Effect of silver nitrate incorporation into heat polymerized acrylic resin on bacterial activity and some mechanical properties

A thesis

submitted to the council of the College of Dentistry at the University of Baghdad, in partial fulfillment of the requirements for the degree of Master of Science in Prosthetic Dentistry

By
Ola Khaleefah Ahmed AL-Husayni
B.D.S

Supervised by
Prof. Dr. Nabeel Abdul Fatah
B.D.S., M.Sc.

2013 A.D 1434 A.H

بِسمِ ٱللَّهِ ٱلرَّحمنِ ٱلرَّحِيمِ

ن والقَلَمِ وَما يَسْطُرونْ*ما أَنْتَ

بِنِعمةِ رَبكَ بِمَجنونْ * وإِنَّ لَكَ

لأجراً غَيرَ مَمْنون*وإنك لَعلَى

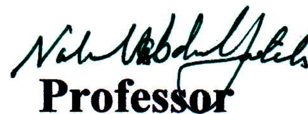
خُلُقٍ عظيم*

صَدَق اللهُ العليُ العَظيم

سوره القلم الايه(1-4)

Declaration

This is to certify that the organization and the preparation of this thesis have been made by the graduated student **Ola Khaleefah Ahmed** under my supervision at the Collage of Dentistry, University of Baghdad in partial fulfillment of the requirement of the Master degree of Science in Prosthodontics.



Professor

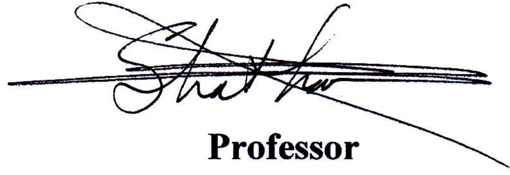
Dr.Nabeel Abdul Fatah

B.D.S., M.Sc.

Supervisor

Committee Certification

We, the members of the examining committee, certify that after reading this thesis and examining the graduated student **Ola Khaleefah Ahmed** in its contents, and that in our opinion it meets the standard of thesis for the **degree of Master of Science in Prosthodontics.**



Professor

Dr. Shatha S. Alameer

B.D.S., M.Sc.

(Chairman of the examining committee)

Assistant Professor

Dr. Intisar J. Ismail

B.D.S., M.Sc., Ph.D.

(Member)

Assistant Professor

Dr. Eman N. Nagi

B.Sc., M.Sc., Ph.D.

(Member)

Approved by the council of the College of Dentistry, University of Baghdad

Professor

Dr. Nabeel Abdul Fatah

B.D.S., M.Sc.

(Dean of the College of Dentistry)

الأهداء

إلى منبع كلي و بعضي........,نبضي و أرضي ....

إلى من تنزف....... فتكابر....... فتداري كل جراحها ....

بغداد................................حفظها الله

إلى الأمر الجلل..........شمعة الأمل.......... التي نورت حياتي.........

أُمي....................................حفظها الله

إلى من بصبره............و سنين حياته..............عطَر حياتي.............

أبي...................................حفظه الله

إلى ياقوت عمري...... و شد أزري.......... ومنبع قوتي وصبري......

ليث خالد نادين رسل عبدالله ....حفظهم الله

إلى كل من آَزر ....و أرشدني أولا وآخر.....و دعا بسر وجهرا.....

لكل هؤلاء أهدي ثمرة عملي

عُلا

ACKNOWLEDGMENTS

Praiseand thanks to **ALLAH HIS MAJESTY** *for his uncountableblessing, I must not forget his kindness and remember always his mercy for us that give me a Great Spirit trust in my life, peace be upon* **Mohammed** *the messenger of* **Allah** *and upon his Relatives.*

I would like to express sincere appreciation to Ministry of High Education and Scientific Research for providing me this opportunity for specialization.

I wish to express sincere thanks andhigh appreciation to Prof. Dr. Nabeel Abdul Fatah, Dean *College of dentistry, University of Baghdad for his continuous supports to postgraduate students.*

I would like to express my sincere gratitude, faithful appreciation and deep respect to my supervisor Prof. Dr.Nabeel Abdul Fatah for the project suggestion, guidance,kindness,continuous advice and encouragement.

I would like to acknowledge with gratitude Assist. Prof .Dr.GhassanAbdulhamed AL-Taai, Head Department of Prosthodontics for his help and support.

Special thanks to Mrs.NahedhaAbdRadhi, Basic Science department, College of Dentistry,MustansiryahUniversity, Dr.KifahA.Jasim, Head Department of Zoonotic disease,Health Laboratory Center.Prof. Dr Abbas AL Mizraqchi,Assist. Prof. Dr.Ghada T. Ibrahim,Assist. Prof. Dr.BatoolHassan AL-Ghurabi,Assist. Prof. Dr.Ghada T. Ibrahim, Dr.Heba Fathil, Microbiology Department, College of Dentistry, Baghdad Universityfor their generous help and invaluable advice.

My sincere thanks toProf. Dr. Ammar AL-Djeli,Head Department of the polymer researches unit, and Ass. Prof.Dr.IssamLatif, Department of Chemistry, College of Education-Ebn Al Haythm, Baghdad University, for thier scientific suggestions and constant advice in conducting this work.

Great admire and high appreciation toAssist. Prof. Dr. Intisar Jamil Ismailfor her help, being ready for cooperation all the time I need her and support.

Grateful thanks toDr. IntidharJ.Mohammed, College of Pharmacology, BaghdadUniversity, for her great assistance, appreciable advice and devoted help and to Miss.HudaJ.Mohammed Department of Bacteriology, College of Pharmacology, BaghdadUniversity, for her kind help.

I would like to express my sincere thanks to Prof. Dr. Shatha S. Alameer, Prof. Dr. Widad Al-Nakkash,Assist. Prof. Dr. BasimaM.A.Hussein,Assist. Prof. Dr. ThekraIsmailand Assist. Prof. Dr. Amir.M.Khamasfor their kind help and advice.

My deep thanks and gratitude to the Iraqi Center for Cancer and Medical Genetic Research, my profound thanks to Dr.Aiman AL-Bayati , Experimental therapy department ,for her help,inetrest,valuable advice and continuous support.

I gratefully acknowledge the help given by all staff member of Spectroscopy Section specially,Miss. JenanH. Mohamed, Head spectroscopy section, Mr. Salam, and Dr.FlayehHasan,Head Department of Chemical Laboratory Diagnosis and therapy for their kindness and help.

Grateful thanks due to all staff members of Bacterial diagnostic unit, Training Laboratories, Medical city (Baghdad), specially Mr. HadiAned, Mrs.Shamam, Haidar, Dr. Israa Adel, Dr. Mohammed Ayad for their kind cooperation and help.

I wish to express my grateful gratitude to Prof. Dr.Harith Ibrahim Physics Department ,College of Science ,Baghdad University ,Dr. MofeedA.Jaleel ,Department of Applied Science ,University of Technology ,Mr. MoaadFoaad, Dr.KasimAbidSaloom,Head Department of Nano metrical Composites, Ministry of Science and Technology,and special thanks to his members Mr .Ali Hadi,MrAubad,Mr. ThaarHadi for their helpful and collaboration .

Sincerely I full agreat urge to present my deepest thanks toDr.Aula KamalRafeeq,Dr.IhabNabeel Safi, Dr.MithaqueRadhi Mohammed, andDr.Assel Abdul Ameer,for theirappreciable help, advice and support.

I would like to thanks Dr.Tuqa Akram,Dr.Rasha Naji,Dr.SanaaRasheed, Dr.Mustafa Mahdi, Dr.Duraid Mohammed, and Dr.Salwan Sami for their kindness and help.

I gratefully acknowledge the helpfrom Mr. Mustafa Khudhair AL- Bayati, Ghasi AL- HareriHospital, Chemical Laboratory Unit, for his kind cooperation and help.

Word of appreciation further extended to all dear friends, Colleagues and Mr. duraidTechnician, Department of Prosthdontics, College of Dentistry, University of Baghdad, who had afford much help.

Deep respect and love to my family specially my father Prof.Dr.Khalifa Ahmed ,my mother Assist .Prof .Souad AL-Bayati ,for being so generously of their time ,their best informed guidance ,constructive remarks ,esteemed advice ,encouraging attitude to overcome the difficulties, and most specially I would thank my lovely brothers Dr.lith ,Dr.Khalid and his wife Dr. Nadin for their effort in progressing the work ,encouragement and generous support.

My hot hugs to my lovers Russul and Abdulla for their patient and duaa'a.

With my love

Ola

Abstract

Recently various inorganic antibacterial materials containing silver have been developed with an effort to be colorless, chemically stable and durable materials which slowly release the silver ions for long period aiming to be used successfully as antimicrobial (medical and dental) biomaterials that can prevent caries and infection of implants.

Different concentrations of silver nitrate(9.375, 15, 30, 60, 120, 150, 300, 600 and 900ppm) were prepared from stock solution of 1000ppm silver nitrate. The Rat Embryonic Fibroblast was exposed to tested concentrations of silver nitrate to evaluate the cytotoxic effect of this material. The Poly methyl methacrylate acrylic resin denture base material was prepared in accordance with the manufacturer's instructions and the tested silver nitrate solution was added to the acrylic resin powder and monomer in a fixed volume(0.2ml). Controls devoid of silver nitrate were included.The specimens for antibacterial experiments and silver ion release study were stored in artificial saliva at 37°C incubation for 30and 90 days. The specimens prepared for antibacterial experiments were tested at baseline (with no treatment with artificial saliva), 30and 90 days of immersion artificial saliva using *mutans streptococci*group as tested microorganism .while,for silver release detection, each solution of artificial saliva for the all tested groups was analyzed using atomic absorption spectrophotometer). Mechanical tests (impact strength, transverse strength, tensile strength)were done for theprepared silver nitrate–loaded resins.

The results showed that the cytotoxic test of silver nitrate concentrations range from 9.375 to 900ppm on rat embryo fibroblastcells was70.8-82.9% inhibition.At baseline, the antibacterial efficacy of

achieved silver nitrate– loaded resin containing 9.375 to 900ppm silver nitrate was 76.7 to 96.6 %. While, after 30 or 90 days immersion there was total inhibition of bacterial growth (antibacterial efficacy = 100%). Silver was not detected in artificial saliva even after 90days of immersion. Fourier transform infra-redconfirmed thatthere was no chemical bond between the Poly methyl methacrylate and silver nitrate.There wasinsignificant increasing ($P=0.05$) in impact strength observed when compared with control group. In transverse strength test, significant reduction was shown when compared with control($P<0.001$). While for tensile strength there was insignificant reduction with 9.375($P=0.05$NS) and 15($P=0.42$NS) ppm silver nitrate.However it was significant above 15 ppm ($P<0.001$). Darkening ofsilver nitrate-loaded resins was shown to be started with concentration of silver nitrateof 300ppm and above.

In conclusion, incorporation of silver nitrate in the acrylic resin was evidenced. Moreover, silver was not detected by the high detection limit of the atomic absorption spectrophotometer used in this study, even after 90 days of storage in artificialsaliva.Furthermore,silver was also not detectedspectrophotometrically in deionized water as different storage media. Silver nitrate is incorporated in the Poly methyl methacrylate denture resin to attain an effective antimicrobial activity to help control common infections involving teeth, and oral mucosal tissues in denture wearers.

List of contents

Subject	Pages
Acknowledgment	I
Abstract	IV
List of contents	VI
List of table	XI
List of figures	XII
List of abbreviation	XV
Introduction	1
Aim of the study	3
Chapter one: Review of Literature	
1.1 polymers	4
1.1.1 Basic nature of polymers	4
1.1.1.1 Chemical composition	4
1.1.1.2 Molecular weight	5
1.1.1.3 Spatial structure	6
1.1.1.4 Chain configuration	6
1.2 Preparation of polymers	7
1.2.1 Addition polymerization	8
1.2.1.1 Initiation	8
1.2.1.2 Propagation	9
1.2.1.3 Termination	9
1.2.2 Condensation polymerization	10
1.3 Denture Base Resins	11
1.4 Composition and structure of acrylic resin	12
1.4.1 Physical form of acrylic resin	12

1.4.2 Chemical composition of acrylic resin	12
1.4.2.1 Liquid (monomer)	12
1.4.2.2 Powder (polymer)	14
1.4.3 Mixing Ratio	15
1.5 Heat activation polymerization reaction of acrylic denture base resin	16
1.6 Antimicrobial polymers	17
1.7 Antimicrobial agent (Silver Nitrate)	18
1.7.1 Silver in medicine	19
1.7.2 Silver in dentistry	21
1.7.3 Application in Medical Tools	24
1.8 Silver exposure	25
1.8.1 Oral exposure	26
1.8.2 Dental exposure	24
1.9 Silver safety	28
1.10 Some mechanical properties for heat –polymerization acrylic resin	31
1.10.1 Flexural strength	31
1.10.2 Tensile strength	32
1.10.3 Impact strength	33
1.11Organisms associated with dental caries	34
1.11.1 Cariogenic bacteria	35
1.12 Bactericidal mechanism of silver	37
Chapter Two: Material& Methods	
2.1 Materials	39
2.1.1.1 Equipment and materials required for cytotoxicity test	39
2.1.1.1 Laboratory equipment and instruments	39
2.1.1.2 Solutions and chemicals	40

2.1.1.3 Tissue culture media	40
2.1.1.4 Rat embryo fibroblast (REF) cell line used for cytotoxic study	40
2-1-2 Equipment and materials required for testing the antibacterial activity of $AgNO_3$ – loaded resins on the growth of *mutans streptococci* group.	41
2-1-2-1 Equipment and Supplies	41
2-1-2-2Solutions, chemicals, and materials	42
2-1-3 Equipment and materials required for *in vitro* Ag release test	44
2-1-3-1 Equipment and Instruments	44
2-1-4 Equipment and materials required for preparation of $AgNO_3$-loaded resins determined for testing some mechanical properties.	44
2-1-4-1 Equipment and Instruments	44
2-1-4-2 Materials	45
2-2 Methods	46
2-2-1 Cytotoxicity of different concentrations of silver nitrate solutions on Rat Embryo Fibroblast (REF) cell line:	47
2-2-2 Preparation of silver nitrate ($AgNO_3$) concentrations for studying samples.	48
2-2-3 Preparation of $AgNO_3$ - loaded resins for testing the antibacterial activity and in vitro Ag release test.	50
2-2-3-1 Mould preparation for fabrication of $AgNO_3$– loaded resins.	50
2-2-3-2 Proportioning, mixing, packing and curing of acrylic resin	51
2-2-3-3 Finishing and Polishing	53
2-2-4 Preparation of culture media for antibacterial activity assay	54
2-2-4-1 Mitis- Salivarius Bacitracin Agar (MSB Agar)	54
2-2-4-2 Brain Heart Infusion Broth (BHI-B)	55
2-2-4-3 Mueller Hinton Agar (MHA)	55
2-2-4-4 CT- Mannitol Agar (CystineTrypticase- Mannitol Agar):	55
2-2-5 Isolation and Purification of mutans streptococci	56

2-2-5-1 Collection of stimulated saliva samples	56
2-2-5-2 Isolation of microorganisms	56
2-2-5-3 Identification of *mutans streptococci*	57
2-2-5-4 Morphological Examination of the microbial Cells	57
2-2-5-5 Biochemical Tests	57
A - Catalase Production Test	57
B - Carbohydrate Fermentation Test for *mutans streptococci*	58
2-2-5-6 Purification and Maintenance of the microbial Isolates	58
2-2-6 Activation of inoculums	59
2-2-7 The antibacterial activity assay	59
2-2-7-1 Determining the antimicrobial activity of different concentrations of silver nitrate solution on the growth *mutans streptococci*	59
2-2-7-2 Inhibitory effects of $AgNO_3$ – loaded resins on the growth of *mutans streptococci*:	60
2-2-7-2-1 Estimation the inhibition zone of $AgNO_3$ – loaded resins	60
2-2-7-2-2 Effect of $AgNO_3$ - loaded resins on viable count of *mutans streptococci* colonies group	60
2-2-8 *In vitro* Ag release test	61
2-2-9 Characterization of $AgNO_3$-loaded resins	64
2-2-10Mechanical tests	65
2-2-10-1 Preparation of test specimens	65
2-2-10-1-1 Pattern preparation	65
2-2-10-1-2 Mould preparation/ proportioning, mixing, packing, finishing and polishing of $AgNO_3$ -loaded resins	66
2-2-10-2 Mechanical tests	66
2-2-10-2-1 Impact strength test	66
2-2-10-2-2 Transverse strength	68
2-2-10-2-3 Tensile strength test	70

2-3 Statistical analysis	72
Chapter Three : Results	
Results	73
3.1 Cytotoxicity test	74
3.1.1 Effect on cell morphology	74
3.1.2 Cell viability	75
3.2 Visual inspectionofof$AgNO_3$ –loaded resins	78
3.3 Identification of microorganisms	78
3.3.1 Identification of *mutans streptococci*	78
3.3.1.1 Colony morphology	78
3.3.1.2 Morphological examination of the microbial cells	79
3.3.1.3 Biochemical test	80
3.4 Antimicrobial activity of $AgNO_3$ on the growth of *mutans streptococci*	81
3.4.1 Determining the level of antimicrobial activity of different concentrations of silver nitrate solution on *mutans streptococci*	81
3.4.2 Inhibitory effect of $AgNO_3$ –loaded resins on the growth of *mutans streptococci*	84
3.4.2.1 Estimation the inhibition zone of $AgNO_3$ –loaded resins	84
3.4.2.2 Effect of $AgNO_3$ –loaded resin on viable counts of *mutans streptococci* colonies.	85
3.5 *In vitro* Ag release test	95
3.6 Characterization of $AgNO_3$ –loaded resin	97
3.7 Mechanical tests	100
3.7.1 Impact strength test	100
3.7.2 Transverse strength test	102
3.7.3 Tensile strength test	104
Chapter Four: Discussion	
4.1 Cytotoxicity test	107
4.1.1 Effect on cell morphology	107
4.1.2 Cell viability	107
4.2 Visual inspectionof $AgNO_3$ –loaded resins	108
4.3 Antimicrobial activity of $AgNO_3$ on the growth of *mutans streptococci*	109
4.4 *In vitro* Ag release test	112
4.5Characterization of $AgNO_3$ –loaded resins	115
4.6 Mechanical tests	116
Chapter Five: Conclusions and Suggestions	
Conclusions	119
Suggestions	120

References	121
Appendices	144

List of tables

Table No.	Title	Pages
2.1	Equipment and materials required for cytotoxicity test.	39
3-1	Percent cell inhibition (cytotoxic effect) (%)	76
3-2	Optical density(OD) for cytotoxic effect (nm)	76
3-3	Descriptive data ofdiameter of inhibition zone (mm)	83
3-4	Descriptive data ofinhibitory effect (%)of $AgNO_3$ loaded resin discs on the growth of *mutansstreptococci* group.	86
3-5	Descriptive data ofColony forming unite /ml	91-92
3-6	Descriptive data ofimpact strength test(KJ/m^2)	101
3-7	Descriptive data oftransverse strength test (MPa)	103
3-8	Descriptive data oftensile strength test (MPa)	105

List of figures

Figure No.	Title	Pages
(2-1)	Equipment and materials required for testing the antibacterial activity of $AgNO_3$ – loaded resins: A- Laminar flow, B -Adjustable micropipette, C- Electronic digital caliper , D- Dissecting microscope, E- Sterile artificial saliva.	43
(2-2)	A-Heat cure acrylic resin for denture (Non – veined acrylic, powder and liquid, B- Dental stone **.**	45
(2-3)	A. Microplate with crystal violates, B. ELISA microplate spectrophotometer, C. Inverted microscope with digital camera.	47
(2-4)	Preparation of silver nitrate ($AgNO_3$) concentrations for studying samples : A- The stock solution of $AgNO_3$,B&C Nine Prepared concentrations of $AgNO_3$, D- Atomic Absorption spectrophotometer	49
(2-5)	Metal pattern in dental flask	50
(2-6)	Preparation of $AgNO_3$ - loaded resin discs: A-(T)-shaped stainless steel metal device, (B&C)Cutted acrylic dough into disk like shape , D- Metal flask under hydraulic press , E- Clamped flask.	52
(2-7)	Test specimens with artificial saliva in Test tubes under incubation.	53
(2-8)	Spectrophotometer.	62
(2-9)	Equipment used for *in vitro* Ag release testA-The specimens incubated under agitation, B and C- Atomic Absorption spectrophotometer.	63
(2-10)	Fourier transform infra –red (FTIR) spectrophotometer.	64
(2-11)	Metal patterns of impact strength, transverse strength, tensile strength.	65
(2-12)	Measurements of specimen used for impact strength.	67
(2-13)	A-Impact testing instrument, B- Impact strength testing specimens (pre and post testing).	68
(2-14)	Measurements of specimens used for transvers strength test.	68

(2-15)	A- Instron testing machine, B- Specimen under testing.	69
(2-16)	Dimensions of tensile strength test specimen.	70
(2-17)	A-Tinius Olsen tensile machine for tensile strength test, B- The location of sample tested in the machine.	71
(3-1)	Rat Embryonic Fibroblasts. A- Cells without treatment. B- Cells treated with silver nitrate.	74
(3-2)	Bar chart showedthe mean inhibitory effect of $AgNo_3$ on the growth of REF cells.	77
(3-3)	Bar chart showed the mean optical density for REF cells of control and the study groups of $AgNO_3$.	77
(3-4)	$AgNO_3$–loaded resinsfrom right to left: control, (9.375, 15, 30, 60, 120, 150, 300, 600, 900 ppm).	78
(3-5)	A-Photographic image for MSB agar with MS colonies, B&C-Microscopic image for Different forms of MS colonies on MSB agar.	79
(3-6)	Gram's stain of MS cells showing the *mutans streptococci* (1000x `magnification).	80
(3-7)	Biochemical identification of *mutans streptococci*, a positive reaction indicated by the change in color of indicator from red(A) to yellow(B).	80
(3-8)	Photograph images of the zone of inhibition of $AgNo_3$ solutions.	82
(3-9)	Bar chart showed the mean diameter of inhibition zone (with its 95% confidence interval)for $AgNO_3$ concentrations, positive and negative control.	84
(3-10A)	Bar chart showed the ABE (%) of $AgNO_3$ loaded resin disks on the growth of *mutansstreprococci* group at baseline (pretreatment).	87
(3-10B)	Bar chart showed the mean inhibitory effect of $AgNO_3$ loaded resin disks on the growth of *mutansstreprococci* (%) at the designed periods.	88
(3-11)	Bar chart showed the mean count of CFU/ml at baseline (pretreatment).	89
(3-12)	Photographic image of MSB agar plate with *mutans streptococci*group colonies as control plate.	89
(3-13)	Photographic images of MSB agar plates represent the baselineviable counts of *matans streptococci*group.	90
(3-14)	Photographic image of MSB agar plate represent	93

	the viable counts at 30 or 90days immersion in artificial saliva, there was complete inhibition of bacterial growth.	
(3-15)	Bar chart showed the mean count of CFU/ml (with its 95% confidence interval)at the three designed periods.	94
(3-16)	A-absorption spectrum found for the artificial saliva with control acrylic specimen (without silver nitrate), B- Spectrum of one of the specimens which was similar for all other specimens. Spectrum clearly shows similar absorption as that of the control.C- shows similar clear solution of artificial saliva (no precipitation of silver halide) only the tested $AgNO_3$ – loaded resins at the bottom of the test tubes. .	96-97
(3-17 A,B,C,D)	FTIR spectrum of A- PMMA and B-$AgNO_3$-loaded resininKBrdisc andCaesiumIodid.	98-99
(3-18)	Bar chart showed the mean impact strength for the concentrations of $AgNO_3$ and control group.	102
(3-19)	Bar chart showed the mean transverse strength for concentrations of $AgNO_3$ and control group.	104
(3-20)	Bar chart showed the mean tensile strength for concentrations of $AgNO_3$ and control group.	106

List of Abbreviations

Abs Absorbance

AGC AGC Flat Glass Europe's AntiBacterial Glass^{TM}

ATP Adenosine triphosphate

BHI-B Brain Heart Infusion Broth

CFU/ml Colony forming unite per milliliter

CSP Colloidal silver preparation

CTA Cystine Trypticase Agar

FTIR Fourier transform infra-red

G_2/M Gap2/ Mitosis

IU International unit

KJ/m^2 Kilo joule per square meter

MHA Mueller Hinton Agar

MPa Mega Pascal

MSB Mitis- Salivarius Bacitracin agar.

nm nanometer

OD Optical density

P/L Powder liquid ratio

PMMA Poly methyl methacrylate

ppm Part per million

REF rat embryo fibroblast

TYC Trypticase yeast extract cystine

µl Microliter

μm	Micrometer
μg	Microgram
d	daily
CSP	Colloidal silver preparation
WHO	World health organization
ppb	part per billion

Introduction

Littleinformation is available about the impact of silver nitrate into heat polymerized acrylic resin to show the effect on the growth of *mutans streptococci* .So this prompted us to shed light on this research.

There is a need for effective broad -spectrum antimicrobial resin materials in dentistry; it is well-known that removable denture bases fabricated from heat-polymerized acrylic resinsmay act as a reservoir for microorganisms andcontribute to re-infection in denture wearers **(keng and Lim, 1996)**. For elderly and institutionalized patients with limited motorskills and special needs, this treatment is further complicated becauseof some factors such as loss of memory, difficulty in rendering appropriate cleaning for their oral cavities. Fifty-sixty percent rate of dental caries occursafter restoration treatment **(Fan*et al.*, 2011**)or in areas around orthodontic brackets bonding agents where effective tooth brushing is difficult **(Major, 1996)**.

Unfortunately, current standers of treatment such as the use of antimicrobial mouthwashes, proper –tooth brushing technique have limited success or side -effects due to problems with patient compliance and the development of antibiotic resistance strains of bacteria. Thus a broad- spectrum antimicrobial resin is needed **(Fan*et al.*, 2011)**.

Silver ions have been reported to inactivate important enzymes and affect the application mechanism of the DNA in bacteria.Ag ions have been reported to attach to the outer membrane and affect the permeability as well as induce structure changes in the cell – ultimately leading to cell death .In addition, Agdoes not cause resistant bacterial strains to develop **(Russell and Hugo, 1994; Lansdowne*et al.,* 2007)**.

For dental application ,the development of other methods of drug elution, such as Ag-Zeolite and SiO_2 filler were incorporated into urethane acrylic monomer in different amount to develop a new temporary filling materials with antibacterial activity against some oral bacterial growth(**Hottal*et al.*,1998)** ,silver containing materials like Novaron,Amenitopand AIS were incorporated into light activated resin composites attended to decrease the frequency of secondary caries around the restorations (**Yoshida *et al.*, 1999**) , The sol-gel derived silica glass powders containing silver are believed to be useful as an antibacterial material for medical applications such as filler of composite resin for dental restoration **(Kawashita*et al.*,2000)**,and the incorporation of nanometer-sized silver-supported antimicrobial agentinto denture base materialsto investigate the distributionand to study the release mode of silver ions from the base**(Casemiro*et al.*,2008; Yu *et al.*,2008)**.

Aim of the study

The aim of the present study is to evaluate the effect ofaddition of silver nitrate ($AgNO_3$)to poly methyl methacrylate indifferent concentrations (9.375, 15, 30, 60,120,150,300,600 and 900 ppm) through:

1. Examining the effectiveness of antimicrobial activity of $AgNO_3$ on the growth of clinically isolated *mutans streptococci*groupthrough: determining the level of antimicrobial activity of different concentrations of silver nitrate solution on the growth of*mutans streptococci*groupand determining the inhibitory effect$AgNO_3$– loaded resinat different periods of immersion in artificial saliva.

2. Testing silver release from PMMA at different timesof immersion in artificialsaliva (T_0= Baseline, $T_{1=}$30day, T_2=90 day).

3. The effect of this additive on impact strength, transverse strength, and tensile strength of $AgNO_3$ – loaded resin.

CHAPTER ONE

REVIEW OF LITERATURE

1.1 Polymers

Plastics and rubbers, as they are generally called in everyday life, have the common property of being polymers. Polymers are long chain molecules, consisting of many repeating units called mers, the name polyethylene is derived from the word “Ploy” meaning many and the basic structure unit on which it is based, ethylene, examples of naturally occurring polymers such as agar, cellulose, DNA, proteins, natural rubber, collagen and silk. The synthetic polymers, which are now everyday household names, are PVC (Polyvinyl chloride), polyethylene, nylon and poly styrene. The most common polymers are those made from the organic compounds of carbon, but polymers can also be made from inorganic compounds, based on silica (SiO_2) **(Noort, 2007).**

1.1.1 Basic Nature of Polymers

1.1.1.1 Chemical composition

The starting material for the production of a polymer is the monomer, Monomers are the molecules that units to form a polymer, and the process by which this occurs is termed polymerization. Methyl methacrylate, a common denture base resin monomer, can form poly (methyl methacrylate). If monomers of two or more different types are joined or in which two more homopolymer are chemically combined, copolymers are formed, copolymers may be either random (mers do not appear in specific order) or block (large number of one type of mer appear arranged in sequence). The block copolymer is composed of relatively long sequence different copolymer segments chemically linked to form a linear molecule. The graft

copolymer is comprised of a linear “back home” polymer molecule containing long braches or (grafts) of a mother polymer species **(O’Brien, 2002).**

1.1.1.2 Molecular weight

The molecular weight of the polymer molecule, which equals the molecular weight of the various mers multiplied by the number of mers, may range from thousands to millions of molecular weight units depending on the preparation conditions. The molecular weight of a polymer is reported as the average molecular weight because the number of repeating units may vary greatly from one molecule to another. As would be expected, the fraction of low-, medium- , and high molecular –weight molecules in a material or, in other word, the molecular weight distribution has a pronounced an effect on the physical properties, therefore, two poly (methyl methacrylate) specimens can have the same chemical composition but greatly different physical properties because one of the specimens has a high percentage of low- molecular –weight molecules , whereas the other has a high percentage of high- molecular –weight molecules. Variation in the molecular weight distribution may be obtained by altering the polymerization procedure for example, the higher the molecular weight, the higher the softening and melting points and the stiffer the polymer **(Power and Sakaguchi, 2006).**

1.1.1.3 Spatial structure

The physical or spatial structure of the polymer molecule is also important in determining the properties of the polymer. There are three basic types of structure: linear, branched, cross- linked.

The liner and branched molecules are separate and discrete, whereas the cross- linked molecules form a network structure that may result in the polymer's becoming one gaint molecule.

The spatial structure of polymers affects their flow properties. In general, the cross- linked polymers flow at higher temperatures than liner or branched polymers and does not absorbed liquids as readily as either the linear or branched materials**(Power and Sakaguchi, 2006).**

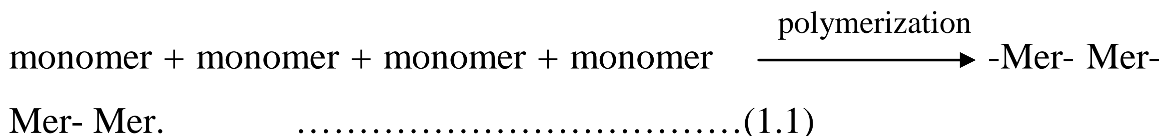
1.1.1.4 Chain configuration

Polymer chains are held together by secondary (or Vander Waal's) bonds, and by entanglement the chain if they are sufficiently long. The higher the molecular weight, the more entanglement, there will be, giving a stiffer and stronger polymer. In a polymer such as polyethylene, which has a linear chain configuration, the weak bonds between the chains can easily be broken by increasing the temperature of the polymer. When this happens the chains can flow past one another so that the polymers soften and readily deforms. On cooling the bonds are re- established, and the polymer becomes hard again, and retain it shape at higher temperature. The temperature at which a plastic softens such that the molecules can begin to flow is defined as its glass transition temperature. They are similar to those for glasses, except that the temperature involved are much lowers in the case of plastics.

A polymer that can be softened and subsequently shaped by heating it above its glass transition.Temperature is known as a thermoplastic polymer, examples of such thermoplastic polymers are polystyrene, polymethyl methacrylate and polyethylene. Those polymers may be softened by heating and solidify on cooling, the process being repeatable. The term thermosetting refers to polymers that solidify during fabrication but cannot be softened by reheating, these polymers decompose on heating without showing a glass transition, typical examples are silicones, and cross- linked PMMA, bis phenol A diacrylates, cis- polyisoprene**(Power and Sakaguchi, 2006; Noort, 2007).**

1.2. Preparation of polymers

Polymers are prepared by a process called polymerization, which consist of monomer units becoming chemically linked together to form high molecular – weight molecules as in the equation**(Noort, 2007).**



monomer + monomer + monomer + monomer $\xrightarrow{\text{polymerization}}$ -Mer- Mer- Mer- Mer. ………………………………(1.1)

The polymerization process may take place by several different mechanisms, but most polymerization reaction fall into two basic types: addition polymerization and condensation polymerization. Important addition polymerization reaction are free- radical, ring- opening and ionic reactions **(Power and Sakaguchi, 2006; Anusavice, 2008).**

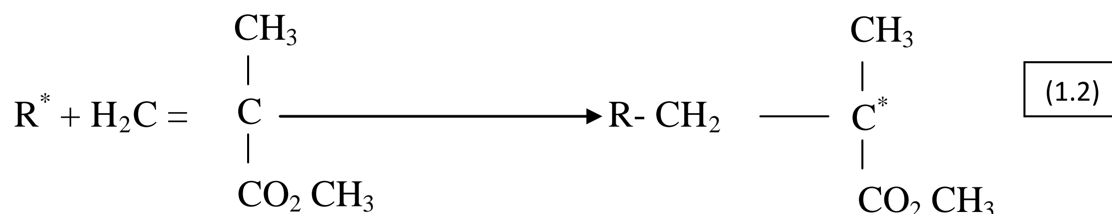
1.2.1 Addition Polymerization

Most dental resins are polymerized by a mechanism in which monomers add sequentially to the end of a growing chain. Addition polymerization start from an active center, adding one monomer at a time to rapidly form a chain. In this type of reaction, no product is obtained and the process is simple, but it is not easy to control **(Anusavice, 2008).**

The reaction takes place in stages called the initiation, propagation, and termination stages. The reaction may be accelerated by heat, light, and traces of peroxides **(Power and Sakaguchi, 2006).**

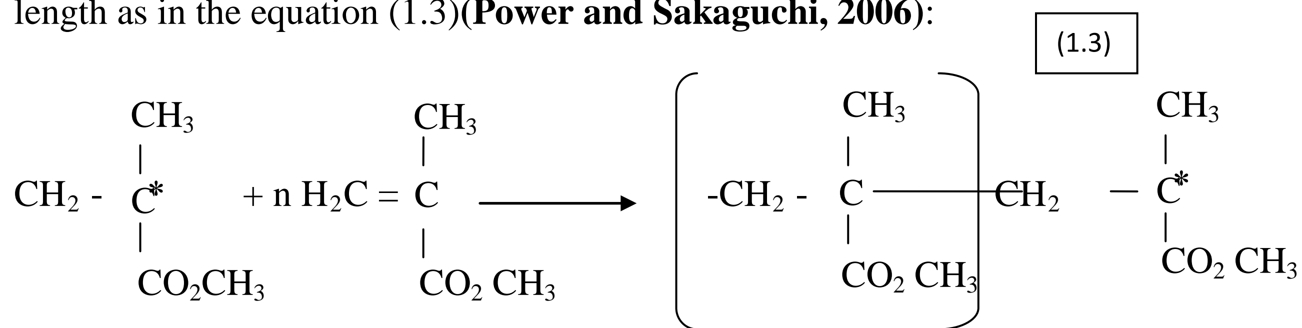
1.2.1.1 Initiation

The initiation step involves the production of free radicals which will encourage a polymer chain to begin growing. Free- radical molecules (R^*) have chemical group with unshared electrons. In chemically activated systems free radical, are generally produced by the reaction of an organic peroxide initiator and an amine accelerator which were the means of production the free radicals which attack the doable bands of available monomer molecules resulting in the shift of the unshared electron to the end of the monomers and the formation of activated monomer molecules. As shown in the equation (1.2)**(Power and Sakaguchi, 2006)**:



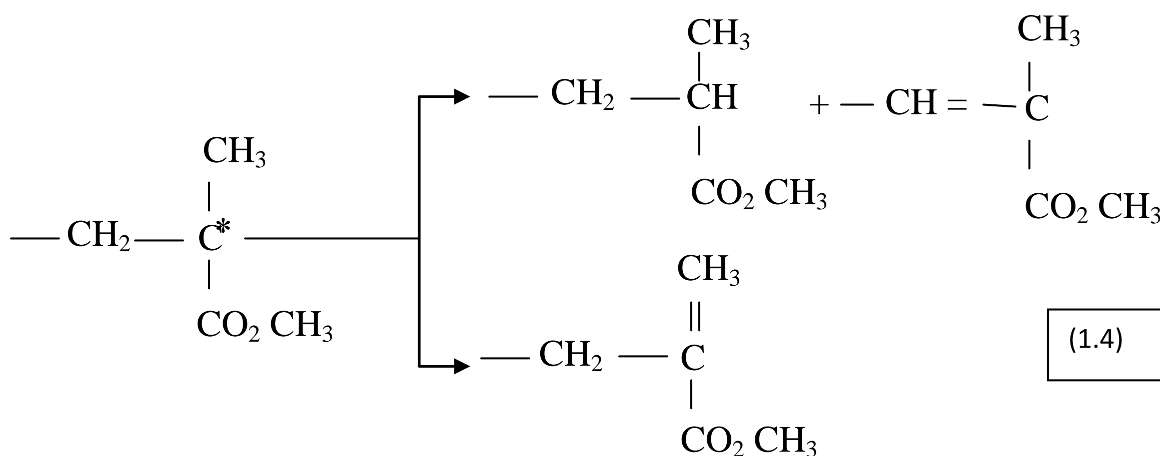
1.2.1.2 Propagation

Activated monomers attack the double bonds of additional available monomer, resulting in the rapid addition of monomer molecules to the free radical. This second stage propagation, continues as the chain grows in length as in the equation (1.3)(**Power and Sakaguchi, 2006**):



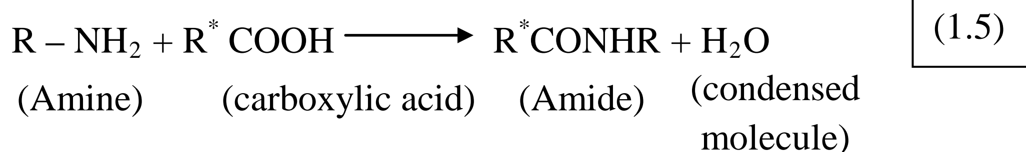
1.2.1.3 Termination

Termination of the growing free radical may occur by several mechanisms and can result in the formation of branches and cross- links. One example of termination is the combination of two growing chains to form one dead chain, or transfer of a single hydrogen ion from one chain to another, referred to as disproportion (**McCabe, 1985; O'Brien, 2002**). The probability of termination reactions occurs at the chain end, the chain length increases rapidly as in the equation (1.4)(**Stevens, 1999).**



1.2.2 Condensation polymerization

Condensation polymerization occurs when two molecules (not usually the same) react to form a larger molecule with the production of a small molecule (often, but not always, water). In this case, monomer units with a carbon- carbon double bond are not necessary, as shown in the following example (1.5):-



$$R - NH_2 + R^* COOH \longrightarrow R^*CONHR + H_2O \qquad (1.5)$$

(Amine) (carboxylic acid) (Amide) (condensed molecule)

This particular process in this equation is used to make polyamides (nylon). The formation of polymers by step- growth is rather slow, because one proceeds in a step- wise fashion from monomer to dimmer to trimmer, and so forth, until large polymer molecules containing many monomer molecules are eventually formed. One major drawback of condensation polymerization is the tendency for the reaction to cease before the chains grow to a sufficient length. This is due to decreased mobility of the chains and the reactant chemical species as polymerization progresses.These results in short chains, step- growth polymerization such as nylon have acquired, their valuable properties when they reach a molecular weight of 10,000 to 20,000 (**Anusavice, 1996).**

1.3 Denture Base Resins

Various materials have been used to construct dentures, including cellulose products, phenol phormaldehyde, vinyl resins and vulcanite. However they have suffered from a variety of problems; cellulose product suffered from warpage, taste of camphor due to its use as plasticizer which leached out of the denture, causing blistering, staining and loss of color within a few months, phenol- formaldehyde was proved to be too difficult to process and also lost its color in the mouth; vinyl resins were found to have a low resistance to fracture and failures were common, possibly due to fatigue; vulcanite was the first material to be used for the mass production of dentures, but its aesthetic qualities are not very good and it has now been replaced by acrylic resins **(Noort, 2007).**

Acrylic resin (poly methyl methacrylate) is now the material of choice; this material has the required aesthetic quality, and is cheap and easy to process. Even so, it is not ideal in all respects. The ideal properties of a denture base material are resistant to bacterial growth; good thermal conductivity; Radiopaque; easy to clean, easy to repair; inexpensive to use; good shelf life; good retention to porcelain and metals; resistant to absorption of oral fluids; absence of (odour, taste); could be disinfected; dimensionally stable, high strength, stiffness, hardness and toughness; natural appearance **(Powers and Sakaguch,2006; Noort, 2007).**

1.4 Composition and structure of Acrylic resin

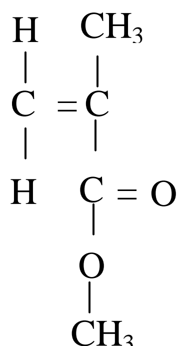
1.4.1 Physical form of acrylic resin

Denture base plastics are commonly supplied in forms: powder and liquid; Gel or plastic cake. The most popular denture base resin is supplied in the form of free running powder and liquid. Because it has long shelf life under normal storing conditions and temperatures**(Powers and Sakaguch, 2006).**

1.4.2 Chemical composition of acrylic resin

1.4.2.1 Liquid (monomer)

An acrylic resin denture is made by the process of free radical addition polymerization to form polymethyl methacrylate (PMMA). The monomer is methyl methacrylate (MMA) as in the scheme (1.6):



(1.6) (Chemical formula of MMA **(Anusavice, 2008)**

Where (me) stands for CH_3. The conversion of the monomer into a polymer involves the normal sequence of initiation, propagation and termination as described in 1.2.1 **(Anusavice, 2008).**

Methyl methacrylate (MMA) is a transparent liquid at room temperature with the following physical properties.

- Molecular weight =100g/mol
- Melting point= -48°C
- Density = 0.945 g/ml
- Heat of polymerization= 12.9 kcal/mol

MMA is stored in a dark bottle because it polymerizes when being activated by heat or exposed to visible or ultraviolet light **(Craig and Powers, 2002; Anusavice, 2008).**

The monomer is polymerized slowly at room temperature, inhibitor such as hydroquinone (C_{16} H_{16} O_4), 0.003% to 0.1% are added to provide the clinician adequate working time (**Powers and Sakaguchi,2006).**

Plasticizers such as dibutyphthalate (Low molecular weight phthalic esters) may be incorporated into the monomer to produce a softer and more resilient polymer (**Craig and Powers, 2002)** and decreases strength, hardness and softening point (**Manappallil, 2007).** The main disadvantage of using plasticizers is that they may gradually leach out of the plastic by oral fluids, resulting in hardening of the denture base. The plasticizers are either low molecular weight phthalic esters such as dibutylphathalate or high molecular weight phthalic ester monomer if the material are used as a soft liners **(Craig and Powers, 2002; Powers and Sakaguch; 2006).**

Acceleratores are included in the liquid of chemically activated resin to speed up the peroxide decomposition such as tertiary amines and the most commonly used are dimethly- para- toluidine and dihydroxyethyl-para- toluidine.These accelerators are included in a concentration of 1% to

the liquid to perform the polymerization reaction at room temperature (**Craig and Powers, 2002; Anusavice, 2008).**

Cross-linking materials may be present in amount of 2% to 14%, are characterized by reactive –CH=CH− groups at opposite ends of the molecules and serve to link long polymer molecules together . The most common cross- linking agent are dimethacylate, either ethylene glycol dimethacylate or 1,4- butylene glycol dimethacrylate**(Dhuru, 2003; Anusavice, 2008).**The accelerators have little effect on the tensile and flexural properties or hardness of acrylic plastics although recovery from indentation such as superficial Hardness indenter is somewhat improved **(Powers and Sakaguchi , 2006).**

1.4.2.2 Powder (Polymer)

Most commercial materials contain particles of pre- polymerized polymethylmethacrylate, which are either spheres or granules of different sizes. Initiator (Benzy Peroxide) 0.5% to 1.5% is included in the polymer to overcome the effect of the inhibitor as well as initiating polymerization reaction**(Craig *et al*., 1996; Powers and Sakaguchi , 2006).**

Plasticizers is also incorporated in the powder beads to assist dough formation, Dibutylphthalate was used for many years as an external plasticizers. Today, internal plasticizers are used instead. Containing various methacrylate or acrylic monomers. They locally soften the beads and allow the monomer to diffuse more rapidly into the beads during the dough stage **(O'Brien, 2002).**

A pure polymer such as poly (methylmethacrylate) is clear and adaptable to a wide range of pigmentations. Inorganic pigments are usually added such as mercuric sulfide, cadmium sulfide, cadmium selenide or ferric oxide (although the use of cadmium salts is questionable because of demonstrated toxicity) to match the shade of the soft tissues **(Powers and Sakaguch; 2006).**

Synthetic Fibers were added to the polymers to simulate the minute blood vessels underlying the oral mucosa; these fibers are made from nylon or acrylic fibers **(Craig and Powers, 2002).**

Elements such as barium or radiopaque glass fibers, bismuth or uranyl salts and zirconyldimethacrylate provide the radio opacity of denture plastics which helps in locating factored fragments in the upper respiratory or digestive tract **(Powers and Sakaguch; 2006).**

1.4.3 Mixing Ratio

The polymer to monomer is usually 3-3.5/1 by volume or 2.5:1 by weight, the accepted polymer to monomer ratio is 3:1 by volume. This provides sufficient excess monomer that would lead to polymerization shrinkage limited to approximately 6% or 5% liner shrinkage **(Anusavice, 2008).**

There must be sufficient monomer to wet polymer bead thoroughly polymerization reaction is to be maximized .Furthermore, if too much polymer powder was used ,less reaction time will be for the polymer and monomer, more unreacted granules of original polymer will be found in the

cured acrylic and less strength will be obtained .On the other hand, too much monomer can result in porosity and longer doughing time in addition to the great curing shrinkage , which will lead to dimensional changes**(Craig ,1997).**

The speed with which the polymer and monomer mixture reaches dough stage depends upon solubility of the polymer beads in the monomer, increasing in the temperature and the size of polymer particles.However a polymer of high molecular weight is more difficult to soften than one of short chain length as the forces of attraction between the chains are greater **(Craig, 1997).**

1.5 Heat activation Polymerization reaction of acrylic denture base resin:

The general method for processing a heat activated acrylic denture base material is the water bath system. In addition there are several methods for supplying heat to accelerate the polymerization reaction for example dry heat, steam, infrared, or dielectric heating and microwave radiation. The results of various processing studies had shown that equally satisfactory clinical results may be obtained with any of these methods compared with the water bath method if adequate temperature control and pressure are maintained **(Craig, 1997; Powers and Sakaguchi, 2006).** The curing temperature at 70 °C for 7 hours followed by 3 hours at 100°C achieved minimal level of residual monomer**(McCabe, 1990).**

On the basis of a large number of studies a satisfactory processing temperature for most products is between 71 and 77 °C, although some products can be proceed at higher temperature without serious difficulty. A satisfactory processing procedure is to cure the polymer in a constant temperature water bath at 74˚C for 8 hours or longer. Longer curing times, such as overnight, will not result in any degradation of properties. Another satisfactory method, which permits curing in a shorter time, is to heat at 74˚C for 1.5 hours and then increases the temperature of the water bath to boiling for an additional hour **(Powers and Sakaguchi, 2006, Anusavice, 2008).**

1.6 Antimicrobial polymers

Polymers are very rarely used in their form, for the same reasons that pure metals are rarely used in comparison to alloys. These modifications are carried out in order to improve the properties of polymers. A composition may be defined as a combination of materials in which the individual components retain their physical identity more importantly, a composite material is multiphase material that exhibits properties at the constituent phases is such a way as to produce a material with a better combination of properties than could be realized by any of the component phases. In two component composites, it is usual to refer to the matrix and filler, the matrix being the component that binds the filler together **(Noort, 2007).**

The antimicrobial polymer is the polymer with antimicrobial activity or the ability to inhibit the growth of microorganisms such as bacteria, fungi or protozoa. The antimicrobial polymers may enhance the efficiency and

selectivity of currently used antimicrobial agents, while decreasing associated environmental hazards because antimicrobial polymer is generally nonvolatile and chemically stable. This makes this material a prime candidate for use in areas of medicine as a mean to decrease infection in the food industry to prevent bacterial contamination, and in water sanitation to inhibit the growth of microorganisms in drinking water**(El-Refaie*et al*., 2007).**

1.7 Antimicrobial agent (Silver Nitrate)

Silver Nitrate is an inorganic compound with chemical formula $AgNO_3$. Silver Nitrate is one of Silver compounds that occur in the environments as a white powder **(Wadhera and Fung, 2005).** It can be prepared by reacting Silver, such as silver bullion or silver foil with Nitric acid as shown in fallowing equation (1.7):

$$3Ag + 4HNO_3 \longrightarrow 3AgNO_3 + 2H_2O + NO \quad (1.7)$$

This is performed under a fumhood because of toxic nitrogen oxide given off in the reaction**.** Silver Nitrate disintegrated at 440˚C in to metallic silver, nitrogen and nitrogen oxides, soly in water at 25°C (g / 100g H_2O) given Ag^+ and No_3^-. Molecular weight 169.87g/mol, Transition temperature 159.8^{o}C, Melting temperature 212°C forming yellowish liquid solidify to white crystal mass on cooling, Density 4.35g/ cm^3 (solid). It is non-hygroscopic, not photosensitive when pure; traces of organic material promote photo reduction **(Neil*et al*., 2006).**

Humans have been exposed to silver and its compounds for centuries via the natural environment, industry, and through the use of silver containing medication **(Lansdown, 2010).**

1.7.1Silverin medicine

Silver and gold acupuncture needle was used in ancient Japanese "Hari therapy" for relief of muscular pain, fatigue and other discomforts. An ophthalmic solution containing 1% of silver nitrate used to be dropped into newborn babies eyes at birth, eye infections and blindness of newborn was reduced by this method; incorrect dosage, however, could cause blindness in extreme cases, this protection was first used by **Crede in 1881(Peter, 2000; Bulletin of the WHO).** In middle Ages, silver nitrate was used for the treatment of nervous system disorders such as epilepsy and tabes dorsal is **(Wadhera and Fung, 2005).**After observing Dr. Halstead of Johns Hopkins University apply silver foil and gauze to wounds to prevent infection, **Crede in 1897** populated the use of silver as an anti- infective measure **(Hill and Shury, 1939).**

Repeated daily application of silver nitrate can induce adequate destruction of cutaneous warts, but occasionally pigmented scars may develop **(Fung and Bowen, 1996; Sterling *et al*., 2001).**

Experimental studies suggest that concentration of 60 ppm Ag^{+} should be sufficient to control the majority of bacterial and fungal pathogens **(Burrell, 2003)**.

Department of chemical engineering found that silver nonoparticles undergo size dependent interaction with HIV-I and inhibit the virus and infectivity in vitro **(Jose *et al*., 2005).**

The antibacterial action of silver is depend on the silver Ion, the effectiveness of silver compounds as an antiseptic is based on the ability of the biologically active silver ion (Ag^{+}) to irreversibly damage key enzyme system in the all membranes of pathogens **(Lansdown, 2006).**

Many research have been done in evaluating the ability of silver ion at inactivating E.coli, a microorganism commonly used as an indicator for fecal contamination and as surrogate for pathogens in drinking water treatment, WHO permissible level is 0.1 mg silver /L as disinfectant agent **(Klaassen, 2008).**

Silver and most silver compounds have been widely used in medical and life care polymers (**Kumar and Munstedt, 2005b; Irzh*et al*., 2007)** and exhibit antimicrobial action against Gram- positive and Gram-negative bacteria and fungi**(Matsuura *et al*., 1997; Panacek*et al*., 2006; Pal *et al*., 2007; Lok*et al*., 2007; Casemiro*et al*., 2008)**.

Silver has proven broad- spectrum antimicrobial activity that includes antibiotic- resistant bacteria with minimal toxicity toward mammalian cell at low concentrations, and has a less likely tendency than antibiotics to induce resistance due to its activity at multiple bacterial target sites **(Hermans, 2006; Tion*et al*., 2007).**

1.7.2 Silver in Dentistry

In response to growing concern of bacterial and fungal contamination high- touch/ high- risk surfaces and foreign body related infection and among current available biocides , silver and inorganic silver salts have potent efficacy against a wide range of microorganisms with low toxicity for human tissue cells **(Chopra, 2007; Hilper*et al*., 2006; Paddock *et al*., 2007)**.

The development of antimicrobial dental material and dental equipment that can effectively prevent and /or inhibit the growth of various microorganisms has attracted considerable research interest such as in introducing polymeric silver sulfadiazines into PMMA(**Zhengbing*et al*., 2009)**.

A two year longitudinal study of over denture patient who were placed on fluoride solution and/or had teeth treated with silver nitrate, had a significant decrease in caries when compared to these who received no treatment or were placed in placebo **(Toolson and Smith, 1978).**

In 1980 a study was carried to assess the relative importence of the silver introducing solutions for topical application with silver nitrate, copper sulfate, silver fluoride and copper fluoride, all metals tested were found to be carried in plaque and inhibit the acidogenicity of plaque which appears to be carried by the cations**(Oppermann and Johansen, 1980).**

Silver nitrate stains the surface layer changes in dental composites resulting from oral temperature changes **(Mair, 1989),** also for detecting the permeability degradation and marginal integrity of dental composite

restoration **(Mair, 1992).** Evaluating the permeability and micro leakage of CL II resin composite restorations was done by **Prati*et al*. in 1994**who used silver nitrate solution to measure microleakage as dye penetration. Also silver nitrate solution was used to evaluate micro leakage and marginal gap of some self-adhesive resin cements **(Hooshmand*et al*., 2011).**

Anusavice*et al*. in 1994 studied the influence of colorants such as $AgNO_3$ and $FeCl_3$ on crystallization and mechanical properties of Lithia- based glass- ceramics, and found that controlling some mechanical properties by the use of Ag NO_3 was more effective than $P2O_5$as a nucleated agent for lithic-based glass ceramic.

The effect of a new type of antibacterial temporary filling materials was evaluated by **Hottal*et al*. in 1998** by adding various ratios of Ag-Zn-Zeolite.

Introducing silver ions into dental restorative material (silica glass) for their slow releasing antimicrobial activitywas done by**Kawashita*et al*. in 2000.**

Ceramics containing silver ions such as silver zeolite, silver zirconium phosphate are of interest for manufacture aiming to apply antimicrobial compounds to their products **(Kourai*et al*., 1994; Miyoshi *et al*., 1998; Kawashita*et al*., 2000; Inove*et al*., 2002).**

Silver and zinc zeolite was added to acrylic resin in different percentages to evaluate the antimicrobial activity for acrylic and to assess whether the addition of zeolite alters the flexural and impact strength of the resins **(Luciana *et al*., 2008).**

The antimicrobial silver ions are utilized to improve the antimicrobial efficacy of endodontic sealers against the remaining bacteria in root canal system **(Kreth*et al*., 2008).**

Zhengbing*et al*. in 2009 synthesized a new PMMA- based polymeric silver sulfadiazine with silver in trade treatment to regenerate any loss of antibacterial and antifungal activity. This recharging can be repeated as needed to achieve long- term protection.

Sliver ions incorporated with orthodontic adhesive gave excellent antimicrobial activity **(Ahn*et al*., 2009).**

Analysis from 50% silver trade tooth immersion provided for assessing marginal leakage at the sealant- enamel interface, showed no statistically significant difference in penetration scores in different times of immersion **(Chen *et al*., 2009).**

One of the surface- originated problems associated with the metallic implants is implantitis. Due to the bacterial adhesion and communization at the implantation site, surface coating with organic and inorganic anti-bacterial agents such as Ag- related agents has been developed**(Guocheng and Zreiqat, 2010).**

Incorporating of silver particles into chemical cure silicon soft liner materials as an *in vitro* study to evaluate the fungal efficacy of these developed composites was performed by**(Chladak*et al*., 2011).**

1.7.3 Application in Medical Tools

Antimicrobial polymers that contain silver represent a great challenge for academics and industry. These materials draw the attention because of their novelty in being long- lasting biocidal material with high temperature stability and low volatility **(Kumar and M"unstedt, 2005b).**

The large increase in the number and occurrence of antibiotic- resistant bacterial strains has prompted a renewed interest in the use of silver as an antimicrobial agent since silver has a less likely tendency than antibiotics to induce resistance due to its activity at multiple bacterial target sites **(Hermans, 2006; Tian*et al*., 2007).**

Since catheter related infection is a common cause of nosocomial infection and bacteremia, analysis clarified discrepant results among earlier trials of silver- coated urinary catheters by reveling silver alloy catheters and significantly more effective in preventing urinary tract infection than are silver oxide catheters **(Saint *et al*., 1998; Roe *et al*., 2008)**, these conclusions are supported by among other Studies by university hospital leuven, Belgium **(Lansdown, 2006)** and the university hospital for anathesiology surgical intensive care, Halle, Germany **(Loertzer*et al*., 2006).**

Ionizable silver is also incorporated into fabrics to reduce the spread of bacteria **(Lansdown, 2006).**

In 2007, AGC Flat Glass Europe introduced the first antimicrobial glass to fight hospital- acquired infection; covered with a thin layer of silver (AGC).Ventilator- associated Pneumonia (VAP) causes substantial

morbidity. **In 2008 a study done by kollef*et al.*** concluded, "Patient receiving a sliver- coated endotracheal tube had a statistically significant reduction in the insider of VAP and delayed time to VAP occurrence compared with those receiving a similar uncoated tube**.** In addition the U.S. food and drug administration (FDA) has recently approved an endotracheal tube with fine coat silver for use in mechanical ventilation **(FDA).**

The use of these devices is contraindicated for persons who are allergenic to silver **(Lansdown, 2006)**, and although they are widely used in hospitals, no thorough testing and standardization of these products has yet been under taken **(Chopra, 2007).**

Sliver ions are also used for a number of non-medical purposes **(Yamanaka *et al*., 2005; Jung *et al*., 2007).**

1.8Sliver exposure

Due to a wide industrial application, a historically high incidence of sliver toxicity has been reported, but new occupational safety regulations have dramatically decreased its toxicity **(Fung and Bowen, 1996)**.

Besides drug or industrial exposure, sliver can be ingested with food and water. The **U.S- EPA (Environmental Protection Agency) in 1994** published a Reference Dose (Rfd) which is an estimate of daily exposures to the entire population (including sensitive subgroups) that is unlike to be associated with an appreciable risk of deleterious effects during a life time. It is based on the presumption that some threshold may exist for certain toxic effects of a chemical such as cellular necrosis independent of

carcinogenicity. The current Rfd for oral silver exposure is 5 μg/ Kg/d with a critical dose estimated at 14μg/Kg/d for the average person based on the current Rfd for a 5Kg infant to 70Kg adult, the maximum daily exposure should be less than 25-350 μg/d.

If the sliver drinking water sources meet EPA guidelines, an average person who drinks (2L/d)is exposed to less than 200 μg of sliver, however a regular daily diet may contain up to about 90 μg of sliver as a back ground level of exposure **(Clayton and Clayton, 1981).**

Current available CSP. Promoted for medicinal or mineral supplement purpose are reported to have an active silver ion concentration of about 1-6 ppm (5-30 μg) per dose**(Health frauadBulpetin, 1995).**

The principle routes for buccal or gastrointestinal Absorption of sliver include: contaminated food, sliver nitrate aerosols, occupational exposure to metallic silver dust, drinking water (including use of sliver copper filters in water purification). Sliver nitrate in oral hygiene and gastro intestinal infection, sliver acetate antismoking therapies, accidental consumption of sliver nitrate or other colorless sliver compound**(Lansdown, 2010).**

1.8.1 Oral exposure

Sliver is absorbed into the human through buccal membranes and gastro- intestinal mucosa which is determined by the ionization of the sliver source and availability of “Free” Ag^+ to interact with protein receptors on cell membrane .Passive uptake is not indicated on account of high reactivity of the silver ion and its binding sulphydryl, carboxyl, hydroxyl, and protein

legends on mucosal surfaces and cell debris. Biologically active Ag^+ readily binds and precipitates like with chloride and phosphate greatly, therefore reducing absorption **(Lansdown, 2010).**

Current estimation suggest that less than 10% of the sliver ingested by humans is absorbed into the circulation **(Fung and Bowen, 1996)** but this can be expected to vary widely according to the age, health, nutritional status, and composition of the diet.

1.8.2 Dermal exposure

The majority of products contain silver or sliver compounds for antibiotic purposes come into contact with human skin at same time, but clinical and experimental studies indicated that percutaneous absorption of sliver is exceedingly low **(Lansdown, 2010).**

The epidermal keratin and phospholipids of the epidermal barrier function provide effective barriers with exposed sulphydryl groups irreversibly binding free Ag^+, in much the same way as other metallic elements ***(*Hostynek*et al*., 1993; Lansdown, 1995).**

Where sever generalized argyria has been reported in occupational situation, it is expected that the greatest proportion of the Ag^+ absorbed occurs through inhalation or through contamination of contaminated food and drinking water **(Bleehen*et al*., 1981).**

The increasing use of metallic sliver, sliver thread, or silver impregnates in textile fibers designed for hygiene purpose might be expected

to lead to percutaneous absorption , increased blood silver, and some accumulation of sliver precipitated in the skin in chronic exposures **(Lansdown, 2010).**

However, risks of argyria through the use of sliver antibiotics in textiles and hygiene clothing are negligible even where the skin is warm and hydrated **(Lansdown, 2006).**

Sliver nitrate is appreciably more astringent than silver sulphadiazine and ionizes more rapidly when applied topically as strong silver nitrate (75%), sliver nitrate sticks, pencils, for warts removal, callus or undesirable granulations, but Ag^+ penetration is very low. Ag^+ binds to epidermal keratin and blackens on exposure to solar radiation to give characteristic brown-black discoloration. local skin discoloration rarely occur following application of sustained silver release wound dressing and occupational contact with silver oxide and other ionisable silver compounds, but are not representative of true argyria which is long lasting **(Lansdown*et al*., 2005).**

In humans, less than 1% of topically applied silver compounds are absorbed through the skin **(Snyder *et al*., 1975).** Once deposit in the layers of the sikn of humans, silver accumulates throughout the ageing process **(Hostynek*et al*., 1993).**

1.9Silver safety

Adverse health effects of silver depend on the dose, the duration of exposure, the rout of exposure (i.e. ingestion, inhalation, or skin contact), also on the exposed individuals characteristic (age, sex, nutritional status, and state of health) **(Wadhera and Fung, 2005).**

The general population is exposed to silver mostly through very low levels of silver present in food and drinking water and some times in the air. **Hamilton et al in 1972** found the daily oral intake of silver from a typical diet to be 27-88 µg per day.

Acute irritation of the respiratory tract can occur from in halation of silver nitrate dust, but generally only at concentrations that produce argyria**(Stokinger, 1981).**

Accidental or intentional ingestion of large doses of silver nitrate caused corrosive damage to the gastrointestinal tract, abdominal pain, diarrhea, vomiting, shock, convulsions, and death. The estimated lethal dose of silver nitrate is l0g, but recoveries have been reported following ingestion of large doses **(U.S. EPA, 1985).**The chronic inhalation or ingestion of metallic silver or ionsable silver compounds can lead to the formation of argyria, which is either localized or generalized. Excessive absorption Ag^+ over a long period of months or years leads to the state of sliver “over load” in the circulation, where absorption exceed the capacity of the liver or kidney to eliminate the metal in bile and urine, respectively. Argyria is characterized by the addition of inert precipitates of silver selenide and silver sulfide in the connective tissue surrounding the vascular tissue and gland of the papillary layer of the dermis but not epidermis **(Bleehen*et al.*, 1981; Sato *et al.*, 1999).**

The black silver sulfide pigment is formed by photo reduction of sliver chloride to metallic silver, the metallic silver is then oxidized by tissue, subsequently forming black silver sulfide in the presence of light and sulfur

containing organic matrix (in the form of amino acids) **(Hill and Shury, 1939).**

The mild to profound blue- gray discoloration of skin and nail bed occur mainly in light- exposed areas and on occasions may be severely disfiguring **(ATSDR, 1990; Bouts, 1999).**

There is no evidence to associate argyria with cellular damage or altered sensory perception in the skin, and even in profound cases, argyria is not life threatening, but can be considered a cosmetic disfigurement **(Lansdown, 2010).** The discoloration may be psychologically disturbing since they are not readily removed chemically or by surgical dermabrasion.

The estimated total dose required to induce argyria by ingestion is in the range of (1-30)g for soluble silver salts **(Nordberg and Gerhardsson, 1988).**

Full preliminary screening for mutagenicity and carcinogenicity for silver and silver compounds has not been completed **(International Agency for Research on Cancer, 1980).** Information of oral, inhalation carcinogenicity of silver in human or animals was not available **(Rosmarie, 1992).**

Published cytotoxicity tests and in vivo experience indicate unequivocally that silver is not carcinogenic in any tissue and should be placed in a “No Risk” category**(U.S Department of health and human resources, 2010).**

1.10 Some Mechanical properties for heat- polymerized Acrylic resin:

1.10.1 Flexural Strength

In the evaluation denture plastics, flexural strength measurement are used to a great extent than either tensile or compressive strength, because this test more closely represent the type loading *in vivo*.

Flexural strength is a combination of compressive, tensile, and shear strength, all of which directly reflect the stiffness and resistance of a material to fracture (**Jagger*et al*., 2002).** In addition, testing the transverse strength and the impact strength of denture base material have been used as methods of comparing performance of denture base material, as also described in international organization for standardization **(Vallittu, 1996; Jagger*et al*., 2002).**

Several factors on which strength of acrylic resin depends such as polymer molecular weight ,polymer bead size , residual monomer level ,plasticizer composition ,amount of cross- linking agents , internal porosity of the polymer matrix ,denture base thickness ,patient factors ,type of polishing ,and action of chemical agents**. (Orsi and Andrade, 2004).**

Measuring the flexural fatigue strength of denture base resin polymerized using short and long curing cycles using different polymerization techniques (water bath ,pressure cooker ,and microwave polymerization) was done by **Banerjee *et al*.in 2010** ,the results revealed that there were no statistically significant difference between water bath processing and pressure cooker processing techniques .In all techniques ,the

long curing cycle proved to be better in producing denture bases with higher flexural strength as compared to the short curing cycle .The water bath technique produced the lowest flexural strength.

Flexural strength is determined by applying an increased load until fracture at the center of a test specimen. The deflection in millimeters at the middle of the plastic specimen is recorded, allowing the flexural modulus to be calculated. The flexural strength varies from 78 to 92 MPa for various denture base resins **(Powers and Sakaguchi, 2006).**

1.10.2 Tensile Strength

The term tensile strength indicates the maximum stress to which a material can be subjected before it begins to fail by localized accelerated deformation. If material continues to have more and more weight applied to it, it will eventually break (**Dhuru, 2003).**

Noortin 2002reported that, the measurement of the tensile strength of brittle resin materials is extremely difficult, and gives rise to a great deal of scatter in the data .The reason for this is that such material are highly susceptible to the presence of internal flows or small cracks in their surface, which are impossible to eliminate .So the tensile strength depends upon the quality of surface finish.

Previous investigations regarding conversional PMMA revealed that the tensile strength of porous specimens was inferior to that of dense once and residual monomer acting as a plasticizer was the cause of inferior tensile strength of acrylic resin **(Jagger,1978).**

The deformation of the material mainly occurs locally by the formation of a neck region which becomes narrower with continued stress application. The localization of deformation is the result of stress concentration under gain force **(O'Brien, 2002).** The tensile strength for acrylic resin is typically no more than 50 MPa(**Noort, 2007).**

1.10.3 Impact Strength

Impacts strength may be defined as the energy required tofracture a material under an impact force. The term impact is used to describe the reaction of a stationary object to a collision with a moving object. It's an important property for acrylic denture base materials which have tendency to fracture if accidently dropped on a hard surface (**McCabe and Walls, 2008**).

The impact strength of acrylic resin can be increased by adding cross linking agent, although the addition of plasticizer may also increase it; the increases are accompanied by decreases in hardness, proportional limit, elastic modulus and compressive strength ***(*Harrison *et al*., 1978; Powers andSakaguchi, 2006).**

Zappini*et al*. (2003)reported that the impact strength test was influenced by loading conditions and specimen geometry.

In general there are two methods of improving impact strength for rigid polymers such as PMMA including: incorporating a rubbery base, and incorporating carbon fibers **(Radford, 1986; Uzun*et al*., 1999).**

A Charpy-type impact tester for unnotched specimens is usually used to measure impact strength .The impact strength of denture acrylic ranges

from 0.26J for a conventional denture acrylic to 0.58J for a rubber modified acrylic resin which indicates that rubber modified acrylic resin absorbs more energy on impact and is more resistant to fracture **(Anusavice, 2008).**

1.11 Organisms associated with dental caries

It is a bacterial disease of the dental hard tissues, occur in certain localized sites in the dentition these sites are the pits and fissures, the proximal contacting surface of the labial, buccal and lingual surfaces of the dentition adjacent to the gingiva. Dental caries is a nonspecific bacterial disease, because the acid produced can be provided mainly by the different types of bacteria found in the plaque flora. However when the number and proportion of acidogenic and aciduric organisms in the plaque flora increase, resulting in the formation of more acid to dissolve the tooth. Caries may be considered also to be caused by specific pathogens invading the oral cavity and when they become in sufficient numbers, they produce the acid causing the disease. The infectious microorganism thought to be the causative agent was *Lactobacillus acidophilus* beside that specific strain of *streptococcus mutans* have been proposed as the infectious agent. Indigenous microorganisms were implicated as the primary etiologic agents of dental caries and periodontal disease **(Bloomquist*et al.*, 1996; Mackeawn*et al*., 2003).**

The predominant cariogenic bacterium was shown to be *streptococcus mutans* and *lactobacillus***(Yu *et al*., 1997; Kidd and Jouyston-Bechal, 2002)**.*Streptococcus mutans* are acid tolerant bacteria that can grow at low

pH. Besides that, it is acidogenic bacteria which are able to produce acid that lead to further drop of the pH in dental plaque **(Almedia*et al*., 2000).**

1.11.1 Cariogenic bacteria

The genus Streptococcus, a member of the family lactobaccillaceae has presented several species members found in the mouth in various oral infection**(Nolte, 1982)** this microorganism makeup a large proportion of the oral flora. It is estimated roughly that around one-quarter of the total flora from supragingival and gingival plaque are of streptococci member also halfe of the isolates from the tongue and saliva are of the genes Streptococcus **(Samaranayake, 2006).**

The Oral streptococci classification (**Russell, 2000**):

Anginosus group including *S.anginosus,S.constellatus,S.intermedius*.species which are frequently isolated from abscesses.Mitis group including,*S.oralis,S.cristaS.mitis,*
*,S.infantis,S.peroris,S.orisratti*specieswhich are pioneer speciesin plaque formation and a common cause of infectious endocarditis.Salivarius group including *S.salivarius*and*S.vestibularis* species;theyare found mainly on mucosal surfaces and rarely pathogenic.Mutans group including *S.mutans*and*S.sobrinus* species ;they are increased in number in association with caries.

The current classification of the oral viridans group (alpha- hemolytic) of streptococci places the bacteria in the six species groups:

mutansgroup,salivarius group,mitis group,bovis group ,urinalis and anginosus groups **(Betty *et al.*,2007).**

Mutans streptococci are Gram–positive,facultative anaerobic bacterium ,catalase negative,non –spore forming ,divided in one plane ;since they do not separate easily after division , they tend to form short or medium chains under microscope **(Baca *et al.*,2002).**

Streptococcus mutans colonies on mitis –salivarius –agar are characterized by their high, convex ,granular ,light blue mucoid circular colonies of 0.5-1.5 mm in diameter, sometimes have drop of glistening polysaccharide beside them or on the top of the surface giving them a characteristic frosted glass appearance **(Arbeit,1999).**On blood agar plates *mutans streptococci* colonies are normally gamma –hemolytic (no agar colour change) at first,but after 24 -48 hours they become hemolytic (greenish colouration of the agar around the colony),sometimes few strains may show beta –hemolysis that appears as a clear zone around the colonies **(Balakrishnan*et al.*,2000).**

S.sobrinus is the primary bacterial pathogen in smooth-surface dental caries. Not commonly detectable in caries-free children present in plaque cultures in 43-60% of children **(Choi, 2009).**

Children with *S.sobrinus and S.mutans* are far more likely to exhibit decay than *S.mutans* alone. *S.sobrinus* is also affiliated with early childhood caries which are responsible for the majority of dental abscess and toothaches in children **(Wu *et al*., 2003).**

In **1971 Ikeda and Sandham** found that *S.mutans* was more prevalent on the pits and fissures, constituting 39% of total streptococci in the oral cavity .Fewer *S.mutans* were found on buccal surface.

Enamel rods are arranged in bundles with a diameter of 5um, streptococcal species are about 1um across. Thermophysiological studies indicated optimal growth at 37°C with no growth outside of 32°- 37°C *.S.mutans and Enterococcushirae* are able to tolerate a much wider temperature range **(Ma and Marquis, 1997).**

In case of *S.mutans* they can grow on most types of culture, the growth occur in anaerobic atmosphere in the presence of 5% Co_2 and 95% N_2 since they are facultative anaerobic bacteria **(Ma and Marquis, 1997)**.**Gold *et al.*(1973)** found a better selective medium which was MitisSalivarius agar containing 20% sucrose and 200 units/L bacitracin.

The therapeutic options for *viridans streptococci* are penicillin or ceftriaxone, with or without an aminoglycoside; vancomycin is used in cases of penicillin allergies and beta- lactam resistance **(Betty *et al.*, 2007).**

1.12 The bacteriocidal mechanism of silver

A widely investigated, effective, biocompatible, broad-spectrum antimicrobial agent is silver (Ag).Since the antimicrobial activity of silver depends on silver ions, which bind strongly to electron donor groups in biological molecules containing sulphur, oxygen or nitrogen. This may result in defect in bacterial cell wall so that cell contents are lost. A complex formation between silver ion and (-SH) bonds of proteins may disturb the

metabolism of bacterial cells and their power functions, such as permeability and respiration. Both effects lead to death of bacterial cells. Furthermore, silver ions can interact with the DNA of bacteria, preventing their proliferation by displacement of hydrogen bonds between adjacent nitrogen of purine and pyrimidines, as a result DNA molecules becomes condensed and loss their ability to replicate upon the infiltration of Ag^{+} ions **(Damm*et al.*, 2008 a; Monteiro*et al*., 2009).**

MATERIALS AND METHODS

Materials & Methods

2-1 Materials

2-1-1Equipment and materials required for cytotoxicity test

2-1-1-1Laboratory equipment and instruments

Instrument	Company(origin)
Agitator	CYAN(Germany)
Autoclave	Harayma(Japan)
Cooling centrifuge	Hitachi(Japan)
Distillator	GFL(Germany)
Electric sensitive balance	Sartorius(Germany)
ELISA multiwell reader plate	Asays(Belgium)
Incubator	Gallenkamp(England), Heraeus (Germany)
Inverted Microscope	Olympus (Japan)
Magnetic stirrer	Gallenkamp
Micropipette	Dragon MED(China)
Microplate (Tissue culture plate flat bottom 96 wells)	IWAKI (Japan)
Milipore filter unit	Corporation(USA), (Ireland)
Nalgene filter units (0.22 µm)	Nalge
pH meter	HANNA (Romani)
Plastic flask for tissue cultures 25 cm^2	Falcon, Nunclon(USA)
Refrigerator	Concord(Lebanon)
Water bath	Memmart (Germany)

2-1-1-2 Solutions and Chemicals

Chemical	Company(origin)
Crystal violet stain	BDH
Fetal calf serum	Flow Lab (U.K.)
PBS (Phosphate Buffer Saline)	Biological(USA)
Sodium bicarbonate(Na_2Co_3)	Difco(USA)
Trypsin-versin	Difco(USA)

2-1-1-3 Tissue Culture media

1. Roswall Park Memorial Institute medium (RPMI-1640). (Iraqi Center for Cancer and Medical Genetic ResearchICCMGR, 2012).
2. Serum Free Media (SFM). (ICCMGR, 2012).

2-1-1-4 Rat embryo fibroblast (REF)cell line used for cytotoxic study

The cell line used in the present study was kindly obtained from Iraqi Center for Cancer and Medical Genetic Research.

Cells of this normal murine cell line were fibroblastic cells with normal chromosomal picture. Tumorigenicity test of this cell line showed no tumor growth in injected rats during three months of monitoring.

2-1-2Equipment and materials requiredfor testing the antibacterial activity of $AgNO_3$ – loaded resins on the growth of *mutans streptococci*goup*:*

2-1-2-1 Equipmentand Suppliesfig. (2-1)

1. Adjustable micropipettes with disposable tips (Dragon MED, China)
2. Anaerobic Jar (Oxiod - UK).
3. Automatic electronic autoclave (HIRAYAMA, Japan).
4. Autovortex (stuart scientific, UK).
5. Bacteriological loop and spreaders (Pastor Pipette).
6. Bristle and Wood Brush. (Italy).
7. Bunsen burner.
8. Clamps (HANUA, Engineering corp. USA).
9. Colony counters (Gallen Kamp, England).
10. Cotton swabs (China).
11. Dental vibrator (Bego, Germany). Plaster (Al- ahliyah co., Iraq).
12. Dissecting microscope (Ken-a-vision/ U.S.A)
13. Electronic balance (Adventurer)
14. Electronic digital caliper (china).
15. Gas packs (Oxiod -England).
16. Glass and plastic petridishes
17. Glass Jar, Watch, thermometer (China)
18. Hydraulic press (Germany).
19. -Incubator (Fisher Scientific, USA).
20. Laminar flow.
21. Lathe polishing machine. (Germany).
22. Magnetic stirrer. (Janke and Kunkel, Germany).
23. Millipore filters size 0.20 µm / 0.45 µm (Inlet Lot, 1824).

24.pH meter (JENWAY, 3510, Belgium).

25.Polyethylene sheet (China).

26.Prosthetic hand piece (W and H elco. Austria).

27.Screw capped bottles.

28.Separating medium (England, 2015).

29.Spectrophotometer CECIL 7200 (France).

30.Stainless steel T- shaped cutter for discs.

31.Test tubes (Gordon).

32.Thermostatically controlled water bath. (Memert, Germany).

2-1-2-2Solutions, chemicals, and materials

1. Arabic chewing gum (Supplied from local supermarkets).
2. Artificial salivafig. (2-1)**:**an electrolyte composition similar to that of human saliva **(Cavalla *et al.*,2001)** which includes:
   1.(1 g) sodium carboxy – methyl –cellulose. 2. (4.3 g) sorbitol .3. (0.1g) potassium chloride. 4. (0.1g) sodium chloride .5. (0.02g) sodium fluoride. 6. (5mg) magnesium chloride.7. (5mg) calcium chloride .8. (40mg) potassium phosphate. 9. (1mg) potassium thio-cyanate. 10. (100ml) deionized water.The solution was sterilized byautoclaving at 121 ºC for 15 min, at 15 pounds then kept in refrigerator until use.
3. Bacitracin powder (AppliChem, Germany).
4. Brain Heart Infusion Broth (Hi-Media Company,India).
5. Ceftriaxone Antibiotic disks (30mcg, Bioanalyse).
6. CT- Mannitol Agar (Bio-Merieux Company, S.A).
7. CystineTrypticase Agar(Bio-Merieux Company, S.A).
8. Gram’s Stain Set (India).
9. Mannitol(Reidel De Haen AG Seelze – Hannover).

10.Mitis- Salivarius Agar (Hi-Media Company,India).

11.Mueller Hinton Agar (Hi-Media Company,India).

12.Sucrose (DIDACTIC,BarcelonaExpana).

A

B

C

D

E

Fig (2-1):Equipment and materials required for testing the antibacterial activity of $AgNO_3$ – loaded resins: A- Laminar flow, B -Adjustable micropipette, C- Electronic digital caliper, D- Dissecting microscope, E- Sterile artificial saliva.

2-1-3Equipment and materials required for *in vitro* Ag release test

2-1-3-1 Equipment, Instruments, and materials

1. Agitator(CYAN, Germany).
2. Atomic Absorption spectrophotometer (Phoenix -986/ AA spectrophotometer, UK).
3. Atomic Absorption spectrophotometer (AA-6800, Shimadzu ,Japan).
4. Incubator (Gallenkamp(England).
5. Artificial saliva.
6. Deionized water (Iraq).

2-1-4Equipment and materials requiredfor preparation of $AgNO_3$-loaded resins determined for testing some mechanical properties

2-1-4-1 Equipment and Instruments

1. Bristle and Wood Brush. (Italy).
2. Clamps (HANUA, Engineering corp. USA).
3. Dental vibrator (Bego, Germany).
4. Electronic balance (accuracy 0.0001g, Sartorius BP 30155, Germany).
5. Electronic Digital caliper (China).
6. Finishing burs (silicon carbide, acrylic, stone, fissure, disc, sand paper bur) (Germany).
7. Flasks (Broden, Sweden).
8. Glass Jar, Watch, thermometer (China).

9. Hydraulic press (Germany).
10. Impact tester (N. 43-1, testing machines, INC. USA).
11. Incubator (Gallenbamp, England).
12. Instron universal testing machine (Instron Corporation, 1122, canton mass).
13. Lathe polishing machine. (Germany).
14. Prosthetic hand piece (W and H elco. Austria).
15. Rubber bowel, stainless steel spatula, wax knife, lacron carver (Germany).
16. Thermostatically controlled water bath. (memert, Germany).
17. Tinius Olsen testing machine (Tensile testing machine, H50KT, UK).

2-1-4-2 Materials fig. (2-2)

1. Deionized water (Iraq).
2. Dental pumice (England).
3. Dental stone (Type III, thixotropic, Zhermack, Italy, 2014).
4. Heat cure acrylic resin for denture (Non – veined acrylic, powder and liquid, IvoclarVivadent AG, Italy, 2013).
5. Plaster (Al- ahliyah co., Iraq).
6. Polyethylene sheet (China).
7. Separating medium (England, 2015)

Fig (2-2): A- Heat cure acrylic resin for denture (Non – veined acrylic, powder andliquid, B- Dental stone .

2-2 Methods

The fallowing tests were performed in the present study:

1-Test the cytotoxicity of different concentrations of $AgNO_3$ solution on the growth of embryo fibroblast cell-line

2-The antibacterial activity assay which include several experiments :

A- Determination the level of antibacterial activity of different concentration of $AgNO_3$ solution on the growth of *mutans streptococci* group.

B- Inhibitory effects of $AgNO_3$ – loaded resin discs on the growth of *mutans streptococci* group through:

B-Effect of $AgNO_3$ –loaded resins on viable count of *mutans streptococci* group colonies.

A-Estimation the inhibition zone of $AgNO_3$ –loaded resins.

3- In vitro Ag release test.

4- Characterization of$AgNO_3$- loaded resins.

5- Mechanical tests including: :

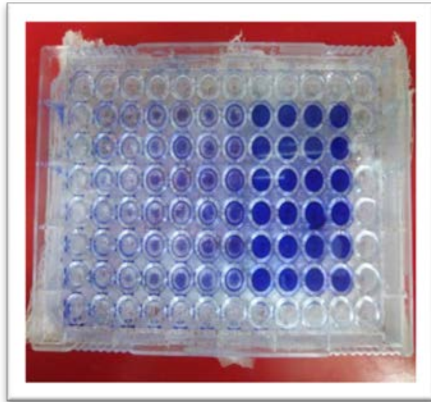
Impact strength

Transverse strength

Tensile strength

2-2-1Cytotoxicity of different concentrations of silver nitrate solutions on rat embryo fibroblast (REF) cell line:

This procedure was done according to **Freshney(2005)** and was done in the Institute of theIraqi Center for Cancer and Medical Genetic Research fig. (2-3).



A B

C

Fig (2-3): A. Microplate with stained cells with crystal violates.B. ELISA microplate spectrophotometer for reading the microplate. C. Inverted Microscope with digital camera for examining the treated REF cells.

The percentages of Inhibitory Rate (IR) were calculated **(Gao, 2003)** according to the equation as below (2.1):

$$IR\% = \frac{C - T}{C} \times 100 \quad (2.1)$$

Since:

IR%: the percentage of Inhibition Rate.

C: the absorbance (optical density) of control (REF cells not exposed to $AgNO_3$ solution).

T: the absorbance (optical density) of the test of each concentration.

2-2-2 Preparation of silver nitrate($AgNO_3$) concentrations for studying samples

Different concentrations of $AgNO_3$solutions wereprepared from stock solution of 1000ppm of $AgNo_3$(Fig.2-4A). Serial concentrations were prepared :(15, 30, 60,120ppm),(9.375, 150,300,600 ppm) and 900ppm (Fig 2-4 B&C)**.**

The preparedconcentrations were confirmed by Atomic Absorption spectrophotometer (Phoenix -986/ AA spectrophotometer, UK) (fig. 2-4D) and stored in dark bottles wrapped with aluminum foil.

A

B

C

D

Fig (2-4):Preparation of silver nitrate ($AgNO_3$) concentrations for studying samples: A- The stock solution of $AgNo_3$,B&C Nine Prepared concentrations of $AgNO_3$, D- Atomic Absorption spectrophotometer.

2-2-3Preparationof $AgNO_3$- loaded resin discsfor testing the antibacterial activity and in vitro Ag release study

2-2-3-1 Mould preparation for fabrication of $AgNO_3$ – loaded resins.

For standardization of acrylic resin specimens, a metallic round molds with compatible thickness (0.6- 0.7 mm) and suitable diameter (50±2mm) were made by cutting stainless steel disks into desirable shape and thickness using turning machine.Following the conventional flasking technique used for complete dentures construction,during the mold preparation ,the metal patterns werecoated with separating medium and allowed to dry ,the lower portion of the metal flask is filled with dental stone that is mixed according to manufacturer instructions at a mixing ratioof 20ml of water to 100g of powder with vibration to get rid of the trapped air .The patterns inserted to approximately one –half of their depth in the stone for easier removal .After setting ,the set stone and specimens were coated with separating medium and allowed to dry ,then the upper half of the metal flask was positioned on the top of the lower portion and filled with dental stone ,again with vibration .The dental stone was allowed to set for one hour before the metal flask was opened in order to remove the metal patters carefully fig.(2-5).The portions of the metal flask were coated with separating medium to be ready for packing with acrylic dough.

Fig (2-5): Metal pattern in dental flask.

2-2-3-2Proportioning,mixing, packing and curing of acrylic resin

The heat cure denture base resin was mixed according to manufacturer instruction P/L ratio: 2.25g of powder was mixed with 1ml liquid (0.8ml monomer + 0.2ml $AgNO_3$ solutionof each concentration). Specimens devoid of silver nitrate were included as controls. In a clean dry glass jar, the mixing of the powder and liquid was done and stirred with a clean wax knife until the monomer and polymer were thoroughly mixed, then the jar was covered until the mixture reached the dough stage. As the acrylic resin reached the dough stage, the resin was removed from the jar, rolled and then packed into the mold previously coated with separating medium. Apolyethylene sheet was used as separating medium between the upper and lower flask during the initial flask closure in a hydraulic pressunder load 850kgf(1 bar) **(Consani et al, 2002).** The flask was removed from the press, opened carefully, then the polyethylene sheet was removed , the acrylic resin access was trimmed with a sharp wax knife, at this stage (before the final closure) with a T -shaped stainless steel metal device fig(2-6 A) with internal diameterof 6 mm, multiple disk like shaped were cutted(fig 2-6 B&C).The discs measurementswere resemblingthe sensitivity discs used in microbial study. During the application of final closure ,metal to metal contact of the flask halves was completed in the press ,the flasks were placed in traditional clamps after final pressing in hydraulic press under a load of 1250 Kgf for 5 min **(Consani et al ,2002)**(fig2-6 D) curing was carried out by placing clamped flasks (fig 2-6 E) in a water bath , the flasks were immersed in water,heat source was operated for 45 min at 74° C then boiled for 45min according to manufacturer instruction .

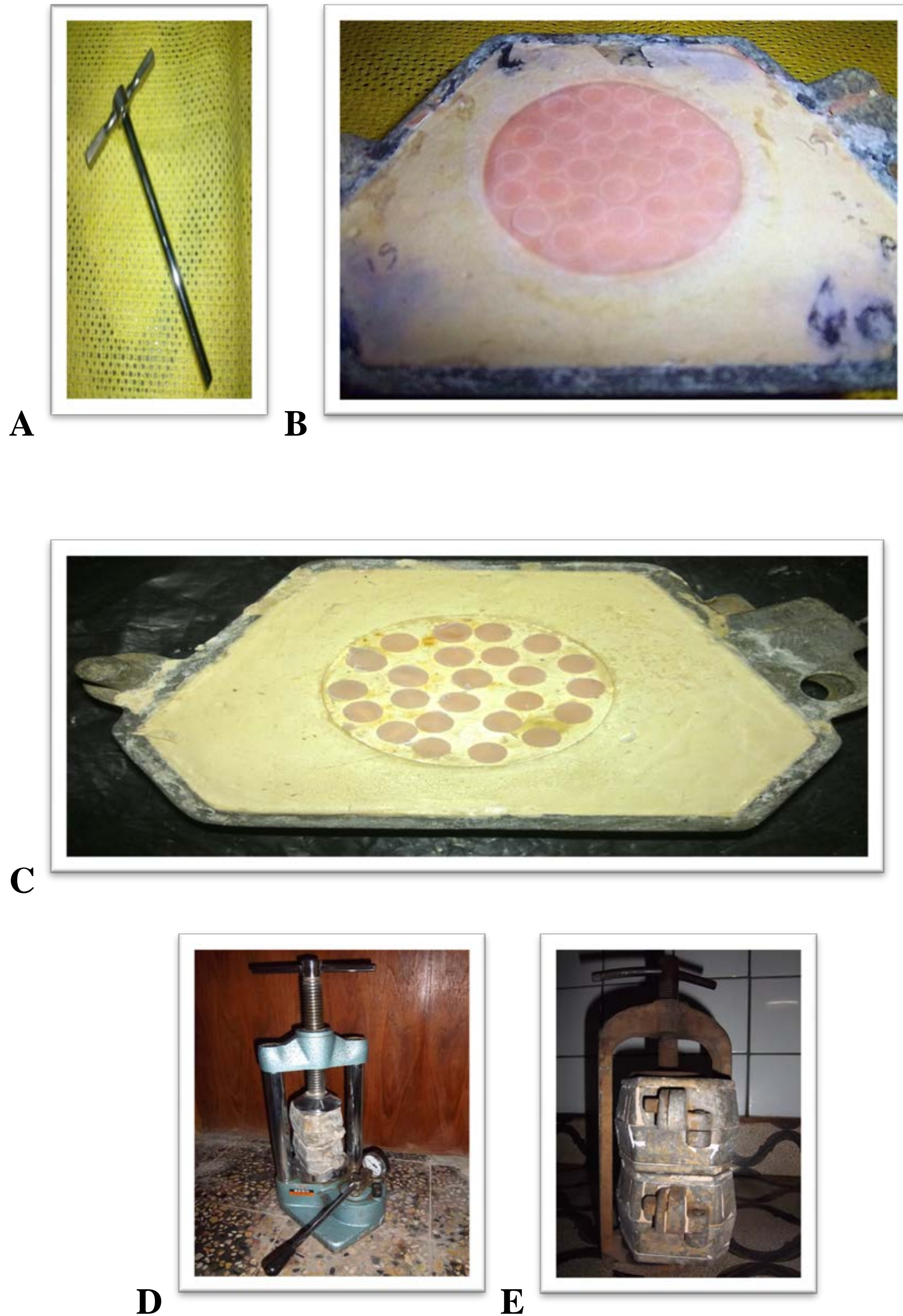


Fig (2-6):Preparation of $AgNO_3$ - loaded resin discs:A-(T)-shaped stainless steel metal device, (B&C)Cutted acrylic dough into disk like shape , D- Metal flask under hydraulic press , E- Clamped flask.

2-2-3-3 Finishing and Polishing

After completing the curing cycle ,the flasks were allowed to cool down slowly in the water bath for 30min then removed from the water bath and allowed to be cool at the bench before deflasking.Then all specimens were carefully deflasked and cleaned , flashes of acrylic were removed with an acrylic bur to get a smooth surface. The specimens were grounded with silicon carbide papers with continuous dipping in water for cooling. Polishing was accomplished by using bristle brush and rag wheel with pumice in lathe polishing machine. A gloss surface was obtained by using chamois buff and polishing soap on dental lathe using low speed (1500 rpm) with continuous dipping in water to ovoid overheating which may lead to distortion of the specimens. They were measured with electronic digital caliper to obtain the standard dimensions of all $AgNO_3$ -loaded resin disks (6 ± 0.8 mm diameter, 0.6-0.7mm thickness). Some of these $AgNO_3$ –loaded resin disks were used in the "Antibacterial Activity Assay"at baseline (without immersion in artificial saliva "T_0"),others were used in the "Antibacterial activity assay"and for "*in vitro* Ag release test" after immersion and incubation with the artificial saliva at 37°C for thirty days (T_1=30) and for ninetydays (T_2= 90) (fig 2-7).

Fig (2-7): Test specimens with artificial saliva in Test tubes under incubation.

2-2-4Preparation of culture media for antibacterial activity assay

All media were prepared under sterile conditions, according to their manufacturing companies. The constituents were dissolved in distilled water (DW), pH was adjusted to 7.2±0.2 and sterilized by autoclaving at 121 ℃ for 15 min, at 15 pounds,thereafter distributed into sterile tubes or plates.Preparation ofthese particular types of culture media which are right for isolation, purification and cultivation for bacteria (*mutans streptococci* group) had been done. These prepared culture media were suitable with the goals and conditions of this study.

2-2-4-1Mitis- Salivarius Bacitracin Agar (MSB Agar):

This agar is the selective medium for the cultivation of *mutans streptococci* group. It was prepared from mitissalivarius agar (MSA) – according to Hi-Media Companyinstructions- with 20% (w/v) sucrose and 200 units/L bacitracin (**Gold *et al.*, 1973; Beighton*et al.*, 1981**). Medium was prepared according to the instructions labeled on the package by suspending 90 gm of the powder in 1000 ml distilled water, mixed well using magnetic stirrer to ensure dissolution of the whole quantity of the powder. To increase the specialty of MSA to the isolation of *mutans streptococci* group, the addition of sucrose in a concentration of 150 g/L (before sterilization) was performed. After autoclaving, the medium was allowed to cool to about 45°C and one ml of bacitracin solutionwhichwas prepared in a concentration of (200IU) by dissolving 0.364gm of Bacitracin antibiotic in 1000ml of sterilized distilled water mixed well by using magnetic stirrer to ensure dissolution of the whole quantity of the antibiotic, the solution was sterilized by Millipore filter

(0.20 µm). then kept in refrigerator until use. A new fresh solution was prepared every 2-3 weeks(**Geigy, 1962**) .The sterile bacitracin was added for each one liter of the agar, then it was poured in petridishes and permitted to cool and set and then stored in the refrigerator until used.

2-2-4-2 Brain Heart Infusion Broth (BHI-B):

The medium was prepared and sterilized according to Hi-Media Companydirections. Thirty seven gram were suspended in 1000 ml distilled water. Sterilization was done by autoclaving at 15 Ibs pressure, 121°C for 15 minutes.

2-2-4-3 Mueller Hinton Agar (MHA):

This was prepared according to the manufacturer instructions.Thirty five gram was dissolved in1000 ml distilled water, when completely dissolved with boiling;sterilization was done by autoclaving at 15 Ibs pressure, 121°C for 15 minutes.

2-2-4-4 CT- Mannitol Agar (CystineTrypticase- Mannitol Agar):

Preparation of CTA was according to the instructions of BioMerieux Company by suspending 28.5 gm powder in 1000 ml distilled water, mixed well using magnetic stirrer to ensure dissolution of the whole quantity of the powder. After the preparation of CTA, mannitol was added (1%) to the CTA media and heated to insure dissolution of the whole quantity of the powder in the CTA medium.

2-2-5Isolation and Purification of *mutans streptococci* group

2-2-5-1 Collection of stimulated saliva samples

Twenty officersfrom Medical City Labs with no medical historyaged (30-38) years were selected to participate in this project. Eachindividual was asked to not to eat any food or drink except water for onehours(**Salimetrics,2009)** before collection of stimulated saliva samples .The collection includes chewing a piece of Arabic chewing gum (0.4-0.5g) for fiveminutes to stimulate salivary collection as much as possible then saliva wascollected in sterilized screw capped bottles.

2-2-5-2 Isolation of microorganisms

Stimulated salivary samples were collected under standard conditionsaccording to **Dasanayake*et al.*,1995.** After that, saliva was homogenizedby vortex mixer for two minutes. Tenfold serial dilutionwas prepared usingnormal saline. Tow dilutionswas selected for each saliva sample and inoculated on the following culture media:MSB-Agar (The selective media for *mutans streptococci* group),0.1ml was withdrawn fromdilutions (10^2 , 10^4) (**Wade *et al.,*1986),** and then spread in duplicate by using sterile microbiological spreader on the plates of MSB agar. The plates wereincubated anaerobically by using a gas pack supplied in an anaerobic jar for48 hrs. at 37°C followed by aerobic incubation for 24hrs. at $37c^0$**(Holbrook and Beighton,1986).**

2-2-5-3 Identification of *mutans streptococci*group

The colonies on MSB agar was examined directly under dissecting microscope (magnification20x). The identification of *mutansstreptococci* group isolates was done according to **(Edwardsson, 1970).**

2-2-5-4 Morphological Examination of the microbial Cells:

A colony was picked up from MSB agar plates separately under sterilized conditions and;a small inoculum was taken from a discrete, singly isolated colony, it was emulsified in a drop of normal saline on glass slide to form suspension which was then spreaded, dried and heat-fixed,and then subjected to Gram stain methodaccording to **Koneman*et al*.** **(1992)**.The slide was examined under light microscope with 100x magnification for cells morphology, arrangement, and staining characteristics.

2-2-5-5 Biochemical Tests:

Bacterial colonies of different morphology were picked up from MSB agar under sterilized conditions using inoculating loop and then inoculated in 10ml of sterilized (BHI-B) and incubated aerobically at 37°C for 18 hrs**(Edwardsson, 1970)**. The following tests were conducted:

A - Catalase Production Test:

This test was performed by using Hydrogen peroxide 3% (H_2O_2) used to detect the activity of catalase enzyme production. A small amount of pure isolates of *mutansstreptococci*groupcultures were transferred separately using a sterile loop to the surface of clean dry glass slide.Three to five Drops of hydrogen peroxide 3% immediately placed onto a portion of

bacterial culture on the slide, absence of gas bubbles indicates the absence of catalase enzyme. This test had been carried out also on the colonies of MSB agar plates directly (**William and Vincent, 2005**).

B - Carbohydrate Fermentation Test for *mutans streptococci*

CTA- mannitol medium had been used to test the ability of*mutans streptococci* group to ferment the mannitol which was added in a concentration of 1% to the CTA-Mannitol medium which was distributed into screw capped bottles (10ml in each bottle) and autoclaved, then stored in the refrigerator until used**.** Each bottle was inoculated with 0.1ml of pure*mutans streptococci*groupisolates and incubated aerobically at 37°C for 48 hrs. Changing in color from red to yellow indicated a positive reaction because of pH reduction as a result of acid production from the fermentationreaction according to **Fingold& Baron (1986)**.

2-2-5-6 Purification and Maintenance of the microbial Isolates

A single colony from *mutans streptococci* group was transferred to 10 ml sterile BHI-B and then incubated for 24 hrs aerobically at 37°C.

The purity of the isolates was checked byreinoculationof 0.1 ml of the isolates from BHI-B suspensions on their selective media by spreader as mentioned before, the *mutans streptococci*groupplates were incubated anaerobically for 48 hours at 37°C followed by incubation aerobically for 24 hrs. at 37°C, then selective colony from each isolate was transferred to 10 ml of sterile BHI-B and incubated for 24 hrs. aerobically at 37°C. One ml from this broth was transferred to 10 ml sterile BHI-B and then 1 ml sterile glycerol was added to the inoculated broth; the tubes were labeled

(the type of inoculum and the date of inoculation) and frozen until use. This procedure was repeated twice monthly **(Beighton, 1985).**

2-2-6Activation of inoculums

Inoculums of *mutans streptococci* groupwere activated by the addition of0.1ml of pure broth culture to 10ml ofsterile BHI-B followed by incubation for 18 hrs. at 37°Cbefore the conduction of each *in vitro* experiment in the study weekly.

2-2-7The antibacterial activity assay

2-2-7-1 Determining the antimicrobial activity of different concentrations of $AgNO_3$solution on the growth*mutans streptococci* group

Disk diffusion test of Kirby –Baure method was performed.For obtaining the inoculum; after activation the inoculums of *mutans streptococci* group, the turbidity of inoculums was adjusted to 0.5 McFarland standards.A sterile cotton swab is dipped into the standardized bacterial test suspension and used to evenly inoculate entire surface of Muller –Hinton agar plate . After the agar surface has been dried for about 5 min, the sterile paper disks were placed on the agar and wetted with a fixed amount of (7ul) of silver nitrate solution .These agarplates were incubated for 24hrs at 37°C.

The zone of inhibition diameters were measured with electronic digital caliper (mm) and this measurement indicated the microbial susceptibility to the different concentrations of the material and compared with sterile distilled water as negative control and Ceftriaxone as positive control **(Joanne et al, 2008)**.

2-2-7-2 Inhibitory effects of $AgNO_3$ – loaded resins on the growth of *mutansstreptococci* group:

2-2-7-2-1 Estimation the inhibition zone of $AgNO_3$– loaded resins

Disk diffusion test of Kirby –Baure method was performed .For obtaining the inoculum; after activation the inoculums of *mutans streptococci* group,the turbidity of inoculums was adjusted to 0.5 McFarland standard .A sterile cotton swab is dipped into the standardized bacterial test suspension and used to evenly inoculate entire surface of Muller –Hinton Agar plate . After the agar surface has been left for about 5 mines, then the $AgNO_3$ –loaded resindisks were placed on the agar and the plateswere kept atroom temperature inside the laminar flow for 120 min for diffusion of theantimicrobial agents **(Moller, 1966).**These agarplates were incubated for 24hrs. at 37° C.
The inhibition zone around the disk (if any) wasmeasured with electronic digital caliper (mm) **(Joanne *et al*., 2008).**

2-2-7-2-2Effect of $AgNO_3$ - loaded resins on viable count of *mutans streptococci*groupcolonies

To examine the antimicrobial efficacy of $AgNO_3$ –loaded resins, *mutans streptococci* group was diluted in 0.9% NaCl and a bacterial suspension of approximately 10^7CFU/ml was prepared using spectrophotometer at (530 nm)(fig.2-8), each specimen was placed in a test tube containing 9.9 ml of sterile BHI-broth, into which were dispensed 100ul of bacterial

suspension .Specimens of PMMA (without $AgNO_3$) in BHI- broth with*mutans streptococci* group were used as controls. All mixtures were incubated at 37°C for 24hrs. After incubation, 0.1 ml of each mixture was transferred to 9.9ml of NaCl (0.9%) and tenfold diluted was performed. From dilution 10^{2-}, 0.1 ml was taken and spread on MSB agar and incubated anaerobically for 48hrs at 37°C then aerobically for 24 hrs at 37°C.The viable counts of all plates were done(**Baron *et al*., 1994**), and the material ABE (Antibacterial efficacy) was calculatedfollowing **Chladek*et a.l,* 2011** using the fallowing equation (2.2):

$$ABE\,(\%) = \frac{V_c - V_t}{V_c} \times 100 \qquad (2.2)$$

Since:

ABE:Anibacterial efficacy**.**

V_c: Number of viable bacterial colonies of control .

V_t : Number of viable bacterial colonies of the test specimens .

Fig (2-8): spectrophotometer.

2-2-8 ***In vitro* Ag release test**

All the disc like specimens were immersed in 10 ml of sterile artificial saliva and incubated at 37˚ Cunder agitation(Fig 2-9-A)for different periods: T_1= 30 days , T_2= 90 days, control specimens containing 0 (zero ppm) $AgNO_3$.The pH of artificial saliva was adjusted also it's volume was reconstituted every 10 days to account for evaporation .One ml of solution of each tube was collected, and Ag dosage was analyzed by Atomic Absorption spectrophotometer (Phoenix -986/ AA spectrophotometer, UK)(Fig 2-9B) with limit for detection of Ag of 0.025ppm andthe Atomic Absorption spectrophotometer (AA-6800 Shimadzu,Japan)(Fig 2-9C)with limit for detection of Ag of 0.01ppb. The amount of Ag^+ released was calculated using a linear calibration curve in the equipment prepared from standard $AgNO_3$ solutions at different concentrations.

A

B

C

Fig.(2-9): Equipment used for *in vitro* Ag release test: A- The specimens incubated under agitation, B- Atomic Absorption spectrophotometer (Phoenix -986/ AA), C- Atomic Absorption spectrophotometer (AA-6800 Shimadzu,Japan).

2-2-9 Characterization of $AgNO_3$-loaded resins

The Fourier transform infra –red (FTIR) spectra was performed (on IR Affinity-1/Shimadzu Corporation/Japan spectrophotometer) using KBr and CaesiumIodid (CsI) pellets to determine whether or not functional groups of the $AgNO_3$ have been attached to the heat cured PMMA by analyzing the characteristic vibrations of functional groups(**Singho*et al*.,2012**)fig.(2-10).

Fig. (2-10): Fourier transform infra –red (FTIR) spectrophotometer.

2-2-10 Mechanical testing

2-2-10-1Preparation of test specimens

2-2-10-1-1Pattern preparation:

Two different metal patterns were constructed by cutting stainless steel platein desired shape and dimension by turning machine according to the required test while the plastic pattern was constructed by cutting plastic plate into desired shape and dimension by laser cutting machine (Fig 2-11).

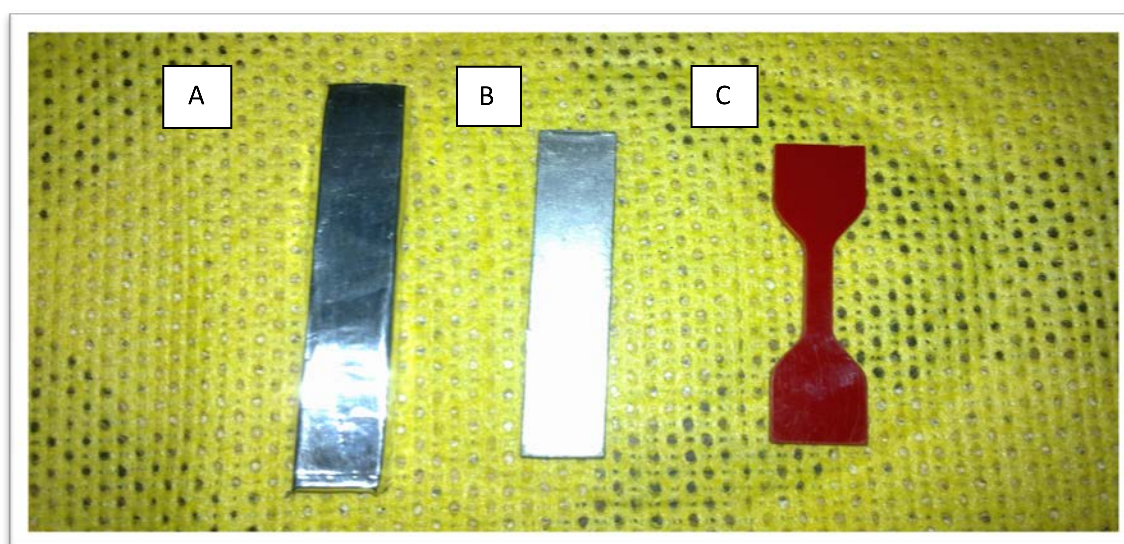


Fig (2-11):

A. Impact strength test: a bar shaped specimen with dimensions of (80mm x 10mmx 4mm) length, width, thickness respectively **(ISO. 179-1, 2000)**.
B. Transverse strength test: a bar shapedspecimen with dimension of (65mm X 10mm X 2.5mm) length, width, thickness respectively **(ADA specification, No. 12, 1999)**.
C. Flat dumbbell shaped specimens with dimensions given by **ISO 527:1993 plastic – determination of tensile properties**(16± 1mm length; 3± 0.2 mm width and 2± 0.2 mm thickness at the parallel segment.

2-2-10-1-2Mould preparation/proportioning, mixing, packing, finishing and polishing of $AgNO_3$ -loaded resins

These procedures were similar of that mentioned in the preparationof $AgNO_3$-loaded resin to determine the antimicrobial activity on the growth of*mutansstreptococci*group (2-2-3-1, 2-2-3-2, and 2-2-3-3).

2-2-10-2Mechanical tests

Evaluation of the mechanical properties of the prepared $AgNO_3$_loaded resinwith conventional denture base (heat cure acrylic resin).

These tests are:

1. Impact strength test.
2. Transverse strength test.
3. Tensile strength test.

2-2-10-2-1 Impact strength test

A. Specimen design

The specimens used were prepared according to**(ISO. 179-1:2000)** with dimensions (80mm length , 10mm width , 4mm thickness ± 0.2mm)(fig.2- 12) for un-notched specimens. Six specimens of each concentration were prepared makingtotal of (60) specimens for impact strength measurements. Specimens were tested after being conditioned in distilled water at 37˚C for 48hours (**ADA specification No. 12, 1999**).

Fig (2-12): measurements of specimen used for impact strength test.

B. Testing procedure

Impact strength test was conducted following the procedure given by the ISO179 with Charpy type impact testing instrument (Fig 2-13A). The specimen was supported horizontally at its ends and strucked by a free swinging pendulum released from a fixed height in the middle. A pendulum of 2 joules testing capacity wasused. The scale reading gave the impact energy absorbed to fracture the specimen in joules when struck by sudden blow (Fig 2-13B) .TheCharpy impact strength ofunnotched specimen was calculated in KJ/m^2 according to **Anusavice, (2008)** as given by the following equation (2.3):

Impact strength=$\times 10^3$(Anus; $\frac{E}{b.d}$) (2.3)

Since:

E: is the impact absorbed energy in joules.

b: is the width in millimeters of the test specimens.

d: is the thickness in millimeters of the test specimens.

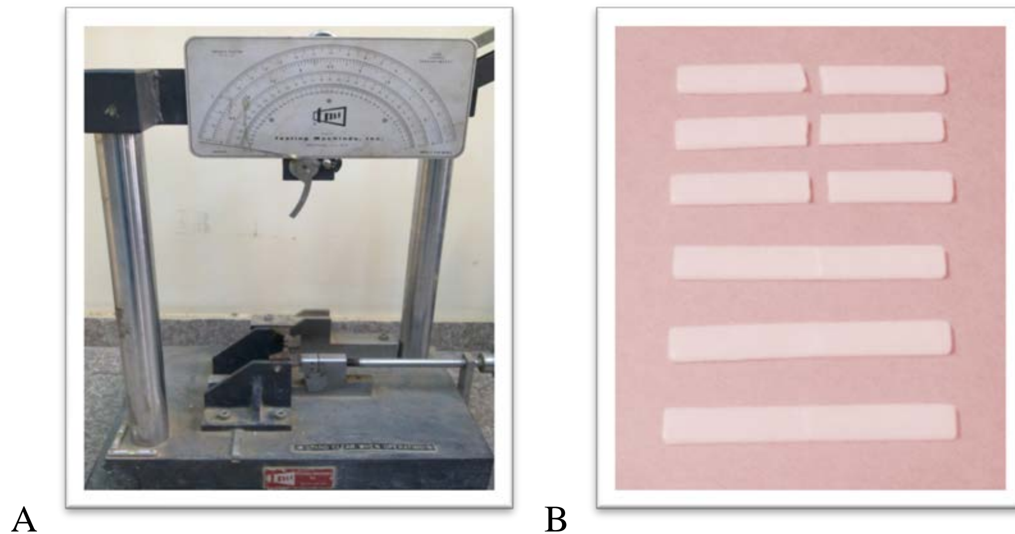


Fig (2-13): A-Impact testing instrument, B- Impact strength testing specimens (pre and post testing).

2-2-10-2-2 Transversestrength

A. specimen design

The specimens used were preparedaccording to **(ADAspecification, No. 12,1999)**withdimensions (65mm length , 10mm width , 2.5mm thickness ± 0.2mm) (fig. 2-14).Six specimens of each concentration make total of (60) specimens formeasurements of transverse strength. All the specimens were immersed in distilledwater at 37˚C for 48 hours before being tested **(ADA No. 12, 1999)**

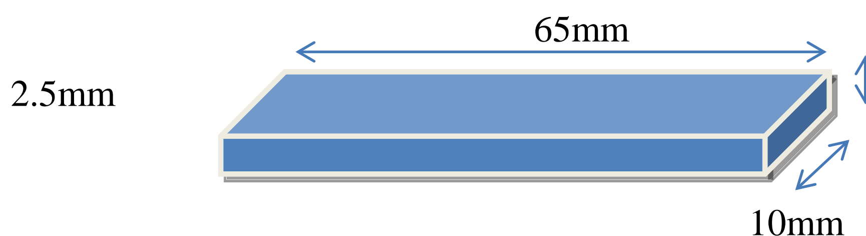


Fig (2-14): measurements of specimens used for transvers strength test.

B. Testing procedure

The test was achieved using instron testing machine, each specimen was positioned on bending fixture, consisting of two parallel supports (50)mm apart, the full scale load was 50kg, and the load was applied with cross head speed of1mm/min by rod placed centrally between the supportsmaking deflection until fracture occurred (Fig 2-15 A& B).

The transverse bend strength was calculated using the following formula (2.4):

(2.4)

$$\text{Transverse strength(MPa)} = \frac{3Pl}{2bd^2}$$ (Anusavice,2008)

Since:

P: is the peak load.

l: is the span length.

b: is the sample width.

d: is the sample thickness.

A

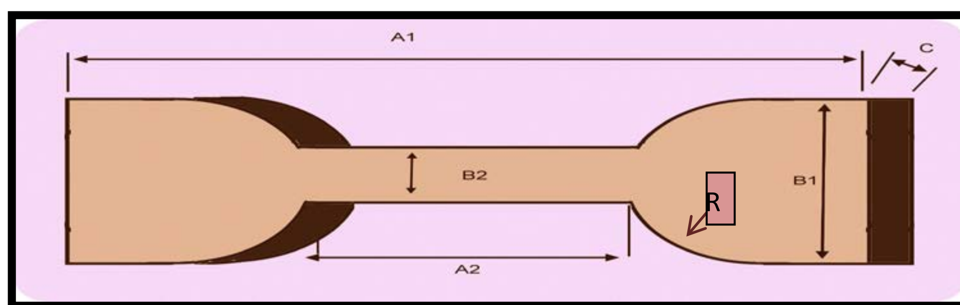
B

Fig (2-15): A- Instron testing machine, B- Specimen under testing.

2-2-10-2-3 Tensile strength test

A.Specimen design

Flat dumbbell – shaped specimen were preparedwithdimension given by (ISO 527: 1993 plastic determination of tensile properties) (Fig 2-16).Six specimens of each concentration were prepared make a total of (60) specimens for tensile strength measurements



A_1: overall length 60 ± 2mm.
A_2: length of narrow parallel –sided portion 16 ± 1mm.
B_1: width at ends 12 ± 1mm.
B_2: width of narrow parallel – sided portion 3 ± 0.2.
C: thickness 2 ± 0.2.
R: large radius 12 ± 1mm.

Fig (2-16): Dimensions of tensile strength test specimen.

B. Equipment and procedure

The specimens stored in distal water for 48 hrs at 37°C before testing **(ADA No. 12, 1999).**The test was measured usingTinius Olsen testing machine (Fig 2-17A) at a cross head speed of 0.5 mm/min and with 50 mm grip – to – grip distance (Fig 2-17B), The force at the failure was recorded in Newton (N) and the tensile strength values were calculated from the following equation (2.5):

$$\text{Tensile strength } (N/mm^2) = \frac{F\ (N)}{A\ (mm^2)} \quad \text{(ASTM,1986)} \qquad (2.5)$$

Since:

F: Maximum load at failure (Newton).

A: Cross sectional area (mm^2).

A B

Fig (2-17): A-Tinius Olsen tensile machine for tensile strength test, B-Specimen under testing..

2-3Statistical analysis

The result of the study was analyzed by SPSS software (version, 20, USA).In the present study, the statistical methods which were used in order to analyze and asses the results are:

1-Descriptive statistic which include:

1) Arithmetic mean(M).
2) Standard deviation (SD).
3) Standard error (SE).
4) Range (min to max).
5) Graphical presentation by (Bar chart).
6) Statistical Tables.

2- Inferential statistics:

ANOVA (one-way analysis of variance test) for assessing differences between more than two groups,LSD (Least Significant Difference test) was used for examining differences between 2 group means when ANOVA model was significant , Cohen's d to evaluate the effect size on the parameter and for comparison between the different parameters(Cohen's d< 0.3=weak,Cohen's d ≥ 0.8= strong, in between the two values= moderate) ,Pearson's r(linear correlation Coefficient) to show the strength ,direction andstatistical significance of linear association between 2 quantitative normally distributed variables, and Beta (ß) to show the amount of change in dependent variable for each unit increase in independent or explanatory effect.

CHAPTER THREE

RESULTS

Results

Results of the conducted tests are presented in this chapter

They includes:-

1. Cytotoxicity test including:
   A. Effect on cell morphology.
   B. Cell viability.
2. Visual inspection of $AgNO_3$ – loaded resin samples.
3. Antibacterial activity assay which include several experiment:
   A. Determining the level of antimicrobial activity of different concentrations of silver nitrate solution on the growth*mutans streptococci*group.
   B. Determining the inhibitory effects of $AgNO_3$ –loaded resins on the growth of *mutans streptococci* group through the below experiments :
   i. Estimation the inhibition zone of $AgNO_3$ –loaded resins.
   ii. Effect of $AgNO_3$ –loaded resinson viable count of *mutans streptococci* group colonies.
4. *In vitro* Agrelease test.
5. Characterization of $AgNO_3$ –loaded resins.
6. Mechanical tests:

These tests are:

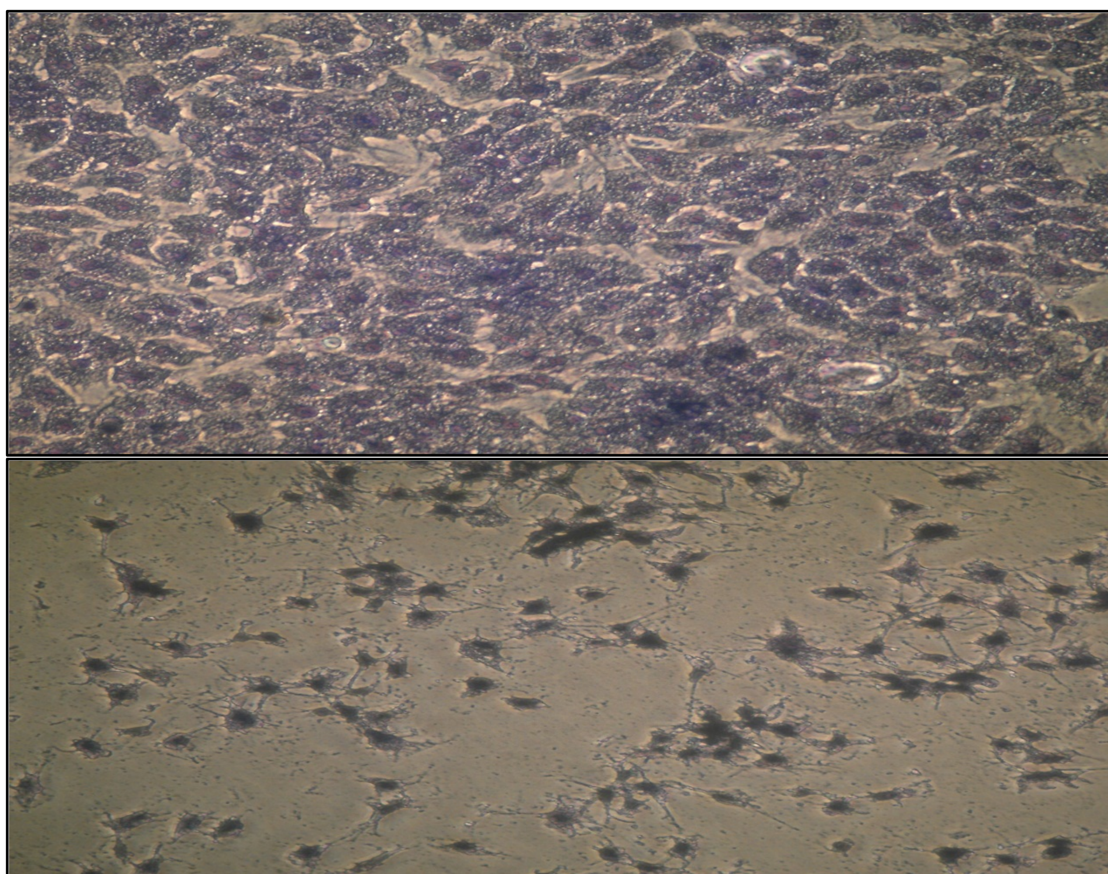
a. Impact strength test.
b. Transverse strength test.
c. Tensile strength test.

3-1 Cytotoxicity test

3-1-1 Effect on cell morphology

The first and most readily noticeable effect fallowing exposure of cells to toxic materials is the alteration in cell shape or morphology in a monolayer culture .Microscopic observation of treated cells showed distinct morphological changes indicatingunhealthy cells, and appeared to be clustered with few cellular extensions, the cell spreading patterns were restricted, whereas the control appeared normal. (Fig 3-1).

A



B

Fig (3-1): A- REF cells without treatment. B- REF cells treated with silver nitrate (100x).

3-1-2 Cell viability

As shown in table 3- 1 and Fig 3-2 the highest mean inhibitory effect of $AgNO_3$ on the growth of REF cells at concentrations of $AgNO_3$ (300 and 600 ppm) was 83.2% and 83.3% respectively. The lowest inhibitory effect was observed at 900 ppm (70.8%).

As shown in table 3- 2 and Fig 3-3 the mean Optical Density (OD) for cytotoxicity effect was highest in the control group (0.33 nm) and lowest in the group with (300, 600 ppm) $AgNO_3$ (= 0.055nm) for both concentrations. The difference observed in mean OD in betweendifferent concentrations of $AgNO_3$ and control group was statistically significant. The strongest cytotoxic effect was observed at 600 ppm $AgNO_3$(Cohen's d=9.12). On the other hand, the lowest effect of $AgNO_3$ regarding the cytotoxic effect was observed at 900 ppm $AgNO_3$ (Cohen's d= 7.05). The effect of $AgNO_3$ concentrations on the growth of REF cells was statistically significant and rated as strong effect.

Table 3-1:Descriptive data of percent cell inhibition % (cytotoxic effect)

	Study groups (concentration of added AgNO3 in ppm)								
	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	300 ppm	600 ppm	900 ppm
Percent cell inhibition % (cytotoxic effect)									
Range	(82.6 to 83.5)	(77.7 to 82.6)	(76.2 to 81.4)	(75.3 to 80.2)	(74.7 to 81.7)	(79.9 to 84.8)	(82 to 85.1)	(82 to 84.1)	(66.8 to 77.7)
Mean	82.9	80.4	78.7	77.5	77.6	82.2	83.2	83.3	70.8
SD	.52	2.48	2.61	2.48	3.64	2.46	1.64	1.14	5.98
SE	.30	1.43	1.51	1.43	2.10	1.42	.95	.66	3.45
N	3	3	3	3	3	3	3	3	3

Table 3- 2:Descriptive data of optical densityOD for cytotoxic effect (nm)

	Study groups (concentration of added AgNO3 in ppm)										
	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	300 ppm	600 ppm	900 ppm	P (ANOVA)
OD for cytotoxic effect											<0.001
Range	(0.233 to 0.415)	(0.054 to 0.057)	(0.057 to 0.073)	(0.061 to 0.078)	(0.065 to 0.081)	(0.06 to 0.083)	(0.05 to 0.066)	(0.049 to 0.059)	(0.052 to 0.059)	(0.073 to 0.109)	
Mean	.328	.056	.064	.070	.074	.073	.058	.055	.055	.096	
SD	.0428	.0017	.0081	.0085	.0081	.0119	.0080	.0053	.0038	.0197	
SE	.0101	.0010	.0047	.0049	.0047	.0069	.0046	.0031	.0022	.0114	
N	18	3	3	3	3	3	3	3	3	3	
Difference in mean compared to control	Reference	-0.272	-0.264	-0.258	-0.255	-0.255	-0.270	-0.273	-0.274	-0.233	
Cohen's d	Reference	-9.07	-8.51	-8.33	-8.21	-8.22	-8.71	-9.11	-9.12	-7.05	
P (LSD)	Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

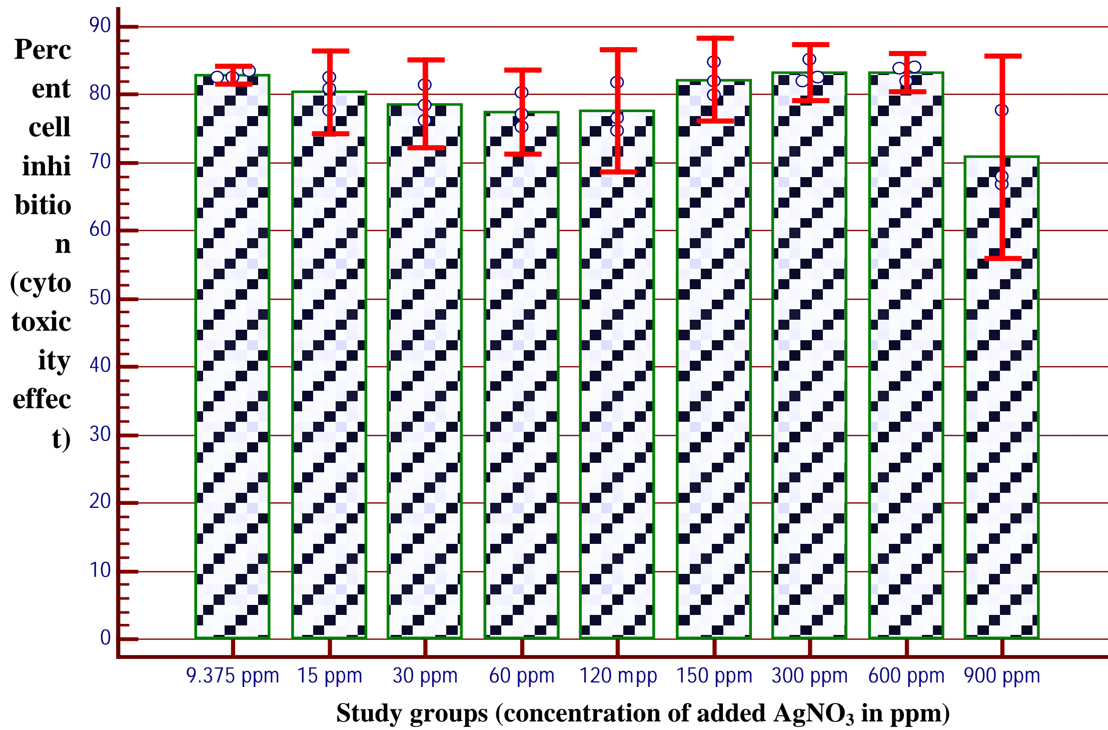


Fig (3-2):Bar chart showedthe mean inhibitory effect of $AgNO_3$(with its 95% confidence interval) on the growth of REF cells

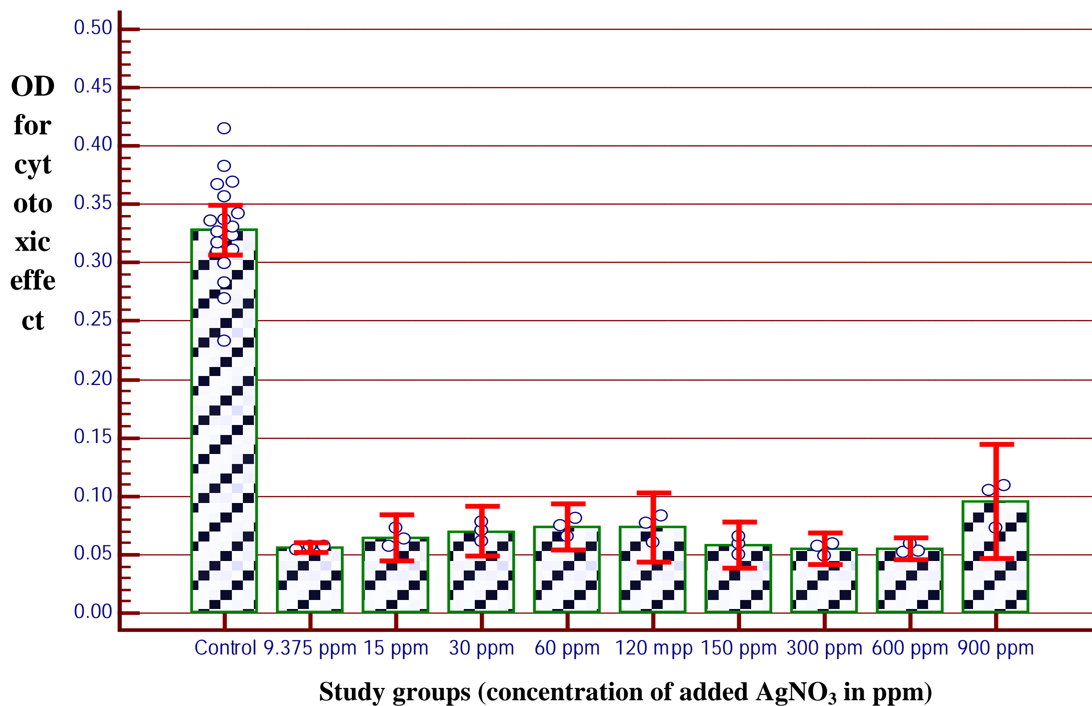
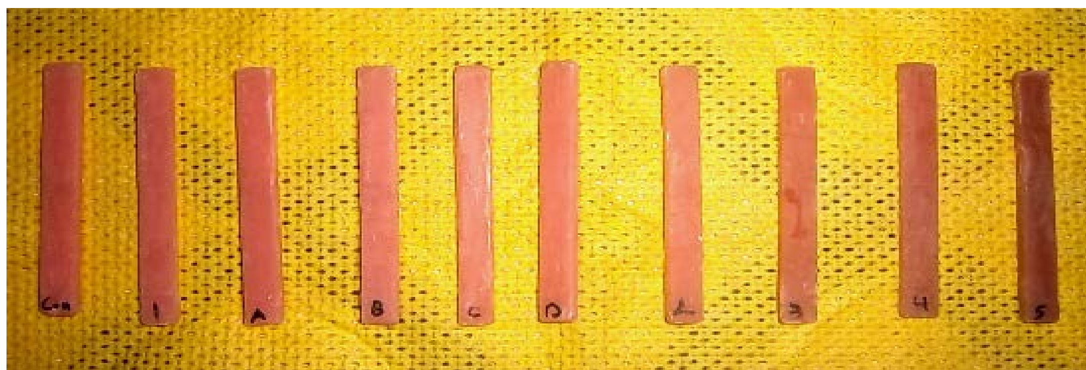


Fig (3-3): Bar chart showed the mean optical density (with its 95% confidence interval) for REF cells of control and the study groups of $AgNO_3$.

3-2 Visual inspection of $AgNo_3$ –loaded resin samples

As the concentration of $AgNO_3$ increased, the prepared $AgNO_3$ –loaded resin samples start to show visually some darkening started at 300 ppm $AgNO_3$ and aboveFig. (3-4).



Control	9.37	15	30	60	120	150	300	600	900ppm

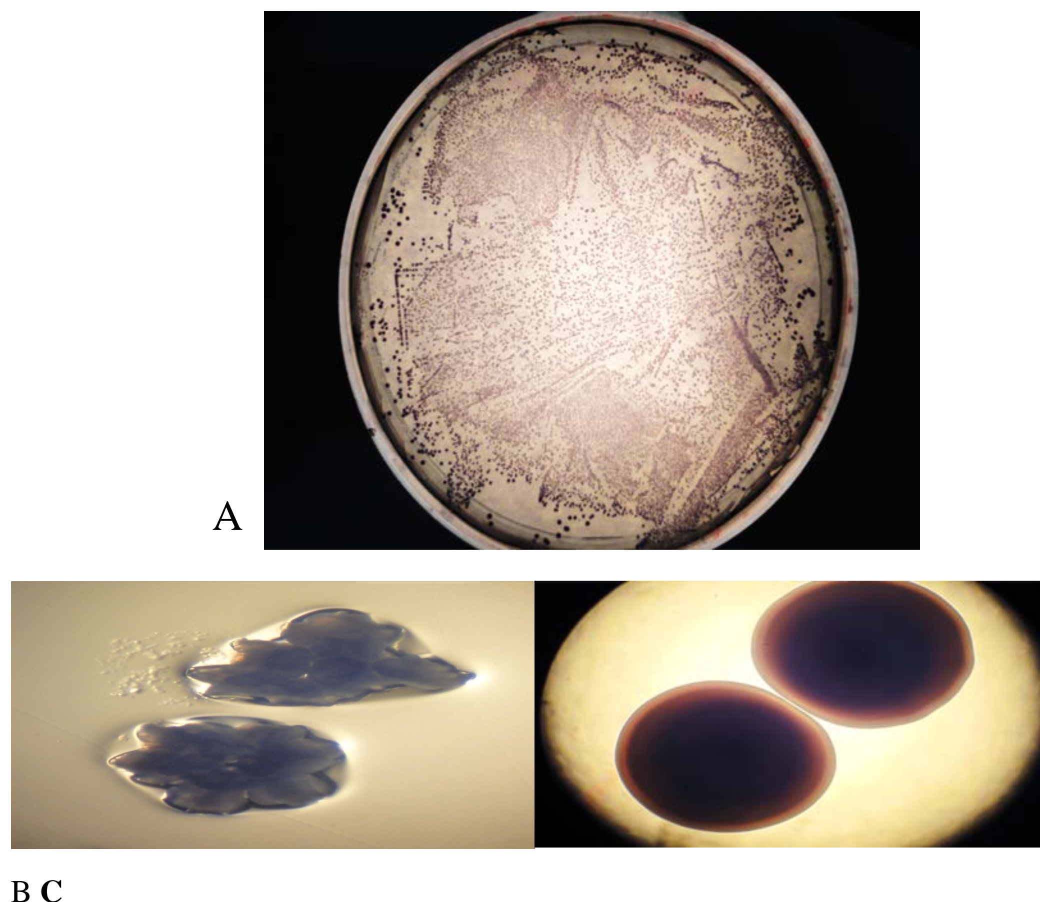
Fig (3-4): $AgNO_3$–loaded resinsfrom left to right: control, (9.375, 15, 30, 60, 120, 150, 300, 600, and 900 ppm).

3-3Identification ofmicroorganisms

3-3-1 Identification of*mutans streptococci*group

3-3-1-1Colony Morphology

On the selective MSB agar plates, *mutans streptococcal*groupcolonies appeared light blue, in color, spherical or ovoid in shape with raised or convex surface, adhered well to the agar surface. Some coloniesappeared as irregular with rough or frosted-glass surface appearance (rough colonies), while others appeared with smooth surface (smooth colonies). Most of *mutans streptococcal*groupcolonies had a depression at the middle of the colony containing a drop of polysaccharide, or sometimes the whole colony submerged in a pool of polysaccharidefig. (3-5).



B **C**

Fig (3-5): A-Photographic image for MSB agar with *mutansstreptococcai*groupcolonies, B&C-Dissecting microscope images for Different forms of *mutans streptococci*group colonies on MSB agar(40x).

3-3-1-2 Morphological examination of the microbial cells

This test had been done to confirm the diagnosis of *mutans streptococci* groupby the gram's staining.*mutansstreptococci* groupcells were gram positive, spherical or ovoid in shape, arranged in short or medium length non spore forming chains(Fig 3-6).

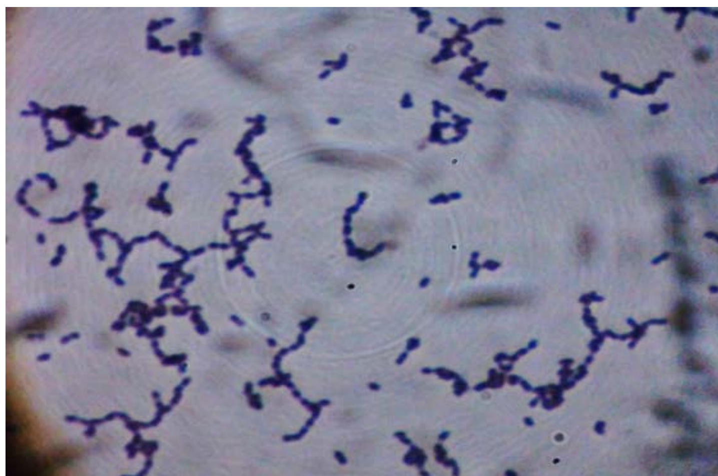


Fig (3-6): Gram's stain showing the *mutans streptococci*group(1000x `magnification).

3-3-1-3 Biochemical tests

All colonies of *mutans streptococci* groupwere catalase negative and had the ability to ferment mannitol. A positive reaction was indicated by the change in color of indicator from red to yellow by the formation of acid after incubation (Fig 3-7).

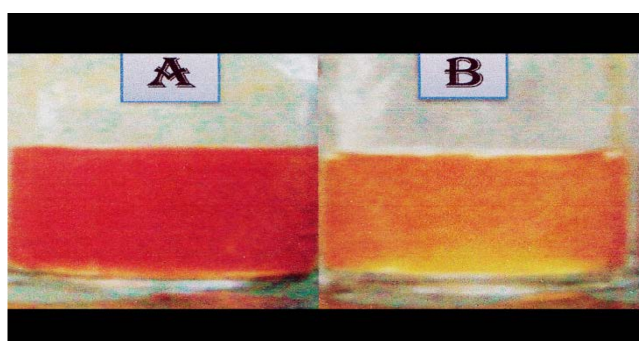


Fig (3-7): Biochemicalidentificationof *mutans streptococci*group, a positive reaction indicated by the change in color of indicator from red(A) to yellow(B).

3-4Antibacterial activity of $AgNO_3$ on the growth of *mutans streptococci*group

3-4-1 Determining the level of antimicrobial activity of different concentrations of silver nitrate solution on *mutans streptococci*group.

As shown in Fig 3-8 and table 3- 3 concentration of $AgNO_3$ had a statistically significant strong positive (direct) liner correlation with diameter of inhibition zone (r = 0.76).

For each one ppm increase in $AgNO_3$ concentration the diameter of inhibition zone is increased by 0.01mm (ß = 0.01).The mean diameter of inhibition zone for the concentrations of $AgNO_3$, positive and negative control is illustrated in Fig.(3-9).

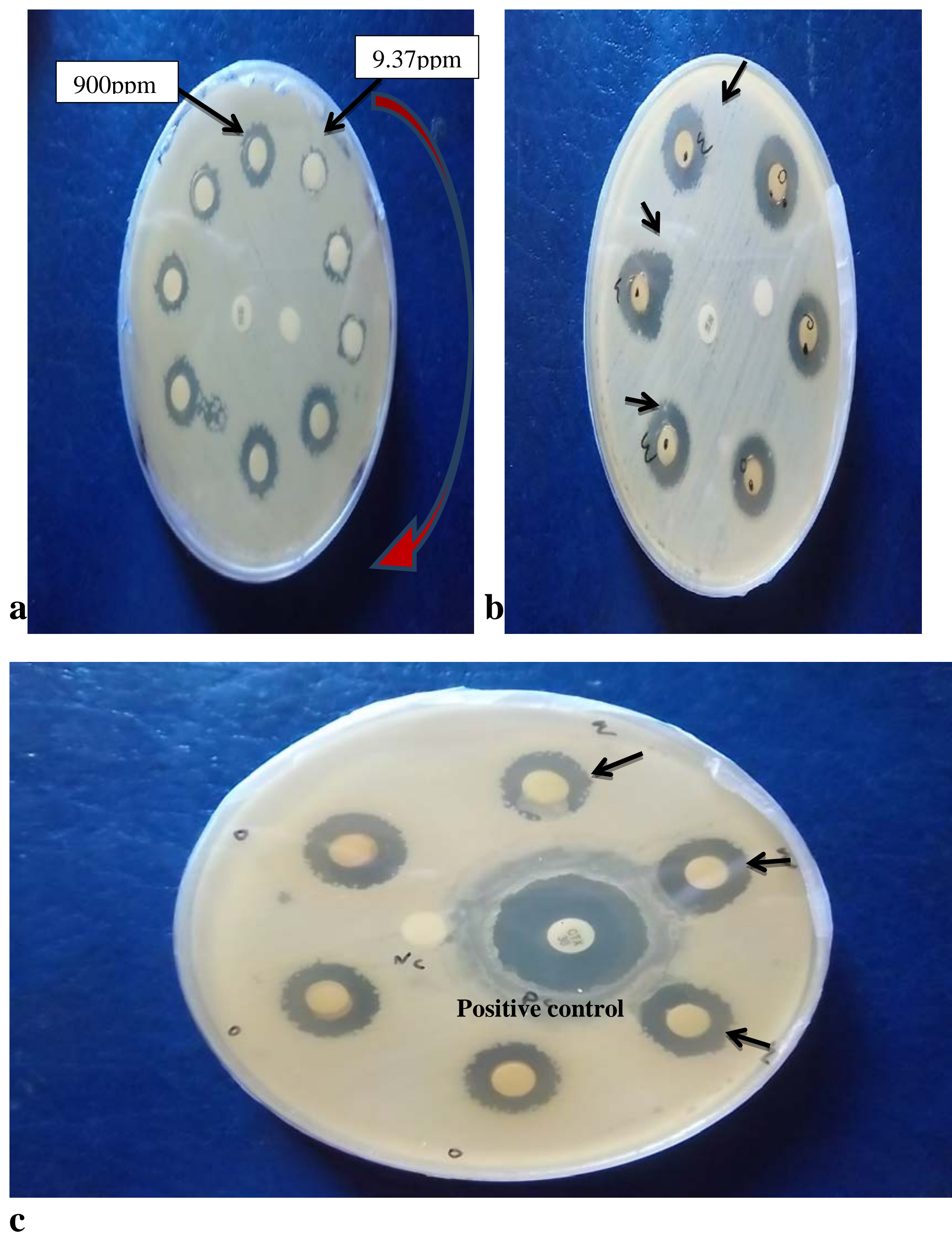


Fig (3-8): Photograph images of the zone of inhibition of $AgNO_3$.solutions:(a)The zone of inhibition of different concentrations of $AgNO_3$solutions compared with deionized water at the center. (b) The zones of inhibition in 600ppmas determined by arrows& 900ppmwere compared with deionized water at the center. (C) The zones of inhibition in 600ppmas determined by arrows& 900ppmwere compared with Ceftriaxone (positive control).

Table 3-3: Descriptive data ofdiameter of inhibition zone (mm)

	Study groups (concentration of added AgNO3 in ppm)											
	Positive control (Ceftriaxone)	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	300 ppm	600 ppm	900 ppm	P (ANOVA
Diameter of inhibition zone (mm)												<0.001
Range	(20 to 23)	(0 to 0)	(6.12 to 6.8)	(6.54 to 7.31)	(7.39 to 7.72)	(7.15 to 8.14)	(8.16 to 8.89)	(9.44 to 11.28)	(10.67 to 12.35)	(11.56 to 13.62)	(12.45 to 14.45)	
Mean	21.2	0.0	6.4	6.9	7.6	7.8	8.7	10.3	11.6	12.8	13.6	
SD	1.05	0.00	.26	.21	.13	.29	.23	.56	.67	.78	.69	
SE	.33	0.00	.08	.07	.04	.09	.07	.18	.21	.25	.22	
N	10	10	10	10	10	10	10	10	10	10	10	
Difference in mean compared to control		Reference	6.4	6.9	7.6	7.8	8.7	10.3	11.6	12.8	13.6	
Cohen's d		Reference	35.56	45.75	84.26	39.24	54.48	25.70	24.58	23.19	27.74	
P (LSD)		Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Difference in mean compared to positive control (Ceftriaxone)	Reference	-21.2	-14.8	-14.4	-13.6	-13.4	-12.5	-11.0	-9.7	-8.5	-7.64	
Cohen's d	Reference	-28.31	-19.26	-18.91	-18.20	-17.38	-16.47	-12.89	-11.00	-9.12	-8.58	
P (LSD)	Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

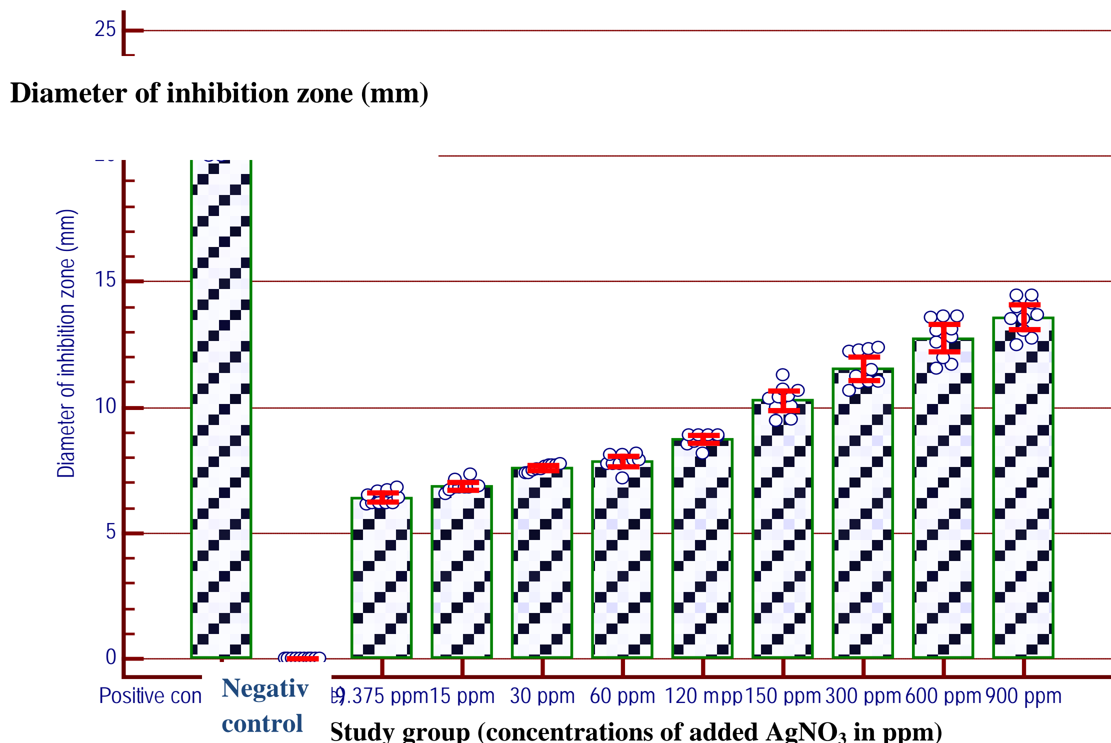


Fig (3-9): Bar chart showed the mean diameter of inhibition zone(with its 95% confidence interval) for $AgNO_3$concentrations, positive and negative control.

3-4-2 <u>Inhibitory effect of $AgNO_3$–loaded resins on the growth of *mutans streptococci*</u>group

3-4-2-1Estimation the inhibition zone of $AgNO_3$ –loaded resins

No inhibition zone detected with $AgNO_3$– loaded resins.

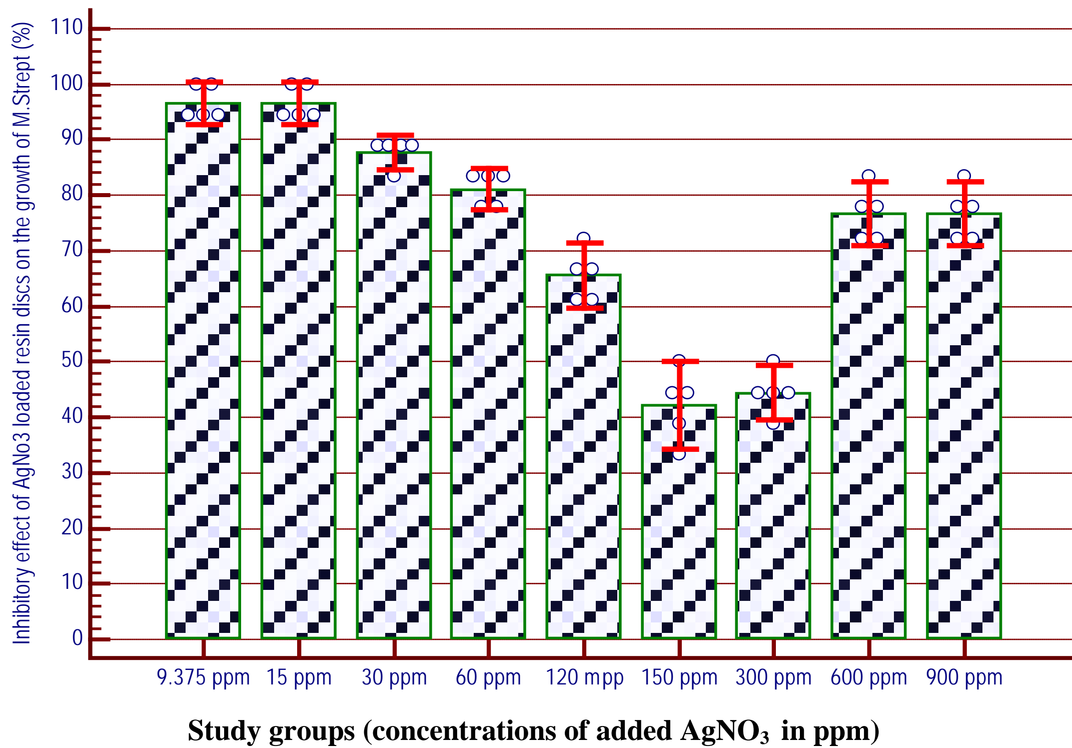
3-4-2-2 Effect of $AgNO_3$ –loaded resinson viable count of *mutansstreptococci* group colonies

As shown in table 3-4and Fig 3-10(A&B) at baseline the mean inhibitory effect of $AgNO_3$ loaded resin disks on the growth of *mutansstreptococci*group(antibacterial efficacy ABE %) at lowest concentration of $AgNO_3$(9.375 and15 ppm) was 96.6% .The lowest inhibitory effect was observed at 150ppm and 300ppm (ABE= 42.2% and 44.4%) respectively.After 30 and 90 days of immersion in artificial saliva the mean inhibitory effect was 100% (ABE) at any concentration of $AgNo_3$.

As shown in Figs 3-11, 3-12, 3-13and table 3- 5at baseline the mean count of CFU/ml was highest in control group (1800 CFU/ml) and lowest in the group with 9.375ppm and 15 ppm of $AgNO_3$(60 CFU/ml).The mean count of CFU/ml was highest in the group 150ppm $AgNO_3$ (1040 CFU/ml). The difference observation in mean CFU/ml between different concentration of $AgNO_3$ and control group was statistically significant.
The strongest effect of $AgNO_3$ compered to control was observed at 9.375ppm and 15 ppm respectively (Cohen's d = 5.46). On the other hand the lowest effect of $AgNO_3$on CFU/ml was observed at 150 ppm (Cohen's d = 2.3) .The effect of any concentration of $AgNO_3$compared to control was statistically significant and rate as strong effect. On the other hand, total inhibition of bacterial growth was observed at 30 or 90 days of immersion in artificial saliva Fig. (3-14 and 3-15).

Table 3-4:Descriptive data of inhibitory effect of $AgNo_3$ loaded resin discs on the growth of *mutansstreptococci*(ABE%)

Inhibitory effect of $AgNo_3$ loaded resin discs on the growth of *mutans.strept.*(ABE%)	Study groups (concentration of added AgNO3 in ppm)								
	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	300 ppm	600 ppm	900 ppm
Follow up time (days) = baseline									
Range	(94.4 to 100)	(94.4 to 100)	(83.3 to 88.9)	(77.8 to 83.3)	(61.1 to 72.2)	(33.3 to 50)	(38.9 to 50)	(72.2 to 83.3)	(72.2 to 83.3)
Mean	96.6	96.6	87.8	81.1	65.6	42.2	44.4	76.7	76.7
SD	3.07	3.07	2.50	3.01	4.65	6.34	3.92	4.65	4.65
SE	1.37	1.37	1.12	1.35	2.08	2.83	1.76	2.08	2.08
N	5	5	5	5	5	5	5	5	5
Follow up time (days) = after 30 days									
Range	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)
Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	5	5	5	5	5	5	5	5	5
Follow up time (days) = after 90 days									
Range	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)	(100 to 100)
Mean	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
SD	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SE	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
N	5	5	5	5	5	5	5	5	5



Study groups (concentrations of added $AgNO_3$ in ppm)

Fig (3-10 A): Bar chart showed the ABE (%) (with its 95% confidence interval) of $AgNO_3$ loaded resin disks on the growth of *mutansstreprococci* group at baseline.

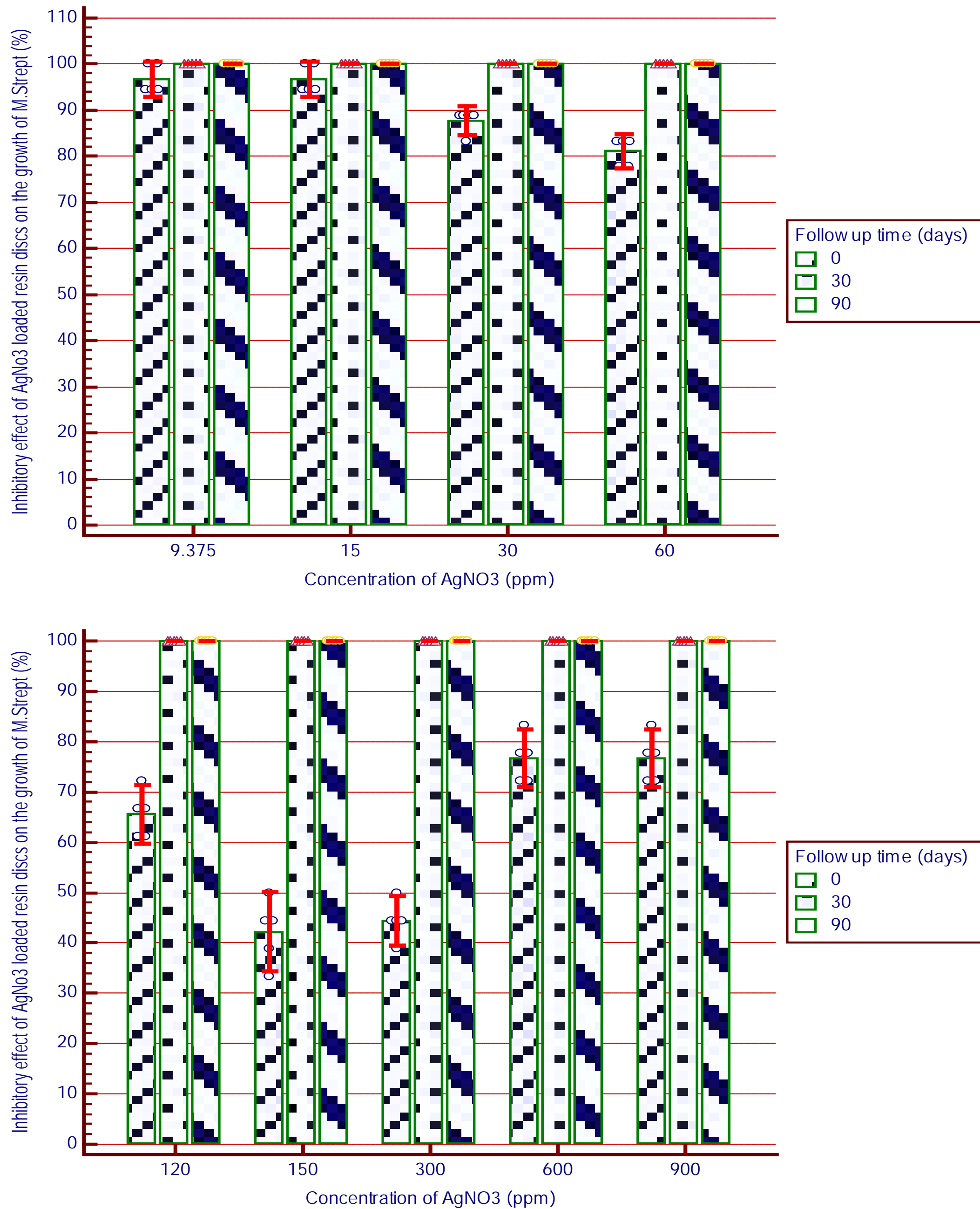


Fig (3-10 B):Bar chart showed the ABE (%) (with its 95% confidence interval) of $AgNO_3$ loaded resin disks on the growth of *mutansstreprococci*group at the designed periods.

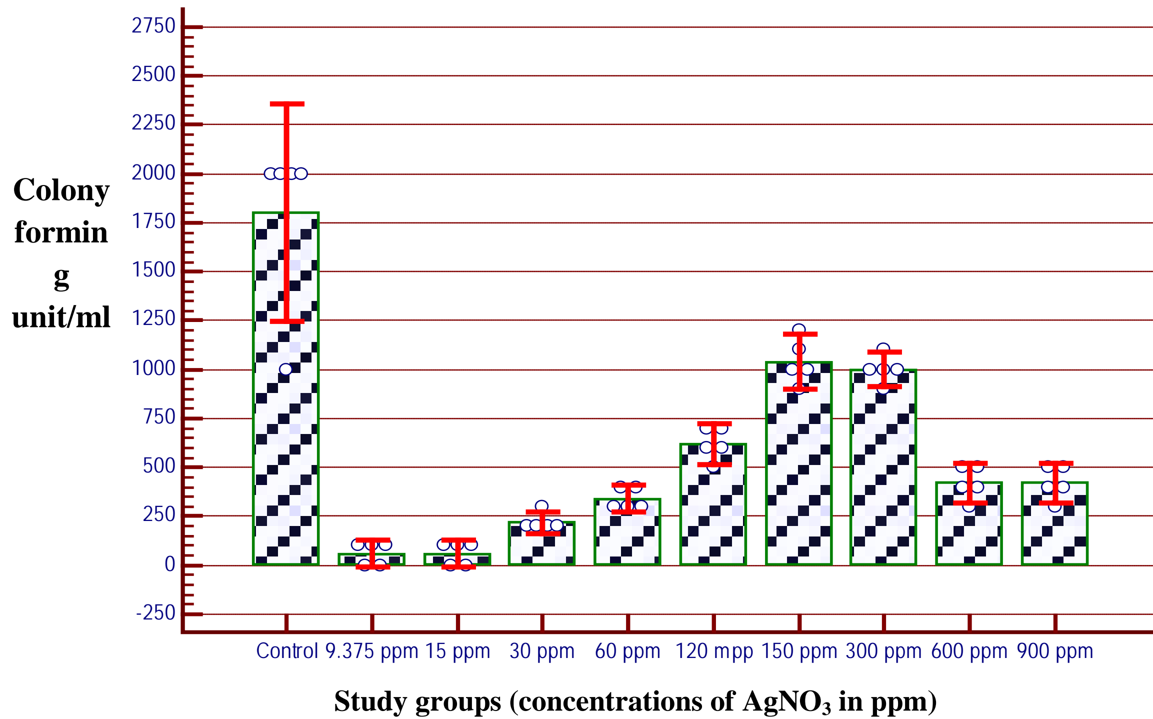


Fig (3-11):Bar chart showed the mean count of CFU/ml (with its 95% confidence interval) at baseline.

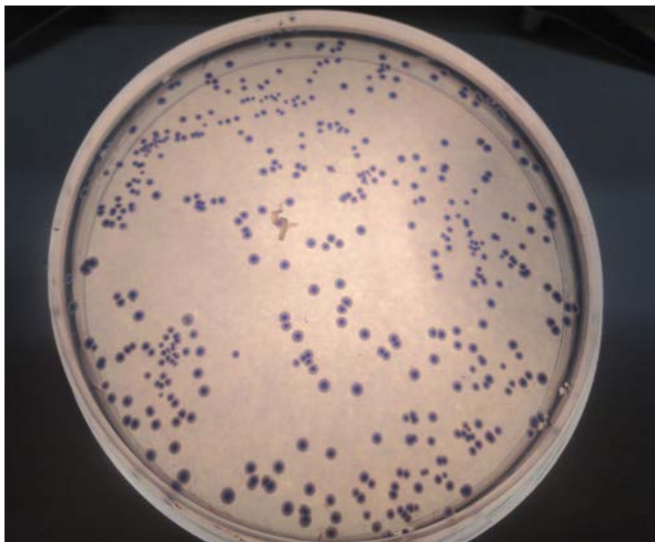


Fig (3-12): Photographic image of MSB agar plate with *mutans streptococci*group colonies as control plate.

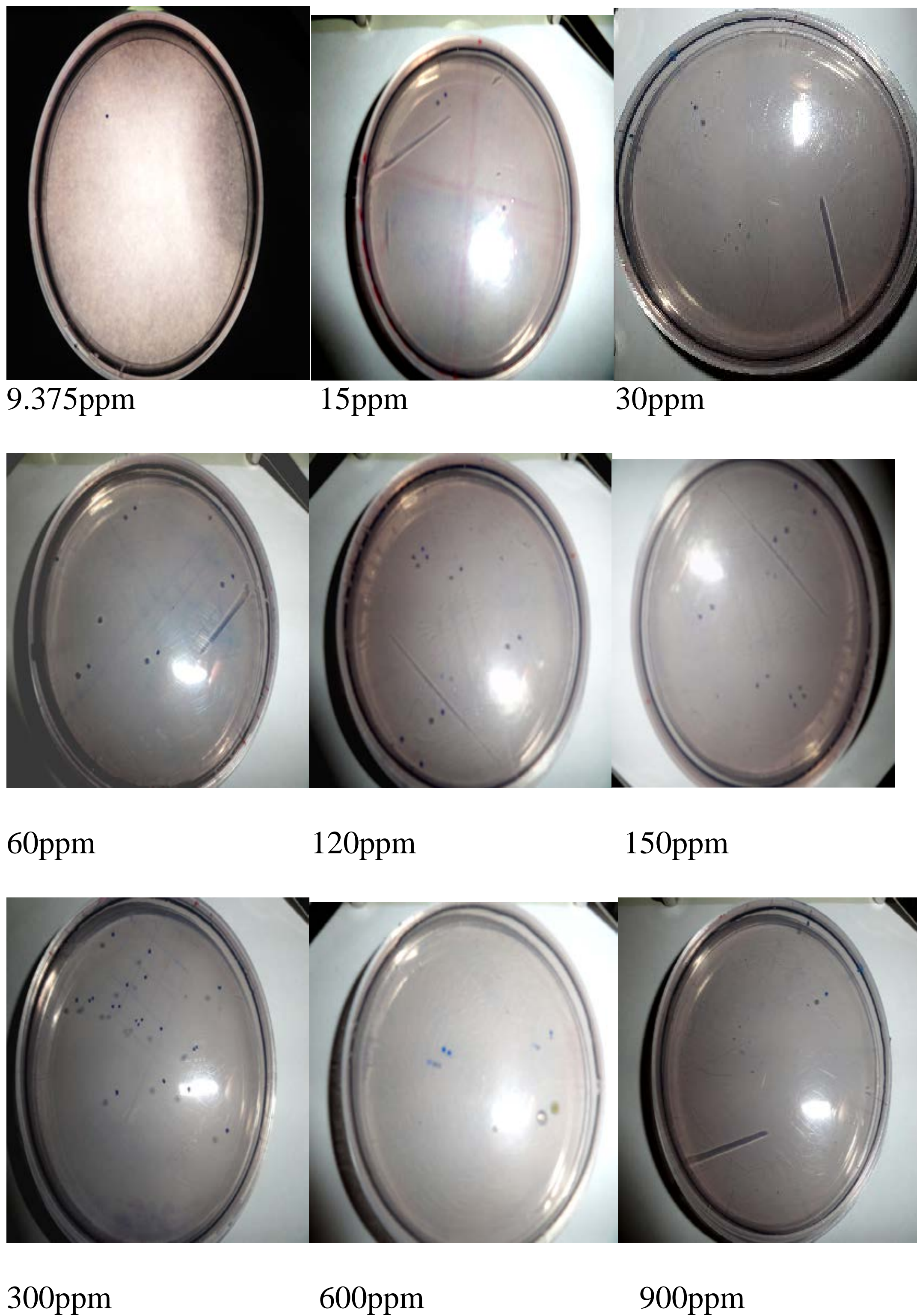


Fig (3-13): Photographic images of MSB agar plates represent the baseline (pretreatment) viable counts of *matans streptococci*group colonies.

Table 3-5:Descriptive data of Colony forming unit/ml

Colony forming unit/ml	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	300 ppm	600 ppm	900 ppm	P (ANOVA)
	Study groups (concentration of added AgNO3 in ppm)										
Follow up time (days) = baseline											<0.001
Range	(1000 to 2000)	(0 to 100)	(0 to 100)	(200 to 300)	(300 to 400)	(500 to 700)	(900 to 1200)	(900 to 1100)	(300 to 500)	(300 to 500)	
Mean	1800	60	60	220	340	620	1040	1000	420	420	
SD	447.21	54.77	54.77	44.72	54.77	83.67	114.02	70.71	83.67	83.67	
SE	200	24.49	24.49	20	24.49	37.42	50.99	31.62	37.42	37.42	
N	5	5	5	5	5	5	5	5	5	5	
Difference in mean compared to control	Reference	-1740.0	-1740.0	-1580.0	-1460.0	-1180.0	-760.0	-800.0	-1380.0	-1380.0	
Cohen's d	Reference	-5.46	-5.46	-4.97	-4.58	-3.67	-2.33	-2.50	-4.29	-4.29	
P (LSD)	Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Follow up time (days) = after 30 days											<0.001
Range	(1500 to 2300)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	
Mean	1940	0	0	0	0	0	0	0	0	0	
SD	288.1	0	0	0	0	0	0	0	0	0	
SE	128.84	0	0	0	0	0	0	0	0	0	
N	5	5	5	5	5	5	5	5	5	5	
Difference in mean compared to control	Reference	-1940.0	-1940.0	-1940.0	-1940.0	-1940.0	-1940.0	-1940.0	-1940.0	-1940.0	
Cohen's d	Reference	-9.52	-9.52	-9.52	-9.52	-9.52	-9.52	-9.52	-9.52	-9.52	
P (LSD)	Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

Continue

Colony forming unit/ml	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 mpp	150 ppm	300 ppm	600 ppm	900 ppm	P (ANOVA)
	Study groups (concentration of added AgNO3 in ppm)										
Follow up time (days) = after 90 days											<0.001
Range	(1600 to 2200)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	(0 to 0)	
Mean	1900	0	0	0	0	0	0	0	0	0	
SD	223.61	0	0	0	0	0	0	0	0	0	
SE	100	0	0	0	0	0	0	0	0	0	
N	5	5	5	5	5	5	5	5	5	5	
Difference in mean compared to control	Reference	-1900.0	-1900.0	-1900.0	-1900.0	-1900.0	-1900.0	-1900.0	-1900.0	-1900.0	
Cohen's d	Reference	-12.02	-12.02	-12.02	-12.02	-12.02	-12.02	-12.02	-12.02	-12.02	
P (LSD)	Reference	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
P (ANOVA) for difference in mean between the 3 time intervals =	0.79[NS]	0.016	0.016	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Effect of after 30 days compared to baseline											
Difference in mean	140.0	-60.0	-60.0	-220.0	-340.0	-620.0	-1040.0	-1000.0	-420.0	-420.0	
Cohen's d	0.37	-1.55	-1.55	-6.96	-8.78	-10.48	-12.90	-20.00	-7.10	-7.10	
P (LSD)	0.52[NS]	0.011	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	
Effect of after 90 days compared to baseline											
Difference in mean	100.0	-60.0	-60.0	-220.0	-340.0	-620.0	-1040.0	-1000.0	-420.0	-420.0	
Cohen's d	0.28	-1.55	-1.55	-6.96	-8.78	-10.48	-12.90	-20.00	-7.10	-7.10	
P (LSD)	0.64[NS]	0.011	0.011	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	

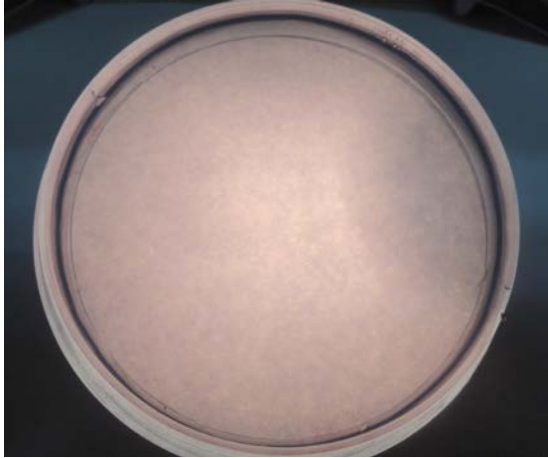


Fig (3-14): Photographic image of MSB agar plate represent the viable counts at 30 or 90days immersion in artificial saliva, there was complete inhibition of bacterial growth.

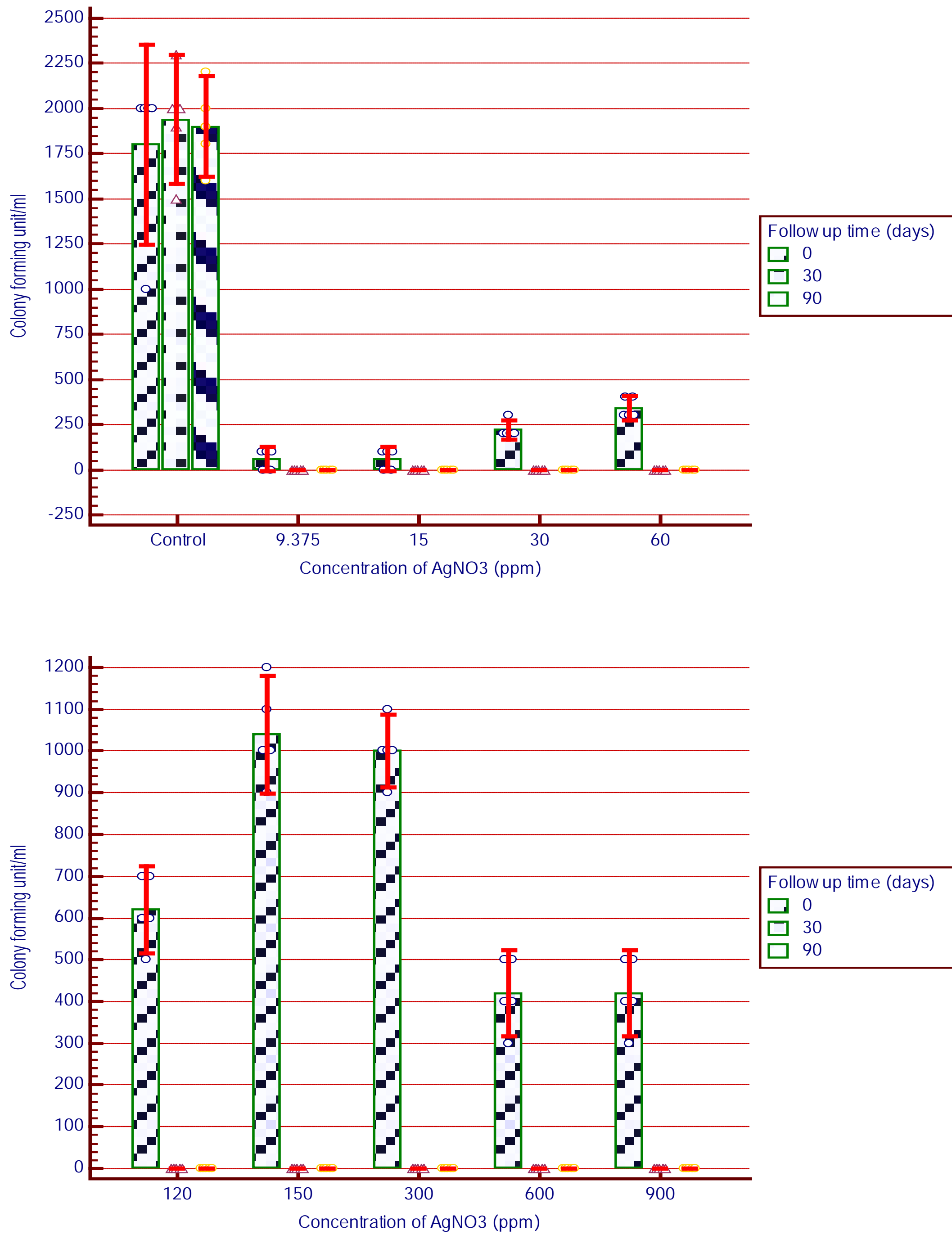
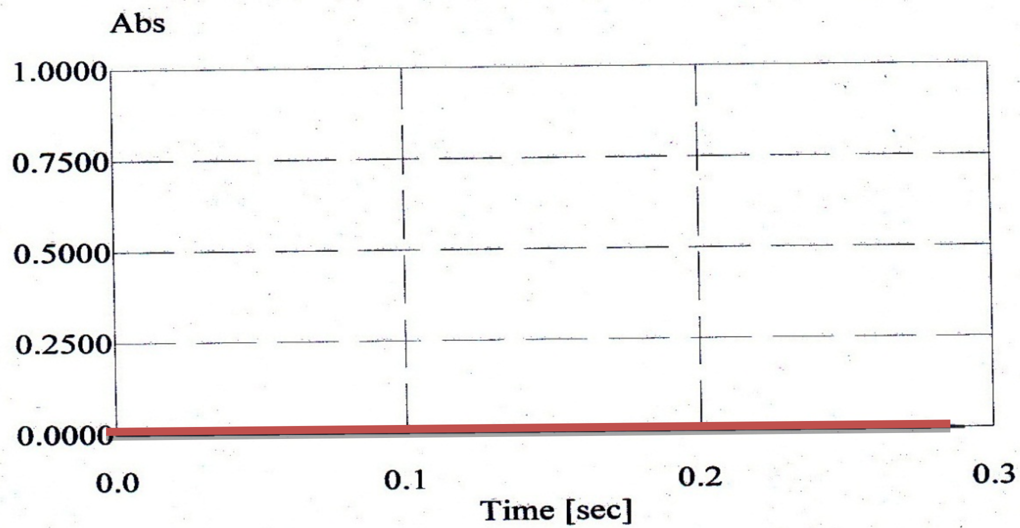


Fig (3-15): Bar chart showed the mean count of CFU/ml (with its 95% confidence interval) at the three designed periods.

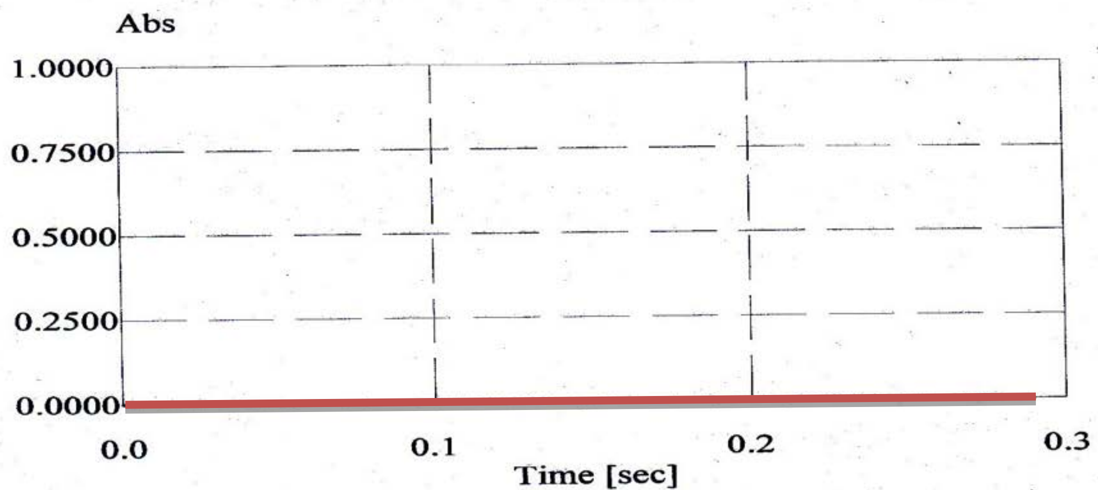
3-5*In vitro* Ag release test

The Artificial saliva that was used as immersion medium for the $AgNO_3$ –loaded resinsregarding all concentrations of $AgNO_3$(9.375, 15, 30, 60,120,150,300,600,900 ppm) and control specimens was analyzed. Despite the high sensitivity of the analytical techniqueused, no Ag was detected in the artificial saliva under all immersion times (30days and 90 days) and concentrations of silver nitrateused (Fig. 3-16 A&B).

Fig.(3-16C) shows similar clear solution (no precipitation of silver halide) in the artificial saliva that used as stored medium for all $AgNO_3$ –loaded resins.



A



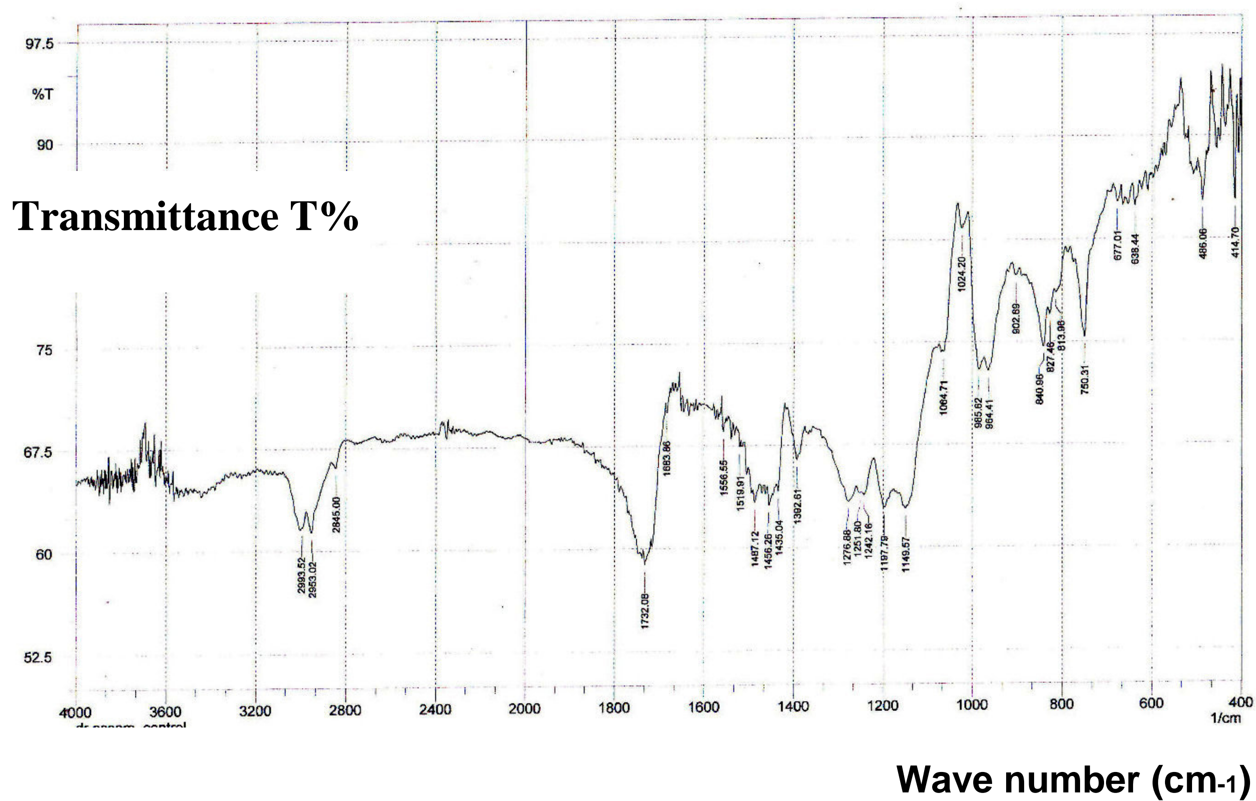
B

Fig (3-16A&B): A-absorption spectrum found for the artificial saliva with control acrylic specimen (without silver nitrate), B- Spectrum of one of the specimens which was similar for all other specimens. Spectrum clearly showssimilar absorption as that of the control.

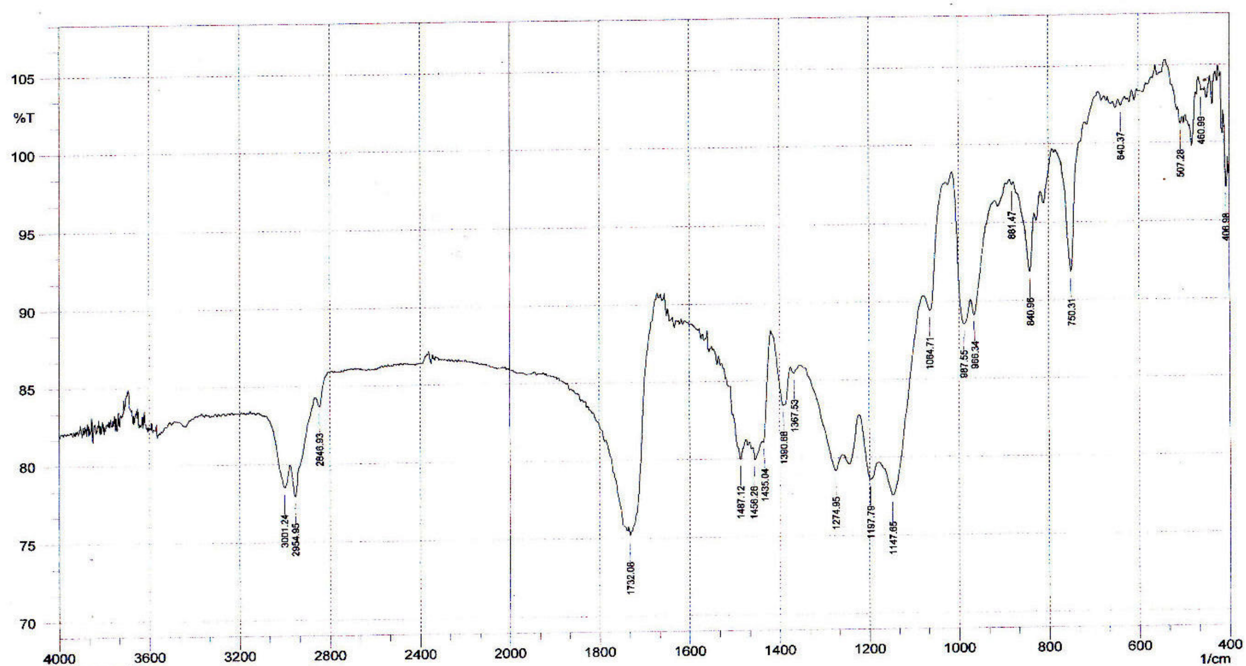
Figure (3-16C) shows similar clear solution of artificial saliva(no precipitation of silver halide) only the tested $AgNO_3$– loaded resin discs at the bottom of the test tubes. .

3-6Characterization of $AgNO_3$ –loaded resins.

The results of FTIR (Fourier transform infra –red) spectra of PMMA and $AgNO_3$ –loaded resins in KBr and CaesiumIodid (CsI) discs,showed no change in the shape of absorption peaks between PMMA(control) and $AgNO_3$ –loaded resinsindicating no chemical bond between thePMMA and $AgNO_3$Figures(3-17A,B,C,D).



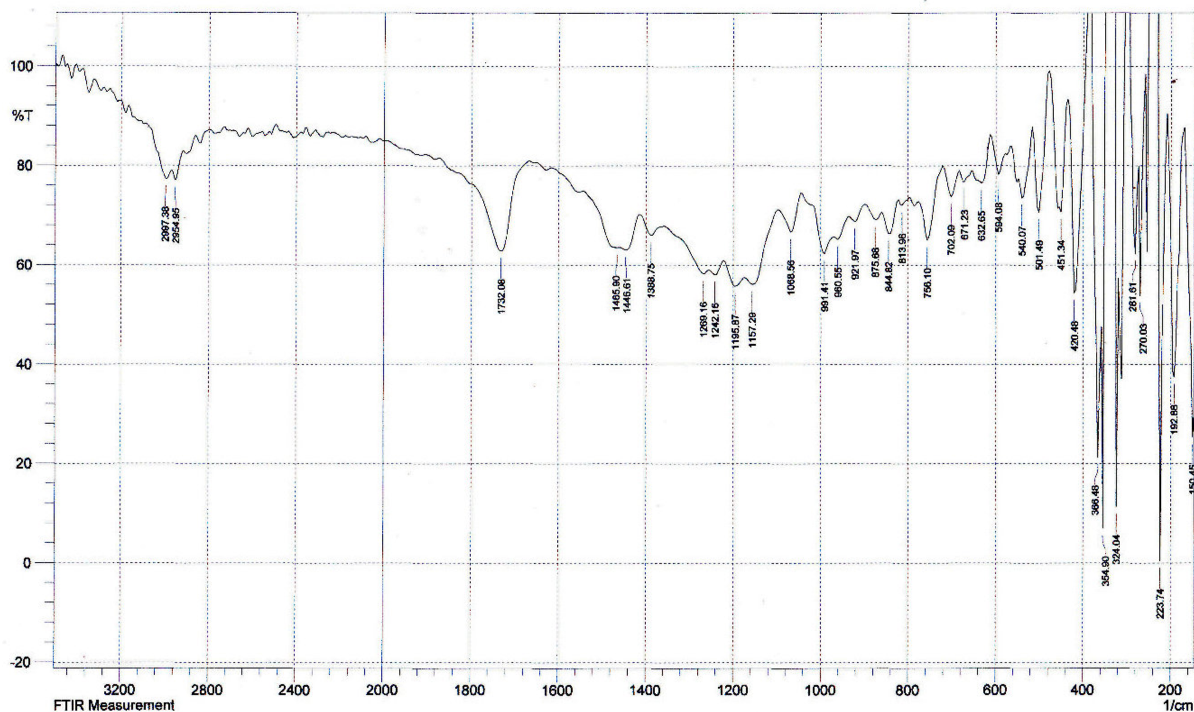
A- PMMA



B-$AgNO_3$-loaded resin

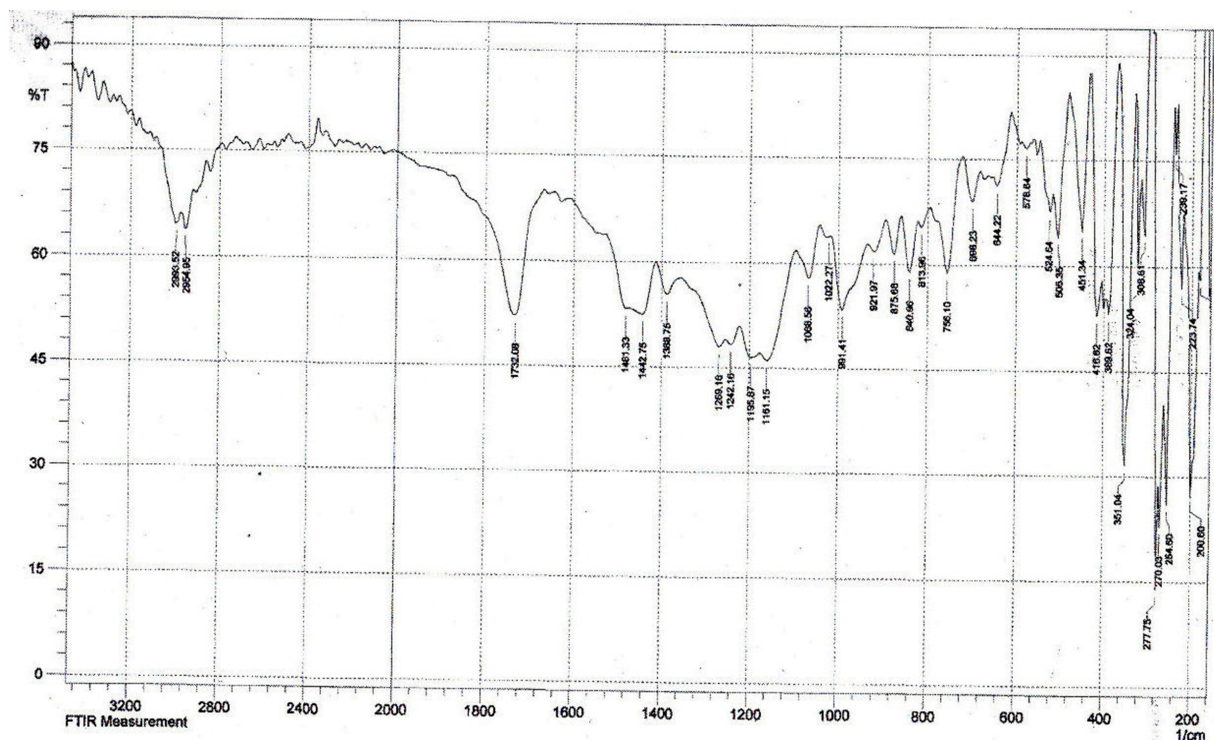
Fig.3-17: FTIR spectrum of A- PMMA and B-$AgNO_3$-loaded resininKBrdisc.

Transmittance T



Wave number (cm-1)

C-PMMA



D-$AgNO_3$-loaded resin

Fig.3-17:FTIR spectrum of C- PMMA and D-$AgNO_3$-loaded resininCaesiumIodid(CsI) disc.

3-7Mechanical tests:

These tests are:

a. Impact strength test.
b. Transverse strength test.
c. Tensile strength test.

3-7-1 Impact strength test

As shown in Table 3- 6 and Fig 3-18 the mean impact strength was highest in the group with 60 ppm $AgNo_3$ (12.8 KJ/ m^2) and lowest in the control group (10.6 KJ/m^2). The difference in mean impact strength between the concentrations of $AgNO_3$ and control group was statistically insignificant. Compared to control the lowest concentration of $AgNO_3$ (9.375ppm) was associated with an average increase in impact strength of $0.1KJ/m^2$. The effect of this very low concentration was evaluated as weak (Cohen's d = 0.17). This effect was statistically insignificant .The 60 ppm $AgNo_3$ was associated with highest increase in mean impact strength of 2.2.This effect was statistically significant which rated as a strong effect (Cohen's d = 2.63) .

Table 3-6: Descriptive data of impact strength test (KJ/m²)

	Study groups (concentration of added AgNO3 in ppm)							
	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	P (ANOVA
Impact strength (KJ/m²)								0.05[NS]
Range	(9.67 to 11.11)	(9.94 to 11.62)	(10.53 to 12.23)	(9.78 to 13.51)	(11.27 to 13.72)	(9.25 to 12.66)	(8.86 to 15.17)	
Mean	10.6	10.7	11.5	11.8	12.8	10.7	11.8	
SD	0.65	0.56	0.63	1.54	0.95	1.57	2.09	
SE	0.27	0.23	0.26	0.63	0.39	0.64	0.85	
N	6	6	6	6	6	6	6	
Difference in mean compared to control	Reference	0.1	0.9	1.2	2.2	0.1	1.2	
Cohen's d	Reference	0.17	1.37	1.03	2.63	0.12	0.78	
P (LSD)	Reference	0.88[NS]	0.24[NS]	0.1[NS]	0.006	0.85[NS]	0.11[NS]	

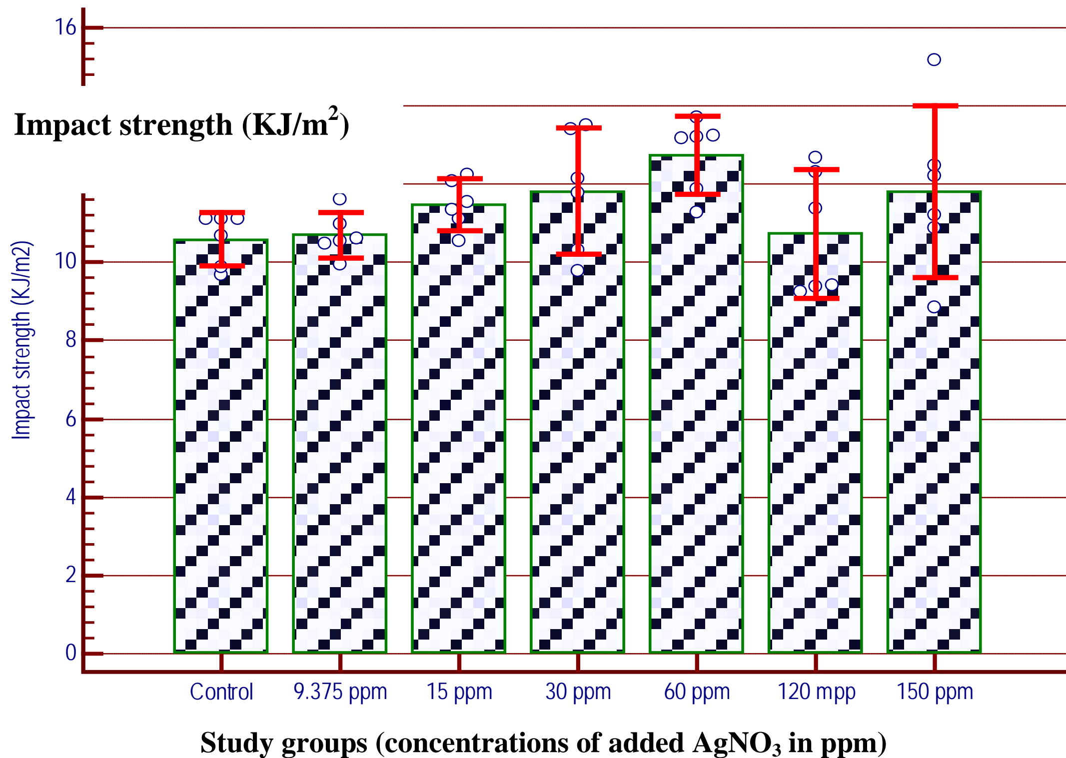


Fig (3-18)Bar chart showed the mean impact strength (with its 95% confidence interval) for the concentrations of $AgNO_3$ and control group.

3-7-2 Transverse strength test

As shown in table 3- 7 and Fig 3-19 the mean transverse strength was highest in the control group (77.8 MPa) and lowest in the group with 120 ppm of $AgNO_3$ (55.4MPa). The difference in mean transverse strength between the concentrations of $AgNO_3$ and control group was statistically significant.

Compared to control the lowest concentration of $AgNO_3$ (9.375 ppm) was associated with an average reduction in transverse strength of (14MPa) ,the effect of this very low concentration was evaluated as strong (Cohen's d = 4.18) . This effect was statistically significant .The strongest effectwas with 120 ppm $AgNO_3$(reduction in transverse strength) (Cohen's d greater than 6).

Table 3-7:Descriptive data oftransverse strength test (MPa)

	Study groups (concentration of added AgNO3 in ppm)							
	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	P (ANOVA
Transverse strength (MPa)								<0.001
Range	(74.4 to 81.6)	(57.6 to 67.2)	(56.4 to 72)	(60 to 74.4)	(58.5 to 79.2)	(48 to 57.6)	(57.6 to 76.8)	
Mean	77.8	63.8	67.0	66.4	64.8	55.4	69.6	
SD	2.78	3.83	5.65	5.76	7.37	3.75	6.57	
SE	1.13	1.56	2.31	2.35	3.01	1.53	2.68	
N	6	6	6	6	6	6	6	
Difference in mean compared to control	Reference	-14.0	-10.8	-11.4	-13.0	-22.4	-8.2	
Cohen's d	Reference	-4.18	-2.42	-2.51	-2.33	-6.77	-1.61	
P (LSD)	Reference	<0.001	0.001	<0.001	<0.001	<0.001	0.012	

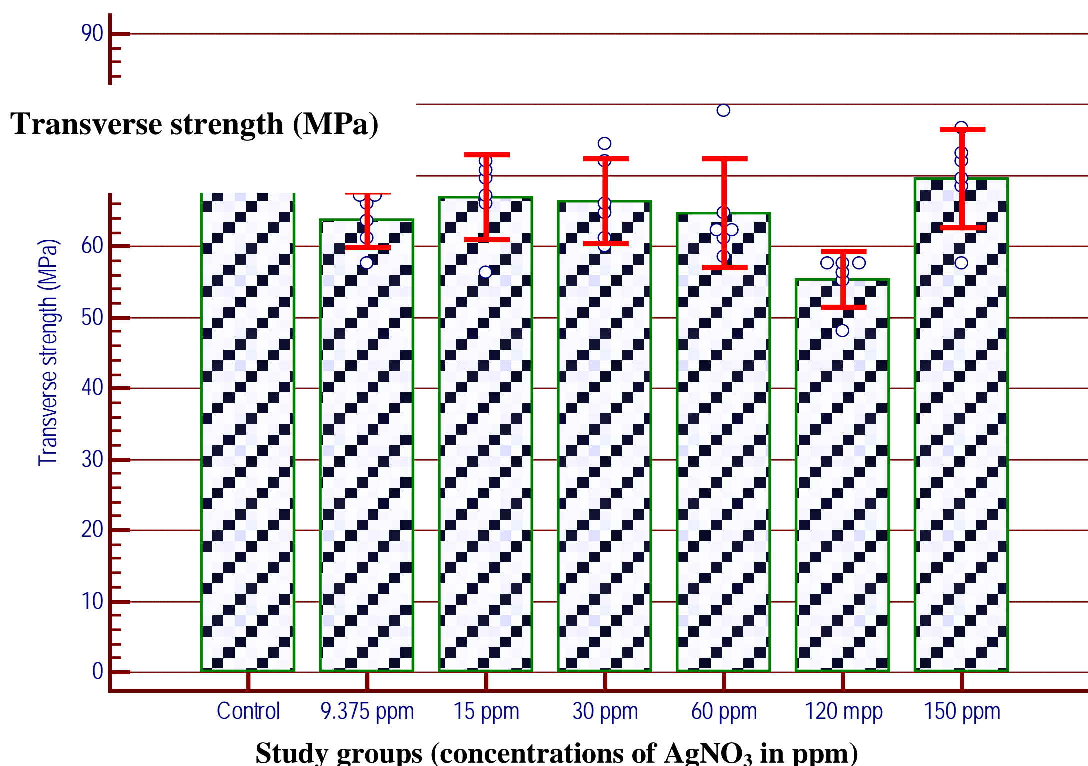


Fig (3-19): Bar chart showed the mean transverse strength (with its 95% confidence interval) forthe concentrations of $AgNO_3$ and control group.

3-7-3 Tensile strength test

As shown in table 3- 8 and Fig 3-20 the mean tensile strength was highest in the control group (54 MPa) and lowest in the group with 60 ppm $AgNO_3$(36.8MPa). The difference in mean tensile strength between the concentrations of $AgNO_3$ and the control group was statistically significant. Compared to control the lowest concentration of $AgNO_3$(9.375ppm) was associated with an average reduction in tensile strength of 5 MPa. However, this effect was statistically insignificant. The 15 ppm $AgNO_3$ was associated with very small and statistically insignificant reduction in tensile strength of 2.1MPa. On the other hand, the $AgNO_3$ concentration associated with strongest effect (reduction in tensile strength) was the 60 ppm (Cohen's d greater than 4).

Table 3- 8: Descriptive data oftensile strength test (MPa)

	Study groups (concentration of added AgNO3 in ppm)							
	Control	9.375 ppm	15 ppm	30 ppm	60 ppm	120 ppm	150 ppm	P (ANOVA
Tensile strength (MPa)								<0.001
Range	(49.54 to 62.5)	(46.06 to 55.2)	(39.71 to 57.8)	(38.84 to 53.7)	(31.48 to 41.69)	(38.25 to 43.4)	(38.3 to 45.56)	
Mean	54	49	51.9	44	36.8	40.7	41.5	
SD	4.82	3.3	6.64	5.18	3.37	2.1	3.34	
SE	1.97	1.35	2.71	2.12	1.38	0.86	1.36	
N	6	6	6	6	6	6	6	
Difference in mean compared to control	Reference	-5.0	-2.1	-10.0	-17.2	-13.3	-12.5	
Cohen's d	Reference	-1.21	-0.36	-2.00	-4.13	-3.58	-3.01	
P (LSD)	Reference	0.05[NS]	0.42[NS]	<0.001	<0.001	<0.001	<0.001	

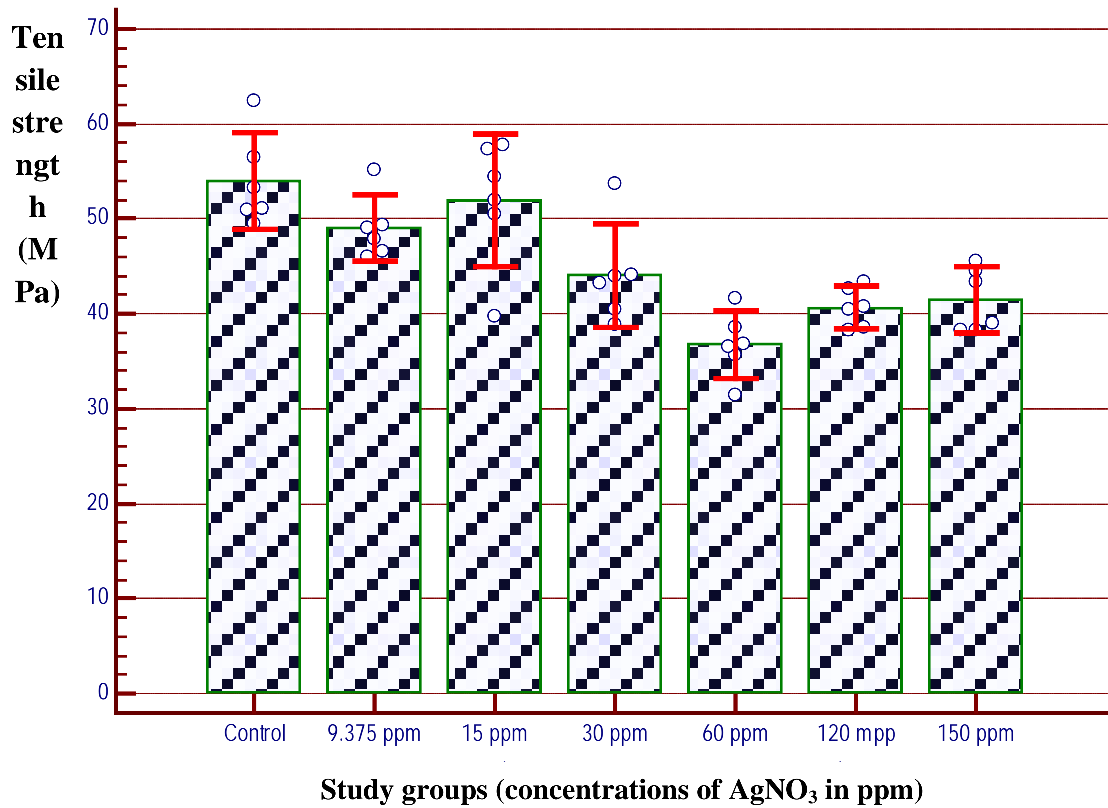


Fig (3-20): Bar chart showed the mean tensile strength for the concentrations of $AgNO_3$ and control group.

CHAPTER FOUR

DISCUSSION

Discussion

4-1 Cytotoxicity test

The *in vitro* assessment of REFcytotoxicityused to be useful method for characterizing cell toxicity mechanism .The embryonic fibroblast had a strong uptake capacity of Ag particles and more sensitive in cytotoxicity screening test thus could be considered a promising candidate for cell model for cytotoxicity screening **(Cao*et al.*, 2008).**

4-1-1 Effect on cell morphology

The first and most readily noticeable effect fallowing exposure of cells to toxic materials is the alteration in cell shape or morphology in a monolayer culture as illustrated in fig. (3-1) that shown distinct morphological changes of treated cells indicating unhealthy cells **(Al Shemry and Al Bayati, personal communication, 2012)**. This could be due to disturbance in cytoskeletal functions as consequence of silver treatment.Similar results were observed by other groups in dermal fibroblast cells treated with citrate –coated gold **(Pernodet*et al.*,2006)**.Only a few floating cells were observed under the microscope suggesting the absence of wide spread cell death due to necrosis.

4-1-2 Cell viability

Viability assay was a vital step in toxicology that explains the cellular responseto a toxicant.Significant decrease ($P<0.001$) in cell viability was observed regarding all concentrations of $AgNO_3$ with the strongest cytotoxic effect was at 300,600ppm $AgNO_3$ as shown in tables (3-1, 3-2) and figures (3-2, 3-3).Probably as a result of reduction in ATP production, generation of reactive oxygen species (ROS), and damage to

the mitochondria respiratory chain.The ROS production is believed to be trigger to DNA damage, followed by cell cycle arrest at G_2/M.All data taken in the present study together suggest that silver particles at range concentrations used result in G_2/M arrest in the cells which might lead to cell death since DNA repair pathway was unsuccessful.Induction of apoptosis especially in low doses of silver nitrate accompanied by proliferation arrest at high concentration 900ppm $AgNO_3$,this could be interpreted as agglomeration and subsequent precipitation , up take rate of Ag particles will drop ; a situation where cells sustain DNA damage and gain resistance to cell death **(Hidalgo and Dominguez,1998 ; Hossain and Huq, 2002).**

4-2 Visual inspection of $AgNO_3$–loaded resins

It is believed thatthediscoloration for $AgNO_3$ loaded resins which was observed at 300ppm $AgNO_3$ and above as illustrated in figure (3-4)was due to the presence of metal oxidesfrom antibacterial metal ions during an oxidation- reductionreaction that occurs during a polymerization reaction, as well as the oxidation of the silver ions on the material surface**(Nakanoda*et al.*, 1995; Yutani and Yamamoto,2002).**It was also reported that by adding such antibacterial agent, due to the Ag^+ in it, the color tone of the denture base resin varies **(Kuroki*et al.*,2010).**

4-3 Antibacterial activity of $AgNO_3$ on the growth of *mutans streptococci*group

*Mutansstreptococcu*group was an appropriate bacterium to use in this study model because the virulence properties of this organism is well established **(Kreth*et al.*, 2008),** and it's the organism associated with dental caries **(Loesche, 1986)**.

This microorganism was grown selectively on MSB agar plates with addition of bacitracin at a concentration of 0.2 units/ml and sucrose at a concentration of 20% **(Gold *et al,* 1973; Wade *et al.,* 1986).** Thismedium supported the growth of *mutans streptococci* group with good suppression of other organisms. This was based on information in **Bergey's Manual of Determinative Bacteriology (9th ed., 1994)** for differential characterization of *mutans streptococci* group. Blood agar with bacitracin added could be used for recovery of *mutans streptococci* group, but other media like TYC agar incorporated with bacitracin and sucrose might be used as an improved selective isolation medium **(Wade *et al.,* 1986).**

It has been generally believed that the mechanism of the antimicrobial effects of silver ions Ag^+ involved their absorption and accumulation by the bacterial cells that would lead to shrinkage of cytoplasm membrane or its detachment from the cell wall**(Feng*et al.*,2003; Gill *et al.*, 2005).** In addition to the ability of Ag^+ to react with sulphydryl groups, other proteins residues, and enzymes associated with cell membrane leading to denaturation, structural damage and mitochondrial dysfunction that seen in bacterial and fungal cells **(Lansdown , 2006).**

No obvious statistically difference were observed in control specimens (fabricated without incorporation of $AgNO_3$) in there antibacterial activity between three time intervals as shown in table (3-5), figure (3-15)

which agrees with**Arizono*et al.*(1992)**and**Casemiro*et al.*(2008)**who reported that no antibacterial activity of tested acrylic resin fabricated without incorporation of antibacterial agent.

It is clear that the inhibitory effect with different concentrations of $AgNO_3$ solutions on the growth of *mutans streptococci*groupis a concentration dependent as shown in table (3-3), fig.(3-8, 3-9).

The outcomes of this study demonstrate the susceptibility of *mutans streptococcus*groupto the lethal effect of tested $AgNO_3$ solutions by observation of inhibition zone.This outcome is in agreement with previous findings which showed the antimicrobial activity of liquid – solubilized silver ions against various oral pathogens(**Burne*et al.*, 1987 ; Spacciapoli*et al.*, 2001).**

The inhibitory effect of $AgNO_3$–loaded resins at baseline period (T_0) illustrate no zone of inhibition was observed indicating that there were no Ag^+ ions leached out of the specimens, only the microbes that made contact with $AgNO_3$- loaded resinswere killed as demonstrated through the total killing of *mutans streptococcus* groupafter 30(T_{30}) and 90(T_{90}) days of immersion $AgNO_3$ –loaded resin discs in artificial salivaas shown in tables (3-4),(3-6) and figures (3-10B),(3-14),(3-15).

These procedures indicated that the $AgNO_3$–loaded resins may provide its biocidal functions mainly through direct contact. Since no Ag ions were detected in the immersion medium that was analyzed spectrophotometrically using atomic absorption spectrophotometer. The result of this study is in agreement withsome other studies that confirmed "contact kill" hypothesis likefor the PMMA- based polymeric silver sulfadiazine against *E.coli and S.aureus*(**Cao *et al.*, 2009**) , and introducing variety of medical tools using silver in ionized and elementary form to create surfaces resistance to bacterial adhesion and

colonization like burns and traumatic wound dressing ,dental work ,scaffold, hip prosthetics , wound sutures, artificial tendons ,surgical masks antimicrobial glass to fight hospital – acquired infection **(stickler, 2000;Balazs*et al.*, 2004; silver *et al.*, 2006; Kim *et al.*, 2007; Thomas *et al.*, 2007; ; AGC Flat Glass Europe, 2007;Balan*et al.*, 2008; Low *et al.*; 2008; Damm*et al.*, 2008; Rai*et al.*, 2009) .**

The increasing in the antibacterial activity of the newly developed resin from the baseline period(T_0) as illustrated in ABE which wasas high as 96.6% (ABE)for 9.375 and 15ppm $AgNO_3$(table 3-4),(fig 3-10A) with a statistical significant reduction($P< 0.001$) in mean count of CFU/ml of any concentration of $AgNO_3$compared to control (table 3-5) ,(figures 3-11, 3-12, 3-13)till reach total inhibition of bacterial growth after 30(T_{30}) and 90(T_{90})days immersion in artificial saliva (ABE 100%) (table 3-4),(figure 3-10B), and the CFU/ml was zero at any concentration of $AgNO_3$(table 3-5),(figures 3-14,3-15)was in agreement with **Damm and M¨unstedt (b) in 2008** who illustrate thattransportation of silver ions from the bulk to the surface more relevant with increases immersion time. The transportation processes through the matrix are influenced by polymer properties and when shown with longer immersion time.

Since the *mutans streptococci* group has the ability to form a biofilm**(Rozen, 2001)**, a high concentrations of $AgNO_3$ in different testing periods were used in this study to show the ability of $AgNO_3$ within the PMMA resin of killing the bacterial cells of the biofilms.Total killing of bacteria after treatment in artificial saliva was observed.This finding is in agreement with **Bjarnshot*et al.*(2007)** who studied the action of silver on mature *in vitro* biofilm of *P.aeroginosa*, concentrationsof 5-10 ppm silver sulphadiazine eradicated the biofilm; whereaslower concentrations(1ppm) had no effect. The antimicrobial

action of silver against the formation of the biofilm is also time dependent **(Stobie*et al.*, 2008).**

The fluctuation in the number of bacterial count as shown in figures (3-10A, 3-11, 3-13)might be due to in low dose of $AgNO_3$ there was inhibition in bacterial growth accompanied by proliferation arrest at high concentrations.It could also be interpreted as a situation where cells sustain DNA damage and gain resistance to cell death **(Hossain and Huq , 2002**).

The result of cytotoxicity assay and antibacterial experiments demonstrated that silver ions exhibit lower toxicity to REF cells which was as high as 83% .While its activity increased about 97% inhibition of bacterial growth. This could be due to the difference in the membrane structure and enzyme properties between normal cells and bacterial cells. Yet prokaryotic cells and eukaryotic cells have entirely different physiological functions which determine sensitivity and survival rate upon exposure to silver ions. Eukaryotic cells have prominent nucleus , a complex DNA repair mechanismand cell cycle pathway to control cell death and survival , which are absent in prokaryotic cells **(AshaRani*et al.* , 2009).**

4-4*In vitro* Ag release test

This study evaluated the incorporation of silver nitrate into an acrylic denture base resin by means of atomic absorption spectroscopy. $AgNO_3$ – loaded resins containingthe different concentrations of silver nitrate used in the present study were kept in artificial saliva, and aliquots of the mediawere collected after 30(T_{30}) and 90(T_{90}) days of storage under 37°C of incubation aiming to assess the influence of time on the release of silver ions. It was found that there were no silver ions released from the

$AgNO_3$ – loaded resins prepared in the period of the time designed as shown in Fig.(3-18A&B).This result depended on the measurementspectrophotometrically.Furthermore, figure(3-18C) shows similar clear solution (no precipitate) for any artificial saliva that used as a stored medium to all $AgNO_3$ – loaded resins indicated no silver halides precipitation presence that can results from interaction of Ag^+ ions(if Ag^+ ions was released from$AgNO_3$ – loaded resins) and halides of the stored mediumproducing a precipitate which colors varies depend on the type of halide**(Svehla,1979;Chemeurope,2012).**

The particlerelease of silver from the surface of the loaded resin should occurduring the initial period. Thus, the rate of release should decreasewith the necessity of water diffusion into the polymericmass. Usually, water diffusion into the PMMA body could resultin the plasticizing of the material, allowing the migration of the particles or Ag^+ toward the surface, which would be released into the water **(Kumar and M¨unstedt, 2005a)**.However, the results of the atomic absorptionspectroscopy, which is very sensitive, did not indicatethe presence of Ag in liquid media, even after the $AgNO_3$ – loaded resins have been immersed for the longest period (T_{90}).Furthermore, other tests were also conducted to evaluate if Ag release would occur in a different medium storage such as deionized water. However, Ag was also not detected by the atomic absorption spectroscopy analysis.

Previous studies indicated that silver was not released from PMMA/Ag nanocompositeinspite using nanocomposite**(Damm andM¨unstedt,2008b; Monteiro*et al.*, 2011)**. The finding of the present study was in agreement with **Monteiro*et al.*(2011)** who evaluated denture base resin containing silver colloidal nano particles stored in artificial saliva, finding that artificial saliva presents substances with potential ionic charge(sodium carboxy – methyl –cellulose, sorbitol ,

potassium chloride ,sodium chloride , sodium fluoride. ,magnesium chloride, calcium chloride , potassium phosphate , potassium thio-cyanate) an attraction between inorganic particles in this liquidand Ag^+ in the PMMA would possibly occur; however, Agwas also not detected by the atomic absorption spectroscopy analysis.

For instance,Fig.(3-16) illustrates theabsorption spectrum for the artificial saliva with control acrylic specimen (without silver nitrate), and spectrum of one of the specimens which was similar for all other specimens regardless of the concentration of silver nitrate(9.37- 900 ppm) and storage period . Spectrum clearly shows similar absorption as that of the control; however, some authors**Damm*et al.*(2007); Damm andM¨unstedt (2008b**)have reported that the release of Ag^+ frompolyamide/silver nanocompositesimmersed in deionized water was proportional to the storageperiod. Obviously, the differences in synthesis method of antimicrobial composite and the different properties of these polymers may attribute to this result.Since polyamide is a more hydrophilic polymer than PMMA used in the present studyand for this reason, allows plasticizing by the action of water. PMMA, on the other hand, is a more hydrophobic materialthan polyamide, which may have generated a barrier for waterdiffusion and consequently Ag^+release**(Damm*et al.*, 2007)**. In addition, thecross-linking agent ethylene glycol dimethacrylate present inthe monomer ofPro Base acrylic resin that used in this study may have restricted the rotation of polymericchains, decreasing the velocity of water diffusion to the polymer **(S¨oderholm ,1984)**.

The glass transition temperature (Tg) is another physicalproperty involved in the diffusion of liquid substances in polymericmaterials. The Tg may be influenced by the polymer thickness, and, for PMMA, it ranges from 97˚C to 125˚C**(Vallittu*et al.*, 1998; Kong and Jang, 2008).**

Thehigher the polymer thickness, the higher the Tg, and the lowerthe plasticization effect. In addition, the velocity of water sorptionand the migration of the particles or $Ag^{+}$to the polymeric surfacewould be reduced**(Kong andJang, 2008).** Since Agwas not detected, it means the silver particles are stronglyfixed on the polymer base.

4-5Characterization of $AgNo_3$ – loaded resins

Since there is no change in the shape of absorption peaks between PMMA(control) and $AgNO_3$ – loaded resin samples as illustrated in the results of FTIR Figures(3-17A,B,C,D) thus there is no chemical bond between the PMMA and $AgNO_3$(**Singho*et al.,*2012**).

.4-6 Mechanical tests

The addition of silver nitrate to heatpolymerizedacrylic resins is consistent with the current trend of incorporating antimicrobials into dental materials(**Abe *et al.*, 2003;Pesci-Bardon*et al.*, 2006).** It is important to evaluate the mechanical properties of acrylic resins containing silver nitrate becauseremovable and complete dentures are subjected to repeated flexural forces. Midline fractures are related to the flexural strength of the resins. On the other hand localized deformation upon stretching is related to tensile strength. The higher impact strength of the base resins reduces the possibility of fracturing when the prosthesis is dropped onto a hard surface **(Jerolimov*et al.*, 1985).**

Because of dark coloration that appeared at 300ppm $AgNO_3$ and above, such kind of concentrations were not taken in consideration of the most mechanical evaluation.

Among the specimens fabricated, the addition of silver nitrate in different concentrations reduces tensile strength(above 15ppm $AgNO_3$) as shown in Table (3-8), Fig.(3-20)and transverse strength Table (3-7)Fig.(3-19) when compared with control as the concentration of silver nitrate increased, this is probably due to Ag^+ ions being reduced as the concentration of Ag increase, generating atom clusters and smaller particle size during the curing process which compete with complete polymerization process(**Fan *et al.*, 2011).** Theplasticization effect of the resultant residual monomer willreduce the molecular binding force. On the other side, the results of FTIR Figures(3-17A,B,C,D)showed no chemical bonding between PMMA and $AgNO_3$.Therefore we suppose that Ag^+ ions attack the double bond in the alkene group of the monomer molecule and will convert it to residual monomer **(Cope and Bach,1973)**. This process will reduce the molecular binding force between the reactant molecules and allows greater deformation upon stretching or flexion

through exhibiting multiple micro fractures that weaken the $AgNO_3$ – loaded resin samples(**Jagger,1978;Jerolimov*et al*., 1985).**

Some other studies also showed that adding an antibacterial agent may affect the material properties,**Kuroki *et al*.(2010)**have reported that there were significant differences of residual monomer in the samples treated by adding antimicrobial agents (Zeomic,BacteKiller,Novaron) although it was insignificant between the control and samples.

Fan *et al*. (2011) found that by adding 0.15% (w/w) AgBz (silver benzoate) and above there was decrease in the degree of curing, result in reduction in Rockwell hardness for light cure resin.**Nakanoda*et al.*(1995)**have reported that, as a result of tensile tests andbending tests, adding Silver-Zeolite to a heat-curing resin tends to decrease the material property dependingon the additive concentrations of antibacterial agent of Zeomic.

There wasin significant reduction in tensile strength with the lowest concentrations of $AgNO_3$ (9.375 and 15ppm) compared with controlas shown in Table (3-8), Fig. (3-20). This outcome is in agreement with **Wakasa*et al*. (1997)** who reported that when the antimicrobial agent (Zeomic) is added to self –cure acrylic resin between 1% and 2%, the polymerization behavior of the resin is not inhibit.

On the other hand the results of impact strength for the different concentrations of $AgNo_3$ shows in significant increase in impact strength (P= 0.05 NS) when compared with control as shown in Table (3-6), Fig.(3-18) .The 60ppm $AgNO_3$ was associated the highest increase in impact strength by 2.2KJ/ m^2 .This could be due to the slow curing process allows greater number of nucleation sites to form and smaller particle sizes, thereby generating more particles(**Fan *et al*., 2011**), the total particle / matrix interfacial surface area available for energy dissipation increase, the critical stress for particle /matrix debonding also

increase **(Chen *et al*.,2007)**.Also The increasing in the impact strength could be due to thepresence of residual monomer **(Cope and Bach,1973;Kuroki *et al*.;2010).**This plasticizing effect renderthe fabricated acrylic resin samples more capable to absorb energy on impact and are more resistant to fracture**(Anusavice, 2008).**

The result of this study disagree with **Casemiro*et al*. (2008)** who added (2.5-10%) by wet. Ag- Zeolite as a powder to acrylic dental resin resulted decrease in impact strength.

CONCLUSIONS & SUGGESTIONS

Conclusions

This study presented a method of $AgNO_3$ incorporation into heat cure PMMA denture base resin and concluded:

1. The cytotoxic study on REF cells demonstrates that there was 70.8-82.9% inhibition with $AgNO_3$ concentrations range from 9.375-900ppm.
2. Antibacterial experiments demonstrated that $AgNO_3$ is effective against *mutans streptococci*group bacteria. However, the concentration and immersion times are important factors.
3. There wasno Ag^+ ions released, even after 90 days of storage in artificial saliva. Furthermore, Ag was also not detected in deionized water as different storage medium by the atomic absorption spectroscopy analysis.
4. Regarding the mechanical tests, the results showed increasing in impact strength compered to control. There was reduction in transverse strength when compared with control. While for tensile strength there was significant reduction above 15 ppm $AgNO_3$.
5. Darkening of $AgNO_3$ – loaded resins was visually detected and shown to be started with concentration of 300ppm $AgNO_3$ and above.

Suggestions

1. The experimental $AgNO_3$– loaded resin should be examined *in vivo* to record any uncommon problems might happen during the period of wearing.
2. Investigation the effect of adding $AgNO_3$ to light cure or microwave resin on mechanical and physical properties.
3. Scanning Electron Microscope study to investigate the morphology of the newly developed denture base resin.
4. Other mechanical and physical properties should be investigated for the $AgNO_3$ – loaded resin such as, surface hardness, fatigue resistance, dimensional stability, porosity, water sorption.

REFERENCES

References

A

- ADA (1999):" American Dental association / American material standers institute, specification No.12 for denture base polymer ". Chicago council on dental matewrials and devices.
- "AGC Flat Glass Europe launches world's first antimicrobial glass, 2007".
- Ahn,S.J;Lee,S.J;Kook, J.K (2009): "Eperimental orthodontic adhesive use innano filler and silver nanoparticles ".Dent Mater ;25:206-213.
- Almedia, A; Roseman, M;Sheff ,M(2000):" Future caries susceptibility in children with early childhood caries following treatment under general anesthesia ". Pediater .Dent, 22:302-306.
- Al-Mizraqchi, A (1998):" Microbiological and Biochemical Studies onAdherence of Mutans Streptococci on the Tooth Surfaces". Ph.D. Thesis, College of Science,Al-Mustansiriya University.
- Al Bayati, A.A, personal communication (2012).The Iraqi Center for Cancer and Medical Genetic Research.
- AL-Nidawi,A(2004):"Effect of siwak extracts of mutans streptococci in compression to selected antimicrobial agents (in vitro and in vivo study)".M.Sc.thesis ,College of Dentistry ,Baghdad University.
- Al Shemry, A. personal communication (2012). TheIraqi Center for Cancer and Medical Genetic Research.

- Anusavice, K.J; Zhang, N.Z; and Moorhead, J.E (1994):" Influence of colorants on cystalization and mechanical properties Lithia – based glass-ceramics ".Dent Mater ;10(2):141-6.
- Anusavice, K.J (1996):"Philip's Science of Dental Materials".10th ed. Chap 7 Elsevier Ltd.
- Anusavice, K.J (2008):"Philip's Science of Dental Materials".11th ed. Ch. 7 Ch. 22. Elsevier Ltd, p: 211,220,235,237,271.
- Arbiet, R.D:"Laboratory procedures for Epidemiologic Analysis of microorganisms". In:"manualof clinical microbiology". 7th ed.Washington ASM press.1999; p: 141-137.
- Arizono,T;Oga,M;andSugioka,Y(1992):"Increased resistance to bacteria after adherence to polymethylmethacrylate".ActaOrthop Scand,63(6):661-664.
- AshaRani, P.V;Mun, G.L.K; Hande, M.P; Valiyaveettil, S(2009):"Cytotoxicity and Genotoxicity of Silver Nanoparticles in Human Cells". ACS Nano, 3 (2): 279-290.
- ATSDR (Agency for Toxic Substances and Disease Registry) (1990):" Toxicology profile for silver .prepared by Clement International corporation, under contact 205-88-0608.U.S.Puplic Health Service .ATSDR/TP-90-24.

B

- Baca, P;Castillo, A.M; Bravo, M; Junco, P; and Lodra, J.C (2002):"Mutans Streptococci and Lactobacilli in saliva after application of fissure sealants".Oper.Dent ,27:107-11.
- Balakrishnan, M; Sinimonds,R.S;Tagg, J.R(2000):"Dental caries is a preventable infectious disease ". Aust.Dent .J. 45(4):235-45.

- Balazs, D.J;TriandaFIlla, K;Wood, P;Chevolot, Y (2004):"Inhibition of bacterial adhesion on PVC endotracheal tubes by RF-oxgen glow discharge, sodium hydroxide and silver nitrate treatments".Biomaterials ,25:213-51.
- Banerjee, R; Banerjee,S;Prabudesai,P.S;Bhide ,S.V (2010):" Influnce of processing Tehnique on the flexural fatigue strength of denture base resins : An in vitro investigation ". Indian Dent Associ, 21:391-5.
- Baron, E.J, Peterson, L.R, Finegold, S.M (1994):" Methods for testing antimicrobial effectiveness. In: Bailey and Scott’s diagnostic microbiology". 9th ed. St. Louis: Mosby-Year Book, Inc., pp 168-193.
- Beighton, D "Streptococcus mutans and other Streptococci from the oral cavity.In:Collins ,C.H.; and Grange ,J.M.(1985):" Isolation and Identification of microorganism of Medical and VenterinaryImportace". London: The academic press.
- Beighton,D; Ruusell, R.R.B; Hayday, H (1981):" The Isolation and characterization of Streptococci Serotype *h* from Dental Plaque of Monkeys (MacacaFascicularis)". J General Microbiol,124: 271-279.
- Berkeley,R.C.W; Bock,D.R;Brenner,D.J; et al(1994):" Bergey's Manual of Determinative Bacteriology".9thed.Williams and Wilkins Philladelphia,USA.
- Betty,A;Daniel, F; and Alice, S (2007):" Diagnostic microbiology "12thed.Chap 17.Mosy., p: 265.
- Bjarnshot, T; Kirketerp- Moller,K;Kristiansen, S (2007):" Silver against P.aeroginosabiofilms ". APMIS, 115:921-8.

- Bleehen, S.S;Gould, D.J; Harrington, C.I(1981):" Occupational argyria;light and electron microscope studies and x-ray micro analysis ". British J.of Dermatology, 104(1):19-26.
- Bloomquist, C.G; Reilly, B.E and Liljemark, W.F (1996):" Adherance ,accumulation and cell division of a natural adherent bacterial population". J. Dent. Res., 69:1205-1210.
- Bouts, B.A (1999):"Images in clinical medicine, Argeria".The new England J.ofmedicine, 340(20):1554.
- Braydich-Stolle, L;Hussain, S;Schlager, J.J; and Hofmann, M.C (2005):"In vitro cytotoxicity of nanoparticles in mammaliangermline stem cells". *Toxicological Sciences*, 88 (2): 412–419.
- Bulletin of the WHO: Crede's method still valid? (http:// www.scielop.org/scielo.php.
- Burne, R.A; Schilling, K; Bowen, W.H; Yasbin, R.E (1987):" Expression , Purification , and Charecterization of an exo-beta0D-fructosidase of Streptococcus mutans ". J Bacteriol, 169(10): 4507-17.
- Burrell,R.E(2003):" Ascientific perspective on the use of topical silver preparation ". Ostomywond management, 49(5): 19-24.

C

- Cao, T, Lu, K, Fu X, and Heng, B.C (2008): “Differentiated fibroblasticprogenies of human embryonic stem cells for toxicology screening”.Cloning and Stem Cells, 10(1): 1–10.
- Cao, Z;Sun, X;Sun, Y; Fong, H (2009):"Rechargeable Antibacterial and Antifungal Polymeric Silver Sulfadiazines". J Bioa. Comp.Polym., 24: 350.

- Casemiro, L.A; Gomes Martins, C.H;Panzeri, F.C; Pires -de –Souza; Panzeri,H(2008):" Antimicrobial and Mechanical properties of acrylic resin with incorporated silver-zinc zeolite –part one .Gerodontology, 25: 187-194.
- Cavalla,V;Reis,A.F;Giannin, M;A Mbrosan, G.M(2001):" The effect of elapaed time of fallowing bleaching on enamel bond strength of resin composite". Operative dentistry, 26: 597-602.
- Chen, X; Cuijpers, V; Fan, M; Frencken, J.E (2009):" Optional use of silver nitrate and marginal leakage at the sealant enamel interface using micro-CT. Am J dent, 22(5):269-72.
- Chen, J; Huang, Z; Zhu, J (2007): "Size effect of particles on thedamage dissipation in nanocomposites." Compos. Sci. Technol, 67(14):2990-2996.
- Chladek, G;Mertas, A; Barszczewska-Rybarek, I; Nalewajek,T; Zmudzki,J; Krol,W;Lukaszczyk,J(2011): "Anti-fungal Activity of Soft Lining Material Modified by Silver Nanoparticles – A Pilot study".Int.J.Mol.Sci.,12:4735-4744.
- Choi, E et al (2009):" Quantitative real –time polymerase chain reaction for streptococcus sobrinus in dental plaque samples and its association in early childhood caries ". Inter J PaedDent, 19:141-147.
- Chopra, I (2007):" Increasing used of silver- based products as antimicrobial agents: a useful development or a cause for concern?" The J of antimicrobial chemotheraby, 59(4): 587-590.
- Clayton, G.D and Clayton,F.E.Patty's(1981):" Industrial Hygiene and Toxicology" .3rd ed .revised edition .New York :JohnEiley and Sons ,p:1881-1894.

- Colloidal silver products".National center for complement and alternative medicine ".(2006).http:// nccaminih.gov/ health / silver /. Retrived2008.
- Consani,R.L.X;Domitti, S.S and Cosani, S (2002):" Effect of new tension system used in acrylic resin flasking on dimensional stability of denture bases ". Braz Dent J, 88(3):285-289.
- Cope,A.C and Bach,R.D (1973):"Trans –Cyclooceten". Organic syntheses,Collected Volume 5:p. 315; Vol.49:p.39.
- Craig, R .G and Powers, J.M (2002):" Restorative dental materials" .11thed .Ch 7, Ch 12 .Elsevier Inc., p: 195-636-656.
- Craig, R.G (1997):"Restorative dental materials".10thed. Polymer and polymerization .Mosby st Louis, p; 127-36.
- Craig, R.G; O'Brein, W.J and Power, J.M (1996):"Dental materials properties and manipulation" .7thed .The C.V.Mosby Co., p: 242-262.
- Crede, C.S.E (1881):" Die Verhurtung der Augenetzundungdeneugeborenen". Archiv fur Ggnaekologie, 17(1):50-53.
- Cucci, ALM ;Vergani, C.E ;Giampaolo,E.T;Afonso, MCFS(1998):" Water sorption, solubility, and bond strength of two auto polymerizing acrylic resin and one heat –polymerizing acrylic resin ".J. Prosthet.Dent ,23:434-438.

D

- Damm,C;Munstedt,H;Rosch, A(2008 a):" The antimicrobial efficacy of polyamide 6/ silver- nano-and microcomposites ". Mater ChemPhys, 108:61-66.

- Damm, C;M¨unstedt,H (2008 b):" Kinetic aspects of the silver ion releasefrom antimicrobial polyamide/silver nanocomposites". ApplPhys A Mater SciProcess; 91:479-486.
- Damm,C;Munstedt,H;Rosch, A (2007):"A long-term antimicrobial polyamide 6/ silver nanocomposites ". J Mater Sci, 42: 6067-6073.
- Dasanayake, A.P; Rosemaa, J.M; andCaufield, P.W (1995):"Distrepution and determination of mutans streptococci African American children and association with selected variables ". Pediatr .Dent, 17(3):192-8.
- Dhuru, V.B(2003). :" Contemporary of dental materials". Oxford University U.K.

E

- Edwardsson, S (1970):" The caries inducing properties of variants of streptococcus mutans .Odent Rev, 21:154-7.
- El –RefaieKenaway; Worley, S.D and Broughton, Roy (2007):" The chemistry and application of antimicrobial polymers: Astate of the Art Review ". Bio Macromolecules (American Chemical Society), 8(5):1359-1384.

F

- Fan, C; Chu, L;H, Ralph Rawls;Norling, B.K; Cardenas,H.l;Whang,K(2011):"Development of antimicrobial resin –Apilot study ". J of Dental Materials, 27:322-328.
- fda.gov.www.fda.gov/bbs/Topics/NEWs/2007/NEW01741/.html.Retrived 2007.
- Feng, Q.L; Wu, J; Chen, G.Q et al (2003). Biomed Mater J Res, 52: 662.

- Fingold, S.Mand Baron, E.J Methods for identification of Etiologic agent of infectious disease .In: Baily and Scott's (1986):"Diagnostic Microbiology" .7thed St Louis.Mosbuy.Co.p: 382-422.
- Freshney, R.I (2005):" Culture of animal cells: Amanual for basic technique".5thed.Wiley-liss, A John Wiley and sons, Inc.PuplicationNewYork. 2005.
- Freshney, R.I (2010):" Application of cell culture to toxicology.CellBiolToxicol, 17:213-230.
- Fung, M.C and Bowen, D.L (1996):" Silver product for medical indications: Risk –Benefit –Assessement ". Clinical Toxicology, 34(1): 119-126.

G

- Gao, Z (2003):" Map –based cloning of the ALK gene,which control the gelatinizatiuon temperature of rice".Sci.China (Ser .C),46:661-668.
- Geigy, S (1962):"Documents scientific tables". .6thed .Basle Switzer.
- Gill, S et al (2005):" Insights on evaluation of virulence and resistance from the complete genome analysis of an early methicillin – resistant Staphylococcus aureus strain and a biofilm producing methicillin – resistant Staphylococcus epidermidis strain". J of Bacteriology , 187(7):2426.
- Gold, O.G; Jordan, H.V;and Van Haute, J (1973):" A selective medium for streptococcus mutans .Archs. Oral Biol, 18:1357-1364.
- Guocheng Wang and HalaZreiquat(2010):"Functional coating or films for hard tissue applications.Materials, 3:3994-4050.

H

- Hamilton, E.I andMinski, M.J(1972/1973):"Abundance of the chemical elements in man's diet and possible relations with environmental factors". Total Environ.Li., 375-394.
- Harrison,A;Hugett, R and Jagger, R.C (1978):" The effect of cross linking agent on abrasion resistance and impact strength of acrylic resin denture base material".J Dent,6:299-304.
- Health Fraud Bulletin # 19 and promotional material, from manufacturers of products .Written communication Feb (1995). Non Traditional Drug compliance Branch , office of compliance ,center for Drug evaluation and Research ,Food and Drug Administration ,Rockville, Mary Land.
- Hermans, M.H (2006):" silver-containing dressings and the need for evidence ". Amer.J. Nur.; 106(12):60-8.
- Hetrick, E.M;Schoenfisch M.H (2006):" Reducing implant related infections: active release strategies". ChemSocRev, 35: 780-9.
- Hidalgo, E; Bartolomé, R; Barroso, C; Moreno, A; Domínguez, C (1998):" Silver nitrate: antimicrobial activity related to cytotoxicity in cultured human fibroblasts".SkinPharmacolAppl Skin Physiol., 11(3):140-51.
- Hidalgo, E andDominguez, C (1998):"Study of cytotoxicity mechanisms of silver nitrate in human dermal fibroblasts".ToxicolLett., 98(3):169-79.
- Hill, W.R and Pill Shury(1939):"Argyria, the pharmacology of silver" .1sted. In: AkhilWadhera and Max Fung. (2005):" Systemic argyria associated with ingestion of colloidal silver ".Dermatology on line Journal; 11(1):12.

- Hipler, U.C; Elsner, P; andFluher, J.W (2006):" Anew silver – loaded cellulosic fiber with antifungal and antimicrobial properties .Curr .Probl.Dermatol, 33:165-178.
- Holbrook,W;Beighton, D (1986):" Streptococcus mutans in saliva and distribution of serotypes 9 years old Ice Landic children .Scan Dent Res,95: 37-42.
- Hooshmand,T;Mohajerfar,M;Keshvad,A;Motahhary, P(2011):" micloleakage and marginal gap of adhesive cements for nobel alloy full cast crown .Oper Dent ,36(3):258-65.
- Hostynek, J.J; Hinz R.S;Lorence ,C.R(1993):" metals and skin". Crit .Rev.Toxicol. 23(2):171-235.
- Hossain, Z and Huq, F (2002):" Studies on the Interaction between Ag (+) and DNA". J. Inorg. Biochem., 91: 398–404.
- Hottal,M;Nakajima,H;Yamamoto,K;Aono,M(1998):" Antibacterial temporary filling materials:The effect of adding various ratio of Ag-Zn-Zeolite .J oral Rehabil ,25(7): 485-9.

I

- Inove,Y.;Hoshino, M.; Takahashi, H.;Noguchi, T.; Murata, T.; Kanzaki, Y.; Hamashima, H.; and Sasatsu, M.(2002):"Bactericidal activity of Ag-Zeolite mediated by reactive oxygen species under aerated condition". J. Inorg. Biochem, 92: 37-42.
- International Agency for Research on Cancer (IARC) (1980):" Long-term and short –term screening assays or carcinogensia critical appraisal ". 1ARC Monographs on the Evaluation of Carcinogenic Risk of chemicals to humans .Supplemented, 2: 1-426.

- Irzh, A;Perkas, N;Gedanken, A (2007);" Microwave assisted coating of PMMA beads by silver nanoparticles .Langmuir ,23: 9891-9897.
- Iso 179-1(2000). International Organization for Standardization. Plastic Determination of charpy impact properties- Part 1: Non-instrumented impact test.
- Iso 527(1993). International Organization for Standardization.Plastic Determination of tensile properties.

J

- Jagger, D.C;Jagger, R.G; Allen, S. M;Harrison, A (2002):" An investigation into the transverse and impact strength of high strength denture base acrylic resins. J oral Rehabil, 29: 263-267.
- Jagger, R.G (1978):" Effect of curing cycle on some propertiesof a polymethylmethacrylate denture base material".J Oral Rehabil, 5: 151–157.
- Jerolimov, V;Huggett, R; Brooks, C.S; Bates, J.F (1985):"The effect of the variations in the polymer/monomermixing ratios on residual monomer levels and flexural properties of denture base materials". Quintessence Int, 9: 431–434
- Joanne, M. Willey;Linda, M; Sherwood and Christopher J Woolverton (2008):" Microbiology" .7th ed .Ch. 4 .McGraw-Hill.com.
- Jose, L. ElcheguerraJusin; Jose,L. B;Morones, R (2005):" Interaction of silver nano-particles with HIV-I .J of Nanobiotechnology ,3:6.
- Jung, W.K; Kim, H; Koo, H.C (2007):" Antifungal activity of silver ion against contaminated fabric ". Mycoses, 50:265-269.

K.

- Kawashita, M;Tsuneyama,S;Miyagif ,K.K (2000):" Antibactrerial silver-containing silica glass prepared by sol –gel method ".Biomaterials ,21(4):393-8.
- Keng, S.B and Lim, M (1996):" Denture plaque distribution and the effectiveness of a perborate –containing denture cleaners .Quintessence Int, 27: 341-345.
- Kidd, M and Jouyston-Bechal, S (2002):" Essentials of Dental Caries". The Disease and its Management ".Oxford.P:1-20.
- Kim, J.S; Kulk,E;Yu, K.N (2007):" Antimicrobial effect of silver nanoparticles ". Nanomedicine nanotechnology , Biology and Medicine ,3(1): 95-101.
- Klaassen C.D (2008):" Toxicology: The Basic Science of Poisons" 7th ed.Ch 5,p: 968 the McGraw-Hill Companies, Inc.
- Kollef,M.H; Afessa, B.; Anzueto,A (2008):' Silver _ coated endotracheal and incidence of ventilator – associated Pneumonia". The NASCENT randomized trial JAMA,300(7): 805-13.
- Koneman E; Schreckenberge, P.C;Allens ,S.D; and Janada ,W.M(1992):"Diagnostic Microbiology.4th ed .J.B.Lippincottco.U.S.A.
- Kong, H; Jang, J(2008):" Antibacterial properties of novel poly (methylmethacrylate)nanofiber containing silver nanoparticles".Langmuir;24:2051-2056
- Kourai, H;Manabe ,Y andYamada, Y (1994):" Mode of bactericidal action of zirconium phosphate ceramics containing silver ions in the crystal structure .J Antibact.Antifung .Agents , 22: 595- 601.

- Kreth, J; Kim,D; Nguyen,M; Hsiao,G; Mito,R (2008):" The antimicrobial effect of silver ion impregnation into endodontic sealer against streptococcus mutans .The Open Dent. J., 2:18- 23.
- Kumar, R and M¨unstedt, H (2005 a):" Silver ion release from antimicrobialpolyamide/silver composites". Biomaterials, 26:2081-2088.
- Kumar, R andM¨unstedt, H. (2005 b):" Polyamide/silver antimicrobials: effectof crystallinity on the silver ion release". PolymInt; 54:1180-1186.
- Kuroki, K;Hayashi, T; Sato,K;Asai,T; Okano,M; Kominami,Y; Takahashi,Y; Kawai,T (2010):" Effect of self-cured acrylic resin added with an inorganic antibacterial agenton *Streptococcus mutans".*Dent Mater J, 29(3): 277–285.

L

- Lansdown, A.B. G(2010):"A pharmacological and Toxicological profile of silver as an antimicrobial agent in medical devices ".Adv. Pharmacol.Sci, 16 pages.
- Lansdown, A.B.G (1995):" Physiological and Toxicological changes in the skin resulting from the action and interaction of metal ions ".Critical reviews in toxicology, 25(5): 397-462.
- Lansdown, A.B.G (2009):" Cartilage and bone as target tissue for toxic materials".In General and applied Toxicology, Ballan Tyne B;Marrs T.C; and SylversenT, Eds 3:1491-1524,John Wiley and sons; Chichester,U.K.
- Lansdown, A.B.G(2006):" Silver in health care: antimicrobial effects and safety in use ". Curentproplems in Dermatology, 33:17-34.

- Lansdown, A.B;Williams, SChanndler; and Benfield, S (2005):"Sliver absorption and antibacterial efficacy of silver dressings". J. of wound care, 14(4):155-160.
- Lansdowne, A.B; Williams, SChanndlerand Benfield, S (2007):" Bacterial resistance to silver in wound care and medical devices ". J.of wound care, 16(1): 15-19.
- Loertzer, H; Soukup, J; Hamza, A (2006):" Use of catheters with the Ag 10N antimicrobial system in Kidney transplant recipient to reduce infection risk". Transplantation proceeding, 38(3): 707-10.
- Loesche, W.J (1986):" Role of Streptococcus mutans in human decay". Microbiol Rev, 50(4): 353-80.
- Lok, C.N; Ho,C.M; Chen, R (2007):" Silver nanoparticles:Prtial oxidation and antibacterial activities .J BiolInorgChem, 12:577-534.
- Low, N; Ansari, S; Livens F.R; Renshaw J.C; Lioyd J .R (2008):" Formation of nanoscale elemental silver particles via enzymatic reduction by Geobacter sulfur reducens ". Appl Environ Microbiol, 74(22): 7090-7093.
- Luciana AssiratiCasemiro;Carlos,H;Gomes,M (2008):"Antimicrobial and mechanical properties of acrylic resins with incorporated silver –zinc- zeolite –part 1.Gerodontology ,25(3):187-194.

M

- Ma, Y and Marquis, R.E (1997):"Thermophysiology of streptococcus mutans and related Lctic –acid bacteria".Antonie Van Leeuwenhook, 72: 91-100.

- Mackeawn, J.M; Cleaton –Jones, P.E;Fatti, P(2003):" Creis and micronutrient intake among urban South African children: acohort study". Comm.Dent .Or. Epidem. , 31:213-223.
- Mair, L.H (1989):" Surface permability and degradation of dental composit resulting oral temperature changes .Dent Mater, 5(4): 247-55.
- Mair, L.H (1992):" The colors with silver and silver nitrate staining in dental materials.Dent Mater, 8(2):110-7.
- Major, I.A (1996):" Glass ionomer cement restoration and secondary caries: a primary report. Quintessence Int, 27: 171-4.
- Manappallil, J.J (2007):"Basic dental materials". 2nd ed. Ch. 8, Ch. 20.New Delhi, p: 99-142, 346-377.
- Matsuura,T.Y.Abe.;Sata, Y;Okamoto, K (1997):" prolong antimicrobial effect of tissue conditioners containing silver –zeolite ". J Dent., 25: 373-377.
- M_cCabe, J.F (1985):"Anderson's applied dental materials".6th ed .Black well scientific publication, London.
- M_cCabe, J.F (1990):"Anderson's applied dental materials ".7thed Ch2, 11, 13.Black well scientific Publication, Oxford London,p: 50-70-88.
- M_cCabe, J.F and Walls, A.W.G (2008):"Applied dental materials". .9th ed .Ch 13.Black well Publication Ltd., p: 110-112.
- Miyoshi,H.; Kourai, H.; Maeda,T.;andShida,T.Y (1998):"Role of cl^-adsorbedon silver-loaded Zirconium phosphate for the photooxidatio of OH^- to OH". J. photochem. Photobiol.A,113: 243-250.
- Moller,AJR(1966):" Microbiological examination of root canals and periapical tissues of human teeth". OdontolTidskr, 74: 1–38.

- Monteiro, D.R; Gorup, L.F; Takamiya,A.S; Camargo,E.R; Ruvolo-Filho,A.C; Barbosa,D.B(2009):" The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver". International J. of Antimicrobial Agents, 34: 104-110.
- Monteiro, D.R; Luiz Fernando Gorup; Takamiya, A.S;Camargo,E.R; Ruvolo- Filho,A.C; Barbosa,D.B (2011):" Silver Distribution and Release from an Antimicrobial Denture Base Resin Containing Silver Colloidal Nanoparticles". American College of Prosthodontics.

N

- Nakanoda, S; Nikawa, H; Hamada, T; Yamamoto, T; Nakamoto, K(1995):" The material and antifungal properties ofantibiotic zeolite incorporated acrylic resin". J JpnProsthodontSoc , 39: 919-926.
- N. Williams (1999):" Longitudinal medical surveillance showing Lack of progression of argyrosis in silver refiner ". Occupational medicine, 49(6):397-399.
- Neil,M.J.O;Heckelman,P.E; Koch,C.B et al(2006):" The MERCK" .14THed.MERCK,Co.,Inc.,Wight house station NJ,USA,p: 8522.
- Nolte, W.A (1982):" Oral microbiology with basic microbiology and immunology ".4thed .The C.V.Mosby Co.
- Noort, R.V (2007);" Introduction to dental materials" .3rded .Elsevier Limited.
- Nordberg, G.F andGerhardsson L (1988):" Silver". In: Seiler H.G.and H. Sigel .Handbook on toxicity of Ionganic Compounds. Marcel Dekker, Inc, New York, p: 619-623.

O

- O'Brien, W.G (2002):"Dental Materials and their selection".3rd ed .Quintessence publishing Co., p: 74-88.
- Oppermann, R.V and Johansen, J.R (1980):" Effect of Fluoride and non – Fluoride salt of Copper, Silver and Tin on acidogenecity of dental plaque in vivo". Scand Dent Res, 88(6):476-80.
- Orsi, I.A and Andrade, V.G (2004):" Effect of chemical disinfection on the transverse strength of heat polymerized acrylic resins submitted to mechanical and chemical polishing ". J of Proshtodontic Dent, 92: 382-8.

P

- Paddock, H.N; Fabia, R; Giles, S; Hayes, J (2007):" Asilver impregnated antimicrobial dressing reduces hospital length of stay for pediatric patients with burns .J Burn care .Res. 28(3): 409-411.
- Pal, S; Tak, Y.K; Song, T.M (2007):" Dose the antibacterial activity of silver nanoparticles depent on the shape of the nanoparticles? Astudy of the Gram –negative bacterium *E.coli*". Appl. Environ Microbiol, 73: 1712-1720.
- Panacek, A; Kvite, K.L; Prucek, R (2006):" silver colloidal nanoparticles:synthesis,charachterization ,and their antibacterial activity". J Phys chem. B, 110: 16248-16253.
- Pariser,R.J (1978):" Generalized argyria:clinicopsyhologic features and histochemical studies .In: AkhilWadhera and Max Fung (2005):" Systemic argyria associated ingestion of colloidal silver". Dermatology Online J, 11(1):12
- Pernodet, N; Fang, X; Sun, Y; Bakhtina, A; Ramakrishnan,

A; Sokolov, J; Ulman, A; Rafailovich, M(2006):" Adverse of Effects Citrate/Gold Nanoparticles on Human DermalFibroblasts". Small, 2(6): 766–773.

- Peter, H (2000):" Dr.CarlCrede (1819- 1892) and the prevention of ophthalmianeonatorum"(http:// www.pupmed central .nih.gov/ article render.polyamide 6/silver-nanocomposites". J Mater Sci; 42:6067-6073.
- Power, J.M and Sakaguchi, R.L (2006):" Graig's restorative dental material".12thed .Ch 7 .Elsevier Inc.,p: 1- 632.
- Prati, C;Taol; Simpson,M; andPashley, D.H (1994):" Permability and microleakage of cl II resin composite restoration ". J. Dent. , 22(1): 49-56.

R

- Rai, M; Yadav, A; Gade,A(2009):" Silver nanoparticles as a new generation of antimicrobials ". Biotechnology Advances, 27:76-83.
- Rodford, R.A (1986):" The development of high impact denture – base materials". J Dent, 14; 214-217.
- Roe, D; Karandikar, B; Bonn-Savage, N(2008):" Antimicrobial surface functionalized of plastic catheters by silver nanoparticles. J Antimicrobchemother, 61: 869-876.
- Rosmarie,A.Faust(1992):" Toxicity summary for silver ". Article review.
- Rozen, R. et al (2001):" Growth rate and biofilm thickness of streptococcus sobrinus and streptococcus mutans on hydroxyapetite ". APMIS, 109:155-160.

- Russell, R (2000):" Pathogenesis of streptococci In: FischettV.A;Novick R.P; Ferretti .J. et al (2000):" Gram –Positive Pathogens ". Washington: ASM press: 212.
- Russell, A.D and Hugo, W.B (1994):" Antimicrobial activity and action of silver". Progress in medical chemistry, 31: 351-370.

S

- Salimetrics, LLC (2009):" Saliva Collection and Handling Advice" Handbook,.Salimetrics Europe.
- S¨oderholm, K.J (1984):" Water sorption in a bis(GMA)/TEGDMA resin".J Biomed Mater Res; 18:271-279.
- Saint, S; Elmore, J.G; Sullevan, S.D (1998):" The efficacy of silver alloy –coated urinary catheter in preventing urinary tract infection: a Meta-analysis. The American J of medicine, 105(3): 236-41.
- Schrenrs, W.J.and H. Rosenberg (1982):" Effect of silver ions on transport and retention of phosphate by *E.coli*". J.Bcteriol, 152:7-13.
- Samaranayake, L (2006):" Essential Microbiology for Dentistry" .3rded. Philadelphia.
- Sato, S; Sue Ki, H; and Nishijima,A(1999):" Two un usual causes of argyria : The application of an improved tissue processing method for x-ray microanalysis of selenium and sulphur in silver –laden granules ". British J of Dermatol , 140(1): 158-163.
- Silver, S; Phung, L.T; Silver, G (2006):" Biocides in burn and wound dressing and bacterial resistance to silver compounds ". J Ind.Microbiol.Biotech., 33: 627-634.

- Singho,N.D;Lah,N.A.C;Johan,M.R;Ahmed,R(2012):"FTIR Studies on Silver –Poly(Methylmethacrylate) Nanocomposites via In-Situ Polymerization Technoque:.Int.J.Elechtrochem.Sci.,7:5596-5603.
- Snyder, W.S et al (1975):"report of task group on Reference man". Pergamon press Oxford, England, p: 407-708. (Cited in ATSDR, 1990).
- Spacciapoli, P; Buxton, D; Rothestein, D; Friden, P(2001):"Antibacterial activity of silver nitrate against Periodental Pathogens". J. Periodental Res, 36(2):108-13.
- Sterling, J.C; Hand Field –Jones, S;Hudson, P.M (2001):" Guide lines for the management of cutaneous warts ". British J of Dermatology, 144(1): 4-11.
- Stevens, M.P (1999):"Polymer Chemistry .An Introduction ". Oxford University Press, Inc, NewYourk.
- Stickler, D.J (2000):" Biomaterials to prevent nosocomial infections: is silver the gold standard?"Curropin infection Dis, 13; 389-93.
- Stobie, N; Duffy B; M_cCormack D.E;Colreavy J (2008):" Prevention of staphylococcus epidermidis biofilm formation using low -temperature processed silver- doped phenyltriethoxysilane sol-gel coating". Biomaterials, 29: 963-9.
- Stokinger,H.E (1981):" Silver In: Patty's IndusterialHygien and Toxicology". Vol.2A, G.D.NewYork, NY, P: 1881-1894.
- Svehla, G. (1979):"Vogel's Text book of macro and semimicro qualitative inorganic analysis".5th ed. Ch.3. Longman Group Limited. New York.

T

- Thomas, V; Yallapu, M.M; Sreedher, B; Bajpai, S.K (2007):" Aversatile strategy to fabricate hydrogel –silver nanocomposite and investigation of their antimicrobial activity ". J. of colloidal interface science, 315:389-395.
- Tian,J; Wong, K.K; Ho, C.M (2007):"Topical delivery of silver nano particles promotes wound healing .Chemmed .chem., 2(1):129-36.
- Toolson, L.B and Smith, D.E (1978):" A two years longitudinal study of over denture patient's .Part 1: incidence and control of caries on over denture abutments ". J ProsthetDent, 40(5): 486-91.

U

- U.S. Department of Health and Human Resources (2010)."12th Report on Carcinogens" National Toxicology, Research Program, Research Triangle Park,NC,USA.
- U.S.EPA (1985):" Drinking water criteria document for silver ", Final Draft ECAO-CIN-026.The office of health and Environmental Assessment,Environmental criteria and Assessment office ,Cincinnati, OH for the office of drinking water ,Washinton ,D C,USA.
- U.S.EPA. (1994a).Integrated Risk Information System (IRIS).Invironmental Criteria and Assessment Office. Office of health and InvironmentalAssessment,Cincinnati. OH.

- USEnvironmental Protection Agency(1991.):"Reference dose for chronic oral exposure of silver".Washington ,D C,Chemical Screening and Risk Assessment Division.CASRN 7440-22-4,
- Uzun,G;Hersek, N and Tincer, T(1999):" Effect of five woven fiber reinforcement on impaqct and transverse strength of denture base resin :. J Prosthet .Dent, 81:616-620.

V

- Vallittu, P.K (1996):" Areview of fiber- reinforced denture base resins". J. of Prosthod, 5(4):270-276.
- Vallittu, P.K;Ruyter, I.E;Buykuilmaz,S(1998):" Effect of polymerization temperature and time on the residual monomer content of denture base polymers". Eur J Oral Sci;106:588-593.

W

- Wade.,W.G; Aldred, M.j; Walker, D.M (1986) :" An improverd medium for isolation of Streptococcus mutans'. J Med Microbiol, 22: 319-323.
- Wadhera, A and Fung, M (2005):" Systemic argyria associated with ingestion of colloidal silver ".Dermatology on line Journal; 11(1):12.
- Wakasa, K;Yosida, Y; Nomura, Y; Ikeda, A; Nakatsuka, A; Ogino,S,;Matsui, H;Shirai, K; Yoshioka, M;Yamaki, M.A(1997):" fundamental study on dental application of silver zeolite". J Hiroshima Univ Dent Soc; 29: 87-98.
- William, F and Vincent, D (2005):" An over review of the genus streptococcus". Thanet press Ltd.,p: 13-22.

- Wu,H;Fan,M;Zhou X(2003):" Detection of streptococcus mutans and streptococcus sobrinus on permanent 1st molars of the mouse people in China ". Caries Res., 37(5): 374-80.

Y

- Yamanaka, M;Hara, K andKudo, J(2005):" Bacteriocidal action of silver ion solution on *E.coli*, studied by energy- filtering transmission electrone microscope and proteomic analysis". Appl.Environ. Microbiol., 71:7589-7593.
- Yoshida,K;Tanagawa,M;Atsuta, M(1999):" Characterization and inhibitory effect of antimicrobial dental resin composites incorporating silver –supported materials ". J. Biomed Mater Res, 47: 516-22.
- Yu, H; Nakano, Y; Yamashita, Y; Hot, O; and Koga, T(1997):" Effect of antibodies against cell surface protein antigen .Glucosyltransferase fusion proteins on glucan synthesis and cell adhesion of streptococcus mutans ". J. Infec. Immun., 65: 2294-2298.
- Yu,R.Y;Zhou,Y.S;Feng, H.L (2008):" Silver ion release and particle distribution of denture base resin containing nanometer-sized silver –supported antimicrobial agent ". Zhonghua Kou Qiang Yi XueZaZhi, 43:54-56.
- Yutani, H; Yamamoto, K (2002):" The antibacterial effect of glassionomer cement containing Ag Silica Glass". Jpn J Conserv Dent, 45: 441-449.

Z

- Zappini, A;Kammann and Wachter, W (2003):" Comparesion of fracture tests of denture base materials". J .Prosth.Dent. 90:578-585.
- Zhenbing Cao; Xinbo Sun; Yuyu and Haofong (2009):" Rechargable antibacterial and antifungal polymeric silver sulphadiazines ". J of Bioactive and compatable polymers, 24:350.

APPENDICES

Appendices

Appendix (1): Data represent the values of impact strength test (KJ/ m^2).

Control	9.375ppm $AgNO_3$	15ppm $AgNO_3$	30ppm $AgNO_3$	60ppm $AgNO_3$	120ppm $AgNO_3$	150ppm $AgNO_3$
11.11	**10.61**	**11.53**	**11.76**	**13.72**	**9.42**	**12.47**
11.10	**10.99**	**11.34**	**13.41**	**13.21**	**12.66**	**8.86**
9.89	**10.49**	**12.23**	**12.15**	**11.89**	**11.38**	**12.20**
9.67	**11.62**	**12.09**	**9.78**	**13.24**	**9.25**	**10.87**
11.10	**10.55**	**10.53**	**10.32**	**13.18**	**12.30**	**15.17**
10.69	**9.94**	**11.11**	**13.51**	**11.27**	**9.39**	**11.20**

Appendix (2): Data represent the values oftransverse strength (MPa).

Control	9.375ppm $AgNO_3$	15ppm $AgNO_3$	30ppm $AgNO_3$	60ppm $AgNO_3$	120ppm $AgNO_3$	150ppm $AgNO_3$
74.4	**67.2**	**56.4**	**60**	**61.2**	**56.4**	**68.4**
76.8	**57.6**	**72**	**64.8**	**62.4**	**57.6**	**76.8**
75.6	**61.2**	**69.6**	**72**	**62.4**	**48**	**57.6**
80.4	**67.2**	**70.8**	**61.2**	**79.2**	**57.6**	**73.2**
81.6	**66**	**66**	**74.4**	**58.5**	**57.6**	**72**
77.7	**63.6**	**67.2**	**66**	**64.8**	**55.2**	**69.6**

Appendix (3): Data represent the values oftensile strength (MPa).

Control	9.375ppm $AgNO_3$	15ppm $AgNO_3$	30ppm $AgNO_3$	60ppm $AgNO_3$	120ppm $AgNO_3$	150ppm $AgNO_3$
51.1	**47.91**	**39.71**	**53.7**	**35.6**	**42.66**	**43.37**
62.5	**55.20**	**50.5**	**43.26**	**38.56**	**38.25**	**39.06**
56.5	**49.35**	**57.3**	**38.84**	**41.69**	**38.51**	**45.56**
53.3	**46.54**	**54.4**	**40.4**	**36.59**	**43.40**	**44.57**
51.0	**46.06**	**57.8**	**44**	**31.48**	**40.70**	**38.31**
49.54	**49.01**	**51.94**	**44.05**	**36.78**	**40.50**	**38.3**

الخلاصة

صنعت في السنين الاخيرة العديد من المواد غير العضوية الحاوية على الفضه والمضادة للبكتريا . وكانت كمحاولة لتكون عديمة اللون , ثابتة كيماءيا وتستمر لفترات طويلة تتحرر منها ايونات الفضه ببطيء على امل ان يتم استعمالها بنجاح كمضادات للبكتريا في المجالات الطبية وطب الاسنان لمنع تسوس الاسنان والتهاب الاسنان المزروعة .

تم تحضير تراكيز مختلفه من نترات الفضه(9,37 ,15 , 30 ,60 ,120 ,150 ,300 ,600 ,900 جزء بالمليون) من محلول قياسي 1000 جزء بالمليون. وعرضت خلايا ليفانيه جنينيه للجرذ لتراكيز من نترات الفضة لتقييم التأثيرات السميه الخلويه لهذه الماده . حضر الراتنج الاكريلي حسب الطريقه المقترحه من قبل المصنع, واظيف محلول نترات الفضه بحجم ثابت (0,2ملليتر) .استخدم ضابط سيطره خاليا من نترات الفضه . حفظت النماذج الخاصه بالتأثير على البكتريا وكذلك تحرير ايون الفضه في محلول اللعاب الاصطناعي على درجه حراره 37درجه مئويه لمده 30 و 90 يوما . فحصت النماذج المحضره في تجارب المقاومه للبكتريا (*mutans streptococc*i group) قبل المعامله وبعد 30 و 90يوما من الغمر في اللعاب الاصطناعي .كما اجري الكشف عن تحرير الفضه في محاليل اللعاب الاصطناعي المستخدم بمختلف الفترات ولكافه المجاميع بأستعمال جهاز مقياس الطيف للامتصاص الذري.

اجريت بعض الاختبارات الميكانيكيه (قوه الصدمه,القوه المستعرضه,وقوه الشد) على الماده المصنعه.

اظهرت النتائج المتعلقه بأختبار التسمم الخلوي على الخلايا الليفانيه الجنينيه للجرذ حصول تاثير منع النمو بنسبه 70,8 - 82,9 % .وكانت كفاءه تاثير الماده المصنعه والحاويه على 9,37- 900 جزء بالمليون قد وصلت الى 76,7 -96,6 % . في حين سبب منع كامل للنمو الجرثومي

(100 %) بعد الغمر في محلول اللعاب الاصطناعي لفتره 30 أو 90 يوما. لم يلاحظ اي اثر للفضه في محلول اللعاب حتى بعد الغمر لمده تسعون يوما.

لم يكن هناك اي اتحاد كيمياوي بين نترات الفضه والبولي مثيل ميثاكريليت . كانت هناك زياده احصائيه غير معنويه (P=0.05NS) في قوه الصدمه بالمقارنه مع مجموعه السيطره . اما اختبار القوه المستعرضه فكانت هناك قله معنويه(P<0.001) مقارنه بالسيطره . أما قوه الشد فقد لوحظ حصول قله غير معنويه في التراكيز 9,37(P=0.05NS) و15(P=0.42NS) جزء بالمليون . إلا إنه كان معنويا في التراكيز التي اعلى من 15 جزء بالمليون . ظهر بدايه تلون باللون الداكن للماده عندما كان تركيز نترات الفضه 300 جزء بالمليون فأعلى.

نستنتج من هذه الدراسه بأن مزج نترات الفضه مع مثيل ميثاكريليت كان ممكنا وبالإمكان اجراءه .لم يلاحظ وجود ايونات الفضه حتى بعد تسعون يوما غمر عينات الراتنج الاكريلي الممزج مع نترات الفضه في محلول اللعاب الاصطناعي وذلك بأستعمال جهاز مقياس الطيف للامتصاص الذري. كما انه لم يتم ملاحظته وقياسه بذات الجهاز بعد الغمر بالماء الفاقد للتأين . وجد بأن لنترات الفضه الممزوجه مع ال مثيل ميثاكريليت تأثير مضاد للبكتريا وهذا يساعد في السيطره على الاصابات الجرثوميه التي تصاب بها الاسنان والانسجه الرخوه الفموية للمرضى مستخدمي طقم الاسنان إظافه لقاعده طقم الاسنان.

تأثير إضافه نترات الفضه على الفعالية البكتيريةفي مادة قاعدة طقم الراتنج الأكريلك الحراري وعلى بعض خواصه الميكانيكية

رسالةمقدمةإلى
مجلس كلية طب الأسنان-جامعةبغداد
كجزءمنمتطلباتنيلدرجةالماجستير
فيصناعةالأسنان

من قبل
عُلا خليفه أحمد الحسيني
بكالوريوس طب وجراحة الفم والأسنان

بإشراف
الأُستاذ الدكتور نبيل عبد الفتاح
بروفيسور

1434هـ -2013 م