



Mechanistic understanding of ragweed pollen for oral vaccine delivery



Vaccine delivery through the oral route, although seemingly simple and easy, has been a long-standing endeavor. Only a few vaccines are commercially available that are orally delivered, and all but one are in the form of live attenuated virus/bacteria, which carry the risk of causing vaccine-associated disease such as polio [1]. The parenteral route continues to be the mainstay of current vaccine administration. Oral vaccination is painless and less expensive, and more importantly, it also can induce both mucosal and systemic immune responses. Because the majority of the pathogens invade through the mucosal surfaces, the mucosal immune response in the form of IgA can provide the first line of defense [1]. Despite these advantages, oral vaccination continues to be challenging, since the vaccine must cross the detrimental acidic and proteolytic enzyme-rich stomach environment before it reaches the intestine. There is a growing necessity to develop a novel delivery system to achieve oral vaccination with subunit proteins.

Pollen particles are nature's microcapsules that safely transport the plant's male gametophyte to the ovary of the flower for fertilization. To perform this important function, pollen is constructed with robust outer walls. Recently, pollen has emerged as a novel microcapsule for encapsulation of therapeutics for oral administration. The primary advantages of pollen as natural microcapsules are their natural chemical toughness (that allows them to pass unscathed through the stomach) and their monodispersity in size. In addition, the presence of unique surface topographical features, such as short spikes, ridges and valleys, is believed to enhance their adhesion to the mucus, which in turn increases their gastrointestinal residence time as compared with smooth spherical microparticles. The first challenge in using pollen for oral vaccination is to evade the allergenicity of pollen. Since the allergic reaction towards pollen is caused by the proteins and biomolecules of plant-origin that are naturally contained in them, and not by the pollen shells themselves, this is easily achieved by removing these allergenic materials through chemical treatment of pollen with organic solvents, acids, and alkali.

Professor Harvinder Gill and his team previously showed that the tough shell of a *Lycopodium clavatum* spore induced mucosal and systemic immune responses in a mouse model against ovalbumin, a model vaccine protein [2]. In the new study in this issue [3], the Gill team describes a dual role of allergen-free ragweed pollen: both as a vaccine carrier and as an adjuvant. In this work, the authors have demonstrated a possible mechanism of how allergen-free ragweed pollen can activate the innate immune system and potentiate an immune response following oral vaccine delivery. First, through chemical treatment, lipids and proteins were removed to prevent allergic reactions. The pollen walls remained intact even after the prolonged acid and alkali treatments, reinforcing the idea that the pollen wall is resistant to acidic degradation. Next, the hollow interior of the intact ragweed pollen shell was filled with the vaccine solution with a facile method based on vacuum-application. Since no organic solvents were used during protein encapsulation it offers a simple, less expensive, and less detrimental process for encapsulating delicate biomolecules such as proteins, as compared to traditional polymeric particles that require organic solvents for protein encapsulation.

The work by the Gill group provides several interesting aspects of allergen-free ragweed shells. Ragweed pollen is safe for oral delivery as demonstrated by its non-toxicity towards Caco-2 cells, which are a widely used model of intestinal epithelial cells (IECs). Ragweed shells activate IECs in a dose-dependent manner, causing them to secrete cytokines with the ability to recruit antigen presenting cells including macrophages. Electron microscopy demonstrated that mouse macrophages attempted to phagocytose ragweed pollen. Further interrogation of the influence of ragweed on mouse bone-marrow derived macrophages and dendritic cells (DCs) showed that these cells produced proinflammatory cytokines when incubated with ragweed pollen, which can further shape the immune response. A small amount of ovalbumin protein was also found to be strongly adsorbed onto the walls of ragweed pollen, which could not be removed even after thorough washing. This suggests that vaccine adsorbed on pollen shells could also be taken up by macrophages and other antigen presenting cells and presented to the immune system. Interestingly, the study has shown that oral delivery of the ragweed shells in mice results in their uptake into the subepithelial region of the mouse intestine. Ragweed pollen is about 15 μm in diameter. This paper thus emphasizes the need to also study larger particulates, which are commonly ignored in vaccine delivery, and to also interrogate the phenomena behind their uptake. Overall, the ability of ragweed pollen to activate IECs, macrophages, and DCs and their uptake into the intestinal wall points to a dual role of ragweed pollen as an adjuvant and a carrier.

The pollen-based delivery system can be further fine-tuned by enteric coatings using an array of pH-responsive polymers that can seal the pores after vaccine loading, thus further securing the delivery cargo in the pollen. A large variety of pollen species are available in nature that can be harnessed for oral vaccination. The importance of the study by the Gill group is that it provides mechanistic insights for understanding how allergen-free pollen interacts with the immune system, and thus, paving the way to augmented oral vaccine delivery. The oral vaccine delivery is expected to renew its vitality through systematic study on many distinctive features, including the pollen surface geometry. Furthermore, the information obtained from the study on pollen can be extended to nano/micro fabrication of even more effective oral vaccine delivery systems.

References

- [1] A.K. Shakya, M.Y.E. Chowdhury, W. Tao, H.S. Gill, Mucosal vaccine delivery: current state and a pediatric perspective, *J. Control. Release* 240 (2016) 394–413.
- [2] S.U. Atwe, Y. Ma, H.S. Gill, Pollen grains for oral vaccination, *J. Control. Release* 194 (2014) 45–52.
- [3] M.J. Uddin, H.S. Gill, Ragweed pollen as an oral vaccine delivery system: mechanistic insights, *J. Control. Release* (2017) .

Kinam Park
Purdue University
Biomedical Engineering and Pharmaceutics
West Lafayette, IN 47907, USA
E-mail address: kpark@purdue.edu