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Biocompatibilty of starch-based films from starch of Andean crops for biomedical applications

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ABSTRACT

In this communication we report the use of starch films as cell substrates. To the best of our knowledge it is the first time that films prepared from native Andean starches are studied as biomaterials. For the present study 3T3 fibroblast cells were seeded in seventeen novel starch based films from different Andean crops. In order to analyze the use of these types of starch as biomedical materials, biocompatibility, viability and cell adhesion studies were performed at the third day of incubation on supplemented DMEM medium. After cultured, films made from starch of "tunta", "muro-huayro" potato and white carrot showed the highest level of living cells and cell viability. These results indicate that native starches from Andean crops can be used for biomedical applications.

1. Introduction

Starch is a natural polymer produced in plants. It is formed by two well packed biopolymers, namely amylose and amylopectin [1,2]. Amylose is a linear polymer of glucose units mainly linked with alpha-1,4-bonds. Amylopectin, which is an extremely high molecular weight polymer, has the same backbone structure of amylose, but with many alpha-1,6-linked branch points [1–4]. Starch from corn, potato, sweet potato, cassava and other sources has been extensively used for the production of biodegradable starch based products [5,6], including starch based films for different applications [7–11].

There are several methods for the development of starch based products for biomedical applications. These methods include phase separation [12,13], electrostatic spinning [14,15], fiber meshing [16,17], three-dimensional solid fabrication techniques [18] and injection molding [19–21].

Starch based products have extensively been used for biomedical applications such as wound dressings in the regeneration of skin [22] and as a carrier of drugs and hormones in delivery systems [23–25]. Also, they have been applied in tissue engineering of bone [26–30], cartilage [16] and vascular regeneration [31].

Biocompatibility is an inherent property of structures derived from organic polymers like starch based materials [32]. In general, starch-based materials exhibit attributes of biocompatibility and have been used in several biomedical applications [33–39] due to the presence of biocompatible structural major components as starch polymer molecules and by-products obtained from partial hydrolysis. Properties such as viability, citotoxicity and cell adhesion of starch based materials have been evaluated with cell lines of osteoblasts [26,27], osteosarcoma cells [29] and human and animal fibroblasts [40,41]. However, there are several plant sources of starch that have not been reported as material for biomedical applications.

The aim of this study was to explore the possibility of producing biomedical biodegradable materials from native starch. All previous reports on the use of starch for biomedical applications have used commercial thermoplastic starch. Our previous research on starch bioplastics has shown that both processability and physico-chemical properties are influenced by the type of starch source. By controlling all components used to produce starch based biomaterials, we can more precisely study the biodegradability of these materials.

2. Materials and methods

2.1. Preparation of the starch based films

Starch was extracted from seventeen varieties of native Andean crops. Starch sources including tubers, roots and seeds are listed in Table 1. Starch films were prepared by casting. Dried starch was diluted in distilled water to form a 5% (w/w) starch solution. This solution was partially hydrolyzed in dilute 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 15 min at 95 °C. Then, the solution was neutralized in dilute sodium hydroxide (0.1 N) adjusting 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 200 μm in thickness were obtained.

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Table 1
Andean crops used for starch based film preparation.

Code	Common name	Part of the plant	Scientific designation
ARR01	White carrot	Root	*Arracacia xanthorriza*
CAM01	Sweetpotato	Root	*Ipomoea batatas*
GAR01	Chickpea	Grain	*Cicer arieticum*
KIW01	Amaranth	Grain	*Amaranthus caudatus*
MAI01	Corn	Cereal	*Zea Mays*
MAS01	Mashua	Root	*Tropaeolum tuberosum*
OCA01	Oca	Tuber	*Oxalis tuberosa*
OLL01	Ulluco	Tuber	*Ullucus tuberosus*
POT01	Gold potato	Tuber	*Solanum gonyocalix*
POT02	Huamantanga potato	Tuber	*Solanum tuberosum sbsp. andigena*
POT03	Mariva potato	Tuber	*Solanum tuberosum sbsp. tuberosum*
POT04	Muru-huayro potato	Tuber	*Solanum tuberosum sbsp. tuberosum*
POT05	Peruanita potato	Tuber	*Solanum tuberosum sbsp. tuberosum*
POT06	Yungay potato	Tuber	*Solanum tuberosum sbsp. tuberosum*
TUN01	Tunta	Tuber	*Solanum ajahuiri*
YUC01	Cassava	Root	*Manihot esculenta*

2.2. Preparation of the culture medium and cell resuspension

3T3 fibroblast cells were used for assessing the biocompatibility and viability of the starch based films. Dulbecco's Modified Eagle's Medium (DMEM, 4.5 g/l D-Glucose, Gibco) supplemented with 10% bovine serum (Gibco), 1% penicillin-streptomicyn (P/S) and 0.1% fungizone was used as culture medium. Cells were trypsinized in 1 ml of 0.5% trypsin solution and 5 ml of fresh culture medium was added. The cell suspension was centrifuged at 1500 rpm for 3 min and the supernatant was removed. 8 ml of fresh culture medium was added for cell resuspension. A volume of 1 ml of resuspended cells was seeded in 25 cm^2 cell culture flask (CCF) (Falcon™, Becton & Dickinson) with 7 ml of fresh culture medium. CCF was incubated in 5% CO_2 atmosphere at 37 °C and examined under phase contrast microscopy at the third day for confluence.

2.3. Cell seeding

Previous to cell seeding, starch films were UV sterilized overnight as described elsewhere [42]. Once sterilized, starch based films were placed into the CCF. For the biocompatibility and citotoxicity assays, 1 ml of 3T3 resuspended cells was seeded directly into the starch based films at a density of 1.73×10^4 cell/ml and covered with 6 ml of fresh culture medium. After 3 days of culture, images were taken for analyzing biocompatibility. A CCF without film was used as a control. Cell adhesion was assessed by removing the films from CCF and putting them into new CCF with fresh culture medium for analyzing if cells were adhered to the surfaces. For the viability analysis, cells of the original CCF were trypsinized with 300 µl of 0.5% trypsin solution. Then, 700 µl of fresh culture medium was used to stop the reaction. 100 µl was put into 2 ml Eppendorf tubes with equal volume of trypan blue. Aliquots of these solutions were placed into a Neubauer camera and analyzed by optical microscopy according to the standard Neubauer equation (Eq. (1))

$$\text{Cell Count}(\text{Cell}/\text{ml}) = \frac{\Sigma C/_4 \times 10^5}{2} \quad (1)$$

where C is the amount of viable (or dead) cells counted in each quarter of the Neubauer chamber. The viability of cells was expressed as the percentage of living cells over the total number of cells (living cells and dead cells). The experiments were carried out two times per triplicate.

3. Results and discussions

3.1. Biocompatibility and cell proliferation

In this study, we have used starch extracted from Andean crops that had not been previously reported as raw material for the preparation of cell substrates. The chemical and physical properties of the starches used as well as their thermal properties have been published elsewhere [43]. The diameter of starch granules is around 40 µm for potato starch and around 10 µm for other root sources such as white carrot. The thermal studies performed showed endothermic peaks associated to gelatinization. The specific enthalpies of gelatinization of these Andean starches were in the range 9.0 J/g to 20 J/g. These results are in agreement with results from other starch sources. The amylose content of the starches studied here is around 70% whereas the amylopectin content is around 30%. The films prepared for this study exhibited good biocompatibility in the tests with 3T3 cell line in agreement with other studies [44,45].

Cell confluence in all the flasks containing the starch films in the presence of 3T3 cells and culture medium was attained after 72 h of cell seeding. Also, cells showed similar morphology among the different types of films seeded and the control group (Fig. 1).

The number of living cells found on each material was counted using a Neubauer camera. Fig. 2A shows that the highest number of living cells was counted on the control group whereas films from starch obtained from “peruanita” potato (POT05) and chickpea (GAR01) showed the lowest ability for hosting living cells.

Fig. 2B depicts the viability of cells in terms of the percentage of living cells over the total number of cells (dead and living cells).

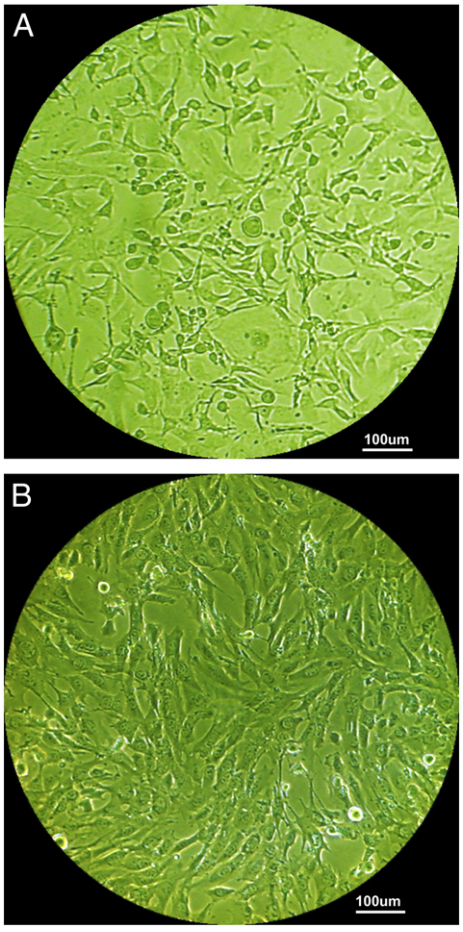

Fig. 1. Proliferation of 3T3 fibroblast cell line after three days in (A) control (CTL) and (B) “muro-huayro potato” (POT04).

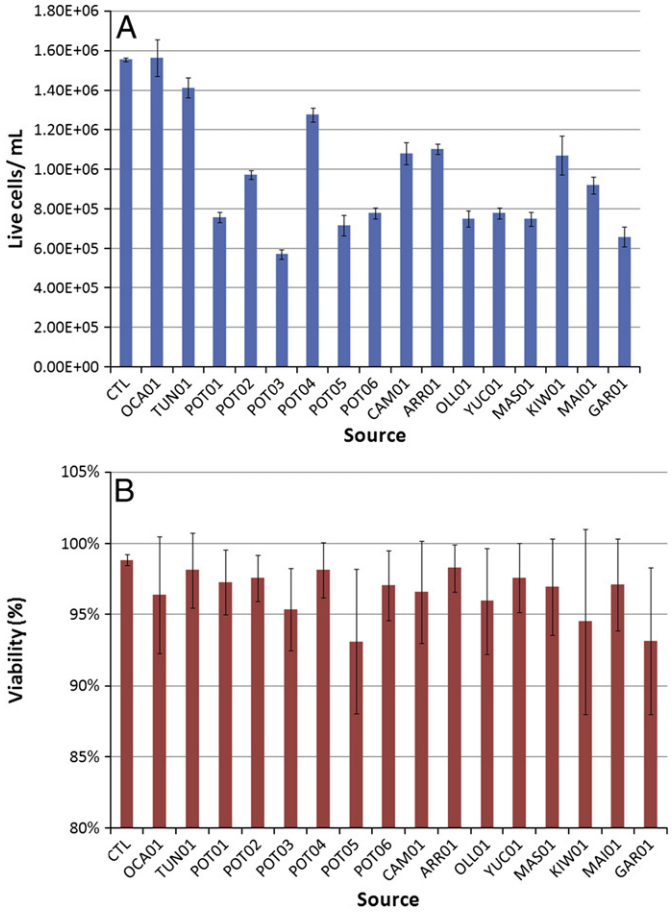

Fig. 2. Response of cells after three days in contact with starch based films. (A) Living cells in the presence of the starch based films and (B) Cell viability.

The films with highest levels of viability were "tunta" (TUN01), "muru-huayro" potato (POT04) and white carrot (ARR01). By contrast, the film with the lowest level of cell viability was made from "Peruanita" potato (POT05). It is worth mentioning that in a previous study from this laboratory POT05 starch was found to be the starch with the highest enthalpy of gelatinization among the starches from Andean crops studied. However, no direct relationship has been established between the enthalpy of gelatinization and the viability of cells yet.

3.2. Cell adhesion

Cells reached total confluence after 3 days. It was after total confluence was reached when cell adhesion was evaluated. The starch-based films were removed from the culture medium and put into new CCF with fresh culture medium. No cells were observed in the new CCF. This confirms that cells were not adhered to the film surfaces, although the samples seemed to have maintained their original dimensions and consistency.

It has been reported that the lack of adhesion between cells and starch-based surfaces could be related to the high level of hydrophilicity of the starch-based films, their topography, chemical properties and the neutral charge of starch [40]. In fact, cell adhesion as well as other functions of cells such as migration and proliferation depends on molecular interactions between cells and the extra cellular matrix (ECM). The ECM is the microenvironment that surrounds the cells and it is formed by a hydrated protein and proteoglycan-based network that provides cell adhesion molecules [46].

It has been shown that some coating reagents can be used to promote cell adhesion on substrates used for cell seeding. The most commonly used coating reagents are positively charged polymers such as poly-L-lysine or biologically purified adhesive molecules such as collagen [47]. Preliminary tests carried out in this laboratory show that polylysine coated starch films enhance cell adhesion response. Further studies in surface modification for improving the adhesion of living cells to the starch based films from Andean crops are necessary for characterizing these materials as potential scaffolds for tissue engineering.

4. Conclusions

Starch based films prepared from 17 different resources of Andean crops have in vitro biocompatibility with 3T3 fibroblast cells. After three days of culture, cells attain total confluence in the flasks containing the films and maintaining their original structure. Results from living cells showed that the films with better response to host cells were those prepared from starch of "muro-huayro" potato, white carrot and "tunta". In spite of the fact that cell adhesion was not achieved, the results showed that the Andean starches used are suitable for producing biocompatible films. As in the case of other biomaterials, cell adhesion on these Andean starch based films can be promoted by adding coating reagents such as polylysine and further studies in this direction are currently being carried out.

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