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Editorial

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Mini review section – Bacterial biofilms are serious global health concern due to their abilities to tolerate antibiotics, host defence systems and other external stresses; therefore, it contributes to persistent chronic infections. Biofilms are immobile microbial communities which colonize and grow on surfaces of medical implants such as sutures, catheters and dental implants, by self-produced extracellular polymeric substances and cause infections which can only be treated by their removal and leads to the unaffordable treatment as well as mental illness to patients. Biofilm comprises of the crammed bacterial population by extra-cellular matrix (ECM) which possesses bacterial secreted polymers such as exopolysaccharides (EPS), extracellular DNA (e-DNA), proteins and amyloidogenic proteins. Biofilms provides the protection to the microorganism not only from altered pH, osmolarity, nutrients scarcity, mechanical and shear forces but also block the access of bacterial biofilm communities from antibiotics and host's immune cell.

Current Trends section – Equipment Sterilization Disinfection and their categories.

In Profile Scientist – Alexander Fleming was a Scottish physician-scientist who was recognized for discovering penicillin. The simple discovery and use of the antibiotic agent has saved millions of lives, and earned Fleming – together with Howard Florey and Ernst Chain, who devised methods for the large-scale isolation and production of penicillin – the 1945 Nobel Prize in Physiology/Medicine.

Bug of the month – Oropouche virus disease is caused by the Oropouche virus (OROV) that can cause fever, headache, joint pain, muscle pain, chills, nausea, vomiting and rash. Most people recover on their own, but the disease can cause severe symptoms in some patients. OROV is a segmented single-stranded RNA virus belonging to the family *Peribunyaviridae,* genus *Orthobunyavirus*, which was first identified in 1955 in Vega de Oropouche, Trinidad and Tobago. The virus is transmitted to people through the bite of an infected insect, usually biting midges but also possibly by mosquitoes. It is thus referred to as an arthropod-borne virus (arbovirus).

Did You Know? – Body weight is determined by the balance between energy intake and energy expenditure. Although energy intake is solely the result of the food that we eat, energy expenditure is a combination of various factors, including physical activity and the basal metabolic rate necessary to maintain vital physiological functions. Most drugs currently used to treat obesity aim to decrease appetite, but increasing basal energy expenditure at the same time to create a calorie deficit has remained a long-lasting quest. In the past few years, obesity- treatment approaches have successfully intervened on both sides of the energy-balance equation by concomitantly targeting different receptors, but attempts to target a single receptor with one drug have so far failed.

Best Practices – 10 simple daily habits to build for a happy life.

Tickle yourself enjoying the jokes in our **Relax Mood section**.

Our JHS team is thankful to all our readers for their ever-increasing appreciation that has served as a reward & motivation for us. Looking forward for your continuous support.

Antibiotics versus biofilm: an emerging battleground in microbial communities

Bacterial biofilms are serious global health concern due to their abilities to tolerate antibiotics, host defence systems and other external stresses; therefore, it contributes to persistent chronic infections.

Biofilms are immobile microbial communities which colonize and grow on surfaces of medical implants such as sutures, catheters and dental implants, by self-produced extracellular polymeric substances and cause infections which can only be treated by their removal and leads to the unaffordable treatment as well as mental illness to patients. Biofilm comprises of the crammed bacterial population by extra-cellular matrix (ECM) which possesses bacterial secreted polymers such as exopolysaccharides (EPS), extracellular DNA (e-DNA), proteins and amyloidogenic proteins. Biofilms provides the protection to the microorganism not only from altered pH, osmolarity, nutrients scarcity, mechanical and shear forces but also block the access of bacterial biofilm communities from antibiotics and host's immune cell.

Therefore, biofilm matrix gives the additional resistance power to bacteria which makes them to not only tolerate harsh conditions but also resistant to antibiotics which lead to the emergence of bad bugs infections like multi drug resistant, extensively drug resistant and totally drug-resistant bacteria.

Ultrastructure of biofilm

Microbial biofilm is the grouping of sessile microbial communities which is attached with substratum and embedded in the self-produced pool of non-crystalline extracellular polymeric matrix. Formation of the three-dimensional structure of biofilm is the dynamic process by heterogeneous bacterial communities. Bacteria living within the biofilms are protected from the varieties of environmental stresses, such as desiccation, antimicrobials attack by the immune system and ingestion by protozoa hence this architecture makes the biofilm communities to advance as compared to planktonic one. Coordination within the biofilm via cell-to-cell communication called quorum sensing (QS) in which accumulation of signalling molecules in extracellular environment leads to regulation of the specific gene's expression. Some bacterial species use QS to coordinate the disassembly of the biofilm community.

Development of biofilms is multi step process. It starts with the initial adherence of bacteria to the substratum and irreversible attachment followed by their colonization in which modification in genes/proteins expression occurs followed by exponential growth phase. The exopolysaccharides (EPS) and water channels formation occur, facilitating nutrient supply which leads to the maturation of the biofilms. Ultimately surface detachment of the cells starts in the environments which again restart/recycle the biofilm formation onto the new surfaces.

Infections associated with biofilms

Approximately 80% of chronic and recurrent microbial infections in the human body are due to bacterial biofilm. Biofilm is formed in diverse environmental niches, including freshwater rivers, rocks, deep-sea vents and hydrothermal hot springs. Biofilm-related infections can be broadly divided into two types. The biofilms may be formed on the abiotic surfaces, especially infections associated with indwelling medical devices and native biofilm infections of host tissue.

Urinary tract and bloodstream infections can be caused by the biofilm initially formed on medical implants, such as heart valves, catheters, contact lenses, joint prostheses, intrauterine devices and dental unit. These infections can only be treated by removal of the implants which not only increasing the cost of the treatment but also it becomes problematic for patients.

Host tissue related biofilm infections are often chronic, including chronic lung infections of cystic fibrosis patients, chronic osteomyelitis, chronic prostatitis, chronic rhinosinusitis, chronic otitis media, chronic wounds, recurrent urinary tract infection, endocarditis, periodontitis and dental caries.

Some of the major biofilm associated infections causing human diseases are listed below:-

Table 1 Bacterial species involved in biofilm associated infection and their adherent surfaces

S. No.	Bacterial Species	Infection/Diseases	Surface
1	*Streptococcus mutans*	Dental caries Endocarditis	Tooth surface Vascular grafts
2	*Enterococcus faecalis*	Endocarditis Root canal infection	Heart valves Urinary catheters Tooth Central venous catheters
3	*Klebsiella pneumonia*	Pneumonia Respiratory tract infection Urinary tract infection Pyogenic liver abscess	Lungs Liver
4	*Pseudomonas aeruginosa*	Nosocomial infection Otitis media Cystic fibrosis	Central venous Catheters Middle ear Prostheses Lungs Contact lenses
5	*Staphylococcus sp (Staphylococcus aureus; Staphylococcus epidermidis).*	Nosocomial infections Chronic wounds Endocarditis Mucoloskeletal Infections Otitis media	Sutures Central venous catheters Arteriovenous shunts Prostheses Surfaces/deep skin Prostheses, Heart valves Bones, Middle ear
7	*Escherichia coli*	Bacterial prostatitis Urinary tract infection Otitis media	Prostheses, Urinary tract Urinary catheters Middle ear
8	*Haemophilus influenza*	Otitis media	Middle ear
9	*Burkholderia cepacia*	Cystic fibrosis	Lungs
10	*Mycobacterium tuberculosis*	Tuberculosis	Lungs

Resistance to antibiotics in biofilms communities

Antibiotic resistance of bacteria in the biofilm communities contributes to the chronic infections. It has been reported earlier that repeated exposure of ceftazidime in biofilm-growing *Pseudomonas aeruginosa* developed the conventional type of intrinsic antibiotic resistance in biofilms infections.

In biofilm communities, antibiotics resistance appears due to various strategies such as slow or incomplete penetration of the antibiotics into the biofilm, an altered chemical microenvironment within the biofilm and a subpopulation of micro-organisms in a biofilm (a type of cell differentiation like to spore formation). These mechanisms are the consequences of the multicellular nature of biofilms which leads to the antibiotic's

resistance of biofilm communities along with the known conventional resistance mechanisms and makes the failure of treatment strategy.

Extracellular polymeric substances (EPS) hold the bacterial cells together and lead to the development of multicellular consortia which makes the heterogenous environment inside the biofilm and initiates the biofilm to function as a multicellular system.

Biofilm development is well organized and during its development intercellular and intracellular signalling occurs. A panel of genes/proteins are upregulated as well as downregulated for attachments of bacteria onto substratum surface and pathways differentiation. Maturation of the biofilm into complex structures is regulated by the signalling among the cells by the quorum sensing process.

Multicellularity nature of biofilm bacterial communities is responsible for antibiotics resistance; if we can disrupt any step in the formation of multicellular structure of the biofilm than antibiotics efficacy as well as the host defences might be increased which leads to quick treatment of this persistent infection.

Antibiotics resistant state of the biofilm cells lead to a treatment complication in the series of human infections which include biofilm formation on various biological implants such as, heart catheters, urinary catheters, joint implants and replacement of heart valves. Biofilms pose a threat to humans because of their persistent nature and plays a major role in certain pathogenic infections.

EPS might quench the activity of antibiotics that diffuse through the biofilms via diffusion–reaction inhibition phenomenon, which may chelate the antibiotics by complex formation or degrade through enzymatically based reactions.

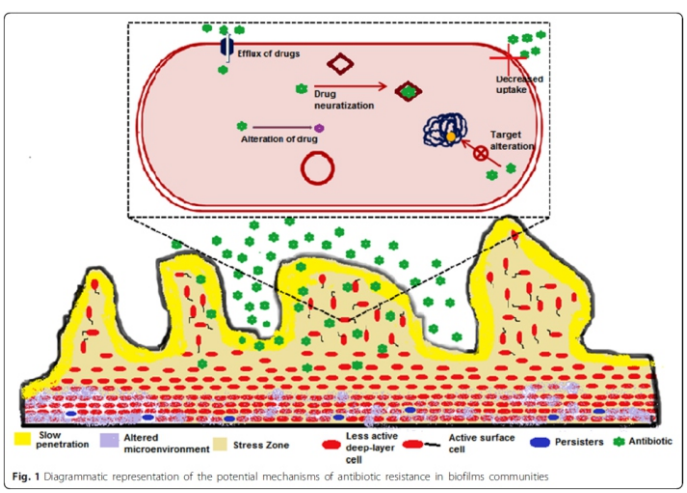


Fig. 1 Diagrammatic representation of the potential mechanisms of antibiotic resistance in biofilms communities

Stationary phase (a slow or non-growth phase of the bacterial life cycle) and viable-but-nonculturable state (VBNC state or a state of dormancy) are the ways of survival for bacterial biofilms communities under antibiotics Stress.

One of the antibiotics resistance mechanisms of biofilms communities is the uptake of resistance genes by horizontal gene transfer. Biofilms provides the compatible conditions for the horizontal gene transfer such as high cell density, increased genetic competence and accumulation of genetic elements or uptake of resistance genes. Conjugation is the only mechanism of horizontal transfer of resistant genes in biofilms and may confirm the resistance to several antibiotics.

At early stage of biofilm development antibiotics treatment was more effective, probably due to the cells which are not completely adapted into biofilm communities.

Alternative approaches to control the biofilm related infections

Naturally produced small molecules by bacterial biofilm communities such as D-amino acids and Polyamine nor spermidine; induced the dispersal of mature biofilms which could prevent biofilm formation in *S. aureus* and *E.coli*. These molecules could be used as antibiofilm agent in the biofilm dispersal strategy. Antibiofilm molecules (N-acetylcysteine/ NAC and Tween 80) alone and in combination with antibiotics were effective against non-pigmented RGM biofilms. Tween 80 is more active against mycobacterial biofilm than NAC because mycobacterial cell wall as well as extracellular matrix possesses high lipid content and suggested that synergistic effect of drugs and anti-biofilm agent may be effective in the treatment of infections associated with mycobacterial biofilms communities.

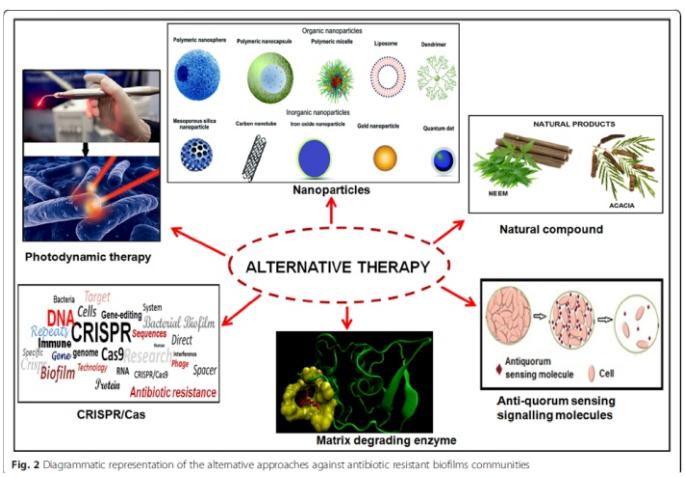


Fig. 2 Diagrammatic representation of the alternative approaches against antibiotic resistant biofilms communities

Degradation of the biofilm matrix by biofilm matrix degrading enzymes (DNase I, Dispersin B (DspB) and a-amylase) is also another promising antibiofilm strategy. Degradation of biofilm structural component allows the increased penetration of antibiotics which enhances the antibiotics efficiency.

Formation of biofilms were controlled by the quorum sensing (QS) signalling genes and their products. Various inhibitors/compounds can disturb the QS signalling cascade and used as alternative therapy for the treatment of biofilm-related infections. Attenuation of bacterial QS signalling by ginseng extract, garlic extract, usnic acid and azithromycin possesses inhibitory activity against bacterial and fungal biofilms. Signalling molecule nitric oxide (NO) disperse the biofilms in P. aeruginosa and enhances the activity of antimicrobial compounds via stimulation of c-di-GMP-degrading phosphodiesterases.

CRISPRi technology was used to knockdown the luxS gene of QS signalling and fimbriae associated gene (fimH) for controlling the biofilm mediated Infections.

Bacterial and actinomycetes have been shown to produce bioactive agents/natural compounds with antibiofilm properties. Methanolic extract of a coral-associated actinomycete helps to reduce biofilm formation of *S.aureus*.

4-phenylbutanoicacid show high antibiofilm activity against Gram positive and Gram-negative bacteria. *Azadiracta indica* (Neem) and Acacia extracts showed antimicrobial effect against *S.mutans* and *S. faecalis*.

Nanoparticles have considered as the alternative of the antibiotics to combat multidrug resistance and biofilm-based infections. Limitations of the conventional antibiotic treatments (reduced penetration and retention in cell or biofilm) were overcome by their nano-formulations which can cross the biological barrier.

Photodynamic therapy (PDT) was used to treat various type of infection like as bacterial, fungal, viral, protozoa or even parasitic infection. It has reported earlier that PDT has sufficiently reduced the clinically relevant microbes, such as drug resistant Gram-positive and Gram-negative bacteria. PDT has significant advantages over conventional treatment owing to its ability of selective binding to the membranes of pathogenic cells and the possibility for accurate delivery of light to the affected tissue for the maximal damage of microbes as well as minimal damage of the host.

EQUIPMENT STERILIZATION DISINFECTION

Sterilization is the complete removal or destruction of all forms of microbial life, including bacteria, viruses, fungi and spores. Sterilization is achieved by steam, dry heat, ethylene oxide gas and liquid chemosterilizers.

The sterility assurance level (SAL) is used as a measure of sterility. It is the probability of survival of a microorganism after a sterilization process. It is expressed as the $\log^{10}$ of the probability of survival. A SAL of '6' means that there is less than one chance in a million (10^{-6}) that a particular item is contaminated. A SAL of 6 is acceptable for a critical item.

Disinfection is a process that eliminates a defined scope of pathogenic microorganisms but not necessarily all microbial forms. Disinfection does not attempt to remove all viable microorganisms. Disinfection's main difference with sterilization is the lack of sporicidal activity, although this is an oversimplification.

Disinfection has been categorized into three levels: high, intermediate and low:

High level disinfection eliminates all pathogenic organisms, but some viable spores may persist on an item disinfected to the high level. The critical distinction between high and intermediate is the elimination of ALL VIRUSES in high disinfection.

Intermediate disinfection eliminates all pathogenic vegetative bacteria, fungi and most viruses but some viruses (particularly small viruses without envelopes), and bacterial spores are not eliminated. The critical distinction between intermediate and low-level disinfection is the elimination of the most resistant bacteria in intermediate level (*Mycobacterium tuberculosis* is used as an indicator because it is relatively resistant to disinfection).

Low level disinfection eliminates most pathogenic bacteria but some of the less sensitive vegetative forms (*M. tuberculosis* for example), the non-lipid viruses and bacterial spores are not eliminated.

Cleaning is the removal of adherent visible soil (blood, protein substance and debris), dust or other foreign material by a manual or chemical process.

Sanitizing is the process that reduces the microbial population on an object to a safe level.

Decontamination is the process that removes pathogenic microorganisms from an object to make it safe to handle.

Antiseptics are chemicals which prevent the growth of a microorganism or destroys it. Antiseptics are used on living tissues.

Disinfectants are chemicals used to carry out disinfection of objects.

Resistance Of Microorganisms

In descending order of resistance:

Sterilization	
Spores bacterial, fungal	*Bacillus stearothermophilus, Bacillus subtilis, Clostridium sporogenes*
High Level Disinfection	
Mycobacteria, TB Bacilli	
Intermediate Level Disinfection	
Hydrophilic viruses	Polio, Cocksackie, Rhinoviruses
Low Level Disinfection	
Vegetative fungi	Trichophyton, Cryptococcus, Candida
Vegetative bacteria	Pseudomonas, Staphylococcus, Salmonella
Lipophilic viruses	HSV, CMV, RSV, HBV, HIV

Wiping /Soaking /Contact time:

When using a germicide-soaked cloth, it is important to consider the time needed by the germicide to kill the microorganisms. All germicides require a minimum time to kill a microorganism. If the wiped surface is dry before the required disinfection time, disinfection cannot be assured. Wiping would remove a large amount of contamination, and the germicide may kill some left-over microorganisms, but there is no assurance that all microorganisms were killed.

Sterilization

Note on D and Z values: Heat inactivation can be better described by using the D-value, which is the time needed at certain temperature to reduce the microbial contamination by one log cycle. D-values are therefore temperature dependent.

The z-value is the increase in temperature needed to lower the D-value by one log. D- and z-values are species dependent, that means *Salmonella enteritidis* or *Bacillus cereus* or *Staphylococcus aureus* will show different values. Moreover, heat resistance depends on the environment, e.g. D-values in humid environments are lower than in dry ones.

1)Steam Sterilization

Steam sterilization is done by saturated steam under pressure. There are four parameters of importance in steam sterilization: **Steam**, **Pressure**, **Temperature and Time.**

To obtain sterilization: Air must be removed, and steam must reach the item for the required time, at the required temperature.

Anhydrous materials (oil, greases, powders) cannot be sterilized by steam because steam will not penetrate the substance, steam condensates on the outside. The correct method for such materials is dry heat.

Steam cannot penetrate hollow needles or instruments packed in moisture resistant materials (test tube, glass) therefore steam sterilization should not be used for those. If an instrument is placed in a glass container for protection, it should not be plugged heretically but with a loose cotton plug that will allow steam to go through. The position of the container should be such that air can easily be removed by steam (place sideways).

Saturated steam (100% relative humidity) has a high heat content and is best to obtain sterilization. Ideally there should be no water in the form of a fine mist. Superheated steam (RH<100%) or wet steam (RH>100%) are much less effective at sterilizing. If saturated steam was used, the pack of equipment should come out of the sterilizer dry**.** Wet packs must be considered as non-sterile.

Surgical dressings are the large bulk of the materials to be sterilized: hand towels, towels, lap sheets, table drapes, gowns, sponges. These materials are arranged in surgical packs. To obtain reliable sterilization, surgical packs should be no larger than 30cm*30cm*50cm and average weight of 5.5kg.

There are several basic types of steam sterilizers:

High speed pre-vacuum sterilizer: A vacuum pump removes the air from the sterilizing chamber and the load. Once the proper

vacuum has been attained (15mm Hg ±1mm, steam is admitted. Steam penetration is very fast into the load. Sterilization time: 4 minutes at 133°C 272°F.

Gravity displacement autoclave: The air is removed by displacement of cool air at the bottom by steam on top. The air is forced down by the steam. When steam enters a material, the air in the shape of a bubble is gradually pushed out. The air bubble prevents sterilization of the material. It takes time for steam to penetrate materials and expel air bubbles.

Small tabletop sterilizers found in dental and medical offices are pressure cookers, reaching a temperature of 121°C.

2) Dry Heat Sterilization

Dry heat is used for materials that cannot be steam sterilized because of damage from steam, lack of penetration, or instruments that cannot be disassembled.

Sterilization takes:

60 minutes at 170°C (340°F)
120 minutes at 160°C (320°F)
150 minutes at 150°C (300°F)
12 hours at 121°C (250°F)

Hot air ovens use gravity convection, or mechanical convection. *Bacillus subtilis* spores should be used as a biological indicator because of their higher resistance to dry heat.

3) Flash Steam Sterilization

Flash steam sterilization is defined as sterilization of an unwrapped object at 132°C for three minutes at 27 to 28 lb of pressure in a gravity displacement autoclave.

4) Ethylene Oxide Sterilization

ETO is a colourless gas that is flammable and explosive; however, mixtures of ETO (10-12%) with carbon dioxide or the fluoridated hydrocarbons reduce the risk. Because of implications of the effect of the halocarbons on the ozone layer, restrictions are emerging. The effectiveness of ETO sterilization is influenced by four essential parameters:

- **gas concentration: 450mg to 1200 mg/l,**
- **temperature: 29°C to 65°C,**
- **humidity: 45% to 85%,**
- **exposure time: 2 to 5 hours.**

5) Liquid Peracetic Acid

This system uses a solution of peracetic acid which contains acetic acid and hydrogen peroxide. This solution is sporicidal at 0.02% in 2 minutes. Peracetic acid disrupts and denatures proteins. The extra oxygen rapidly inactivates many cell systems. All products are harmless to the environment and very safe for personnel. Peracetic acid remains effective with hard water and organic matter.

6) Hydrogen Peroxide Plasma Sterilization

Radio frequency emissions are applied to the hydrogen peroxide substrate. The electric field creates a gas plasma. A deep vacuum is generated to avoid using excessive heat and to facilitate maximum dispersion of the hydrogen peroxide vapor around the equipment. It does not produce any harmful substances: water and oxygen are the final products.

+ recommended, xxx Not recommended, ± depending on type

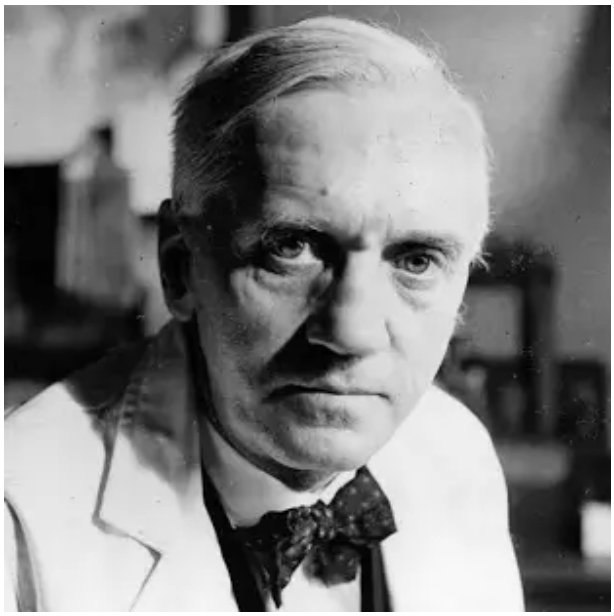
STERILIZATION: contact with vascular space and tissue	Disposable	Steam	ETO- Ethylene Oxide	Glutaraldhyde (10 hrs)	ClO_2 - Demand Release Chlor. (6 hrs)	H_2O_2 - Hydrogen Peroxide (6%)	Steris® - Peracetic acid	Sterrad® - Gas Plasma System
Surgical metallic instruments, smooth surface		+	+	+	+	+	+	+
Instrument with electric connections		xxx	+				xxx	+
Implantable devices		+	+	+	+	+	+	
Catheters, rubber tubing	+	+	+	-	+	+	xxx	xxx
Catheters, polyethylene tubing	+	+	+	+	+	+	xxx	xxx
Needle, IV lines	+	+	+	+	+	+	xxx	xxx
Dental instruments		+	+	+	+	+	+	+
Dental handpieces		+	+				+	+
Urethral catheter		+	+	xxx	+	+	+	xxx
Internal Scopes: Lensed instrument		xxx	+	+	+	+	+	±
Arthroscope, Culdoscope		xxx	+	+	+	+	+	±
Cystoscope, Peritoneoscope		xxx	+	+	+	+	+	±
Ureteroscope		xxx	+	+	+	+	+	±
All GI/UR rigid endoscopes		-	+	+	+	+	+	±
Endoscopic biopsy forceps		+	+	+	+	+	+	+
Cannulas, guidewires		+	+	+	+	+	+	+
Vaginal speculum (after rupture of membranes)		+	+	+	+	+	+	+
Material, gauze, swabs, linen	+	+		xxx	xxx	xxx	xxx	

INTERMEDIATE LEVEL DISINFECTION	
Ethyl alcohol	10 minutes
Isopropyl alcohol	10 minutes
Chlorine 1000 ppm	10 minutes
Phenolic germicidal solution	10 minutes
Iodophor germicidal solution	10 minutes
Cl1= 500ppm ClONa	10 minutes
Cl2=5000ppm	xxx

LOW LEVEL DISINFECTION	Non-critical	Contact With **Intact Skin**	Ethyl alcohol 70-90	ClONa 100 ppm	Phenolic	Iodophor	Quaternary NH4
Ethyl alcohol	≤ 10mn	Thermometer (single patient)	+				
Isopropyl alcohol	≤10mn	Bathtub					
Chlorine 100 ppm	≤10mn	Hydrotherapy tank		+			
Phenolic germicidal solution	≤10mn	Blood pressure cuff	+				
Iodophor germicidal solution		Earphones	+				
Quaternary germicidal	≤10mn	Ventilation bag	+				
		Furniture: Bedpan, bedrail Bathtub Examination table,countertops					
		Food utensils					

HIGH LEVEL DISINFECTION	
Glutaraldehyde (2%)	Unstable 45minutes
Demand relchlorine dioxide	Corrode 20 minutes
Hydrogen peroxide (6%)	Corrode 20 minutes
Wet pasteurization 75°C	30 minutes
Chlorine 1000 ppm	Corrode 20 minutes

Alexander Fleming



Alexander Fleming was a Scottish physician-scientist who was recognised for discovering penicillin. The simple discovery and use of the antibiotic agent has saved millions of lives, and earned Fleming – together with Howard Florey and Ernst Chain, who devised methods for the large-scale isolation and production of penicillin – the 1945 Nobel Prize in Physiology/Medicine.

On August 6, 1881, Alexander Fleming was born to Hugh Fleming and Grace Stirling Morton in Lochfield Farm, Scotland. Initially schooled in Scotland, Fleming eventually moved to London with three brothers and a sister, and completed his youth education at the Regent Street Polytechnic. He did not enter medical school immediately after; instead, he worked in a shipping office for four years. When his uncle John died, he willed equal shares of his estate to his siblings, nieces and nephews, and Fleming was able to use his share to pursue a medical education. In 1906, he graduated with distinction from St Mary's Medical School at London University.

Fleming did not intend to begin a career in research. While serving as a private in the London Scottish Regiment of the Territorial Army, he became a recognised marksman. Wishing to keep Fleming in St Mary's to join its rifle club, the club's captain convinced him to pursue a career in research rather than in surgery, as the latter choice would require him to leave the school. The captain introduced him to Sir Almroth Wright, a keen club member and a pioneer in immunology and vaccine research, who agreed to take Fleming under his wing. It was with this research group that Fleming stayed throughout his entire career.

When World War I broke out, Fleming served in the Army Medical Corps as a captain. During this time, he observed the death of many of his fellow soldiers, not always from wounds inflicted in battle, but from the ensuing infection that could not be controlled. The primary means to combat infection was antiseptics, which frequently did more harm than good. In an article he wrote during this time, Fleming discussed the presence of anaerobic bacteria in deep wounds, which proliferated despite antiseptics. Initially, his research was not accepted, but Fleming continued undaunted and in 1922, he discovered lysozyme, an enzyme with weak antibacterial properties. History tells us that, while infected with a cold, Fleming transferred some of his nasopharyngeal mucus onto a Petri dish. Not known for fastidious laboratory organisation, he placed the dish among the clutter at his desk and left it there, forgotten, for two weeks. In that time, numerous colonies of bacteria grew and proliferated. However, the area where the mucus had been inoculated remained clear. Upon further investigation, Fleming discovered the presence of a substance in the mucus that inhibited bacterial growth and he named it lysozyme. He also discovered lysozyme in tears, saliva, skin, hair and fingernails. He was soon able to isolate larger amounts of lysozyme from egg white, but in subsequent experiments found that this enzyme was effective against only a small number of non-harmful bacteria. Nevertheless, this would lay the groundwork for Fleming's next great discovery.

In 1928, Fleming began a series of experiments involving the common staphylococcal bacteria. An uncovered Petri dish sitting next to an open window became contaminated with mould spores. Fleming observed that the bacteria in proximity to the mould colonies were dying, as evidenced by the dissolving and clearing of the surrounding agar gel. He was able to isolate the mould and identified it as a member of the *Penicillium* genus. He found it to be effective against all Gram-positive pathogens, which are responsible for diseases such as scarlet fever, pneumonia, gonorrhoea, meningitis and diphtheria. He discerned that it was not the mould itself but some 'juice' it had produced that had killed the bacteria. He named the 'mould juice' penicillin. Later, he would say: "*When I woke up just after dawn on September 28, 1928, I certainly didn't plan to revolutionize all medicine by discovering the world's first antibiotic, or bacteria killer. But I suppose that was exactly what I did.*"

Although Fleming published the discovery of penicillin in the British Journal of Experimental Pathology in 1929, the scientific community greeted his work with little initial enthusiasm. Additionally, Fleming found it difficult to isolate this precious 'mould juice' in large quantities. It was not until 1940, just as he was contemplating retirement, that two scientists, Howard Florey and Ernst Chain, became interested in penicillin. In time, they were able to mass-produce it for use during World War II.

Fleming received many awards for his achievements. In 1928, he became Professor of Bacteriology at St Mary's. He was elected a Fellow of the Royal Society in 1943 and elevated to the level of Emeritus Professor of Bacteriology at the University of London in 1948. A recipient of some thirty honorary degrees, in 1945, he won the most prestigious award, the Nobel Prize in Physiology/Medicine. He was made a Knight Bachelor by King George VI in 1944 and a Knight Grand Cross of the Order of Alfonso X the Wise in 1948. Time Magazine named Fleming one of the 100 most important people of the 20th century.

On March 11, 1955, Alexander Fleming suddenly died of coronary thrombosis at home. He had been suffering from what he perceived to be gastric upset for some weeks. When his wife called their family physician regarding the onset of nausea on March 11, he reassured them that a house visit was not necessary. However, within minutes, he succumbed to the coronary event. His cremated ashes were placed in St Paul's Cathedral, and "*he died as he wished; quietly, without a gradual decline in physical or mental capacity, and even without inconveniencing his physician*".

Jokes

Wife: "How would you describe me?"

Husband: "ABCDEFGHIJK."

Wife: "What does that mean?"

Husband: "Adorable, beautiful, cute, delightful, elegant, fashionable, gorgeous, and hot."

Wife: "Aw, thank you, but what about IJK?"

Husband: "I'm just kidding!"

Teacher: "Kids, what does the chicken give you?"

Student: "Meat!"

Teacher: "Very good! Now what does the pig give you?"

Student: "Bacon!"

Teacher: "Great! And what does the fat cow give you?"

Student: "Homework!"

Is Google male or female?

A: Female, because it doesn't let you finish a sentence before making a suggestion.

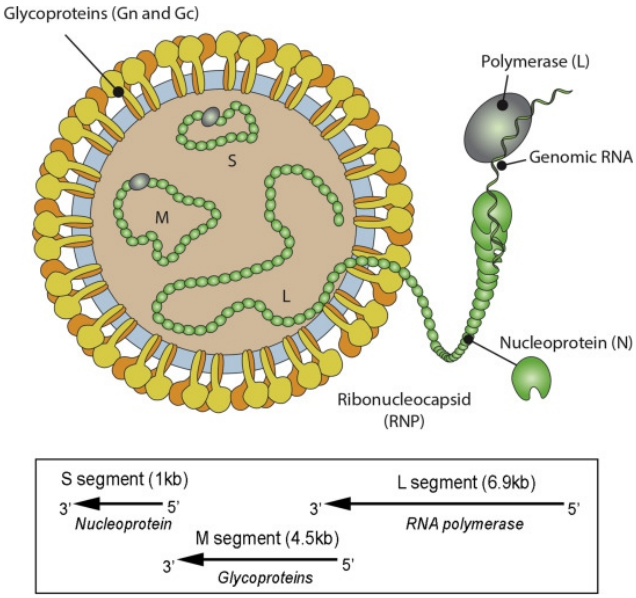
A science teacher tells his class, "Oxygen is a must for breathing and life. It was discovered in 1773." A blonde student responds, "Thank God I was born after 1773! Otherwise, I would have died without it."

Three friends stranded on a deserted island find a magic lamp. Inside it is a genie who agrees to grant each friend one wish.
"I want to go home," says the first friend. The genie grants her wish.
"I want to go home, too," says the second friend. And the genie sends her back home.
"I'm lonely," says the third friend. "I sure wish my friends were back here."

A photon walks into a hotel.
The desk clerk says, "Can we help you with your luggage?"
The photon says, "No, thanks. I'm traveling light."

The teacher asked little Johnny if he knew his numbers.
"Yes," he said. "My father taught me."
"Good. What comes after three?"
"Four," answered the boy.
"What comes after six?"
"Seven."
"Very good," said the teacher. "Your dad did a good job. And what comes after 10?"
"Jack."

Oropouche virus



Oropouche virus disease is caused by the Oropouche virus (OROV) that can cause fever, headache, joint pain, muscle pain, chills, nausea, vomiting and rash. Most people recover on their own, but the disease can cause severe symptoms in some patients. OROV is a segmented single-stranded RNA virus belonging to the family *Peribunyaviridae,* genus *Orthobunyavirus*, which was first identified in 1955 in Vega de Oropouche, Trinidad and Tobago.

The virus is transmitted to people through the bite of an infected insect, usually biting midges but also possibly by mosquitoes. It is thus referred to as an arthropod-borne virus (arbovirus). Prior to late 2023, reported cases of Oropouche virus disease were limited to South America, mostly near the Amazon rainforest, and the Caribbean. However, since December 2023, cases have been detected in other areas and have become more severe. In 2024, outbreaks have been documented outbreaks in nonendemic areas, two fatal cases with confirmed infection, and the possibility of mothers transmitting the disease to their babies while pregnant.

Oropouche virus disease was the second most common arboviral disease in South America (after dengue) before the emergence of chikungunya and Zika viruses in 2013 and 2015. Prior to late 2023, Oropouche virus disease was reported in Brazil, Bolivia, Colombia, Ecuador, Haiti, Panama, Peru, Trinidad and Tobago, French Guiana and Venezuela; most cases were reported near the Amazon rainforest area. However, since December 2023, there has been an increase in the number of cases reported, including in areas where transmission had not been previously documented.

In 2024, locally transmitted Oropouche virus disease was reported in seven countries in Latin America and the Caribbean: Brazil, Bolivia, Colombia, Cuba, Guyana, Peru and the Dominican Republic. Additionally, Oropouche virus disease cases were reported among travellers returning from countries with local transmission to the United States, Canada, Spain, Italy and Germany.

The Oropouche virus is primarily transmitted to humans through the bite of *Culicoides paraensis* midges. *Culex quinquefasciatus, Coquillettidia venezuelensis* and *Aedes serratus* mosquitoes can also act as possible vectors. The virus is believed to circulate in both a sylvatic cycle in forested areas, and in an urban epidemic cycle between insects and people. In the sylvatic cycle, non-human primates, sloths and perhaps birds serve as vertebrate hosts, although a definitive arthropod vector has not been identified.

Further studies are underway to better understand the insect vectors and transmission cycles in the current outbreaks. Previously, there had been no confirmed reports of human-to-human transmission. However, there were reports in Brazil in 2024 of possible fetal infection with Oropouche virus, transmitted from mothers infected during pregnancy. The incubation period (the time from the bite of an infected insect to first symptoms) of the Oropouche virus is typically 3 to 10 days. Symptoms of disease include fever, headache, joint pain (arthralgia), muscle pain (myalgia), chills, nausea, vomiting and rash.

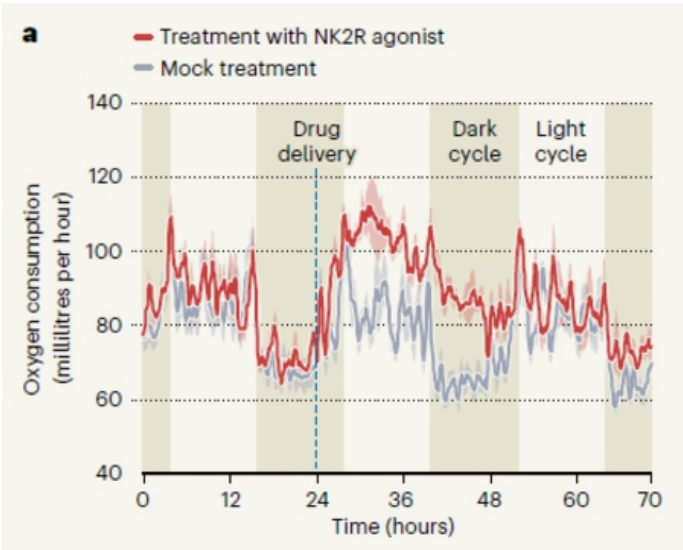
Most cases recover completely within 7 days after the onset of symptoms. However, recovery can take weeks in some patients, and severe complications like aseptic meningitis may occasionally occur. Though deaths from OROV infection were not previously described, in 2024 there were two reports of deaths in previously healthy young adults with Oropouche virus infection. Given the similar clinical presentation to other arboviruses like dengue and chikungunya, Oropouche virus disease is often unrecognized or misdiagnosed.

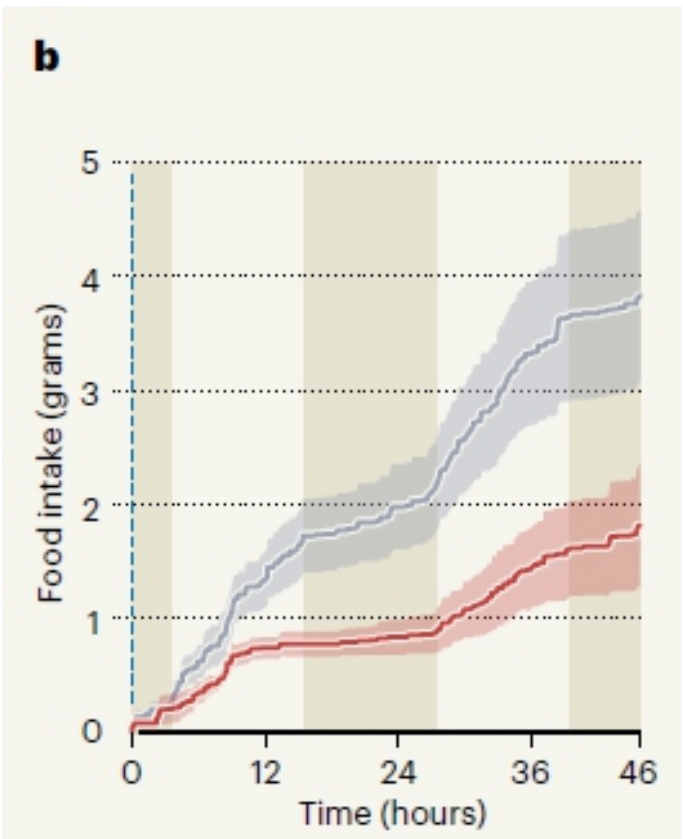
Diagnosis of Oropouche virus disease is made by reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR *(9)*. Serologic assays can be used to aid diagnosis; however, they should be conducted by highly trained personnel and in laboratories equipped with appropriate containment facilities. There are no available commercial diagnostic or rapid tests based on antigens or immunoassays (e.g. ELISA, immunochromatography) available.

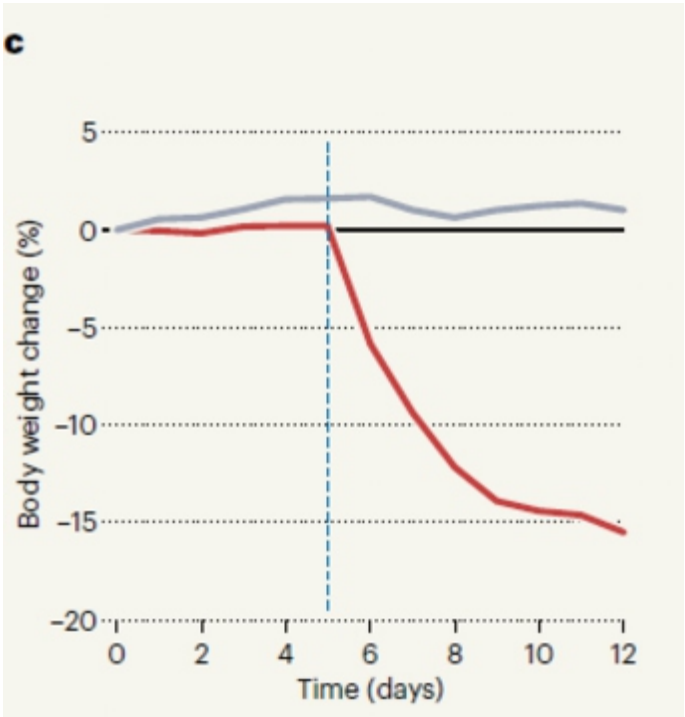
There is no specific treatment available for Oropouche virus disease. Treatment is primarily supportive and focuses on relieving symptoms. The understanding of complications from Oropouche virus disease is limited. Occasionally, aseptic meningitis may occur. Recently, there were reports from Brazil describing five cases of possible Oropouche virus transmission during pregnancy (four stillbirth and one spontaneous miscarriage) as well as four cases of newborns with microcephaly detected via retrospective investigations. Despite the detection of viral RNA by reverse transcription polymerase chain reaction (RT-PCR) testing of fetal tissues, it cannot be concluded that OROV infection was the cause of fetal deaths, and investigations are still ongoing.

There is no vaccine available to prevent Oropouche virus disease. Vector control and personal protective measures are key in reducing the spread of the virus. Standard bed nets are less effective against the biting midge, as these insects are small and can pass through the netting. In contrast, fine mesh bed nets and chemical insecticides used as residual spray on internal and external walls of infested premises have been shown to be effective. Personal protective measures, such as wearing protective clothing and using insect repellents containing DEET, IR3535 or icaridin, are recommended to minimize the risk of infection.

Two for one: candidate obesity drug boosts energy use and curbs calorie intake







Body weight is determined by the balance between energy intake and energy expenditure. Although energy intake is solely the result of the food that we eat, energy expenditure is a combination of various factors, including physical activity and the basal metabolic rate necessary to maintain vital physiological functions. Most drugs currently used to treat obesity aim to decrease appetite, but increasing basal energy expenditure at the same time to create a calorie deficit has remained a long-lasting quest. In the past few years, obesity- treatment approaches have successfully intervened on both sides of the energy-balance equation by concomitantly targeting different receptors, but attempts to target a single receptor with one drug have so far failed. A single receptor as a pioneering therapeutic target that not only suppresses appetite but also enhances energy expenditure in animal models. By analysing human genetic data, the authors uncovered a striking correlation between levels of haemoglobin bonded to glucose — a measure of blood glucose and a clinical indicator of type 2 diabetes — and variations in the gene that encodes the neurokinin 2 receptor (NK2R). NK2R is expressed throughout the body, but is known for its role in the central nervous system and gastrointestinal tract. To assess the potential of targeting NK2R for the treatment of obesity, the researchers first administered the natural ligand for NK2R, neurokinin A, to obese mice. This led to substantial weight loss through decreased food intake, and, remarkably, increased energy expenditure. Building on this proof-of-concept work that describes the dual role of NK2R as a regulator of appetite and energy expenditure, the authors engineered an improved activator (agonist) of NK2R with two key features: an extended half-life in the body so that the activator remains in the blood for longer and only one dose is

needed per day (or potentially per week), and an increased selectivity for NK2R to avoid it binding to other receptors and circumvent off-target effects. When given to obese mice, a single dose of this NK2R-specific long-acting agonist strongly increased energy expenditure and decreased food intake, causing substantial weight loss. Repeated daily administration further reduced fat mass, resulting in a 10–15% weight loss, commensurate with established obesity medications such as semaglutide (marketed as Ozempic and Wegovy), which targets the receptor for the appetite-regulating hormone GLP-1. This insight into the central role of NK2R in regulating energy balance holds transformative potential for obesitytherapeutics. To bridge the translational gap between pre-clinical research and human applications, the authors next tested the efficacy of their candidate drug in obese and diabetic rhesus macaques (*Macaca mulatta*). Consistent with the weight loss seen in rodents, the treatment lowered food intake and body weight in these primates. Although energy expenditure was not measured, the weight loss triggered by NK2R agonism in obese primates emphasizes its translational potential. The drug also counteracts several secondary medical conditions associated with metabolic disorders. In both macaques and mice, NK2R agonism greatly mitigated high blood glucose and resistance to the hormone insulin — the main hallmarks of type 2 diabetes. Similarly, treatment reduced high levels of lipids such as cholesterol and triglycerides in the blood, a condition that is associated with obesity. These findings could pave the way for long awaited new strategies for metabolic therapies, but they also have limitations. The drug led to only around 4% weight loss in primates after 8 weeks of treatment, which is noticeably less than that achieved with the latest generation of obesity drugs. Nevertheless, NK2R activation might show promise as part of a medication that targets multiple pathways. A successful example of this strategy is the recently marketed tirzepatide (sold as Mounjaro), which targets receptors for both GLP-1 and another gut hormone called GIP, and resulted in participants in clinical trials losing around 20% of body weight5. Obesity drugs in clinical use often have unwanted side effects for the gastrointestinal system. Sass and colleagues' NK2R agonist might not be exempt from this issue. Given the role of NKR2 in gut motility6, the reports of loose stools in both mice and macaques at the highest dose will prompt further investigations. Although the authors did not report vomiting and nausea in primates, the drug was seen to activate the area postrema, the brain's vomiting centre. This also warrants further study. How might NK2R activation improve metabolic health? The authors compared the outcomes of drug administration to the brain (central) with that to the whole body (peripheral). They found that the reduction in feeding behaviour is centrally evoked, whereas the increase in energy expenditure is peripherally driven. Both routes led to weight loss, but only peripheral administration of the drug reduced blood glucose levels, raising questions about the organ-specific and cell-specific mechanisms that underlie its therapeutic effects. Receptors from the NKR family, including NK2R, are involved in mood and stress regulation7. Perhaps unsurprisingly, Sass and colleagues saw activation of brain regions associated with these processes. Conducting thorough behavioural studies should resolve whether the observed metabolic improvements are accompanied by adverse psychological effects. In any case, the beneficial effects of the drug on the periphery suggests that targeting peripheral NK2R, but not NK2R in the central nervous system, could be an effective strategy for treating metabolic diseases. The discovery of a single-receptor-targeting agonist that curbs appetite, stimulates energy expenditure and counteracts metabolic disorders could have considerable implications for human health. Because basal energy expenditure markedly declines as an individual ages, and this decline is particularly prominent after weight loss, owing to loss of lean mass, finding long-term and durable obesity treatments is challenging. Crucially, NK2R-targeting drugs could help to treat the subset of people with obesity whose food intake is low but who also have a low metabolic rate. Harnessing the dual benefits of this therapeutic option, assuming that metabolic improvements could be achieved safely, should open new avenues for obesity and type 2 diabetes treatment.

10 simple daily habits to build for a happy life

1) Meditation

Meditation has endless benefits. It helps reduce stress, improves overall emotional health, increases attention span, and helps sleep better. All these things directly correlate with a happier life. There are different ways to meditate (breath meditation, vipassana, transcendental meditation, mantra meditation, zen, etc).

How to inculcate the meditation habit:

- Sit comfortably and start with a few deep breaths. Then let your breathing become normal and observe the sensations of each breath.
- Start small – maybe with 10-15 min of meditation.
- Fix a time and place for meditation.

2) Workout

Workout not only helps raise your good cholesterol and lowers blood pressure, but it also helps fight stress, depression, and anxiety. All you need is at least 30 minutes of exercise, 3 to 5 times per week.

How to include exercise in your life:

- Choose to walk or cycle if possible. Take staircase instead of elevators
- Make workout as sacrosanct as a doctor's appointment – make exercising your number one priority. Nothing else should come in the way.
- Go for a 10-15 mins walk after dinner.
- Try different types of workouts to see what interests you and suits your body.
- Incorporate physical activities, e.g., take up classes for tennis, badminton, skating, dancing, or anything else that you enjoy.

3) Journaling

Journaling is the most underrated and yet, the simplest **way to bring joy into your life.** It allows you to develop clarity about your thoughts, identity patterns in your behaviour and then, take corrective steps. Journaling lifts your mood and almost always makes you feel better.

The most popular types of journaling techniques are:

- **Stream of consciousness**, where you allow your thoughts to flow on a paper – have a conversation with yourself about whatever is on your mind
- **Gratitude journaling**
- **Unsent letters** (to get closure to certain events)
- Journaling **with prompts**

How to start journaling for mental health:

- Pick a journaling technique that works for you.
- Schedule 10 mins into your calendar for journaling.

4) Break smartphone addictions

While smartphones make our lives easy, using them too much is as bad as compulsive gambling and drug abuse. It leads to anxiety, depression, behavioural issues, fear of missing out, and disturbed sleep patterns – all of which snatch away your happiness.

How to break phone addiction:

- Uninstall all the unnecessary apps. For example, shopping sites, games, or even social media.
- Whenever you feel the urge to pick up your phone, ask yourself if you *really* need to.
- Do not take your phone to the dining table or to your bedroom
- Turn off the notifications for all apps, as far as possible.
- Focus on pursuing hobbies that don't need a phone.

5) Do focused work every day – experience flow

Deep work enhances the sense of well-being by giving us a sense of progress, improving our comprehension, and boosting our productivity manifold.

Hence, recommend doing 1-2 hrs of deep work every day.

How to begin doing focused work every day:

- Decide on the time slot for each day one day in advance. If possible, set the schedule for the whole week.
- For each session, set achievable goals. Think through them in advance
- Before you start your focus session, allow your mind to calm down – you could meditate for 5 minutes if you like
- Switch on a timer for between 30 to 50 minutes and start your deep work.
- During the deep work session, do not switch your attention to any other task.
- After the session, take a 10-minute break – you have earned it.

6) Fix your sleep

You need between 7-9 hrs of sleep (eight hours on average) to combat stress, anxiety, snoring, and a fall in productivity.

How to start fixing your sleep:

- Reduce your screen time, especially in the evening. Avoid using gadgets at night, at least 1-2 hrs prior to sleep. Instead, read a book or spend time with family.
- Dim the brightness and blue light element in your screen by using Flux.
- Cut down on your caffeine intake, especially in the evening. Anyone who finds it difficult to fall asleep should avoid coffee post-lunch.
- Meditate for 10 minutes before sleeping. You can also write an evening journal.
- Set a time to go to bed every day and follow it.

7) Waking up early

Research has shown that people who wake up an hour earlier than usual, while sleeping adequately, have lower risks of major depression.

In fact, there are studies suggesting that waking up early can make you happy and healthier.

How you can start waking up earlier:

- To wake up earlier, start sleeping earlier so that you get ample sleep – there is no way around that.
- Open your curtains and allow maximum light exposure in the morning. It helps reduce the formation of melatonin (the sleep-inducing hormone).

Do the opposite at night, ensure everything is dark to stimulate the production of melatonin.

- Stick to your schedule and avoid using gadgets in bed.
- Avoid eating late at night or drinking caffeine/alcohol.
- Stop snoozing your alarm. Keep your clock far away for you to get up from bed in the morning.

8) Eating non-processed food

Did you know that eating highly processed food can result in chronic health problems like diabetes, obesity, cancer, and fatty liver etc. On the other hand, those who consume fruits and vegetables experience better moods.

How to start cutting processed food:

- Say goodbye to packaged juices, sodas, etc., and start drinking more water. One way to make it happen is to take a lot of veggies and fruits.
- Cut down on sugar intake in all forms.
- Pick whole grains over processed ones.
- Keep healthy snacks to munch on (e.g., nuts, carrots, and fruits).

9) Reading books for 30 min each day

If you read just 30 min every day, in two weeks, you can finish a 300-page book. Reading books is like a software upgrade for your brain. Also, reading is enriching and soothing like a walk or having a warm cup of tea.

How to include reading in your life:

Immersing yourself in a much-loved book is one of the best habits for a happier life.

- Start replacing your screen time with a book you like
- Schedule your reading time every day
- Use your idle time to read. You can make it a habit to read before going to bed.

10) Spend time on hobbies or learning something new

We often get so caught up in work and day-to-day life that we forget about having fun. And a great way to have fun is to pursue hobbies or learn something new. The important thing is to spend time doing things you find enjoyable for their own sake.

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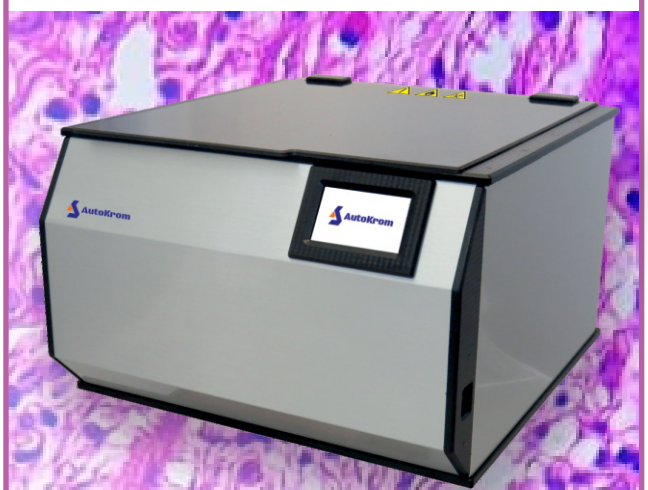

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