A 3D-Bioprinted Dermal-Like Scaffold Incorporating Fibroblasts and DRG Neurons to investigate Peripheral Nerve Regeneration

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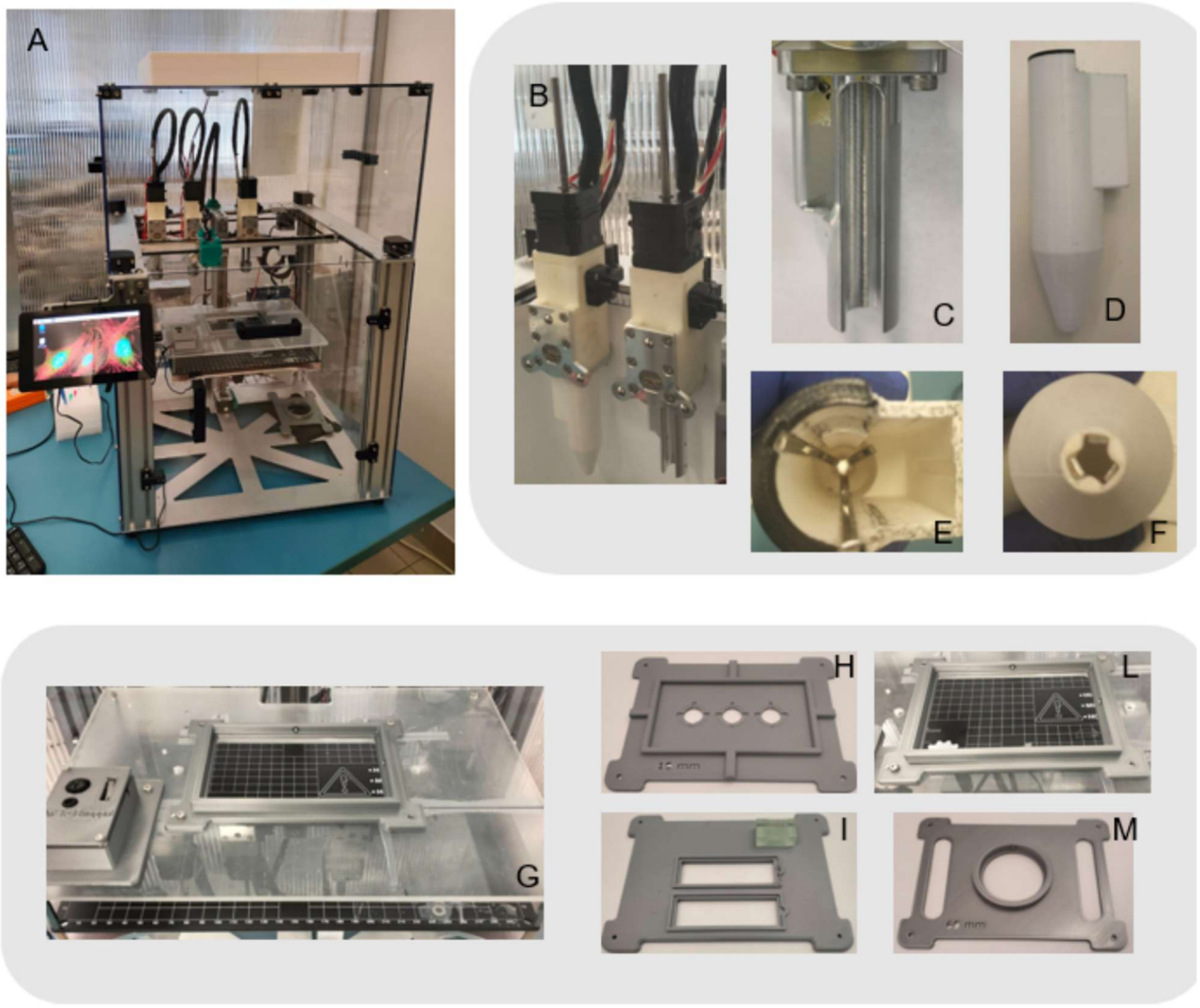

Figure S1: (A) Open-source bioprinter used in this study. (B) Microtool thermally controlled extruder. (C) Syringe heating system, including (D-E-F) cap for the tips and syringes. (G) Bioprinter plate designed to accommodate various supports, such as (H) 19 mm circular glass, (I) multiwell plates, (L) microscope slides, and (M) 60 mm Petri dishes.

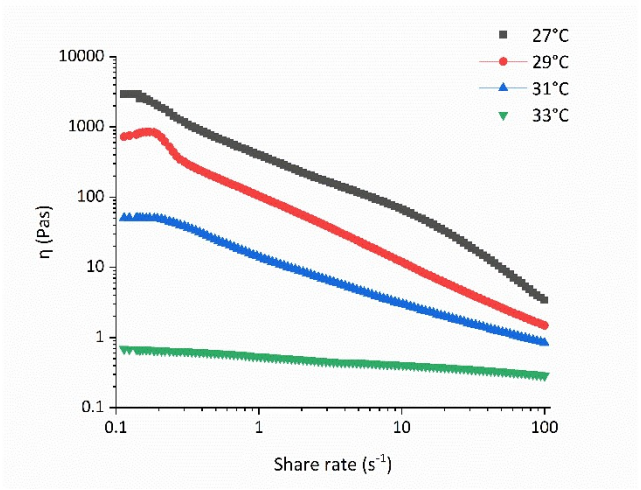

Figure S2: Logarithmic relationship between viscosity and shear rate for the bioink at various temperatures.

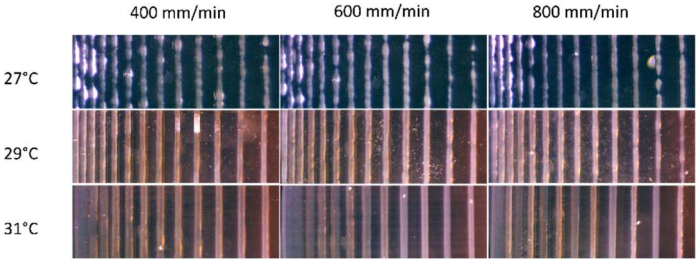

Figure S3: Images of printed samples at different temperatures and printing speeds used to study filament fusion during the printing.

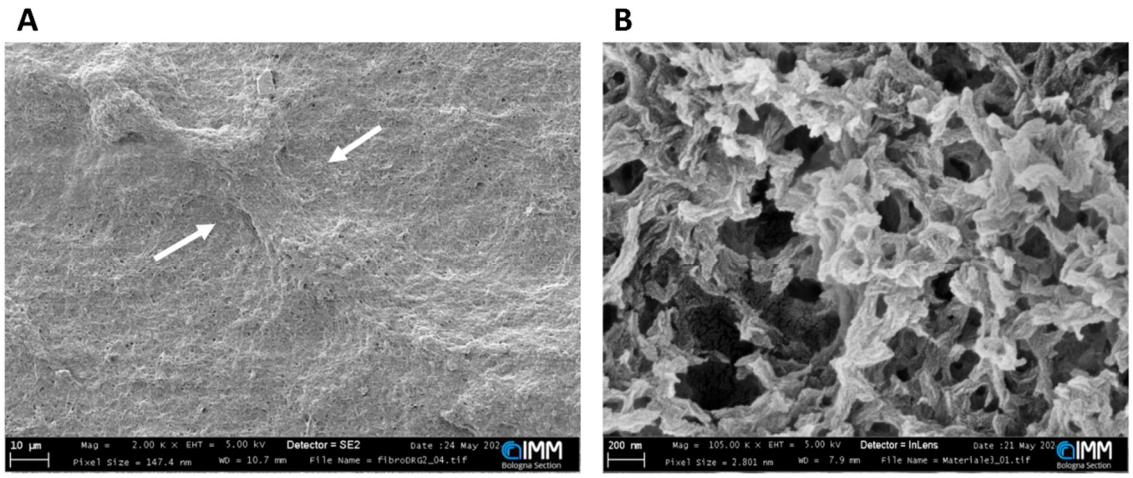

Figure S4: (A) SEM image at 5 DIV showing a 3T3 fibroblast embedded in the superficial layer of the scaffold surface (scale bar: 10 µm). The image demonstrates efficient adhesion and interaction with the bioink matrix. White arrows highlight the typical boundaries of healthy, adherent fibroblasts. The observed cell morphology reflects the behavior of fibroblasts in vitro. (B) SEM image of the scaffold surface without cells at 5 DIV (scale bar: 200 nm), revealing pronounced nanoscale porosity and a fibrillary texture. These features are considered fundamental for enhancing cell–material interactions and promoting cellular adaptation to the matrix topography.

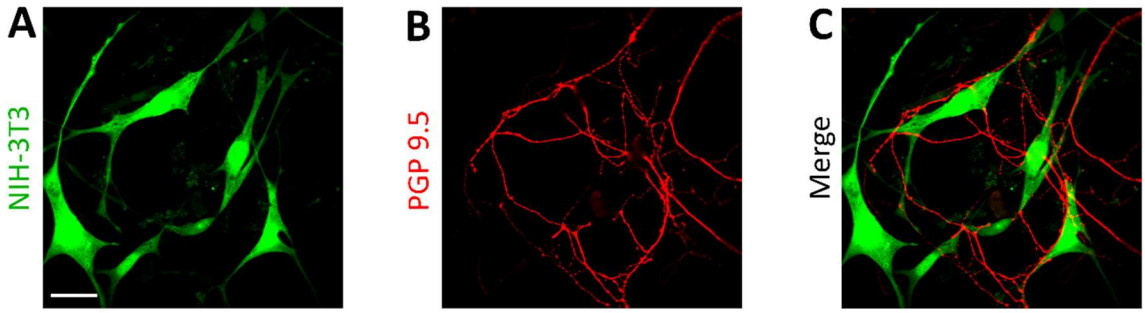

Figure S5: Single plane confocal images showing the interaction between 3T3 fibroblasts (A, green) and DRG (B, red) in a bioprinted construct, at 7 DIV after neuronal seeding. Scale bar is 20 µm. The merged image (C) depicts DRG neurites entering the artificial dermis making contacts with the fibroblast cell soma. Neurites alignment was also observed with elongated fibroblasts processes.

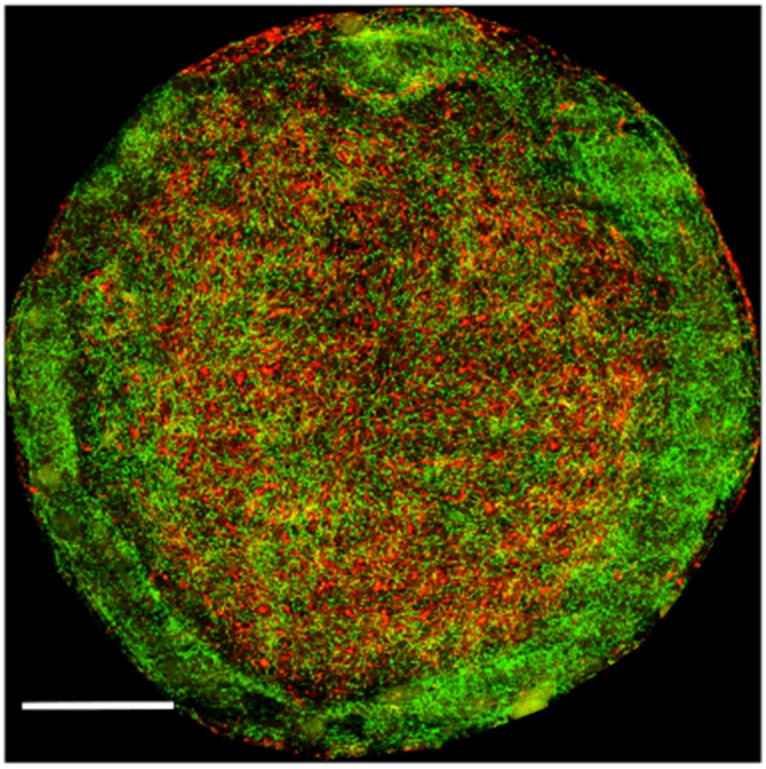

Figure S6: Single-plane confocal image showing 3T3 fibroblasts (green) and DRG neurons (red) within the entire bioprinted construct at 10 DIV (10 days of fibroblast culture and 7 days after neuronal seeding). Scale bar: 2 mm.

Bioink Preparation Protocol:

The bioink formulation consisted of a mixture of 2% (w/v) fibrinogen from bovine plasma (Sigma-Aldrich, F8630), 0.5% (w/v) sodium alginate (Sigma-Aldrich, A2158), and 10% (w/v) gelatin derived from porcine skin (Sigma-Aldrich, G1890). All components were dissolved in Dulbecco's Modified Eagle's Medium (DMEM) 1X ([+] 4.5 g/L D-glucose, [+] L-glutamine, [-] sodium pyruvate; Gibco). The preparation steps were as follows: Fibrinogen powder was gradually added to DMEM prewarmed at 37°C, under continuous gentle stirring using a magnetic stirrer. The solution was maintained at 37°C and stirred for 1 hour to ensure complete fibrinogen dissolution, avoiding bubble formation. Once a clear and homogeneous fibrinogen solution was obtained, sodium alginate powder was slowly incorporated into the mixture. Care was taken to prevent the formation of aggregates by sprinkling the powder gradually onto the surface of the solution. Stirring was continued for an additional 3 hours at room temperature to guarantee complete alginate solubilization. Subsequently, gelatin powder was added to the fibrinogen-alginate blend. The mixture was stirred for 4 hours at 37°C until a fully homogeneous and viscous solution was obtained. The temperature was carefully controlled to avoid fibrinogen agglomeration. For cell incorporation, 900 µL of the prepared bioink were transferred into a sterile syringe. Separately, 100 µL of a cell suspension at concentration of 1×10^7 cells/mL were prepared in sterile conditions (to achieve a final concentration of 1×10^6 cells/mL). The 100 µL of cell suspension were then added to the 900 µL of bioink, reaching a total volume of 1 mL. To ensure a homogeneous distribution of the cells within the viscous bioink, the mixture was gently and repeatedly transferred back and forth between two syringes connected through a sterile Luer lock connector (internal diameter of 2 mm suitable to avoid excessive shear stress) for approximately 20 passes. This double-syringe mixing method minimized bubble formation and mechanical damage to the cells, resulting in a uniform cell-laden bioink suitable for printing. The cell-laden bioink was kept at 37°C until use to maintain optimal viscosity and cell viability."