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CASE STUDY

SKIN REGENERATION AFTER SCALP TRAUMA THROUGH AUTOLOGOUS TRANSPLANT OF ACELLULAR DERMIS: A MEXICAN CASE STUDY

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ABSTRACT

Human acellular dermis and human stem cells have been used in clinical applications, including wound healing, soft tissue reconstruction, and sports medicine procedures. A variety of methods exist to prepare these useful types of biomaterials. However, in Mexico, their use remains an unconventional therapy. This article illustrates a case study in the management of a wound with cranial soft-tissue loss (scalp tissue) through the use of an autologous transplant of acellular dermis and mesenchymal cells. We suggest that based on this case study, human acellular dermis may be a useful scaffold for the healing, reconstruction, and growth of soft tissues.

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INTRODUCTION

In current international research, there have been a number of achievements in a variety of biomaterial applications, including the use of an acellular dermal matrix (ADM) obtained from fragments of full-thickness skin whose cells are removed but whose native dermal structures are preserved. This technique has received attention in many fields of research[1-4].

Recent studies have presented different methodologies for producing an ADM from pig skin and from the submucosa of the intestine[5]. Walter *et al* have documented that methodologies to produce ADMs using trypsin treatments, cycles of freezing and thawing, and prolonged incubation with enzymes generated highly antigenic biomaterials when implanted in recipients, in whom the graft induced immunological reactions, resulting in low survival rates of the graft[6]. Two methodologies of more controlled extraction have been reported to produce ADMs with lower antigenicity and excellent stability while also retaining their native dermal structures. One method uses hypertonic solutions of sodium chloride (NaCl) followed by sodium dodecyl sulfate (SDS) and

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freeze-drying[1,7], while the other uses serial treatments with dispase followed by Triton X-100[8,9].

Walter *et al* compared these two methods and concluded that both methodologies result in ADMs with extensive extraction of cells and cellular components while still retaining the basic skin structure. Lee *et al* also published an alternative method for producing ADM from pig skin[10]. This excellent aseptic method for preparing ADM required pieces of skin with 0.03-cm^3 total thickness to be combined with 0.25% trypsin at 4ºC for 24 hours, followed by serial treatments with 0.1% Triton X-100 for 8 hours at room temperature, dispase (500 U/L) at 4ºC for another 24 hours, and finally a thorough washing in ultrapure water.

Recently, scaffolds derived from xenogenic acellular matrix have been shown to be effective in the repair and reconstruction of certain parts of the body, including the urinary tract, the dura mater, the esophagus, muscle tendons, and blood vessels[11,12]. The primary characteristic of these scaffolds is their ability to induce a host cellular response of reconstruction or remodeling, as opposed to one of scart issue formation.

Mesenchymal stem cells were initially discovered in bone marrow by Friendenstein[13]; however, they can be found in other tissues. In vitro, these cells have demonstrated the ability to differentiate into multiple cellular lineages, including chondrocytes, adipocytes, myoblasts, and osteoblasts[14,15]. In recent years, mesenchymal stem cells have been used in orthopedic clinics for the treatment of various pathologies, such as cartilage reconstruction and extensive bone loss, in plastic surgery to re-epithelialize ulcers, in cardiology for the management of dilated and ischemic cardiomyopathies, in rheumatology for the management of autoimmune diseases, and in hematology for the management of graft-versus-host disease[16-27].

In the present study, a case study, in Mexico, of managing a lesion with cranial soft-tissue loss (scalp) is presented. This paper describes the process of an autologous transplant of acellular dermis (Fig 1) and mesenchymal stem cells (Fig 2), from harvesting the autologous dermal material and mesenchymal stem cells from bone marrow, to the decellularization treatment of the autologous matrix[13], and finally to the growth of the mesenchymal cells. In sum, this paper describes the application of the therapy, following the clinical course of the patient (Fig. 7).

MATERIALS AND METHODS

Patient clinical data

A 54-year-old male with no known pathologies was a victim of a hit-and-run on a public street, with soft-tissue loss exposing cranial bone tissue. Initial wound-care management included debridement, followed by proposed management with autologous acellular dermis and autologous mesenchymal stem cells[28], which the patient accepted, signing an informed consent (Fig. 1).

Bone marrow harvesting for isolation and growth of mesenchymal stem cells

The day of surgical wound debridement, 60 mL of bone marrow was extracted from the iliac crest by aspiration with a needle and syringe containing citrate in a concentration of 1 mL per 10 mL of marrow blood. The sample was taken to the lab, where mononuclear cells were obtained using a density-gradient method (Histopaque, 1077 Sigma), seeded in 25-cm^2 flasks (430168 Corning) at a concentration of 10,000 cells per cm^2 in a mesenchymal-cell-culture medium[28] (Mesencult 04459, Stem Cell Technologies), and placed in an incubator with 5% CO_2 and 95% humidity for 72 hours.When the medium was changed, only adherent cells were left behind. Four more passages were performed to reach the required dose, verifying the cellular morphology at each passage (Fig 2). Finally, flow cytometry was used to verify the cellular identity (Fig 3).

Flow cytometry

After their growth, mesenchymal stem cells were processed via flow cytometry in a BD FACSCANTO (Beckman Coulter). Briefly, the markers used were CD105-PE Cy5 (BD Pharmigen 560839), CD90-FITC (BD Pharmigen 555595), CD73-PE (BD Pharmigen 550257), and CD44-PE Cy7(BD Pharmigen 555479;Fig. 3).

ADM (Acellular Dermal Matrix)

The same day that bone marrow was obtained, a skin biopsy was also performed via surgical removal of an area of approximately 10 cm^3 from the left thigh (Fig. 1). The skin was then processed in the lab as described below. The first step was to clean the skin of its subcutaneous adipose tissue, washing it thoroughly, and freezing it at -20ºC. Once treatment was to begin, the skin sample was placed at 4ºC and divided into two parts.

The treatment performed is that described in 2002 by Takamiand collaborators[12]. Briefly, it consists of the following: 1) Freezing and thawing in 1 M NaCl with phosphate buffer(PBS) for 48 hours at -20ºC, followed by 30 min at room temperature. 2) Freezing and thawing in 1 M NaCl with 0.5% Triton X-100 (v/v) for 24 hours at 22-24ºC. 3) Freezing and thawing in 1 M NaCl with 0.1% SDS at 25ºC for 12 hours. 4) Freezing and thawing with trypsin in PBS at 25ºC for 24 hours. 5) Freezing and thawing with trypsin plus Triton X-100 at 25ºC for 12 hours and at 37ºC for 7 hours.

To confirm decellularization and dermal authenticity, a specimen was examined via light microscopy and electron microscopy (Fig 4, Fig 5). The ADM was frozen at -70ºC until use. By the fourth step in processing the mesenchymal stem cells, the acellular dermis had been cultured for 72 hours with these same cells. The ADM was implanted in the patient after being washed with PBS and resuspended in autologous-platelet-rich plasma.

Implantation of the acellular dermis co-cultured with mesenchymal stem cells

The patient was scheduled for wound debridement as had been previously performed. The wound was debrided, and the acellular dermis co-cultured with mesenchymal cells was affixed with simple Vicryl sutures.

RESULTS

Clinical data and cutaneous biopsy

Mesenchymal stem cells harvesting and a cutaneous biopsy were performed on the patient (Fig. 1). The cutaneous biopsy was performed as previously described, and the sample was characterized as acellular dermis. Light and electron microscopy were used to confirm cell removal. For light microscopy, the samples were fixed in 10% formaldehyde, immediately embedded in paraffin, and then cut into 5-μm sections. The sections were stained with hematoxylin and eosin (Fig. 4). Electron microscopy performed at the National Autonomous University of Mexico in its cell and tissue biology lab revealed that the sample contained only collagen fibers with their characteristic three-dimensional structure (Fig. 5).

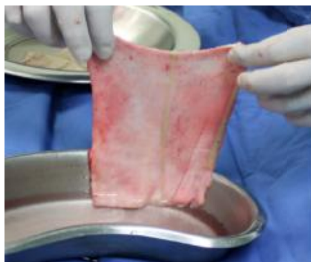

Figure 1 Biopsy of human skin, obtained by quirurgical extraction of one area of 10 cm^3.

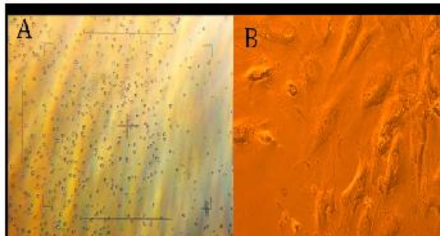

Figure 2 A culture *in vitro* of bone marrow of 4 days and; B mesenchymal stem cells *in vitro* with fibroblast morphology and 4 pass of expansion.

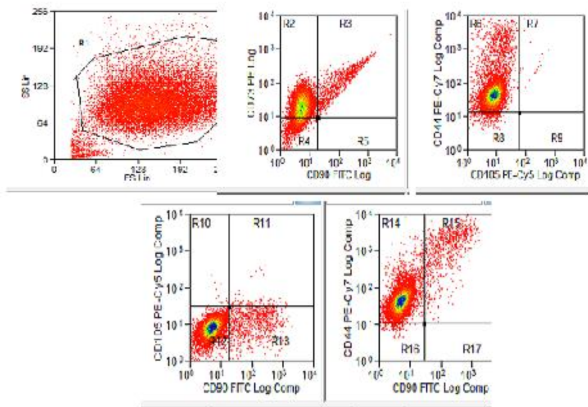

Figure 3 Flux cytometry of mesenchymal stem cells, show markers CD73, CD90, CD44, CD105. They markers all positives a mesenchymal stem cells.[28]

Analysis of residual DNA

Genetic material that may have been present in the acellular dermis was quantified. The DNA content was quantified with a Quant-iTTM PicoGreen dsDNA kit (Life Technologies, Inc., Carlsbad, CA), and the ng/mg-tissue was calculated, using five sections from the donor sample. Comparing the processed and unprocessed dermis, a minimum of 97% of the DNA content was removed after the decellularization process. The DNA content was quantified in the ADM (12.37 ± 0.5 ng/mg ww). A t test was performed to establish significance (Fig. 6).

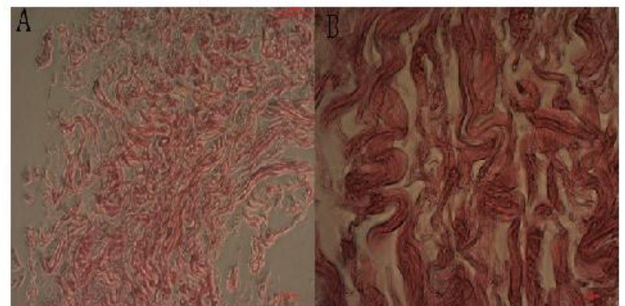

Figure. 4 Histological analysis overview. The ADM process is designed to remove cellular remnants from tissue. Evidence of decellularization by histological analysis is demonstrated by the absence of cell nuclei and cell membrane components. In A) show the fibers of collagen (200um); and B) show the fibers of collagen (50 um). Not evidence of cells.

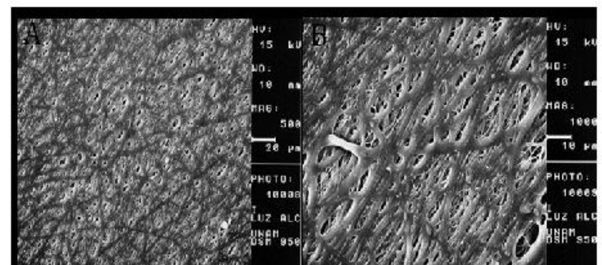

Figure 5 Scanning electron microscope images of human acellular dermal matrix (**A, B**), Then acellular dermal matrix possesses a three-dimensional porous structure with channel diameter of about 50–100 μm. Not evidence of cells.

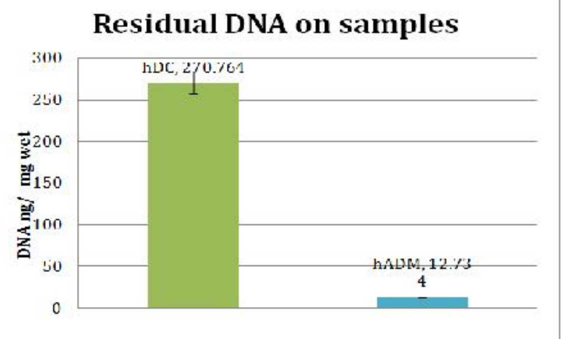

Figure. 6 Comparison of residual DNA content in five pieces of cut acellular dermal matrix (ADM) vs five pieces of human dermal cells (hDC). All DNA content results are presented as ng/mg of wet weight of material. We show that are the significative differences.

Before patient intervention was performed, the integrity of the invitro mesenchymalstem cells was verified through flow cytometry (Fig 3), as previously described regarding isolation and growth (Fig. 2).

Finally, the acellular dermis co-cultured with mesenchymal stem cells was affixed to the patient, which was then covered

with petrolatum gauze and bandages. After one week, the wound was uncovered, and tissue integration and vascularization were observed. Weekly follow-ups were performed, and after one month, nearly the entire wound had epithelialized. Likewise, the patient's donor area was evaluated to ensure its progress (Fig. 7).

Figure 7 Clinical evaluation and donor site 15 days after. In A, placing the acellular dermal matrix and B, vascularization created by the added mesenchymal stem cells is observed. In C, the wound bed is shown. In D, closing the injury and we observed growth hair in some areas. In E, the site of the autologous donor tissue is observed.

DISCUSSION

Extensive soft-tissue injuries are common wounds in trauma and are typically managed with debridement, flaps, and various surgeries, all of which represent a significant expenditure to the health system and to the population using these services. Regrettably, individuals who suffer these injuries are often of a social stratum with poor access to healthcare services, which impedes care and can occasionally result in complications. Similarly, soft-tissue injuries, such as metabolic, traumatic, and vascular ulcers, are frequent ailments in our population, mainly due to diabetes. In 2011, the Mexican healthcare system spent US$20,426,025 on peripheral vascular disease secondary to diabetes alone, one of the principal causes of temporary and permanent disabilities, on which the system also spent US$16,224,794 and US$4,092,058,480, respectively (US$1 = 19.15 Mexican pesos)[29].

These ailments mainly affect the working-age population, creating a burden on society and the healthcare system. The primary problem with these injuries is compromised microvasculature that complicates wound re-epithelialization[30]. In recent years, the use of mesenchymal stem cells has been shown to promote revascularization and epithelialization in various tissues and wounds, which has made them a therapeutic option for a variety of injuries, including those of soft tissues[30,31].Concurrently, the use of scaffolds has furthered the therapeutic effects of different cell sources, including mesenchymal stem cells; these scaffolds have been shown to improve recovery from various injuries *in vitro* and in vivo, both with and without cells[30-32].This type of technology is widely established throughout the world and has been seen to be cost-effective in caring for patients with complications or where current therapeutic modalities are not having desired results[33].Thus, this patient's case study demonstrates the potential of implementing these technologies effectively, safely, and efficiently for both healthcare systems and patients, returning patients to work as quickly as possible and alleviating financial burdens on healthcare systems.

CONCLUSIONS

In conclusion, we report that the use of ADM for the treatment of scalp wounds produces optimal results due to the matrix serving as a bridge to achieve wound healing. We propose further evaluations of the use of this methodology as a clinical tool in medical protocols in Mexico.

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