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PATENT EVALUATION

Site-specific delivery of polymeric encapsulated microorganisms: a patent evaluation of US20170165201A1

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ABSTRACT

Introduction: Probiotics inculde live microorganisms therapeutically effective in the treatment of wide range of diseases. Probiotics possibly stimulates the growth of preferred microorganisms, crowds out potentially harmful microorganisms, and reinforces the body's natural defense mechanisms. Microencapsulation of probiotic microorganisms protects them from the destructive environment and prolongs their survival. Use of mucoadhesive and pH responsive polymers could impart extended retention, pH sensitive release and mucoadhesive properties to the system. The probiotic formulations could be used for therapeutic, diagnostic, and prophylactic purposes.

Areas covered: Layer-by-layer techology was developed for encapsulating Bacillus coagulans employing chitosan and alginate as mucoadhesive polymers (for attachment to the gastrointestinal mucosa) and Eudragit EPO and Eudragit L100 as pH responsive polymers (for site-specific delivery). The formulation was evaluated for layer stability, mucoadhesion capability, protection of microorganisms from biological insults, pH responsive layer removal, in vitro evaluation in three-dimensional intestinal tissue model, probiotic bacterial delivery.

Expert opinion: In this patent, a unique layer-by-layer assembly of two differently charged polymers (mucoadhesive and pH repsonsive) was achieved for encapsulating the probiotic microorganism. For assessing the clinical applicability of the invention, further studies may be needed since the conclusions are drawn solely based on in vitro data.

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Probiotics; microrganisms; layer-by-layer; mucoadhesive polymer; pH responsive polymer

1. Introduction

The gastrointestinal microbiome has been shown to play a vital role in regulation and progression of various diseases ranging from ulceratives colitis through allergies to cancers. Modulating the microbiome population either via decreasing disease-causing microorganisms and/or increasing probiotic microorganisms has been shown to be effective in the treatment of a numbers of diseases [1]. The presence of certain microbes or absence of normal microbes or alteration in the proportion of microbes has shown to be responsible for certain diseases or disorders. In Crohn's disease, concentration of *Eubacteria, Bacterioides,* and *Peptostreptococcus* is increased whereas that of *Bifidobacteria* is decreased. In ulcerative colitis, the number of facultative anaerobes is increased. Bacterial vaginosis is characterized by the presence of *Gardnerella* and *Mobiluncus* spp. of bacteria and absence or reduction in number of *Lactobacilli* [1–4]. Probiotic bacteria *Lactobacillus fermentum* and *Bifidobacterium lactis* have been proved to reduce gliadin induced cellular damage. Enteric pathogens like *Escherichia coli, Salmonella enteriditis, Yersina pseudotuberculosis, Listeria monocytogenes* are associated with diseases like diarrhea, irritable bowel syndrome, and intestinal hemorrhages. Hence, live microorganisms (or probiotics) can improve the microbial balance of the host for curing respective diseases or disorders [1–5]. Several bacterial strains have been proven in clinical studies to be therapeutically effective against various diseases/disorders and are listed in Table 1 [1].

Recent findings suggest that specific alterations in gut microbiota could significantly enhance the efficacy of anticancer therapy. Probiotics have exhibited promising results in animal intestinal tumor models [2,3]. Chemotherapeutic agents used in anticancer therapy, being toxic to the gut microbiota, alters its composition either directly or through the activation of immune response. Thus, selective manipulation of gut microbiota may limit the incidence of specific tumors and/or improve the therapeutic efficacy of various anticancer agents [5]. Genetically engineered modified microorganisms have found applications in agriculture, human health, and bioremediation. Genetic modification offers the advantages of improving chemical selectivity and increasing molecular diversity. Genetically modified probiotics have been successfully used as vectors for the delivery of immunostimulatory molecules, tumor associated antigens, or enzymes that limit the toxicity of conventional chemotherapy [4]. Microorganisms could be genetically modified to secrete the

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Table 1. Microorganisms shown to be useful in curing diseases/disorders [1].

S. No.	Microorganism	Disease/Disorder
1.	*Lactobacillus plantarum* 299v, *Bacillus coagulans* ATCC No. 31,284, *Lactobacillus acidophilus* L1	Hypercholesterolemia and cardiovascular disease
2.	*Lactobacillus rhamnosus GG*	Atopy
3.	*Lactobacillus rhamnosus GG, Bifidobacterium lactis* *Lactobacillus paracasei*	Food allergies Eczema
4.	*Lactobacillus rhamnosus, Bifidobacterium lactis* *Lactobacillus acidophilus, Lactobacillus johnsonii*	Lowered immunity
5.	*Lactobacillus rhamnosus GG, Lactobacillus reuteri* MM53, *Lactobacillus acidophilus* CRL730, *Lactobacillus paracasei* CRL431, *Lactobacillus johnsonii* La1, *Bifidobacterium lactis* Bb12, *Lactobacillus plantarum* 299v, *Lactobacillus paracasei*	Gastroenteritis
6.	*Lactobacillus acidophilus, Lactobacillus johnsonii* La1	Lactose intolerance
7.	*Lactobacillus rhamnosus GG, Saccharomyces cerevisiae*	Crohn's disease
8.	*Lactobacillus rhamnosus GG, Lactobacillus acidophilus, Lactobacillus delbrueckii* ssp. *bulgaricans*	Colon cancer
9.	*Lactobacillus acidophilus, Lactobacillus johnsonii* La1, *Lactobacillus rhamnosus*	Peptic ulcer Non-erosive gastritis
10.	*Lactobacillus plantarum* 299, *Escherichia coli* Nissle 1917	Ulcerative colitis

therapeutics or diagnostic product, e.g. microorganisms could be engineered to act as 'drug factories' for secreting insulin in the treatment of diabetes or to secrete fluorescent proteins for detecting/diagnosing certain GI diseased conditions [4–6].

2. Mucoadhesion and mucoadhesive polymers

Mucoadhesion may be defined as the property of polymers to adhere to the moist mucosal membranes present in various regions of the body like ocular and nasal cavities as well as respiratory, gastrointestinal, and urogenital tracts. Mucoadhesion is a two-step process involving contact and consolidation stage. In contact stage, the mucoadhesive polymer is activated due to wetting and subsequent hydration leading to intimate contact between the polymer and the mucosal membrane. The consolidation stage involves interpenetration of the chains of mucoadhesive polymer and the mucopolysaccharide (mucin). These processes lead to the formation of bonds between the mucoadhesive polymer and the mucosal surface predominantly by weak van der Waals and hydrogen bonding, although electrostatic interactions can also occur in some cases [7].

Mucoadhesive polymers play an important role in the design and development of mucoadhesive drug delivery systems and may be classified on the basis of generation, source, charge, and solubility (Table 2). Potential advantages of mucoadhesive drug delivery systems include prolonged residence time of the dosage form, increased local and/or systemic availability and therapeutic efficacy of the drug and increased patient compliance [8].

Table 2. Classification and examples of different mucoadhesive polymers [**Ref. 8**; Reproduced with permission from John Wiley and Sons © 2017].

Classification basis	Category	Examples
Generation	First-generation	Cationic, anionic, nonionic
	Second-generation	Lectins, thiomers, bacterial adhesions, amino acid sequences
Source	Natural	Agarose, chitosan, gelatin, pectin, sodium alginate, various gums (guar, xanthan, gellan, carrageenan)
	Synthetic	*Cellulose derivatives* (CMC, sodium CMC, thiolated CMC, HEC, HPC, HPMC, MC, methylhydroxyethylcellulose) *Poly(acrylic acid)-based polymers* (CP, PC, PAA, polyacrylates, poly (methylvinylether-co-methacrylic acid), poly(2-hydroxyethyl methacrylate), poly (acrylic acid-co-ethylhexylacrylate), poly(methacrylate), poly (alkylcyanoacrylate), poly (isohexylcyanoacrylate), poly (isobutylcyanoacrylate), copolymer of acrylic acid and PEG)
Charge	Cationic	Aminodextran, chitosan, trimethylated chitosan, dimethylaminoethyl dextran,
	Anionic	Chitosan-EDTA, CP, CMC, pectin, PAA, PC, sodium alginate, sodium CMC, xanthan gum
	Nonionic	Hydroxyethyl starch, HPC, poly (ethylene oxide), PVA, PVP, scleroglucan
Solubility	Water soluble	CP, HEC, HPC, HPMC, PAA, sodium CMC, sodium alginate
	Water insoluble	Chitosan, EC, PC
Mucoadhesive interaction	Electrostatic interaction	Chitosan
	Covalent bonding	Cyanoacrylate
	Hydrogen bonding	Acrylates [hydroxylated methacrylate, poly(methacrylic acid)], CP, PC, PVA

3. pH responsive polymers

pH responsive polymer responds to changes in the pH of the medium and generally exhibit changes in physicochemical properties such as solubility, chain conformation/configuration, and surface activity. A change in pH may cause de-protonation of functional groups of the polymer. In some cases, it may cause flocculation, precipitation, and chain collapse/extension. It may also lead to self-assembly such as formation of micelles, gels, unimers, vesicles, swelling/deswelling, etc. [9]. A specific application of pH responsive polymers being targeted cancer chemotherapy wherein the inherent challenges such as non-specific multidrug resistance, tissue distribution, and tumor heterogeneity could be overcome by developing targeted drug delivery systems that sharply respond to specific pH environment in solid tumors. pH responsive polymers can be employed for various applications such as, but not limited to, gene delivery, drug delivery, sensors, and chromatography [10]. Typical pH responsive polymers may be classified as acidic polymers viz. *poly(carboxylic acid)s* (poly(acrylic acid), poly (methacrylic acid), poly(propylacrylic acid), poly(4-vinyl benzoic acid)), *poly(phosphoric acid)s* (poly(vinyl phosphonic acid), poly (ethylene glycol acrylate phosphate), poly(ethylene glycol

methacrylate phosphate)), *poly(sulfonic acid)s* (poly(vinyl sulfonic acid), poly (4-styrene sulfonic acid)), *poly(amino acid)s* (poly aspartic acid), poly(L-glutamic acid), poly(histidine)), *poly (boronic acid)s* (poly(vinylphenylboronic acid), poly(3-acrylamidophenyl boronic acid)); and basic polymers viz poly [(2-dimethylamino)ethyl methacrylate], poly[(2-N-morpholino) ethyl methacrylate], poly(acryloylmorpholine), poly(4-vinylpyridine), poly(N-vinylimidazole), poly(propylenimine) dendrimer, poly(ethylenimine) dendrimer, and poly(amidoamine) dendrimer [9].

4. Microencapsulation of microorganisms

Microorganisms may be entrapped in the polymer matrix by spray drying, emulsion, and extrusion techniques. The solvent systems used in encapsulation must be nontoxic and process conditions should be selected with an aim to maintain maximum viability of the microorganisms. The microencapsulation provides extended retention, prolonged survival, and pH sensitive release of microorganisms at the target sites of gastrointestinal tract [11].

4.1. Extrusion technique for encapsulation of microorganisms

It involves preparation of hydrocolloid solution of alginate followed by subsequent addition of probiotic microorganisms. The suspension of probiotic microorganisms in polymeric solution was passed through a hypodermic syringe into cationic hardening solution of calcium leading to the formation of three-dimensional lattice structure of polymer entrapping the probiotic microorganisms. The microorganism entrapped polymeric beads were further dripped in chitosan solution for providing a coat of chitosan on the beads [12].

4.2. Emulsion technique for encapsulation of microorganisms

A small volume of probiotic microorganisms and polymer slurry (dispersed phase) were added to the continuous phase of vegetable oil (e.g. soya oil, sunflower oil, corn oil) or liquid paraffin. Once emulsification is done, it was gelified by ionic, enzymatic, or interfacial polymerization techniques [13].

4.3. Emulsification and ionic gelification technique for encapsulation of microorganisms

First, under emulsification step, a single-phase emulsion is formed. Water soluble polymers become insoluble after the addition of calcium chloride leading to the formation of cross-linked gelatinous particles in the oil phase of the emulsion [14].

4.4. Layer-by-layer technique for encapsulation of microorganisms

The layer-by-layer approach for encapsulating living cells was first reported by Diaspro and co-workers [15]. Layer-by-layer procedure involves alternate adsorption of oppositely charged polymer on the surface of microorganisms leading to the formation of nanocages. Thickness, strength, permeability, and morphology of the layers could be tailored to provide tunable properties to the system. For oral delivery of probiotic microorganisms, this technique offers the advantages of 1) protection against acidic conditions, 2) digestive enzymes and bile salts of gastric environment, and 3) enhanced mucoadhesion and in vivo survival of microorganisms [16]. Layer-by-layer procedure is a promising method for enhancing the viability of microorganisms during storage, processing, and gastrointestinal transit. Priya and co-workers (2011) used layer-by-layer self-assembly employing chitosan and carboxymethyl cellulose as polyelectrolyte polymers for encapsulating *Lactobacillus acidophilus*. The enhanced survival rate of encapsulated microorganism was attributed to the impermeability of polyelectrolyte nanolayers to pepsin and pancreatin enzymes. Layer-by-layer encapsulation of microorganism resulted in enhanced stability in gastric and intestinal environment. Moreover, it also reduced viability losses of the microorganism during freezing and freeze drying [17].

5. Expert opinion

The US patent application (US 2017/0165201 A1) by Anselmo and co-inventors (2017) provided a unique method for encapsulating microorganisms [6]. Although polymer coating approaches were reported earlier to this invention and were capable of protecting the probiotic from harsh GI environment (gastric acid and intestinal bile salts); these approaches prevented direct contact between the encapsulated probiotic and the GIT wall. As shown in Figure 1; the mucoadhesive characteristic was imparted to the delivery system by first coating the microorganism with a cationic polymer (such as chitosan) followed by layered coating with an anionic polymer (such as sodium alginate) – this was assigned as one bilayer. This layer-by-layer (LbL) approach was achieved via electrostatic interactions between the constituent polyelectrolytes thereby allowing minimal polymer coating. The process was repeated to give two- or three-bilayered systems. The rationale for using a cationic polymer as the first layer was the negatively charged nature of cell membrane. Furthermore, given the inherent presence of polysaccharides on the cell membrane, the use of natural polymers such as chitosan and alginate persevered the surface topography and morphology of the probiotic [6,16]. The current patent employed a minimal amount of polymer to coat the microorganism and further disclosed the targeted enteric delivery of microorganisms accompanied by 1) prolonged survival of microorganisms within the coating and in the gastrointestinal environment; 2) extended retention on the enteric mucosa due to mucoadhesion; and/or 3) a pH-sensitive release of the bioload (Figure 2).

In an additional embodiment, a pH sensitive polymer such as Eudragit L100 (anionic) or Eudragit EPO (cationic) was/may be employed to impart localized delivery of the probiotic in the GI tract. In a preferred embodiment, the terminal layer polymer exhibited the pH responsiveness while the next layer came into play when the terminal layer is shed. Anselmo et al., 2016, tested and reported the in vitro and in vivo performance of the delivery system. For example, in case of a chitosan/L100

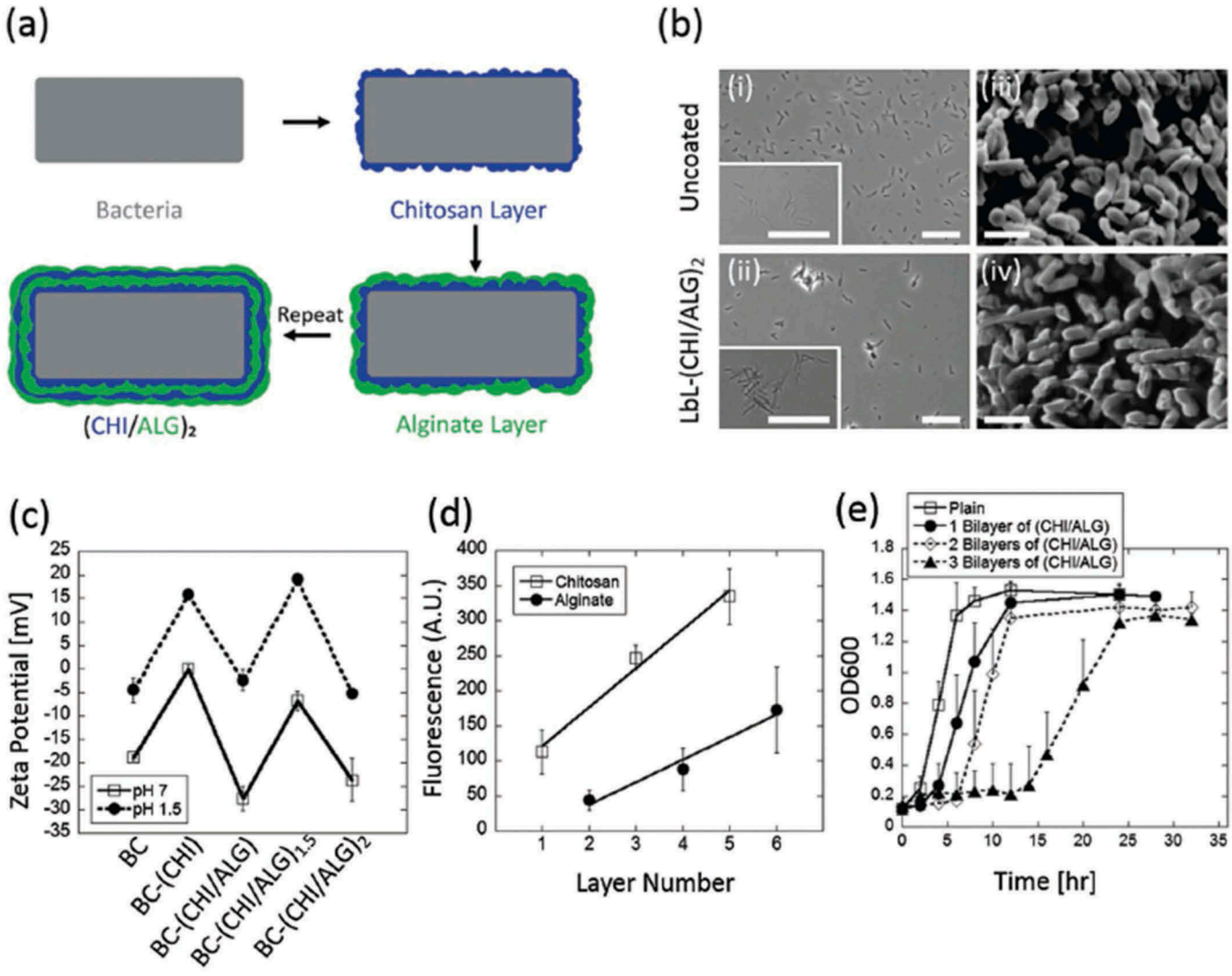

Figure 1. Layer-by-layer encapsulation of probiotics. (a) Schematic LbL templating of chitosan and alginate on probiotic. (b) Brightfield images of (i) uncoated-BC and (ii) LbL-(CHI/ALG)2-BC. SEM images of (iii) uncoated-BC and (iv) LbL-$(CHI/ALG)_2$-BC. (c) Zeta potential at each sequential layer, for up to two chitosan and alginate bilayers, $(CHI/ALG)_2$, at pH 1.5 and 7. (d) Uniform layer templating for up to three bilayers of chitosan and alginate was confirmed via measuring fluorescently labeled chitosan and alginate. (e) Bilayer number modulates probiotic growth. As bilayer number increases, the time taken to reach the exponential growth phase is shifted to the right. Error bars represent standard deviation (n = 3). Bright field scale bars = 25 μm. SEM scale bars = 2 μm [**Ref. 16**; Reproduced with permission from John Wiley and Sons © 2016].

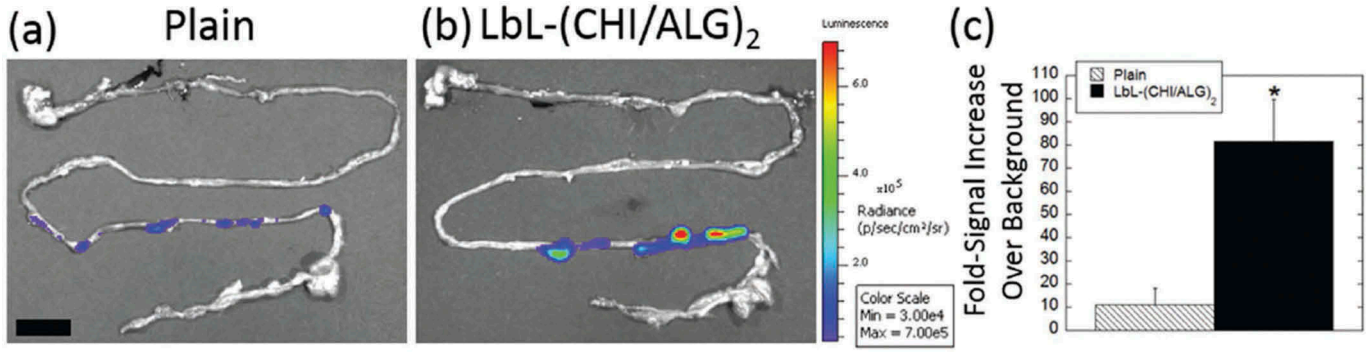

Figure 2. LbL coatings lead to enhanced survival of probiotics in vivo. Representative IVIS images of (a) plain-BC and (b) LbL-BC 1 h after oral gavage. (c) Fold-signal increase over background for plain (hatched) and LbL (black) BC 1 h after oral gavage of an identical number (8.5×10 [8] CFU) of BC. Error bars represent standard deviation (n = 4). *denotes statistical difference ($P < 0.05$) using Student's t-test between plain and LbL groups. Scale bar = 1.5 cm [Ref. 16; Reproduced with permission from John Wiley and Sons © 2016].

bilayered encapsulation, the L100 layer will protect the system from the acidic gastric environment while dissolving under intestinal conditions and hence, exposing the chitosan layer to the intestinal wall leading to adhesion of the encapsulated probiotic. However, such chitosan/L100 bilayer was not able to protect the probiotic from high concentration bile salts. On the contrary, the CHT/ALG bilayered system demonstrated dual protection from acidic gastric conditions and intestinal bile salts given the robust electrostatic interaction between the component polyelectrolytes. The probiotic effectively maintained proliferation and growth after encapsulation with all three growth phases – stationary, lag and exponential – present w.r.t. the number of layers. Such growth becomes even more effective when the probiotic is present in the direct vicinity of the intestinal membrane. In the in vivo studies, it was observed that the terminal alginate layer was released from the $(CHT/ALG)_2$ system as soon as in 30 min exposing the chitosan coating and hence the mucoadhesion (Figure 2). This observation was very important as mucoadhesion at short time points allowed for effective replication of the probiotic on the intestinal wall and the exponential growth phase was achieved faster. However, the achievement of exponential phase was delayed with an increase in number of bilayers. Three bilayers $(CHT/ALG)_3$ being the threshold as more than 10 h were required to reach the exponential phase (Figure 1). Zeta potential measurements and fluorescence imaging was performed for layer-by-layer templating of microorganism. Linear increase in fluorescent intensity indicates uniform layer building of the mucoadhesive polymers. The polysaccharide layering of the microorganisms was also evaluated for layer stability in simulated gastric and simulated intestinal conditions [6,16].

The results revealed stability and durability of the mucoadhesive layers on the microorganisms. In vitro mucoadhesion (using porcine small intestine) was evaluated by spectrum bioluminescent and fluorescent imaging systems. Terminal coating of the microorganisms with pH sensitive polymers (Eudragit EPO and Eudragit L100) ensured the stability and survival of the microorganism through the stomach allowing for dissolving and subsequent exposure of mucoadhesive layers in the pH regulated areas of the gastrointestinal tract. EpiIntestinal™, a three-dimensional intestinal tissue model, was employed for comparing the plain microorganisms with the layer-by-layer microencapsulated probiotics. Layer-by-layer encapsulated microorganisms exhibited significant adhesion and growth kinetics on in vitro live mammalian intestinal tissues [6,16].

The microorganism was selected from the group consisting of *Bacillus coagulans, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum, Lactobacillus fermentum, Lactobacillus rhamnosus, Streptococcus thermophiles, Bifidobacterium breve, Lactobacillus reuteri*, and *Saccharomyces boulardii*. Apart from probiotic microorganisms layer-by-layer technique could be used to encapsulate genetically transformed microorganisms, vaccines, and other therapeutic, prophylactic, and diagnostic agents. The diseases or conditions were selected from the group consisting of Crohn's disease, ulcerative colitis, gluten insensitivity, lactose intolerance, obesity, asthma, allergies, metabolic syndrome, diabetes, psoriasis, eczema, rosacea, atopic dermatitis, gastrointestinal reflux disease, cancers of the gastrointestinal tract, bacterial vaginosis, neurodevelopmental conditions, and general lowered immunity following a course of antibiotics or chemotherapy [6,16]. One of the major advantages of this technology is the flexibility of delivery as it is a platform technology wherein the microorganisms or cells were individually coated with the said polymers and hence can be incorporated as or into various delivery forms ranging from capsules through tablets to suspensions. Additionally, the layer-by-layer technique employed for encapsulation is industrially scalable with several layers attainable employing minimal equipment and infrastructure. Furthermore, the technique requires minimal polymer amount as only the electrostatically interacting components will be retained on the microorganism and, hence, can reduce the wastage during manufacturing.

In summary, this invention provides methods for encapsulating probiotic microorganisms or their components for targeted enteric delivery offering the advantages of prolonged survival of the encapsulated microorganisms, extended retention, and pH sensitive release characteristics. The probiotic formulations could be employed used for therapeutic, diagnostic, and prophylactic applications.

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A peer reviewer on this manuscript declares that they are an inventor on the patent that is the topic of this paper but have no other relevant financial relationships to disclose.

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References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Solanki HK, Pawar DD, Shah DA, et al. Development of microencapsulation delivery system for long-term preservation of probiotics as biotherapeutics agent. Biomed Res Int. 2013;2013:620719.
2. Liong MT. Roles of probiotics and prebiotics in colon cancer prevention: postulated mechanisms and in-vivo evidence. Int J Mol Sci. 2008;9:854–863.

•• **This article highlights the role of probiotics, prebiotics and synbiotics in the prevention of colon cancer.**

3. Capurso G, Marignani M, Delle Fave G. Probiotics and the incidence of colorectal cancer: when evidence is not evident. Dig Liver Dis. 2006;38:S277–S82.
4. Bermúdez-Humarán LG, Aubry C, Motta JP, et al. Engineering lactococci and lactobacilli for human health. Curr Opin Microbiol. 2013;16:278–283.
5. Zackular JP, Baxter NT, Iverson KD, et al. The gut microbiome modulates colon tumorigenesis. M Biol. 2013;4:e00692–e13.
• **This article correlates the changes in the gut microbiome with tumorigenesis.**
6. Anselmo AC, Langer R, Jaklenec A pH responsive mucoadhesive polymeric encapsulated microorganisms. US Patent US20170165201A1.
•• **The patent describes methods for encapsulating probiotics for enteric delivery.**
7. Mathiowitz E, Chickering DE. Definitions, mechanisms and theories of bioadhesion. In: Mathiowitz E, Chickering DE, Lehr CM, editors. bioadhesive drug delivery systems: fundamentals, novel approaches and development. New York, NY: Marcel Dekker; 1999. p. 11–23.
8. Singh I, Pawar P, Sanusi EA, et al. Mucoadhesive polymers for drug delivery systems. In: Mittal KL, Etzler FM, editors. Adhesion in pharmaceutical, biomedical and dental fields. Beverly, MA, USA: Scrivener Publishing LLC; 2017. p. 87–112.
9. Kocak G, Tuncer C, Bütün V. pH responsive polymers. Polym Chem. 2017;8:144–176.
10. Huh KM, Kang HC, Lee YJ, et al. pH-sensitive polymers for drug delivery. Macromol Res. 2012;20:224–233.
11. Champagne CP, Kailasapathy K. Encapsulation of probiotics In: Garti N, editor. Delivery and controlled release of bioactives in foods and nutraceuticals. Cambridge, UK: Woodhead; 2008. p. 344–369.
12. Gbassi GK, Vandamme T. Probiotic encapsulation technology: from microencapsulation to release into the gut. Pharmaceutics. 2012;4:149–163.
• **Biomaterials based microencapsulation of probiotic microorganisms for delivering them into the gut has been described.**
13. Lacroix C, Paquin C, Arnaud JP. Patch fermentation with entrapped growing cells of Lactobacillus casei. Optimization of the Rheological Properties of the Entrapment Gelmatrix. Appl Microbiol Biotechnol. 1990;32:403–408.
14. Heidebach T, Forst P, Kulozik U. Microencapsulation of probiotic cells by means of rennet-gelation of milk proteins. Food Hydrocolloids. 2009;23:1670–1677.
15. Diaspro A, Silvano D, Krol S, et al. A single living cell encapsulation in nano-organized polyelectrolyte shells. Langmuir. 2002;18:5047–5050.
16. Anselmo AC, McHugh KJ, Webster J, et al. Layer-by-layer encapsulation of probiotics for delivery to the microbiome. Adv Mater. 2016;28:9486–9490.
•• **The article reported layer-by-layer approach for encapsulation of probiotics to protect against GIT insults and improve their adhesion on the intestine.**
17. Priya AJ, Vijayalakshmi SP, Raichur AM. Enhanced survival of probiotic Lactobacillus acidophilus by encapsulation with nanostructuredpolyelectrolyte layers through layer-by-layer approach. J Agric Food Chem. 2011;59:11838–11845.