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Decoration of RGD-mimetic porous scaffolds with engineered and devitalized extracellular matrix for adipose tissue regeneration

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ABSTRACT

Fat grafting is emerging as a promising alternative to silicon implants in breast reconstruction surgery. Unfortunately, this approach does not provide a proper mechanical support and is affected by drawbacks such as tissue resorption and donor site morbidity. Synthetic scaffolds can offer a valuable alternative to address these challenges, but poorly recapitulate the biochemical stimuli needed for tissue regeneration. Here, we aim at combining the positive features of a structural, synthetic polymer to an engineered, devitalized extracellular matrix (ECM) to generate a hybrid construct that can provide a mix of structural and biological stimuli needed for adipose tissue regeneration. A RGD-mimetic synthetic scaffold OPAAF, designed for soft tissue engineering, was decorated with ECM deposited by human adipose stromal cells (hASCs). The adipoinductive potential of the hybrid ECM-OPAAF construct was validated *in vitro,* by culture with hASC in a perfusion bioreactor system, and *in vivo*, by subcutaneous implantation in nude mouse. Our findings demonstrate that the hybrid ECM-OPAAF provides proper mechanical support and adipoinductive stimuli, with potential applicability as *off-the-shelf* material for adipose tissue reconstruction.

Statement of Significance

In this study we combined the functionalities of a synthetic polymer with those of an engineered and subsequently devitalized extracellular matrix (ECM) to generate a hybrid material for adipose tissue regeneration. The developed hybrid ECM-OPAAF was demonstrated to regulate human adipose stromal cells adipogenic commitment *in vitro* and adipose tissue infiltration *in vivo*. Our findings demonstrate that the hybrid ECM-OPAAF provide proper mechanical support and adipoinductive stimuli and represents a promising *off-the-shelf* material for adipose tissue reconstruction.

We believe that our approach could offer an alternative strategy for adipose tissue reconstruction in case of mastectomy or congenital abnormalities, overcoming the current limitations of autologous fat based strategies such as volume resorption and donor site morbidity.

1. Introduction

Breast cancer is the most common form of female tumors worldwide. Patients usually undergo mastectomy or lumpectomy as part of the surgical treatment for cancer resection [1]. Reconstruction after tumour resection is currently an optional treatment, which helps women to recover from the physical and psychological point of view [1,2]. Current reconstructive approaches, including the use of silicon prosthesis and the autologous flap implantation, are still limited by severe drawbacks such as incompatibility with radiotherapy and significant donor site morbidity [2,3]. In the last years fat grafting, which consists in the transfer of patient's fat tissue from liposuction to the site of the defect, is emerging as promising reconstructive technique [4]. This approach relies on the adipogenic and adipoinductive potentials of human adipose

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stromal cells (hASCs) that are an abundant source of multipotent progenitors [5]. Unfortunately, there are still some significant concerns for the use of fat grafting after mastectomy, such as the potential associated risk of hASCs with tumour seed activation, neoplastic formation and the volume loss over time that occurs because of limited vascular supply [4,6,7].

In the past years, different biomaterials, of synthetic or natural origins, have been tested and combined with several cell sources in adipose tissue engineering [8–13], but a strategy clinically acceptable to efficiently and repeatedly reconstruct adipose tissue is still missing. Biological scaffolds composed of ECM are commonly used for a variety of reconstructive surgical applications and are increasingly been tested in regenerative medicine strategies for tissue and organ replacement [14,15]. ECM represents the collection of structural proteins secreted by cells resident in each tissue and has been shown to provide cues that affect cell migration, proliferation, and differentiation in a tissue specific manner [15–17]. Recent studies demonstrated that biomaterials obtained by the ECM of adipose tissue could provide an adipoinductive substrate for the differentiation of hASCs, in the absence of additional differentiation factors [9,11,18,19]. Although the ECM is by definition the nature's ideal biological scaffolding material, its fast degradation rate and poor mechanical properties make its clinical application difficult [10,20]. On the other hand, synthetic scaffolds can offer a tuneable range of degradation and mechanical properties [21–23]. Our group recently developed a promising synthetic porous scaffold, a RGD-mimetic poly(amidoamine) oligomer macroporous foam (OPAAF), designed to provide suitable biological and mechanical cues for adipose tissue engineering applications [24]. We also demonstrated that OPAAF is able to support adipogenic differentiation of preadipocytic cell lines *in vitro*, while promoting adipose tissue infiltration *in vivo* [24]. In addition, OPAAF is characterized by a progressive hydrolitic degradation compatible with the implantation requirement in the first phase of the regenerative process [24]. Despite the efficiency of our material in supporting adipogenesis, its implementation with adipoinductive features would be instrumental for a possible clinical application.

This study aimed at combining the functionalities of a structural synthetic polymer with those of an engineered, devitalized ECM to generate a hybrid construct for adipose tissue regeneration. Towards this goal, OPAAF was decorated with adipose ECM, deposited by human hASCs *in vitro,* that were subsequently devitalized. The adipoinductive potential of such hybrid ECM-OPAAF was investigated *in vitro* and *in vivo*. The resulting hybrid construct represents an *off-the-shelf*, ready-to-use material for adipose tissue reconstruction, providing both proper mechanical support and adipoinductive stimuli.

2. Materials and methods

2.1. OPAAF fabrication

OPAAF was synthesized starting from RGD-mimetic poly(amidoamine) oligomers through gas foaming technique, as previously reported in our previous work [24]. Briefly, the OPAA oligomers were crosslinked by free radical polymerization using ammonium bicarbonate (NH_4HCO_3) as porous agent and ammonium persulfate (APS) as cross-linker. This reaction generated a hydrophilic 3D foam with an interconnected porous network.

2.2. Cell source and expansion

Samples of human adipose tissue were collected either as lipoaspirates or as dermolipectomies obtained during routine surgical procedures after informed consent from the patient and following protocol approval by the local ethical committee. The tissue was processed as reported in literature [25] in order to retrieve hASCs. hASCs were then plated for expansion (seeding density of 3×10^3 cells/cm^2) and cultured in complete medium (CM) which consisted of α-Modified Eagle's Medium, 10% fetal bovine serum, 100 mM HEPES buffer solution, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 mg/ml streptomycin and 292 mg/ml L-glutamine (GIBCO, Switzerland) supplemented with 5 ng/ml fibroblast growth factor-2 (FGF-2; R&D systems, USA). Cells were cultured at 37 °C in 5% CO_2 95% air-humidified incubator, harvested by using 0.05% trypsin, centrifuged and suspended in basal medium. Medium was changed twice a week. At confluence, cells were enzymatically retrieved and counted for experimental use. Human bone marrow-derived mesenchymal cells (hBMCs), used as a control condition, were cultured and expanded as reported in previous studies [26].

2.3. In vitro 3D model for OPAAF decoration

Using a previously developed perfusion bioreactor system [27] for cell seeding and culture of 3D scaffolds, hASCs were perfused overnight through OPAAF (8 mm diameter and 3 mm thickness) at a superficial velocity of 1000 μm/s to obtain a homogeneous cell seeding and distribution. After 24 h, the superficial velocity was reduced to 100 μm/s for perfusion culture. For the cell-polymer interaction experiments, cells were seeded and cultured in perfusion for 7 days in CM supplemented with FGF-2. For adipogenic differentiation, cells were cultured in perfusion for two weeks in DMEM supplemented with 10 μg/ml insulin, 1 μM dexamethasone, 100 μM indomethacin, and 500 μM 3-isobutyl-1-methyl xanthine (IBMX) (adipogenic induction medium) and one additional week in Dulbecco's modified Eagle medium (DMEM) supplemented with 10 μg/ml insulin (adipogenic mantainance medium). Culture medium was changed twice a week. This protocol was also used for decoration of OPAAF with adipose ECM.

For the decoration of OPAAF with a stromal ECM (Supplementary Fig. 2), hASCs and hBMCs were cultured for two weeks in CM supplemented with FGF-2 and 0.1 mM L-ascorbic acid-2-phosphate (AA, R&D systems, USA).

2.4. Hybrid ECM-OPAAF constructs decellularization

After ECM deposition, adipogenic and stromal constructs were devitalized according to previous protocols [28] in order to obtain the hybrid ECM-OPAAF constructs. Briefly, samples underwent three freeze and thaw (F/T) cycles in liquid nitrogen and 37 °C water bath (10 min each), respectively. Samples were rinsed in sterile Phosphate Buffered Saline (PBS) after each thawing step as well as in double distilled water after the second thawing in order to create a hypotonic shock to lyse remaining cells. To eliminate cellular debris, a perfusion-based washing step was introduced after the last F/T cycle: the constructs were placed into the bioreactor system and perfused at 100 μm/s in PBS for 30 min at room temperature (RT).

2.5. Evaluation of hASCs-OPAAF interaction in vitro

2.5.1. Live/Dead assay

Cellular viability was investigated by using a live/dead assay solution containing 1 μM Calcein-AM (Invitrogen) and 0.1 mM ethidium homodimer (Invitrogen). Briefly, samples were rinsed with PBS, and incubated with the staining solution for 15 min at 37 °C. The staining solution was removed and samples washed twice with PBS before imaging. Live imaging was performed with a confocal microscope (Zeiss LSM 710).

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