In Vivo Delivery of Macrophage Subtypes via Genipin-Crosslinked Collagen Biotextile

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Introduction:

Emerging approaches to control the immune response is a universal challenge in the clinical translation of tissue-engineered scaffolds [1]. The objective is now to develop smart scaffolds and materials for tissue engineering applications that can exert an enhanced positive action on the implant integration and healing process. Genipin-crosslinked collagen scaffolds were recently reported by our group to stimulate pro-regenerative M2 macrophage subtype in vitro [2]. The establishment of advanced biomaterials to induce early tissue remodeling and integration by preventing fibrous capsule formation may be a beneficial application for tissue regeneration. Controlling or avoiding the initial phase of the inflammatory response can provide a broad range of advancements in the implantation of biomaterial or engineered tissue scaffolds. Accordingly, the aims of this study were: 1) to determine the polarization status of M0, M1, and M2 macrophages in response to genipin-crosslinked collagen biotextile in vivo, 2) assess the long-term tissue response and post-surgical mechanical properties of implanted genipin-crosslinked collagen biotextile in comparison to M0, M1, and M2 seeded scaffolds in a subcutaneous rat model.

Materials and Methods:

Scaffold Fabrication: Electrochemical compaction was employed to convert collagen solutions (type I bovine collagen, 3 mg/mL) to aligned threads in continuous length on spools as we described previously [3]. Collagen scaffolds were crosslinked in genipin solution (2% w/v in 90% ethanol) for 72 h at 37°C (Fig.1)
Allogeneic Rat Macrophage Culture: Macrophages were harvested from rat bone-marrow and polarized into M1 and M2 macrophages. Primary antibodies against clusters of differentiation CD68, CD86, CD206, Arginase 1, and iNOS were used to identify the macrophage subtypes by flow cytometry, immunocytochemistry, and western blotting. Cell-seeded, genipin-crosslinked collagen scaffolds were assessed for cell attachment and proliferation. Experimental groups included M0, M1, and M2 (N/group=8) macrophages (Figure 2).
Surgery and Euthanasia: Cell-seeded scaffolds were implanted subcutaneously and harvested at three weeks and three months' time points.
Histology: Dissected tissue blocks were trimmed and processed histologically. Hematoxylin-eosin and Masson's trichrome stains were utilized to visualize cells and collagen deposition, respectively. Measurements of thickness of fibrous capsule, and percentage of collagen thread and interstitial collagen were obtained quantitively. Histology slides were examined by a senior pathologist (J. Anderson) for host response and tissue integration.
Immunohistochemistry (IHC): IHC was also performed for M1 macrophage (iNOS, IL-1β), M2 macrophage (Arginase 1, Ym-1), collagen type 1 (COL1A) and collagen type 3 (COL3A1).
Mechanical testing: Samples were kept hydrated with PBS until mechanical testing was performed within 24 h after sacrifice. Load and displacement data were recorded accordingly. Sample width and thickness were recorded along with this data to calculate stress, strain, and modulus values for each sample.
Statistical analysis: A Kruskal Wallis test was used to test the differences in the mechanical properties with the significance set at $p<0.05$.

Materials and Methods:

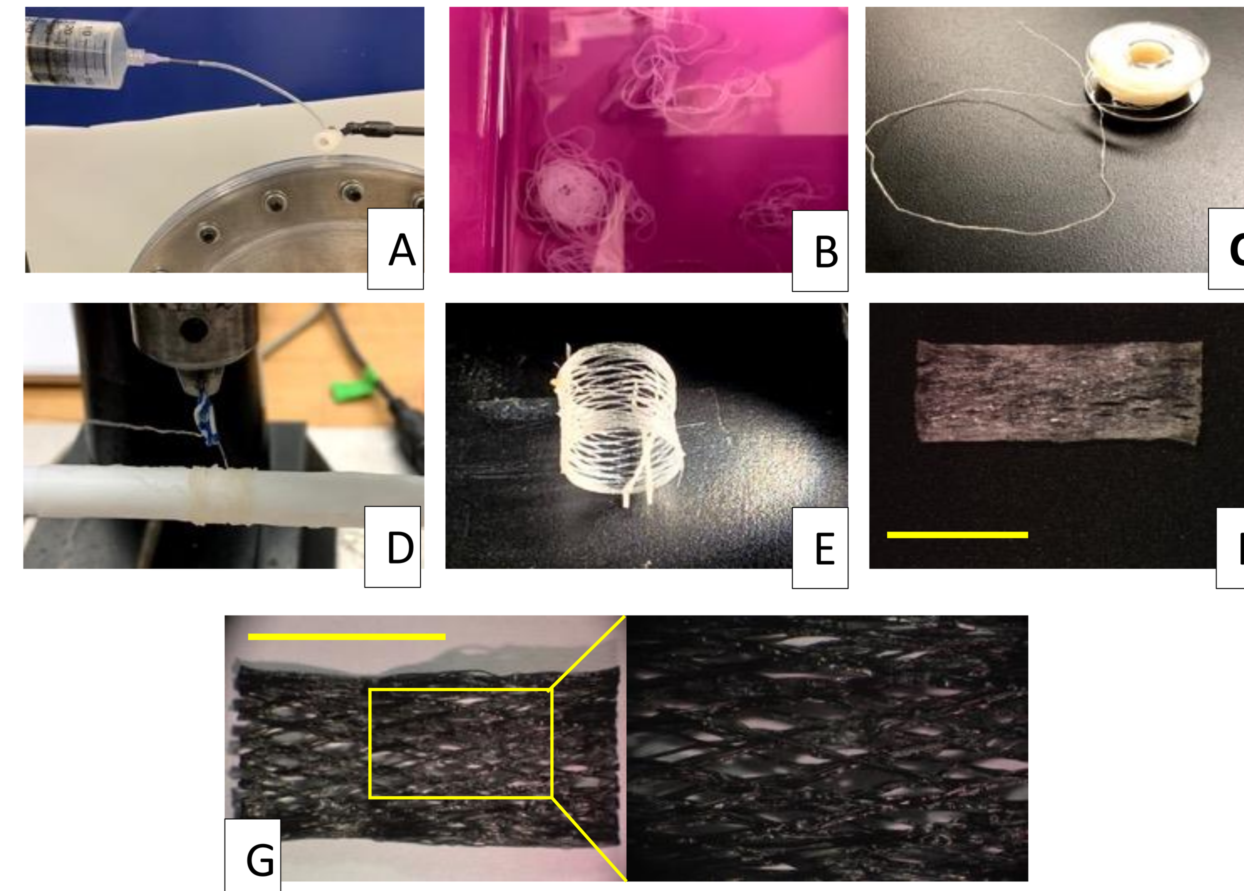

Figure 1. A) Fabrication of filament wound collagen scaffolds A) Electrochemical compaction of type 1 collagen solution B) Collection of collagen threads in isopropanol jar C) Collection of collagen yarns in continuous lengths onto spool D) Computer numeric controlled (CNC) filament winding of collagen yarns as scaffolds E) Recovery of collagen scaffold from the mandrel F) Rectangular-shaped uncrosslinked collagen scaffold, scale bar is 10 mm G) Light microscopy image of rectangular-shaped genipin crosslinked collagen scaffold.

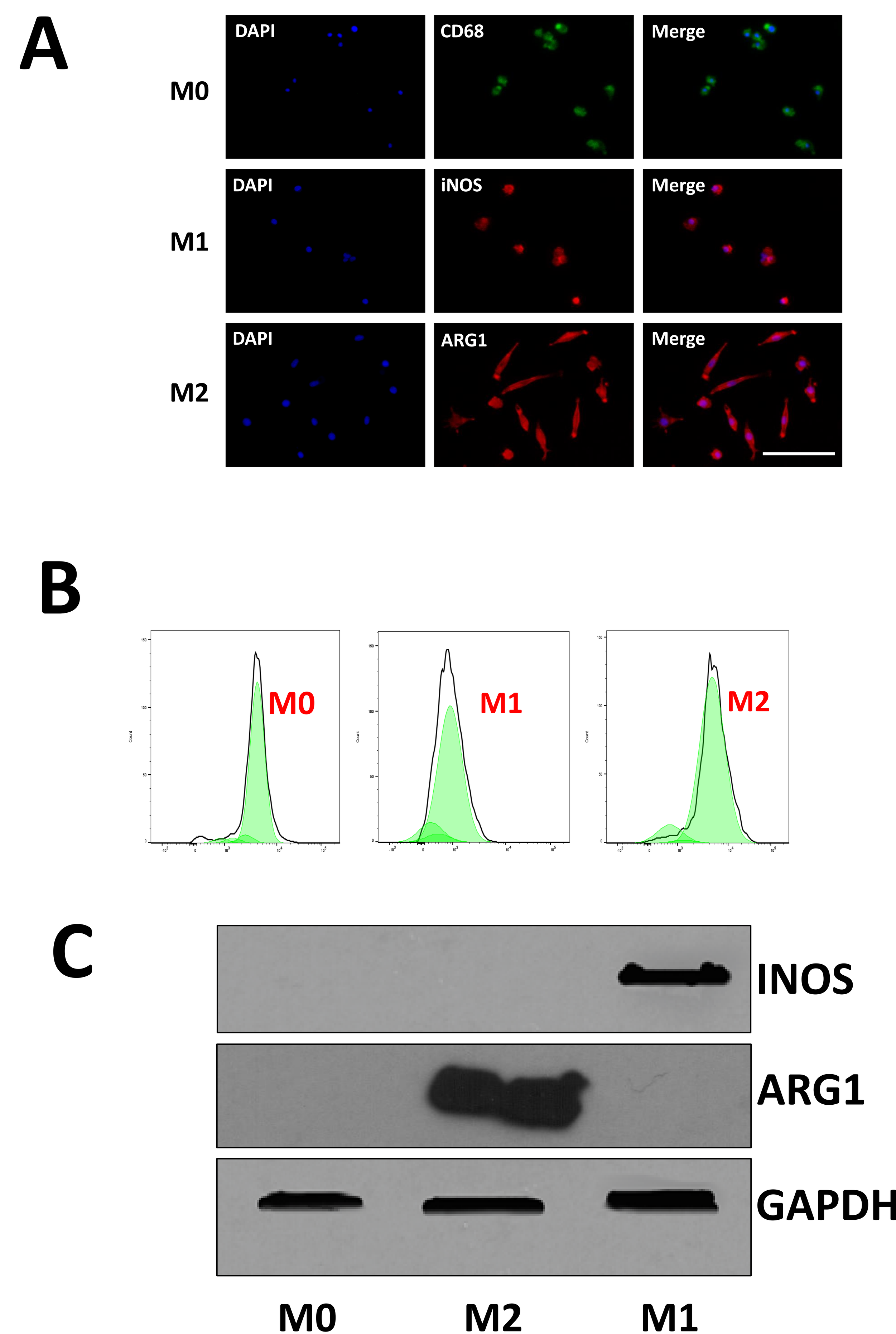

Figure 2. Characterization of macrophage subtypes using A) Immunocytochemistry- Representative immunofluorescent images of CD68 (green), iNOS (red), and Arginase 1 (orange-red) expression in M0, M1, and M2 macrophages. Blue DAPI stain identifies nuclei., B) Flow Cytometry and C) Western Blotting.

Results:

M2 delivery induced greater amount of interstitial collagen deposition while limiting fibrous encapsulation of the scaffold. Arginase-1 and Ym-1 have been classically considered M2 markers which were found abundant in GES+M2 group. iNOS and IL-1β, historically M1 markers, were absent in all groups. Increased collagen type 1 to collagen type 3 ratio in the interstitial matrix observed in the GES+M2 group (Fig.3). Furthermore, GES+M2 group showed a higher modulus than that of cell-free scaffolds (GES) ($p<0.05$), whereas moduli of GES+M0 or GES+M1 did not differ significantly from that of cell-free scaffolds.

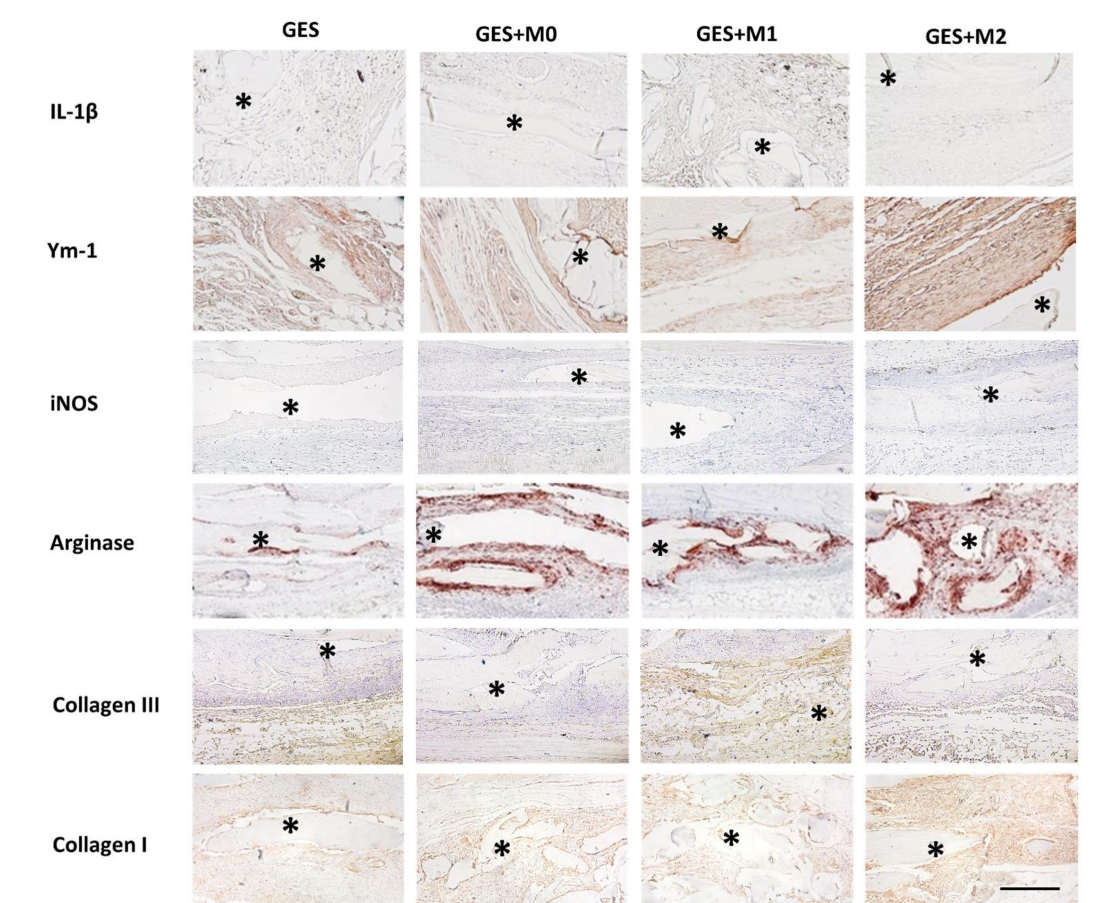

Figure 3. Immunohistochemistry results of IL-1β (M1 marker), Ym-1 (M2 marker), iNOS (M1 marker), Arginase (M2 marker), collagen type III and collagen type 1 at three months. An asterisk (*) indicates collagen threads of the collagen scaffold. GES = Genipin Scaffold, GES+M0 = M0 cells seeded on genipin scaffold, GES+M1 = M1 cells seeded on genipin scaffold, GES+M2 = M2 cells seeded on genipin scaffold. Scale bar is 150 µm.

Conclusions:

This study demonstrates the improved biomechanical and histological effects following the incorporation of M2 macrophage subtype into a genipin crosslinked collagen biotextile for tissue repair and remodeling. M2 macrophage delivery via genipin-crosslinked collagen scaffolds may help address clinical applications that require structural augmentation; applications such as hernia repair, orthopedic soft tissue repair, or urogynecological repair for stress urinary incontinence or pelvic prolapse could benefit significantly from this approach. Future studies are needed to evaluate the efficacy and safety of macrophage delivery for clinical applications.

Acknowledgments: NIH grant: R21HD095439

References:

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