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Multifunctional implantable particles for skin tissue regeneration: Preparation, characterization, in vitro and in vivo studies

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Abstract

The transplantation of cell-polymer constructs has been developed as a novel approach to curing tissue defects. However, a number of methodological problems remain to be solved, including the loss of a proper cellular milieu, the relatively long period of culture time and the complexity of the application. The aim of the present article is to evaluate the feasibility of porous gelatin-based implantable particles as a novel strategy for delivery of cultured cells and bioactive molecules to correct dermal defects. For this purpose, implantable porous gelatin particles (100–230 μm) encapsulating proliferative growth factors were prepared and characterized, and their influence on fibroblasts was assessed. In vivo examinations were undertaken to observe guided dermal tissue regeneration after the transplantation of the implantable particles. Our results indicate the feasibility of transplanting multifunctional implantable particles as a culture substrate, as a protein transplantation vehicle or as a biodegradable implant for skin regeneration, thus giving an indication of the possible applications in tissue engineering.

Keywords: Implantable particles; Basic fibroblast growth factor (bFGF); Delivery; Fibroblast; Tissue regeneration

1. Introduction

Current treatments for burns, chronic ulcers, pressure ulcers and reconstructive surgery include tissue transfer from a healthy site in the same or another individual, the use of medical devices to support the function of the lost tissue, and pharmacologic supplementation of the metabolic products of the lost tissue. Problems with these treatments include the limited number of tissue donors and potential tissue complications, such as imperfect matches and dependence on immunosuppressant, and may have a functional impact (or usually an aesthetic impact) on the patient. To address these problems, grafting autologous or non-autologous cells is a promising strategy to repair diseased organs. In this regard, cell-transplantation approaches for tissue repair, notably transplantation of cultured autologous cells with polymer, have produced promising results [1]. However, for all these approaches, the short- but also long-term survival and functional state of the cell implants still need to be improved [2].

The major drawback of these polymers is that they are not completely functional and are weak relative to native compounds. For this reason, considerable efforts have made to develop matrices as transplant vehicles [3]. Some reports have suggested that cytodexs or glass microcarriers

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enhance the survival of rat adrenal chromaffin cells, rat and human fetal mesencephalic cells and human retinal pigmental epithelial cells grafted in the striatum of hemiparkinsonian rats [4,5]. Moreover, some researchers demonstrated that PLGA, which is a commercially available product currently used for human application, could be employed as a cell scaffold [6]. Gelatin is the derivative of collagen and has been proved to possess a similar chemical structure to glycosaminoglycan and collagen. It has been developed and used as the supporting scaffolds to mimic the natural ECM in skin tissue-engineering [7–9]. In this study, we utilized gelatin to form the biocompatible and biodegradable particles, which act as cell carriers for transplantation. Implantable porous gelatin particles have several attractive characteristics for cell-transplantation, tissue-engineering and reconstructive surgery. They are easily manufactured and relatively cheap, and allow a large number of cells to be cultured on them [10,11].

Growth factors may improve survival and differentiation of the cells, and may also affect the immediate environment, thus allowing better graft integration. Nevertheless, the administration of these factors still remains a technological challenge, due to their short half-life. To overcome these difficulties, some groups have developed a site-specific delivery approach, using implantation of biodegradable microparticles and allowing a controlled and sustained release of a growth factor [12,13]. Additionally there are many cases where gelatin-based vehicle has proved to be highly beneficial to controlled delivery growth factors [14–16]. It is thus of interest to associate these two strategies – drug delivery vehicle and cell-transplantation vehicle – to improve graft integration. In the present study, basic fibroblast growth factor (bFGF) is applied as it is a multifunctional protein that promotes angiogenesis and regulates many aspects of cellular activity.

Additional drawbacks of reconstituted cell-seeded constructs are the need for reseeding the retrieved cells into a scaffold delivery system and not having a sufficient number of cells with the appropriate phenotype for tissue repair in the short term [17]. The result can also be discouraging as the cells must be harvested with trypsin prior to transplantation in this procedure. It is well known that trypsinization damages cells, e.g., by degrading anchor proteins. It has been suggested that this damage results in mechanical instability of the sheet grafts and insufficient dermal–epidermal reconstitution [18]. In the present study, we aimed to prepare multifunctional implantable particles to act as scaffolds for guided tissue regeneration, as this appears to be a simple and rapid strategy to repair dermal tissue defects. It was envisaged that a small number of autologous cells isolated from the patient would be cultivated to a sufficient cell density and seeded within biodegradable vehicles, the resultant structurally and mechanically functional cell-vehicle construct then being implanted into the defect site. By this method, cells cultured on the implantable particles could be subsequently transported and transplanted without being detached from the matrix. In this fashion, trypsinization could be eliminated and the cells could be transported to grow in a more natural state.

By combining these objectives, multifunctional implantable particles would play a triple role in this study. As the culture substrate, it offers the advantage of providing a large surface area for cell growth during propagation. The porous structure also facilitates more efficient gas–liquid oxygen transfer and the maintenance of the crucial physical, biological and chemical milieu. As a beneficial drug delivery vehicle, bFGF can be introduced to promote cell growth onto polymer surfaces in cell culture and induce cell ingrowth and vascularization during the initial stages of wound healing. A more important factor is that biodegradable implantable particles can be delivered directly to the site that needs repair, thus eliminating the trypsinization and reseeding process. Due to their unique beneficial effects for tissue-engineering applications, it could be hypothesized that multifunctional implantable particles might substantially improve skin regeneration.

2. Materials and methods

2.1. Materials

Gelatin (type A) was obtained from Sigma (USA), as were all other reagents and chemicals, which were of analytical grade. All were used without any further treatment or purification. Double-distilled water was used for all the experiments.

2.2. Preparation of bFGF-incorporated porous particles

The particles with porous structures were prepared by freeze-drying. A typical preparation is as follows: 2 ml of 10 wt.% gelatin aqueous solution was added dropwise into 30 ml of paraffin oil while the mixture was mechanically stirred at 800 rpm to form a water-in-oil emulsion. The solution was then rapidly cooled by immersing in ice-water. The formed gelatin particles were filtered, washed with acetone and dried at room temperature. The non-cross-linked particles prepared were then placed in a 2 wt.% glutaraldehyde aqueous solution containing 0.1 wt.% Tween 80 and the cross-linking was allowed to proceed at 4 °C for 12 h. After cross-linking, the particles were placed in 100 ml of 10 mM glycine aqueous solution for 30 min to block any residual unreacted glutaraldehyde. The sample was swollen by adding double-distilled water and, after being quenched in liquid nitrogen, was freeze-dried using a CHRIST ALPHA2-4 (Germany) freeze-drying instrument at −55 °C for 12 h. Gelatin particles with porous structures were finally obtained. In order to measure the surface pore size of particles, five samples were analyzed using Image J software. The total number of the surface pores was 100. Because the pore size of particles had a binary distribution, small size surface pores (below 5 μm) were ignored. Thus, particles with a diameter of 100–230 μm were separated by standard sieves and used for the following studies.

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