Biomaterials 33 (2012) 3615–3625

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Leading opinion

# Strategies to reduce dendritic cell activation through functional biomaterial design☆

Patrick S. Hume [a], Jing He [b], Kathryn Haskins [c], Kristi S. Anseth [a,c,*]

[a] Department of Chemical and Biological Engineering, University of Colorado, 424 UCB, Boulder, CO 80309, USA
[b] Integrated Department of Immunology, University of Colorado Denver School of Medicine and National Jewish Health, Denver, CO 80206, USA
[c] The Howard Hughes Medical Institute, University of Colorado, Boulder, CO 80309, USA

ARTICLE INFO

*Article history:*
Received 29 January 2012
Accepted 2 February 2012
Available online 21 February 2012

*Keywords:*
Immunomodulation
Hydrogel
Surface modification
Adhesion molecule
Photopolymerization

ABSTRACT

Dendritic cells play a key role in determining adaptive immunity, and there is growing interest in characterizing and manipulating the interactions between dendritic cells and biomaterial surfaces. Contact with several common biomaterials can induce the maturation of immature dendritic cells, but substrates that reduce dendritic cell maturation are of particular interest within the field of cell-based therapeutics where the goal is to reduce the immune response to cell-laden material carriers. In this study, we use a materials-based strategy to functionalize poly(ethylene glycol) hydrogels with immobilized immunosuppressive factors (TGF-β1 and IL-10) to reduce the maturation of immature dendritic cells. TGF-β1 and IL-10 are commonly employed as soluble factors to program dendritic cells *in vitro*, and we demonstrate that these proteins retain bioactivity towards dendritic cells when immobilized on hydrogel surfaces. Following stimulation with lipopolysaccharide (LPS) and/or cytokines, a dendritic cell line interacting with the surfaces of immunosuppressive hydrogels expressed reduced markers of maturation, including IL-12 and MHCII. The bioactivity of these immunomodulatory hydrogels was further confirmed with primary bone marrow-derived dendritic cells (BMDCs) isolated from non-obese diabetic (NOD) mice, as quantified by a decrease in activation markers and a significantly reduced capacity to activate T cells. Furthermore, by introducing a second signal to promote BMDC-material interactions combined with the presentation of tolerizing signals, the multifunctional PEG hydrogels were found to further increase signaling towards BMDCs, as evidenced by greater reductions in maturation markers.



## 1. Introduction

Dendritic cells (DCs) bridge the innate and adaptive immune systems and are crucial to initiating and guiding the adaptive immune response. Immature dendritic cells (iDCs) survey the body's periphery in search of foreign and self antigens [1]. Upon antigen uptake, DCs present antigen to lymphocytes, but have the unique capacity to induce either immunity or tolerance to antigen [1,2]. For example, when iDCs encounter antigen in the presence of stimulatory factors such as "danger signals" or pathogen associated molecular patterns (PAMPs), iDCs undergo maturation into mature DCs and initiate an adaptive immune response [3]. Upon maturation, DCs increase expression of MHC stimulatory molecules, B7 family co-stimulatory molecules (i.e., CD80 and CD86), and inflammatory cytokines, including IL-12, which enable the activation of naïve $CD4^+$ helper T cells to initiate an immune response [1]. Conversely, if iDCs encounter antigen under conditions which prevent full DC maturation, they express an altered pattern of surface proteins, preventing an unfavorable adaptive immune response [2,4].

Because of their critical importance in dictating the fate of the adaptive immune response, understanding interactions between iDCs and implanted biomaterial surfaces has gained considerable interest in recent years [5]. For example, several commonly-used biomaterials including poly(lactic-co-glycolic acid) (PLGA) and chitosan [6], as well as surface modifications with common extracellular

☆ *Editor's Note*: This paper is one of a newly instituted series of scientific articles that provide evidence-based scientific opinions on topical and important issues in biomaterials science. They have some features of an invited editorial but are based on scientific facts, and some features of a review paper, without attempting to be comprehensive. These papers have been commissioned by the Editor-in-Chief and reviewed for factual, scientific content by referees.

* Corresponding author. HHMI Investigator, Department of Chemical and Biological Engineering, University of Colorado, Boulder, CO 80309, USA.
*E-mail address:* Kristi.Anseth@Colorado.edu (K.S. Anseth).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.02.009

matrix (ECM) proteins [7] and integrins [8], have been identified as regulators that can induce iDC maturation and initiate an adaptive immune response. The induced maturation of iDCs by biomaterials, or adjuvant effect, is desirable for vaccines against infection and tumors where an immune response is favorable [9]. However, in the design of biomaterials for cell delivery, such as the encapsulation of tissues for transplantation, ideal biomaterial cell carriers would prevent the full maturation of iDCs. Babensee and coworkers recently demonstrated that hyaluronic acid and agarose have the capacity to limit immunogenicity compared to surfaces with known adjuvant effect [6,10], but in general, the investigation of strategies to actively limit iDC maturation via controlled modification of biomaterial surfaces has been limited. In solution, however, iDCs have been cultured in the presence of one or more soluble factors such as transforming growth factor β-1 (TGF-β1) and/or interleukin 10 (IL-10) [11–14]. Resulting DCs have been reported to undergo incomplete maturation upon immune stimulation and can promote T cell anergy or induce regulatory T cell production [2]. For example, Torres-Aguilar et al. recently cultured iDCs with IL-10 and TGF-β1 in the presence of insulin to generate DCs that could induce antigen-specific insulin tolerance in humans [15].

Numerous proteins, peptides and other molecules of interest have been previously incorporated onto biomaterial surfaces and remained bioactive for cell signaling [16,17]. We have previously investigated PEG-based surfaces for the purposes of immune signaling, and demonstrated that PEG coatings containing immobilized anti-fas are capable of interacting with T cells and inducing apoptosis [18–20]. Notably, Mann et al. tethered TGF-β1 within PEG hydrogels to signal vascular smooth muscle cells and demonstrated that immobilized TGF-β1 maintained bioactivity and increased ECM protein synthesis [21]. Further, it is known that DCs have the capacity to receive biological cues from tethered signaling proteins, as Leclerc et al. immobilized granulocyte-macrophage colony stimulating factor (GM-CSF) upon surfaces to promote the development of iDCs from isolated bone marrow tissue [22]. In the study we describe herein, a general approach to modify biomaterial surfaces with thiolated proteins, specificallyTGF-β1 and/or IL-10, to create immunomodulatory surfaces that signal iDCs and reduce maturation upon stimulation with LPS. A poly(ethylene glycol) (PEG) hydrogel platform, which limits immunogenicity and allows facile modification for incorporation of proteins, was chosen as a basis for tethering anti-inflammatory molecules for iDC signaling. By introducing a second signal that promoted cell–material interactions, along with the immunomodulatory signals, multifunctional PEG hydrogel surfaces could be tailored to suppress iDC maturation to a greater degree than either signal alone.

## 2. Materials and methods

### 2.1. Dendritic cell culture

Initial studies were conducted with the cytokine-dependent, immortalized immature dendritic cell line, JAWSII. The JAWSII dendritic cell line was originally isolated from the bone marrow of $p53^{-/-}$ C57BL/6 mice and has been previously shown to mimic the capacity of primary iDCs to undergo maturation in response to immune stimuli [23–26]. JAWSII cells, an immortalized dendritic cell line of murine bone marrow origin (ATCC, Manassas, VA), were cultured in α-MEM media (Invitrogen, Carlsbad, CA) supplemented with 20% heat inactivated FBS (Invitrogen), 1% penicillin/streptomycin (Invitrogen), and 5 ng/ml GM-CSF (Peprotech, Rocky Hill, NJ). JAWSII were cultured in tissue culture flasks and media was changed weekly. Additionally, primary bone marrow-derived DCs (BMDCs) generated from bone marrow isolated from non-obese diabetic (NOD) mice were evaluated with immunomodulatory hydrogels. Primary BMDCs were harvested from femurs isolated from NOD mice (4–10 weeks old). The ends of femurs were cut and the marrow was rinsed with 10 ml RPMI media 1640 (Invitrogen) with a 27 gauge syringe needle. Freshly isolated samples were then mixed in an 18 gauge syringe to dissociate clumps and the resulting cell suspension was cultured in media consisting of RPMI 1640 supplemented with 1.5% mouse serum (Invitrogen), 20 ng/ml GM-CSF, and 1% penicillin/streptomycin. BMDCs were seeded onto tissue culture polystyrene (TCPS) in 6-well plates or hydrogels in 96-well plates and 50% fresh media volume was changed daily.

### 2.2. Thiolation of proteins

To incorporate TGF-β1 and IL-10 as covalent pendant functional groups within hydrogels, proteins were rendered polymerizable via modification by Traut's reagent (Thermo Scientific, Rockford, IL), which conjugates to free amines to create thiols. In brief, proteins were reconstituted in phosphate buffered saline (PBS, pH 7.4, Invitrogen) containing 2 mM EDTA (Sigma) and a 5-fold molar excess Traut's reagent per mol protein. Samples were mixed and reacted at room temperature for 1 h. Following thiolation, unreacted Traut's reagent was removed via filtration through Zeba™ Spin Desalting Columns (7 K MWCO, Thermo Scientific), yielding the final thiolated product of TGF-β1-SH or IL-10-SH. Samples were diluted to a final concentration of 25 μg/ml in PBS with 2 mM EDTA and immediately placed in a −80 °C freezer. Prior to use, protein solutions were rapidly thawed and added to pre-polymer solutions in concentrations ranging from 0 to 1 μg/ml for gel formation via photopolymerization.

### 2.3. PEG hydrogel formation

The synthesis of poly(ethylene glycol) (PEG) diacrylate (PEGDA, 10 kDa) macromolecular monomers from hydroxyl-PEG (Sigma) has been described in detail elsewhere [27]. Briefly, hydroxyl PEG was dissolved in anhydrous toluene by heating to 60 °C with mixing. After the dissolved PEG was allowed to cool to room temperature (RT), triethylamine (TEA, 4-fold molar excess per hydroxyl group) and acryloyl chloride were added and reacted overnight at RT with stirring. Next, TEA was removed via filtration through neutral alumina. PEGDA was precipitated in cold diethyl ether and desiccated to dryness overnight. To ensure high levels of purity, PEGDA was dialyzed against deionized water overnight (1 kDa MWCO membrane) with >3 dialysis volume changes.

Pre-polymer solutions were prepared consisting of 10 wt% PEGDA in PBS with 0.05 wt% of the photoinitiator 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (Irgacure-959, Ciba Specialty Chemicals) and thiolated proteins (0–1 μg/ml). For selected studies, poly-L-lysine (PLL, 10 μM, Sigma), laminin (200 μg/ml, BD Biosciences, Bedford, MA) or fibronectin (200 μg/ml, BD Biosciences) were also included in pre-polymer solutions. Pre-polymer solutions were loaded into the tips of 1 ml syringes (30 μl) and polymerized for 10 min under ultraviolet light (6 mW/cm$^2$, centered at 365 nm) for gel formation. Following polymerization, hydrogels were incubated overnight in either PBS or cell culture media (4 °C, with orbital shaking) with at least two solution changes to ensure complete removal of residual photoinitiator and untethered proteins. The final, swollen hydrogels were disks with a diameter of ~5.5 mm and height of ~2 mm.

### 2.4. Measurement of incorporated proteins

TGF-β1 was added to pre-polymer solutions in concentrations ranging from 0 to 1 μg/ml. Following photopolymerization, functionalized hydrogels were rinsed overnight in 3 ml PBS with >2 solution changes to ensure complete removal of non-covalently bound protein. The following day, a modified ELISA was employed to quantify the concentration of protein on the gel surface. Hydrogels were incubated with anti-TGF-β1 monoclonal antibody from mouse (5 μg/ml, Peprotech) in ELISA buffer (sterile filtered PBS with 0.1% BSA and 0.05% TWEEN-20) for 1.5 h and then rinsed three times with ELISA buffer. Next, samples were incubated with horseradish peroxidase-conjugated goat anti-mouse secondary antibody (100 ng/ml, Jackson ImmunoResearch, West Grove, PA) for 30 min, followed by three rinses with PBS. Samples were then incubated for 60 min in ELISA buffer, rinsed three times, and incubated another 15 min in ELISA buffer to ensure complete removal of unbound antibodies. Hydrogels were distributed into individual wells of a 96-well plate containing 70 μl PBS (30 μl hydrogel plus 70 μl PBS resulted in roughly 100 μl total volume/well), and the TMB ELISA substrate (100 μl/well, Thermo Scientific) was added for 20 min. Finally, the reaction was quenched via the addition of 100 μl $H_2SO_4$ 100 μl combined TMB/$H_2SO_4$ and the absorbance was measured at 450 nm and compared to that of standards prepared from known quantities of secondary antibody, TMB, and $H_2SO_4$.

### 2.5. PE-25 TGF-β reporter cell assay

The presence of biologically active TGF-β1 on hydrogel surfaces was verified using a PE-25 TGF-β reporter cell line (i.e., mink lung epithelial cells (Mv1Lu or CCL-64)) containing a stably transfected luciferase reporter gene for TGF-β which were generously donated by Dr. Xuedong Liu [28]. PE-25 cells were seeded atop functionalized hydrogels at a density of $1 \times 10^5$ cells/ml for 24 h in 200 μl Dulbecco's Modified Eagle Medium (Invitrogen). The following day, the media was gently removed and samples were lysed via the addition of 200 μl passive lysis buffer (Promega, Madison, WI) and incubated for 10 min at 37 °C, with shaking, and finally stored at −80 °C for >2 h. Next, the samples were thawed, transferred to