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Polyurethane sheet impregnated with Arabinogalactan can lead to increase of attachment of promastigotes and Amastigote of *Leishmania major* (MRHO/IR/75/ER) by *GP63* and *HSP70* genes

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ABSTRACT

The aim of this study was to investigate the effect of polyurethane sheet (PUS) and polyurethane sheet impregnated with Arabinogalactan (PUSIAG) on the cell attachment and viability of Promastigotes and Amastigotes of *Leishmania major* (MRHO/IR/75/ER), and mouse macrophages, and its whole skin cells (WSCs). In a sterile condition, 10 mL of Arabinogalactan 5% *w/v* was poured into a falcon. Then, a piece of PUS was placed inside it, and incubated at 37 °C for 24 h. Next, it was washed, and cut. Then, one piece of PUS and PUSIAG was separately added to 1 mL of cell suspension (Promastigotes, Amastigote, and WSCs), and then incubated for 1, 2, 3, and 4 days at 37 °C. After incubation times, the quantity of adhered cells was counted, and cell viability was measured by MTT assay. Also, for WSCs and macrophages, the expression of *integrin, fibronectin* and *GAPDH* was investigated, and for Promastigotes and Amastigotes, the expression of *GP63*, *Cpb*, and *18s rRNA* was measured. This study showed that with increase of exposure time, the percentage of attached cells was increased. There was a significant difference between attached cells to PUSIAG and PUS in case of Promastigotes and Amastigotes. It seems that Promastigotes and Amastigotes have higher interest to PUSIAG than WSCs and Macrophages. Also, this study showed with increase of exposure time, the percentage of viable cells was decreased. There were significant differences between cell viability of Promastigotes and Amastigotes when exposed to PUSIAG and PUS, especially in long time incubation. Also, when incubation time was increased the relative expression of *integrin* and *fibronectin* in WSCs and macrophages, and *GP63* and *HSP70* in Promastigotes and Amastigotes were increased.

1. Introduction

Leishmaniasis refers to a wide range of conditions caused by the intracellular flagellate protozoan transmitted by sand fly. It has a worldwide distribution observed in > 90 countries. Cutaneous Leishmaniasis (CL) is rendered as a dominant public health problem and induces a wide spectrum of disorders from self-healing infections to several chronic diseases. Currently, there is no effective vaccine and pharmaceutical treatment available for CL. However, no new strategies have been introduced for controlling CL. Of course, some new approaches for manufacturing vaccines which contribute to the prevention of CL and immunotherapy are under way due to increased knowledge concerning the cells involved in its etiology. [1]. A few weeks after the incidence of CL ulcer, the secondary bacterial and fungal infections are superimposed on it. In the course of time, bacteria and fungi replace the parasite Amastigote in the Leishmaniasis ulcer [2]. Cysteine Peptidases play a pivotal role in the regulation of GP63 gene expression and both GP63 and CP are key factors in the virulence of CL [3]. GP63 is a useful metalloprotease which can rapidly regulate the cellular signaling pathways and hosts predisposed to their challenge [4]. GP63 is one of the most prominent superficial proteases of which > 500,000 transcriptions (replication) (% 0.5-%1 of the total cellular protein content) are expressed. Since its significant role in the innate resistance of macrophages infected with the parasite has been demonstrated, it is rendered a suitable candidate for the development of anti-Leishmaniasis vaccine [5].

Polyurethane is composed of organic units joined by urethane links. Polyurethane are traditionally formed by reacting of di- or poly-isocyanate and polyol. Mostly, this polymer has thermosetting property that does not melt when heated. Because of high chemical and physical

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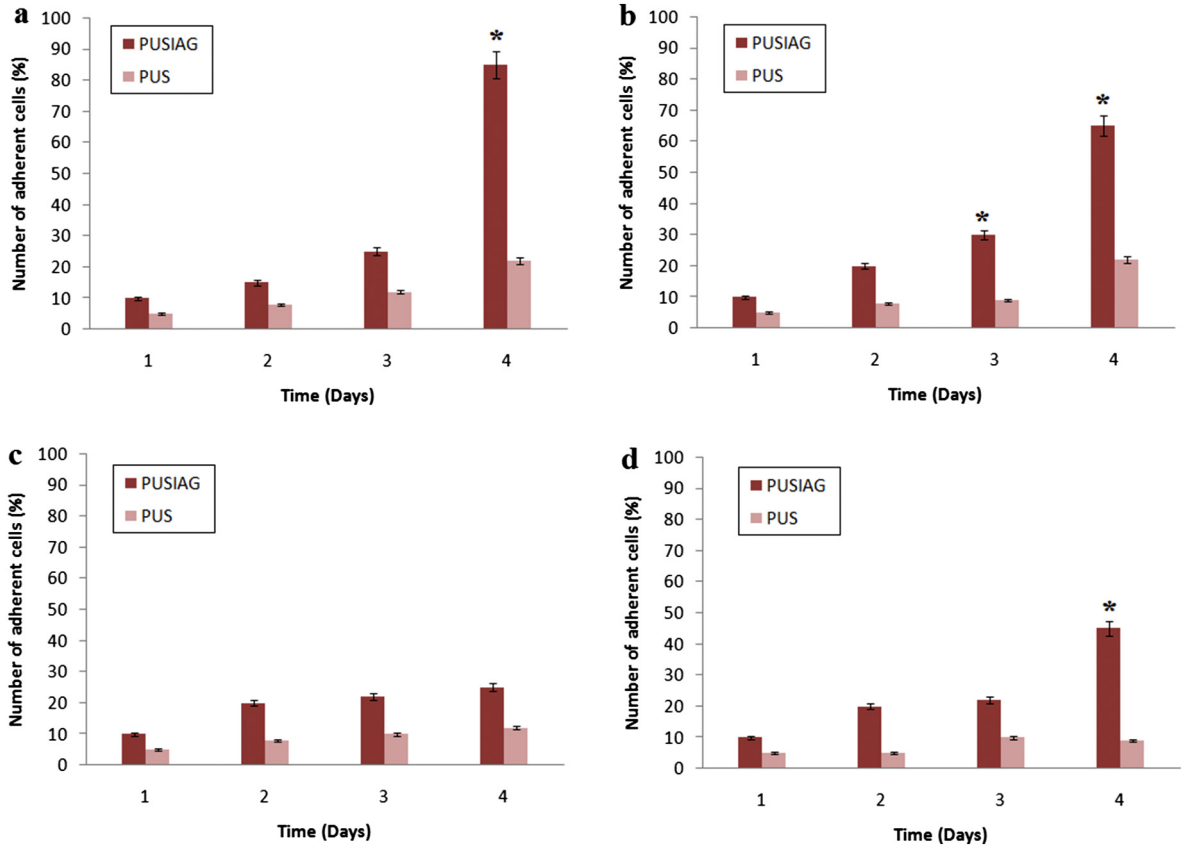

Fig. 1. The percentage of attached Amastigotes (a), Promastigotes (b), WSCs (c), and Macrophages (d) when exposed to PUSIAG and PUS. * $P < 0.05$ compared with PUS at same day, $n = 3$.

properties of polyurethane, it is used in the manufacture for different applications, e.g., high-resilience foam seating, microcellular foam seals, durable elastomeric wheels, etc. Moreover, polyurethane can be used in medical applications such as the artificial heart, intra-aortic balloons, pacemaker leads, heart valves, and hemodialysis membranes [6].

The aim of this study was to investigate the effect of polyurethane sheet (PUS) and polyurethane sheet impregnated with Arabinogalactan (PUSIAG) on the cell attachment and viability of Promastigotes, Amastigotes, Macrophages, and skin cells.

2. Methods

2.1. Materials

Arabinogalactan, polyurethane, NNN medium, RPMI1640, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), isopropanol, chloroform were purchased from Sigma-Aldrich Chemical Co, (St Louis, MO, USA). RNA extraction buffer (RiboX) and cDNA mastermix were purchased from GeneAll Company, South Korea. The real-time Mastermix was provided by Applied Biosystems Company, USA. Also, all primers were sourced from Takapoo Zist Company, Iran.

2.2. Preparation of PUSIAG

Five mL of Arabinogalactan 5% w/v was poured into a falcon containing 10 pieces of PUS (1 cm × 1 cm). Then, they were incubated at 37 °C for 24 h. Next, they were washed by distilled water to remove excess Arabinogalactan. After washing, all were kept in a sterile condition at 4 °C.

2.3. Preparation of Promastigotes of L. major

The standard isolates of *L. major* (MRHO/IR/75/ER) was sourced from Shahid Sadoughi University of Medical Sciences, Yazd, Iran. First, it was incubated in NNN medium enriched by RPMI1640 for 48 h at 37°C. After incubation, it was centrifuged at 3000 rpm, and washed by cold RPMI1640. Finally, the density of Promastigotes was adjusted to 2×10^5/mL.

2.4. Preparation of Amastigotes of L. major

Two mL of *L. major* Promastigotes (2×10^5/mL) were injected to peritoneum of male BALB/c mouse. After 5 days, 3 mL of RPMI1640 was injected to its peritoneum, and then aspirated. Preformed cell suspension was three times aspirated in sterile tube. Next, all were aseptically washed by RPMI1640, and then its density was adjusted to 2×10^5/mL.

2.5. Preparation of mouse peritoneal Macrophages

First, cold RPMI1640 was injected to peritoneum of male BALB/c mouse. Then, the peritoneal fluid was aspirated, and centrifuged at 3000 rpm for 10 min. Then, Macrophages were suspended in RPMI1640, and finally adjusted to 2×10^5/mL.

2.6. Preparation of whole skin cells (WSCs) of mouse

Ten male BALB/c mice with weight of 18–20 g were anesthetized, and then their dorsal skin was biopsied (a piece of 2 × 2 cm) and rinsed by normal saline. One mL of trypsin enzyme (0.025 mM) was added to each skin piece, and incubated at 37 °C for 5 min with mild agitation. After enzymatic digestion, all skin pieces were crushed with a mortar