), I cannot forget your support and what you have given me, no matter how many words of gratitude I say to them, I will never give them what they deserve.

Elaf

Summary:

The present study sheds light on *Entamoeba gingivialis* (*E. gingivalis*) and *Trichomonas tenax* (*T. tenax*) two anaerobic protozoan species that reside in the human oral cavity. Orthodontics can facilitate plaque accumulation and hinder oral hygiene, so Poor oral hygiene can increase the risk of infection with these parasites. to treat these oral parasites in orthodontic patients a hydrogel-based smart material has been developed as a local drug delivery platform by a smart sustained release mechanism.

The first part of the current study aims to examine the infection rates of oral parasites in orthodontic patients compared to non-orthodontic individuals by wet mount and using the culture method.

The second part of the current study examines the dominant strains of oral parasites in Maysan Province, using the nested polymerase chain reaction to detect the oral parasites and performing the DNA sequencing to detect the strains of the two studied species *E. gingivalis* and *T. tenax*.

The third part of the research focuses on developing a hydrogel-based smart material as a localized drug delivery platform. The purpose is to effectively treat oral parasites in orthodontic patients by employing a sustainable and intelligent release mechanism.

The study included 200 participants, 100 (76 females and 24 males) orthodontics patients, and 100 (75 females and 25 males) non-orthodontics participants. Three specimens were collected from each participant, one was used to prepare wet mounts, the second was used to prepare a smear for Giemsa staining, and the third was used for culture. Later, the hydrogel was prepared, and the treatment

metronidazole was mixed, to monitor the release time drug concentration was determined using a UV-visible spectrophotometer. Three different concentrations of hydrogel: 10, 15, and 20% (w/v) have been prepared and the drug release has been measured. To detect any association significant between infection with parasites and orthodontic applications, Chi-square (χ^2) and analysis of variance (ANOVA)\F and using $p \leq 0.01$ as criteria for probability.

Infection rates with these parasites were higher in orthodontic patients, with *E. gingivalis* infection being only 47.0% in orthodontic patients, while it was 25.0% in non-orthodontic individuals. The infection rate of *T. tenax* was 2.0% in the orthodontic patients while it was 1.0% in the non-orthodontic individuals. The infection rates of both parasites (*E. gingivialis* and *T. tenax*) were highest in the orthodontic patients at 19.0% while it was 16.0% in the non-orthodontic individuals. The study found a significant relationship between infection and orthodontic applications at $p=0.001$.

The results demonstrate that the hydrogel concentration is a critical factor affecting the pore structure of the hydrogel as shown in SEM. The drug release profile indicates that the drug diffuses from the network of the three hydrogels and the release appears to be diffusion controlled. After 14 days (9– 10) % of the incorporated drug was released from the 10% hydrogel concentration discs, (3-4) % from the 15% hydrogel, and (1-2) % from the 20% hydrogel disc. There were significant differences observed in cumulative release percentages between the 10% hydrogel and both 15% and 20% hydrogel ($p<0.001$), but only small differences (not statistically significant) between the 15% hydrogel and the 20% hydrogel.

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List of Abbreviations

Abbreviation	Title
%	Percent
<	Less than
A	Adenine
bp	base pair
C	Cytosine
CMC	Carboxymethyl cellulose

DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
EDC	ethyl carbodiimide hydrochloride
et al.	et alia (and others)
G	Guanine
gm	Gram
GSB	Gel sample buffer
HCl	hydrochloric acid
HPA	hydroxyphenyl propionic acid
IgA	Immunoglobulin A
IgG	Immunoglobulin G
M	Molarity
M	Molality
MC	Methylcellulose
ml	milliliter
mRNA	messenger RNA
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NHS	N-hydroxy succinimide
Ni-Ti	Nickel-Titanium

No.	Number
nPCR	Nested polymerase chain reaction
ºC	Degree Celsius
p	Probability value
PCR	Polymerase chain reaction
RNA	ribonucleic acid
siRNA	Small interfering ribonucleic acid
SEM	Scanning Electron Microscope
SS	Stainless steel
SSU-rRNA	Small subunit ribosomal ribonucleic acid
T	Thymine
TBE	Tris Borate EDTA
TLR	Toll-like receptor
TMJ	Temporomandibular joint
TYR	Tyramine hydrochloride
U	Uracil
UV	Ultraviolet
WSP	white spot plaques
µl	Microliter
µg	Microgram

Chapter one

Introduction

1. Introduction:

Different species of microorganisms live in human mouth mucosa that play a major role in changing the ratio of health to illness, the oral is a home to bacteria, archaea, protozoa, fungi, and viruses, most of which have distinct "fingerprints" that typically coexist peacefully with their hosts (Nearing *et al.,* 2020).

Organisms that reside in host tissue and induce somatoform disorders are known as parasites. Not all parasitic infections are associated with symptoms, which sometimes only manifest in cases of immunodeficiency, such as AIDS, there are internal and exterior parasites that are distinguished. The three different types of somatoform disorders include skin, tissue, and intestinal diseases (Hadaś and Derda, 2015).

The mouth cavity is a natural repository for numerous bacterial, fungal, and protozoan species that are in equilibrium and frequently serve as a window into the body's overall health. Lesion development comes from the disruption of the natural equilibrium in the mouth cavity to, dental exams, especially of the mucosal surface, may be necessary for the early detection of systemic disorders because many of them are present in the buccal mucosa (Derda *et al.,* 2014). In the oral cavity, two important parasites found associated with mouth diseases are *Entamoeba gingivalis* and *Trichomonas tenax* (Badri, 2021).

Entamoeba gingivalis is one of the many oral pathogens that live in humans' mouths and are particularly common in plaque, periodontal or tooth surfaces, adjacent teeth gaps, and various diseases (Badri, 2021).

The two parasites are presented in 50% of people with a healthy mouth and 95% of those with gingivitis. The viable parasite is trophozoite, and parasite response is caused directly through the kiss, droplet sprays, and shared dining utensils (Mielnik-Błaszczak *et al.,* 2018).

Trichomonas tenax is a motile-flagellated anaerobic protozoan. It takes the form of trophozoite with varying forms (Morozińska-Gogol, 2016). This 12-20 μm in length and 5-6 μm broad creature is pyriform to oval shape and contains four varying lengths of anterior flagella. May be involved in the pathophysiology of periodontal disorders. *T. tenax* is a protozoan belonging to the same Genus as *Trichomonas* (Yazar *et al.,* 2016; Rashidi Maybodi *et al.,* 2016).

It is protozoa often found in the human oral microbiota and colonizes on the edges of the gums, in the interdental spaces, pathological periodontal pockets, and cavities, as well as on the diseased oral mucosa. In either past study, individuals with gingivitis had a frequency of *E. gingivalis* of (47.9-88.9) % in asymptomatic individuals. The frequency of *T. tenax* was less at 25.6 % in the gingivitis cohort and 3.2% among healthy participants (Yaseen *et al.,* 2021).

Individuals that use orthodontics devices, this orthodontics are constructed of different metals bonded to the enamel surface in a specified location by composite glue. Orthodontic rings are typically bonded to the primary molars, with the major component of the device located in the mouth's septum. The device features inhibit salivary self-cleaning, encourage meal particle storage, and degrade dental health, promoting calcification (Perkowski *et al.,* 2016).

Among the dangers of orthodontic therapy are Periodontal damage, discomfort, root resorption, tooth devitalization, TMJ (temporomandibular joint) condition, cavities, speech difficulties, and enamel damage. The type of appliance, force vectors, and length of therapy are generally treatment parameters that might affect risk, whereas significant patient factors are biological and behavioral in nature. Therefore, there are differences in risk due to the inherent variance in orthodontic treatment programs and individuals (Wishney, 2017).

Therefore, the use of orthodontic appliances may increase the risk of it possible to infection of *E. gingivalis* and *T. tenax* infections that are caused by poor oral care, as evidenced by the findings of the current investigations. Everyone needs an orthodontic device to preserve excellent oral health (Abbass *et al.,* 2020).

According to many researchers, poor dental hygiene is frequently associated with a greater occurrence of protozoa in the oral cavity (*T. tenax* and *E. gingivalis*), and age > 40 years is a major factor impacting the immune response and, consequently, the development of parasites in the oral (Derda *et al.,* 2014). The length of time spent cleaning your teeth is a significant aspect that influences transmission. Investing less than a minute in these tasks and cleaning too little often harms the health of the buccal mucosa and encourages the growth of parasites (Wantland and Lauer, 1970). But on the other hand, using floss or chopsticks improperly or in combination with other technical dental hygiene tools might be harmful. Protists are more likely to be found in individuals who use orthodontics devices. This is most likely a result of the

mishandling of these gadgets, which can hurt the periodontal ligament and lead to pain.

Result of the prevalence of these protozoa in the oral cavity in individuals that used orthodontics devices (fixed and detachable), the development of polymeric drug delivery systems as an alternative to conventional drug formulations has grown steadily for several decades now, mostly trying to address inadequate local drug availability and challenges associated with delivery sites. Thermoplastic- and in particular hydrogel-based scaffolds are attractive for controlled drug delivery as their properties can be tuned during manufacturing while also being amenable to safe implantation, release, and degradation (Calori *et al.,* 2020).

Hydrogels are of interest among polymeric drug delivery methods because of their great biocompatibility. Hydrogels are networks of hydrophobic polymers that resemble cells' extracellular matrix (Sivakumaran *et al.,* 2013).

As a result, it is preferable that now the simulated model be (1) biodegradable, in which the chemical bonds that formed the polymer structure can break; (2) disassemble and its fragments are dissolvable, however, its covalent bonding could indeed tear; as well as (3) single, in which the covalent bonding does not easily accommodate and thus stay unchanged in the skin. Micro-sized polymers can be employed in situations 1 and 2. Yet, nano-sized monomers should be used for renal clearance of hydrogels that are neither biodegradable nor disassembled. The technique through which these polymers are removed from the body is a critical issue. They are most likely taken down immediately from the kidneys (renal elimination) or by biodegradability, in which

they are reduced to protein pieces and afterwards eliminated from the body (metabolic clearance). Transmission across the renal tubules is limited to molecules with a molar mass of fewer than 50 kDa, but this number varies depending on molecular makeup. A further point to consider in the architecture of hydrogels is their concentrations, which include the rating of hydrophobic nature and chemical bonding between monomers, because the speed and conditions of degradation, and thus the quantity and placement of release of drugs, can be adapted and determined by the chemical properties and polymer structure used (Vilar *et al.,* 2012).

1.1 The Aim of the Study:

The first part of the current study aims to examine the prevalence of oral parasites in orthodontic patients compared to non-orthodontic individuals through the following objective:

1 -Diagnosis of the oral parasites in all samples that have been taken from all participants by wet mount smear and microscopic examination.

2 -Using the culture method to detect the oral parasites to confirm what has been recorded in the above point.

The objective of the second part of the current study is to examine the dominant strains of oral parasites in Maysan Province, located in South of Iraq. The research methodology employed to accomplish this is outlined as follows:

a) Using the nested polymerase chain reaction to detect the oral parasites.
b) Performing the DNA sequencing to detect the strains of the two studied species *E. gingivalis* and *T. tenax*.

3- The third part of the research focuses on developing a hydrogel-based smart material as a localized drug delivery platform. The purpose is to effectively treat oral parasites in orthodontic patients by employing a sustainable and intelligent release mechanism.

Chapter two

Literatures Review

2. Literatures Review

2.1 *Entamoeba gingivalis* and *Trichomonas tenax*

2.1.1 History view:

Entamoeba gingivalis name is from Greek – enteron (intestine), Amo (change), gingivalis (belonging to gingiva) (Bonner *et al.,* 2014).

A parasitic protozoan called *E. gingivalis* is primarily present in the oral cavity (Foda and El-Malky, 2012). It was first discovered in 1849 using swabs of tooth plaque, consisting of both intracellular compartments and amoebic motion (Gros, 1849), Although its harmful relationship with humans was not confirmed until 1914, that was the first amoeba discovered in humans (Barrett, 1914). Throughout all 46, instances of pyorrhea (periodontitis) that were included in the investigation, amoebae were found. The researchers subsequently stated that they did not find amoebae in "the debris gathered around the throat of the tooth" which included 7 healthy people from the same group of patients in the "Insane Department of the Philadelphia Hospital" (Smith and Barrett, 1915a). Additionally, the treatment of pyorrhea in 13 individuals who received emetine led to the removal of amoebae (Barrett, 1914).

Smith and Barrett renamed periodontal "amoebic pyorrhea" (Barrett, 1914) or "mouth endamebiasis" because they hypothesized that emetine, a particular amoebicidal alkaloid having low antibacterial action, was responsible for *E. gingivalis* associated periodontal (Smith and Barrett, 1915b).

Since Otto Friedlich Müller first named this microbe *Cercamonas tenax* in 1773, it has gone by several other names. Other names for this flagellated protozoan of the oral cavity later developed. This is what Virgulina tenax was referred to as by Moquin and Tandon in 1860, *Trichomonas biflagellate*, *Trichomonas caudata*, and *Trichomonas elongate* were the four separate species of *Trichomonas* mentioned by Steinberg in 1862, Weynon renamed the last species as the flagellate protozoan of the oral cavity in 1926. When Clifford Dobell disagreed in 1939 that this protozoan was called *Trichomonas buccalis*, he cited the fact that for many years he had been well persuaded that the microorganisms of the Genus *Trichomonas* were found in the human oral cavity (Dobell, 1939).

At the San Quentin jail in California, advanced periodontitis affected inmates at a frequency of about 90% in the early 1900s. This was caused, at least in part, by a high prevalence of *T. tenax* infections. It was hypothesized in the Journal of the American Dental Association that the overcrowding and subpar food of offenders were to blame for this fast frequency of transmission. Age significantly affected these inhabitants, and severe inflammation was seen (Kofoid *et al.,* 1929).

2.1.2 morphology:

The gingival edges, gingival pockets, necrotic tissue around the teeth, and the spaces between tooth cavities are all places where this aggressive species may be found (Ghapanchi *et al.,* 2015). They are also present in dental plaque (Derda *et al.,* 2014).

The trophozoites are generated only, there is no cyst development. which range in size from 10 to 35 μm (Bogitsh *et al.,* 2013). The length of their globular nuclei ranged from 2 to 4μm, bacteria, blood cells, and metabolic debris are present in several food vacuoles **Figure (2-1)** (Bonner *et al.,* 2014).

Even so, behind human ribosomal RNA (rRNA), *E. gingivalis* contributes the second-highest amount of rRNA here (Deng *et al.*, 2017). *E. gingivalis* has at least two subtypes, ST1 and ST2 may coexist and, although information is limited, appear to demonstrate no intrinsic demographic, geographical, or clinical grouping across the rRNA gene (Zaffino *et al.*, 2019).

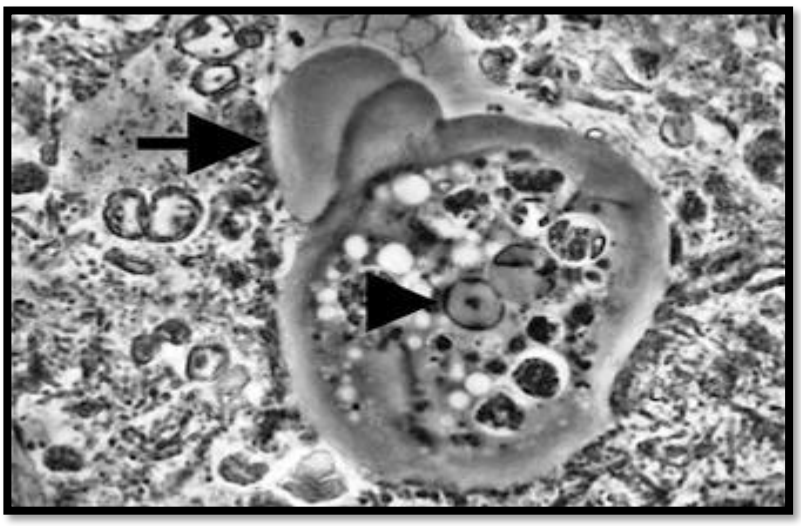

Figure (2-1): The trophozoite of *E. gingivalis* (Bonner *et al.*, 2014).

Trichomonas tenax is a motile-flagellated anaerobic parasitic 12–20 μm in length and 5–6 μm in breadths and has a pear-shaped (Roberts and Schmidt, 2009).

Samples may be recognized by their long axons and tails, four frontal flagella, and flagella that raise an undulating membrane that extends two-thirds of the length of the body**,** this membrane's waves may give the impression of legs, due to its shape and potential for increased size, it can occasionally be mistaken for *T. vaginalis*, since the parasite is rapidly spread by direct contact with mucosal membranes, the presence of an oral or vaginal parasite must be established in such situations (Dobell, 2009).

Flagella originate from structures called blepharoplasty. Similarly, there is a kind of thin rod with a uniform diameter that is parallel to the undulating membrane and is known as the coast, around this rod, and along a series of cytoplasmic granules are observed, which are called hydrogenosomes (Lifeder, 2023).

Like other protozoa, it has an axostyle, this is a rigid structure made up of microtubules that give the cell some rigidity and run along its entire anteroposterior axis, the axostyle protrudes from the posterior end of the protozoan, various organelles, such as the endoplasmic reticulum, ribosomes, Golgi apparatus, vacuoles, and lysosomes, as well as starch granules, can be seen in the cell cytoplasm, within the vacuoles, it is possible to find remains of phagocytosed bacteria or WBCs (Lifeder, 2023).

As expected, a series of digestive enzymes are contained in lysosomes, which help to process the food particles ingested by the protozoan, the

nucleus of the cell is arranged at the anterior end and is surrounded by an endosome which is a system of vesicles that contains phagocytosed material that will subsequently be subjected to the action of lysosome enzymes (Lifeder, 2023). **Figure (2-2)**

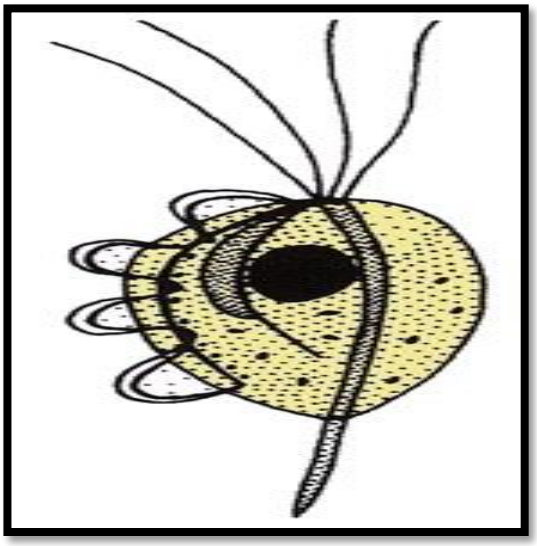

Figure (2-2): *T. tenax* (Mehlhorn, 2015).

2.1.3 Classification of *E. gingivalis* and *Trichomonas tenax*

Entamoeba gingivalis classification as following:

Domain: Eukaryota

Kingdom: Protista

Phylum: Sarcomastigophora

Class: Archamoebae

Order: Amoebida

Family: Entamoebidae

Genus: *Entamoeba*

Species: *E. gingivalis* (Gros, 1849)

According to Honigberg and Lee (1959), the *Trichomonas* genus classification to more than one. The flagellated protozoan of the oral cavity should be included in any future categorization of members of the Family Trichomonadidae, as human oral *Trichomonas* (*T. tenax*) is morphologically nearly identical to *T. vaginalis.* According to taxonomy (Honigberg and Lee, 1959), *T. tenax* is classified as follows:

Domain: Eukaryota

Kingdom: Protista

phylum: Sarcomastigophora

subphylum: Mastigophora

Class: Zoomastigophorea

Order: Trichomonadida

Family: Trichomonadidae

Genus: *Trichomonas*

Species: *T. tenax* (Dobell, 1939).

2.1.4 life cycle

Two cellular stages of most *Entamoeba* species have been recognized in essence: the reproductive cell with the ability to divide, known as trophozoites, which develops from the early embryogenesis of cysts consumed by the host, and the cyst, which is the contaminating form found in the environment (Mi-Ichi *et al.,* 2016).

There is no sexual reproduction in *E. gingivalis*; it reproduces via binary division, when a vulnerable person is exposed, directly or indirectly, to saliva tainted with the parasite, the cycle starts, and the trophozoite starts to divide after the *Entamoeba* enters the new host, if the conditions are right, they establish themselves in different ecological habitat and stay there, **Figure (2-3)** (Bradbury *et al.,* 2019).

It is found in the mouth within the periodontal pocket and in the biofilm near the base of the teeth. *E. gingivalis* is found in 95% of people with gum disease, and rarely in people with healthy gums (Trim *et al.,* 2011).

Entamoeba gingivalis has pseudopodia that allows it to move rapidly and engulf the practice of polymorphonuclear leukocytes by external crystallization in periodontal disease (Bonner, 2013).

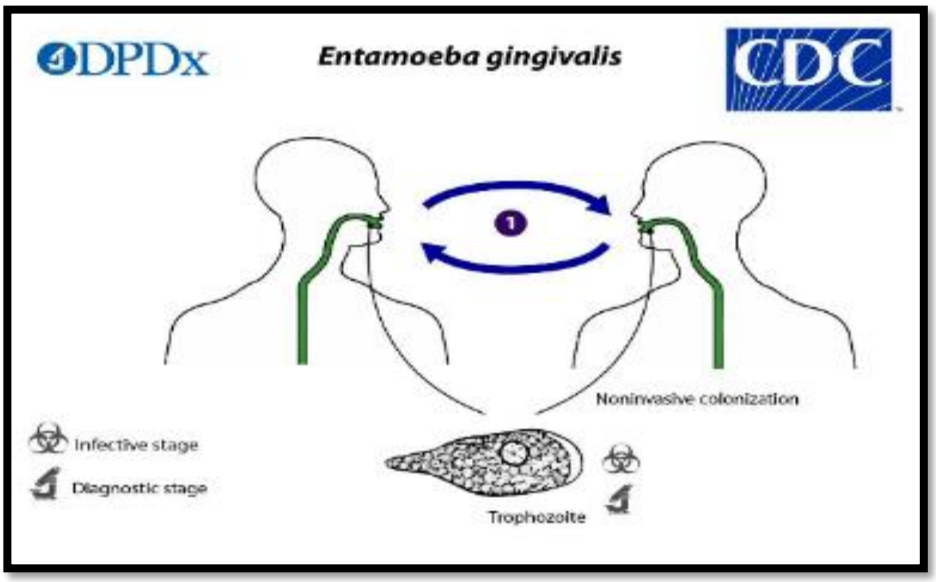

Figure (2-3): Life cycle of *E. gingivalis*.

https://www.cdc.gov/dpdx/entamoebagingivalis/index.html

Trichomonas tenax is mono auxin because it requires one host, which can be humans or domestic animals such as cats, dogs, and horses (Dybicz *et al.,* 2018). *T. tenax* reproduces mostly asexually by longitudinal binary division, the binary division starts with the nucleus splitting, then the neuromotor apparatus, and ultimately the cytoplasm, resulting in two daughters' cells, **Figure (2-4)** (Faust *et al.,* 1974).

It has been discovered that *T. tenax* has a moderate emission of protoplasmic pseudopods, which engulf various nutrients from their environment, including solid particles, bacteria, blood cells, and occasionally *E. gingivalis*. Once engulfed, these nutrients are transported to the cytoplasm of the protozoan, where they will later be metabolized (Feki and Molet, 1990).

The parasite *T. tenax* respires either directly by consuming oxygen when it is found and exhaling carbon dioxide or indirectly by utilizing molecular oxygen generated from complex substances by the activity of several enzymes (Brown *et al.,* 1985).

However, the protozoan's anaerobic metabolism is mediated by cytoplasmic hydrogenases (Poirier *et al.,* 1990).

The trophozoite multiplies through longitudinal divisions. They consume glucose residues in addition to the bacterium flora that naturally exists in the mouths to eat. Both the gut and the vaginal area are inhospitable to them, and they cannot live there. However, if mouth hygiene is inadequate, many of them might appear in the mouth. In these circumstances, more than 50% of infection rates are typical, and cell death was seen (Lyons *et al.,* 1980).

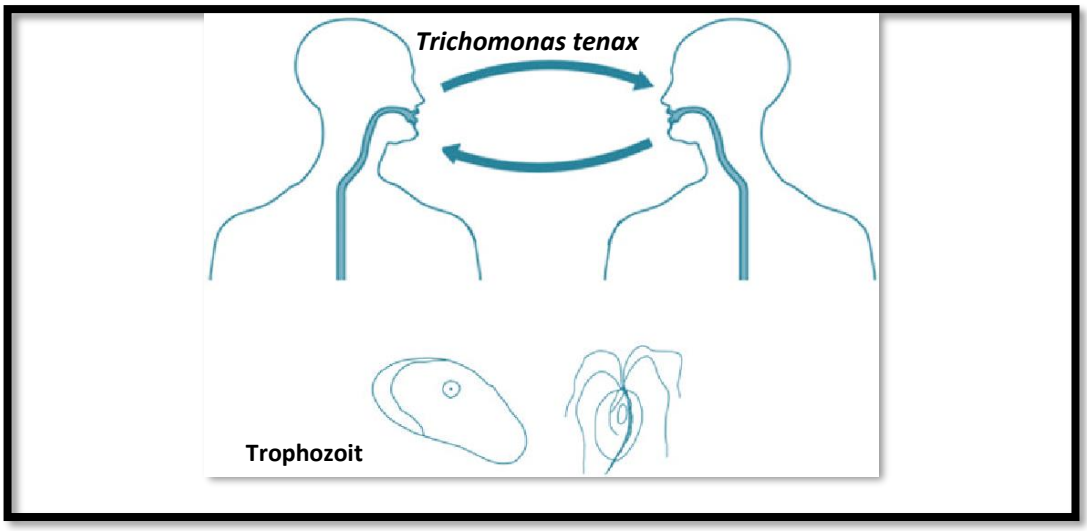

Figure (2-4): life cycle of *T. tenax*

https://www.tekportal.net/trichomonas-tenax/

2.2.5 Pathology

Entamoeba gingivalis and *T. tenax* are largely reported as oral commensal. These pathogenic organisms were reported to be often related to periodontal disease and gum alterations and their identification in host tissues is often related to oral hygiene, global and communal patient health, geographical factors, and QOL (quality of life) status. This comprises water consumption, environmental pollution, and water filtering systems (Fanuli *et al.,* 2018).

Among the parasites found in dental plaque, *T. tenax*, an anaerobic motile-flagellated protozoan, may play a role in the pathophysiology of periodontal diseases. It is 12–20 μm long and 5–6 μm wide organism is either ellipsoidal or ovoid and has four anterior flagella of unequal lengths (Maybodi *et al.,* 2016). *E. gingivalis* is found in the oropharynx, but rarely in the head and neck lesions. This microorganism is commonly found in patients with poor dental health and oral hygiene, periodontal disease, and immune suppression. It was the first commensal found in the human oral cavity (Bao *et al.,* 2020).

Entamoeba gingivalis can usually be observed on the surface of the teeth and gum tissues. The inflammatory process produces a propitious anaerobic environment for parasite growth, researchers previously demonstrated that *E. gingivalis* infects the oral mucosa using an in vitro infection model with a ruptured live vivo mucous membranes biopsy, there, it travels and consumes pieces of live host cells (Bao *et al.,* 2020).

Here, the main body of *E. gingivalis* developed a tube that extended outward and pierced the host cellular membranes, trogocytosis-like behavior can also be seen in *E. gingivalis* infected leukocytes from plaques of the periodontium, where pieces of the interior of the host cells were consumed through the duct of this tube (Bonner *et al.,* 2018).

Pathogenic bacteria involved in periodontal host colonization and immune subversion use complement and toll-like receptor (TLR) bacteria–like signaling pathways, parasites are recognized by TLR, stimulation of the innate immune system via TLR4 by *T. vaginalis* has been reported, and a similar mechanism within the periodontium was also hypothesized for *T. tenax*, massive neutrophil recruitment found in human periodontitis can be explained by observation of patients infected by *T. vaginalis* in which a similar development of predominant tissue recruitment of neutrophils can be identified (Marty *et al.,* 2017) .

Given this pathogenic property, host tissue disruption and lysis may be induced by *T. tenax* secretion of peptidases such as cathepsin B-like proteinases for material type 1 collagen and gelatine hydrolyses or hemolysins for erythrocytes, recently reporters confirmed the ability of the flagella to adhere to periodontal epithelial cells, after only 6 h, *T. tenax* in co-culture caused significant direct damage, disrupting some of the cells in the tissue, inducing membrane damage and cell apoptosis, in contrast, *T. vaginalis* has been found in the oral cavity but failed to elicit in vitro damage on periodontal cells (Yazar *et al.,* 2016).

2.1.6 Transmission

Geographically, these protozoa are widely distributed throughout the world, *T. tenax* is in the oral cavity and forms part of tartar and dental plaque because it feeds on food residues that remain in the mouth after chewing, it is common in people with poor oral hygiene (López and Beatriz, 2020).

Trichomonas Tenax is a parasite that lives in the dental biofilm and is most frequently detected in those who have poor dental hygiene and severe periodontitis. Saliva, droplet sprays, kissing, or the use of infected water supply or tableware are all (Mallat *et al.,* 2004).

It is disseminated by close contact with people who have *E. gingivalis* in their mouths and saliva. This suggests that deep kissing, drinking, and eating with cups or cutlery contaminated with saliva from persons who have trophozoites in their mouths are the main ways *E. gingivalis* is passed from person to person because toothbrushes are so widely used (Zaffino *et al.*,2019; Sharifi *et al.,*2020).

2.1.7 Epidemiology

In population research, especially in regions with poor access to care and developing countries, several authors have studied improvements and statistical analyses since 1992.The global prevalence of 37% was predicted to be of *E. gingivalis*. In terms of various nations, Portugal had an *E. gingivalis* infection prevalence of 3%, whereas Jordan had an infection prevalence of 87% (El-Dardiry and Shabaan, 2016).

In France, Feki *et al.* (1990) found that *T. tenax* distribution was 28% and *E. gingivalis* distribution was 50.7% (Feki and Molet, 1990).

According to research conducted in Lublin, Poland, *E. gingivalis* was present in individuals with some gum disease 81,4% and in patients without oral problems 62,5% (Hussian, 2017).

In another research conducted in south India, *E. gingivalis* was found in 88% of gingivitis sufferers, 76% of periodontitis patients, and just 4 % of healthy individuals (Ramamurthy *et al.,* 2018). In different research by Yazar *et al.,* in Kayseri-Turke, they discovered a high prevalence of *E. gingivalis* (34.3%) and *T. tenax* (2.9%) in those with periodontitis and gingivitis diseases (Yazar *et al.,* 2016).

In research done in Iran in 2015 among individuals with Down syndrome, 18.8% of oral *T. tenax* infections were detected in the individuals, compared to 3% in the control group (Atabank, 2015). As shown in a study conducted in Egypt in 2022, the prevalence of dental protozoa was considerably higher in patients with chronic conditions. *E. gingivalis* was found in 80 % of patients with diabetes, 76 % of patients with kidney disease, and 74 % of patients with liver disease, compared to 20 % in the regulated healthy subjects, as opposed to 16% in the control group, *T. tenax* was found in 70%, 62, and 64% of cases, respectively (Meabed and Henin, 2022). Regarding the epidemiology of these parasites in Iraq, in Basrah (south of Iraq) analyzed the saliva of 143 individuals with poor oral care and *T. tenax* was found in 8.4%. However, additional research revealed that saliva was an unsuitable medium for the detection of parasites (Kikuta *et al.,* 1997).

In 2022 a study was conducted in the city of Kufa to assess the prevalence of *E. gingivalis* among patients with periodontitis and healthy individuals. It was found that 71% of patients of different ages suffered from periodontal disease infected with *E. gingivalis,* male

employees had a higher risk of amoebiasis than female patients, *E. gingivalis* was discovered in the control group as well (Salman, 2022).

Abbas *et al.* (2020) have conducted research in the city of Al-Muthanna to determine the prevalence of *E. gingivalis* and *T. tenax* in children with orthodontics devices fixed and mobile. The presence of *E. gingivalis* was found in 9.47 % of samples and 15.78 % of samples by the microscopical approach and PCR technique, respectively. *T. tenax* has not been discovered by either diagnostic test. Using the microscopical approach, the parasite percentage based on the orthodontic device treatment was comparable (9.58%) in the fixed and 9.09%) in the detachable, whereas by PCR technique it was (12.32%) in the fixed and 27.27%) in the detachable (Abbas *et al.,* 2020).

in Kirkuk City, the prevalence of *E. gingivalis* was found to be 16.6% and *T. tenax* to be 11% (Hamad *et al.,* 2012). Jaffer *et al.* (2019) in Duhok City reported a prevalence of *T. tenax* at 4.32% *T. tenax* was only found in the female group of 184 individuals, with all the males testing negative (Jaffer *et al.,* 2019).

2.1.8 Treatment

Metronidazole has been prescribed to treat infections for over a century and continues to be helpful in the therapy of amoebiasis, metronidazole was known as the most effective drug for human trichomoniasis, metronidazole is a cost-effective medication because of its low price, few adverse effects, and favorable pharmacokinetics and pharmacodynamic properties (Dighriri *et al.,* 2021).

Nonsurgical periodontal therapy may lower the amount of *E. gingivalis* in the aqueous cavity of people with chronic periodontitis, metronidazole kills *E. gingivalis in vitro* at a dosage of 4 mg/L.

Eloufir and his colleagues *in vivo* clinical trial found that oral metronidazole, 750 mg daily for 7 days, reduced *E. gingivalis* in periodontal disease from 64% to 26% (Eloufir *et al.,* 2014).

Metronidazole has been proven to effectively combat anaerobic bacteria and parasites at a molecular level (Löfmark *et al.,* 2010). Hussain and his colleagues showed that 85% of *E. gingivalis* were killed with metronidazole (Hussain *et al.,* 2017). Research has shown that metronidazole is effective in eliminating oral parasites, a group of researchers discovered in 2019 that using undiluted metronidazole resulted in a 100% elimination of *E. gingivalis* after 1 minute, 94% after 2 minutes, and 96% after 3 minutes, meanwhile, the death rate to *T. tenax* was 85% after 1 minute, 70% after 2 minutes, and 80% after 3 minutes (Moroz *et al.,* 2019).

2.2 Orthodontic

Orthodontics has been of considerable benefit to humankind. Orthodontics has been practiced since the early 1800s. Orthodontics is the science of preventing and correcting misaligned teeth in the jaws, as well as the right alignment of the jaws in the face (dentofacial orthopedics). The major reasons for seeking orthodontic treatment are aesthetics and function. Metal appliances have been used to shift teeth since the early twentieth century. Attachments to the tooth (brackets) and wires engaged within a specified slot contained within the brackets comprised these. This clinical arrangement is also known as fixed orthodontic appliances (Mewman,1964; Redlich and Tenne, 2013).

The metal bands were glued to the teeth. This arrangement enables the orthodontist to manage tooth movement during therapy. Orthodontists occasionally employed gold, platinum, silver, steel, gum rubber, vulcanite, zinc, copper, and brass in the early 1900s. Stainless steel (SS) was introduced in orthodontics in the 1950s and quickly became a preferred material for producing brackets and archwires. For the first time in orthodontics, nickel-titanium (Ni-Ti) archwires were utilized in the early 1970s (Andreasen *et al.,* 1974; Redlich and Tenne, 2013).

Stainless steel brackets and archeries constructed of SS and Ni-Ti metal alloys with varied sizes and forms (round and rectangular) are now used by orthodontists, wires constructed of cobalt-chromium-nickel and -titanium is also available for orthodontic application, to begin orthodontic tooth movement, force must be given to the tooth, this force is applied by archwires inserted into bracket slots, springs, and elastics connected to brackets, or extra-oral devices such as headgear, aside from the metallurgical element of orthodontics, tooth movement is

dependent on biological changes happening inside the tissues enclosing the teeth, primarily the periodontal ligament and the alveolar bone. Without the metal fixed appliance setup, a force magnitude of 40-60 g placed directly on the tooth for a suitable continuous duration is sufficient to move the tooth in the jaw. However, employing fixed appliances to apply orthodontic force necessitates a large increase in force level to overcome the friction caused by the bracket-wire contact (Redlich and Tenne, 2013).

2.2.1 Hazard of Orthodontic Appliances

2.2.1.1 Gum Disease

Gum disease, gum regression, and a wide periodontal embracing can all be made worse by orthodontic therapy. It's well-recognized that orthodontic devices can make it harder to keep plaque under control, which can cause gingivitis (Naranjo, 2006).

Individuals with active periodontitis, and gingivitis during orthodontic treatment may cause a periodontal collapse (Boyd *et al.,* 1989; Wishney, 2017). and where the alveolar adopts a variation, a thin layer of bacteria covers it (Zachrisson, 2008).

Patients who have received orthodontics are more likely to suffer gingival recession than people who have not, according to several previous studies (Renkema *et al.,* 2013). Even if this result is not typical (Gomes *et al.,* 2007) In this regard, a comprehensive assessment of the literature indicated that the orthodontic treatment causes the periodontal to experience 0.03 mm of regression, 0.23 mm of higher deep periodontal, and 0.13 mm of alveolar bone (Bollen *et al.,* 2008).

Although mean bone loss on primary teeth was 0.54 mm, almost one-third of patients had bone loss surpassing 2 mm, and this was significantly connected with age, according to a radiological follow-up study of 343 adult orthodontic patients. By relocating teeth's roots outside of their alveolar housing and weakening the connected gingiva, orthodontic treatment might jeopardize the integrity of the periodontal tissues (Wennstrom, 1996; Wishney, 2017).

2.2.1.2 Black Triangles

The aesthetic zone is when the interproximal tissue equivalent is removed. Although they may be caused by periodontitis, they frequently have a different etiology than gingiva. Age, tooth morphology, proximal contact length, proximal bone height, and interproximal gingival thickness have all been connected to the existence of the papilla (Chow *et al.,* 2010). According to Tarnow and his colleague's research, open gingival embrasures are more prone to develop when there is more than 5 mm of space between both the alveolar bone and the tooth contact site (Tarnow *et al.,* 1992; Wishney, 2017). Hence, orthodontic tooth movement may cause an open gingival embrasure by diverging roots (Kurth and Kokich, 2001). Other risk factors include triangular crown morphology, and teeth being in a pretreatment Therefore, diverging roots from orthodontic tooth movement may result in a black triangle position where the papilla has not completely formed, and the embrasure morphology itself (Sharma and Park, 2010). In addition to being unsightly, exposed embrasures encourage food impaction. Crown reshaping, bracket repositioning, and restorative procedures are all examples of management techniques.

2.2.1.3 Ache

The possibility of aches exists at every step of orthodontic therapy. Starting orthodontics might be hindered by a patient's fear of discomfort (Oliver and Knapman, 1985; Wishney, 2017). During treatment, the pain has been shown to lessen patient compliance (Sergl *et al.,* 2000). It has furthermore been mentioned as a typical excuse for ending prematurely (Brown and Moerenhout, 1991; Wishney, 2017). In general, two forms of ache frequently occur during orthodontics: mucosal pain from an injury to the oral mucous membranes caused by the appliance and periodontium ache from the use of orthodontic pressures on the teeth.

2.2.1.4 Mouth Mucosa

Most patients undergoing treatment with fixed appliances experience oral mucosal pain at some point and for some people, this can rate as the most annoying part of treatment (Kvam *et al.,* 1987; Wishney, 2017). However, this topic has not been well studied. Baricevic et al. reported that orthodontic brackets tended to cause mucosal erosions and desquamations whereas arch wires caused ulcerations (Baricevic *et al.,* 2011). Unsurprisingly, the pattern of mucosal ulceration reflects the location of the appliance: lingual appliances tend to ulcerate the tongue whilst buccal appliances tend to ulcerate the cheeks (Caniklioglu and Ozturk, 2005). The location of the ulceration greatly impacts morbidity; the constant activity of the tongue makes lingual ulcerations more debilitating than buccal ones (Shalish *et al.,* 2012).

Mucosal irritation and discomfort can also arise during treatment with clear aligners (e.g., Invisalign) although this does not seem to rate as a significant concern to patients (Fujiyama *et al.,* 2014).

2.2.1.5 Enamel Calcification

Lead encouraging plaque development and inhibiting dental care, orthodontic equipment leads to an increased risk of caries (Chatterjee and Kleinberg, 1979). One of the most typical side effects of orthodontic therapy is white spot plaques (WSP) (Heymann and Grauer, 2013). The fourth-most referenced article in orthodontic research focused on the prevalence of WSP in orthodontic patients (Hui *et al.,* 2013). Due to the minimal danger of development with portable items, most studies on WSP focused on permanent devices (Alexander, 1993). To properly visualize WSP when orthodontics, it is essential to extract plaque and fully dry the enamel. According to the research, the prevalence of WSP in orthodontics patients varied between 2-97% (Heymann and Grauer, 2013). Teenagers (10-19) years are more susceptible to poor dental hygiene practices and a lack of enamel development (Haydar *et al.,*1996). Some data suggests that male orthodontic individuals are more susceptible to WSP than female ones (Al Maaitah *et al.,* 2011). The production of WSP is prevented by saliva. As a result, the top jaw is often more prone to WSP than the bottom jaw teeth (Ogaard, 1989).

2.2.1.6 Speech Problems

Orthodontic appliances may affect speech directly by impeding the articulation of sounds or indirectly by affecting the physical and mental health of a person. One study involving bonded palatal expanders found no relationship between patient age and the time for speech adaptation. The impact of Invisalign, labial, and lingual appliances on quality of life is also well-recognized (Wishney, 2017).

2.2.1.7 Tooth Devitalization

The dental pith may alter because of orthodontic pressure. Numerous research has suggested that the pith is irritated and altered histopathological because of orthodontic pressure (von Böhl *et al.,* 2012). Modern research into the impact of orthodontics pressure on the pith concluded that more research is needed in this domain. strangling the pith might result in soft tissue apoptosis (Butcher and Taylor, 1952). While soft tissue ischemia and calcifications can be brought on by orthodontic pressure, current research shows that the pith is extraordinarily robust even in the face of strong forces. A very uncommon occurrence is orthodontic therapy (Han *et al.,* 2013). While receiving orthodontics, mouths with a history of trauma may have an insufficient flow of blood, which increases their risk of rotting (Bauss *et al.,* 2010). Moving the root tips out of the periodontal tissues may also result in the loss of the vasculature (Proffit *et al.,* 2013).

2.3 Hydrogels

Hydrogels are polymeric networks, are hydrophilic, and have substantially enlarged pores that can hold a significant amount of water (Wang, 2018). Due to their great biocompatibility with live cells, such biological substances have been used in bioengineering (Vermonden and Klumperman, 2015). Hydrogels act like living tissues because of their very high porosity and plusher surface. These hydrogels qualify as "biomaterials" due to their responsiveness to stimuli including temperature, pressure, *p*H, and magnetoelectric charges. Such materials have a high capacity to release and absorb protons in response to minute *p*H changes in the swelling medium (Liu *et al.,* 2015).

The hydrogels' unique properties make them dependable for use as regulated delivery systems for the distribution of medicines, proteins, and peptides to the intended places, a very wide variety of structural pieces, including protein, peptide, and polymer (which are often categorized as either organic or artificial), are used to create hydrogels, depending on their desired design (Wu *et al.,* 2019).

Varying chemicals, arrangement of the basic components, and types of connections (bend) results in a variety of macro and nano shapes, dimensions, and structural characteristics roles these factors decide which pharmaceuticals are enclosed (for example, tiny medications (Mayr *et al.,* 2018), and cells (Wang *et al.,* 2018) or proteins (Foster *et al.,* 2017)) and the method of delivery. Hydrogels can be classified as "bulk"/macro gels (ideal for the transepithelial approach, implantation, or infusion into the body) or microgels (Agrawal and Agrawal, 2018) (Size μm in diameter, ideal for lung and intrabody administration (Li

and Mooney, 2016)) as well as nano gels (10-100 nm) (Hajebi *et al.,* 2019), Direct interaction between both the gel network and the medication, if covalently bound toward the polymeric as well as reacting via mechanical links, also influences release on a lower length scale (hydrophobic, van der Waals, electrostatic, etc.). The capability to inject hydrogels is a very appealing characteristic (Thambi *et al.,* 2017). **Figure (2-5)**

Conventional medication administration entails ingesting an active chemical repeatedly to sustain therapeutic levels in the body; this is harmful to adherence to treatment and effectiveness and can result in adverse impacts at excessive dosages. Drug carrier studies have concentrated on attaining regulated and targeted drug release using nanomaterial structures such as liposomes, nanoparticles, membranes, and hydrogels (Asai et al., 2017).

Hydrogels are primarily composed of water, and they provide an ecosystem system like the natural body tissue, tunable mechanical properties compatible with soft tissues, and the ability to encapsulate drugs, slow or prevent their degradation, aggregate, and extend their lifespan while providing sustained diffusion-controlled release. Several hydrogels have an aqueous locus of solubility and are thus particularly appealing to biopharma, a quickly evolving category of licensed novel medications that includes synthetic protein molecules (Asai *et al.,* 2017) as well as DNA or RNA and targeted therapy (Leach *et al.,* 2018).

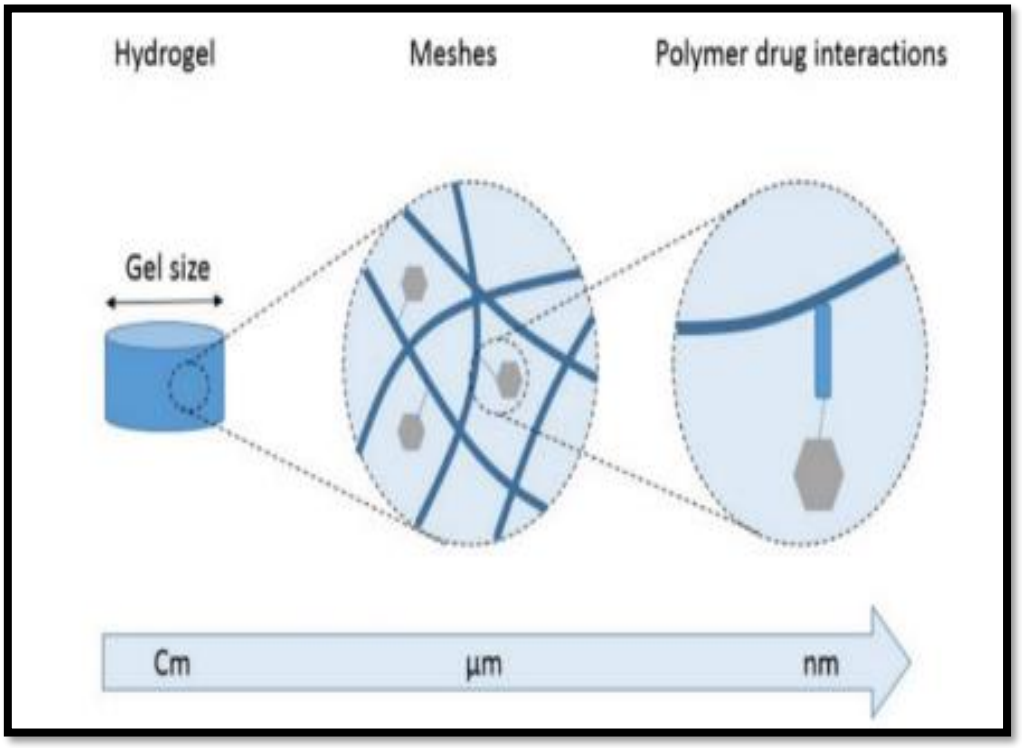

Figure (2-5): Hydrogels' dynamical characteristics (Chamkouri and Chamkouri, 2021).

2.3.1 Hydrogel Fabrication

With high flexibility, changeable binding sites, low cytotoxicity, and such qualities, polymers are carbohydrate materials frequently employed to create physical/chemical hydrogels (Mathew *et al.,* 2018). By choosing the kind of monomer or polymer and the hydrogel formation processes, hydrogels may be created specifically for a certain purpose. Two processes—chemical linkage and physical linkage—are used to create hydrogels (Wang and Han, 2017).

2.3.1.1 Chemical linkage

One kind of hydrogel which could be changed from a liquid to a solid by covalent bonding is a chemical cross-linked hydrogel. Additionally, *in situ*, hydrogel systems employ this technique. This approach uses a variety of processes to create hydrogels, including laser polymers, enzyme processes, and click events. The techniques for creating these hydrogels will be addressed as follows (Jundika *et al.,* 2012). Since of their high stability, chemically crosslinked hydrogels could be given consideration (Akhtar *et al.,* 2016).

2.3.1.2 Physical linkage

Hydrogels created by physical attachment may be produced by altering intra-molecular strengths such as hydrogen bonding, hydrophobic interaction, and electrostatic ionic strength. This approach enables the manufacture of hydrogel using straightforward & secure procedures, preventing a potential rise inside the crosslinker's cytotoxicity in the sol-gel process. Ionic temperature-dependent, and

pH-dependent methods are examples of physiological bridge techniques (Guaresti *et al.,* 2018).

2.3.2 Hydrogel Applications

The unique physical features of hydrogels have created a particular interest in using them in pharmaceutical delivery applications. The hydrogel porosity can easily be adjusted by regulating the density of cross-links in the gel matrix and the tendency of the hydrogels to swell in the aqueous medium. The presence of porosity in hydrogels also allows the loading of drugs into the gel matrix and subsequently provides the release of the drug at a rate dependent on the diffusion coefficient of a variety of molecules, either small or macromolecular through the gel network.

Originally, the advantages of hydrogels for drug delivery systems may be caused by specific pharmacokinetics that retain a high local concentration of drug in the surrounding tissues over a long period, however, they can also be used for systemic release. Hydrogels are commonly extremely biocompatible, as shown in their successful usage in Periton and further sites *in vivo*. The biocompatibility of hydrogels increases with the higher water content of hydrogels and the physicochemical similarity of the hydrogels to the intercellular matrix, both in composition (especially for hydrogels based on carbohydrates) and in mechanical terms.

Biodegradation or dissolution of the hydrogel may be designed through enzymatic, hydrolytic pathways or environmental factors (e.g., pH effect or electric field strength); although, depending on the period and location of the delivery, degradation is not always favorable. Hydrogels are also partly deformable and can adapt to the shape of the surface which is required. In the further field, the much- or bio-adhesive attributes of some hydrogels can be useful in inactivating them at the

site of usage or in using them on surfaces that are not horizontal (Desai and Harrison, 2010).

2.3.3 Method for Applying Medication to Hydrogels.

There are two ways to fill hydrogels with drugs:

The first technique involves adding the drug, an initiator, a cross-linker, or none, to the hydrogel monomers and allowing the mixture to polymerize, locking the drugs inside the polymer. The technique involves allowing a prepared hydrogel to stabilize in a drug carrier mixture. The gadget is made once the hydrogel substance has dried (Altinişik, 2011).

The second technique has certain benefits over the first since polymerization processes may negatively affect drug characteristics and because it is sometimes difficult to purify devices after loading and polymerization. Traditional Flory-Huggin's swelling theory may be used to predict swelling levels, which depend on the bonding strength of the polymer network, the polymerization circumstances, and the polymer-solvent interaction parameter. The amount of swelling may also be affected by the drug's presence in the solvent (Altinişik, 2011).

2.3.4 The Way that Hydrogels Discharge Their Contents.

2.3.4.1 Diffusion

The most prevalent mechanism of drug release from hydrogels is diffusion release. Fickian diffusion theory is employed for dynamical modeling in this form of drug release (Lin and Anseth, 2009). Medication spread through porosity hydrogels with pore diameters bigger than drug molecule dimensions may be linked to hydrogel porous or texture, diffusion-release hydrogels have the potential to operate as reservoirs or matrices because drugs in reservoirs' drug delivery systems are enclosed and surrounded by polymeric hydrogels, drug release generally follows the first rule of Fickian diffusion (Lin and Metters, 2006). Drugs are homogeneously disseminated within polymer hydrogels in matrices drug carriers because the release of drugs is primarily governed by the second law of Fickian diffusion.

2.3.4.2 Swelling

Once the prevalence of drug diffusion is higher than the rate of hydrogel swelling, the bulging release of the drug may happen. The zero-order theory of releasing may provide the best match for medication administration that is solely regulated by edema (Lin and Anseth, 2009). When hydrogels expand, they can change more quickly from a crystalline to an elastic range (at the melting point), which speeds up drug diffusion and release from polymeric chains. The rate of hydrogel swelling in swelling-controlled delivery systems is inversely correlated with the increase in drug delivery; therefore, the speed and capacity of hydrogel moisture content as well as the thicknesses of polymeric gels are crucial variables (Siepmann and Peppas, 2012).

2.3.4.3 Chemically

Chemically speaking, the drug release mechanism shows how reactions happen inside the gel network. The drug loaded in these processes is caused by the enzymatic or hydrolytic breakdown of the polymer structure. The trapped or tether medication will be delivered by hydrogels as just a result of the breakage of the polymer network by mass or outer layer degradation in chemically controlled delivery methods (Lin and Anseth, 2009). The chemical delivery method's percentage phase is polymerization breakage, and in this system, drug molecular diffusion has no discernible impact on medicine release **(Figure 2-6)**.

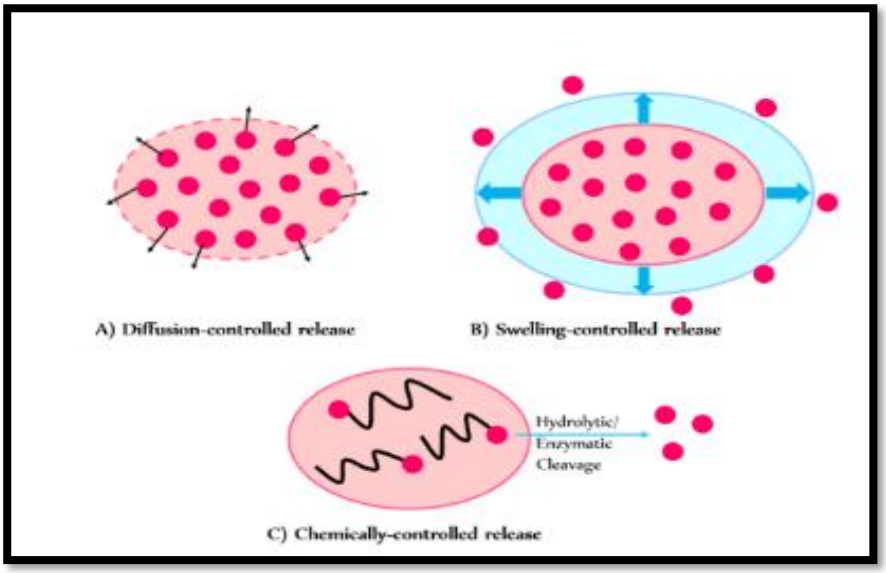

Figure (2-6): Schematics show how hydrogels discharge their contents (Ghasemiyeh and Mohammadi-Samani, 2019).

Chapter Three

Materials

&

Methods

3. Materials and Methods:

3.1 Materials:

3.1.1 Stock

Table (3-1): The equipment that was used in the current research experiments.

Equipment	Company	Country
Autoclave	Hirayama	Japan
Biosafety Cabinet	Human Lab	Korea
Camera	Nikon	Japan
Distillation	Lab Teach	Korea
Electrophoresis	Bioneer	Korea
Exispin vortex centrifuge	Bioneer	Korea
High-speed Cold Centrifuge	Eppendorf	Germany
Incubator	Binder	USA
Labconco	Kansas	USA
Magnetic stirrer	Heidolph	Germany
Micropipettes (different volumes)	Eppendorf	Germany
Microscope	Olympus	Japan
Microwave	Shownic	China
Nanodrop	Thermo Scientific	UK
pH meter	HANNA	Romania
Refrigerator	Concord	Lebanon
Sensitive balance	Sartorius	Germany
SEM	FEI	

T100 Thermal Cycler PCR	Bio-Rad	USA
U.V transilluminator	Wised	Korea
Vortex	CYAN	Belgium
Water bath	Memmert	Germany

3.1.2 Tools

Table (3-2): The tools that were used in the experiments.

Tools	Company	Country
Cover glass	TRUST LAB	China
Disposable tubes	Beijing Hanbaihan Medical Co.	China
Eppendorf tubes	Biobasic	Canada
Gloves	Mumu	Malaysia
Micro Pipette tips	Bio Tek	USA
Rack	TRUST LAB	China
slides	TRUST LAB	China
Standard Wire loop(1m)	Hi- Media	India
Swab	TRUST LAB	China

3.1.3 Chemical Materials

Table (3-3): The chemical materials that were used in the experiments.

Chemical Materials	Company	Country
10X TBE buffer	iNtRON	Korea
Absolute Ethanol	BDH	England
Agarose	iNtRON	Korea
Ethanol 75%	Aljoud	Iraq
Ethidium Bromide	BioBasic	Canada
free Nuclease water	Biolab	UK
Giemsa stain	Aspenbio	China
immersion oil	Alhannof factory for medical &lab supplies	Jordan
Marker or DNA ladder (100bp)	iNtRON	Korea
Metronidazole (pure)	MERCK	Australia
Normal saline 0.9%	Pioneer	Iraq
Phosphate Buffer Saline (P B S)		
TYGM-9 Medium	ATCC	USA

3.1.4 Kits

Table (3-4): The type of kits used in the Experiment.

kit	Company	Country
CO_2 Gen™	Thermo Scientific™ CD0025A	England
gSYNCTM DNA Extraction	Genaid	Taiwan
Taq G2® green master mix	Promega	USA

3.1.5 The PCR primers

The Nested PCR primers for the detection of *E. gingivalis* and *T. tenax* based on the *18S ribosomal RNA* gene were designed in this study using NCBI-Genbank sequence and primer 3 plus design. These primers were provided by ScientificResercher. Co. Ltd, Iraq as the following table:

Table (3-5): The nested PCR primer for *E. gingivalis* with their sequence and product size:

Primers	Sequence 5'-3'		Product size	GenBank Reference code
Inner Nested PCR primer	F	GGGTTTGACATCGGAGAAGGA	521bp	KX061779.1
	R	TGCTTTCGCTCTCGTTATCT		
Outer Nested PCR primer	F	ACAGAAAGAGGTAGTGACGACA	338bp	KX061779.1
	R	TCAAATCTCCTTCTTATTGTCCCA		

Table (3-6): The nested PCR primer for *T. tenax* with their sequence and product size.

Primers	Sequence 5'-3'		Product size	GenBank Reference code
Inner Nested PCR primer	F	CCTAGCAGAGGGCCAGTCTA	793bp	JX943582.1
	R	TCTAAAGGGCATCACGGACC		
Outer Nested PCR primer	F	AACGCCCGTAGTCTGAATTG	467bp	JX943582.1
	R	CCTTCAAGTTTCAGCCTTGC		

3.1.6 Hydrogel Preparation:

Table (3-7): Synthesis of gelatin-HPA conjugate materials.

Chemical material	Weight/ Concentration
HPA	3.32g
NHS	3.2g
EDC	3.82g
HCl	10M
NaOH	1M, 5M, 10M
Gelatin	10g
MilliQ	150M

Table (3-8): Synthesis of CMC-Try conjugate materials.

Recipe	0.5-unit C.D.	0.25-unit C.D.	1 unit C.D.	2-unit C.D.
CMC	5g	5g	5g	5g
TYR	0.8648g	0.4324g	1.7296g	3.4592g
NHS	0.5732g	0.2866g	1.1464g	2.2926g
EDC	0.9547g	0.47735g	1.9094g	3.8187g

3.1.7 Drug Release Experiment:

1. Metronidazole, MERCK/USA (Analytical standard)
2. GTN-HPA/CMC-TYR hydrogel
3. Sterile H_2O_2 (stock = 30% w/w)
4. Sterile Horseradish Peroxidase (HRP) (stock = 25 unit/ml)
5. Sterile PBS, pH 7.4

3.1.6 Design of experiment

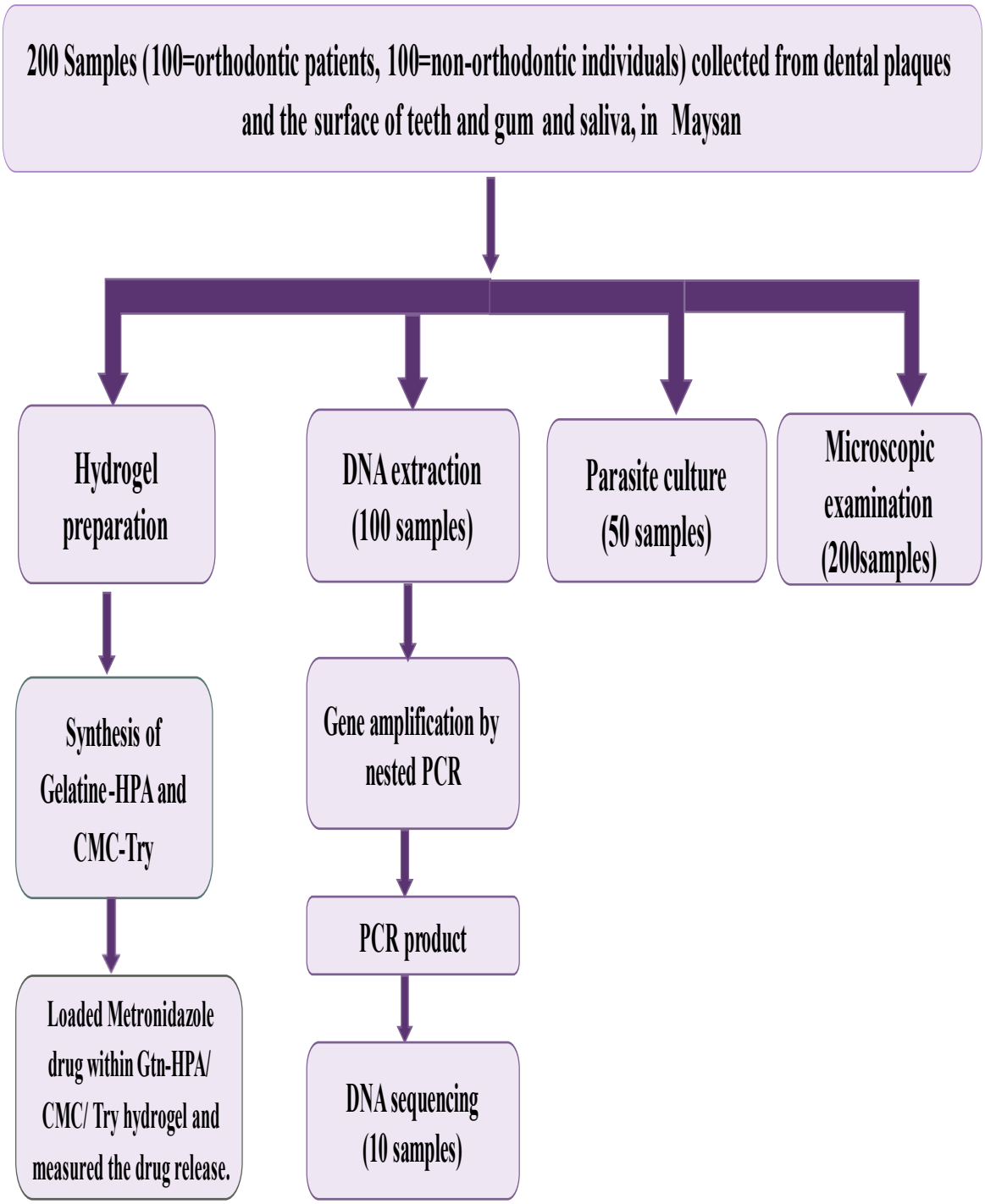

Figure (3-1): Main steps of the current research.

3.2 Methods

3.2.1 Samples Collection and Preparation

In this study, 200 people participated, split into two groups 100 individuals received orthodontic treatment (with fixed and removable), while 100 people did not receive orthodontic therapy. In contrast with the control group, which included 25 males and 75 females aged between 11 and 50 years old, the orthodontic treatment group had 24 males and 76 females.

According to Misan University's ethical guidelines, the study procedure was accepted. Swab samples were taken from the participants at the Orthodontic Industry Center in Maysan, Amarah South of Iraq 31.833ºN 47.15ºE between October 2022, and May 2023. The subjects' verbal consent was taken before sample collection.

The orthodontic group determined that the patient met the diagnostic criteria for a jaw deformity, the subjects were all undergoing either fully fixed orthodontic appliances or removable appliances. The orthodontic group fitted the patient with braces with straight archwires and positioned on the outer surface of the teeth, emphasizing the importance of maintaining good oral hygiene. Swab samples were collected from plaque, saliva, tooth surface, and tonsil crypts using sterile cotton swabs (**Appendix 1**).

All the participants were given a questionnaire to complete, the key criteria in the questionnaire were age, gender, dental health, smoking, tooth type, tooth decay, gum inflammation or tooth loss, and braces (fixed or removable) for the orthodontic patients group.

For the current study, participants with conditions such as salivary gland and duct diseases, oral mucosal disease, systemic diseases, and those who had taken antibiotics or were following a special diet within the last three months were not included.

Additionally, the control group did not have these conditions and had not undergone orthodontic treatment. The orthodontic and control group participants collected swab samples in the morning. Participants were instructed to fast for at least two hours before their samples were collected.

3.2.2 Microscopic Examination

Each specimen was divided into three parts: one was used to prepare wet mounts, the second was used to prepare a smear for Giemsa staining, and the third was used for culture.

3.2.2.1 Wet Mount Examination

Using a sterile Pasteur pipette, a drop of diluted specimen was placed on clean microscopic slides (25.4x76.2 mm) a coverslip (24x50 mm) was applied on top, and the material was spread by applying pressure to the coverslip. This process created a thin film which was then examined immediately with a light microscope at 100X and 400X magnifications. *E. gingivalis* and *T. tenax* were identified based on their shapes, including the presence of pseudopodia formation and sluggish movement (Ibrahim and Abbas, 2012).

3.2.2.2 Giemsa Staining

1. Thick smears were prepared on a clean microscopic slide and allowed to dry (wet swab).
2. The samples were fixed with methyl alcohol for 5 minutes.
3. The samples were then stained with commercially available Giemsa stain for 40 minutes.
4. Then wash with tap water.
5. Swabs were then examined directly under a light microscope (Olympus, Japan) at 100x, 400x, and 1000x magnifications, depending on the morphological characteristics of the vegetative stage (Garcia *et al.,* 2016).

The characteristics that set *E. gingivalis* apart included its size of 10 to 20 µ, a single nucleus with a tiny central Kary some, noticeable lamellipodia, and slow motility. *T. tenax* was recognized as a flagellated trophozoite with an ovate body and distinctive motility, measuring 5 to 16µ (Meabed and Henin, 2022).

3.2.3 parasite Culture

The oral swabs, after collection from the study participants, were transferred to the parasitology laboratory for culturing on TYGM-9 (ATCC Medium 1171) medium to culture *E. gingivalis* and *T. tenax* (Benabdelkader *et al.,* 2019; Bao *et al.,* 2021) under anaerobic conditions using the CO^2 Gen™ kit (Thermo Scientific™ CD0025A, England) and incubated them at 37˚C for 5 days. To confirm the growth of parasites, the samples were examined directly by light microscopy in 400x and 1000x magnifications.

3.2.4 Nested PCR

The Nested PCR technique was performed for the detection of *E. gingivalis* and *T. tenax* based on the *18S ribosomal RNA* gene from the surface of gum and teeth samples. This method was carried out according to the following steps:

3.2.4.1 DNA Extraction

Genomic DNA was extracted directly from oral samples or culture by using the gSYNCTM DNA Extraction Kit (Geneaid. Taiwan) done according to company instructions as following steps:

1. A 200µl sample was transferred to a sterile 1.5ml microcentrifuge tube and then added 20µl of proteinase K and GST lysis buffer then, mixed by the vortex. And incubated at 60℃ for 15 minutes.
2. After that, 200µl of GSB cell binding buffer was added to each tube and mixed by vortex vigorously, and then all tubes were incubated at 60℃ for 15 minutes and inverted every 3 minutes through incubation periods.
3. 200µl absolute ethanol was added to the lysate and immediately mixed by shaking vigorously.
4. The DNA filter column was placed in a 2 ml collection tube and all the mixtures (including any precipitate) to the column. Then centrifuged at 10000rpm for 5 minutes. The 2 ml collection tube containing the flow-through was discarded and placed the column in a new 2 ml collection tube.

5. 400µl W1 buffer was added to the DNA filter column, then centrifuged at 10000rpm for 30 seconds. The flow-through was discarded and the column was back in the 2 ml collection tube.
6. 600µl Wash Buffer (ethanol) was added to each column. Then centrifuged at 10000rpm for 30 seconds. The flowthrough was discarded, and the column was back in the 2 ml collection tube.
7. All the tubes were centrifuged again for 3 minutes at 10000 rpm to dry the column matrix.
8. The dried DNA filter column was transferred to a clean 1.5 ml microcentrifuge tube and 50 µl of preheated elution buffer was added to the canter of the column matrix.
9. The tubes were left to stand for at least 5 minutes to ensure the elution buffer was absorbed by the matrix. Then centrifuged at 10000 rpm for 30 seconds to elute the purified DNA.

3.2.4.2 Genomic DNA Estimation

The extracted genomic DNA was checked by using a Nanodrop spectrophotometer (THERMO. USA), which checks and measures the purity of DNA by reading the absorbance at (260 /280 nm) (Scientific, 2009).

3.2.4.3 Nested PCR master mix preparation

The nPCR master mix was prepared using (**Green PCR Master Kit**) and this master mix was conducted according to the company's instructions as shown in the following tables.

Table (3-9): First round PCR mixture reaction.

PCR-Mixture reaction	Volume
DNA template 5-50ng	5µL
PCR *18S ribosomal RNA* Forward primer (20*p*mol)	2µL
PCR *18S ribosomal RNA* Reverse primer (20*p*mol)	2µL
PCR master mix	12.5µL
Nuclease free water	3.5 µL
Total volume	25µL

The PCR master mix components that mentioned above in **Table (3-9)** were transferred into the Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in a PCR Thermocycler.

Table (3-10): Second round Nested PCR Mixture reaction.

PCR Mixture reaction	Volume
First-round PCR product	3µL
Nested PCR outer Forward primer (20*p*mol) for	2µL
Nested PCR outer Reverse primer (20*p*mol)	2µL
GoTaq Green PCR Master Mix	12.5µL
Free nuclease water	7.5 µL
Total volume	25µL

The PCR master mix components mentioned above in **Table (3-10**) were transferred into the Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in a PCR Thermocycler.

3.2.4.4 PCR Thermocycler Conditions

PCR thermocycler conditions were completed using a conventual PCR thermocycler system as presented in the following **Table (3-11)**:

Table (3-11): First Round PCR Thermocycler Condition for *E. gingivalis* and *T. tenax.*

PCR steps	*E.gingivalis*		Cycles	*T.tenax*		Cycles
	Temperature ºC	Time		Temperature ºC	Time	
Initial denaturation	95º	5min.	1	95	5min.	1
Denaturation	95	30sec.	35 cycles	95	30sec.	35 cycles
Annealing	60	30sec		58	30sec	
Extension	72	2min.		72	2min.	
Final extension	72	1min.	1	72	5min.	1
Hold	4	Forever	-	4	Forever	-

Table (3-12): Second Round PCR Thermocycler Condition for *E. gingivalis* and *T. tenax*:

PCR steps	*E.gingivalis*		Cycles	*T.tenax*		Cycles
	Temperature ºC	Time		Temperature ºC	Time	
Initial denaturation	95	5min.	1	95	5min.	1
Denaturation	95	30sec.	35 cycles	95	30sec.	35 cycles
Annealing	60	30sec		58	30sec	
Extension	72	2min.		72	1min.	
Final extension	72	1min.	1	72	5min.	1
Hold	4	Forever	-	4	Forever	-

3.2.4.5 Nested PCR Product Analysis

The Nested PCR products were analyzed by agarose gel electrophoresis following the below steps:

1- 1.5% Agarose gel was prepared using 1X TBE and dissolved in a water bath at 100 °C for 15 minutes, then left to cool at 50°C.

2- 3µl of ethidium bromide stain was added into an agarose gel solution.

3- The agarose gel solution was poured into the tray after fixing the comb in the proper position after that, left to solidify for 15 minutes at room temperature, and then the comb was removed gently from the tray.

4- The gel tray was fixed in the electrophoresis chamber and filled with 1X TBE buffer.

5- 10µl of the PCR product were added into each comb well and 3µl of (100bp Ladder) in the first well.

Then electric current was performed at 100 volts and 80 mA for 1 hour.

6- Nested PCR products were visualized using a UV Transilluminator.

3.2.4.6 DNA Sequence:

After confirming the amplification of the samples, we sent 20 microliters of the nested PCR product to Macrogen Company in South Korea to obtain the actual sequences of the nitrogenous bases for the necessary gene fragments**.**

Once we received the results, we checked the sequence's identity in the Gene Bank by using multiple alignment analyses constructed using (the ClustalW alignment tool. Online).

3.2.5 Hydrogel Preparation

3.2.5.1 Synthesis of Gelatine-hydroxyphenyl propionic acid:

Day 1: Mixing and Adjusting *p*H.

1. The *p*H meter was calibrated using a pH buffer.
2. hydroxyphenyl propionic acid (HPA) and N-hydroxy succinimide NHS were dissolved in 150 ml Milli-Q water and 100 ml dimethylformamide (DMF) in a 1000 ml beaker with a stir bar.
3. The *p*H probe was placed in the mixture.
4. ethyl carbodiimide hydrochloride (EDC) was added, so that the *p*H was 5 plus, the *p*H was immediately adjusted to 4.7 with 10M HCl.
5. The *p*H will start to drop, add NaOH continuously to maintain the *p*H of 4.7 Maintain *p*H closely for 5 hours after step (4). The *p*H should stabilize at *p*H 4.7 after 5 hours.
6. After 5 hours of step (4), the dissolved gelatine solution was added, then the *p*H was continued to be maintained at 4.7 for another hour.
7. The Gelatine-HPA solution was stirred overnight, covered with parafilm and the *p*H scale was removed.

Day 2: Harvest and Dialysis NaCl

8. After 24 h of reaction (counting from step 4), the *p*H was adjusted to 7.
9. The inside and outside of the dialysis membrane were rinsed with water, and one end of the dialysis tubing was double-clamped.

10. The gelatine-HPA solution was poured into either a MW1000/3500 dialysis membrane and double-clipped to seal the other end of the tubing.
11. Haemodialysis against sodium chloride solution (29 g NaCl + 5L Milli-Q water), the sodium chloride solution was changed twice a day.

Day 3 Dialysis NaCl

Dialysis was continued against the NaCl solution (29 g NaCl + 5L Milli-Q water) and the NaCl solution was changed twice a day.

Day 4 Dialysis 25% Ethanol.

Dialysis against 25% ethanol solution (1.25 L ethanol + 3.75 L Milli-Q water), the ethanol solution was changed twice a day.

Day 5 Dialysis 25% Ethanol

Dialysis against 25% ethanol solution (1.25 L ethanol + 3.75 L Milli-Q water), the ethanol solution was changed twice a day.

Day 6 Milli-Q Water

Dialysis against Milli-Q water (5 L Milli-Q water) water was changed twice a day.

Day 7 Milli-Q Water

Dialysis against Milli-Q water (5 L Milli-Q water) and water was changed twice a day.

Day 8 Freeze-Drying

Dialysis tubes were opened, and gelatin-HPA was poured into falcon tubes, frozen in a −21°C freezer overnight, and freeze-dried (Labconco, Kansas/ USA) for 3-4 days (Al-Abboodi, 2014).

3.2.5.2 Carboxymethyl Cellulose -Tyramine Synthesis Protocol:

1. The *p*H was calibrated with a standard solution (*p*H 4, 7, 9).
2. 250 ml of Milli-Q water was added to a 500 ml beaker and then placed in the beaker over the magnetic stirrer.
3. carboxymethyl cellulose sodium salt (CMC) was added slowly into the Milli-Q water and allowed CMC to dissolve by stirring continuously with a stir bar.
4. a pH probe was put inside the mixing solution.
5. Tyramine hydrochloride (TYR) was added to the dissolved CMC solution.
6. N-hydroxy succinimide (NHS) was Immediately added and N-ethyl-N'-3-dimethyl aminopropyl carbodiimide (EDC).
7. The *p*H of the solution was immediately adjusted to *p*H 4.7 by adding concentrated hydrochloric acid dropwise.
8. The *p*H was maintained at *p*H 4.7 for the next 5 h, by first adding the 10M NaOH solution dropwise, then adjusting the *p*H with the 1M NaOH solution once the *p*H was more stable.
9. The beaker was covered, and the resulting solution was left to stir overnight under ambient conditions.
10. The *p*H was slowly adjusted to *p*H 7 by adding 10M NaOH overnight (24 h after reaction) while stirring.
11. The contents of the vial were transferred to a dialysis bag (MWCO 1000-3500, Spectrapore), and both ends of the dialysis acupuncture were sealed with dialysis closure clips.

12. Dialysis was done with 5 L of 10 mM NaCl solution (29.22 g in 5 L H-Q) 4 times, ~12 hours each.
13. Dialysis was done with 5 L of 25% v/v ethanol solution (1.25 L in 3.75 L Water-Q) 4 times, ~12 h each.
14. Dialysis was done with 5 L of Milli-Q water 4-6 times, ~ 12 hours each.
15. After dialysis, the solution was transferred to 50 ml Falcon tubes, frozen at −80°C for 5 h, and freeze-dried at −60, 40 m Torr for 4 days.
16. CMC-Tyr 5mg was taken for NMR analysis (Al-Abboodi, 2014).

3.2.6 *In vitro* Metronidazole Drug Release from Gtn-HPA/CMC-Tyr Hydrogels:

In vitro, release testing of metronidazole drug loaded on Gtn-HPA/CMC-Tyr hydrogels are critical in assessing the drug's release profile from the hydrogel. This information is essential for understanding drug delivery kinetics, optimizing drug formulations, and ensuring the effectiveness of the drug delivery system. The release medium was a phosphate buffer solution (PBS) that simulates the physiological conditions. Purified metronidazole was purchased from MERCK, Australia (Analytical standard, MERCK) to be conjugated within the Gtn-HPA/CMC-Tyr hydrogels. The drug release experiment was started by loading Gtn-HPA/CMC-Tyr hydrogel with 25 µg/ml of metronidazole by mixing 500 µl of Gtn-HPA/CMC-Tyr at three different concentrations: 10, 15 and 20 % (w/v) (to gain soft gel, medium gel, and stiff gel) with 500 µl of drug solution to give a final concentration of metronidazole drug of 25µg/ml. Then 5 µl each of 15.5

unit/L of Horseradish peroxidase Enzyme (HRP) and 49.8 µM of Hydrogen peroxide (H_2O_2) were added and the mixture was vortexed gently before it was injected between two parallel glass plates clamped together with 1mm spacing (**Appendix 3**). The crosslinking reaction was allowed to proceed at 37ºC for 1 h. Round hydrogel disks with diameters of 1 cm were then cut out from the hydrogel slab using a circular mold. The hydrogel disks moved to a well plate and were immersed in 5 ml of PBS at pH 7.4 and 37ºC on an orbital shaker at 100 rpm. At selected time points, 2 mL was withdrawn from the release medium and replaced by 2 mL of fresh PBS solution which was added to the hydrogel disk to maintain sink conditions and incubated under the same conditions. The collected samples were stored in microcentrifuge tubes at -20 ºC, and the metronidazole drug concentrations released in the collected samples were determined using a UV-visible spectrophotometer, where metronidazole release was monitored by the wavelength at 320 nm. Finally, the cumulative percentage of drug release was plotted against time to create a release profile. This experiment was conducted in three independent triplicates. The drug release profile is illustrated in **Figure 3-2**.

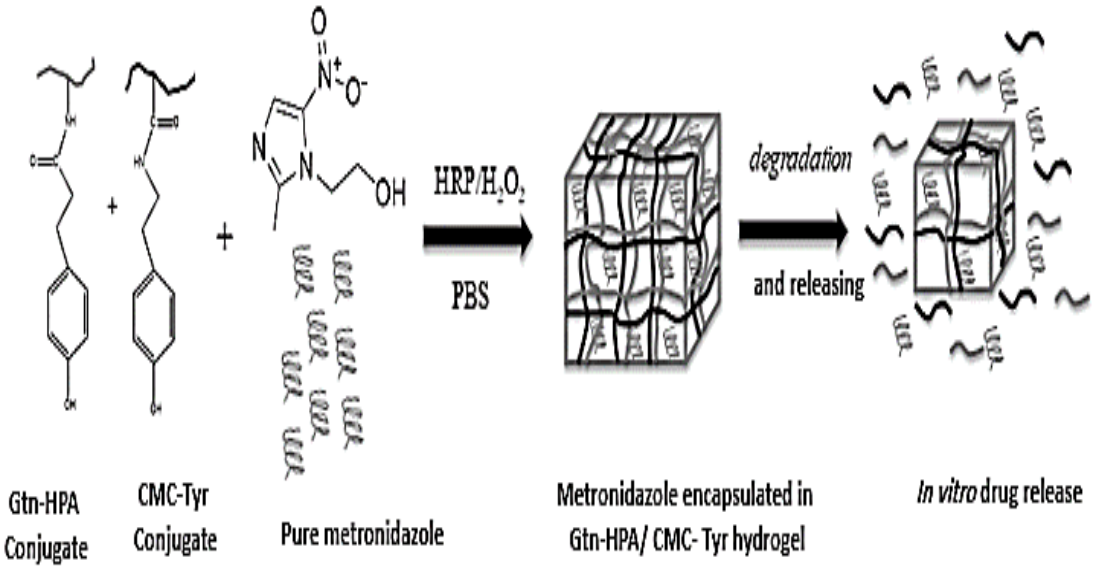

Figure (3-2): Drug release profile: The figure illustrates a metronidazole drug release profile encapsulated within the scaffold of the hydrogel drug delivery system. A hydrogel matrix is created by combining the Gln-HPA and CMC-Tyr conjugates; this matrix encapsulates the metronidazole within its porous structure. The hydrogel's crosslinking process begins with the addition of HRP/H2O2. To provide its therapeutic effect, metronidazole is released into the surrounding environment as the hydrogel degrades. This method exemplifies the promise of hydrogels as medication delivery vehicles, which could one day enable the targeted and regulated delivery of therapeutic drugs to oral parasites.

3.2.6 Statistical Analysis:

SPSS (version 21) software was used to conduct the data analysis for the current study. Additionally, the association among the variables (use of orthodontic, type of orthodontic, sex, age, gingivitis, dental calcification, dental decay, toothache, floss, mouthwash, brushing, smoking habits) and the prevalence of parasites were examined using the analysis Chi-square test (χ^2) and variance (ANOVA)\F and using $p \leq 0.01$ as criteria for probability.

Chapter Four

Results

&

Discussion

4: Results and Discussion:

4.1 The Wet Mount studies of oral parasites and Orthodontic Patients:

Table 4-1 shows the infection rate of *E. gingivalis* alone was 47.0%, compared to 25.0% among non-orthodontic participants. In comparison, the infection rate of *T. tenax* alone among orthodontics was 2.0% and 1.0% among non-orthodontics.

It was shown that the infection rate among orthodontic patients, with mixed infection with *E. gingivalis* and *T. tenax* was 19.0%, while the infection rate for non-orthodontic participants was 16.0%. there is a significant relationship between orthodontic treatment and infection, χ^2=13.67, p<0.01, (**Table 4-1**).

Table (4-1): The infection rates of *E. gingivalis* and *T. tenax* among orthodontic patients compared to non-orthodontic individuals.

Participant status	Result				Total NO. (%)	*p*-value
	E. gingivalis alone	*T. tenax* alone	*E. gingivalis* + *T. tenax*	Non-infection NO. (%)		
Non-orthodontic participants	25(25.0%)	1(1.0%)	16(16.0%)	58(58.0)	100(50.0)	0.001
Orthodontic patients	47(47.0%)	2(2.0%)	19(19.0%)	32(32.0)	100(50.0)	
Total	72(36.0%)	3(1.5%)	35(17.5%)	90(45.0)	200(100.0)	
χ^2=13.67, p<0.01						

Out of the 100 orthodontic treatments, it was found that 47.0% had *E. gingivalis*, this result agrees with Garcia *et al.,* (2018). This high infection rate may be attributed to the orthodontics devices' help of fixed appliances required for the correction of dental and skeletal discrepancies, despite its obvious advantages in improving aesthetics, occlusal balance, and function, orthodontic treatment carries some side effects that sometimes occur due to poor oral hygiene maintenance. In these patients, oral hygiene may be more difficult to maintain due to the presence of attachments like brackets, wires, and bands, which may lead to plaque accumulation and gingival inflammation (Bollen *et al.,* 2008; Selvaraj *et al.,* 2020). This may provide a good environment for the parasite to thrive.

Based on the findings of this study, it appears that using orthodontic appliances may contribute to an increased risk of oral parasites because of poor hygiene. It is crucial for patients who utilize orthodontic appliances to maintain their optimal dental hygiene. The findings in this study align with those of (Garcia *et al.,* 2018).

4.1.1 The Relationship Between the Infection of the Oral Parasites and Type of Orthodontic Applications:

The current results **(Table 4-2)** show there are two types of appliances used: fixed and mobile. The infection rate of *E. gingivail*s and *T. tenax* was 63.6% (56/88) of those with fixed appliances, while 100.0% (12/12) of those with mobile appliances. A significant relationship exists between the type of appliance used and parasite infection (χ^2=19.29, p<0.01).

Table (4-2): The infection rates of *E. gingivalis* and *T. tenax* among the orthodontics patients (fixed and removable).

Participant status	Infection		Total	*p*-value
	Positive	Negative		
Fixed	56(63.6%)	32(36.4%)	88(80.0%)	0.01
Removable	12(100.0%)	0(0.0%)	12(12.0%)	
Total	(68.0%)	32(32.0%)	100(100.0%)	
χ^2=19.29, *p*<0.01				

In the current study, it is shown that the percentages of infection rates with (*E. gingivalis* and *T. tenax*) depended on whether orthodontic appliances were used fixed or removable at 63.6% and 100.0%, respectively, these results are high than that found by Abbass *et al.,* (2020). The risk of developing gingivitis and creating an environment that is conducive to the growth of parasites (e.g., "a moist, nutrient-rich environment") refers to the conditions created by plaque retention due to the use of orthodontic appliances (Abbass *et al.,* 2020).

Statistical analysis indicated a significant relationship between infection and the type of orthodontic appliance used with a level of significance. Conversely, a study conducted by Abbass *et al*. (2020) did not find any significant relationship between infection and the type of orthodontic appliance used.

4.1.2 The Relationship Between the Infection of the Oral Parasites and the Period Orthodontic:

Figures (4-1) show Patients who were treated with orthodontics had varied infection rates associated with the duration of their treatment. For patients with orthodontic periods ranging from 1-6 months, the infection rate with *E. gingivalis* alone was 33.3% (12\36), while the infection rate with *T. tenax* alone was 0.0%. The infection rate with mixed (*E. gingivalis* and *T. tenax*) is 19.4% (7\36).

Patients with periods from the 7-12 months group had an infection rate of *E. gingivalis* alone at 58.3% (7\12), while the infection rate with *T. tenax* alone was 17.0% (2\12), and the infection rates with mixed (*E. gingivalis* and *T. tenax*) 25.0% (3\12).

Finally, patients with periods from 13-18 months group had an infection rate with *E. gingivalis* alone at 54.0% (28\52), while the infection rate with *T. tenax* alone was 0.0% (0\52), and the infection rates with mixed (*E. gingivalis* and *T. tenax*) 17.3% (9\52). There is a significant relationship between the period orthodontic and infection rates of oral parasites at $\chi^2=23.86$, $p<0.01$, **(Figure 4-1)**.

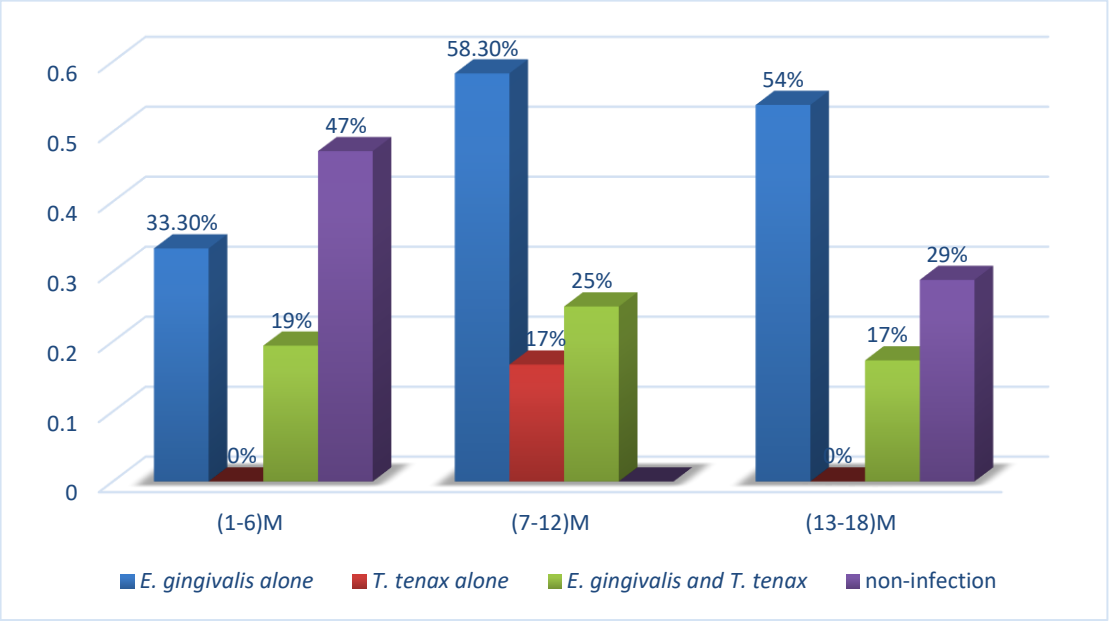

Figure (4-1): The infection rates of oral parasites according to orthodontic periods in patients treated with orthodontics.

Orthodontic appliances, which are prosthetic devices placed in the mouth, can have a significant impact on oral health by allowing plaque and food debris to accumulate, they can also increase the number of microorganisms such as parasites (Zheng *et al.,* 2016). This study finds an increase in infection rates over 1-6 months. The study suggests that the increased rates of microorganisms during the first six months of orthodontic appliance treatment may be due to extensive dental movements, which can exacerbate gingivitis, because of extensive dental motions that occurred during this period, which may enhance gingival disease (Bollen *et al.,* 2008).

fixed appliances might induce changes in the local environment that could be a transient advantage for these periodontal pathogens, after twelve months of orthodontic treatment the number of pathogenic microorganisms may return to a dynamic equilibrium this new equilibrium may favor organisms adapted to the altered environment,

possibly due to competitive pressures thus potentially reducing the rates of oral diseases (Bollen *et al.,* 2008) .

The current study shows an increase in infection rates of oral parasites in patients treated with orthodontics for 13-18 months, according to a study by Jing and his colleagues, patients undergoing orthodontic treatment for more than eighteen months experienced a significant increase in microorganisms (Jing *et al.,* 2019).

4.1.3 The Relationship Between the Infection of the Oral Parasites and the Sex of All Participants:

Table 3-4 shows that the non-orthodontic infection rate with alone *E. gingivalis* was 28.0% (7\25) for males compared with 24.0% (18\75) for females. On the other hand, the infection rate with alone *T. tenax* was 4.0% (1\25) in males, while 0.0% (0\75) in females and mixed *E. gingivalis* and *T. tenax* 14.7% (11\75) in females compared to 20.0% (5\25) in males.

Among orthodontic patients **(Table 4-3)**, it was found that the infection rates of *E. gingivalis* alone were 39.4% (39\76) in females compared to 37.5% (9\24) in males, while *T. tenax* alone showed no infections in females and only 8.3% (2\24) in males, while the infection rates with mixed *E. gingivalis* and *T. tenax* were 18.4% (14\76) in females and 20.8% (5\24) in males.

There is a significant relationship between parasite infection and sex in orthodontic groups $\chi^2=26.36$, $p<0.01$.

Table (4-3): The infection rates of *E. gingivalis* and *T. tenax* among males and females.

participant status	Sex	Results				*p*-value
		E. gingivalis alone	*T. tenax* alone	*E. gingivalis* and *T. tenax*	Total NO. (%)	
Non-orthodontic participant	Female	18(24.0%)	0(0.0%)	11(14.7%)	75(75.0)	0.02
	Male	7(28.0%)	1(4.0%)	5(20.0%)	25(25.0)	
	Total	25(25.0%)	1(1.0%)	16(16.0%)	100(50.0)	
Orthodontic patients	Female	30(39.4%)	0(0.0%)	14(18.4%)	76(76.0)	0.0001
	Male	9(37.5%)	2(8.3%)	5(20.8%)	24(24.0)	
	Total	39(39.0%)	2(2.0%)	19(19.0%)	100(50.0)	
Total		64(32.0%)	3(1.5%)	35(17.5%)	200(100.0)	
$\chi^2=26.36$, $p<0.01$						

The current study finds the infection rate with *E. gingivalis* in females 39.4%, which is consistent with the findings of another study by (Garcia *et al.,* 2018). The infection rates in the current original study among females at sexually active ages may be attributed to the fact that sexual activity has a significant effect on the immune system and fluctuations in sex hormones even during menstruation increase infection (Lorenz *et al.,* 2015). There is a study that found the relationship between sexual activity and cycle-related changes in two humoral immunity markers, mucosal (salivary) Immunoglobulin A and circulating (serum) Immunoglobulin G, in healthy women. During menstruation, the IgA

and IgG levels of sexually active and abstinent women were comparable. Additionally, sexually active women showed higher IgG but lower IgA levels at the time of ovulation as compared to sexually abstaining women. Higher sexual activity was linked to a greater ovulation-related fall in Ig, and the frequency of sexual activity moderated cycle-related changes in IgA (Lorenz *et al.,* 2015). Increased fluctuation of salivary IgA levels may promote the growth of protozoa and contribute to infections of the buccal mucosa.

It was discovered in this study that the presence of oral parasites in orthodontic patients is notably related to significance χ^2=26.36 p<0.01 with sex. However, previous research conducted by Garcia and their team did not find any significant association between the infection and sex (Garcia *et al.,* 2018).

4.1.4 The Relationship Between the Oral Parasites Infection and the Age:

Table 4-4 shows Among non-orthodontics, the infection rates with mixed (*E. gingivalis* and *T. tenax*) were 100.0% (4\4) in the age group of (41-50), while the infection rate with *E. gingivalis* alone was 50.0% (5\17) in the age group of (11-20). The infection rate for *T. tenax* alone is 1.6% (1\6) in the age group 21-30 years.

Among orthodontic patients, the infection rates with mixed *E. gingivalis* and *T. tenax* were 25.0% (9\34) age group 31-40 years old. The infection rate with *T. tenax* alone 5.6% (2\34) was found in 31-40 years, while the age group with the infection rate for *E. gingivalis* alone was 31-40 years, with a rate of 58.3% (21\34).

There are no significant differences between age group and infection rates of oral parasitic among orthodontic patients χ^2= 47.06, p<0.01.

Table (4-4): The infection rates of *E. gingivalis* and *T. tenax* in different participants age groups.

Participant status	Age	Results				p-value
		E. gingivalis alone	*T. tenax* alone	*E. gingivalis* and *T. tenax*	Total NO. (%)	
Non-orthodontic	11-20	10(50.0%)	0(0.0%)	0(0.0%)	20(20.0)	0.0001
	21-30	10(16.9%)	1(1.6%)	10(22.2%)	59(59.0)	
	31-40	5(29.4%)	0(0.0%)	2(11.8%)	17(17.0)	
	41-50	0(0.0%)	0(0.0%)	4(100.0%)	4(4.0)	
	Total	15(15.0%)	1(1.0%)	16(16.0%)	100(50.0)	
Orthodontic patients	11-20	19(52.8%)	0(0.0%)	3(8.3%)	34(34.0)	0.4
	21-30	7(23.3%)	0(0.0%)	7(32.3%)	30(30.0)	
	31-40	21(58.3%)	2(5.6%)	9(25.0%)	36(36.0)	
	41-50	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	
	Total	47(47.0%)	2(2.0%)	19(19.0%)	100(50.0)	
Total		72(36.0%)	3(1.5%)	35(17.5%)	200(100.0)	
χ^2= 47.06, p>0.01						

The current study finds a higher infection rate of *E. gingivalis* in the 31-40 age group this finding is like AlJubory and AlHamairy, (2021). *T. tenax* was 1.6% among the 31-40 age group, this result is less than that found by (AlJubory and AlHamairy, 2021). Although a relationship has been demonstrated between the increased occurrence of this protozoan and the progression of this disease. These results agree with other studies (AlJubory and AlHamairy, 2021; Malaa *et al.,* 2022).

The infection rate with *T. tenax* was 1.6% among the 21–30 years old age group of non-orthodontic participants. These results are comparable to those found in a study by (Jaffer *et al.,* 2019).

During the investigation, it was determined that there was no significant relationship between the age of orthodontic patients and the occurrence of oral parasites. These results are consistent with (Garcia *et al.,* 2018).

On the other hand, the study by Abbass *et al.* (2020) found a significant relationship between the presence of oral parasites and the age of orthodontic patients.

4.1.5 The Relationship Between the Infection of the Oral Parasites and Gingivitis:

Table 4-5 In non-orthodontic participants, it was shown that severe gingivitis had the infection rates for mixed (*E. gingivalis* and *T. tenax*), at 75.0% (9\12). The infection rates of *E. gingivalis* alone are showing among patients with moderate gingivitis, at 57.9% (22\38), while the infection rate of *T. tenax* alone was observed in those with severe gingivitis, at 8.3% (1\12).

The orthodontic patients with severe gingivitis had the highest infection rate with oral parasites, with the mixed infection *E. gingivalis* and *T. tenax*, at 54.5% (12\22). Patients with moderate gingivitis had the highest infection rate of *E. gingivalis* alone at 78.6% (32\41), while the highest rate of *T. tenax* alone infection was in those with severe gingivitis, at 9.1% (2\22).

There is a significant relationship between oral parasite infection and gingivitis in orthodontic patients $\chi^2=209.66$, $p<0.01$.

Table (4-5): The infection rates of oral parasites among all participants based on gingivitis.

participant status	Gingivitis	Results				*p*-value
		E. gingivalis alone	*T. tenax* alone	*E. gingivalis* and *T. tenax*	Total NO. (%)	
Non-orthodontic participant	Moderate	22(57.9%)	0(0.0%)	7(18.4%)	38(38.0)	0.0001
	Severe	2(16.7%)	1(8.3%)	9(75.0%)	12(12.0)	
	Mild	1(3.8%)	0(0.0%)	0(0.0%)	26(26.0)	
	Healthy	0(0.0%)	0(0.0%)	0(0.0%)	24(24.0)	
	Total	25(25.0%)	1(1.0%)	16(16.0%)	100(50.0)	
Orthodontic patients	Moderate	32(78.6%)	0(0.0%)	7(35.0%)	41(41.0)	0.0001
	Severe	8(36.4%)	2(9.1%)	12(54.5%)	22(22.0)	
	Mild	7(30.4%)	0(0.0%)	0(0.0%)	23(23.0)	
	Healthy	0(0.0%)	0(0.0%)	0(0.0%)	15(15.0)	
	Total	47(47.0%)	2(2.0%)	19(19.0%)	100(50.0)	
Total		72(36.0%)	3(1.5%)	35(17.5%)	200(100.0)	
χ^2=209.66, p<0.01						

In this study fins the high infection rate with *E. gingivalis* was 78.6% among patients with moderate gingivit, while Abbass *et al.* (2020) found that infection rates with *E. gingivalis* were in mild gingivitis.

Gingivitis is one of the most prevalent chronic diseases worldwide. Gingival is characterized by reduced microfloral diversity and increased prevalence of *E. gingivalis*, *E. gingivalis* colonization can lead to infiltration of the dental cavity and oral mucosa, destroying gingival tissue, this destruction leads to additional bacteria invading the host tissue, exacerbating swelling and pain (CUB, 2020).

After adhering to the gums, the parasites migrate inside the tissues, feasting on and destroying host tissue, according to their studies. The *E. gingivalis* infection decreases cell growth and induces apoptosis (CUB, 2020).

Recent research has characterized the pathophysiological role of *T.tenax* in gingival disease*, T. tenax* disrupts the production of cytokines and has an impact on human macrophages (Govro and Stuart, 2016). Additionally, one *in vitro* study revealed that *T. tenax* had cytotoxic activity in normal tissues (Ribeiro *et al.,* 2015).

These findings were in agreement with several studies on the effect of oral parasite infections on the prevalence of gingivitis illnesses (AlJubory and AlHamairy, 2021; Bao *et al.,* 2021).

4.1.6 The Relationship Between the Infection of the Oral Parasites and The Level of Dental Calcification:

This study **(Table 4-6**) shows infection rates for non-orthodontic participants, the highest infection rate of mixed *E. gingivalis* and *T. tenax* was 56.3% (9\16) at the strong calcification level. The highest rate of alone *E. gingivalis* infection was observed at the level of middle calcification, with a rate of 51.9% (14\27). The infection rate of alone *T. tenax* was 6.3% (1\16) at a strong calcification level.

It was found that the degree of tooth surface calcification relationship with infection rates with *E. gingivalis* and *T. tenax*, the high infection rates were observed at the level of strong calcification at 46.3% (19\41). Additionally, the infection rate of alone *E. gingivalis* was high at the level of middle calcification at 89.5% (17\19). The infection rate of alone *T. tenax* was high at the level of strong calcification at 4.9%

(2\41). There is a significant relationship between infection rates with oral parasites and calcification levels at χ^2=174.91, $p\leq$0.01, as shown in **(Table 4-6)**.

Table (4-6): The relationship between the infection rates of *E. gingivalis* and *T. tenax* and dental calcification among all the participants.

participant status	Dental calcification	Results				*p*-value
		E. gingivalis alone	*T. tenax* alone	*E. gingivalis* and *T. tenax*	Total No. (%)	
Non-orthodontic participant	Simple	5(11.1%)	0(0.0%)	0(0.0%)	45(45.0)	0.0001
	Middle	14(51.9%)	0(0.0%)	7(25.9%)	27(27.0)	
	Strong	6(37.5%)	1(6.3%)	9(56.3%)	16(16.0)	
	No	0(0.0%)	0(0.0%)	0(0.0%)	12(12.0)	
	Total	25(25.0%)	1(1.0%)	16(16.0%)	100(50.0)	
Orthodontic patients	Simple	10(40.0%)	0(0.0%)	0(0.0%)	25(25.0)	0.0001
	Middle	17(89.5%)	0(0.0%)	0(0.0%)	19(19.0)	
	Strong	20(48.9%)	2(4.9%)	19(46.3%)	41(41.0)	
	No	0(0.0%)	0(0.0%)	0(0.0%)	15(15.0)	
	Total	47(47.0%)	2(2.0%)	19(19.0%)	100(50.0)	
Total		72(36.0%)	3(1.5%)	35(17.5%)	200(100.0)	
χ^2=174.91, p<0.01						

According to the study, there was a variation in the occurrence of oral parasite infection based on the degree of dental calcification. Patients with strong dental calcification who underwent orthodontic treatment had the highest infection rate for mixed *E. gingivalis* and *T. tenax* parasites. The reason can be explained by that plaque will accumulate more since it is challenging to maintain good oral hygiene with fixed

orthodontic gear. Plaque accumulation is particularly severe around the borders of brackets, buccal tubes, bands, and the incisal edges of teeth. It consequently puts the patient at a higher risk for gingivitis and calcification (Shrestha *et al.,* 2016)

Fixed orthodontic appliances are a common and effective tool used to treat malocclusion, but can be associated with secondary effects, such a change in the microbiota and subsequent infections. The complicated undercut shape of orthodontic appliances makes teeth cleaning more difficult and induces plaque accumulation (Kado *et al.,* 2020).

Therefore, it has been suggested that the risk of white-spot lesions, dental caries, and periodontal complications is due to the change in the oral microbiome. The infection rates of white spot lesions in patients undergoing orthodontic treatment are high (Kado *et al.,* 2020). The results from this investigation revealed that plaque had infection rates of *E. gingivalis* and *T. tenax*. The findings of this are in line with those of other previous studies (Li *et al.,* 2010; AlJubory and AlHamairy, 2021).

4.1.7 The Relationship Between the Infection of the Oral Parasites and Dental Decay:

Table 4-7 shows the non-orthodontic group with dental decay, the infection rate was lower than that of orthodontic patients at 54.7% (35\64). Non-orthodontic participants without dental decay had a lower infection rate of 19.4% (7\36) compared to orthodontic patients without dental decay. In orthodontic patients with dental decay, the infection rate with parasites (*E.gingivalis* and *T.tenax*) is 78.8% (52\66), this rate is the highest compared to a 32.4% (11\34) infection rate in those

without dental decay. There is a significant difference (χ^2= 30.98, p<0.01) between the infection with oral parasites and dental decay among both groups (orthodontic and non-orthodontic).

Table (4-7): The relationship between the infection rates of *E. gingivalis* and *T. tenax* and Dental Decay among both groups.

participant status	Dental decay	Result		Total	*p*-value
		Infection	Un infection		
Non-orthodontic participant	Yes	35(54.7%)	29(45.3%)	64(64.0%)	0.001
	No	7(19.4%)	29(80.6%)	36(36.%)	
	Total	42(42.0%)	58(58.0%)	100(50.0%)	
Orthodontic patients	Yes	52(78.8%)	14(21.2%)	66(66.0%)	0.0001
	No	11(32.4%)	23(67.6%)	34(34.0%)	
	Total	63(63.0%)	37(37.0%)	100(50.0%)	
Total		105(52.5%)	95(47.5%)	200(100.0%)	
χ^2= 30.98, p<0.01					

The current study found there is a significant association between dental decay and the infection rates of oral parasites, these results are in agreement with the findings of another study (Malaa *et al.,* 2022). Dental decay is the localized destruction of tooth structure (enamel, dentin, and cementum) by acids produced by microorganisms that metabolize consumed carbohydrates (Pitts *et al.,* 2017).

Orthodontic appliances, such as braces and archwires, can increase plaque accumulation, thereby raising the risk of gingivitis and tooth decay, which raises the risk of gingivitis and dental decay in patients (Cantekin *et al.,* 2011).

This study has shown a strong association between protozoa, dental decay, and gingivitis (Santos and Roldán, 2023).

It has been proven through studies that both *E. gingivalis* and *T. tenax* can not only lead to periodontal diseases but also play a role in the development of dental decay (Mahmoudvand *et al.,* 2018).

4.1.8 The Relationship Between the Infection of the Oral Parasites and the Use of Floss:

Table 4-8 shows that non-orthodontic participants who used floss had low infection rates of oral parasites at 14.3%, while participants who did not use floss had higher infection rates at 46.5%. The rate of participants without infection who use floss is higher than don't use floss at 85.7%. The infection rates were highest in orthodontic patients who did not use floss at 70.9%, while the patients who used floss had low infection rates of oral parasites at 33.3%.

There is a significant difference between the infection with oral parasites and the use of floss among orthodontic patients and non-orthodontic at $\chi^2 = 12.206$, $p<0.01$.

It was observed that only a small percentage used dental floss every day when the treatment was long-term, and this is related to the fact that patients undergoing long-term treatment reduce their motivation for oral care and orthodontic appliances can complicate the use of dental floss (Almuhana and Al-Hamairy, 2021).

Table (4-8): The relationship between the infection rates of *E. gingivalis* and *T. tenax* among orthodontics and non-orthodontic participants based on used floss.

participant status	floss	Result		Total	*p*-value
		Infection	Un infection		
Non-orthodontic participant	Yes	2(14.3%)	12(85.7%)	14(14.0%)	0.021
	No	40(46.5%)	46(53.5%)	86(86.0%)	
	Total	42(42.0%)	58(58.0%)	100(50.0%)	
Orthodontic patients	Yes	7(33.3%)	14(66.7%)	21(21.0%)	0.002
	No	56(70.9%)	23(29.1%)	79(79.0%)	
	Total	64(64.0%)	37(37.0%)	100(50.0%)	
Total		105(52.5%)	95(47.5%)	200(100.0%)	
χ^2= 12.206, p<0.01					

The influence of hygiene on various disorders can be kept in mind; poor hygiene increases the number of oral parasites. The defense mechanisms ineffectiveness and lack of exposure to these parasites are the causes of decreased resistance (Almuhana and Al-Hamairy, 2021).

This study finds a significant relationship between flossing and a lower prevalence of oral parasites χ^2= 12.206, p<0.01. These findings are consistent with another study by Malaa *et al.,* (2022). In contrast, Azadbakht and his colleagues found no significant difference between the infection with oral parasites and the used floss (Azadbakht *et al.,* 2022).

4.1.9 The Relationship Between the Infection of the Oral Parasites and the Use of Mouthwash:

The present results **(Table 4-9)** show that non-orthodontic participants who used mouthwash had a low infection rate of oral parasites at 8.3% (1\12), while participants who didn't use mouthwash had higher infection rates at 46.6% (41\88). The infection rate in orthodontics patients who didn't use mouthwash was 77.3% (58\75), while the infection rate of oral parasites who used mouthwash was 20.0% (5\25). There is a significant association between oral parasite infection and the use of mouthwash χ^2= 23.96, p<0.01, (**Table 4-9)**.

Table (4-9): *E. gingivalis* and *T. tenax* among two groups (orthodontic and non-orthodontic) that used mouthwash or not.

participant status	Mouthwash	Result		Total	*p*-value
		Infection	Un infection		
Non-orthodontic participant	Yes	1(8.3%)	11(91.7%)	12(100.0%)	0.01
	No	41(46.6%)	47(53.4%)	88(100.0%)	
	Total	42(42.0%)	58(58.0%)	100(50.0%)	
Orthodontic patients	Yes	5(20.0%)	20(80.0%)	25(100.0%)	0.0001
	No	58(77.3%)	17(22.7%)	75(100.0%)	
	Total	63(63.0%)	37(37.0%)	100(50.0%)	
Total		105(52.5%)	95(47.7%)	200(100.0%)	
χ^2= 23.96, p<0.01					

The current study finds a higher rate of oral parasite infection in patients who do not use mouthwash. This may be attributed to the fact that periodontitis and mucus irritation are encouraged through poor oral hygiene. Additionally, poor oral hygiene encourages the buildup of food particles and the formation of dental calcification, which serves as the breeding ground for oral parasites (Afara and Binsaad, 2023).

Mouthwash aids in the treatment of oral diseases, and oral parasite infection decreases when oral hygiene improves. Washing the teeth and the mouth is thought to be crucial for preventing oral parasite diseases (Malaa *et al.,* 2022).

Using mouthwash can help get rid of oral parasites such as *E. gingivalis* and *T. tenax*. The researchers found that after 10 minutes, *T. tenax* had a mortality rate of 50%, while after 30 minutes, it increased to 70%. Meanwhile, *E. gingivalis* had a mortality rate of 10% after 10 minutes and 20% after 30 minutes (Moroz *et al.,* 2019).

These findings were consistent with another study that dealt with the effect of the use of mouthwash on the incidence of oral parasites and various oral diseases (Moroz *et al.,* 2019; Malaa *et al.,* 2022).

Some studies don’t find a significant association between oral parasites and the use of mouthwash (Azadbakht *et al.,* 2022)

4.1.10 The Relationship Between the Infection of the Oral Parasites and the Brushing of the Teeth:

Table 4-10 shows that non-orthodontic participants who used teeth brushing had low infection rates of oral parasites at 21.1% (15\71), while participants who didn't use teeth brushing had higher infection rates at 93.1% (27\29).

The infection rates were highest in orthodontics patients who do not brush their teeth at 100.0% (20\20), while the patients who used teeth brushing had low infection rates of oral parasites at 53.8% (43\80). There is a significant difference between oral parasite infection and the use of brush χ^2= 49.06, p<0.01 **(Table 4-10)**.

Table (4-10): The relationship between the infection with oral parasites and daily brushing teeth among orthodontic & non-orthodontic groups.

participant status	Brushing	Result		Total	*p*-value
		Infection	Un infection		
Non-orthodontic participant	Yes	15(21.1%)	56(78.9%)	71(71.0%)	0.0001
	No	27(93.1%)	2(6.9%)	29(29.0%)	
	Total	43(43.0%)	58(58.0%)	100(50.0%)	
Orthodontic patients	Yes	43(53.8%)	37(46.3%)	80(80.0%)	0.0001
	No	20(100.0%)	0(0.0%)	20(20.0%)	
	Total	63(63.0%)	37(37.0%)	100(50.0%)	
Total		110(55.0%)	90(45.0%)	200(100.0%)	
χ^2= 49.06, p<0.01					

Brushing is regarded as a crucial practice for maintaining dental hygiene since it is the best tool for removing food debris and controlling dental plaque (Kaneyasu *et al.,* 2022).

Without proper cleaning and preservation, the regular use of the toothbrush might encourage the growth of dangerous germs and cross-contamination by parasites (protozoa), viruses, fungi, and bacteria (Cunha *et al.,* 2018). Based on our research, it has been found that there is a high association between the regularity of teeth brushing and the infection rates of *E. gingivalis* and *T. tenax*, which is consistent with the studies conducted by Derikvand *et al.* (2018) and Mahmoudvand *et al.* (2018) and Afara and Binsaad, (2023). This highlights the importance of maintaining good oral hygiene practices to prevent the proliferation of harmful oral parasites in the mouth. (Derikvand, 2018; Mahmoudvand *et al.,* 2018; Afara and Binsaad, 2023).

4.1.11 The Relationship Between the Infection of the Oral Parasites and Smoking:

Table 4-11 shows nonsmoking non-orthodontic participants had low infection rates of mixed *E. gingivalis* and *T. tenax* at 31.8% (27\85), while participants who smoked had higher infection rates at 100.0% (15\15).

The infection rates were highest in smoking orthodontics patients at 100.0% (12\12), while the infection rate in non-smoking orthodontic patients with mixed *E. gingivalis* and *T. tenax* at 63.6% (56\88). There is a significant relationship between oral parasite infection and smoking χ^2= 6.05, p<0.01 **(Table 4-11)**.

The infection rate of non-smoking non-orthodontic participants with mixed *E. gingivalis* and *T. tenax* was 31.8%, this result is less than AlJubory and AlHamairy (2021).

The present findings were consistent with those of previous studies and demonstrated that the effect of the smoking factor affects the infection rates with oral parasites. According to several investigators, the smoking variable is among the variables that determine the incidence of oral parasites. Their findings are in line with several previous studies that showed the impact of smoking on the prevalence of chronic oral diseases and oral parasitic infections (Mathieu *et al.,* 2017).

Table (4-11): The relationship between the oral parasites & smoking among orthodontic & non-orthodontic groups.

Participant statute	smokers	Result		Total	*p*-value
		Infection	Un infection		
Non-orthodontic participant	Yes	15(100.0%)	0(0.0%)	15(100.0%)	0.2
	No	27(31.8%)	58(68.3%)	85(100.0%)	
Orthodontic patients	Yes	12(100.0%)	0(0.0%)	12(100.0%)	0.01
	No	56(63.6%)	32(36.4%)	88(100.0%)	
Total		110(55.0%)	90(45.0%)	200(100.0%)	
χ^2= 6.05, *p*=0.01					

This may be because non-smokers neglect mouth or periodontal care, have higher levels of dental plaque, tooth decay, and oral microorganisms than non-smokers, and have greater levels of microorganisms than non-smokers (AlJubory and AlHamairy, 2021).

4.2 The culture studying of oral parasites among orthodontic treatment:

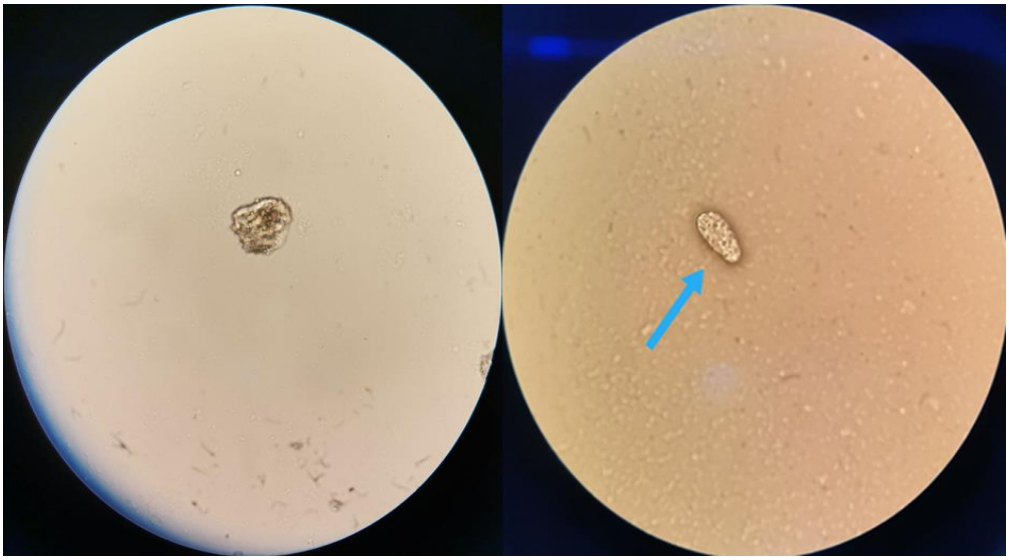

Figure (4-2): *E. gingivalis* culture in TYGM-9 medium under 100x power.

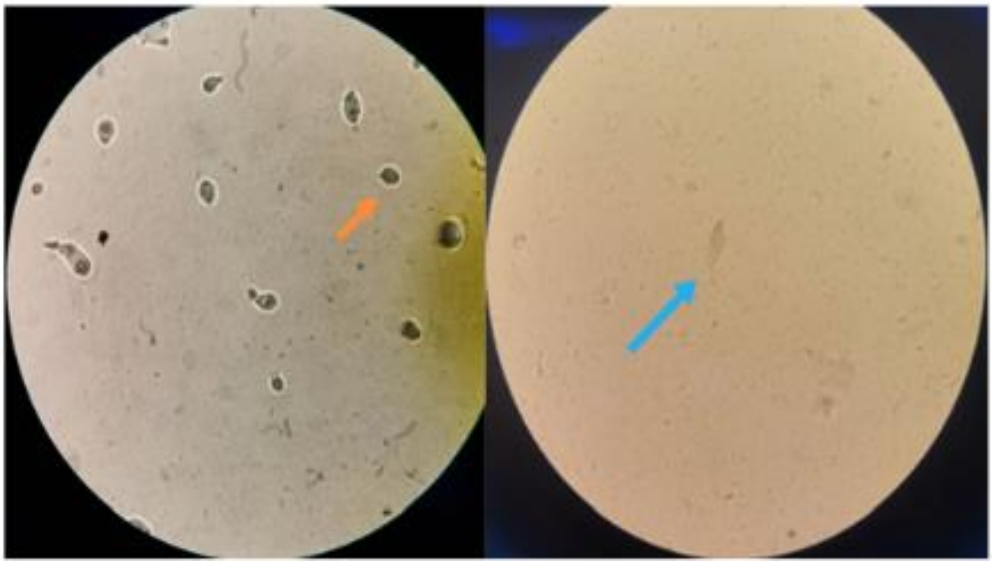

Figure (4-3): *T. tenax* culture in TYGM-9 medium under 100x power.

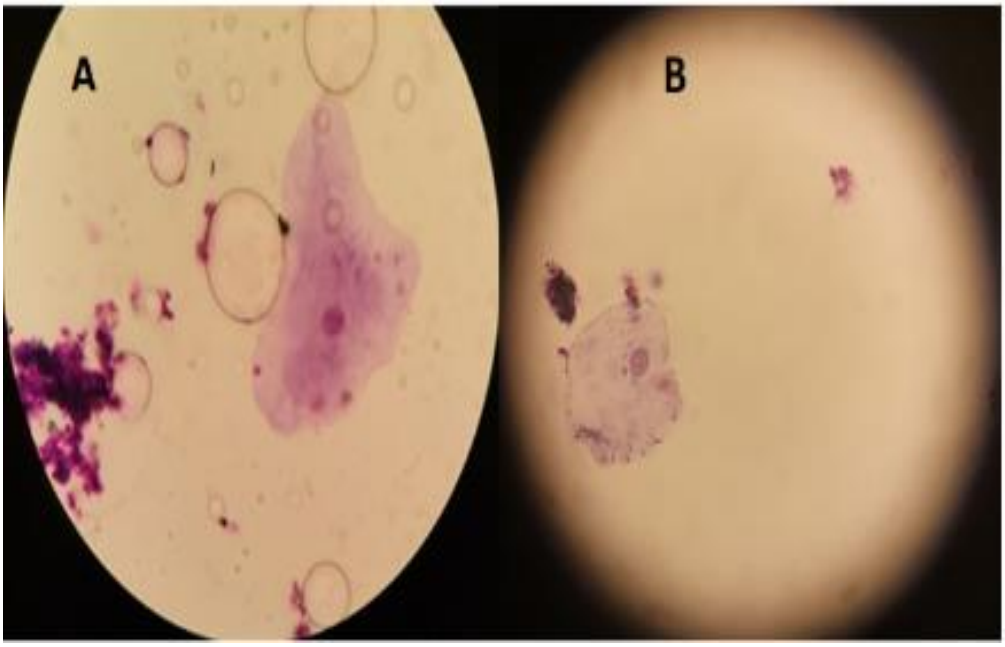

Figure (4-4): *E. gingivalis* extend of pseudopods, with Giemsa staining under 400x power.

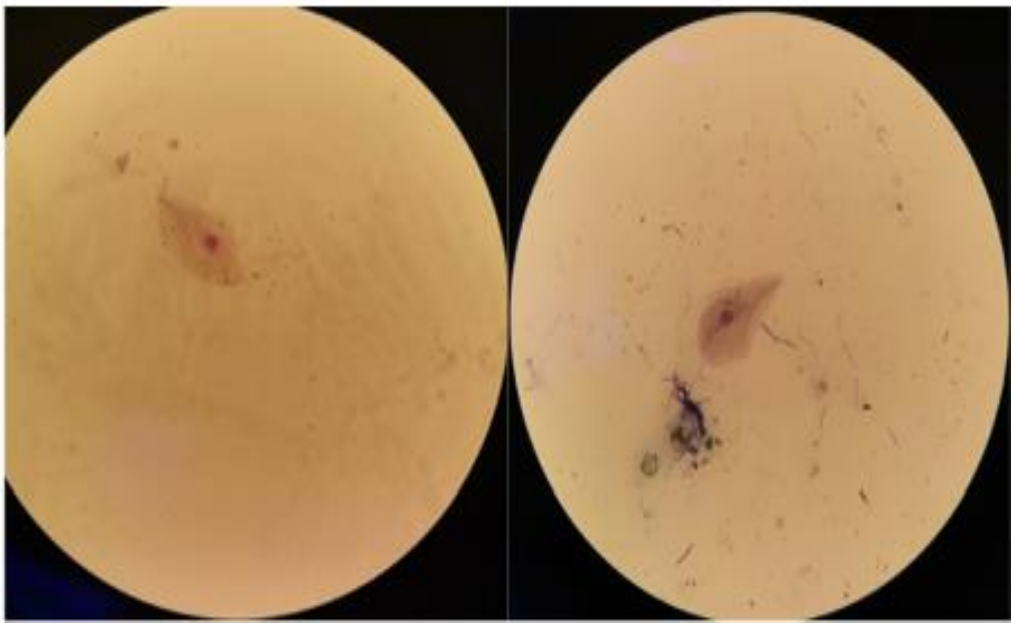

Figure (4-5): *T. tenax* with Giemsa staining under 400x.

4.3 Results of Nested Polymerase Chain Reaction:

In the current study, we adopted this technique by using two pairs of primers for each of the parasites (*E. gingivalis* and *T. tenax*) to amplify a region (*18S ribosomal RNA*) extracted from the rDNA of the cells of the oral parasites. The sequence of bases in region 18S rRNA differs from one species to another, so this difference is used in the team process between parasitic strains, and this piece was used by researchers to detect oral parasites (Garcia *et al.,* 2018; Sharifi *et al.,* 2020)

The results of the current study showed amplification of the DNA strand of oral parasites using the Nested Polymerase Reaction technique. Primers amplified DNA and ranged in size of the amplified bands in *E. gingivalis* (521 bp) as shown in **Figure (4-5)**, while the size of the amplified bands in *T. tenax* was (467 bp) as shown in **Figure (4-6)**.

As shown in Figures shows the electrophoresis of the PCR product obtained from samples examined through microscopy and culture. **Figure (4-5)** and **Figure (4-6)**.

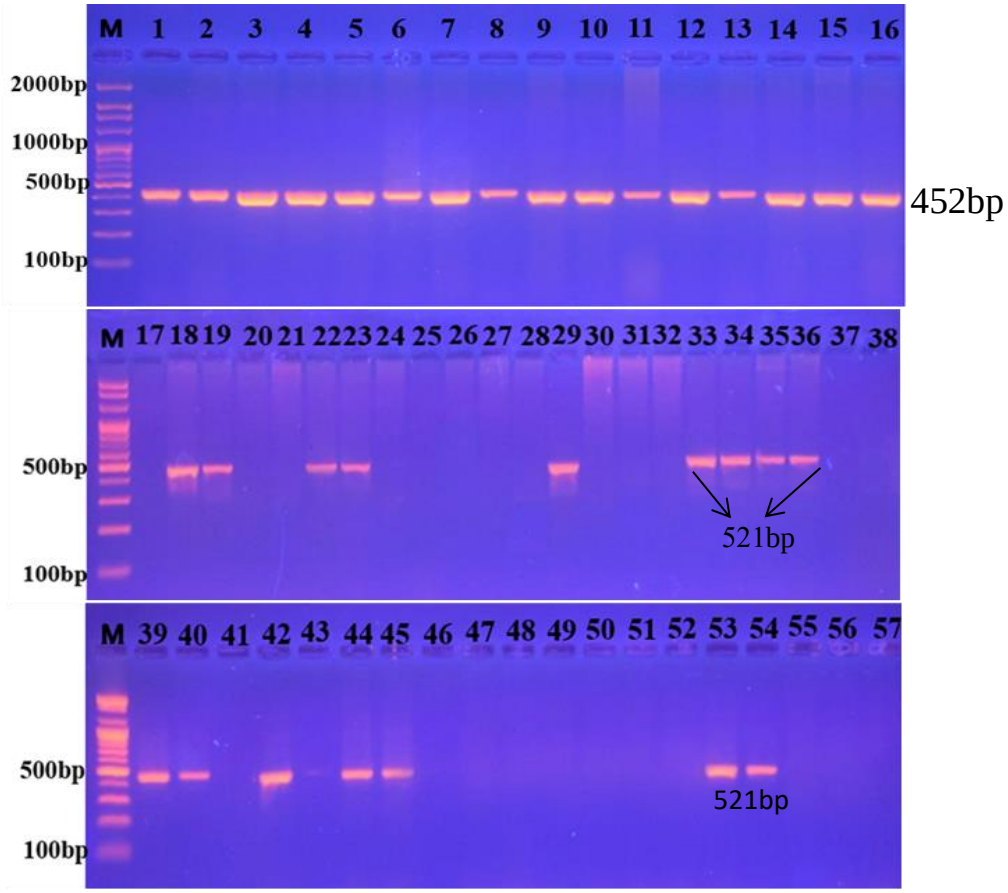

Figure (4-6): Electrophoresis of DNA products obtained from the nested PCR of *E. gingivalis*

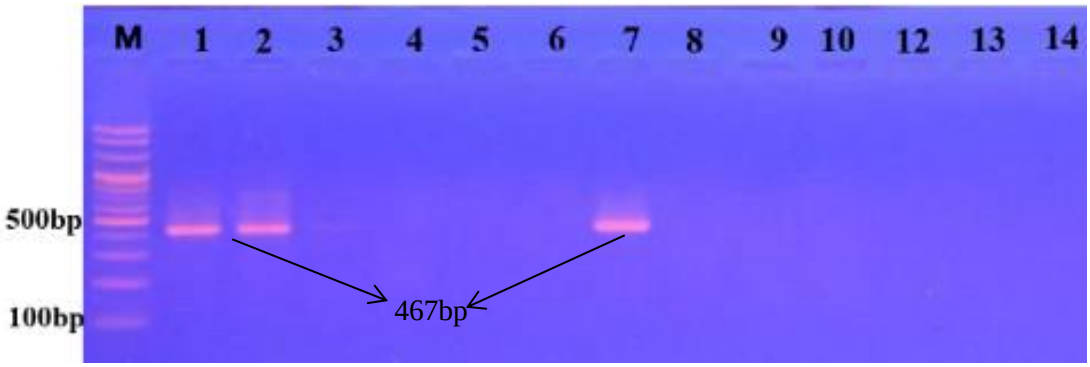

Figure (4-7): Electrophoresis of DNA products got from the nested PCR of *T. tenax*

4.4 DNA Sequencing:

In the percent study, the DNA sequencing method was carried out for genetic relationship analysis in the B1 gene of local *Entamoeba gingivalis* IQ isolates and *Trichomonas tenax* that were compared with NCBI-Blast country-related isolates.

The phylogenetic tree analysis showed that local *Entamoeba gingivalis* IQ.No.1- Tox. No.10 isolates showed closed related to NCBI-BLAST *Entamoeba gingivalis* Brazil isolate at total genetic changes (0.0050-0.0010%). The local *Trichomonas tenax* IQ.No.1-No.3 isolates were showed closed related to NCBI-BLAST *Trichomonas tenax* Brazil isolate at total genetic changes (0.0030-0.0010%) as shown in **figure (4-8)** and **figure (4-10).**

The homology sequence identity between local *Entamoeba gingivalis* IQ No.1- No.7 isolates and NCBI-BLAST related *Entamoeba gingivalis* Brazil isolate showed genetic homology sequence identity ranging from (99.60- 99.96). The homology sequence identity between local *Trichomonas tenax* IQ No.1- No.3 isolates and NCBI-BLAST related *Trichomonas tenax* Brazil isolate showed genetic homology sequence identity ranged from (99.40- 99.45) as shown in **Table (4-12)** and **Table (4-13)**.

Finally, the local *Entamoeba gingivalis* IQ.No.1- Tox. No.10 isolates and local *Trichomonas tenax* IQ No.1- No.3 has been submitted to Genbank with the recorded accession numbers (OR405080.1-OR405089.1)

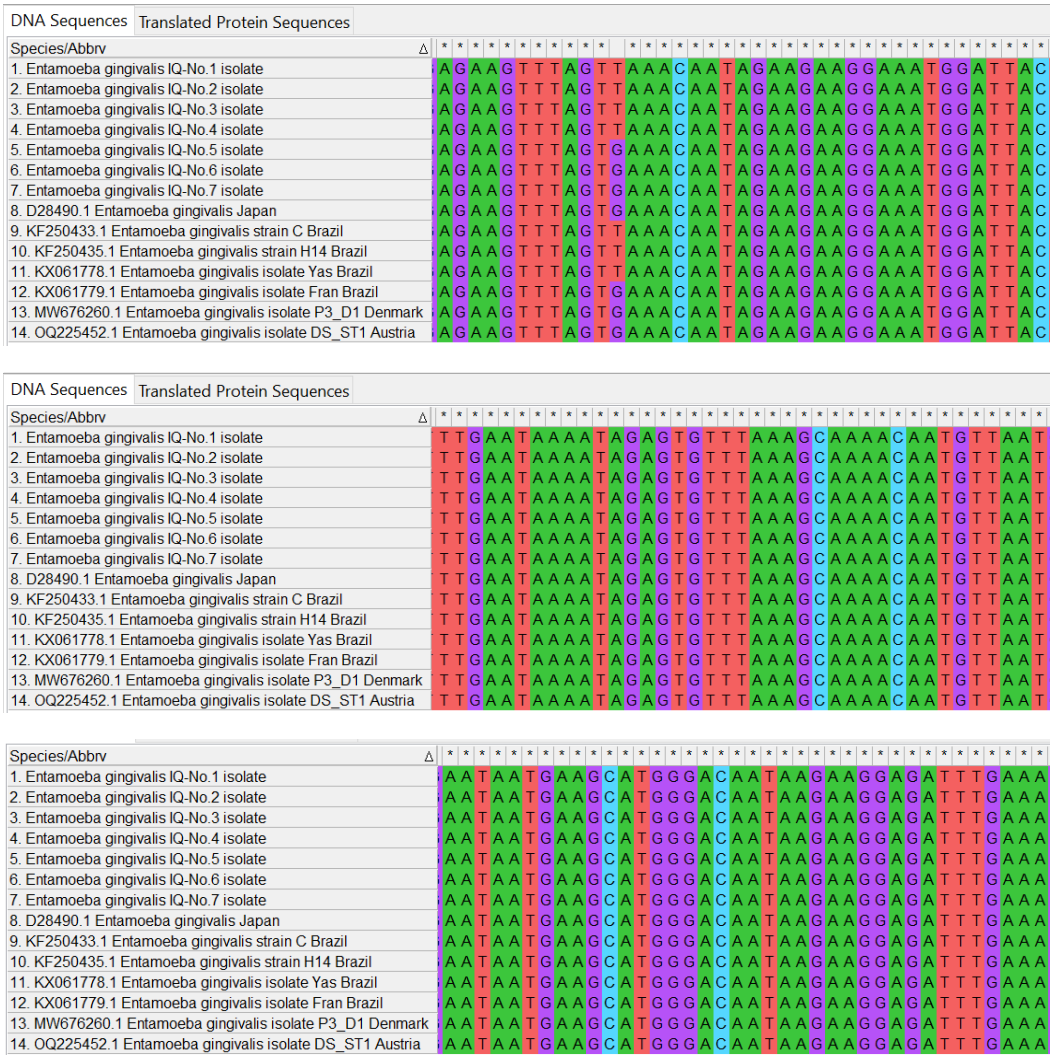

Figure (4-8): Multiple sequence alignment analysis of *18S Ribosomal RNA* Gene in local *Entamoeba gingivalis* IQ isolates and NCBI-Genbank *Entamoeba gingivalis* country-related isolates. The multiple alignment analysis was constructed using (the ClustalW alignment tool. Online). That alignment analysis showed the nucleotide alignment similarity as (*) and substitution mutations in the 18S Ribosomal RNA Gene between isolates.

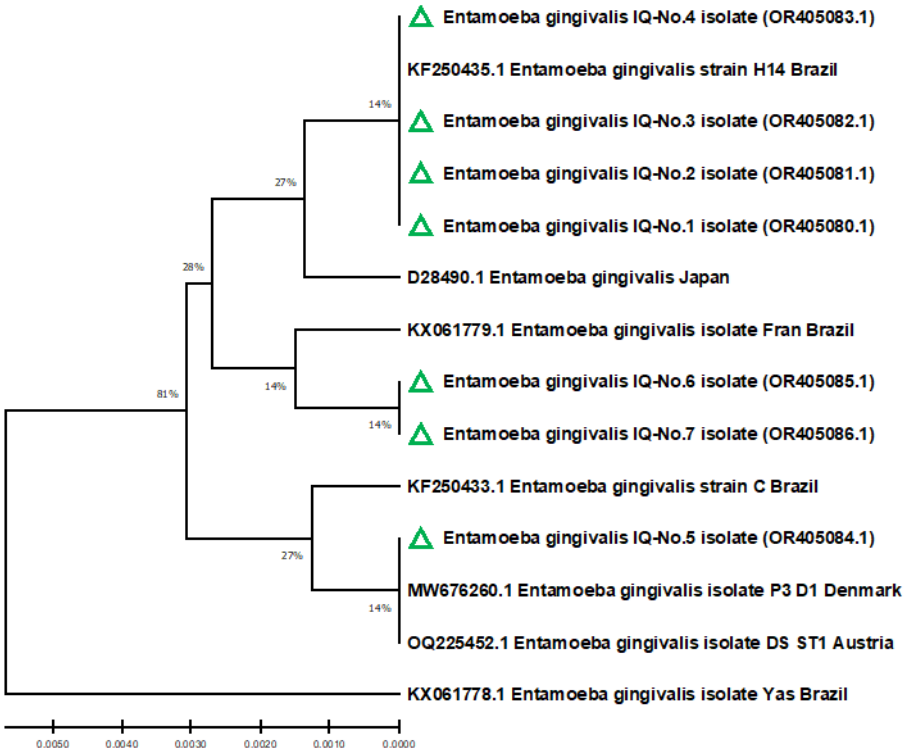

Figure (4-9): Phylogenetic Tree Analysis Based on *18S Ribosomal RNA* Gene partial sequence in local *Entamoeba gingivalis* IQ Isolates that were Used for Genetic Relationship Analysis. The Phylogenetic Tree was Constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA tree method) and the evolutionary distances were computed using the Maximum Composite Likelihood method in (MEGA 6.0 version). The Local *Entamoeba gingivalis* IQ.No.1- No.7 Isolates Showed Closely Related to NCBI-BLAST *Entamoeba gingivalis* Brazil Isolate at Total Genetic Differences (0.0050-0.0010%).

Table (4-12): The NCBI-BLAST homology sequence identity percentage between local *E. gingivalis* isolates and NCBI-BLAST closely related *E. gingivalis* Isolates:

local *Entamoeba gingivalis* isolate	Accession number	Homology sequence identity (%)	
		Country related NCBI	Identity (%)
***Entamoeba gingivalis* IQ No.1 isolates**	OR405080.1	Brazil	99.96
***Entamoeba gingivalis* IQ No.2 isolates**	OR405081.1	Brazil	99.76
***Entamoeba gingivalis* IQ No.3 isolates**	OR405082.1	Brazil	99.63
***Entamoeba gingivalis* IQ No.4 isolate**	OR405083.1	Brazil	99.64
***Entamoeba gingivalis* IQ No.5 isolates**	OR405084.1	Brazil	99.76
***Entamoeba gingivalis* IQ No.6 isolates**	OR405085.1	Brazil	99.60
***Entamoeba gingivalis* IQ No.7 isolates**	OR405086.1	Brazil	99.96

Figure (4-10): Multiple sequence alignment analysis of *18S Ribosomal RNA* Gene in local *Trichomonas tenax* IQ Isolates and NCBI-Genbank *Trichomonas tenax* country-related isolates. The multiple alignment analysis was constructed using (The ClustalW Alignment Tool. Online). That alignment analysis showed the nucleotide alignment similarity as (*) and substitution mutations in the *18S Ribosomal RNA* Gene between isolates.

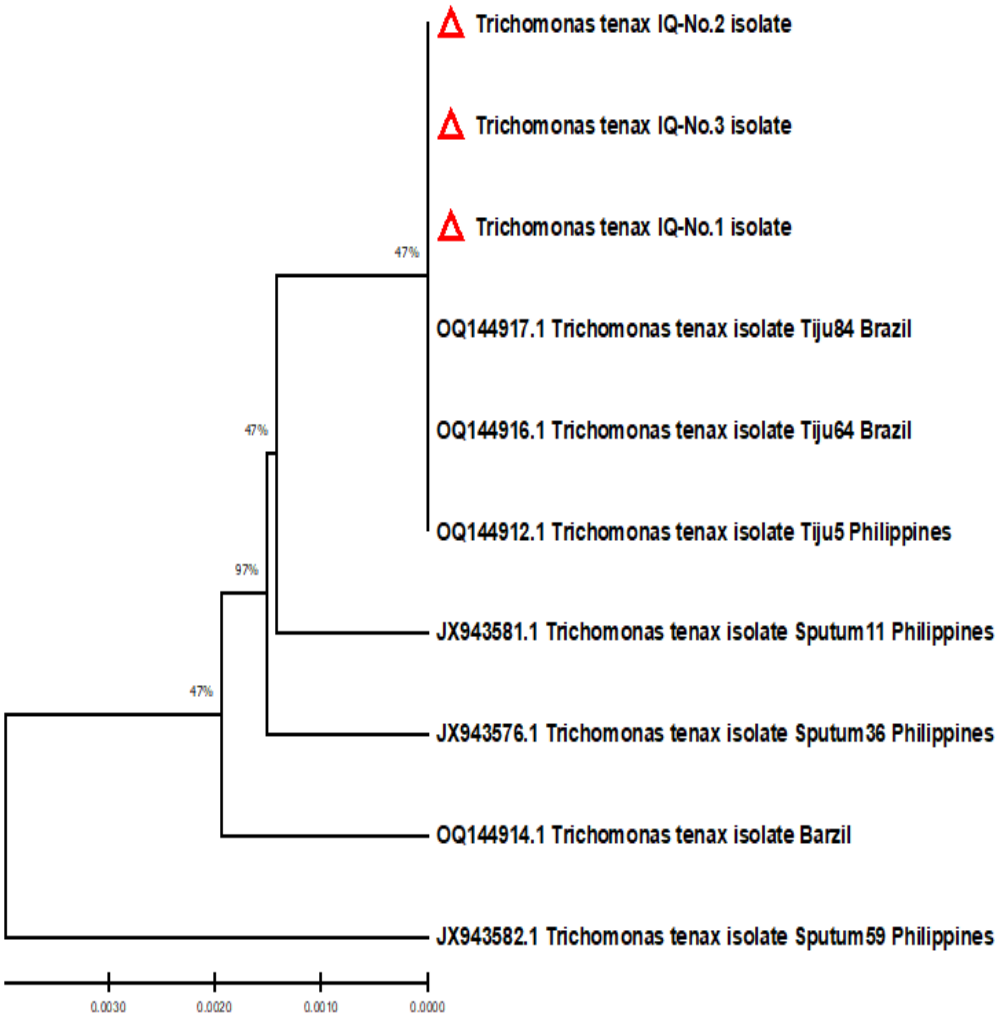

Figure (4-11): Phylogenetic tree analysis based on *18S Ribosomal RNA* Gene Partial Sequence in Local *Trichomonas tenax* IQ Isolates that were Used for Genetic Relationship Analysis. The phylogenetic tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA Tree Method) and the evolutionary distances were computed using the maximum composite likelihood method in (MEGA 6.0 version). The local *Trichomonas tenax* IQ.No.1-No.3 Isolates were showed closed related to NCBI-BLAST *Trichomonas tenax* Brazil isolate at total genetic changes (0.0030-0.0010%).

Table (4-13): The NCBI-BLAST homology sequence identity percentage between local *T. tenax* Isolates and NCBI-BLAST closed-related *T. tenax* isolate:

local *Trichomonas tenax* isolate	Accession number	Homology sequence identity (%)	
		Country related NCBI	Identity (%)
***Trichomonas tenax* IQ No.1 isolates**	OR405087.1	Brazil	99.40
***Trichomonas tenax* IQ No.2 isolates**	OR405088.1	Brazil	99.45
***Trichomonas tenax* IQ No.3 isolates**	OR405089.1	Brazil	99.45

4.5 The results of Metronidazole Drug Release from Gtn-HPA/CMC-Tyr:

It is essential for an *in situ* hydrogel system to crosslink quickly as slow gelation causes delocalized hydrogel formation due to diffusion of the hydrogel precursors away from the injection site (Lee *et al.*, 2009; Duceac and Coseri, 2022). In addition, it may cause undesired leakage of the encapsulated biological molecules such as proteins and drugs to the nearby tissues and compromise vaccination or therapeutic outcomes. Using the HRP enzyme to accelerate the rate of formation of tyramine crosslinks, resulted in decreasing the gelation time (Lee *et al.*, 2009). Here, whether changes in the concentration of the Gtn-HPA/CMC-Tyr hydrogel could modify the release rate of the drug has been investigated. Three different concentrations of hydrogel: 10, 15, and 20% (w/v) have been prepared and the drug release has been measured. The results demonstrate that the hydrogel concentration is a critical factor affecting the pore structure of the hydrogel as shown in SEM images in **Figure (4-12)** and involving the pore sizes as shown in **Figure (4-13)**. Most likely through its effect on phase separation during hydrogel formation.

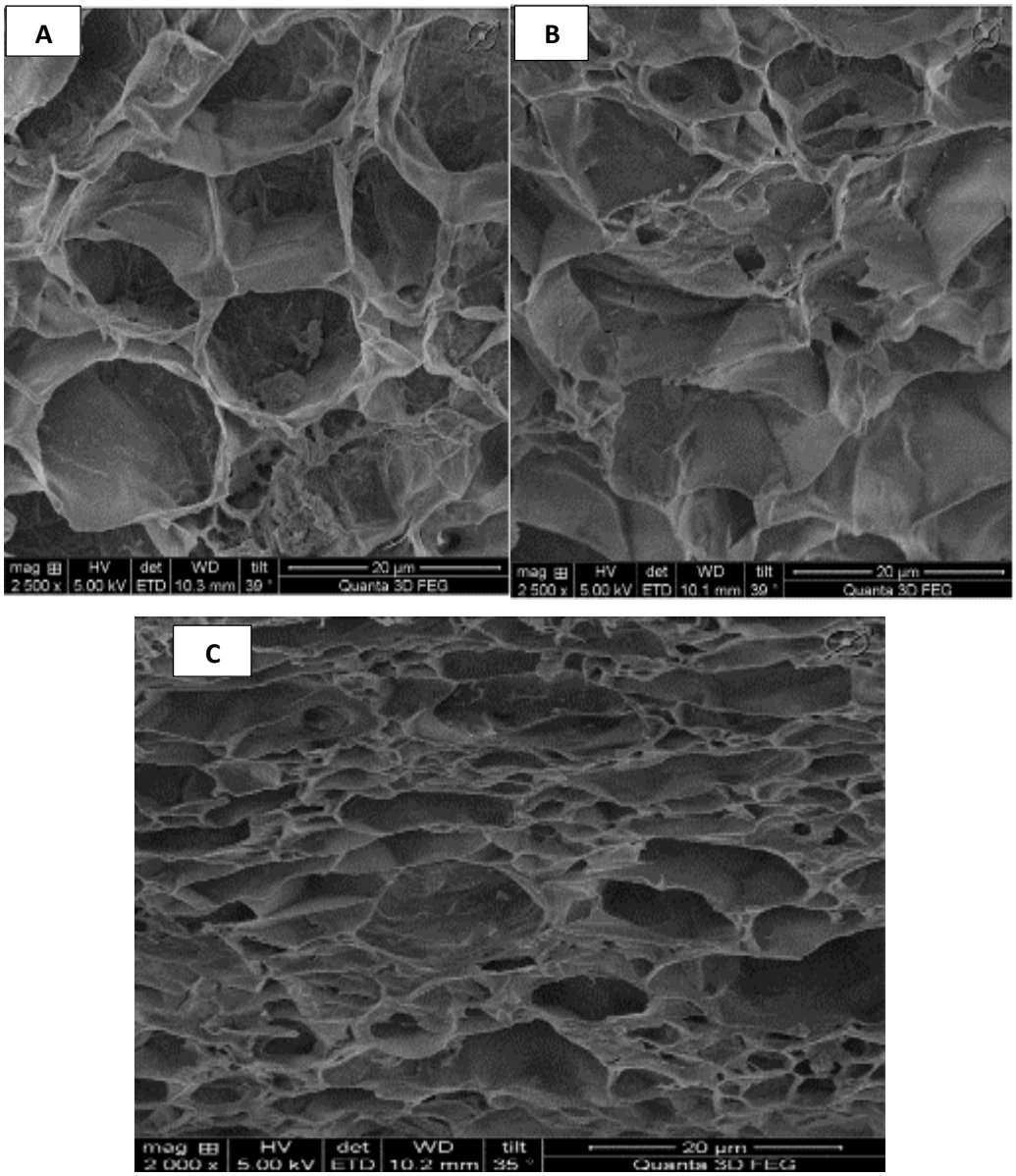

Figure (4-12): SEM images of Gtn–HPA/CMC–Tyr hydrogel Scaffold. A: Hydrogel of 10% Concentration; B: Hydrogel of 15% Concentration; C: Hydrogel of 20% Concentration. The Scale bar is 20µm. Quanta DualBeam 3D (FEI Company, Hillboro, OR) and Helios 600 instrument (FEI company).

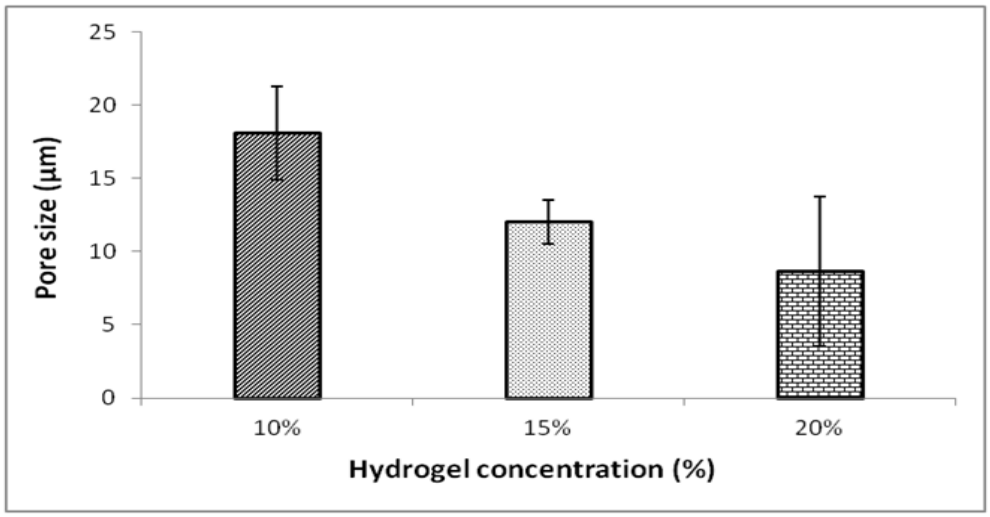

Figure (4-13): Comparison of the Pore Size Measurements between the Three Different Concentrations of Gtn–HPA/CMC–Tyr hydrogel 10%, 15% and 20%.

The mixture of Gtn-HPA and CMC-Tyr for the three different concentrations is initially homogenous and miscible. Upon polymerization, phase separation occurs as the fraction of molecules with high molecular weight increases, causing the morphology of the mixture to separate into discrete domains (Yang *et al.*, 2022). Thereafter the rate of polymerization probably allows time for these discrete domains to increase in size and undergo a phenomenon known as spinodal decomposition (Elbert, 2011) so that a porous matrix with relatively large voids is formed.

Figure (4-14) depicts the cumulative release of metronidazole drug from the three concentrations of Gtn-HPA/CMC-Tyr hydrogels.

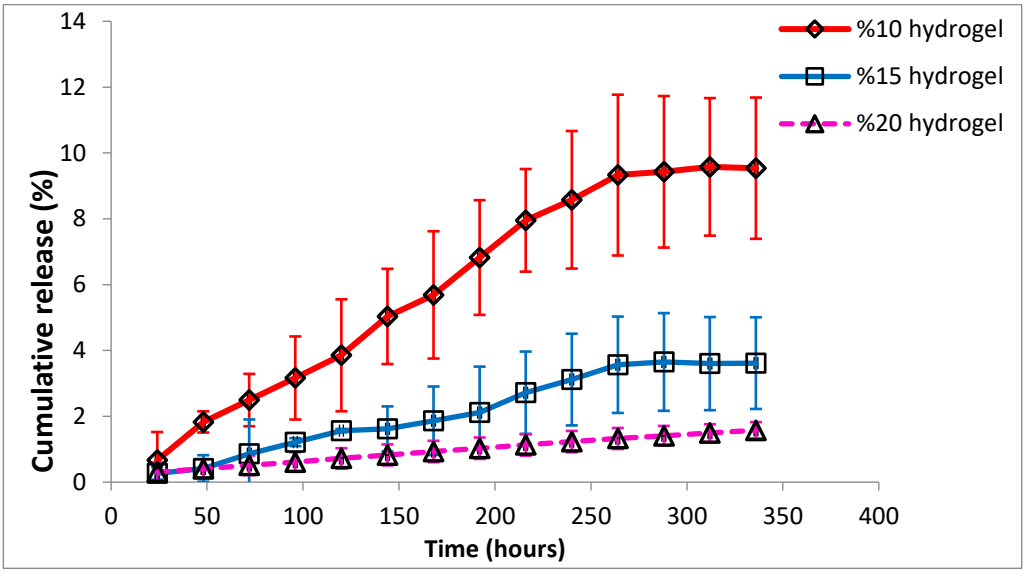

Figure (4-14): Cumulative Release Profiles of Metronidazole Drug from Three Different Concentrations of Gtn-HPA/ CMC-Tyr hydrogels (10%, 15% and 20%) in PBS Buffered Saline (pH 7.4) at 37 °C. The Results were Expressed as Means ± SD (n=3).

The drug release profile in **Figure (4-14)** indicates that the drug diffuses from the network of the three hydrogels and the release appears to be diffusion-controlled.

By plotting the release of the drug during the first eight hours as a function of the square root of time, a linear plot was obtained which indicated a first-order release typical of Fickian diffusion, followed by a sustained **Figure (4-15)**. Progressive degradation of the hydrogel network may likely play a vital role in drug release. More importantly, no burst release was observed.

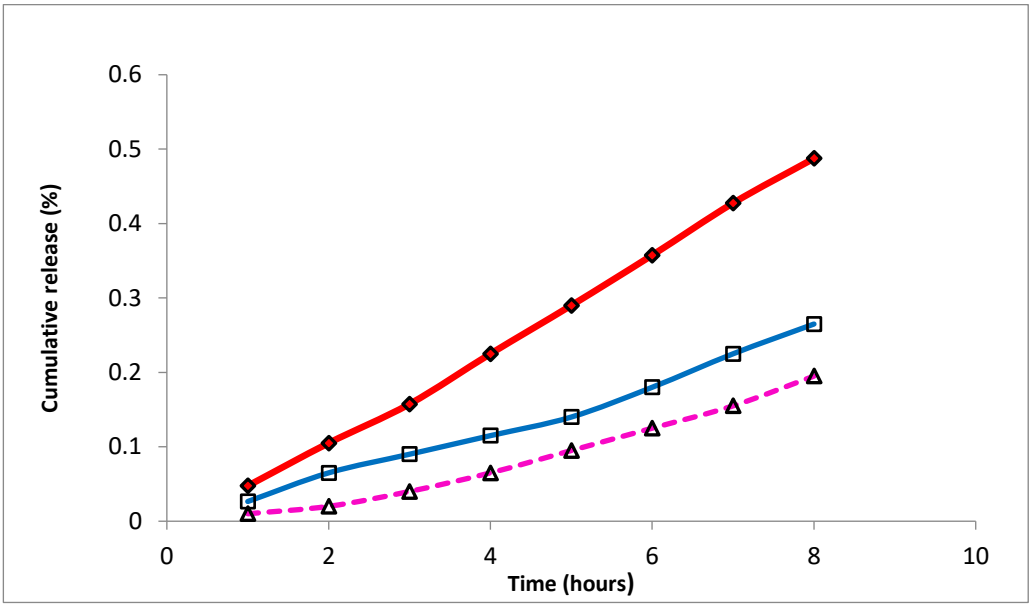

Figure (4-15): shows Cumulative Release at the First Eight Hours of the Experiment Time. For the Sake of Clarity Error bars are Not Shown.

After 14 days (9– 10) % of the incorporated drug was released from the 10% hydrogel concentration discs, (3-4) % from the 15% hydrogel, and (1-2) % from the 20% hydrogel disc. This slow release from the highest hydrogel concentration suggests that the 20% hydrogel has a higher network density compared to 15% or 10% hydrogels. These findings may be explained by differences in the porous structure of the three concentrations of gel-forming polymers associated with correspondingly different degradation properties of the corresponding hydrogels. Increasing hydrogel density may make the pore size relatively smaller as well as decrease the rate of degradation by hydrolysis or oxidation reactions (through enzyme and body fluids activity). Degradation rates particularly *in vivo* are difficult to determine as they depend not only on the composition and crosslink density of the hydrogel (Rouwkema *et al.*, 2008). but also on the prevailing physical, chemical, and biological conditions. As shown in **Figure (4-14)**, there

were significant differences observed in cumulative release percentages between the 10% hydrogel and both 15% and 20% hydrogel ($p<0.001$), but only small differences (not statistically significant) between the 15% hydrogel and the 20% hydrogel. The *in vitro* release results provide some insight into how hydrogel concentrations and their porous structures would affect the release profiles *in vivo* that we aim to work on in the future. However, the period of release in the *in vitro* experiments could not be translated directly to *in vivo* conditions.

4.6 Hydrogel Degradation

To elucidate the degradation kinetics of (Gtn-HPA/CMC-Tyr) hydrogels, a series of hydrogels were synthesized with varying concentrations (10%, 15%, 20%) and subjected to systematic degradation assays. Quantitative degradation analysis was conducted by assessing the weight loss of the Gtn-HPA/CMC-Tyr hydrogels over time, thus providing an empirical measure of their structural integrity and stability under simulated physiological conditions. This weight loss is indicative of the hydrogel's degradation, substantiating its potential for sustained drug release capabilities.

The degradation rate of the Gtn-HPA/CMC-Tyr hydrogels was observed to escalate with the prolongation of incubation in an artificial salivary solution, formulated to mimic the ionic composition and pH of human saliva (ranging approximately between 6.75 and 7.25), and supplemented with Amylase enzyme (refer to **Figure 4-16**). This accelerated degradation in the enzyme-augmented salivary analogue suggests an enhanced potential for expedited drug liberation from the hydrogel matrix.

A temporal analysis of the hydrogel degradation was conducted over 21 days. This involved the measurement of the residual dry weight of the hydrogels post-oven drying at a temperature range of 50–60ºC until a constant weight was achieved. In this experimental setup, A control sample was used consisting of the hydrogel in Phosphate-buffered saline (PBS, pH 7.4) to simulate normal physiological conditions.

This comprehensive approach underscores the degradation dynamics of Gtn-HPA/CMC-Tyr hydrogels, providing crucial insights into their potential as efficacious drug delivery systems in a biomimetic oral environment. At predetermined time intervals, the hydrogel samples were removed from the incubation medium, washed, and dried to a constant weight at 50–60ºC. Measure and record the dry weight of each sample to assess degradation.

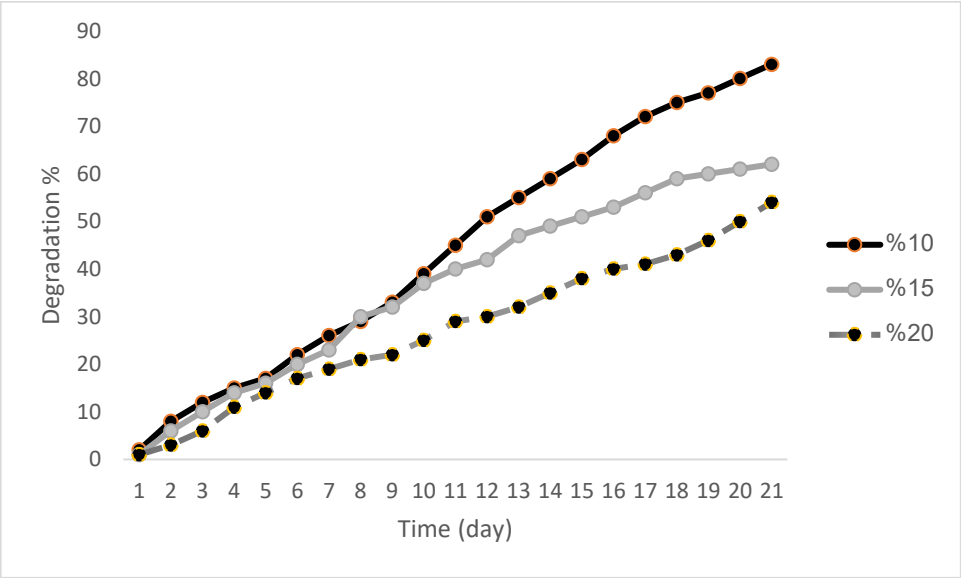

Figure (4-16): Degradation Profile of Gtn-HPA/CMC-Tyr Hydrogels in Artificial Salivary Solution.

Chapter Five

Conclusions

&

Recommendations

5:1 Conclusions

1. The outcomes gleaned from this comprehensive study shed light on the significant presence of *Entamoeba gingivalis* and *Trichomonas tenax* within the oral milieu, with a pronounced prevalence observed among individuals who exhibit subpar oral hygiene habits and concurrently employ orthodontic appliances.
2. This elucidation underscores the potential repercussions of microbial accumulation in orthodontic apparatus, potentially precipitating the onset of various ailments including periodontal diseases and gingivitis.
3. The culture method proved effective showing positive results in growing oral parasites on TYGM-9 medium.
4. In the current study, the conventional PCR technique failed to detect oral parasites.
5. The nested polymerase reaction technique is highly sensitive for diagnostic species of oral parasites.
6. There is a significant relationship between orthodontic treatment and the incidence of oral parasite infection.
7. There is a significant relationship between the use of mouthwash, flossing, and smoking and the oral parasite infection.
8. The damage resulting from treatment with orthodontic appliances is due to plaque accumulation, which hinders the cleaning process and provides a suitable environment for oral parasites.
9. The chemical and physical properties of hydrogel have proven its efficiency as a nano-biomaterial for treatment delivery in the field of orthodontics.

10. There are significant differences observed in cumulative release percentages between the 10% hydrogel and both 15% and 20% hydrogel.

5:2 Recommendations:

1. A complementary study on hydrogel and its applications as a drug delivery platform.
2. A complementary study that includes the investigation of other pathogenic microorganisms, especially bacteria, because they are frequently observed in orthodontic patients.
3. Attention to oral hygiene and using toothbrushes, mouthwash, and floss because it reduces the risk of infection with oral parasites in orthodontic patients.
4. The imperative for fostering meticulous oral hygiene practices and regular dental examinations becomes paramount in the context of orthodontic treatment, serving as a bulwark against the emergence of such unwarranted conditions (e.g., oral infections, and periodontal diseases).
5. In the future, researchers should use culture methods and nested polymerase chain reaction technology to isolate and diagnose oral parasites.
6. The use of hydrogels is not only in the delivery of treatment in the field of orthodontics but in other applications.
7. Regular Monitoring and Assessment of Oral Health in Orthodontic Patients: Implementing a structured schedule for

oral health assessments could help in the early detection and management of parasitic infections.

8. Educational Programs for Orthodontic Patients: Developing educational materials and programs about the importance of oral hygiene and the risks associated with orthodontic appliances could increase patient awareness and compliance.
9. Collaboration Between Dentists and Microbiologists: Encouraging interdisciplinary collaboration could enhance the understanding and management of oral parasites in orthodontic patients.

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Appendices

Appendix I: Questionnaire template that is used in this study.

Oral parasites study questionnaire

Residential area: =	☐ male ☐ Female	Age:

Education level

Name	The level
	uneducated ☐ primary education ☐ medium education ☐ high school education ☐ institute education ☐ university education ☐ postgraduate ☐
University:	

Do you suffer from chronic disease?

diabetic ☐ heart disease ☐ liver problems ☐ no ☐

other disease ☐ blood pressure ☐ asthma ☐

Do you suffer from Gingivitis?

severe ☐ moderate ☐ mild ☐ Healthy mouth ☐

Do you brush your teeth?

:how many times yes ☐ No ☐

Do you use Floss?

yes ☐ No ☐

Do you use mouth wash?

Yes ☐ No ☐

Do you suffer from toothache?

yes ☐ No ☐

Do you suffer from Dacey?

how many teeth: yes ☐ No ☐

Are there calcifications?

☐ strong ☐ medium ☐ simple ☐ No

Tooth type?

scattered ☐ Overlapping ☐ ☐ equal

Do you wear braces?

yes ☐ no ☐

Type of braces?

removable ☐ fixed ☐

are you smoking?

Yes ☐ No ☐

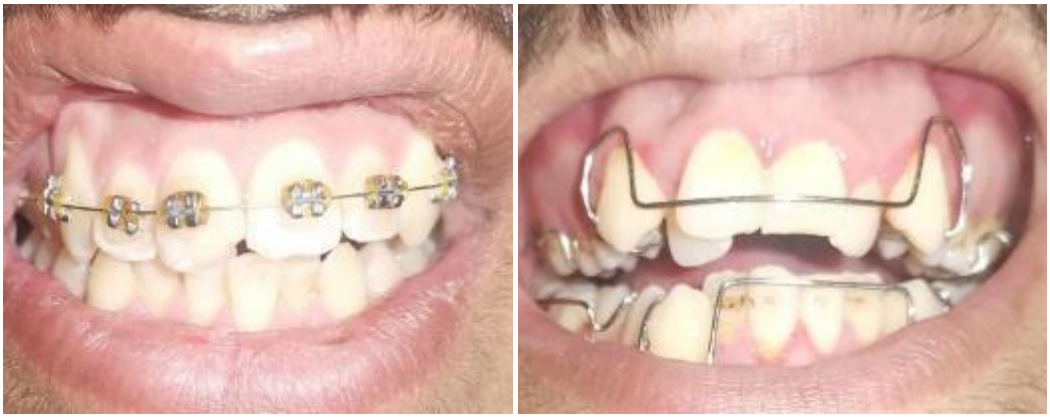
If female, are you pregnant?

Yes ☐ No ☐

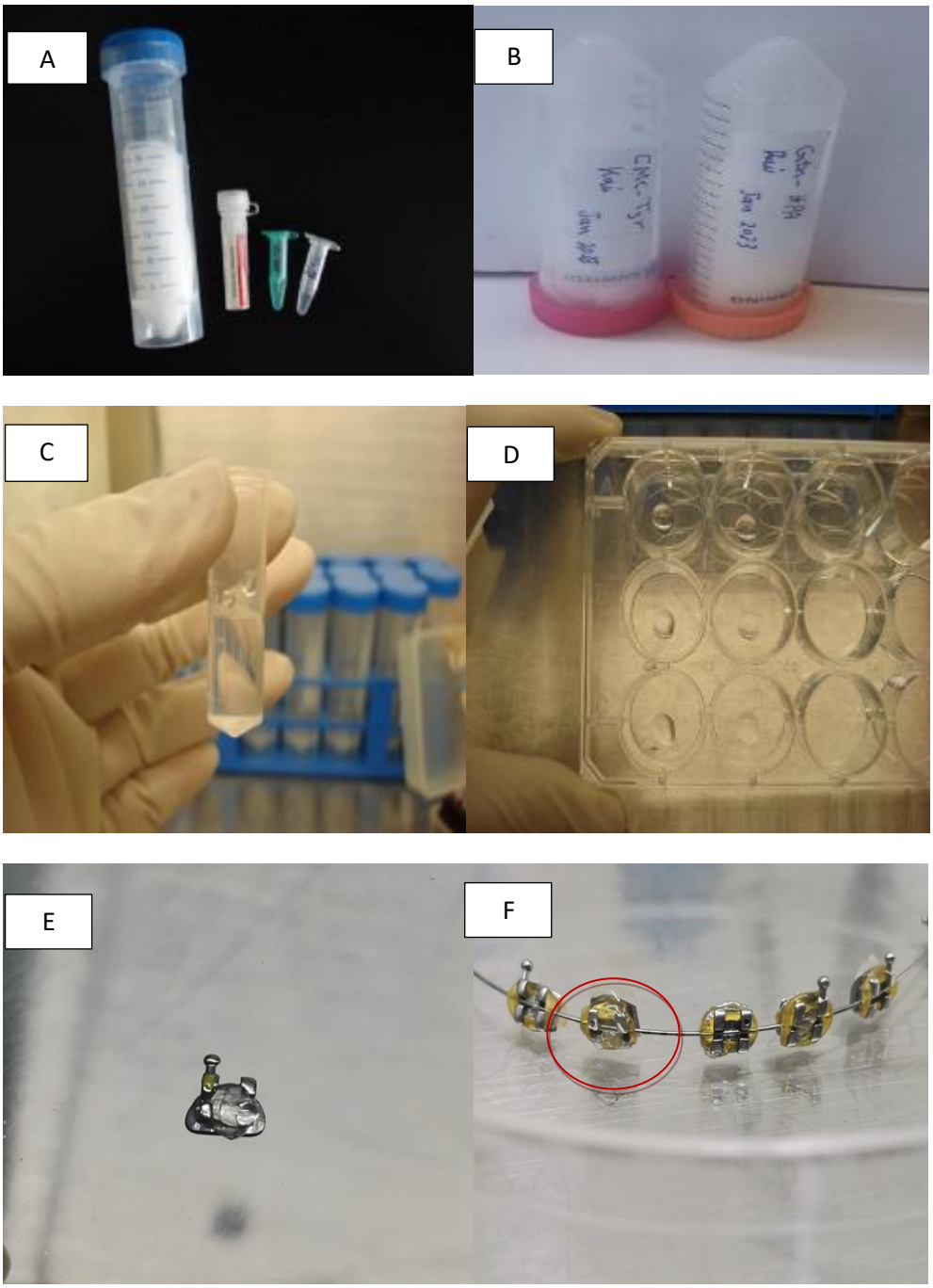
Have you been taking antibiotic during the bast three months?

what is it? ☐ yes no ☐

Appendix 2: Sample collection from orthodontic patients in this study.



Appendix 3: A: main materials needed, B: Gtn-HPA and CMC-Tyr hydrogel, C: due to rapid gelation, HRP and H2O2 are placed by the side of Eppendorf tube before mixing, D: the hydrogel disks, E: the hydrogel with drug injectable in a single brace, F: the orthodontic with hydrogel.



Appendix 4: Preparation of SEM Samples:

For Scanning Electron Microscopy (SEM) investigations, three different concentrations of hydrogel precursors (10%, 15%, 20%) were polymerized using HRP and H2O2, once the hydrogels gelled on microscope slides, were frozen rapidly by plunging into liquid nitrogen (approximately (196ºC) and then freeze-dried for 2 days for vacuum-based analysis. Additional aliquots for high resolution imaging were stained with osmium tetroxide (OsO4).

الخلاصة:

Trichomonas tenax (*T. tenax*) و *Entamoeba gingivialis* (*E. gingivalis*) نوعان من الاوليات اللاهوائية ، يقيمان في تجويف الفم البشري. في الآونة الأخيرة ، كانت هناك حاجة لاستخدام تقويم الأسنان ، على الرغم من الفوائد التي يوفرها ، ولكن لا يمكن التغاضي عن الأضرار التي يسببها تقويم الأسنان. يساعد تقويم الأسنان على تراكم البلاك كما أنه يعيق عملية تنظيف الفم ، لذا فإن سوء النظافة يزيد من الإصابة بالطفيليات الفموية. لعلاج هذه الطفيليات الفموية في مرضى تقويم الأسنان ، تم تطوير مادة ذكية تعتمد على الهيدروجيل كمنصة توصيل العلاج من خلال آلية إطلاق مستدامة ذكية.

يهدف الجزء الأول من الدراسة الحالية إلى تقيم انتشار طفيليات الفم لدى مرضى تقويم الأسنان مقارنة بالأفراد الذين لا يخضعون لتقويم الأسنان بواسطة المجهر واستخدام طريقة الزرع للكشف عن طفيليات الفموية لتأكيد ما تم تسجيله سابقا. يهدف الجزء الثاني من الدراسة الحالية هو فحص السلالات السائدة للطفيليات الفموية في محافظة ميسان ، باستخدام تقنية تفاعل البلمرة المتسلسل المتداخل للكشف عن الطفيليات الفموية, إجراء تسلسل الحمض النووي للكشف عن سلالات من النوعين *E. gingivalis* و *T. tenax*. يركز الجزء الثالث من البحث على تطوير مادة ذكية قائمة على الهيدروجيل كمنصة توصيل الأدوية الموضعية والغرض من ذلك هو معالجة الطفيليات الفموية لدى مرضى تقويم الأسنان من خلال استخدام آلية تحرر مستمره وذكية.

تضمنت الدراسة 200 مشارك منها 100 (76 إناث و 24 ذكر) مريض تقويم أسنان و 100 (75 إناث و 25 ذكر) مشاركين بدون في تقويم الأسنان. جمعت ثلاث عينات لكل مشارك ، للفحص المسحة الرطبة وطريقة الزرع واستخدام تقنية تفاعل البوليميراز المتسلسل المتداخل. في وقت لاحق ، تم تحضير الهيدروجيل ، وتم مزجه مع علاج الميترونيدازول ، من أجل مراقبة وقت التحرر وتركيز الدواء تم تحديدها باستخدام مقياس الطيف الضوئي المرئي للأشعة فوق البنفسجية، عند الطول الموجي للميترونيدازول 320 نانومتر. تم تحضير ثلاثة تراكيز مختلفة من الهيدروجيل: 10 و15 و20% (وزن/حجم) وتم قياس تحرر الدواء. للكشف عن أي ارتباط كبير بين الإصابة بالطفيليات وتطبيقات تقويم الأسنان ، تم إجراء مربع كاي Chi-square واختبار F-test و كانت على الأرجحية عند مستوى دلالة $p \leq 0.01$.

كانت معدلات الإصابة بهذه الطفيليات أعلى في مرضى تقويم الأسنان ، حيث بلغت الإصابة ب *E. gingivalis* ٪47.0 فقط في مرضى تقويم الأسنان ، بينما كانت 25.0٪ في مجموعة الضابطة. كانت نسبة الإصابة بـ *T. tenax* 2.0 ٪ فقط في مرضى تقويم الأسنان بينما كانت 1.0٪ في المجموعة الضابطة. كانت معدلات الإصابة لكلا الطفيلين (*E. gingivialis* و *T. tenax*) الأعلى في مرضى تقويم الأسنان بنسبة 19.0٪ و 16.0٪ في المجموعة الضابطة. وجدت الدراسة علاقة معنوية بين الاصابة بالطفيليات وتطبيقات تقويم الأسنان عند مستوى دلالة $P < 0.01$.

توضح النتائج أن تركيز الهيدروجيل هو عامل حاسم يؤثر على التركيب المسامي للهيدروجيل كما هو موضح في المجهر الماسح الالكتروني. يشير التحرر التراكمي للدواء إلى أن الدواء ينتشر من شبكة الهلاميات المائية ويبدو أن الإطلاق يتم التحكم فيه. من خلال التخطيط لتحرر الدواء خلال الساعات الثماني الأولى كدالة للجذر التربيعي للوقت، تم الحصول على مخطط خطي يشير إلى تحررمن الدرجة الأولى نموذجي لانتشار فيكيان، يليه تحررمستمر. من المحتمل أن يلعب التحلل التدريجي لشبكة الهيدروجيل دورًا حيويًا في تحررالدواء. والأهم من ذلك، لم يلاحظ أي تحررانفجاري. بعد 14 يومًا، تم إطلاق (9 – 10)% من الدواء المدمج من أقراص تركيز هيدروجيل 10%، و(3 – 4)% من هيدروجيل 15%، و(1 – 2)% من قرص هيدروجيل 20%. كانت هناك اختلافات كبيرة ملحوظة في نسب التحررالتراكمي بين هيدروجيل 10% وكلا من هيدروجيل 15% و20% ($p<0.01$)، ولكن هناك اختلافات صغيرة فقط (ليست ذات دلالة إحصائية) بين الهيدروجيل 15% والهيدروجيل 20%.

وزارة التعليم العالي والبحث العلمي

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كلية العلوم

قسم علوم الحياة

الهيدروجيل المحمل بالميترونيدازول كنظام إيصال دوائي واعد لعلاج بعض طفيليات الفم في مرضى تقويم الأسنان

دراسة مقدمة الى

مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير في علوم الحياة

من قبل الطالبة

إيلاف مشتاق طالب

بكالوريوس علوم الحياة/ كلية العلوم

جامعة ميسان 2020

بأشراف

أ.م.د. اسوان كاظم جبر

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