Abstract
Immunologically compromised patients can suffer from mild superficial lesions to disseminated full blown infections caused by several fungal pathogens including *Aspergillus fumigatus*, *Candida albicans* and *Cryptococcus neoformans* etc. Several advancements made in the field of medical sciences like, the introduction of antibiotics, newer chemotherapeutic option in treatment of cancer organ transplantation were intended to make tremendous impact on general public health. Paradoxically, some of these stipulated opportunity to infections, mainly those of fungal origin to establish themselves in the host. In fact, human subjects have become more susceptible to a range of opportunistic infections for last two or three decades. Besides HIV infection, individuals who have undergone organ transplantation or remain on treatment with anticancer drugs are prime targets of such infections. Similarly, patients receiving broad-spectrum antibacterial therapy or those subjected to invasive procedures are also highly prone to infection by fungal pathogens. The high frequency of life threatening systemic fungal infections could be attributed to the impaired immune system of the host because of human immunodeficiency virus infliction or due to the use of immunosuppressive drugs during organ transplantation or usage of anticancer agents in treatment of different types of malignancies. Limitations associated both with diagnosis as well as lack of effective treatment protocols for most of the fungal diseases by conventional means argue strongly in favour of pursuing the development of preventive strategies rather relying on antifungal chemotherapy.
Efforts will be made to evaluate the fully biocompatible and biodegradable delivery system made up of fibrin microsphere/Liposome for sustained delivery of anti fungal drugs/immunomodulators for efficient elimination of the fungal pathogens from systemic circulation. Besides, a novel strategy for immunization of animals against pathogenic *Candida* *spp* is also envisaged in the proposed study. Candida cytosolic proteins with immunogenic potential will be trapped in the fibrin network directly or after their encapsulation in appropriate liposome. Finally, the potential of the vaccine will be evaluated against experimental murine candidiasis/cryptococcosis in balb/c mice.

The proposed study envisages the development of new strategies for imparting protection against highly pathogenic fungal disease viz. candidiasis/cryptococcosis. We wish to attempt the development of sustained antigen delivery system using a combination of immunomodulators and cytosolic extract (*Candida albicans* and *Cryptococcus neoformans*) along with antifungal drugs and incorporating them in fully physiological and biodegradable fibrin microspheres. A parallel study involving incorporation of *C. Albicans*/*C. neoformans* immunogenic proteins (cytosolic/membrane proteins) in fibrin microspheres as candidate vaccine will be undertaken. Both free as well liposome encapsulated antigen will be incorporated in the fibrin microspheres and evaluated for its potential to impart protection against experimental murine candidiasis.

Nanomedicine is now within the realm of reality starting with nanodiagnostics and drug delivery. In the last 30 years there has been an explosive growth of nanotechnology in the purview of medicine which has ushered in challenging innovations in pharmacology. These discoveries have been highly instrumental in revolutionizing the temporal and spatial site-specific delivery of biologically active
compounds (Couvreur and Vauthier 2006). Nanoengineered materials can be
designed to exhibit very specific and controlled bulk chemical and physical
properties as a result of the control over their molecular synthesis and assembly.
Nanotechnology will be applied at all stages of drug development—from
formulations for optimal delivery to diagnostics applications in clinical trials.
Many of the assays based on nanotechnology will enable high-throughput
screening. The most important pharmaceutical applications of nano-particles are
in drug delivery. Besides offering a solution to solubility problems,
nanoparticulate systems provide efficient means of intracellular delivery.

_E. coli_ membrane comprises a great majority of anionic phospholipids that play a
pivotal role in membrane-membrane fusion. In the present study, we report that
the liposomes made of _E. coli_ lipid vesicles (escheriosomes) readily fuse with the
plasma membrane, and successfully deliver the encapsulated antigen to cytosol of
the target cells. In vivo administration of escheriosomes encapsulated antigen
(cAg) induced antigen specific strong CTL responses in the immunized mice. In
contrast, the antigen encapsulated in egg PC-liposomes, in a manner similar to the
antigen-IFA emulsion, had limited access to the cytosolic pathway of MHC-I
dependent antigen presentation and failed to generate antigen specific cell
mediated immune response.

Protection against intracellular fungal infections, in a manner similar to viral
challenges necessitates activation of both humoral and cell mediated immune
responses in unison. Most of the presently available antigen delivery vehicles
including egg phosphatidyl-choline (egg-PC) liposomes can evoke mainly humoral
immune responses in the immunized animals. Keeping this fact into
consideration, we earlier developed Escherichia coli membrane lipid vesicles
(escheriosomes) and demonstrated that escheriosomes successfully fuse with the plasma membrane of macrophages ensuing in effective cytoplasmic delivery of entrapped antigen, a pre-requisite for inducing CD8+ T cell response against antigens. In the present study, we report the ability of escheriosomes encapsulated Candida albicans (C. albicans) cytosolic antigens (cAg), to generate protective immunity against systemic C. albicans infection in BALB/c mice. The immunization schedule using escheriosome encapsulated cAg induced strong antigen-specific CD8+ T-cell responses, which were markedly higher than that observed in mice immunized with IFA-antigen emulsion, or antigen encapsulated in egg PC liposomes. Interestingly, immunization with cAg delivered in escheriosomes was also successful in complete elimination of C. albicans infection in Balb/c mice. The study suggests that escheriosomes may function as a novel immunoadjuvants and emerge as an effective tool for generating protective immunity against C. albicans infection.

The lack of early and effective diagnostic procedure, toxicity displayed by the most commonly used fungicidal drugs and emergence of resistant strains responsible for high morbidity and mortality requires an urgent need for vaccination against intracellular pathogens. In the present study, we report the use of γ-irradiated pathogen primed macrophages as an immunoprotective agent against disseminated cryptococcosis. The T-cell proliferation analysis clearly showed that the γ-irradiated pathogen primed macrophages proved to be a better immunostimulatory agent than the cytosolic fraction of C. neoformans. Mice immunized with different vaccine formulations developed CD8+ T-cell mediated Th-1 response as was assessed from the cytokine profiling and IgG isotyping. Protective studies in immunized animals challenged with live C. neoformans showed improved survival rates. However, the protective efficacy was highest in case of
animals immunized with xenovaccines as was evaluated with increased survival rate (80%) and decreased fungal burden in the vital organs of the animals compared with control groups and groups of mice immunized with allovaccines or for that matter synvaccines. Together, these suggest that γ-irradiated pathogen harbouring xenovaccines could play an active role in imparting protection against experimentally disseminated cryptococcal infection.

The development of a prophylactic vaccine against systemic cryptococcosis employing Cryptococcus neoformans cytosolic proteins (Cp) as antigen and biodegradable and biocompatible fibrin beads as an antigen carrier system. Groups of mice were administered either with free Cp, or Cp entrapped in fibrin beads, or liposome encapsulated Cp further co-entrapped in fibrin beads. Humoral immunity was studied by measuring the anti-Cp antibody titers in the sera of the immunized animals. Induction of cell mediated immunity was measured by delayed type hypersensitivity-(DTH), NO production, up-regulation of co-stimulatory molecules viz. CD80, CD86 on APCs on one hand and T-cells proliferation as well as induction of IFN-γ and IL-4 on the other. The efficacy of various vaccines in protecting mice against a lethal challenge with C. neoformans was assessed by determining animal survival rate and fungal burden in the systemic circulation and vital organs. Among various Cp based vaccines the preparation containing liposomised Cp co-entrapped in fibrin beads imparted better protection in the immunized mice as compared to other antigens delivery systems.

We can conclude that nanotechnology offers very promising tools that can be used for passive targeting. Various nanoparticle and liposome based approaches can be applied to overcome the solubility and bioavailability issues of
immunogenic protein. We found that escheriosomes based vaccine successfully protect mice against C. albicans infection and pave the way for its use against other infection also. γ irradiated pathogen primed macrophages of different types (synvaccine, allovaccine, xenovaccine) showed greater efficacy in combating infection than the cytosolic fractions of the pathogen and amongst these types xenovaccine established itself an upperhand to all these vaccine. Thus it proved to be a better candidate for vaccine against intracellular pathogen, C. neoformans. In last, the fibrin bead based liposomal vaccine successfully protects mice against C. neoformans and may emerge as a model system for use against various other infections.