



Micap™ topical antifungal formulation has 5 x greater efficacy than commercial equivalent Pevaryl®

The Micap™ microencapsulation technology based on the use of non-viable natural yeast cells and cell walls commonly used in the brewing and baking industry has been found to be a highly effective way in enhancing the efficacy of the antifungal active econazole nitrate (EN) commonly used to treat the skin infection 'thrush' caused by the fungus *Candida albicans*.



Figure 1
Micap E (bottom) and Pevaryl® against Candida albicans

Micap encapsulated econazole nitrate (Micap E) and a commercial product, Pevaryl® containing the same concentration of active were compared in laboratory trials. Initial results on *Candida albicans* cultures grown on agar demonstrated that Micap E (Figure 1 bottom left) has as much activity in inhibiting the growth of *Candida albicans* as the commercial product, Pevaryl® (Figure 1 top right).

Quantifiable results were obtained using an assay for viable cells based on ATP measurement. ATP is the basic energy source in living organisms and its level is directly proportional to the number of living organisms. Stock solutions of *Candida albicans* at the same concentration were incubated for ten minutes with the same quantity of EN in both Pevaryl® and Micap E formulations.

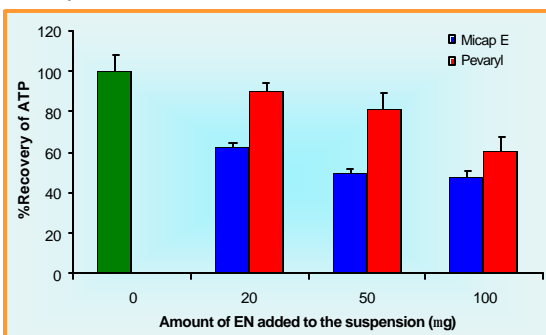


Chart 1
Concentration dependant effect of Micap E and Pevaryl®

Chart 1 shows that the % ATP (i.e. number of viable *Candida albicans*) recovered with 20mg of EN in a Micap E formulation is equivalent to that recovered with 100mg of EN in a Pevaryl® formulation. This shows the Micap E formulation having 5x greater efficacy than the Pevaryl®. Similarly the Micap E was also found to act faster than the Pevaryl® - at 5 minutes the kill rate achieved was better than that with the commercial product after 30 minutes.

'Infected skin model'

The results found in the plate assay and in suspension were confirmed when skin samples infected with *Candida albicans* were held in a Franz cell and treated with Micap E and Pevaryl®. The Franz cell uses skin samples taken from the abdomen and provides a more realistic representation of the skin. *Candida albicans* cultures were dried onto these skin samples and the formulations applied as 10% aqueous suspensions. Using the ATP assay the viability of *Candida albicans* was tested after ten minutes incubation (Chart 2).

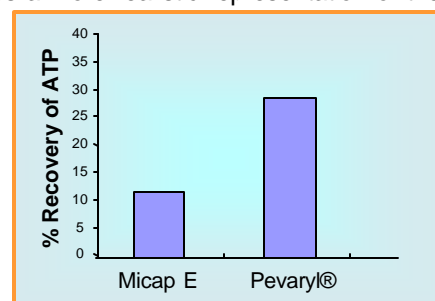


Chart 2
Infected skin model bioassay

Whilst both formulations showed activity the Micap E proved to be considerably better than the commercial formulation.

'Triggered release'

At this stage it is difficult to determine the mechanism for the enhanced effect- it appears that the improvement in activity may be due to direct contact between the yeast capsules and the target microorganism. In the course of the experiment it was found that the active ingredient EN remained in the capsules unless *Candida* was present. Control experiments using skin or aqueous based formulations alone did not result in release of EN. Clearly EN must be released for fungicidal activity to be detected and the 'trigger' appears to be the presence of live growing fungal cells.

Formulation

In developing a pharmaceutical formulation, Micap E showed good stability and bioavailability in a range of water based gels such as hyaluronic acid. Further Franz cell work showed that availability of the econazole from these water based gels was significantly greater than from conventional cream formulations.

Conclusion

Using the Micap™ yeast microencapsulation system, bioavailability of a topical drug such as econazole nitrate is improved considerably. The efficacy of the drug is enhanced in terms of both active required and speed of action. With a simple formulation, Micap™ encapsulation technology gives the opportunity to develop cost effective topical applications that are not only more effective but also kinder to the skin.

Fungal infections

Fungi are widespread microbes that can be found living in every conceivable habitat in the world; anywhere there's a little moisture, a source of carbon and nitrogen and the barest hint of mineral nutrients. Given their adaptability, it's not surprising that many fungi have found a way to grow on or in animal and human hosts. Some of these fungi survive without causing us any problems, forming part of the normal gut and skin 'flora'. Others are more invasive and aggressive and can cause irritating and sometimes dangerous infections if left unchecked.

Fungi can grow as spreading networks (mycelia) made of fine tubes (hyphae) or as single-celled, budding yeasts. Mycelial fungi known as dermatophytes are responsible for many skin, nail and hair infections including the well-known 'athlete's foot' and 'ringworm'. Skin



infections show up as spreading patches of itchy, flaking or inflamed skin; nail infections can cause discoloration, cracking and thickening of the nail. Dermatophyte spores are spread in flakes of skin, allowing the infection to travel to new sites or hosts. Dermatophyte infections are generally more irritating than dangerous, but can be persistent and open the skin up to bacterial infections. Thrush is a common fungal infection of the mouth or vagina typically caused by *Candida*

albicans; thrush appears as white patches or coatings accompanied by intense itching or burning sensations. *Candida* are capable of causing more dangerous deep-seated or blood infections, especially vulnerable are people with HIV-AIDS, transplant and chemotherapy patients and premature babies. Those with weak immunodefences may also be vulnerable to infection, particularly of the lungs, by the *Aspergillus* family of mycelial fungi. Fortunately there are drugs that are effective against fungal infections. Many of these drugs target the fungal

cell membrane; polyene drugs puncture the membrane and kill the cell whereas azole drugs prevent the fungal cell from making ergosterol, a vital membrane component which is the fungal version of cholesterol. One problem with the azole drugs is that they have poor water-solubility, making

them difficult to formulate resulting in poor bioavailability.

Micap has recently shown that the azole drug econazole can be encapsulated and formulated into a topical antifungal cream with superior effectiveness; other azoles also encapsulate well. So, at Micap we are looking forward to the exciting prospect of using fungi, in the form of yeast-encapsulated azole formulations, to fight fungi. I hope that my athlete's foot appreciates the irony!

Dr Jonathan Crowe



Jonathan's background in yeast physiology gives him a key role in developing the Micap™ technology for industrial applications. Presently his work is concentrating on Micap™ pharmaceutical applications for both oral and topical drug delivery.

He has considerable experience in yeast physiology gained at some of the leading research institutes in the world. He first spent two years at the Royal Agricultural and Veterinary University in Copenhagen working on fungal pathogens of potatoes. Jonathan followed this up working on biomedical aspects of fungal science at the University of Aberdeen. Here his work addressed problems associated with infection by *Candida albicans* which causes both thrush and deeper infections and is the biggest fungal pathogen of humans.

His work concentrated on how *Candida* interacts with human blood proteins and also on developing 'cell suicide' of the organism as a potential therapy. After his experience in academia, Jonathan is now looking forward to bringing his experience of yeast physiology to Micap where he will be looking at using encapsulated drugs to

Micap™ applications

The Micap™ technology of microencapsulation uses micro-organisms in particular yeast cells as naturally preformed microcapsules. The mechanism of microencapsulation and release and the natural surface properties of the yeast combine to give a wide range of benefits suitable for exploitation in various business sectors including food, pharmaceutical and agrochemical sectors.

The microencapsulation process and applications in key sectors are protected by a number of international patents and patent applications controlled by Micap plc.

Micap plc

Micap plc is a publicly quoted technology licensing company that uses yeast cells as capsules to deliver an active substance. The patented microencapsulation technology, which covers the use of bacteria as a capsule as well as yeast, is already licensed for flavours, with Firmenich SA, and drug delivery applications with SkyPharma plc.

For further information please visit www.micap.co.uk

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