Development of Extracellular Matrix-laden Piezoelectric Scaffolds to Guide Nerve Repair

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Statement of Purpose: While current intervention strategies for traumatic peripheral nerve injuries involve autografts or direct repair, these surgeries have drawbacks. Therefore, conduits meant to guide regeneration across injury gaps have been introduced. No conduit thus far has managed to possess the necessary physical, electrical, and biochemical signaling capabilities required to promote adequate functional recovery. To address these shortcomings, this study develops a piezoelectric biomaterial functionalized with decellularized extracellular matrix (dECM) capable of directing cell function through physical and chemical cues while possessing endogenous electrical signaling to direct nerve regeneration.

Methods: Polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) scaffolds were electrospun and biofunctionalized from a polymer solution containing PVDF-TrFE powder in DMF-acetone incorporated with a decellularized, cell-derived extracellular matrix (dECM). Prior to electrospinning, dECM was lyophilized, digested, and mixed with precursor solution to form a homogeneous mixture. Four scaffold groups containing different w:v of dECM to PVDF-TrFE were electrospun: 0 %, 0.2 %, 0.3 %, and 0.4 % dECM. Scaffolds were characterized with scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDS) to confirm dECM presence. *In vitro*, immunofluorescent (IF) microscopy was used to identify changes in cell phenotype due to dECM content. For *in vivo* studies, scaffolds were subcutaneously implanted into rats and removed after 7 and 28 days to analyze the resulting host immune response.

Results: Primary Schwann cells grown on biofunctionalized scaffolds were analyzed for morphological changes due to dECM content. At 24 h, cells on 0.4 % scaffolds maintained highly aligned structures and had larger cellular aspect ratios compared to other scaffold groups. Due to this regenerative-like behavior on 0.4 % scaffolds, this group was used for *in vivo* studies. When implanted, M1 macrophage polarization denoting a pro-inflammatory response were limited across 0 % and 0.4 % scaffolds (**1A**, **B**). Consistent with M1 percentages seen within our FDA-approved control, polycaprolactone (PCL), standard and blended PVDF-TrFE scaffolds maintained a minimal foreign body response (FBR) after 7 days (**1C**). To gauge pro-regenerative, anti-inflammatory responses, M2 phenotype analysis showed slight increases in 0.4 % scaffolds, but overall low percentages across all groups (**1D-F**). After 28 days post-surgery,

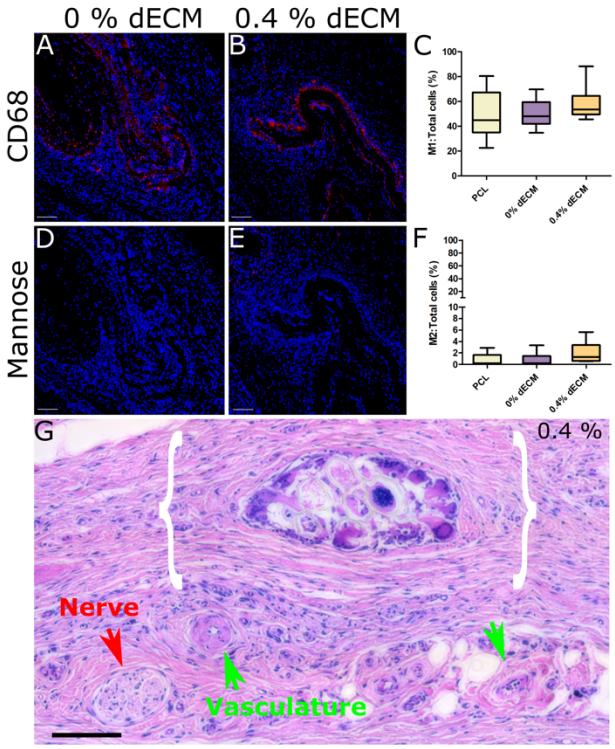

Figure 1: (A-C) Visualization of CD68 details M1 macrophage percentages after 7 days. (D-F) Mannose receptor details the M2 cell response after 7 days. (G) Area of inflammation following a 28-day implantation includes slight capsule formation (white braces) with nerve and vasculature presence in piezoelectric scaffolds.

biofunctionalized, piezoelectric implants possessed collections of nerve and vasculature networks not visible in PCL groups (**1G**). Understanding electrical activity accelerates wound healing and facilitates optimal axonal outgrowth, we attribute the electrical and bioactive components of our scaffold to drive these regenerative behaviors.

Conclusion: Early immune response towards our biofunctionalized, piezoelectric scaffold showed bioactive scaffolds promoted a minimal immune reaction at 7 days. Limited capsule formation observed across all groups is consistent with a typical response to foreign body implants; however, the strong presence of nerves and vasculature surrounding our 0 % and 0.4 % scaffolds are attributed to the bioactive material and piezoelectric nature of our scaffolds attracting these nerves and vessels indicating these scaffolds promote advanced regenerative behaviors in surrounding tissue.