A Laminated Polymer Film Formulation for Enteric Delivery of Live Vaccine and Probiotic Bacteria

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ABSTRACT: Live bacterial cells (LBCs) are administered orally as attenuated vaccines to deliver biopharmaceutical agents and as probiotics to improve gastrointestinal (GI) health. However, LBCs present unique formulation challenges and must survive GI antimicrobial defenses including gastric acid after administration. We present a simple new formulation concept, termed polymer film laminate (PFL). LBCs are ambient dried onto cast acid-resistant enteric polymer films that are then laminated together to produce a solid oral dosage form. LBC of a model live bacterial vaccine and a probiotic were dried directly onto a cast film of enteric polymer. The effectiveness at protecting dried cells in a simulated gastric fluid (SGF, pH 2.0) depended on the composition of enteric polymer film used, with a blend of ethylcellulose plus Eudragit L100 55 providing greater protection from acid than Eudragit alone. However, although PFL made from blended polymer films completely released low-molecular-weight dye into intestinal conditions (pH 7.0), they failed to release LBCs. In contrast, PFL made from Eudragit alone successfully protected dried probiotic or vaccine LBC from SGF for 2 h, and subsequently released all viable cells within 60 min of transfer into simulated intestinal fluid. Release kinetics could be controlled by modifying the lamination method. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci

Keywords: oral drug delivery; enteric delivery; vaccine delivery; probiotics; polymer film; polymers; dissolution; drying

INTRODUCTION

Recently, there has been a growing need to formulate and deliver increasingly complex biological therapeutics, from peptides, recombinant proteins, and monoclonal antibodies to *in vitro* cultured eukaryotic cells, such as stem cells, and therapeutic live bacterial cells (LBCs). Therapeutic LBCs are being explored for various applications, each of which requires a tailored formulation to be delivered effectively. Attenuated live bacterial vaccines either injected (Bacillus Calmette–Guerin) or oral (Ty21a, Vivotif) can closely mimic natural infection and typically promote potent, long-lasting protective immune responses.[1] Genetically engineered LBC vaccines can deliver heterologous antigens and induce an immune response to both the attenuated strain and the vector, thus protecting against a wide range of infections.[2] Some orally administered therapeutic LBCs are classed as probiotics, and are currently under intense development to modulate the gut microbiota in health and disease.[3,4] Commensal enteric bacteria have also been genetically engineered to deliver biopharmaceutical agents such as IL-10 to treat inflammatory bowel disease[5] or insulinotrophic proteins such as GLP-1.[6] For all of these fields to advance, oral formulations are needed of LBCs that offer the potential for controlled delivery of known doses of viable organisms, maintain stability for long-term storage preferably without refrigeration, and allow cost-effective manufacture.

For LBC to be therapeutically active, cells must be kept alive during formulation and delivery. For example, dead bacteria are less immunogenic than live cells,[7,8] and although some health benefits have been suggested for dead probiotic cells, only live cells can replicate within and colonize the colon.[9] Mammals have evolved a highly antimicrobial gastrointestinal (GI) tract as a defense against food- and water-borne microbial enteric pathogens, and LBCs administered by the oral route confront multiple challenges after ingestion, including gastric acid, enzymatic degradation, antimicrobial peptides, bile acids, and secreted innate and adaptive immunoglobulins. A successful oral formulation of LBCs typically requires enteric delivery to protect from the strong acidic environment in the stomach and target release into the small intestine or beyond, depending on the therapeutic target site. Thus, LBC vaccines may be targeted to the ileum rich in Peyers patches, whereas some probiotics are targeted to the large intestine to modulate the intestinal microbiota. Enteric coatings are typically acid-insoluble films that stay intact during the transit of solid oral doses through the stomach and subsequently dissolve and release contents as pH increases in the intestine. Tablets and granules are the most common solid oral enteric formulations but capsules can also be effectively enterically coated.[10] Enteric coatings can be used for three distinct purposes: first, to protect gastric mucosa from irritant or toxic active pharmaceutical ingredient (API); second, to deliver an API to a lower GI site; and third, to protect acid-labile API from gastric acid (e.g., omeprazole). Protection of gastric mucosa and intestinal delivery are both achieved by the enteric film layer remaining intact in acid preventing dose disintegration, whereas API protection is achieved by the enteric polymer film blocking the ingress of acid into the solid formulation. Although most enteric polymers are acid insoluble, they do typically swell in acid with the degree of swelling

Abbreviations used: TPY, tryptone–phytone—yeast; LBCs, live bacterial cells; SIF, simulated intestinal fluid; SGF, simulated gastric fluid; CFU, colony forming unit; MLF, multilayer laminated film; TF, thick film; EC, ethylcellulose; PFL, polymer film laminate.

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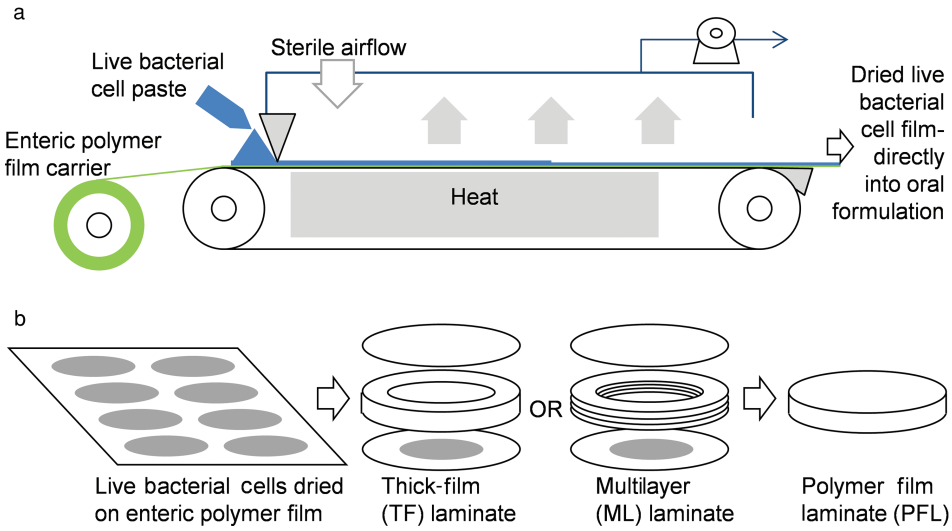

Figure 1. Polymer Film Laminate concept. (a) diagram of proposed continuous manufacture method to produce LBCs dried directly onto pharmaceutical polymer film web. Cells mixed with lyoprotectants are spread onto a continuous web of enteric polymer film. (b) Alternative methods for laminating enteric formulations from film-dried bacterial cells together with enteric polymer film spacers to produce PFL formulations.

and permeability of enteric polymer films varying dependent on polymer composition.[11,12] LBC formulations must achieve delivery to an intestinal site but protection from acid toxicity is also vital given the high sensitivity of dried bacteria to acid.[13,14] Research in enteric delivery has developed a spectrum of approved enteric polymers coupled to a broad range of coating methodologies. Further improvement of pharmaceutical polymer properties can be achieved by blending polymers to combine distinct desired properties and provide endlessly tunable coating performance.[12] For example, improved protection of API from acid was achieved by blending enteric polymers with insoluble polymers.[11,15,16]

Live bacterial cells have been formulated using enteric-coated tablets,[17] capsules,[18] and granules.[19] Protection from acid using coadministration with buffer solutions has also been used,[18] and microencapsulation has been explored for acid protection and controlled delivery.[20,21] In all cases, drying is required to preserve cell viability during long-term storage. However, the dehydration process can damage cells through osmotic and oxidative stress and also denaturation of biomolecules.[22] This can be prevented by sugar glasses formation, an effective and well-understood approach to stabilization of biomacromolecules during drying.[23] Although much work has focused on freeze-drying, many bacteria and related complex biological payloads are more robust when freezing is avoided.[24] A continuous, ambient temperature drying process would also be more cost-effective than freeze-drying, where major costs are incurred by the slow batch turnaround and high cost of large-scale freeze-drying equipment. Other drying methods, including spray, fluidized bed, foam, vacuum, and air convective drying have been explored for the industrial mass production of dried microorganisms.[25] Perhaps the simplest ambient temperature drying method is to spread material in a thin film to provide a large surface area for evaporation. Ambient temperature drying on a flexible belt is used for continuous, scaleable drying of sensitive food products.[26,27] Ambient temperature drying onto polymer films has historically been used for the preservation and storage of microbial reference samples,[28] but sophisticated film drying methods has recently been adopted for advanced biotechnology applications ranging from biocatalysis and biosensing to food technology. Flickinger et al.[29] developed a sophisticated and robust method for painting and printing live bacteria, using polymer latex plus nontoxic adhesive films to permanently entrap cells, preserving cell viability and metabolic activity on surfaces at ambient temperatures. Others have incorporated live lactic acid bacteria into an edible, flexible casein film, with high cell survival after drying and storage, as an antimicrobial meat-packaging material.[30]

Here, we propose a novel approach to oral formulation of LBCs that exploits the benefits of ambient film drying technology and inspired by a novel yet simple and powerful web formulation technology recently developed for oral delivery of small molecule APIs.[31,32] In the Sticky Web approach, a carefully metered dose of API in dry powder form adheres to an adhesive patch printed on a polymer film web. We propose that a cast film of enteric polymer can be used as a web for drying LBC allowing continuous and scaleable ambient temperature lyophilization and combination with a protective enteric film in a single-unit operation (Fig. 1a). The web comprising dried cells on enteric polymer film can then be directly laminated using edible adhesives to produce an oral solid dosage form (Fig. 1b). The resulting polymer film laminate (PFL) formulation can be made using a variety of lamination methods, polymers, and film thicknesses to provide fully customized LBC delivery. Here, we investigate whether bacteria can be directly dried onto enteric polymer films, whether this material is sufficient for protecting dried bacteria from gastric acid, and whether a prototype oral PFL formulation can be used to deliver live vaccine and probiotic bacteria in simulated GI fluids.

MATERIALS AND METHODS

Materials

Eudragit L100 55 (Eudragit L, methacrylic acid–ethyl acrylate copolymer, 1:1) was a kind gift from Evonik (Milton Keynes, UK). Water Blue dye, Ethyl cellulose [ethoxyl content 48%, 8–11 cPs at 5% (w/v) in 80:20 toluene/ethanol], Triethyl citrate, LB

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