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3D modelling of human brain in combination with reprogramming and genome editing technologies represent powerful tools for understanding and treating human neurological diseases. Despite the advances in these technologies there is still little known about neuronal network function and plasticity in brain organoids. To study human neuronal network function, we developed a defined, Matrigel-free 3D cell culture system termed human bioengineered neuronal organoids (BENOs). Neural differentiation of pluripotent stem cells (iPSC) embedded in a collagen matrix was directed under defined serum-free conditions to derive BENOs of an approximate diameter of 2 mm. RNA-sequencing at different time points of BENO-development (n=3-6/time-point) showed that similar to human brain development, in our model neurogenesis (d30-40) precedes gliogenesis (d50-60). Whole mount immunofluorescence revealed the presence of dopaminergic (TH), glutaminergic (VGLUT) and gabaergic (GABA) neurons. Calcium imaging revealed spontaneous waves of neuronal activity propagating throughout the organoid by d22 with fast tetradotoxin (1 µM)-sensitive signals by d30. To test spontaneous neuronal network activity, BENOs (day 30-60) were subjected to calcium imaging under GABAergic (picrotoxin, 58 µM; saclofen, 330 µM) inhibition. Interestingly, spontaneous Ca2+ signals of synchronized neurons became asynchronous upon GABAR inhibition (2 independent experiments). Antagonist washout restored synchronicity suggesting the presence of functional GABAergic networks. Using a stimulation electrode (injected current: 20-100 µA) we could evoke Ca2+ influx in remote regions (distance from electrode 0.5 to 1.5 mm) suggesting a strong neuronal network that extends throughout the organoid. Multi-pulse stimulation demonstrated a Ca2+ influx pattern similar to paired pulse depression (PPD), indicating short term plasticity. The PPD-like Ca2+ signal pattern was alleviated by a GABA-A inhibition (picrotoxin 58 µM) and was restored upon washout (2 independent experiments). Taken together, BENOs from human pluripotent stem cells contain electrically active neuronal networks that demonstrate typical forms of short-term plasticity mediated by functional interneurons.

TISSUE ENGINEERING

W-2087

HETEROCELLULAR COUPLING MEDIATES PRO-CONTRACTILE EFFECTS OF CARDIAC PROGENITOR CELLS IN HUMAN ENGINEERED CARDIAC TISSUE

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The promising benefits for treating heart failure by delivering human bone marrow-derived mesenchymal stem cells (hMSCs) and c-Kit-positive cardiac progenitor cells (hCPCs)-in isolation or in combination-warrant a better understanding of underlying mechanisms of action. Previous work showed paracrine signaling, not heterocellular coupling, as primarily responsible for hMSC-mediated pro-contractile effects on human engineered cardiac tissues (hECTs); however, the effects of hCPCs on hECTs have not been tested. This study aims to distinguish the relative contributions of paracrine signaling and heterocellular coupling in hCPC-mediated contractility enhancement of hECTs created using cardiomyocytes derived from human induced pluripotent stem cells. We studied three experimental groups in our force-sensing, multi-tissue bioreactor system: (1) control hECTs without hCPC supplementation (-hCPC), or (2 and 3) alternating hECTs without or with hCPC supplementation (10% of total cell number added during tissue creation) cultured in a shared paracrine media bath (p.-hCPC and p.+hCPC, respectively). Functional testing of hECT twitch force at 0.5-Hz electrical pacing was performed on culture day 6. The hECTs directly supplemented with hCPCs (p.+hCPC) had a two-fold increase in developed force ($p<0.05$) compared to non-hCPC-supplemented counterparts sharing the paracrine media bath (p.-hCPC), as well as non-hCPC-supplemented negative controls (-hCPC),

with no significant difference between p.-hCPC and -hCPC groups. The spontaneous beat rate and beat rate variability (analyzed without electrical pacing) showed no significant difference between groups. Similar results were obtained on culture days 8 and 10. In addition, treatment of -hCPC hECTs with hCPC-conditioned media had no significant effect on developed force. Altogether, these findings demonstrate that unlike hMSCs, contractile potency of the hCPC secretome is minimal, whereas direct heterocellular coupling is critical for hCPC-mediated contractile enhancement in human cardiac tissues. These findings may open new avenues of research to exploit the contrasting and possibly synergistic cardioactive mechanisms of hMSCs and hCPCs to improve hECT performance in vitro, and optimize future stem cell based-cardiotherapies in vivo.

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W-2089

HUMAN VENTRICULAR CARDIAC TISSUE STRIPS ENGINEERED FROM PLURIPOTENT STEM CELLS ACCURATELY PREDICT INOTROPIC DOSE RESPONSES IN A BLINDED VALIDATION STUDY

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Traditional discovery and development of novel drugs and therapeutics for heart diseases continue to be an inefficient and expensive process. Although traditional animal models such as rodents, dogs and pigs are accessible, major species differences in anatomy and physiology limit their ability to predict pharmacological responses in humans. Cardiomyocytes derived from human pluripotent stem cells can potentially fill this gap, but conventional 2D cultures and experiments with single cells or disorganized clusters inadequately recapitulate the human cardiac phenotype. In this study, we systematically examined the pharmacological responses of our engineered human ventricular cardiac tissue strips (hvCTS), with a morphological appearance resembling that of native human trabecular muscle, to different categories of well-defined cardioactive drugs. Under baseline conditions, hvCTS developed a mean developed force of 76 micronewtons. As a validation step, we measured developed force in hvCTS (n=70) subjected to 13 drugs with known cardiovascular effects. When treated with known negative inotropes (verapamil, nifedipine, mibefradil and bepridil) and Class I antiarrhythmics (disopyramide and flecainide), the developed forces of hvCTS dose-dependently decreased. By contrast, positive inotropes including beta-agonists and phosphodiesterase inhibitors dose-dependently increased the developed forces. Drugs with no known inotropic effects (procainamide and tocainide) elicited no significant contractile responses. To test the predictive capacity of hvCTS, we next performed a blinded study of 17 drugs on 128 hvCTS, categorizing their effects on developed force into positive, negative or no inotropic effects and estimating their EC50 values. Upon unblinding of the results, 100% of negative inotropes, 86% of positive inotropes and 80% of drugs without known effects were accurately identified. We conclude that hvCTS can recapitulate known effects of cardiovascular drugs with prediction of potency via accurate estimation of EC50, and can be used as a highly efficient in vitro human model for screening of pharmacologically active agents.

W-2091

THE EFFECT OF STRUCTURAL OFFSET AND GRADIENT PORE SIZE ON OSTEOBLAST DIFFERENTIATION IN PCL SCAFFOLDS

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Melt elctrospinning writing (MEW) is a solvent free strategy allowing precise control of the configuration of electrospun fibers in 3D printed scaffolds 1. By controlling fibre diameter and geometry and the distribution and size of pores, scaffolds with increased surface area allowing, greater cell penetration and proliferation, nutrient/waste/gas exchange and improved mechanical properties, can be produced 2. This study describes the effect of 1) pore size, 2) pore size gradient and 3) fibre offset on osteogenesis in MEW 3D -poly caprolactone (PCL) scaffolds. The highest surface area was seen with offset scaffolds and calcium phosphate coating improved scaffold wettability. Mineralization, bone-specific gene and protein expression were assessed following 30 days cell culture with human osteoblasts. The 50% offset scaffold showed higher mineralization in response to osteogenic growth factors while high levels of expression of opn and ocn markers of bone differentiation were observed in the gradient scaffold.

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