https://www.scientificarchives.com/journal/journal-of-experimental-pathology

Journal of Experimental Pathology Mini Review

Application of Encapsulated Probiotics in Health Care

Esmat Alemzadeh[1,2], Ahmad Oryan[3*]

[1]Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran

[2]Department of Medical Biotechnology, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran

[3]Department of Pathology, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]Correspondence should be addressed to Ahmad Oryan; oryan@shirazu.ac.ir, oryan1215@gmail.com

Received date: April 25, 2020, **Accepted date:** September 02, 2020

Copyright: © 2020 Alemzadeh E, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Probiotics are generally defined as nonpathogenic living organisms that have beneficial effects on host health. The term "probiotic" means "for life" that is derived from Greek language. At first, probiotics was defined as substances that are produced with microorganisms and promote the other microorganisms. Then probiotics were described as tissue extracts that contribute to grow microbial and regulate the intestinal flora balance [1]. For the first time, the word probiotic was introduced by Gibson and Roberfroid in 1995 and described food supplements that were able to stimulate growth or activity of microorganisms [2].

Adequate amount of probiotics can prevent and treat diseases. However, probiotic effects are different based on the type of their strain [3]. Hence, some criteria such as identification of the genus, species, and strain level, safe for clinical use, able to adapt with intestine environment, product effective substances are necessary to these microorganisms qualify as probiotics [4]. Today diverse forms of probiotic products including capsules or lyophilized probiotics, heat-dried culture supernatants, and mixed in dairy foods are available. However, how to use and the type of probiotic used are the general problems with the use of probiotics [5]. Two genera *Lactococcus* and *Bifidobacterium* are the most common type of probiotics.

Today, probiotics gained special attention in the treatment of diseases. Several researches have shown that the effective role of probiotics in the treatment of cutaneous inflammatory, management of diabetes, prevention of infections, and gastro-intestinal disorders [6-8]. Beneficial effects of probiotics are exerted through multiple mechanisms including improvement of lymphocyte proliferation, stimulation of host immune responses and regulation of anti-inflammatory cytokine production [9,10].

It is shown that probiotics can be considered as a good alternative for conventional antibiotics in the treatment of skin disorders [11,12,13]. Findings of Puch et al. showed that oral consumption of probiotics could improve the stratum corneum barrier function [14].

The probiotic microorganisms indicate their beneficial properties on wound healing via two mechanisms: direct effects of the live microbial cells [15] or indirect effects through metabolites of these cells (biogenics). Peptides as the most important biogenics are derived from microbial activity that can affect on various immune responses such as increased IgA-producing cells in a dose-dependent way, increased macrophage activity, and increased specific antibody responses during infections [15]. Probiotics are able to help to normalize disruptions in human microbial communities and bacteria-host interactions that contribute to non-healing wounds [16]. Sekhar et al. proposed a logical hypothesis that topical application of probiotics promotes the healing of diabetic ulcer and prevents the diabetic foot infection. Probiotics activate TLRs and the production of beta-defensins by penetrating the inter-cellular lipid matrix into the dermis. Beta defensins increases the skin's immune functions by its anti-microbial and anti-inflammatory properties [17].

Infection is a big challenge in wound healing processes which delays wound repair in primary closure, traumatic wounds, burns, and chronic skin ulcers [18,19]. Chronic wounds can also show symptoms such as low transcutaneous oxygen tension, development of necrotic tissue, foul odor and wound breakdown [20]. A clinical study showed that oral treatment with a probiotic drink can change *P. aeruginosa* from a multi-drug resistant (MDR) to multi-drug sensitive strain. They suggested that probiotics could provide a therapeutic option for

combating MDR *P. aeruginosa* in patients colonized with this organism [21]. Many species of *lactobacilli* have S-layer proteins which are aligned in unit cells on the outermost surface of many prokaryotic microorganisms, can provide a protective action by inhibiting the growth of bacteria [22-24]. The initial theory behind the use of probiotics was thought to be a competitive blockage of pathogens, but it was also concluded that local immunity had been enhanced. Excessive use of human and agricultural antibiotics has raised the incidence of multidrug-resistant bacteria therefore, application of alternative methods such as probiotics is a new approach in managing infections [25].

Probiotics especially lactic acid bacteria and bifidobacteria have close association with gut epithelial cells and are ideal candidates for pathogen inhibition [26]. Lactic acid bacteria and bifidobacteria with inducing host mucosal defense systems activate tissue repair mechanisms [27]. Production of antimicrobial peptides such as bacteriocins, co aggregation and quorum sensing are the main mechanisms by which probiotics directly inhibit pathogens' growth. In addition, probiotics have high affinity binding to epithelial cell receptors in comparison with pathogens and it enable probiotics to displace pathogens in skin and mucosal surfaces [27].

Although the beneficial effects of probiotics on wound healing in the gastrointestinal tract have been confirmed through various experimental models, the problem of acid-sensitive probiotic strains is a big challenge for probiotic therapy in intestine [28]. Probiotic bacteria during transit of gastrointestinal trace and in stomach encounter with a very harsh environment that reduce viability of probiotics. Therefore, it is necessary to protect probiotics against the acidic and protease-rich conditions of the stomach [29]. Hence, increasing survival rate of probiotics is a priority for the treatment of intestinal wounds. Microencapsulation is one of the newest methods for promoting viability of probiotics [30].

Microencapsulation is a process in which solids, liquid or gaseous material are packaged with thin polymeric coatings and form small capsules. A microcapsule is a small sphere with a core and wall. Core is referred to as the internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. The stability of microparticles depend on wall. In addition, the wall composition may also determine functional properties and potential applications of the encapsulated components [31]. Microencapsulation has numerous applications such as oral drug delivery, encapsulation of biocides and pesticides, subcutaneous and intra-muscular delivery of analgesics, arterial and intra-tumoral delivery of anticancer agents, incorporation in coatings (anti-fungal, anti-microbial and anti-fouling), controlled release of special chemicals for wound healing [32,33]. Since some materials have short half-time and are rapidly degraded or their systemic administration in large doses cause harmful side effects, microencapsulation technology can deliver desired levels of bioactive materials within extended periods and maintain the biological and functional characteristics of natural products. The controlled release system has many applications in tissue engineering and wound healing studies [34].

Several factors have been reported to affect the viability of probiotics, including pH, hydrogen peroxide, oxygen, storage temperature, and so on. Microencapsulation is one of the most efficient methods to maintain the beneficial effects of probiotics [35]. Studies have already shown the potential benefits of probiotics in skin repair and healing [36-39]. It is hypothesized that microencapsulation of probiotics increases the stability and viability of probiotic bacteria and promotes the wound healing processes.

Microencapsulation of probiotics can be done with polysacaridic or lipid-beased materials such as alginate, starch, gelatin, celloluse, and chitosan. Microencapsulation, in fact, prevents dissolving coating materials in the acidic environment of the stomach, while in the alkaline environment such as gut with high pH starts dissolving coating materials [40]. Many studies have been reported the microencapsulated bacteria have higher stability and viability than uncoated bacteria [41-43]. Findings of Ghorbani-Choboghlo et al. showed that microencapsulation could increase the survival rate of *Saccharomyces cerevisiae* in the gastrointestinal tract [44].

There are several approaches to encapsulate the probiotics; these include spray-drying, spray-cooling, fluid-bed agglomeration and coating, freeze and vacuum-drying, emulsion-based techniques, coacervation, and finally the extrusion techniques that are used to encapsulate the microspheres [45]. The advantages and disadvantages of these techniques have been summarized in Table 1. However, extrusion and emulsion are the most two common encapsulation techniques [46,47]. In the extrusion technique, the capsules are made with hydrocolloids and the mixture of hydrocolloids and probiotics is fed into an extruder, typically a syringe. The pressure on the syringe results in extruding the cell suspension in the form of droplets to drip into a gelling solution. The viscosity of the hydrocolloid, diameter of the needle, and the distance between the needle and the setting bath are important and influential factors in size and shape of the droplets [46]. Alginate is a supporting material for extrusion technique. In this approach, the cell suspension is mixed with sodium alginate solution and the mixture free-fell into $CaCl_2$.

Probiotic encapsulation technique	Advantages	Disadvantages
Spray-drying	- suitable for large-scale, industrial applications - the most economic and effective drying method in industry - low operating costs	- low survival rate during drying of the bacteria - low stability upon storage
Spray-cooling	- low melting point for encapsulation - higher survival rate than spray-drying	- capsules are not soluble in water
Fluid-bed agglomeration and coating	- uniform coating - the most applicable technique for the coating of probiotics in industrial productions since it is possible to achieve large batch volumes and high throughputs	
Freeze and vacuum-drying	- avoid the water phase transition and oxidation - higher survival rate - higher water content - lowest inactivation upon storage	- very expensive technology
Emulsion-based techniques	- small size of capsules - enhance the viability of microorganism cells	- expensive technology - emulsion instability - need for vigorous stirring - random incorporation of cells into the capsules - inability to sterilize vegetable oil
Coacervation	- a relatively simple low-cost process - allow the incorporation of a large amount of micro-organisms in relation to the encapsulant	- it is a batch process - need to additional drying process
Extrusion techniques to encapsulate in microspheres	- simple and easy implementation - allow the retention of a high number of cells - useful in order to produce probiotic encapsulation in microspheres	---

Table 1: Advantages and disadvantages of encapsulation techniques of probiotic.

However, the mixture of cell and discontinuous phase of polymer, in the emulsification technique, is dispersed in a vegetable oil and homogenized by stirring to form a water-in-oil emulsion. The water-soluble polymer must be insolubilized to form the capsules [47]. In comparison to extrusion, the emulsification technique is more expensive because it requires the emulsifiers to stabilize the emulsion. Besides, emulsification shows some difficulties in preparing process including vigorous stirring and emulsion instability [46,48]. On the other hand, emulsification is a relatively new technique and easy to be scaled up for large-scale production. The size of capsules in emulsification is smaller than the beads produced by the extrusion technique [47].

Infection is a major challenge in chronic wounds and the emergence of antibiotic resistance has become a serious problem in health care and delay wound healing. Today, probiotics have been considered as a potential alternative treatment method. However, incompatibility of probiotics with antibiotics has been diminished their therapeutic utility. Microencapsulation can be an effective approach to protect probiotics. In this regard, Li et al. encapsulated probiotics with alginate and exposed to the antibiotic tobramycin. The results of this study showed that the growth and metabolic activity of encapsulated probiotics was not affected by tobramycin. In addition, they showed that using tobramycin combined with encapsulated probiotic could inhibit the growth of methicillin-

resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* in co-culture, the two important bacteria in chronic wounds [49]. In another study, Singh et al. encapsulated *Lactobacillus acidophilus* and ginger extract simultaneously and individually in alginate floating beads. The results showed that encapsulated *Lactobacillus acidophilus* and ginger extract were released slowly and significantly influenced gastric ulcer healing in rats [50].

Certainly, microencapsulation can be considered as an effective method for increasing the viability of probiotics over the course of the gastrointestinal tract. However, there are still challenges such as choosing non-toxic materials and developing microencapsulation procedures. Future research also needs to evaluate release profile of probiotics from capsules. In addition, new polymers need to be investigated to select the best protection materials. Finally, a large number of *in vivo* studies and then clinical trials need to be carried out in order to examine the efficacy of microencapsulated probiotics in health care. The size of capsules or beads is one of the main challenges which should be controlled. Various sizes of particles can impact the results. Selection of emulsifier is also an important subject because it can cause toxicity for probiotic cells. Development of new strategies to delete the emulsifier from the technology can be a good strategy for researchers. It is important to select encapsulation materials which can show different behavior under various pH conditions. The use of polymers that dissolve in high pH can help to design specific vehicles which are used in treatment of intestinal diseases. Finally, it should be noted again that encapsulation is a promising approach in which probiotics have gained higher viability and stability than the non-encapsulated bacteria. This strategy can also be used to deliver the probiotics into intestine for reducing the infections [46,47].

References

1. Shi LH, Balakrishnan K, Thiagarajah K, Ismail NIM, Yin OS. Beneficial Properties of Probiotics. Trop Life Sci Res. 2016 Aug; 27(2): 73–90.

2. Kechagia M, Basoulis D, Konstantopoulou S, Dimitriadi D, Gyftopoulou K, Skarmoutsou N, et al. Health benefits of probiotics: a review. ISRN Nutr. 2013 Jan 2;2013:481651.

3. Anadón A, Martínez-Larrañaga MR, Ares I, Martínez MA. Probiotics: Safety and Toxicity Considerations. In:Nutraceuticals; Efficacy, Safety and Toxicity. Hill-Parks E, (Ed). London: Academic Press. 2016; pp. 777–798.

4. Kunes M, Kvetina J. Probiotics: Preclinical Testing for Verification of Their Gastrointestinal Effectiveness. In:Nutraceuticals; Efficacy, Safety and Toxicity. Hill-Parks E, (Ed). London: Academic Press. 2016; pp. 799-810.

5. McFarland LV. Common Organisms and Probiotics: Saccharomyces boulardii. In: The microbiota in gastrointestinal pathophysiology: implications for human health, prebiotics, probiotics, and dysbiosis. Floch MH, Ringel Y, Walker WA, (Eds). London: Academic Press. 2017; pp. 145-164.

6. Hacini-Rachinel F, Gheit H, Le Luduec JB, Dif F, Nancey S, Kaiserlian D. Oral probiotic control skin inflammation by acting on both effector and regulatory T cells. PLoS One. 2009; 4(3):e4903.

7. Rad AH, Sahhaf F, Hassanalilou T, Ejtahed HS, Motayagheni N, Soroush AR, et al. Diabetes management by probiotics: Current knowledge and future perspectives. Curr Diabetes Rev. 2016

8. Bongers ME, Van den Berg MM, Liem O, Benninga MA. The role of a probiotics mixture in the treatment of childhood constipation: a pilot study. Nutrition Journal. 2007 Dec;6(1):1-6.

9. Manuel PM, Elena B, Carolina MG, Gabriela P. Oral probiotics supplementation can stimulate the immune system in a stress process. Journal of Nutrition & Intermediary Metabolism. 2017 Jun 1;8:29-40.

10. Ashraf R, Vasiljevic T, Day SL, Smith SC, Donkor ON. Lactic acid bacteria and probiotic organisms induce different cytokine profile and regulatory T cells mechanisms. Journal of Functional Foods. 2014 Jan 1;6:395-409.

11. Oryan A, Jalili M, Kamali A, Nikahval B. The concurrent use of probiotic microorganism and collagen hydrogel/scaffold enhances burn wound healing: An in vivo evaluation. Burns. 2018 Nov 1;44(7):1775-86.

12. Oryan A, Alemzadeh E, Eskandari MH. Kefir accelerates burn wound healing through inducing fibroblast cell migration in vitro and modulating the expression of IL-1ss, TGF-ss1, and bFGF genes in vivo. Probiotics and antimicrobial proteins. 2019 Sep 15;11(3):874-86.

13. Salaran M, Oryan A, Nikahval B, Kamali A, Ghaemi M, Abbasi-Teshnizi F, et al. Topical Application of Lactobacillus Plantarum on Burn Wound Healing in Diabetic Rats. Iranian Journal of Veterinary Surgery. 2019 Apr 1;14(1):60-72.

14. Puch F, Samson-Villeger S, Guyonnet D, Blachon JL, Rawlings AV, Lassel T. Consumption of functional fermented milk containing borage oil, green tea and vitamin E enhances skin barrier function. Experimental Dermatology. 2008 Aug;17(8):668-74.

15. Vinderola CG, Duarte J, Thangavel D, Perdigón G, Farnworth E, Matar C. Immunomodulating capacity of kefir. Journal of Dairy Research. 2005 May;72(2):195-202.

16. Wong VW, Martindale RG, Longaker MT, Gurtner GC. From germ theory to germ therapy: skin microbiota, chronic wounds, and probiotics. Plastic and reconstructive surgery. 2013 Nov 1;132(5):854e-61e.

17. Sekhar MS, Unnikrishnan MK, Vijayanarayana K, Rodrigues GS, Mukhopadhyay C. Topical application/ formulation of probiotics: will it be a novel treatment approach for diabetic foot ulcer?. Medical Hypotheses. 2014 Jan 1;82(1):86-8.

18. Augustine H, Gillis J, Williams J. Pseudomonas aeruginosa wound infections: a critical appraisal of topical antiseptics. Dalhousie Medical Journal. 2015; 42.

19. Oryan A, Alemzadeh E, Moshiri A. Burn wound healing: present concepts, treatment strategies and future directions. Journal of Wound Care. 2017 Jan 2;26(1):5-19.

20. Gardner SE, Frantz RA, Doebbeling BN. The validity of the clinical signs and symptoms used to identify localized chronic wound infection. Wound Repair and Regeneration. 2001 May;9(3):178-86.

21. Thomson CH, Hassan I, Dunn K. Yakult: a role in combating multi-drug resistant Pseudomonas aeruginosa?. Journal of Wound Care. 2012 Nov;21(11):566-9.

22. Rodrigues KL, Caputo LR, Carvalho JC, Evangelista J, Schneedorf JM. Antimicrobial and healing activity of kefir and kefiran extract. International Journal of Antimicrobial Agents. 2005 May 1;25(5):404-8.

23. Mobili P, de los Ángeles Serradell M, Trejo SA, Puigvert FX, Abraham AG, De Antoni GL. Heterogeneity of S-layer proteins from aggregating and non-aggregating Lactobacillus kefir strains. Antonie Van Leeuwenhoek. 2009 May 1;95(4):363-72.

24. Nole KL, Yim E, Keri JE. Probiotics and prebiotics in dermatology. Journal of the American Academy of Dermatology. 2014 Oct 1;71(4):814-21.

25. Howard JC, Reid G, Gan BS. Probiotics in surgical wound infections: current status. Clinical and Investigative Medicine. 2004 Oct 1;27(5):274.

26. Li H, Limenitakis JP, Fuhrer T, Geuking MB, Lawson MA, Wyss M, et al. The outer mucus layer hosts a distinct intestinal microbial niche. Nature communications. 2015 Sep 22;6(1):1-3.

27. Lukic J, Chen V, Strahinic I, Begovic J, Lev-Tov H, Davis SC, et al. Probiotics or pro-healers: the role of beneficial bacteria in tissue repair. Wound Repair and Regeneration. 2017 Nov;25(6):912-22.

28. Khoder G, Al-Menhali AA, Al-Yassir F, Karam SM. Potential role of probiotics in the management of gastric ulcer. Experimental and Therapeutic Medicine. 2016 Jul 1;12(1):3-17.

29. Del Piano M, Carmagnola S, Ballarè M, Sartori M, Orsello M, Balzarini M, et al. Is microencapsulation the future of probiotic preparations? The increased efficacy of gastro-protected probiotics. Gut Microbes. 2011 Mar 28;2(2):120-3.

30. Zanjani MA, Ehsani MR, Tarzi BG, Sharifan A. Promoting probiotics survival by microencapsualtion with Hylon starch and genipin cross-linked coatings in simulated gastro-intestinal condition and heat treatment. Iranian Journal of Pharmaceutical Research: IJPR. 2018;17(2):753.

31. Mano JF, Silva GA, Azevedo HS, Malafaya PB, Sousa RA, Silva SS, et al. Natural origin biodegradable systems in tissue engineering and regenerative medicine: present status and some moving trends. Journal of the Royal Society Interface. 2007 Dec 22;4(17):999-1030.

32. López AF, Deladino L, Alba SN, Miriam NM. Encapsulation of bioactive compounds with alginates for the food industry. @ limentech, Food Science and Technology. 2011 Nov 7; 10 (1).

33. Silva PT, Fries LL, Menezes CR, Holkem AT, Schwan CL, Wigmann ÉF, Bastos JD, Silva CD. Microencapsulation: concepts, mechanisms, methods and some applications in food technology. Ciência Rural. 2014 Jul;44(7):1304-11.

34. Elçin YM, Dixit V, Gitnick G. Extensive in vivo angiogenesis following controlled release of human vascular endothelial cell growth factor: implications for tissue engineering and wound healing. Artificial Organs. 2001 Jul 1;25(7):558-65.

35. Martín MJ, Lara-Villoslada F, Ruiz MA, Morales ME. Microencapsulation of bacteria: A review of different technologies and their impact on the probiotic effects. Innovative Food Science & Emerging Technologies. 2015 Feb 1;27:15-25.

36. Nasrabadi H, Ebrahimi T. Comparison of the effects of Lactobacillus brevis and Lactobacillus plantarum on cutaneous wound healing in rats. African Journal of Microbiology Research. 2011 Oct 30;5(24):4226-33.

37. Huseini HF, Rahimzadeh G, Fazeli MR, Mehrazma M, Salehi M. Evaluation of wound healing activities of kefir products. Burns. 2012 Aug 1;38(5):719-23.

38. Mayes T, Gottschlich MM, James LE, Allgeier C, Weitz J, Kagan RJ. Clinical safety and efficacy of probiotic administration following burn injury. Journal of Burn Care & Research. 2015 Jan 1;36(1):92-9.

39. Zoccali G, Cinque B, La Torre C, Lombardi F, Palumbo P, Romano L, et al. Improving the outcome of fractional CO2 laser resurfacing using a probiotic skin cream: Preliminary clinical evaluation. Lasers in Medical Science. 2016 Nov 1;31(8):1607-11.

40. Sarao LK, Arora M. Probiotics, prebiotics, and microencapsulation: A review. Critical Reviews in Food Science and Nutrition. 2017 Jan 22;57(2):344-71.

41. Zanjani MA, Tarzi BG, Sharifan A, Mohammadi N. Microencapsulation of probiotics by calcium alginate-gelatinized starch with chitosan coating and evaluation of survival in simulated human gastro-intestinal condition. Iranian Journal of Pharmaceutical Research: IJPR. 2014;13(3):843.

42. Ding WK, Shah NP. An improved method of microencapsulation of probiotic bacteria for their stability in acidic and bile conditions during storage. Journal of Food Science. 2009 Mar;74(2):M53-61.

43. D'Orazio G, Di Gennaro P, Boccarusso M, Presti I, Bizzaro G, Giardina S, et al. Microencapsulation of new probiotic formulations for gastrointestinal delivery: in vitro study to assess viability and biological properties. Applied Microbiology and Biotechnology. 2015 Nov 1;99(22):9779-89.

44. Ghorbani-Choboghlo H, Nikaein D, Khosravi AR, Rahmani R, Farahnejad Z. Effect of microencapsulation on Saccharomyces cerevisiae var. boulardii viability in the gastrointestinal tract and level of some blood biochemical factors in wistar rats. Iranian Journal of Microbiology. 2019 Apr;11(2):160.

45. Chávarri M, Marañón I, Villarán MC. Encapsulation technology to protect probiotic bacteria. InProbiotics 2012 Oct 3.

46. Gbassi GK, Vandamme T. Probiotic encapsulation technology: from microencapsulation to release into the gut. Pharmaceutics. 2012 Mar;4(1):149-63.

47. Krasaekoopt W, Bhandari B, Deeth H. Evaluation of encapsulation techniques of probiotics for yoghurt. International Dairy Journal. 2003 Jan 1;13(1):3-13.

48. Mortazavian A, Razavi SH, Ehsani MR, Sohrabvandi S. Principles and methods of microencapsulation of probiotic microorganisms. Iran Journal of Biotechnology. 2007; 5(1):1-18.

49. Li Z, Behrens AM, Ginat N, Tzeng SY, Lu X, Sivan S, et al. Biofilm-inspired encapsulation of probiotics for the treatment of complex infections. Advanced Materials. 2018 Dec;30(51):1803925.

50. Singh PK, Kaur IP. Synbiotic (probiotic and ginger extract) loaded floating beads: a novel therapeutic option in an experimental paradigm of gastric ulcer. Journal of Pharmacy and Pharmacology. 2012 Feb;64(2):207-17.