ORAL MICRCAPSULES IMPACT ON GASTROINTESTINAL MICROBIAL FLORA: AN IN-VITRO ANALYSIS

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Microencapsulation, objectives and aspects

The technology of microencapsulation has created the possibility for immobilization of various compounds in an immunoprotective barrier. In addition to immunoisolation, the entrapment of bioactive compounds in a polymeric ultra-thin membrane can provide controlled release and continuous delivery of therapeutic products including drugs, enzymes, live bacteria and cells in order to provide the objectives of microencapsulation including replacing deficient organs by encapsulating therapeutic cells, slow release of therapeutic materials from the semi permeable membrane and protecting the active agents from biodegradation[1].

Microencapsulation, oral delivery

This technique is a potential solution to make the oral delivery of therapeutic agents possible[2]. As a result of the practical advantages over other administration routes, considerable attention has been focused on oral delivery. However the bioavailability of active materials in the gastrointestinal (GI) tract is normally low due to several barriers in GI tract including proteolytic enzymes and high acidic conditions in the stomach[3]. Through microencapsulation, active agents are isolated from the surrounding environment including the immune system of host and harsh condition of GI tract. Many researches have been performed to optimize different membrane formulation using various modified polymers[4]. However, the lack of a stable membrane for the oral delivery of live cells and bacteria has not been solved.

Novel microcapsule, biomaterials

A novel microcapsule has been designed consisting of a multi-layer membrane using alginate, polylysine, and pectin[5]. Alginate and polylysine are very commonly used biomaterials for encapsulation. Alginate is an anionic polysaccharide containing D-mannuronic acid and L-guluronic acid. it is normally used for the core of microcapsules. Polylysine is used as the polycation to form the membrane and reduce the parasity of the gel[6]. Pectin is a natural polysaccharide present in the cell wall of most plants. Pectin increases the stability of microcapsules in acidic pH of GI tract[7,8]. The combination of these materials may constitute a new chemical entity which needs performing in-vitro analysis to prove biomaterials composing the membrane does not lower the microbial population significantly hence it does not affect the functionality of the GI tract.

The present work analyzed the effects of the oral delivery of the novel microcapsules on the microbial contents of the GI tract. For this purpose, a GI tract model consisting of five vessels has been applied as a simulator of the human GI tract. Each vessel represents a different region. The stomach, small intestine and colon can be simulated using this model[9,10] and valuable information can be potentially obtained in the early steps of characterizing the novel microcapsule.

Materials and methods:

Microcapsules were prepared using an INOTECH Encapsulator. Alginate solution (1.65% (w/v)) (Sigma-Aldrich, low viscosity) was loaded in a 60ml syringe and extruded through a 300 μm nozzle at a frequency of 1052HZ and a voltage of 1.000kv. Alginate droplets were collected in 0.1 M $CaCl_2$ solution and stirred for 10 minutes for gel hardening. Ca-alginate beads were incubated sequentially in 0.1% (w/v) poly-l-lysine (Sigma, MW 27400), 0.1% (w/v) pectin (Sigma, degree of esterification 25%), 0.1% poly-l-lysine, and 0.1% alginate solution for 10 minutes in each. After each coating the capsules were washed with saline (0.85% w/v) and stored at 4°C. Solutions were prepared in saline 0.85% w/v.

Experimental setup of GI model reactor system

This apparatus has been designed to simulate human GI microbial ecosystem. It consists of 5 double layer vessels; each vessel represents a part of GI tract (Figure 1). The reactor was set up as described by Molly et al. In each vessel the condition of temperature, pH, volume and retention time are simulated and controlled by a computer. The first vessel serves as the stomach is fed by sterilized GI model medium. Then the medium is passed to the next vessel, which represents the small intestine, while simulated pancreatic juice is added (Oxgall 6g/l, Difco; Pancreatin 0.9g/l Across; $NaHCO_3$ 12g/l Fisher). Vessel 3 simulates the colon ascendans with a volume of 400 ml, pH of 5.5-6 and retention time of 9 hours. Vessel 4 and vessel 5, simulate the colon transversum and colon descendans, respectively. Total volume of vessel 4 is 800 ml, pH is 6-6.4 and retention time is 18 hours and for vessel 5 total volume is 500 ml with the pH of 6.6-6.9 and retention time of 11 hours. All vessels are maintained in anaerobic conditions by flushing daily with nitrogen for 20 minutes; the temperature is maintained at 37°C and the pH is regulated by the addition of 0.1mol/l HCl or 0.1mol/l NaOH.

Microbial analysis of GI model:

During the setup of the GI model, the last three vessels were inoculated with a fecal suspension. In order to perform microbial analysis, a range of different agar media were prepared to enumerate colonies formed of various bacteria. Considering the ratio of food materials and human bodily fluids a suitable amount of APPPA microcapsules was weighed and exposed to liquids of each vessel and stored in anaerobic conditions. One container was considered to be the control, which contained liquids without microcapsules. At specific time intervals, liquid samples were taken from each container and serially diluted in physiological solution (0.85%). The plates were inoculated with 0.1 ml sample of suitable dilution and were incubated in related conditions.

Results:

The results of the influence of APPPA microcapsules on the microbial flora of the colon ascendans simulator are represented in table 1. As it is shown the difference between microcapsules and the control was not very obvious. The numbers also indicate the reduction of bacteria in total anaerobe, however the microcapsules and the control had almost the same reduction. Table 2 represents the results of microbial analyses of the colon transversum simulator. The amount of total aerobes has shown a slight reduction over the 24 hours. Numbers show there was a clear reduction in the amount of *Escherichia coli* after 24 hours. The amount of total anaerobes has been clearly lowered after 24 hours. *Lactobacillus sp.* exposed to APPPA microcapsules lowered after12 and 24 hours as compared with the control.The influence of microcapsules on the microbial population of the colon descendans simulator is shown in table 3. Generally, significant variation between samples was not observed.

Conclusion:

This research indicates that biomaterials used in the new membrane do not affect bacterial flora of GI tract significantly. Based on the present study, APPPA microcapsule has shown encouraging results for the oral delivery; however, supplementary research is required to evaluate this membrane for therapeutic application. In-vivo experiments in experimental animal models are particularly required to consider for further research.

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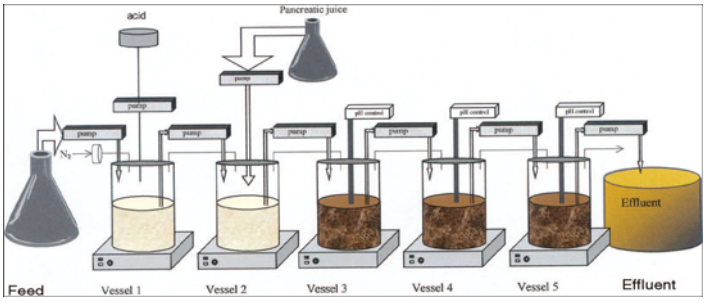

Figure1: Schematic representation of the dynamic simulated human gastro-intestinal (GI) model. Vessel1: Stomach; Vessel 2: Small intestine; Vessel3: Ascending colon; Vessel 4: Transverse colon; Vessel 5: Descending colon

	APPPA			Control		
	0h	6h	12h	0h	6h	12h
Aerobes	3.16±0.1	2.93±0.01	2.94±0.03	3.16±0.1	2.86±0.04	2.91±0.11
Ecoli	3.28±0.09	3.17±0.09	3.4±0.03	3.28±0.09	2.83±0.2	3.29±0.18
Anaerobes	3.11±0.05	2.99±0.04	3.03±0.01	3.11±0.05	3.0±0.03	2.97±0.03
Lactobacilli	2.63±0.04	2.56±0.06	2.58±0.6	2.63±0.04	2.67±0.1	2.55±0.02

Table1: The difference between bacterial populations of colon ascendance simulator in samples exposed to APPPA microcapsules and samples without any microcapsules at designated time intervals. The values have been shown in Log colony-forming unit/ml (log CFU/ml) unit versus time

	APPPA				Control			
	0h	6h	12h	24h	0h	6h	12h	24h
Aerobes	3.4±0.04	3.27±0.02	3.3±0.002	3.21±0.02	3.4±0.04	3.36±0.01	3.37±0.03	3.16±0.1
Ecoli	3.28±0.04	3.27±0.06	3.24±0.02	3.15±0.04	3.28±0.04	3.17±0.06	3.31±0.02	3.07±0.01
Staph*	2.97±0.05	2.49±0.007	2.74±0.02	2.6±0.08	2.97±0.05	2.79±0.08	2.77±0.08	2.54±0.07
Anaerobes	3.41±0.04	3.36±0.01	3.29±0.03	3.2±0.05	3.41±0.04	3.4±0.05	3.4±0.03	2.99±0.01
Lactobacilli	3.29±0.03	3.29±0.02	3.22±0.04	3.21±0.06	3.29±0.03	3.28±0.08	3.38±0.04	3.35±0.03

Table2: The difference between bacterial populations of colon transversum simulator in samples exposed to APPPA microcapsules and samples without any microcapsules at designated time intervals. The values have been shown in Log colony-forming unit/ml (log CFU/ml) unit versus time

	APPPA			Control		
	0h	6h	12h	0h	6h	12h
Aerobes	3.16±0.09	2.97±0.02	3.0±0.08	3.16±0.09	3.02±0.05	3.06±0.05
Ecoli	3.0±0.13	3.03±0.04	2.84±0.02	3.0±0.13	3.19±0.001	2.86±0.05
Staph.*	3.11±0.009	2.86±0.1	2.75±0.03	3.11±0.009	3.09±0.01	2.79±0.01
Anaerobes	3.22±0.12	3.26±0.15	2.9±0.02	3.22±0.12	3.02±0.19	2.88±0.12
Lactobacilli	2.91±0.01	2.89±0.02	2.71±0.05	2.91±0.01	2.87±0.005	2.88±0.09

Table3: The difference between bacterial populations of colon descendans simulator in samples exposed to APPPA microcapsules and samples without any microcapsules at designated time intervals. The values have been expressed in Log colony-forming unit/ml (log CFU/ml) unit versus time

* Staphylococcus sp.