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INDUCED NEURONAL DIFFERENTIATION OF HUMAN EMBRYONIC STEM CELLS ON THREE-DIMENSIONAL POLYMER SCAFFOLDS

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INTRODUCTION: Human embryonic stem (hES) cells have the potential to form various cell types. Because neurons have a limited capacity to regenerate, the derivation of neurons from hES cells holds promise to treat neurological pathologies of the central and peripheral nervous system, such as Parkinson's disease, Spinal cord injury, and Glaucoma. However, growth and differentiation of hES cells into complex, viable 3D neural-like tissue is challenging.

It was hypothesized that porous biodegradable polymer scaffolds support the formation of complex 3D tissues during differentiation of hES cells. The scaffold provides physical cues for cell orientation and spreading, and pores provide space for remodelling of tissue structures [1]. The chemical cues to induce and direct neuronal differentiation were hypothesized to be the Neurotrophins [2] and Retinoic Acid [3]. So far, the influence of the Neurotrophins on the differentiation of hES cells on 3D polymer scaffolds has not been described.

METHODS: Cell culture: Human embryonic stem (hES) cells (H9 clone) were grown in an undifferentiated status on mouse embryonic fibroblasts in knockout medium. To induce differentiation, hES cell colonies were dissociated with 1mg/ml collagenase type IV and suspended in differentiation media without lymphocyte inhibitory factor (LIF) and basic Fibroblast growth factor (bFGF). **Scaffold Preparation:** The scaffold consisted of a 50/50 blend of PLGA/ PLLA. The PLGA was selected to degrade quickly (≈3 weeks) to facilitate cellular ingrowth, whereas the PLLA was chosen to provide mechanical stiffness to support 3D structures. The pore size of 250μm-500μm was chosen to facilitate the seeding and ingrowth of cells. The sponges were cut into rectangular pieces of ≈4x4x1mm^3. **hES cell differentiation:** 4- and 9d-old Embryoid bodies (EB) were trypsinized and 0.9x10^6 cells were mixed of a 50% (vol/vol) medium and matrigel. The differentiation medium was supplemented with the Neurotrophins Brain Derived Neurotrophic Factor (BDNF) (20ng/ml), Nerve Growth Factor (NGF) (20ng/ml), Neurotrophin-3 (NT-3) (20ng/ml) each alone and combined with Retinoic Acid (RA) (300ng/ml). For all experiments EBs were grown on the PLLA/PLGA scaffolds for 14d. **Immunohistochemical Staining:** The cell-scaffold constructs were stained with Hematoxylin and Eosin and the primary antibodies Anti-human β_{III}-tubulin (1:500), nestin (1:1000), Cytokeratin-7 (1:25), CD31 (1:20) and SSEA-4.

RESULTS: The results show that neuronal differentiation of hES cells on three-dimensional polymer scaffolds can be directed by Neurotrophins such as BDNF, NGF, NT-3 and RA. The presence of capillary-like networks throughout the scaffolds especially those treated with NGF was shown.

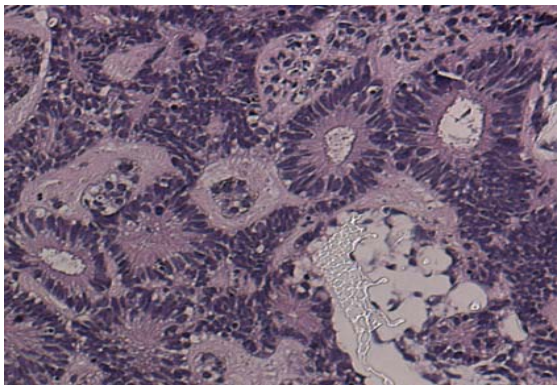

Fig. 1: Neural-like tissue derived from human embryonic stem cells on 3D polymer scaffolds

DISCUSSION & CONCLUSIONS: This approach provides a potential mechanism for creating viable human neural tissue structures for future therapeutic applications in neural pathologies such Parkinson's disease, Spinal cord injury, and Glaucoma.

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