Saccharomyces Cerevisiae (brewer's yeast) and Oral Vaccine Development

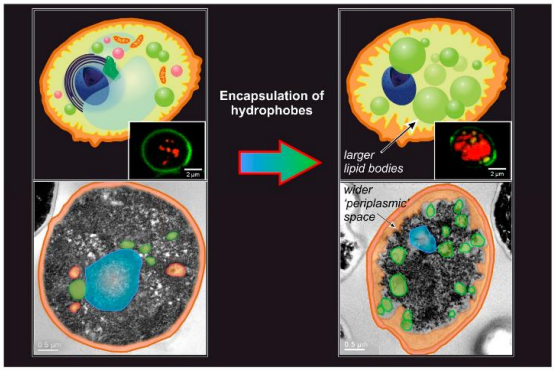


Image shows how the yeast ulstrastruture is dramatically altered by the encapsulation of the active ingredient. For example, there is a large increase in the number and dimensions of bodies that can be coloured by the lipid stain Nile Red. The periplasmic space is also increased. Cytoplasmic organisation also appears more granular.

What is Yeast and What Makes It Special?

Yeasts are uni or multicellular eukaryotic organisms that reproduce asexually. They can thrive in aerobic and anaerobic conditions, and are considered remarkable because of their metabolic activity, which has made them very useful for a number of applications. Yeast is commonly used in the food and fermentation industry, and has more recently been used within the field of microcapsules. Yeast cells can be employed (either living or dead), intact or permeabilized, and even emptied completely of their cytoplasmic contents, in some cases. Yeast is a suitable organism for microencapsulation because it has a cell wall (a physically rigid structure); it is an appropriate size (S.cerevisae cells, for example, most commonly range from 5-10um, and a microcapsule works at a 1-1000um scale); and they are known for their ability to absorb hydrophobes. . Furthermore, yeast makes for a superior 'capsule' because of it's low cost, the biodegradability and biocompatibility of the capsules, and the sustainable origin of the organism. Another advantage of yeast is that, unlike with newly developed polymers, the safety of yeast for application via the oral an d topical route, is well established, which makes for easier acceptance of the material in drug delivery application.

Microcapsules with Microbes – What!?

Microencapsulation is a process in which tiny particles are coated to produce small capsules, with useful properties. These particles may be solid, liquid, or glass, and through various techniques are encapsulated into microparticles with a diameter of 1-1000um – which have a variety of applications, including in textiles, cosmetics, food, medicine, and advanced materials. The unique advantage of microencapsulation lies in that the core material is completely coated and isolated from external environment. More importantly, microencapsulation would not affect the properties of core materials, provided that proper shell material and preparing method are chosen. Microencapsulation as a process began in the 1930s, but grew in popularity in the 1970s, which was when Joseph Shank first proposed microbes, in particular yeast, for the encapsulation of active ingredients. He concentrated on industrial uses of yeast cells, however (the first seminal patent was in 1976, but there have been many more, each specific to the exact encapsulation method, as this depends on the exact use). Later, researchers began to consider yeast-based microencapsulation technology for targeted drug delivery.

The Microencapsulation Process?

The process involves lipophilic (soluble in lipids)/hydrophobic active ingredients being captured into the central core of the yeast cell, driven by processes within the lipid bilayer membrane of the yeast

cell. How exactly this stabilises has not been determined, but it appears that lipid material from the membrane and cell organelles change in structure during the process.

In practice, a yeast slurry is prepared and stirred, and then the active ingredient is added. Stirrer speed is carefully controlled to obtain correct droplet size for optimum diffusion, and the vessel is heated to around 40 degrees, which, without destroying the yeast cell membrane's structure, 'opens' it so that it becomes permeable for the active ingredient. The yeast cell wall acts as a molecular sieve, so only very tiny molecules may enter. At the end of the process, the yeast cells are harvested by centrifugation, washed, and then treated as necessary (for example, are freeze dried), to produce a dry powder, or other suitable form, of microcapsule.

Upon meeting appropriate stimuli, the active ingredient is released.

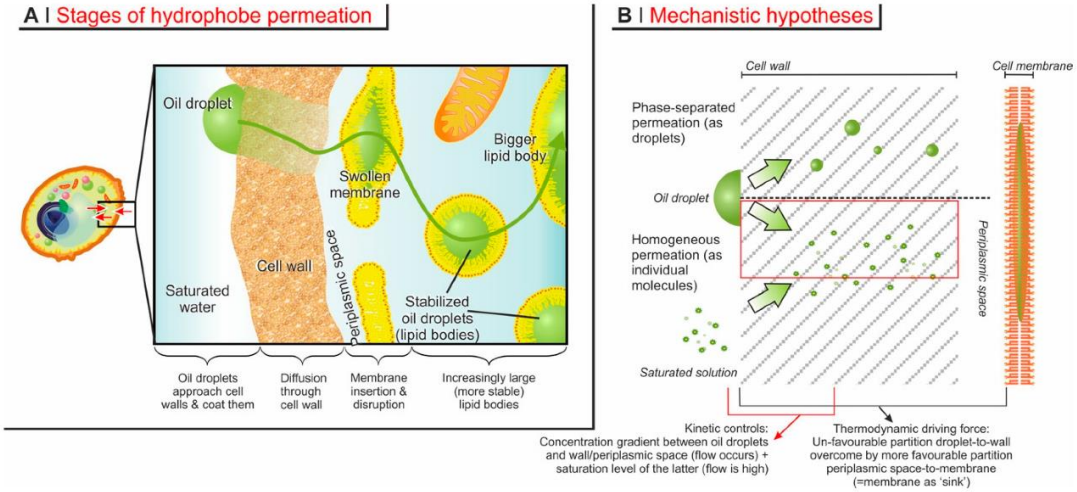


Diagram on left shows the four main stages of permeation of the active ingredient into the yeast cell. Diagram on right shows the process on a mechanistic level (diffusion process).

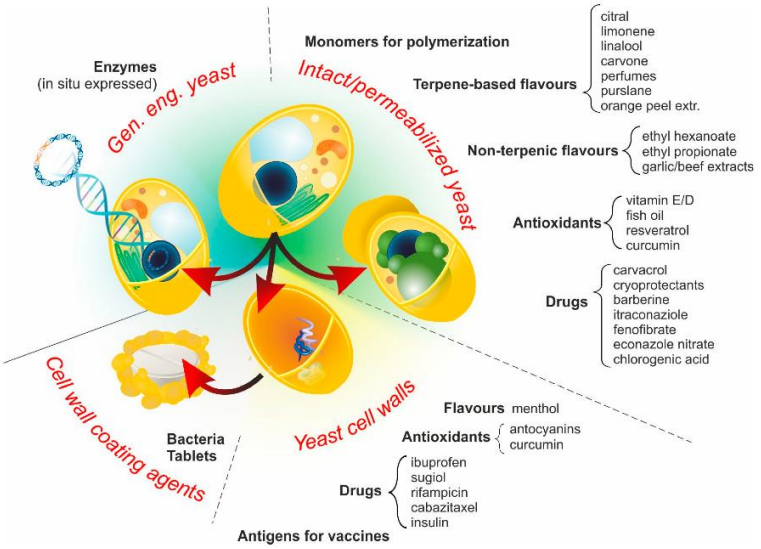
Drug Delivery Applications

Oral Delivery? Yeast cell based microcapsules are able to deposit a localised concentrated active ingredient on mucosal surfaces of choice. There are already established coating materials, such as enteric (intestine related) coating systems, which can allow targeted delivery of the yeast and therefore the lipophilic active ingredient, anywhere from the mouth to the lower bowel. At the desired mucosal surface, the yeast cells deliver their contents for absorption into the appropriate systemic circulation system.

Topical Delivery? Developed after oral delivery approaches, topical delivery relies on other biological materials stimulating the release of the active ingredient. Release has not been detected with epidermal (three outermost) skin layers, but live microbes can trigger release. Yeast-encapsulated antimicrobials can therefore be used to target conditions, and has successfully killed bacteria including Candida albicans (thrush) and methicillin-resistant Staphylococcus aureus (MRSA) in the laboratory. It was found that, in comparison with an antifungal topical cream, the yeast microencapsulated formulation containing the same concentration of active ingredient was at least five times more effective, and the speed of microbe-death was also much faster. The yeast microcapsules can also easily be incorporated into a variety of conventional cream bases.

Why are Oral Vaccinations so useful? Antibiotics have dramatically reduced deaths and sufferings from microbial vaccinations since their widespread application in the 1960s, however they remain a huge threat. Present vaccine preparation and application regimes have many drawbacks, still. Many conventional vaccines require multiple injections to induct the required immune response, and there is the risk of pathogenicity with live attenuated vaccines (reduced virulence). There is also a steady rise in allergic responses to many vaccines, or hypersensitivity. Administration of live or attenuated vaccines in immunocompromised individuals is also an issue. Thus there is an urgent need for novel vaccines with alternative administration routes, preferably that are low cost and suitable for resource-poor settings, as many infections are most prevalent in less developed countries. Oral vaccination is considered a very useful route of vaccination, as it provides social advantages (e.g. patients may have fears of needles and want to avoid pain) as well as economic (cost of the injection process, practically). Needle free vaccine administration also eliminates the risk of transmitting blood-borne pathogens and can be performed by health workers without medical training. Oral vaccines also do not require extensive antigen purification, because the gut is already heavily colonised by microbiota.

A Bit More About Yeast Based Microencapsulated Cells, and their Uses:



There are four main approaches to yield YBMCs. The classes depend on whether intact or permeabilised yeast is used, or if cell wall remnants are used for passive/diffusion encapsulation, or whether they are genetically engineered to produce active compounds intracellularly.

Intact yeast cells mean the yeast is not pre treated, and has been used for the encapsulation of terpenes as well as perfumes, and water insoluble drugs, however studies have shown the encapsulation in these cells is less efficient in many actives. Permeabilised yeast cells, through plasmolysis (a treatment weakening the cell membrane) or partial enzymatic degradation, or organic solvents, is one way of counteracting the lack of efficiency in intact yeast cells.

Yeast cell walls involve a much more aggressive approach, in which the cell wall is reduced to it's glucan components, which are likely to be the least immunogenic constituents. Though they may not present an intact barrier, nanoparticles can be trapped inside of them, but the retention of this material is a critical issue and needs addressing in each specific case. In terms of applications, YCWs are often considered as pharmaceutical carriers; despite their potentially lower immunogenicity in comparison to their parent cells, YCWs are often employed as an avenue to specifically direct a payload to inflammatory cells such as macrophages in or through oral administration in the intestinal tract. It is worth mentioning that YCWs can also be employed as coating agents.

Finally, there is the possibility of genetically engineered yeast. In this approach, the natural yeast barriers are circumvented by having the cells producing specific (bio)molecules within their own body, an approach sometimes referred to as a 'biodrug'. Since the 1980s, yeast is one of the favourite microorganisms—and the preferred eukaryote—for the expression of recombinant, exogenous, and in particular, human, which in turn can intracellularly produce small molecules such as ascorbic acid and antioxidant amino acids.

Sources:

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