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REVIEWS

Special Issue: Plasma Biotechnology

Spotlight

Cold Atmospheric Plasmas: A Novel and Promising Way to Treat Neurological Diseases

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Cold atmospheric plasmas (CAPs) can enhance neural cell differentiation into neurons both *in vitro* and *in vivo*, which is of great interest for medical treatment of neurodegenerative diseases like Alzheimer's disease and traumatic injuries of the central nervous system. CAPs represent a promising method for future neurological disease therapy.

A neurological disease is any disease that affects the central, peripheral, and vegetative nervous system, including brain trauma, spinal injury, and Alzheimer's and Parkinson's diseases [1]. With the isolation of neural stem cells (NSCs) and the development of cloning technology, central nervous tissue (CNS) transplantation is considered to be one of the most promising therapies for neurodegenerative and neurotraumatic diseases. However, the methods for preparing the desired nerve cells are limited by the drawbacks of the existing methods, which include chemicals, Chinese medicine, hormones, and a few other treatments. These treatments may cause chemical toxicity, form glial scars after transplantation, and suffer from insufficient selectivity of specific cell type differentiation. Recently, a novel and promising method using cold atmospheric plasmas (CAPs) has been proposed to overcome these disadvantages.

Plasma is the fourth state of matter, following solid, liquid, and gas. With the development of CAPs in the past two decades, plasma medicine as a new research field has attracted significant attention worldwide [2]. Highly reactive species (reactive oxygen and nitrogen species), electrical fields, UV radiation, and charged particles generated by plasma enable this mixture to be applied in cancer therapy, skin wound healing, root canal treatment, and other biological applications, even including cell proliferation and differentiation [3]. In a recent study, Jang and colleagues [4] showed that a CAP based on a dielectric barrier discharge (DBD; an electrical discharge method used to create plasma between two electrodes separated by an insulating dielectric barrier) successfully induced neural differentiation both *in vitro* and in zebrafish, and the results showed promise for the future treatment of neurological disease (Figure 1). They also investigated the physicochemical and biological connection between the CAP cascade and the TrK/Ras/ERK signaling pathway that causes neural differentiation.

This study used mouse neuroblastoma Neuro 2A (N2a) cells for the *in vitro* investigation and a zebrafish (*Danio rerio*) transgenic embryo Tg(Huc:GFP) for the *in vivo* investigation. The treatment was conducted with a DBD plasma, using a mixture of N_2 and O_2 as a working gas, with input power ~1 W. The CAP-treated Neuro 2A cells increased in size to more than four times that of untreated cells, exhibiting a maximum neurite length of around 70 μm and average of 46.3 ± 1.5 μm after 24 hours under optical treatment. The neural progenitor cells terminally differentiated into mature neurons, specifically including catecholaminergic dopaminergic (DA) neurons, which are the main source of dopamine in the CNS and play an important role in the control of multiple brain functions. Furthermore, the loss of DA neurons is strongly related to Parkinson's disease.

In vivo 1-minute CAP treatment of transgenic zebrafish embryos expressing GFP only in postmitotic mature neurons showed that the *in vitro* results are also physiologically interesting. GFP started to increase in intensity after a 6-hour incubation and maintained intensity for up to 33 hours. GFP^+ mature neurons in the developing zebrafish were clearly visible in the CNS within 6 hours after 36-hours postfertilization (hpf) embryos were exposed to CAP. In later-stage embryos (60 hpf), a 1.17-fold increase in GFP^+ mature neurons was observed in the CAP-treated group.

In terms of the biological mechanism of the plasma treatment, the authors found that NO served as an upstream extracellular messenger, while mitochondrial $\cdot O_2$ and cytosolic H_2O_2 cooperatively acted

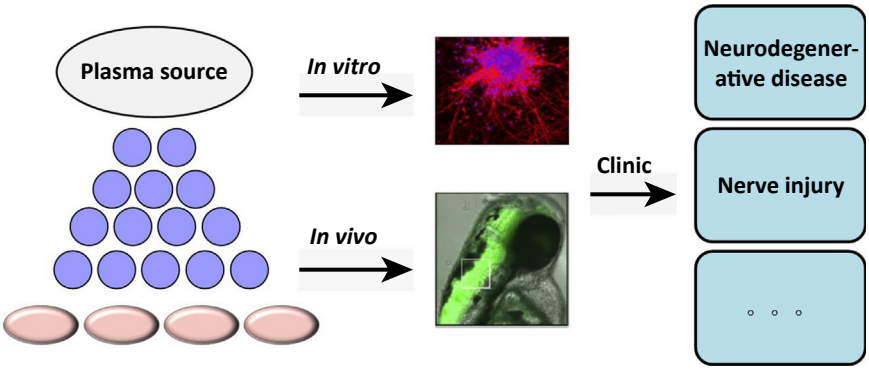

Figure 1. Promising Cold Atmospheric Plasma (CAP) Treatment for Neurological Disease. Both nerve cells and the *in vivo* nervous system treated by CAPs showed accelerated differentiation and selective differentiation into neurons, both of which may have great potential for future clinical treatment of neurological disease.

as messenger reactive oxygen species that played critical roles in communicating with cells during CAP-induced neural differentiation.

In a similar study, another research team successfully induced NSC differentiation *in vitro* by using a different CAP source: a micro-plasma jet device [3]. This earlier study showed rapid and selective NSC differentiation in C17.2-NSC murine NSCs (with ~75% of NSCs differentiating into neurons) and in primary rat NSCs. However, the investigation by Jang and colleagues further enhanced the neural differentiation in animals to a specific class of mature neurons, which is an important step for the future treatment of neurological diseases using CAPs.

CAP-enhanced NSC and progenitor cell differentiation offers several advantages compared with typical chemical methods that employ retinoic acids, resveratrol, or serum starvation. According to this new finding, differentiation with CAP was 2–3 times faster and showed 2–2.5-fold better differentiation efficiency. Moreover, 70% of the mature neurons induced by CAP were catecholaminergic DA neurons, which are especially desired in treating Parkinson's disease. Furthermore, plasmas are readily controllable by changing the electrical power supply, input power, working gas, or plasma source device. Plasma biomedical effects depend tremendously on plasma dosage: generally, low plasma dosage can enhance proliferation, differentiation, and migration, while high plasma dosage leads to cell death. Under certain conditions, CAPs have been observed to not harm treated cells, either *in vitro* or *in vivo* [5]. Therefore, this fast, controllable, one-step, and safe method for enhanced and selective neural cell differentiation will be a promising option in the future treatment of neurological disease.

CAP enhancement and selectivity of neural cell differentiation represents a promising advance for the future therapy of neurological disease, but this is a new field of application that still has a long way to go. Future work should focus on optimizing CAP treatments for neural cell differentiation, using CAP to treat more realistic models of neurological disease, and studying the response of the nervous system to CAP, while also considering side effects such as mutagenicity or cell death induced by overdose.

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Forum

Plasma in Dentistry: Brief History and Current Status

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We briefly discuss the history of cold atmospheric plasma (CAP) applications in dentistry. The reasons for seeking innovative solutions in dentistry are reported, highlighting results showing the potential of plasma along with some still-open questions. Finally, we suggest the next steps on the road from the laboratory to the dental chair.

The Year Zero of CAP in Dentistry

The idea of using CAP – an ionized gas where the temperature of heavy particles, responsible for the macroscopic plasma temperature, is much lower than the electronic temperature – for innovative dental procedures was first proposed by Stoffels and colleagues. In their most relevant paper on the subject [1], they demonstrated the capability of CAP to kill one of the most significant microorganisms promoting the development of tooth decay (e.g., dental caries), *Streptococcus mutans*, and envisioned the use of CAP in clinical practice to battle dental caries. Despite the early state of their research, the authors of this study felt rightfully compelled to support their proposal with at least some considerations of treatment safety, running the experiments under low-plasma-power operating conditions to maintain the temperature of the treated samples below a safe threshold value and to avert the formation of destructive filamentary electrical discharges.

CAP-Assisted Endodontic Therapy: A Dream Becoming Reality?

Since this initial demonstration of inactivation of oral pathogens by CAP, many researchers have started to investigate its potential for various dental treatments; the area that has been most extensively studied involves a clinically and economically relevant practice in dentistry: endodontic therapy. Also known as root canal treatment, this practice is required when the pulp of a