Injectable neural hydrogel as *in vivo* therapeutic delivery vehicle

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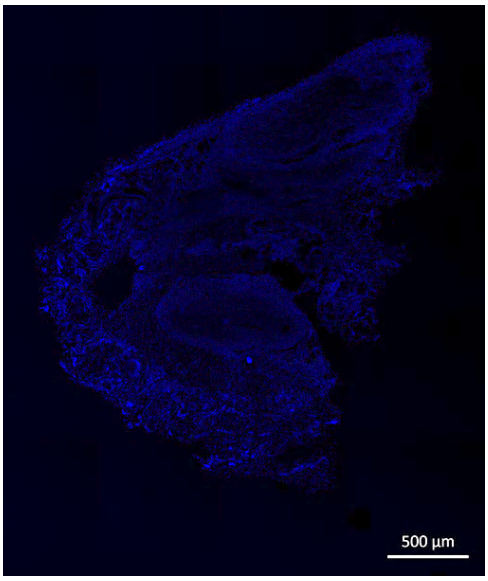
Supplemental Data

iPN hydrogel integrity *in vivo*

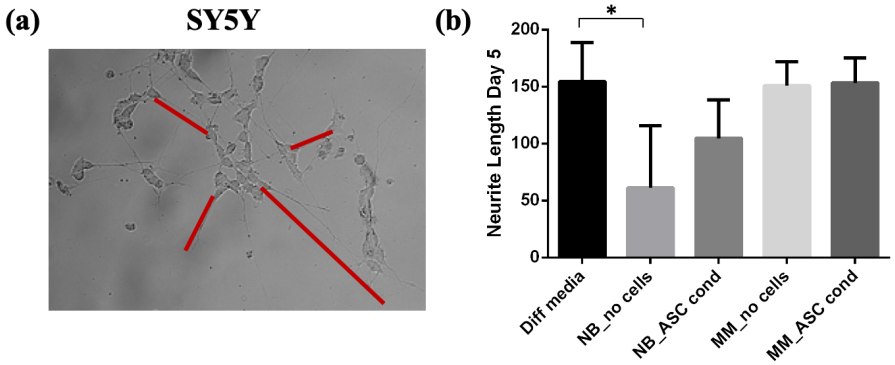
The iPN hydrogel implants from the main study were harvested from rats at three days post-injection. To provide a visual representation of iPN's molded structure following *in vivo* injection, the implants were fixed in 4% paraformaldehyde, cyrosectioned and stained. Mounted sections were stained for DAPI (Thermo Fisher) and imaged on a Zeiss Axio Observer. A representative 10X image is shown in Figure S1, in which there is consistent cellular infiltration around the perimeter of the molded hydrogel shape.



Supplementary Figure S1. A representative image of an extracted iPN hydrogel implant three days after subcutaneous injection. The hydrogel section was stained with DAPI to demonstrate cellular infiltration in the decellularized matrix. Image was acquired at 10X and the scalebar is 500 µm.

SY5Y neurite extension analysis

A neurite outgrowth assay was performed to assess neural-specific regenerative properties of adipose-derived stem cell (ASC) secretome. SH-SY5Y neuroblastoma cells were obtained from ATCC (CRL-2266) and were cultured by recommended procedures. SY5Y were seeded in 48 well plates and grown for two days before differentiation prior to assay analysis. To differentiate the SY5Y cells (i.e., prime the cells for neurite extension), the media was switched to EMEM/F12 containing 3% fetal bovine serum and 10 µM retinoic acid formulation. After two days of differentiation, cells were assigned to one of the following groups: (1) differentiation media (Diff media, positive control, containing retinoic acid), (2) neurobasal media (NB_no cells, negative control), (3) neurobasal conditioned by ASCs (NB_ASC cond, i.e., secretome), (4) modified media as described in the main text and (5) modified media conditioned by ASCs (MM_ASC cond). Brightfield images were taken on a Ziess Axio Observer after 5 days of media incubation. Analysis included randomly selecting 10 cells per well (n=6 wells/group) using a random ROI function in ImageJ and measuring the longest neurite outgrowth (examples in Figure S1A of each cell from its body. Figure S2B shows a significant loss of neurite extension when SY5Y were incubated in plain neurobasal (NB_no cells). However, ASC conditioning (NB_ASC cond) increased average neurite length approximately double that of NB_no cells and was not significantly different from the positive control. This indicated a potentially positive influence of ASC secretome on neurite extension. Additionally, both modified media conditions (with and without ASCs) performed identically to the positive control. Other analyses, perhaps with primary neural cells, may be necessary to determine if ASC conditioning of the MM enhances its neural regenerative properties, as effects were saturated in the SY5Y assay.



Supplementary Figure S2. An SH-SY5Y neurite outgrowth assay was used to test the regenerative properties of ASC secretome. (a) Example approximations for straight-line neurite measurements extending from SY5Y bodies. (b) Neurite length following 5 days of incubation in differentiation media (Diff media), neurobasal media (NB_no cells), neurobasal media with ASC conditioning (NB_ASC cond), modified media containing growth factors (MM_no cells), or modified media with ASC conditioning (MM_ASC cond) was determined via 10 individual cells per well (n=6 wells/group), *p-value<0.05