

Unlocking the Potential of Stem Cells in Neuroscience Research

By Erik A Miljan^a and Dominique Fauvin^b

^aSimply Cells Ltd, 37 Elm Hayes, Corsham, UK, www.SimplyCells.co.uk;

^bAMS Biotechnology, 184 Milton Park, Abingdon, UK; Tel +44 (0) 1235 828 200; info@amsbio.com; www.amsbio.com

ABSTRACT

Stem cells have revolutionized our approaches and understanding of neuroscience. The advances within the field have been at an alarming pace since the early descriptions of *in vitro* differentiated electrophysiological active human neural stem cells in 2007⁽¹⁾. Today fully differentiated neuronal cell types such as astrocytes and ready-to-use complete kits are commercially available. Human stem cell neural differentiation is no longer an art since both 2D and 3D extracellular matrices and scaffolds and differentiation tools ensure robust and physiological relevant differentiation into mature neural cell types.

Introduction

Stem cells have provided the ideal model system in which to investigate diverse aspects of neural cell biology. The main reason for the widespread applicability of stem cells in neuroscience is the renewable and consistent supply of physiologically functional human cells within a controlled *in vitro* environment. There is an increasing trend in neuroscience to switch from biochemical assays, which have high target specificity but low physiological relevance, to stem cell based assays with multiple outputs and high physiological relevance. Currently, neural stem cell assays are widely performed by researchers on flat (2D) surfaces. However, there is increasing demand for more complex structures. Three-dimensional (3D) extracellular matrices and scaffolds address this demand by providing models for which stem cells are able to form more complex “organ-like” functions.

In nature, neural stem cells exist within a specialized niche within the brain. The niche represents the extracellular environment in which the stem cell resides. *In vitro*, the “niche” is comprised of the medium components and matrix

within the culture conditions. It is easy to understand that the *in vitro* culture conditions have a direct impact on the fate and functionality of the stem cells. AMSBIO supplies the industry with a comprehensive range of stem cell tools that promote stem cell growth and potent neuronal differentiation in both 2D and 3D matrices, in addition to the most potent stem cell neuronal differentiation agent, the synthetic retinoid ec23[®].

Neural Stem Cell Culture

Different stem cell and cell types have strong preferences for specific extracellular matrices that best mimic their *in vivo* environment. Neural stem cells and neurons are no exception and display a high affinity for the extracellular matrix protein laminin. Neural stem cells can be continuously renewed by expanding the culture on laminin coated tissue culture surfaces in the appropriate culture medium.

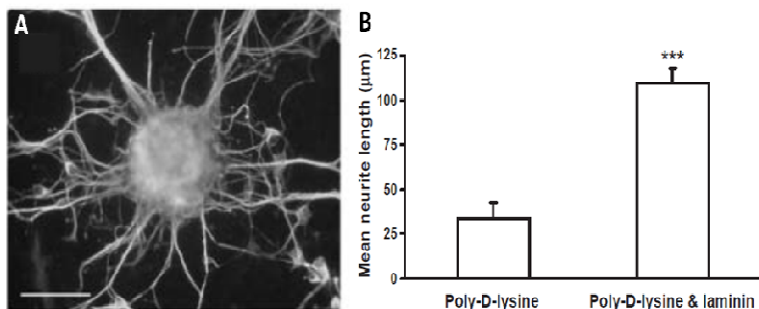


Figure 1: Enhancing the differentiation of neurons from neural stem cells and human pluripotent stem cells.

(A) Intact neurospheres formed from human pluripotent stem cells plated onto poly-D-lysine (10ug/ml) and laminin (10ug/ml) and grown for a further 7 days, sprout many neurites that radiate from the central aggregation of neuronal perikarya. Cell aggregates and their processes are highly positive for the neuronal marker β -tubulin-III as shown by immunofluorescent staining⁽⁴⁾. (B) The effect of coating culture surfaces with poly-D-lysine or poly-D-lysine and laminin on the growth of neurites from human neurons. Mean neurite length shown, $P=0.001$ ⁽³⁾.

Laminin coating of tissue culture surfaces could be carried out as described ⁽²⁾. Briefly, defrosted laminin (1mg/ml) should be diluted to 10µg/ml in basal medium. The entire culture surface is covered with the diluted laminin solution and the culture vessel incubated at 37°C for approximately 2 hours, followed by two equal volume washes with pre-warmed basal medium. The coated surface is now ready to accept the stem cells and pre-warmed medium. Note that a poly-D-lysine pre-coating may be required prior to laminin coating when using non-tissue culture treated (uncharged) plasticware. AMSBIO supplies the highest quality Cultrex® Pathclear Laminin, Cultrex® Stem Cell Qualified Laminin and recombinant MAPTrix™ Laminin mimetics to support neural stem cell culture.

Culturing neural stem cells is straightforward with ready to use medium and animal free culture medium components that include ecoHSA™ recombinant human serum albumin and growth factors, basic fibroblast growth factor (bFGF) and Epidermal growth factor (EGF), essential to sustain consistent stable growth.

Neuronal Stem Cell Differentiation

Differentiated neural cell types are equally at home in laminin as their predecessor stem cells. Pluripotent stem cells must be first “neutralized” prior to promoting neural differentiation. In order to achieve this, pluripotent stem cells are first grown in suspension culture to form neurospheres

and then plated on laminin coated tissue culture surfaces to induce differentiation. Neurons distinctly form following 7 days incubation after plating neurospheres on the laminin coated culture surface (Fig 1A). Neural stem cells are readily differentiated directly on laminin coated surfaces in the absence of growth factor mitogens, bFGF and EGF. As shown in Fig 1B, laminin significantly increases the neural differentiation of human neural stem cells as shown by an increase in neurite length, while the charged surface of Poly-D-lysine alone is not sufficient to support neuronal outgrowth ⁽³⁾.

Enriching Stem Cell Neural Differentiation

Controlling cell differentiation in a predictable way is a major challenge in stem cell research. As described above, stem cells may spontaneously differentiate into neurons simply by culturing on laminin in the absence of growth factors. However, neural differentiation is modest and uncontrolled in a spontaneous paradigm, as shown in Fig 2A, B that compares undifferentiated to control (spontaneously) differentiated. Why leave it up to chance - AMSBIO supplies a range of factors to drive and enhance stem cell differentiation. The stem cell fate regulator set II contains a set of eight small molecule modulators that are found to promote stem cell differentiation. These modulators are of interest to discover novel differentiation pathways in stem cell research.

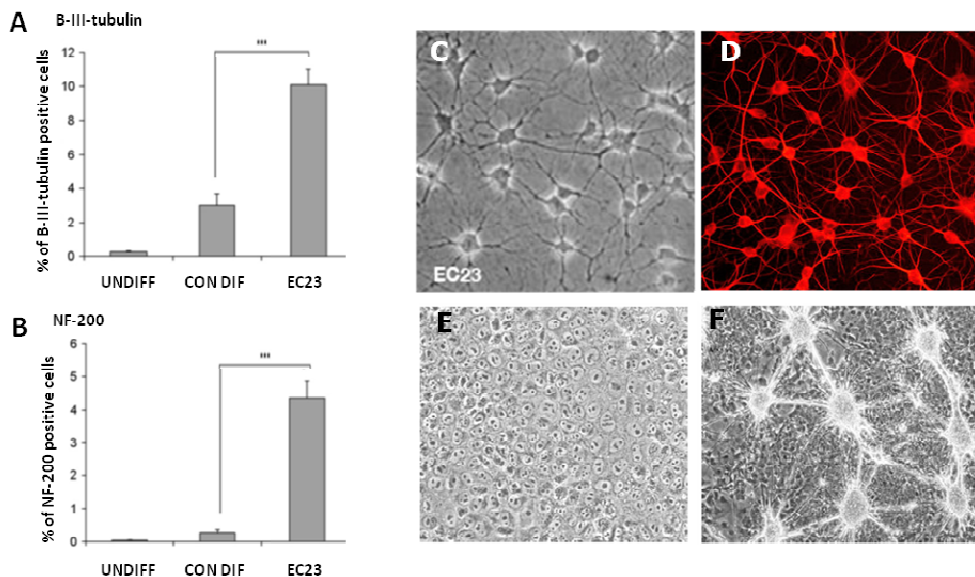


Figure 2: Synthetic retinoid ec23® induced differentiation: ec23® is a potent inducer of neurogenesis in adult neural stem cells and pluripotent stem cells. (A,B) Plots showing quantification of immunological data as performed by counting positively labelled cells. The increased number of β-III-tubulin-positive cells (A) and NF-200-positive cells (B) in retinoid-supplemented cultures was highly significant compared to results obtained with the standard differentiation protocol.

Values shown represent mean+ SEM, n = 9. ***_p = 0.0005 ⁽²⁾. (C, D) Rat adult hippocampal progenitor cells exposed to 10µM ec23® differentiate into neural cell types. Differentiation induced by exposure to ec23® was consistent and reproducible. Images show ec23® treated cells, phase contrast (C) and stained with β-tubulin-III (D). (E) Phase contrast micrograph of undifferentiated human TERA2.cl.SP12 EC stem cells. (F) Phase contrast micrograph of neural differentiated human TERA2.cl.SP12 EC cells treated for 28 days with ec23®. Note phase bright aggregations of neurons linked to each other by bundles of axons. Undifferentiated control cells displayed no expression of the neuronal marker; whereas, ec23-treated cultures contained areas of high expression. Scale bar: 50 µm ⁽⁴⁾.

Of particular interest to stem cell neural differentiation is the synthetic retinoid ec23[®]. Naturally occurring retinoids, such as All Trans-Retinoic Acid (ATRA), have long been known to be intimately involved in controlling stem cell neural differentiation pathways. However, compounds like ATRA are notoriously difficult to work with and lead to inconsistent results. The main reason is the highly unstable and light sensitive nature of naturally occurring retinoids. Degradation during storage and even during the experiment leads to unacceptable levels of variation. The synthetic retinoid ec23[®] provided by AMSBIO overcomes these challenges and delivers consistent results due to its long-term stability. The synthetic retinoid ec23[®] has been modified to increase the stability of the chemical structure, yet retains the potent activity of naturally occurring retinoids. The removal of growth mitogens and addition of synthetic retinoid ec23[®] to the culture medium (at 10 μ M concentration) significantly enhances neuronal differentiation of human neural stem cells at least 5 fold as shown by an increase in β -tubulin-III and Neurofilament 200 (NF200) positive neural cells (Fig 2 A, B). Neural stem cells differentiated with ec23[®] display extensive neurite processes and mature neural morphology (Fig 2 C, D). Neural differentiation of pluripotent stem cells is equally potent with ec23[®]. Undifferentiated pluripotent stem cells display typical “cobblestone” morphology (Fig 2E); whereas, the addition of 10 μ M ec23[®] drives pluripotent stem cells to a neural fate (Fig 2F).

3D Neural Differentiation – Forefront of Structure & Function

The potential of stem cells to differentiate into a multitude of cell types makes them extremely sensitive to their environment. Conversely, controlling a stem cell's extracellular environment can determine its fate. The ideology of early 20th century architects was that “form follows function”; however, contemporary stem cell biology is clearly opposite to this principle. Stem cells and differentiated cell types naturally exist within three dimensional structures. There are a number of 3D matrices and scaffolds currently available that provide physiologically relevant *in vitro* model platforms.

Recombinant and synthetic 3D matrices offer excellent alternatives to naturally occurring proteins. MAPTriX[™] HyGels[™] is semi-synthetic 3D extracellular matrices that are tailored to mimic specific ECM properties, without any animal derived components. These hydrogels are composed of two components: MAPTriX[™] ECM mimetics, which are small peptide mimics of extracellular matrices fused with recombinant adhesive protein (there are currently four different laminin motifs) and a multi-arm polyethylene glycol

derivative linker. The elasticity and pore size of the HyGel[™] may be easily engineered by adjusting the concentration of MAPTriX[™] ECM or MAPTriX[™] Linker. Use of MAPTriX[™] Laminin HyGel[™] generates a well-controlled and reproducible cellular environment for your 3D neural cell culture.

The 3D alvetex[®] synthetic scaffold is a thin membrane made from inert polystyrene with a well-defined and uniform micro-porous architecture (Fig 3A). The unique pore structure of alvetex[®] enables stem cells to migrate and differentiate within a 3D structure that is ideally suited for stem cell neuronal differentiation. The culture device is pre-fabricated, sterile, is ready to use off-the-shelf and can be handled in a similar manner as standard 2D plastic ware. Alvetex may be coated with laminin or recombinant matrices using the same protocols as with 2D plastic ware.

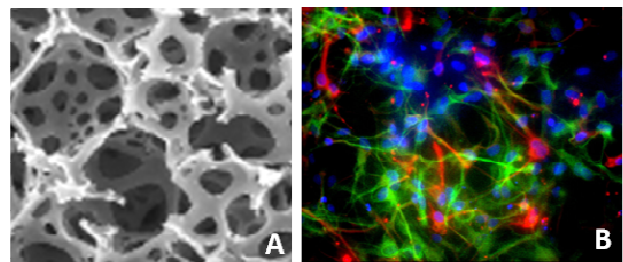


Figure 3: Neural stem cell differentiation on alvetex[®].

(A) Scanning electron micrograph of alvetex[®]. Large numbers of controlled sized interconnecting pores are formed during the manufacturing process, providing increased surface area and greater opportunity for 3D cell growth and differentiation⁽³⁾. (B) Neural stem cells differentiated on alvetex[®] scaffold. Immunocytochemistry performed for astrocytes GFAP (red); neurons β -tubulin-III (green), and Hoechst nuclear counterstain shown in blue. Images provided courtesy of Lara Stevanato, PhD, ReNeuron Limited, United Kingdom.

In addition to its ease of use, alvetex[®] provide an excellent platform to differentiate stem cells to neurons. Differentiated human neural stem cells form extensive projections and neural networks within the alvetex[®] structure (Fig 3B). Neural differentiation of pluripotent human ES cells within alvetex[®] is equally impressive. High magnification electron microscope imaging of ES cell differentiated with alvetex[®] shows with exquisite detail the extensive neural processes and extent of neural maturation within the scaffold (Fig 4, A-C)⁽⁵⁾.

Summary

Stem cells are powerful tools in Neuroscience research. The key to research success is the ability to control and direct differentiation into physiologically relevant neural cell types. Stem cell qualified reagents are pre-tested and remove lot-to-lot variability. The synthetic retinoid ec23[®] and animal-free reagents provide a defined *in vitro* assay system.

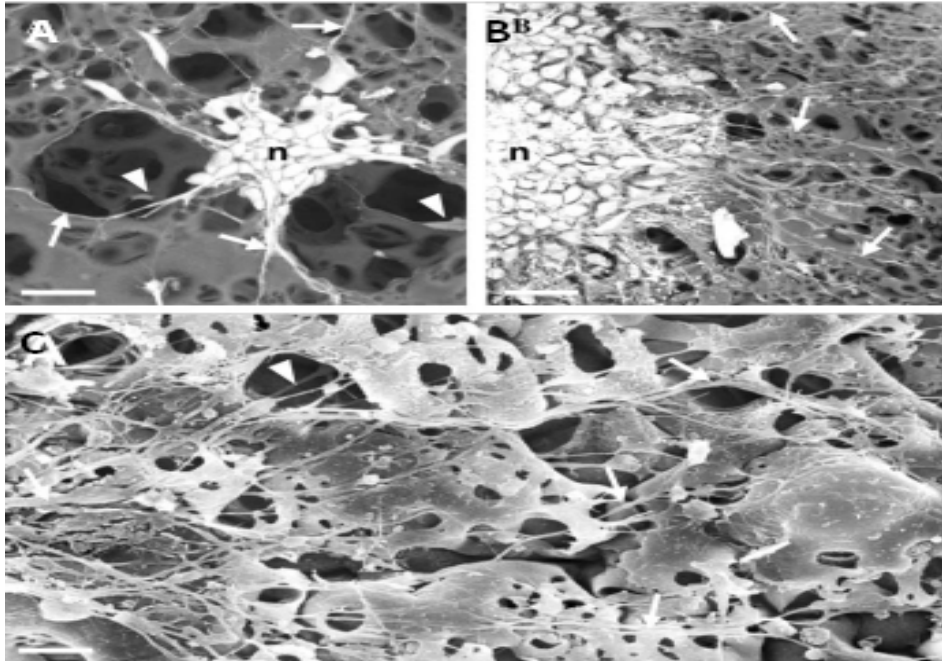


Figure 4: Examination of neurons differentiated from human pluripotent stem cells on alvetex® scaffolds using environmental scanning electron microscopy.

(A) Small aggregate of neuronal perikarya (n) expressing neural processes (arrows) are visible. Arrowheads indicate individual neurons sprouting neurites and located within voids of the matrix.

(B) Large aggregation of neuronal cells (n) radiating high numbers of nerve processes (arrows).

(C) High power scanning electron microscopic analysis of gold-coated samples of human neurons grown on alvetex® matrix. Neurite outgrowth over the surface (arrows) and within the voids of the polymer (arrowhead) is shown.

Scale bars: (A, B) 20 µm; and (C) 3 µm. Source ⁽⁵⁾.

Cultrex® natural extracellular matrices, recombinant MAPTrix™ extracellular matrix mimetics, and synthetic alvetex® scaffold provide excellent platforms to differentiate adult neural stem cells and pluripotent ES cells alike.

Discover today the difference the environment makes to your neural differentiation experiments, thanks to the cutting-edge stem cell reagents and tools that AMSBIO provides, from stem cell source to cryopreservation.

Supplementary Information

AMSBIO is the global source for the following products. alvetex® and ec23® are registered trade mark of and manufactured by Reinnervate. MAPTrix™ is a trade mark of Kollodis Bioscience Inc. Cultrex® products are trade mark of Trevigen Inc.

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www.amsbio.com - info@amsbio.com

 <small>UK and rest of the world</small>	<p>Tel: +44 (0) 1235 828 200 Fax: +44 (0) 1235 820 482</p>	 <small>DE</small>	<p>Tel: +49 (0) 69 779099 Fax: +49 (0) 69 13376880</p>
 <small>CH</small>	<p>Tel: +41 (0) 91 604 55 22 Fax: +41 (0) 91 605 17 85</p>	 <small>US</small>	<p>Tol Free: +1 800 987 0985 Tel: +1 949 265 7717 Fax: +1 949 265 7703</p>

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