The Soluble and Particulate Form of Alginates Positively Regulate Immune Response

Fei Ge[1], Longbao Zhu[1], Liangjun Yang[1], Wanzhen Li[1], Shenghua Wei[1], Yugui Tao[1*], Guocheng Du[1,2]

[1]School of Biochemical Engineering, Anhui Polytechnic University, Wuhu, Anhui, [2]Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, Wuxi, China

ABSTRACT

Background: Alginate materials have been widely employed for biomedical applications ranging from wound healing to cancer treatment. However, how alginate materials affect the immune system is largely unknown. **Objective:** To explore the impact of alginate materials on immune system. **Methods:** The effect of three types of alginate materials, low viscosity, high viscosity and particulate alginate, were examined by both *in vivo* and *in vitro* analyses. C57BL/6J (B6) mice were treated with alginate and peripheral blood was tested by ELISA for cytokine production. Dendritic cells, macrophages and splenocytes isolated from mice were analyzed for the response to alginate treatment. Administration of alginates by intra lymph node injection (I.L.N.) yielded more potent cytokines productions than other injection routes. **Results:** Alginate materials did not affect the viability of lymphocytes. Particulate alginate induced the most potent inflammatory reaction as determined by the production of cytokines, such as, IL-1β, IL-8, TNF-α and IFN-γ. Low viscosity and particulate alginates are more effective than high viscosity alginates in activating dendritic cells as indicated by the expression of dendritic cells surface markers (CD80, CD86 and CD40). Similarly, the level of G-CSF was slightly higher in particulate alginate treated macrophages. **Conclusion:** Alginate materials could affect immune response through different ways, including promoting inflammatory cytokine production, and activating dendritic cells. Therefore, alginate materials, especially in particulate form, have the potential to be applied in inflammation related diseases.

Ge F, et al. Iran J Immunol. 2018; 15(3): 228-238.

Keywords: Alginate, Cytokines, Dendritic Cells, Immune Modulation, Macrophages

---

*Corresponding author: Dr. Yugui Tao, School of Biochemical Engineering, Anhui Polytechnic University, Wuhu, Anhui, China, e-mail: apu_tao@126.com

INTRODUCTION

Biomaterials are widely employed to treat multiple diseases including infectious conditions, traumatic injuries and cancer (1). They possess unique advantages, such as biosafety, low-cost and easy accessibility (2). Using biomaterials as carriers or implants for delivering therapeutic cargos to modulate the host responses in a controlled manner is a new direction in biomedical field (3,4). Alginate is a biodegradable polysaccharide with negative charges commonly extracted from seaweed. It is composed of 1,4-linked β-d mannuronic acid (M) and α-l-guluronic acid (G) units, that are assembled at variable numbers and sequence distributions (5). Alginate is characterized by its bio-compatibility, low cost and non-toxicity. Upon exposure to divalent cations, alginate can form hydrogels that are similar in structure to extracellular matrix. These hydrogels can maintain a certain degree of moisture and reduce infection by pathogens, hence promoting wound healing. Therapeutic cargos, such as macromolecular proteins, can be loaded onto the gel and released in a controlled manner (6). Alginate hydrogels have been applied *in vivo* for tissue engineering, drug delivery, and encapsulating live cells in order to protect therapeutic agents from rejection by host immune system. For example, human cadaveric pancreatic islets have been encapsulated into alginate microsphere to treat diabetic patients, where glycemic correction has been achieved (7,8). On the other hand, studies have shown that alginates are able to elicit strong innate immune responses that lead to fibrotic deposition and tissue necrosis (9,10). In other studies, innate immune response was observed in preclinical diabetic mouse models and nonhuman primate models when alginate gel encapsulated xenogeneic islets or pancreatic progenitor cells were implanted (11,12). These studies suggest that it is of great significance to investigate the impact of alginate material on immune system in order to favor their clinical application. The effect of alginate materials on immune cells has been reported by several groups. In one study, sodium alginate and poly-L- lysine were assembled onto a sacrificial solid core and then dissolved in order to generate hollow microcapsules. After that, these microcapsules were allowed to interact with macrophages and lymphocytes. They found that the alginate and poly-L-lysine together promoted the production of cytokines, such as IL1-β and TGF-β (13). In another study, similar hollow capsules composed of alginate and poly-L-lysine were employed to treat lymphocytes; these capsules were able to promote the proliferation of lymphocytes and enhance the production of IL-1 and TNF-α (14). Although these two studies revealed that alginate and poly-L-lysine promote inflammation by activating lymphocytes and producing inflammatory cytokines, the role of each component in this process is yet to be fully elucidated. Moreover, it is reported that alginate can promote the productions of pro-inflammatory cytokines in macrophages in a time- and dose-dependent manner. Furthermore, alginate is capable of inducing innate immune responses through activating the same pathways as pathogen recognition (15).

In the present work, three forms of alginates (i.e. soluble high viscosity alginate (H.V.), soluble low viscosity alginate (L.V.) and particulate alginate) were employed *ex vivo* and *in vivo* to assess their impact on immune response. Specifically, these alginates were administrated *in vivo* to test their impact on the production of cytokines through ELISA. Further analyzed was the effect of different ways of administration. Additionally, the alginate materials were employed to interact with specific immune cells, i.e. macrophages and dendritic cells, two major antigen-presenting cells that

bridge innate and adaptive immunity, so as to examine their impact on immune response *in vitro*.

MATERIALS AND METHODS

Materials. Low and high viscosity alginates were purchased from VWR (Radnor, USA). The average molecular weight (MW) was reported to be 1.43×10^5 g/molin low viscosity alginate and 3.5×10^5 g/mol in high viscosity alginate. $CaCl_2.2H_2O$ was purchased from Sigma, and phosphate buffered saline (PBS 1x) was purchased from VWR. ACK (Ammonium-Chloride-Potassium) Lysis Buffer was obtained from Thermo scientific (San Diego, USA), and DAPI (4′,6-diamidino-2-phenylindole) from Invivogen (San Diego, USA). RPMI cell culture medium, fetal bovine serum, penicillin and streptomycin were obtained from VWR (Radnor, USA).

Animals and Cells. All animal experiments were approved by the Animal Care and Use Committee of Jiangnan University. C57BL/6J (B6) mice, 8-10 weeks old, and ~25g, were purchased from Animal center in Jiangnan University (Wuxi, China). Mice were injected with alginate materials through different injection routs. To test the cytokine levels in serum, peripheral blood was drawn from mice 24 hours following alginate treatment. The blood was centrifuged at 20000 rpm for 5 min at 4ºC. Serum was collected and stored at -80ºC prior to ELISA test. Dendritic cells were cultured in RPMI medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL).

Preparation of Particulate Alginate. Particulate alginate was prepared by ionic gelation method as reported in the literature. Briefly, $CaCl_2$ (5 mL, 0.5 mM) was added into alginate solution dropwise. The alginate was stirred by a magnetic bar at 800 rpm (Velp Scientifica, Usmate, Italy) at room temperature. After 2 hours of reaction, the nanoparticles were collected by centrifugation at 17500 g for 25 min (Bio-Rad, San Diego, USA). The particles were then filtered through a 40 μm filter (Stemcell, Vancouver, Canada) to remove the large particles, washed with water for three times and collected for further use.

Splenocyte Isolation. The mice, treated with different samples for 48 hours, were sacrificed via carbon dioxide, followed by cervical dislocation to ensure complete sacrifice. Spleen was collected from the mice and the tissue was minced into pieces smaller than 1 mm^3, and treated with dissociation medium (Stemcell Technologies, Vancouver, Canada) for 30 min. After that, the cells were twice washed with PBS buffer (1x) and cultured in a cell culture medium for further use.

Dendritic Cell Isolation: Similar to splenocytes isolation, mice spleen was collected from the mice sacrificed by carbon dioxide and cervical dislocation. The spleen was minced into small pieces and treated with dissociation medium for 30 min to process the tissue into cell suspension. EDTA buffer (2 mM) was added to preclude cell aggregation. The cells were magnetic beads for dendritic cell isolation, where dendritic cells were selected positively. The collected cells were twice washed with PBS and then cultured in medium.

Viability Test. The viability of cells were assessed through staining with trypan blue (1:100 dilution), followed by cell viability analysis with a cell counter (Countess™ automated cell counter).

Macrophage Isolation. Macrophages were isolated from mice bone marrow based on

well-established protocols reported elsewhere (16). Briefly, abdomen and hind legs were removed from the sacrificed mice. The femur and tibia were separated by cutting the knee joint. Next, the bones were flushed with lymphocytes medium by use of a syringe with needle (5 mL syringe and 25gauge needle). The cells were subsequently cultured in a humidified incubator with 5% CO2 at 37°C and washed twice every 2 days with PBS buffer. The macrophage progenitors were not washed away since they were attached to the petri dish surface. After 6 to 7 days, the macrophages were employed for further analysis.

Dendritic Cell Activation and Staining. Dendritic cells were employed to interact, for 24 h, with three types of alginate, L.V., H.V. and particulate alginates. After that, DCs were collected, washed with PBS and treated with Fc antibody (BD bioscience, CA, USA) to block non-specific binding. These cells were then stained with antibodies against surface markers (i.e. CD80+, CD86+ and CD40+, BD bioscience, CA, USA) followed by flow cytometry analysis.

Flow Cytometry Analysis. For activation tests, dendritic cells were stained with fluorescence labeled antibodies against the surface markers, CD80 (FITC) CD86 (APC) and CD40 (PECy7) (BD bioscience, CA, USA). Briefly, the cells were treated with different samples for a certain amount of time. After the treatment, the cells were collected and washed with PBS to remove the debris. Subsequently, they were treated with Fc block to prevent non-specific binding of antibodies. Then, the cells were stained with the fluorescence labeled antibodies in order to test the expression of these markers.

ELISA. The production of cytokines by co-cultured macrophage and alginate samples were analyzed by ELISA, which kits were employed for IL-1β, IL-6, TNF, IFN-γ (BD Bioscience, San Diego, USA). All the operations were performed by following the producer's instructions and read by Microplate Photometer (Fisher Scientific, San Diego, USA). Employed for each test was 10 μL of supernatant. The level of cytokine concentration was calculated via comparison with standard curves established from the standards provided by the producer. Samples were examined on Microplate Absorbance Reader (Bio-Rad, San Diego, USA).

Statistical Analyses. All statistical data were analyzed using GraphPad Prism 6 software. In each experiment, significant difference ($P<0.05$) is indicated in the results and/or figure legends. Data are representative of results obtained in at least three independent experiments.

RESULTS

To test the *in vivo* inflammatory response induced by alginate materials, the mice were treated with or without three types of alginates, L.V., H.V., and particles. Compared with the controls, only particulate alginate treated mice had a slightly reduced activity. Mice serum was collected from each group for ELISA analysis: all three types of alginate yielded an enhanced production of Interleukin-1β (IL-1β), a key pro-inflammatory cytokine that defends body against infections and injury (Figure 1A). The particulate alginate induced a higher level of IL-1β compared with L.V. and H.V. alginates which were in a soluble form (Figure 1A). A similar trend was also found in the production of another inflammatory cytokine, IL-8 (Figure 1B and Figure 1C). As for TNF-α, a similar level of production was achieved by L.V., H.V. and particulate alginate (Figure 1C).

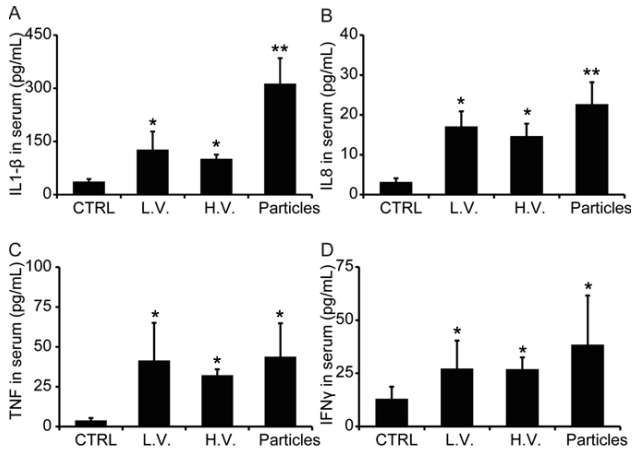

Figure 1. Soluble and particulate alginates modulate cytokines production *in vivo*. Production of (A) IL1-β, (B) IL-6, (C) TNF and (D) IFN-γ were examined after administrating alginate materials *in vivo* with the dose of 50 mg/kg weight. The mice with no treatment (CTRL) were employed as controls. Injection was performed via tail vein. After 48 hours of injections, peripheral blood was collected from mice and serum was isolated from the blood and employed for ELISA test. H.V., high viscosity alginate; L.V., low viscosity alginate; Particles, particulate alginate. Data are obtained from at least 3 independent experiments. Data represent means ± SD from three independent experiments. *p<0.05, **p<0.01.

Further examined was the production of interferon gamma (IFN-γ), the only member of type II class of interferon that regulates both innate and adaptive immune responses. All three types of alginates produced IFN-γ; alginate in the particulate form, on the other hand, generated a slightly higher level of IFN-γ, compared to those in soluble forms; L.V. and H.V. alginates had a similar potency in inducing IFN-γ (Figure 1D).

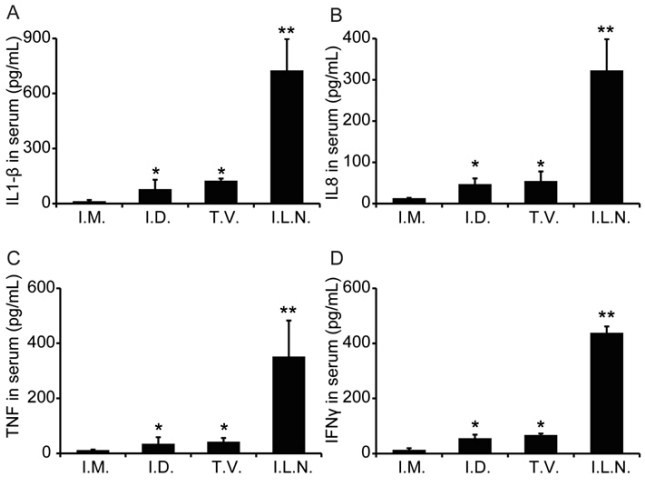

Figure 2. Injection routs of alginate materials impact cytokine production level in mice. Production of (A) IL1-β, (B) IL-6, (C) TNF and (D) IFN-γ after administrating alginate materials *in vivo*. Four types of injection routs were employed: I.M. I.D., T.V. and I.L.N., H.V. and L.V alginate was used at 50mg/Kg weight. Peripheral blood was collected from mice and serum was isolated from the blood and employed for ELISA test 48 hours after injections. I.M. (intra muscular), I.D. (intra dermal), T.V. (tail vein) and I.L.N. (intra lymph node injections). Data are obtained from at least 3 independent experiments. Data represent means ± SD from three independent experiments. *p<0.05, **p<0.01.

After testing the production of cytokines in serum, the impact of injection routs on cytokine productions was investigated. L.V. alginate was injected via four different injection routs: intramuscular (I.M.), intradermal (I.D.), tail vein (T.V.) and intra-lymph node (I.L.N.) (Figure 2A to 2D). Among all the injection routs, I.L.N. yielded the most potent inflammatory responses, strongly producingIL-1β, IL-8, TNF-α and IFN-γ (Figure 2A to 2D). T.V. injection resulted in the second highest level of IL-1β and IL-8 cytokine production among the four injection routs (Figure 2A to 2D). I.D. injection resulted in a slightly lower level of TNF-α and IFN-γ production compared to T.V. injection. I.M. injection had the weakest cytokine production among the selected injection routs (Figure 2A to 2D).After the *in vivo* test, we assessed the impact of alginate on the viability of lymphocytes, a mixture of different immune cells such as macrophages, T cells and dendritic cells. The lymphocytes were collected from mice spleen and processed into single cells through dissociation medium. In the present study, three types of alginates were applied at different doses, namely 5 µg/mL, 50 µg/mL and 500 µg/mL. No significant reduction was observed in the lymphocyte viability of the three types of alginates, even when a relatively high concentration of alginate (i.e. 500 µg/mL) was employed (Figure 3). Consistent with the findings by other studies, alginates, either in soluble or particulate form, were non-toxic with regards to lymphocytes.

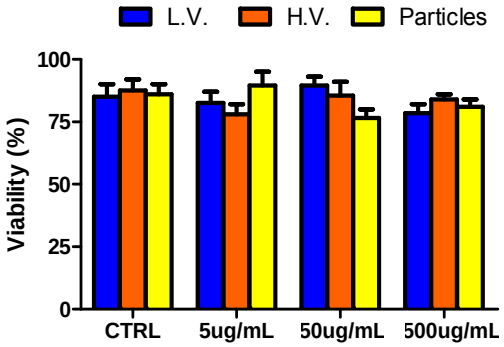

Figure 3. Impact of three types of alginate materials on lymphocyte viability. Lymphocytes were isolated from mice spleen. Cells were treated with the alginate materials at the indicated dose for 48 hours before viability test. Data are obtained from at least 3 independent experiments.

These alginates were further employed to interact with dendritic cells, a major cell type that functions at the interface of innate and adaptive immunity. The dendritic cells were collected from mice spleen through selecting the CD11c positive group. Three types of alginates (L.V., H.V. and particulate) were used to interact with dendritic cells for 24 hours, where flow cytometry was employed to test the activation of surface markers on these cells. The analysis showed that all three types of alginates activated surface markers such as CD80, CD86 and CD40 (Figure 4A to 4C). Particulate and L.V. alginates had a similar level of potency in inducing CD80 and CD86; H.V. alginate was the weakest in activating these two markers (Figure 4A and 4B). As far as CD40 expression is concerned, all three alginates were equally strong (Figure 4C). In all three tests, LPS and polyIC, two Toll like receptor agonists that can act as “danger signals”

for dendritic cell activation, induced a high level of CD80, CD86 and CD40, indicating that dendritic cells were able to respond to the right stimuli (Figure 4A to 4C).

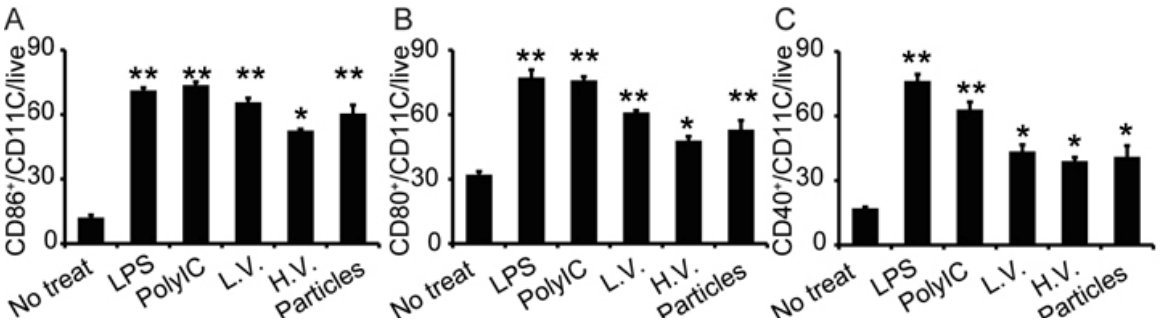

Figure 4. Soluble and particulate alginate materials activate surface markers on dendritic cell surface. Expression levels of (A) CD86, (B) CD40 and (C) CD40 on dendritic cell surface were checked. 50ug/ml of alginates were used to treat dendritic cells, LPS at 1ng/ml and poly IC at 5ug/ml were used as positive control. The dendritic cells were isolated from mice spleen via positive selection. The cells were treated with the alginate materials for 24 hours, followed by staining the markers, and assessed under flow cytometry. Data are obtained from at least 3 independent experiments. Data represent means ± SD from three independent experiments. *p<0.05, **p<0.01.

The comparison between alginate samples, polyIC, LPS and negative (no treatment) controls demonstrated that all three types of alginate materials can stimulate dendritic cell activation at different degrees. Macrophages are a group of immune cells that function in nonspecific immune defense by increasing inflammation and secreting cytokines. They are also involved in specific defense by activating lymphocytes and other immune cells. Therefore, it is of great importance to investigate the effect of alginates materials on macrophages. Based on the results, all three types of alginates similarly enhanced the production of inflammatory cytokines, such as IL-6 and TNF-α(Figure 5A and 5B).

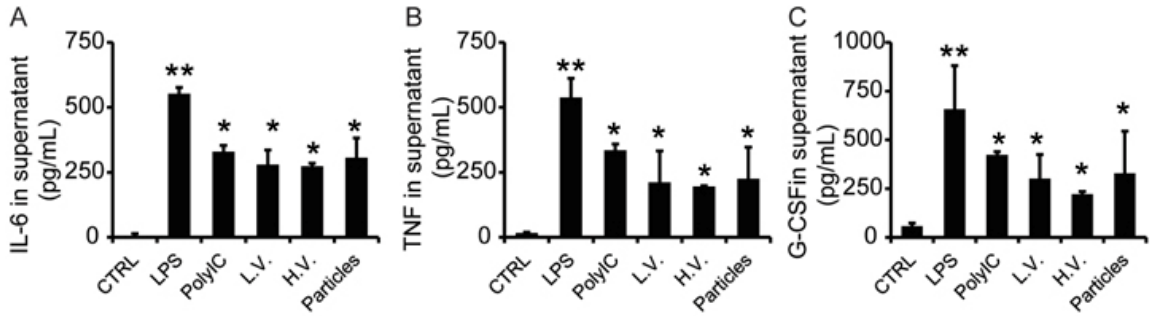

Figure 5. Soluble and particulate alginate materials promoted the secretion of cytokines from macrophages. The macrophages were isolated from mice bone marrow. After interacting the cells with the alginate materials (50ug/ml), and LPS (1ng/ml) or poly IC (5ug/ml), the supernatant was collected for examining (A) IL-6, (B) TNF and (C) G-CSF cytokines by ELISA. Data are obtained from at least 3 independent experiments. Data represent means ± SD from three independent experiments. *p<0.05, **p<0.01.

Further examined was granulocyte-colony stimulating factor (G-CSF), another important cytokine secreted by macrophage, and commonly employed as a marker of activation and proliferation. The alginate materials, either soluble (H.V. or L.V.) or particulate, promoted G-CSF production (Figure 5C). The L.V. and particulate alginates resulted in a slightly higher level of G-CSF production comparisons with H.V. alginate (Figure 5C).

DISCUSSION

As a naturally occurring polymer, alginate has received extensive attention in biomedical application. This material has been generally considered as biocompatible, low-toxic and low-cost (17). In particular, the most significant advantage of alginate is its ability to form hydrogels (with a structure similar to extracellular matrix of living tissues) when encountering divalent cations (e.g. Ca^{2+}) (18). Accordingly, this polymer has been widely employed for tissue engineering or wound healing, where therapeutic cargos such as drugs and proteins can be implanted in the hydrogel (18-20). Moreover, alginate hydrogel can maintain moist environment when implanted and can reduce bacterial invasion at the wound sites. Upon wound healing, the material will degrade by themselves, without inducing significant toxicity (21-22). Moreover, alginate has been explored in delivering peptide antigen material in biomaterial vaccine development (23) and developing oral vaccine (25). Diphtheria toxoid loaded onto alginate particles has recently been employed for the treatment of diphtheria, a contagious, acute and bacterial disease (24). This study also found that alginate is able to induce potent humoral response. In other studies, alginate was employed in a free soluble form to interact with macrophages, where strong inflammatory responses were induced (15). While these studies partially reveal that alginate can activate innate immunity, there is no systematic evidence as to how alginate, in different viscosity forms (i.e. soluble or particulate), impacts the immune system. In this regard, we investigated the role of three types of alginate materials, namely L.V., H.V. and particulate in immune response via both *in vitro* and *in vivo* approaches. Equivalent doses of L.V., H.V. and particulate alginates were utilized for *in vivo* test of cytokine production. Four types of cytokines were selected, and IL-1β, IL-8, and TNF-α were tested because they represent the most commonly induced inflammation factors (26). Our results showed that particulate alginate induced the highest level of inflammatory cytokines compared to the other two types. This is probably because a controlled degradation of particulate alginate can prolong the inflammation process, thereby inducing a higher level of inflammatory cytokines. L.V. alginate induced a slightly higher level of inflammatory cytokines, compared with H.V., possibly because the former enters more easily into circulation *in vivo* due to its low viscosity. The only member of type II class of interferon, IFN-γ, was further examined in the present research. IFN-γ is a very important cytokine because it regulates both innate and adaptive immune responses (27,28). Based on the present results, all three types of alginate materials promoted IFN-γ production, indicating its universal role in immune response. Similarly, particulate alginate had the highest potency in producing IFN-γ, while L.V. alginate yielded a higher production than H.V. All these data suggest that alginate is capable of potentially affecting both innate and adaptive immuneresponses. The administration routes of certain types of drugs play very important roles in their *in vivo* effect (29). A broad biomedical applications of

alginate may require the administration of the materials through different routes. For instance, for wound healing applications, where I.M. or I.D. has to be selected, alginate materials are generally administered directly into skin or muscle. In particular, skin is an important immune organ that contains multiple types of immune cells such as leukocytes, mast cells, and tissue macrophages (30). Therefore, we compared the effect of four injection routs: I.M., I.D. T.V. and I.L.N. The T.V. injection was selected because it is one of the most efficient ways for alginates to get into circulation system *in vivo*. Both I.D. and T.V. injection of alginate promoted inflammatory (IL-1β and IL-8) and effector (TNF and IFN-γ) cytokine production. I.L.N injection was chosen because lymph node is a major organ for immune modulation, in which multiple vital immune cells (i.e. macrophages, T cells, and dendritic cells) reside i (31). The present results revealed that I.L.N. yielded the most potent cytokine productions (i.e. IL1-β, IL-8, IFN-γ and TNF), which might be explained by the fact that a high density of immune cells reside in lymph nodes and once these cells are in touch with alginate materials, robust inflammatory responses are induced. Multiple studies have demonstrated that alginate does not affect cell viability (5). In this work, a high concentration of alginates was employed to test their impact on immune cell viability *in vitro*. Consistent with the reported studies (15,32,33), alginates, either in soluble or particulate form, did not affect immune cell viability. Further investigated, in the present work, was the impact of alginate, in soluble or particulate form, on the activation of dendritic cells; all three types of alginates were found to be able to activate DC surface markers such as CD40, CD80 and CD86. Dendritic cells are the most important antigen-presenting cells in mammalian immune system. They process the antigens and present them to T cells to elicit adaptive immunity, during which activation, the above-mentioned surface markers are required (34,35). In the present study, particulate and L.V. alginate induced the highest level of CD80 and CD86 markers and all the three types of alginates induced a similar level of CD40 marker. Therefore, alginate materials have the potential for modulating adaptive immunity. Macrophages are a type of white blood cell that digest pathogens, microbes and even cancer cells. They are also involved in the adaptive immunity by presenting antigens to T cells (36). The impact of alginate materials on macrophage was assessed through measuring the production of cytokines from the cells. It was shown here that alginate promoted the production of inflammatory cytokines such as IL-6, with a production level similar to polyIC, a toll like receptor 3 agonists, indicating that alginate (i.e. L.V., H.V. or particulate) can induce inflammatory responses in macrophages. These results are consistent with the previous studies, in that alginate materials can activate inflammatory responses similar to pathogen-associated molecular patterns (37). Altogether, alginate materials can elicit innate and adaptive immune responses when employed *in vivo* and *in vitro*. These immune responses, if well-modulated, can be utilized in the development of novel biomaterials-based vaccines that combat challenging diseases such as cancer. These studies further remind us of the fact that the inflammatory responses induced by alginates are a potential concern for their biomedical applications.

In conclusion, alginates are associated with both innate and adaptive immune responses, given their influence on the activation of two major antigen-presenting cell types, namely dendritic cells and macrophages. Alginate materials generate inflammatory cytokines without affecting the viability of immune cells. These impacts are associated with their injection routs. Overall, our data provide significant approaches for the application of alginates in clinical practice.

ACKNOWLEDGEMENTS

This work is supported by Anhui Science Foundation (Grant #:1308085MC51) and Anhui Science Foundation for National Institutes and Colleges (KJ2012A034).

REFERENCES

1. Weber JS and Mule JJ. Cancer immunotherapy meets biomaterials. Nat Biotechnol. 2015; 33:44-5.
2. Mitragotri S and Lahann J. Physical approaches to biomaterial design. Nat Mater. 2009; 8:15-23.
3. Nilsson B, et al. Can cells and biomaterials in therapeutic medicine be shielded from innate immune recognition? Trends Immunol. 2010; 31:32-8.
4. Ali OA,Mooney DJ. Immunologically Active Biomaterials for Cancer Therapy. Curr Top Microbiol Immunol. 2011;344:279-97.
5. Lee KY,Mooney DJ. Alginate: properties and biomedical applications. Prog Polym Sci. 2012; 37:p.106-126.
6. Hoffman AS. Hydrogels for biomedical applications. Ann N Y Acad Sci. 2001;944:62-73.
7. Jacobs-Tulleneers-Thevissen D, et al. Sustained function of alginate-encapsulated human islet cell implants in the peritoneal cavity of mice leading to a pilot study in a type 1 diabetic patient. Diabetologia. 2013; 56:1605-14.
8. Soon-Shiong P, et al. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. Lancet. 1994; 343: p.950-1.
9. Schneider S, et al. Long-term graft function of adult rat and human islets encapsulated in novel alginate-based microcapsules after transplantation in immunocompetent diabetic mice. Diabetes. 2005; 54: p.687-93.
10. Lum ZP, et al. Prolonged reversal of diabetic state in NOD mice by xenografts of microencapsulated rat islets. Diabetes. 1991; 40: p.1511-6.
11. Tuch BE, et al. Safety and Viability of Microencapsulated Human Islets Transplanted Into Diabetic Humans. Diabetes Care. 2009; 32:1887-9.
12. De Groot M, Schuurs TA,van Schilfgaarde R. Causes of limited survival of microencapsulated pancreatic islet grafts. J Surg Res. 2004; 121:141-50.
13. Robitaille R, et al. Inflammatory response to peritoneal implantation of alginate-poly-L-lysine microcapsules. Biomaterials. 2005; 26:p.4119-27.
14. Orive G, et al. Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy. Biomaterials. 2006; 27:p.3691-3700.
15. Yang D, Jones KS. Effect of alginate on innate immune activation of macrophages. J Biomed Mater Res A. 2009; 90:p.411-8.
16. Weischenfeldt J, Porse B. Bone Marrow-Derived Macrophages (BMM): Isolation and Applications. CSH Protoc. 2008; 2008:p.pdb prot5080.
17. Nilsson B, et al. Can cells and biomaterials in therapeutic medicine be shielded from innate immune recognition? Trends Immunol. 2010: 31:p.32-8.
18. Rahimnejad M, Derakhshanfar S,Zhong W. Biomaterials and tissue engineering for scar management in wound care. Burns Trauma. 2017; 5:p.4.
19. Chaudhari AA, et al. Future Prospects for Scaffolding Methods and Biomaterials in Skin Tissue Engineering: A Review. Int J Mol Sci. 2016; 17. pii:E1974.
20. Wang K, Nune KC, Misra RD. The functional response of alginate-gelatin-nanocrystalline cellulose injectable hydrogels toward delivery of cells and bioactive molecules. Acta Biomater. 2016; 36:p.143-51.
21. Dabiri G, Damstetter E, Phillips T. Choosing a Wound Dressing Based on Common Wound Characteristics. Adv Wound Care (New Rochelle). 2016; 5:p.32-41.
22. Straccia MC, et al. Alginate hydrogels coated with chitosan for wound dressing. Mar Drugs. 2015; 13:p.2890-908.
23. Sarei F, et al. Alginate nanoparticles as a promising adjuvant and vaccine delivery system. Indian J Pharm Sci. 2013; 75:p.442-9.

24. Sarei F, et al. Alginate Nanoparticles as a Promising Adjuvant and Vaccine Delivery System. Indian J Pharm Sci. 2013; 75:442-9.
25. Joosten PHM, et al. Oral vaccination of fish against Vibrio anguillarum using alginate microparticles. Fish Shellfish Immunol. 1997; 7:p.471-485.
26. Turner MD, et al. Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta. 2014; 1843:p.2563-2582.
27. Todd PA,Goa KL. Interferon gamma-1b. A review of its pharmacology and therapeutic potential in chronic granulomatous disease. Drugs. 1992; 43:p.111-22.
28. Schoenborn JR,Wilson CB. Regulation of interferon-gamma during innate and adaptive immune responses. Adv Immunol. 2007; 96:p.41-101.
29. Turner PV, et al. Administration of substances to laboratory animals: routes of administration and factors to consider. J Am Assoc Lab Anim Sci. 2011; 50:p.600-13.
30. Salmon JK, Armstrong CA, Ansel JC. The Skin as an Immune Organ. West J Med. 1994; 160:146-52.
31. Komori J, et al. The mouse lymph node as an ectopic transplantation site for multiple tissues. Nat Biotechnol. 2012; 30:976-83.
32. Ning L, et al. Influence of Flow Behavior of Alginate-Cell Suspensions on Cell Viability and Proliferation. Tissue Eng Part C Methods. 2016; 22:p.652-62.
33. Zhu L, et al. Alginate Particles with Ovalbumin (OVA) Peptide Can Serve as a Carrier and Adjuvant for Immune Therapy in B16-OVA Cancer Model. Med Sci Monit Basic Res. 2017; 23:p.166-172.
34. Bodey B, Bodey B Jr,Kaiser HE. Dendritic type, accessory cells within the mammalian thymic microenvironment. Antigen presentation in the dendritic neuro-endocrine-immune cellular network. In Vivo. 1997; 11:351-70.
35. Hespel C,Moser M. Role of inflammatory dendritic cells in innate and adaptive immunity. Eur J Immunol. 2012; 42:p.2535-43.
36. Harvey BP, et al. Antigen presentation and transfer between B cells and macrophages. Eur J Immunol. 2007; 37:p.1739-51.
37. Paredes-Juarez GA, et al. The role of pathogen-associated molecular patterns in inflammatory responses against alginate based microcapsules. J Control Release. 2013; 172:p.983-92.