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Immunological properties of Andean starch films are independent of their nanometric roughness and stiffness

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ABSTRACT

Starch is a natural material extracted from roots, seeds, stems and tubers of different plants. It can be processed as a thermoplastic to produce a variety promising products for biomedical applications, including foams, sheets and films. In the present work, we investigated the immunological properties of microfilms prepared with starches extracted from six different types of Andean potatoes and their relationship with the different film-surface features. We confirmed the biocompatibility of all the films using THP-1 human monocytes, noticing only slight decrease in cell viability in two of the tested starches. We also analyzed pro-inflammatory cytokine release and immune cell surface receptor modulation on THP-1 plated onto the films. Our data show differences in the immunological profile of the same cells cultured onto the different starch films. Furthermore, we examined whether the dissimilar stiffness or the nanometric roughness of the films might influence the immune stimulation of the THP-1 monocytes. Our results demonstrate no correlation between cultured THP-1 immune activation and surface film characteristics. We conclude that different Andean native potato starch films have specific ability to interact with cell membranes of immune cells, conceivably due to the different spatial localization of amylose and amylopectin in the diverse starches.

1. Introduction

Starch is a combination of natural polymers that can be extracted from roots, seeds, stems and tubers of different plants, such as corn, potato, wheat, rice and other vegetables. Starch granules are formed by two polymers, amylose and amylopectin. Amylose is a linear polymer and comprises 20–30% of the starch granule. It is made by long chains of α-(1,4)-linked D-glucose units with a degree of polymerization ranging 3×10^2–1×10^4 [1]. By contrast, amylopectin is a branched polymer and has α-(1,4)-linked glucose chains, joined by -(1,6)-linkages with a degree of polymerization of about 10^8 [2].

Starch can be processed as a thermoplastic using the right amount of heat and water in order to produce a variety of products, *e.g.* foams, sheets, films, *etc.* Water diffusion, granular expansion, gelatinization, melting and crystallization are some chemical and physical reactions that may occur during starch processing. Gelatinization is a particularly important phase transition because it is the basis of the conversion of starch thermoplastics [3].

Biocompatibility is an inherent property of materials produced from natural polymers such as starch [4]. Biocompatibility of starch based products is accounted for the presence of major biocompatible structural components. Previous studies from our group [5] confirmed the biocompatibility of starch films when used as substrates for cell growth.

A variety of biomedical applications has been studied for starch-based materials, such as substrates for cell seeding [5], scaffolds for tissue engineering [6–9], drug delivery systems [10–14] and bone replacement implants [15]. Nevertheless, some disadvantages limit the use of starch in a variety of applications, where high mechanical properties and no hydrophilic character are needed.

The use of starch based materials in biomedicine could be limited by the host immune response toward the starch-based tool in contact with cells and tissues [16]. Specific immune response is elicited by the direct interaction between the material surface and the immune cell surface or as a secondary effect due to the molecular immune factors absorbed onto the material, once in the host body. The latter effect is very challenging to be unraveled, since the mechanism producing an immune reaction may be

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tissue-specific and depends on the different immune cell subpopulation patrolling the specific body organ.

However, the first immune response in the majority of the tissues is mediated by the innate immunity [17]. This is a coordinated system that includes different cellular and biochemical mediators, which are able to produce an immediate non-specific response to the "non-self" material, as immunology defines each organism or material from external source. Phagocytic cells, like neutrophils or monocyte/macrophages, are the major cell types that remove the foreign pathogen by encapsulating and destroying it by specific enzymatic reaction cascades. The trigger to the response is based on the cell recognition of unique molecular structures present on the surface of microbial pathogens, called Pathogen-Associated Molecular Patterns (PAMPs), by means of specific pattern-recognition receptors. Well known examples of PAMP-receptors are the Gram-negative bacteria wall component lipopolysaccharide (LPS), the lipoteichoic acid (present on the wall of Gram-positive bacteria) receptor or the receptors for mannose-rich oligosaccharides present on fungi. Innate immune system differs from the adaptive immune system, a second line of defense that counteracts the pathological organisms with specific responses, and whose major cellular type is represented by lymphocytes.

Among the immune cell populations, monocytes represent the key players of the innate immunity implicated in inflammatory processes in different tissues [18]. This cell type is able to sense environmental signals and rapidly migrate to the site of inflammation, where they differentiate into macrophages that dynamically phagocytize non-self-molecules. Moreover, through the release of specific molecular messengers, called cytokines, they regulate growth and differentiation of the other immune cells and link the innate and adaptive immune responses.

In view of potential biomedical application of natural materials, we show in this paper the immunological aspects of microfilms prepared with starches extracted from six different types of Andean potatoes. We focused our attention on the immune reaction of human monocytes plated onto starch films, following the release of monocyte-derived pro-inflammatory cytokine release and modulation of cell surface receptors involved in immune responses. Furthermore, we studied the stiffness and the nanometric roughness of the starch films and their possible effects to the monocyte immune response.

2. Materials and methods

2.1. Starch extraction

Starches used in this study were extracted from the six varieties of Andean potatoes listed in Table 1. The procedure used for starch extraction was followed from Torres et al. [19]. Briefly, the potatoes were cleaned with tap water and rinsed with distilled water. In a blender sleeve the samples were homogenized and left for decantation using distilled water for 4 h. The supernatant was discarded and the precipitates were dissolved again. White precipitates were degreased in a suspension of methanol and water. The result was decanted again for 1 h while the remaining starch precipitated at the bottom of the container. Finally, the resulting starch was dried at 40 °C for 48 h and stored in a desiccator at room temperature.

Table 1
Code used in the paper, common name and scientific designation of the starch extracted from Andean crops used in this study.

Code	Common name	Scientific designation
T-POT01	Golden potato	*Solanum tuberosum*
T-POT02	"Negra" potato	*Solanum tuberosum* sbsp. *andigena*
T-POT03	"Peruanita" potato	*Solanum goniocalyx*
T-POT04	"Yungay" potato	*Solanum tuberosum*
T-POT05	"Huamantanga" potato	*Solanum tuberosum* sbsp. *andigena*
T-POT06	White potato	*Solanum tuberosum* sbsp. *andigena*

2.2. Preparation of starch films

Starch films were prepared by casting. The procedure reported by Torres et al. [20] was used. Briefly, starch was mixed with distilled water to form a 5% (w/w) starch solution. This solution was partially hydrolyzed in diluted hydrochloric acid (0.1 N) adjusting pH to 2.0. Glycerol was added at ratio of 2:5 (glycerol:starch (dry basis)). The starch solution was homogenized by stirring for 25 min at 95 °C. Then, the solution was neutralized in diluted sodium hydroxide (0.1 N) adjusting the pH to 10 to stop hydrolysis. Finally, the starch solution (7%, w/w) was spread on Petri dishes and placed in an oven at 40 °C. After 16 h of drying, films of about 250 μm in thickness were obtained (Fig. SI 1a).

Supplementary Fig. SI 1 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2015.02.008.

2.3. Endotoxin test

To determine endotoxin in starch films (T-POT01 to T-POT06 see Table 1) both quantitative and qualitative assays were used. We quantify the amount of endotoxin Gram negative bacterial endotoxin by QCL-1000™ assay (Fig SI 1b) with a sensitivity range of 0.1–1.0 EU/ml (Lonza Walkersville, Inc.) Patches of each film approximately of 0.5 cm × 0.5 cm were aseptically cut soaked in 300 μl of endotoxin free (EF) water for 12 h in and EF glass tubes. Supernatant samples of 100 μl were dropped in double in EF 96-microwell plate and mixed with the LAL and chromogenic substrate reagents. After 16 min incubation absorbance reading at 450 nm was performed by using Fluo Star Optima. Data were collected by Control Software and elaborated with MARS Data Analysis Software (BMG LABTECH) and expressed as mean ± standard deviation. Not statistically significant deviation from the negative control was found in all the tested starch film samples. Statistically significant difference was determined by performing one-way ANOVA analysis followed by Bonferroni *post hoc* test. P value <0.05 was considered significant (Fig. SI 1b). To further verify with higher sensitivity the presence of endotoxin in starch films we employed PYROGENT™ Gel Clot LAL Assay (Lonza Walkersville, Inc.), a qualitative LAL test with sensitivity ranging from 0.125 to 0.0015 EU/ml. Patches of each film were incubated in 1 ml of EF water in parallel with negative, positive controls and endotoxin standard. The gel clot assays were run in EF tubes that are placed in dry heat block at 37 °C. After 1 h incubation the tubes were flipped 180°. A firm clot that stays in the bottom of the tube indicates a positive reaction. On the other hand, liquid flow down the tube the result is negative for endotoxin. Also in this case the presence of endotoxin was not detectable for all starch films.

2.4. THP-1 cell culture

Human THP-1 monocytic cells were purchased from American Type Culture Collection (ATCC Manassas, VA, USA). Cells were maintained as a monocytic cell suspension in RPMI-1640 ATCC modified (Sigma, St. Louis, MO #) supplemented with 10% fetal bovine serum (FBS, Life Technologies cat. no. #10108-165), 1% antibiotics and 0.05 mM 2-mercaptoethanol (Life Technologies cat. no. #21985) at 37 °C in 5% CO_2, and cultures were split every 3 days. For each experiment cells were cultured in 24-well plates at a concentration of 6×10^5 cells/ml on the different starch-based films for 24 h.

Cells were stimulated with 100 ng/ml of lipopolysaccharide (LPS) as positive control.

2.5. Cell viability

Cell viability was assessed by Trypan blue assay. After being cultured for 24 h on starch-based films, the medium was removed and the cells were stained by 0.4% Trypan blue (Sigma–Aldrich) solution for 3 min. The cells with damaged cell membranes were stained by Trypan blue and counted under the optical microscope.

2.6. Cytokine release

MCP-1, MIP-1β, RANTES, IL-10, IFN-γ, TNF-α, IL-1β and IL-6 levels released by THP-1 cultured for 24 h on starch-based films were assessed by Bio-Plex Magpix System (Bio-Rad) following manufacturer's instructions. LPS (100 ng/ml) was used as positive control (see Supporting Information).

2.7. Monocyte receptor expression and flow cytometry

After 24 h treatment, supernatants were collected and cells washed with RPMI at 8000 rpm for 4 min. Then, the pellets were resuspended in RPMI-1640/0.5% BSA and incubated for 30 min on ice, in the dark, with the respective fluorescently labeled antibodies. After the incubation time, cells were washed with ice-cold RPMI at 8000 rpm for 4 min and resuspended in an appropriate volume with MACS Running buffer (Miltenyi, cat. no. #130-092-747). Cell associated fluorescence was analyzed by flow cytometry (MACSQuant Analyzer, Miltenyi Biotec, Bergisch, Germany) and 20,000 events for each sample were acquired. Live cells used for the analysis were gated based on forward light scatter (FSC) and side light scatter (SSC) and further analyzed using the MACS Quantify software (Miltenyi Biotec). The specific antibodies used in flow cytometric experiments were: FITC conjugated mouse anti-human CD195/CCR5 (BD Pharmigen, cat. no. #555992), AlexaFluor 647 conjugated mouse anti-human CD192/CCR2 (BD Pharmigen, cat. no. #558406), PE conjugated mouse anti-human CX3CR1 (Miltenyi Biotec, cat. no. #130-096-432), APC conjugated mouse anti-human CD16 (Miltenyi Biotec, cat. no #130-091-246), VioBlue conjugated mouse anti-human CD14 (Miltenyi Biotec, cat. no. #130-094-364), PE conjugated mouse anti-human CD11b (Miltenyi Biotec, cat. no. #130-097-336). All the antibodies were used in the concentrations suggested by the suppliers.

2.8. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) images were acquired at room temperature working in dynamic tapping mode. Commercially available silicon cantilevers (nominal spring constant 2.8 N/m) were used working at low oscillation amplitudes with half free amplitude set-point. High-resolution images (10 μm × 10 μm, 512 × 512 pixels frames) were acquired at 0.6–1 lines/s scan speed. Agilent Pico Image software was used for data processing. Root mean squared roughness [21] values were calculated as average sampling three different regions of each film. The error is the standard deviation.

Mechanical properties of the materials were evaluated by AFM nanoindentation measurements. Commercially available silicon cantilevers (nominal spring constant 2.8 N/m, Agilent technologies) were used. The analyses were performed in cell medium sampling 32 × 32 points of 30 μm × 30 μm regions of each film. Young's modulus was calculated with the Hertz's model fit of each loading force–distance curve [22] with an assumed 0.5 Poisson ratio. The average and the standard deviation were calculated from the values distribution. For the data analysis Agilent Pico image and Origin Pro software were used.

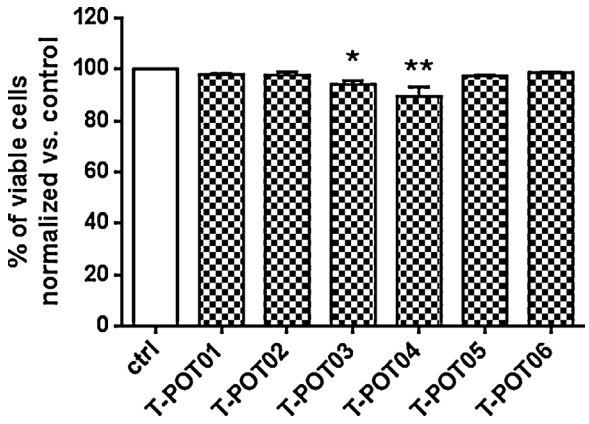

Fig. 1. Cell viability of THP-1 monocytes. Columns represent the percentage of viable THP-1 cells plated onto the starch films *vs.* control plated onto culture dishes. Trypan blue staining has been used to subtract the dead cells in each sample. Three aliquots per sample have been counted. Data are expressed as means ± SD of three independent experiments. One way ANOVA analysis followed by Bonferroni *post hoc* test has been performed ($^{*}P < 0.05$, $^{**}P < 0.01$)

2.9. Statistical analysis

Each experiment was performed in triplicate. Data are expressed as fold increase compared to control. Statistically significant difference was determined by performing one-way ANOVA analysis followed by Bonferroni *post hoc* test. A P value of <0.05 was considered significant.

3. Results

3.1. Biocompatibility of starch films

We previously demonstrated the biocompatibility of native Andean starch films as cell culture support using fibroblasts [5]. Here, we show that all the six starches listed in Table 1 do not affect the viability of THP-1 monocytes cultured onto the films for 24 h, evaluated by Trypan blue-exclusion counting (Fig. 1). Albeit statistically significant in respect to the control, we observed only a slight viability decrease (<10%) in cultures with T-POT03 and T-POT04.

3.2. Pro-inflammatory cytokine release by THP-1 monocytes cultured onto starch films

THP-1 monocytes have been plated onto starch films in culture medium conditions (see Section 2). Culture medium has been harvested after 24 h and the levels of TNF-α, IL-1β, IL-6 and CCL2 were evaluated (Fig. 2). Those cytokines were present at different concentrations in the media of the six starch films tested. Namely, THP-1 cells cultured onto starch film extracted from T-POT03 and T-POT06 did not induce any significant release of the pro-inflammatory cytokines tested. The chemokine CCL2 was the only cytokine detected in the medium from monocytes plated onto T-POT05 for 24 h. On the other hand, high amount of TNF-α, IL-1β, IL-6 and CCL2 was measured in the sample cultures of T-POT02 and T-POT04. T-POT01-culture plates of THP-1 produced intermediate amount of TNF-α, IL-1β and CCL2, but not statistically significant concentration of IL-6 was noticed.

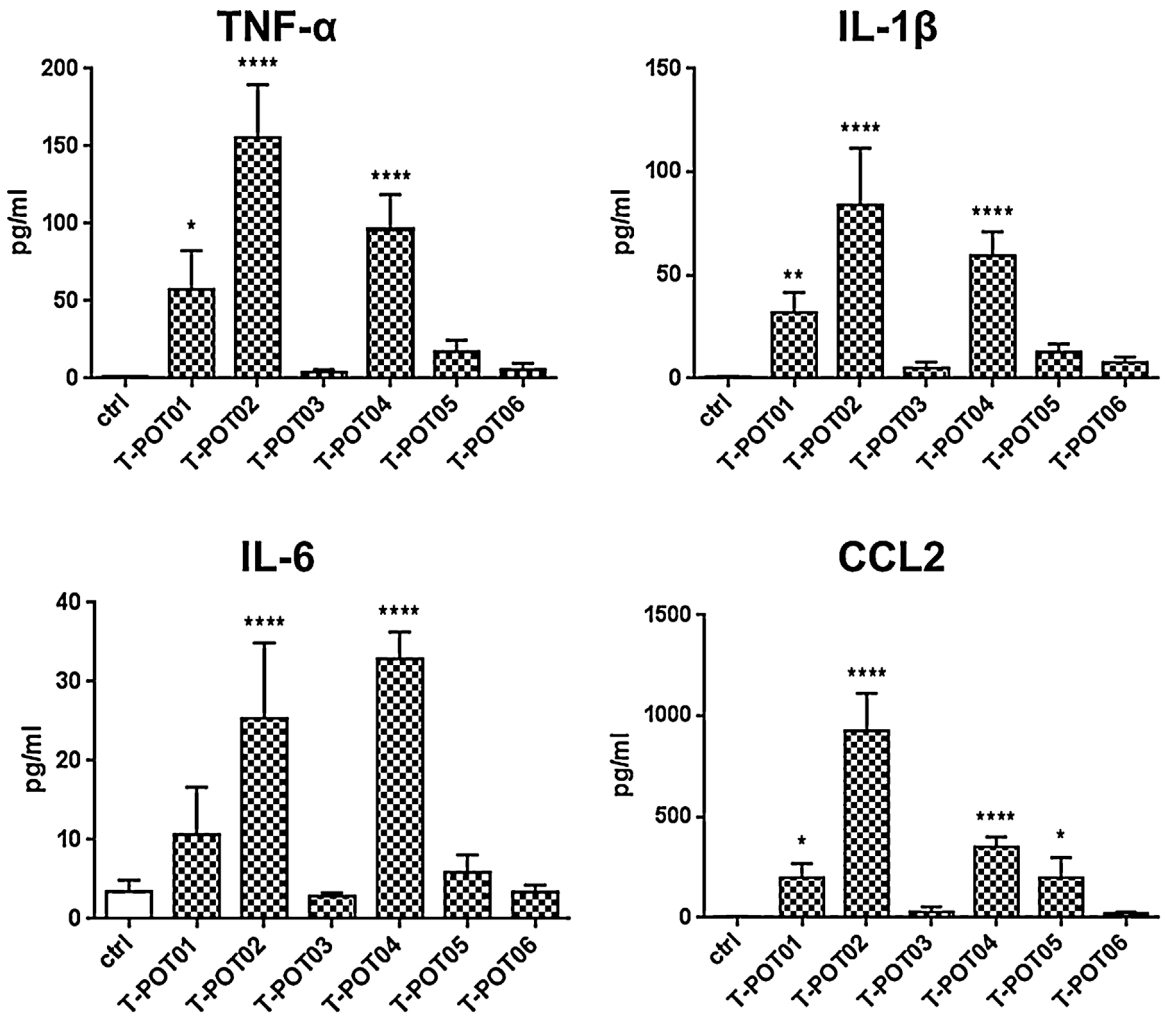

Fig. 2. Pro-inflammatory cytokine release. Column graphs show the release of TNF-α, IL-1β, IL-6 and CCL2 expressed in pg/ml induced by culturing THP-1 cells onto the different starch films for 24 h. The Control column refers to THP-1 cells plated into culture dishes without starch films. Data are expressed as means ± SD of three independent experiments. One way ANOVA analysis followed by Bonferroni *post hoc* test has been performed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

3.3. Immune receptor expression on THP-1 monocytes cultured onto starch films

In order to understand whether the contact with the starch films could stimulate THP-1 monocytes to differentiate toward an activated-monocyte or macrophage profile, we measured by flow cytometry the cell membrane expression of immune receptors (Fig. 3). Our results show that THP-1 cells cultured onto T-POT04 derived starch films up-regulate CD11b, also known as Macrophage-1 antigen (MAC-1), and the Fc receptors FcγRIII (CD16). Moreover, only in presence of this specific starch film the monocytes over-expressed the pro-inflammatory chemokine receptors CCR2 and CCR5. On the other hand, T-POT01 provoked the up-regulation of both CD11b and CD16, while T-POT03 and T-POT05 only prompted the increase in the expression of a single membrane immune protein. CD11b in the case of T-POT03 starch film and CD16 when cells were plated onto films derived from T-POT05. It is worth to be mentioned that CD14 and CX_3CR1, also constitutively expressed on THP-1 cells, did not change their expression in a statistically significant way when monocytes were plated onto all the starch films (Fig. SI 2).

Supplementary Fig. SI 2 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2015.02.008.

3.4. Surface characterization of starch films

Monocyte activation in the blood stream is mediated by soluble molecules as well as endothelial cell-surfaces. The blood vessel walls regulate monocyte polarization and differentiation by changing the morphology of their luminal structure, and often exposing specific molecular ligand for cell receptors [17]. To investigate possible correlation between the observed THP-1 immune activation and the characteristics of the starch film surfaces facing the plated cells, we analyzed our films by atomic force microscopy (AFM). Fig. 4 shows the three-dimensional topographical images of the investigated samples. Root mean squared roughness was used to compare sample topography (Fig. 5a). The results highlight differences among the samples. In particular, it is possible to observe the presence of peaks and asperities in the case of sample T-POT04 reporting the highest roughness value (Rq = 42.4 ± 8.2 nm). Also the sample T-POT02 (Rq = 29.6 ± 4.1 nm) presents a high roughness compared to the others. On the contrary, T-POT01 (Rq = 4.71 ± 0.9 nm) and T-POT03 (Rq = 11.7 ± 0.6 nm) result to be the flattest.

Furthermore, the elastic moduli of the films were calculated by AFM nanoindentation. It was possible to extrapolate the Young's modulus of each film performing the measurements in the cell medium, in order to reproduce the cell substrate conditions. The

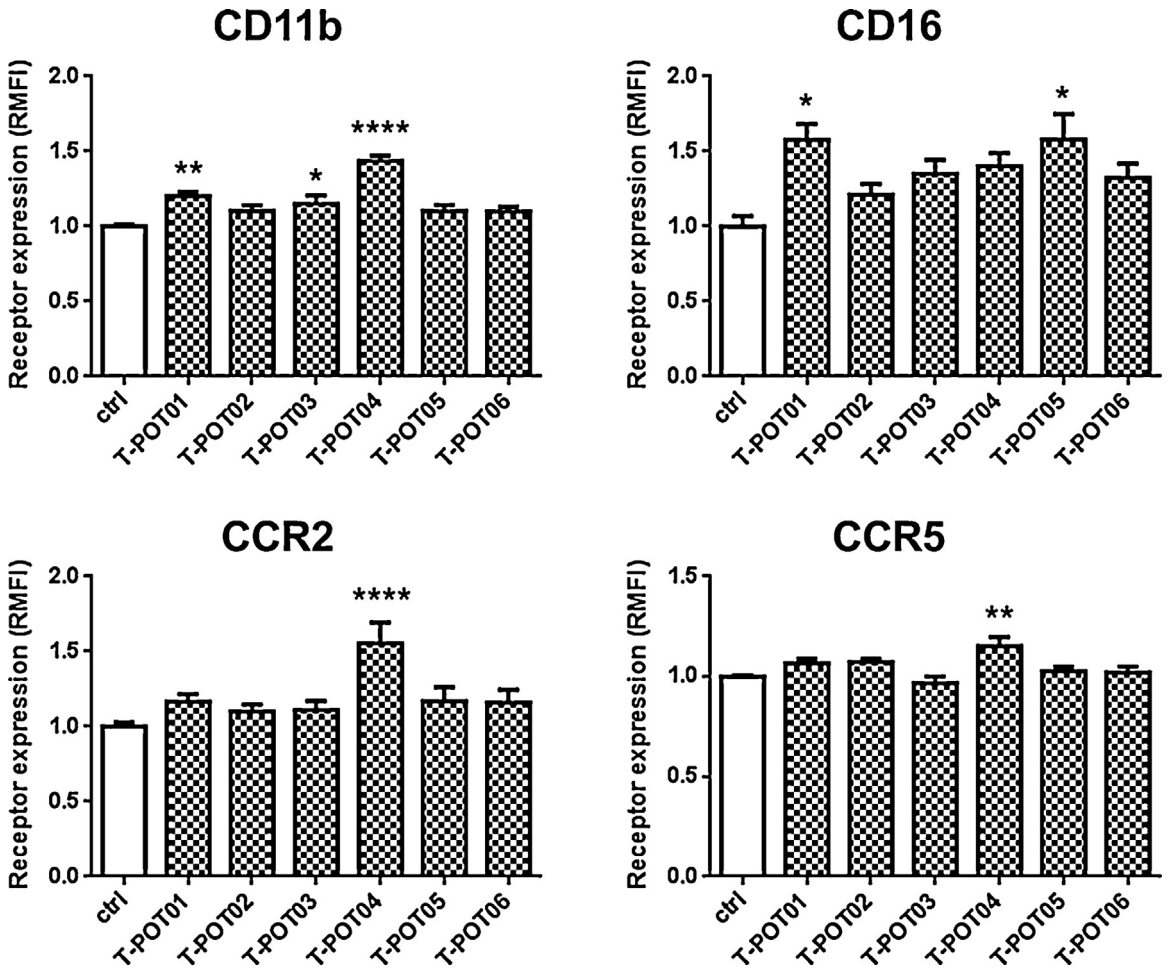

Fig. 3. THP-1 pro-inflammatory receptor expression. THP-1 monocytes expression of pro-inflammatory receptors CD11b, CD16, CCR2 and CCR5 after 24 h culturing onto the different starch films. The Control column refers to THP-1 cells plated into culture dishes without starch films. Data are expressed as means ± SD of three experiments. One way ANOVA analysis followed by Bonferroni *post hoc* test has been performed (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

data show that T-POT05 films have the highest Young's modulus (99.35 ± 4.1 MPa) with regard to T-POT01 (13.34 ± 1.3 MPa), T-POT02 (13.3 ± 1.1 MPa) and T-POT06 (17.5 ± 1.8 MPa) films (Fig. 5b).

4. Discussion

Bio-engineers and bio-medical scientists have increased the interest in starch-based materials fabrication, including films and particles, due to the mechanical properties and bio-degradability of the material [23,24]. At the same time, bio-nanotechnologies and the possibility to characterize materials at the nanometric scale are prompting interdisciplinary research aimed at life technology applications. In this scenario, we investigated the interaction that peculiar Andean native starches may have with human immune cells, in view of potential bio-applications.

We follow the ongoing research on the biocompatibility of Andean starches [5] expanding the investigation to the potential immune reaction that could be triggered by the contact between the immune cells and the starches. To appropriately address this issue, we focused on the human monocyte cell population, since it plays a key role in the immune response linking the innate to the adaptive immunity [17]. Monocyte/macrophage cell lineage coordinates the immune cell communication by the release of signaling cytokines. Furthermore, they modify their ability to receive specific environmental signaling by reprogramming monocyte-specific membrane protein receptors on their surface.

In the present work we evaluated the immune activity of monocytes plated onto the Andean native starch films by measuring the release of pro-inflammatory cytokines and the cell surface receptor expression. All the six tested starch films confirmed the high degree of biocompatibility (Fig. 1) as already pointed out in our preceding publication [5] using a different cell line. Actually, a minor (<10%) variation of *in vitro* viability previously seen with T-POT03 and T-POT04 has been confirmed with THP-1 monocytes (Fig. 1), possibly related to the cell adhesion properties of this specific starch. On the other hand, the pro-inflammatory cytokine release (Fig. 2) showed independent effects of immune response for each tested starch film. Indeed, T-POT03 did not induce any release of cytokine, whereas T-POT04 seemed to be immunogenic. Besides, T-POT04 starch was able to induce the up-regulation of all the considered receptors, while T-POT03 induced a slight variation of CD11b (Fig. 3). Other Andean native starch films used as cell plating substrates, like T-POT01, 02 and 05 induced an increase of the Fc receptors FcγRIII (CD16). This receptor is involved in the phagocytosis of opsonized (covered with antibodies) pathogens, suggesting a monocyte polarization toward macrophage/phagocyte phenotype.

We investigate a possible correlation with the specific surface morphology or stiffness of the starch films used in this study. Atomic force microscopy results demonstrated that starch immunogenicity does not properly correlate either with the nanometric roughness or with stiffness of the films (Fig. 4). For example, culturing THP-1 monocytes onto the T-POT01 films, which displayed a relatively low roughness and Young's modulus

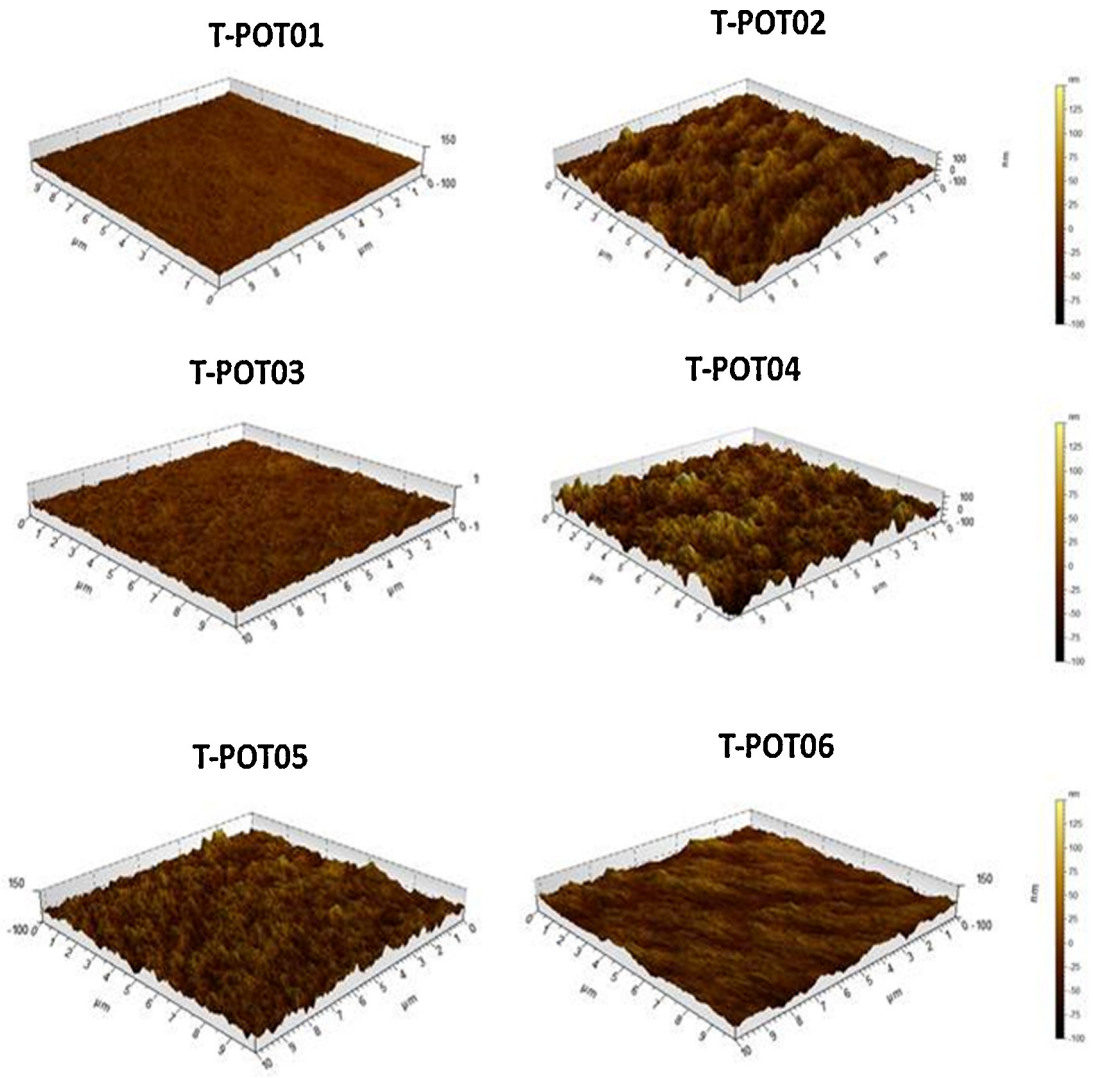

Fig. 4. Topography of starch film surfaces by atomic force microscopy. Representative three-dimensional AFM topography of starches films (10 μm × 10 μm), 250 nm full *Z*-scale.

Table 2
Summary of the monocyte immune activation induced by starch films.

Starch	Released cytokine	Up-regulated receptor	Surface roughness	Surface stiffness (Young's modulus)
T-POT01	TNF-α* IL-1β** CCL2*	CD11b** CD16**	Rq = 4.71 ± 0.9 nm	13.3 ± 1.3 MPa
T-POT02	TNF-α**** IL-1β**** IL6**** CCL2****	CD11b** CD16**	Rq = 29.6 ± 4.1 nm	13.3 ± 1.1 MPa
T-POT03		CD11b*	Rq = 11.7 ± 0.6 nm	38.5 ± 3.4 MPa
T-POT04	TNF-α**** IL-1β**** IL6**** CCL2****	CD11b**** CD16* CCR2**** CCR5**	Rq = 42.4 ± 8.2 nm	54.9 ± 1.8 MPa
T-POT05	CCL2*	CD16**	Rq = 23.9 ± 1.4 nm	99.3 ± 4.1 MPa
T-POT06			Rq = 17.7 ± 0.9 nm	17.5 ± 1.8 MPa

(Fig. 5, Table 2 and Fig. SI 3), provoked the cell production of TNF-α, IL-1β and CCL2 and the over-expression of CD11b and CD16. No immunogenic influence was detected using the stiffer and more rigid T-POT06 as cell substrate. On the contrary, the T-POT04 films demonstrated immune activation of THP-1 cells, with strong release of all the pro-inflammatory cytokines and the increased expression of all the receptors. It is worth to mention that T-POT04 was the only starch film able to induce CCR2 and CCR5 upregulation, two chemokine receptors directly involved in monocyte/macrophage migration to the sites of inflammation.

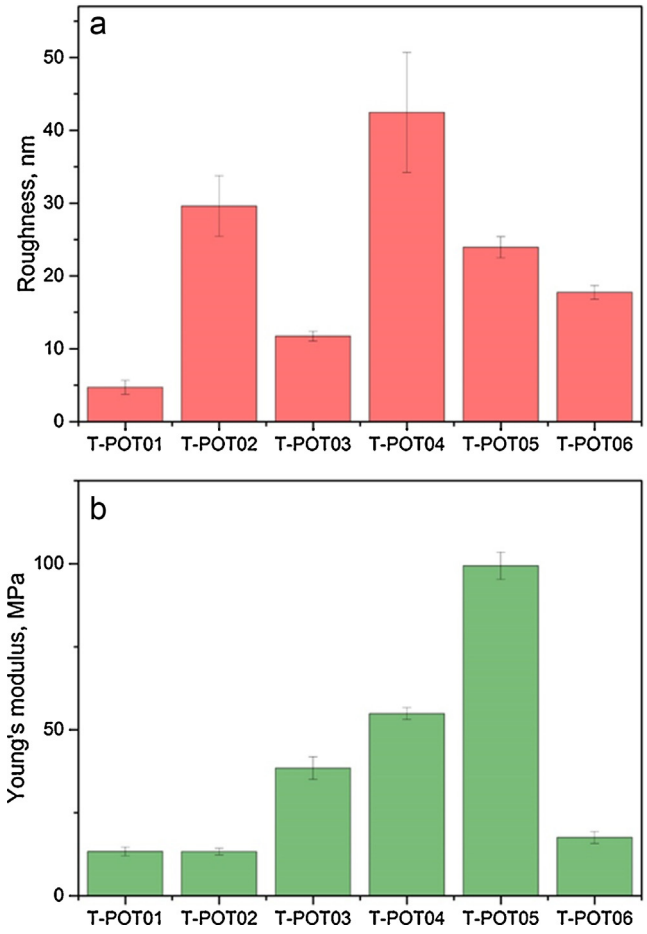

Fig. 5. Evaluation of the roughness and the stiffness of the starch films. (a) Average roughness values of starch films. (b) Average Young's modulus values of starch films. Data are expressed as means ± SD of three experiments.

Supplementary Fig SI 3 related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijbiomac.2015.02.008.

Previous work reported elsewhere has provided data for other mechanical properties of these native starch films [20]. It is worth noting that the values of the elastic modulus obtained from AFM measurements presented here are in agreement with the values obtained from previous uni-axial tensile tests performed in this laboratory and reported by Torres et al. [20]. The tensile elastic modulus found for native starch films ranged 15–106 MPa. The ultimate tensile strength (UTS) and the maximum elongation were also assessed by uni-axial tensile tests. Both UTS and maximum elongation displayed very similar ranges and showed no dependence on the starch source, with UTS varying in the range 2.68–3.99 MPa while maximum elongation was in the range 18.05–24.18%.

Furthermore, the thermal properties of the starches used in the present research are also analogous and have been assessed by our group using differential scanning calorimetry (DSC) and previously reported [19]. We determined that the temperature at which gelatinization takes place for a variety of native starches was in the range 62.5–65.5 °C. Based on this, no correlation can be established between the gelatinization temperature of the starches used in the present work and their immunological response, since cell culturing temperature (37 °C) is far from starch gelatinization-temperature range.

These observations could open the way to novel applications of such materials as, for instance, immune activating cell chambers for *in vitro* experimental settings. The preparation of biomedical research tools or clinical devices, based on different starches could rely on their biocompatibility and biodegradability, but offering several immune-modulating possibility.

5. Conclusions

We can conclude that some Andean native starches differ in their capacity to induce immune activation of THP-1 monocytes. Furthermore, the nanometric differences of the starch surfaces and the stiffness of the material do not correlate with their immunogenic activity. It is likely that the different Andean native potato starch films have specific ability to interact with cell membranes of immune cells, plausibly due to the diverse spatial localization of amylose and amylopectin in the dissimilar starches. It is intriguing to speculate that differences in the molecular structure of the single starch could interact with the cell membrane proteins and induce a specific signal to the cell. However, the study of starch film-surface interaction with cells, leading to immune activation, is still at the beginning. Although biocompatibility of these materials has been proved, more *in vitro* and *in vivo* research is required before any bio-medical application.

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