Australian Endodontic Journal
The Journal of the Australian Society of Endodontology Inc., the Australian and New Zealand Academy of Endodontists and the Asian Pacific Endodontic Confederation

Aust Endod J 2018; 44: 186–194

INVITED REVIEW

Cell-based therapies and natural compounds for pain

Rachel Shparberg, BMedSci (Hons)[1]; and Edward R. Vickers, PhD, MDSc, MScMed, MArt, BDS, FFPMANZCA, Dip Herbal Medicine[2]

1 Bosch Institute, Discipline of Physiology, School of Medical Sciences, University of Sydney, Sydney, New South Wales, Australia
2 Sydney Medical School, University of Sydney, Sydney, New South Wales, Australia

Keywords
endocannabinoids, polyamines, polyphenols, stem cells.

Correspondence
Rachel Shparberg, Clinical Stem Cells: PO Box 6471, Alexandria, NSW 2015, Australia.
Email: rshp1808@uni.sydney.edu.au

doi: 10.1111/aej.12256

(Accepted for publication 12 January 2018.)

Abstract

Cell-based therapies and natural compounds are increasingly being acknowledged for their potential roles in managing a variety of conditions, including pain. Stem cells have gained attention over the years for their contribution to our understanding of normal and abnormal physiology, their capacity to assist in efficient drug design and screening and their role in regenerative medicine. This chapter will review our current understanding of the complex pathophysiology of pain using adult, embryonic and induced pluripotent stem cells as models. In addition, this chapter outlines several natural compounds that are used in the treatment of neuropathic pain, with a focus on evidence-based information of the polyphenols, polyamines and endocannabinoids derived from plants and food sources. Advantages of these compounds are the higher margins of safety, low incidence of side effects. Recent evidence shows several polyphenols can induce differentiation of healthy stem cells yet inhibit cancer stem cells.

Stem Cells

An introduction to stem cells

Stem cells are undifferentiated cells that have the potential to both self-renew whilst maintaining a normal karyotype, and, when the appropriate signals become available, are able differentiate into a variety of mature cell types. Their capacity to differentiate, or potency, depends on where the stem cell population has been derived from. If a stem cell is defined as *totipotent*, it has the ability to produce all the cells that are required for multicellular organism development including cells of both the embryonic and extraembryonic lineages, for example the zygote. Stem cells that are *pluripotent* (such as embryonic and induced pluripotent stem cells), have the ability to differentiate into somatic, germ and some extraembryonic tissue, but have lost the ability to form the placental lineage. If a stem cell is defined as being *multipotent*, this means it is restricted into differentiating into cells that give rise to the cell lineage that it was derived from. For example neural stem cells have the capacity to give rise only to cells of the neural lineage. Finally, when a cell is said to be *terminally differentiated*, it has reached its developmental capacity and can no longer undergo further differentiation, including mature somatic cells such as neurons.

There are three main types of stem cells: Embryonic, adult and induced pluripotent (iPSCs), which we will now discuss in more detail in regards to their individual characteristics and uses in the laboratory and clinic.

Embryonic stem cells

Embryonic stem cells (ESCs) are those that are derived from the inner cell mass (ICM) of the blastocyst-stage embryo from a variety of species, including the 4–5 day human embryo (1,2). At this stage of development, the blastocyst contains a population of cells known as the inner cell mass (ICM). This population of ICM cells transition into a second pluripotent population known as the primitive ectoderm. Following gastrulation of the primitive ectoderm, the three multipotent germ layers (definitive mesoderm, endoderm and ectoderm) arise. This is then followed by the differentiation of mature cell types (for a detailed review on early mammalian embryology, see (3)). Similarly, ESCs, like the ICM are able to transition to a population of cells analogous to the primitive

© 2018 Australian Society of Endodontology Inc

ectoderm (early primitive ectoderm-like cells) (4), followed by differentiation into cells of the three germ layers and germ cells (5–7) (Fig. 1).

Embryonic stem cells were first successfully derived from the mouse ICM and maintained in culture in 1981 (8), followed by the derivation of human ESCs in 1998 (1). ESCs express markers of pluripotency (including Oct4, Nanog and Sox2), giving them the ability to differentiate when the appropriate differentiation signals become available (9–11). This means that is it possible to manipulate the culture conditions to either (i) keep ESCs as a self-renewing, pluripotent, rapidly dividing population (12,13), or, (ii) culture them in an environment conducive to cell-specific differentiation, also known as "directed differentiation" (14–17). Due to these unique properties, ESCs have been widely used to study the molecular mechanisms that underlie normal and abnormal development, disease progression, the stem cell niche, therapeutic screening and drug design (18,19) (see below).

Adult stem cells

Adult stem cells, also known as somatic stem cells, are undifferentiated, multipotent cells that can differentiate into lineage-specific cell types. They retain the ability to self-renew over extended periods of time, but have lost the capacity to differentiate into cells of other developmental germ layers (20) (Fig. 1). Adult stem cells are defined by their tissue of origin, and thus express lineage-specific markers. They are often located in a stem cell niche within their organ of origin. It is the constituents of this niche (including, but not limited to, growth factors, cytokines, cell–cell interactions, etc.) that control the behaviour of an adult stem cell, and thus forms the basis of many experimental and therapeutic stem cell studies (21–24). Dental pulp stem cells for example reside specifically in the dental pulp tissue, and can give rise to cells of the neural crest lineage including peripheral neurons and cells involved in craniofacial structures (25). They are thus a possible candidate for regenerative-based medicines due to their capacity to differentiate into a number of cells types (26,27), as well as their abilities to produce signals required for cell survival and differentiation (23,26).

The primary role of adult stem cells *in vivo* is to repair and rejuvenate damaged tissue. Like that of ESCs, adult stem cells have been used *in vitro* to study the molecular mechanisms of cell-specific adult cell development and disease, the stem cell niche as well as drug design and

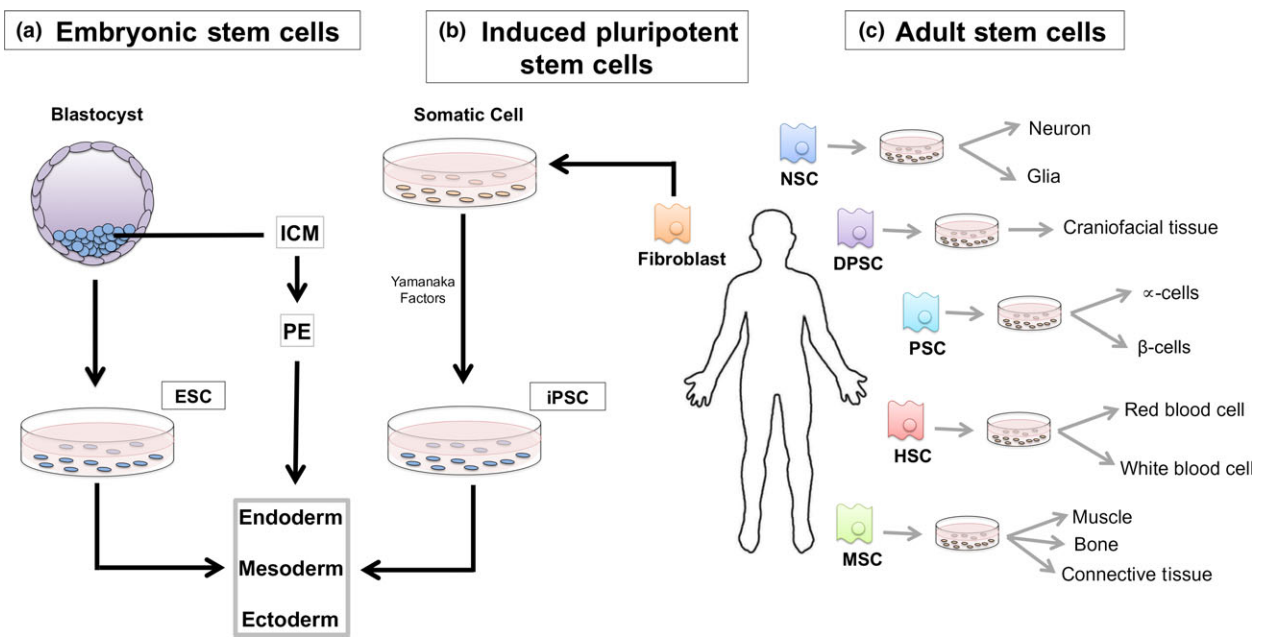

Figure 1 Derivation of human stem cells. (a) Embryonic stem cells are derived from the inner cell mass (ICM) of the blastocyst-stage embryo. In vivo, the ICM transitions into the primitive ectoderm (PE), and following gastrulation, the formation of the three primary germ layers are formed, followed by differentiation into mature cells. Similarly, ICM cells can be cultured in vitro as ESCs, which can spontaneously differentiate into cells of the three primary germ layers. (b) Induced pluripotent stem cells (iPSCs) are derived from mature human cell types (such as fibroblasts) and are reprogrammed back into a pluripotent cell type that can be cultured indefinitely in vitro. Like ESCs, iPSCs can differentiate into mature cell types of the three multipotent germ layers. (c) Adult stem cells such as neural stem cells (NSCs), dental pulp stem cells (DPSC), pancreatic stem cells (PSCs), haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) are multipotent (and thus lineage restricted) cells that are derived from mature adult and embryonic organs.

© 2018 Australian Society of Endodontology Inc

screening. In recent years, adult stem cells, in particular, mesenchymal stem cells (MSCs), have generated much excitement for their potential role for autologous cell-based therapies (28–31) (discussed below).

Induced pluripotent stem cells

Whilst ESCs and adult stem cells have provided us with a platform for understanding development and disease in the culture dish, their use in cellular therapies is not as well accepted or well-studied. hESC lines are often derived from unused *in vitro* fertilisation (IVF) embryos (that would otherwise be discarded). However, some people believe there are ethical issues in using them (32,33). In Australia, stem cell research and therapies are regulated under strict laws that protect those involved in the process (including the patient, researchers and doctors) (34). The other major problems associated with stem cell therapies from donor sources (allogeneic therapy), is the concept of tissue rejection. These issues, amongst others, have meant that stem cell research has been difficult to translate into clinical medicine.

A technology that is currently at the forefront of medical research is induced pluripotent stem cells (iPSCs) (35,36). iPSCs are laboratory manufactured cells that are derived from mature adult cells. These mature adult cells (most commonly fibroblasts), are reprogrammed back to an 'ESC-like' state (37,38). They contain the genomic DNA from the organism from which they were originally derived from. Therefore, if injected back into the host (as an autologous cell therapy), an immune response is evaded, as the body recognises these cells as 'self'. This technology has the potential to overcome the medical and ethical issues surrounding ESC and allogeneic adult stem cell therapies (39,40).

The first iPSCs were made in 2006, and were derived from mouse fibroblasts (35). A year later, iPSCs were developed from human fibroblasts (36). This discovery revolutionised the way we think about cell biology: The ability to revert a mature cell 'backwards' to a pluripotent embryonic-like cell was once thought to be impossible. However, with the addition of just four transcription factors into the genome of mature adult cells (Fig. 1), Yamanaka and his team have changed the face of regenerative stem cell biology and regenerative medicine, winning the Nobel Prize for Physiology and Medicine in 2012 (35,36). These factors, known as Yamanaka Factors, include Oct4, Nanog, Klf4 and c-Myc – each of which are expressed in the pluripotency networks within ESCs. Whilst we are still very much at the experimental stages, it may be likely that iPSC technology will be the future of personalised regenerative medicine (39,41,42).

Understanding the pathophysiology of nerve damage and pain using stem cells: The translation of basic cell science into cell therapies

The ultimate goal of stem cell research is to generate homogeneous cell populations in large enough quantities to be used to regenerate damaged and/or diseased tissue. Whether the cells themselves rejuvenate the tissue, or whether they secrete factors that recruit endogenous supplies of stem and mature cells, is relatively poorly understood. Being able to understand how stem cells remain in their undifferentiated state, as well as having a thorough understanding of what prompts them to migrate, proliferate and differentiate is a key area of research leading to the development of successful, safe and innovative stem-cell-based therapies. The interaction between cells and their local environment, both *in vivo* and *in vitro,* is thus a fundamental aspect that must be considered if cell therapies are to be efficiently and successfully developed for clinical use.

Turning our focus to the stem cell niche, a study conducted by Nosrat *et al.* (23) showed that when co-cultured in the presence of rat dental pulp cells, rat trigeminal neurons produce extensive neurite networks compared to those co-cultured in the presence of fibroblasts. It was later shown that these dental pulp cells secreted factors including brain-derived neurotrophic factor (BDNF), glial-derived neurotrophic factor (GDNF), neural growth factor (NGF) that guide neuronal growth and survival (23). Similarly, injection of dental pulp cell grafts into the spinal cords of these rats promoted motor neuronal regeneration (23). The authors suggested that, rather than the stem cells differentiating and forming new tissue, it is the release of these neurotrophic factors that supported the growth and survival of the neurons. The release of these neurotrophic factors (which are naturally secreted into the dental pulp niche) of the developing tooth is what is likely to cause tooth innervation during development (23). Along the lines of this research, two studies conducted by Sasaki *et al.* (43,44), similarly used dental pulp cells to regenerate nerve tissue in a rat model of facial nerve damage (43). The dental pulp cells were seeded in collagen-embedded silicon tubing, and following 2 weeks post-surgery, facial nerve regeneration had occurred (43). Presumably, these same neurotrophic signals secreted by the dental pulp cells facilitated the growth and survival of the endogenous neuronal population (45–47). Coupled with improved biomaterial technology, this same group designed a Poly-DL-lactide-co-glycolide (PLGA) scaffold to replace the former non-biodegradable silicon tubing. Similar to their initial study, facial nerve regeneration was achieved in

 © 2018 Australian Society of Endodontology Inc

the presence of the dental pulp cells, and the PLGA tubing was resorbed after 2 months, preventing the need for secondary removal surgery (44).

Whilst animal studies have provided considerable insight into the normal physiology of nerve development, disease and nociception, their role in understanding chronic neuropathic pain are not as well characterised. The lack of available human tissue for experimentation has confounded our ability to assess pain experimentally. A major gap still lies in our ability to translate this 'benchside' pain research from animal models, into safe and effective human therapies and intervention, due in part to the subtle differences of mice and humans (48). As a result, very few drug developments have arisen from animal studies that target the cause of pain. The ideal scenario would be to use human tissue *in vitro*, to produce large quantities of mature cells of interest (in this instance, neurons and associated tissue) to assess the pathophysiology of pain.

Stem cells provide a scalable system for modelling normal mammalian physiology and disease. With the discovery of human-derived stem cells, they provide a system for assessing human- (and potentially down to patient-) specific drug targets for combatting disease. Understanding the normal biology of human cells, organs and systems as a whole entity is a crucial aspect if stem cell therapies are to be developed for human disease prevention and treatment.

The use of human-derived stem cells has thus been a key driver in understanding these molecular mechanisms underlying many conditions, including pain. Based on an understanding of normal embryonic nociceptor development, a differentiation protocol was developed by Chambers *et al.* (49) to produce a near-homogeneous population of nociceptors from human ESCs (hESCs). Step-wise small-molecule inhibition firstly produced Sox10+ neural crest cells, followed by NTRK1+ nociceptors of the peripheral nervous system (49). This protocol provided the first efficient model for studying nociceptor development. These nociceptors were shown to be phenotypically, genetically and functionally equivalent to those found in the adult dorsal root ganglion (49): They expressed high levels of glutamate (excitatory neurons) as well as voltage-gated sodium channels (SCN9A (Nav1.7), SCN10A (Nav1.8), SCN11A (Nav1.9) and noxious-stimuli-responsive vanilloid receptors (such as TRVP1). These cells had thus undergone differentiation from unspecialised hESCs to functionally active nociceptors. A follow-up study using electrophysiology techniques later showed that these cells expressed functional ion channels that have been implicated in the pathophysiology of pain and sensory disorders (including HCN1, KCNQ2/3 and $GABA_AR$) (50). This protocol, amongst others, has subsequently been used to screen for neurotoxic agents and therapeutic drugs to block hyperexcitability of these known targets associated with pain (49–53).

Cell-based therapies for neuropathic pain and nerve damage

Cell-based therapies are those which rely on intact, living cells to be used on affected patients to promote repair and rejuvenation of damaged tissue. Currently, most cell therapies are still in experimental phases, with very few available for commercial use. As neuropathic pain responds poorly to pharmaceutical intervention, mesenchymal stem cell (MSC) and adipose-derived stem cell (ADSC) therapy has been suggested as a means of potential autologous therapy for patients with neurological-based diseases (28,29,54,55). MSCs and ADSCs are found endogenously within the body and have been shown to reduce inflammation by releasing cytokines involved moderating inflammation. They also retain the ability to differentiate into a vast variety of cell types involved in tissue healing (56). Similarly, they have also been shown to express neuotrophic factors such as BDNF, GDNF and NGF to promote neuronal growth and survival (30). MSCs have been used in a variety of animal model studies to successfully repair damaged neurons, whereby injections of MSCs have shown to improve motor recovery, allodynia and hyperalgesia symptoms (57). Similarly, intravenous injections of bone marrow stromal cells (BMSCs) (58) and neural stem cells (NSCs) (59) have been shown to alleviate pain-associated symptoms in rat models. However, very few published articles are available on the use of human stem cell therapies for the treatment of pain. Of those available, they are mostly in clinical trial phases. A recent Australian study involving a cohort of female patients (aged between 22 and 80 years of age) were treated with a single dose of autologous MSC therapy for chronic neuropathic pain (28). This preliminary study demonstrated reduction in patient pain scores, accompanied by a significant reduction in daily pain medication requirements, indicating biological (rather than psychological) effects of the treatment. The researchers suggested that rather than the MSCs themselves differentiating and rejuvenating damaged tissue, MSCs secrete factors that promote the growth, repair and survival of endogenous tissue, as described in section 'Understanding the pathophysiology of nerve damage and pain using stem cells: The translation of basic cell science into cell therapies'. This is the first successful autologous stem cell therapy study that has been shown to alleviate peripheral neuropathic pain in human patients.

© 2018 Australian Society of Endodontology Inc

Natural compounds

Recent developments in molecular technology have yielded the discovery of new analgesic compounds from our environment in fruits, vegetables, plants, insect and spider venoms and several aquatic species including fish and cone shell venoms. Several of these compounds are of specific interest for treating neuropathic pain.

Endocannabinoids

The endocannabinoids system is found in humans and mammalian species and serve a number of critical roles in pain, the immune system, appetite and mood. The cannabinoid receptors, abbreviated as CB1 and CB2, are expressed in the CNS and PNS (mainly CB1) and the immune system (CB2). Palmitoylethanolamide (PEA) is a major compound researched for analgesia and has substantial published documentation for its use to treat neuropathic pain (60) and with a high margin of safety (61). The compound is a fatty acid amide and has been known for its analgesic activities for 60–70 years and is a well-known antineuropathic agent in Europe but virtually unheard of in North America and Australia. It is safe, virtually free of side effects and has few drug interactions compared with typically used conventional antidepressants and anticonvulsants (62). Humans synthesise PEA endogenously and the chemical is found and processed from natural sources of egg yolk, soybean and peanut oil. It is a white powder, insoluble in water (soluble in methanol for mass spectrometric chemical analysis) and classified as a nutraceutical or food supplement.

It targets several receptors including CB1, CB2, PPAR-alpha receptor and TRPV. It is one of the few compounds available for pain physicians to reduce noradrenaline release and sympathetic nervous system phenomena (sympathetically maintained pain). Capsaicin, the bioactive compound from chillies and capsicum used in topical formulations to treat oral mucosal and dermal neuropathic pain is structurally similar to the endocannabinoids. PEA is used therapeutically in both the oral systemic dose form and topical cream form. It has a high margin of safety with nil effects reported from a daily dose of 4.5 g in healthy volunteers. In addition to PEA, there are a further recently identified group of endogenous lipid molecules termed maresins, resolvins and protectins. These bioactive compounds are anti-inflammatory mediators first reported a decade ago (63). They are very potent and active at picogram concentrations and have several distinctive chemical isomers named D-series resolvins (Resolvin D1 to Resolvin D6), protectins (including protectin D1-neuroprotectin D1) and maresins (MaR1 and MaR2) (64). They have both acute and chronic anti-inflammatory action (65).

Polyphenols (phytotherapeutic compounds)

The polyphenol compounds are recognised as a source of molecules with potential antinociceptive and antineuropathic actions (66). Polyphenols are plant-derived bioactive compounds with an estimated 8000 chemical variants and consumed daily by humans as they are in fruit, vegetables, grains, herbal condiments and herbal therapeutic compounds. The chemical structure is multiple phenolic hydroxyl groups attached to a number of aromatic rings. Considering the long presence of plants on the planet and the need to counter viral, bacterial, fungal inflammation and infections over tens of millions of years they together have developed an extraordinary repertoire of defensive mechanisms. Modern pharmaceutical drug design utilises large libraries of (in part) naturally occurring chemicals to develop new drugs. In addition, modern technology using separation phase chromatography combined with rapid mass spectrometric identification is able to purify the individual chemicals to assess respective therapeutic targets. Many plant and herbal compounds demonstrate potent anti-inflammatory, anticancer activities and broad spectrum antimicrobial defence properties. They are generally well tolerated with less or negligible side effects in humans due to our exposure from thousands of years of food intake. Several of these polyphenols are now the subject of intensive research and several key compounds of capsaicin, curcumin, resveratrol and quercetin are briefly discussed.

Capsaicin is derived from hot chillies and capsicum and is consumed throughout the world, especially a popular food ingredient in Asia. The first use of capsaicin to treat neuropathic pain was arguably Professor James T. Kent an American homeopathic physician who interestingly described its use to treat ‘constant burning pains’ of the gingivae and tongue in 1895. Since its rediscovery by pain researchers in the 1980s it has been extensively researched. It has been shown to have safety and efficacy to treat neuropathic trigeminal pain and dermal neuropathy (67,68) with topical concentrations ranging from 0.025% (67) to 8% (69). Curcumin is derived from turmeric is another widely used food ingredient in Asia. It has anticancer (70), anti-inflammatory (71) and also antineuropathic pain activity (72). Curcumin has multiple effects and inhibits the TNF-α and nitric oxide release in a dose dependent manner (73). Resveratrol has shown to attenuate neuropathic pain in a dose dependent model and has biphasic pharmcologic effects where it prevents expression of proinflammatory molecules and promotes expression of anti-inflammatory molecules (animal

 © 2018 Australian Society of Endodontology Inc

model) (74). It is found in berries and red wine. Quercetin is generally recognised as a first line anti-inflammatory compound in the complementary medicine field. It is widely distributed in vegetables and herbal preparations and found in high concentrations in broccoli and capers. It is also found in high concentrations (with kaempferol) in raspberry leaf herbal extract and recently has shown safety and efficacy to treat oral lichen planus (28) and an animal model of neuropathic pain (75).

Further research has also shown a direct effect of polyphenols on both healthy and cancer stem cells. Multiple in vitro studies reveal the anticancer stem cell activity of quercetin, kaempferol, curcumin, apigenin and resveratrol. Conversely for healthy stem cells, baicalin from the herb skullcap promotes neuronal differentiation and resveratrol promotes expansion and differentiation of osteoblasts from mesenchymal stem cells.

Polyamines

Polyamines (PAs) are naturally occurring compounds found in plants, animals and humans. They are critical for cellular function and survival. Chemically the polyamines have 2 or more-NH2 primary amino groups and can be of linear or cyclic structure. The major biological PAs are putrescine, spermine, spermidine and cadaverine. The multiple -NH2 groups give the compounds a distinctive odour. For example cadaverine is the smell in decomposing cadavers and putrescine ('putrid' odour) is from the breakdown of fatty acids in putrefying tissue of dead animals. Chemosignaling from the compounds have been shown to be involved in the 'threat' survival experience. Even small amounts of sweat from humans exposed to a fear experience elicited sympathetic responses when exposed to a control group via the olfactory system (termed necrophobic behaviour and is estimated to have evolved 420 million years ago (76). Cadaverine is a similar foul-smelling compound produced by bacterial decarboxylation of lysine. Spermine and spermidine are interesting biomolecules in a number of physiological mechanisms. Spermine was so termed from its original discovery in sperm in its crystalline form in the 17th century and is responsible for the identifiable odour of sperm. It is derived chemically from its precursor ornithine and intermediary putrescine. Serum ornithine is found in higher concentrations in patients with persistent musculoskeletal pain (77). Spermidine is also derived from putrescine. The roles of these compounds have been to provide essential mechanisms in maintaining longevity of cells through their anti-inflammatory properties, regulating cell metabolism and proliferation. From a pain perspective there are several processes regulating calcium, potassium and sodium ions, and ion passage through the NMDA receptor which can have substantial effects on pain therapy. Polyamines are found in foods such as broccoli, cauliflower and citrus fruits. A recent study showed a polyamine-deficient diet resulted in a rapid reduction of high intensity pain in acute pain (78,79). In addition, subcutaneous injections of spermidine or spermine triggered mechanical allodynia and oedema (rat model) (80). In contrast, co-administration of fentanyl and spermine vastly increases the analgesic efficacy (15×) of fentanyl (81). The effect of spermine on stem cells is to induce osteogenic differentiation, and spermidine enhances autophagy in anti-ageing processes.

Conclusions

Stem cell science is a fast moving field, at the forefront of medicine. With the advancements in gene editing techniques, biotechnology and bioengineering capabilities, our understanding of cell biology is becoming increasingly more defined (82–87). Since the establishment of ESCs in 1981, our understanding of the complexity of human development and disease has improved, to a point where we are starting to harness the regenerative capabilities of stem cells for potential therapies. Whilst most stem cell therapies are predominantly still in laboratory phases (animal- and cell-based research), there is a growing number of human clinical trials available, some of which show promising potential for reversing and/or treating disease (28,31). Understanding the basic biology of how stem cells choose their fate (of which we still have much to learn about) is key to developing successful, efficient and safe cell-based therapies in the field of regenerative medicine. In addition, naturally occurring compounds comprise a vast new source of targeted therapeutics for analgesic, anti-inflammatory and anticancer medicine. Incubating natural compounds with stem cells in the laboratory has produced surprising discoveries for 21st century regenerative medical and oral health.

Acknowledgements

None.

Authorship declaration

(i) All authors have contributed significantly, and (ii) all authors are in agreement with the manuscript.

Disclosure statement

The authors declare no financial or conflict of interest.

© 2018 Australian Society of Endodontology Inc

References

1. Thomson JA. Embryonic stem cell lines derived from human blastocysts. Science (80-) 1998;282 (5391):1145–7.
2. Pera MF, Reubinoff BE, Fong C-Y, Trounson A, Bongso A. Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro. Nat Biotechnol 2000; 18 (4): 399–404.
3. Loebel DAF, Watson CM, De Young RA, Tam PPL. Lineage choice and differentiation in mouse embryos and embryonic stem cells. Dev Biol 2003; 264(1): 1–14.
4. Rathjen J, Lake JA, Bettess MD, Washington JM, Chapman G, Rathjen PD. Formation of a primitive ectoderm like cell population, EPL cells, from ES cells in response to biologically derived factors. J Cell Sci 1999; 112(Pt 5): 601–12.
5. Poh Y-C, Chen J, Hong Y *et al.* Generation of organized germ layers from a single mouse embryonic stem cell. Nat Commun 2014; 5: 4000.
6. van den Brink SC, Baillie-Johnson P, Balayo T *et al.* Symmetry breaking, germ layer specification and axial organisation in aggregates of mouse embryonic stem cells. Development 2014; 141(22): 4231–42.
7. West JA, Park I-H, Daley GQ, Geijsen N. In vitro generation of germ cells from murine embryonic stem cells. Nat Protoc 2006; 1(4): 2026–36.
8. Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. Nature 1981; 292(5819): 154–6.
9. Kalkan T, Smith A, Nichols J *et al.* Mapping the route from naive pluripotency to lineage specification. Philos Trans R Soc Lond B Biol Sci 2014;369(1657):487–92.
10. Hackett JA, Surani MA, Abranches E *et al.* Regulatory principles of pluripotency: from the ground state up. Cell Stem Cell 2014;15(4):416–30.
11. Medvedev SP, Shevchenko AI, Mazurok NA, Zakian SM. OCT4 and NANOG are the key genes in the system of pluripotency maintenance in mammalian cells. Russ J Genet 2008; 44(12): 1377–93.
12. Ying QL, Nichols J, Chambers I, Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 2003; 115(3): 281–92.
13. Hall J, Guo G, Wray J *et al.* Oct4 and LIF/Stat3 additively induce Krüppel factors to sustain embryonic stem cell self-renewal. Cell Stem Cell 2009; 5(6): 597–609.
14. Bouhon IA, Kato H, Chandran S, Allen ND. Neural differentiation of mouse embryonic stem cells in chemically defined medium. Brain Res Bull 2005; 68(1–2): 62–75.
15. Pankratz MT, Li XJ, Lavaute TM, Lyons EA, Chen X, Zhang SC. Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. Stem Cells 2007; 25(6): 1511–20.
16. Murry CE, Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. Cell 2008; 132(4): 661–80.
17. Ying QL, Stavridis M, Griffiths D, Li M, Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. Nat Biotechnol 2003; 21 (2): 183–6.
18. Prajumwongs P, Weeranantanapan O, Jaroonwitchawan T, Noisa P. Human embryonic stem cells: a model for the study of neural development and neurological diseases. Stem Cells Int 2016; 2016: 1–9.
19. Pouton CW, Haynes JM. Embryonic stem cells as a source of models for drug discovery. Nat Rev Drug Discov 2007; 6 (8): 605–16.
20. Burgess R. Stem cells: a short course. USA: John Wiley and Sons, Incorporated; 2016. 338 p.
21. Ferraro F, Celso Lo C, Scadden D. Adult stem cells and their niches. Adv Exp Med Biol 2010; 695: 155–68.
22. Fuentealba LC, Obernier K, Alvarez-Buylla A. Adult neural stem cells bridge their niche. Cell Stem Cell 2012; 10 (6): 698–708.
23. Nosrat IV, Widenfalk J, Olson L, Nosrat CA. Dental pulp cells produce neurotrophic factors, interact with trigeminal neurons in vitro, and rescue motoneurons after spinal cord injury. Dev Biol 2001; 238(1): 120–32.
24. Gurok U, Steinhoff C, Lipkowitz B, Ropers H-H, Scharff C, Nuber UA. Gene expression changes in the course of neural progenitor cell differentiation. J Neurosci 2004; 24(26): 5982–6002.
25. Gilbert SF. Developmental biology, 8th ed. Massachusetts, USA: Sinauer Associates; 2006. 373–400 p.
26. Tatullo M, Marrelli M, Shakesheff KM, White LJ. Dental pulp stem cells: function, isolation and applications in regenerative medicine. J Tissue Eng Regen Med 2015; 9 (11): 1205–16.
27. Ashri NY, Ajlan SA, Aldahmash AM. Dental pulp stem cells. Biology and use for periodontal tissue engineering. Saudi Med J 2015; 36(12): 1391–9.
28. Vickers ER, Karsten E, Flood J, Lilischkis R. A preliminary report on stem cell therapy for neuropathic pain in humans. J Pain Res 2014; 7: 255–63.
29. Mazzini L, Mareschi K, Ferrero I *et al.* Mesenchymal stromal cell transplantation in amyotrophic lateral sclerosis: a long-term safety study. Cytotherapy 2012; 14(1): 56–60.
30. Dadon-Nachum M, Sadan O, Srugo I, Melamed E, Offen D. Differentiated mesenchymal stem cells for sciatic nerve injury. Stem Cell Rev Rep 2011;7(3):664–71.
31. Mackay-Sim A, Féron F, Cochrane J *et al.* Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain 2008;9:2376–86.
32. Lo B, Parham L. Ethical issues in stem cell research. Endocr Rev 2009;30(3):204–13.
33. Leist M, Bremer S, Brundin P *et al.* The biological and ethical basis of the use of human embryonic stem cells for

© 2018 Australian Society of Endodontology Inc

in vitro test systems or cell therapy. Altex 2008; 25(3): 163–90.
34. Stem cells, cloning and related issues | National Health and Medical Research Council [Internet]. 2014 [cited 2017 Aug 27]. Available from: https://www.nhmrc.gov.au/about/nhmrc-committees/embryo-research-licensing-committee/human-embryos-and-cloning/stem-cells-clon
35. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4): 663–76.
36. Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 2007;131(5):861–72.
37. González F, Boué S, Belmonte JCI. Methods for making induced pluripotent stem cells: reprogramming à la carte. Nat Rev Genet 2011; 12(4): 231–42.
38. Ban H, Nishishita N, Fusaki N *et al.* Efficient generation of transgene-free human induced pluripotent stem cells (iPSCs) by temperature-sensitive Sendai virus vectors. Proc Natl Acad Sci 2011; 108(34): 14234–9.
39. Ohnuki M, Takahashi K. Present and future challenges of induced pluripotent stem cells. Philos Trans R Soc Lond B Biol Sci 2015;370(1680):20140367.
40. Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. Nat Rev Drug Discov 2015; 14(10): 681–92.
41. Kim C. iPSC technology–Powerful hand for disease modeling and therapeutic screen. BMB Rep 2015; 48(5): 256–65.
42. Inoue H, Nagata N, Kurokawa H, Yamanaka S.. iPS cells: a game changer for future medicine. EMBO J 2014;33 (5):409–17.
43. Sasaki R, Aoki S, Yamato M *et al.* Tubulation with dental pulp cells promotes facial nerve regeneration in rats. Tissue Eng Part A 2008; 14(7): 1141–7.
44. Sasaki R, Aoki S, Yamato M *et al.* PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. J Tissue Eng Regen Med 2011;5(10):823–30.
45. Fine EG, Decosterd I, Papaloïzos M, Zurn AD, Aebischer P. GDNF and NGF released by synthetic guidance channels support sciatic nerve regeneration across a long gap. Eur J Neurosci 2002;15(4):589–601.
46. Lee AC, Yu VM, Lowe JB *et al.* Controlled release of nerve growth factor enhances sciatic nerve regeneration. Exp Neurol 2003; 184(1): 295–303.
47. Sakamoto T, Kawazoe Y, Shen J-S *et al.* Adenoviral gene transfer of GDNF, BDNF and TGFβ2, but not CNTF, cardiotrophin-1 or IGF1, protects injured adult motoneurons after facial nerve avulsion. J Neurosci Res 2003; 72(1): 54–64.
48. Lin S, Lin Y, Nery JR *et al.* Comparison of the transcriptional landscapes between human and mouse tissues. Proc Natl Acad Sci 2014; 111(48): 17224–9.
49. Chambers SM, Qi Y, Mica Y *et al.* Combined small-molecule inhibition accelerates developmental timing and converts human pluripotent stem cells into nociceptors. Nat Biotechnol 2012; 30(7): 715–20.
50. Young GT, Gutteridge A, Fox HDE *et al.* Characterizing human stem cell-derived sensory neurons at the single-cell level reveals their ion channel expression and utility in pain research. Mol Ther 2014; 22(8): 1530–43.
51. Eberhardt E, Havlicek S, Schmidt D *et al.* Pattern of functional TTX-resistant sodium channels reveals a developmental stage of human iPSC- and ESC-derived nociceptors. Stem Cell Rep 2015;5(3):305–13.
52. Cao L, McDonnell A, Nitzsche A *et al.* Pharmacological reversal of a pain phenotype in iPSC-derived sensory neurons and patients with inherited erythromelalgia. Sci Transl Med 2016;8(335):335ra56.
53. Hoelting L, Klima S, Karreman C *et al.* Stem cell-derived immature human dorsal root ganglia neurons to identify peripheral neurotoxicants. Stem Cells Transl Med 2016;5 (4):476–87.
54. Fortino VR, Pelaez D, Cheung HS. Concise review: stem cell therapies for neuropathic pain. Stem Cells Transl Med 2013;2(5):394–9.
55. Oh K-W, Moon C, Kim HY *et al.* Phase I trial of repeated intrathecal autologous bone marrow-derived mesenchymal stromal cells in amyotrophic lateral sclerosis. Stem Cells Transl Med 2015; 4(6): 590–7.
56. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells – current trends and future prospective. Biosci Rep 2015;35(2):e00191.
57. Yousefifard M, Nasirinezhad F, Shardi Manaheji H, Janzadeh A, Hosseini M, Keshavarz M. Human bone marrow-derived and umbilical cord-derived mesenchymal stem cells for alleviating neuropathic pain in a spinal cord injury model. Stem Cell Res Ther 2016;7:36.
58. Guo W, Wang H, Zou S *et al.* Bone marrow stromal cells produce long-term pain relief in rat models of persistent pain. Stem Cells 2011; 29(8): 1294–303.
59. Franchi S, Valsecchi AE, Borsani E *et al.* Intravenous neural stem cells abolish nociceptive hypersensitivity and trigger nerve regeneration in experimental neuropathy. Pain 2012; 153(4): 850–61.
60. Skaper SD, Facci L, Fusco M *et al.* Palmitoylethanolamide, a naturally occurring disease-modifying agent in neuropathic pain. Inflammopharmacology 2014;22(2):79–94.
61. Paladini A, Fusco M, Cenacchi T, Schievano C, Piroli A, Varrassi G. Palmitoylethanolamide, a special food for medical purposes, in the treatment of chronic pain: a pooled data meta-analysis. Pain Physician 2016; 19(2): 11–24.
62. Gatti A, Lazzari M, Gianfelice V, Di Paolo A, Sabato E, Sabato AF. Palmitoylethanolamide in the treatment of chronic pain caused by different etiopathogenesis. Pain Med 2012; 13(9): 1121–30.

© 2018 Australian Society of Endodontology Inc

63. Serhan CN, Yang R, Martinod K *et al.* Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. J Exp Med 2009; 206(1): 15–23.
64. Serhan CN, Dalli J, Colas RA, Winkler JW, Chiang N. Protectins and maresins: new pro-resolving families of mediators in acute inflammation and resolution bioactive metabolome. Biochim Biophys Acta - Mol Cell Biol Lipids 2015; 1851(4): 397–413.
65. Dalli J, Serhan C. Macrophage proresolving mediators-the when and where. Microbiol Spectr 2016; 4(3): 37–54.
66. Boadas-Vaello P, Vela J, Verdu E. New pharmacological approaches using polyphenols on the physiopathology of neuropathic pain. Curr Drug Targets 2016; 18(2): 160–73.
67. Vickers ER, Cousins MJ, Walker S, Chisholm K. Analysis of 50 patients with atypical odontalgia. A preliminary report on pharmacological procedures for diagnosis and treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998; 85(1): 24–32.
68. Pak D, Hung JC. Post-herpetic neuralgia. In: Yong R, Nguyen M, Nelson E, Urman R, eds. Pain medicine. Cham: Springer International Publishing; 2017. pp. 553–5.
69. Dey S, Belgrade M, Macedo F. (392) Postherpetic neuralgia treatment with high concentration capsaicin patch: three Cases demonstrating reduction in pain receptive field without associated reduction in pain intensity. J Pain 2017;18(4):S72.
70. Shehzad A, Lee YS. Curcumin in human cancer prevention and treatment. In: Mahabir S, Pathak YV, eds. Nutraeuticals and health: review of human evidence. CRC Press; 2013. p. 27–42.
71. Hussain Z, Thu HE, Amjad MW, Hussain F, Ahmed TA, Khan S. Exploring recent developments to improve antioxidant, anti-inflammatory and antimicrobial efficacy of curcumin: a review of new trends and future perspectives. Mater Sci Eng, C 2017; 1(77): 1316–26.
72. Ceyhan D, Kocman AE, Yildirim E, Ozatik O, Aydin S, Aydan K. Comparison of the effects of curcumin, tramadol and surgical treatments on neuropathic pain induced by chronic constriction injury in rat. Turk Neurosurg 2017; 27: 1–8.
73. Sharma S, Kulkarni SK, Agrewala JN, Chopra K. Curcumin attenuates thermal hyperalgesia in a diabetic mouse model of neuropathic pain. Eur J Pharmacol 2006; 536(3): 256–61.
74. Tao L, Ding Q, Gao C, Sun X. Resveratrol attenuates neuropathic pain through balancing pro-inflammatory and anti-inflammatory cytokines release in mice. Int Immunopharmacol 2016; 34: 165–72.
75. Ji C, Xu Y, Han F *et al.* Quercetin alleviates thermal and cold hyperalgesia in a rat neuropathic pain model by inhibiting Toll-like receptor signaling. Biomed Pharmacother 2017; 94: 652–8.
76. Wisman A, Shrira I. The smell of death: evidence that putrescine elicits threat management mechanisms. Front Psychol 2015; 28(6): 1274.
77. Mäntyselkä P, Ali-Sisto T, Kautiainen H *et al.* The association between musculoskeletal pain and circulating ornithine: a population-based study. Pain Med 2016;18 (6):pnw285.
78. Estebe J-P, Degryse C, Rezzadori G *et al.* Tolerance and efficacy of a polyamine-deficient diet for the treatment of perioperative pain. Nutrition 2017; 36: 33–40.
79. Ferrier J, Bayet-Robert M, Pereira B *et al.* A polyamine-deficient diet prevents oxaliplatin-induced acute cold and mechanical hypersensitivity in rats. Price TJ, editor. PLoS ONE 2013;8(10):e77828.
80. Silva MA, Klafke JZ, Rossato MF *et al.* Role of peripheral polyamines in the development of inflammatory pain. Biochem Pharmacol 2011; 82(3): 269–77.
81. Serdyuk SE, Gmiro VE. IEM-1460 and spermine potentiate the analgesic actions of fentanyl and analgin in rats. Neurosci Behav Physiol 2015;45(6):680–3.
82. Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. Science 2014; 345(6194): 1247125.
83. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nat Cell Biol 2016; 18(3): 246–54.
84. Gjorevski N, Ranga A, Lutolf MP. Bioengineering approaches to guide stem cell-based organogenesis. Development 2014; 141(9): 1794–804.
85. Yin X, Mead BE, Safaee H, Langer R, Karp JM, Levy O. Engineering stem cell organoids. Cell Stem Cell 2016; 18 (1): 25–38.
86. McMurray RJ, Dalby MJ, Tsimbouri PM. Using biomaterials to study stem cell mechanotransduction, growth and differentiation. J Tissue Eng Regen Med 2015; 9(5): 528–39.
87. Horii T, Hatada I. Production of genome-edited pluripotent stem cells and mice by CRISPR/Cas [Review]. Endocr J 2016; 63(3): 213–9.

 © 2018 Australian Society of Endodontology Inc