

Acellular Human Amniotic Membrane Scaffold Loaded with Nanoparticles Containing 15d-PGJ2: A New System Local Anti-Inflammatory Treatment of Eye Diseases

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Abstract

The pathogenesis of chronic inflammatory eye diseases is multifactorial and includes factors as tissue injuries, metabolic disorder and autoimmune diseases. The 15-deoxy- Δ 12, 14-PG J2 is known for its anti-inflammatory, antioxidant and immunomodulatory properties. *In vivo* adhesions between cells and the extracellular matrix play a crucial role in cell differentiation, proliferation, and migration as well as tissue remodeling. Here, we present a simple method to incorporate 15d-PGJ2 nanoparticles in acellular human amniotic membrane (HAM) scaffold, as potential local anti-inflammatory delivery system. After completely removing the cells on the amniotic membrane with a sodium dodecyl sulphate and mechanical approach, we seeded Vero cells incorporate 15d-PGJ2 nanoparticles on it. The morphology of the Vero cells and nanoparticles was observed by scanning electron microscopy (SEM). The cells cultivated observed by scanning electron microscopy (SEM) presented the incorporation of the nanoparticles smooth surface and spherical shape. Our results indicate that the HAM may be an ideal candidate as a nanoparticle-matrix adhesion substrate to study a new system for local anti-inflammatory therapy.

Keywords: Human amniotic membrane, Nanoparticles, 15d-PGJ2, Eye diseases

Introduction

The incidence of chronic inflammatory diseases (CID) has increased worldwide in the past few decades, threatening human health. The inflammatory process is a key component of chronic and acute diseases of the eye. The pathogenesis of CID is multifactorial and includes factors as tissue injuries, metabolic disorders and autoimmune diseases [1].

Among the already identified anti-inflammatory prostaglandins (PG), the 15-deoxy- Δ 12,14-PG J2 (15d-PGJ2) has recently been described as an anti-inflammatory molecule due to its protective activity in a variety of inflammatory mediated diseases, including rheumatoid arthritis, neural damage, and myocardial infarction [2]. The nanotechnology is an emerging field that is changing the diseases treatment methods through new nanoparticles delivery systems [3]. Furthermore, novel treatments models based on PG, have been increased the interest for new biomaterials in the field of nanotechnology, drug delivery systems, and regenerative medicine [4].

Current advances in biotechnology and related areas are aiding the discovery of many new scaffolds, in which it is crucial to improve specific drug delivery approaches [5] Human amniotic membrane (HAM), a biocompatible material that has been extensively investigated in several reconstructive medical areas, shows great potential for drug delivery [6]. In this study, we aimed to incorporate 15-deoxy- Δ 12, 14-PG J2 nanoparticles in acellular human amniotic membrane scaffold as a potential local anti-inflammatory delivery system.

Methods

Preparation of acellular human amniotic scaffold (AHAS)

The study was approved by the Hospital Pequeno Príncipe Ethical Committee for the usage of biological material for research purposes. All materials were used in compliance with ethical guidelines by the Brazilian National Health Council. 0948-11. HAM was obtained with informed consent from mothers before delivery. In brief, the human placenta was obtained immediately after delivery with negative serologic tests for human immunodeficiency virus, human hepatitis type B and C, and syphilis. The acellular human amniotic scaffold (AHAS) was prepared as described by Riaux et al. [7].

Nanoencapsulation of (15d-PGJ2-NC) and culture and seeding of VERO cells

The 15d-PGJ2-NC were prepared by the nanoprecipitation method, as described by Fessi et al. and supplied by Dr. Napimoga from Laboratory of Immunology and Molecular Biology, São Leopoldo Mandic Institute and Research Center [8,9]. Vero cells (ATCC[®] CCL-81[™]) were prepared as previously described [10]. The Vero cells were seeded on plastic plate with 15d-PGJ2-NC (at 1 μ M concentration) for 24 h in order to incorporate the nanoparticles to the AHAS.

Scanning electron microscope (SEM)

The morphology and structure of the acellular human amniotic scaffold (AHAS) with 15d-PGJ2-NC (AHAS-NC) were examined in a JEOL 1200EX II microscope (Jeol Ltda, Akishima) operating at 80 kV. In order to perform the SEM analysis, the AHAS-NC was fixed on top

coverslip, dried, mounted on a stub for SEM, fixed in 2.5% (v/v) glutaraldehyde (Sigma-Aldrich) in PBS and post-fixed with 1% (v/v) and 0.1 M sodium cacodylate trihydrate (Sigma-Aldrich).

Results

SEM images of AHAS-NC showed a typical aggregate composed of many smaller nanoparticles; these aggregates could be defined as

nano-complexes of 15d-PGJ2 (at 1 μ M concentration). The cellular adhesion of the 15d-PGJ2 with scaffold, however, that may occur during a study period of 72 hours, which indicating its good compatibility with HAM (Figure 1).

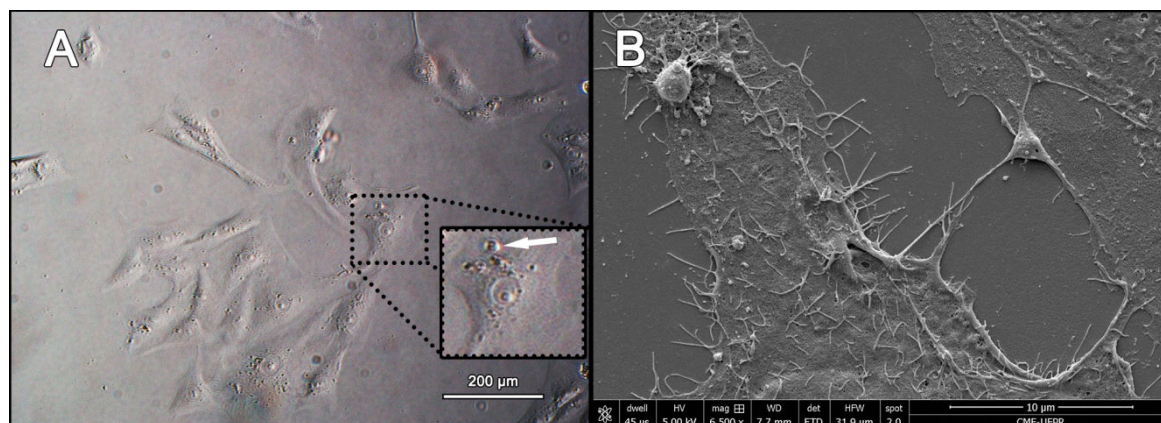


Figure 1: Phase image of Vero cell cultured with 15d-PGJ2-NC (arrow), (B) Scanning electron microscopic image of Vero cell cultivated with nanoparticles. The presence of cellular adhesion on the surface of the human amniotic membrane with 15d-PGJ2-NC.

Discussion

The main objective of this study was to incorporate 15-deoxy- Δ 12,14-PG J2 nanoparticles in acellular human amniotic membrane scaffold as a potential local anti-inflammatory delivery system. This '*in vivo* assay is' based on the fact that the (15d-PGJ2) contributes to its anti-inflammatory activity at micromolar concentrations and the HAM has many characteristics that are desirable for a biomaterial [9-12].

The results showed that there was cellular interaction with the acellular human amniotic scaffold surface were clearly observed on Scanning image. In the presence of Vero cells with PG J2 nanoparticles the cells could stretch and were able to adhere to the scaffold (Figure 1).

The use of nanotechnology in delivery system has received considerable attention the past decade. Nanoparticles have a wide range of pharmaceutical applications since their physical and chemical characteristics, for example, shape, surface charge and hydrophobicity, can be adjusted accordingly to their target [10]. Recent studies have shown the use of polymer based nanoparticles in the reformulation of 15-deoxy- Δ 12,14-PG J2 nanoparticles for ophthalmic and colonic us with promising results [13].

Acellular amniotic membrane human scaffolds have recently become the focus of interest mainly due to the possible beneficial and applications in regenerative medicine [5,14,15]. Tissue engineering using acellular scaffolds has introduced a new field of repair in the treatment of wounds tissues or diseases, with special focus in ophthalmology [16].

Amniotic membrane has played an important role in ocular surface reconstruction for decades [14]. A number of different types of human cells have been cultured using acellular human amniotic membrane as

a substrate [17]. *In vitro* culture of VERO cells which have led to the microenvironment for incorporation 15d-PGJ2 nanoparticles in acellular human amniotic membrane scaffold. In summary, we demonstrate that acellular human amniotic membrane loaded with nanoparticles containing 15d-PGJ2 is a biomaterial with acceptable biocompatibility for local delivery applications anti-inflammatory.

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