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Surgical meshes coated with mesenchymal stem cells provide an anti-inflammatory environment by a M2 macrophage polarization

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ABSTRACT

Surgical meshes are widely used in clinics to reinforce soft tissue's defects, and to give support to prolapsed organs. However, the implantation of surgical meshes is commonly related with an inflammatory response being difficult to eradicate without removing the mesh. Here we hypothesize that the combined use of surgical meshes and mesenchymal stem cells (MSCs) could be a useful tool to reduce the inflammatory reaction secondary to mesh implantation.

In vitro determinations of viability, metabolic activity and immunomodulation assays were performed on MSCs-coated meshes. Magnetic resonance imaging, evaluation by laparoscopic optical system and histology were performed for safety assessment. Finally, flow cytometry and qRT-PCR were used to elucidate the mechanism of action of MSCs-coated meshes.

Our results demonstrate the feasibility to obtain MSCs-coated surgical meshes and their cryopreservability to be used as an 'off the shelf' product. These biological meshes fulfill the safety aspects as non-adverse effects were observed when compared to controls. Moreover, both *in vitro* and *in vivo* studies demonstrated that, local immunomodulation of implanted meshes is mediated by a macrophage polarization towards an anti-inflammatory phenotype.

In conclusion, the combined usage of surgical meshes with MSCs fulfills the safety requirements for a future clinical application, providing an anti-inflammatory environment that could reduce the inflammatory processes commonly observed after surgical mesh implantation.

Statement of Significance

Surgical meshes are medical devices widely used in clinics to resolve hernias and organs' prolapses, among other disorders. However, the implantation of surgical meshes is commonly related with an inflammatory response being difficult to eradicate without removing the mesh, causing pain and discomfort in the patient. Previously, the anti-inflammatory, immunomodulatory and pro-regenerative ability of mesenchymal stem cells (MSCs) have been described.

To our knowledge, this is the first report where the anti-inflammatory and pro-regenerative ability of MSCs have been successfully applied in combination with surgical meshes, reducing the inflammatory processes commonly observed after mesh implantation. Moreover, our *in vitro* and *in vivo* results highlight the safety and efficacy of these bioactive meshes as a 'ready to use' medical product.

1. Introduction

Surgical meshes have replaced the sutures for hernia repair and are indicated for the wall reinforcement of all kinds of abdominal hernias. The reinforcement given by the prosthesis does not occur due to the material but caused by the tissue produced around the mesh fibers [1]. After surgical implantation, different steps are involved such as coagulum formation and platelet adherence followed by migration of fibroblasts, polymorphonucleocytes and macrophages. These macrophages fuse into multinucleated giant cells and remain in the meshes for an undetermined period of time. The failure to resolve the inflammatory response leads to foreign body reaction, granulation tissue formation and encapsulation of

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the implant, preventing its functional integration with the surrounding host tissue.

Newly designed light-weight meshes and new materials have been developed to reduce the complications (adverse inflammatory reaction, chronic pain or mesh erosion) attributed to mesh implantation [2]. For this, the improvement of surgical meshes is focused on the biocompatibility of materials [3] and the mechanisms to permit the transmigration and localization of beneficial host cells [4]. In this sense, different coatings such as autologous plasma [5] and titanium [6] have been clinically tested in propylene meshes to reduce foreign body reaction and increase biocompatibility.

In this work we hypothesize that surgical meshes combined with mesenchymal stem cells (MSCs) may favor the biocompatibility of material reducing the inflammatory reaction after surgical implantation. Supporting the use of MSCs as a therapeutic agent, numerous papers have previously demonstrated the immunomodulatory capacity of MSCs which is, at least in part, mediated in a paracrine manner for the regulation of NK cell cells [7,8], invariant NKT cells [9], cytotoxic T lymphocytes [10], γδ T cells [11], dendritic cells [12,13] and macrophages [14,15]. Here we demonstrate that MSCs and surgical meshes can be easily co-administered using MSCs-coated meshes to be used as an 'off the shelf' product. Finally, MSCs-coated meshes fulfill the safety aspects and both *in vitro* and *in vivo* studies evidenced a local immunomodulation which is mediated by a macrophage polarization towards an anti-inflammatory phenotype.

2. Materials and methods

2.1. Isolation and expansion of human adipose mesenchymal stem cells for in vitro assays

The human adipose mesenchymal stem cells (hASCs) were isolated from lipoaspirates obtained from human adipose tissue from healthy adult donors. Lipoaspirates were washed with phosphate buffered saline solution (PBS), and digested with collagenase type I in PBS. The digested sample was washed with 10% of fetal bovine serum (FBS), treated with ammonium chloride 160 mM, suspended in DMEM containing 10% FBS, and filtered through a 40 μm nylon mesh. Cells were seeded onto tissue culture flasks and expanded at 37 °C and 5% CO_2, changing the culture medium every 7 days. Cells were passed to a new culture flask when cultures reached 90% of confluence. Cell lines from three healthy donors were used in the study. The biological samples were obtained after informed consent under the auspices of the appropriate Research and Ethics Committees. These hASCs were used for all the *in vitro* studies.

2.2. Isolation and expansion of murine mesenchymal stem cells for in vivo assays

The murine bone marrow-derived MSCs were isolated from femurs of three euthanized B6D2 mice aged between 2 and 6 months and weighted between 20 and 25 g. The cells were flushed via needle and syringe. Cell suspension was filtered through a 40 μm nylon mesh and mononuclear cells were isolated by centrifugation over Histopaque-1077 (Sigma, St. Louis, MO, USA). Mononuclear cells were recovered and washed twice with PBS. Finally, mononuclear cells were resuspended in DMEM containing 10% FBS, seeded onto tissue culture flasks and expanded at 37 °C and 5% CO_2. Following 48 h in culture, the non-adherent hematopoietic cells were removed. Adhered cells were passaged at 80–90% confluence by 0.25% trypsin solution (Lonza Walkersville, Inc., Walkersville, MD, USA) and seeded to a new culture at a density of 5000–6000 cells/cm^2. Culture medium was changed every 7 days. The murine MSCs were used at passages 10–15 for *in vivo* experiments.

2.3. Mesh pretreatments and mesenchymal stem cells coating

In order to promote cell adhesion to the inert support, the surgical meshes were pre-treated with different compounds and incubated with different cell doses at different time points. For that, meshes were pre-coated with gelatin from porcine skin or poly-L-Lysine (PLL) hydrobromide (Sigma) both at 1 mg/ml for 10 min in sterile distilled water before incubation with cells as previously described by Casado et al. [16]. Non-treated meshes were used as control.

The human or murine MSCs were detached from flasks with 0.25% trypsin solution, counted and adjusted at 1, 2 and 4×10^6 - cells/ml. A total of 2, 4 and 8×10^6 cells, respectively, in 2 ml of DMEM containing 10% FBS were placed in a Nunc CryoTube vial (Nunc, Roskilde, Denmark) together with a 2 × 2 cm polypropylene surgical mesh (Surgimesh® Easy Plug® Patch System, Aspide Medical, St. Etienne, France) under continuous rotation for 1, 2 or 4 h at 37 °C.

2.4. Phenotypic analysis of MSCs adhered to surgical meshes by flow cytometry

For flow cytometric analysis by Fluorescent Activated Cells Sorting (FACS), MSCs were detached from meshes with 0.25% trypsin solution. The phenotypic analysis was performed as follows: 2×10^5 cells were incubated for 30 min at 4 °C with appropriate concentrations of monoclonal antibodies (mAbs) in the presence of PBS containing 2% FBS. The cells were stained with FITC-conjugated human mAbs against CD29, CD44, CD90, CD105, HLA-I (human leukocyte antigen class I) and HLA-II (human leukocyte antigen class II) from Serotec (Kidlington, United Kingdom). After incubation with antibodies, the cells were washed and resuspended in PBS. The flow cytometric analysis was performed on a FACScalibur cytometer (BD Biosciences, San Jose, CA, USA) after acquisition of 10^5 events. Cells were primarily selected using forward and side scatter characteristics and fluorescence was analyzed using CellQuest software (BD Biosciences). Isotype-matched negative control antibodies were used in all the experiments. The mean relative fluorescence intensity (MRFI) was calculated by dividing the mean fluorescent intensity (MFI) by the MFI of its negative control.

2.5. Cryopreservability of MSCs-coated meshes

The MSCs-coated meshes were frozen at −80 °C in Nunc CryoTube vials using a solution of CryoStor® CS10 freezing media (BioLife Solutions Inc., Bothell, WA, USA). After 15, 30 and 45 days, MSCs-coated meshes were thawed, centrifuged at 300×*g* for 5 min and CryoStor® solution was replaced by DMEM containing 10% FBS. These thawed MSCs-coated meshes were subsequently cultured for 1 day and 7 days. Control meshes (non-frozen) were compared with cryopreserved meshes (frozen–thawed). The viability of control and cryopreserved meshes was calculated by trypan blue dye-exclusion using the Countess® Automated Cell Counter (Thermo Fisher Scientific Inc., Waltham, MA, USA).

2.6. Lymphocyte proliferation and IFN-γ in vitro assays

Peripheral blood lymphocytes (PBLs) from healthy human donors were obtained after informed consent under the auspices of the appropriate Research and Ethics Committees by centrifugation over Histopaque-1077 (Sigma) and washed twice with PBS. The isolated PBLs were frozen and stored in liquid nitrogen until

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