Three-Dimensional Tendon Biomimetic Scaffold Exerts a Boosted Immune Inductive Effect on Amniotic Epithelial Stem Cells

Mohammad El Khatib[a], Valentina Russo[a], Giuseppe Prencipe[a], Annunziata Mauro[a], Maria Rita Citeroni[a], Adrián Cerveró-Varona[a], Arlette A. Haidar-Montes[a], Melisa Faydaver[a], Matthias Schnabelrauch[b], Barbara Barboni[a]*

[a] Unit of Basic and Applied Sciences, Faculty of Biosciences and Agro-Food and Environmental Technologies, University of Teramo, 64100, Teramo, Italy
[b] Department of Biomaterials, INNOVENT e. V., 07745, Jena, Germany.
**e-mail: melkhatib@unite.it*

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Introduction

Tendinopathies still represent a public health concern both in human and veterinary medicine due to the unsatisfactory outcomes of the available conventional treatments [1]. In the last decades, relatively to the limited regenerative potential of tendons, much attention has been given to tendon tissue engineering (TE) that emerged as an advanced approach to deal with tendinopathies. TE aims to stimulate tendon regeneration through the production of functional implantable scaffolds [2]. Taking into consideration the crucial impact that the immune system has in tendon tissue regeneration, this latter can be achieved by designing scaffolds able to control the immune signalling through the creation of pro-regenerative environment [2]. Nowadays, immunoengineering has been introduced as a new discipline aiming at applying different immunoregenerative approaches to enhance the interaction between the transplanted scaffolds and the host tissues, which in turn could modulate the immune response and hinder the formation of fibrotic scar tissue within the regenerated site [2]. In fact, an ideal scaffold for tendon TE must be designed not only to mimic tendon architecture but to also switch the tendon pro-inflammatory response towards an anti-inflammatory one enhancing hence its regeneration [2]. This less explored feature in TE, especially for tendinopathies, might allow the modulation of the host inflammatory response and the stimulation of tissue regeneration or the potentiation of the immunomodulatory functions of stem cells. In this context, given the notable immunomodulatory properties of stem cells as the amniotic epithelial (AECs) ones demonstrated *in vivo* [3] together with their high teno-differentiative ability both *in vitro* and *in vivo* [4], the combination of this stem cell source together with tendon biomimetic scaffolds might represent an ideal microenvironment to modulate the immune cells and the regenerative process within the tendon injury site.

Starting from these premises, this study aimed at verifying the immune inductive potential of the fabricated tendon biomimetic 3D scaffold with highly aligned fibres on AECs by assessing the gene expression profile of anti- and pro-inflammatory cytokines as well as the released immunomodulatory molecules within the conditioned media (CM).

Materials and Methods

Bundled 3D PLGA tendon-like scaffolds were obtained by rolling up manually fleeces with highly aligned fibres fabricated through a rotatory collector using a commercial E-Spintronic electrospinning apparatus. The resulted constructs were characterized for their ultrastructure and biomechanical properties using scanning electron microscope (SEM) and uniaxial tensile test, respectively. The immune inductive potential of the fabricated constructs on the engineered AECs was assessed after 48h of culture by analysing the gene expression profiles of IL-10 and IL-12, anti- and pro-inflammatory cytokines, respectively, through real time qPCR. Moreover, the CMs were collected and analysed for the simultaneous expression profile of 40 immunomodulatory molecules, primary cytokines and chemokines, using Inflammation Antibody Array Membrane.

Results and Discussion

The fabricated 3D scaffold resembles macroscopically and microscopically the architecture of tendon tissues. In fact, the 3D scaffold was characterized by the high fibre alignment (SEM), as the native tissue. Moreover, the produced constructs had had significantly higher biomechanical properties in terms of Ultimate Tensile Strength and Young's modulus with respect to fleeces ($p<0.05$). The high fibre alignment of the fabricated constructs exhibited an immune inductive potential on AECs as demonstrated by the significant upregulation of IL-10, the anti-inflammatory cytokine, with boosted upregulation for 3D scaffolds at 48h ($p<0.05$). Interestingly, the expression of the pro-inflammatory IL-12, instead, was maintained at its basal levels within the 3D scaffolds. These results were confirmed by the analysis of IL-10/IL-12 ratio which showed a favourable pro-regenerative potential especially on 3D constructs ($p<0.05$) indicating the possibility of a better stimulation of macrophage skewing towards M2 phenotype when transplanted *in vivo*. Moreover, the immune-inductive role of the

developed scaffolds on AECs was also confirmed by analysing the CM, which revealed a modulation of AECs' secretion profile of immunomodulatory molecules. In detail, CM collected from AECs engineered 3D scaffold showed an overall enhancement in all analysed immunomodulatory factors, whereas a general down expression was revealed within the CM derived from engineered fleeces. In particular, in the CM collected from AECs engineered 3D scaffolds, all the analysed factors were mostly higher (1,5-fold) compared to the corresponding target expression levels in CM derived only from AECs (used as a control), with the only exception of the anti-inflammatory I-309 and TGF-1β and of the pro-inflammatory IL-8, MCP-1, IFN-γ, IL-7 and TIMP-2. On the contrary, CM derived from cells seeded on fleeces showed a down regulation of all the immunomodulatory factors with the only exception of the anti-inflammatory ones (IL-10 and IL-12 p40) and the pro-inflammatory ones (s TNF RI, MIP-1b, GCSF, ICAM-1, IL-17 and M-CSF). Interestingly, some of the upregulated immunomodulatory molecules within CM derived from AECs engineered 3D scaffolds, classified as pro-inflammatory ones including PDGF-BB, RANTES, IL-1α, IL-1b, IL-2, IL-6aR, IL-8, MCP-1, TNF-α, and IFN-γ are also known for their angiogenic role opening new insights on better understanding their involvement in the tendon resolution and regeneration.

Conclusions and/or Outlook

The presented data demonstrated the biomimicry properties of the fabricated 3D tendon-like scaffolds in terms of architecture and biomechanical properties. Moreover, the developed 3D scaffolds exhibited an immune inductive potential on AECs verified by the upregulation of anti-inflammatory cytokines as well as the release of immunomodulatory molecules within the CM. In future experiments, it will be explored the biological role of the secreted molecules within the CM on immune cells, as macrophages, to confirm their favourable immunomodulatory role.

These results might represent an innovative approach to design immune-informed scaffolds able to blunt pro-inflammatory response and improve tendon regeneration.

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