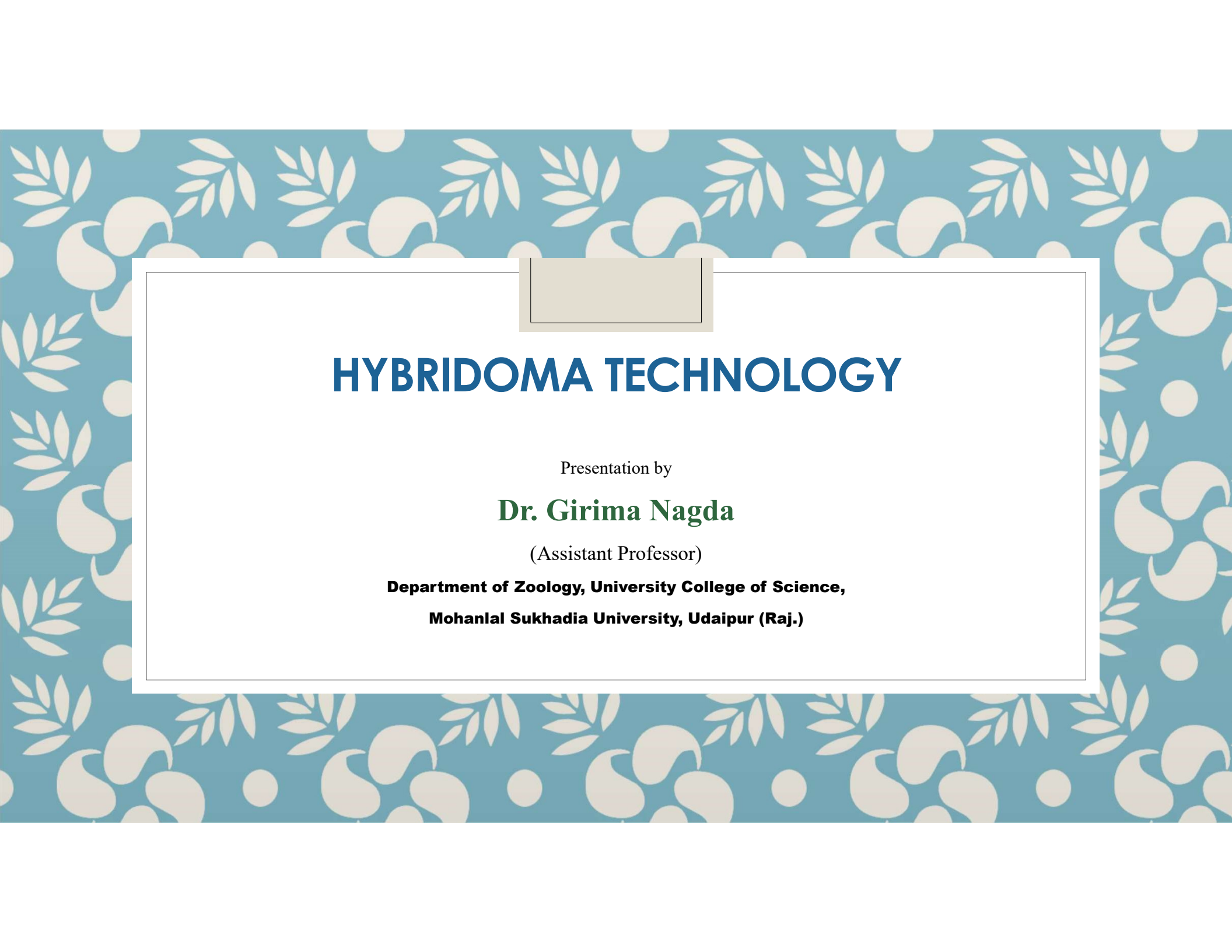




HYBRIDOMA TECHNOLOGY

Presentation by

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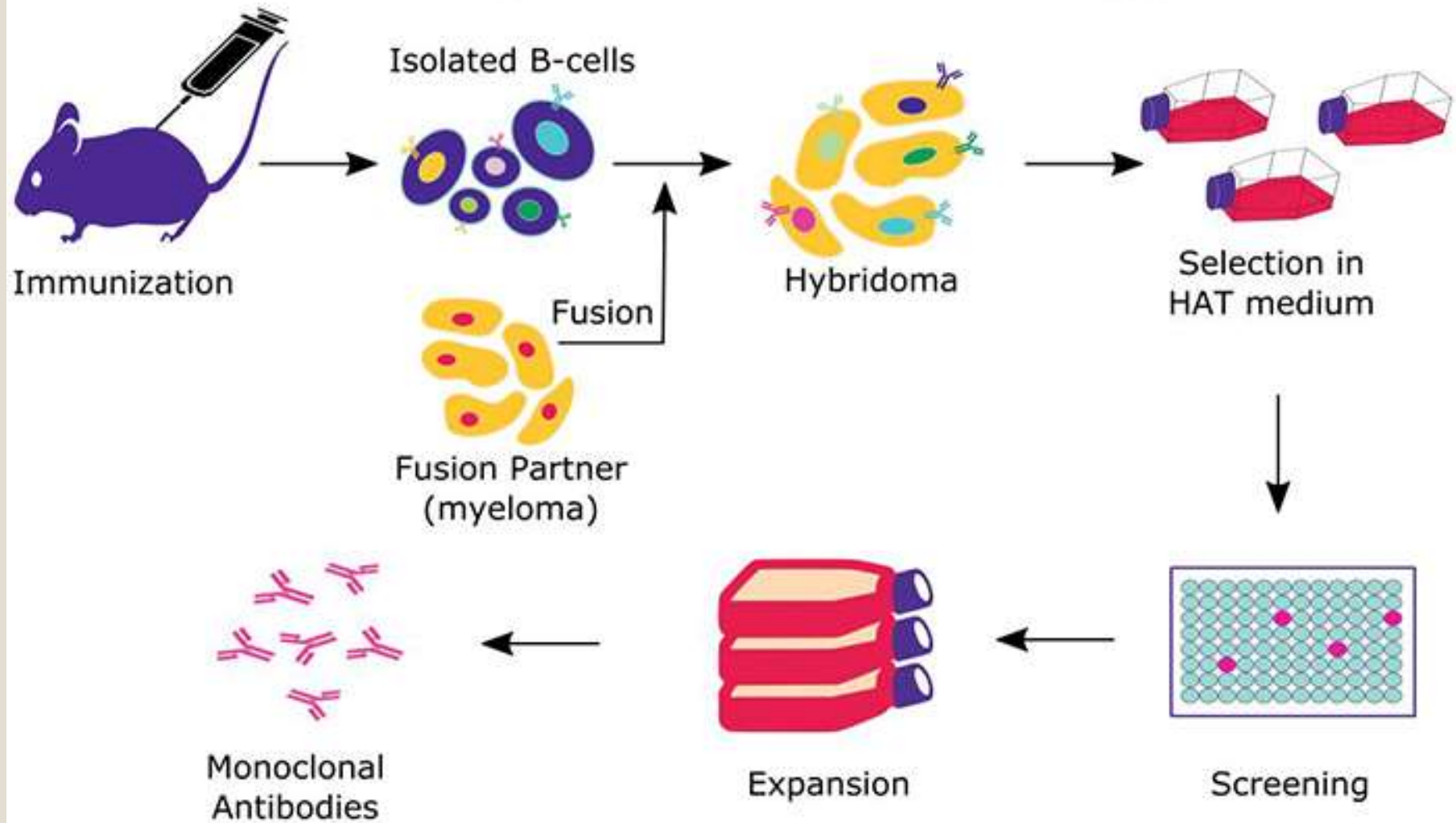
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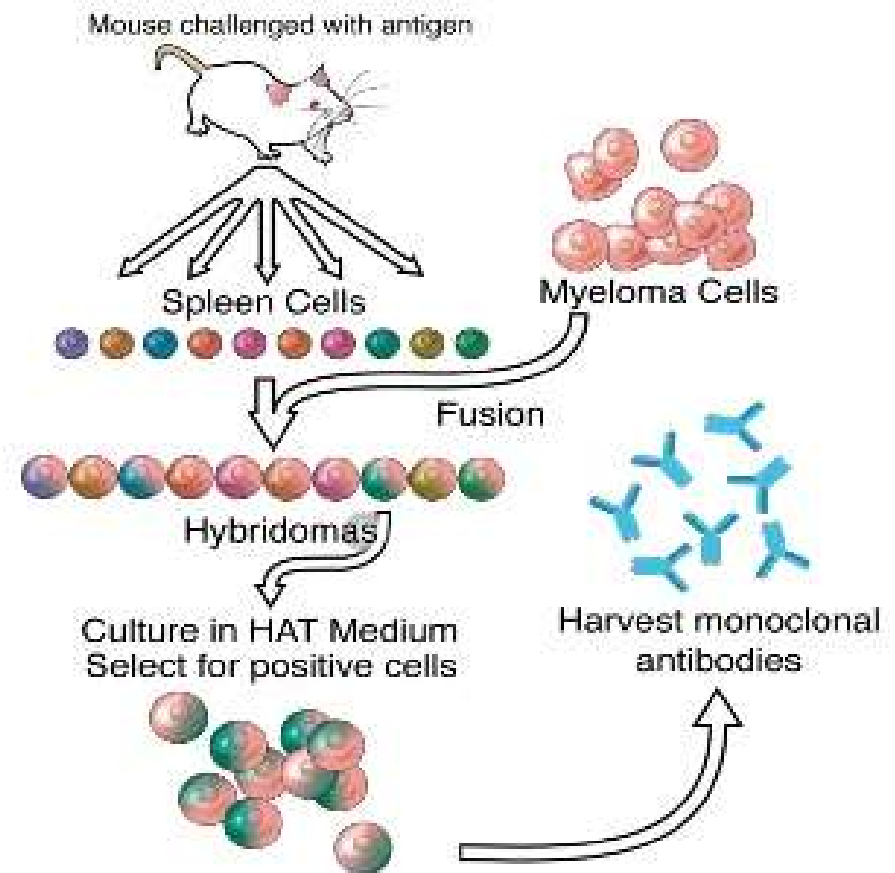
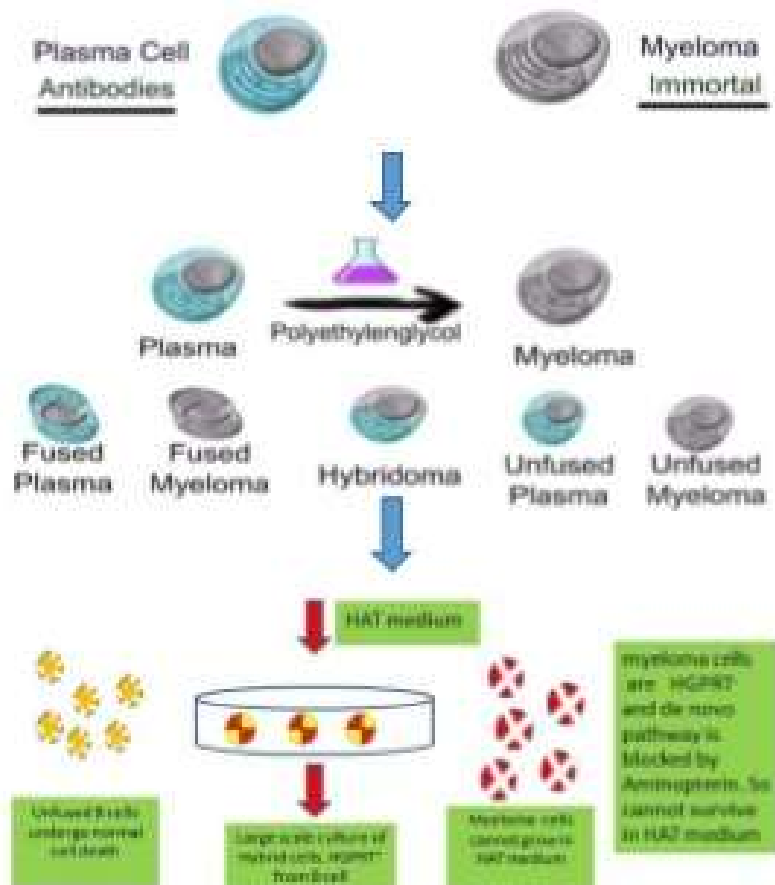
HYBRIDOMA TECHNOLOGY

- Hybridoma technology is a method for producing large number of identical antibodies called monoclonal antibodies.
- It was discovered by **G. Kohler and C. Milstein** in 1975. they were awarded nobel prize for physiology and medicine in 1975. • The hybrid cells are produced by fusing B- lymphocyte with myeloma cells or tumour cells.
- The B-lymphocyte have the ability to produce large number of antibodies and tumour cells have indefinite growth.
- This is why two cells are used for the production of hybrid cell.

Hybridoma Technology



HYBRIDOMA TECHNOLOGY



Procedure

1. The mouse is immunised by specific antigen injection against which monoclonal antibodies have to be produced.
2. After 72 hrs of immunisation spleen is collected from mouse.(antibody producing B cells).
3. The B cells are fused with immortalised myeloma cells by polyethylene glycol (PEG) or sendai virus.
4. The B cells are fused with immortalised myeloma cells.
5. The fused cells are incubated in the HAT medium.

HAT medium

- The hybridoma cells or fused cells are selected using selective media are called HAT medium.
- It contains Hypoxanthine, Aminopterin & Thymidine.
- The unfused B cells will die due to their short life span.
- The myeloma cells can synthesise DNA nucleotides using two pathway : Denovo pathway and Salvage pathway.

- In HAT medium, the myeloma cells are unable to replicate because the denovo pathway is blocked by Aminopterin in the medium.
- When denovo pathway is blocked, the cell will utilise salvage as an alternative pathway. But it cannot take place due to the lack of HGPRT (Hypoxanthine-guanine phosphoribosyl transferase) so it is contributed by B cell and is rich in HGPRT+.
- The salvage pathway is also inhibited due to the mutation of Thymidine kinase(TK), an enzyme that catalyses the phosphorylation reaction.
- The resulting clones of hybridoma cells secrete large quantities of monoclonal antibodies.

Applications

- It is used for the early detection of pregnancy.
- Detection and treatment of cancer.
- Diagnosis of leprosy.
- Treatment of autoimmune diseases.
- Radiolabelled monoclonal antibodies are used in vivo for detecting or locating tumour antigen.

Thank you