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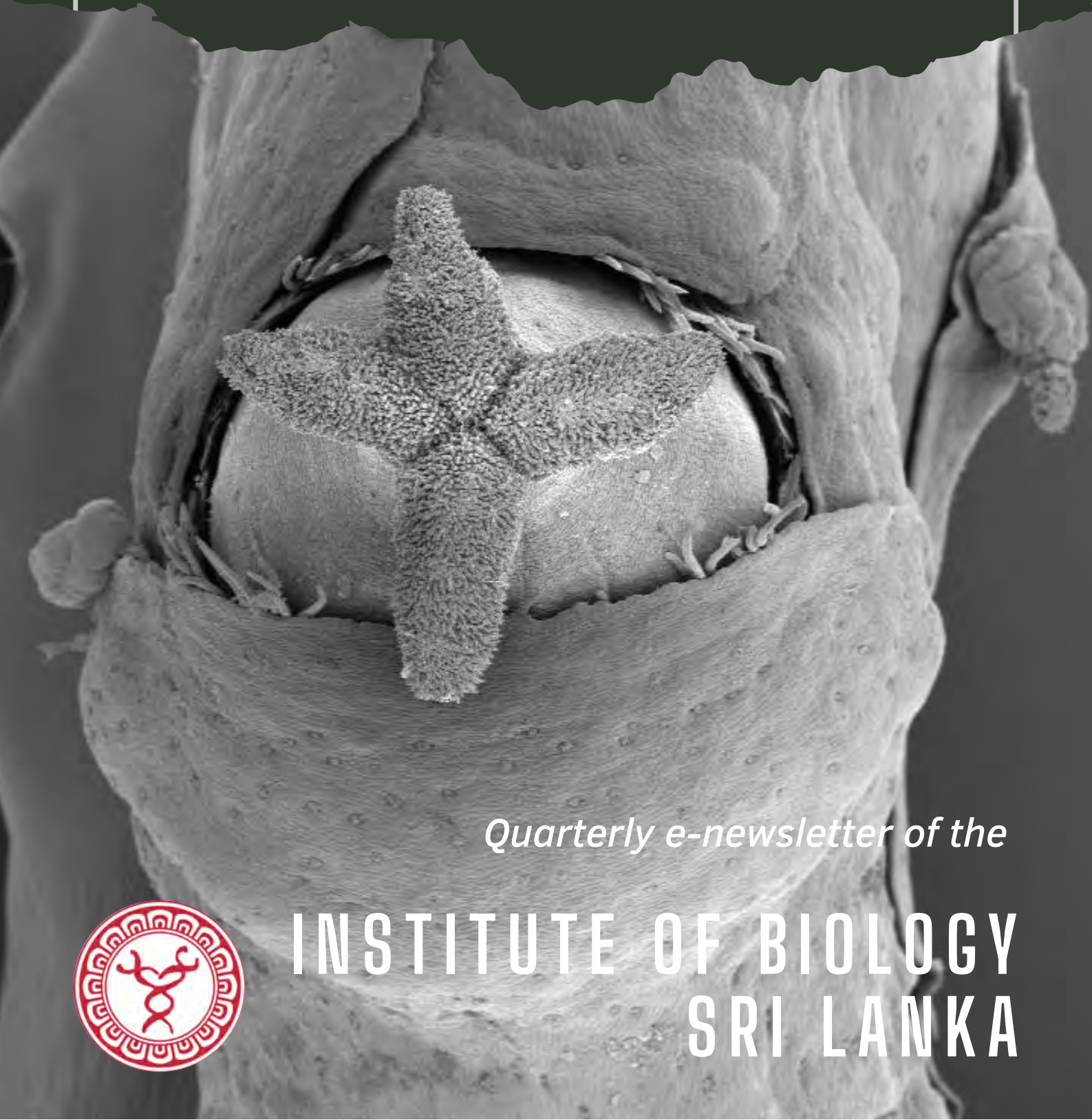
E-NEWSLETTER



Quarterly e-newsletter of the

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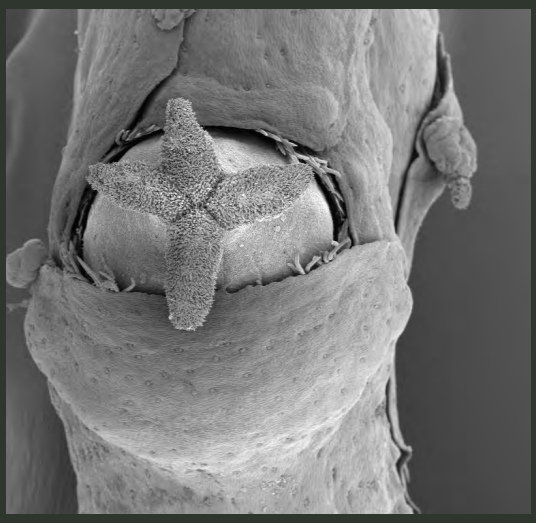

COVER STORY

The floral morphology of black pepper (*Piper nigrum*) observed by scanning electron microscopy

Scanning electron microscopy (SEM) is a useful method that allows the examination of the topology or three-dimensional appearance of floral and inflorescence structures. A scanning electron micrograph of a female flower of *P. nigrum* is presented here. A solitary ovary with the four-lobed stigma subtended by a copular bract can be observed. The flower is without a perianth.

Photograph by Ms. Nilni Wimalarathna
Department of Plant Sciences, Faculty of Science
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Sri Lanka

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PRESIDENT'S MESSAGE

Dear Colleagues,

It indeed is a great pleasure for me to issue this message as the President of the Institute of Biology, Sri Lanka (IOBSL), the premier professional organization for biologists in Sri Lanka.

As we go through a crucial period in the post-independence history of Sri Lanka, we as biologists do have an important role to play for the betterment of our people and our nation. The membership of the IOBSL comprises a highly competent group of scientists who are well-qualified in their respective sub-disciplines in biology. Each one of these sub-disciplines under the main 'Biology umbrella' can contribute greatly to combat and overcome some of the main crises of the nation at the present time. The vast and diverse field of biology can greatly contribute to relieve the pains of food crisis, production of essential drugs and even energy crisis to name a few. Keeping in parallel with the current situation of the country, the IOBSL declared '*Biological Wealth for Economic Prosperity*' as the theme for the year 2022-2023.

A series of activities and programmes on the selected theme have been planned by the IOBSL and we expect the fullest cooperation and active contribution of our membership in these activities. The target groups of the activities are also diverse from school children, undergraduates, scientists, entrepreneurs and the general public at large. The thematic publication of the year will also be focused on the same theme and we invite the membership to respond positively by contributing chapters which would be useful for the readership.

This e-newsletter will keep the membership and the general public updated on the events of IOBSL in addition to providing useful information on current biological issues and related research. Further, the newsletter is intended to strengthen and maintain the bonds between the IOBSL and the interested parties. While wishing all our stakeholders a fruitful and productive year, I would like to extend a warm invitation for you to forward your ideas to us and to stay connected with the IOBSL.

Thank you.

Prof. S. A. Chandrika N. Perera
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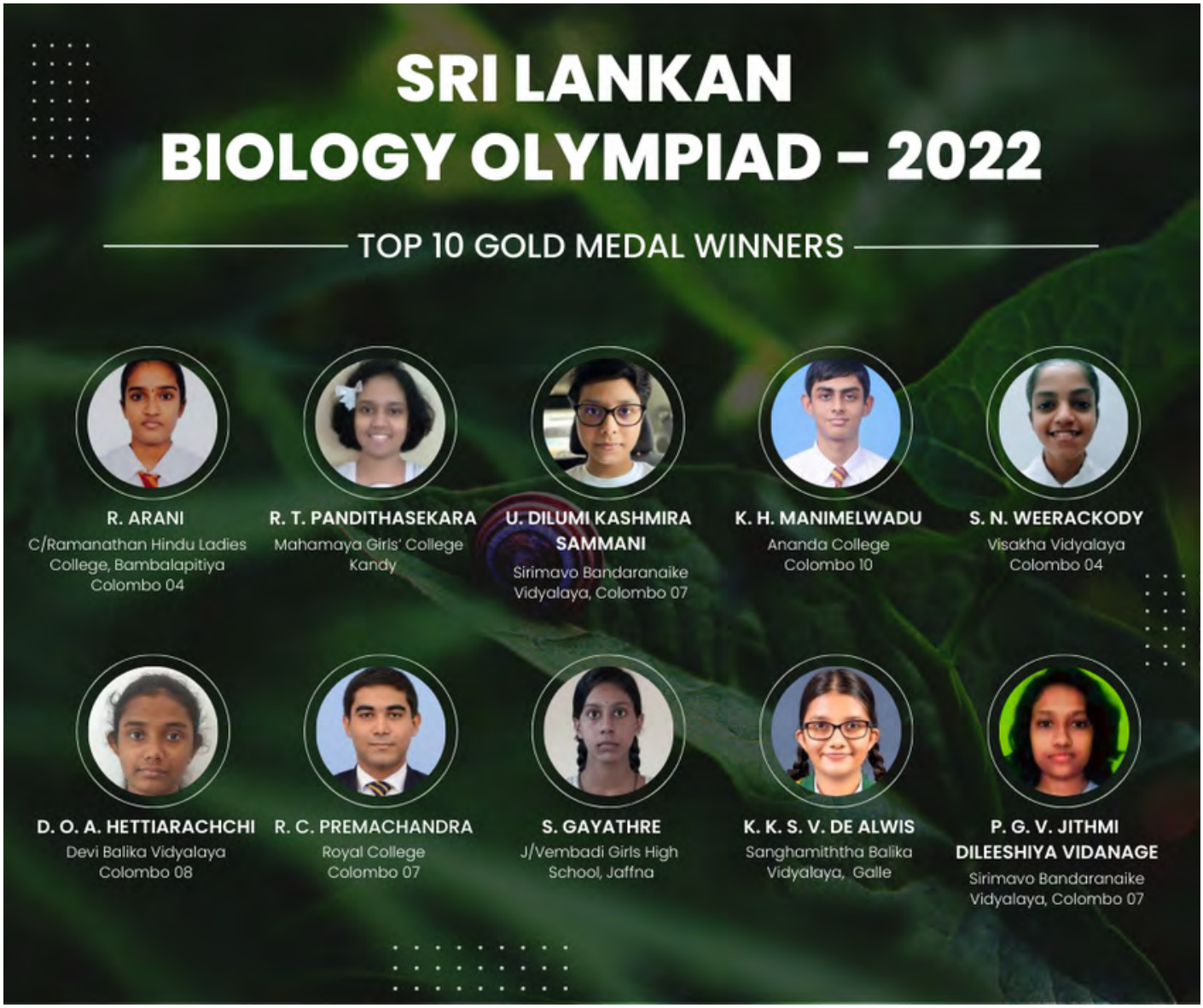
IOBSL NEWS AND EVENTS



The Sri Lankan Biology Olympiad 2022 examination was held on 11th December 2022 as an online examination. Twenty medal winners will participate in the second round of the International Biology Olympiad (IBO) selection training program. The 34th IBO will be held from 3rd July to 11th July 2023, in Al Ain, United Arab Emirates.

For more information, please visit http://www.iobsl.org/

Our heartiest congratulations to all medal winners!

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Institute of Biology Sri Lanka

UPCOMING EVENTS 2023

INTER-UNIVERSITY BIOLOGY QUIZ COMPETITION

E-PHOTOGRAPHY COMPETITION

INTER-UNIVERSITY BIOLOGY CHALLENGE

YOUNG SCIENTIST AWARD

ENTREPRENEURIAL TRAINING PROGRAMS/ WORKSHOPS

IOBSL ORATION

THEMATIC PUBLICATION

ANNUAL SESSIONS

COURSES WORKSHOPS WEBINARS

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Molecular Chaperone ClpB Becomes a Novel Antimicrobial Target

Introduction

Proteins are the most versatile and complicated biological macromolecules. Protein aggregation is defined as a non-physiological association of misfolded/partially folded polypeptides. Proteostasis prevents or minimizes protein aggregation and keeps proteins soluble and active. In live cells, molecular chaperones and their regulators facilitate protein quality control and proteostasis. A molecular chaperone is a protein that helps other proteins reach their physiologically active native conformation without being present in the client protein's final functional structure. These proteins help *de novo* protein folding and refolding of misfolded/partially folded proteins under cellular stresses and thereby inhibit protein aggregation.

Evolution and biological functions of molecular chaperones

It is interesting to note that certain types of cells have developed a particular type of molecular chaperone with the unique capacity to disassemble protein aggregates and transform them into unstructured polypeptides (a reversal of protein aggregation).

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Generally, nascent polypeptides fold to become functionally active (native state) proteins as they emerge from ribosomes. However, some polypeptides may misfold during this process leading to immediate protein aggregation. Under stress, specific proteins might misfold and lose their native structure. Cells have molecular chaperones to help polypeptides/proteins fold into their native state or guide misfolded polypeptides/proteins back into the functionally active native structure.

Cells produce chaperones GroEL/Hsp 60, DnaK/Hsp 70, DnaJ/Hsp 40, and GrpE/nucleotide exchange factor (NEF) for this purpose. Once protein aggregates are formed, they might concentrate in the cytoplasm and form inclusion bodies. Inefficient aggregate clearance can build up enormous clumps of proteins inside cells, leading to protein toxicity or cell death (Figure 1).

In bacteria, molecular chaperones are referred to as Caseinolytic peptidase B (ClpB), whereas yeast and plants are referred to as heat-shock protein (Hsp)104 and Hsp101, respectively. On the other hand, metazoans do not possess ClpB.

Molecular chaperon ClpB is a member of the Hsp100 family, and the proteins that belong to Hsp100 are members of the AAA+ superfamily. AAA+ superfamily proteins are associated with diverse cellular processes. Therefore, to perform their cellular operations, they need the energy provided by the hydrolysis of ATP. ClpB plays a vital role in bacterial protein homeostasis by reactivating aggregated proteins, which is essential for maintaining optimal cellular functions in a cellular environment. The proper balance of protein homeostasis is critical for maintaining optimal cellular processes.

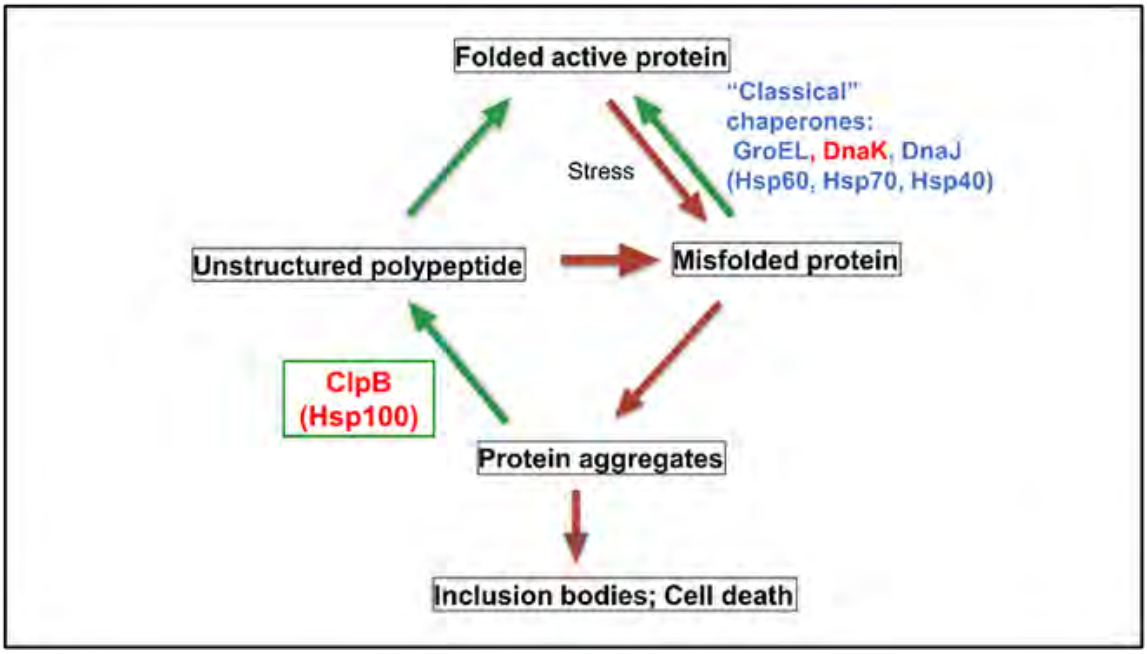

Figure 1 Folding, misfolding, and aggregation of proteins in a cellular milieu
Source: modified from Ranaweera (2021)

Proposed mechanism of the ClpB mediated protein disaggregation

ClpB-mediated protein aggregation reactivation and breakdown are associated with two other molecular chaperones, DnaK and DnaJ, and a NEF/GrpE that works in cycles. However, the exact ClpB-mediated protein aggregate reactivation and breakdown mechanism is still a mystery. Biologically active bacterial ClpB is a 575 kDa hexamer. In the presence of nucleotides (ATP or ADP), six ClpB monomers (each 95 kDa) combine into an active hexametric ClpB unit. The self-associating monomers form a narrow channel at the center of the hexamer. ClpB hydrolyses ATP to release polypeptides from aggregates. ClpB disassociates protein aggregates by threading either polypeptide ends (N or C terminus) or exposed loops of polypeptides through its central channel via ATP hydrolysis. DnaK is needed for ClpB to recognize protein aggregates and target them within a cell. ClpB can pull the peptide by both arms of the loop or switch to a single-arm translocation if resistance is encountered when extracting a polypeptide loop.

As the extracted polypeptide exits ClpB's central channel, the substrate can refold on its own or be transferred to other co-chaperones (Dnak, DnaJ-GrpE or GroEL-GroES). If peptide extraction from aggregates is unfeasible, a ClpB hexamer dissociates into its monomers and reassembles to engage a new loop or terminal. Multiple ClpB machines can work simultaneously on the same aggregate making aggregate breakdown fast and efficient (Figure 2).

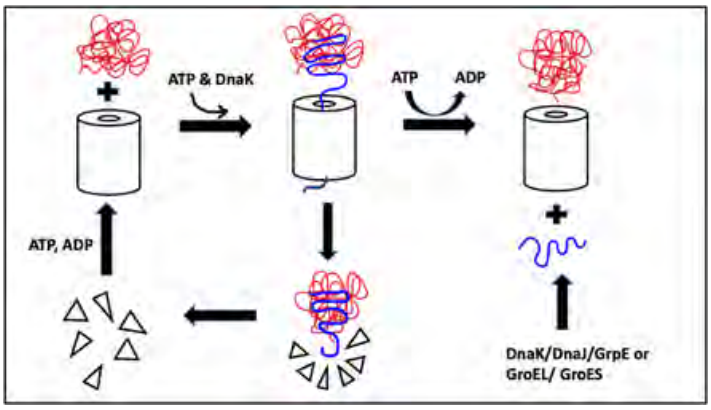

Figure 2 Proposed ClpB disaggregation mechanism. Six monomers are shown as triangles. Hexameric ClpB is shown as a cylinder with a central channel. Aggregated polypeptides are shown in red, and the extracted polypeptide is shown in blue.
Source: modified from Zolkiewski et al. (2012)

Therapeutic potential of ClpB as a novel antimicrobial target

To be considered as antimicrobial targets, targets must be essential for microbe life, have no analogous targets in humans, and be druggable *in vitro* and *in vivo* with small compounds. Few research groups have shown that the host-pathogen stress response mechanism can be a unique antibacterial target for medication development. Hsp100 chaperones are required for invasiveness and/or in-host survival of multiple significant bacterial and protozoan pathogens, including the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species).

Moreover, bacterial heat shock response has emerged as a distinct target for antibiotic development. Pathogens endure heat shock and oxidative stress during infection, and their survival depends on molecular responses. The pathogen stress response is key to generating new antimicrobials.

No successful suppression of pathogen stress-response machinery has been developed due to the highly conserved nature of the Hsp sequence across many domains of life.

Loss of ClpB activity is deleterious to the survival of many pathogens, and no mammalian ClpB orthologs are known. Inhibiting Hsp100 may lower the infectivity and survival of therapeutically important pathogens without harming the harboring host. So far, no Hsp100-selective high-affinity inhibitors exist. Therefore, AAA+ ATPase inhibitors unrelated to Hsp100 could be exploited to generate Hsp100-selective ligands.

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Nutmeg: A Spice Worth to be in the Limelight

Figure 1 Nutmeg mace and kernel
Photo credit: Thiranya Wanigarathna, Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka

The story of nutmeg begins in the Banda Islands of Indonesia, where nutmeg (*Myristica fragrans* Houtt.) grows as a native perennial tree. Nutmeg was traded by the Banda Islands' natives, the Orangkaya and then by their colonizers, the Portuguese, followed by the Dutch and British. Nutmeg derives from the Latin term *nux muscatus*, which means 'musky nut', attributing to its characteristic aroma. In no time, nutmeg established itself as a major spice all over the world, influencing many ethnobotanical uses, especially in local cuisines.

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Nutmeg was first introduced to Sri Lanka in the 19th century, and gradually, the crop has gained popularity as a minor export crop. In the local dialect, nutmeg is commonly referred to as '*Sadikka*' in Sinhalese and '*Jaathikai*' in Tamil. The economically important plant parts of nutmeg are the kernel (seed) and the red-coloured aril, commonly known as the mace (Figure 1). Both mace and kernel are used in their dried forms as spices in curries, confectioneries, bakery products and beverages. It is also a commercial source of essential oil and butter and is widely used in the pharmaceutical and cosmetic industries, ayurvedic/Chinese medicine, and aromatherapy.

Today, with a market dealing of 2,451 mt production (mace and kernel) and an export value of LKR 5,379 million in 2021, nutmeg is a thriving revenue-generating crop in the Sri Lankan agricultural sector. With our current production status, Sri Lanka only caters to around 5% of the global demand for nutmeg, exporting mainly to India, UAE, the USA, Germany, and Pakistan.

However, driven by the intricate chemical composition and flavour-base acquired due to the unique climate, Ceylon nutmeg carries a great export market potential. Currently, nutmeg is exported to global markets as whole kernel and mace, ground powder of kernel and mace, and nutmeg essential oil.

Nutmeg is a dominant tree species in the Kandyan home gardens (Figure 2) in Matale, Kandy and Kegalle districts, with a cultivation extent of nearly 2,788 ha, mainly owned by small and mid-scale farmers. Kandy, home to more than 80-85% of the plants in cultivation is the leading nutmeg producing district. Nutmeg is a popular choice for a perennial tree crop in these home gardens giving an additional household income with minimal agronomic management and inputs.

For an introduced crop, nutmeg germplasm in Sri Lanka harbors a great diversity captured across tree architecture, leaves, fruits, mace, kernel, and flowers.

Figure 2 Nutmeg tree and fruits

Photo credit: Thiranya Wanigarathna, Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka

A key issue in nutmeg cultivation globally is the difficulty in determining the sex of a plant (male: a plant carrying only male flowers, female: a plant carrying only female flowers or monoecious: a plant carrying both male and female flowers) prior to attaining the flowering age (Figure 3). While many have researched this aspect, no definitive method to determine the sex at an immature stage is available. As a result, the farmers undergo an opportunity cost as they maintain trees without knowing the exact sex of the plant for nearly 5 to 8 years until it reaches the reproductive stage. At that age, even though the recommendation is to maintain a 1:10 sex ratio (male: female), farmers are reluctant to cut down trees for which they have cared for years. Therefore, knowing the sex of the seedling planted is very beneficial for nutmeg growers and it remains the most pressing issue concerning the crop. In addition, from an ecological standpoint, one of the major concerns with nutmeg cultivation is the loss of undergrowth as a result of the well-spread, thick canopy of nutmeg trees. This has led to the depletion of plant diversity in many of the Kandyan home gardens where nutmeg is grown widely. Further, it is also a concern for the farmers given they are unable to practice intercropping with nutmeg and hence, they tend to choose alternatives.

Currently, a multidisciplinary research project is underway, funded by the University of Peradeniya to assess the genetic diversity and biochemical profile of Sri Lankan nutmeg and to determine the sex of nutmeg plants at an immature stage using molecular, morphological, and biochemical means. In brief, the genetic diversity assessment is carried out at the morphological level by sampling nutmeg plants representing Kandy, Matale and Kegalle districts and observing their morphological characteristics. DNA markers will be used to assess the allele diversity at the genome level. Moreover, analysis of essential oil and antioxidant content in nutmeg will be performed via gas chromatography-mass spectrometry (GC-MS) and chemical assays, respectively. The sex determination of nutmeg will be investigated by observing differences in leaf morphology and profiling biochemicals in leaf extracts representing the different sexes. Further, DNA markers are assayed to identify potential target loci on the genome to differentiate the sexes of nutmeg at the molecular level. Our research team is comprised of scientists from the University of Peradeniya, South Eastern University, Rajarata University of Sri Lanka, Department of Export Agriculture, and Food Research Unit, Gannoruwa.

Figure 3 Nutmeg flowers
Photo credit: Dr. Kanishka Ukuwela, Faculty of Applied Sciences, Rajarata University of Sri Lanka

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Investigating the Wound Healing Potential of Sri Lankan Medicinal Plants

Wounds and Wound Healing

Chronic wounds have become a major global public health issue, and their severity has been exacerbated by the rise in diabetes, metabolic syndrome, obesity, and aging. Wound healing refers to the complex and multifactorial process in response to a disruption of the normal anatomical structure and function of skin tissue. It is primarily composed of a series of overlapping processes namely inflammatory reaction, proliferation, and remodelling. The inflammatory phase involves vascular responses characterized by exudation, blood coagulation, and hemostasis. During this process, varieties of immune cells from the blood vessels are attracted to the wound lesion and secrete pro-inflammatory cytokines. During the proliferative phase, there is a formation of the epithelium to cover the wound surface with concomitant growth of granulation tissue to fill the space of the wound. In this phase, the existing cells and newly formed cells migrate to the surface of the wound. Management of wounds with western drugs is expensive and involves many side effects, such as allergic reactions and drug resistance. Hence, it is important to investigate cost-effective alternatives with fewer side effects.

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Medicinal plants and wound healing

From ancient times, plants have been extensively used for the treatment and management of wounds. Plant natural products have anti-inflammatory, antioxidant, antibacterial, and pro-collagen synthesis properties that synergistically facilitate the wound healing process. The medicinal properties might be contributed by the bioactive phytochemical constituents of the various chemical families, such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins, and phenolic compounds. Each bioactive agent may have a specific functional role in wound healing properties.

In Sri Lanka, nearly 145 plant species are frequently used for the treatment of various types of wounds. Medicinal plants such as *Ficus racemose* (Attikka), *Cryptolepis buchananii* (Wal ruk attana), *Glycyrrhiza glabra* (Wel mee), *Ageratum conyzoides* (Hulanthala), *Vernonia zeylanica* (*Jeffreycia zeylanica*) (Pupula), *Holoptelea integrifolia* (Godakirilla), *Argemone Mexicana* (Dumukeiya or Rankiri gokatu), *Ziziphus oenoplia* (Heen Eraminiya) and *Leea indica* (Burulla) have found to be effective in the treatment of wounds. The leaves and bark of these plants, in particular, have been used for the treatment of wounds. However, these traditional beliefs have not been scientifically validated. Hence, our study aimed to validate the wound healing activities of different plant parts using *in vitro* wound healing assays.

Wound healing assays

Traditionally, wound healing activity is investigated using both *in vitro* and animal models. The Scratch Wound Assay (SWA) has been established as a simple and reliable *in vitro* wound healing assay. This simple and low-cost tool is used for obtaining preliminary insights into how plant preparations or their secondary metabolites can enhance the formation of new tissue by unidirectional cell migration. During this SWA, the scratched cell monolayer responds to the disturbance of cell-cell contacts by increasing the concentration of growth factors and cytokines at the wound edge. The wound healing activity of plant extracts was expressed in terms of mean percentage wound closure within 24 h of the incubation period.

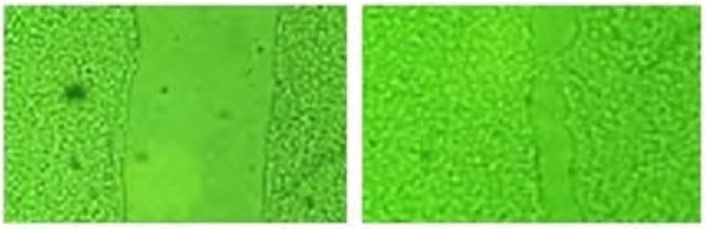
The Chick Chorioallantoic Membrane (CAM) assay is another method used to investigate the wound healing process. The process of wound healing is tightly regulated by multiple growth factors. The formation of new cells and the development of new blood capillaries in the inflammatory phase, releasing pro-inflammatory cytokines at the wound site have been recorded. To determine the growth of blood vessels in the wound healing process, the CAM assay was developed. This assay facilitates the study of the process of blood vessel sprouting during wound healing in response to angiogenic agents. The angiogenic response of the CAM assay is generally expressed as the vascular index.

In our laboratory at the Open University of Sri Lanka (OUSL), we established both SWA and CAM assays to investigate the wound healing activity of traditional medicinal plants in Sri Lanka. *In vitro* assays were conducted in cell cultures of baby hamster kidney (BHK21) and Madin-Darby canine kidney (MDCK) cell cultures. These cell cultures were established in the laboratory at the OUSL using standard *in vitro* methods.

In our study, bioactive phytochemical constituents from the aforementioned plant materials were prepared by sequential extraction with hexanes, dichloromethane, ethyl acetate, and methanol, separately, from the leaves and barks of selected plants. SWA (cell migration) and CAM (angiogenic potential) assays were conducted with purified chemical extracts. Further, the structure of bioactive compounds was determined by NMR spectroscopic data.

Wound healing activity of medicinal plants

As shown in Figure 1, *in vitro* SWA demonstrated that the experiment treated with medicinal plant extracts exhibited induced cell migration and accelerated the wound healing process. Further, findings of the CAM assay revealed that these plant extracts accelerated the development of blood capillaries in Chick embryos (Figure 2).



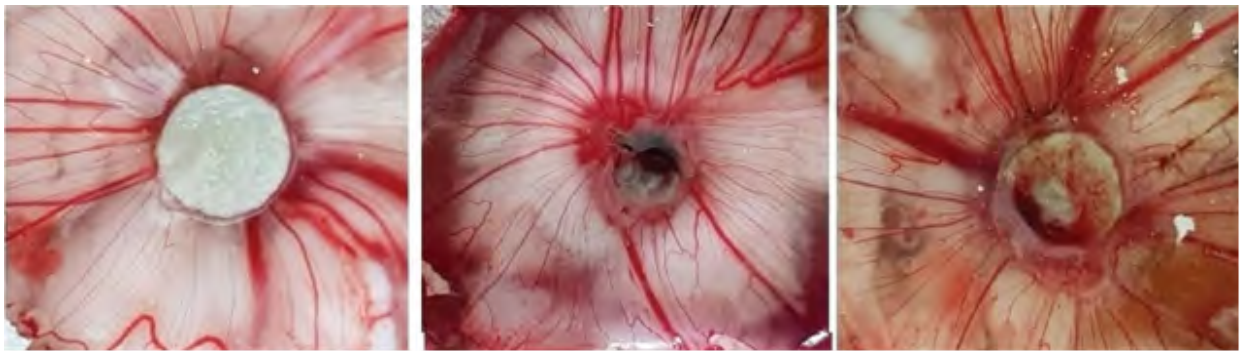
(a) (b)

Figure 1 SWA *in vitro* wound healing assay. (a), untreated cell cultures; (b), treated cell cultures.

The most abundant chemical constituents isolated were β-amyrin and lupeol. In addition, ethuliacoumarin, stigmasterol, glut-5-en-3β-ol, friedelin-3β-ol, stigmast-5-en-3β,7α,22α-triol and botulin showed enhanced cell migration in the SWA assay and significant angiogenic potential in the CAM assay.

Although *in vitro* studies have proven different levels of effectiveness in wound healing, *in vivo* studies are recommended to confirm the findings. Documented clinical trials are still lacking, and therefore, to ensure safety and effectiveness in human application, clinical trials need to be conducted. Currently, the challenge lies in the identification of the active compounds responsible for the wound healing properties and their mechanisms of action. Our study reports the use of selected plant natural products in promoting wound healing and their mechanisms. The findings of this study validate the traditional use of medicinal plants in wound care management.

This study was carried out as a collaborative research project between the Department of Zoology and the Department of Chemistry of the Faculty of Natural Sciences at the OUSL, led by Emeritus Prof. K. B. Gunaherath (Department of Chemistry), Dr. Kithsiri Jayawardana (Department of Zoology) and Dr. Chandani Ranasinghe (Department of Chemistry). Mr. Priyantha Samarasinghe, the research assistant working on this project, is currently reading for his PhD.



(a) (b) (c)

Figure 2 Angiogenic potential in the CAM assay. (a), control; (b) and (c), treated.

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Microplastics: Origin, Fate, and Behavior in the Environment and on Human Health

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Plastics in modern society

It was not until after the 1950s that the large-scale production of plastic and synthetic organic polymers was brought to life. Polymers of many types are commercially available, to allow for variable function and abundance in applications such as construction, medicine, textile, agriculture, packaging, and food industries. Despite there being more than 50 chemically distinct plastic polymer categories, only a few polymer types are produced in high quantities for commodity use. These include polypropylene, polyethylene, polystyrene, polyvinyl chloride, and polyethylene terephthalate. Although first thought to be harmless, overproduction of these polymer types led by consumerism as well as prolonged improper waste disposal has now introduced a mass array of environmental problems.

The aquatic environment receives the most plastic waste entering in from land-based or ocean-based sources (Figure 1). Plastics, before entering and in the seabed, are exposed to different abiotic and biotic factors which affect polymer structural integrity, making them degradable to form smaller debris. Plastic debris can be classified according to their size and can degrade into components as small as <1 µm. Micro-sized plastics of various shapes are persistent and are invisible to the human eye. They are a threat to human health and to the environment and have, in the recent decade, been an issue of global concern.

Figure 1 Illustration of microplastics and plastics in the ocean
Source: Dottedyeti. *Plastic pollution in ocean water, bottles floating in the current* [online]. Available at: https://stock.adobe.com/ (Accessed: 4 February 2023)

Defining microplastics

Microplastics (MPs) can be defined as "synthetic polymeric matrices", ranging in size from 1 µm to 5 mm, consisting of regular or irregular shapes, which are insoluble in water. MPs can be classified according to their manufactured origin; primary or secondary. Primary MPs are intentionally manufactured microscopic plastics for use in industrial applications such as when producing personal care products like cosmetics, toothpaste, etc., nurdle manufacturing, and in synthetic plastic-fiber textiles. Secondary MPs are produced from the degradation of larger plastic particles that are already prolonging in the environment.

Microplastic affecting marine life

MPs are found along the coastlines, deep in sediment and on the sea surface (Figure 2). The problems associated with MPs in marine ecosystems are that their removal is difficult and the degree of perpetuality of plastic-presence in the ocean leads to degradation. It has been discovered that MPs have entered the marine animal food chain. The presence of such MPs is challenging for environmental recovery and destructive to organisms in the ocean, as it contributes to fatalities through its ingestion. MP ingestion has been reported to affect many marine species including turtles, fish, seabirds, amphipods, and barnacles. It moves through the food chain via tiny organism exposure to larger consumed fish species by trophic transfer. In terms of an ecotoxicological aspect, MPs have an indirect effect on organisms as well, which includes a reduction in reproduction, growth, and behavioral changes. When investigating the effects of MPs like polystyrene on fish, it was found that it disrupted the reproductive endocrine and prenatal development and displayed oxidative stress.

MPs have previously caused digestive system blockages, nutritional and growth problems, inflammation and lipid accumulation. Organic pollutants such as endocrine disruptors, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons can absorb onto MPs and then be transferred into the food-chain. In this regard, MP contaminated seafood has the potential to contaminate cultured fish species marketed for human consumption.

Microplastics on human health

The primary route of human exposure to MPs is through ingestion of food and water contamination (Figure 3). Commercial fish and crustaceans retaining MPs from polluted waters are not the only origins of human MP exposure, but other sources such as salt, bottled water, inhaling synthetic textiles, and skin contact, can be too. Yet, those from seafood and the environment may present a higher threat due to plastic additive leaching, weathering, toxic pollutants, and pathogen interactions caused by absolute MP exposure. MPs have been observed to cause potential human health risks such as inflammation, obstruction, and accumulation in organs, as researched using *in-vitro* testing and analyzing cellular changes in fish. Exposure to MPs has introduced risks of oxidative stress, disrupted immunity and translocation to distinct tissues. Further prolonged exposure may lead to chronic irritation. Following MP ingestion, particles less than 150 μm travel to the lymph and circulatory system but absorption is expected to occur to only 0.3% of the ingested particles and only particles <20 μm in size, particularly nano plastics, could pass through into organs, passing the blood-brain barrier. Particles less than 10 μm can induce local or systemic immune responses.

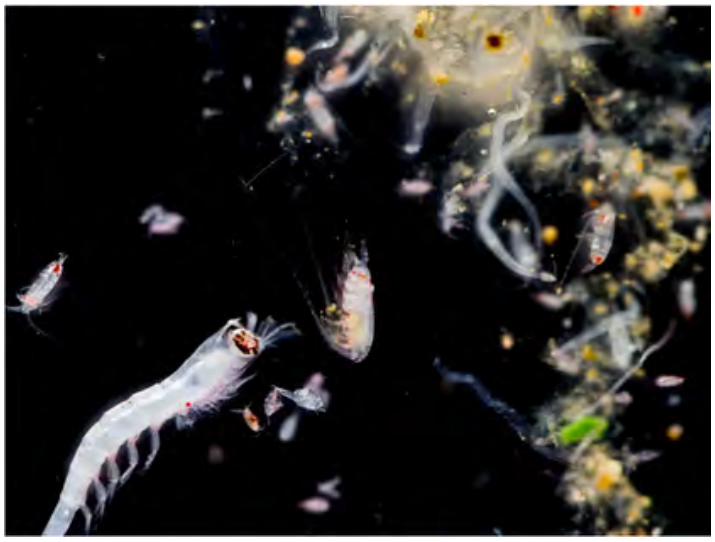

Figure 2 A sample of South Atlantic water containing plankton and microplastics
Source: Trimble, M. (2017) *Plankton sample contaminated by microplastics collected by marine biologists aboard oceanographic research cruise in the south Atlantic Ocean* [online]. Available at: https://www.alamy.com/ (Accessed: 4 February 2023)

An *in-vivo* study using mice reported that temporary immunosuppression can occur due to increased levels of inflammatory cytokines interleukin-6 (IL-6), IL-1β, tumor necrosis factor (TNF-α), T-helper cell suppression and reduction of T-effector cell production. MP-induced autoimmune disorders may lead to chronic damage in cells, incorrect immune cell stimulation and production of immune modulators. This could be linked with developing autoimmune rheumatic illness and systemic lupus erythematosus.

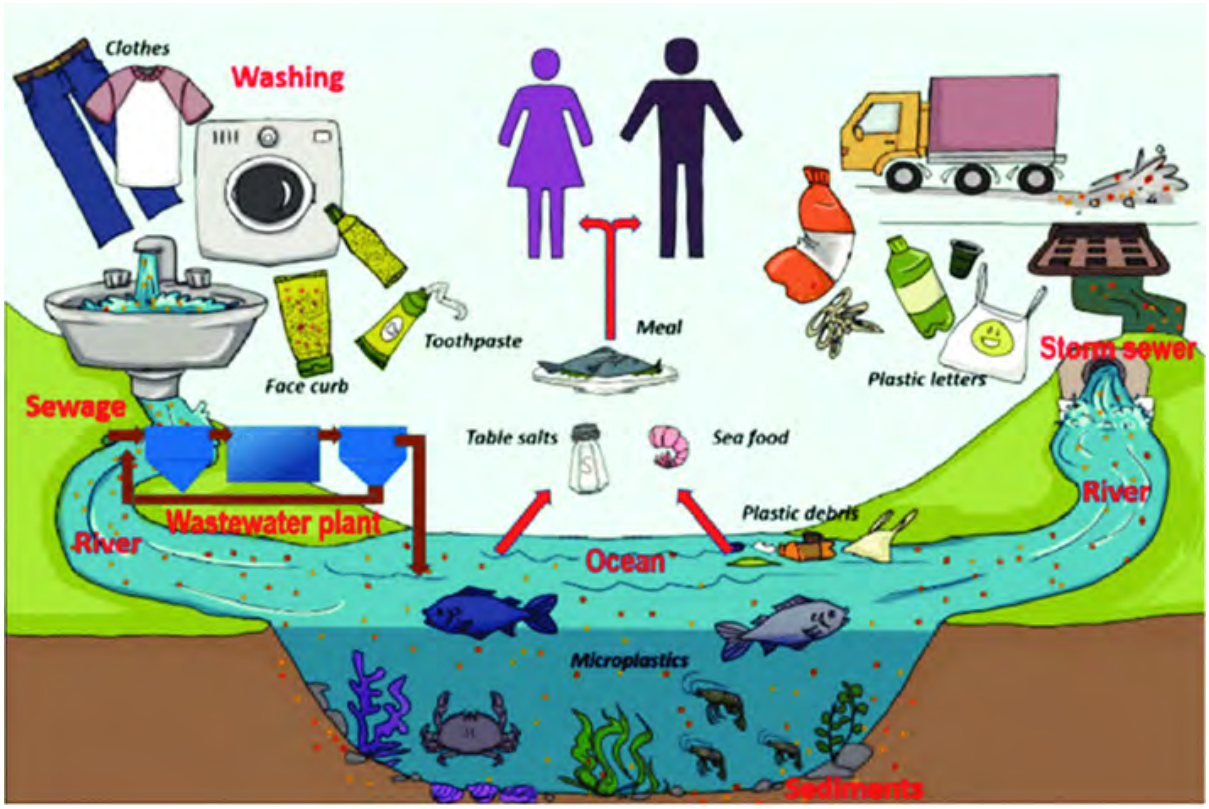

Figure 3 Microplastic pollution in aquatic environments and impact on food chains
Source: Wu et al. (2017)

In addition, MPs can release reactive oxygen species as a product of polymerization. The generation of free radicals can cause destruction of target tissues as indicated through mice and zebrafish studies. MPs present in limb and joint prostheses in humans were found to release acute toxicants and radicals caused by an inflammatory response because of the induced hydrolysis of the polymer, thus potentially leading to rejection from the body.

Moreover, MPs have been demonstrated to impact neuronal function and behavior when undergoing *in vivo* toxicity studies. Acetylcholinesterase enzyme release was reduced, lipid peroxidase levels increased, and energy production anaerobically was induced upon exposure to MPs in the brain of a sea bass (*Dicentrarchus labrax*), thus indicating that the presence of neurotransmitters may get affected.

Plastics in the nano-size category have been reported to promote tumor formation because of chronic inflammation and irritation, where pro-inflammatory mediators were released to induce angiogenesis.

Knowledge about the impact of MPs on human health is limited. Therefore, there is a need for a better understanding about the different MP exposure concentrations and its individual susceptibility linked with causing immune disruption, oxidative stress, translocation to tissues, neurotoxicity, reproduction scarcity, carcinogenicity, and more. Further research on MP ecotoxicology in aquatic and terrestrial environments and on human health, can lead to awareness on the drastic consequences of long-term plastic pollution.

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Immunotherapy: The Future of Medicine

Introduction

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Personalized medicine or precision medicine is considered as a promising approach for treating enigmatic diseases. Immunotherapy has been recognized as an emerging and effective therapeutic modality in precision medicine that aims at strengthening the immune system to help with the body's battle against cancer, infection, and other disorders. It targets cells, components and specific pathways in the human immune system. Cytokines, vaccines, Bacille Calmette Guerin (BCG), and some monoclonal antibodies are examples of immunotherapeutic agents currently used in therapeutic applications.

History and advances in immunotherapy

The origin of immunotherapy goes back to China's Qin dynasty, in the third century BC. However, the number of documented evidence on immunotherapy applications are limited. Intentional inoculation of variola minor virus to healthy, non-immune individuals to prevent smallpox disease can be considered as a successful immunotherapeutic application from the past. A report on intentional inoculation with variola minor virus was presented to the London Medical Society by Dr. John Fewster in 1765. Further, in 1796, Edward Jenner demonstrated protective immunity against smallpox through inoculation with common cowpox virus which is considered as the beginning of the history of vaccines.

This has drawn significant attention to immunotherapy as a novel therapeutic approach with the potential of saving millions of lives all around the world.

The earliest systematic study of cancer immunotherapy was recorded in 1981 by the father of immunotherapy, William B. Coley. He injected a mixture of live and inactivated *Streptococcus pyogenes* and *Serratia marcescens* to patients afflicted with inoperable bone and soft tissue sarcoma. This has triggered a strong antitumor response in patients. Since then, cancer immunotherapy has continued to evolve and has begun to provide an efficacious therapeutic approach to conventional cancer care.

In 1986, the first immunomodulatory cytokine, interferon-α (IFN-α) was approved by the Food and Drug Administration (FDA) for leukemia. In 1990, BCG, a tuberculosis vaccine, was licensed by the FDA for the treatment of bladder cancer. The CD20-binding specific chimeric mouse/ human monoclonal antibody, Rituximab, became the first licensed therapeutic antibody for treating lymphoma in 1997. Sipuleucel-T is the first autologous cellular immunotherapy licensed in 2010 for the treatment of patients with prostate cancer. In 2011, the first immune checkpoint inhibitor to treat advanced melanoma was approved for clinical use. So far over thirty different cancer immunotherapies have been approved by FDA for the treatment of patients. Marking a milestone in cancer immunotherapy, the Nobel Prize for Physiology and Medicine in 2018 was awarded to Dr. James P. Allison and Dr. Tasuku Honjo in recognition of their discovery of cancer therapy via inhibition of negative immune regulation.

Research on human immune checkpoints namely cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein 1 (PD-1) has revealed that these checkpoints act as 'brakes' on the immune system. Inhibition of these checkpoint pathways allows T-cells to eliminate cancer cells more effectively. The first humanized anti-CTLA-4 antibody, ipilimumab, was approved by FDA in 2011 for clinical use in metastatic melanoma. Under treatment, the survival rate of metastatic melanoma patients has almost doubled. This has made a paradigm shift in cancer care.

Types of immunotherapies

Immunotherapy can be either passive or active. Active immunotherapy involves vaccination, non-specific immunomodulation, or targeting specific antigen receptors that stimulate the self-immune system to attack target cells. On the other hand, passive immunotherapy is the administration of agents such as monoclonal antibodies, and lymphocytes to enhance the existing immune response. These immunotherapies target at enhancing the immune system's ability to fight in a number of ways. Non-specific immunotherapy stimulates the immune system in a more general way leading to a better immune response against cancer or infectious cells. This approach is also used to suppress immune reactions to help treat autoimmune diseases or to minimize bone marrow or organ transplant rejections.

Cytokines are proteins secreted by some immune system cells to control the growth and activity of other immune system cells. Interferons are a type of cytokine that encourage the activity of immune cells in the fight against cancer. For instance, leukemia, sarcoma, lymphoma, and melanoma are treated by IFN-α. Immunomodulators such as imiquimod (Aldara, Zyclara), lenalidomide (Revlimid), lenalidomide (Revlimid), pomalidomide (Pomalyst), thalidomide (Thalomid) and BCG are some of the drugs that stimulate the immune system to fight cancer and infection.

In addition, immune checkpoint proteins such as PD-1, PD-L1, and CTLA-4 sometimes prevent the arousal of a strong immune system and thereby tend to protect cancer cells.

Checkpoint inhibitors (i.e., anti-PD-1, anti-PD-L1, and anti-CTLA-4) are designed to block the immune checkpoint proteins and facilitate T-cell mediated cell death of target cells. Natural killer (NK) cells are immune cells that destroy foreign invaders in the body such as tumor cells and virus-infected cells. This cellular immunotherapy is known as NK cell therapy and it is capable of efficiently killing multiple adjacent cancer cells. Clinical trials of NK cell infusion in patients with hematological disorders and solid tumors are currently underway with promising results.

Tumor-infiltrating lymphocyte (TIL) therapy is an experimental cell therapy targeting the treatment of solid tumors. Here, T-cells in a tumor are separated and are grown in huge quantities *in vitro*. These cells known as TILs are then reintroduced into the body to enhance the immune attack.

Engineered T-cell receptor (TCR) therapy is performed by removing T-cells from the blood and reprogramming them in a lab to quickly detect cancers like sarcoma and late-stage melanoma skin cancer. This field has grown significantly in recent years with the FDA approval of chimeric antigen receptor (CAR) T-cell therapies for blood cancers. CAR T-cells are engineered to recognize specific targets on cancer cells. In cell-based immunotherapies, immune cells are infused into patients' bodies through sophisticated cell transplants and bone marrow transplants.

Viral particles or attenuated/killed viruses are commonly used in conventional vaccines. Similarly, engineered oncolytic viruses are used in cancer therapy to preferentially infect and/or replicate in cancer cells and cause them to burst. This treatment modality uses a virus to infect and kill cancer cells while avoiding healthy cells. T-VEC (ImlgicTM) is developed from a genetically modified herpes virus, commonly known as the cold sore virus. It was approved in 2015 for the treatment of patients with advanced melanoma.

Modified T-cell treatments or identification of novel tumor antigens through next-generation sequencing has marked a new era in cancer immunotherapy. Next-generation vaccines such as malaria vaccines and COVID-19 vaccines are focused on increased efficacy, durability or protection and reduced number of doses required, with the potential of providing a greater impact in preventing diseases. Severe acute respiratory syndrome coronavirus (SARS-CoV) was discovered in 2002 and next-generation vaccinations for SARS-CoV were developed six months after SARS-CoV-2 was discovered. Pfizer BioNTech vaccine is based on the virus's genetic materials (mRNA) for building the spike protein. mRNA vaccines are worked by introducing a piece of mRNA that is corresponding to the viral protein. Figure 1 summarizes the types of immunotherapies.

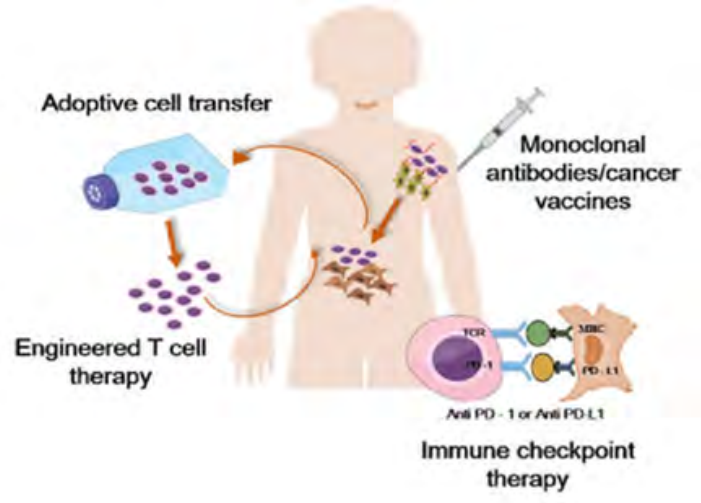

Figure 1 Types of immunotherapies and mechanism of programmed cell death.
Source: Surendran et al. (2018)

Immunotherapy: advantages and disadvantages

Immunotherapy supports the self-immune system to fight cancer or infected cells more effectively. It has proven to increase the efficacy of conventional treatments like chemotherapy and has significantly improved the long-term survival rate of patients. Since immunotherapy only targets the immune system, it has shown fewer negative effects compared to traditional therapies. Further, immunotherapy has high accuracy and specificity and is effective for a long time period. Immunotherapy treatments have shown wide adaptability; immunotherapy can control and kill multiple types of tumors. Moreover, the treatments can restore and improve the body's immune function and help to prevent tumor recurrence and metastasis. Further, residual tumor cells and microscopic lesions can be removed from the body thoroughly.

Although immunotherapy has been shown to be efficacious, the overall response rate of immunotherapy is nearly 15-20%. Generally, immunotherapy works for less than half of the people who try it. Also, it takes a longer time to work than other treatment methods. In addition, a variety of side effects may occur in some patients. Some of the side effects caused by immunotherapy treatments include fatigue, itchy rash, diarrhea, nausea, vomiting, and decreased thyroid hormone levels. In some cases, after immunotherapy treatments, hyper progressive disease may occur, decreasing the overall survival. Furthermore, some drugs that are used in immunotherapy can cause the self-immune system to attack organs like the heart, liver, lungs, and kidneys. The immune checkpoint inhibitors used in cancer therapy may also lead to autoimmune diseases or even the death of patients due to the negative regulation of immune checkpoint inhibitors. Another limitation of immunotherapy is that the treatment costs are high.

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NATURE CAPTURES

Photograph by Dr. Sandun J. Perera, M. I. Biol. (Sri Lanka)
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"Maha Eliya Tenna", among the largest highland plateaus of Sri Lanka with a habitat mosaic of wet patana grasslands, cloud forests, and dwarf bamboo marshes, also known as the Horton Plains National Park, provides a pristine habitat for the largest deer species found in Sri Lanka, *Rusa unicolor unicolor* the sambar. The female on your right and the sub adult spike male with its velvet single tine antler shown here, makes a part of a twenty-five strong sambar herd one could only see grazing in the Maha Eliya Tenna, as the sambar is a predominant solitary browser elsewhere in the forests of Sri Lanka.

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