ISSN: 2394-0182

Vol:17 E D I T I O N: 2023

ZOON

ANNUAL RESEARCH JOURNAL

DEPARTMENT OF ZOOLOGY
B. BOROOAH COLLEGE
GUWAHATI

Cover designed by: Dr. Archana Borah

Back cover designed by: Dr. Sabina Langthasa

Editor's Desk

Zoon earlier published as a research journal is now open completely for faculties and students to express their knowledge, talents and thoughts on the subject. In addition, it now carries every latest essential information of the department. This issue is being published after a long gap of 5 years and it is indeed an honour to be an editor of this revived issue. This issue comprises of reviews, research work, research techniques, art and general articles on important issues. Theis issue also contains recent events of the department and students' achievements.

This issue and the forthcoming ones will serve as a platform and ice breaker for upcoming young researchers and academicians to exhibit their ideas and thoughts on the subject thereby preparing them further research.

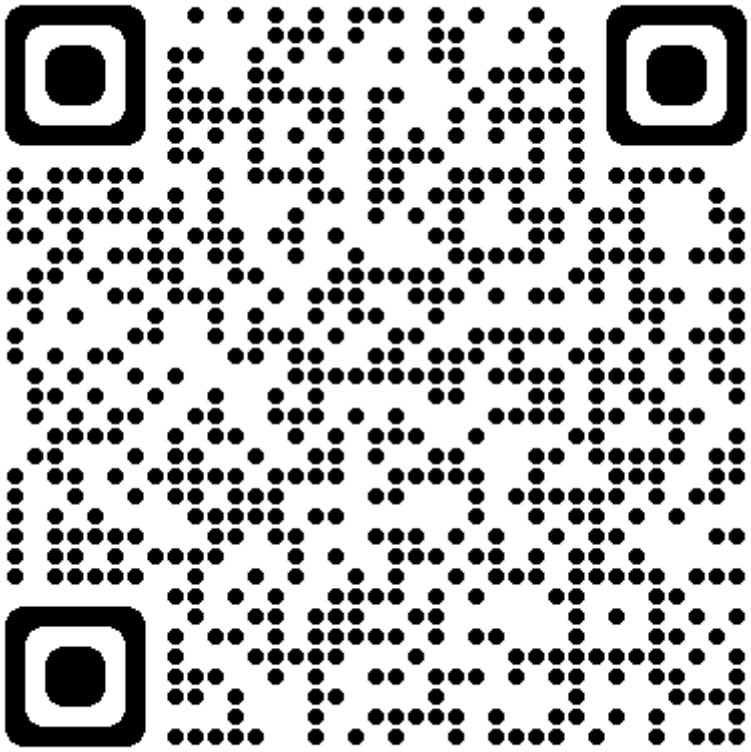
My special thanks to the Department for entrusting me with the portfolio of Editor for this memorable issue. I deeply acknowledge the contribution of all the authors and for the gentle support and advice from my learned colleagues.

Finally, a huge shout out to all the students and staff of the Department for their cooperation and assistance. Errors if any, they are mine.

Dr. Amar Deep Soren

Editor

CONTENTS



DEPARTMENT OF ZOOLOGY

B. BOROOAH COLLEGE

DEPARTMENT AT A GLANCE

BRIEF HISTORY

The Science section at B. Borooah College started in the year 1964 offering one year Pre-University Science course. Later, Zoology was offered as a general subject in the degree course from 1971. Following this, Honours (major) course in the subject was started from the academic session 1980-81 with a strength of 12 students. All of them secured honours in the final examination held in 1982. The Department of Zoology was continuing in the old building of the college since its inception till 2001. In the year 2001, the Department was shifted from the old building to its present new R.C.C. building. The PG course was introduced from 2014 (Vide letter No.GU/AFF/2013/4241 dated 21-10-2013). Since then, the Department had produced numerous graduates who now hold several portfolios in government and non-governmental sectors. The Department proudly boasts of several students who have cleared NET, GATE and SLET conducted by UGC/CSIR, ICAR, IIT and SLET Commission.

FACULTY

The department has a group of well qualified faculty members specialized in their own fields..

Sl No	Name and designation	Specialization	Publications	Books/book chapters
1	Dr. Mridul Kumar Borthakur Ph.D., Head and Assistant Professor	Ecology and Wildlife Biology	28	12
2	Dr. Janardan Pathak, B.Ed., Ph.D., Assistant Professor	Fish and Fishery Biology	2	2
3	Dr. Sabina Langthasa, B.Ed., Ph.D., Assistant Professor	Insect Physiology	10	3
4	Dr. Archana Borah, Ph. D., SLET., Assistant Professor	Biochemistry	10	5
5	Dr. Amar Deep Soren, Ph.D., SLET, Assistant Professor	Genetics	22	5
6	Dr. Anurupa Goswami, Ph.D.,SLET, Assistant Professor	Cell and Molecular Biology	4	1
7	Mr. Sahil Zaman Seikh, M.Sc., NET-JRF, Assistant Professor	Ecology		
8	Dr. Salma Mazid, Ph.D., Assistant Professor (Guest faculty)	Entomology	12	2
9	Dr. Anjumani Ojah, Ph.D., Assistant Professor (Guest faculty)	Animal Physiology and Biochemistry	4	1
10	Ms. Barsha Sarma, SLET, GATE, Assistant Professor (Guest faculty)	Cell and Molecular Biology	3	3

NON-TEACHING STAFF

1. Mr. Prabhat Barman, Laboratory Bearer

2. Mr. Manoj Deka, Laboratory Bearer

3. Mr. Bitul Gogoi, Laboratory Bearer

COURSES OFFERED

Sl. No.	Course	Seats	Strength
1	Higher Secondary		
2	B Sc (Honours)	35 per semester	39 + 32 + 36 = 107
3	M Sc (Specializations: i) Entomology, ii) Animal Physiology and Biochemistry)	15 per semester	15 +14 = 29
4	Ph. D.	As per vacancy	9

LABORATORY

The Department has a well-equipped UG and PG laboratory. The lab houses several sophisticated instruments, some of which are, Gradient PCR, Gel Doc. System, -20 ◦C Deep Freezer, Horizontal Electrophoresis, Refrigerated Centrifuge, Phase contrast microscope, Compound microscope, Dissecting microscope, Binocular microscope, SDS PAGE electrophoresis equipment, Digital balance, Electronic single pan balance, Digital Colorimeter, Digital pH meter, Digital Spectrophotometer, Double distilled water plant, Microtome, Serological water bath, Rotary Vacuum evaporator, Paraffin wax bath, Centrifuge, Magnetic stirrer, Hot air oven, Water analyzing kit, Occular micrometer, Kymograph, Electric shaker, Binoculars, B.O.D. incubator, Paper chromatography, Thin layer chromatography, Column chromatography, Dissection microscopes, Chromatographic apparatus, Chromatographic plate drier, Altimeter, GPS, Hair Hygrometer, Conductivity meter, Dissecting magnifier, Soil thermometer, Atomic Absorption Spectrometer, FT-IR Spectrophotometer, and UV-Vis Spectrophotometer

LIBRARY

The Department has a separate library for UG and PG students and the library houses more than 1000 books in the various fields of Zoology. These include important publications on General Zoology, Genetics, Cell Biology, Molecular biology, Developmental Biology and Embryology, Evolution, Physiology, Biochemistry, Endocrinology, Parasitology, Entomology, Ecology and

Wildlife Biology, Fish and Fishery Science, Biotechnology, Biostatistics, Taxonomy and Animal behaviour. The Department has also subscribed to various journals as well, such as, Indian Journal of Experimental Biology, Resonance, Science Reporter, Bijnan Jeuti, Bijnan Prasar, Indian Journal of Biotechnology, Indian Journal of Entomology, Bioscience, Current Science, and Indian Journal of Environmental Biology. The department has also been publishing an annual Newsletter "Zoon" since 2002 and an annual wall magazine "Sakhiotee" since 1998.

RESEARCH

The laboratory of the Department has been recognized as a research lab to carry out Ph. D. level research under Gauhati University (vide Memo No.GU/Acad/Ph.D/Recog.lab./2012/1854-56 dated 5.12.2012). Two faculty members of Zoology Department has been recognized as Ph. D Guides by Gauhati University. The Department has produced 7 Ph. D. scholars and currently 9 scholars are working towards their Ph. D. degree. Several projects have been completed by faculty members of the Department and a few projects are still undergoing completion.

Ph. D. GUIDES

1. Dr. Mridul Kr. Borthakur

2. Dr. Amar Deep Soren

RESEARCH AREAS

Reproduction and fertility, Toxicology, Parasitology, Ethnopharmacology, Sericulture, Antimicrobial activity, Endocrinology, Diabetes, Liver and kidney protection, Ecology

Ph D AWARDED

Sl No	Scholar	Title	Supervisor
1	Dr. Parag Moni Baruah	Toxicity evaluation and analysis of caffeine profile of unprocessed and processed tea marketed in Sonitpur (Tezpur) and Biswanath (Gohpur) districts of Assam	Dr. Sunayan Bardoloi
2	Dr. Asha Rani Borah	Studies on the effects of Genistein on endocrine pancreas in albino rat (*Rattus albicans*)	Dr. Kamal Choudhury
3	Dr. Palki Hazarika	Effects of *Saraca indica* Linn. bark extracts on reproductive system of Levonorgestrel treated female *Rattus albicans*	Dr. Kamal Choudhury
4	Dr. Pramathesh Kalita	A study in toxicological impact of herbicide Pendimethalin on hepato-gonadal functions in *Channa punctata* (Bloch)	Dr. Kamal Choudhury

5	Dr. Angana Das	Hepatoprotective effect of *Chenopodium album* Linn. in albino rats	Dr. Mridul Kumar Borthakur
6	Dr. Alisha Nasreen	Studies on the effect of *Enhydra fluctuans* Lour. on alloxan induced diabetic albino mice	Dr. Kamal Choudhury
7	Dr. Anjumani Ojah	Effect of methanolic root extract of *Persicaria hydropiper* (L) Delarbre on fertility in albino mice	Dr. Mridul Kumar Borthakur Co-guide: Prof. Jogen Chandra Kalita (Gauhati University)

RESEARCH SCHOLARS

Sl. No	Scholar	Title	Supervisor
1	Ms. Sanghamitra Saharia	Effect of selected plant extracts against the white muscardine disease in muga silkworm, *Antheraea assamensis* Helfer	Dr. Sunayan Bardoloi
2	Mrs. Shibani Kalita	Studies on *Bacillus thuringiensis* induced low molecular weight peptides of *Antheraea assamensis* Helfer for their potential antimicrobial activity	Dr. Sunayan Bardoloi
3	Mrs. Jilimani Sarma	Studies on the Anuran species as bio-indicator of wetland health in Nalbari district, Assam, India	Dr. Sunayan Bardoloi
4	Ms. Barsha Sarma	A study on the effect of methanolic leaf extract of *Manihot esculenta* Crantz in Streptozotocin induced diabetic mice	Dr. Mridul Kumar Borthakur
5	Ms. Dimpi Moni Kalita	Isolation, characterization, identification and pathogenicity assessment of gut microbes of diseased eri silkworm, *Samia ricini* (Donovan)	Dr. Sunayan Bardoloi
6	Mrs. Sanjana Sharmin	A study on the effects of juvenile hormone analogue Methoprene on the biochemical and commercial parameters of silkworm *Antheraea assamensis* Helfer	Dr. Sunayan Bardoloi
7	Mr. Kunal Chanda	Breeding biology of stump tailed macaque in Gibbon Wildlife Sanctuary	Dr. Mridul Kumar Borthakur
8	Ms. Kakali Deka	Study on the effects of methanolic leaf	Dr. Mridul Kumar

		extract of *Streblus asper* on the fertility status in female albino mice	Borthakur
9	Ms. Tapashi Sutradhar	Ameliorative effect of methanolic extract of *Heliotropium indicum* in adenine induced chronic kidney disease in Wistar rat	Dr. Mridul Kumar Borthakur
10	Mr. Sonot Deori	Phytoremediation of waste water of Guwahati city, Assam	Dr. Mridul Kumar Borthakur
11	Ms. Tanushree Dutta	NEWLY ADMITTED	Dr. Amar Deep Soren

ADD-ON COURSES

The Department offers several add-on courses for the benefit of the students.

1. Certificate course on Sericulture and its prospect in NE India
2. Certificate course on Basics of Wild Life Photography
3. Certificate course on aquarium keeping and ornamental fish breeding
4. Certificate course on Non-mulberry sericulture and its prospect in NE India
5. Identification and monitoring of Avian species

CHAMELEON: THE COLOUR CHANGING SPECIES

Dr. Jagadindra Raychoudhury
Ex Head, Department of Zoology

Nature has luminously decorated the world with a mixture of habitats of flora and fauna which are totally unraveled with any other invention of human being. We can spend hours together in an around nature without thinking of hungriness or feelings of dehydration but the situation has been reversed with a remarkable speed by destroying natural recourses for the pseudo progress of mankind towards development. The state of affairs happened to occur only for the greediness of human being however, without the bed of nature people will never survive for a long time. A handful of nature's lover always try to raise their voices for the conservation of nature and sometimes government machinery also extend their help, but a strong legislation is also required for protection of nature especially for the welfare of future generation.

Many flora and fauna have already been extinct, some are endangered and others are vulnerable yet a few species are still survived with their entire trait intact. We will try to express one of the lizards which is very much popular due to its charismatic changing of colour and the species is nothing but chameleon. Chameleons are a distinctive and highly specialized clade of old world lizard with 202 species described as of 2015. Chameleons have their long, sticky tongue; and their eyes, which can be moved independently of each other. Chameleons are under the Class of –Reptelia, Family- Chamaeleonidae and Order- Squamata.

It is to be noted that chameleons have some specific characteristics besides changing their colours. They are distinguished by their zygodactylous feet, their prehensile tail, literally compressed bodies with casqued head, projectile tongues along with swaying gait. They also have a horn or crest on their brow and snout. The eyes of chameleon are of independent nature with mobility where two separate individual images formed and that is analyzed by the brain for its environment. The eyes are able to move laterally 180 degree and vertically 160 degree.

Chameleons are mostly aerborial and it helps because of their compressed body for which adaptability generated for climbing and visual hunting. The practice of climbing is also adhered for their prehensile tail which supports the stability especially during movement while on a branch in the canopy; hence it is often referred to as 'Fifth limb'. Chameleons live in warm habitats that range from rainforests to desert like conditions where various species are available in Africa, South Europe, Madagascar and southern Asia.

The most vital feature of chameleon is changing of skin colors and this coloration varies from species to species. The colour combination spark on chameleon is blue, pink, red, orange, green, black, brown, yellow and purple etc. The skin colours are changing because of a superficial layer present on the skin which contains pigment and under the layer, a small cell is present with guanine crystals. The tuning, by an unknown molecular mechanism, changes the

wavelength of light reflected off the crystals which changes the colour of the skin. It is to be noted that chameleon colour palettes have evolved through evolution and the environment. Chameleons living in the forest have a more defined and colourful palette compared to those living in the desert.

The most important observation on chameleon is that why they change their colour? It is due to social signaling and reactions to regulate temperature. It's a physiological change, either to a darker colour to absorb light or to a lighter colour to reflect light and heat to stabilize or lowering their body temperature. Mostly, chameleons tend to show brighter colours displaying aggression to other chameleon and on the other hand darker colour when they submit or 'give up'. It is to be noted that some other species especially in Madagascar and South Africa have their blue florescence in their skull tubercles.

The feeding habits of chameleons are something special because of their technique. No doubt, they are normally insectivores but for capturing food they use their long tongues from some distance away. This method is possible because chameleon's tongues are two times longer than their bodies. The long tongue consists of highly modified hyoid bones has an elongated, parallel sided projection, called the entoglossal process, over which tubular muscle, the accelerator muscle sits. The drawing back of tongue into the mouth could be possible due to hyoid and accelerator muscle. The thermal sensitivity of tongue retraction is not a problem because chameleons have very effective mechanism of holding into their prey once the tongue has come into contact with it because of wet adhesion and interlocking and suction.

Besides chameleon, there are many more species with differences in their features ranging from terrestrial to aquatic, even in snow bedded mountain. The amazing nature of various species which attracted to us are losing their habitat for the hardcore encroachment of human being for their unwanted need. This greediness of acquiring more land in every aspect indirectly reflects in the verse of extinction of many more species.

PLANKTON - THE LIVING CAPSULES

Dr. Janardan Pathak
Assistant Professor

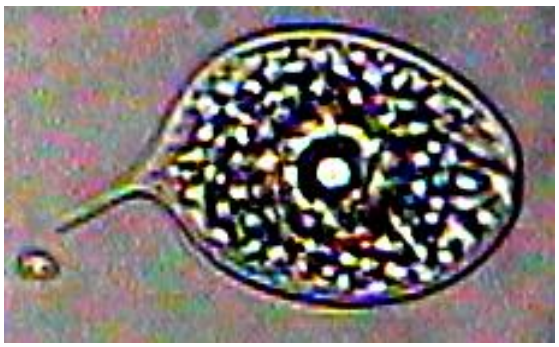
Successful fish production in aquaculture ponds depends on the availability of live food which provides the natural nourishment for proper growth of the fishes at different stages of their development. Now-a-days live food organisms particularly the plankton are called as the living capsules of nutrition for the fish larvae. There is a direct relationship between plankton production and fish yield. Some species of fish are plankton feeders throughout their life while others subsisted on plankton at some particular stage of their life cycle. It has been established that carp fry in their early stages of development mainly feed on the plankton. The term plankton has been used to describe freely-floating and weakly-swimming marine and freshwater organisms. These 'wandering' or 'drifting' organisms can be defined as free floating animal and plant organisms, whose intrinsic power of locomotion, if present, is so feeble that they remain almost at the mercy of water currents and waves.

Qualitatively plankton can be broadly classified as phytoplankton and zooplankton. Phytoplankton include the chlorophyll bearing organisms capable of photosynthesis and non - photosynthetic plants or saproplankton. The plankton of animal origin are included in zooplankton. In the aquatic ecosystem phytoplankton stand on the baseline of different food webs and are considered to be one of the most important primary producers. On the other hand, as the primary consumers zooplankton play a significant role in conversion of plant protein into animal protein. On the basis of their size plankton may be termed as Pico plankton (< 2 µm), Nano plankton (2 - 20 µm), Micro plankton (20 – 200 µm), Macro plankton (200 – 2000 µm) and Mega plankton (> 2000µm). On the basis of site of occurrence plankton are classified as limnoplankton or the lake plankton, rheoplankton or running water plankton, heleoplankton or pond plankton, halioplankton or salt water plankton and hypalmyroplankton or brackish water plankton.

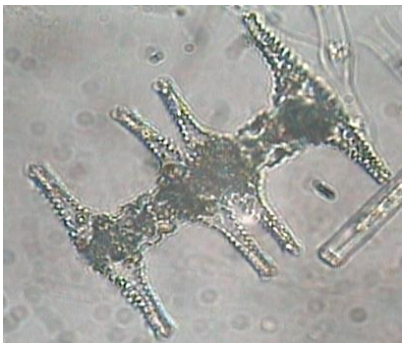
In general, the freshwater phytoplanktonic forms are contributed by a number of algal groups namely Myxophyceae, Chlorophyceae, Euglenophyceae, Xanthophyceae, Chrysophyceae, Dinophyceae and Bacilleriophyceae, whereas, the zooplanktonic forms of freshwater regime belong to the free living non photosynthetic protist - the Protozoans, to the Rotifera and to the Crustacea. Although most of the planktonic groups are widely distributed, the Myxophyceae plankters are found to flourish in the warm and nutrient - rich water with some exceptions. The planktonic Chlorophyta represents a number of organisms of very different nature among which the Volvocales and Chlorococcales seem to occur most abundantly in ponds and small productive lakes. Another important group belonging to Chlorophyta which spectacularly develop in the regions of dilute acid waters is the Desmid. Besides, the Euglenophyta are often abundant in the water rich in organic matter. On the other hand,

Bacilleriophyceae is considered as one of the dominant freshwater groups which present in significant numbers in aquatic systems.

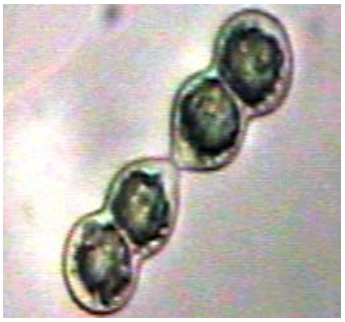
The Rotifers, along with the two Crustacean groups namely Cladocera and Copepoda share the major part of the freshwater zooplankton. The majority of planktonic species of rotifers show a wide range of adaptability to varying environment and are potentially cosmopolitan, while a few exhibit geographical limitations.



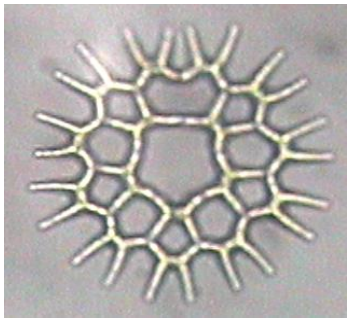
Phacus sp.



Staurastrum sp.



Cosmarium sp.



Pediastrum sp.

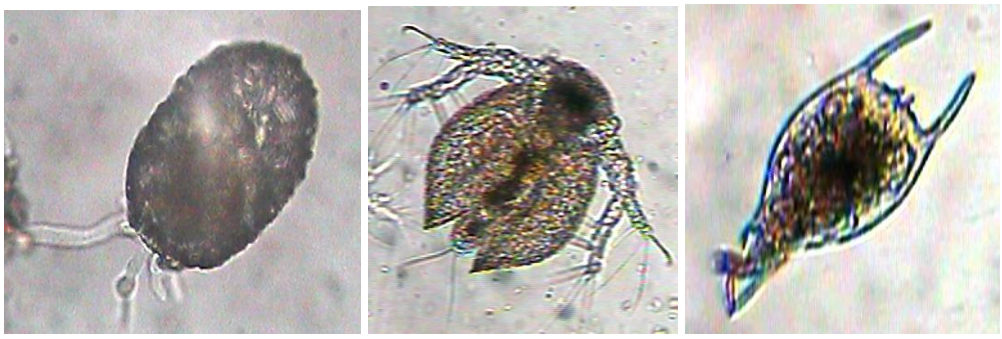
Xanthidium sp.

Figure 1: Some species of Phytoplankton

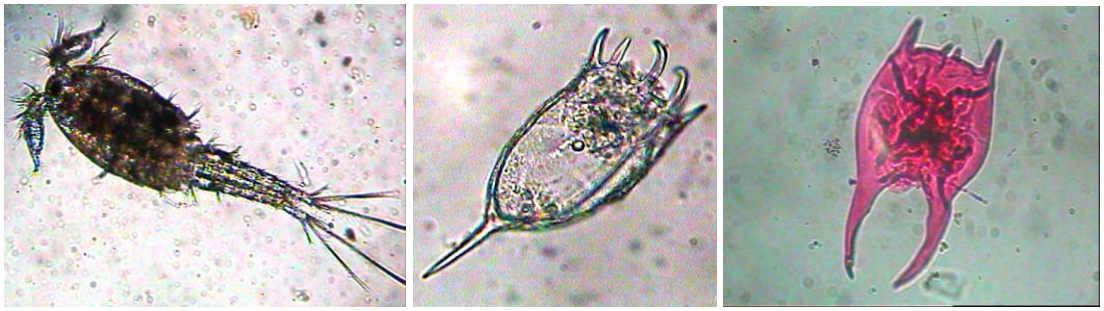
Amongst different characteristics of plankton, it is interesting to observe their periodic fluctuation qualitatively and quantitatively in almost all types of aquatic habitats. Certain planktonic populations found to disappear at specific periods and reappear during others. Such temporary disappearances may be due to physiological activity of plankton or environmental factors such as temperature, light, inorganic and organic nutrients etc. The planktivorus fishes in aquaculture system play an important role in the regulation of plankton community. Selective consumption of large zooplankton by the planktivorus fishes shifts zooplankton community towards dominance by smaller species.

Besides seasonal variation, it is interesting to observe the diurnal fluctuation in plankton. Most of the phytoplankters are found abundantly in the surface water during the day hours while zooplankton are copiously found in the night hours. Diurnal variation of plankton is guided by a number of physico-chemical parameters and biological components of an aquatic system. The

light has been considered as the most important factor triggering the diel vertical migration of plankton. However, other environmental factors such as temperature, dissolved oxygen and availability of food are also involved in regulating such behaviours of plankton. Many zooplankters exhibit diel vertical migration as a defense to avoid predation by larger insects and fishes.



Difflugia sp. ***Diaphanosoma sp.*** ***Brachionus diversicornis***



Mesocyclop leuckarti ***Keratella coachlearis*** ***Brachionus forficula***

Figure 2: Some species of Zooplankton

Recently various techniques are innovated to culture plankton to provide a good supplement of nutrition to the young fishes. Among such culture methods, mass culture of rotifers in earthen ponds is very simple and effective which can be done in our aquaculture farms. In this method, 5 to 10 rectangular ponds with suitable size of about 1000 sq m. surface area and of about 1 m depth are to be constructed. After filling up with water each pond is to be treated with about 500 kg of fermented bean cake or mustard oil cake and chicken manure (in 1:4 ratio). Since rotifer culture practice require a large volume of phytoplankton as the source of food for rotifers, 3 to 4 of the constructed tanks should be used as algal culture ponds. The ponds for rotifer culture are inoculated with rotifer resting eggs which can be obtained from other water bodies. After hatching of the resting eggs, the algal water is to be pumped into rotifer ponds. So, algal ponds are required to enrich with water and mustard oil cake/ fermented bean cake and chicken manure as applied earlier. About 10 days later after hatching, the rotifers can be harvested and supplied to the fish ponds.

ARRIVAL OF AMUR FALCON *Falco amurensis* IN DIMA HASAO DISTRICT, ASSAM

Dr. Sabina Langthasa
Assistant Professor

Amur Falcon, *Falco amurensis* is a small and migratory bird of the falcon family. They are renowned for their long migratory route in large numbers. The males are dark grey with reddish-brown thighs and under-tail coverts, reddish-orange eye-ring and feet. Females are duller above, with dark scaly markings on white underparts, an orange eye ring and legs. Only a pale wash of rufous is visible on their thighs and undertail coverts. Their diet mainly comprises insects, such as termites.

This bird mainly feeds on insects. Amur Falcon breeds in the Eastern Siberia region of Russia and Northern China and migrates to Southern Africa during winter season. While migrating, Amur Falcon covers a distance of 22,000 km making it one of the longest distances covered by migratory birds. Amur Falcon during their migration journey make a stop-over in India, particularly in Nagaland and some parts of Manipur and Assam before starting off for the most difficult part of their migration route, flying over the Arabian Sea to finally winter in South Africa. In Assam, every year flocks of Amur falcon starts landing in Umrangso, Dima Hasao district. In Umrangso, known as the industrial town in Dima Hasao district, this bird roosts at Tumbung area near the Kopili reservoir of Dima Hasao. The village is mainly a forest area with pine trees, bamboos and diverse trees as well. Every year groups of birds come in huge numbers encircling the area making peculiar sounds. From one to huge numbers this bird usually makes their appearance in the late evening hours and roosts at the particular site. Thousands of these birds' frolic in the roosting site for about two months before they head for warmer climates in Kenya and South Africa in a continuous flight over the Arabian Sea. Roosting takes place for about a month particularly from the last week of October or first week of November till December.

Appearance of the huge number of birds is a sight to behold which has attracted of spectators and tourists as well from all over the place. Roosting takes place for about a month particularly from the last week of October or first week of November till the end of December.

Falcon Festival in Umrangso

In earlier days, as millions of Amur Falcons came to roost they were mercilessly targeted by local villagers. As such with a view to safeguard the Amur Falcons during their roosting period in the region as well as for the public awareness, various steps have been adopted by the district officials, forest department, local NGOs and the local people as well to ensure there are no trapping or killing of the birds. Various conservation activities have also been initiated.

One of the biggest festivals of Dima Hasao is now the Falcon festival which has been celebrated mainly to safeguard this particular fauna. The first Falcon Festival was organized in

2015 at Karbi Club ground in the heart of Umrangso from October 31 to November 1, 2015. This was organized by the Blue Hills Society supported by Assam Forest Department, 38 Assam Rifles, Charities Aid Foundation, India and Wildlife Trust of India. This festival is celebrated with two main ideas in mind. The first is to create awareness among the people about the longest travelled migratory birds and to develop tourism sector of the district and the second is to promote the traditions and culture of the Dimasa people of the state. The festival is celebrated with great pomp and splendor including the cultural presentation of the local people in terms of music and traditional dances which saw participation of people from all ages from different parts of the district as well as from neighbouring state. With the success of the first Falcon Festival, it became an annual event which happens every year at Umrangso.

On 21st and 22nd November 2020 in a bid to boost tourism in Dima Hasao district and to create awareness about the bird's conservation, the North Cachar Hills Autonomous Council in collaboration with Dima Hasao Tourist department, Dima Hasao Forest department, District Information & public relation department along with Falcon Festival Organizing Committee organized the sixth edition of the Falcon festival at the Golf Course near Tumbung village at Umrangso. The two days festival included academic such as seminars, campaigns as well as cultural programs displaying the tribes living in Dima Hasao district. This programme was attended by hundreds of bird lovers from various parts of the state. Till date 8th Edition falcon festival has been organized.

Fig. 1 Amur falcon

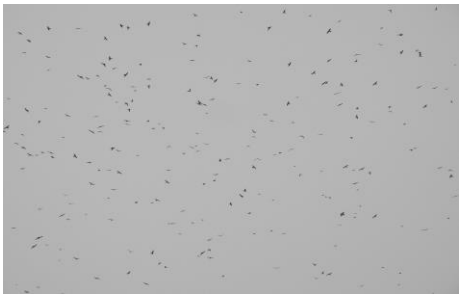


Fig. 2 Arrival of Amur falcons

Fig. 3 Falcon Festival

Fig. 4 Children participation in the Festival

REARING OF MUGA SILKWORM

Dr. Anurupa Goswami
Assistant Professor

Muga silkworm rearing is an age-old practice in Assam. These silkworms are semi domesticated in nature as only cocooning and grainage performed indoor and reared on outdoor host plants. Though the primary host plants of this silkworm are som (*Persea bombycina*) and soalu (*Litsea monopetala*) but many other secondary host plants like dighaloti (*Litsea salicifolia*), Mejankari (*Litsea cubeba*) etc. are also consumed by them. Rearing of muga silkworm done in six seasons round the year but mainly May-June and October- November are considered as commercially profitable crop season and rest of the seasons are seed crop season. This rearing practice is an environment-controlled affair due to outdoor process of doing it.

For rearing purpose, the seed cocoons are mainly collected from commercial rearers or from Government grainage farms. The cocoons are then placed in a single layer in trays to enable the moth emergence. The emerged moths are mated and the moth pair is tied securely with a cotton thread to an approximately 1.5 feet long stick made up of a dried straw locally known as "Kharika". The female moth lays approximately 150-250 eggs on the Kharika. Then the eggs are collected and disinfected with 2% Formalin for about 5 minutes to avoid any risk of getting diseases. Generally, the eggs hatch in the morning time in about 9-10 days. The newly hatched larva is transferred by a soft brush to the tender leaves of dwarf host plants. The rearing area host plants are covered with a big net to prevent the potential predators from harming the larva. The larvae instantly crawl to the leaves and feeding started. When the leaves are finished in a host plant, the larvae crawl down the plant and then they are collected on triangular bamboo sieves locally known as "Chaloni". The Chaloni's are hanged on a fresh host plant. To stop the silkworms from crawling down the ground, a band of straw with some ash is tied around the host plant trunk on 1-1.2 m above the ground. The muga silkworms are voracious eater and complete 4 moults to reach the matured phase. In the final phase, larvae develop into greenish blue in colour with prominent tubercles. The larval period takes 30-35 days. The 'ripe' or matured silkworms come down the plants in search of an appropriate place for spinning the cocoons. Then they are collected by rearers and kept in the baskets containing dry leaves to set a cocoonage known as "Jali" for spinning of the cocoons. The Jali's are then hung in a secure room and left undisturbed till cocoons are formed. Spinning of the cocoons takes about 2-3 days in summer and 5-6 days in winter.

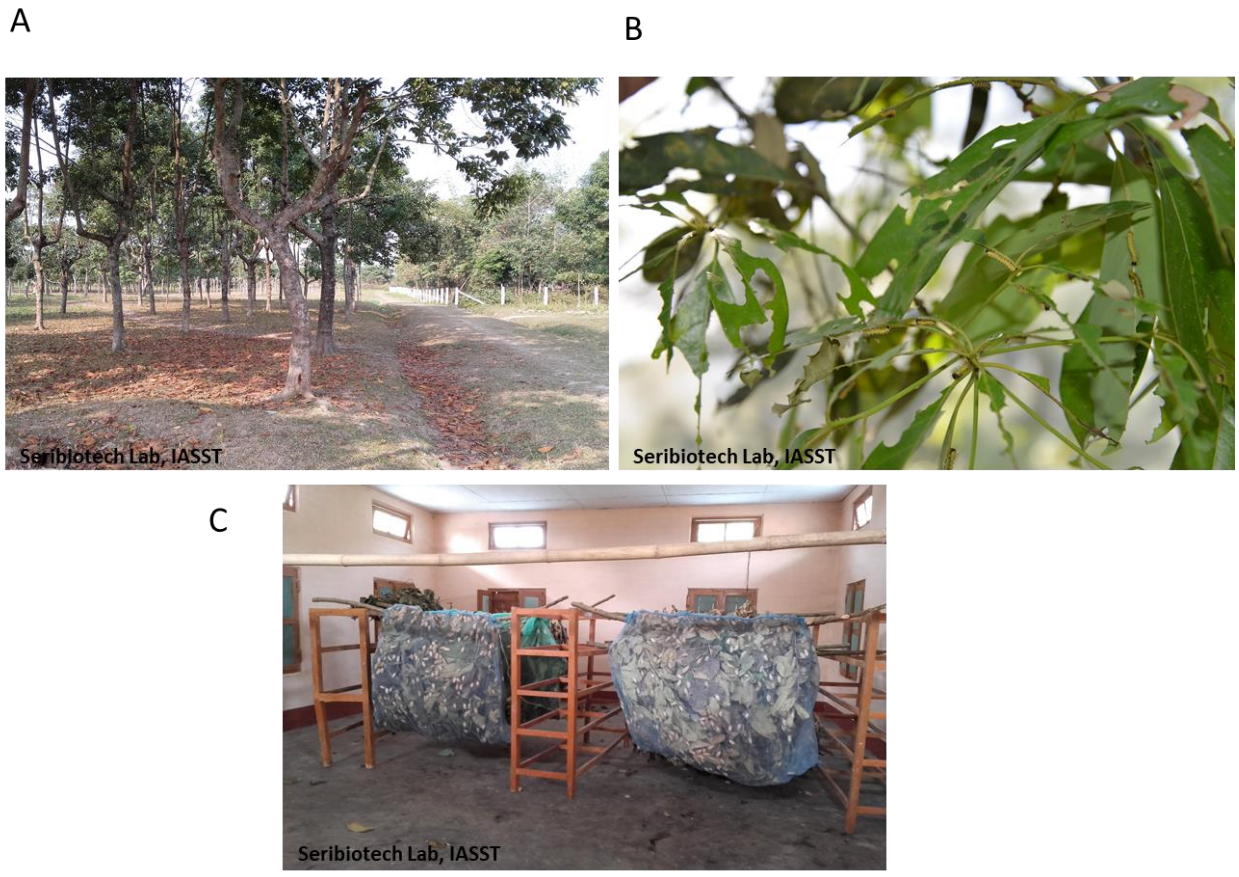


Fig: A. Som plantation for rearing of muga silkworm; B. Muga silkworms in a som plant during rearing; C. Cocooning

BIODIVERSITY AND CONSERVATION

Meghashree Borah
B Sc Zoology (H)

Biodiversity refers to the diversity of life- on a planet, in a watershed or in a single stream. It is often used to describe how many different species live in a certain area. But biodiversity can just as easily refer to diversity within a single species or diversity among the entire ecosystem. Conservation of biodiversity means the practice of protecting and preserving the wealth and variety of species, habitats, ecosystems and genetic diversity on the planet.

The word 'conservation' can evolve many powerful images. For some, it involves protecting forests and national parks. Some picture rescuing endangered species from extinction and others envision harvesting food, energy or water in a way that conserves them for future generations. Conservation at its core encompasses all these aspects and several others. Biodiversity provides us with various services like supply of oxygen to the surrounding, pollination of plants, pest control, wastewater treatment, cycling of nutrients and many ecosystem services.

It also provides us with some cultural services like recreational, spiritual benefits derived from ecosystems etc. These are the reasons why one should conserve biodiversity. If biodiversity of an area is not preserved it can lead to environmental disasters such as forest fires and floods. It can lead to soil erosion and habitat loss of plants and animals which ultimately will cause the extinction of many vulnerable species. Hence, to protect the critically endangered and other threatened animal and plant species, laws and policy initiatives, which are as follows-

- The central government has enacted the Wildlife (Protection) act, 1972. The act, inter alia, provides for the creation of protected areas for the protection of wildlife and also provides for punishment for hunting of specified fauna in the schedules (I) to (IV) thereof.
- The Wetland (Conservation and Management) Rules 2010 have been framed for the protection of wetland in the states.
- The centrally sponsored scheme of National Plan for Conservation of Aquatic Ecosystem also provides assistance to the states for the management of wetlands including Ramsar sites in the country.
- Wildlife Crime Control Bureau has been established for control of illegal trade in wildlife, including endangered species.
- Wildlife Institute of India, Bombay Natural History Society and Salim Ali Center for Ornithology and Natural History are some of the research organizations undertaking research on conservation of wildlife.

Apart from these government measures, many NGOs as well as many people are working hard in order to conserve biodiversity and preserve its aesthetic beauty along with the endangered species of plants and animals.

FROM THE PEA PLANT EXPERIMENT TO GENE EDITING: THE JOURNEY OF GENETICS

Ayaan Borthakur
B Sc Zoology (H)

Genetics, the study of heredity and the variation of inherited traits, has been a captivating field of scientific exploration for centuries. The journey of genetics has witnessed remarkable advancements, from Gregor Mendel's famous pea plant experiment to the revolutionary gene editing technologies of today. This article delves into the milestones of this captivating journey, highlighting the key discoveries and breakthroughs that have shaped our understanding of genetics.

Gregor Mendel, an Austrian monk, who is called the 'Father of Genetics', conducted a series of experiments with pea plants in the middle of the 19th century. Mendel carefully bred various pea plant varieties together and tracked the inheritance patterns in the progeny. He developed the inheritance rules, now known as Mendelian genetics. Through his research, basic ideas including dominant and recessive traits, as well as the ideas of segregation and independent assortment, were unearthed. Mendel's innovative study, however, received little attention during his lifetime; it wasn't until the early 20th century that it was acknowledged and established as the foundation for contemporary genetics. Building upon Mendel's work, scientists began to unravel the mysteries of chromosomes and genes.

In 1902, Walter Sutton and Theodor Boveri independently proposed the chromosomal theory of inheritance, which stated that genes are the units of heredity and are located on chromosomes. This breakthrough concept enabled people to get a deeper understanding of how traits are passed from one generation to the next one. This field of genetics received a very significant boost in the 1950s with the discovery of the structure of DNA. James Watson and Francis Crick, along with Maurice Wilkins and Rosalind Franklin, elucidated the double-helix structure of DNA, which revealed how genetic information is stored and transmitted. This discovery paved the way for the subsequent deciphering of the genetic code and also resulted in the birth of molecular genetics. In the 1940s, transposons were discovered which are known as the jumping genes by American geneticist Barbara McClintock, who conducted research on corn plants. Transposons are genetic elements that have the ability to move themselves within a genome. That means that certain genetic traits can appear or disappear in the corn plants. Today, as far as we know transposons play a very significant role in movement of genetic material, promoting genetic diversity and also influencing gene expressions. Marshal Nirenberg and Har Govind Khorana had significant roles to our understanding of genetic code. In 1960s, Nirenberg along with his colleagues conducted a series of experiments to determine the relationships of specific sequences of the nucleotides that encodes the particular amino acids. Meanwhile, around the same time Khorana and his team were working on synthesizing RNA molecules with specific nucleotides sequences. These studies were able to determine that there are sequences of three

nucleotides called codons that correspond to each of the twenty amino acids. The genetic code describes how the DNA and RNA sequences are translated into the protein sequences.

In the late 20th century, scientists achieved a major milestone with the completion of the Human Genome Project. The objective of this huge worldwide project was to sequence and map the whole human genome, which contains roughly three billion base pairs of DNA. The Human Genome Project, which was completed in 2003, produced a full catalogue of human genes and created the groundwork for a new era of genetic study.

We have become more proficient at modifying and editing genes as technology has improved. Genetics experienced a revolution in the 1970s following the introduction of recombinant DNA technology, frequently referred to as genetic engineering. As a result of being able to add, remove, or alter genes in different organisms, scientists are now better able to understand how genes work and to create novel biotech, agricultural, and medical applications. The creation of the ground-breaking gene editing tool CRISPR-Cas9 (CRISPR- Clustered Regulated Interspaced Short Palindromic Repeats) has been one of the most important developments in recent years. It has two components: Cas-9 which is a DNA cutting protein and an RNA molecule called the guide RNA. CRISPR-Cas9 accurately edits genes by utilising a built-in defence system present in bacteria. It performs the role of a pair of molecular scissors that can target particular genes and precisely alter their nucleotide sequence. This technology has opened up new possibilities for treating genetic diseases, engineering crops with enhanced traits, and advancing our understanding of gene function.

This journey consisting of research and development of genetics has also resulted in societal and ethical concerns. As our understanding of how to change DNA has increased, discussions about the morality of genetic engineering and gene editing have surfaced. Global conversations have been inspired by topics like designer babies, genetically modified organisms, and their unforeseen consequences. Genetic research and its uses continue to be the topic of continuing discussion and regulation over its ethical implications. The future of genetics holds a lot of promise for new discoveries and breakthroughs. Personalised healthcare based on a person's genetic profile is gradually becoming a reality as our knowledge of the human genome builds up. A promising approach to treating genetic illnesses, gene therapies provide an intriguing possibility of reversing disease-causing mutations. Gene therapies are emerging as a promising avenue for treating genetic disorders, offering the potential to correct disease-causing mutations. Additionally, advancements in gene editing technologies, such as base editing and prime editing, hold promise for even more precise and efficient gene modifications.

From Gregor Mendel's pioneering experiments with pea plants to the revolutionary gene editing technologies of today, the journey of genetics has been marked by curiosity, perseverance, and remarkable discoveries. The field continues to develop rapidly, transforming our understanding of life's building blocks and offering new routes for improving human health, agriculture and in many other fields.

THE SOCIAL LIFE OF HONEYBEES

Bipasha Deb
B Sc Zoology (H)

Nature teaches us the laws of a functioning society. The survival of life consists of breeding, communication, gathering food, and defence which are performed efficiently by several different species in nature. One such organisms which has established one of the most exceptional structures of society in terms of functions is the Honeybee. The social life is one of the most studied phenomena. The colony of honeybees consists of a queen, a few dozens male fertile bees called drones and a large number of female sterile bees called workers. Workers constitute the largest percentage of the colony. The Queen lays all the eggs for the colony. The normal fecundity of the queen is about 600 eggs per day. Fertilised eggs grow into females while unfertilised ones grow into males. The female larvae can be developed into queen by feeding them with royal jelly, the rest are fed pollen or honey to develop them into worker bees. The Queen secretes chemicals called pheromones to keep the colony together. The worker bees lick the secretions of the various glands of the Queen. These secretions are then passed to the other members of the colony through a process of food exchange called trophallaxis. The worker bees play a large role in the sustainability of the hive. They secret the royal jelly and feed it to the upcoming queen. They take care of the larvae, build the hive, become foragers to collect nectar, locate the food source, conversion of nectar to honey is done by them, secrete wax, and defend the hive.

One of the most fascinating aspects of their biology is the bee dance. The various forms of dances by the workers bees have been observed by scientists throughout years. They are round dance, sickle dance and waggle dance. These are performed by the worker bees in order to locate the food source. The round dance is performed when the food source is less than 50m, the sickle dance is performed when the food source is located at intermediate distance of 50–150m. The waggle dance is performed when the food source is located more than 150m from the hive. The bee dance is also performed when they plan to move to a different location to set up a new hive. Lastly, comes the drones. They are the fertile male bees which try to chase the queen during her nuptial flight. One of them gets to mate with the queen and dies in the process. The drones are then driven out of the hive once the queen gets fertilised. Another fascinating trait of the bee species is that bees have the capacity to reverse their age. This ability comes to play in times of destruction and threat to the hive and bee colony. They reverse age, rear a new queen, and repair the hive.

The honey bees have developed mechanisms to benefit the structure and functions of the hive. The evolution of their way of working together has given rise to a social life based on intelligence and efficiency. The hierarchy of the honey bees, the communication gestures such as secretions and dances, the systematic foraging for nectar and shelter, building of the hive and taking care of the larvae; all play a significant role in maintaining the social functioning of the hive in the right way.

COULD THE HUMAN BABY LOOK LIKE AN ALIEN?

Rajib Shah
B Sc Zoology (H)

THE SPACE! A place which surprises the whole human race since evolution the continuous things that fascinates the most is the uniqueness of the space, as how it looks, why it is dark and many more. Most of these questions and curiosities were solved by some of the legendary characters of the history like Sir Issac Newton, and Abert Eienstien. With all these curiosities 20th century marks some of the most innovative and exploring instruments and techniques that leads the concept of space exploration easier. Apart from all these the main milestone achieve by the humans were the "first steps of humans on the surface of Moon" which literally inspires so much that they start thinking that they are the species which could go beyond the concept of anything by their ability of problem solving. In most recent times they have proved this by building a'' Re-useable rockets'' to understand the importance of this development, just take an example- to send 1 kg of mass to the space, we have to spend almost US $ 18500 per kilogram but after the use of re-useable rockets it is reduced to the cost of same object up to 30% per kilogram.

These all developments were like stairs or connecting dots which build a pathway that could give humans a new tag from *homo sapeins* to interstellar species, and this is one of the biggest ambition of humans to settle its base on the planet like Mars and all. Wait guys! These all sounds like a script of some science fiction movies, but this is the reality and the major problem that is waiting for humans is the process of Reproduction, the concept of fertilization and the implantation of embryo in the uterus. Is this possible in anti- gravity space?

With the advancement of technology, some scientists have been paying attention to how to pregnancy in animal is affected in space. All sorts of critters, from salamanders to fish and rats with various projects abuzz exploring the possibility of future space colonies, there is a chance that one- day human might actually give birth in Zero gravity. What would that be like? Would it have any effect on development? Some scientists believe that space babies would have a few very distinguishing features.

Is it even possible to conceive in space?

Conceiving a baby in zero gravity could be very challenging for us, if somehow implantation was successful, other problems would continue to arise. If space conception somehow achieved a successful fertilization, we now need to worry about the maturation of the embryo, there could be a problem. At the time of birth, the mother will also be at a major health risk due to the loss of bone density caused by the absence of gravity. Studies shows that for every month spent in space Astronauts lose 1% to 2% of their bone density. This is especially dangerous for childbearing at the pelvis could probably fracture during the process of delivery. During such condition normal delivery is not possible!

Appearance of physical changes

It is a known fact that the way we gave birth has a significant influence on our anatomy. For instance, the size of our heads is restricted by the size of our mother's birth canal. Therefore, the use of more C-sections, our descendants head would be larger due to lack of restriction. Normally on Earth, all the fluids in our bodies are pulled downward due to the impact of gravity. Since it is not possible in space, so it is speculated that space babies would develop bloated bodies and puffy faces, their blood pressure would also increase in the upper body part due to zero gravity, which results in bulging of eyes, and losing their brain efficiency, paler skin and weakness could also occur due to loss in blood content as the atrophies while not having to work against gravity.

Lastly some more speculative points are there like-

- Space babies could possibly have a new type of skin pigment, this is due to the UV radiation coming from which adversely affect humans health, Specially skin of baby as there is no such protection shield present like Ozone in Earth.

So, summing up all the changes, a baby might be like the alien emoji i.e Bigger heads, Bulging eyes, Deformed bones, and totally different skin colour would be quite enough for us Earthling to consider them almost a new species! With the current pace of technology, migrating to space is very much within reach. Time to mentally prepare for a future family that is truly out of this world.

APPLICATION OF CRISPR GENE EDITING IN TRANSLATIONAL MEDICINE

Swagat Baruah
B Sc Zoology (H)

Abstract

Translational medicine and CRISPR gene editing technology has recently gained a lot of attention, due to which, significant research and clinical trials have been conducted in this field. This review records the latest research and development work in CRISPR gene editing. Resources of NCBI and PubMed were used to briefly understand the field of diagnosis and treatment and where CRISPR fits in it. The limitations of CRISPR gene editing depends on different factors such as specificity, PAM sequence and delivery, off-target mutations, and Gene-toxicity. Besides having its own advantages and drawbacks, CRISPR have the potential to improve its shortcomings and could be seen as a viable technology in biomedical research and application.

Key words: Biomedical, CRISPR, Diagnostics, Gene editing, Translational Medicine, Treatment

Introduction

Every new discovery and research start with scientists trying to answer questions about nature followed by complex experiments in the laboratory finally giving us some meaningful results. This laboratory research takes years to get translated into clinical applications resulting in new methods of diagnosis, treatment, or prevention (Jia, 2016). This is where Translational medicine plays a significant role. According to the European Society of Translational Medicine (EUSTM) (Cohrs et al., 2014), translational research is an interdisciplinary branch of a biomedical field with three main pillars: bench-side, bedside, and community. Translational medicine "translates" the basic science research and applies it to medical practices and enhances human health and well-being. The concept of translational medicine has been there for a long time but only recently gained popularity and now has a wide array of different research under its name.

CRISPR (clustered regularly **interspaced short palindromic repeats**) gene editing is another research topic that has gained popularity. CRISPR was initially observed in different parts of the world as an accident and was not given that much attention (Ishino et al., 1987; van Soolingen et al., 1993). The scientists observed clusters of repeated sequences which were unusual. A repeated strand is generally continuous with no other sequences interrupting it (Mojica et al., 2000). Mojica continued the studies and proposed the acronym CRISPR. Tang et al. (2002) showed in *Streptococcus thermophilus* that CRISPR sequences were being transcribed in RNA strands which were further processed to form unit-length RNAs. Jansen observed the CRISPR sequences were accompanied by a set of homologous genes called CRISPR-associated enzymes or Cas enzymes. He observed that Cas enzymes had helicase and nuclease motifs, which formed

the dynamic structure of CRISPR loci. Three independent groups of researchers in the year 2005 (Bolotin et al., 2005; Mojica et al., 2005; Pourcel et al., 2005) found that spacers of the sequence were DNA fragments of the bacteriophages that tried to infect the bacteria, which hinted at a potential source of adaptive immunity in bacteria.

Mojica first proposed the role of CRISPR-Cas immunity in bacteria and predicted the role of transcribed spacer RNA in targeting specific sequences. His studies were further extended by Makarova, who proposed mechanisms of actions of different CRISPR-Cas subtypes (Makarova et al., 2006). Later experiments were conducted with a simpler CRISPR system in *Streptococcus pyogenes* that relies on the Cas9 enzyme (Deltcheva et al., 2011). The major breakthrough came when Doudna and Charpentier re-engineered the Cas9 enzyme (Jinek et al., 2012) from a 4-component system to a 2-component system by fusing two RNA strands (crRNA and tracrRNA) into a single-guide RNA (gRNA) which when combined with Cas9 enzyme can locate and cut the target DNA sequence complementary to the gRNA. The nucleotide sequence could be manipulated, and the programmed Cas9 protein could target any DNA sequence for cleavage.

Table 1 Advantages and disadvantages of gene editing nucleases (González-Romero, Martínez-Valiente et al. 2019)

Gene editing nucleases	Advantages	Disadvantages	Main dates
Meganucleases	• Large recognition site for DNA • Highly specific • DSB repaired by HDR or NHEJ	• Very low design flexibility • Low specificity of the enzyme/off-target possibility	1988
ZFN	• Possibility of engineering nucleases • Highly efficient • DSB repaired by HDR or NHEJ • Biallelic changes are possible	• Off-target effect possible but less than with CRISPR • Still harder to design than TALEN	1996
TALEN	• Easy design than ZFN • Highly efficient • DSB repaired by HDR or NHEJ • Biallellic changes obtained with efficiency • Works in different cell types and species	• Off-target effect possible but less than with CRISPR • Still harder to design than CRISPR	2009
CRISPR/Cas9	• Easy design and optimization • Highest efficiency • DSB repaired by HDR or NHEJ • Biallellic changes obtained with efficiency • Works in different cell types and species	• More off-target effects than TALEN and ZFN (though there are ways to reduce them dramatically) • PAM sequence limits target selection (though, many CRISPR systems available, and more to come)	2013

Researchers were able to program the Cas9 protein and repurpose it for other purposes. Mutating the Cas9 enzyme makes it inactive, but still able to bind to the target site. The inactive protein was named dead Cas9 (dCas9) (Qi et al., 2013). dCas9 allowed researchers to perform gene screening, silencing, and activation (Konermann et al., 2015). It can also be fused with other RNPs, fluorescent proteins, inhibitors, and other proteins to perform numerous desired effects such as CRISPR inference (CRISPRi) similar to RNAi (Gilbert et al., 2013). dCas9 with fluorescently labelled CRISPR components can also be used to study epigenetic modifications (Polstein and Gersbach, 2015).

CRISPR gene editing has been preferred over other gene editing techniques such as TALENs (Transcription- activator-like effector nucleases) and Zinc Finger Nucleases (ZFNs) because the latter ones are expensive, slow, and difficult in comparison to CRISPR (González-Romero et al., 2019). It is also very reliable and flexible in comparison to other techniques. CRISPR-Cas system also provides scaling far beyond what ZFNs and TALENs can offer (Table 1). Due to all these reasons, thousands of research articles based on CRISPR are listed in PubMed showing the high adaptability of the technique in different species and applications. Through this study, an effort has been made to list the research and experiments that are already being adapted or have a high potential to be applied in translational medicine for diagnosis and treatment. This review also highlights the limitations that still exists for CRISPR-Cas system during clinical trials.

Biomedical Applications

Diagnosis

Current diagnosis of diseases at the genetic level includes the detection of single nucleotide biomarkers associated with the disease, by the methods such as quantitative polymerase chain reaction (qPCR). It enables highly specific gene detection by amplification of a trace amount of sequence. The versatility, robustness, and sensitivity of PCR allowed it to be used in laboratories for the detection of DNA or RNA biomarkers, helpful in detecting viral and bacterial infections (Yang and Rothman, 2004; Kubista et al., 2006; Bustin et al., 2009). The recent application includes the detection of SARS-CoV-2 (Santiago-Frangos et al., 2021a). However, non-specific amplification can result in lower detection specificity. The reagents used in PCR are expensive and require sophisticated laboratories and experienced technicians (Mahony et al., 2009). CRISPR-based diagnostics have the potential to combine the ease of use and cost efficiency of isothermal amplification with diagnostic accuracy down to the specificity of a single nucleotide.

Table 2 Summary of CRISPR-Cas systems (Xu and Li 2020)

Class	Type	Subtype	Effector	Target	Nuclease domains	TracrRNA requirement	PAM/PFS
1 (multi-Cas proteins)	I	A, B, C, D, E, F, U	Cascade	dsDNA	HD fused to Cas3	No	–
1	III	A, B, C, D	Cascade	ssRNA	HD fused to Cas10	No	–
1	IV	A, B	Cascade	dsDNA	unknown	No	–
2 (single-Cas protein)	II	A	SpCas9	dsDNA	RuvC, HNH	Yes	NGG
2	II	A	SaCas9	dsDNA	RuvC, HNH	Yes	NNGRRT
2	II	B	FnCas9	dsDNA/ssRNA	RuvC, HNH	Yes	NGG
2	II	C	NmCas9	dsDNA	RuvC, HNH	Yes	NNNNGATT
2	V	A	Cas12a (Cpf1)	dsDNA	RuvC, Nuc	No	5′ AT-rich PAM
2	V	B	Cas12b (C2c1)	dsDNA	RuvC	Yes	5′ AT-rich PAM
2	V	C	Cas12c (C2c3)	dsDNA	RuvC	Yes	5′ AT-rich PAM
2	VI	A	Cas13a (C2c2)	ssRNA	2xHEPN	No	3′PFS: non-G
2	VI	B	Cas13b (C2c4)	ssRNA	2xHEPN	No	5′PFS: non-C; 3′PFS:NAN/NNA
2	VI	C	Cas13c (C2c7)	ssRNA	2xHEPN	No	–
2	VI	D	Cas13d	ssRNA	2xHEPN	No	–

CRISPR-Cas systems are currently classified into 2 classes, 6 subtypes, and several subtypes (Makarova et al., 2015). Class 2 systems are simpler to reconstitute because of which they have primarily been applied for diagnostics. The collateral activity of enzymes acts as the fundamental of many CRISPR-based diagnostic assays. Type III effector nuclease Csm6 or Cas10 (class 1

system) have also been engineered to work with components of class 2 or with the native type III complex for diagnostics (Gootenberg et al., 2018a; Santiago-Frangos et al., 2021). A summary of CRISPR-Cas systems is depicted in Table 2.

NASBACC [nucleic acid sequence-based amplification (NASBA)-CRISPR cleavage] is based on Cas-9 and is used for the detection of Zika virus (ZIKV) in infected monkey plasma. It combines nucleic acid sequence-based amplification for the isothermal preamplification of targets (Walker et al., 1992a), Cas9 cleavage for PAM-dependent target detection, and a toehold sensor for the readout. First, a toehold trigger is integrated into the NASBA-amplified RNA fragment by reverse transcription. If Cas9-mediated cleavage leads to a truncated RNA without the trigger sequence, it indicates the presence of a PAM sequence in the RNA fragment; if absent, the trigger containing full-length RNA activates the toehold switch, detected as a colour change. By sensing the strain-specific PAM sites, it detected ZKIV (Compton, 1991; Pardee et al., 2016).

Diagnostic methods such as SHERLOCK/ SHERLOCKv2 (specific high-sensitivity enzymatic reporter unlocking) and DETECTR (DNA endonuclease-targeted CRISPR trans reporter) are based on CRISPR type V (Cas12) and type VI (Cas13) systems, which is different from type II as it has the ability to trigger non-specific collateral cleavage (trans cleavage) on target recognition. The CRISPR-Cas system detects the target DNA (Cas12) or target RNA (Cas13), by cis-conformation after which the collateral activity of the Cas enzymes allows the cleavage of non-targeted single-stranded DNA (ssDNA; Cas12) or single-stranded RNA (ssRNA; Cas13) by trans-conformation. This enables the sensing of nucleic acids through signal amplification and allows various readouts through the addition of functionalized reporter nucleic acids (Abudayyeh et al., 2016; East-Seletsky et al., 2016). In the SHERLOCK assay (based on Cas13), using Recombinase Polymerase Amplification (RPA) or Reverse Transcription RPA (RT-RPA), DNA or RNA is first isothermally amplified. A forward primer is also used that adds a T7 promoter to the amplicon. This promoter allows reverse transcription of the target. A Cas13a- crRNA will recognize and bind to the target having the complementary sequence to the crRNA strand. The activated Cas13 will cleave the on-target RNA sequence by cis-cleavage, in a target dependant manner, and will cleave the ssRNA reporter molecules by trans-cleavage. The ssRNA reporter molecule consists of a fluorophore and a quencher linked together by a short RNA, which when cleaved separates the fluorophore from the quencher, resulting in fluorescence. SHERLOCK enables the detection of viral RNA, bacterial DNA, Human Single nucleotide polymorphism (SNPs), and cancer-associated mutations with attomolar (10^{-18} M) sensitivity. The SHERLOCKv2 increases this sensitivity even further with target detection at zeptomolar (10^{-21}M) concentrations (Gootenberg et al., 2018b; Kellner et al., 2019). Figure 1 shows the two strategies for CRISPR-based diagnostics.

DETECTR (DNA endonuclease-targeted CRISPR trans reporter**)** was one of the earliest CRISPR-based diagnostic assays using Cas12. DETECTR is designed to target dsDNA with a

complimentary crRNA. Once recognized and paired with the target dsDNA, it triggers the collateral cleavage of the reporter molecule carrying fluorophore, a quencher, and ssDNA. Like SHERLOCK, target detection and cis-cleavage also trigger the trans-cleavage of reporter molecules which results in fluorescence (Chen et al., 2018; Yan et al., 2019). CRISPR-based diagnostics have also been applied to non-infectious diseases. It can detect RNA relevant to the disease-causing genes. For example, with a sensitivity of 93% and a specificity of 76%, CRISPR-Cas13a-based sensing of human CXCL9 mRNA was used to detect acute cellular kidney-transplant rejection from 31 Urinary cell pellets (Kaminski et al., 2020). CRISPR has also been used to detect miRNA. For example, the detection of miR-19b in serum samples of patients with medulloblastoma using Cas13, miR-17 for breast cancer using Cas13, and miR-21 using Cas12 (Bruch et al., 2019; Shan et al., 2019; Wang et al., 2020).

Treatment

The treatments of infectious and non-infectious (monogenic, autoimmune, cancer, etc) majorly consist of pharmaceutical drugs and medicines. Which are capable of slowing down the diseases but don't have the ability to cure them. Gene editing has the potential to cure diseases that are generally incurable. Gene editing facilitates the treatment via gene therapy. Due to ethical and security concerns, only somatic cell gene therapy is practiced (Gao et al., 2019). Target cells are either genetically modified in vivo or in vitro. However, the prior one is difficult to execute and the success rate is very low compared to the latter. Homologous gene recombination or lentiviral delivery was used in traditional gene therapy (Krejci et al., 2012; Cai and Giehm Mikkelsen, 2016). CRISPR gene editing is now currently used instead due to low cost, higher specificity, and success rates. The methodology is similar to that of diagnostics, but a different set of Cas proteins, and delivery methods are used in gene therapy (Sahel et al., 2019). Different types of viral vectors have also been used for delivering and the selection depends on the size of the delivery. Non-viral vectors such as Polymeric vectors such as HPAE-EB, provide an alternative to due comparatively large packaging capacity (O'Keeffe Ahern et al., 2022).

Mutations of the DMD gene (encoding dystrophin) an X-linked recessive disorder, causes the inhibition of muscle isoform dystrophin (Dp427m) production resulting in progressive muscle wasting (Duan et al., 2021). Premature termination or frameshift mutations in DMD results in non-functional and unstable dystrophin production. Mutations occur when one or more exons of the DMD gene are deleted hence normal dystrophin is not produced (Nelson et al., 2016). Using the CRISPR/Cas9 method a functional shortened dystrophin protein is produced by removing the mutant transcripts. Generation of a single large deletion can rectify up to 62% of DMD mutations (Ousterout et al., 2015; Nelson et al., 2019; Moretti et al., 2020).

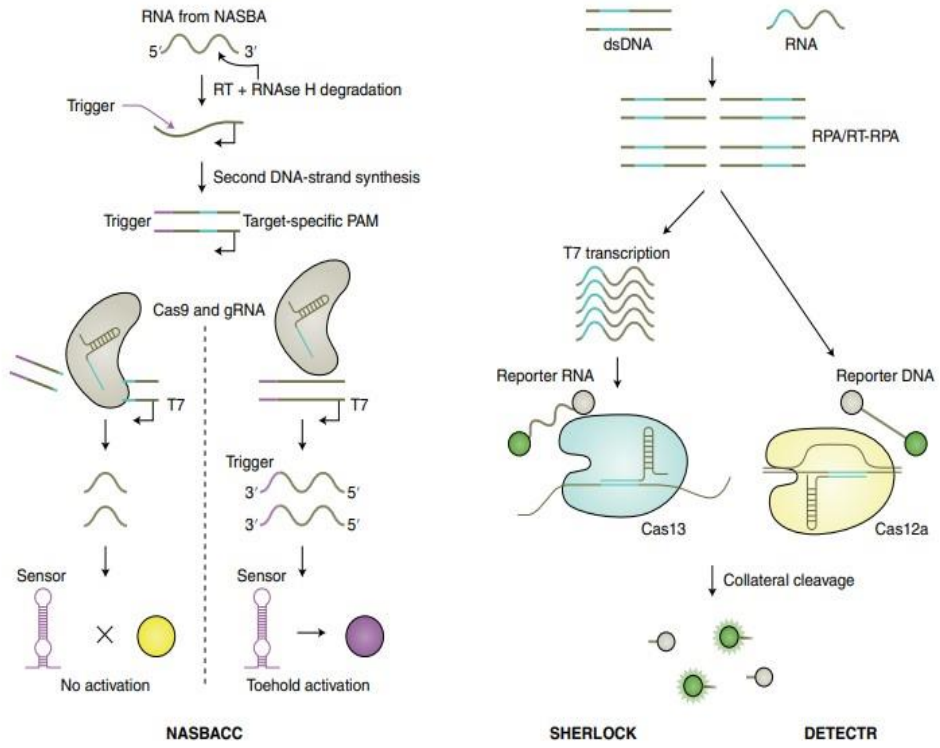


Fig. 1 | Two strategies for CRISPR-based diagnostics. Left: RNA targets are amplified through NASBA, which starts with reverse transcription (RT) to complementary DNA using a sequence-specific primer that appends a trigger sequence (magenta) for the toehold sensor. The RNA from the RNA/DNA hybrid is destroyed by RNase H, enabling a primer that contains a T7 promoter to bind and create a complementary second DNA strand. T7 transcription of the dsDNA template creates the target RNA sequence, which can be used as starting material for a new NASBA cycle or detected by the toehold sensor. If a PAM sequence (blue) is present in the dsDNA amplicon, Cas9-mediated cleavage leads to a truncated template for T7 transcription, which generates a shorter target RNA that cannot activate the toehold sensor. In the absence of the PAM sequence, a full-length target RNA containing the trigger is transcribed, which activates the toehold sensor and produces a visible change in colour. Right: DNA or RNA are amplified by RPA or RT-RPA, respectively. For RNA-targeting CRISPR enzymes (including Cas13a), the amplified RPA product is T7-transcribed into RNA. Binding of the crRNA to the complementary target sequence activates the Cas enzyme and triggers collateral cleavage of quenched fluorescent reporters. Thereby, Cas13a (used in SHERLOCK) or Cas12a (used in DETECTR) indicate the presence of RNA or DNA target sequences, respectively.

Sickle cell anaemia disorder (SCD) occurs due to point mutation of the haemoglobin- β at the 6th position changing the codon from glutamic acid to valine; resulting in abnormal haemoglobin (HbS). It causes the erythrocytes to become stiff and sickle-shaped. The RBCs pile up in the blood vessels and lead to serious organ and tissue damage. An evidence-based study suggested that CRISPR is safe for treating SCD by correcting the β-globin gene mutation or reactivating HbF expression (Sato et al., 2016; Tasan et al., 2016; Demirci et al., 2019). In another study, cellular functional reinstitution was achieved by using CRISPR/Cas9-based system for HbS gene correction in hematopoietic stem and progenitor cells (HSPCs) of sickle cell anaemia patients (Wen et al., 2017).

Studies on the treatment of viral infectious diseases based on the CRISPR/ Cas9 system also has been conducted. In the case of HIV, HIV-1 can be completely eradicated from the genome of infected host cells by targeting long terminal repeats (LTR). Additionally, resistance towards HIV-1 infection can be generated in CD4 T-cells by knocking down the HIV co-receptors CCR5 gene (Doitsh et al., 2014). Earlier cancer studies documented the link between cancer and

autoimmune diseases. In contrast, many autoimmune diseases and immunosuppressant therapy, have been related to an increased risk of cancer (Giat et al., 2017). CRISPR has been to use to correct oncogenic mutations in tumour cells and animal models, resulting in tumour cell growth inhibition and cell apoptosis, therefore tumour growth control.

Immunotherapy, particularly Chimeric antigen receptor – T (CAR-T) has been modified into universal CAR-T cells by simultaneously deleting the native T-cell receptor gene and the HLA class 1 expressing a gene from T cells from healthy donors. This allowed it to be used in a variety of patients without triggering graft vs host disease (GVHR). CRISPR has also been used to knock down genes encoding signalling molecules or T-cell inhibitory receptors such as programmed cell death protein (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) which improved the CAR-T cell performance (Lee et al., 2022).

Limitations

One of the significant limitations is the off-target gene mutations. Comparatively CRISPR is more precise than the other gene editing methods; it still has a high frequency of off-target effects (OTEs) which have been observed at a frequency of ≥50% (Zhang et al., 2015). Attempts have been made to reduce off-targets effects of RNA-guided Endonucleases (RGENs) below the detection limits of deep sequencing by choosing unique target sequences in the genome and modifying both the RNA and Cas protein (Cho et al., 2014).

Requirement of Protospacer Adjacent Motif (PAM) near the target site has also been a limitation. One of the most extensively used Cas9 is SpCas9 variant derived from *Streptococcus pyogenes* which have a relatively short canonical PAM recognition site: 5'NGG3', where N is any nucleotide. However due to its large size it is difficult to pack it in AAV vectors, which is the most common vector for gene therapy (Lau and Suh, 2017; Lino et al., 2018). On the other hand, Cas9 variant SaCas9 derived from *Streptococcus aureus* can be easily packed in AVV vector but have a larger PAM sequence: 5'NNGRRT3' or 5'NNGRR(N)3' where R is any purine, which further narrows the window of therapeutic targeting sites.

The DSBs induced by CRISPR can often trigger apoptosis rather than gene edit (Hu et al., 2014). It was also revealed that when using this tool with human pluripotent stem cells (hPSCs), p53 activation in response to the toxic DSBs introduced by CRISPR often triggers subsequent apoptosis (Ihry et al., 2018). The use of catalytically inactive dCas9 can transiently manipulate expression of specific genes without introducing DSBs through fusion of transcriptional activating or repressing domains or proteins to the DNA-binding effector (Xu and Qi, 2019).

Conclusions

With all the advantages and limitations of CRISPR we can conclude that it still has scopes of improvement, in regards to specificity, efficiency, PAM size, toxicity, off-target mutations. It has a lot of potential in the field of Translational Medicine with diagnosis and treatments. Due to

ethical reasons, scientists and researchers have to take extreme precautions while carrying out clinical trials. However, the application of CRISPR in the field of diagnostics has the highest scope of implementation in biomedical fields as it does not have to follow the strict protocols as gene editing based treatment. It does not have to deal with catastrophic side effects, such as off-target mutations or cell apoptosis leading to cancer in patients.

References

Abudayyeh, O. O., Gootenberg, J. S., Konermann, S., Joung, J., Slaymaker, I. M., Cox, D. B. T., Shmakov, S., Makarova, K. S., Semenova, E., Minakhin, L., Severinov, K., Regev, A., Lander, E. S., Koonin, E. v., & Zhang, F. (2016). C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science*, *353*(6299). https://doi.org/10.1126/science.aaf5573

Bolotin, A., Quinquis, B., Sorokin, A., & Ehrlich, S. D. (2005). Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. *Microbiology*, *151*(8), 2551–2561. https://doi.org/10.1099/mic.0.28048-0

Bruch, R., Baaske, J., Chatelle, C., Meirich, M., Madlener, S., Weber, W., Dincer, C., & Urban, G. A. (2019). CRISPR/Cas13a-Powered Electrochemical Microfluidic Biosensor for Nucleic Acid Amplification-Free miRNA Diagnostics. *Advanced Materials*, *31*(51), 1905311. https://doi.org/10.1002/adma.201905311

Bustin, S. A., Benes, V., Garson, J. A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M. W., Shipley, G. L., Vandesompele, J., & Wittwer, C. T. (2009). The MIQE Guidelines: Minimum Information for Publication of Quantitative Real-Time PCR Experiments. *Clinical Chemistry*, *55*(4), 611–622. https://doi.org/10.1373/clinchem.2008.112797

Cai, Y., & Giehm Mikkelsen, J. (2016). Lentiviral Delivery of Proteins for Genome Engineering. *Current Gene Therapy*, *16*(3), 194–206. https://doi.org/10.2174/1566523216666160527143702

Chen, J. S., Ma, E., Harrington, L. B., da Costa, M., Tian, X., Palefsky, J. M., & Doudna, J. A. (2018). CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science*, *360*(6387), 436–439. https://doi.org/10.1126/science.aar6245

Cho, S. W., Kim, S., Kim, Y., Kweon, J., Kim, H. S., Bae, S., & Kim, J.-S. (2014). Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases. *Genome Research*, *24*(1), 132–141. https://doi.org/10.1101/gr.162339.113

Cohrs, R. J., Martin, T., Ghahramani, P., Bidaut, L., Higgins, P. J., & Shahzad, A. (2014). Translational Medicine definition by the European Society for Translational Medicine. *European Journal of Molecular & Clinical Medicine*, *2*(3), 86. https://doi.org/10.1016/j.nhtm.2014.12.002

Compton, J. (1991). Nucleic acid sequence-based amplification. *Nature*, *350*(6313), 91–92. https://doi.org/10.1038/350091a0

Deltcheva, E., Chylinski, K., Sharma, C. M., Gonzales, K., Chao, Y., Pirzada, Z. A., Eckert, M. R., Vogel, J., & Charpentier, E. (2011). CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, *471*(7340), 602–607. https://doi.org/10.1038/nature09886

Demirci, S., Leonard, A., Haro-Mora, J. J., Uchida, N., & Tisdale, J. F. (2019). *CRISPR/Cas9 for Sickle Cell Disease: Applications, Future Possibilities, and Challenges* (pp. 37–52). https://doi.org/10.1007/5584_2018_331

Doitsh, G., Galloway, N. L. K., Geng, X., Yang, Z., Monroe, K. M., Zepeda, O., Hunt, P. W., Hatano, H., Sowinski, S., Muñoz-Arias, I., & Greene, W. C. (2014). Cell death by pyroptosis drives CD4 T-cell depletion in HIV-1 infection. *Nature*, *505*(7484), 509–514. https://doi.org/10.1038/nature12940

Duan, D., Goemans, N., Takeda, S., Mercuri, E., & Aartsma-Rus, A. (2021). Duchenne muscular dystrophy. *Nature Reviews Disease Primers*, *7*(1), 13. https://doi.org/10.1038/s41572-021-00248-3

East-Seletsky, A., O'Connell, M. R., Knight, S. C., Burstein, D., Cate, J. H. D., Tjian, R., & Doudna, J. A. (2016). Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature*, *538*(7624), 270–273. https://doi.org/10.1038/nature19802

Gao, Q., Dong, X., Xu, Q., Zhu, L., Wang, F., Hou, Y., & Chao, C. (2019). Therapeutic potential of CRISPR/Cas9 gene editing in engineered T-cell therapy. *Cancer Medicine*, *8*(9), 4254–4264. https://doi.org/10.1002/cam4.2257

Giat, E., Ehrenfeld, M., & Shoenfeld, Y. (2017). Cancer and autoimmune diseases. *Autoimmunity Reviews*, *16*(10), 1049–1057. https://doi.org/10.1016/j.autrev.2017.07.022

Gilbert, L. A., Larson, M. H., Morsut, L., Liu, Z., Brar, G. A., Torres, S. E., Stern-Ginossar, N., Brandman, O., Whitehead, E. H., Doudna, J. A., Lim, W. A., Weissman, J. S., & Qi, L. S. (2013). CRISPR-Mediated Modular RNA-Guided Regulation of Transcription in Eukaryotes. *Cell*, *154*(2), 442–451. https://doi.org/10.1016/j.cell.2013.06.044

González-Romero, E., Martínez-Valiente, C., García-Ruiz, C., Vázquez-Manrique, R. P., Cervera, J., & Sanjuan-Pla, A. (2019). CRISPR to fix bad blood: a new tool in basic and clinical hematology. *Haematologica*, *104*(5), 881–893. https://doi.org/10.3324/haematol.2018.211359

Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., Joung, J., Collins, J. J., & Zhang, F. (2018a). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, *360*(6387), 439–444. https://doi.org/10.1126/science.aaq0179

Gootenberg, J. S., Abudayyeh, O. O., Kellner, M. J., Joung, J., Collins, J. J., & Zhang, F. (2018b). Multiplexed and portable nucleic acid detection platform with Cas13, Cas12a, and Csm6. *Science*, *360*(6387), 439–444. https://doi.org/10.1126/science.aaq0179

Hu, Z., Yu, L., Zhu, D., Ding, W., Wang, X., Zhang, C., Wang, L., Jiang, X., Shen, H., He, D., Li, K., Xi, L., Ma, D., & Wang, H. (2014). Disruption of HPV16-E7 by CRISPR/Cas System Induces Apoptosis and Growth Inhibition in HPV16 Positive Human Cervical Cancer Cells. *BioMed Research International*, *2014*, 1–9. https://doi.org/10.1155/2014/612823

Ihry, R. J., Worringer, K. A., Salick, M. R., Frias, E., Ho, D., Theriault, K., Kommineni, S., Chen, J., Sondey, M., Ye, C., Randhawa, R., Kulkarni, T., Yang, Z., McAllister, G., Russ, C., Reece-Hoyes, J., Forrester, W., Hoffman, G. R., Dolmetsch, R., & Kaykas, A. (2018). p53 inhibits CRISPR–Cas9 engineering in human pluripotent stem cells. *Nature Medicine*, *24*(7), 939–946. https://doi.org/10.1038/s41591-018-0050-6

Ishino, Y., Shinagawa, H., Makino, K., Amemura, M., & Nakata, A. (1987). Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *Journal of Bacteriology*, *169*(12), 5429–5433. https://doi.org/10.1128/jb.169.12.5429-5433.1987

Jia, X. (2016). Translational Medicine: Creating the Crucial Bidirectional Bridge between Bench and Bedside. *International Journal of Molecular Sciences*, *17*(11), 1918. https://doi.org/10.3390/ijms17111918

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A Programmable Dual-RNA–Guided DNA Endonuclease in Adaptive Bacterial Immunity. *Science*, *337*(6096), 816–821. https://doi.org/10.1126/science.1225829

Kaminski, M. M., Alcantar, M. A., Lape, I. T., Greensmith, R., Huske, A. C., Valeri, J. A., Marty, F. M., Klämbt, V., Azzi, J., Akalin, E., Riella, L. v., & Collins, J. J. (2020). A CRISPR-based assay for the detection of opportunistic infections post-transplantation and for the monitoring of transplant rejection. *Nature Biomedical Engineering*, *4*(6), 601–609. https://doi.org/10.1038/s41551-020-0546-5

Kellner, M. J., Koob, J. G., Gootenberg, J. S., Abudayyeh, O. O., & Zhang, F. (2019). SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nature Protocols*, *14*(10), 2986–3012. https://doi.org/10.1038/s41596-019-0210-2

Konermann, S., Brigham, M. D., Trevino, A. E., Joung, J., Abudayyeh, O. O., Barcena, C., Hsu, P. D., Habib, N., Gootenberg, J. S., Nishimasu, H., Nureki, O., & Zhang, F. (2015). Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature*, *517*(7536), 583–588. https://doi.org/10.1038/nature14136

Krejci, L., Altmannova, V., Spirek, M., & Zhao, X. (2012). Homologous recombination and its regulation. *Nucleic Acids Research*, *40*(13), 5795–5818. https://doi.org/10.1093/nar/gks270

Kubista, M., Andrade, J. M., Bengtsson, M., Forootan, A., Jonák, J., Lind, K., Sindelka, R., Sjöback, R., Sjögreen, B., Strömbom, L., Ståhlberg, A., & Zoric, N. (2006). The real-time polymerase chain reaction. *Molecular Aspects of Medicine*, *27*(2–3), 95–125. https://doi.org/10.1016/j.mam.2005.12.007

Lau, C.-H., & Suh, Y. (2017). In vivo genome editing in animals using AAV-CRISPR system: applications to translational research of human disease. *F1000Research*, *6*, 2153. https://doi.org/10.12688/f1000research.11243.1

Lee, M. H., Shin, J. il, Yang, J. W., Lee, K. H., Cha, D. H., Hong, J. B., Park, Y., Choi, E., Tizaoui, K., Koyanagi, A., Jacob, L., Park, S., Kim, J. H., & Smith, L. (2022). Genome Editing Using CRISPR-Cas9 and Autoimmune Diseases: A Comprehensive Review. *International Journal of Molecular Sciences*, *23*(3), 1337. https://doi.org/10.3390/ijms23031337

Lino, C. A., Harper, J. C., Carney, J. P., & Timlin, J. A. (2018). Delivering CRISPR: a review of the challenges and approaches. *Drug Delivery*, *25*(1), 1234–1257. https://doi.org/10.1080/10717544.2018.1474964

Mahony, J. B., Blackhouse, G., Babwah, J., Smieja, M., Buracond, S., Chong, S., Ciccotelli, W., O'Shea, T., Alnakhli, D., Griffiths-Turner, M., & Goeree, R. (2009). Cost Analysis of Multiplex PCR Testing for Diagnosing Respiratory Virus Infections. *Journal of Clinical Microbiology*, *47*(9), 2812–2817. https://doi.org/10.1128/JCM.00556-09

Makarova, K. S., Grishin, N. v, Shabalina, S. A., Wolf, Y. I., & Koonin, E. v. (2006). A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. *Biology Direct*, *1*(1), 7. https://doi.org/10.1186/1745-6150-1-7

Makarova, K. S., Wolf, Y. I., Alkhnbashi, O. S., Costa, F., Shah, S. A., Saunders, S. J., Barrangou, R., Brouns, S. J. J., Charpentier, E., Haft, D. H., Horvath, P., Moineau, S., Mojica, F. J. M., Terns, R. M., Terns, M. P., White, M. F., Yakunin, A. F., Garrett, R. A., van der Oost, J., … Koonin, E. v. (2015). An updated evolutionary classification of CRISPR–Cas systems. *Nature Reviews Microbiology*, *13*(11), 722–736. https://doi.org/10.1038/nrmicro3569

Mojica, F. J. M., Díez-Villaseñor, C., García-Martínez, J., & Soria, E. (2005). Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *Journal of Molecular Evolution*, *60*(2), 174–182. https://doi.org/10.1007/s00239-004-0046-3

Mojica, F. J. M., Diez-Villasenor, C., Soria, E., & Juez, G. (2000). Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. *Molecular Microbiology*, *36*(1), 244–246. https://doi.org/10.1046/j.1365-2958.2000.01838.x

Moretti, A., Fonteyne, L., Giesert, F., Hoppmann, P., Meier, A. B., Bozoglu, T., Baehr, A., Schneider, C. M., Sinnecker, D., Klett, K., Fröhlich, T., Rahman, F. A., Haufe, T., Sun, S., Jurisch, V., Kessler, B., Hinkel, R., Dirschinger, R., Martens, E., … Kupatt, C. (2020). Somatic gene editing ameliorates skeletal and cardiac muscle failure in pig and human models of Duchenne muscular dystrophy. *Nature Medicine*, *26*(2), 207–214. https://doi.org/10.1038/s41591-019-0738-2

Nelson, C. E., Hakim, C. H., Ousterout, D. G., Thakore, P. I., Moreb, E. A., Rivera, R. M. C., Madhavan, S., Pan, X., Ran, F. A., Yan, W. X., Asokan, A., Zhang, F., Duan, D., & Gersbach, C. A. (2016). In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. *Science*, *351*(6271), 403–407. https://doi.org/10.1126/science.aad5143

Nelson, C. E., Wu, Y., Gemberling, M. P., Oliver, M. L., Waller, M. A., Bohning, J. D., Robinson-Hamm, J. N., Bulaklak, K., Castellanos Rivera, R. M., Collier, J. H., Asokan, A., & Gersbach, C. A. (2019). Long-term evaluation of AAV-CRISPR genome editing for Duchenne muscular dystrophy. *Nature Medicine*, *25*(3), 427–432. https://doi.org/10.1038/s41591-019-0344-3

O'Keeffe Ahern, J., Lara-Sáez, I., Zhou, D., Murillas, R., Bonafont, J., Mencía, Á., García, M., Manzanares, D., Lynch, J., Foley, R., Xu, Q., Sigen, A., Larcher, F., & Wang, W. (2022). Non-viral delivery of CRISPR–Cas9 complexes for targeted gene editing via a polymer delivery system. *Gene Therapy*, *29*(3–4), 157–170. https://doi.org/10.1038/s41434-021-00282-6

Ousterout, D. G., Kabadi, A. M., Thakore, P. I., Majoros, W. H., Reddy, T. E., & Gersbach, C. A. (2015). Multiplex CRISPR/Cas9-based genome editing for correction of dystrophin mutations that cause Duchenne muscular dystrophy. *Nature Communications*, *6*(1), 6244. https://doi.org/10.1038/ncomms7244

Pardee, K., Green, A. A., Takahashi, M. K., Braff, D., Lambert, G., Lee, J. W., Ferrante, T., Ma, D., Donghia, N., Fan, M., Daringer, N. M., Bosch, I., Dudley, D. M., O'Connor, D. H., Gehrke, L., & Collins, J. J. (2016). Rapid, Low-Cost Detection of Zika Virus Using Programmable Biomolecular Components. *Cell*, *165*(5), 1255–1266. https://doi.org/10.1016/j.cell.2016.04.059

Polstein, L. R., & Gersbach, C. A. (2015). A light-inducible CRISPR-Cas9 system for control of endogenous gene activation. *Nature Chemical Biology*, *11*(3), 198–200. https://doi.org/10.1038/nchembio.1753

Pourcel, C., Salvignol, G., & Vergnaud, G. (2005). CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. *Microbiology*, *151*(3), 653–663. https://doi.org/10.1099/mic.0.27437-0

Qi, L. S., Larson, M. H., Gilbert, L. A., Doudna, J. A., Weissman, J. S., Arkin, A. P., & Lim, W. A. (2013). Repurposing CRISPR as an RNA-Guided Platform for Sequence-Specific Control of Gene Expression. *Cell*, *152*(5), 1173–1183. https://doi.org/10.1016/j.cell.2013.02.022

Sahel, D. K., Mittal, A., & Chitkara, D. (2019). CRISPR/Cas System for Genome Editing: Progress and Prospects as a Therapeutic Tool. *Journal of Pharmacology and Experimental Therapeutics*, *370*(3), 725–735. https://doi.org/10.1124/jpet.119.257287

Santiago-Frangos, A., Hall, L. N., Nemudraia, A., Nemudryi, A., Krishna, P., Wiegand, T., Wilkinson, R. A., Snyder, D. T., Hedges, J. F., Cicha, C., Lee, H. H., Graham, A., Jutila, M. A., Taylor, M. P., & Wiedenheft, B. (2021a). Intrinsic signal amplification by type III CRISPR-Cas systems provides a sequence-specific SARS-CoV-2 diagnostic. *Cell Reports Medicine*, *2*(6), 100319. https://doi.org/10.1016/j.xcrm.2021.100319

Santiago-Frangos, A., Hall, L. N., Nemudraia, A., Nemudryi, A., Krishna, P., Wiegand, T., Wilkinson, R. A., Snyder, D. T., Hedges, J. F., Cicha, C., Lee, H. H., Graham, A., Jutila, M. A., Taylor, M. P., & Wiedenheft, B. (2021b). Intrinsic signal amplification by type III CRISPR-Cas systems provides a sequence-specific SARS-CoV-2 diagnostic. *Cell Reports Medicine*, *2*(6), 100319. https://doi.org/10.1016/j.xcrm.2021.100319

Sato, M., Saitoh, I., & Inada, E. (2016). Efficient CRISPR/Cas9-based gene correction in induced pluripotent stem cells established from fibroblasts of patients with sickle cell disease. *Stem Cell Investigation*, *3*, 78–78. https://doi.org/10.21037/sci.2016.11.05

Shan, Y., Zhou, X., Huang, R., & Xing, D. (2019). High-Fidelity and Rapid Quantification of miRNA Combining crRNA Programmability and CRISPR/Cas13a *trans* -Cleavage Activity. *Analytical Chemistry*, *91*(8), 5278–5285. https://doi.org/10.1021/acs.analchem.9b00073

Tang, T.-H., Bachellerie, J.-P., Rozhdestvensky, T., Bortolin, M.-L., Huber, H., Drungowski, M., Elge, T., Brosius, J., & Hüttenhofer, A. (2002). Identification of 86 candidates for small non-messenger RNAs from the archaeon *Archaeoglobus fulgidus*. *Proceedings of the National Academy of Sciences*, *99*(11), 7536–7541. https://doi.org/10.1073/pnas.112047299

Tasan, I., Jain, S., & Zhao, H. (2016). Use of genome-editing tools to treat sickle cell disease. *Human Genetics*, *135*(9), 1011–1028. https://doi.org/10.1007/s00439-016-1688-0

van Soolingen, D., de Haas, P. E., Hermans, P. W., Groenen, P. M., & van Embden, J. D. (1993). Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. *Journal of Clinical Microbiology*, *31*(8), 1987–1995. https://doi.org/10.1128/jcm.31.8.1987-1995.1993

Walker, G. T., Fraiser, M. S., Schram, J. L., Little, M. C., Nadeau, J. G., & Malinowski, D. P. (1992a). Strand displacement amplification—an isothermal, *in vitro* DNA amplification technique. *Nucleic Acids Research*, *20*(7), 1691–1696. https://doi.org/10.1093/nar/20.7.1691

Walker, G. T., Fraiser, M. S., Schram, J. L., Little, M. C., Nadeau, J. G., & Malinowski, D. P. (1992b). Strand displacement amplification—an isothermal, *in vitro* DNA amplification technique. *Nucleic Acids Research*, *20*(7), 1691–1696. https://doi.org/10.1093/nar/20.7.1691

Wang, G., Tian, W., Liu, X., Ren, W., & Liu, C. (2020). New CRISPR-Derived microRNA Sensing Mechanism Based on Cas12a Self-Powered and Rolling Circle Transcription-Unleashed Real-Time crRNA Recruiting. *Analytical Chemistry*, *92*(9), 6702–6708. https://doi.org/10.1021/acs.analchem.0c00680

Wen, J., Tao, W., Hao, S., & Zu, Y. (2017). Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing. *Journal of Hematology & Oncology*, *10*(1), 119. https://doi.org/10.1186/s13045-017-0489-9

Xu, X., & Qi, L. S. (2019). A CRISPR–dCas Toolbox for Genetic Engineering and Synthetic Biology. *Journal of Molecular Biology*, *431*(1), 34–47. https://doi.org/10.1016/j.jmb.2018.06.037

Xu, Y., & Li, Z. (2020). CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Computational and Structural Biotechnology Journal*, *18*, 2401–2415. https://doi.org/10.1016/j.csbj.2020.08.031

Yan, W. X., Hunnewell, P., Alfonse, L. E., Carte, J. M., Keston-Smith, E., Sothiselvam, S., Garrity, A. J., Chong, S., Makarova, K. S., Koonin, E. v., Cheng, D. R., & Scott, D. A. (2019). Functionally diverse type V CRISPR-Cas systems. *Science*, *363*(6422), 88–91. https://doi.org/10.1126/science.aav7271

Yang, S., & Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *The Lancet Infectious Diseases*, *4*(6), 337–348. https://doi.org/10.1016/S1473-3099(04)01044-8

Zhang, X.-H., Tee, L. Y., Wang, X.-G., Huang, Q.-S., & Yang, S.-H. (2015). Off-target Effects in CRISPR/Cas9-mediated Genome Engineering. *Molecular Therapy - Nucleic Acids*, *4*, e264. https://doi.org/10.1038/mtna.2015.37

MICROFABRICATED CELL CULTURE MODELS FOR STUDYING THE HIPPOCAMPUS

Eshaan Banerjee
B Sc Zoology (H)

Abstract
Primary cell culture methods of hippocampal neurons have been inadequate for studying the functional (shooting of action potentials) and microanatomic (intracellular interactions) aspects of the hippocampus. Advancements in microfabrication technologies and their application in cell culture have addressed these issues with newer micropatterned cell culture models. This paper is a review of some of these models.

Key words: hippocampus, microfabricated Cell Culture

Introduction

The characterization of hippocampal involvement in human disease has been of great value to neuroscientists and clinicians. Disorders in which the role of the hippocampus has been extensively investigated are Alzheimer's disease and temporal lobe epilepsy. The commonest neuropathological lesion identified in temporal lobectomy series in patients with mesial temporal lobe epilepsy (TLE) is hippocampal sclerosis (Berkovic et al., 1991). A strong correlation between MRI determined hippocampal volumes and neuronal numbers in the hippocampus in case of Alzheimers Diseease exists (Bobinski et al., 1999). Hippocampal cell cultures are therefore used as a test bed for drug screening for these diseases in preclinical trials.

Before the integration of microfabrication technologies in cell culture, hippocampal neuronal culture was done either on Either 16 mm diameter tissue culture plastic wells (Costar 3424) or 12 mm diameter sterile glass coverslips (Brewer and Cotman,1989). Several limitations of these included a) lack of a patterned neuronal network between the regions of the hippocampus b) 2-dimensional structure of the culture c) Action potential firing rates cannot be studied qualitatively. These led to the development of cell culture models with the use of microfabrication techniques to stimulate similarity to their in-vitro setup and the concept of brain on a chip (Brewer and Wheeler, 2010).

Microfabrication

Microfabrication techniques are used to generate patterns of cells on surfaces. This cellular patterning is a necessary component for cell-based biosensors, cell culture analogues, tissue engineering, and fundamental studies of cell biology (Park and Shuler, 2003). These microfabrication technologies can be adapted to better suit their biological applications (Kim et al., 1995). Soft lithography (Kane et al., 1999), microcontact printing (Lopez et al., 1993), microfluidic patterning using microchannels (Folch el al., 1998), Laminar flow patterning (Takayema et al., 1999), Stensil patterning (Jimbo el at., 1993), MIMIC (Chiu et al.,2004) are

techniques used for micropatterning of cell cultures. A summary of these techniques has been listed in a table below

Patterning technique	Summary
Photolithography	Micropatterns are generated and cell adhesion proteins are applied; chemicals used in this method are toxic to cells. Equipments for photolithography is expensive.
Soft Lithography	Is a modification of photolithography. More suitable for biological applications. Uses soft elastomeric material instead (PDMS)
Microcontact printing	Provides good flexibility in pattern shapes; Ligand number is limited.
Laminar Flow Patterning	Can patterns spatial relationship of medium to patterned cells; Multiple ligands can be used.
Stensil Patterning	Allows for cell patterning without chemical modification of substrate.
MIMIC	Allow making of channels of arbitrary complexity in 3D

Hippocampal studies using microfluidic cultures

Application of microfluidics in cell culture can be used to precisely control, manipulate and monitor cellular microenvironments (Tourovskaia el at., 2005). The first work in this area was done with the use of "Camenot" chambers (Campenot, 1977) using rat hippocampal neurons (Figure 1). A three-chamber culture system was made in which the local fluid environment of the portions of the neurons (distal and proximal) could be altered without affecting the other regions. Parallel scratches were made on the dish surface connecting the three chambers. These scratches guided the growth of neurons. Experiments were conducted by altering the concentration of NGF (Nerve Growth Factors) in the three chambers. Alterations to NGF concentration was done in two ways; a) The neurons were plated on central chamber which contained NGF and growth on the side chambers with little or no NGF was compared b) Neurons were allowed to grow on all three chambers with NGF and subsequently it was withdrawn from one side/or the central chamber. It was observed that only in the chambers where NGF was present in sufficient concentration(1µg/ml) neurons would grow. Withdrawal of NGF lead to the stoppage of neuron growth and subsequent degeneration. This phenomenon was not observed in neurons whose somas were associated with neurite bundles that crossed into chambers in which NGF was present.

Taylor et al. (2005) used soft lithography and replica moulding in PDMS (polydimethylsiloxane) to create a microfluidic culture platform (Figure 2). The PDMS mould contains pattern of somal and axonal compartments separated by microgrooves. Microgrooves

function include a) allow only neuritic processes to grow between compartments. b) development of unique chemical microenvironment between compartments by establishing volume differences. Hippocampal dissociated neurons from embryonic mouse (E17) were plated on the somal side. Study of Axonal mRNA in developing neurons was done using RN aqueous-Micro kit (Ambion) in both the axonal and somal sides. This model also allows directed growth of axons without the use of neurotropins. Co-culture with oligodendrocytes and neurons to study myelination is also possible. Oligodendroctyes are added to a compartment after 7 days of neuron growth and myelination was studied using confocal microscopy.

Barbati et al. (2012) designed a microfluidic system which is capable of delivering soluble factors to specific locations of a culture of Hippocampul neurons (Figure 3). Local signals arising from only one part of a neuron cell mediate certain functional aspects (Oliva et al. 2003). The microfluidic device is two layered. Upper Fluid delivery layer which can confine solute delivery to certain segments of the axonal channel. Lower layer where the cells are plated and axons grow in an array fashion. The solute disposition to certain segments is controlled by direction and magnitude of flow in the fluid network with resistances present in the upper layer. Primary hippocampal culture from F18 embryonic rats were introduced in the culture layers by holes cut through the delivery layer. Phase contrast microscopy showed dendrite and axon growth at day in vitro (DIV) 7. By DIV 11 the cells had reached the fluidic channels allowing confined solute delivery.

Robertson et al. (2014) has defined a microfluidic system in which cellular activity can be observed with the help of calcium imaging. Hippocampal neurons are grown with astrocytes and their synaptic communication are studied. Distinct cellular microenvironments can be created using this model. Their model consists of two parallel chambers which are fed by open inlet and outlet wells, with microchannels allowing neurite growth connecting the two chambers (Figure 4). Primary hippocampal cells were prepared and loaded into the inlet wells of each chamber. 200µl of culture medium was used. Neurite growth across microchannel barrier was observed day 10 after plating using immunocytochemistry, following synapse formation at day 12. Calcium imaging was done to confirm functionality of synaptic communication between the two co-cultures. This was achieved by chemical stimulation of neurons in one chamber and results were located in the adjacent chambers. KCl and Glutamate were used to produce these simulations. This Culture model allows us to study signaling between neuronal co-culture without the use of MEA's (Micro Electrode Arrays).

Integration of MEA (Microelectrode Arrays) in patterned Cultures.

Hippocampal cells are bio-electrically active cells. This electrical activity of these cells can be studied with the help of MEAs integrated into a cell culture system. Integration is done by microfabrication techniques. Thomas et al. (1972) created a model integrating MEA's on a cell culture of E (10) chick heart tissues. Electrical activity could be observed after 3 ½ h after cell plating. These electrical activities were measured with oscilloscopes connected to the micro electrodes on the cell culture. Pine (1979) cultured embryonic rat neurons in a culture platform

which included a microelectrode array of 16 horizontal gold conductors across a petri dish in which the cultures were grown. These conductors became wider as they appeared the edge of the dish and where contact was made with external circuitry. A differential amplifier with usual bandwidth was used for recording. Change in network topology and functional connectivity of neurons is associated with diseases such as Alzheimer's (Sanz-Arigita et al., 2010) and epilepsy (Netoff et al., 2004).

Marconi et al. (2012) for the first time developed a neuronal network of hippocampus with a defined topology in vitro (Figure 5). Functional property analysis of this network was done with the help of MEA's. These recordings were then compared with random cultures to ascertain if functional connectivity depends on imposed topology. Rates of spontaneous firing and bursting of action potential was also compared with random neuronal cultures. It was found that synchronized bursting activity appeared more precariously in patterned networks. Stronger links with shorted delays and lengths emerged along vertical and horizontal direction in grid networks.

Shmoel et al. (2016) has used micrometer sized, extracellular gold mushroom shaped electrodes (gMµEs) to record synaptic action potentials of intracellular recordings (Figure 6). The spacing between the gMµE and effective recording area was increased. This allowed the rat hippocampal neurons (E17) to form functional networks. These gMµEs were prepared on 300µm glass wafers by means of photolithography and electroplating techniques. Signal from the gMµEs were passed through (MEA-1060-Inv-BC, MCS) amplifier with frequency limits set to 1-10,000Hz and sampling rate of 10kHZ. Measurements of spike amplitudes and durations were done using Clampfit software. AP recording potentials between planar MEA's and gMµEs significantly differ in the number of high peak amplitudes.

Dworka and Wheeler (2009) integrated MEA's with MEMS (Micro-electromechanical systems) to achieve the goal of recording AP from isolated bundles of axons in-vitro system. Their design consisted of five 1.5-mm square culture wells connected through microtunnels only allowing axon and dendrite growth in them. Non-Platinized MEA's were of gold electrodes were then patterned to lie at bottom of each of the four groups of microtunnels (Figure 7). Three different widths of 25,50 and 75 µm were chosen and results were compared for signal amplitude variations. E18 rat cortical tissue were prepared and plated on the growth wells. Axons freely entered into the microtunnels after 48 hours plating. Unidirectional growth reaching the adjacent culture well within 5 to 6 days. Cultures at 10 to 14 DIV exhibited synchronous bursting activity from all active electrodes. All cultures were therefore synaptically interconnected. At 10 to 14 day division Drug experiments were conducted with mepivacine which verified changing conduction velocity and spike rate. This culture was not done on hippocampal neuronal cells but cells of the different regions of the hippocampus (CA1, CA2, CA3 and dentate gyrus) can be plated on different microwells and drug screening tests along with electophysical activity can be measured and therefore has been kept as a part of this review.

Brewer et al. (2013) created a microfabricated device in which cells from major areas of the hippocampus (EC, DG, CA3, CA1) were plated in compartments (Figure 8). These cells would be connected through axons growing through microscale tunnels. MEA's were integrated into the device to monitor electrical activity. Postnatal day 4 rats hippocampus was dissected as a source of these cells. Single cell suspensions from each region were plated in different compartments of the microfabricated PDMS device. Qualitative PCR of region restricted gene expression showed that gene expression is maintained between compartments both with same and different subregions. This proved that the hippocampal subregions were able to maintain their specific identity in the culture platform. Electrophysiological studies were done with the MEA's and these observations were consistent with a synchronously connected network shown in in-vitro conditions. Narula et al. (2017) compared the width of micro tunnels used in the above platform. 2.5µm and 5µm micro tunnel widths were compared with more common 10µm wide tunnels. It was overall shown that 2.5µm wide tunnels were the best choice for studying of electrophysiological data. 2.5µm tunnels allow less axons to grow which leads to lesser variation in spike amplitude and width contributing to precise estimates of spike timings. Poli et al. (2021) used a similar model to culture different neurons of rat hippocampus (EC, DG, CA-3-CA-1). Electrophysiological studies showed that high activity in the axons that provide input in the feed-forward native direction of EC-DG and CA3-CA1 co culture evolve much more target output than in feed-back direction. This result is similar to in-vivo conditions (Rolls et al., 1996).

Conclusions

This review has summarized some micropatterned models which have been used to study hippocampal function in vitro. The future of cell culture will be 3D in nature allowing neurons to be grown in 3D geometric patterns. These models will further develop the "Braininachip" technologies.

References

Barbati, A. C., Fang, C., Banker, G. A., & Kirby, B. J. (2013). Culture of primary rat hippocampal neurons: design, analysis, and optimization of a microfluidic device for cell seeding, coherent growth, and solute delivery. Biomedical Microdevices, 15(1):97-108. https://doi.org/10.1007/s10544-012-9691-2

Berkovic, S.F., Andermann, F., Olivier, A., Ethier, R., Melanson, D., Robitaille, Y., Kuzniecky, R., Peters, T. and Feindel, W. (1991), Hippocampal sclerosis in temporal lobe epilepsy demonstrated by magnetic resonance imaging. Annals of Neurology, 29:175-182. https://doi.org/10.1002/ana.410290210

Brewer, G. J., Boehler, M. D., Leondopulos, S., Pan, L., Alagapan, S., DeMarse, T. B., & Wheeler, B. C. (2013). Toward a self-wired active reconstruction of the hippocampal trisynaptic loop: DG-CA3. Frontiers in Neural Circuits, 7:165. https://doi.org/10.3389/fncir.2013.00165

Brewer, G. J., & Cotman, C. W. (1989). Survival and growth of hippocampal neurons in defined medium at low density: advantages of a sandwich culture technique or low oxygen. Brain Research, 494(1):65-74. https://doi.org/10.1016/0006-8993(89)90144-3

Campenot R. B. (1977). Local control of neurite development by nerve growth factor. Proceedings of the National Academy of Sciences of the United States of America, 74(10):4516-4519. https://doi.org/10.1073/pnas.74.10.4516

Chiu, D. T., Jeon, N. L., Huang, S., Kane, R. S., Wargo, C. J., Choi, I. S., Ingber, D. E., & Whitesides, G. M. (2000). Patterned deposition of cells and proteins onto surfaces by using three-dimensional microfluidic systems. Proceedings of the National Academy of Sciences of the United States of America, 97(6):2408-2413. https://doi.org/10.1073/pnas.040562297

Dworak, B. J., & Wheeler, B. C. (2009). Novel MEA platform with PDMS microtunnnnels enables the detection of action potential propagation from isolated axons in culture. Lab on a Chip, 9(3): 404-410. https://doi.org/10.1039/B806689B

Folch, A., & Toner, M. (1998). Cellular micropatterns on biocompatible materials. Biotechnology progress, 14(3):388-392. https://doi.org/10.1021/bp980037b

Gabriel P. Lopez, Mark W. Albers, Stuart L. Schreiber, Reed Carroll, Ernest Peralta, and George M. Whitesides. (1993). Convenient methods for patterning the adhesion of mammalian cells to surfaces using self-assembled monolayers of alkanethiolates on gold. Journal of the American Chemical Society ,115(13):5877-5878. https://doi.org///10.1021/ja00066a0

Jimbo, Y., Robinson, H. P., & Kawana, A. (1993). Simultaneous measurement of intracellular calcium and electrical activity from patterned neural networks in culture. IEEE transactions on Bio-medical Engineering, 40(8):804-810. https://doi.org/10.1109/10.238465

Kim, E., Xia, Y. & Whitesides, G. (1995). Polymer microstructures formed by moulding in capillaries. Nature, 376:581-584. https://doi.org/10.1038/376581a0

Marconi, E., Nieus, T., Maccione, A., Valente, P., Simi, A., Messa, M., & Benfenati, F. (2012). Emergent functional properties of neuronal networks with controlled topology. PloS One, 7(4): e34648. https://doi.org/10.1371/journal.pone.0034648

Narula U, Ruiz A, McQuaide M, DeMarse TB, Wheeler BC, Brewer GJ. (2017). Narrow microtunnel technology for the isolation and precise identification of axonal communication among distinct hippocampal subregion networks. PLoS One 12(5):e0176868. https://doi.org/10.1371/journal.pone.0176868

Oliva, A. A., James, C. D., Kingman, C. E., Craighead, H. G., & Banker, G. A. (2003). Patterning axonal guidance molecules using a novel strategy for microcontact printing. Neurochemical Research, 28(11):1639-1648. https://doi.org/10.1023/A:1026052820129

Park, T. H., & Shuler, M. L. (2003). Integration of cell culture and microfabrication technology. Biotechnology progress, 19(2):243-253. https://doi.org/10.1021/bp020143k

Pine J. (1980). Recording action potentials from cultured neurons with extracellular microcircuit electrodes. Journal of Neuroscience Methods, 2(1):19-31. https://doi.org/10.1016/0165-0270(80)90042-4

Poli, D., Wheeler, B. C., DeMarse, T. B., & Brewer, G. J. (2018). Pattern separation and completion of distinct axonal inputs transmitted via micro-tunnels between co-cultured hippocampal dentate, CA3, CA1 and entorhinal cortex networks. Journal of Neural Engineering,15(4):046009. https://doi.org/10.1088/1741-2552/aabc2

Kane, R. S., Takayama, S., Ostuni, E., Ingber, D. E., Whitesides, G. M. (1999). Patterning proteins and cells using soft lithography, Biomaterials, 20(23–24):2363-2376. https://doi.org/10.1016//S0142-9612(99)00165-9.

Robertson, G., Bushell, T. J., & Zagnoni, M. (2014). Chemically induced synaptic activity between mixed primary hippocampal co-cultures in a microfluidic system. Integrative Biology, 6(6):636-644. https://doi.org/10.1039/c3ib40221e

Rolls, E. T. (1996). A theory of hippocampal function in memory. Hippocampus, 6(6):601-620. https://doi.org/10.1002/(SICI)1098-1063(1996)6:6%3C601

Sanz-Arigita, E. J., Schoonheim, M. M., Damoiseaux, J. S., Rombouts, S. A., Maris,E., Barkhof, F., Scheltens, P., & Stam, C. J. (2010). Loss of 'small-world' networks in Alzheimer's disease: graph analysis of FMRI resting-state functional connectivity. PloS One, 5(11):e13788. https://doi.org/10.1317/journal.pone.0013788

Shmoel, N., Rabieh, N., Ojovan, S. M., Erez, H., Maydan, E., & Spira, M. E. (2016). Multisite electrophysiological recordings by self-assembled loose-patch-like junctions between cultured hippocampal neurons and mushroom-shaped microelectrodes. Scientific reports, 6(1), 1-11. https://doi.org/10.1038/srep2711

Takayama, S., McDonald, J. C., Ostuni, E., Liang, M. N., Kenis, P. J., Ismagilov, R. F., & Whitesides, G. M. (1999). Patterning cells and their environments using multiple laminar fluid flows in capillary networks. Proceedings of the National Academy of Sciences of the United States of America, 96(10):5545-5548. https://doi.org/10.1073/pnas.96.10.5545

Taylor, A. M., Blurton-Jones, M., Rhee, S. W., Cribbs, D. H., Cotman, C. W., & Jeon, N. L. (2005). A microfluidic culture platform for CNS axonal injury, regeneration and transport. Natural Methods, 2:559-605. https://doi.org/10.1038/nmeth777

Theoden I. Netoff, Robert Clewley, Scott Arno, Tara Keck, John A. (2004). White epilepsy in small-world networks. Journal of Neuroscience, 24(37):8075-8083. https.//doi.org/10.1523/JNEUROSCI.1509-04.2004

Thomas, C. A., Jr, Springer, P. A., Loeb, G. E., Berwald-Netter, Y., & Okun, L. M. (1972). A miniature microelectrode array to monitor the bioelectric activity of cultured cells. Experimental Cell Research, 74(1):61-66. https://doi.org/10.1016/0 14-4827(72)9 0481-8

Tourovskaia, A., Figueroa-Masot, X., & Folch, A. (2005). Differentiation-on-a-chip:a microfluidic platform for long-term cell culture studies. Lab on a chip, 5(1):14-19. https://doi.org/10.1039/b405719h

Wheeler, B. C., & Brewer, G. J. (2010). Designing Neural Networks in Culture: Experiments are described for controlled growth, of nerve cells taken from rats, in predesigned geometrical patterns on laboratory culture dishes. Proceedings of the IEEE. Institute of Electrical and Electronics Engineers, 98(3):398-406. https://doi.org/10.1109/JPROC.2009.2039029

Fig 1. Campenot chamber a) Central chamber where neurons were plated. b) Side chamber on the left. c) Side chamber on the right. Scratches present guide the growth of neuron (Campenot, 1977).

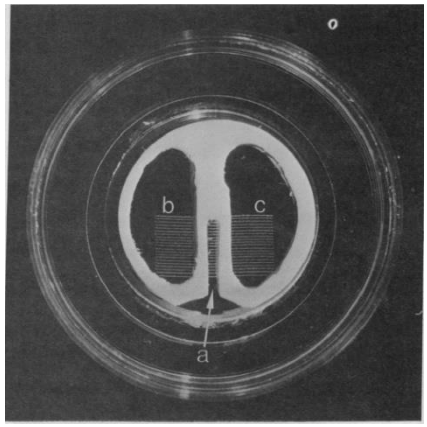
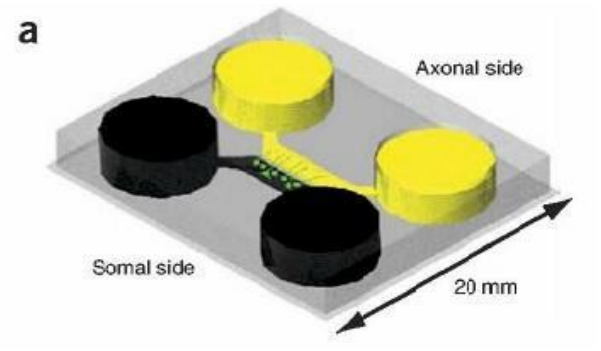


Fig 2. Microfluidic hippocampus culture platform a) The culture chamber consists of a PDMS mold containing a relief pattern of somal and axonal compartments (1.5 mm wide, 7 mm long, 100 mm high) connected by microgrooves (10 μm wide, 3 μm high). The optically transparent PDMS adheres to apolylysine-coated coverslip. Rat CNS neurons (green) are added to the somal-side reservoir and are drawn into the somal channel (black) by capillary action. Within 3–4 d, axonal growth is guided into the axonal side (yellow) through the microgrooves (Taylor et al., 2005).



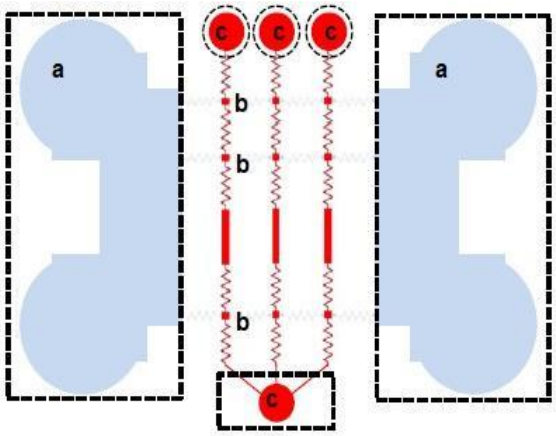


Fig 3. Microfluidic device for hippocampal neuron seeding a) Lower layer where cells are plated and grown, shown in blue. b) Axons pass through these microchannels. c) Upper fluid delivery layer, coloured red. Resistors are areas of hydraulic resistances. Fixed pressure zones are outlined by dashed lines (Barbati et al., 2013).

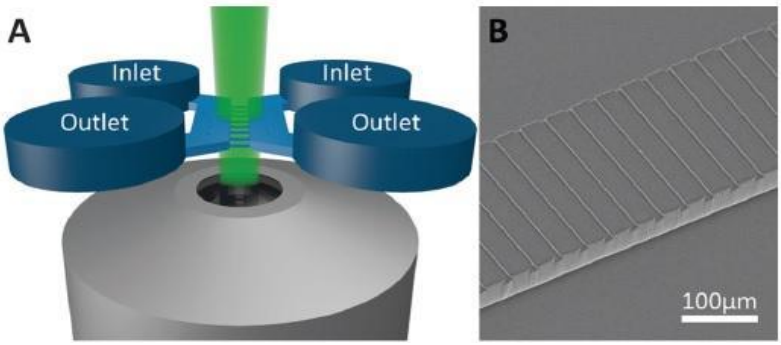


Fig 4. Mixed primary hippocampal co-culture in microfluidic system a) The device consists of two parallel chambers (8 mm long, 2 mm wide and 90 mm deep) which are fed by open inlet and outlet wells at each end of the chamber. The area between the two parallel chambers is bridged by an array of 200 microchannels (250 mm long, 10 mm wide and 7 mm deep) allowing both axons and dendrites to enter and form synapses with the opposing culture. b) Scanning electron microscope image of the barrier of microchannels between the two chambers (Robertson et al., 2014).

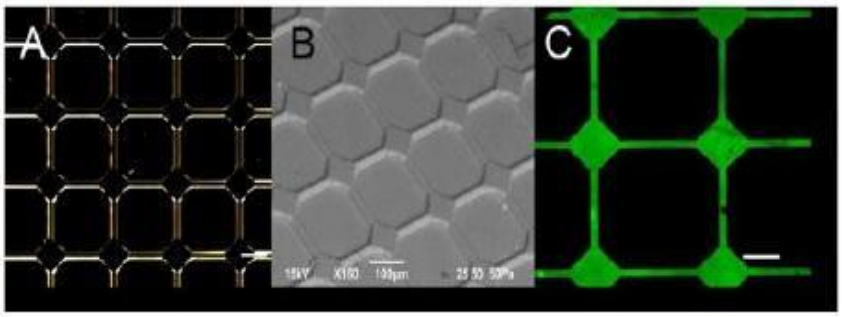
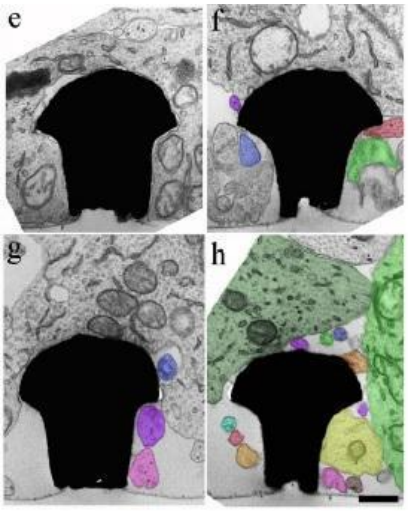


Fig 5. Neuronal network of hippocampus with a defined topology a) Microphotograph of the Si-etched master. b) ESEM picture of an area of the PDMS stamp replicated from the Si master. c) Fluorescence image of a representative FITC-PLL printed area on a glass coverslip. Scalebar: A and C, 50 mm; B, 100 mm (Marconi et al., 2012).

Fig 6. Micrometer size gold mushroom shaped electrodes (gMμEs) e–h). Cell body profiles are shown in gray, different neurites are labeled in different colors. The black mushroom shaped is the electrode. The cell bodies and neurites were identified using low magnification images (Shmoel et al., 2016).



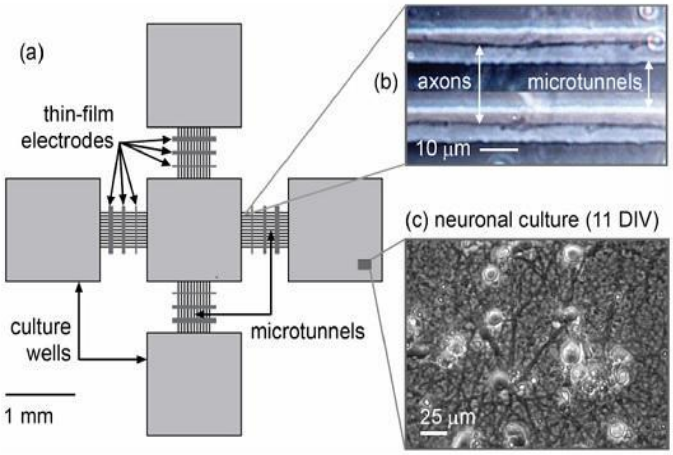


Fig 7. MEA platform with PDMS microtunnels a) Schematic of the design configuration. b) Phase-contract image of axonal growth inside microtunnels. c) Healthy neuronal growth inside culture well (Dworak et al., 2009).

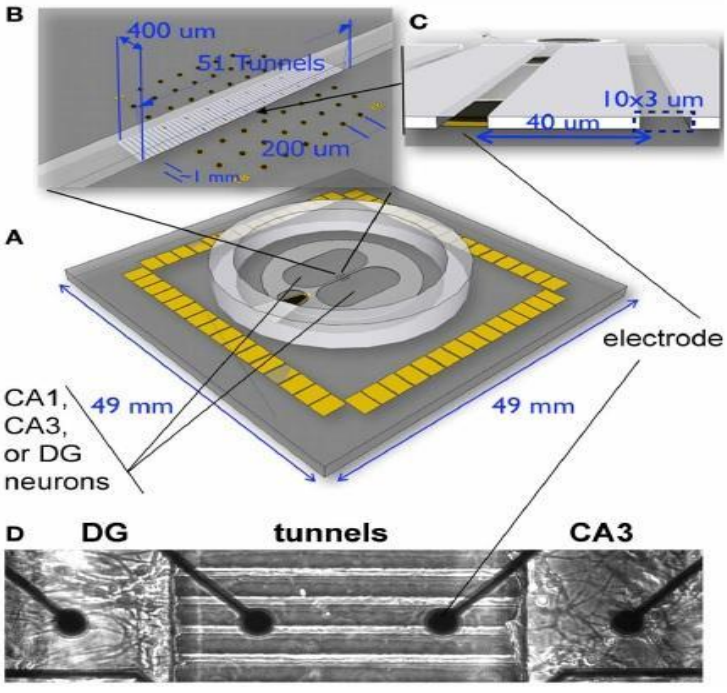


Fig 8. Hippocampal trisynaptic loop grown in microtunnels a) Dual culture chamber on micro electrode array separated by microtunnels. Note ground electrode on lower left for common culture medium. b) 51 tunnels of 400μm length aligned to the 8 columns of electrodes. c) Fifty-one tunnels of 3 × μ10 m cross section were separated by 40um with alignment over one pair of dark electrodes shown. d) Phase contrast imaging of live neurons shows how the tunnels promoted selective growth of axons from one compartment into another.

SNAPSHOTS

Ayaan Borthakur
B Sc Zoology (H)

STUDENTS' ACHIEVEMENTS

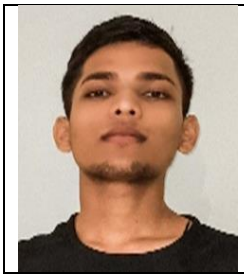
COMPETITIVE EXAMS AND RESEARCH

Year	NET	SLET	GATE	Ph D
2018	Bhabna Das Bikash Rabha Nitul Ali Kamal Adhikari	Sankranti Devi Kamal Adhikari Bhabna Das	Anuradha Kalita	Dr. Parag Moni Baruah
2019	Radhika Rimal	Musfica Saikia Dimpimoni Kalita Papori Deka Taslema Begum Prajjwalita Patir Radhika Rimal Babli Das	Bhanita Bora Musfica Saikia Sankranti Devi	
2020			Taslema Begum Sanjana Sharmin Padmanav Kaushik	Dr. Asha Rani Borah Dr. Palki Hazarika
2021	Dipamani Sarmah Bikash Rabha Titikhya Borah	Sangita Das Shibani Kalita Trishna Deka Seujpriya Borah Bhanita Borah	Arpita Bardoloi Juri Konwar Tanveera R Sarhadi Khaleda Parbin Manish Buragohain	
2022	Kakali Deka Sonot Deuri	Juri Konwar Arpita Bordoloi		Dr. Angana Das Dr. Pramathesh Kalita
2023	Bishal Das Nabila Akhtara	Bishal Das Nabila Akhtara Iffat Khan Suman Mehjebin Sanjana Sharmin Khaleda Parbin Ipsah Akhtar Sachin Saha	Bishal Das Nabila Akhtara Iffat Khan	Dr. Alisha Nasreen Dr. Anjumani Ojah

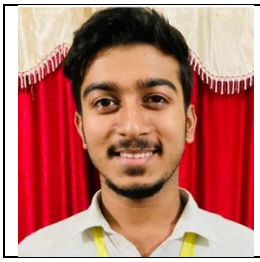
STUDENTS' INTERNSHIP

Name	Topic
	Mr. Ayaan Borthakur carried out a study titled "Production of wine from Averrhoa carambola" under the supervision of Prof. Utpal Bora, Centre for the Environment, IIT, Guwahati
	Ms. Debashree Sanyal carried out a study titled "Study on MSA and pairwise alignment of, Introduction to Pymol and insight on various biological databases" under the supervision of Dr. Anupam Nath Jha, Department of Molecular Biology and Biotechnology, Tezpur University
	Ms. Manjeeta Baro carried out a study titled "Assessment of pathogenic vaginal microbes" under the supervision of Prof B. K. Konwar, Department of Molecular Biology and Biotechnology, Tezpur University
	Ms. Meghashree Borah carried out a study titled "Introduction to Bioinformatics, Research literature and literature survey and Introduction to HPLC" under the supervision of Dr. Pankaj Barah, Department of Molecular Biology and Biotechnology, Tezpur University

SPORTS

	**Vijendra Pratap Singh Rajput** received Best Ground Position Player in International Basketball Championship Organized by the International Basketball Federation held in March 2020 (The Philippines). He had been selected for the Indian Team for International tournament in 2021 held in Stanford Maples Pavilion 655 Campus Drive, Stanford, CA 94305, United States. Recently he has been nominated for Paris Olympic, 2024.

EXTRA-CURRICULAR ACTIVITIES

	**Vargav Medhi** received the best debater award in Bhola Borooah intercollege debate competition event, 2022. He also received the best debater award (Team) in youth zonal festival under Gauhati University, 2022.

	**Dikshita Sarma** received the second prize in the Youth Festival, 2022 under Guwahati University and received total of 12 awards in different singing event in Annual College Week 2022. She has been the second best singer in 2023 in Annual College Week and received a total of 14 awards in different singing competition.
	**Neha Talukdar** received the 1st position in classical dance competition at Simanta Sankardev Nrityatsav, 2023. She had participated in dance event during G20 event, 2023 and also during the visit of Indian President Droupadi Murmu to Assam in 2023.

ANNUAL COLLEGE WEEK

Event/activity	Name of the student
Best clean department, 2nd Prize	Department of Zoology
Mr. B. Borooah, 1st runner up	Vargav Medhi
Letter Writing (English), 1st position	Mira Swagata R Marak
Relay Race, 2nd position	Mira Swagata R Marak
Mehendi, 2nd position	Sonia Nag
Cricket, 1st position	Radha Joishi
Kabaddi, 1st position	Radha Joishi
Short Story, 1st position	Jharnali Deka
Poem, 2nd position	Jharnali Deka
Cricket, 1st position	Kritika Sharma
Arm Wrestling, 2nd position	Kritika Sharma
Bongeet, 1st position	Dikshita Sarma
Group song, 1st position	Dikshita Sarma
Classical song, 2nd position	Dikshita Sarma
Tokari, 2nd position	Dikshita Sarma
Sugam sangeet, 2nd position	Dikshita Sarma
Bisnu rabha sangeet, 2nd position	Dikshita Sarma
Bhupendra Sangeet, 2nd position	Dikshita Sarma
Borgeet, 3rd position	Dikshita Sarma
Lokogeet, 3rd position	Dikshita Sarma
Semi classical, 3rd position	Dikshita Sarma
Bihu geet, 3rd position	Dikshita Sarma
Jyoti Sangeet, 3rd position	Dikshita Sarma
Rabindra Sangeet, 3rd position	Dikshita Sarma
Classical Dance, 3rd position	Neha Talukdar

Rangoli, 1st position	Banashree Kakati, Dikshita Kalita
Salad making, 1st position	Banashree Kakati
Salad making, 1st position	Naznin Sultana
Spot poetry writing, 1st position	Tanushree Biswas
Salad making, 2nd position	Tonmoyee Bhuyan
Arm wrestling, 1st position	Manjusa Choudhury
Spot article writing, 2nd position	Manjusa Choudhury
Salad making, 2nd position	Manjusa Choudhury
News reading competition	Satarupa Baishya
Best library user	Rajib Shah

STUDENTS' ACTIVITIES

Students trip to Kaziranga National Park

Students visit to College of Fisheries, Raha

Students visit to State Zoo

Students visit to CIFA, Bhubaneshwar

Students visit to ZSI, Shillong

Students visit to Poultry farm

Students visit to NEHU, SAIF

Students visit to NEHU, Zoology Department

Students visit to Pobitora Wildlife Sanctuary

Add on course on Sericulture

Students visit to Regional Sericulture Institute

Students visit to harbour, Visakhapatnam

DEPARTMENT EVENTS

WELCOME

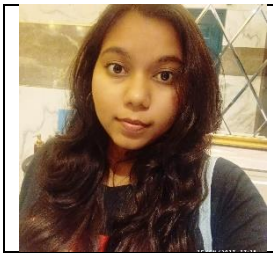
The Department welcomed new faculty members, talking the total strength to 9 permanent faculties.

	**Dr. Anurupa Goswami** completed her M Sc from Gauhati University and her Ph D from IASST, Guwahati. She earlier served at Diphu Government College and then joined B. Borooah College on 1st of June, 2023
	**Mr. Sahil Zaman Seikh** completed his M Sc from Gauhati University and after clearing NET-JRF, he had joined NEIST, Jorhat for his Ph D. Later he joined B. Borooah College on 1st of August, 2023

CONGRATULATIONS

	**Dr. Anjumani Ojah**, PG faculty of the Department has been awarded the Ph D degree for her work on "Effect of methanolic root extract of *Persicaria hydropiper* (L) Delarbre on fertility in albino mice". She worked under the supervision of Dr. Mridul Kr. Borthakur and Professor Jogen Ch. Kalita (Gauhati University).

GUEST FACULTY

	**Ms. Aishwarya**, M Sc (Biotechnology) research scholar at IIT Guwahati is serving as a guest faculty in the Department as part of the Prime Minister Research Fellowship Program (PMRF)

WORKSHOP

A workshop on pearl culture in freshwater mussel was conducted from May 26-27, 2023. Dr. Akhalesh Kr. Verma, Assistant Professor, Department of Zoology, Cotton University served as the resource person.

ALUMNI MEET

Alumni meet was held on 13th of August, 2023 after a long gap. Ex faculty members, Dr. Rekha Deka (Ex-Principal), Prof. Karabi Dutta, Dr. Dinesh Baishya (Ex-Principal), Tariful Islam, Dr Jagadindra Raychoudhury, Dr. Kamal Choudhury, Dr. Rashmimala Kakati, and Dr. Sunayan Bordoloi. Students from earlier batches serving in different institutions and departments participated in the memorable event.

MOU

The Department of Zoology has signed MOU with several institutions for teaching exchange and collaborative research namely, Don Bosco University (Guwahati), Pachhunga University College (Aizawl, Mizoram), Science College (Kokrajhar), and Debray Roy College (Golaghat).

MEMORANDUM OF UNDERSTANDING

Between

B. Borooah College

Ulubari, Guwahati-781007

Assam

and

Assam Don Bosco University

Tepesia Garden, Sonapur-782402

Assam

Agreement of Collaboration

This Agreement of Collaboration is made and executed on this the 1st of February, 2022

Between

Department of Zoology, Debraj Roy College, Golaghat, Assam, 785621, which expression shall include and deemed to include their executers, successors or administrators and assigns of the ONE PART.

And

Department of Zoology, B. Borooah College, Guwahati, Assam, 781007 which expression shall, include and deemed to include their executers, successors or administrators and assigns as the TWO PART

1. Purpose of the Agreement:

The purpose of this Agreement is to create a framework for building a platform for joint research between faculty members. Further expertise of faculty members and facilities available in both the institutions will be utilised.

2. Terms and Conditions:

Department of Zoology, B. Borooah College and Department of Zoology, Debraj Roy College will carry out the necessary research work as per the facilities and expertise available in their respective departments. Softwares or any other necessary assistance or expertise will be made available to each other as per the convenience of each department.

3. Publications:

It is contemplated that results of the Research jointly worked on will be published with co-authorship. In case of non-collaborative research, Department of Zoology, Debraj Roy

Agreement of Collaboration

This Agreement of Collaboration is made and executed on this the 11th of January, 2021

Between

Department of Life Sciences, Pachhunga University College, Aizawl, Mizoram, 796001, which expression shall include and deemed to include their executers, successors or administrators and assigns of the ONE PART.

And

Department of Zoology, B. Borooah College, Guwahati, Assam, 781007 which expression shall, include and deemed to include their executers, successors or administrators and assigns as the TWO PART

1. Purpose of the Agreement:

The purpose of this Agreement is to create a framework for building a platform for joint research between faculty members. Further expertise of faculty members and facilities available in both the institutions will be utilised.

2. Terms and Conditions:

Department of Zoology, B. Borooah College and Department of Life Sciences, Pachhunga University College will carry out the necessary research work as per the facilities and expertise available in their respective departments. Softwares or any other necessary assistance or expertise will be made available to each other as per the convenience of each department.

3. Publications:

Agreement of Collaboration

This Agreement of Collaboration is made and executed on this the 6th of January, 2020

Between

Department of Zoology, Science College, Kokrajhar, BTC, Assam, 783370, which expression shall include and deemed to include their executers, successors or administrators and assigns of the ONE PART.

And

Department of Zoology, B. Borooah College, Guwahati, Assam, 781007 which expression shall, include and deemed to include their executers, successors or administrators and assigns as the TWO PART

1. Purpose of the Agreement:

The purpose of this Agreement is to create a framework for building a platform for joint research between faculty members. Further expertise of faculty members and facilities available in both the institutions will be utilised.

2. Terms and Conditions:

Department of Zoology, B. Borooah College and Department of Zoology, Science College will carry out the necessary research work as per the facilities and expertise available in their respective departments. Softwares or any other necessary assistance or expertise will be made available to each other as per the convenience of each department.

3. Publications:

DEPARTMENT PEOPLE

Teaching staff

Non-teaching staff

4 year UG programme (1st year)

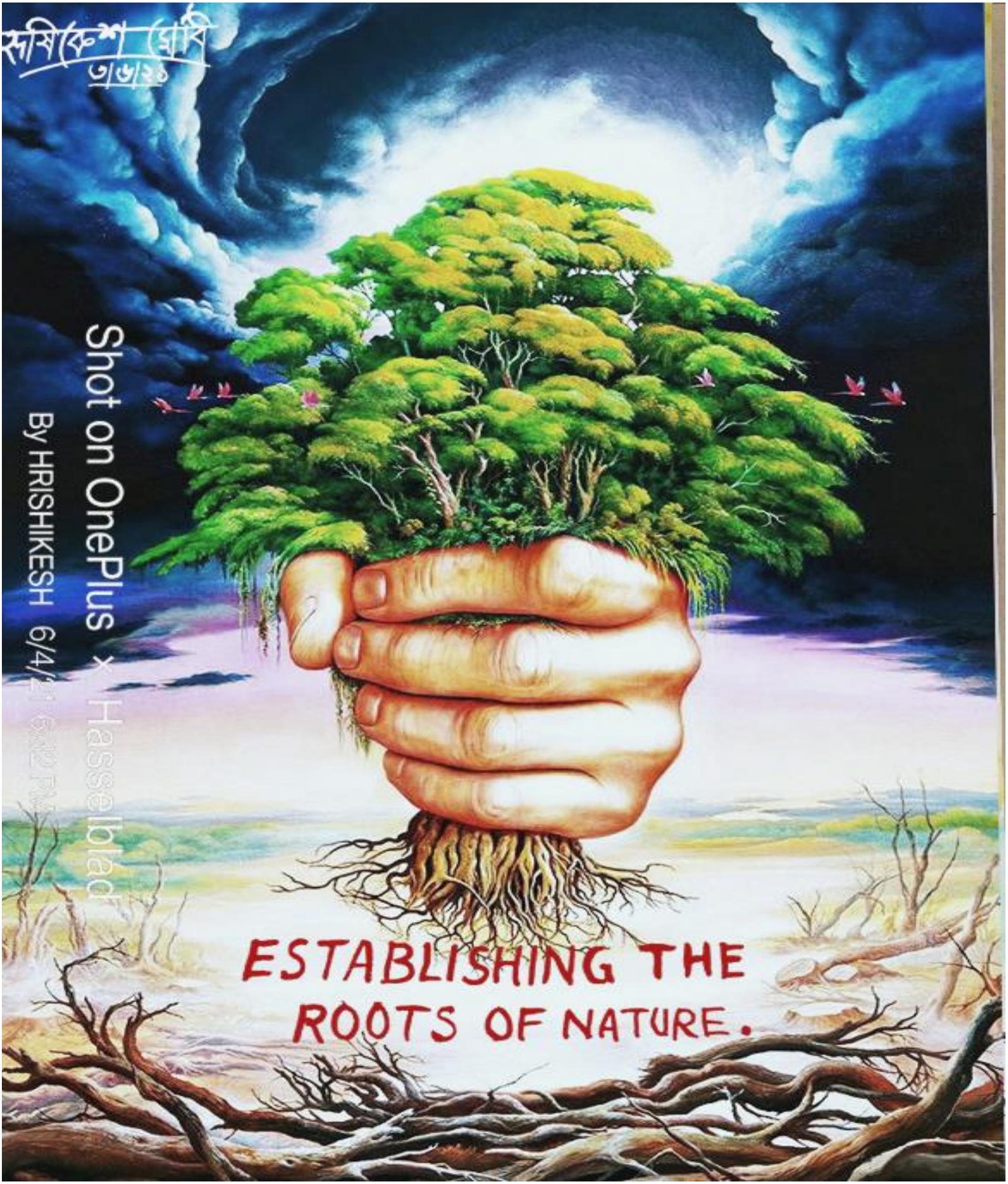
UG 3rd semester

UG 5th semester

Research scholars

PG 1st semester

PG 3rd semester



PUBLISHED BY

DEPARTMENT OF ZOOLOGY

B. BOROOAH COLLEGE, GUWAHATI, 781007