Nanofiber Scaffolds Functionalized with Mesenchymal Stem Cell Conditioned Media for Enhanced Human Tendon Regeneration

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INTRODUCTION

Human tendon injuries are a significant clinical challenge due to the limited natural regenerative capacity of tendon tissue. Tissue engineering approaches utilizing nanofiber scaffolds combined with mesenchymal stem cell (MSC)-derived factors represent promising strategies to enhance tendon healing.

For this purpose, we developed a novel oriented nanofiber scaffold composed of a mixture of polylactic acid (PLA), polycaprolactone (PCL), hyaluronic acid (HA), and collagen (Col), functionalized with conditioned medium (CM) derived from human Wharton's jelly mesenchymal stem cells (hWJ-MSCs).

In this study, we present the results of an in vitro study evaluating the tenogenic potential of these innovative functionalized nanofiber scaffolds in the presence of human tenocytes. Additionally, we evaluated the effects of hWJ-MSC-derived conditioned medium (CM-hWJ-MSCs) on the viability and cellular function of hWJ-MSCs themselves, which represent a highly potent and versatile cell source for cell-based therapies and scaffolds, with applications extending beyond tendon repair

MATERIALS AND METHODS

The PLA/PCL/HA/Col based nanofibers were fabricated by electrospinning using a specialized yarn-spinning chamber (KOPRIS 1A) under controlled conditions resulting in a final membrane density of 47 g/m². Subsequently, the scaffolds were functionalized by post-spinning impregnation with hWJ-MSC-derived conditioned medium (cell passage P4, cultured in serum-free medium for 24 hours).

Cell viability, morphology, proliferation, and expression of specific markers were evaluated. Tendon neotissue formation potential was assessed by immunohistochemical visualization of selected tendon matrix proteins (collagen type I, biglycan), together with gene expression analysis of selected tenogenic markers (collagen type I, biglycan, tenascin C) determined by RT-PCR. Collagen type I was also used as a general indicator of cellular biosynthetic activity to evaluate the influence of WJ-MSC-derived conditioned medium on hWJ-MSCs viability and matrix deposition.

RESULTS

Influence of functionalization with hWJ-MSC-Derived Conditioned Medium on Tendon Neotissue Formation Capacity:

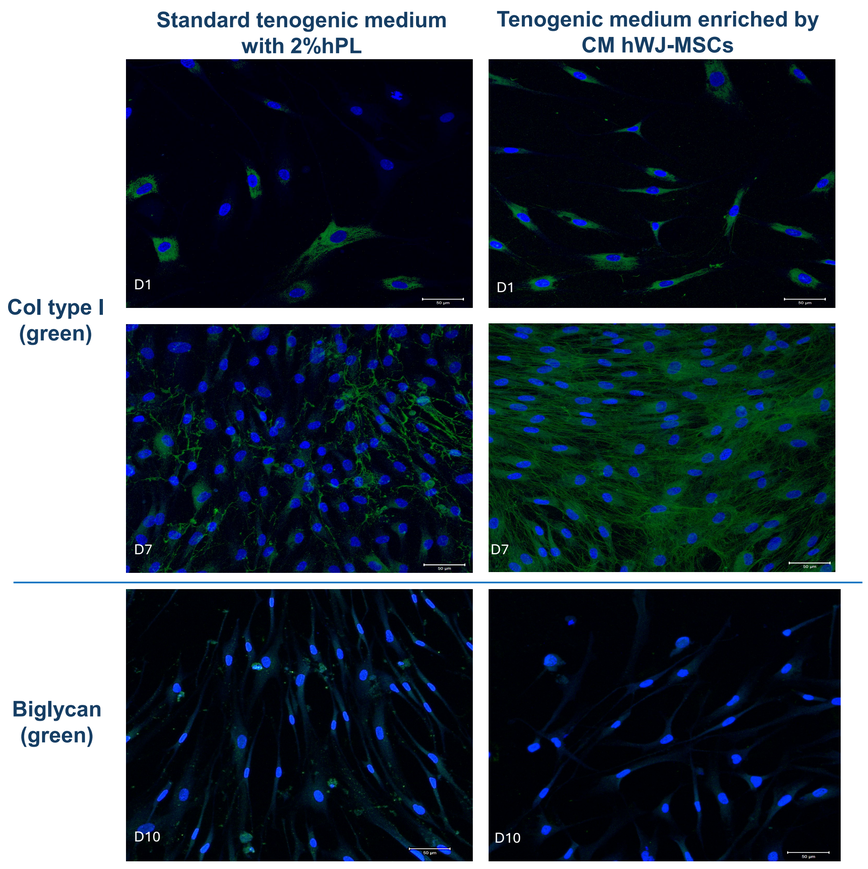


Fig. 1: Immunohistochemical staining of collagen type I and biglycan produced by human tenocytes cultured on tissue culture plastic in standard tenogenic medium (Innoprot) supplemented with 2% human platelet lysate (hPL) (left); and in the tenogenic medium enriched with hWJ-MSC-derived conditioned medium (right). Confocal microscopy; scale bar: 50 µm.

Influence of hWJ-MSC-Derived Conditioned Medium on hWJ-MSCs Viability and Collagen type I expression:

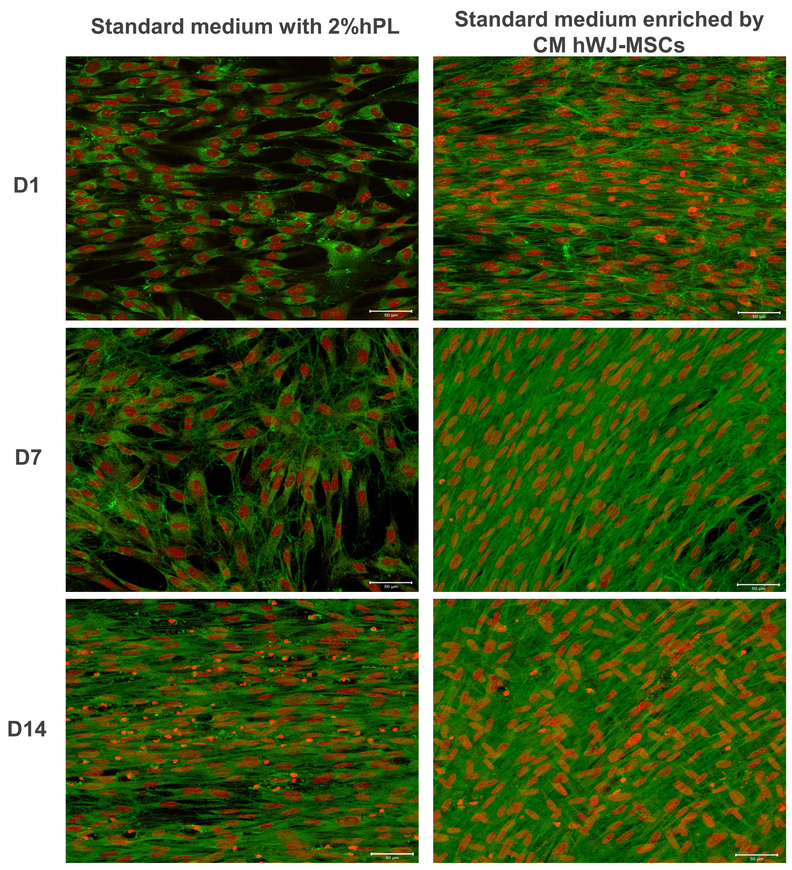


Fig 2: Immunohistochemical staining of collagen type I produced by hWJ-MSCs cultured on tissue culture plastic in standard cultivation medium (DMEM low glucose with Glutamax™ (Thermo Fisher Scietific), supplemented with 2% hPL (left); and in the medium supplemented with hWJ-MSC-derived conditioned medium (right). Confocal microscopy; scale bar: 50 µm

Tenogenic Potential of Newly Designed PCL/PLA/COL/HA Nanofiber Scaffolds Functionalized with WJ-MSC-Derived Conditioned Medium:

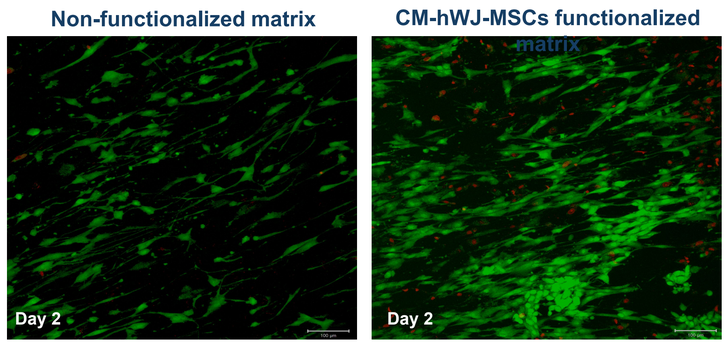


Fig. 3: Live/dead staining of human tenocytes seeded on PCL/PLA/COL/HA nanofiber scaffolds at a density 18,000 cells per cm²: non-functionalized (left); and functionalized with hWJ-MSC-derived conditioned medium (right), on day 2. Confocal microscopy; scale bar: 100 µm.

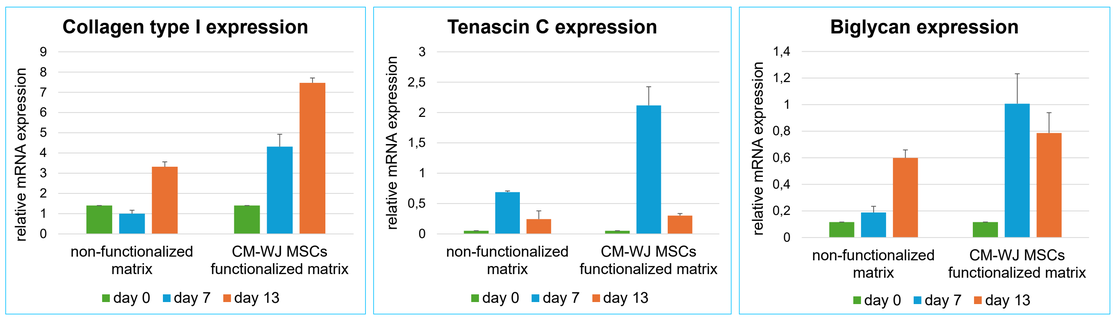


Fig. 4: RT-qPCR analysis of the relative mRNA expression of tenogenic markers in human tenocytes seeded on non-functionalized and CM-hWJ-MSC-functionalized PCL/PLA/COL/HA nanofiber scaffolds.

CONCLUSION

The results obtained so far demonstrate that our newly designed CM-hWJ-MSC-functionalized nanofiber scaffold effectively supports cell viability, appropriate cell morphology and proliferation, while promoting the expression of tendon-specific extracellular matrix proteins essential for effective tendon regeneration. These findings suggest that the innovative scaffold design, combining oriented nanofiber architecture with biochemical functionalization by hWJ-MSC-derived conditioned medium represents a promising biomaterial for tendon injury treatment and potential improvement of tendon regenerative therapies.

Moreover, the results demonstrate that WJ-MSC-derived conditioned medium exerts a significant positive effect on cell viability, cellular biosynthetic activity and matrix deposition. These findings indicate its strong potential for a wide range of applications in regenerative medicine and tissue engineering.

TERMIS EU-Chapter 2026 PALMA DE MALLORCA 21-24 APRIL / SPAIN

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