Nociception and neural sprouting are dependent on multicellular organization in a dorsal root ganglia model system stimulated with human herniated intervertebral disc conditioned media

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INTRODUCTION: Back pain affects ~80% of the world population, causing an enormous socioeconomical burden with >$134.5B of annual health care costs in the US.[1] Back and neck disability are associated with intervertebral disc (IVD) degeneration (IVDD), inflammation and nerve ingrowth deep into IVD tissues.[2] These nerves express neuropeptides calcitonin gene related peptide (CGRP) and substance P[3,4], which are markers of nociception.[5] The nociceptors' cell bodies that innervate the IVD are located in the dorsal root ganglia (DRGs), nodule-like structures proximal to IVDs. The impact of human IVDD on nociceptor phenotype and neurite outgrowth remains understudied. *In vitro* models are a useful tool to simplify complex mechanisms, however, current *in vitro* models mostly use dissociated DRG neurons that lose the multicellular architecture of naïve DRGs when cultured in traditional monolayer culture. We developed a bio-assembly approach using sound-induced hydrodynamic forces where neurons can be re-assembled into multicellular architecture within seconds to preserve important DRG structural features.[6,7] We compared this assembled multicellular DRG model to typical randomly oriented monolayer DRG model to investigate the influence of conditioned media (CM) generated from human herniated IVDs on nociceptor phenotype and neurite sprouting. We hypothesize that the neuronal response of the pro-inflammatory environment of IVDD will depend on DRG multicellular organization.

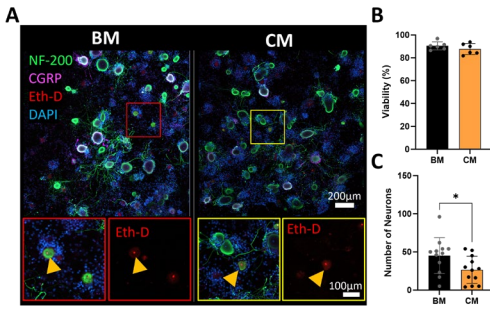

Fig. 1: *High neuronal viability was confirmed in random culture in* ***(A)*** *using Eth-D (with dead cells shown with yellow arrowhead)* ***(B)*** *in both BM and CM, although* ***(C)*** *number of neurons was lower in CM.*

METHODS: DRGs were collected from adult bovine spines (C1-C7, obtained at a local abattoir) that were minced and digested for 2 hrs (4mg/mL collagenase P) under agitation. Isolated DRG neurons were seeded at 5000 neurons/cm^2 either randomly ("random") or assembled into ring-shape aggregates under dynamic waves using a mechanical wave driver at 80Hz, for 1 minute ("assembled"). Neurons of each setup (random / assembled) were stimulated with either basal media ("BM") or with human herniated IVD CM ("CM"), generated as described.[8] After 72 hours, neuron viability was evaluated using ethidium homodimer (Eth-D) and immunofluorescent (IF) labelling of neurofilaments (NF-200). For nociception and neurite sprouting, DRG neurons were labelled for NF-200 and CGRP. Neurite outgrowth analysis was performed using 'ImageJ SNT' plugin. Statistical analyses were done using GraphPad Prism 10.

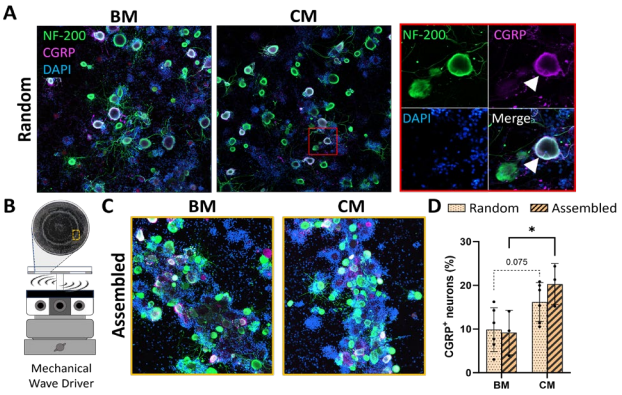

Fig. 2: *Nociception of bDRG neurons in* ***(A)*** *random culture and* ***(B-C)*** *assembled culture using CGRP IF (pink) shows* ***(D)*** *significant increase of CGRP$^+$ neurons using CM in assembled culture.*

RESULTS: Bovine DRG neurons stimulated by BM and CM showed equally high viability after 72hrs (Fig. 1A & 1B), although CM resulted in significantly fewer neurons which may be explained by less neuronal adhesion with CM (Fig. 1C). CM significantly increased the proportion of CGRP$^+$ nociceptors (Fig. 2, white arrow indicates CGRP$^+$ neuron), suggesting a nociceptor phenotype switch associated with pain sensitization. Interestingly, CM increased CGRP$^+$ nociceptors more prominently in assembled than random culture (Fig. 2B-D), highlighting a greater sensitivity of nociceptors in the assembled model that restored certain multicellular architecture features. CM did not affect mean neurite outgrowth length compared to BM in either random or assembled models (Fig. 3B). CM increased the number of neurite branches compared to BM in random culture, while the number of branches in the assembled model remained high regardless of media (Fig. 3C).

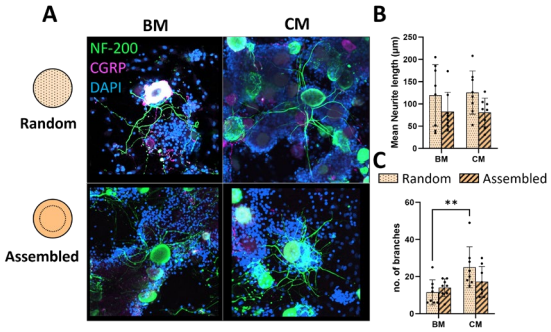

Fig. 3*Neurite outgrowth in* ***(A)*** *random vs. assembled shows* ***(B)*** *no significantly reduced mean neurite length, but a* ***(C)*** *significant increase of branches in random culture.*

DISCUSSION: CM significantly increased the proportion of CGRP$^+$ nociceptors, suggesting a phenotype switch from non-nociceptors to nociceptors. The significant increase in CGRP$^+$ in the assembled model suggests a greater propensity for neurons to exhibit a nociceptive shift when assembled. CM increased the number of neuronal branches significantly for the random model, suggesting these neurons may be exhibiting non-nociceptive DRG cell communications in response to the cytokines mixture in CM. Meanwhile assembled DRGs has high levels of branches in all conditions. We believe if CM was provided from a localized source instead of a dispersed media condition such as here, that assembled DRGs may increase the neurite length and/or make the numbers of branches technically easier to detect and measure since another study on the assembled DRG model stimulated neurite growths towards the source of a cytokine gradient.[7] Together with the literature, this assembly study highlights that DRG nociception and sprouting behaviors are dependent on proximity of neighboring cells, the presence of cytokines, and the cytokine gradient. The importance of DRG assembly in neuronal responses may also have implications in DRG remodeling responses known to occur in vivo in response to stimuli in neuropathy models. We conclude that cytokines in the IVDD CM can promote nociception and neural sprouting, and the DRG neuronal response is highly dependent upon the presence and proximity of neighboring cells.

SIGNIFICANCE: Back and neck pain are highly dependent on neuronal nociception and sprouting. This novel assembled DRG cell culture model shows that CM can switch neuronal phenotype and promote neural sprouting in a way that depends on DRG architecture. This multicellular platform can be used to study pain-related responses to disease-related stimuli and to screen for new therapeutics intervening into the neuronal signaling.

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